The Effect of Ayurvedic Plant Extracts-- *Mucuna pruriens* and *Brassica oleracea*--on the Delay of Motor Symptoms in PINK1 *Drosophila melanogaster*: A Model of Parkinson's Disease

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"Parkinson's Disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic (DA) neurons in the *substantia nigra,"* negatively affecting motor control and causing symptoms such as dyskinesia, or uncontrollable, involuntary muscle movement[1]. The purpose of this study was to explore a safe, affordable, and accessible method of treatment for such symptoms. It was hypothesized that when *Mucuna pruriens* (*Mpe*) and *Brassica oleracea (B. oleracea)* extracts are administered to PINK1 *Drosophila, Brassica oleracea* would delay the loss of motor ability in the PINK1 flies the longest because it contains sulforaphane which activates the Nrf2 pathway, promoting antioxidant activity and countering oxidative stress. To quantify the climbing ability of the PINK1 *Drosophila*, which display PD-like symptoms, a climbing assay was conducted on the treatment groups, where the flies were administered either 32mg/100g *Mpe* or *B. oleracea,* and the control group, which was administered nothing. After running an ANOVA and t-test on the results of this experiment, it was determined that only *Mpe* had a significant effect on the climbing ability of PINK1 *Drosophila.* The t-test displayed that the difference between the Mpe treatment group and the control group was significant, but the difference between the *B. oleracea* treatment group and the control was not statistically significant. Therefore, only *Mpe* effectively delayed the loss of climbing ability in PINK1 *Drosophila*, meaning it can potentially be used to treat the motor symptoms of PD in the future.

Introduction

"Parkinson's disease (PD) is the second most prevalent neurodegenerative disease in the world"[1]. Seven to ten million people suffer from this disease everyday[2]. PD is a "characterized by the selective loss of dopaminergic (DA) neurons in the *substantia nigra,"* a basal ganglia structure of the midbrain that is responsible for the regulation of movement and reward[1]. With both motor and non-motor symptoms, PD is a chronic progressive disease that impairs quality of life, health, and movement. Motor symptoms discussed in this paper include tremor, bradykinesia, and rigidity, which are characterized by the loss of controllable movement. Because there is no known cure for PD, most therapeutic treatments are aimed at alleviating symptoms. However, there is a lack of treatments that aim towards the reversal or regression of the disease[3]. Therefore, there is concern regarding the availability and effectiveness of the common symptomatic treatments for Parkinson's disease. Although clinical studies have been performed to explore a cure, many developing countries would still not be able to access it. According to scholars in the Department of Elderly Medicine at North Tyneside General Hospital, "the majority of people with PD in sub-Saharan Africa are undiagnosed and untreated, resulting in impaired quality of life and increased mortality rates"[4]. Therefore, finding a safe, affordable, and effective method of treatment using Ayurvedic extracts is the aim of this comparative study.

The conducted study targets various properties possessed by Aryuvedic extracts and their preventative abilities. Traditional Ayurvedic medicine, commonly practiced in Asian countries, exercises the use of herbal treatments to treat many ailments, such as wound healing and even liver disorders[5]. Because of the availability of these natural therapeutic agents, they are a favorable alternative to those who can not afford sustainable healthcare. Clinical studies have been conducted on various Ayurvedic agents with healing properties, but because *Brassica oleracea* and *Mucuna Pruriens* have so rarely been studied on PINK1 drosophila, and seldom compared, this study fills that gap by exploring the differences in their properties that would lead to their success or failure in trying to slow the regression of the flies' impaired climbing ability. This study compares various properties of each plant extract in order to determine the factors that most contribute to the prolonging of loss of motor symptoms. This study will benefit further exploration of herbal treatments to alleviate the motor impairments caused by PD.

Literature Review

Disadvantages of Current Treatments for Parkinson's Disease

Although PD is mainly caused by genetic factors, mitochondrial dysfunction, and inflammation are main causes of the strenuous motor symptoms[3]. Apoptosis (selective cell death) and oxidative stress, or the imbalance between antioxidants and free radicals in one's body also play a role. Many researchers have been working to find treatments throughout the years, but with these causes, disorders such as bradykinesia, rigidity, tremor-at-rest, flexed posture arise, and loss of postural reflexes continually cause uncontrollable movement of the body[6].

