From Hive to Hope: A Proposal on Honey's Antioxidant Impact in Mitigating Oxidative Stress in Alzheimer's

Valerie Nunez Martinez, Ornina Shneker, Alondra Castillo, Ferhat Ozturk

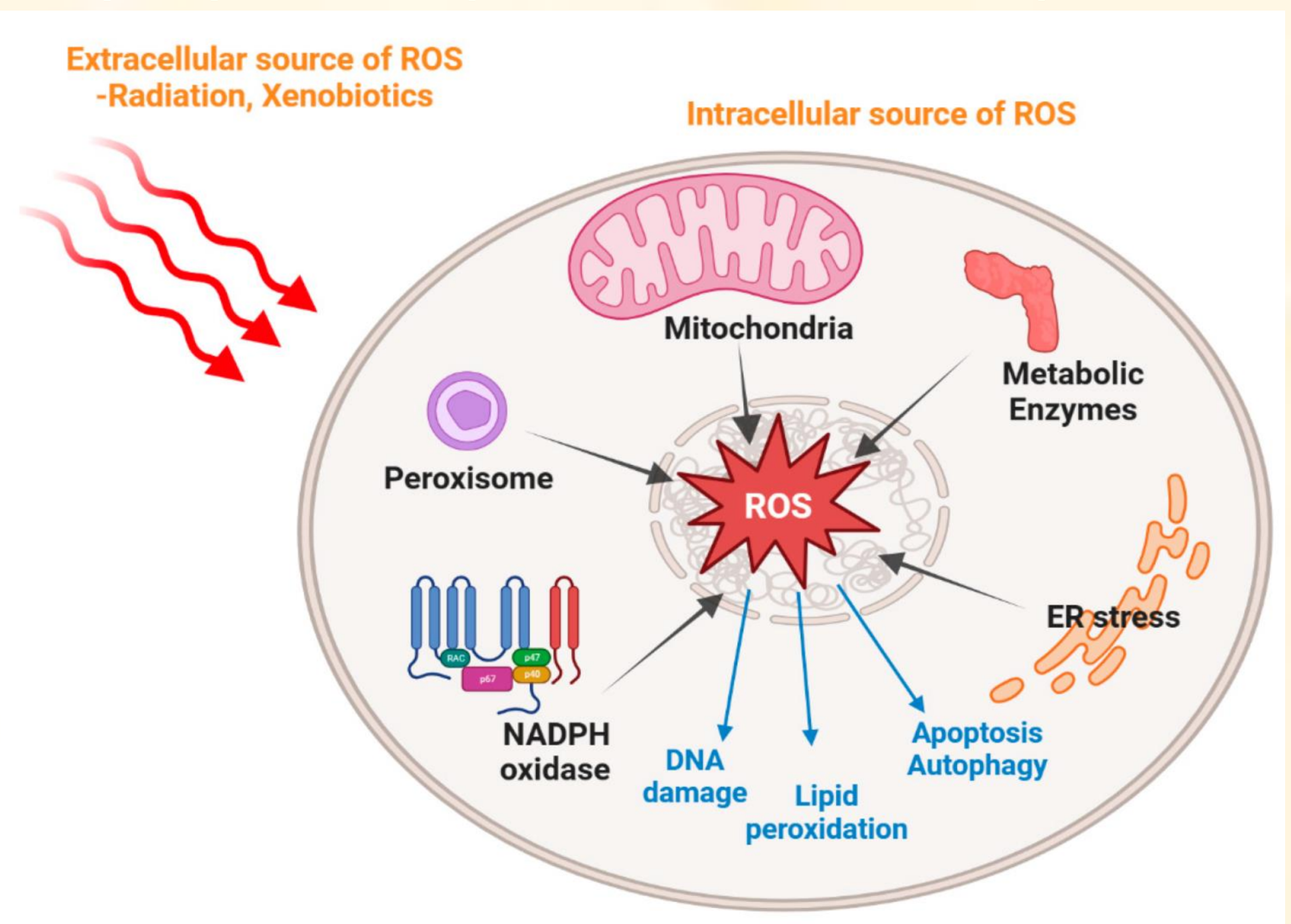
The University of Texas at San Antonio, San Antonio TX, 78249

