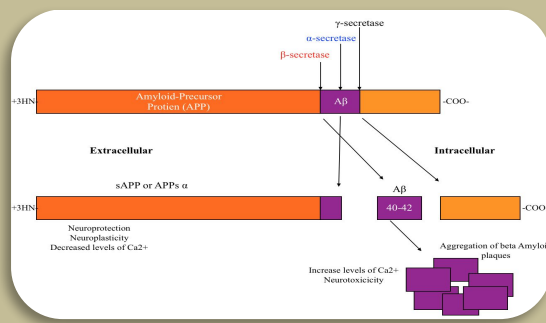
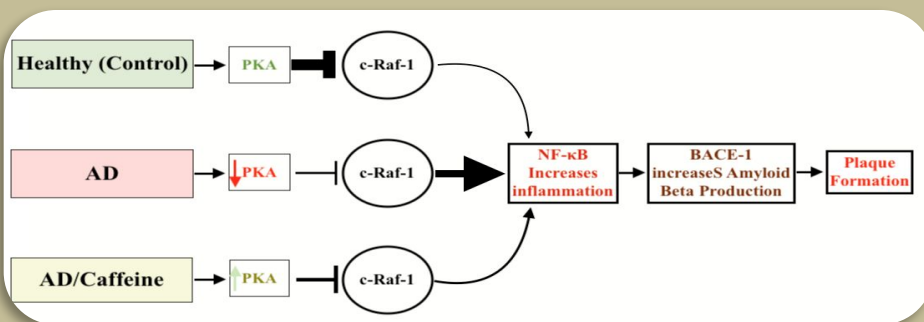


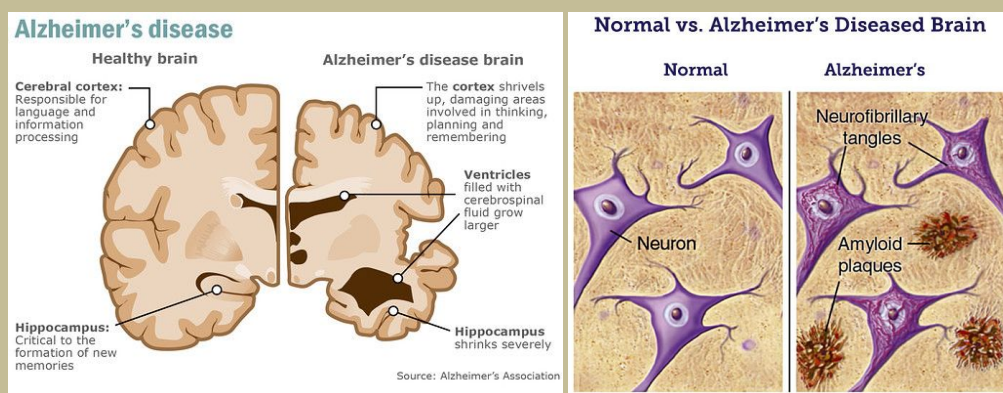
Abstract

Epidemiological data and recent scientific publications provide support for the neuroprotective effects of coffee against AD⁽¹⁾. The proposed mechanisms of therapeutic benefits include inhibition of an enzyme known as alpha secretase which results in lower levels of Amyloid beta plaques and increase in a hematopoietic factor known as GCSF⁽²⁾. The aim of this study is to find the amount of coffee and type of coffee which ensures cell survival while reducing amyloid beta levels. N₂APP are neuroblastoma cells from the APPsw mouse model with mutated genes for developing Alzheimer's disease. N₂APP cells can be treated with various drugs and the cytotoxicity of these drugs on their neurons can be measured using MTT assay. The survival rate of cells at various concentrations of coffee treatment can be quantified. 24 hours after drug treatment, the supernatant was collected, and amyloid beta levels were measured using ELISA. The data in this study is based on treatment of N2APP cells with Instant coffee and common commercial coffee solutions.



Background

Alzheimer's disease (AD) is a progressive neurodegenerative disease that impairs memory and other important mental functions⁽²⁾. More than 5 million Americans are living with AD. These numbers are expected to dramatically increase as the baby boomer population transitions into the late-adulthood age group. Currently, the treatment for AD includes FDA drugs that focus on treating its symptoms. These types of drugs lose their efficacy as the disease progresses. Therefore, preventive measures are very critical in decreasing the incidence rate of AD. Many epidemiological and scientific studies have indicated the connection between moderate-high coffee consumption reduces the risk for Alzheimer's Disease (AD). More studies in AD mice models indicate that consistent caffeine consumption is able to reduce amyloid beta plaques in the brain¹. Furthermore, non-caffeinated portions of coffee have proven to be effective in combating major causes of AD, such as inflammation and oxidative stress.



