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Effects of Vitis vinifera L. Seed Extract on Short-Term Memory of Amyloid-Beta-Mediated Neurodegeneration in Transgenic Caenorhabditis elegans

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Effects of *Vitis vinifera L.* Seed Extract on Short-Term Memory of Amyloid-Beta-Mediated Neurodegeneration in Transgenic *Caenorhabditis elegans*

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By

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Abstract

Alzheimer's disease (AD) is a neurodegenerative condition that is a common cause of dementia and a growing concern worldwide with no effective treatment or cure. Two pathological AD hallmarks include buildup of amyloid beta (Aβ) plaques and neurofibrillary tangles in the brain. Recent attention has turned to exploring natural products and compounds for AD symptom relief and treatment. *Vitis vinifera* grape seed extract (GSE) contains many beneficial substances. GSE has been tested in several animal models and has shown to improve memory and is even being examined in an AD treatment human clinical trial. In this study, the aim was to examine the potential effects of *V. vinifera* GSE on Aβ-mediated neurodegeneration in transgenic *Caenorhabditis elegans*. A transgenic *C. elegans* strain expressing human Aβ1-42 in glutamatergic neurons that displays progressive, age-dependent neuron loss was used. Behavior and memory were measured utilizing a short-term memory assay (n = 100 per group). Experimental groups included N2 wildtype control, transgenic Aβ1-42 expressing strain, and the transgenic plasmid control strain. Each experimental group was treated with 2 concentrations of GSE (1 mg/mL and 10 mg/mL) or nematode growth media without treatment. The results of the memory assay of the baseline controls showed a trending difference between the N2 and UA198 strains. And when the treatment group at 1 mg/mL were compared to the control groups there was significance with a P value at 0.0343.

Acknowledgements

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that is a common form of dementia. One of the first symptoms is the inability to store new memories (Lopez et al., 2019). The most common early symptom of AD is sporadic memory loss, but it progresses to the inability to carry out everyday life. Some common early symptoms are repeating conversations, asking questions repeatedly, and difficulty remembering details of current events (Lopez et al., 2019). There are non-memory related symptoms, but these are uncommon (Lane et al., 2018). There is currently not a cure for AD and only the symptoms are treated. There are some medications available, but a successful prevention of amyloid beta plaque buildup is not available (Lane et al., 2018; Lopez, 2019). The first treatment is usually a cholinesterase inhibitor which only addresses one of the many causes of AD (Lopez et al., 2019), leaving all the other pathways unchecked.

There are many causes of AD that include genetic mutations across three known genes (amyloid protein precursor, presenilin-1, and presenilin-2) (Oliveira, 2021) and a buildup of amyloid beta plaques (Lane et al., 2018; Ho et al., 2009; Mahdipour et al., 2022). The buildup of amyloid beta (Aβ) plaques is the result of an impaired or damaged protein proteostasis (Mahdipour et al., 2022). Tau aggregation and neurofibrillary tangles are another cause (Lane et al., 2018; Ho et al., 2009; Mahdipour et al., 2022). Tau, in excess, clusters together creating inflammation in the brain along with the decrease of acetylcholine. This inflammation causes AD progression (Mahdipour et al., 2022). Some other proposed causes are the breakdown of the cholinergic neurotransmitter system (Shahat et al., 2015) and the abundance of free radicals (Uttara et al., 2009). Regardless of the cause, activation of inflammatory pathways is consistent

with most AD cases. Neuroinflammation is directly correlated with cognitive impairment (Solfrizzi et al., 2006). Aβ is shown to activate the inflammatory cytokines and the microglia (Garcez et al., 2017). This neuroinflammation and cytokine presence increases tau phosphorylation which leads to neuronal death (Oliveira et al., 2021). The microglia activate the pattern recognition receptor which in turn trigger an immune response (Oliveira et al., 2021). The immune response then starts an inflammasome assembly (Heneka et al., 2014) which leads to cell death. Therefore, prevention or treatment of neuroinflammation might be one solution to the AD problem.

Recent attention has turned to exploring natural chemicals and methods for symptom relief. Many plants contain substances that are used medicinally today. *Citrullus colocythis,* also known as bitter apple, and *Emex spinosa,* also known as devil's thorn, have shown to inhibit acetylcholinesterase. It breaks down the cholinergic system which is a cause of AD (Shahat et al., 2015). *Vitis vinifera* grape seed extract (GSE) contains many beneficial substances including polyphenols, proanthocyanidins, antioxidants (Nallathambi et al., 2020), catechin, flavonols, and gallic acid (Harbeoui et al., 2019). The seeds produce the most phenolics, flavanols, ferric reducing-antioxidant power, and antioxidants at a higher concentration compared to the fruit (Rockenbach et al., 2011; Martin et al., 2020; Mahdipour et al., 2022). These different substances have been shown to have many health benefits including antiulcer, antioxidant, antimicrobial, anticancer, neuroprotective, and bone healing properties (Martin et al., 2020; Mahdipour et al., 2022). GSE has been shown to decrease the cytokines in RAW264.7 microphages (Harbeoui et al., 2017). GSE has increased some antioxidant enzymes in the presence of lipopolysaccharide (LPS) induced stress in mitochondria and reduced the amount of

cytokines in Caco-2 colon cells under the LPS induced stress (Nallathambi et al., 2020). Some polyphenols found in grape seeds can cross the blood brain barrier making them a candidate for treatment in neurodegenerative diseases (Gates et al., 2022). In animal model experiments, GSE has been shown to improve the intestinal barrier integrity, performance and wound closure in IPEC-J2 cells and prolong the lifespan in N2 *Caenorhabditis elegans* (Sander et al., 2023). It has also prolonged the lifespan of *C. elegans* with gonad cancer (Ozpinar et al., 2017), and it improved the memory of aged rats (Sarkaki et al., 2007).

Since memory loss is the earliest symptom of AD, studies started looking into the extract as a possible treatment. Additionally, some polyphenols present in *Vitis vinifera* GSE inhibited amyloid beta build up in a mouse model (Gaamouch, 2021) which is one of the causes of AD. GSE has shown to block some amyloid beta production and has the potential to interfere with the formation of tau (Pasinetti & Ho, 2010). Grape seed polyphenolic extract produced by Meganatural-Az® has shown to slow the build-up of specific tau peptides (Ho et al., 2009). It has been used as a treatment for multiple conditions. The results found that grape seed extract does reduce the oligomerization of amyloid beta peptides and increased the monomeric peptides (Wang et al., 2008). Grape seed oil was proven to prevent the degradation of acetylcholine, but not as well as the donepezil, the current medication used for treatment. However, the results were only significant when the mice were treated before the scopolamine, or the AD onset. So, grape seed extract cannot restore tissue damage (Berahmand et al., 2020). All APP/PS1, or AD, treatment groups showed less degradation compared to the control group, however, the higher concentration of grape seed proanthocyanin showed less degradation than the lower concentration. Grape seed proanthocyanin also decreased amyloid precursor protein and tau

expression compared to the APP/PS1 control group (Lian et al., 2016). Another study investigated the polyphenols present in grape seed extract on AD mice models. The polyphenol group showed less amyloid beta deposition in the brain when compared to the control with the polyphenol group being the most effective compared to curcumin (Wang et al., 2009).

