A Combination of Epigallocatechin gallate and Ginkgo biloba delays the onset of Alzheimer's disease symptoms in a Drosophila model

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ABSTRACT: Alzheimer's disease (AD) is the leading cause of dementia; approximately 5 million people are impacted annually. Popular drugs to treat AD, like Donepezil and Rivastigmine, have significant side effects, so there is a need for natural treatments to prevent or reduce AD symptoms. There are studies on preventing dementia using natural products; however, no systematic research exists. I studied five natural products — Moringa oleifera, Sesamum indicum, Vaccinium, Epigallocatechin gallate (EGCG), and Ginkgo biloba — in preventing AD using Tau and Aβ Drosophila melanogaster lines. Our data showed that these natural products could not be used to treat AD once it has progressed beyond the early stages of the disease; however, our data confirmed that some of these natural products could be used to delay AD onset when treated early. EGCG and G. biloba extracts treatment groups were less symptomatic than the control samples. As the concentrations in EGCG and G. biloba increased, the number of flies with smooth eyes and uniform ommatidia decreased, with a 10% concentration having the largest decrease in the smooth eye. A combination of EGCG and G. biloba showed very good endpoints, with 10% EGCG + 10% G. biloba being the best and very close to the healthy wild-type flies (with smooth eye and uniform Ommatidia) for both Tau and Aβ lines. The combination extracts showed better endpoints and delayed the onset of AD in the flies by approximately 20 days, preventing the production of all the pro-inflammatory cytokines.

KEYWORDS: Biomedical and Health Sciences, Alzheimer's; Drosophila; Epigallocatechin gallate; Ginkgo biloba.

Introduction
Alzheimer's disease (AD) is characterized by an irreversible chronic and progressive degeneration of the nervous system, especially neurons in the brain, resulting in loss of behavioral and intellectual capabilities over time. Some symptoms include increased memory loss and confusion, difficulty with language, inability to learn new things, problems with reasoning or judgment, shortened attention span, and disorientation. It has recently been shown that AD is the leading cause of dementia, a general term of memory loss enough to interfere with daily life. Approximately 70% of individuals experiencing dementia do so due to AD, and around 5 million people are impacted. Some studies reveal that the degeneration in the brain cells may initiate several years before the onset of cognitive impairment, during which abnormal augmentation of amyloid and tau proteins in the brain happens. When such degeneration of brain cells begins, it leads to the malfunction of some neurons, eventually causing the death of brain cells. The greatest known risk factor is age, and the second factor is preexisting genetic conditions.

There are many common side effects to the popular drugs available in the market, such as donepezil, galantamine, and rivastigmine. Common side effects are diarrhea, heart and lung damage, dizziness, raised blood pressure, bladder damage, and high fertility impairment at high doses. Therefore, finding alternate treatments, such as treatments with natural products for AD, is necessary to avoid such side effects. Moringa oleifera has several antioxidants, anti-inflammatory, and antidiabetic properties and is used to treat age-related dementia. The leaf extracts improve and enhance cholinergic spatial memory and have been shown to decrease acetylcholine esterase (AChE) activity. Acetylcholine is a chief neurotransmitter of our nervous system that contracts muscles, dilates blood vessels, and slows the heart rate. AChE is the enzyme that hydrolyzes acetylcholine to choline and acetate and, as a result, causes several brain disorders.

Previous studies have shown that Sesamum indicum lignans, present in Sesame seeds and oil, decrease age-related dementia due to their antioxidant and anti-inflammatory properties and have neuroprotective effects in several models of brain dysfunction. Sesamum indicum lignans suppress age-related cognitive decline in senescence by reducing lipid peroxide and inducing anti-oxidative enzymes. EGCG, a polyphenol catechin, is abundant in green tea leaves and has antioxidant and anti-inflammatory properties. EGCG is also found in small trace amounts in apple skin, plum, onion, and hazelnut. Several studies suggest that drinking tea reduces the risk of memory-related disorders. The leaves of the Ginkgo biloba trees are used in treating older adults with neurodegenerative diseases, peripheral vascular diseases, and other memory problems. Some studies also showed that Vaccinium bilberry extract (BBE) could treat developmental and behavioral defects and slow the progression of cognitive decline in mice by...
BBE-induced enhancement of memory-associated neuronal signaling.\textsuperscript{16}  

