Evaluating the Effects of Curcumin-Coated Gold Nanoparticles on Survival and Motor Functions in a Parkinson's *C. elegans* Model

Grant Proposal

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Abstract (RQ)

Parkinson's disease is a neurodegenerative disease that is caused by the death of dopamine neurons and a-synuclein protein aggregation. PD's neurodegeneration is characterized by motor and non-motor defects, which worsen as the disease progresses. Although there are currently individual treatments synthesized for the symptoms of Parkinson's such as Levodopa, the drugs have several side effects and wear off easily. The present study utilizes the synthesis of gold nanoparticles and curcumin to explore their neuroprotective effects on a Parkinson's model of *Caenorhabditis elegans*. Curcumin and gold nanoparticles were chosen for their neuroprotective properties, ability to reduce oxidative stress, and efficient drug delivery across the BBB. In our study, we used the model organism C. elegans N2 Wildtype and NL5901 Parkinson's strain. To further simulate oxidative stress and neurodegeneration, the neurotoxin 6-OHDA is exposed to the Parkinson's model. This study examines the effects of curcumin-coated gold nanoparticles on locomotion, basal slowing response, lifespan, and *a*-synuclein levels of the nematodes, and compares the results with the control groups of the wild type and 6-OHDA-induced Parkinson's model. The expected results are that the locomotion of the Parkinsonian C. elegans will recover by 45% after Cur-AuNPs treatment in body bends. In addition, the average body bends of the worms for the basal slowing response assay will improve by 60% compared to the treatment groups with the control. The *a*-synuclein levels will also be reduced, demonstrating the therapeutic potential of Cur-AuNPs in alleviating Parkinson's associated neurotoxicity.

Keywords: Parkinson's disease, neurotoxicity, curcumin, gold nanoparticles, locomotion, basal slowing response, *a*-synuclein, lifespan, ROS

Evaluating the Effects of Curcumin-Coated Gold Nanoparticles on Survival and Motor Functions in a Parkinson's *C. elegans* Model

Parkinson's disease (PD) is an age-related neurodegenerative disease that is the second most common neurodegenerative disorder globally after Alzheimer's (Córneo et al., 2020). PD is characterized by various motor and non-motor symptoms, which include low blood pressure, mood swings, tremors in the body, slower movement, and posture instability (Thirugnanam & Santhakumar, 2022). Moreover, these symptoms majorly result from the death of dopamine-producing (dopaminergic) neurons, leading to a significant decline in dopamine levels as the disease progresses. While the exact cause of the disease is still majorly unknown, researchers believe that several genetic and environmental factors are involved (Warner & Schapira, 2003). Thus, several biological processes contribute to the development of Parkinson's such as apoptosis (programmed cell death), *α*-synuclein aggregation, mitochondrial dysfunction, neuroinflammation, and the development of reactive oxygens species (ROS) (Thirugnanam & Santhakumar, 2022). At the cellular level, the overexpression of *α*-synuclein (SNCA), disrupts homeostasis and causes neuronal death, leading to toxicity and loss of dopaminergic neurons in both, in vivo and in vitro models (Hu et al., 2018). Although there are individual treatments synthesized for the symptoms of Parkinson's, there are no specific treatments or drugs that have been discovered to halt the progression of the disease completely. Thus, current research is focused on utilizing natural compounds instead of drugs as a therapeutic treatment for Parkinson's disease.

Curcumin, also known as diferuloylmethane, is a natural compound used in India and Asia for medical treatments. It is found in turmeric, a common spice, and has homeopathic properties such as being an antioxidant and anti-inflammatory (Monroy et al., 2013). It has the striking ability to cross the blood brain barrier (BBB) and protects against mitochondrial dysfunction (Liu et al., 2011). Recent studies have shown that testing curcumin on PC12 cells that are induced with A53T *α*-synuclein, a mutant form linked to Parkinson's disease, may have neuroprotective effects (Liu et al., 2011).

Neuroprotective agents prevent or slow the progression of neuronal damage. As a result, the study highlights how curcumin significantly reduced the ROS levels and protected the cells against neuronal death. Due to its multifaceted protective properties, curcumin is currently being utilized in many treatments for pathological disorders.