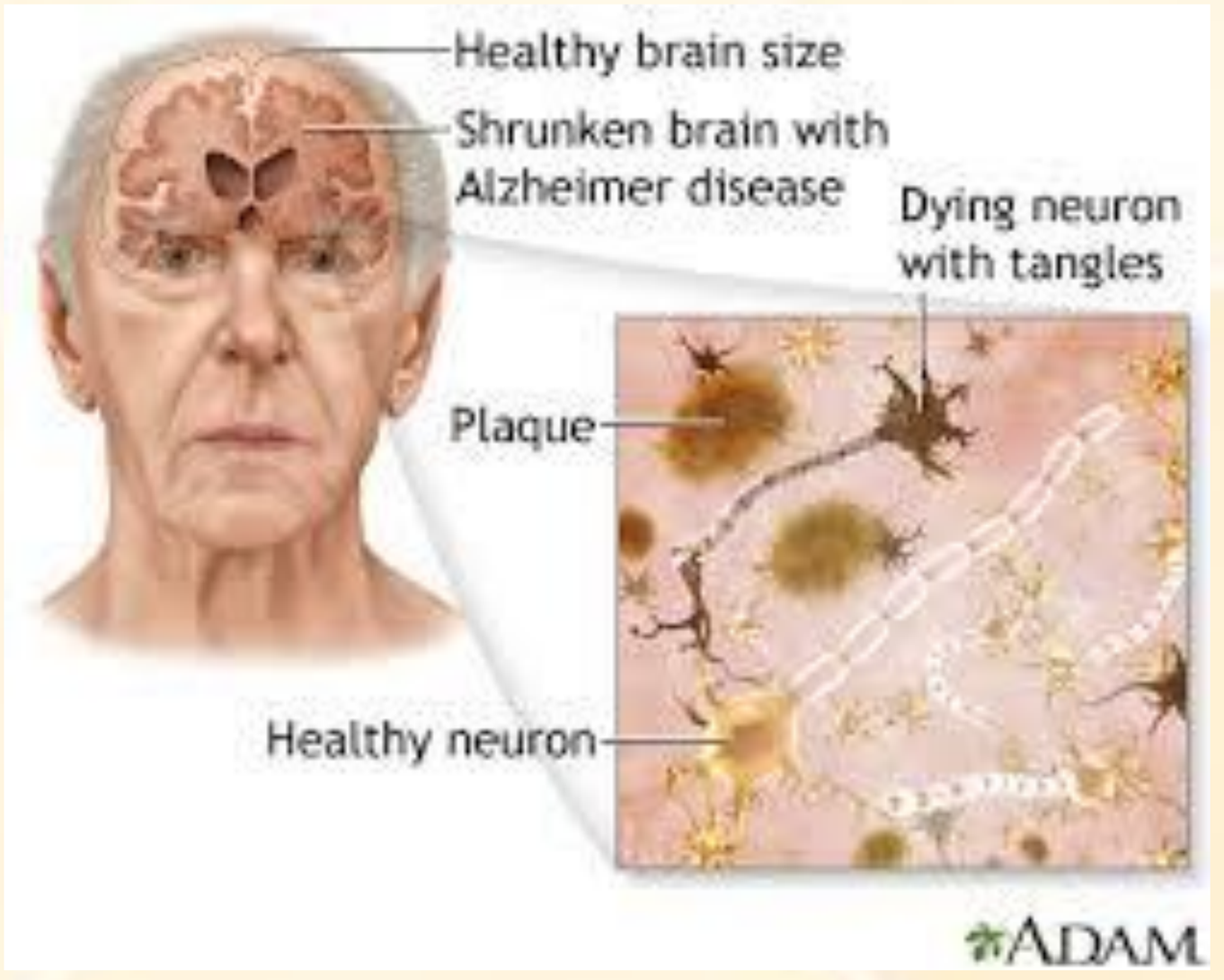
Abstract

Alzheimer Disease develops through the accumulation of β-amyloid plaques due to excessive reactive oxygen species in the brain, leading to development of its symptomatology. The purpose of this study is to examine the potential therapeutic role of honey in mitigating against the spread of reactive oxygen species through analyzing its antioxidant properties within the human brain, thus slowing down the aggregation of amyloid plaques and mitochondrial/DNA dysfunction which contribute to Alzheimer's Disease (AD). To assess the antioxidant impact, three local Texas honey samples were tested for their antioxidant and antimicrobial potential which include DPPH radical scavenging activity and zones of inhibition. Mature neuron cells derived from Alzheimer patients will be used to induce β-amyloid plaque formation that marks the neurological disease in the brain tissue, incorporate a honey solution and examine their resulting pathological effect. Through this analysis, we expect a reduction of peptide precursors for β-amyloid plaque formation in the neuron cells through protection against oxidative stress and mitochondrial DNA damage. As such, it will encourage more research for a development of a natural therapeutic approach for neurological diseases and provide novel insight into honey's antioxidant properties. As a result, this study will contribute to brain health research and alternative medicine.