*Drosophila melanogaster* has been used as a model system to study cellular and physiological, and behavioral traits of human neurodegenerative diseases. *Drosophila* has been used for several decades to study the effects of various drugs.\textsuperscript{17-20} Nearly 75% of human disease-causing genes are present in the *Drosophila* genome.\textsuperscript{21} The *Drosophila* compound eye contains approximately 700–750-unit eyes known as ommatidia.\textsuperscript{22} Ommatidia can be seen under the microscope and evaluated for uniformity qualitatively. Glass is a transcription factor present in eye cells during eye development and can exert control over various genes by turning them on or off. I used a transgenic *Drosophila* line where the glass multimer reporter (GMR) promoter drove the expression of tau in the developing eyes. Tau expression in the fly eye causes a moderately reduced eye size and roughened surface (non-uniform ommatidia), a phenotype that can be visibly seen under the microscope. In the tau line, a decrease in the size of the eye is one of the symptoms of AD.

Amyloid beta 42 (A\textbeta\textbeta\textbeta\textbeta)I, generated from proteolytic processing of amyloid precursor protein, is neurotoxic and directly linked to AD development. I measured the climbing efficiency (the number of flies that pass a certain point) in *Drosophila* using a Gal4-UAS expression system to express A\textbeta\textbeta\textbeta\textbeta within the nervous system to determine the disease progression.\textsuperscript{23} Another system is the tau line, which expresses “rough eye” on the flies’ eyes that is easy to detect and measure via simple microscopes.\textsuperscript{24}

While several studies use natural products to treat AD, no systematic research compares the effects of these specific natural products in preventing or treating AD. I hypothesized that the standard natural products *M. oleifera*, *S. indicum*, *V. accini*, EGCG, and *G. biloba* would treat and prevent AD using a *Drosophila* model. Unfortunately, our data showed that these natural products could not be used to treat AD once it has completely formed. However, our data supported that some of these natural products can delay the onset of AD when treated early.

### Materials and Methods

**D. melanogaster Care:**

I purchased *Drosophila* wild-type flies from Carolina Biological Supply Company and mutant strains (RetMEN2B and UAS-GAL4 driver) from Bloomington *Drosophila* Stock Center at Indiana University. I followed all the necessary guidelines provided by the supply center.\textsuperscript{24} I fed all the flies with Formula 4-24\textsuperscript{20} Instant *Drosophila* Medium food and water. Six to eight grains of yeast were added to each of the vials.

**Drosophila lines:**

I used two different *Drosophila* models (Tau and A\textbeta system). In the first model, I used a GMR- MAPT.V337M (Stock# 51367) line where tau containing a p.V337M missense variant is driven in the eye under the direct control of GMR. The endpoints I checked are rough eye, size of the eye, ommatidia, and fly survival rate. I fed the flies and the larvae with various natural extracts and compared the endpoints with the control sample, which was treated with standard food (Formula 4-24\textsuperscript{20} Instant *Drosophila* Medium).

In the second model, I used the GAL4/UAS system to express A\textbeta\textbeta\textbeta\textbeta carrying the "Arctic" mutation (p.E693G, a familial AD mutation) in the nervous system. I crossed a nSyb–Gal4 line (Stock# 39171) to a UAS-A\textbeta\textbeta\textbeta\textbeta line (Stock# 33774) in this model. After about three weeks, these flies go from being typically mobile to being unable to climb in the space of a few days. I fed the flies and the eggs with various experimental food treatments and compared the endpoints with the control sample which was provided standard food.

Vials were flipped every 2 to 4 days to amplify the stocks until I had enough flies to work with. I used 6–8 females and 4–5 males per vial. Then, I flipped the adults to new food every 2 to 4 days (depending on how fast they laid eggs) approximately 2 to 3 times total.

#### Climbing assay:

I used a clear vial to watch the flies move up the vial walls and recorded using a video. I used ten flies per vial and counted the number of flies that climbed about 3 inches in 10 seconds. I tested each vial ten times at one-minute intervals, i.e., I tapped the flies down, counted the number of flies above the line after ten seconds, tapped them down again, counted again, repeated one more time, and then averaged the results.

