Effects of *Panax ginseng* extract on Alzheimer's Disease patients through Paralysis Assay of CL4176 (Amyloid-Beta Protein Transgenic) *C. elegans*.

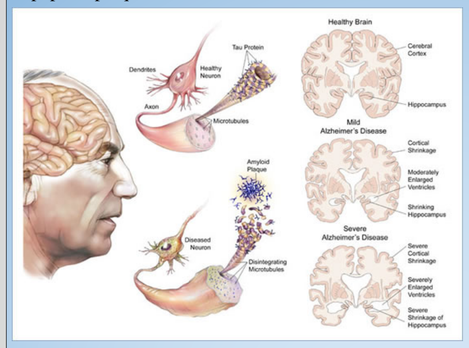
David Shau[1], Larry Chen[1], Dr. Hahn[1], Angela Merchant[1]
Henry M. Gunn High School[1]

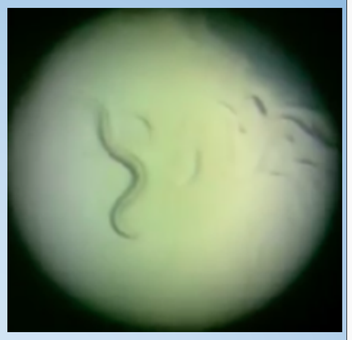
INTRODUCTION

We will be testing the effects of *Panax ginseng* on Aβ toxicity in Alzheimer's Disease patients, looking for a method of alleviating symptoms. To do this, we will be testing these effects by using engineered transgenic *C. elegans* as a model for Alzheimer's Disease. We collected evidence indicating that there is a significant effect of ginseng in alleviating symptoms in C. elegans.

BACKGROUND AND SIGNIFICANCE

Alzheimer's is a prevalent disease affecting more than 3 million people in the united states. It causes eventual brain decay to a point where certain common functions can no longer be performed (D). While it is known to be a neurodegenerative disease, the true root cause is still debated. The three most prominent hypotheses are the Cholinergic Hypothesis, Amyloid Hypothesis, and Tau Hypothesis (A). We will be building off of the amyloid Hypothesis, which states that the primary cause of AD is from an over accumulation of beta-amyloid peptide plaques in brain tissue.





Panax ginseng is a root, often classified under Chinese Medicine, that has been used for centuries to treat many diseases and injuries. Recently, there have been results showing many neurological benefits from *Panax Ginseng,* such as maintaining homeostasis, and such as anti-inflammatory, anti-oxidant, anti-apoptotic, and immunostimulatory activities. Some studies show that AD patients who take significant *Panax Ginseng* treatments tend to perform better on AD rating tests after the treatment period; there is also evidence that the pharmaceutical components of ginseng, ginsenosides, have the potential to lower Aβ (beta-amyloid) levels and toxicity (C).

Alzheimer's disease, the most common cause of dementia in older people, is characterized by Aβ clearance capability, mitochondrial function, synaptic dysfunction, down-regulation of antioxidant up-regulation. Current therapeutic strategies only address the symptoms, and not the root cause. For example, many drugs can interrupt tau hyperphosphorylation, but no drug has yet been found to prevent progressive loss of neurons in AD patients.

There have been some previous studies on the effect of Ginseng Extracts on AD Patients (in vivo).
Korean red ginseng powder showed significant improvement on the AD assessment scale, the mini mental state examination scores, and the clinical dementia rating after 12 weeks of ginseng therapy compared to the control group. Furthermore, the extract showed significant prevention of memory loss in SAMP8 mice.

C. elegans

C. elegans are a very thoroughly studied species of nematodes that can be easily genetically engineered to express a variety of phenotypes. They are microscopic, and resemble the shape of a worm. In our experiment, we will be using a strain of *C. elegans* (CL4176) that express the Aβ peptide at 25 degrees Celsius. When expressed, the peptide gradually paralyzes the worms over time.

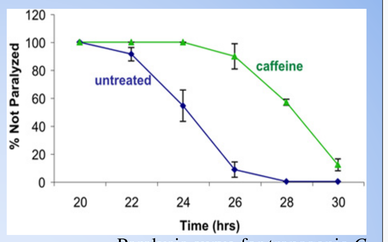
Some of the benefits of using C. elegans as our test organism include short lifespan for quick testing periods, easy of genomic manipulation, and equipment necessary to handle.

These strains (CL4176 and N2) were created by Dr. Christopher D. Link of the University of Colorado in Boulder.

RESEARCH METHODOLOGIES

1. Previous research methods

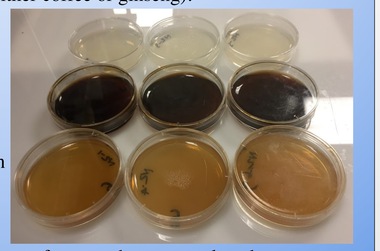
Many laboratory experiments using transgenic rats have shown that treatments with Panax Ginseng extracts have led to improvements in memory (E). Previous studies have also studied the effects of coffee on Aβ peptide expressing *C. elegans*, where *C. elegans* exposed to coffee exhibited a decrease in their rate of paralysis (F). This implies that coffee, or some substance in coffee, could reduce the severity of Alzheimer's Disease.



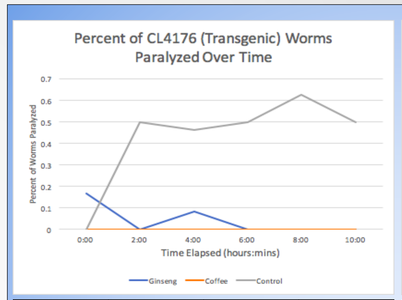
Paralysis curve for transgenic *C. elegans* treated and untreated with caffeine.

