Supplementation of Antioxidants to Reduce Dopaminergic Neurodegeneration and Alpha-synuclein Accumulation Associated with Parkinson's

Research Plan

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A. Rationale:

Parkinson's disease (PD) is a progressive neurodegenerative disorder that diminishes motor functioning and affects over 10 million people worldwide. (Centers for Disease Control, 2019) Patients diagnosed with PD can experience a wide range of symptoms including tremors, slowness of movement, impaired balance and coordination, stiffness, and several other symptoms that can severely affect one's life. (National Institute of Neurological Disorders and Stroke, 2019) Rates of PD have increased from 18.2 between 1976 and 1985 to 30.4 between 1996 and 2005 with the greatest increase in men over 70. (Savica et al. 2016) Parkinson's risk and prevalence increases with age and with life expectancy on the rise, PD diagnosis is projected to increase.

Parkinson's disease symptoms worsen as the disease progresses, which is due to the neurodegenerative effects of the disorder. Motor degeneration caused by PD leads to symptoms typically beginning on one side of the body, but as the disease progresses it can affect both sides and eventually lead to the loss of motor function. The two main factors in the pathology of PD is the loss of dopaminergic neurons in the substantia nigra and the development of Lewy bodies in the brain tissue. (National Institute of Neurological Disorders and Stroke, 2019)

Movement control is established by interactions between nerve cells in the central nervous system. (Triarhou, 2013) The neurons that are specifically designed for movement communication are located in the substantia nigra. The substantia nigra is a major region of the brain that controls balance and movement. The neurotransmitter dopamine, also known as 3,4-Dihydroxytyramine, is a chemical released by neurons to send signals to other neurons. Dopamine controls movement and is used by the substantia nigra to send signals to the corpus striatum which results in smooth, purposeful movement. Dopamine is produced by dopaminergic neurons in the brain from tyrosine through the addition of a hydroxyl group which transforms it to L-dopa and then removes a carboxylic acid group resulting in dopamine. Once produced, dopamine is stored in a synaptic vesicle until action potentials, which is the change in electrical potential associated with the passage of an impulse along the membrane of a neuron, induce the release of dopamine. (Triarhou, 2013)

The cause of motor related symptoms in Parkinson's patients is largely due to the progressive loss of dopamine-producing neurons in the substantia nigra. (National Institute of Neurological Disorders and Stroke, 2019) Dopaminergic neurons (DA) begin to die when the cell's energy producer, mitochondria, fail. The loss of dopaminergic neurons decreases dopamine signaling to the striatum which contributes to the motor symptoms of PD. (Cooper et al, 2018) The average Parkinson's patient loses over 60 to 80

percent or more of the dopamine-producing cells in the substantia nigra before symptoms appear. People with Parkinson's do not develop symptoms until later in life which indicates that age plays a role in the development of the disease. As patients age, mitochondrial function and cellular metabolism decline resulting in dopamine neurons dying. (Parkinson, 2019)

In affected brain cells of people with PD, there is a strong presence of Lewy bodies, which are abnormal aggregates of protein that develop inside nerve cells and contribute to the progression of the disease. (U.S. National Library of Medicine) The protein that is deposited in these Lewy bodies is alpha-synuclein, which is a protein that is abundant in the brain and found mainly at the endings of neurons. Alpha-synulcien is encoded by the SNCA gene which is a gene that can make PD heritable. (Fu et al. 2014) The function of alpha-synuclein is not completely understood, however, it may play a role in the release of dopamine. In Parkinson's, the alpha-synuclein protein misfolds and begins to accumulate forming Lewy bodies. (The Cure Parkinson's Trust, 2019)

This accumulation is very damaging to cellular components and causes cell death, including dopaminergic neurons. Alpha-synuclein accumulation spreads from diseased cells to healthy cells through synapses, a connection between two cells, resulting in the exacerbation of Parkinson's. (Longhena et al. 2017) It has been found that abnormal alpha-synuclein deposition occurs early in PD and may play a key cause in the pathogenesis of PD. A promising strategy in treating PD could be reducing and altering alpha-synuclein to nonpathogenic. Alpha-synuclein accumulation is harmful to cellular structure because it attacks synaptic vesicles leadings to leaking of neurotransmitters like dopamine which results in oxidative stress. (Stefanis, 2012)