Many studies have outlined the potential therapeutic applications of gold nanoparticles (AuNPs) that may be effective in treating neurodegenerative disorders (Grancharova et al., 2024). Gold nanoparticles are synthesized in nanoscale sizes with tunable surfaces, which allow them to deliver drugs in a controlled manner across the blood brain barrier. For instance, the *Paeonia moutan* extract-coated gold nanoparticles were tested in Parkinson-induced mice, whose dopaminergic (DA) neuronal levels were depleted due to exposure to MPTP (a neurotoxin). Figure 1 shows signs of increased DA levels with reduced neurotoxicity (as shown in grip strength and footprint of the mice) to approximately normal levels after nanoparticle treatment. Additionally, ROS levels of the mice were reduced, suggesting that gold nanoparticles are anti-inflammatory and can be used as therapeutic agents (Xue et al., 2019).

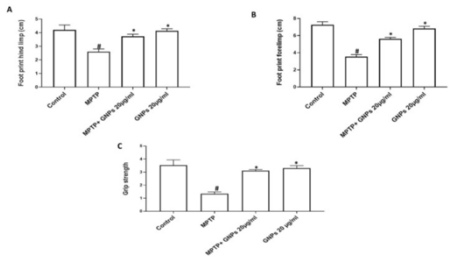


Figure 1. Effect of gold nanoparticles synthesized from *Paeonia moutan extract* (PM-AuNPs) against neuronal dysfunction in Parkinson's induced mice after treatment. The mice were tested for their footprint and grip strength with their behaviors analyzed with t-tests.

6-OHDA, also known as 6-hydroxydopamine, is widely used in preclinical research to model Parkinson's disease. Its primary effects include the degeneration of dopaminergic neurons, induction of neuronal damage, generation of cytotoxic species, and the onset of various motor dysfunctions (Simola et al., 2007). Moreover, 6-OHDA triggers oxidative stress by increasing ROS levels (reactive oxygen species) to amplify the overall neurotoxicity, making it the ideal neurotoxin to create a Parkinson's model. Furthermore, recent research has shown that administering curcumin in a 6-OHDA-induced PD model reduced oxidative stress and lowered the α-

synuclein levels (Grancharova et al., 2024). These findings highlight curcumin's potential as a therapeutic agent for Parkinson's disease in a 6-OHDA-induced model.

The current study utilizes the worm model *Caenorhabditis Elegans* as a model organism to test the therapeutic effects of curcumin-coated gold nanoparticles (Cur-AuNPs). *C. elegans* is an effective model organism due to its small size (1-2 nm), short lifespan (2-3 weeks), and a genome similar to a human, with 302 neurons that have similar functionality (Cooper & Raamsdonk, 2018). Furthermore, this study investigates the effects of curcumin-coated gold nanoparticles (Cur-AuNPs) on motor-related functions in a Parkinson's model of *C. elegans*. We hypothesize that the behavior and locomotion of the Parkinsonian C. *elegans* will recover to near-normal levels after Cur-AuNPs treatment. In addition, we anticipate that the a-synuclein levels will be reduced, demonstrating the therapeutic potential of Cur-AuNPs in alleviating PD-associated neurotoxicity.

Section II: Specific Aims

This proposal's objective is to evaluate the effects of curcumin-coated gold nanoparticles in a Parkinson's C. *elegans* model induced by the neurotoxin 6-OHDA. Our long-term goal is to explore the potential of gold nanoparticle therapy to treat or mitigate the severity of other neurodegenerative diseases. The central hypothesis of this proposal is that curcumin-coated gold nanoparticles will enhance nematode survival by reducing neurotoxicity and restoring motor-related behaviors to normal levels. The rationale behind this approach is that gold nanoparticles reduce oxidative stress and when coated with curcumin, can simultaneously lower α-synuclein levels**.** The work we propose here will determine whether Cur-AuNP treatment is effective in a Parkinson's model of C. *elegans*.

Specific Aim 1: Induce the PD worms with 6-OHDA to further simulate Parkinsonian symptoms

Specific Aim 2: Conduct behavioral assays and an *a*-synuclein assay in C. *elegans*

The expected outcome of this work is that the Cur-AuNPs are non-toxic to the worms and subsequently, the levels of a-synuclein are restored to near-normal.

Section III: Project Goals and Methodology

Relevance/Significance

This proposal will provide scientific evidence regarding the success and safety of using curcumin gold nanoparticles as a potential targeted therapy for Parkinson's disease. If successful, these findings could pave the way for future research into nanoparticle-based therapies for other neurodegenerative disorders. Moreover, Parkinson's disease is a significant public health problem that millions of people suffer from, and my project explores a strategy by using a natural antioxidant and combining it with nanoparticles to decrease oxidative stress and other neuronal dysfunctions. Coating the nanoparticles with curcumin enhances the particle's ability to deliver therapeutic agents effectively. In addition, this contributes to our understanding of the toxicity of gold nanoparticles as the safety of nanomedicine will ultimately be tested in this study.