One model often used for research, *Caenorhabditis elegans* is a great model to use for age-related neurodegenerative diseases like AD. It has a short life span and a fully defined neuroanatomy that makes it a good model for animal studies. The organism does not have a higher ordered brain but does have an intricate system of neurons. There is a total of 302 neurons throughout the nematode body, but they are mostly concentrated in the head and tail (Hobert, 2018). Also, the body of the nematode is transparent which makes the viewing of neuronal fluorescence easier. In addition, it also has the same cellular mechanisms, molecular pathways, and major functions in synaptic transmissions as mammals (Caldwell et al., 2020). Some transgenic nematode strains that overexpress human Aβ can be used for studies exploring compounds that impact this important hallmark of AD. For this study the utilized AD model is the UA198 strain because the Aβ 1-42 is concentrated in the neurons (Griffin et al., 2017). This potentially makes the results easier to be translated to humans (Griffin, 2017). Multiple transgenic models for AD exist. One model focuses on the buildup of Aβ due to the human amyloid precursor protein and another one contains human apolipoprotein E (ApoE) (Caldwell et al., 2020). Using the ApoE model, it was concluded that out of the 4 different ApoE the ApoEε2 was protective against Aβ neurodegeneration (Griffin et al., 2019). Multiple aspects of AD have been studied using *C. elegans* including amyloid beta toxicity, protein quality control, relationship of amyloid beta and aging, neurobehavior, and many more (Griffin et al., 2017).

This study aimed to examine the effects of GSE on the memory of different strains of *C. elegans*: wildtype (N2), Aβ 1-42 (UA198), and the plasmid control for Aβ 1-42 (DA1240) at different concentrations of GSE. *C. elegans* were exposed to the GSE after the expression of human Aβ and neuron loss to see if the GSE could treat and rescue memory loss in the AD nematode strain. All experimental groups were examined using a short-term memory behavioral assay reported by Kauffman and colleagues (Kauffman et al., 2011). Next, a chemotaxis assay was completed to quantify the short-term memory of the strains (Kauffman et al., 2011).

Materials and Methods

All experiments detailed in this study were conducted in accordance with established standard operating protocols and procedures as set forth by the research laboratory of Dr. Ashley Turner.

Polymerase Chain Reaction and Gel Electrophoresis

DNA was extracted from each nematode strain using a nematode lysis solution. The lysis solution was made up of 500 µL of 1 M KCl, 100 µL of 1 M Tris (pH 8.3), 25 µL of 1 M $MgCl_2$, 45 µL of NP40 and Tween 20, 50 µL of 2% gelatin, and 10 mL of molecular water. A total of 450 µL of lysis solution along with 50 µL of proteinase K (stock solution of 10 mg/mL) was used for the final lysis solution. Next, 40 µL of the final lysis solution was added to a PCR tube along with 10-15 nematodes for each nematode strain (Table 1). Lysis tubes were then placed at –80℃ overnight followed by heat denaturation.

A PCR master mix was created for Aβ and GFP primer sets using 2.5 µL of each 10 µM forward and reverse primer, 62.5 µL of Taq 2X master mix (BioLabs, Lot #10083502, Vial

#M0270S), and 42.5 µL of nuclease free water. Each PCR reaction was carried out at a volume total of 11.5 µL of PCR master mix and 1 µL of DNA template. The primer sets used are outlined in Table 2. PCR reaction tubes were placed in a thermocycler and ran on the following cycling program: 95℃ for 2 minutes, 95℃ for 30 seconds, 55℃ for 1 minute, 68℃ for 1 minute with final three steps being repeated for 30 cycles. PCR reactions were stored at -20℃ until gel electrophoresis could be carried out.

All PCR reactions were resolved on 6% TBE-polyacrylamide gels alongside a 100 bp DNA ladder (Promega, Lot #0000447665, Reference #G2101). Each poly gel was ran at 150 V for 45 minutes and then stained with GelRed (Biotium, Catalog #41003), exposed to UV, and imaged with a gel documentation system.

Nematode Strains and Culture

Wildtype strain of *C. elegans*, N2, was obtained from the Caenorhabditis Genetics Center (CGC). UA198 (glutamatergic Aβ expression - *baIn34*[P*eat-4*::A*β*, P*myo-2*::mCherry]; *adIs1240*[P*eat-4*::GFP] and DA1240 (GFP expression - *adIs1240*[P*eat-4*::GFP+lin-15(+)] were obtained in collaboration with Drs. Guy and Kim Caldwell at the University of Alabama (Griffin et al., 2019; Griffin et al., 2017). GRU101 and GRU102 were also obtained from the CGC. The pharyngeal muscle GFP expressing mutant strain was obtained in collaboration with Drs. Melissa LaBonty and Mike Miller at the University of Alabama at Birmingham. Table 1 outlines all strains used in the study.

Escherichia coli OP50-1 was obtained from the CGC and grown in liquid cultures of LB broth. Nematode growth media (NGM) plates were made following standard protocols and were seeded with live OP50-1 (Stiernagle, 2006).

All nematode strains were maintained on seeded NGM plates at 20°C in a refrigerated incubator. Nematodes were transferred with a platinum wire tool. The tool was sterilized before each use by flame and 70% EtOH.

Grape Seed Extract

GSE from Meganatural®-BP was obtained as GSE 150 mg extended-release tablets. The tablets were crushed into powder using a mortar and pestle. The crushed powder was then dissolved in 200 proof 100% ethanol (Decon Laboratories Inc., CAS #64-17-5, Bottle #3916EA) at a stock concentration of 100mg/mL. The GSE solution was vortexed for 2-3 minutes. Next, the solution was filtered with a 0.45 μm vacuum filter system (Thermo Scientific, Catalog #166-0045). The stock GSE solution was added directly to NGM after autoclaving and cooling to 55°C to generate plates at two concentrations (10 mg/mL and 1 mg/mL).

Timed Egg Lay

Ten adult hermaphrodite nematodes were transferred to two 35 mm seeded NGM plates for each strain. Nematodes were left to lay eggs for 4 hours at 20 °C for timed egg lay. Adult hermaphrodites were then removed from each egg lay plate. Egg lay plates were then wrapped in parafilm and placed at 20 °C incubator.

Short-term Memory Behavioral Assay

The assay was conducted on day 3 after the timed egg lay. Nematodes are removed from the plates by washing with 500 μL M9 buffer. The M9: nematode suspension was pipetted into a 15 mL tube. Note, the micropipette filter tip was cut off to avoid damaging the nematodes during transfer. This process was repeated twice with each 35 mm plate for each nematode strain. The

nematodes were allowed to settle to the bottom of the tube by gravity and the supernatant was removed with a micropipette.

