

## **Synergistic potential of combined tocotrienols-astaxanthin in addressing inflammation caused by IL-1 $\beta$ induced in A549 cell lines**

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### **Structured Abstract**

**Background:** NSAIDs are commonly used to treat inflammatory diseases but are associated with adverse side effects. The combination of astaxanthin and tocotrienols has been found to have anti-inflammatory and antioxidant properties in reducing inflammation.

**Methods:** In this study, A549 cell cultures were prepared by adding fetal bovine serum to complete growth medium. Astaxanthin extracted from *Haematococcus pluvialis* and Tocotrienol Rich Fraction were dissolved in soya bean oil and were diluted in various concentrations according to Box-Behnken Design. These compounds were seeded in 96-well plates into three conditions: Healthy A549 cells, IL-1 $\beta$  infected A549 cells, and IL-1 $\beta$  infected A549 cells with astaxanthin-tocotrienols treatment. The results were evaluated by performing cell viability via MTT Assay and NO production via Griess Assay.

**Results:** The cell viability result showed that 10 ng/mL of IL-1 $\beta$  did not affect the cells' viability; no cytotoxicity effects were observed in all treatments. The cells were still viable even at higher concentrations of combined astaxanthin-tocotrienols. The NO production result showed that when astaxanthin and tocotrienols were administered in varying combined concentrations, NO production decreased by 21% to 10%. The synergistic effects analysis revealed that the combination of 200  $\mu$ g/mL of astaxanthin and 50  $\mu$ g/mL of tocotrienols was the optimum formulation due to its low CI value of 0.98, indicating synergism. Therefore, it can prevent adverse effects from high-dose administration and ability to reduce the emergence of drug resistance.

**Conclusion:** The combination of astaxanthin 200  $\mu$ g/mL and TRF 50  $\mu$ g/mL is the optimal cocktail formulation as it exhibited a synergistic effect, and the findings of this study can be useful for future researchers to determine the extensive applicability of the combined treatment in addressing various inflammatory conditions.

**Keywords:** Astaxanthin, tocotrienols, synergistic, inflammation, IL-1 $\beta$

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