Innovation

Researchers are beginning to explore the potential applications of utilizing gold nanoparticles as a novel therapeutic strategy for Parkinson's. Due to the strategy being recent, there aren't many similar studies conducted yet. Although gold nanoparticles can exhibit toxicity under certain conditions, they have shown therapeutic outcomes in the field of nanomedicine (Hornos Carneiro & Barbosa, 2016). Similarly, research on curcumin for Parkinson's has shown positive results, such as reducing inflammatory symptoms and oxidative stress, when tested against the neurotoxin 6-OHDA in mice (Bhowmick et al., 2021). Thus, my project combines curcumin with AuNPs to evaluate their efficacy and innovation in a Parkinson's model using the nematode C. *elegans*.

Methodology

This study utilizes *C. elegans* as the model organism due to its simplicity, short lifespan, and similarity to the human genome. Through behavioral assays, the toxicity of Cur-AuNPs and their effects on motor functions can be effectively measured.

First, C. *elegans* are grown on NGM agar plates until they reach the adult L4 stage. The worms are then synchronized to ensure that they are all the same age. This is done using a bleaching method, which isolates embryos from worms of all ages. There are two control groups: Wildtype (healthy, untreated worms) and Parkinson's model (NL5901 strain is induced with 6-OHDA). Next, the experimental groups include plain gold nanoparticles, plain curcumin, and Cur-AuNPs. C. *elegans* will be treated overnight through their food source, E. coli bacteria.

Four major behavioral assays will be conducted to determine the effects of worms' dopamine response and a-synuclein levels: Worm population, Basal Slowing Response, Locomotion, and a-synuclein levels. Worm population assay monitors the survival rate of the worms over time by counting the number of worms alive; this is especially targeted towards the nanoparticle-based treatment group as they may have toxic effects. Next, the basal slowing response assay measures how the worms' locomotion changes before and after they encounter food. Normally, the nematodes tend to slow down when they encounter food; however, in the case of Parkinson's, they move faster in the presence of bacteria due to dopamine dysfunction. Additionally, the locomotion assay studies the general movement of worms, and the body bends per 20 seconds will be counted manually. Lastly, the a-synuclein levels assay will evaluate the levels of a-synuclein through the use of a fluorescent microscope. After performing the assays, the experimental groups will be compared to both the control groups (wildtype and 6-OHDA-induced PD) strain.

Specific Aim #1:

The objective is to induce 6-OHDA into the NL5901 strain of PD. Our approach is to mix 6-OHDA into E. coli bacteria so that the worms can uptake the neurotoxin. Our rationale for this approach is that having the worms' uptake the 6-OHDA through their food source would ensure that the neurotoxin would be in their system.

Justification and Feasibility. C. elegans consume OP50 normally as their food source, making it efficient for drug delivery. By mixing 6-OHDA with OP50 and seeding onto the NGM plates, the neurotoxin can be naturally ingested by C. elegans when they are feeding. This approach also avoids stressing the worms into paralysis when inducing them with 6-OHDA via liquid media.

Summary of Preliminary Data. My data consists of locomotion data for the Wildtype, 6-OHDA-induced PD worms, and its vehicle control Ethanol+PD worms. Worms' locomotion is measured in body bends, and since PD affects motor functions, locomotion is an important assay to perform. Due to 6-OHDA being a solid, we had to dissolve the 6-OHDA in ethanol. Thus, the vehicle control for the 6-OHDA PD group is a PD group exposed to plain Ethanol. According to Figure 2, when comparing the wild type to the 6-OHDA-induced PD worms, the number of body bends is approximately 15 bends lower – a drastic reduction. Student t-tests show that there is a significant difference between the wild type and the 6-OHDA induced worms after the uptake of the neurotoxin ($p<0.001$). Therefore, there is sufficient evidence that the uptake of 6-OHDA did not have any toxic effects and further simulates a PD model.

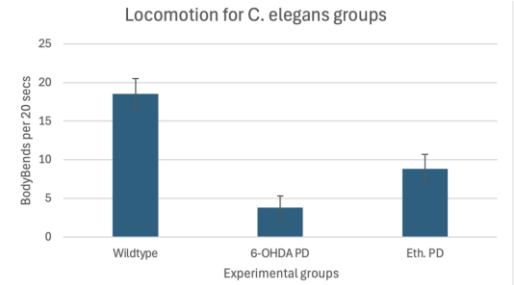


Figure 2. Locomotion for the control groups. Decrease in bodybends per 20 secs after 6-OHDA treatment on PD worms. ($p<0.001$)

Expected Outcomes. The overall outcome of this aim is to confirm that the nematodes have ingested the 6-OHDA neurotoxin safely. This knowledge will ensure that the 6-OHDA-induced worms can

be utilized as a control group for the treatment groups. Because if the effects of 6-OHDA were absent, there must have been an error when exposing the worms to the neurotoxin.

