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# The Remedial Effect of *Mucuna pruriens* extract on Copper-induced Parkinsonian Behaviors in *Daphnia magna*

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***Abstract*—In 2018, the Parkinson's Foundation estimated healthcare for Parkinson's Disease (PD) (treatment, social security payments, lost income) to be a $52 billion industry. PD is a neurodegenerative disorder that induces difficulty in movement and reduced motor skills. The aim of this study was to determine the effects of exposure to *Mucuna pruriens* extract (MPE) on reduced locomotion as a result of copper-induced Parkinsonism in *Daphnia magna*. Further study of *M. pruriens* on *D. magna* served to validate its efficacy on multiple model organisms. Parkinsonian behavior was induced by exposing *D. magna* to copper sulfate (15.9 mg/L) for 96 hours. It was hypothesized that exposure to MPE would increase locomotion in *D. magna* that had previously exhibited Parkinsonian behaviors. *D. magna* were treated with MPE (20, 40 µg/mL) for 96 h. Their movement was then recorded microscopically for 1 min after each exposure. Movement was quantified with the use of wrmTrck, an ImageJ plugin. The distance each daphnid traveled and the frequency at which they spun in terms of bends was measured. Analysis of the data using IBM SPSS v. 25 ANOVA followed by a post hoc Scheffe ($p < .05$) showed that both groups treated with MPE significantly increased movement from the group treated with only copper sulfate. Frequency of spinning significantly decreased in groups exposed to MPE compared to daphnids only exposed to $CuSO_4$. It was concluded that *M. pruriens* shows efficacy as a treatment for impaired behaviors found in Parkinson's disease in *D. magna*.**

## I. Introduction

It is estimated that 930,000 Americans will be diagnosed with Parkinson's disease (PD) by 2020. [1] This number is predicted to further increase to 1.2 million in 2030. PD is a neurodegenerative disorder that develops when the neurons of the substantia nigra in the brain die or become damaged, resulting in the loss of ability to produce dopamine. [2] The most widely prescribed medication for PD is levodopa (L-DOPA) and is converted by the brain into dopamine. [3] However, it causes unwanted effects, such as nausea and vomiting, and even dyskinesia or motor fluctuations. An alternative to L-DOPA that does not have these consequences would be beneficial.

*Mucuna pruriens* (MP), also known as the velvet bean, is a subtropical to tropical legume that has been used to treat neurodegeneration, like Parkinsonism and tremors. Its seeds are a source of L-DOPA, but previous studies have presented that pure *M. pruriens* is a safe alternative to L-DOPA. [4,5] To confirm the efficacy of *M. pruriens* as a possible remedy for PD across multiple model organisms, *D. magna* were utilized for their sensitivity to changes in water chemistry, as well as them being cost-effective organisms.

wrmTrck is an ImageJ plugin used for C. elegans motility analysis. [6] However, it can also be used on other experimental organisms, including *D. magna*. A dopamine receptor signaling pathway, mediated by putative D2-like receptors, was found to be involved in the control of *Daphnia* swimming behavior. [7] Using wrmTrck, the movement of *D. magna* was monitored in terms of distance traveled and number of body bends, in which their circular movement was quantified. Improved movement in *D. magna* would suggest the efficacy of *M. pruriens* as treatment for copper-induced Parkinsonism.

## II. Materials and Methods

Copper sulfate (CuSO4) was used to induce *D. magna* with Parkinsonism. *D. magna* were obtained from Carolina Biological. A concentration of 15.9 mg Cu/L of copper sulfate was used to induce each experimental group of Daphnia magna with Parkinsonian-like symptoms. [8] Each group of *D. magna* was exposed to 500 µL of copper sulfate in a 100 mL container for development of PD for 96 h. *M. pruriens* in its form of *M. pruriens extract* (MPE) was used to remediate the symptoms of PD from Daphnia magna. 20 ug/mL and 40 ug/mL concentrations of MPE were utilized in this study. [9] Each experimental copper sulfate-exposed group was exposed to 120 mL water with dissolved pure MPE from BulkSupplements. All experimental groups were exposed to MPE for 96 h in a controlled environment, and examined after exposure.

