**ORIGINAL CONTRIBUTION**

# A comprehensive topical antioxidant inhibits oxidative stress induced by blue light exposure and cigarette smoke in human skin tissue

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## Abstract

**Objective:** Skin damage from visible light predominantly results from exposure to the blue light spectrum (400-500 nm) which generates Reactive Oxygen Species (ROS) causing a cascade of harmful effects to skin. Topical antioxidants reduce the effects of free radical damage caused by environmental exposures. This study evaluated a comprehensive topical antioxidant's ability to inhibit ROS production induced by blue light and cigarette smoke (CS) in human skin.

**Methods:** Two experiments were conducted utilizing human skin (Fitzpatrick Skin Types III and V; N = 3, each). After confirmed reactivity of untreated tissues at 412 nm, 20J/cm$^2$, untreated and pretreated (WEL-DS, 2 mg/cm$^2$) skin tissue was exposed to blue light and blue light plus CS and left overnight. A nonfluorescent probe (DCFH-DA) was added to skin and exposed to blue light (412 nm, 20J/cm$^2$) and blue light plus CS. Fluorescent 2',7'-DCF was generated upon enzymatic reduction and subsequent oxidation by ROS.

**Results:** ROS increased at least tenfold following initial exposure to blue light and blue light plus CS in untreated skin. Pretreatment with WEL-DS decreased ROS in FST III exposed to blue light by 51% and 46% in skin exposed to blue light plus CS vs. untreated skin (both, $P < .001$). In FST V, pretreatment with WEL-DS decreased ROS exposed to blue light by 54% ($P < .001$) and 50% in skin exposed to blue light plus CS vs. untreated skin ($P < .0001$).

**Conclusion:** WEL-DS demonstrated significant reduction in ROS induced by blue light and blue light in combination with CS compared with untreated, exposed skin.

**KEYWORDS**
antioxidants, environmental stresses, formulation/stability, high-energy visible light, skin barrier, skin physiology/structure

# 1 | INTRODUCTION

The deleterious effects of ultraviolet radiation on skin are well known. Recently, investigators have begun elucidating the damaging consequences of exposure to Infrared Radiation (IR) and High Energy Visible Light (HEVL), as well as to environmental stressors such as pollution, ozone, and cigarette smoke (CS).[1-7] Greater than half of the solar energy reaching skin is from the IR range (700-14,000 nm), and 7% is ultraviolet light (UVB, 5-315 nm; UVA, 315-400 nm; Figure 1). The remaining 39% is from HEVL (400-760 nm), which includes blue-violet light (400-490 nm).[8-9] Different solar wavelengths induce varying photobiological effects.[1,10] Skin is a major target of oxidative stress with recent data reporting that HEVL, the only portion of the solar spectrum visible to the human eye, causes some of the same physiological effects in skin as UV light including skin barrier disruption, inflammation and premature skin aging.[11] The majority of skin damage associated with HEVL occurs as a result of exposure to the blue light spectrum, which represents the shortest and highest HEVL wavelengths capable of penetrating deep into skin.[1,12, 13] Within skin cells, exposure to blue light induces the enzymes matrix metalloproteinases (MMPs) that have been shown to degrade collagen and prevent future collagen formation.[12] While the biological effects have not been fully elucidated, widespread use of light-emitting diode (LED) devices, including the use of smart phones, tablets and computers, have contributed to cumulative exposure and growing concerns regarding long-term consequences to the health of eyes and skin.[12,14-16] Emerging data suggest exposure to artificial visible light substantially induces mitochondrial damage and other cellular changes in human dermal fibroblasts.[16] This may be of even greater concern owing to the substantial increased use of LED devices during the current COVID-19 pandemic. Recent studies have suggested that even short exposure times to light emitted from electronic devices increases the production of Reactive Oxygen Species (ROS).[17]

