Natural molecules limit effect of daily blue light

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ABSTRACT

Artificial light is increasingly used in our modern daily life, composing a new kind of pollution called light pollution, considered an important health issue. Artificial light sources are more concentrated in high energy blue light than natural sunlight. Despite beneficial properties reported in the literature, blue light has been shown to also exert deleterious effects on skin, leading to skin premature ageing, this negative effect depending on doses and/or conditions of exposure. In this study, we evaluated the protective capacity of a phenylpropanoid-rich *Buddleja officinalis* flower extract in reducing the deleterious effects of blue light on skin. By using *in vitro* and *ex vivo* models, this concentrated extract was proved to significantly limit the effect of blue light exposure: oxidative stress, DNA damages, extracellular matrix degradation, and hyperpigmentation by modulating Opsin 3 in melanocytes. Our natural active ingredient seems to prevent hyperpigmentation induced by blue light that could be of great interest for several hyperpigmentary disorders such as melasma.

Natural light is essential for the entrainment of circadian clocks. However, emerging evidence suggests that the increasing exposure to artificial light is a risk factor for sleep and circadian disorders.[1]

With the prevalent use of LED lighting and device displays, humans are subjected to an increasing amount of light in the blue spectrum since commonly used LEDs emit a high fraction of blue light, often peaking at 460 nm. More precisely, blue light is a high energy visible (HEV) light with a wavelength ranging from 400 nm to 495 nm that could significantly influence biological systems and act on our health.[2]

In fact, several publications reported blue light exposure as being beneficial.[3] For instance, it seems to be able to improve *in vivo* wound healing by affecting keratin gene expression.[4] On the other hand, authors have also shown that exposure to blue light can disrupt the circadian rhythm as well as induce hyperpigmentation and oxidative stress in skin.[5,6] Interestingly, Zastrow *et al.*, 2009 [7] noted that blue light may also contribute to skin ageing similarly to UVA. It penetrates into skin and reaches the dermis and hypodermis, where it seems to exert cytotoxic effects, inducing several biological consequences within human skin cells.[8] Production of proinflammatory cytokines (IL-1, IL-6, IL-8...) and increased expression of extracellular matrix-degrading proteins MMP-1 and MMP-9 in human skin were also reported.[9] All these changes could contribute to a gradual loss of skin elasticity and an increase in wrinkles.

The photoreceptors associated with blue light are called opsins (OPN). They were primarily found in retina cells where they transduced the light signal. OPN1 short wavelength, OPN2 (rhodopsin) and non-visual OPN3 (panopsin or encephalopsin) are expressed in human skin, in melanocytes and keratinocytes. Opposite increase in opsin expression and damage of opsin structure were described after blue light exposure,[10] OPN3 being considered as a sensor of pigmentation in melanocytes[11] and as a trigger of MMP-1 up-regulation.[12]

This combination of the seemingly positive and negative effects on skin arising from exposure to blue light raises a question as to how blue light can have such opposing impacts on skin? We can put forward the hypothesis that different doses or conditions of exposures explain these different effects on skin.[4]

While ultraviolet (UV) filters effectively reduce UV-induced Reactive Oxygen Species (ROS), they cannot prevent blue light-induced deleterious effects on skin. Assuming that natural molecules with antioxidant and anti-inflammatory properties can be found in plants, the aim of this study was to evaluate the effect of a phenylpropanoid-rich extract from *Buddleja officinalis* (BO) on skin exposed to blue light. Phenylpropanoids (also known as cinnamic acids) are secondary metabolites composed of thousands of different compounds.[13] Among them, verbascoside has been shown to possess several biological properties including photoprotection and antioxidant activity.[14]

Thus, using our experience in vegetal extraction, we obtained a *Buddleja officinalis* flower extract (BOFE; trade name: Soliberine®), highly concentrated in verbascoside (10-20%/dry matter) and echinacoside (1.5-3.5%/dry matter). Firstly, we examined the blue light protective capacity of BOFE in human keratinocytes and fibroblasts regarding the ROS production and lipofuscin formation. Secondly, we evaluated the effect of BOFE on skin explants exposed to blue light, regarding DNA damages, extracellular matrix degradation, and skin pigmentation by focusing on Opsin 3.

