

Examining the Effects of the Homeopathic Supplement, Nux Vomica, on Metabolism in *Tetrahymena thermophila*

Mila Duncan, Hannah Kolb, and Dr. Stefanie Otto-Hitt
Department of Biology, Carroll College

Introduction

- Nux Vomica a homeopathic supplement traditionally used in Chinese medicine to treat a variety of conditions, from hangover to Liver Cancer.
- Nux Vomica is thought to possess anti-tumor properties that disrupt the replication of tumor cells.
- These properties are believed to come from the two active alkaloids in Nux vomica, namely Strychnine and Brucine.
- CDK3* and *CAM1* genes are both involved in regulating and promoting the cell cycle.
- Hypothesis:** In the model organism *T. thermophila*, Nux Vomica treatment would result in increased expression of *CDK3* and *CAM1* genes, due to its anti-tumor properties, while also limiting cell growth and increasing metabolic rate and response to chemical stimulation.

Methods

- Dilution:** 6.6 mg of Nux vomica (15 pellets) was diluted in 10 ml of water. Further dilutions were taken to accommodate for the cell size. The final concentration is 0.00066 mg/ml.
- Cell Culturing:** *T. thermophila* cultures were maintained in NEFF media. 3 ml of cells were transferred into SPP media and exposed to 1 ml of diluted Nux vomica for 48 hours.
- Primers:** Primers for *CAM1* and *CDK3* were designed using the NCBI database and purchased from IDT.
- RNA extraction:** RNA extraction was done using Qiagen's RNeasy Mini Kit.
- Reverse Transcription:** cDNA was synthesized using ThermoFisher's RevertAid kit.
- Quantitative-PCR:** Was performed for *CDK3* and *CAM1* genes, as well the control *BTU1* gene, using BioRad's iTAQ SYBR Supermix.
- Cell counts:** Performed using hemocytometers and 5% glutaraldehyde.
- Deciliation:** *T. thermophila* cilia were degraded with Dibucaine and cell movement was monitored.
- Chemotaxis** was performed using Percoll-PP solution and spectrophotometer at 550nm.