In a study conducted utilizing human epidermal skin equivalents, exposure to visible light (400-700 nm) induced production of ROS, proinflammatory cytokines (IL-6, IL-1, TNF-α), and MMP-1 expression in a dose-dependent manner (65, 130 and 180 J/cm$^2$).[11] Additionally, previous studies have shown that blue light generates the same amount of ROS in the skin as does UVA plus UVB.[10,18] The resulting generation of ROS from blue light exposure, which is likely superoxide and not singlet oxygen, triggers a cascade of harmful effects in skin inducing oxidative stress preferentially in mitochondria.[19,20] Within the blue light spectrum, wavelengths between 412 nm to 415 nm have been shown to induce significant and long-lasting hyperpigmentation, degrade carotenoids, and activate inflammation and apoptosis.[1,19,20] A study conducted on the backs of twenty healthy individuals exposed to 400-700nm (40J/cm$^2$) of visible light demonstrated that pigmentation induced by blue light compared with UVA was darker and more sustained in volunteers with Fitzpatrick Skin Types (FST) IV-VI compared with FST II.[19] Histopathology in volunteers exposed to blue light versus nonirradiated controls showed a redistribution of melanin pigments from the basal cells to the upper layers of the epidermis.[21] Doses used in reported studies have varied in range from 5J/cm$^2$ to greater than 240J/cm$^2$.[11,21] One study used doses of 40-240 J/cm$^2$ to approximate exposure for 15-90 minutes during midsummer sunlight in Houston TX.[11]

In addition to blue light exposure, there is also growing evidence demonstrating the harmful effects of environmental influences on skin, such as air pollution (ozone and particulate matter) and CS. Coupled with exposure to solar radiation, these environmental insults result in cumulative damage to skin.[3,22] Highly lipophilic compounds contained in CS readily penetrate skin cells, directly altering the structural and functional integrity of the skin barrier, as well as indirectly affecting skin through the induction of proinflammatory mediators.[23] Cigarette smoke causes production of hydrogen peroxide in the skin, which in turn alters the uptake of cellular cholesterol.[24] As more than 25% of epidermal skin is composed of cholesterol, reductions in cholesterol uptake can lead to impairments in skin barrier function.[22] Similar to observations following exposure to ozone and particular matter, exposure to CS alters redox homeostasis and increases ROS production and oxidative stress in the skin.[25] Generation of ROS overwhelms the natural defense systems of the skin and induce DNA damage.[26] One consequence of continued exposure to these noxious pollutants is production of 4-hydroxy-2-nonenal (4-HNE), a marker of lipid oxidation which has been shown to accelerate skin aging.[25]

Growing evidence highlights the benefits of topical antioxidants to protect skin from damage associated with environmental stressors.[11,12,19,26-29] Sunscreen active ingredients afford protection against exposure to ultraviolet radiation, but do not defend
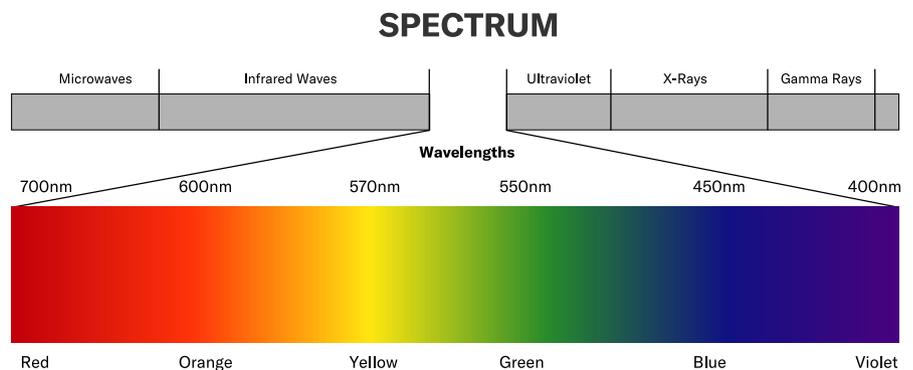
## SPECTRUM



