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ORIGINAL ARTICLE

The protective effect of a novel sunscreen against blue light

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Abstract

Background and objective: Premature skin ageing, and skin hyperpigmentation are influenced by exogenous factors, such as ultraviolet radiation and blue light. In this study, we assess the protective effect of a sunscreen (TDF® Blu Voile Sunscreen) in protecting the skin against the harmful effects of blue light irradiation in vivo and through the in situ quantitative and qualitative evaluation of protein carbonylation in human skin explants.

Methodology: The protective effect of the test product against blue light was first evaluated ex vivo on human skin explants. The treated and non-treated explants were exposed to 14 J/cm^2 of blue light 460 nm following which the protein carbonylation was evaluated by in situ epifluorescence imaging and separation by high-resolution gel electrophoresis. To determine whether the test product could also protect against the immediate and persistent pigmenting effect of blue light, two randomized in vivo studies were conducted, which included respectively 17 subjects with a skin phototype of IV and V (Fitzpatrick classification) and 22 subjects with a skin phototype of IV, V, and VI (Fitzpatrick classification). The duration of the study for each subject was 2 days (D1 and D2) for immediate observations and 5 days (D1–D5) for persistent observations. Specific zones on the subjects' back were either left non-treated or treated with the test product and were then exposed to a unique dose of blue light 415 nm. The onset of pigmentation between the treated and exposed zones was then assessed relative to the non-exposed treated zone through colorimetric measurements of the Individual Typology Angle (ITA°).

Results: Human skin explants treated with test product showed significantly lower levels of accumulated carbonylated proteins, with a protection of 82%, following exposure to blue light 460 nm. Findings of the in vivo studies also indicated that the test product presented significantly better protective efficacy against immediate and persistent pigmentation induced by blue light 415 nm.

Conclusion: Hence, it can be concluded that the test product can protect against the oxidative stress as well as the immediate and persistent pigmentation induced by blue light.

KEYWORDS
blue light, melasma, pigmentation, protein carbonylation, sunscreen

Résumé

Contexte et objectif: Le vieillissement prématuré de la peau et l'hyperpigmentation cutanée sont influencés par des facteurs exogènes, tels que les rayons ultraviolets et la lumière bleue. Dans cette étude, nous évaluons l'effet protecteur d'un écran solaire (TDF® Blu Voile Sunscreen) en matière de protection de la peau contre les effets nocifs de l'irradiation à la lumière bleue in vivo et par l'évaluation quantitative et qualitative in situ de la carbonylation des protéines dans des explants cutanés humains.

Méthodologie: L'effet protecteur du produit testé contre la lumière bleue a d'abord été évalué ex vivo sur des explants cutanés humains. Les explants traités et non traités ont été exposés à 14 J/cm^2 de lumière bleue à 460 nm, après quoi la carbonylation des protéines a été évaluée par imagerie par épifluorescence in situ et séparation par électrophorèse sur gel à haute résolution. Afin de déterminer si le produit testé pouvait également protéger contre la pigmentation immédiate et persistante dues à lumière bleue, deux études in vivo randomisées incluant respectivement 17 sujets ayant un phototype cutané IV et V (classification de Fitzpatrick) et 22 sujets ayant un phototype cutané IV, V et VI (classification de Fitzpatrick) ont été menées. La durée de l'étude pour chaque sujet était de 2 jours (J1 et J2) pour les observations immédiates et de 5 jours (J1 à J5) pour les observations persistantes. Des zones spécifiques du dos des sujets ont été laissées non traitées ou bien traitées avec le produit testé, et ont ensuite été exposées à une dose unique de lumière bleue à 415 nm. L'apparition de la pigmentation entre les zones traitées et exposées a ensuite été évaluée par rapport à la zone traitée non exposée par des mesures colorimétriques de l'angle typologique individuel (Individual Typology Angle, ITAo).

Résultats: Les explants cutanés humains traités avec le produit testé ont montré des taux significativement plus faibles de protéines carbonylées accumulées, avec une protection de 82 %, après une exposition à la lumière bleue à 460 nm. Les résultats des études in vivo ont également indiqué que le produit testé présentait une efficacité protectrice significativement meilleure contre la pigmentation immédiate et persistante induite par la lumière bleue à 415 nm.

Conclusion: Par conséquent, on peut conclure que le produit testé peut protéger contre le stress oxydatif ainsi que contre la pigmentation immédiate et persistante induite par la lumière bleue.