Next, the lid of a new seeded 35mm plate was streaked with 2 µL of 10% butanone diluted in 95% EtOH. Stock reagents of butanone (100%, Thermoscientific, Catalog L13185.AU, Lot Q19I034) and ethanol (100%, Decon Laboratories, Catalog #3916EA, Lot 252016) were used. Then, 100-200 µL of nematodes were transferred to this plate using a pipette and incubated at room temperature for 1 hour. The plates were washed with about 500 µL of M9 buffer and transferred into new 15 mL tubes, repeating the washing process twice. These nematodes were used for the downstream chemotaxis assay. Each experiment was completed three times for each group.

Chemotaxis Assay

All chemotaxis assay plates were unseeded NGM plates. 60 mm plates were marked with three dots equal distance from each other with the following designations: “O” is the origin of the nematodes/ where the nematodes are first pipetted and placed, “B” is where the butanone was pipetted, and “E” is where the EtOH was pipetted (Figure 3). 1 µL of 1 M sodium azide solution was added 10-15 minutes before the assay at the EtOH and butanone points. Next, 1 µL of 10% butanone and 95% ethanol were added in specific spots. The butanone dilution was made on the day of the assay. 15 µL of nematode suspension was delivered in 5 µL intervals at the origin spot, again cutting the pipette tip. Excess M9 buffer was blotted up with the twisted corner of a paper towel. Presence of nematodes was confirmed with dissecting microscope. Plates were then incubated at room temperature for 1 hour.

Following the incubation period, the number of worms at B, E, and O were counted and compared to the total number of nematodes on the plate, including those paralyzed around the points. The plate was imaged and used to count nematodes a second time following each experiment. Each experiment was completed three times for each group.

Chemotaxis Index

A chemotaxis index was used to calculate the overall memory within each experimental group. The number 1 on the index represents the strongest association of the odor to food with 100% choosing the correct odor. The formula is provided below:

$$\frac{(Number\ of\ nematodes\ at\ butanone - number\ of\ nematodes\ at\ ethanol)}{Total\ nematodes}$$

Results

DNA was extracted from 6 nematode strains and used in PCR (Table 1). N2, UA198 and DA1240 were experimental groups being confirmed for the study. There were 3 additional strains used for controls for two PCR experiments.

Table 1

List of C. elegans strains used with brief description

Nematode Strains	**Description**
N2	Wildtype
DA1240	Plasmid control for UA198 with GFP expression *adIs1240*[P*eat-4*::GFP+*lin-15*(+)]

UA198	Aβ 1-42 and GFP expression; concentrated in the neurons; glutamatergic Aβ expression *baIn34*[P*eat-4*::A*β*, P*myo-2*::mCherry]; *adIs1240*[P*eat-4*::GFP]
GRU101	Plasmid control for GRU102 with YFP expression *gnaIs1* [*myo-2*p::yfp]
GRU102	Aβ 1-42 and YFP expression; concentrated in the neurons. *gnaIs2* [*myo-2*p::YFP + *unc-119*p::Abeta1-42]
GFP	GFP expression strain; pharyngeal muscle GFP expression

The two primer sets obtained from IDT and used are provided in Table 2. The GFP primer set was used to confirm the presence of GFP. The Aβ primers were used to confirm the presence of Aβ.

Table 2

List of primers with brief description

Target	**Forward Sequence**	**Reverse Sequence**	**Target length**
GFP	5' GGTCCTTCTTGAGTTTGTAAC 3'	5' CCATCTAATTCAACAAGAATTGGGACAAC 3'	1500 bp

Aβ	5' CCGACATGACTCAGG ATATGAAGT 3'	5' CACCATGAGTCCAAT GATTGCA 3'	100 bp

Figure 1 shows the resolved Aβ PCR reactions by gel electrophoresis. PCR reactions loaded in lanes 4 and 7 showed amplification of the target Aβ band around 100 bp. These two include the experimental group UA198 containing Aβ and the control group GRU102 containing Aβ. There were no other nematode strains that showed positive amplification of Aβ which was consistent with what was expected.

Figure 1

Gel results of Aβ PCR experiment confirming the genotypes of the nematode strains

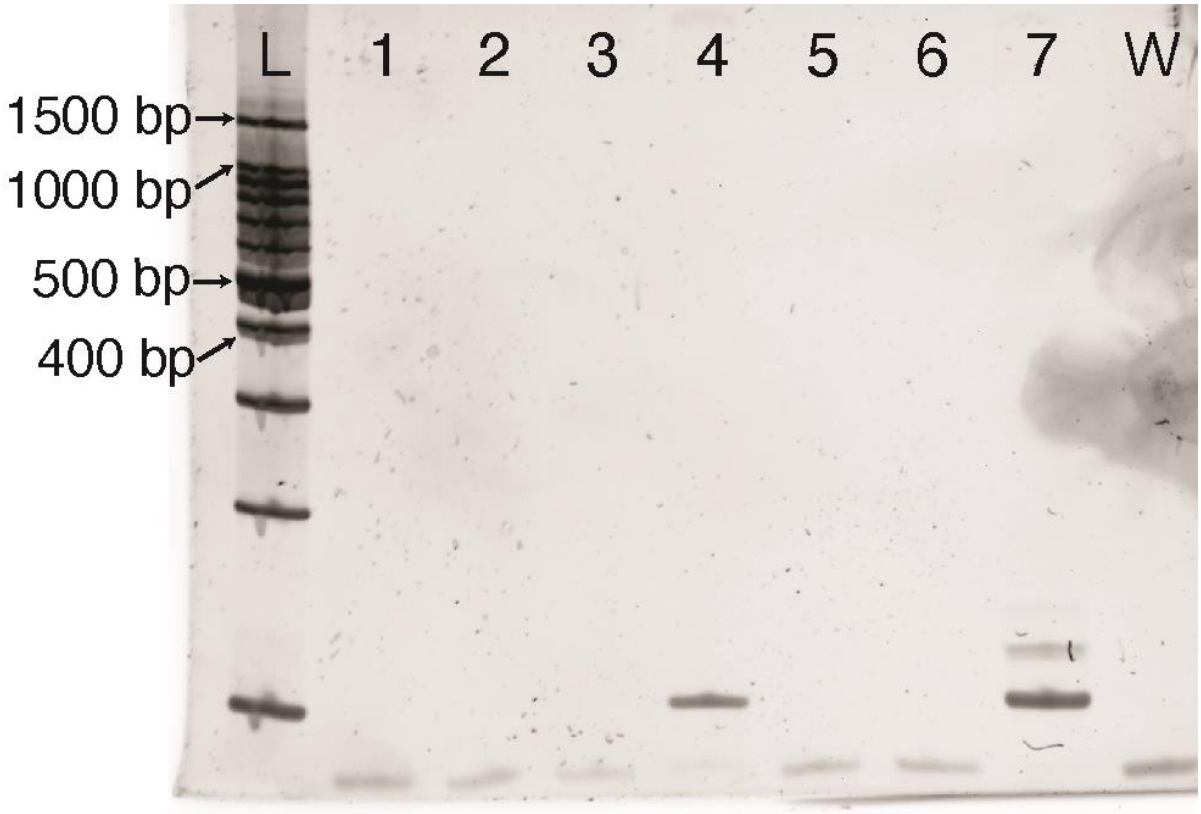


Image of Gel Red stained polyacrylamide gel showing initial PCR amplification for Aβ primer set using a 55 ºC annealing temperature. L = 100 bp ladder, 1 = N2 (positive control), 2 = N2 experimental (extract), 3 = GFP, 4 = GRU102, 5 = GRU101, 6 = DA1240, 7 = UA198, and W = water (no template control).

