

Introduction

- *Tetrahymena thermophila* is a single-celled eukaryotic model organism that quickly reproduces and is cost-effective.
- Methylcellulose in media acts as a mechanical stressor to affect *T. thermophila* behavior.
- Turkesterone is a naturally occurring plant steroid that has gained attention for its potential medicinal and biological properties without the adverse effects typically associated with traditional anabolic steroids.
- *OXR1* is a gene linked to oxidative stress responses.
- *AAC1* encodes an ADP/ATP carrier protein involved in mitochondrial energy regulation.
- *IFT122* plays a role in ciliary function and motility.
- **Hypothesis:** *T. thermophila* supplemented with Turkesterone will exhibit reduced expression of *OXR1*, *AAC1*, and *IFT122* compared to control and mechanically stressed groups, indicating enhanced oxidative stress resistance, improved metabolic efficiency, and stable ciliary function under stress conditions.

Methods

- **Culturing:** *T. thermophila* were cultured in NEFF media and transferred to SPP media for experimentation. Turkesterone was added to the treatment group at a final concentration of 0.194 mg/mL (2.035 mg per 10.5 mL culture). 1% methylcellulose was added to both experimental groups.
- **RNA Extraction:** RNA was extracted using a Qiagen RNeasy Mini Kit.
- **Quantitative PCR:** cDNA was amplified using BioRad's iTAQ SYBR SuperMix. *BTU1* was used as a positive control.
- **Motility Assay:** *T. thermophila* were added to 1 μ L microcaps, and the number of cells crossing the midline from left to right within 30 seconds was recorded.
- **Deciliation Assay:** Cells were deciliated with dibucaine, and the percentage of motile cells was recorded every 10 minutes.
- **Phagocytosis Assay:** Feeding activity was assessed by mixing cultures with 1% India ink, fixing with glutaraldehyde, and counting vacuoles in 10 cells per group at 0 and 5 minutes under 40x magnification.
- *Descriptive statistics* was used to calculate standard deviation and standard error using Excel.