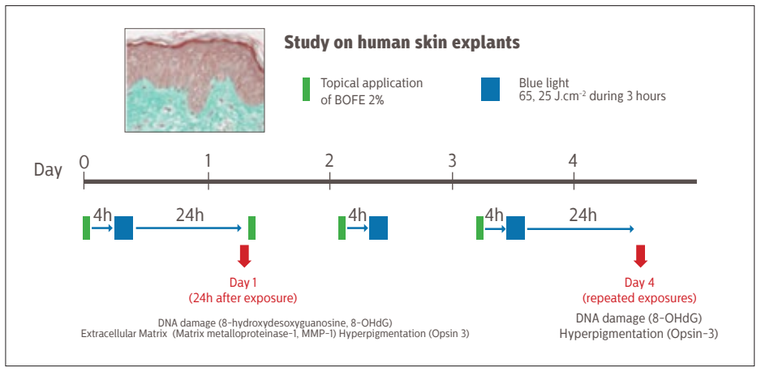

Figure 1: Study design for the *ex vivo* evaluation of the effects of BOFE on DNA damages, extracellular matrix and hyperpigmentation in skin explants exposed to blue light.

Material and methods

Effects of BOFE in counteracting oxidative stress induced by blue light in keratinocytes

Normal human epidermal keratinocytes (NHEK) were exposed to blue light to investigate the response of the cells to the induced oxidative stress and evaluate the potential of BOFE in counteracting ROS production. Four experimental replicates (treated with BOFE at 0.01%, 0.03% and 0.1%, or controls during 24h) were evaluated for ROS accumulation after exposure to blue light. After treatment, cells were exposed to blue light in the cell culture plate for 20 minutes. Light source was at 6 cm from cell surface resulting in an approximate dose of 7 mW.cm^{-2} that corresponds to 8.4 J.cm^{-2}, at a wavelength of 420 nm. Immediately after blue light exposure, the ROS detection buffer was added into the cell culture medium and incubated for 1 hour. The intracellular ROS accumulated reacted with a fluorogenic probe localised in the cytoplasm, resulting in a fluorometric product in amounts proportional to the amount of ROS present. Fluorescence quantification was measured at λex = 490 nm / λem = 525 nm. The normalisation used the difference between ROS levels at the 'Control + Blue light' and the 'Control' as a reference, to determine the efficacy of the treatment upon the blue light-induced oxidative stress.

Effects of BOFE in counteracting lipofuscin formation induced by blue light in fibroblasts

Normal human dermal fibroblasts (NHDF) were exposed to blue light to investigate the response of the cells to the induced senescence by lipofuscin quantification. Three experimental replicates (treated with 0.06% BOFE, or controls) were evaluated for lipofuscin accumulation after exposure to blue light. After 24h treatment, cells were exposed to 110 J.cm^{-2} (35 mW.cm^{-2}, 52.4 min), at a wavelength of 416 nm. A second 24h incubation was performed with BOFE or controls. The cells were then fixed with formalin and stained with a lipid-specific colorant: Sudan Black B. Staining was imaged with standard microscope (LEICA® DFC 280) to provide qualitative result.

Effects of BOFE on DNA damages, extracellular matrix and hyperpigmentation in skin explants exposed to blue light

Human skin explants were exposed to blue light to evaluate a protective effect of a topical application of BOFE 2%. Topical applications with BOFE at 2% (2 mg.cm^{-1}) were performed at day 0, 1, 2 and 3 (Fig 1). Then, 4 hours after each application of BOFE, skin explants were irradiated with blue light at 65.25 J.cm^{-2} for 3 hours using a Solarbox®.

Blue light-induced DNA damages were evaluated by the immunodetection and quantification of the 8-hydroxydesoxyguanosine (8-OHdG).

Extracellular matrix degradation induced by blue light was evaluated by the immunodetection and quantification of the matrix metalloproteinases-1 (MMP-1) in the epidermis.

The impact of blue light and a protective effect of BOFE on skin hyperpigmentation was assessed by immunodetection and quantification of Opsin 3 (OPN3) protein.

For all the evaluated markers, the covering area of the staining was determined by image analysis and expressed as percentage of the area of interest. The percentage obtained after blue light exposure, either treated or untreated with BOFE ("Untreated + Blue light" and "BOFE 2% + "Blue light" conditions), were compared to the condition without exposure ("Control" condition).

Results

Effects of BOFE in counteracting oxidative stress induced by blue light in keratinocytes

Blue light exposure for 20 minutes induced an increase in Reactive Oxygen Species (ROS) levels in human keratinocytes by 24%, compared to the "Control" condition (data not shown). When human keratinocytes were treated with BOFE and exposed to blue light, results showed that BOFE at 0.01%, 0.03% and 0.1%, significantly decreased ROS levels respectively by 37%, 42%, and 43%, compared to the "Control + Blue light" condition (Figure 2).