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia, affecting an estimated 6.7 million Americans aged 65 and older—a number projected to rise to 13.8 million by 2060 (Alzheimer's Disease Facts and Figures, 2023). A key hallmark of AD pathology is the aggregation of amyloid-β (Aβ) plaques, which contribute to tau hyperphosphorylation, neurofibrillary tangle (NFT) formation, and neuronal death (Shaikh et al., 2023). Growing evidence implicates oxidative stress, primarily from mitochondrial dysfunction and excessive reactive oxygen species (ROS) production, as a major driver in the initiation and progression of plaque formation (Zhao and Zhao, 2013). As the search for affordable and effective treatments continues, honey has emerged as a promising natural therapeutic agent due to its rich content of antioxidant flavonoids and phenolic acids, long recognized for their neuroprotective properties (Khan et al., 2014).





Objective

The study sought to examine the effect of the most potent honeys' antioxidant potential from Texas beekeepers on oxidative stress in brain cells through the analysis of its role in slowing the development of β-amyloid plaque formation in brain tissue. The goal is to determine the potential to prevent mitochondrial DNA dysfunction and other markers which function in Alzheimer's Disease.

Materials and Methods

1.1 Honey Samples

Table 1. Properties of Texas Local Honey Samples and their Bioactivity Results (BAL)

Sample Name	Nectar	Antioxidant Potential (DPPH) Avg	Antibacterial (ZOI) Avg
24H-2	Wildflower	77.00%	28.41
24H-35	Wildflower	90.00%	23.35
24H-81	Unknown	86.30%	26.1