Figure 2 shows the resolved GFP PCR reactions by gel electrophoresis. PCR reactions loaded in lanes 6 and 7 showed amplification for the target GFP band around 1,500 bp. These include the two experimental groups DA1240 and UA198. containing GFP in their plasmid inserts. There were no other nematode strains that showed positive amplification of GFP, including the GFP nematode strain.

Figure 2

Gel results of GFP PCR experiment confirming the genotypes of the nematode strains

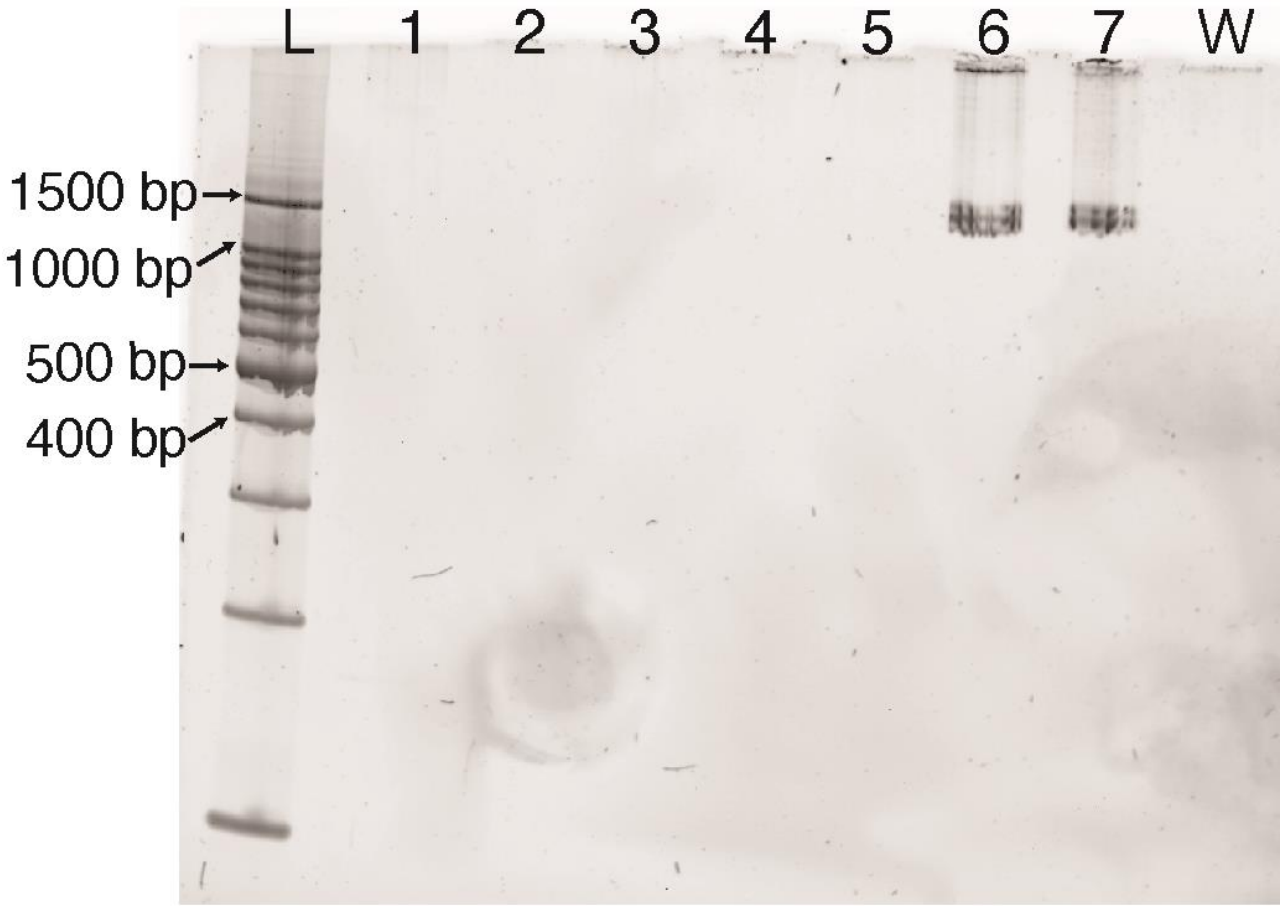


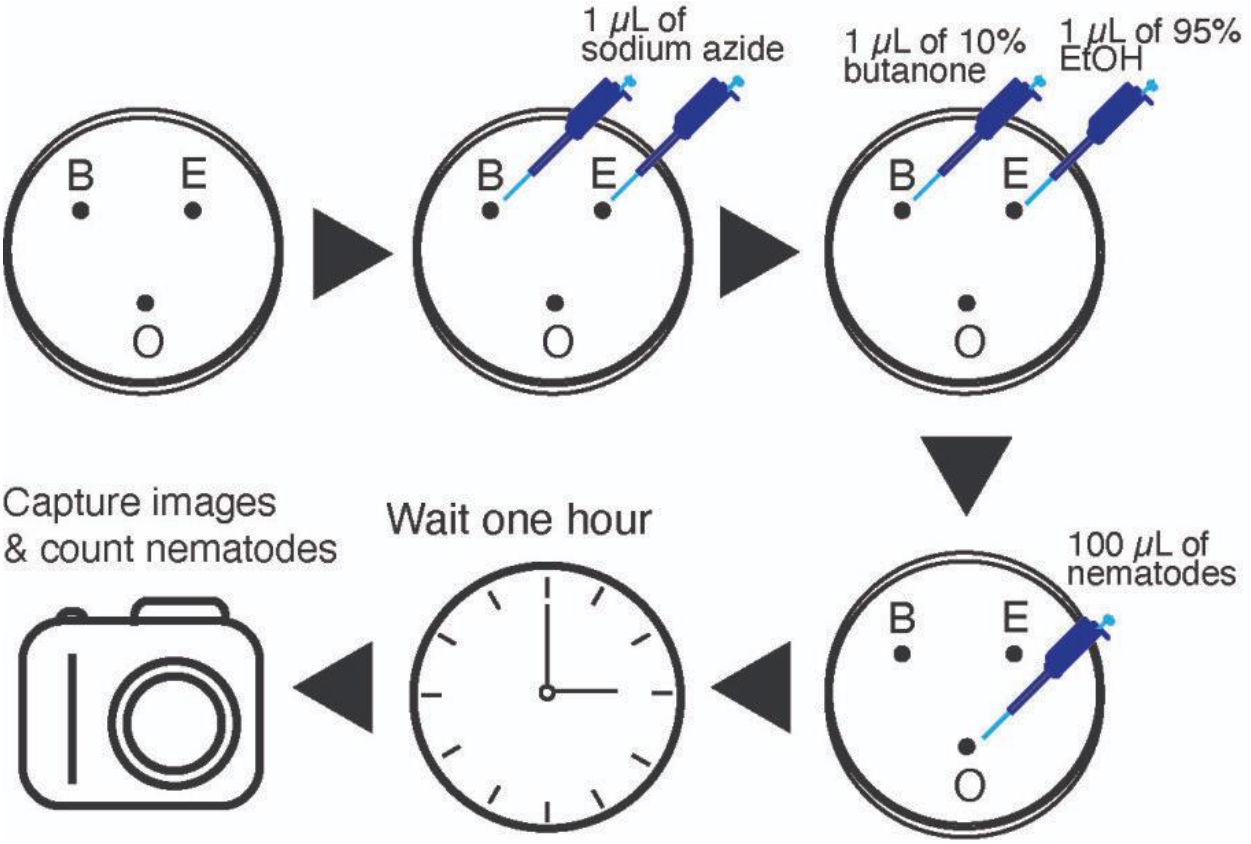
Image of Gel Red stained polyacrylamide gel showing initial PCR amplification for GFP primer set using a 55 ºC annealing temperature. L = 100 bp ladder, 1 = N2 (positive control), 2 = N2 experimental (extract), 3 = GFP, 4 = GRU102, 5 = GRU101, 6 = DA1240, 7 = UA198, and W = water (no template control).