**FIGURE 1** Solar Spectrum

against free radical damage resulting from visible light and environmental exposure.[3,30] Previous studies have demonstrated that when broad spectrum sunscreens have been exposed to blue light, there was no change in photon emission. In contrast, broad spectrum sunscreens containing antioxidants resulted in substantial reductions in free radicals generated and in the release of ROS, MMP-1 and proinflammatory cytokine mediators.[11] Exposure to antioxidants—topically or orally—can prevent blue light oxidation both before and after exposure.[19] Utilizing deliberate combinations and ratios of antioxidants ensures broad-based defense against a variety of stressors and promotes synergistic interaction to counteract free radical damage.[31] *Alto Defense Serum*™ (WEL-DS) is a comprehensive topical antioxidant that combines a balanced and optimized ratio of 19 water-soluble, enzymatic, and lipid-soluble antioxidants to inhibit oxidative stress at all cellular levels of the skin. The aim of this current study was to evaluate the ability of WEL-DS to inhibit ROS production induced by blue light exposure and CS in human skin explants obtained from both light and dark-skinned individuals.

## 2 | METHODS

Two independent experiments were conducted utilizing human skin explants obtained from two separate donors (N = 3, each) following facial plastic surgery (Fitzpatrick Skin Type [FST] III) and abdominoplasty (FST V) in which skin would have otherwise been discarded. Upon receipt of the fresh full skin, each section was separated into 3 disks with 8 mm skin disk punches and plated in 24 well plates containing sufficient volume of complete medium. The well plates were incubated overnight at 37° and 5% $CO_2$ to ensure skin tissue adaptation. The skin sections were re-examined the following day, and fresh medium was added; the sections were incubated a second night at 37° and 5% $CO_2$.

An initial baseline experiment was conducted to confirm reactivity and generation of ROS in untreated, nonexposed tissues (dark) versus tissues exposed to blue light and blue light plus the smoke from 3 cigarettes. Skin tissues were either untreated or pretreated with WEL-DS (2 mg/cm$^2$) and left overnight. A nonfluorescent probe (dichloro-dihydro-fluorescein diacetate; DCFH-DA) was added to the skin tissue for 3 hours at 37° and 5% $CO_2$. A transparent exposure chamber designed to accommodate a 24-well plate, and a cigarette connected to an air pump was used. CS was obtained by the combustion of cigarettes using the pump. A cigarette was connected to a pump which mimics the aspiration of a smoker and the smoke released in the chamber corresponds to exhaled smoke. One cigarette is lighted at the start of the exposure, and then one every 10 minutes for a total of 3 cigarettes per exposure of 30 minutes.

The blue light source was produced by a set of LED bulbs (412 + 5 nm) arranged in an aluminum casing. The blue light output was measured with a broadband thermopile detector (Sciencetech Broadband Thermopile Detector, Ontario Canada). The light source

was placed at the top of the exposure chamber to ensure that the skin tissue in the disks was exposed to CS in combination with blue light. Tissues were then exposed to blue light (412 nm, 20 J/cm$^2$) and blue light (412 nm, 20 J/cm$^2$) plus CS for 30 minutes. Following the exposure period, 400 µL of lysis solution was added to the explants. Fluorescent 2',7'-DCF was generated upon enzymatic reduction and subsequent oxidation by ROS. Fluorescence was measured to assess the ability of WEL-DS to scavenge or exacerbate ROS using a multimode microplate reader. The fluorescence intensity directly correlates to the test item's ability to scavenge ROS. The higher the percentage of ROS inhibition, the higher the antioxidant potential of the test item.

Data were analyzed using one-way ANOVA uncorrected Fisher's LSD test. Data presented in the graphs represent the mean ± Standard Error Mean (SEM). All results were considered significant for a $P < .05$.