Methods

Cell Culture: 10,000 N₂APP cells were grown in 96 well plate in 200 μl RPMI 10% FBS medium

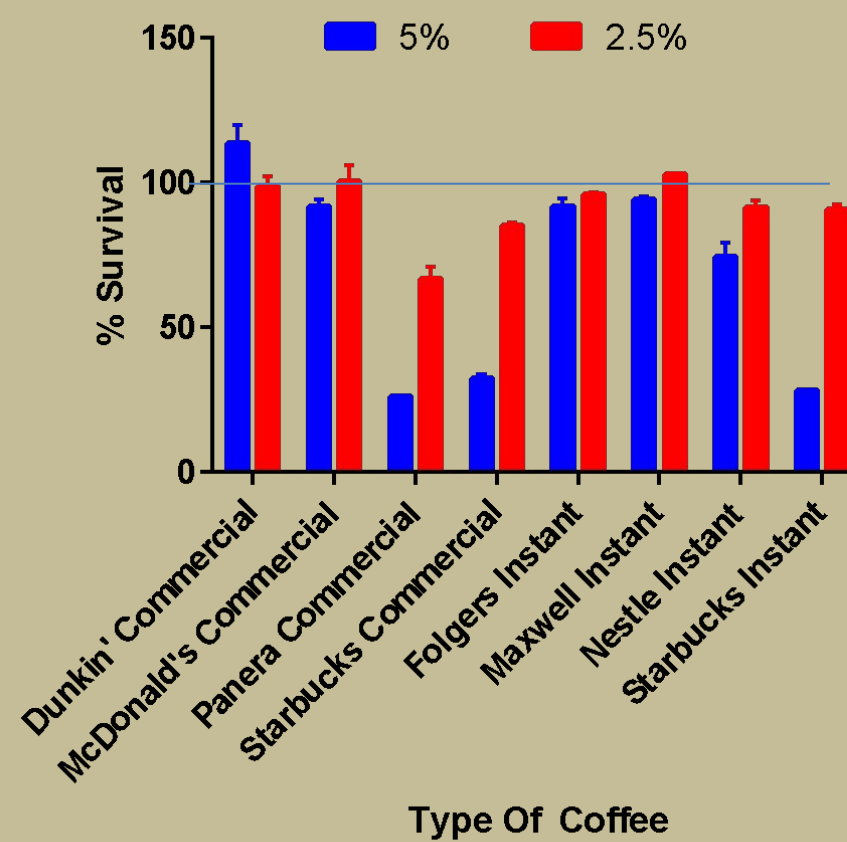
Coffee Treatment: N₂APP cells in 96 well plate were treated with 5% and 2.5% coffee solution, performed in triplicate with controls.

ELISA: 100 μl of supernatant from 96 well plate was collected 24 hours after the drug treatment. The ELISA plates were used to measure amyloid beta levels 1-40 and 42.

MTT Assay: After 24 hours of drug treatment, 25 μl of MTT(5mg/mL) solution was added to all 96 well pates. After 4 hours, 96 well plate was emptied and 100 μl of MTT solvent was added to the wells. After 15 minutes, absorbance was measured at 590 nm to quantify cell survival.