The full experiment has two main components, the short-term memory assay and the chemotaxis assay. The first one conditions the nematodes to associate a certain chemical odor (10% butanone) with available food and eating. The second one tests the short-term memory of

the nematodes. So, the chemotaxis assay is completed directly following the short-term memory assay (Figure 3).

Figure 3

Chemotaxis assay design

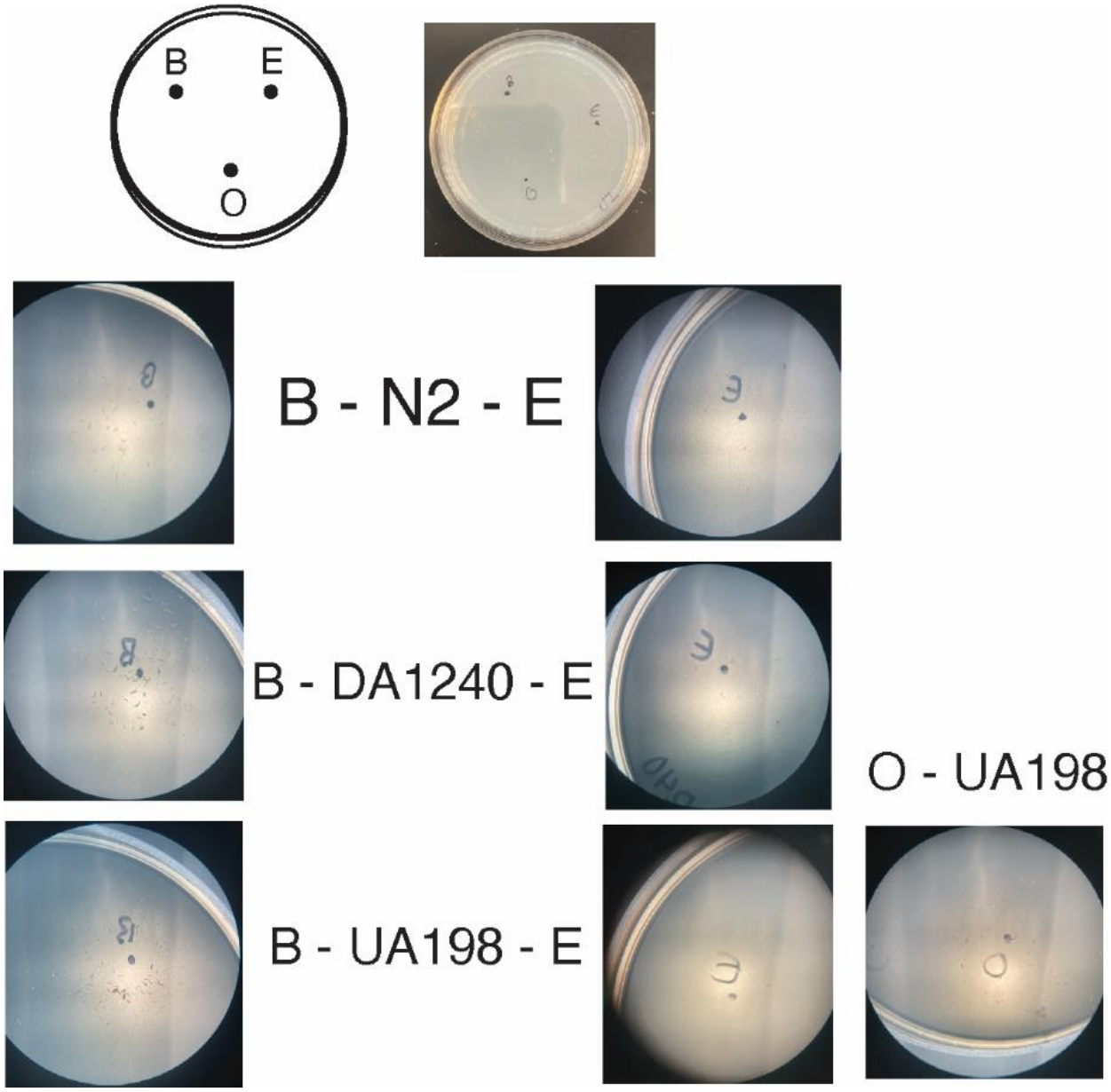


The first set of experiments were examining the baseline behavior and short-term memory for the experimental groups of N2, UA198, and DA1240. Three experimental groups were tested in each experiment. The experiment was repeated independently 3 times. The plate and B/E/O spots of one of the replicates is displayed in Figure 4. The images show the nematodes paralyzed at the odor they were drawn towards. The N2 and DA1240 have most of the nematodes present in the sample at the butanone spot showing correct odor association.

However, the AD model, UA198, shows a significant number of nematodes at the O, or origin spot, which shows no odor association with food.