Results

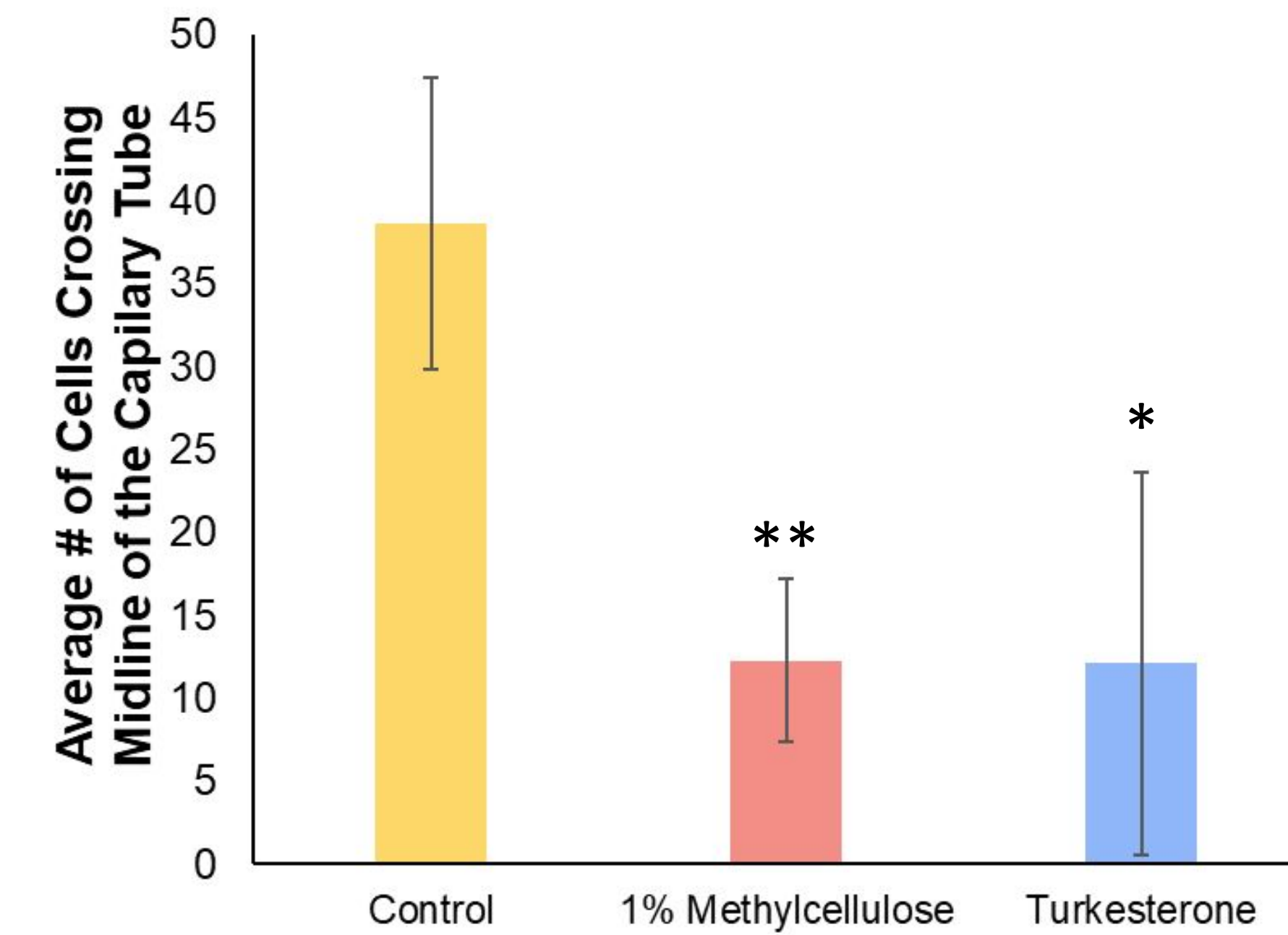


Figure 1: Motility assay comparing average number of *T. thermophila* crossing a marked capillary line in 30 seconds across control, 1% methylcellulose, and Turkesterone-treated groups (* $p < 0.01$, ** $p < 0.001$, $n = 7$). Error bars represent \pm SD.