*Daphnia* movement was examined through microscopy with the Motic SMZ-171, an industrial stereo microscope. *D. magna* were placed into a small petri dish filled halfway with water. The petri dish was then placed on the stage of the microscope, where the light source illuminated the specimens. Using an iPhone XS, the movement of daphnids per group was recorded for 1 min. After a video was recorded, wrmTrck was used to quantify the data. First, a video of daphnid motility was opened in ImageJ. The background was then subtracted using the rolling ball method in order to remove the uneven background, and the "Rolling ball radius" was adjusted. Next, the movie was converted to binary, where adjusting the threshold indicated what pixels

Figure 1. D. magna exposed to different concentrations of copper sulfate and/or MPE were observed under a microscope. (Photo by Author)

were daphnids and what pixels were background. Afterwards, ImageJ was informed of the real-world scale of the animals in the movie. The "wrmTrck" plugin was then be run, and factors such as minimum (minSize) and maximum (maxSize) worm area in pixels, maxVelocity, "maxAreaChange," "minTrackLength," "FPS," and "bendThreshold," were adjusted. A Results window popped up, containing the tracking analysis of all detected specimens. Movement was quantified based on distance traveled in a span of 1 min by each daphnid, and the frequency of spins in terms of bends.

Data was analyzed using IBM SPSS v. 25 One-way ANOVA followed by a post hoc-Scheffe test, where $p<.05$ and was graphed using Excel.

## III. Results and Discussion

The purpose of this study was to investigate the effects of MPE on movement in *D. magna* as a Parkinson's model. It was hypothesized that *M. pruriens* would be effective in treating Parkinson's symptoms induced by copper sulfate in *D. magna*.

| | **Average Distance Traveled by *D. magna* (mm)** | | | |
|---|---|---|---|---|
| | **Control** | **$CuSO_4$** | **MPE (20 ug/mL)** | **MPE (40 ug/mL)** |
| **Distance** | 18.4 ± 2.40 | 4.63 ± 2.82 | 16.5 ± 4.34 | 18.5 ± 9.59 |

*Figure 2. D. magna exposed to MPE exhibited significantly greater movement than daphnids exposed solely to copper sulfate. (Table by Author)*

Statistical analysis using IBM SPSS one-way ANOVA followed by a post-hoc Scheffe test where $p<0.05$ showed that both groups treated with MPE significantly increased movement from the group treated with only copper sulfate. The groups exposed to MPE demonstrated similar movement to that of the untreated control. The frequency of spinning was greatest with exposure to only $CuSO_4$, and decreased from exposure to MPE. However, spinning increased as the concentration of MPE increased, which could have been due to a higher amount of natural levodopa. These results suggest similar findings to the climbing assay in Johnson et al (2018), where *D. melanogaster* treated with MPE exhibited significantly increased climbing distance compared to flies exposed only to toxins. In the current study, daphnids treated with MPE after $CuSO_4$ exposure exhibited a 256% (20 ug/mL) and a 300% increase in movement compared to those exposed solely to $CuSO_4$. Thus, the alternate hypothesis was supported.

## IV. Conclusion

Significant improvement in movement was exhibited by a novel model organism, *D. magna*, after MPE treatment. With exposure to increased concentrations of MPE (20, 40 ug/mL), daphnids demonstrated the ability to travel greater distances, signifying remediation of PD-like symptoms. However, decreased spinning exhibited at the lower MPE concentration indicated that higher concentrations of MPE exhibited higher concentrations of L-DOPA, which may have resulted in dyskinesia-like symptoms. The overall success of MPE in remediating PD-like symptoms in *D. magna* suggests that it may exhibit similar success in other model organisms for which MPE treatment has not been studied, and more importantly, humans. A cheaper alternative to the current treatment process of PD that could be provided by MPE would enable a greater population of PD patients to have access to treatment. *M. pruriens* is a legume, which can be easily grown in a variety of climates and regions. Its use in the future as a treatment for PD would be to act as a natural, cost-effective alternative to current treatments. Due to the COVID-19 pandemic, full data collection could not be completed. Future study of *M. pruriens* on PD model organisms would provide further support for its efficacy as an alternative treatment for PD.

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