## 3 | RESULTS

Prior to conducting the experiments, initial exposure to blue light (412 nm, 20 J/cm$^2$) and blue light (412 nm, 20 J/cm$^2$) plus CS in untreated skin tissue confirmed that the level of ROS increased by at least 10 times (Figure 2). Pretreatment with WEL-DS decreased the level of ROS in FST III exposed to blue light alone by 51% ($P <.0001$) vs. untreated skin, and by 46% exposed to blue light plus CS ($P < .001$) vs. untreated skin (Figure 3). In FST V, pretreatment with WEL-DS decreased the level of ROS in skin tissue exposed to
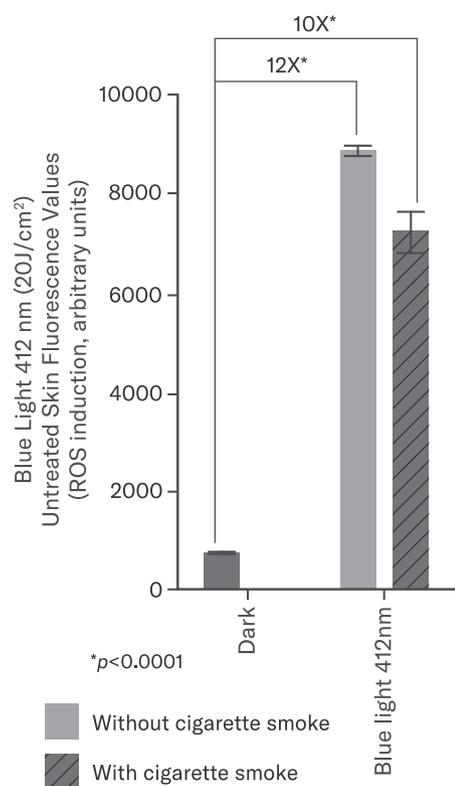


**FIGURE 2** Untreated Skin Fluorescence Values

**FIGURE 3** WEL-DS Inhibited ROS in FST III Exposed to Blue Light Alone and Blue Light Plus Cigarette Smoke
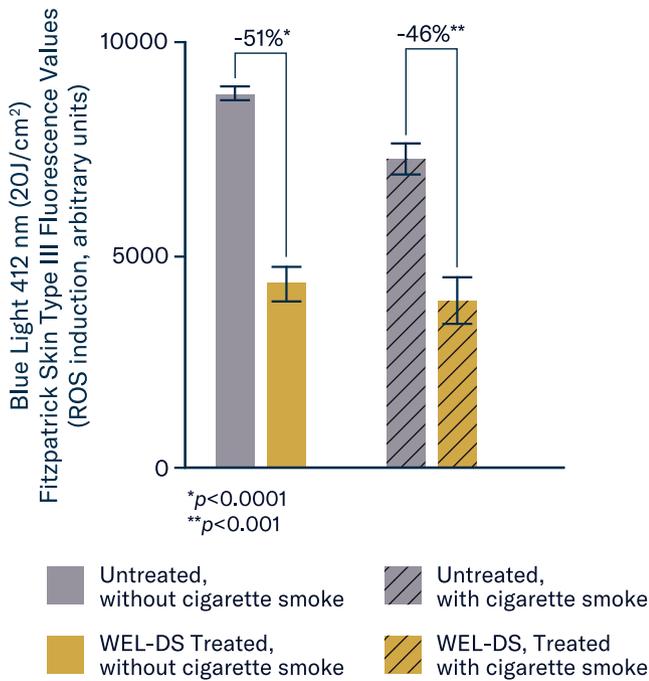


**FIGURE 4** WEL-DS Inhibited ROS in FST V Exposed to Blue Light Alone and Blue Light Plus Cigarette Smoke

blue light alone by 54% ($P < .001$) vs untreated skin, and by 50% in skin tissue exposed to blue light plus CS ($P < .0001$) vs untreated skin (Figure 4).

## 4 | DISCUSSION

Accumulation of ROS has long been a known contributor to the aging process, particularly in the skin.[12,19,32] The ROS load in skin is greater than that in any other human organ and has been shown to clearly correlate with aging consequences. Intrinsic aging, which is influenced by genetic factors and other physical alterations owing to the aging process, causes the epidermis and dermis to thin and impairs the skin's natural barrier function against environmental damage. To manage the generation of ROS, the skin utilizes its many anti-oxidative defense mechanisms, such as the enzymes superoxide dismutase and catalase, and organic compounds such as L-ascorbate, beta-carotene, and glutathione.[32]