Oxidative stress occurs when an imbalance is formed between production of reactive oxygen species (ROS) and cellular antioxidant activity. (Hwang, 2013) Oxidative damage is the harm sustained by cells and tissues that are unable to keep up with free radical production. The presence of ROS generating enzymes including tyrosine hydroxylase and monoamine oxidase makes dopaminergic neurons prone to oxidative stress. The major sources of oxidative stress generated for dopaminergic neurons is believed to be the ROS produced during dopamine metabolism, mitochondrial dysfunction, and neuroinflammation. (Hwang, 2013) Due to the cellular activity of oxidative enzymes, free radicals are produced which are unstable atoms that can damage cells. The accumulation of oxidative stress caused by aging and free radicals can cause accumulation of alpha-synuclein which exacerbates PD. (Kumar et al. 2012)

As of right now there is no effective cure for PD. (Fu et al. 2014) Currently,the most popular treatment for PD is levodopa, which is converted to dopamine in the brain. Levodopa, however, become less effective

over time and is limited by significant side effects. The most effective therapeutic route for PD involves finding compounds that can both improve the disease and prevent its symptoms. Natural products could potentially protect dopaminergic degeneration while acting as therapeutic agents in the pathogenesis of alpha-synuclein. (Stefanis, 2012)

Curcumin, a compound ($C_{21}H_{20}O_6$) in the plant turmeric, has been linked to reducing inflammation (Woodman et al, 2016). Turmeric is made up of 4 curcuminoids including curcumin, demethoxycurcumin, bisdemethoxycurcumin, and cyclocurcumin. Curcumin which is a curcuminoid of turmeric, is suggested to reduce inflammation by being intervened with the upregulation of the peroxisome proliferator-activated receptor-y (PPAR-y) activation. (Jacob et al, 2008) Curcumin also exhibits antioxidant and neuroprotective properties in neurological disorders. Since the imbalance between reactive oxygen species (ROS) generation and cellular antioxidant activity is a leading cause of oxidative stress which leads to PD, curcumin's antioxidant properties may decelerate the progression of PD. (Nguyen et al. 2018)

Quercetin, a compound ($C_{15}H_{10}O_7$) is a flavonoid that is found in fruits and vegetables. (Warnsmann et al. 2018) Quercetin actively balances cellular reactive oxygen species (ROS) levels and has a cyto-protective function. Costa et al. (2016) found that quercetin has neuroprotective effects and is able to scavenge free radicals, reducing ROS. Antioxidant properties have been shown to reduce oxidative stress in *C. elegans* strains BZ555 and OW13. (Cooper et al, 2018)

L-glutathione reduced is an antioxidant present in almost every cell in the body, playing a role in the detoxification of drugs and xenobiotics. Reduced glutathione (GSH) acts as a hydrogen donor in the detoxification of hydrogen peroxide. As a dietary supplement, GSH possesses various systemic effects such as improvement of liver abnormalities, improvement of diabetic complication, protection from viral infection, and antitumor activity. (Weschawalit, 2017) Morgan et al. 2010 found that L-glutathione prevented oxidative stress and suppressed ROS formation in Caenorhabditis elegans.

Testing the therapeutic value of possible treatment compounds is made efficient through the use of inexpensive in vivo assays involving *Caenorhabditis elegans*. (Fu et al, 2014) *Caenorhabditis elegans* are nematodes or roundworms that live in the soil and feed on bacteria (Alton, 2006). A versatile cuticle surrounds *C. elegans* on the outside with neurons and the hypodermis separated from the musculature by a thin extracellular matrix known as the basal lamina. The muscles receive contribution from the neurons by sending muscle arms to motor neuron processes in the nerve ring. The somatic muscular system consists of striated tissue. The non-striated muscle consists of tissue that don't have sarcomeres. The adult

body is anatomically simple with about 1000 somatic cells. *C. elegans* are well studied, have 8 dopaminergic neurons and have a PD-related homologous gene. (Alton, 2006)