**Experimental procedure:**

To test the effectiveness of the natural products (*Sesamum indicum*, *Moringa oleifera*, *Vaccinium*, EGCG, and *Ginkgo biloba*) at the maximum concentrations, I took five vials with 12–15 AD flies as decided by the climbing assay. I compared the AD flies’ eyes to that of the wild type. Pure natural products are denoted as 100% concentration.

I took 18 vials, four vials for each of the natural products, and two vials for the control samples with varying concentrations ranging from 20% to 80% concentrations. I added 12–15 AD flies to each of the vials. I set up a GMR-Tau cross for the Tau line and an A\textbeta\textbeta\textbeta\textbeta–Gal4 cross for the A\textbeta line in those 18 vials (Figure 2). I diluted all the natural products with water. I also included water control and wild-type control samples.

I separated all the parent flies from the vials to clearly distinguish adults from the progeny. I let the larvae that fed on the natural products grow into adults and checked their eyes for the “rough eye” and “uniform ommatidia.” It took 4–5 days for all the larvae to turn into adult flies. I examined the eyes of all the flies on the microscope and measured endpoints such as length and width of the eye, number of flies with uniform ommatidia, and climbing efficiency. I fed all the larvae for 4–5 days and flies afterward for around 30 days.

**Dosage curve:**

For the adult flies’ dose-response, I took four vials for each natural product and added 10%, 20%, 30%, and 40% concentrations of each product I prepared to the vials. I maintained the total volume of the natural products and water at 15 mL.

**Measuring the size of the eyes:**

The microscope I used in the study is AmScope SE306R-PZ-LED Stereo Microscope with 20X/40X/80X Magnification. I
captured all the images with the same 40X magnification and the same microscope lens. I printed out the images and used ratio and proportions to calculate the sizes of eyes for each sample using the wild-type flies’ eyes as approximately 0.6 mm long and 0.35 mm wide from the literature for comparison.25

I took around 9–10 flies and averaged to get the length and width of the eyes of flies in each vial.

**Statistical analysis:**
I used a simple two-sample t-test to test the difference between the length and width of the eyes of the test samples against the control flies. If the p-value was < 0.05, I rejected the null hypothesis that there is no difference between the means and concluded that a significant difference does exist.

### Results

**Testing the effects of 100% concentrated natural products on AD and fly viability:**

I compared the effects of each natural product — *Moringa oleifera*, *Sesamum indicum*, *Vaccinium*, *EGCG*, and *Ginkgo biloba* — at 100% concentration on AD using the GMR-Tau system to express tau in the developing *Drosophila* eye and the Aβ system that shows climbing deficiency. I placed 14–15 flies from these two systems in five test vials, each containing one of the five natural products. I also set up three control vials: one with GMR-Tau, one with Aβ-Gal4 systems (without any natural product), and the last one with just the healthy flies (without any natural product). Four to five days later, all the flies in the test vials died, whereas control and reference samples were alive, demonstrating that a 100% concentration of any natural product is lethal to the flies. Then, I determined the concentrations at which the flies would survive.

Dose-response curves can determine the concentrations for each natural product at which flies remain viable. I sought to generate dose-response curves and use these concentrations for the experiment using the Tau and Aβ line flies. All the flies died in the Vaccinium-treated vials at all concentrations tested, so I did not use Vaccinium going forward. The flies from the rest of the all natural products (*Moringa oleifera*, *Sesamum indicum*, *EGCG*, and *Ginkgo biloba*) died for all concentrations greater than 40%. Based on our results, I selected 10%, 20%, 30%, and 40% concentrations for all the natural products.

**Treating AD using dosage curve concentrations (on adult flies):**

Next, I aimed to compare the effects of each of the various concentrations of the natural products (i.e., *Moringa oleifera*, *Sesamum indicum*, *EGCG*, and *Ginkgo biloba*) in preventing AD. Vials of the selected concentrations were prepared for each natural product, and control sample vials were for both Tau and Aβ systems. I added 14-15 flies in each natural product and control sample vial. All the flies were treated with food around 30 days after they eclosed from the pupa. I examined the eyes of the tau line flies in each vial under the microscope at 45 days of age and the Aβ flies for climbing efficiency. All the flies in all the natural products treated sample vials showed rough eyes on the tau lines (Figure 1) and no improvement in the flies’ eyes on the Aβ lines compared to the control samples flies (Figure 2), which implies that the natural products may not be able to cure AD once it is formed in the adult stages.