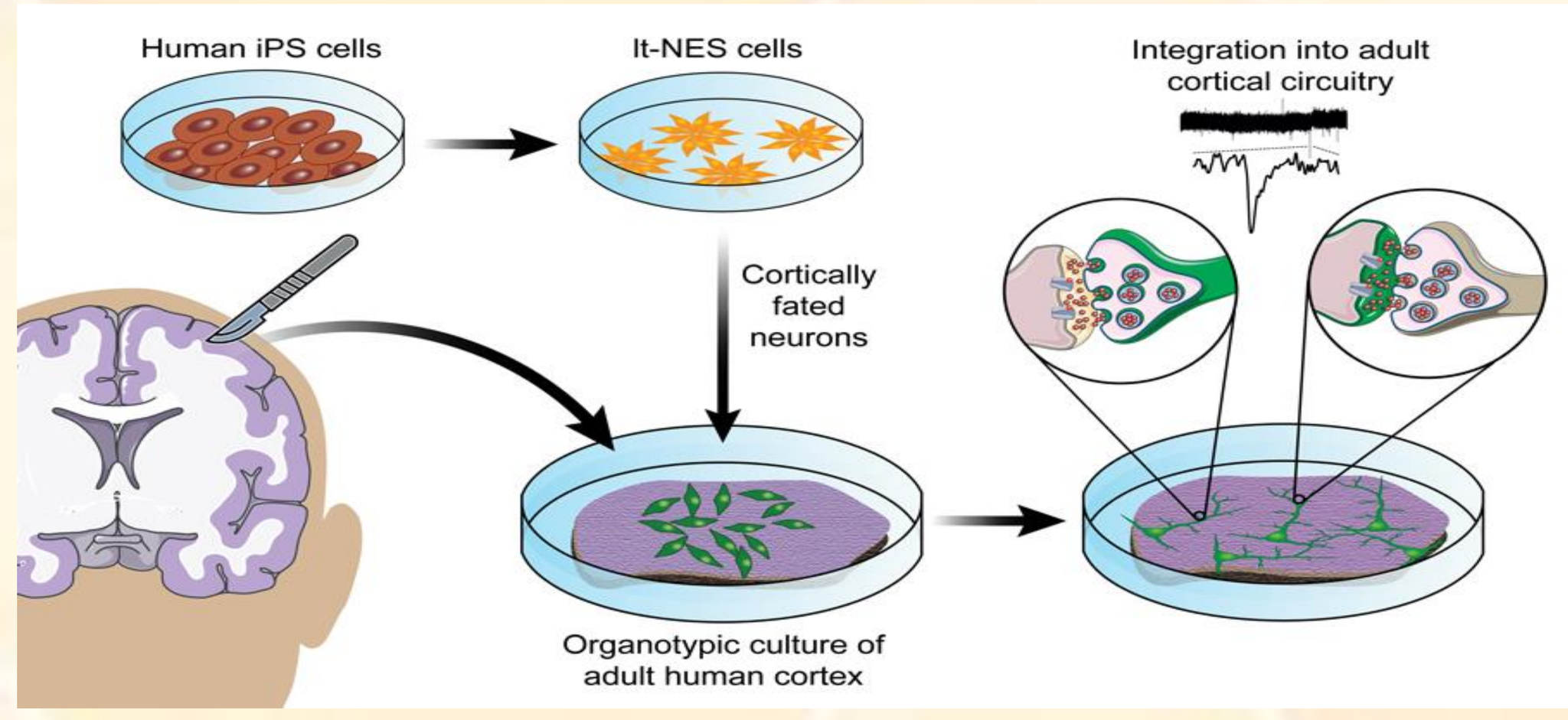
1.2 Honey Preparation & Filtration

The honey samples will be prepared by dilution in media for neuron culture. An initial 5% dilution will be prepared, with subsequent concentrations (2.5%, 1% and 0.1%) until optimal concentration is reached. Honey samples will be filtered to eliminate potential environmental contaminants.

1.3 Preparing Neuron Cell Lines

Mature neuron cells derived from iPSCs of patients with Alzheimer's will be used. Training will be undertaken to culture, feed and freeze neuronal cells under sterile conditions. The cells will be grown and maintained in optimal-neuron media.

Figure 1. iPSCs Collection Procedure for Culture



1.4 Treatment of Neurons

Mature neurons will be treated with the filtered honey for a set defined period. Experimental groups will thus include the untreated control group and the honey treated cells.

1.5 Cytotoxicity Testing

To determine optimal, non-toxic concentration for neuronal treatment, Cytotoxicity assays with cells grown in multicell plates and exposed to each dilution (5%, 2.5%, 1%, and 0.1%) for 24-72 hours. Cell viability will be assessed using a viability assay.

1.6 Cellular Health Assessment and Oxidative Stress Markers

The peptide concentrations will be quantified and mitochondrial function and other oxidative stress markers, such as ROS, will be assessed.

Expected Results

Through the implementation of this project, we expect to see change in the following properties compared to the control group:

- A reduction in reactive oxygen species in brain cells
- The observational decrease of β-amyloid plaque peptide precursor concentration
- An improvement in mitochondrial DNA stability
- A significant decrease in other oxidative stress markers.
- Increased Cell viability