C. elegans are also transparent and can be transgenically produced to be fluorescent to express human alpha-synuclein and dopaminergic neurons. *C. elegans* can be exposed to neurotoxins to induce Parkinson's which results in a useful pharmacological model of PD. Neurotoxins cause toxicity by increasing ROS leading to cellular toxicity, damage, and neuronal loss. (Cooper et al. 2018) Copper sulphate ($CuSO_4$) for example, is a toxin used by Mashock et al. (2016) to induce neurodegenerative effects in *C. elegans*. Food-borne exposure of neurotoxins has been shown to be the primary, and most toxic, route of exposure in *C. elegans* (Mashock et al. 2016)

B. <u>Research Question(s), Hypothesis(es), Engineering Goal(s), Expected Outcomes:</u>

- Research Question: If curcumin (25µM, 50µM, 100µM), quercetin (50µM, 100µM, 200µM), and L-glutathione (0.1mM, 1mM, 10mM) are exposed to Parkinson's induced *C. elegans*, will dopaminergic neuron degeneration, alpha-synuclein accumulation, and decreased motility be reduced?
- Alternate Hypothesis: Based on the research of Satapathy et al. (2016), Javed et al. (2019), and Morgan et al. (2010), it is hypothesized that curcumin, quercetin, and L-glutathione will each significantly restore dopamine levels, reduce alpha-synuclein accumulation, and improve motility in Parkinson's induced *C. elegans* due to their potent antioxidant properties.

  Null hypothesis: Curcumin, quercetin, and L-glutathione will not affect dopamine levels, alpha-synuclein accumulation, and motility of Parkinson's induced *C. elegans*.

- Expected Outcomes: Both BZ555 strain *C. elegans* expressing green fluorescent protein tagged dopaminergic neurons and OW13 strain *C. elegans* expressing yellow fluorescent protein tagged Alpha-synuclein will be exposed to 10mg/L copper sulphate ($CuSO_4$) for 24 hours and then treated for 72 hours with either curcumin, quercetin, or L-glutathione. PD is exacerbated by an imbalance between reactive oxygen species (ROS) and cellular antioxidant activity which increases oxidative stress. This oxidative stress leads to dopaminergic neurodegeneration, alpha-synuclein accumulation and decreased motility due to unstable free radicals damaging and denaturing cellular structure. It's postulated that *C. elegans* treated with curcumin, quercetin, and L-glutathione will experience a balance between ROS and cellular antioxidant activity which could reduce oxidative stress, a main factor in the pathology of Parkinson's.

C.

- **Methodology:**

Caenorhabditis elegans Strains: Strains BZ555 and OW13 will be acquired by the Caenorhabditis Genetics Center (CGC). *C. elegans* strain BZ555 is tagged with green fluorescent proteins expressed specifically in dopaminergic neurons. *C. elegans* strain OW13 is tagged with yellow fluorescent proteins expressed specifically in alpha-synuclein.

Culturing and Maintenance of *Escherichia coli* OP50: Luria broth powder (Carolina Biological) will be dissolved in water in a ratio of 250g/L. The solution will be stirred with a glass rod in an Erlenmeyer flask over a hot plate. Luria broth will be sterilized in the autoclave for 25 minutes at 121°C at STE. Once fully sterilized, the broth will be transferred into previously sterilized vials. Next, an inoculating loop will be sterilized by heating it in a Bunsen burner flame, and utilized to transfer a small amount of OP50 *E. coli*, that will be donated from the CGC, from the culture vial to a Luria broth vial. These vials will be incubated at room temperature (37°C) overnight. (Carolina, 2018).

***Caenorhabdistis elegans* Food Source Preparation:** The new *E. coli OP50* luria broth culture will be removed from the incubator and the mouth of the vial will be sterilized using a Bunsen burner. A sterile cotton tip applicator will be opened and used to swab 1.0mL fluid inoculum onto a nematode growth media (NGM) plate. The NGM plate will then be placed in the incubator at 37 °C (Lab-Line Imperial II Incubator) overnight to ensure growth. This process will be done on several NGM petri dishes (Carolina, 2018).