![Figure 1](https://www.ijhighschoolresearch.org)

**Figure 1:** Representative images of the eyes for Tau lines from each natural product. All flies treated with 100% concentration of natural products showed rough eyes. (a) 100% *Moringa oleifera*-treated flies, (b) 100% *Sesamum indicum*-treated flies, (c) 100% *EGCG*-treated flies, (d) 100% *Ginkgo biloba* treated flies, (e) control flies, and (f) healthy wild type flies.

For the length of the eyes, 10% *EGCG* and 10% *Ginkgo biloba* samples were significantly higher than the control samples, with p-values of 0.031 and 0.049, respectively. For the samples, 10%, 20%, 30% *Sesamum indicum*, and 30% *Moringa oleifera*, the length of the eyes is significantly lower than the control samples with the p-values 0.018, 0.034, 0.014, and 0.048, respectively. For the samples 10%, 20% *Moringa oleifera*, 20%, 30% *Ginkgo biloba*, and 30% *EGCG* the lengths of the eyes are not significantly different from the control samples with the p-values 0.7, 0.73, 0.46, 0.34, 0.44 and 0.43.

![Figure 2](https://www.ijhighschoolresearch.org)

**Figure 2:** The average length and width of the flies’ eyes. Flies treated with 10% and 20% *EGCG* and 10% *Ginkgo biloba* were promising and showed longer sizes (lengths and widths) than the control sample but smaller sizes than the healthy wild-type flies. The error bar represents the standard deviation of the distribution within each sample.

For the width of the eyes, 10% *Ginkgo biloba*, and 10%, 20%, and 30% *EGCG* did show smooth eyes and uniform ommatidia on the flies’ eyes with the p-values 0.44, 0.47, 0.24, 0.49, and 0.042, respectively. For the samples, 10%, 20%, 30% *Sesamum indicum* and 10%, 30% *Moringa oleifera*, the lengths of the eyes are significantly lower than the control samples with the p-values 0.01, 0.034, 0.013, 0.048 and 0.045 respectively. For the samples, 20% *Moringa oleifera*, 20%, 30% *Ginkgo biloba*, and 30% *EGCG* are not significantly different from the control samples with the p-values 0.22, 0.42, 0.25, and 0.51.

**Testing if I can reduce AD using dosage curve concentrations (on larvae):**

Nine to ten flies for each treatment group were analyzed, and each sample’s average length and width were calculated (Figure 3A-B). Flies treated with *Moringa oleifera*, *Sesamum indicum*,
and 30% *Ginkgo biloba* showed smaller eye sizes than the control sample (width: 0.33mm vs. 0.325mm vs. 0.33mm, respectively). Flies treated with *Moringa oleifera* had bigger eye sizes than those treated with *Sesamum indicum*. Flies treated with 20% *Ginkgo biloba* and 30% *EGCG* showed the same size eyes (length and width) as the control sample, so I did not successfully prevent AD. Flies treated with 10% and 20% *EGCG* and 10% *Ginkgo biloba* showed longer length and width than the control sample but smaller length and width than healthy wild-type flies.

I performed a simple t-test to compare each natural treatment condition to the control sample to determine if the treatment significantly affected the length and width of the flies’ eyes. For the size of the eyes, 10% *EGCG*-treated and 10% *Ginkgo biloba*-treated samples were considerably higher than the control samples with *p*-values < 0.05. For the samples, 10%, 20%, 30% *Sesamum indicum*-treated, and 30% *Moringa oleifera*-treated samples, the length of the eyes was significantly lower than the control samples with *p*-values < 0.05. The remaining treatments were not very different from the control samples, with *p*-values > 0.05. For the width of the eyes, 10%, 20% *EGCG*-treated, and 10% *Ginkgo biloba*-treated samples were significantly higher than the control samples with

![Figure 3](image)