Results

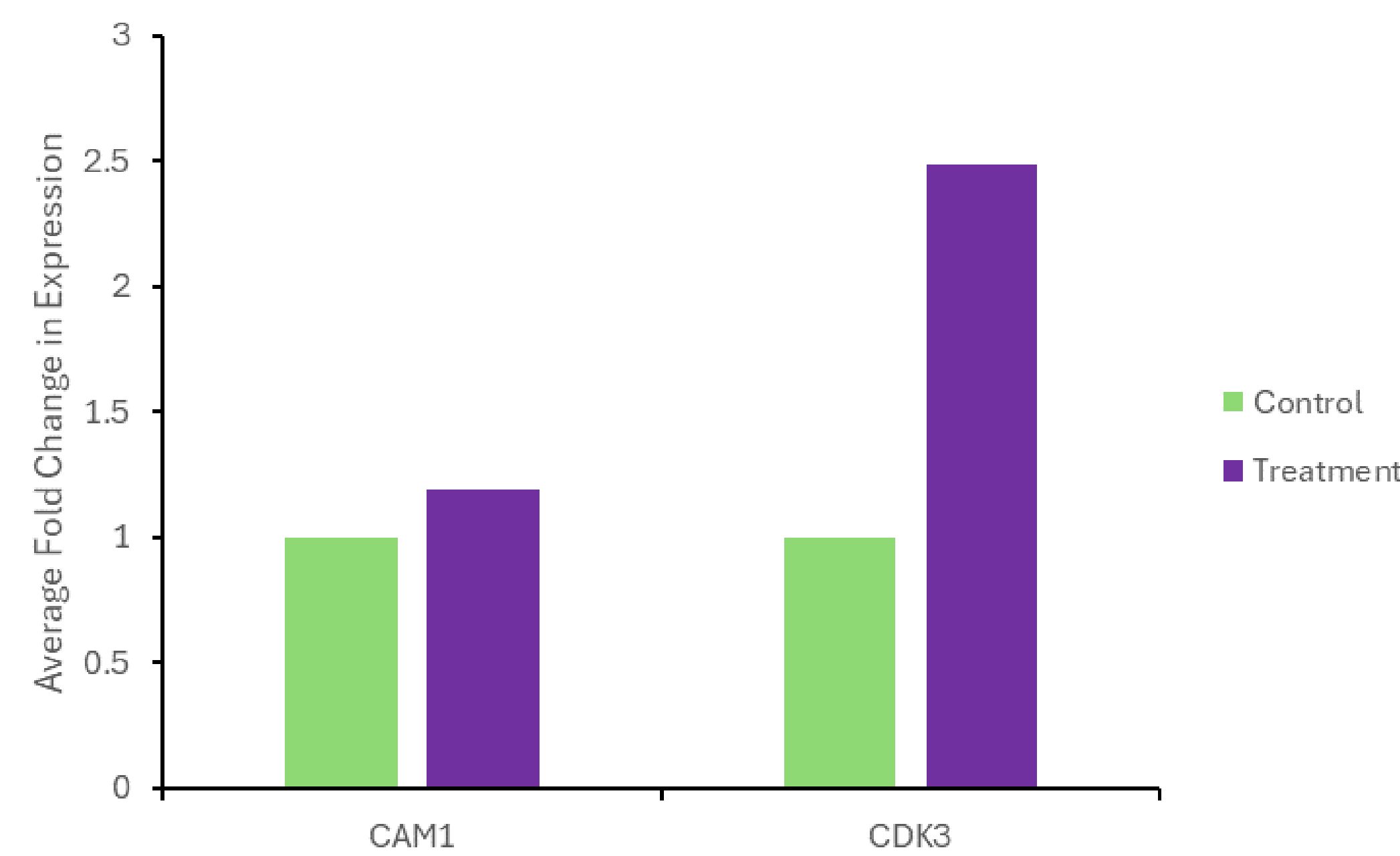


Figure 1: Fold change in expression of *CDK3* and *CAM1* genes in Nux-Vomica treated *T. thermophila* (p-value>.05; n=3 replicates).