Culturing and Maintenance of *Caenorhabditis elegans*: To culture the *C. elegans*, *E. coli* OP50 inoculated NGM plates will be removed from the 37 °C incubator. A sterile disposable scalpel will be removed from its package. The *C. elegans* culture will be cut into blocks of $1cm^3$. The blocks will then be transferred to one of the inoculated plates and placed upside down so that the worms can directly contact the agar. This process will be repeated for the other inoculated plates. Then, the scalpel will be disposed into the autoclave disposal bag. The *C. elegans* plates will be wrapped with parafilm and cultures will be placed in an area with no air drafts and at room temperature. The original *C. elegan*s stock will be kept as an extra stock. (Carolina, 2018).

Age Synchronization: In order for results to be comparable, each assay will have to be done exactly the same. Age synchronization will be performed on *C. elegans* before all assays. First, 6.0 mL of M9 solution will be pipetted onto a plate of *C. elegans* to dislodge the worms from the plate. Then, 5.0 mL of worms will be transferred into a tube where it will be centrifuged (1,000 x g for 1 minute) to create a worm pellet at the bottom of the tube. There will be a blank micro test tube in the centrifuge to balance

out the circular motion, if needed. After centrifugation, the supernatant will be taken out carefully. Next the worms will be washed with 20% alkaline hypochlorite (5.0mL) and after being centrifuged, the supernatant will be removed again. Next the worms will be washed with 10 mL M9 buffer in the centrifuge (1,000 x g for 1 minute). The supernatant will be removed and new M9 will be inserted and then the tube will be put onto an orbital shaker (Cole Parmer) for 24 hours at 80rpm. 24 hours later, after embryonic development, *C. elegans* (L4 stage) will be poured onto a NGM agar plate and will be ready to use in assays (Satapathy et al. 2016).

Copper Sulphate Exposure: Age synchronized *C. elegans* will be placed onto unseeded NGM agar plates containing 10mg/L copper sulphate for 24 hour exposure. *C. elegans* that are not receiving treatment will be placed on an unseeded NGM agar plate for 72 hours. Groups that are receiving treatment will then be transferred onto NGM agar plates with specific variables and concentrations for 72 hours.

Chemical Administration: Age synchronized *C. elegans* will be placed onto the specific NGM agar plates. Curcumin, quercetin, and L-glutathione treated groups will be placed onto agar plates that will be made with the associated chemical. Curcumin, quercetin, and L-glutathione will all be purchased from Sigma-Aldrich. Curcumin and quercetin will be diluted with Dimethyl Sulfoxide and l-glutathione will be diluted with distilled water. To treat groups of copper sulphate exposed *C. elegans*, chemicals will be integrated into the agar using a micropipette. In this experiment *C. elegans* groups will be treated for 72 hours by being exposed to 25µM, 50µM, and 100µM of curcumin, 50µM, 100µM, and 200µM of quercetin, and 0.1mM, 1mM, and 10mM of L-glutathione reduced.

Fluorescence Microscopy Dopaminergic Neurodegeneration Assay: Assay of dopaminergic degeneration will be performed in *C. elegans* exposed to either copper sulphate alone or treated with a concentration of curcumin, Quercetin, and L-glutathione. A single L4 *C. elegans* will be transferred using a sterilized platinum wire into 1 mL of ethanol (40%) on a microscope well slide to paralyze the specimen, and a cover slip will be placed on top of the ethanol and specimen. After 15 minutes, the slide will be viewed under the fluorescence microscope. In this assay a ZEISS Axiovert 40 CFL fluorescence microscope with a green fluorescence protein filter having an excitation wavelength of 500 nm and an emission wavelength of 487 nm will be utilized to observe fluorescently tagged dopaminergic neurons in *C. elegans* at 250x magnification. A Canon DSLR camera with an 8” shutter speed and 3200 ISO will be also used to capture photographs.

Fluorescence Microscopy Alpha-synuclein Accumulation Assay: Assay of alpha-synuclein accumulation will be performed in animals exposed to either copper sulphate alone or treated with a concentration of curcumin, Quercetin, and L-glutathione. A single L4 *C. elegans* will be transferred using a sterilized platinum wire into 1 mL of ethanol (40%) on a microscope well slide to paralyze the specimen, and a cover slip will be placed over the ethanol and specimen. After 15 minutes, the slide will be viewed under the fluorescence microscope. In this assay a ZEISS Axiovert 40 CFL fluorescence microscope with a yellow fluorescence protein filter having an excitation wavelength of 490 nm and an emission wavelength of 535 nm will be utilized to observe fluorescently tagged Alpha-synuclein in OW13 strain *C. elegans* at 250x magnification. A Canon DSLR camera with an 8" shutter speed and 3200 ISO will be also used to capture photographs.