As of now, the "golden standard" medication for PD is L-DOPA, which can cross the protective blood-brain barrier where the central nervous system converts it into dopamine, increasing its concentrations in the brain[7]. However, long term use of L-DOPA can even induce dyskinesia, or the impairment of voluntary movement. This problem has been mitigated by using dopamine agonists to lessen the use of L-DOPA, but his could cause a greater risk of drug reaction and has been known to cause side effects, such as ankle and leg oedema, impulse control disorders, hallucinations, confusion and psychosis. Similarly, Amantadine, another dopamine agonist, has been used as an antidyskinetic agent to reduce the L-DOPA induced dyskinesia, but the effects of this also lessen over time[6]. However, current treatments are not easily accessible, nor are they affordable for those who lack resources. There is currently a lack of universal L-DOPA availability, which can be presently seen in various countries[8]. Therefore, this study will compare a more natural remedy (*Brassica oleracea)* with a potential L-DOPA replacement (*Mucuna pruriens*), in order to further explore safer, long-term treatment options for the motor symptoms of PD.

Antioxidant and Protective Properties of *Brassica oleracea*

Oxidative stress, a major cause of PD, is expressed as a factor that "leads to a decrease in antioxidant pathways, resulting in altered oxygen consumption and interrupted redox homeostasis" [9]. It is characterized by an imbalance of the free radicals and the body's ability to detoxify and

repair the damage caused by the unstable atoms. There is evidence suggesting that increased damage to lipids, proteins, and DNA is caused by oxidative stress[10]. However, sulforaphane is a phytochemical found in *Brassica oleracea* (*B. oleracea*) that is said to prevent oxidative stress-induced cytotoxicity through the activation of the Nrf2-antioxidant responsive pathway, which can protect the body against free radicals[11]. A study completed in 2010, by professors at the University of Ulsan College of Medicine, in the Department of Biochemistry and Molecular Biology, suggests that Sulforaphane can also benefit the alleviation of symptoms by protecting dopaminergic cells from cytotoxicity through the removal of intracellular Dopamine quinones and degrading misfolded proteins[12]. Similarly, as demonstrated by previous researchers in a study that used Rotenone to induce neurodegeneration, Sulforaphane inhibited apoptosis of neurons through the restoration of the mTOR pathway. Furthermore, it reduced dopaminergic neurodegeneration and corrected oxidative damage by activating the Nrf2 pathway[13]. By regulating the cells and allowing for antioxidant activity, *B. oleracea* presents as a promising future treatment for PD.

L-DOPA Containing Herbal Remedy: *Mucuna pruriens*

Unlike the previously mentioned agent, *Mucuna pruriens (Mpe)* can be used to combat PD because they contain L-DOPA, which increases dopamine concentration, making it one of the most effective extracts for alleviating PD symptoms[7].In a study done by professors of biotechnology, health sciences, and neuroscience, using *Drosophila and Caenorhabditis elegans, Mpe* was shown to have reduced hydrogen peroxide-induced cytotoxicity and ameliorated dopaminergic concentration, due to its L-DOPA content[14]. However, in a 2014 study done by Poddighe et al., from the department of Biomedical Sciences at the University of Cagliari, *Mpe* was found to have increased the tyrosine hydroxylase levels and restored damaged mitochondria, independently of L-DOPA. They also discovered that the administration of 0.1% *Mpe* greatly reduced *PINK1* mutants' motor impairment[7]. *Mpe* has a reduced level of L-DOPA than the "golden standard" L-DOPA, but redeems its worth through the other biological agents that improve the motor symptoms of PD. In another 2014 study by Jansen et al., "L-DOPE treated flies had a significantly decreased climbing ability compared with Zandopa," which contains *Mpe*[6]. This further induces the notion that *Mpe* has intrinsic properties that act to delay loss of motor ability. However, according to a 2015 article, long term consumption of *Mucuna pruriens* has also been linked to vomiting, nausea, abdominal distention, spermatogenic loss, etc.[15]. In this study, it will be determined if *Mpe* can act as an alternative for direct administration of L-DOPA, since it is associated with less serious long term side effects and can potentially mitigate the issue of the L-DOPA unavailability.