**Figure 3:** Flies’ eyes of the 10%, 20%, and 30% concentrations treated natural extracts. (a) 10% *Ginkgo biloba*, (b) 20% *Ginkgo biloba*, (c) 30% *Ginkgo biloba*, (d) 10% *Moringa oleifera*, (e) 20% *Moringa oleifera*, (f) 30% *Moringa oleifera*, (g) 10% *EGCG*, (h) 20% *EGCG*, (i) 30% *EGCG*, (j) 10% *Sesamum indicum*, (k) 20% *Sesamum indicum*, (l) 30% *Sesamum indicum*, (m) 10% Control and (n) Healthy wild-type flies. None of the *Moringa oleifera*, *Sesamum indicum*, and 30% *Ginkgo biloba*-treated flies lead to smooth-eye or uniform ommatidia for any of the flies.

10%, 20% *Ginkgo biloba*, and all concentrations of *EGCG* did show smooth eyes and uniform ommatidia on the flies’ eyes with *p*-values < 0.05. For the samples, 10%, 20%, 30% *Sesamum indicum*-treated samples, 10%, and 30% *Moringa oleifera*-treated samples, the width of the eyes is significantly lower than the control samples with the *p*-values < 0.05. The remaining treatment groups were not significantly different from the control samples. Sometimes, small *p*-values (less than 5%) happen by chance, which could lead to incorrect rejection of the true null hypotheses.²⁵ In future work, I want to address this by incorporating Benjamin–Hochberg correction to avoid errors (false positives).²⁵

*Moringa oleifera*-treated, *Sesamum indicum*-treated, and 30%-*Ginkgo biloba*-treated samples did not lead to smooth-eye or uniform ommatidia for any of the flies (Figure 3A–N). Ten percent and 20 percent *Ginkgo biloba*-treated and all concentrations of *EGCG*-treated samples did show smooth eyes and uniform ommatidia on the flies’ eyes. The number of flies with smooth eyes and those with uniform ommatidia was significantly higher than the control samples for both *Tau* and *Aβ* lines, including the control sample, were plotted (Figure 4A–B). For *EGCG*-treated and *Ginkgo biloba*-treated samples, the number of flies with smooth eyes and uniform ommatidia decreased with natural product concentration. So, lower concentrations of *Ginkgo biloba* and *EGCG* showed better performance in preventing AD. Our study revealed that 10% *EGCG* is the best candidate, among those tested in our research, to prevent AD in the Tau line of the *Drosophila* fly model. We plan to perform further studies with even lower concentrations of the treatments.

![Figure 4](image)

**Figure 4:** The number of flies with smooth eyes and the number of flies with uniform ommatidia for each of the vials, including the control sample, were plotted. (a) number of flies with the smooth eye from each of the 10%, 20%, and 30% concentrations of the natural extracts, including the control sample and healthy wild-type flies. (b) the number of flies with the uniform ommatidia from each of the 10%, 20%, and 30% concentration of the natural extracts including the control sample and healthy wild-type flies. For *EGCG* and *Ginkgo biloba*, the number of flies with smooth eyes and uniform ommatidia decreased with natural product concentration.

I plotted the climbing efficiency of flies for each of the samples, including the control sample, for the *Aβ* system (Figure 5). As the number of days increased, the climbing efficiency decreased. All the flies in all the experimental vials except the healthy wild-type flies showed a sharp drop in the climbing efficiency between 10 to 20 days, indicating the onset of neurodegeneration. Flies at all ages showed better climbing efficiency at low concentrations of *EGCG* and *Ginkgo biloba* compared to the control sample but not as good as the healthy wild-type flies. 10% *EGCG* showed the best result showing the plot of climbing rate with age very close to that of the healthy flies compared to all the other samples. 20% *EGCG* and 10% *Ginkgo biloba* results are the next, with climbing efficiency curve slopes flatter than the rest of the samples.

![Figure 5](image)

**Figure 5:** The climbing efficiency of flies for each sample, including the control sample for the *Aβ* system. As the number of days increased, the climbing efficiency decreased. All the flies in all the experimental vials except the healthy wild-type flies showed a sharp drop in the climbing efficiency between 10 to 20 days, indicating the onset of neurodegeneration.