Quantification of Fluorescence: Obtained fluorescence images will be uploaded to an Apple Macbook Pro with ImageJ software. Images will be uploaded to ImageJ to analyze fluorescence intensity where the fluorescence represents the level of dopaminergic neurons in GFP tagged BZ555 *C. elegans* and Alpha-synuclein accumulation in YFP tagged OW13 *C. elegan*s. With the numbers generated from ImageJ analysis, fluorescence will be quantified through the corrected total cellular fluorescence (CTCF), equation 1.

[CTCF = Integrated Density - (Area of Selected Cell × Mean Fluorescence of Background Readings)] (Equation 1)

This will allow for both a qualitative measurement of mutant SOD1 aggregation in the images and a quantitative measurement of the CTCF equation outputs.

Locomotion Assay: First, age synchronized groups of BZ555 control, 24 hr copper sulphate exposed, 72 hour quercetin treated, 72 hour curcumin treated, and 72 hour L- glutathione treated will be placed on an unseeded NGM agar plate. Then, worms in resting position, will be stimulated using a platinum worm pick. The *C. elegans* will be tapped with a platinum worm pick for one second. The worms performed body bends which will be then recorded in a 60 second trial. A 60 second video of *C. elegans* will be recorded using a video phone with the SnapZoom attachment.

Liquid Thrashing Assay: BZ555 groups will be tested in the liquid thrashing assay. Groups of age synchronized BZ555 control, 24 hr copper sulphate exposed, 72 hour quercetin treated, 72 hour curcumin treated, and 72 hour L- glutathione treated will be placed on an unseeded petri dish. Next, 3 mL of M9 buffer will be placed in the center of the dish and spread to the entirety of the dish. *C. elegans* then thrashed showing stimulation as a result of the application of the M9 buffer. The number of thrashes

performed will be recorded during a 60 second video of *C. elegans* using a video camera with the SnapZoom attachment to the compound microscope.

- **Risk and Safety:**
  1. *Human participants research: N/A*
  2. *Vertebrate animal research: N/A*

  3. Potentially hazardous biological agents research:

     As per ISEF rules and guidelines, this study involves BSL-1 organisms that are exempt from prior SRC review and require no additional forms: (ISEF Rulebook Page 16, section 2f.)
     As such, all organisms outlined below will be listed on ISEF Form 3.

     Organism Name: ***Escherichia coli* OP50***
     **E. coli OP50 will be used as a food source for the C. elegans*
     a. Source of Organism: *Caenorhabditis Genetics Center (CGC)*
        BSL assessment determination: BSL- 1
     b. Safety precautions: The student researcher will be trained by the designated supervisor in all safety aspects associated with working with bacteria, sterile technique and proper handling and disposal of the bacteria. The designated supervisor will directly supervise the student researcher when working with the bacteria. Goggles, lab apron and nitrile gloves will be worn during experimentation. Prior to use, all surfaces will be wiped with 10% bleach solution. All surfaces will also be wiped down with 10% bleach after experimentation.
        Methods of disposal: 10% bleach will be used to to kill the bacteria. 10% bleach will be incorporated onto the NGM agar plate and the plate will be sealed with parafilm and disposed of.

     Organism Name: **BZ555 *Caenorhabditis elegans***
     a. Source of Organism: *Caenorhabditis Genetics Center (CGC)*
        BSL assessment determination: BSL- 1
     b. Safety precautions: The student researcher will be trained by the designated supervisor in all safety aspects associated with working with *C. elegans* and bacteria, sterile technique and proper handling and disposal of the bacteria and c. elegans. The

designated supervisor will directly supervise the student researcher when working with the bacteria. Goggles, lab apron and nitrile gloves will be worn during experimentation. Prior to use, all surfaces will be wiped with 10% bleach solution. All surfaces will also be wiped down with10% bleach after experimentation.

Methods of disposal: 10% bleach will be used to to kill the bacteria and *c. elegan*s. 10% bleach will be incorporated onto the NGM agar plate and the plate will be sealed with parafilm and disposed of.