Figure 4

Images of chemotaxis results of control baseline experiments

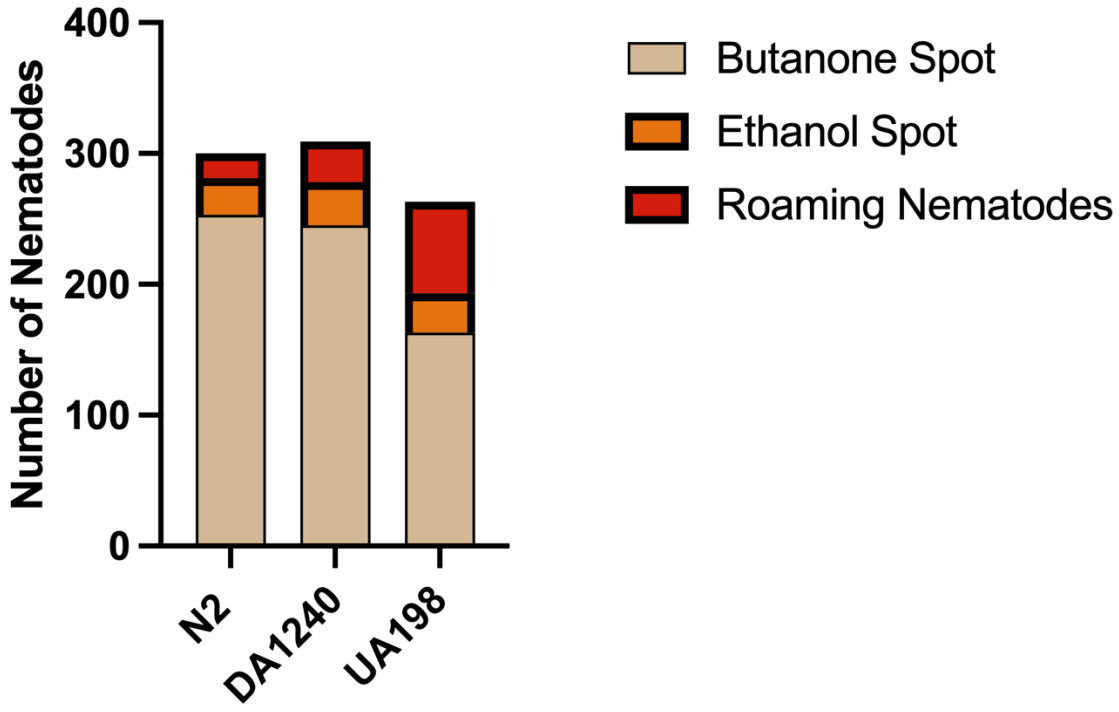


Chemotaxis assay images at B, E, and O spots of the experimental groups after the hour waiting period.

Figure 5 displays the combined data from the three independent experiments. The graph shows that the data is trending towards UA198 group having a lower number of nematodes at the B spot compared to N2 and DA1240 groups. The calculated chemotaxis index for each group showed a similar trend with a lower chemotaxis index for UA198 group compared to the two control groups (Figure 6). A Kruskal-Wallis test was performed across the groups for the chemotaxis index data set and it showed no statistical significant difference across the groups.

Figure 5

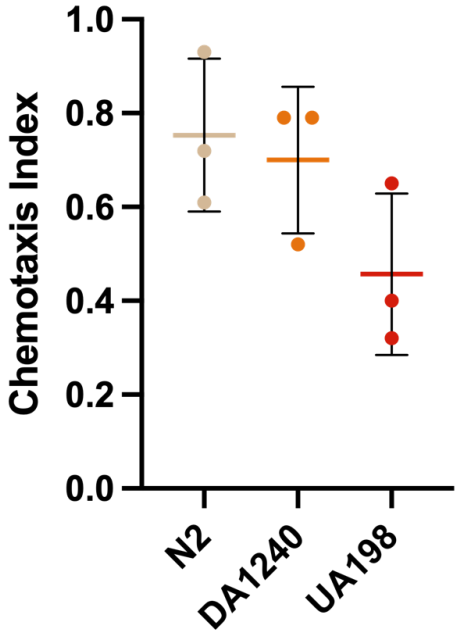
Bar graph outlining the results of the chemotaxis assay for the control baseline study



Graph showing the combined totals from the chemotaxis assay of the experimental groups across three independent experiments.

Figure 6

Scatter plot outlining the chemotaxis index for the control baseline study



Scatter plot displaying the chemotaxis index of the experimental groups across three independent experiments along with mean ±standard deviation (SD). A Shapiro-Wilk test revealed a non-parametric distribution for the data. A Kruskal-Wallis test was performed resulting in a p-value of 0.2143.

GSE treatment experiments followed the same protocol as the baseline experiments. There were two GSE concentrations tested (1 mg/mL and 10 mg/mL). The GSE treated groups were placed on their respective GSE concentration plates on day two following the egg lay. The complete experimental design is shown in Figure 7.