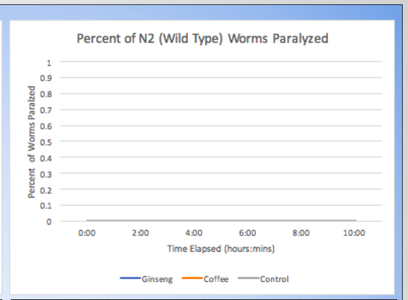
2. Research Methodology (Procedure)

General Timeline (total days: ~12days):
Day 1: Make big plates- Use NGM on 10 cm petri dishes to create large plates in order to pick gravid adults. We added solutions of CaCl2 and MgSO4 and some cultures of OP50.
Day 2: Chunk the worms from the original plates onto the large plates made prior. The worms will then be allowed to grow into gravid adults.
Day 3: Pick the gravid adults off the large plates and place them on separate large plates, so that the ages of the worms are synchronized. The worms will then be given 7 days for an egg laying period.
Day 6-9: Make smaller plates (at least 3 caffeine, 3 ginseng, 3 control). This step will take two days total. One day is required for the NGM to solidify, and then another day is required to spread the treatment group extract (either coffee or ginseng).
Day 10: Remove "second day" progeny and put them on new plates to grow worms to use for paralysis assay. We did this step through a bleaching solution that effectively gave us a solution of just C. elegans eggs. Place the plates in an incubator for 16 degrees C.
Day 12: Upshift the temperature to 25 degrees in order to begin the paralysis process.
Day 13: Perform the paralysis by periodically (every 2 hours) use a worm picker to count the number of paralyzed worms, and record the percent of worms that are paralyzed.



DATA ANALYSIS AND RESULTS





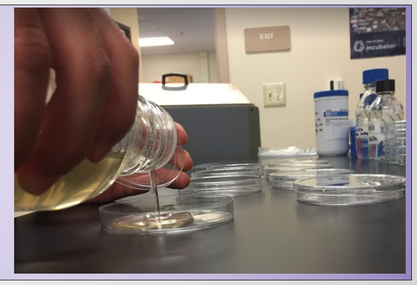
CL4176	Ginseng	Coffee	Untreated
8:00 AM	1/6	0/3	0/3
10:00 AM	0/6	0/3	3/6
12:00 PM	1/12	0/4	6/13
2:00 PM	0/10	0/5	6/12
4:00 PM	0/7	0/4	5/8
6:00 PM	0/9	0/5	5/10

(N2)	Ginseng	Coffee	Untreated
8	0/10	0/10	0/22
10	0/14	0/13	0/33
12	0/16	0/26	0/20
2	0/19	0/16	0/22
4	0/24	0/11	0/28
6	0/24	0/18	0/26

After conducting a one-sided proportional t-test, there is less than 1% chance that the worms on the plates treated with ginseng reflect the worms on the untreated place. This result gives us statistically significant evidence to state that the Ginseng extract did affect the Cl4176 worms. Because this type of transgenic worm employs the beta-amyloid peptide plaques also found in Alzheimer's disease patients, we can reasonably conclude that some of the chemicals found in the Panax ginseng extract have positive effects in alleviating symptoms of the disease. The plates treated with coffee were used as a positive control, while the untreated plates were used as a negative control.

CONCLUSION

Ginseng does significantly reduce the rate of paralysis in Aβ peptide expressing *C. elegans*. No solid conclusions could be made about the distinction between the effects that ginseng and coffee have, however. Assuming that the effects of ginseng are the same in *C. elegans* as they are in humans, we can conclude that ginseng could reduce Aβ peptide toxicity in Alzheimer's Disease patients, and could therefore be used as part of a treatment for the neurological diseases.



ACKNOWLEDGEMENTS / REFERENCES

Special thanks to Angela Merchant, Dr. Hahn and Dr. Christopher D. Link.

Works Cited:

A. Calcul, Laurent, Bo Zhang, Umesh K. Jinwal, Chad A. Dickey, and Bill J. Baker. "Natural Products as a Rich Source of Tau-targeting Drugs for Alzheimer's Disease." Future Medicinal Chemistry 4.13 (2012): 1751-761. Web.
B. De Calignon, Alix, Manuela Polydoro, Marc Suárez-Calvet, Christopher William, David H. Adamowicz, Kathy J. Kopeikina, Rose Pitstick, Naruhiko Sahara, Karen H. Ashe, George A. Carlson, Tara L. Spires-Jones, and Bradley T. Hyman. "Propagation of Tau Pathology in a Model of Early Alzheimer's Disease." Neuron 76.2 (2012): 461. Web.
C. Cho, Ik-Hyun. "Effects of Panax Ginseng in Neurodegenerative Diseases." Journal of Ginseng Research 36.4 (2012): 342-53. Web.
D. Calcul, Laurent, et al. "Combinatorial Tau Pseudophosphorylation" *PMC*, NCBI, 2 Feb 2011, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3077627/. Accessed 4 Nov. 2016.
E. Song XY1, Hu JF, Chu SF, Zhang Z, Xu S, Yuan YH, Han N, Liu Y, Niu F, He X, Chen NH, 2013 "Ginsenoside Rg1 Attenuates Okadaic Acid Induced Spatial Memory Impairment by the GSK3β/tau Signaling Pathway and the Aβ Formation Prevention in Rats." *PubMed*, NCBI, 15 June 2013, ww.ncbi.nlm.nih.gov/pubmed/?term=Alzheimer%27s+disease+tau+protein+natural+ingredients. Accessed 5 Oct. 2016.
F. Dostal, V., Link, C. D. Assaying β-amyloid Toxicity using a Transgenic *C. elegans* Model. *J. Vis. Exp.* (44), e2252, doi:10.3791/2252 (2010).