**Combination of EGCG and Ginkgo biloba extracts to prevent AD:** Since both *EGCG* and *Ginkgo biloba* showed better endpoints than the control samples for both *Tau* and *Aβ* lines, I tested the combination of these two natural extracts
for preventing AD using the Drosophila model. This study was done on larvae as well. I picked different combinations of 10% and 20% concentrations of EGCG and Ginkgo biloba and evaluated the Tau and Aβ lines for eye roughness, eye size, and climbing efficiency. A combination of 10% EGCG and 10% Ginkgo biloba showed the best performance compared to other combinations and the control sample. Flies treated with 10% EGCG + 10% Ginkgo biloba showed a completely smooth eye and uniform ommatidia, similar to the healthy wild-type flies’ eyes (Figure 6A-E). The other combinations still showed some rough eye and non-uniform ommatidia. Fly eye length and width and the number of flies with smooth eyes with a combination of 10% EGCG + 10% Ginkgo biloba were comparable to the healthy wild-type flies (Figure 7A-B). All the combinations of EGCG and Ginkgo biloba concentrations showed better climbing efficiency than the control samples (Figure 8). As the number of days increased, the climbing efficiency decreased. The climbing efficiency curve of the eyes treated with a combination of 10% EGCG + 10% Ginkgo biloba was similar to that of the healthy wild-type flies. Though there was still a drop in the climbing efficiency for the flies treated with the combination, the drop shifted from 10–20 days for the individual extracts to 30–40 days for the combined samples.

![Figure 6: Eyes of the flies treated with the combination of EGCG and Ginkgo biloba. (a) 10% EGCG + 10% Ginkgo biloba, (b) 20% EGCG + 10% Ginkgo biloba, (c) 10% EGCG + 20% Ginkgo biloba, (d) Control sample and (e) healthy wild-type flies. 10% EGCG + 10% Ginkgo biloba showed a completely smooth eye and uniform ommatidia, similar to the healthy wild-type flies’ eyes. The other combinations still showed some rough eye and non-uniform ommatidia.](image)

![Figure 7: Fly eye sizes (length and width) and a number of flies with smooth eyes with a combination of EGCG and Ginkgo biloba compared to control and healthy samples (a) The average length of the flies’ eyes in each of the vials treated with a combination of EGCG and Ginkgo biloba was plotted to compare to each other and the control and healthy flies’ samples. (b) The average width of the flies’ eyes in each of the vials treated with a combination of EGCG and Ginkgo biloba was plotted to compare to each other and the control and healthy flies’ samples. Flies treated with 10% and 20% EGCG and 10% Ginkgo biloba showed longer sizes (lengths and widths) than the control sample but smaller sizes than the healthy wild-type flies.](image)

![Figure 8: The climbing efficiency of flies for each of the samples treated with a combination of EGCG and Ginkgo biloba, including the control sample for the Aβ system. As the number of days increased, the climbing efficiency decreased. The climbing efficiency curve of the flies treated with a combination of 10% EGCG + 10% Ginkgo biloba was closest to that of the healthy wild-type flies. Though there was still a drop in the climbing efficiency for the flies treated with the combination, the drop shifted from 10–20 days for the individual extracts (shown in Figure 5) to 30–40 days for the combined samples.](image)

**Discussion**

I hypothesized that the common natural products Moringa oleifera, Sesamum indicum, Vaccinium, EGCG, and Ginkgo biloba would help treat or prevent AD in Drosophila. This study showed that some of these natural products could prevent AD when treated during the early stages. Our study indicated that these natural products could not treat AD once it is completely formed during the adult stage of a fly’s life cycle. The Drosophila wing imaginal disc is a sac-like epithelial structure found inside the larva of insects that undergo metamorphosis and later turn into the external structures of the head, thorax, limbs, and genitalia. Several studies on these discs are done to experimentally address how cells and tissues respond to the gain or loss of specific gene functions. Imaginal discs of Drosophila eyes are formed during the initial larval stage and hence the rough eyes. Most of the food the flies eat during their life is consumed during the larval stage. I hypothesized that these natural products could help prevent AD if the larvae feed on the experimental food before they close from pupa into an adult fly. Therefore, I concluded that these natural products could not prevent AD once it forms in adult flies. Since most of the food consumption in the flies was done during the larval stages, as per our observation, I hypothesized that treating flies with natural compounds before the initial larval stages may prevent AD.