Figure 7

Treatment experimental design

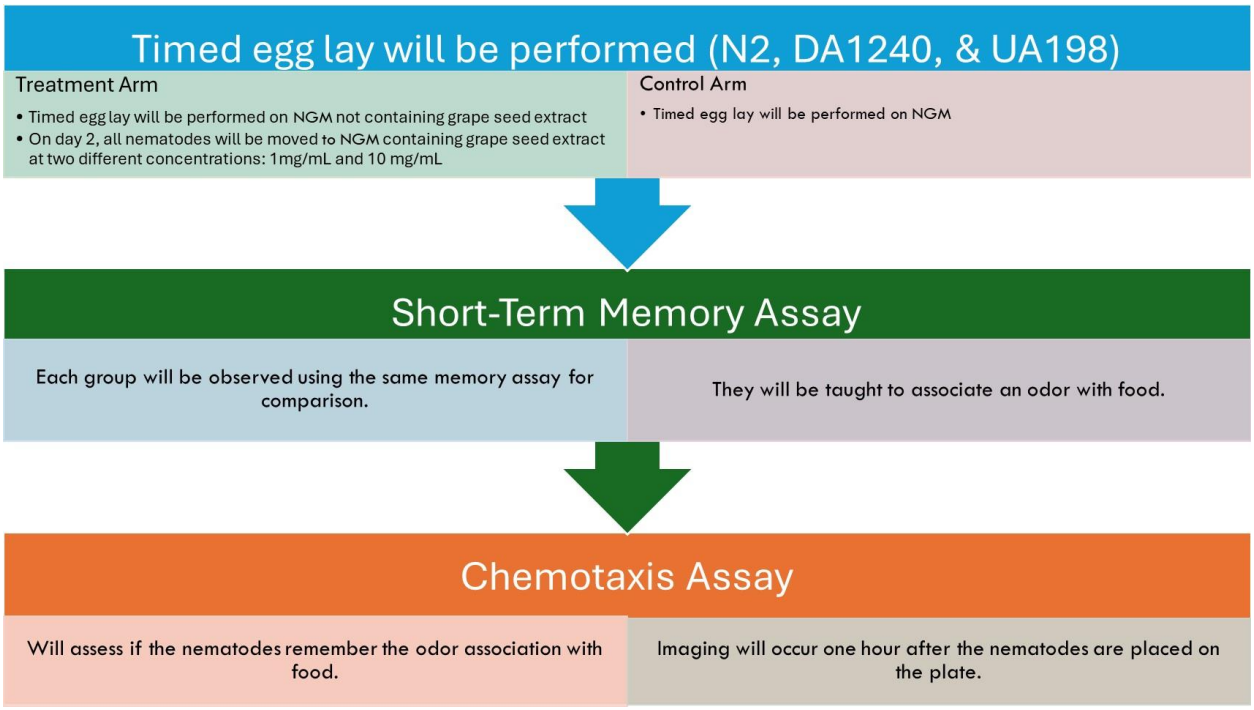
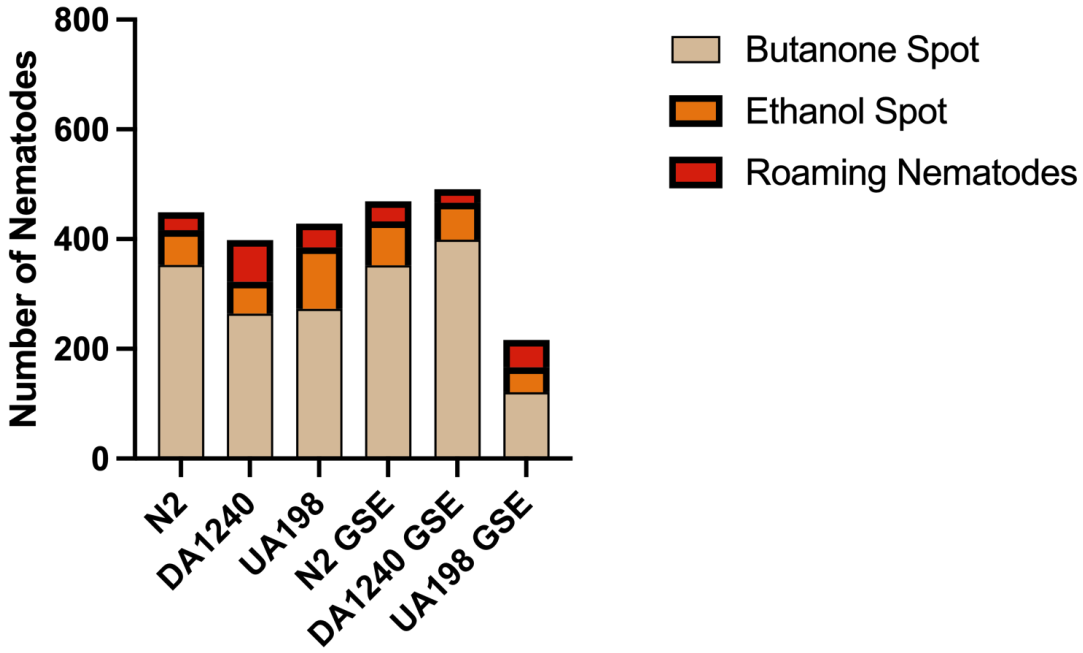


Figure 8 displays the combined data from the three independent treatment experiments. The graph shows that the data is similar across the treatment groups and the control groups. The GSE treated groups at 10 mg/mL are not shown on the graph. Within 30 minutes of transfer to the GSE 10 mg/mL NGM plate, nematodes across all the groups were not moving or crawling around on the plate. They were assumed to be dead due to no response from probing head and tail with nematode pick tool. To confirm this further, around 10 nematodes from each treatment group that were observed to be not moving were transferred to a new NGM plate and incubated from the second and third independent treatment. Twenty-one hours later the nematodes were observed and some of the nematodes from the N2 and UA198 strains were alive. However, the

10 mg/mL GSE group was not tested using the short-term memory and chemotaxis assay. The size of the groups was consistent except with for the UA198 GSE treated group.

Figure 8

Bar graph outlining the results of chemotaxis assay for the treatment study

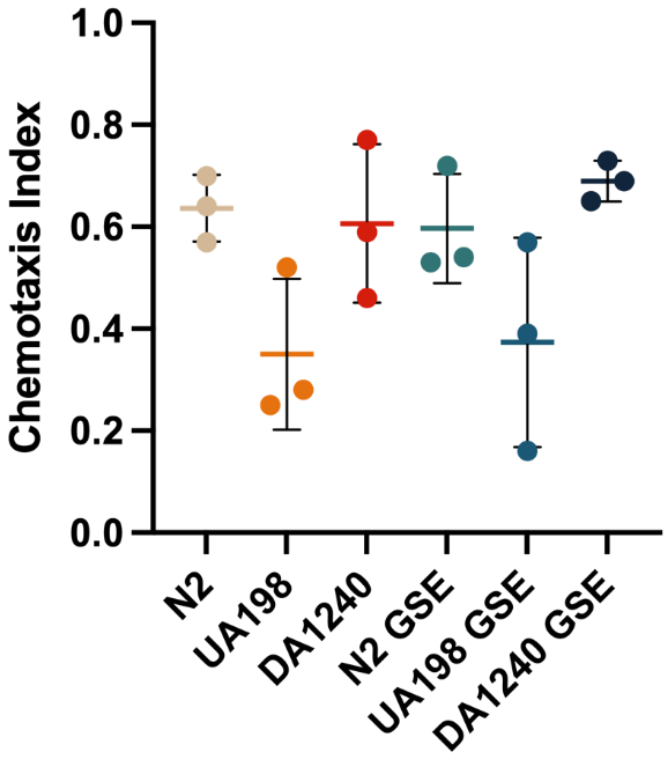


Graph showing the combined totals from the chemotaxis assay of the experimental groups across three independent experiments. All experimental groups were exposed to grape seed extract (GSE) at 1 mg/mL or 10 mg/mL. Only data for 1 mg/mL GSE treatment is included on the graph.

When comparing the data, the EtOH spot has more of an effect on the chemotaxis index when compared to the roaming nematodes. The EtOH spot represents no odor association, while the roaming nematodes have no preference to either odor. However, the nematodes at the butanone spot retain the odor association with food. When comparing the chemotaxis indexes across three independent experiments, there is a lot of overlap of the data. A one-way ANOVA was conducted. It showed a significant p-value at 0.0343.

Figure 9

Scatter plot outlining the chemotaxis index for the treatment study



Scatter plot displaying the chemotaxis index of the experimental groups across three independent experiments along with the mean ± standard deviation (SD). A Shapiro-Wilk test revealed a parametric distribution for the data. A one-way ANOVA was performed resulting in a p-value of

0.343. All experimental groups were exposed to grape seed extract (GSE) at 1 mg/mL or 10 mg/mL. Only data for 1 mg/mL GSE treatment is included on the graph.

Raw data from control and treatment experiments is presented Table 3. Graphs displayed in figures 5, 6, 8 and 9 display the data shown in Table 3.