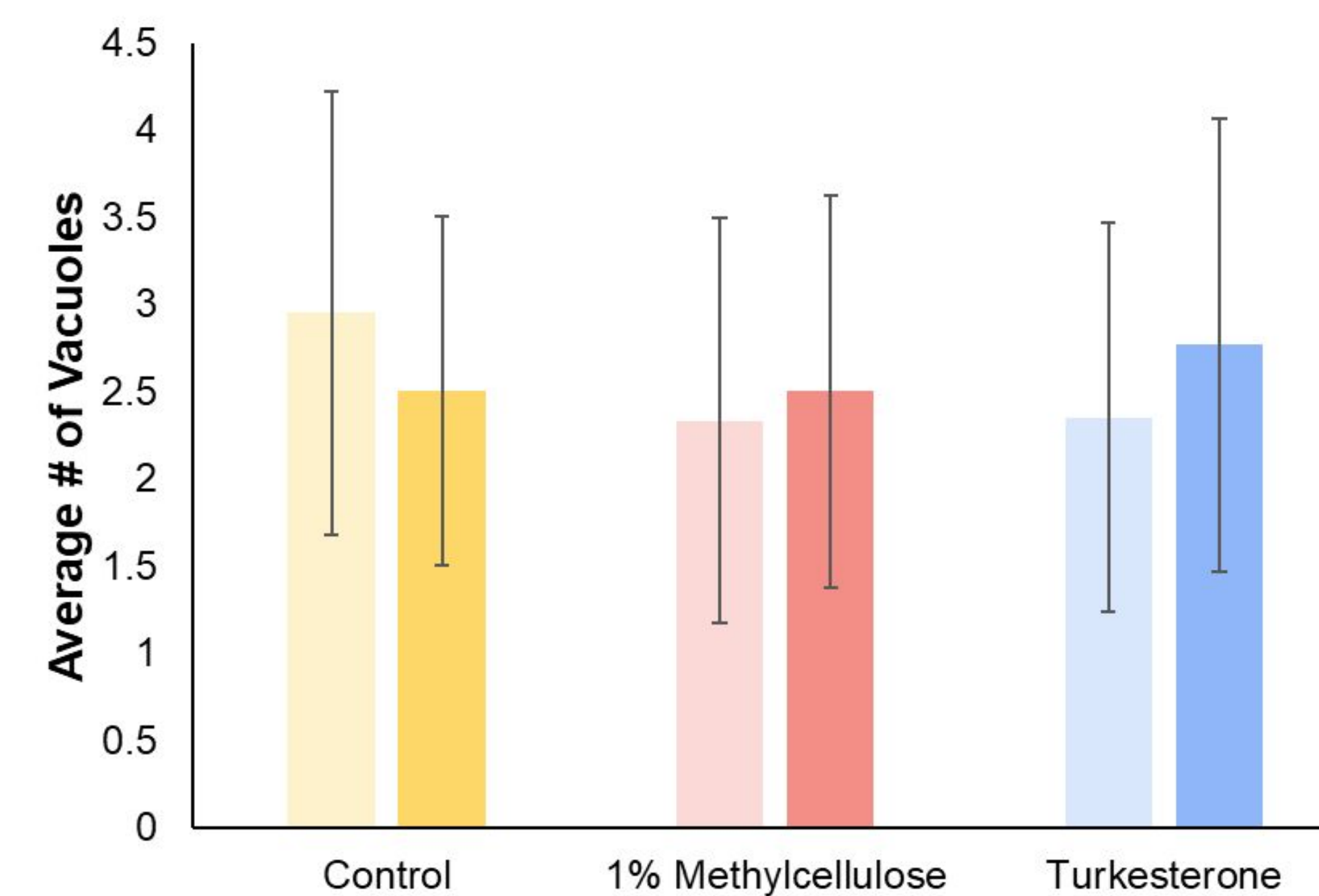


Figure 2: Phagocytosis assay comparing average number of vacuoles per *T. thermophila* at 0 (light-colored bars) and 5 (dark-colored bars) minutes post-India ink exposure ($p > 0.05$ for all comparisons, $n = 6$).