Results

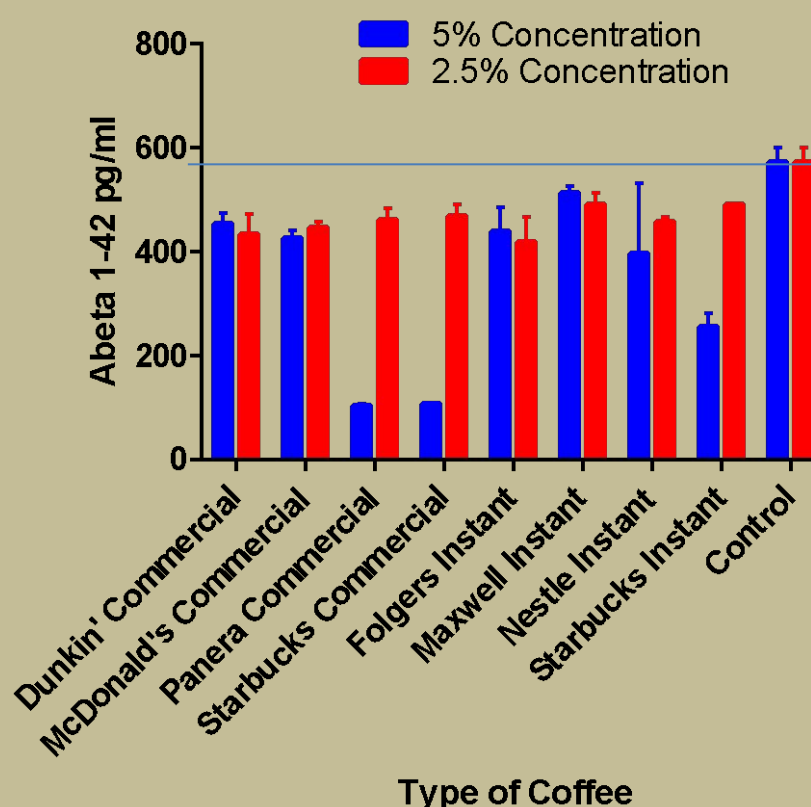
MTT Assay Instant And Commercial Coffee



Type Of Coffee

Figure 1: **MTT Assay results.** This assay was performed to measure the viability of cells and the optimal concentrations for drug treatment

ABETA42 concentration for Instant/Commercial

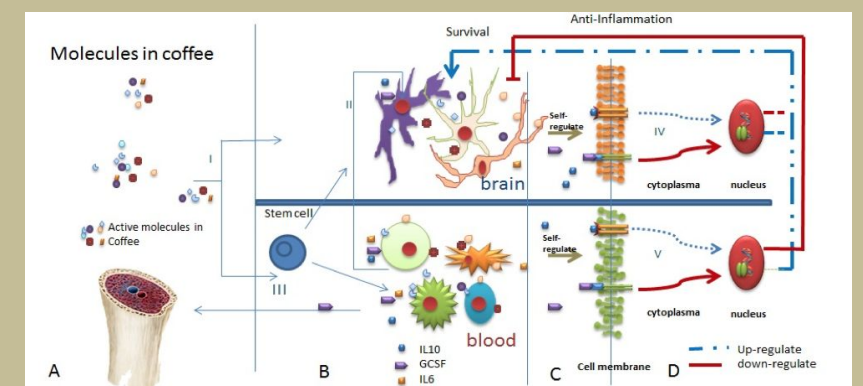


Type of Coffee

Figure 2: **ELISA results for A-beta levels.** This assay was performed to measure the concentration of amyloid beta 1-42 after drug treatment of N2APP cells.

Discussion

- The MTT data shows that the 5% concentration of coffee was toxic to the N₂APP cells and decreased the viability of the cells significantly.
- The coffee treatment was significantly less toxic at a concentration of 2.5%.
- All coffees (except Dunkin's and Panera commercial coffee) induced a very small amount of toxicity at the 2.5% concentration.
- In the future, the cells should be treated at various lower concentrations to find the optimal dosage of coffee at which cell viability is close to 100%.
- ELISA results also showed significant reduction in the amount of amyloid beta 1-42 after drug treatment compared to the control group.
- This reduction in toxic amyloid-beta shows the potential neuroprotective effects of coffee.
- Dunkin's commercial coffee significantly reduced amyloid-beta levels from approximately 600 pg/ml to 400 pg/ml without being toxic to the N₂APP cells.
- McDonald's Commercial, Folgers Instant, Maxwell Instant, and Nestle Instant coffee showed a similar trend of reducing amyloid-beta levels with minimal toxicity to the neuronal cells.



Conclusion

This study found that coffee solutions treated at 5% are toxic to neurons. However, the toxicity reduced significantly at 2.5%. While the toxicity was significantly less allowing for more cells to survive at 2.5%, the levels of toxic amyloid beta 1-42 significantly reduced. This reduction in amyloid beta is associated with improvement in cognitive performance, as the presence of these toxic peptides is one of the characteristics of AD pathophysiology. The data is consistent with the claims from various epidemiological and scientific studies. In fact, coffee treatment significantly reduced the levels of beta amyloid protein in the cell culture supernatant.

References

- Cao C, Wang L, Lin X, et al. (2011) Caffeine synergizes with another coffee component to increase plasma GCSF: Linkage to cognitive benefits in Alzheimer's mice. J Alzheimers Dis 25: 323-335.
- Khan UN, Cao C (2018) Coffee Consumption Provides Therapeutic Benefits against AD through Increasing Plasma GCSF Levels and Improving Cognitive Performance. J Clin Neurol Neurosurg 1: 103.