PINK1 *Drosophila melanogaster* as a Model Organism for PD

D. melanogaster PINK1 mutants display several phenotypic characteristics of PD, including dopaminergic neurodegeneration, mitochondrial dysfunction, and locomotor defects[16]. The PINK1 gene, which is responsible for encoding mitochondrially targeted protein kinase, plays a key role in mitochondrial quality control through phosphorylation of chaperones (assistants of conformational folding or unfolding), and regulation of mitophagy, or the elimination of dysfunctional mitochondria to protect cells from damage. Once PINK1 gathers on the outer membrane of a damaged mitochondria, Parkin (another gene) is activated, which triggers selective autophagy, where damaged cells are eliminated[17]. Consequently, mutations in the PINK1 gene can lead to an increase in damaged protein/mitochondria, which can further cause oxidative stress or death of healthy cells. PINK1 is commonly associated with PD because it displays these qualities. Therefore, this study utilized PINK1 mutant *Drosophila* as a model of Parkinson's disease. It was hypothesized that when *Mucuna pruriens* (*Mpe*) and *Brassica oleracea (B. oleracea)* extracts are administered to PINK1 *Drosophila, Brassica oleracea* would delay the loss of motor ability in the PINK1 flies the longest because it contains sulforaphane which activates the Nrf2 pathway, promoting antioxidant activity and countering oxidative stress. A climbing assay was conducted to quantify the effect of the extracts on the climbing ability of PINK1 *Drosophila*.

Methods

The PINK1 *Drosophila* from the Bloomington Stock center were transferred into a culture chamber upon arrival. PINK1 *Drosophila* were used because of their mutation that causes them to have Parkinson-like symptoms[9]. The following ratio of the *Drosophila* food/water/yeast was adapted from Carolina Biological's instructions to account for the dosage that was later administered. 0.75 oz of *Drosophila* food medium and 0.75 oz of tap water were placed at the bottom of the culture chamber [6]. 5-10 grains of Fleischmann's Active Dry Yeast were also placed on the medium[18]. Furthermore, netting was placed into the chamber. Two minutes after the medium settled, the container that the PINK1 *Drosophila* had initially come in was tapped against a surface to cause the flies to fall to the bottom of the container, thus preventing them from escaping. Then, the plug was removed, and the container was flipped and tapped to transfer the flies to a new container. Larvae were left in the container that they had arrived in for growth to adulthood. Once flies emerged, they were transferred to the new chamber as well. The culture chamber was left out of direct sunlight, in a shaded environment at 23 degrees Celsius[19].

After their period of growth, which lasted 12 days, the flies were anesthetized with FlyNap. Gloves and safety goggles were used during this process. The wand provided by Carolina Biological was dipped into the FlyNap solution once. The plug for a fly chamber was removed and the wand was left in the top corner of the chamber for 4 minutes. The wand and plug were removed. After being anesthetized, the flies were counted and equally distributed into three separate culture chambers with at least 20 flies in each container. The procedures for creating the initial culture chamber were used to set up the next three. In two separate chambers, 8 milligrams of *Mucuna pruriens* and *Brassica oleracea* extract were mixed in with fly medium[6]. In a 2014 experiment done by Jansen et al., from the School of Agriculture, Food and Rural Development at Newcastle University, dosages that have been used in human trials were equated to the dosages used for the flies per 100g of medium. Since 12.3–31.8 mg of *M. prurien* extract were suggested for use on *Drosophila*, the ratio of 32mg/100g was used for the *M. prurien* extract. In a similar study, Bellew-Dunn administered *B. oleracea* extract at 1% w/w. However, because high doses of *B. oleracea* extract can be detrimental to *Drosophila* and 32mg/100g is a ratio that is less than 1% w/w, the *B. oleracea* extract was administered at 32mg/100g to control the confounding variable of dosage difference[20]. The last chamber contained a standard fly medium.