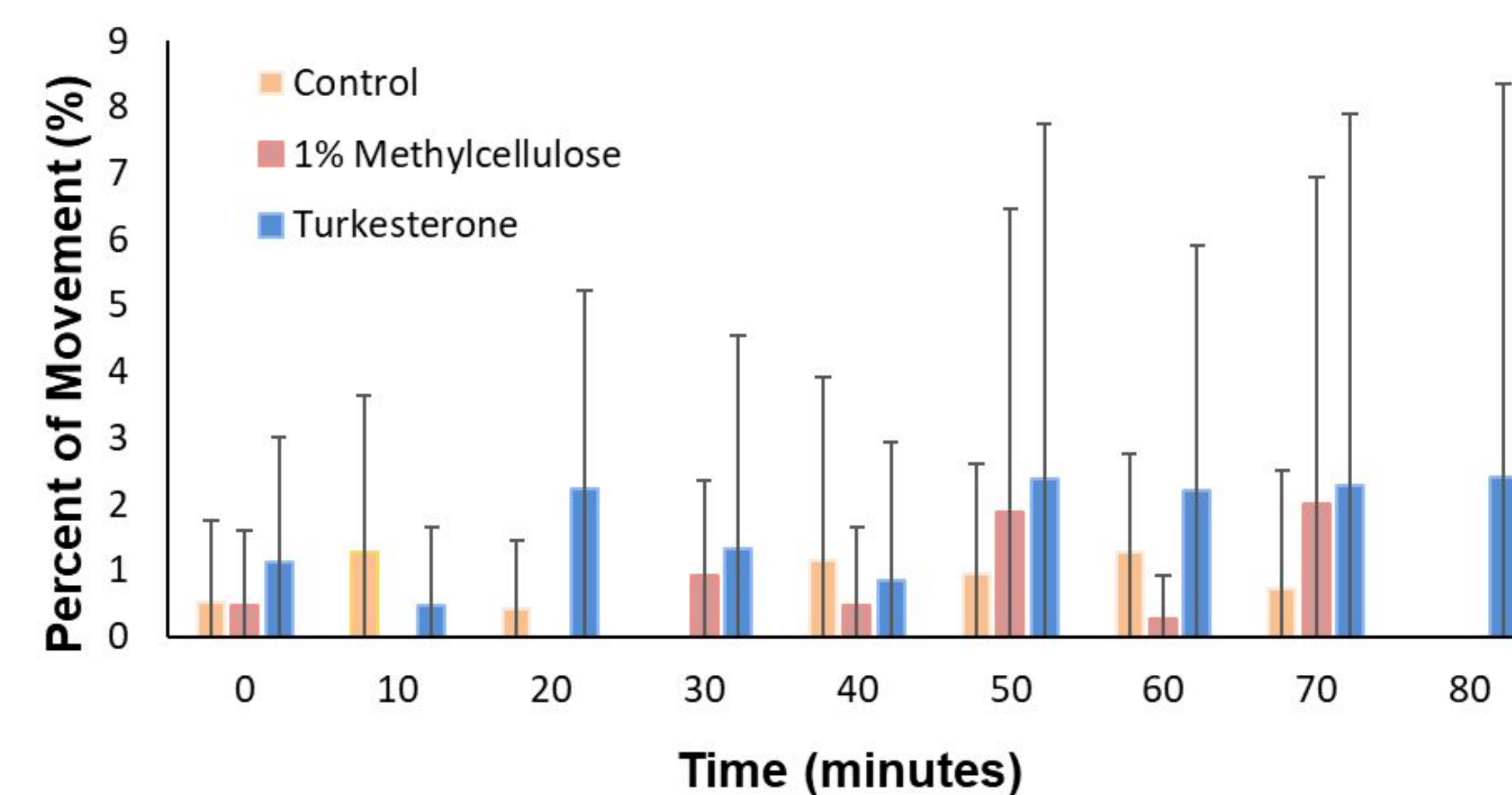


Figure 3: Deciliation recovery assay comparing percentage of motile *T. thermophila* measured at 10-minute intervals following dibucaine-induced deciliation ($p > 0.05$ for all comparisons, $n = 6$).