INTRODUCTION

The skin plays a primordial role in protecting against environmental stressors to which it is routinely subjected to, and this often leads to premature cutaneous ageing [1]. Indeed, the detrimental impact of sun irradiation on the skin has been well-documented. For instance, ultraviolet (UV) light exposure has been reported to result in 80% of premature skin ageing on the face resulting in significant extrinsic skin ageing or photoaging [2].

Until recent years, most photodermatological studies were primarily focused on the effects of UV light which are known to produce significant biological effects in the skin [3]. Since these niche studies focused on the cutaneous effect of UV, a vast majority of the commercially available sunscreen on the market contains UV-A and UV-B filters only.

However, it was recently discovered that as a major component of solar radiation, high-energy visible (HEV) light, also referred to as blue light with a wavelength of 400–500 nm was also an important contributor to photoaging [4, 5]. Mahmoud et al. conducted a study to assess the effect of visible light on immediate pigmentation and delayed tanning when compared with UV-A1 irradiation. Results reported that visible light can induce pigmentation in skin types IV to VI, and the pigmentation induced by visible light was darker and more sustained compared with UV-A1. It was further reported that the quality and quantity of pigment induced by visible light and UV-A1 were different. Pigmentation induced by UV-A1 was initially grey before turning brown whereas pigmentation induced by visible light was already dark brown from the start [6]. Other studies have also reported that blue light penetrates deeper into the skin to cause hyperpigmentation, such as melasma. Due to its higher penetrating power in the skin compared with UV radiation (UVR), it was also proven to cause a more acute onset of inflammation than the latter [7].

Similar to UV, blue light has been proven to affect the molecular structure of the skin by inducing oxidative stress, inflammation, and DNA damage. In particular, carbonylated proteins (CPs) generated through lipid peroxidation leading to DNA damage have been reported to be found in corneocytes and are frequent in sun-exposed skin [8]. CPs exposed to blue light generates more oxygen-free radicals ($\bullet O_2$-) in the skin which re-synthesized new CPs through lipid peroxidation, thereby creating a loop. A previous histological study of the sun-exposed skin showed an accumulation of CPs in the epidermis and the dermis [9]. A previous study by Yamawaki et al. investigated the role of CPs in the dermis of photoaged skin and how to counteract its synthesis. It was reported that CPs disrupted the formation of the dermal matrix and thus accelerated the process of skin ageing. CPs have also been found to upregulate matrix metalloproteinase-1 (MMP-1) in the dermal fibroblast leading to collagen degradation and thus skin photoaging [10]. Furthermore, as a result of sun exposure, disruption of the skin barrier occurs as well as changes in skin colour, decreased wound healing capabilities, and increased risk of skin cancer [11]. Studies on doses of blue light affecting pigmentation were reported to play a key role in worsening some skin disorders following sun exposure despite the use of sunscreen with UV-A and UV-B protection [12, 13].

While the skin can protect itself against stress to a certain level, persistent exposure to blue light surely impacts this protective ability. Thus, regular application of a sunscreen that acts as an efficient protective shield against solar irradiation is greatly desired. As such, a complete sun protection that acts as a barrier and can scavenge oxidative stress is key towards maintaining healthy skin. Here, our research is centred around evaluating the photoprotective ability of a blue light protection sunscreen against the oxidative stress and blue light-induced pigmentation on human skin.

MATERIALS AND METHODS

Test compounds

The test product (TDF® Blu Voile Sunscreen) is a SPF50/PA+++ sunscreen containing zinc oxide (14.25%), titanium dioxide (7%), polymethylsilesquioxane, and HDI/Trimethylol hexyllactone crosspolymer (1%) which act as a proprietary blue light blocking ingredient. The conventional broad-spectrum sunscreen contained avobenzone (3%), homosalate (10%), octisalate (5%), and octocrylene (10%).

Blue light doses

For the ex vivo and in vivo evaluation, the 415 nm and 460 nm blue light lamps contained 10 identical LEDs (Honglitronics, Guangzhou, PRC), respectively. This emitted a continuous visible radiation embedded in a reflector and covered by a transparent glass window. A single peak of wavelength 415 ± 5 nm and 460 ± 5 nm was observed for each lamp. The aperture of the light source was 4.5×4.5 cm and a thermopile detector (Gentec-EO Inc., Lake Oswega, OR, USA) was used to measure the precise intensity of the light source in watt/cm^2 on the appropriate zone. The distance between the blue light lamps was adjusted to 14 J/cm^2 for the 460 nm blue light lamp used in the ex vivo study and 60 J/cm^2 or 87.5 J/cm^2 for 415 nm blue light lamp used in the in vivo study.