Effects of BOFE in counteracting lipofuscin formation induced by blue light in fibroblasts

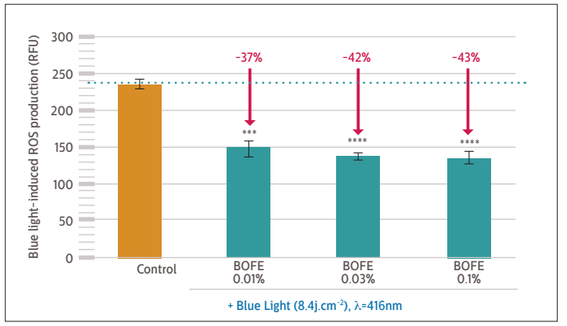

Figure 2: Protective effect of BOFE against blue light-induced ROS accumulation in keratinocytes. Mean ± SEM, statistical significance of "BOFE + Blue light" conditions vs "Control + Blue light" condition, t test: *** p <0.001, **** p<0.0001.

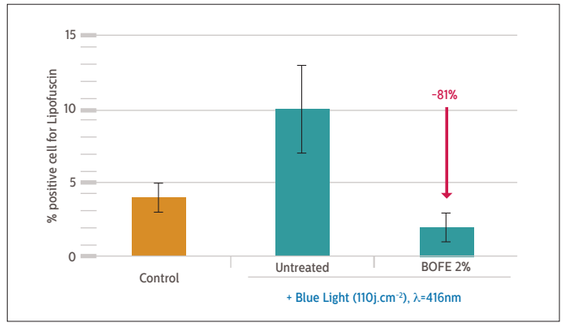

Figure 3: Protective effect of BOFE against blue light-induced lipofuscin accumulation in keratinocytes. Mean ± SEM, statistical significance of "BOFE + Blue light" condition vs "Control + Blue light" condition, Mann-Whitney test: * p < 0.05.

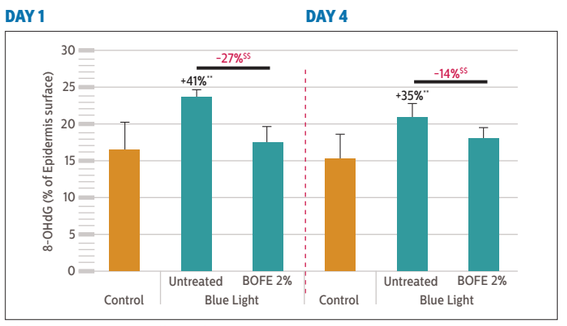

Figure 4: Protective effect of BOFE against blue light-induced DNA damages in skin explants. Mean ± SD, statistical significance of "Untreated + Blue light" condition vs non-exposed "Control" condition, t test : **p <0.01; and "BOFE 2% + Blue light" condition vs "Untreated + Blue light" condition, t test: $$p<0.01.

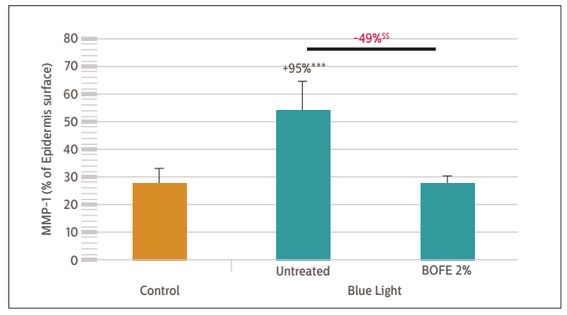

Figure 5: Protective effect of BOFE against blue light-induced MMP-1 in skin explants at day 1. Mean ± SD, statistical significance of "Untreated + Blue light" condition vs non-exposed "Control" condition, t test: ***p<0.001; and "BOFE 2% + Blue light" condition vs "Untreated + Blue light" condition, t test: $$p<0.01.

BOFE treatment was able to prevent lipofuscin formation in fibroblasts exposed to blue light (Fig 3) with a significant inhibition of 81% ($p<0.05$) at 0.06%.

Effects of BOFE on DNA damages, extracellular matrix and hyperpigmentation in skin explants exposed to blue light

At day 1 and day 4, 8-OHdG was significantly induced in the epidermis by blue light exposure (+41%: $p<0.01$, and +35%: $p<0.01$, respectively) (Fig 4). The treatment with BOFE at 2% protected skin explants against DNA damages induced either by a single blue light exposure or after 4 days of repeated exposures.

Blue light exposure induced a significant increase of MMP-1 in the epidermis by 95% ($p< 0.001$) (Fig 5). The treatment with BOFE at 2% significantly prevented MMP-1 induction by blue light (-49%: $p<0.01$ as compared to "Untreated + Blue light" condition).

Compared to "Untreated + Blue light" condition, either after a single or a repetitive blue light exposure, BOFE 2% significantly reduced OPN3 epidermal content (-29%: $p<0.05$, and -45%: $p<0.01$, respectively) (Fig 6).