Potential Pitfalls and Alternative Strategies. One potential pitfall is that the 6-OHDA could kill the worms. Because a neurotoxin is meant to promote neurodegeneration, it often amplifies neuronal death. At higher concentrations, the neurotoxin could result in significant toxicity, killing the worms rather than modeling Parkinson's disease effectively. An alternative strategy is to perform a MIC to find the minimum inhibitory concentration with the bacteria. This would essentially help find the highest concentration that will not kill the bacteria – which is how the worms are going to uptake 6-OHDA.

Specific Aim #2:

The objective is to investigate survival and motor functions by looking at toxicity and protective applications of curcumin-coated gold nanoparticles. Our approach is to conduct behavioral assays and an a-synuclein assay on the PD model of C. elegans. Our rationale is that 6-OHDA causes the death of dopaminergic neurons and increases levels of oxidative stress in the worms. Thus, any positive changes that appear in the motor functions and a-synuclein levels will indicate the levels of dopamine improving and returning to normal.

Justification and Feasibility. Several assays, such as locomotion, worm population, basal slowing response, and a-synuclein levels need to be conducted to provide evidence that the Cur-AuNPs have medicinal properties. For instance, Tapia-Arellano et al. (2024) performed a swimming assay, which is part of locomotion, on a PD model of mice. The neurotoxin MPTP is induced in mice and one of the experimental groups includes gold nanoclusters (GNCL). As Figure 3 illustrates, the combination of GNCL and MTPT improves the swimming distance and duration time.

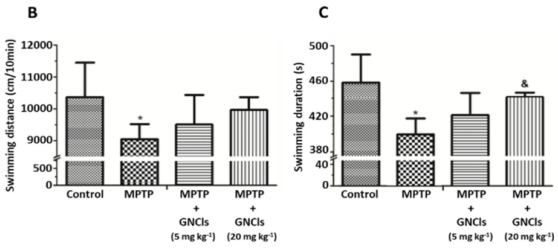


Figure 3. Study encompasses GNCIs (Gold nanoparticles clusters) in a PD model. B) The effects of GNCIs on motor functions of mice in a mouse PD model using a swimming test, measuring swimming distance. C) Evaluation of swimming duration from a swimming test (Tapia-Arellano et al., 2024)

With the gold nanoclusters treatment, the swimming duration and distance are very similar to the control. Through the locomotion assay, the study highlights the potential therapeutic applications of gold nanoparticles in a PD model.

Summary of Preliminary Data. Preliminary data for this aim includes practicing the assays on the C. elegans Wild Type. Performing the preliminary assays ensures that I can handle the practices outlined in the study and can move forward to handling gold nanoparticles and other chemicals. According to Figure 4, the basal slowing response assay was performed on the wild type. The basal slowing response is a worm's response to food, and its locomotion is measured before and after encountering food. For the wild type, it is normal that the worms move slower with food than without as they slow down when they are approaching OP50. The data supports this conclusion, the wildtype strain moving approximately 21 body bends per 20 secs on plain agar and moving 13 body bends per 20 secs when feeding. A student's t-test demonstrates that there is a significant difference between the groups ($p<0.05$).

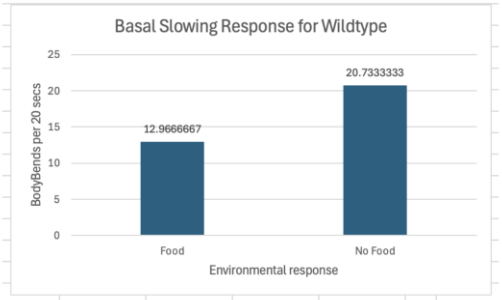


Figure 4. Basal slowing response for Wildtype. There is a significant difference between the food and the absence of food groups after 3 trials. ($p<0.05$)

Expected Outcomes. The overall outcome of this aim is to provide evidence that Cur-AuNPs are non-toxic, enhance survival, and improve motor functions in a PD model. This knowledge will be used to form connections between the data recorded and the degeneration of dopaminergic neurons. Because the control group encompasses the decrease of dopamine levels and Parkinson's symptoms due to 6-OHDA, it should be clear from observing the motor functions in the independent groups if the treatment is working and successful.

