The Effect of Ginkgo Biloba Extract on Beta-Amyloid Aggregation in C. elegans

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***Abstract*— In 2019, the Alzheimer's Association estimated that approxima tely 5.6 million people in the United States alone are affected by Alzheimer's disease. Alzheimer's is a neurodegenerative disorder associated with the accumulation of beta-amyloid proteins, resulting in inflammation that disrupts synaptic functioning. The purpose of this experiment was to determine the extent to which *Ginkgo biloba* extract could be used to remediate beta-amyloid-induced paralysis in C. elegans. *Ginkgo biloba* is an antioxidant containing ginkgolides, which reduce inflammation by regulating cytokines. Additionally, *Ginkgo biloba* contains flavonoids, which reduce oxidative stress by limiting the buildup of free radicals, another characteristic of Alzheimer's. Transgenic strain CL2120 worms, which express beta-amyloid paralysis, were exposed to either 50µg/mL, 100µg/mL, 150µg/mL, or 0µg/mL (control) of *Ginkgo biloba* extract. After 48 hours, paralysis was determined by prodding individual worms with a platinum worm pick, with full body movement indicating the worm was not paralyzed, and no movement or only head movement indicating paralysis. Statistical analysis of the data using IBM SPSS v. 25 ANOVA followed by a Post Hoc Scheffe with $p<0.05$ showed that there was a statistically significant reduction in paralysis in worms treated with *Ginkgo biloba* extract when compared to the control. Overall, there is a correlation between the addition of *Ginkgo biloba* extract and a reduction in beta-amyloid-induced paralysis. This data may lend itself in the future to studies observing not only the use of *Ginkgo biloba* extract as a treatment for Alzheimer's symptoms, but also as a method of Alzheimer's prevention.**

I. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes cognitive impairment, including memory loss and confusion. On a cellular level, AD develops by the enzymatic activities of β-secretase and γ-secretase, rather than α-secretase and γ-secretase, on Amyloid Precursor Protein (APP). This amyloidogenic pathway leads to the formation of amyloid-beta (Aβ) peptides. Aβ aggregation causes inflammation, which can disrupt synaptic functioning, resulting in neurological damage and disruption of cell communication. (Bali et al, 2010). This describes the widely accepted amyloid cascade hypothesis that states that the aggregation of Aβ peptides results in neurotoxicity, senile plaque development, and Alzheimer's-related dementia [1].

Another common pathology of AD is oxidative stress, caused by Aβ aggregation. Oxidative stress is characterized by the formation of free radicals, namely reactive oxygen species (ROS), which are highly reactive molecules that lack a valence electron. Due to this, they are unstable, and oxidize many compounds in the brain, including DNA and proteins, causing significant neurodegeneration [2].

AD is also associated with the formation of neurofibrillary tangles (NFT) of tau proteins in neurons. Aβ releases kinases into neurons, which are enzymes that allow for the hyperphosphorylation of tau, which is when all phosphorylation sites are saturated. This causes the tau proteins to change structure, thus resulting in their loss of function, which is necessary for maintaining the structural integrity of microtubules in neurons [3].

Ginkgo biloba is an antioxidative chemical with many properties contributing to its ability to combat neurodegenerative disorders. One component of *Ginkgo biloba* extract is ginkgolides, which is a platelet-activating factor (PAF) receptor antagonist that is able to limit the binding of the platelet-activating factor (PAF) to the platelet activating factor receptor (PAFR), a G-protein-coupled-receptor (GPCR). Through the PAF signaling cascade, inflammation is caused by PAF binding to PAFR, which results in the synthesis of mediators such as leukotrienes, pro-inflammatory chemicals. By preventing PAF binding to PAFR, ginkgolides are able to reduce inflammation that is characteristic of AD [5]. The flavonoid component of *Ginkgo biloba* extract is also responsible for limiting the production of free radicals by donating electrons to ROS, inhibiting them from destabilizing compounds in the brain. Additionally, *Ginkgo biloba* extract is associated with the upregulation of protein phosphatase, which is able to reduce the hyperphosphorylation of tau [1].

II. Methods

C. elegans strain CL2120 (donated by the CGC) was separated into 4 groups, one control and three experimental groups (0, 50, 100, and 150 µg/mL of *Ginkgo biloba* extract). *C. elegans* were cultured in petri dishes containing nematode growth medium (NGM) agar and *E. coli* OP50 as a food source, and prior to conducting trials, all *C. elegans* were age synchronized through centrifugation [6].