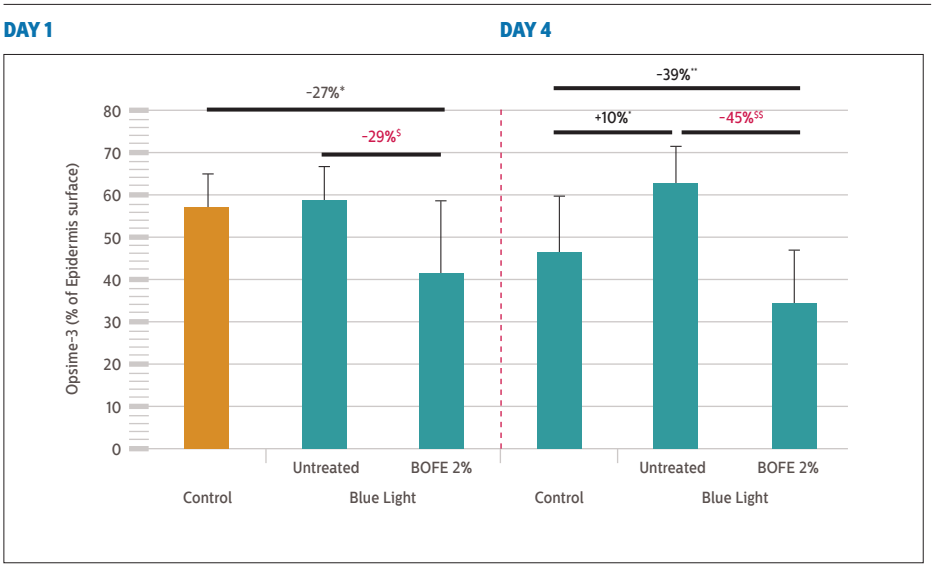

Figure 6: Protective effect of BOFE against blue light-induced Opsin-3 in skin explants at day 1 and at day 4.Mean ± SD, statistical significance of "Untreated + Blue light" condition or "BOFE 2% + Blue light" condition vs non-exposed "Control" condition, t test: *p <0.05; **p<0.01; and "BOFE 2% + Blue light" condition vs "Untreated + Blue light" condition, t test: $p<0.05, $$p<0.01

Discussion and conclusion

Blue light, as a part of the visible light spectrum, is commonly described within a range from 380 to 495 nm, including violet to green wavelengths, and these specific wavelengths are also reported to have opposite biological effects. Indeed, it is well known that skin exposure to blue light results in antimicrobial, antibacterial, and anti-inflammatory effects leading to the increasing development of photobiomodulation therapies.[15] However, blue light has been shown to increase ROS production, leading to mitochondrial DNA damage, resulting in a delay of skin barrier recovery. Plant-derived compounds featuring antioxidant and anti-inflammatory activities could be efficient solutions to counteract these deleterious effects on skin. Our results show that verbascoside and echinacoside included in a *Buddjela officinalis* flower extract (BOFE) significantly limited oxidative stress, DNA damages, extracellular matrix degradation induced by blue light exposure. Moreover, it also significantly decreased OPN3. This last result is of great interest. In fact, OPN3 is a G-protein coupled membrane receptor expressed in the human eyes but also in the brain, liver, kidneys, and skin. Regazzetti et al. (2018)[11] showed that OPN3 serves as the sensor for blue light in melanocytes. Indeed, OPN3 activates CREB, extracellular signal-regulated kinase (ERK), and p38, leading to the phosphorylation of MITF and, ultimately, to the increase of the melanogenesis enzymes tyrosinase and dopachrome tautomerase (DCT). Hence, targeting OPN3 activation could prevent blue light-induced hyperpigmentation that could be of great interest for several hyperpigmentary disorders such as melasma.

To summarise, blue light significantly increases oxidative stress in human skin, inducing a cascade of events that involves a variety of cell/molecular signalling pathways that ultimately leads to the production of MMPs that degrade collagen and elastin in the dermis, as well as the expression of opsin 3 that promotes melanogenesis. By adapting themselves to hostile environments, plants produce a wide variety of secondary metabolites with biological activities. Based on its ethnopharmacological and pharmacognostic knowledges, Greentech Research proposed the photoprotective effect of a phenylpropanoid-rich extract (particularly in verbascoside) from *Buddleja officinalis* (a shrub in the Buddlejaceae family), phenylpropanoids being known for their anti-inflammatory, antiviral and antibacterial properties. It appears that this active ingredient provides a natural protection against blue light-induced hyperpigmentation that could be of great interest for several hyperpigmentary disorders.

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