

Abstract

Natural Killer (NK) cells play a critical role in immune defense against cancerous and infected cells through cytotoxic activity. NK cell activity is often regulated by a variety of signalling molecules, and release of these molecules can be governed by a multitude of compounds and stimulants. This study investigated specifically the independent and tandem effects of caffeine and cortisol on NK cell function, using an in vitro fluorescence-based cytotoxicity assay. NK cells were exposed in varying concentrations (1-10 uM) of caffeine, cortisol, and combined caffeine-cortisol concentrations in co-culture with target cells for 48 hours. Cytotoxicity was quantified via relative fluorescence reduction and analyzed. Results indicated that both stimulants independently reduced cytotoxicity across all concentrations tested. Caffeine exposure was relatively consistent, while cortisol exhibited dose-dependent decreases. Combined treatment resulted in lower cytotoxicity overall, but a two-way ANOVA test revealed no statistically significant interaction between the two compounds (p=0.83). These findings indicate that both stimulant and stress-associated compounds can independently impair NK cell function in vitro, emphasizes the importance of considering multiple stressors when evaluating immune function, and suggest that further research is needed to explore the interactions of the two compounds under different conditions.

Introduction

- Natural Killer cells make up 5-10% of all lymphocytes and play a major role in cancer suppression
- Chronic stress reduces immune function by altering the gene expression of immune cells, and also affects a large majority of the teenage population (Alotiby, 2024), (Zakowska et al., 2022)
- Caffeine alters signalling pathways that reduce the release of IL-2, a critical immune signalling molecule (Kloubert et al., 2021)
- Effects of both compounds in tandem has yet to be studied

Research Question / Hypothesis

How does caffeine and cortisol affect Natural Killer cell cytotoxicity in tandem?

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Cytotoxicity should drop linearly with both compounds.

Method and Materials

- Similar existing studies:
 - Chromium assays to study cytotoxicity (Feighner et al., 2014)
 - LDH absorbance assay (Chava et al., 2018)
- Prepared two cell lines, an NK cell line and HeLa cells modified with GFP
- Used a Synergy HTX reader to find relative fluorescence units (RFUs) of each well

Results

- Results show that caffeine and cortisol result in a significant decrease in cytotoxic ability.
- Cytotoxicity % drops as concentration increases
- Caffeine results in overall decrease, cortisol results in non-linear interaction
- Compounds do **not** result in a linear increase, suggesting a non-linear relationship

Discussion

- Caffeine and cortisol do in fact have tandem effects on NK cell cytotoxi^{cty}, but the interaction is not strictly nonlinear.
- This warrants investigation into the specific interactions between caffeine and cortisol.

Implications

- Cytotoxicity drops around 10-30% depending on dosage
- Teens should reduce caffeine intake and stress
- High risk individuals should also avoid said factors, such as:
 - Firefighting
 - Radioactive environments
 - Rubber industry