On the day of administration of the extracts, a negative geotaxis, or climbing assay was conducted. For this procedure, 20 flies from an experimental group were placed in a 20 cm × 2.5 cm glass graduated cylinder. They were tapped down in a vial before starting to climb[21]. The flies were allowed 10 seconds to acclimate per trial. The number of flies passing the 8 cm mark in this time was recorded for each trial[22]. These flies were labeled as "escaped" flies. In a study done by Rose et al., from the Department of Life and Environmental Sciences, at the University of Cagliari, the percentage of flies that crossed a certain mark in a vial was recorded for each trial[22]. A study done by Siddique et al. viewed the climbing ability of *Drosophila* over time, by doing similar trials every 3 days for 24 days[5]. The methods of these studies were adapted in the conducted study. Therefore, in the current study, there were 5 trials for each experimental group. The mean percentage of flies that had passed the 8cm mark in ten seconds was calculated. Due to the limitation of time, this process was repeated every other day, for only 16 days, in order to determine the effectiveness of the extracts in delaying the loss of motor ability over time[5]. This methodology for experimentation allows for the deterioration or rescue of motor ability in *Drosophila* over time to be viewed. A two-way ANOVA and a post-hoc t-test were conducted using Excel to determine the significance of the results[7].

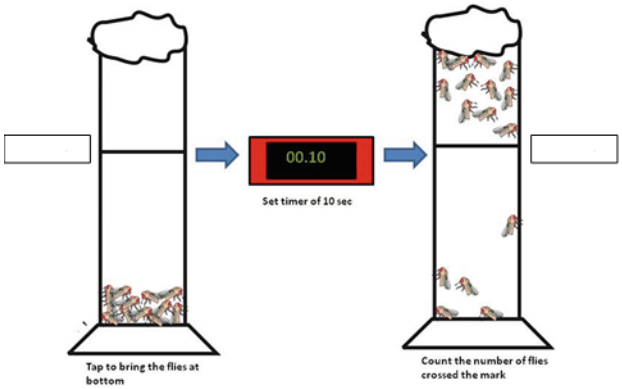


Figure 1 This is a graphic of the climbing assay, where the flies are initially tapped down to the bottom and are given ten seconds to climb past the 8cm mark. Adapted from: Dhar G. et al. (2020) Various Behavioural Assays to Detect the Neuronal Abnormality in Flies. In: Mishra M. (eds) Fundamental Approaches to Screen Abnormalities in *Drosophila*. Springer Protocols Handbooks. Springer, New York, NY. https://doi.org/10.1007/978-1-4939-9756-5_18

Results

To quantify the climbing ability of PINK1 *Drosophila*, A climbing assay was conducted using a 20 cm × 2.5 cm graduated cylinder. After data was collected, the results of the climbing assays of the controlled group were compared to those of the experimental groups. After 5 trials were conducted each day, using 20 flies in each trial, the average number of flies and the percentage of flies that "escaped" per trial was recorded in Tables 2 and 3. The data in these Tables were adapted from Appendix 1, which displays the raw data collection. In this study, the flies that had been administered with *Mucuna pruriens* extract had displayed a greater average percentage of escaped flies over the course of the 16 day period. However, the flies that received the *Brassica oleracea* treatment, had shown results that were similar to the control group.

Table 2 - Average number of flies "escaped" after the administration of *Brassica oleracea* and *Mucuna pruriens* every other day for 16 days

Days after treatment	Average % of flies passed after 10 seconds (no treatment)	Average % of flies passed after 10 seconds (*Mucuna pruriens* treatment)	Average % of flies passed after 10 seconds (*Brassica oleracea* treatment)
0 days after treatment	12.6	12.4	12.8
2 days after treatment	11	12	11.2
4 days after treatment	9.4	11.6	9.8
6 days after treatment	7	12.2	8.8
9 days after treatment	5.8	11.2	6.6
10 days after treatment	4.8	10.6	6
12 days after treatment	3.6	9.6	4.2
14 days after treatment	3	8.6	3.6
16 days after treatment	2.6	8	3

The statistics summary, seen in Table 4, was calculated to observe differences in the sum and average amounts in the percentage of flies "escaped." The average percentage of escaped flies for the control group is 33.22, which is similar to that of the *B. oleracea* treatment group's average of 36.55. However, the average percentage of flies that escaped in the *Mpe* treatment group was around 53.44, which is a higher average than those of the other two groups. Similarly, the sum of escaped flies for the control group is 299, which is similar to that of the *B. oleracea* treatment group's sum of 329. Both of these values are lower than the sum of flies escaped after being treated with *Mpe* extract (481). The variance level for the *Mpe* treatment group is 65.03, which is much lower than that of the control group (328.69) and the *B. oleracea* treatment group (307.03). The climbing ability of the *Mpe* treatment group did not decrease as drastically as those of the flies in other groups.