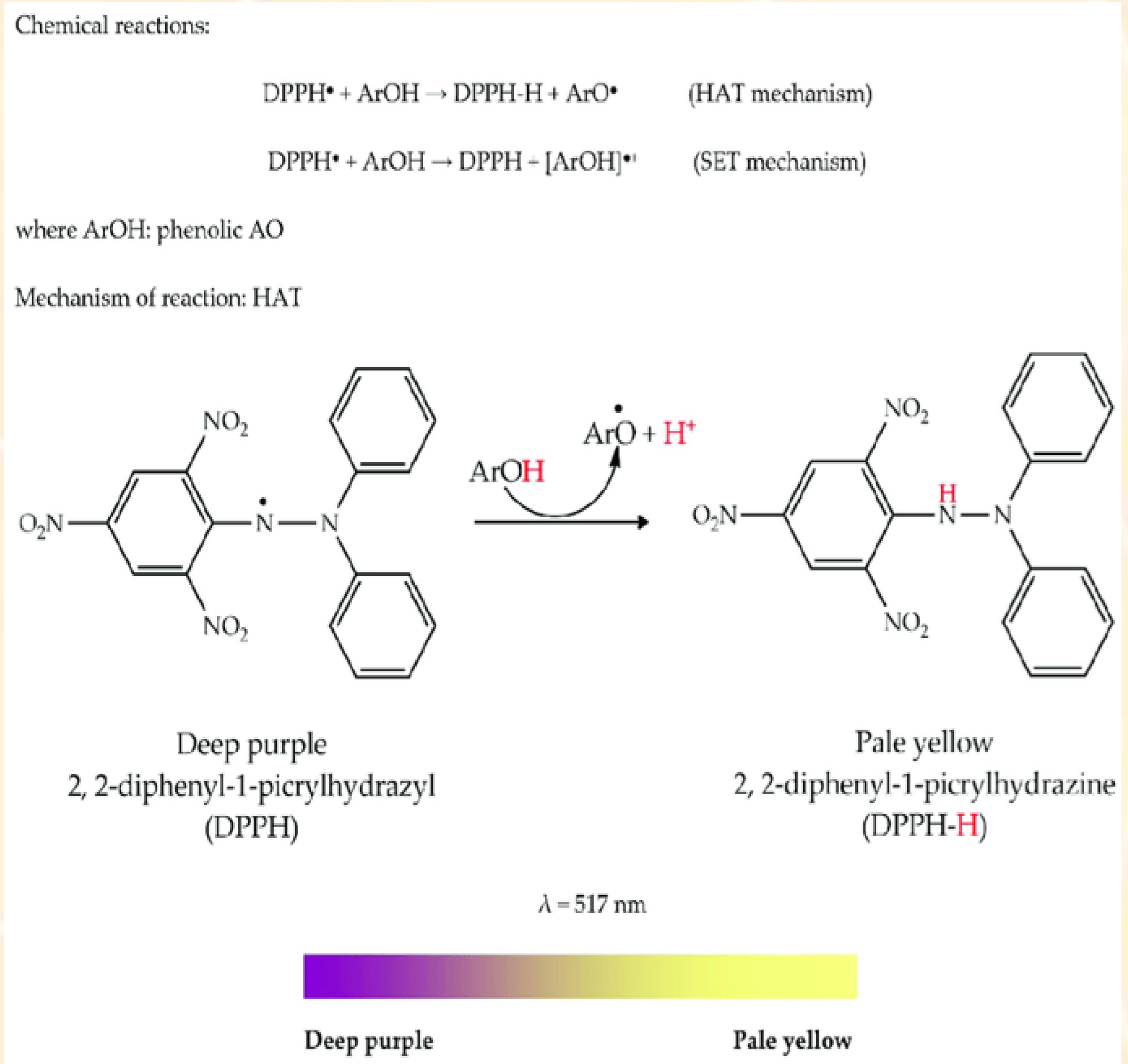