References

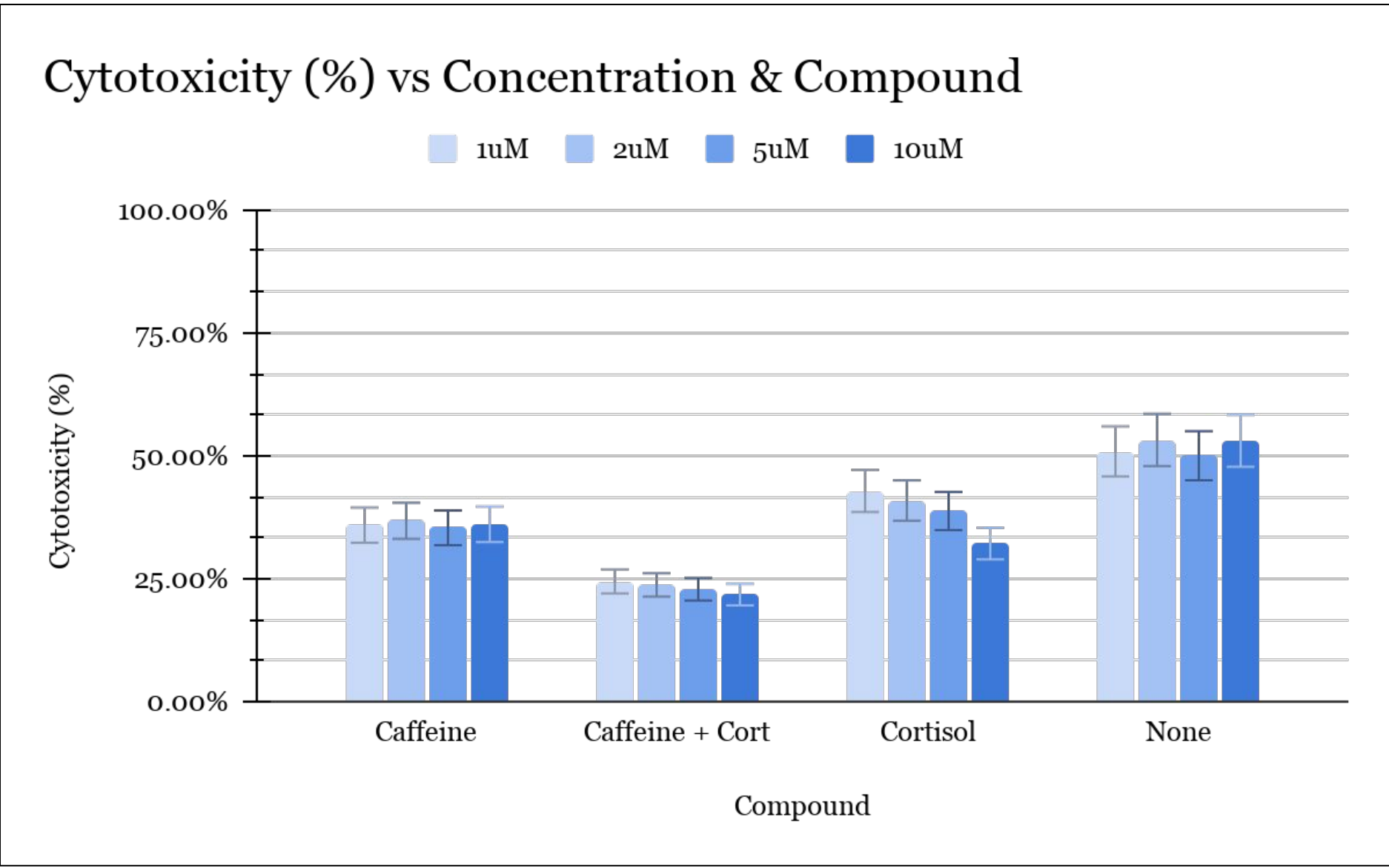
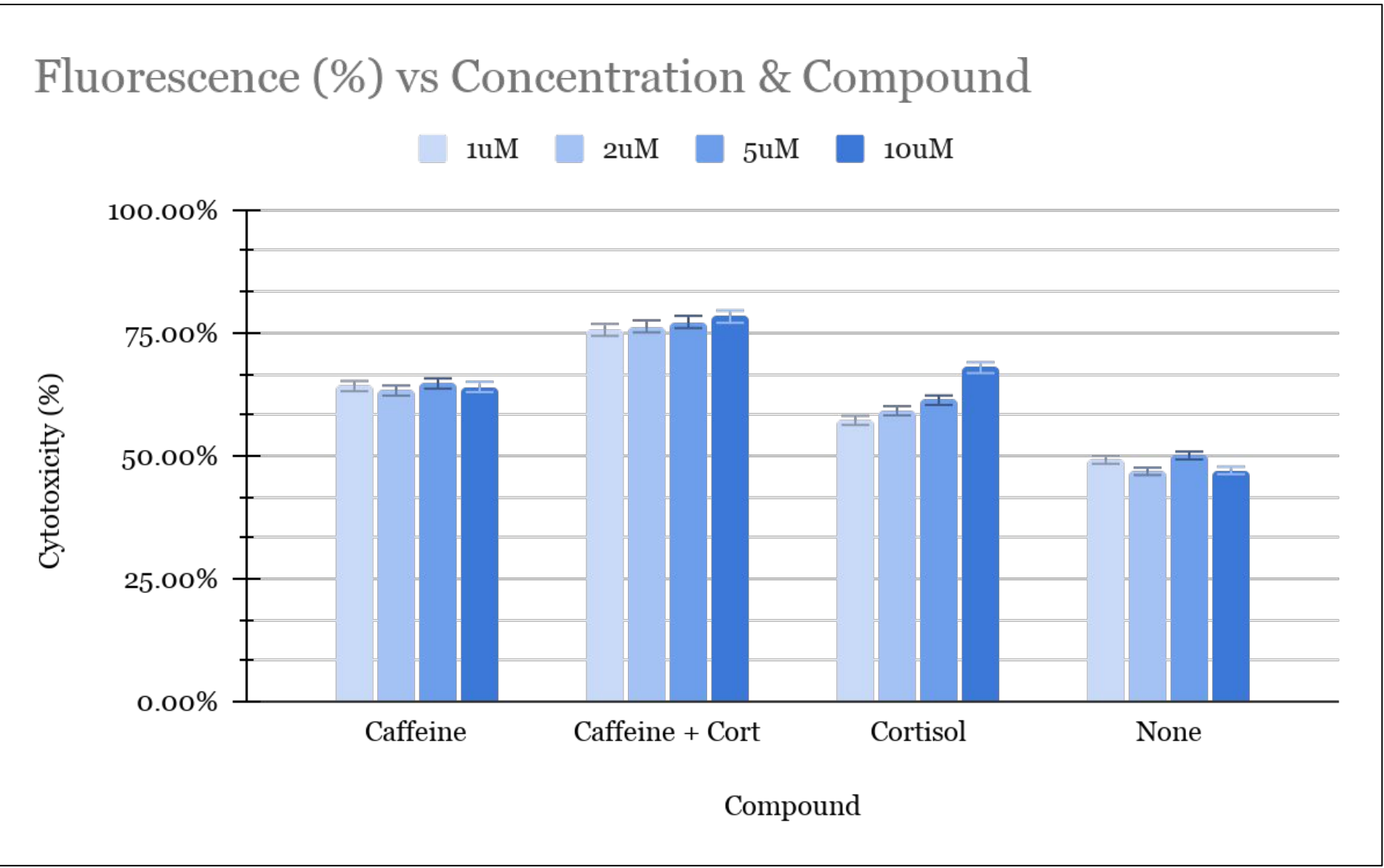
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Effects of Caffeine and Cortisol on NK cell function

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