Ex vivo protein carbonylation study

The ex vivo studies were conducted by OxiProteomics SAS (Creteil, France) using human skin explants.

Human skin explants preparation

Human skin explants were obtained, with research consent, from the residual skin of a 40-year-old female Caucasian donor of phototype III following abdominal surgery and trimmed to remove any subcutaneous fat. The explants were maintained under air-liquid interface culture conditions by culturing them on metal grids into standard 12-well plates in contact with skin culture media. Gibco™ 1X DMEM medium with high glucose content of 4.5 g/L and glutaMAX (Thermo Fisher, Waltham, MA, USA) supplemented with foetal bovine serum (10%) and penicillin–streptomycin (1%) was used as maintenance medium. The skin was maintained at 37°C in a 5% CO_2 atmosphere for sufficient adaptation time.

Following adaptation period, the human skin explants were distributed into four experimental groups (Table 1). The explants were topically treated with test product (TDF® Blu Voile Sunscreen; at 2 mg/cm^2) or with vitamin E analogue (Trolox; solution at 1% w/v) and incubated for 1 h before exposure to blue light (460 nm; 14 J/cm^2). The stress group received blue light exposure and the medium renewal only. The control group did not receive any treatment except for the medium renewal. After blue light exposure, the culture medium was renewed, and the explants incubated for 2 h at 37°C in 5% CO_2 humidified air. After 2 h of incubation, one half of the skin explants was included in OCT for cryopreservation and the other half was snap-frozen in liquid nitrogen. The samples were stored at −80°C until analysis.

In situ visualization and semi-quantification of carbonylated proteins

Explant sections of 4 µm thickness were obtained using a cryostat (Leica Biosystems, Wetzlar, Germany) and fixed with a solution containing 95% ethanol and 5% acetic acid. Carbonylated proteins were labelled using a specific fluorescent probe (λEx = 488 nm / λEm = 530 nm) and nuclear labelling using the reagent DAPI (4′,6-diamidino-2-phenylindole). Fluorescent images were collected with an epifluorescent microscope Evos M5000 (Thermo Fisher, Waltham, MA, USA) and analysed with ImageJ software [14]. Image comparisons of different conditions were achieved using identical conditions of acquisition (resolution, exposure, and 40× objective).

For each image, the semi-quantification of the carbonylation level was performed independently for the total skin and for the different anatomical compartments (stratum corneum, epidermis, dermis). The carbonylation rate was calculated with respect to the basal fluorescent signal intensity of the control and the specific fluorescent intensity of the blue light stress of each skin compartment. Three images per treatment condition were analysed to obtain a mean value and a standard deviation.

Extraction and absolute quantification of carbonylated proteins

Explants were subjected to protein extraction. The quantification of the total proteins was carried out using the Bradford method. The oxidized (carbonylated) proteins were labelled with a specific fluorescent probe and then separated by high-resolution electrophoresis (SDS-PAGE – gradient 4–20%). After their migration, the total proteins were in gel stained with the fluorescent SyproRuby™ reagent (Life Technologies, Carlsbad, CA, USA). The values of carbonylated proteins (Carbonyl Score) were obtained by densitometric analysis of the specific fluorescence signal, normalized to the content of total proteins.

Statistical analysis

Experiments were independently repeated in biological triplicate. Error bars in the graphical data represent standard estimation of the mean (SEM). A one-way ANOVA was used for the statistical analysis using the software GraphPad Prism Version 7 (GraphPad Software Inc., San Diego, CA, USA), and a statistical

TABLE 1 Experimental group and design

Group	Description	Treatment	Number of explants
CONTROL	Control	Non-treated and unexposed	3
STRESS	Blue light exposure	Blue light irradiation (460 nm; 14 J/cm^2)	3
TDF® Blu Voile Sunscreen	Test product + blue light exposure	Test product for 1 h at 2 mg/cm^2 + blue light irradiation	3
Vitamin E	Vitamin E + blue light exposure	Vitamin E for 1 h at 1% w/v + blue light irradiation	3

significance was claimed when the *p*-value was lower than 0.05 ($p < 0.05$).

In vivo clinical study with volunteers

These studies were conducted as an intra-individual comparative trial by the Centre International de Développement Pharmaceutique (CIDP) in Mauritius. Two independent clinical studies were conducted and for both studies, volunteers gave their informed consent to participate in the study, and the general principles of the Declaration of Helsinki guidelines were applied. The studies were approved by an independent ethics committee. Adverse effects, if any, were recorded.