Organism Name: **OW13 *Caenorhabditis elegans***

a. Source of Organism: *Caenorhabditis Genetics Center (CGC)*
   BSL assessment determination: BSL- 1

b. Safety precautions: The student researcher will be trained by the designated supervisor in all safety aspects associated with working with *C. elegans* and bacteria, sterile technique and proper handling and disposal of the bacteria and c. elegans. The designated supervisor will directly supervise the student researcher when working with the bacteria. Goggles, lab apron and nitrile gloves will be worn during experimentation. Prior to use, all surfaces will be wiped with 10% bleach solution. All surfaces will also be wiped down with 10% bleach after experimentation.

   Methods of disposal: 10% bleach will be used to to kill the bacteria and *c. elegan*s. 10% bleach will be incorporated onto the NGM agar plate and the plate will be sealed with parafilm and disposed of.

4. Hazardous chemicals, activities & devices:

Ethanol (40%, 1mL) from Sigma Aldrich

a. Risk assessment: Classified as a hazardous chemical: highly flammable, strong irritant
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked “flammables” cabinet. This will be kept away from heat/sparks.open flames/hot surfaces.
d. Methods of disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company
   https://www.sigmaaldrich.com/catalog/product/mm/818760?lang=en&region=US

Isopropyl alcohol (70%, 3mL) from Sigma Aldrich

a. Risk Assessment: Classified as a hazardous chemical: highly flammable, strong irritant
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked "flammables" cabinet
d. Methods of disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/substance/isopropylalcohol60106763011?lang=en&region=US

Sodium Hypochlorite (10%, 3mL) from Sigma Aldrich

a. Risk Assessment: Classified as a hazardous chemical: can cause serious skin and eye burns, strong irritant
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked cabinet when not in use
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/product/sigald/425044?lang=en&region=US

Alkaline hypochlorite (20%, 3 mL) From Sigma Aldrich

a. Risk Assessment: Classified as a hazardous chemical: strong irritant
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked cabinet
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/product/sigma/a1727?lang=en&region=US

M9 buffer solution (5mL) from Sigma Aldrich

a. Risk Assessment: Not Classified as a hazardous chemical
b. Supervision: Will be handled only under direct supervision by a mentor

c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked cabinet
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/product/sial/63011?lang=en&region=US

Copper sulfate (1.32mM, 150µL diluted to 10mg/L in agar)

a. Risk assessment: Harmful if swallowed, can cause skin irritation, can cause serious eye irritation, and is toxic to aquatic life.
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked cabinet
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/substance/copperiisulfate15961775898711?lang=en&region=US

Quercetin (1mM, 2mM, 4mM, 150µL diluted to 50µM, 100µM, 200µM in agar; DMSO as solvent) from Sigma Aldrich

a. Risk assessment: May cause eye and skin irritation if excessive exposure.
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: Will not be inhaled. This chemical will not be handled without personal protection. When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked flammables cabinet.
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/product/sigma/q4951?lang=en&region=US

L-glutathione (0.1mM, 10mM, 100mM, 340µL diluted to 0.1mM, 1mM, 10mM in agar; distilled water as solvent) from Sigma Aldrich

a. Risk assessment: Not Classified as a hazardous substance or mixture
b. Supervision: Will be handled only under direct supervision by a mentor

c. Safety precautions: Will not be inhaled. This chemical will not be handled without personal protection. When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked cabinet
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/product/sial/g4251?lang=en&region=US

Curcumin (0.5mM, 1mM, 2mM, 150µL diluted to 25µM, 50µM,100µM in agar; DMSO as solvent) from Sigma Aldrich

a. Risk assessment: Will not be inhaled and should be kept away from eyes and face to avoid irritation
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: Will not be inhaled. This chemical will not be handled without personal protection, When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container at -20°C
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/catalog/substance/curcumin3683845837711?lang=en&region=US

Dimethyl Sulfoxide (25 mL) from Sigma Aldrich

a. Risk assessment: Classified as a hazardous chemical: highly flammable and combustible liquid.
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When handling, safety goggles, nitrile gloves, and apron will be worn and when storing, will be kept in a sealed container in a locked flammables cabinet. This will be kept away from heat/sparks.open flames/hot surfaces.
d. Disposal: Surplus or unrecyclable solutions will be transferred to a licensed disposal company https://www.sigmaaldrich.com/chemistry/solvents/products.html?TablePage=17292420