An ANOVA Two-Factor Without Replication was conducted to analyze the significance of this data. The ANOVA in Table 5 was conducted instead of the ANOVA Two-Factor with Replication because the conducted study compares multiple groups of individuals performing one task. There was substantial evidence to reject the null hypothesis that the ayurvedic extracts did not affect the climbing ability of PINK1 *Drosophila melanogaster*. The F-value of 24.34 on the first row was greater than the critical value of 3.63, supporting that the treatment did have a significant effect on the flies' climbing ability. With a p-value of 1.4E-05, which is <0.00001, and an alpha value of 0.05, it can be determined that there was a statistically significant difference between effects that the extracts had on the percentage of flies that "escaped." Also, the F-value of 14.12 on the

Table 3 - Average percentage of flies "escaped" after administration of *Brassica oleracea* and *Mucuna pruriens* every other day for 16 days

Days after treatment	Average % of flies passed after 10 seconds (*Mucuna pruriens* treatment)	Average % of flies passed after 10 seconds (*Brassica oleracea* treatment)	Average % of flies passed after 10 seconds (no treatment)
0 days after treatment	63%	62%	64%
2 days after treatment	55%	60%	56%
4 days after treatment	47%	58%	49%
6 days after treatment	35%	61%	44%
9 days after treatment	29%	56%	32%
10 days after treatment	24%	53%	30%
12 days after treatment	18%	48%	21%
14 days after treatment	15%	43%	18%
16 days after treatment	13%	40%	15%

Table 4: Statistic summary of the average percentage of flies "escaped" after administration of *Brassica oleracea* and *Mucuna pruriens* every other day for 16 days

SUMMARY	Count	Sum	Average	Variance
Control	9	299	33.22	328.69
Mpe	9	481	53.44	65.03
B. oleracea	9	329	36.56	307.03
Day 0	3	189	63	1
Day 2	3	171	57	7
Day 4	3	154	51.33	34.33
Day 6	3	140	46.67	174.33
Day 8	3	117	39	219
Day 10	3	107	35.67	234.33
Day 12	3	87	29	273
Day 14	3	76	25.33	236.33
Day 16	3	68	22.67	226.33

Table 5: Anova - Two-Factor Without Replication on the effects of *Brassica oleracea* and *Mucuna pruriens* on climbing ability of *Drosophila melanogaster*

Source	SS	D.F.	MS	F	P-value	F crit
Treatment	2115.85	2	1057.93	24.34	1.4E-05	3.63
Time	4910.52	8	613.81	14.12	6.52E-06	2.59
Error	695.48	16	43.47			
Total	7721.85	26				

second row was greater than the critical value of 2.59, supporting the notion that time had a significant effect on the flies' (loss of) climbing ability. This can also be deducted from the p-value of 6.52E-06, which indicates statistical significance when compared to an alpha value of 0.05, since the p-value is less than the alpha value. Following the ANOVA, a post-hoc t-test was conducted.