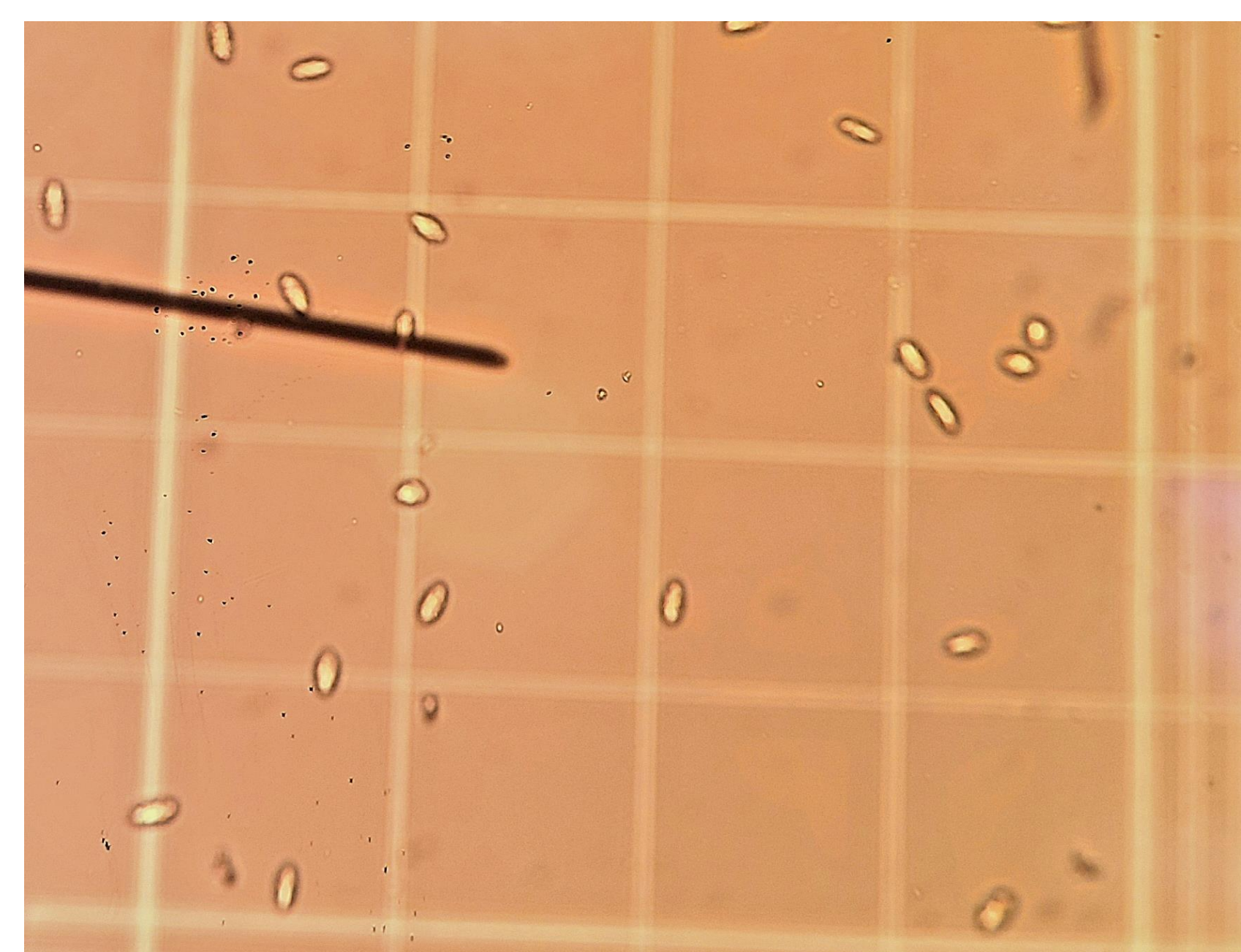


Figure 3: Image showing *T. thermophila* cells used for cell counting.