150 mL of *Ginkgo biloba* extract were added to 3mL of agar in 35 mm petri dishes, with concentrations varying per experimental group. After 48 hours of exposure to the respective concentrations, the paralysis assay was

conducted. In *C. elegans,* paralysis is a common symptom of beta-amyloid aggregation, suggesting the presence of AD, and thus testing for paralysis indicates whether beta-amyloid plaques are present in the worms. The paralysis assay involved using a platinum wire pick to transfer individual *C. elegans* onto a new petri dish containing only NGM agar. Following this, paralysis was tested using a platinum wire worm pick, where individual worms were stimulated lightly on the head. Following stimulation, worms were determined to be paralyzed or not paralyzed, based on whether they exhibited any body movement. Three trials of the paralysis assay were performed, and 15 worms per group were tested per trial.

Following paralysis assay completion, averages were compiled per group in Microsoft Excel. Statistical analysis was performed using a One-Way Anova followed by a Post-Hoc Scheffe test with $p<0.05$ in IBM SPSS v. 25 to determine significance between groups.

III. Results and Discussion

After performing statistical analysis, it was determined that all three concentrations of *Ginkgo biloba* extract (50, 100, 150 μg/mL) significantly reduced beta-amyloid induced paralysis. 75% of the control group exhibited paralysis, compared to only 10% of the 50 μg/mL group, 3.33% of the 100 μg/mL group, and 20% of the 150 μg/mL group, as shown in Figure 1. While the three experimental groups were statistically significant from the control, they were not statistically significant from each other.

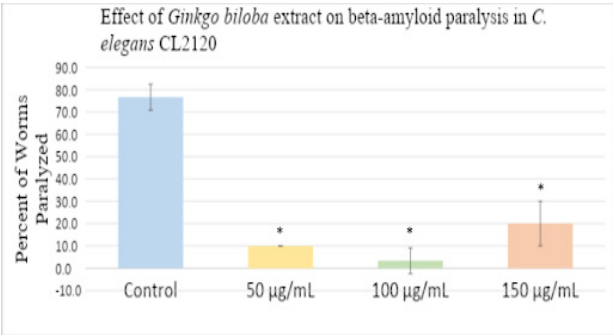

Figure 1: Percentage of CL2120 C. elegans paralyzed after 48 hours, n=15. Statistical significance determined by a One-Way ANOVA with a post-hoc Scheffe test ($p<0.05$). Error bars represent a 95% confidence interval. Significance is indicated by asterisks. (Graph by Author)

The probable causes for this significant reduction in paralysis are the ginkgolide and flavonoid components of the *Ginkgo biloba* extract. The ginkgolide component, which regulates platelet activation via limiting PAF binding to PAFR, allows for reduction of inflammation caused by the aggregation of platelets and the release of inflammatory chemicals. The limitation of beta-amyloid aggregation results in reduced paralysis, as evidenced by the results and data collected. Additionally, the flavonoid component is able to limit the production of ROS through its ability to donate a valence electron, thus negating the threat of ROS oxidizing easily-oxidizable compounds in the brain. This reduces neurodegeneration, and thus prevents paralysis from occurring in *C. elegans* [1].

IV. Conclusion

To conclude, the purpose of this experiment was to analyze the effects of *Ginkgo biloba* extract on reducing beta-amyloid paralysis in transgenic *C. elegans* strain CL2120. After performing the paralysis assay, it was determined that all experimental concentrations of *Ginkgo biloba* extract significantly reduced the percentage of *C. elegans* paralyzed. In addition, this study suggests that, pending further research, *Ginkgo biloba* extract, along with other similar antioxidative chemicals, may be applied in a human medical context as treatment for AD. In the future, *Ginkgo biloba* extract could be studied as a potential treatment contributing to the prevention of AD onset, rather than a remedy for AD symptoms. Additionally, further testing may show that *Ginkgo biloba* extract could be combined with other anti-inflammatory or antioxidative chemicals such as epigallocatechin gallate (EGCG) to test the effect of multiple treatments on AD symptom remediation.

V. Acknowledgments

I would like to thank Mrs. Flanagan, Mrs. Zhu, Ms. Cheryl, and Ms. Huenger for their support throughout the commencement of my research, along with the Manhasset Secondary School and the Manhasset Secondary School Science Research Program for allowing me to conduct my research.

VI. References

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