Though these natural products may not cure or treat AD, they could potentially be used for preventing AD. Sesamum indicum- and Moringa oleifera-treated flies showed worse endpoints on the Tau line eye sizes and Aβ line climbing efficiency than the control sample flies, though the reason is unclear. Moringa oleifera and Sesamum indicum have antioxidant and anti-inflammatory properties but don't have anti-neuroinflammatory specific properties and hence may not be able to prevent AD in the flies.

Though I used 10%, 20%, 30%, and 40% concentrations for the natural products based on the dosage curve, all the flies in the 40% concentration did not survive more than a week. So, I only selected 10%, 20%, and 30% concentrations of all the natural products for testing. The flies in the Vaccinium vials...
died at all concentrations tested. In addition, the smell of the Vacinium extract was more potent than other extracts tested and may have contributed to increased mortality. Because of this, I could not use the Vacinium extract further in this study.

Both EGCG and Ginkgo biloba extracts showed better endpoints compared to the control samples for the Tau and Aβ lines. As the concentrations in EGCG and Ginkgo biloba increased, the number of flies with smooth eyes and uniform ommatidia decreased, showing the best performance at 10% concentrations. I did not test less than 10% concentrations in this study, which will be explored in future work. EGCG at all concentrations (10% - 40%) of the extracts showed better endpoints than the Ginkgo biloba extracts.

A combination of EGCG and Ginkgo biloba showed very good endpoints, especially 10% EGCG + 10% Ginkgo biloba being the best and showing the endpoints very close to the healthy wild-type flies. This was the case for both the Tau and the Aβ lines. The combination extracts authenticated better endpoints and delayed the incidence of AD in the flies by approximately 20 days. One thing to note is that even among the EGCG- and Ginkgo biloba-treated flies, none of the vials showed 100% of flies with smooth eyes. Some of the larvae may not have consumed or absorbed the extracts fully. Hence, when those larvae turned into adult flies, they displayed rough-eye symptoms.

Microglia are the immune cells of the central nervous system that regulate brain development, maintenance of neuronal networks, and injury repair. Microglial cells help remove damaged neurons and infections. In AD patients, microglia steadily become less efficient at these processes, which may lead to neuronal damage such as neuroinflammation (associated with deposition of Aβ in the brain), oxidative stress, and neurodegeneration. LPS is bound by surface receptors on the microglia and causes the production of pro-inflammatory cytokines (IFNγ, TNFa, IL-6, IL16, IL1β), oxidative stress species (reactive oxygen species and lipid peroxidation), and neurodegeneration through NF-κB and JNK/ERK/P38 signaling pathways.

Ginkgo bilobaide B, an active ingredient of Ginkgo biloba, suppresses the JNK/ERK/P38 signaling pathways and inhibits the production of IFNγ, IL-6, and IL-8 cytokines. EGCG suppresses NF-κB, and MAPK activation inhibits the production of TNFα and IL1β cytokines and prevents oxidative stress and neurodegeneration. Both EGCG and Ginkgo biloba dampen the production of several pro-inflammatory cytokines.

We want to focus on translating this study to humans in our future work. Based on the current study in Drosophila eye development occurred during the larval stage. We surmise that it may occur in the womb when it translates to humans. Therefore, we would have to administer the supplements during the human pregnancy stage.

There are some limitations in this study. The microscope used had low resolution, limiting the eye measurements’ accuracy. The flies were anesthetized, but slight movements made capturing sound and high-quality images difficult. I compared the fly eye size with approximate values obtained from the literature and compared the test samples to that as a reference. Since I compared the size of the eyes among each sample, the actual numerical values were not deemed as important for the study.

In this study, I only extensively studied concentrations of natural products lower than 10%. In future research, I would like to analyze concentrations of EGCG-treated and Ginkgo biloba-treated samples lower than 10% and compare them with the control and healthy wild-type flies for both Tau and Aβ lines. I want to test the lowest concentration at which I could see the effect of these natural products. I would also like to combine these natural products with medicines that are already FDA-approved for the treatment of AD, such as Aducanumab.

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