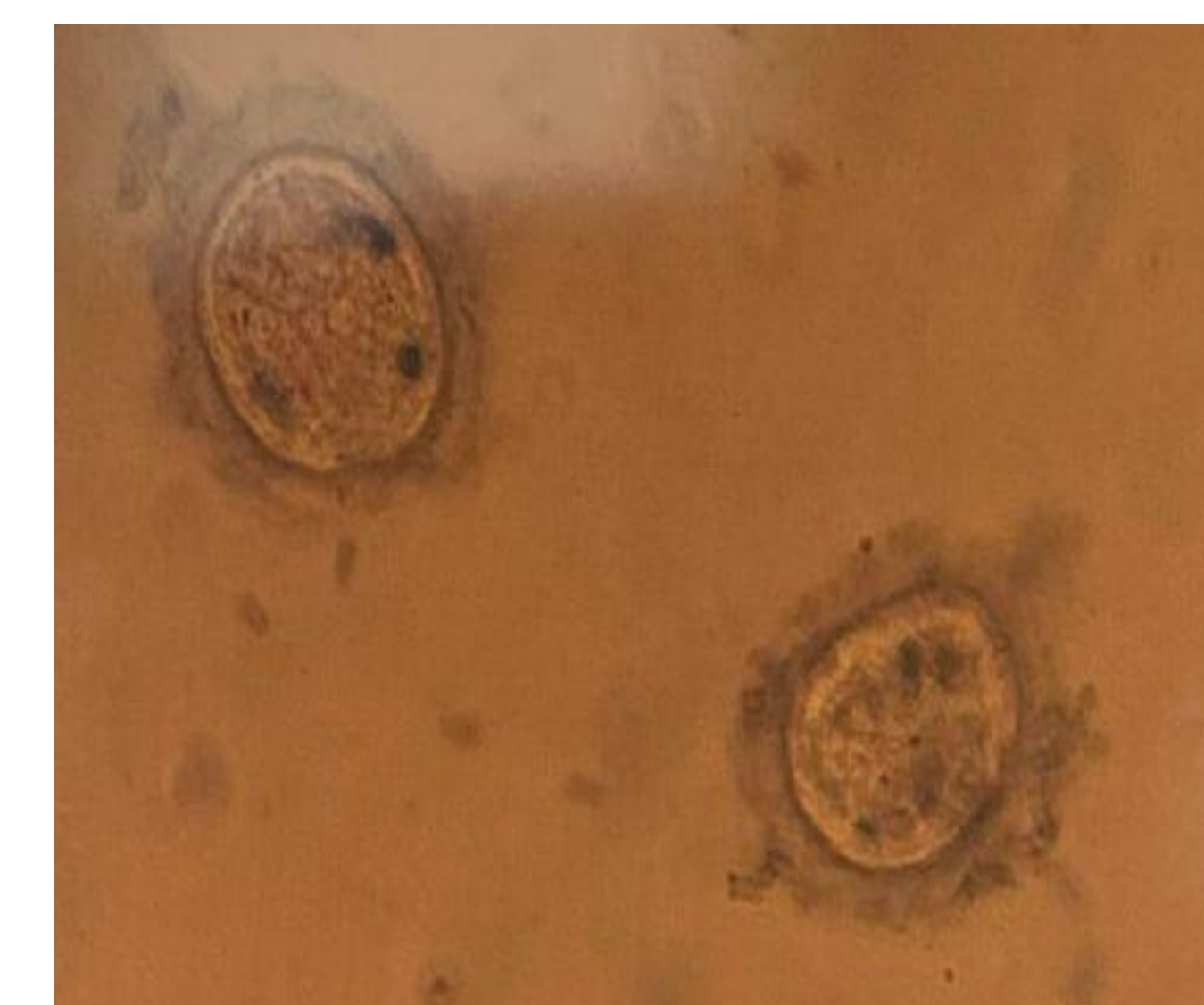


Figure 4: Image of *T. thermophila* during the phagocytosis assay, under a microscope at a magnification of 40x.