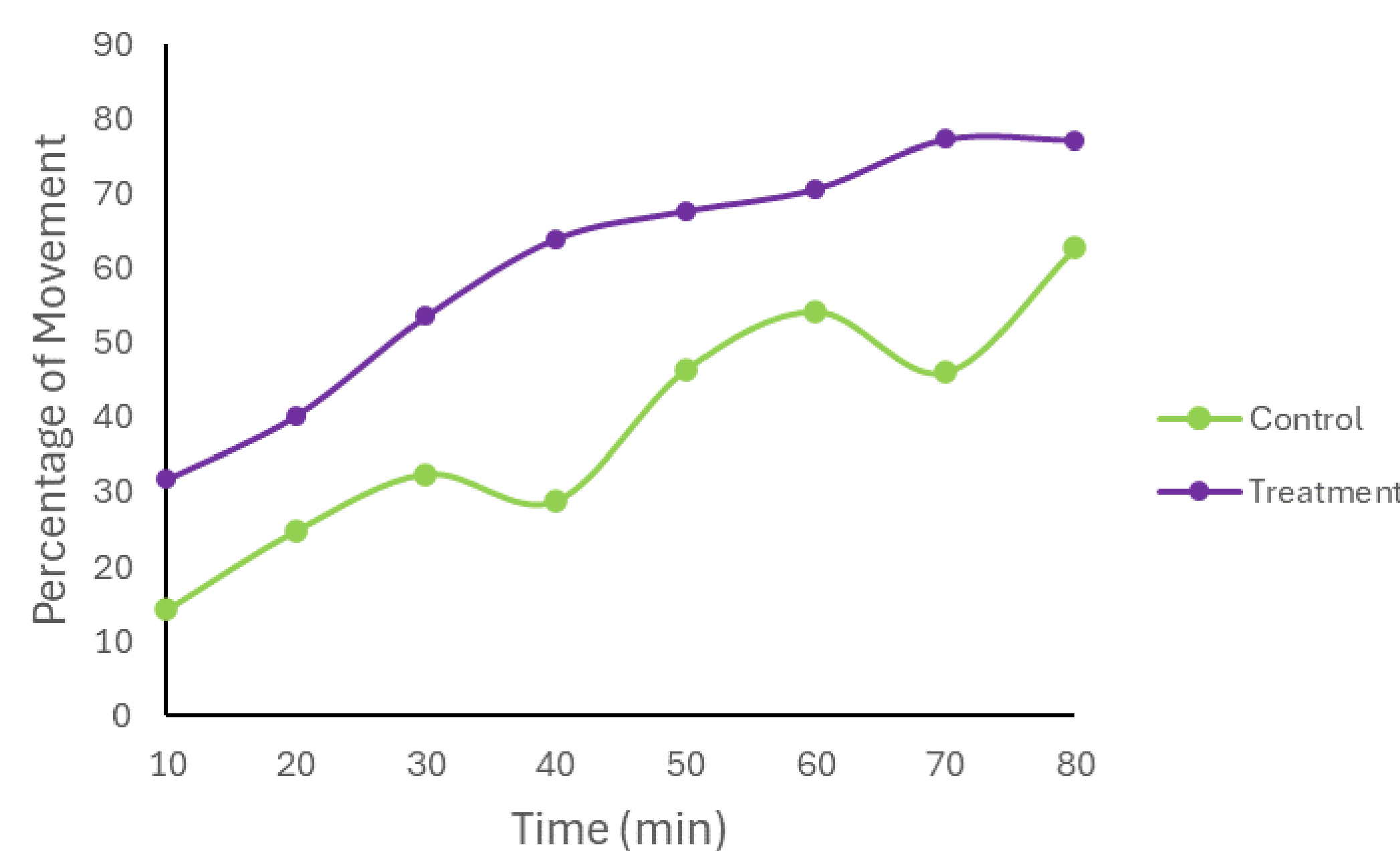


Figure 5: Deciliation Assay showing metabolic activity of Nux-Vomica treated cells compared to controls (p-value>.05; n=6 replicates).