Microcentrifuge

a. Risk assessment: Usage may be hazardous if not used properly. If not used properly, centrifuge can malfunction
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When Handling, safety goggles, nitrile gloves, and apron will be worn, the experimenter will be trained through the instruction manual and by mentor demonstration in proper usage of equipment

Bunsen burner

a. Risk assessment: Can cause serious burns to skin and can ignite clothing or hair
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When Handling, safety goggles, heat resistant gloves, and apron will be worn, the experimenter will be trained through the instruction manual and by mentor demonstration in proper usage of equipment, will be used with extreme caution and flammable objects will be kept away at all times

Scalpel

a. Risk assessment: Can cause cuts and puncture wounds that can lead to infection, if not handled properly
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When Handling, safety goggles and apron will be worn, the experimenter will be trained through the instruction manual and by mentor demonstration in proper usage of equipment, will be used with extreme caution and materials will be cut in a direction away from the body.

Autoclave

a. Risk assessment: Can potentially risk steam burns if autoclave is opened prior to depressurization
b. Supervision: Will be handled only under direct supervision by a mentor
c. Safety precautions: When Handling, safety goggles, heat resistant autoclave gloves, and apron will be worn, the experimenter will be trained through the instruction manual and by mentor demonstration in proper usage of equipment, will be used with extreme caution

- **Data Analysis:**

For the Locomotion assay, each scored *C. elegans* will be considered as a trial, and the mean number of body bends across all trials of a group will be calculated. Similarly, for the Liquid Thrashing assay, each scored *C. elegans* will be considered as a trial, and the mean number of body thrashes across all trials of a group will be calculated. Each *C. elegans* in both the dopaminergic neurons and alpha-synuclein fluorescence microscopy assays will also be considered a trial, and the mean of all corrected total cell fluorescence (CTCF) output values of a group will be calculated. (Fitzpatrick, 2014) Mean ± SD will be presented on bar graphs using Microsoft Excel for each assay. All data will be analyzed using a One Way ANOVA followed by a post-hoc Scheffe test ($p < 0.05$) using the IBM SPSS version 25 software.

D. Bibliography:

Altun, Z.F, and D.H Hall. "CAENORHABDITIS Elegans AS A GENETIC ORGANISM." *Wormatlas Homepage*, 2006, www.wormatlas.org/ver1/handbook/anatomyintro/anatomyintro.htm.

Costa, Garrick, Roque, Pellacini. "Mechanisms of Neuroprotection by Quercetin: Counteracting Oxidative Stress and More." *Oxidative Medicine and Cellular Longevity*, Hindawi Publishing Corporation, 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4745323/.

Cooper, Jason F, and Jeremy M Van Raamsdonk. "Modeling Parkinson's Disease in *C. Elegans*." *Journal of Parkinson's Disease*, IOS Press, 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC5836411/.

Fitzpatrick, Martin. "Measuring Cell Fluorescence Using ImageJ." *Measuring Cell Fluorescence Using ImageJ - The Open Lab Book v1.0*, 2014, theolb.readthedocs.io/en/latest/imaging/measuring-cell-fluorescence-using-imagej.html.

Fu, Harn, Liu, Chen, Chang, Chen, Huang, Li, Tsai, Hung, Shyu, Lin, Wang. "n-Butylidenephthalide Protects against Dopaminergic Neuron Degeneration and α-Synuclein Accumulation in Caenorhabditis Elegans Models of Parkinson's Disease." *PloS One*, Public Library of Science, 8 Jan. 2014, www.ncbi.nlm.nih.gov/pubmed/24416384.

Hwang, Onyou. "Role of Oxidative Stress in Parkinson's Disease." *Experimental Neurobiology*, The

Korean Society for Brain and Neural Science, Mar. 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC3620453/.

Javed, Meeran, Azimullah, Adem, Sdek, Ojha. “Plant Extracts and Phytochemicals Targeting α-Synuclein Aggregation in Parkinson's Disease Models.” *Frontiers*, Frontiers, 20 Dec. 2018, www.frontiersin.org/articles/10.3389/fphar.2018.01555/full.