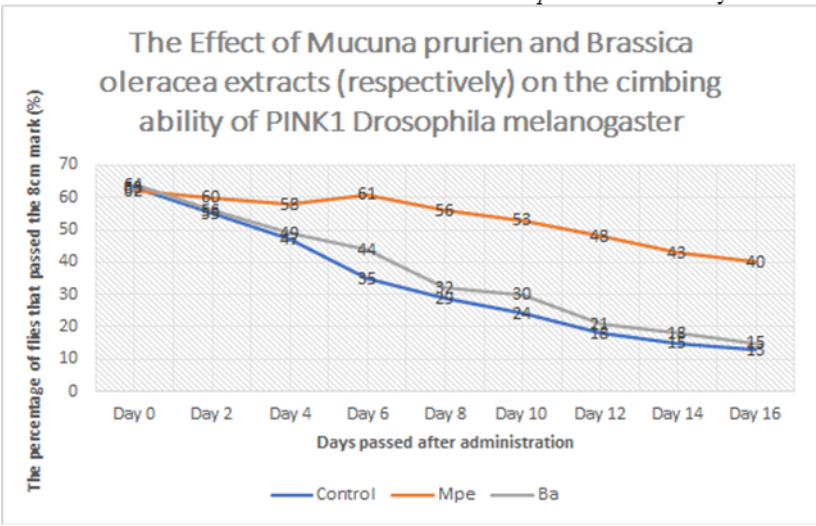
The t-test shown in Table 6 was conducted to determine if there was a significant difference between the climbing ability of the control group and the *Mpe* treatment group, the control group and the *B. oleracea* treatment group, and the *Mpe* treatment group fescue and *B. oleracea* treatment group. The resulting p-value when comparing the control group and the *Mpe* treatment group was 0.003761, which is less than the alpha value of 0.5, meaning that the difference between these two conditions is significant. However, the resulting p-value when comparing the control group and the *B. oleracea* treatment group was 0.348447, which is larger than the alpha value of 0.5, meaning that the difference was not significant. Finally, when comparing the *Mpe* treatment group fescue and *B. oleracea* treatment group the resulting p-value was 0.009158, which is less than the alpha value of 0.5, meaning that the difference was significant. From this t-test, it can be observed that *Mpe* treatment had more of an impact on the climbing ability of PINK1 *Drosophila* than the *Mpe* treatment did, since it displayed the longest delay in loss of motor ability.

Table 6: T-test to on the effects of *Brassica oleracea* and *Mucuna pruriens* on climbing ability of *Drosophila melanogaster*

Treatment Pairs	p-value	inference
Control and *Mpe*	0.003761	*p<0.05; significant
Control and *B. oleracea*	0.348447	*p>0.05; not significant
B. oleracea and *Mpe*	0.009158	*p<0.05; significant

As can be seen in Figure 2, the line graph, and in Table 3, the PINK1 flies that were administered *Mpe* extract displayed a significant delay in the loss of climbing ability, as compared to the control group. Conversely, the flies that were administered *B. oleracea* extract did not display a significant delay or acceleration of loss in climbing ability. Therefore, the B. oleracea extract did not have an effect on the flies. Furthermore, there was a significant difference between the experimental groups, signifying the difference in effect that *Mpe* had, in comparison to *B. oleracea*. Figure 1 provides a visual representation of the delaying effect that *Mpe* had on the climbing ability of the PINK1 flies.

Figure 2- Line graph of the average percentage of flies "escaped" after 10 seconds after administration of *Brassica oleracea* and *Mucuna pruriens* for 16 days



Similarly, it can be seen in Figure 3 and Table 7 that on the last day of data collection, the climbing ability of the group that was administered *Mpe* was significantly affected, when compared to those of the *B. oleracea* treatment group and the control group. In the box plot, the X that displays the mean number of flies escaped is similar between the *B. oleracea* treatment group (3) and the control group (2.6). However, the value of the mean number of flies that escaped in the *Mpe* treatment group (8) was significantly higher. The box plot provides a visual representation of the effectiveness of the *Mpe* treatment. The relative location of the box for the Mpe treatment group is higher than that of the other groups as well.

Discussion

This study was conducted to delay and alleviate the motor symptoms of Parkinson's Disease (PD) in *Drosophila melanogaster* through the administration of ayurvedic extracts. By measuring the climbing ability of the flies, over the course of 16 days, and comparing those results, the effectiveness of *Brassica oleracea* and *Mucuna pruriens extracts* was determined. As this model serves to represent PD in humans, it is acknowledged that it may be applicable to future trials on humans. As many developing countries do not have access to the advanced healthcare that is in the United States, the purpose of this experiment was to find a safe and affordable method of treatment for this disease.

The hypothesis of the conducted study was that when *Mucuna pruriens* and *Brassica oleracea* extracts are administered to PINK1 *Drosophila, Brassica oleracea* would delay the loss of motor ability in the PINK1 flies the longest because it contains sulforaphane which activates the Nrf2 pathway, promoting antioxidant activity and countering oxidative stress. However, the hypothesis was not supported. The t-test displayed that the *B. oleracea* treatment had no significant effect on the climbing ability of PINK1 *Drosophila.* On the other hand, the *Mpe* treatment did significantly

Figure 3 - Box plot of the five-number summary statistics for the flies that "escaped" within 10 seconds for the control group, *Mpe* treatment group, and *B. oleracea* treatment group on day 16