Photoprotection has traditionally focused on the prevention of acute or chronic damage owing to UV radiation exposure through the application of sunscreens. Increasingly, evidence emphasizes the importance of additional skin protection from HEVL and IR exposure with the application of topical antioxidants.[29,33,34] Sunscreens and topical antioxidants differ mechanistically. Sunscreens scatter, absorb, or block UV prior to the formation of free radicals in the skin, whereas topical antioxidants penetrate the skin to stabilize or neutralize free radicals, thereby inhibiting their ability to effect cellular damage.[3,35-39] Topical antioxidants counteract free radical damage caused by environmental insults not neutralized by sunscreen
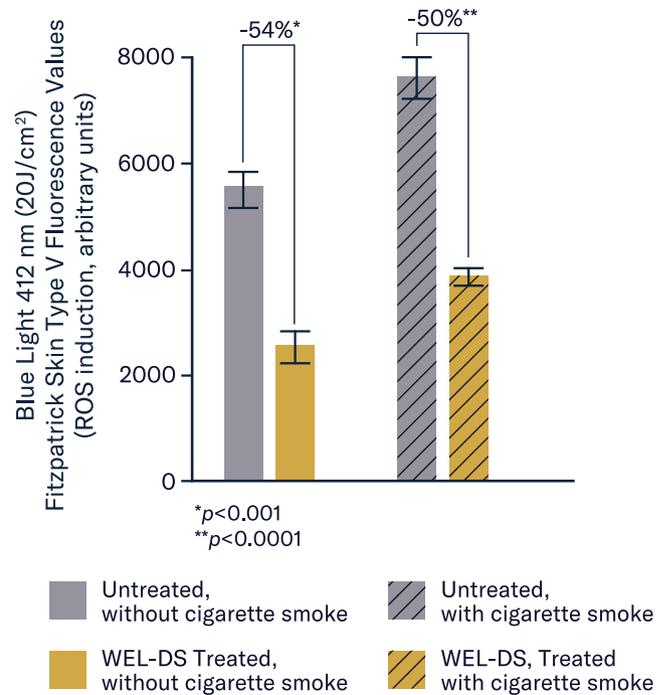
actives[40] and are complementary to broad spectrum sunscreens providing the skin with comprehensive protection against blue light, IR, and environmental stressors such as ozone, particulate matter, and CS.[12,19,36,38-40]

Prior studies have demonstrated the ability of WEL-DS to inhibit the effects of UVA/UVB and ozone-induced damage.[5,35] A recent study conducted utilizing a reconstructed human epidermal skin model examined the effects of pretreatment with WEL-DS compared with untreated skin tissue exposed to ozone ($O_3$).[5] In $O_3$-exposed groups, WEL-DS significantly inhibited ROS formation versus untreated skin tissues. Pretreatment with WEL-DS significantly inhibited $H_2O_2$ production and decreased NF-κB p65 transcription factor signal as compared with untreated, exposed skin tissues. Oxidative stress induction in $O_3$-exposed skin tissues was confirmed by increased levels of 4-HNE protein adducts (marker of lipid peroxidation); WEL-DS application reduced this effect. Additionally, pretreatment with WEL-DS inhibited structural damage in epidermal tissues exposed to $O_3$.

In the current study, pretreatment with WEL-DS inhibited ROS production in FST III and FST V from blue light exposure (412 nm, 20J/cm$^2$) with and without exposure to CS, compared with untreated, exposed skin tissue. The benefits were significant in both light and dark skin, despite the propensity for greater damage to dark skin.[12] As has been demonstrated in other studies, measurement of ROS can be effectively used to evaluate the effect of blue light on the integrity of extracellular matrix.[19] Prior methodologies reported in the literature measuring the effects of visible and blue light exposure on skin vary considerably with regard to models, doses and energy levels utilized. We believe that 412 nm, 20J/

cm$^2$ represents realistic dose ranges obtained from daily sun exposure, and conducting the experiments using viable skin grafts from both light- and dark-skinned donors allowed us to identify potential differences. The study may have been limited by the use of one donor each with FST III and FST V skin types, and by photo-exposed facial skin versus relatively photo-protected abdominal skin. Nevertheless, this study demonstrated the benefits of a comprehensive topical antioxidant's ability to inhibit ROS production in both FST III and FST V caused by exposure to blue light and blue light in combination with CS.

## 5 | CONCLUSION

Utilizing human skin tissue obtained from FST III and FST V donors, WEL-DS significantly reduced levels of ROS induced by blue light exposure alone and in combination with exposure to cigarette smoke compared with untreated, exposed skin.

### DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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