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Protective effects of *Dunaliella salina* – a carotenoids-rich alga – against ultraviolet B-induced corneal oxidative damage in mice

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Purpose: Ultraviolet B (UVB) radiation from sunlight is known to be a risk factor for human corneal damage. The purpose of this study was to investigate the protective effects of *Dunaliella salina* (*D. salina*) on UVB radiation-induced corneal oxidative damage in male imprinting control region (ICR) mice.
Methods: Corneal oxidative damage was induced by exposure to UVB radiation at 560 μW/cm^2. Animals were orally administered (gavage) *D. salina* at doses of 0, 123, and 615 mg/kg bodyweight/day for eight days. Corneal surface damages were graded according to smoothness and the extent of lissamine green staining. Corneal glutathione (GSH) and malondialdehyde (MDA) levels, as well as the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-Rd) in cornea were measured to monitor corneal injury.
Results: UVB irradiation caused significant damage to the corneas, including apparent corneal ulcer and severe epithelial exfoliation, leading to decrease in the activities of SOD, catalase, GSH-Px, GSH-Rd, and GSH content in cornea, whereas there was increased corneal MDA content as compared with the control group. Treatment with *D. salina* could significantly ($p<0.05$) ameliorate corneal damage and increase the activities of SOD, catalase, GSH-Px, GSH-Rd, and GSH content, and decrease the MDA content in corneas when compared with the UVB-treated group.
Conclusions: The studies demonstrate that *D. salina* exhibits potent protective effects on UVB radiation-induced corneal oxidative damage in mice, likely due to both the increase of antioxidant enzyme activity and the inhibition of lipid peroxidation.

Ultraviolet (UV) irradiation is the most common cause of radiation injury to the eye. The cornea has the physiologic capacity to absorb the majority of UVB radiation, and protects the inner eye against UVB-induced oxidative damaging effects. A recent study has suggested that the cornea absorbs 92% of UVB and 60% of UVA radiation and is most sensitive to UVB damage [1]. The corneal effects of excessive exposure to UVB radiation may include photokeratitis, damage to the epithelium, edema, and several biochemical changes, including DNA modification, protein cross-linking, enzyme inactivation, and the production of excessive reactive oxygen species (ROS) [2-4]. Previous reports suggested that natural antioxidants can effectively prevent and cure UVB-induced cell damage in cornea [5]. Antioxidants appear to act against oxidative stress by raising the levels of endogenous defense (e.g., by upregulating gene expressions of the antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase [6].

Dunaliella salina (*D. salina*) is a unicellular biflagellate green alga of the Chlorophyceae class. The algal cells are surrounded by a thin elastic membrane and can yield three major valuable products: glycerol, β-carotene, and proteins [7]. Due to the abundance of β-carotene, which is an antioxidant as well as a vitamin A precursor, *D. salina* has been used as a food coloring agent, a pro-vitamin A food supplement, an additive to food and cosmetics, and a health food product [8-12]. Recently, our group demonstrated that the major carotenoids in *D. salina* include all-*trans*-β-carotene and 9- or 9'-*cis*-β-carotene. Specifically, the 9-cis isomer has demonstrated a higher antioxidant activity due to the higher reactivity of the cis bond compared to trans. By trolox equivalent antioxidant capacity assay, reducing power, and 2, 2-diphenyl-2-picrylhydrazyl hydrate radical scavenging assay, we also found that our carotenoid-rich algal extract had significantly higher antioxidant activity than pure all-trans-β-carotene, α-carotene, lutein, and zeaxanthin [13]. Both lutein and zeaxanthin are major constituents of the retinal macular region of humans [14]. Increased dietary intake of lutein and zeaxanthin was found to result in increased plasma levels, which were positively associated with a reduced risk for age-related macular degeneration [15]. Additionally, the major precursor of vitamin A is β-carotene, which quenches excited sensitizer molecules and singlet oxygen. Documented evidence has been reported that vitamin A deficiency is known to cause a high degree of damage in ocular surfaces [16].

Based on the excellent antioxidant activities of *D. salina* found in vitro, it was of interest to us to evaluate its

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