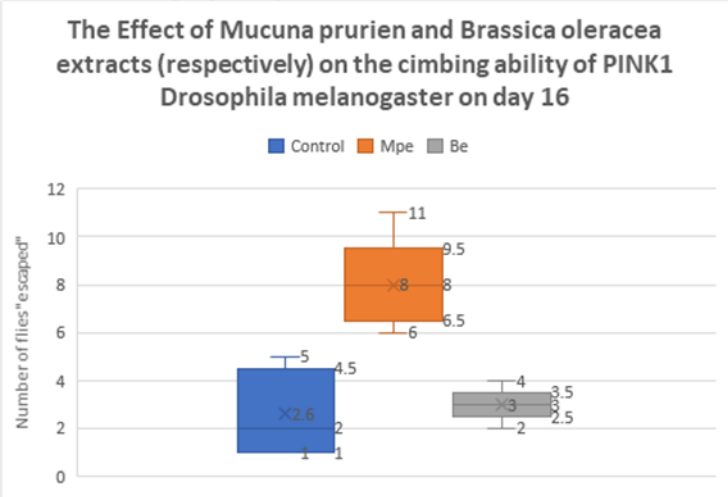


Table 7 - Descriptive statistics for the number of flies that "escaped" within 10 seconds for the control group, *Mpe* treatment group, and *B. oleracea* treatment group on day 16

	Minimum	Q1	Mean	Q2	Q3	Maximum
Control	1	1	2.6	2	4.5	5
Mpe	6	6.5	8	8	9.5	11
B. oleracea	2	2.5	3	3	3.5	4

affect the climbing ability of PINK1 *Drosophila,* as it slowed the loss of motor ability. This finding can be applied to human clinical trials, where *Mpe* treatment could potentially be used to delay the loss of motor ability that is caused by PD. Since *Mpe* is more accessible to those in developing countries, those who cannot afford professional treatment could use this as an alternative aide.

In a 2014 study conducted by professionals in the fields of nanochemistry, molecular science, neuroscience, pharmacology, etc, it was found that *Mpe* (0.1% L-DOPA) treatment significantly improved the climbing activity by 76% in *PINK1B9* mutant flies by reducing the trend of worsening climbing rate[7]. However, in another 2014 study, done by scholars in the departments of Agriculture, Biology, and Biomedicine, "*Mpe* treatment did not result in a significant effect on climbing ability in the PINK1 flies," only increasing the climbing ability by 31.7%[6]. The results of the conducted study displayed that *Mpe* did significantly delay the loss of climbing ability in PINK1 Drosophila, although it did not increase it. By the end of the 16 day period, the *Mpe* treatment group had 27% more escaped flies than the control group. These results of the conducted study can be justified by the properties of *Mpe,* which prevent hydrogen peroxide-induced cytotoxicity and regulate dopaminergic concentration through the chemical, L-DOPA[14]. As *Mpe* is a plentiful source of L-DOPA, it is known to be used to increase dopamine concentration in the brain, which PD depletes. Because *Mpe* helped replenish the dopaminergic concentration in the *substantia nigra,* it showed a steady delay of loss of climbing ability, in comparison to the control group of flies, which lost climbing ability much more rapidly. However, it is believed that Mpe contains healing properties that are unrelated to its L-DOPA content, which may have contributed to its success in delaying the motor symptoms of PD[15].

In a study conducted by a bachelor of science at Lancaster University, it was found that when flies were administered with *B. oleracea*, "there was no significant change in fly performance at any given time point, nor any change in performance decline over time" [20].The conducted experiment produced similar results, displaying an insignificant effect of *B. oleracea* on climbing ability. However, another study conducted by scholars in the department for life quality studies, claim that sulforaphane, which is plentiful in *B. oleracea*, is preventative against oxidative stress-induced cytotoxicity through the activation of the Nrf2-antioxidant pathway and removal of intracellular DA quinone and degrading the misfolded protein[11]. It is believed that the *B. oleracea* treatment was unsuccessful in slowing the regression of climbing rate in *Drosophila* because the dosage level and administration were not sufficient. This study was unable to administer a dosage of *B. oleracea* that is proportionate to the ratio that would be used in humans, due to the lack of studies regarding its use on *Drosophila.* As a result, the same dosage used for *Mpe* was used for *B. oleracea*, in hopes of controlling the variable of unequal dosage. However, as a model of Parkinson's disease, using the same concentration was not representative of the dosage that would likely have been used in human trials. This error can justify the lack of motor improvement that the flies administered with *B. oleracea* displayed. Similarly, it is believed that the chemical Sulphoraphone, when administered directly, could delay the symptoms of PD better than it's oral administration through *B. oleracea.* It is also possible that the Nrf2-antioxidant pathway has no effect on the gene mutation.