Potential Pitfalls and Alternative Strategies. We expect to utilize curcumin-coated gold nanoparticles for three independent groups; however, since the AuNPs were pre-synthesized, it may not

be possible to coat the particles with curcumin. Thus, an alternative strategy is to remove the Cur-AuNPs from the experimental groups and work with different concentrations of plain AuNPs for the independent groups. In addition, another pitfall could be if the treatment indicates no difference from the data of the control group. It demonstrates that the Cur-AuNPs did not work and do not have any therapeutic applications in the PD model. One potential alternative is to increase or decrease the concentrations of AuNPs to alter the overall effectiveness of the treatment.

Section III: Resources/Equipment

This study requires several resources, including C. elegans N2 Strain, C. elegans NL5901 Parkinson's strain, curcumin, 6-OHDA, and AuNPs. It will also use NGM Agar plates that are seeded with OP50 E. coli as the food source. Mass Academy will provide all other standard lab materials required, such as microscopes.

Section V: Ethical Considerations

Consideration #1: In the process of exposing C. Elegans to gold nanoparticles and the neurotoxin 6-OHDA, the worms may die.

Consideration #2: Gold nanoparticles (AuNPs) could pose risks to researchers handling them and the environment if improperly disposed of. If released into the environment, any nanomaterials will have ecological risks because of their toxicity. Proper disposal, containment, and safety protocols will be followed in the lab.

Consideration #3: 6-OHDA is a hazardous chemical that is a risk to the researcher, and the environment, if not handled with safety precautions. These concerns will be addressed by following the Standard Operating Protocol for 6-OHDA in Animals (Tel-Aviv University). Protective equipment will be used when

exposing the neurotoxin to *C. Elegans* and proper disposal of the chemical and contaminated materials will be strictly followed according to hazardous waste regulations.

Section VI: Timeline

1. Phase 1: August-November
2. Phase 2: November- December
3. Phase 3: December – January
4. Phase 4: January – February

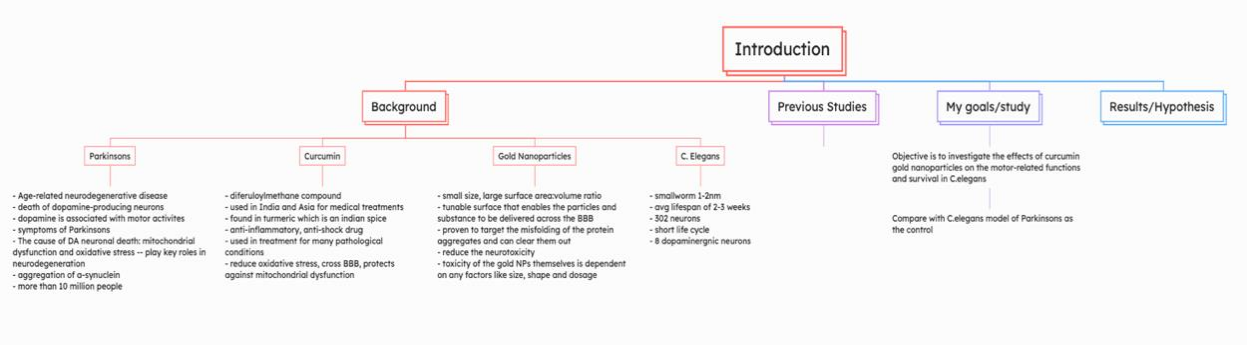
Phase 1: Phase 1 includes initial brainstorming and research potential topics of interest for the STEM project. Once the list of topics is narrowed down to one, reading published works and journals is important to understand the subject of interest.

Phase 2: In Phase 2, the goal is to move forward from reading to starting to develop a potential methodology for preliminary testing. For my project, several professors and labs were contacted to clarify further questions after reading respective journals. In this phase, the ultimate objective was to find a lab that can assist with the complexities and the equipment required for my project.

Phase 3: Phase 3 entails collecting preliminary data and adjusting the methodology of my experiment if needed. This includes growing C. Elegans and practicing conducting behavioral assays on the worm.

Phase 4: In this phase, the goal is to work with nanoparticles and expose them to the nematode. Then, we repeat the assays practiced during preliminary testing and analyze the data reported from the behavioral assays. Results will be used to determine future work and research.

Section VII: Appendix



Appendix 1. Mind map of the Introduction of the grant proposal which specifically outlines the Background section.

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