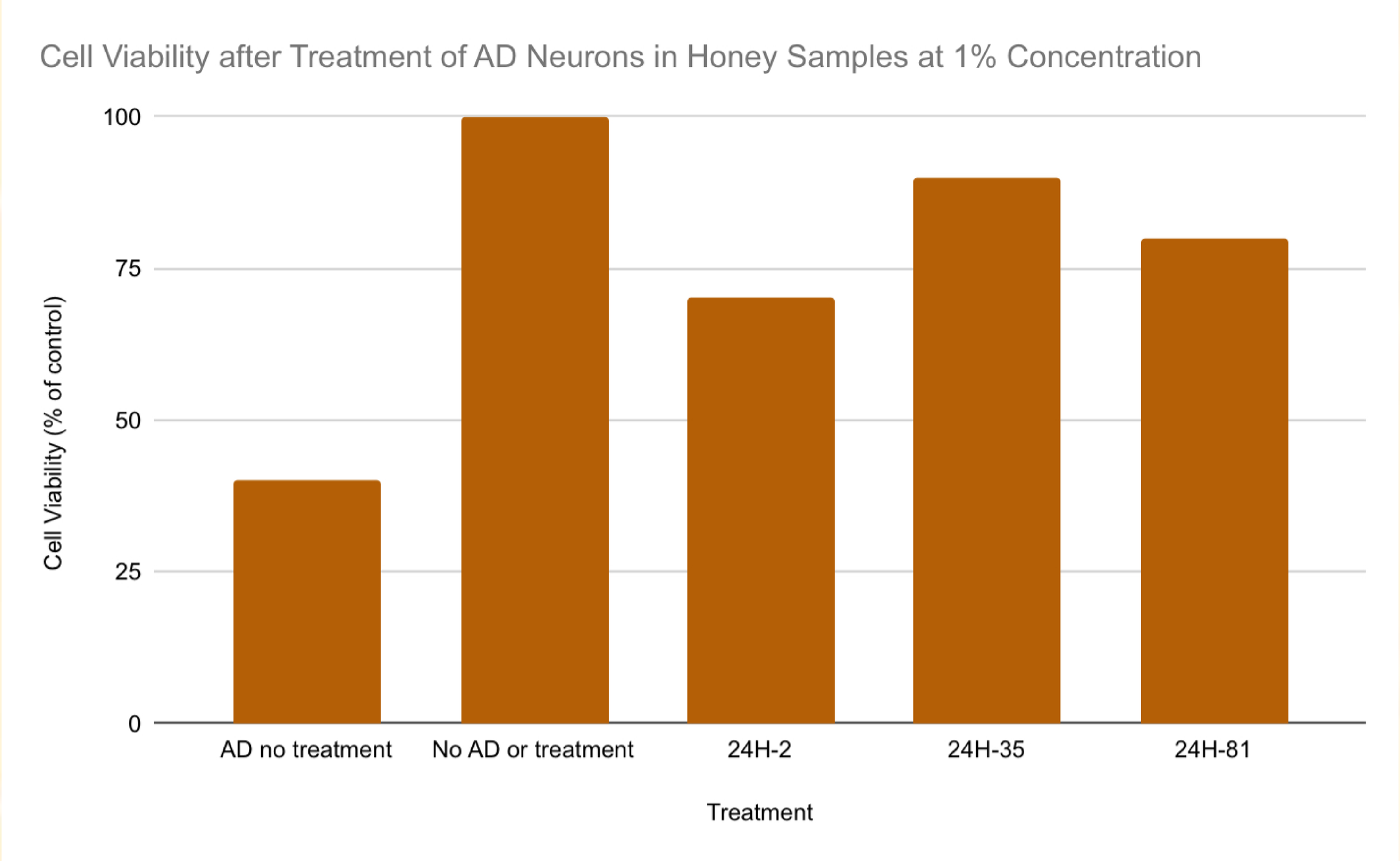


