

Comparative Analysis of Anti-Inflammatory Capabilities of Combined Astaxanthin and Tocotrienols in CALU-3 and RAW264.7

Athirah Mohd Jamil^a, Khairul Adzfa Radzun^{ab*}

Structured Abstract

Background: Chronic inflammation is a major contributor to numerous diseases, such as cancer, neurological disorders and cardiovascular diseases. It has been proved that astaxanthin and tocotrienols are two naturally occurring compounds with anti-inflammatory and antioxidant properties that inhibit inflammatory responses and reduce oxidative stress. If there is a reduced in inflammation in cells, it indicates that the combination of both inflammatory agents effectively reduces inflammation. This study used CALU-3 and RAW264.7 cells were used to evaluate the anti-inflammatory effects of astaxanthin and tocotrienols. This study aims to investigate the effects of combined astaxanthin and tocotrienols on the inflammatory responses of CALU-3 human lung cells and RAW264.7 murine macrophage cells.

Methods: Both CALU-3 and RAW264.7 cells were retrieved from ATCC. Following the preparation of combined treatment, astaxanthin (which was extracted from *Haematococcus pluvialis* obtained from the University of Texas) and Tocotrienol-Rich Fraction (which extracted from palm fruit oil obtained from Sime Darby) were mixed in ethyl alcohol using analytical balance. The prepared solutions were kept at 20°C before used. To evaluate inflammatory markers, MTT assay was used to assess the cell viability, while Griess assay was used to quantify nitric oxide levels in supernatant in RAW264.7 and CALU-3 cells. The data were presented as mean SD, and statistical significance was assessed using ANOVA.

Results: The findings indicate that the combination of astaxanthin and tocotrienols effectively reduced inflammatory markers, including NO production. Astaxanthin and tocotrienols, at a concentration of 1-25 µg/mL, were found to be non-toxic to the cells, indicated by high cell viability. At the same time, the significant reduction in NO production demonstrated its effectiveness in reducing inflammation. Meanwhile, the concentrations used for the combination treatment were based on a specific ratio of astaxanthin, tocotrienols and soybean. The combination treatment demonstrated a stronger inhibitory effect in NO production than individual treatments.

Conclusion: In conclusion, the findings indicate that the combination treatment of astaxanthin and tocotrienols significantly enhances the inflammatory responses in stimulated CALU-3 and RAW264.7 cells. The results suggest that these compounds work synergistically to reduce inflammation.

Keywords: Anti-inflammatory, astaxanthin, tocotrienols, CALU-3, RAW264.7

*Correspondence: khairuladzfa@uitm.edu.my

^a School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia

^b Industrial Waste Conversion Technology Research Group, Universiti Teknologi MARA, Shah Alam, Malaysia