

## **The Analysis of The Synergistic Anti-inflammatory Effect of The Combination of Chlorella Extract – Tocotrienols in LPS Stimulated RAW 264.7 Macrophages**

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### **Structured Abstract**

**Background:** Tocotrienols and Chlorella extracts are natural substances known to be antioxidants with anti-inflammatory capabilities. Each substance has different levels of anti-inflammatory activity. The research on the synergistic anti-inflammatory activity of chlorella extract and tocotrienols in LPS-stimulated RAW 264.7 macrophages yet to be reported. Distinct combinations of the antioxidant compounds; astaxanthin-tocotrienols, and chlorella extract-tocotrienols create varying efficiency levels of synergistic anti-inflammatory action.

**Methods:** In this study, RAW 264.7 cells were stimulated by LPS and treated with 15 different concentration treatments. Cell viability was tested using the MTT assay, and nitrite oxide (NO) was determined using the Griess assay. An isobologram was utilised to confirm that the combination treatments have a synergistic impact.

**Results:** The result showed that the combination of astaxanthin and TRF exhibited inflammatory markers such as NO production. The concentration of chlorella extract ranging from 31.25 to 125 µg/mL and TRF at 25-100 µg/mL had no toxicity and achieved higher cell viability. In the most tested parameter, the combination of chlorella extract – tocotrienols showed a synergistic anti-inflammatory effect and was superior to each component's solo effect. Combination Index (CI) resulted in synergism values of 0.80 and 0.63.

**Conclusion:** The combination of chlorella extract and TRF exerted anti-inflammatory activities in LPS-stimulated RAW 264.7 macrophages cell. Two concentration treatments showed the best proximity. Therefore, it is suggested to utilise this two-concentration combination treatment for future studies to conduct RT-PCR and for the detection of inflammatory markers. The positive outcomes will be further studied, and new anti-inflammatory therapeutic products could be developed.

**Keywords:** Synergistic anti-inflammatory effect, chlorella extract - TRF

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