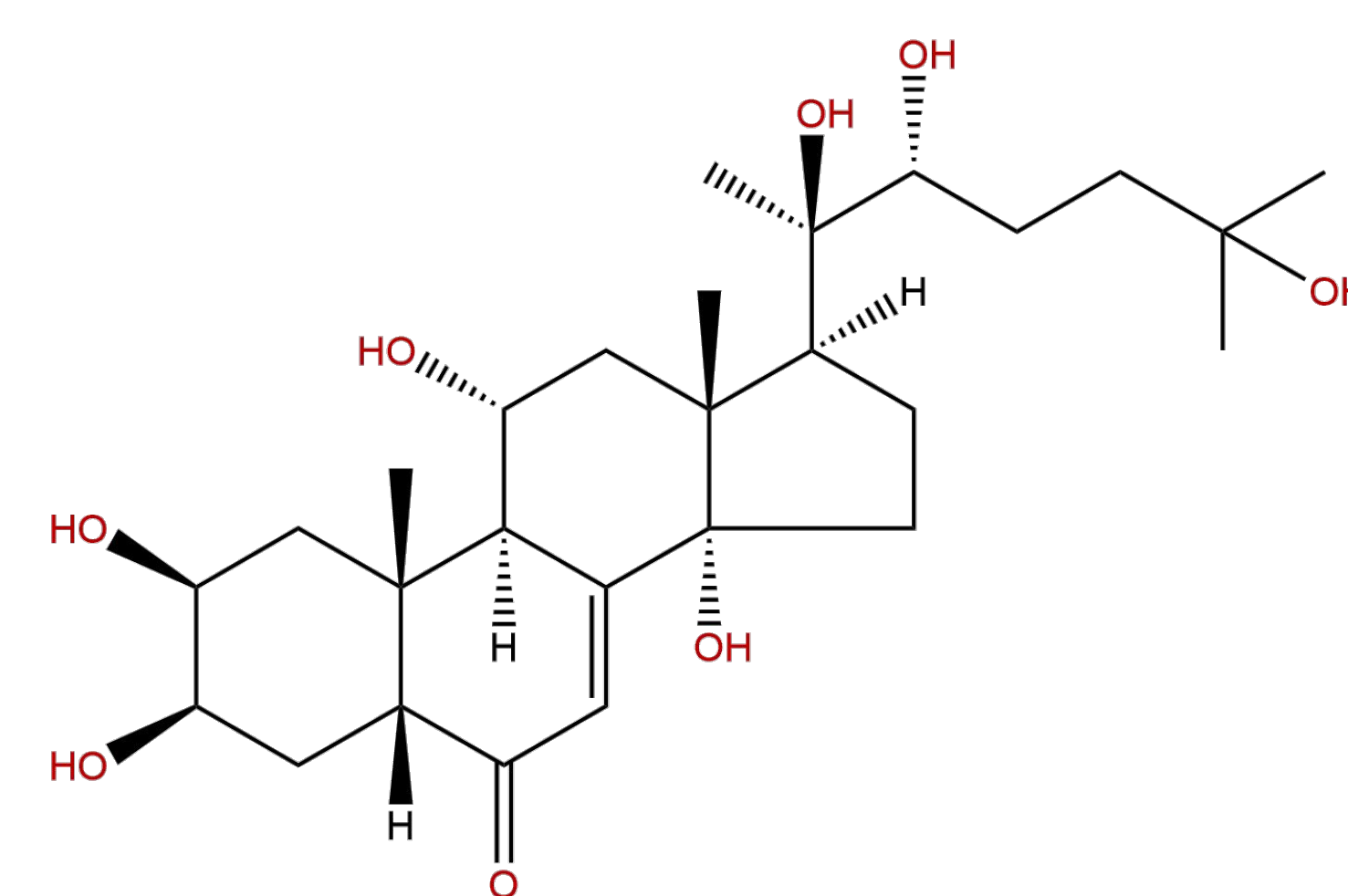


Figure 5: Chemical Structure of Turkesterone.