Table 3

Raw data compiled from all assays and replicates

	N2	**DA1240**	**UA198**	**N2 GSE**	**DA1240 GSE**	**UA198 GSE**
CB1: Nematodes at B	101	36	13	-	-	-
CB1: Nematodes at E	3	2	3	-	-	-
CB1: Total Nematodes	105	43	25	-	-	-
CB2: Nematodes at B	57	126	120	-	-	-
CB2: Nematodes at E	8	10	22	-	-	-
CB2: Total Nematodes	68	146	150	-	-	-
CB3: Nematodes at B	95	83	30	-	-	-
CB3: Nematodes at E	17	21	5	-	-	-
CB3: Total Nematodes	127	120	78	-	-	-
T1: Nematodes at B	155	35	80	82	30	25
T1: Nematodes at E	23	2	42	23	3	16
T1: Total Nematodes	189	71	134	110	37	56
T2: Nematodes at B	96	150	59	176	236	76
T2: Nematodes at E	22	16	31	46	37	25
T2: Total Nematodes	129	174	111	244	290	132

T3: Nematodes at B	101	158	134	94	133	20
T3: Nematodes at E	18	40	39	11	27	4
T3: Total Nematodes	130	200	183	115	164	28

Note: CB = control baseline; T = treatment; B = butanone spot; E = EtOH spot; GSE = 1 mg/mL grape seed extract

Conclusions

This study aimed to determine the potential impact of GSE treatment on a physiological marker of Aβ toxicity in a nematode. This was tested using a short-term memory assay. The overexpression of Aβ was obtained using the human protein Aβ 1-42 in *C. elegans*. For scientific rigor of the study, the transgenic genotypes of each strain were confirmed through PCR experiments detecting Aβ and GFP. Next, baseline short-term memory experiments were conducted for the experimental groups to be examined in the downstream GSE treatment experiments. There was a trending difference of the number of nematodes at the B spot and the chemotaxis indexes between the wildtype N2 and the DA1240 groups compared to the UA198 group.

PCR genotyping results confirmed the genotypes for each nematode strain based on the presence or absence of Aβ and GFP amplification. Accordingly, both strains used in this study were confirmed to have both the Aβ protein and GFP. When confirming the contents of the strains, there was an outcome that was not expected. The GFP primer set did not amplify the positive control, or GFP strain. The primers did amplify a GFP fragment in the UA198 and DA1240 strains with the GFP plasmid insert. It could be that the GFP plasmid insert in the GFP positive control has a different sequence in the regions these GFP DNA primers bind compared

to the UA198 and DA1240 strains. This could result in the primers not attaching and being able to detect GFP in this particular GFP strain, therefore, resulting in the negative PCR result.

When determining how to obtain the GSE for this study multiple methods were considered. Creating GSE from grapes at the local grocery store, buying a powered GSE from a vineyard, or using a supplement from a vitamin company were all used in the past. However, the Meganatural brand was used because that is the specific brand used in the ongoing human clinical trials. The concentration of 10 mg/mL and 1 mg/mL was chosen because they were the most effective concentrations in another nematode study examining cancer. However, the 10 mg/mL concentration in this study appeared to shock and stun the nematodes until moved to another NGM plate without any GSE present. Even when stroked and probed on the 10mg/mL plate the nematodes had no response to move. Some nematodes recovered while others did not. It took multiple hours for the GSE treated nematodes to start moving again and recover. Perhaps, the higher GSE concentration was toxic to the nematodes. The nematodes could start moving again only after being removed from the GSE for hours. Future studies could include a toxicity study of GSE across a wide range of concentrations for N2 nematodes.

Out of all the nematode strains used the UA198 strain posed some experimental difficulties. The development of the nematodes appeared to be slower when compared to the N2 and DA1240 strains. The adults of the UA198 strains appeared to be smaller than the adults of the N2 and DA1240 strains. When carrying out the timed egg lays, the UA198 adults appeared to lay less eggs compared to the N2 and DA1240 strains. This could be due to the presence of the Aβ 1-42 protein present in that strain. A future study could be a lifespan assay of the UA198

when compared to the N2 and DA1240 with comparisons at a set time interval throughout the study period.

The strains N2, DA1240, and UA198 were used in the baseline study. This study showed that there was a slight difference between N2, the wildtype, and DA1240, the plasmid control. There was also a trending difference between the wildtype and plasmid control when compared to the UA198 group. The plasmid control was expected to be similar to the wildtype group, but there was a slight difference. The UA198 strain was consistent with what was expected. It does appear that the Aβ protein expression in the nematode does affect the short-term memory of the organism. However, the sample sizes for the baseline experiments were small across groups. These experiments should be carried out again to observe the repeatability of this study's observations. A future study could have at least three independent experiments with larger sample sizes for the groups. A recommendation would be doubling the target sample size during the timed egg lays, so around 200 nematodes per group. This would make the study more robust.

The same nematodes strains were used for the treatment study, but they were exposed to 1mg/mL and 10 mg/mL of GSE. The treatment study showed the most improvement in the DA1240 strain, or the plasmid control strain following GSE treatment. This was unexpected but it was probably due to the number of roaming nematodes around both treatment groups. The number of roaming nematodes might have occurred in one experiment due to the odorants getting down the sides of the agar plate and confusing the nematodes on where to go. The GSE treated UA198 group showed some improvement, but not to the extent that was observed for the DA1240 group. Also, the memory of the N2, or wildtype nematode was negatively affected. We did not find any mention of any negative effects of GSE on memory. Although shown in many

studies that GSE improves the memory of as a preventative method, it was not shown as an effective treatment. Previous studies found was that it improved memory, however, this study did not have the large sample size that the other studies had. So, this study needs to be repeated with either larger group sizes, and more replicates to come to a more accurate conclusion.

The treatment study also had a smaller sample for the GSE treated UA198 group across three independent experiments. Since the 10 mg/mL appeared to have toxic effects on all nematode strains, the UA198 group might have experienced the toxic effects at 1 mg/mL. This could be due to the Aβ protein present in the strain. These effects could lead the nematodes to crawl up the sides or the lid of the media plates. The nematodes crawling away from the media are not transferred, resulting in a smaller sample size. Future studies could include a toxicity study of GSE across a wide range of concentrations for UA198 nematodes.

Finding any type of treatment for AD would be groundbreaking, but finding a natural treatment for AD would be even better. There would be no need to look for negative side effects if the natural treatment is successful. No negative side effects have been reported for GSE, just effectiveness is mentioned. It would also be easy to obtain because you could just buy the fruit for consumption. The source of the treatment would be easily obtainable, so hopefully shortages would not be as long, or the alternative could be to eat more grapes. The quickness of the arrival at human trials shows that there is promise for GSE. Plus, the jump from the mouse model to the clinical trials shows that there were minimal negative side effects present, or they would not have expedited the process.

Natural treatments can come from diet. Certain diets are less likely to develop AD. Diet and other connected fields has the ability to find more prospective treatments. The gut

microbiome and diet are connected new fields of study that have the potential to offer a new perspective on medication and treatment for complex diseases. Every individual's gut microbiome is unique. Certain genera of microorganisms are common in guts across human populations. For example, resveratrol can only be metabolized by *Bifidobacterium infantis* and *Lactobacillus acidophilus* from grape juice. Resveratrol has many health benefits including anticancer effects (Gates et al., 2022; Martin et al., 2020). Introduction of antioxidants into diet has shown some success in minimizing the number of free radicals within the body, however, this conclusion is controversial (Uttara et al., 2009).

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