A source of uncertainty during the experiment was the age of flies. Because of the minimal number of flies that arrived upon order, time had to pass for the next generation of *Drosophila* to reach adulthood. Due to this factor, it was not guaranteed that all of the flies were the same age, although all of them were in the adult stage. It is possible that some flies from the previous generation survived long enough to endure the first trial of data collection.

The conducted study could improve its procedure by using a *B. oleracea* extract dosage that is most proportionate to drug dosages that would be used in humans. In future experimentation, it would also be preferable to have a more plentiful number of flies, for a larger number of trials to

make the current experiment more accurate. Similarly, a positive and negative control for this experiment should have been implemented. Rather than just having untreated PINK1 flies, using Wild Type flies would allow for a model of normal fly behavior (without the gene mutation).

In the future, having multiple tests, such as a crawling assay, survival rate, along with the climbing assay, would allow one to discover the effects of the extracts on multiple factors. Moreover, another potential study would be to be able to experiment with more extracts, specifically ones that target apoptosis and inflammation, rather than mitochondrial damage or oxidative stress (which were focused on in this experiment). Similarly, comparing the treated flies to those treated with L-DOPA treatment would demonstrate the applicability of the Mpe treatment used in this experiment. Models of PD have been done on *Drosophila* of the following strains: LRRK2, α-synuclein, GBA, VPS35, parkin, DJ-1, or PINK1.

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Table 1 - Experimental Design Matrix

Title of the Experiment: The Effect of Ayurvedic Plant Extracts-- *Mucuna pruriens* and *Brassica oleracea*-- on Delaying the Loss of Motor Ability in PINK1 *Drosophila melanogaster:* A Model of Parkinson's Disease.			
Hypothesis: When *Mucuna pruriens* and *Brassica oleracea* extracts are administered to PINK1 *Drosophila, Brassica oleracea* would delay the loss of motor ability in the PINK1 flies the longest because it contains sulforaphane which activates the Nrf2 pathway, promoting antioxidant activity and countering oxidative stress.			
Independent Variable: The type of ayurvedic extract that is administered to the flies (mg)			
Levels of Independent Variable	8 mg *Mucuna pruriens* administered	8 mg *Brassica oleracea* administered	untreated
Number of Repeated Trials	20	20	20
Dependent Variable: The percentage of flies that have crossed the 90ml line every 10 seconds, for a minute. (mean %)			
Control Group: The group of untreated PINK1 flies (without being administered anything).			
Constants: Temperature of the environment, type of standard food that is administered, time of day that data is collected, age of the flies.			

Appendix A - Number of flies (out of 20) that climbed passed the 8cm mark (escaped) after administration of *Brassica oleracea* and *Mucuna pruriens* everyday for 16 days

	Control	***Mpe***	***B. oleracea***
Day 0	10	12	14
	12	14	14
	15	11	12
	14	13	12
	12	12	12
Day 2	11	11	12
	12	14	14
	9	12	10
	10	10	9
	13	13	11
Day 4	9	13	7
	11	15	8
	8	10	12
	7	9	14
	12	11	8
Day 6	9	14	9
	7	12	7
	7	11	9
	6	11	6
	6	13	13
Day 8	8	12	8
	7	10	8
	4	10	6
	5	13	6
	5	11	5
Day 10	5	10	8
	4	9	6
	5	12	6
	6	11	5

	4	11	5
Day 12	3	9	5
	4	8	5
	4	10	4
	3	12	3
	4	9	4
Day 14	4	7	4
	2	10	4
	3	7	3
	3	10	2
	3	9	5
Day 16	5	8	4
	4	8	3
	2	7	3
	1	6	2
	1	11	3