For the two studies conducted, the protective effect of the test product was evaluated against either the immediate or the persistent pigmenting effect of blue light 415 nm. Prior to their enrollment in the studies, a dermatologist performed a clinical evaluation of the skin at back to ensure that there were no dermatological conditions that would interfere with the study results and objectives. Subjects with skin conditions, such as erythema, hyperpigmentation, skin rash, eczema and/or wound on the studied zones were excluded.

Evaluation against the immediate pigmenting effect of blue light

Seventeen healthy subjects aged 20–59 participated in the study. The volunteers were of mixed ethnicity and of skin phototype IV and V according to Fitzpatrick classification. The subjects came to the study site without applying any product on the investigational zones (back). The study design is described below in Table 2. Three test zones were identified on the back, a control zone that was left untreated, a second zone which was treated with the test product under investigation and a third zone tested with a conventional broad-spectrum sunscreen. Each test zone had an exposed part of 3 × 2 cm and a lateral non-exposed part of 3 × 2 cm. All subjects were acclimatized for 15 min in a room at 24 ± 2°C. Prior to treatment with the test product on D1, photography, and chromametric measurement on all the test zones were performed on the back. 2 mg/cm^2 of the test product was then applied on the treated zone and 30 min after product application, the treated exposed zone and the non-treated exposed zone were exposed to 60 J/cm^2 of blue light 415 nm. About 30 min after blue light exposure, the product was removed, photography and chromametric measurement of all the test zones were then performed on the back. Photography and chromametric measurement of all the test zones were repeated on the back 24 h after the exposure to blue light (D2).

Evaluation against the persistent pigmenting effect of blue light

Twenty-two healthy subjects aged 22–56 participated in the study. The volunteers were of mixed ethnicity and skin phototype IV, V, and VI according to Fitzpatrick classification. The subjects came to the study site without applying any product on the investigational zones (back). The study design is detailed below in Table 3. Two test zones were identified on the back, a control zone that was left untreated and a second zone which was treated with the test product under investigation. Each test zone had an exposed part of 3 × 2 cm and a lateral non-exposed part of 3 × 2 cm. All subjects were acclimatized for 15 min in a room at 24 ± 2°C. Prior to treatment with the test product on D1, chromametric measurement on all the test zones was performed on the back. 2 mg/cm^2 of the test product was then applied on the treated zone and 30 min after product application, the treated exposed zone and the non-treated exposed zone were exposed to 87.5 J/cm^2 of blue light 415 nm. After exposure, the subjects were advised not to wet their back till the next day just prior to their visit. For the next three subsequent days (D2–D4), the subjects came to the study site without applying any product on the investigational zones (back), acclimatized for 15 min in a room at 24 ± 2°C, the test zones were identified on the back and a clinical examination by an investigator was performed to assess any local intolerance. Chromametric measurement was performed on all the test zones. Test product was then applied and 30 min after product application, the treated exposed zone and the non-treated exposed zone were exposed to 87.5 J/cm^2 of blue light 415 nm. 24 h after the last exposure (D5), chromametric measurement of all the test zones was conducted.

Colorimetric measurement

Measurements using a Chromameter (Minolta CR400, Konica Minolta, Tokyo, Japan) were taken in the middle of the zones. The recorded L*a*b* values (CIELab colour system) were used to calculate the Individual Typological Angle (ITA°) (characterizing skin clarity and the pigmentation value) using the following formula:

$$\mathrm{ITA}^{\circ} = \left[\mathrm{Arc\ Tangent}\left(\left(\mathrm{L}^{*} - 50\right)/\mathrm{b}^{*}\right)\right] 180/\pi$$

Delta ITA° (ΔITA°) represents the data obtained after removing the effect of the non-exposed zone from its corresponding exposed zone for each condition. The Delta E (ΔE*) was also calculated using the following formula on differences between exposed and non-exposed zone:

$$\Delta E = \sqrt{\left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)}$$

Statistical analysis

For each parameter in the analysis of immediate pigmentation, the evolution across time was assessed for each exposed zone and non-exposed zone, using either the Student's paired *t*-test or the Wilcoxon signed rank test, depending on normality of the difference data. The latter was tested using a Shapiro–Wilk test at 1% level of significance.