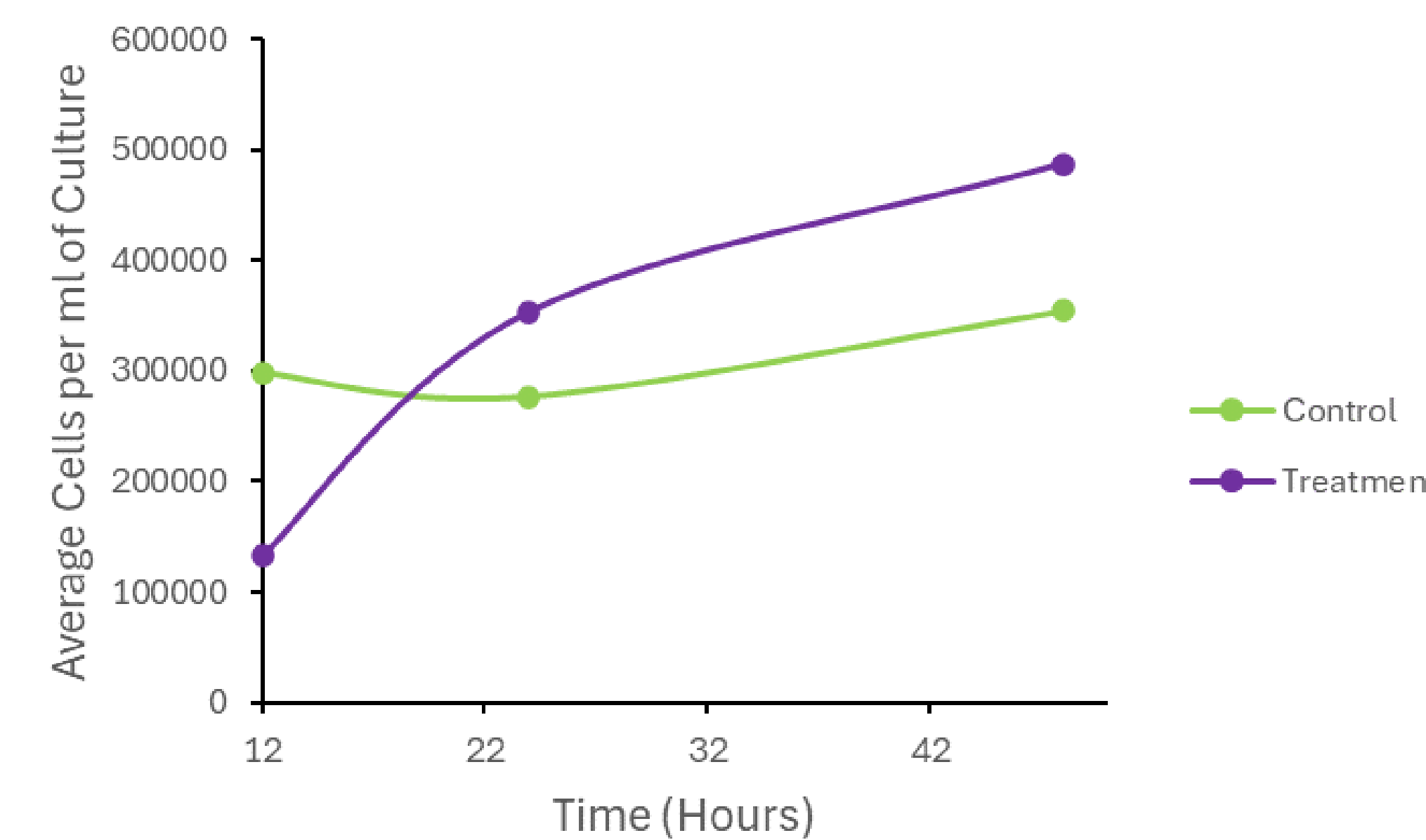


Figure 2: Cell growth over a 48hr time period of Nux-Vomica treated *T. thermophila* (p-value>.05; n=7 replicates).

