

# Evaluation of Carotenoid Extract from *Dunaliella salina* against Cadmium-Induced Cytotoxicity and Transforming Growth Factor $\beta 1$ Induced Expression of Smooth Muscle $\alpha$ -Actin with Rat Liver Cell Lines

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## ABSTRACT

The carotenoid extract from *Dunaliella salina* was identified and quantitated. The effects against cadmium (Cd)-induced hepatotoxicity and transforming growth factor  $\beta 1$  (TGF- $\beta 1$ )-induced activation of hepatic stellate cells (HSCs) were evaluated. All-*trans*- $\beta$ -carotene (474.82 mg/g extract) and 9- or 9'-*cis*- $\beta$ -carotene (425.64 mg/g extract) were major carotenoids in the extract, accounting for 90.30% of total carotenoids (997.17 mg/g extract). Other carotenoids were all-*trans*-lutein (22.77 mg/g extract), all-*trans*-zeaxanthin (39.26 mg/g extract), 13- or 13'-*cis*- $\beta$ -carotene (17.18 mg/g extract), all-*trans*- $\alpha$ -carotene (9.26 mg/g extract) and 9- or 9'-*cis*- $\alpha$ -carotene (8.24 mg/g extract). The algal extract had a higher effect ( $p < 0.05$ ) than all-*trans*- $\beta$ -carotene in the reduction of cytotoxicity for CdCl<sub>2</sub>-treated Clone 9 cells and expression of smooth muscle  $\alpha$ -actin ( $\alpha$ SMA) for TGF- $\beta 1$ -treated HSC-T6 cells. These effects were shown to be concentration-dependent.

Key words: cadmium, carotenoid, cytotoxicity, *Dunaliella salina*, HSC-T6 cells, Clone 9 cells, HPLC analysis, smooth muscle  $\alpha$ -actin,  $\alpha$ SMA, transforming growth factor  $\beta 1$ , TGF- $\beta 1$

## INTRODUCTION

*Dunaliella salina* is unicellular and belongs to the class *Chlorophyceae* and the order *Volvocales*<sup>(1,2)</sup>. It is a type of halophile micro-algae that lacks a cell wall but has a mucus surface coat<sup>(2-5)</sup>. Algae gained commercial interest because it contains large amounts of  $\beta$ -carotene and is widely used in cosmetics and dietary supplements<sup>(6,7)</sup>. Carotenoids can reduce lipid peroxidation and singlet oxygen (<sup>1</sup>O<sub>2</sub>) formation<sup>(8,9)</sup>. Many reports indicate that carotenoids could lower the incidence of certain types of cancers, coronary heart disease and other degenerative diseases<sup>(10-15)</sup>. Raja *et al.*<sup>(2)</sup> found that *Dunaliella salina* could protect rats against fibrosarcoma. However, no thorough report has been conducted on the effect of

algal carotenoid extract against cadmium (Cd)-induced cytotoxicity and transforming growth factor  $\beta 1$  (TGF- $\beta 1$ ) induced activation of hepatic stellate cells (HSCs).

Cd is a toxic heavy metal, as well as an important industrial pollutant and food contaminant. It influences cellular functions such as enzyme activities, DNA repair systems, redox state of the cell and signal transduction. Cd also increases the risk of lung, prostate, kidney, liver, pancreatic and stomach cancer<sup>(16,17)</sup>. It induces both apoptosis and necrosis of the normal cells<sup>(18)</sup>. Shaikh *et al.*<sup>(19)</sup> reported that oxidative stress plays a major role in chronic Cd-induced hepatotoxicity, and antioxidants could protect animals against Cd-induced hepatotoxicity.

HSCs have important functions in the liver, such as the production of cytokines and growth factors, remodeling of extracellular matrix (ECM), and retinoid storage<sup>(20)</sup>. Nevertheless, after the liver has sustained

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