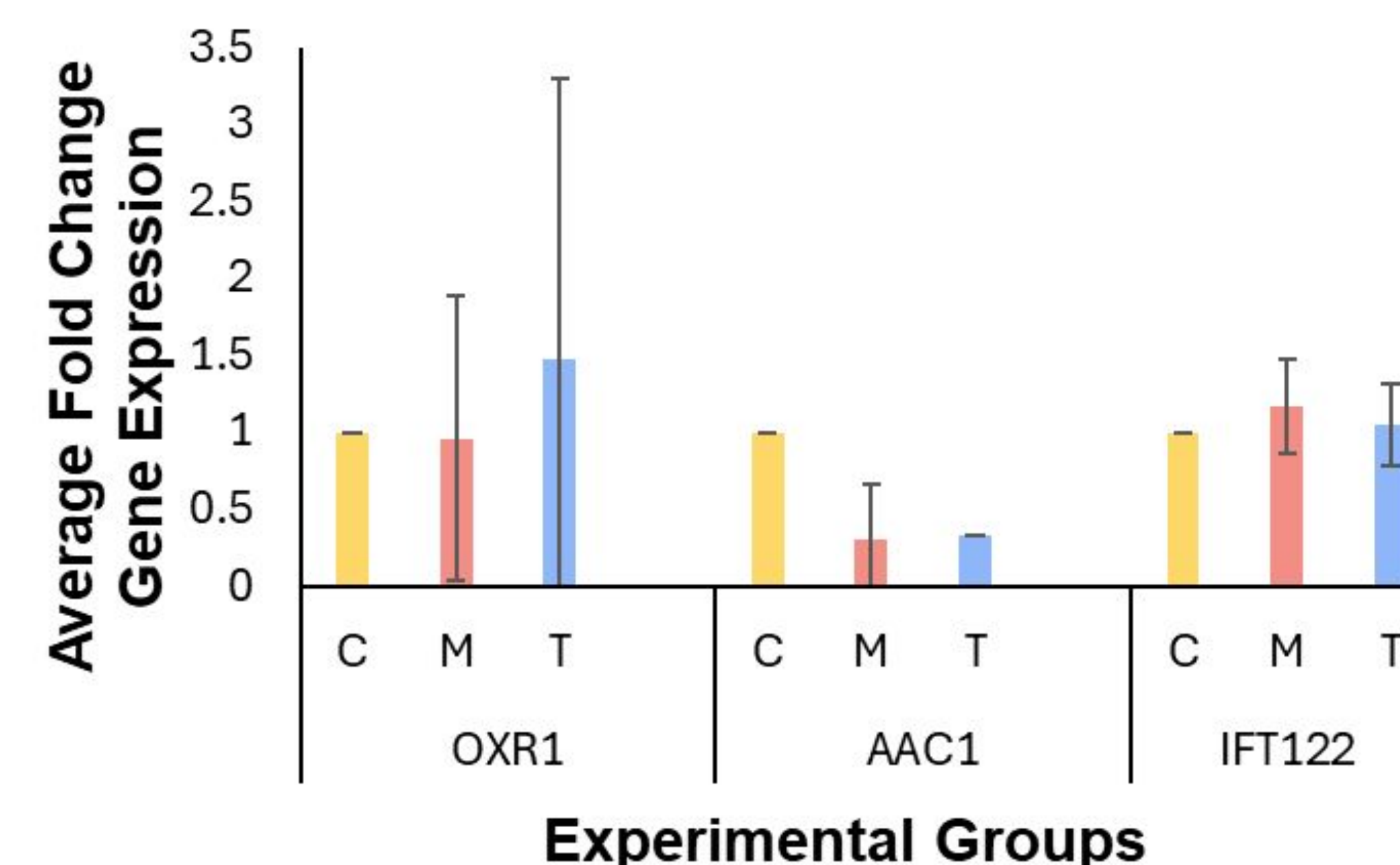


Figure 6: Fold change in expression of *AAC1*, *OXR1*, and *IFT122* in *T. thermophila* ($p > 0.05$ for all comparisons, $n = 6$). C = Control, M = 1% Methylcellulose, T = Turkesterone.