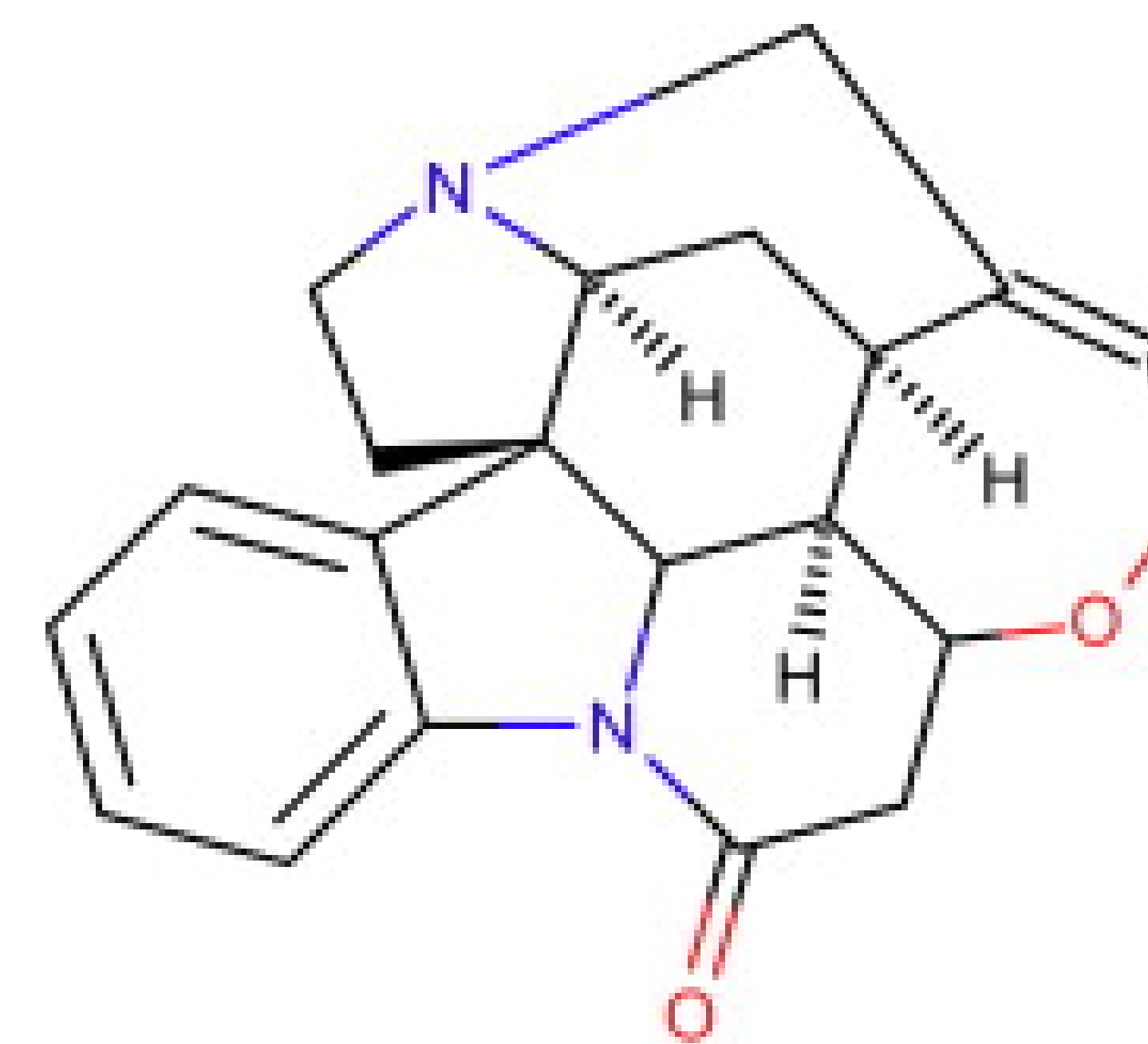


Figure 4: Chemical structure of strychnine, an active ingredient in Nux Vomica.

(<https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/strychnos-nux-vomica>)

