

Effects of *Bryonia alba* on Oxidative Stress in *Tetrahymena thermophila*

Introduction

- The protist *Tetrahymena thermophila* was utilized as the model organism.
- Prior research indicates the presence of flavonoids in *Bryonia alba*, which have been implicated in the reduction of oxidative stress.
- Oxidative stress is caused by reactive oxygen species (ROS) that cause DNA damage. ROSs are a by-product of normal metabolic function.
- The genes *RAD51* & *OXR1* play a role in DNA damage repair and cellular response to oxidative stress, respectively, in *T. thermophila*.
- **Hypothesis:** *T. thermophila* cells that were exposed to the ROS, hydrogen peroxide, in the presence of *Bryonia alba* would exhibit reduced expression of *RAD51* and *OXR1* and increased cell growth, feeding, and responsiveness to a chemoattractant

Methods

- **Primer synthesis:** Primers for *RAD51* & *OXR1* were purchased from IDT.
- **Culturing:** Stock *T. thermophila* cultures were maintained in NEFF media while experimental cultures were maintained in SPP media. Oxidative stress was initiated by adding 3.4 μ L of hydrogen peroxide. The *Bryonia alba* dose was 10 μ L of a 10⁻⁷ dilution of a human dose.
- **RNA extraction:** RNA was extracted using Qiagen's RNeasy Mini Kit.
- **Reverse transcription:** ThermoFisher's RevertAid RT Kit was utilized to prepare cDNA.
- **Quantitative PCR** for *RAD51* and *OXR1* was performed using BioRad's iTAQ SYBR Supermix.
- **Cell Counts** were performed using 5% Glutaraldehyde and a hemocytometer.
- **Vacuole Counts** were performed using 1% India ink, 5% Glutaraldehyde and a hemocytometer
- **Chemotaxis** was performed used a Percoll: Protease Peptone solution overlaid with *T. thermophila*.



Figure 1. Hemocytometer with *T. thermophila* under 10x magnification.

Hunter Smith, Hunter Wetherelt, and Dr. Stefanie Otto-Hitt
Department of Biology, Carroll College

Results

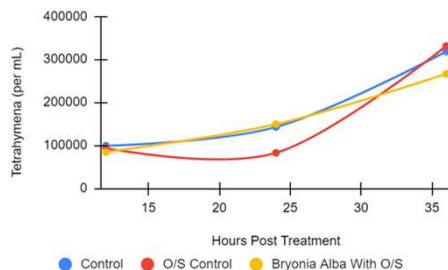


Figure 2. Cell count assay showing the average number of *T. thermophila* (cells/ml) at 12, 24, and 36 hours post treatment ($p > 0.05$ for all comparisons; $n=7$).

Figure 3. Feeding assay showing the average number of feeding vacuoles in each treatment group ($p > 0.05$ for all comparisons; $n=7$).

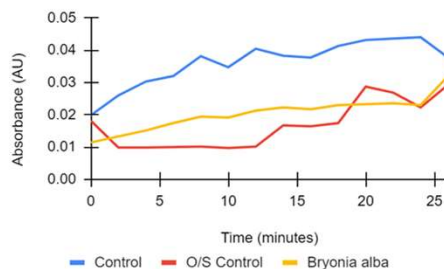


Figure 5. Fold change in *RAD51* expression across the three treatment groups ($p > 0.05$ for all comparisons; $n=6$)

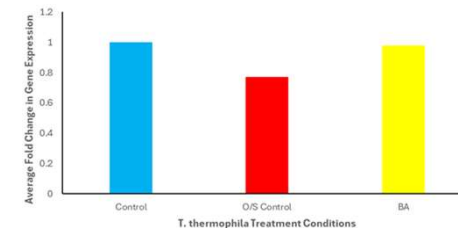


Figure 6. Fold change in *OXR1* expression across the three treatment groups ($p > 0.05$ for all comparisons; $n=6$)

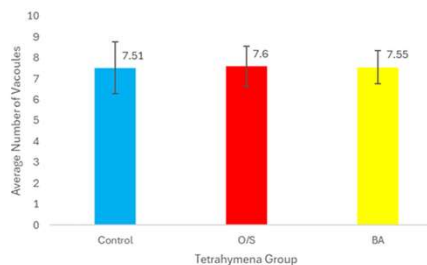
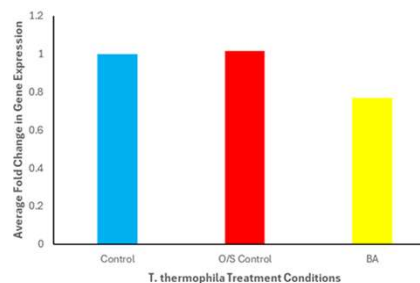


Figure 4. Chemotaxis assay showing average absorbance of treatment groups over 26 minutes ($p > 0.05$ for all comparisons; $n=7$).



Conclusions

- Cell counts showed no statistically significant difference in cell growth across the different treatment groups.
- The results of the feeding assay show there is no change in the number of feeding vacuoles across the different treatment groups.
- For the chemotaxis assay, the control group appeared to move further and more quickly towards the chemoattractant compared to the oxidative stress groups with/without *Bryonia alba*.
- There was no statistically significant difference in fold change for both *RAD51* and *OXR1* across the three groups
- The results from this study do not support our hypothesis as there was no significant change in gene expression or behavior in *T. thermophila* that were exposed to oxidative stress and treated with *Bryonia alba*.
- **Future Studies:** Utilizing different oxidative stressors, varying *Bryonia alba* dosages, and different behavioral assays.

References

- Bouchab et al. (2023) *Molecules*
- Campbell et al. (1998) *Nucleic Acids Research*
- Ielciu et al. (2019) *Antioxidants*
- Kuawska et al. (2019) *Ethnobiol Ethnomed*
- Mar et al. (2020) *Journal of King Saud University-Science*
- Smith et al. (2004) *Nucleic Acids Research*
- Yang et al. (2014) *Free Radic Biol Med*
- Yang et al. (2015) *Scientific Reports*
- Ye et al. (2018) *PeerJ*