TABLE 2 The study flow of the in vivo trial for immediate pigmentation effect of blue light

Days of study	D1	D2
Informed consent	X	–
Verification and confirmation of inclusion/ exclusion criteria	X	–
Demographic data	X	–
Subject acclimatization	X	X
Identification of investigational zones	X	X
Cutaneous examination of the back	X	X
Photography	X	X
Chromametric measurements	X	X
Product application	X	–
Exposure of specific zones to blue light	X	–
Adverse event/local tolerance monitoring	X	X

The comparison between zones of immediate pigmentation was conducted on the difference between the exposed zone and non-exposed zone at each evaluation time point, using the Univariate ANOVA procedure whereby 'zones' was a fixed factor and 'subjects' was a random component. This was followed by Tukey's procedure for pairwise comparisons between the zones. The analysis was conducted on rank transformed data whenever the normality assumption of the residuals generated from the model was rejected by the Shapiro–Wilk at 1% level of significance.

The comparison between zones of persistent pigmentation was conducted using the Univariate ANOVA procedure with 'zones' as fixed factor and 'subjects' as random component. The analysis between the test product and untreated zone was conducted based on the area under curve two-way ANOVA with special contrast, computed as the sum of trapezoids for each subject first. Analyses were conducted on original data since the normality assumption of the residuals generated from the model was not violated by the Shapiro Wilk at 1% level of significance.

RESULTS

Prevention against accumulation of carbonylated proteins in human skin explants

The protective effect of the test product against the oxidative stress induced by blue light was first evaluated using an ex vivo model, human skin explants. In situ detection of protein carbonylation (stained in red) was performed by epifluorescence microscopy (Figure 1) and the signal intensity was quantified and represented in bar charts in Figure 2. As shown in Figures 1 and 2, blue light irradiation induced protein carbonylation (comparing Figure 1a,b

TABLE 3 The study flow of the in vivo trials for persistent pigmentation effect of blue light

Days of study	D1	D2	D3	D4	D5
Informed consent	X	–	–	–	–
Verification & confirmation of inclusion/ exclusion criteria	X	–	–	–	–
Demographic data	X	–	–	–	–
Subject acclimatization	X	X	X	X	X
Identification of investigational zones	X	X	X	X	X
Cutaneous examination of the back	X	X	X	X	X
Chromametric measurements	X	X	X	X	X
Product application	X	X	X	X	–
Exposure of specific zones to blue light	X	X	X	X	–
Adverse event/local tolerance monitoring	–	X	X	X	X

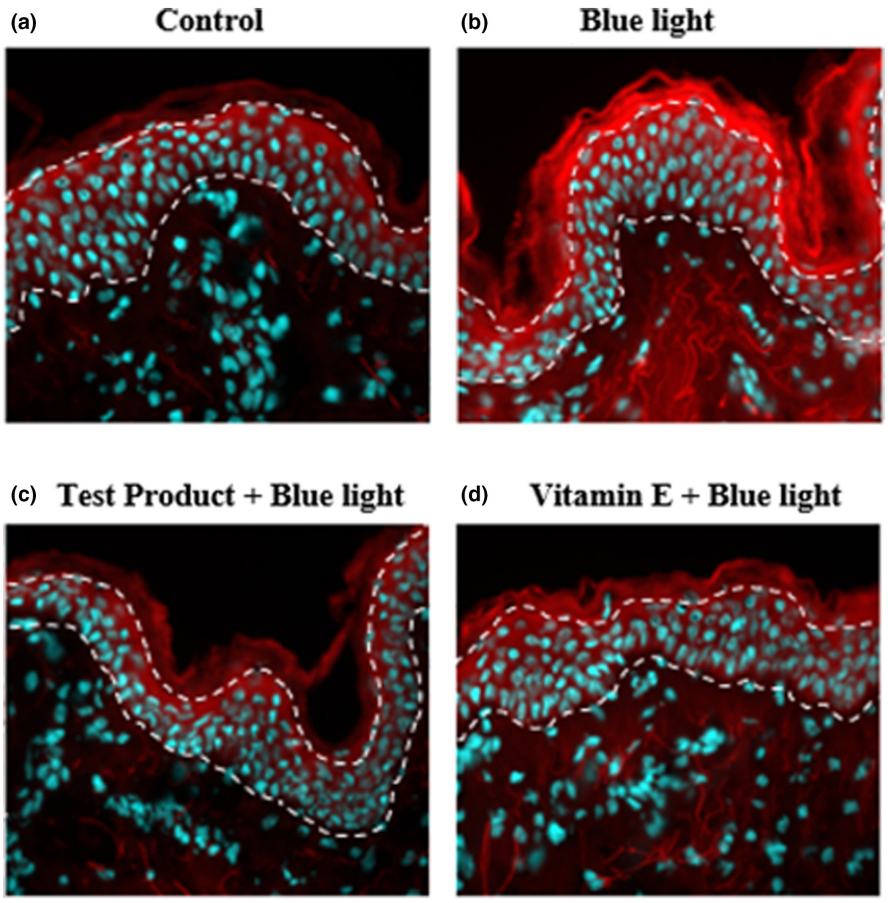

FIGURE 1 In situ visualization of oxidized proteins (red). Representative images for carbonylated proteins were obtained by using a functionalized fluorescent probe (λEx 488 nm/λEm 530 nm). White dotted lines separate the anatomical compartments of skin

