Evaluation of the Anti-Senescence Effects of E3 Topical Cream (ExoBlue™) on Human Dermal Fibroblasts: Protein Markers (Part 2)

SUMMARY: E3 topical cream (ExoBlue) was evaluated in an oxidative stress-induced senescence model using human dermal fibroblasts, with protein expression of key senescence markers analyzed by western blot to assess cell cycle regulation, nuclear integrity and DNA repair. The results of this study demonstrate that the E3 topical cream (PCCA Formula #15843) successfully modulated critical mechanisms associated with cellular aging.

Introduction:

PCCA ExoBlue is a next-generation dermatological base design to support skin function through a multi-mechanistic approach. It integrates advanced peptides, exosomes and essential metal cofactors, designed to influence key pathways involved in cellular signalling, structural integrity and moisture balance, contributing to improved skin resilience and overall performance. Given these properties, a topical formulation including estriol (E3) 0.3% in ExoBlue (PCCA Formula #15843) was selected to assess its potential to modulate cellular senescence and support skin cell recovery. Cellular senescence is a key contributor to skin aging, characterized by irreversible cell cycle arrest, altered protein expression and decreased regeneration capacity.

Methodology:

The ExoBlue topical formulation was evaluated in an oxidative stress-induced senescence model using human dermal fibroblast BJ cells (ATCC, CRL-2522). Cellular senescence was induced by exposure to hydrogen peroxide (H_2O_2), a well-established oxidative stressor. Following induction (0.2 mM H_2O_2 for 2 hours), cells were allowed to recover overnight and then treated for 5 days with E3 0.3% in ExoBlue. Post-treatment, cells were washed with ice-cold PBS and lysed with cell lysis buffer. Protein concentrations were determined using a BCA (bicinchoninic acid) Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL). Western blot analysis was performed by resolving proteins in SDS-PAGE gels, transferring them onto nitrocellulose membranes (Li-Cor Biotechnology, Lincoln, NE), and probing with primary antibodies. Signals were detected using IRDye secondary antibodies; images and band densities were analyzed using the Odyssey DLx imaging system (Li-Cor Biotechnology, Lincoln, NE).

Results and Discussion:

Protein expression of key senescence markers was analyzed by western blot to assess molecular pathways involved in cell cycle regulation, nuclear integrity and DNA repair. Phosphorylated p53 (p-p53) and p21 were evaluated as indicators of cell growth arrest, while Lamin B1 served as a marker of nuclear structure, and Poly ADP-Ribose Polymerase-1 (PARP-1) as a key regulator of DNA repair. Collectively, these markers provide insight into the cellular mechanisms underlying senescence and the impact of treatment on restoring normal cellular function. β-Actin served as loading control to validate the reliability and accuracy of the western blot results.

Human dermal fibroblasts exposed to H_2O_2 were the positive control for senescence induction, whereas the untreated cells were the negative control (Figure 1). The western blot analysis demonstrated that oxidative stress markedly altered the expression of key protein markers. Specifically, H_2O_2-exposure significantly increased the expression of p21 and p-p53, central mediators of stress-induced cell cycle arrest and an early driver of cellular senescence. Concurrently, a substantial reduction in Lamin B1 was observed, indicating disruption of nuclear architecture and chromatin organization, along with a marked decrease in PARP-1 expression, reflecting impaired DNA repair capacity and genomic instability.

Treatment with E3 0.3% in ExoBlue partially reversed these molecular alterations, as evidenced by downregulation of p21 and p-p53, suggesting attenuation of stress-induced cell cycle arrest signaling. In parallel, Lamin B1 expression was restored, indicating preservation of nuclear structure and chromatin integrity, while PARP-1 levels were increased, supporting improved DNA repair activity and maintenance of genomic stability.

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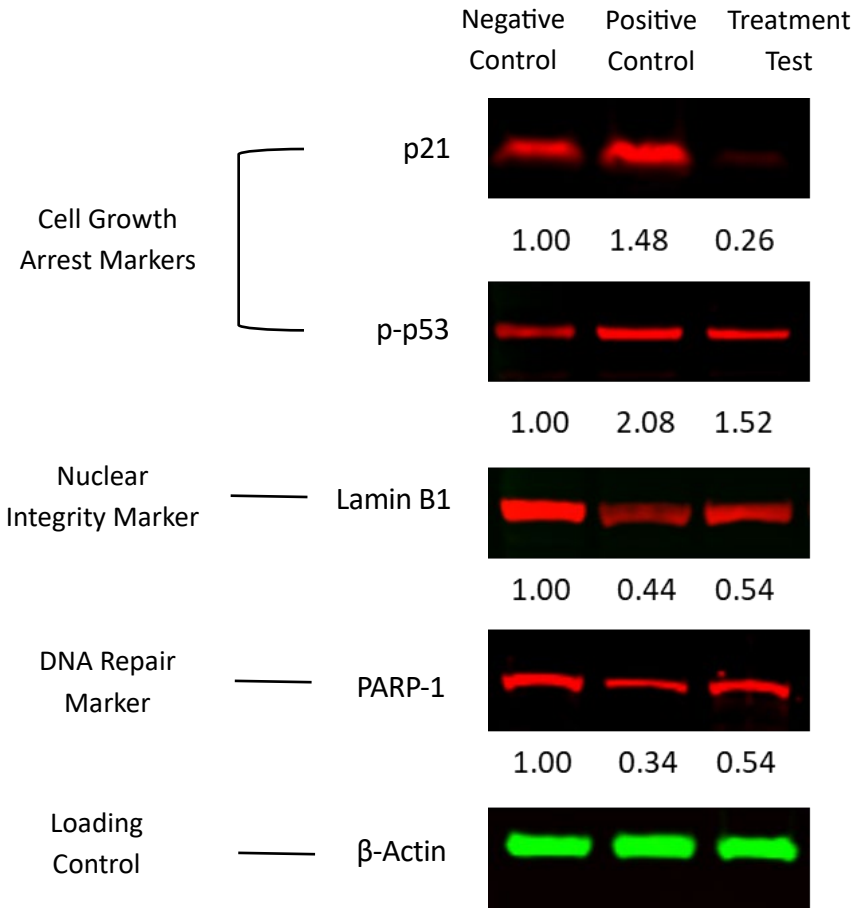

Figure 1. Western blot analysis of senescence-associated protein markers and β-actin (loading control) in human dermal fibroblasts under oxidative stress (H_2O_2) and treatment conditions (E3 in ExoBlue).

In this study, the human dermal fibroblast cells treated with E3 0.3% in ExoBlue (PCCA Formula #15843) supported the restoration of normal cellular function by reducing stress-induced growth arrest while preserving nuclear organization and enhancing the cell's ability to recover from damage. These effects suggest a coordinated modulation of pathways involved in cellular resilience and homeostasis under stress conditions. Collectively, these findings highlight the ability of the ExoBlue formulation to protect against senescence and promote recovery of normal cellular function.