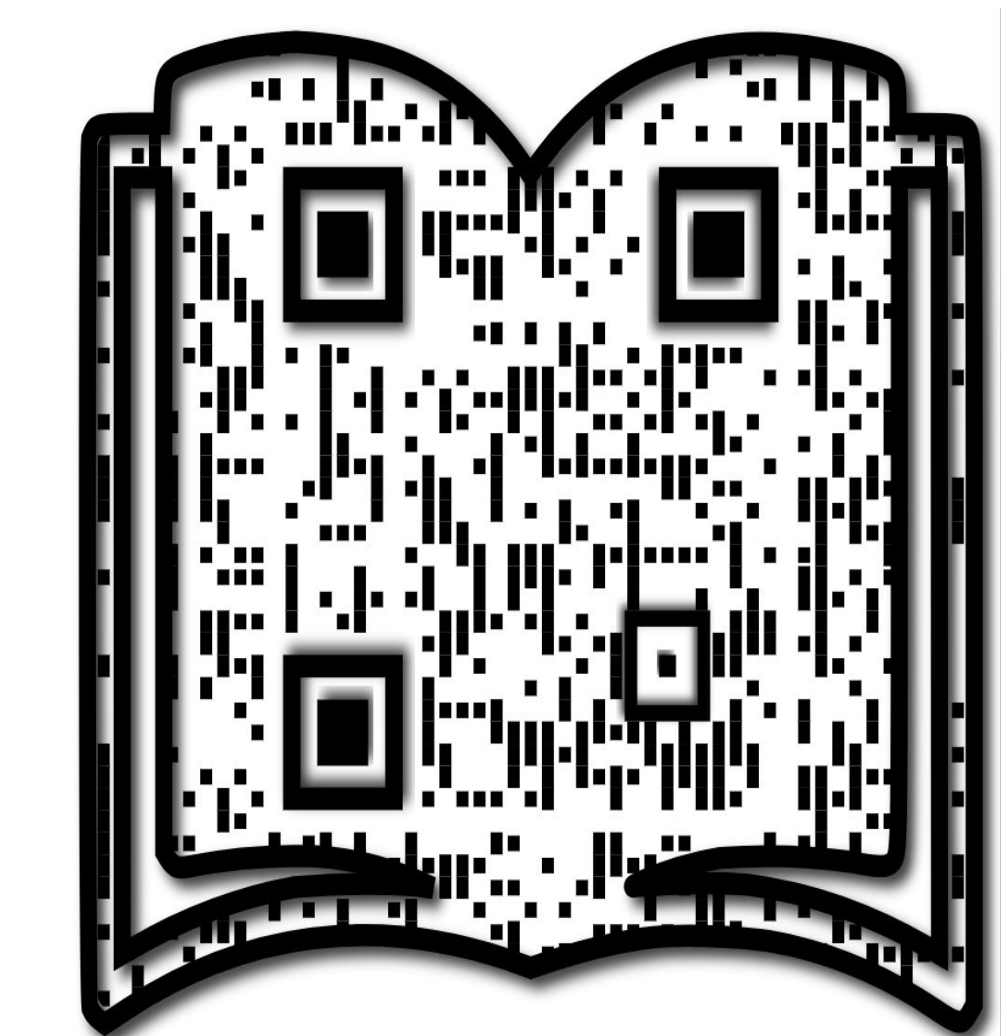
Conclusion

- 1% methylcellulose significantly impaired motility in *T. thermophila*, and treatment with Turkesterone did not restore movement to control levels. This suggests that, under the tested conditions, Turkesterone does not rescue or enhance locomotion in mechanically stressed cells.
- Deciliation recovery and feeding activity trends were consistent with potential metabolic benefits of Turkesterone, but results were inconclusive due to high variability.
- RT-qPCR analysis showed no significant changes in *OXR1*, *AAC1*, or *IFT122* expression in response to 1% methylcellulose or Turkesterone treatment, suggesting negligible impact on gene expression.
- Further replication and refinement of assays are needed to assess behavioral and genetic differences.

Future Directions

- Conduct a dose-response study by testing multiple concentrations of Turkesterone to evaluate its effects on metabolic activity in *T. thermophila*.
- Include longer incubation periods to evaluate delayed or cumulative effects of Turkesterone on cellular behavior and gene expression.
- Use alternative behavioral assays (e.g., chemotaxis) to detect differences in metabolic performance.

References



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