and Figure 2a) at the level of the whole skin (comprised of the stratum corneum, epidermis, and dermis). When skin explants were treated with the test product prior to exposure to blue light, a protection of 82% ($p = 0.0017$) could be observed (Figure 1c and 2a). A significant protection against carbonylated protein could also be observed in skin explants treated with a comparator, vitamin E prior to exposure to blue light (66% protection [$p = 0.0050$]; Figure 1d and 2a).

The signal intensity of the fluorescence emitted by the carbonylated proteins was then quantified in each skin compartment that is the stratum corneum, epidermis, and dermis (Figure 2b, c). As shown in Figure 2b, an increase in oxidation level when normalized to control skin explants (non-treated and unexposed samples) could be observed in all skin compartment evaluated. The highest oxidation level was measured in the dermis followed by the stratum corneum and epidermis (Figure 2b). A statistically significant prevention in protein carbonylation accumulation could only be observed in the epidermis and the dermis of skin explants treated with either the test product or the comparator prior to exposure to blue light. The protection level in protein carbonylation was more prominent in the dermis at 100% ($p = 0.0003$) and 77% ($p = 0.0012$) for test product and vitamin E, respectively.

These results suggest that the test product can prevent accumulation of carbonylated proteins in all anatomical compartment of the skin evaluated (stratum corneum, epidermis, and dermis). Moreover, the results show that the test product was more effective than the comparator, vitamin E, to protect against the oxidative stress induced by blue light.

The protective effect of test product was further confirmed by absolute quantification of carbonylated proteins. As shown in Figure 3, when skin explants were treated with the test product prior to exposure to blue light, a 56% ($p = 0.0021$) protection can be observed. Only a statistically non-significant 16% protection was observed with the comparator, vitamin E. These results confirm the protective efficacy of the test product as well as its higher efficacy compared with the comparator, vitamin E.

Protection against the immediate pigmenting effect of blue light

In addition to oxidative stress, blue light induces immediate as well as a more persistent pigmentation in vivo (Figure 4). As shown in Figure 4a, b, immediately after exposure to blue light both the treated and non-treated zones showed a decrease in the ΔL* and ΔITA° parameter