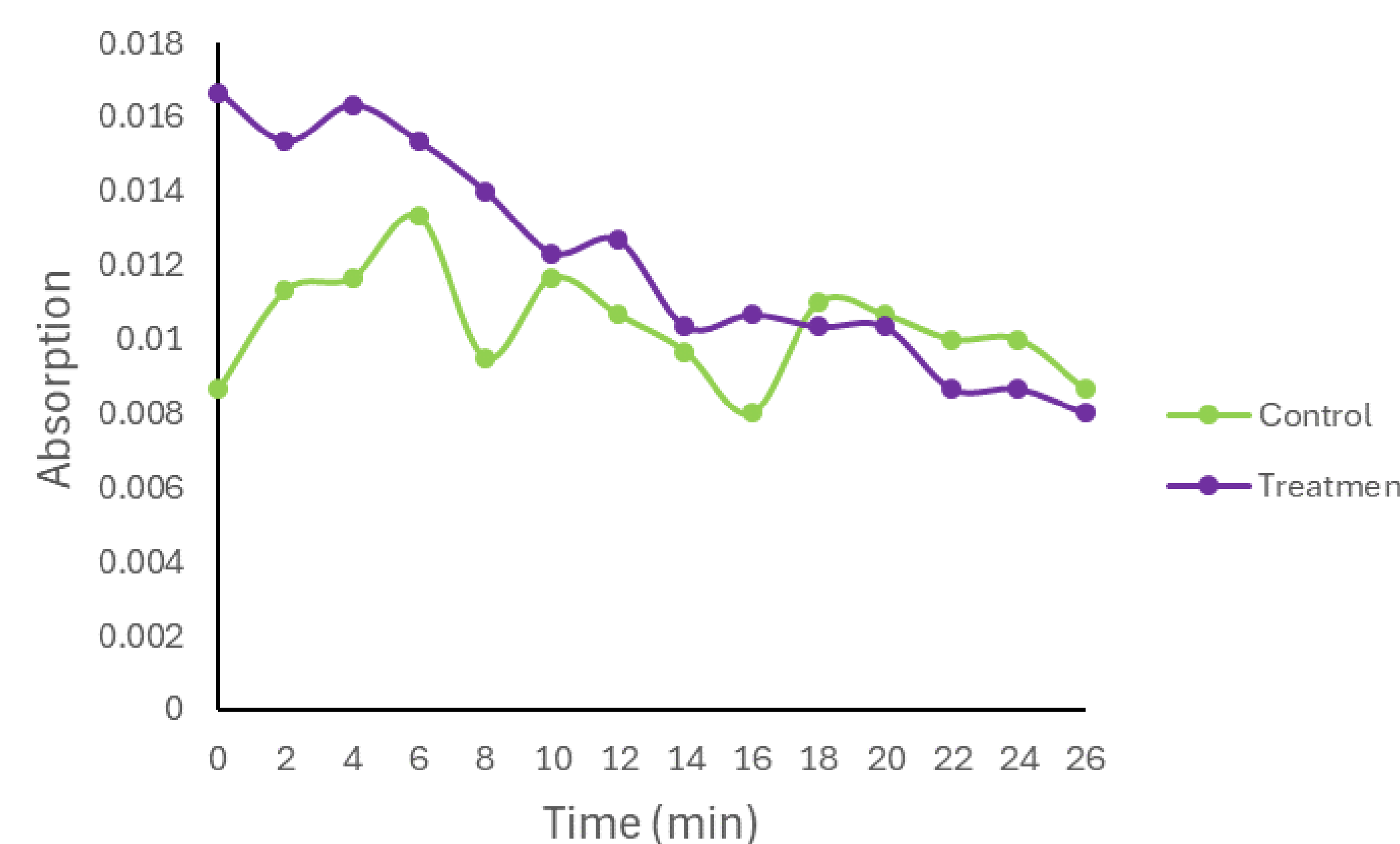


Figure 6: Chemotaxis Assay showing cell movement toward a chemoattractant in Nux-vomica treated cells compared to controls (p-value>.05; n=3 replicates).

Conclusions

- There was no significant change in the expression of *CDK3* and *CAM1* in Nux Vomica treated cells compared to controls.
- No significant difference was observed in cell growth, metabolic activity, and chemoattraction in Nux Vomica treated cells compared to controls.
- General trends indicate an increase in cell growth, metabolic activity, and *CDK3* and *CAM1* expression in Nux-Vomica-treated *T. thermophila* compared to control cells.
- General trends also showed no attraction towards the Protease Peptone for control or Nux-Vomica-treated *T. thermophila*.
- Future Direction:** To determine whether the trends we observed during the seven weeks of data collection hold statistical significance, additional data is needed. Future studies should focus on refining the behavioral and gene expression assays so as to obtain more replications for each experiment.

References

- Ren H, et al. (2019). *Phytother Res.*
- Yin, W, et al. (2007). *Food Chem Toxicol.*
- Luo H, et al. (2018). *Toxicol Res (Camb).*

Acknowledgments

We would like to thank Dr. Stefanie Otto-Hitt for all her support and help with the project. We would also like to thank the *Tetrahymena* for their honorable sacrifice.