Figure 2. Free radical scavenging mechanism of DPPH by phenolic acid example.



Graph 1. Cell viability after treatment of AD neurons in honey samples at 1% concentration. Samples include the 3 honey samples collected as well as positive control with no AD reatment (normal neurons) and negative control with AD and no treatment.

Research Significance

When designing this project, there was no other thesis proposals conducted in the University of Texas at San Antonio that examines the antioxidant impact of medical grade honey against oxidative stress and β-amyloid plaque formation in Alzheimer's Disease. Thus, this study will call further research concerning brain health and the potential of honey use as a therapeutic approach for Alzheimer's Disease and other pathologies marked by oxidative stress. We look forward for the development of a natural therapeutic treatment in which is accessible for the increasing population of Alzheimer's Disease patients and can be used in conjunction with current treatments. Given these expected outcomes, they will contribute to the novel insights into honey's neuroprotective properties and alternative medicine in brain health research.

Impact

Future impact of our research include:

- investigating honey's influence on the expression of antioxidant genes and the modulation of transcription factors involved in inflammatory pathways that exacerbate oxidative stress.
- Prompt deeper studies into the gut-brain axis and honey's potential role in preserving blood-brain barrier integrity.
- Understanding the mechanism behind the
- Exploring the use of local honey with high Bioactivity levels (BAL) as an alternative or additional therapy and advancing honey's candidacy as a promising pharmacological agent for neurodegenerative disease intervention.

References

- **Al-Abd, N. M., Kassim, M., & Zajmi, A. (2017).** The inhibitory effect of galangin on cytokines and nitric oxide in microglia BV2 cell line. *Malaysian Journal of Science, 36*(3), 145–156. https://doi.org/10.22452/mjs.vol36no3.2
- **Ali AM, Kunugi H. (2019).** Bee honey protects astrocytes against oxidative stress: A preliminary in vitro investigation. *Neuropsychopharmacol Rep, 39*(4), 312–314. https://doi.org/10.1002/npr2.12079
- 2023 Alzheimer's disease facts and figures. (2023). *Alzheimer's & dementia : the journal of the Alzheimer's Association*, *19*(4), 1598–1695. https://doi.org/10.1002/alz.13016
- **Baranowska-Wójcik, E., et al. (2020).** Honey as the Potential Natural Source of Cholinesterase Inhibitors in Alzheimer's Disease. *Plant Foods for Human Nutrition, 75*(1), 30–32. https://doi.org/10.1007/s11130-019-00791-1
- **Fadzil MAM, Mustar S, Rashed AA. (2023).** The Potential Use of Honey as a Neuroprotective Agent for the Management of Neurodegenerative Diseases. *Nutrients, 15*(7), 1558. https://doi.org/10.3390/nu15071558
- **Hossen, M. S., et al. (2017).** Beneficial roles of honey polyphenols against some human degenerative diseases: A review. *Pharmacological Reports, 69*(6), 1194–1205. https://doi.org/10.1016/j.pharep.2017.07.002
- Khan, I. U., Dubey, W., & Gupta, V. (n.d.). *Medicinal Properties of Honey: A Review* [Review of *Medicinal Properties of Honey: A Review*]. School of Pure and Applied Science, JECRC University, Jaipur-303905, Rajasthan . Retrieved April 10, 2025, from https://www.researchgate.net/profile/Ishan-Khan-2/publication/333103599_Medicinal_Properties_of_Honey_A_Review/links/5cdbb30c299bf14d959885e8/Medicinal-Properties-of-Honey-A-Review.pdf
- **Namsi, A., et al. (2018).** Induction of Neuronal Differentiation of Murine N2a Cells by Two Polyphenols Present in the Mediterranean Diet Mimicking Neurotrophins Activities: Resveratrol and Apigenin. *Diseases, 6*(3), 67. https://doi.org/10.3390/diseases6030067
- **Shaikh, A., et al. (2023).** Honey and Alzheimer's Disease—Current Understanding and Future Prospects. *Antioxidants, 12*(2), 427. https://doi.org/10.3390/antiox12020427
- Tariot, Pierre N.*; Federoff, Howard J.†. Current Treatment for Alzheimer Disease and Future Prospects. Alzheimer Disease & Associated Disorders 17():p S105-S113, July 2003.
- Zhao, Yan, Zhao, Baolu, Oxidative Stress and the Pathogenesis of Alzheimer's Disease, *Oxidative Medicine and Cellular Longevity*, 2013, 316523, 10 pages, 2013. https://doi.org/10.1155/2013/316523

Acknowledgements

- Dr. Melanie Carless
- Dr. Eddie Hernandez
- Dr. Ferhat Ozturk
- The Honors College
- Dr. Hector Aguilar and Counselor Jennifer Riddick