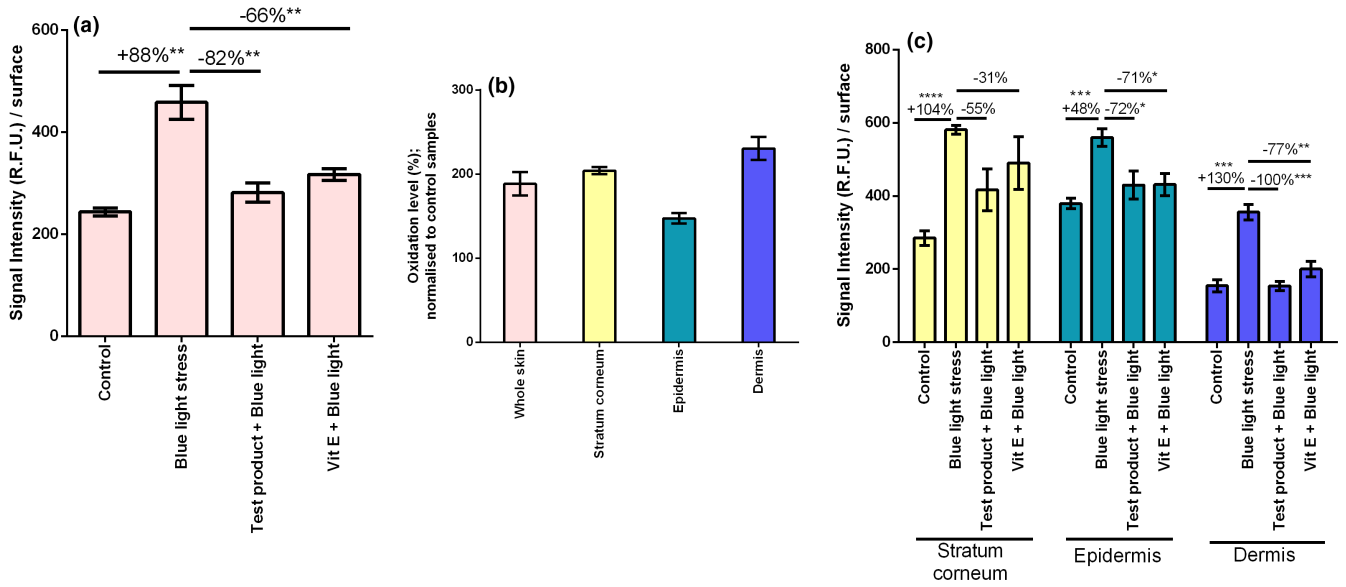

FIGURE 2 Signal intensity and oxidation levels of whole skin or by skin compartment. (a) Signal intensity of in situ oxidation of whole skin. (b) Oxidation level as represented in percentage and normalized to control samples (untreated and unexposed samples). (c) Signal intensity of in situ oxidation as per skin compartment analysis (stratum corneum, epidermis, dermis). The bar charts represent MEAN +/− SEM. Statistical analysis was performed by ANOVA analysis with Fisher's LSD multiple comparison; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

which demonstrates an increase in pigmentation after blue light exposure. However, 24 h after a single exposure to blue light, both ΔL* and ΔITA° increase showing a decrease in pigmentation compared with the pigmenting effect observed immediately after exposure.

Zone comparison for the ΔL* parameter, between the zone treated with the test product and the non-treated zone showed a mean significant difference of 2 units ($p = 0.009$) immediately after exposure to blue light and a mean significant difference of 1 unit ($p = 0.014$) 24 h after exposure to blue light. Zone comparison between the conventional broad-spectrum sunscreen and the untreated zone showed a mean difference of 1 unit ($p = 0.249$) immediately after exposure to blue light and mean difference of 1 unit ($p = 0.188$) 24 h after exposure to blue light. These changes were however not significant.

Zone comparison for the ΔITA° parameter, between the zone treated with the test product and the non-treated zone showed a mean significant difference of 6 units ($p = 0.012$) immediately after exposure to blue light and a mean significant difference of 3 units ($p = 0.019$) 24 h after exposure to blue light. However, when a comparison was drawn between the zone treated with conventional broad-spectrum sunscreen and the untreated zone, a mean difference of 3 units ($p = 0.245$) was observed immediately following exposure while a mean difference of 1 unit ($p = 0.325$) was observed 24 h following exposure to blue light. These differences were not significant.

The ΔE* parameter which characterizes the total colour difference was also investigated (Figure 4c). As shown in Figure 4c and reminiscent to what was observed with the ΔL* and ΔITA°, the mean ΔE* increases immediately after exposure for all conditions indicating a darkening of the skin following blue light exposure. Zone comparison between the zone treated with the test product and the non-treated exposed zone revealed a mean significant difference of 2 units ($p = 0.012$) immediately after exposure and mean significant difference of 1.2 units ($p = 0.028$) 24 h after exposure. However, zone comparison drawn between the conventional broad-spectrum sunscreen and the untreated zone showed a mean difference of 1 unit ($p = 0.321$) immediately after exposure to blue light and mean difference of 0.5 unit ($p = 0.419$) 24 h after exposure to blue light. These changes were not significant. These results suggest that the test product protects against the immediate pigmentation induced by blue light.

Illustrative photographs of immediate pigmentation

Illustrative pictures of the upper back of four subjects, namely, subject 5, 6, 12, and 13 were captured via a multi-featured Nikon D7000 (Nikon, NY, USA) on D1 before exposure, D1 after exposure and D2. These photographs were compared against the untreated section of each investigational zone (Figure 5). It is confirmed through the photographs that there is a visible clinical decrease in immediate pigmentation following the application of the test product. Indeed, a small induction in pigmentation visible