Morgan, Estevez, Mueller, Cacho-Valadez, Miranda-Vizuete, Szewczyk, Estevez. “Glutaredoxin Elegans.”GLRX-21 Functions to Prevent Selenium-Induced Oxidative Stress in Caenorhabditis *OUP Academic*, Oxford University Press, 10 Sept. 2010, academic.oup.com/toxsci/article/118/2/530/1652832.

Nguyen, Vuu, Huynh, Yamaguchi, Tran, Dang. “Curcumin Effectively Rescued Parkinson's Disease-Like Phenotypes in a Novel Drosophila Melanogaster Model with DUCH Knockdown.” *Oxidative Medicine and Cellular Longevity*, Hindawi, 3 July 2018, www.hindawi.com/journals/omcl/2018/2038267/.

Kumar, Lim, More, Kim, Koppula, Su Kim, Choi. “The Role of Free Radicals in the Aging Brain and Parkinson's Disease: Convergence and Parallelism.” *International Journal of Molecular Sciences*, Molecular Diversity Preservation International (MDPI), 2012, www.ncbi.nlm.nih.gov/pmc/articles/PMC3431873/.

Longhena, Faustini, Missale, Pizzi, Spano, Bellucci. “The Contribution of α-Synuclein Spreading to Parkinson's Disease Synaptopathy.” *Neural Plasticity*, Hindawi, 3 Jan. 2017, www.hindawi.com/journals/np/2017/5012129/.

Satapathy, Pankaj, and Chinnu Salim. “Attenuation of Dopaminergic Neuronal Dysfunction in Caenorhabditis Elegans by Hydrophilic Form of Curcumin.” *Neurochemistry & Neuropharmacology*, vol. 2, no. 1, 2016, doi:10.4172/2469-9780.1000111.

Savica, Rodolfo. “Time Trends in the Incidence of Parkinson Disease.” *JAMA Neurology*, U.S. National Library of Medicine, 1 Aug. 2016, www.ncbi.nlm.nih.gov/pubmed/27323276.

Stefanis, Leonidas. “α-Synuclein in Parkinson's Disease.” *Cold Spring Harbor Perspectives in Medicine*, Cold Spring Harbor Laboratory Press, Feb. 2012, www.ncbi.nlm.nih.gov/pmc/articles/PMC3281589/.

Weschawalit, Sinee. “Glutathione and Its AntiAging and Anti Melanogenic Effects.” *Clinical, Cosmetic and Investigational Dermatology*, Dove Medical Press, 27 Apr. 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5413479/.

Triarhou, Lazaros C. “Dopamine and Parkinson's Disease.” *Madame Curie Bioscience Database [Internet]*., U.S. National Library of Medicine, 1 Jan. 1970, www.ncbi.nlm.nih.gov/books/NBK6271/.

“Centers for Disease Control and Prevention.” *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 2019, www.cdc.gov/.

CureParkinsonsT. “Alpha-Synuclein and Parkinson's.” *The Cure Parkinson's Trust*, 2019, www.cureparkinsons.org.uk/why-alpha-synuclein.

“MilliporeSigma | United States.” *Sigma-Aldrich*, the Journal of Pharmacy and Pharmacology, 2018, www.sigmaaldrich.com/united-states.htm.

“National Institute of Neurological Disorders and Stroke.” *National Institute of Neurological Disorders and Stroke*, U.S. Department of Health and Human Services, 2019, www.ninds.nih.gov/.

“National Library of Medicine - National Institutes of Health.” *U.S. National Library of Medicine*, National Institutes of Health, 2019, www.nlm.nih.gov/.

Staff, Science X. “Treating with Antioxidants Early in Parkinson's Disease Process May Halt Degeneration and Improve Neuronal Function.” *Medical Xpress - Medical Research Advances and Health News*, Medical Xpress, 7 Sept. 2017, medicalxpress.com/news/2017-09-antioxidants-early-parkinson-disease-halt.html.

“Welcome to Carolina Biological Supply.” *Carolina Biological Supply*, 2019, www.carolina.com/.

Research Plan: Project Summary

No addendums exist. No changes were made.