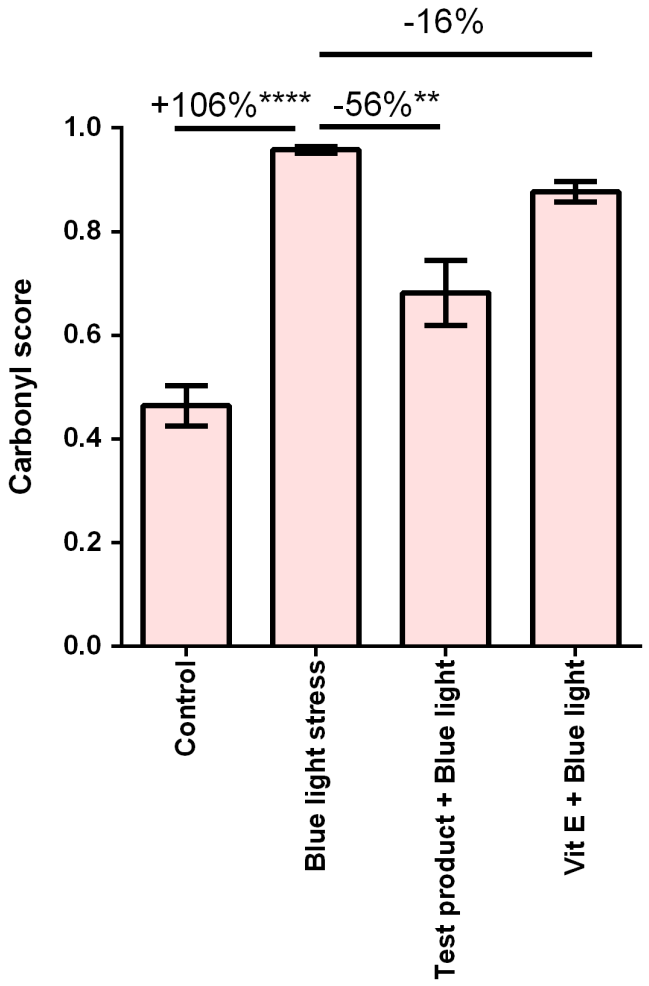

FIGURE 3 Absolute quantification of protein carbonylation. The carbonylated proteins of each sample were presented as a bar chart (MEAN +/− SEM). Statistical analysis was performed by ANOVA analysis with Fisher's LSD multiple comparison; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

on D1 was markedly less pigmented compared with the exposed control zone immediately following exposure and progressed to display very little visibility 24 h following exposure compared to the untreated control zone and the zone treated with conventional broad-spectrum sunscreen.

Protection against the persistent pigmenting effect of blue light

Since the test product is capable of protecting against the immediate pigmenting effect of blue light, we investigated whether it could also protect against the persistent pigmenting effect of blue light. As shown in Figure 6a,b, a decrease in ΔL* and ΔITA° parameters could be observed after blue light exposure. A statistical analysis of the area under the curve of the ΔL* and ΔITA° parameters for the treated and the non-treated zones showed a statistically significant difference ($p = 0.038$) between these two zones for ΔL* but no statistically significant results for the ΔITA° parameter ($p = 0.108$). When the ΔE* parameter was monitored, analysis of the area under the curve for the treated and the non-treated zone showed a limit significant difference ($p = 0.051$). Looking at the overall results obtained for all parameters investigated, in particular ΔL* and ΔE*, there are indications that the test product shows the ability to protect against persistent pigmentation.

DISCUSSION

It is well documented that exposomes such as blue light which can be found mainly in solar radiation contributes to vulnerability in the skin and this in turn can lead to hyperpigmentation [15]. Blue light can penetrate the deeper layers of the skin where it triggers a chain of reaction which will promote free radicals' generation, oxidation of lipids and proteins, inflammation, cutaneous ageing, and hyperpigmentation [16]. In fact, blue light has a key role in hyperpigmentation disorders, such as melasma [17]. It was demonstrated that visible light of shorter wavelength such as 415 nm can cause lasting hyperpigmentation in healthy subjects as compared with visible light of longer wavelength [12, 13]. Skin pigmentation, being dependent on melanogenesis, has been confirmed to be more pronounced in darker skin tones (phototype IV–VI) while mostly absent in lighter skin tones. However, the mechanism involved has not been completely elucidated.

In this study, an oxidative stress marker, carbonylated proteins were monitored in human skin explants at different anatomical compartment (stratum corneum, the epidermis, and the dermis). As a type of protein oxidation induced by reactive oxygen species [18], protein carbonylation was confirmed to be increased in untreated human skin explants exposed to blue light through the in situ visualization and carbonyl score. When treated with sunscreen containing blue light blocker, the level of protection was later reported to be more pronounced in the dermis and significantly higher than the positive control, vitamin E. This increased protection efficiency was inferred to be caused by components in TDF® Blu Voile Sunscreen incorporating a unique blue light blocking agent along with zinc oxide (ZnO) and titanium dioxide (TnO_2) which are frequently employed as an inorganic physical sunblock. Since TnO_2 has been shown to be more effective against UV-B and ZnO against UV-A, utilizing both ensures a broad-spectrum protective effect against UVR in general [19].

The blue light blocking agent comprised of polymethylsilsesquioxane (PMSQ) and HDI/Trimethylol

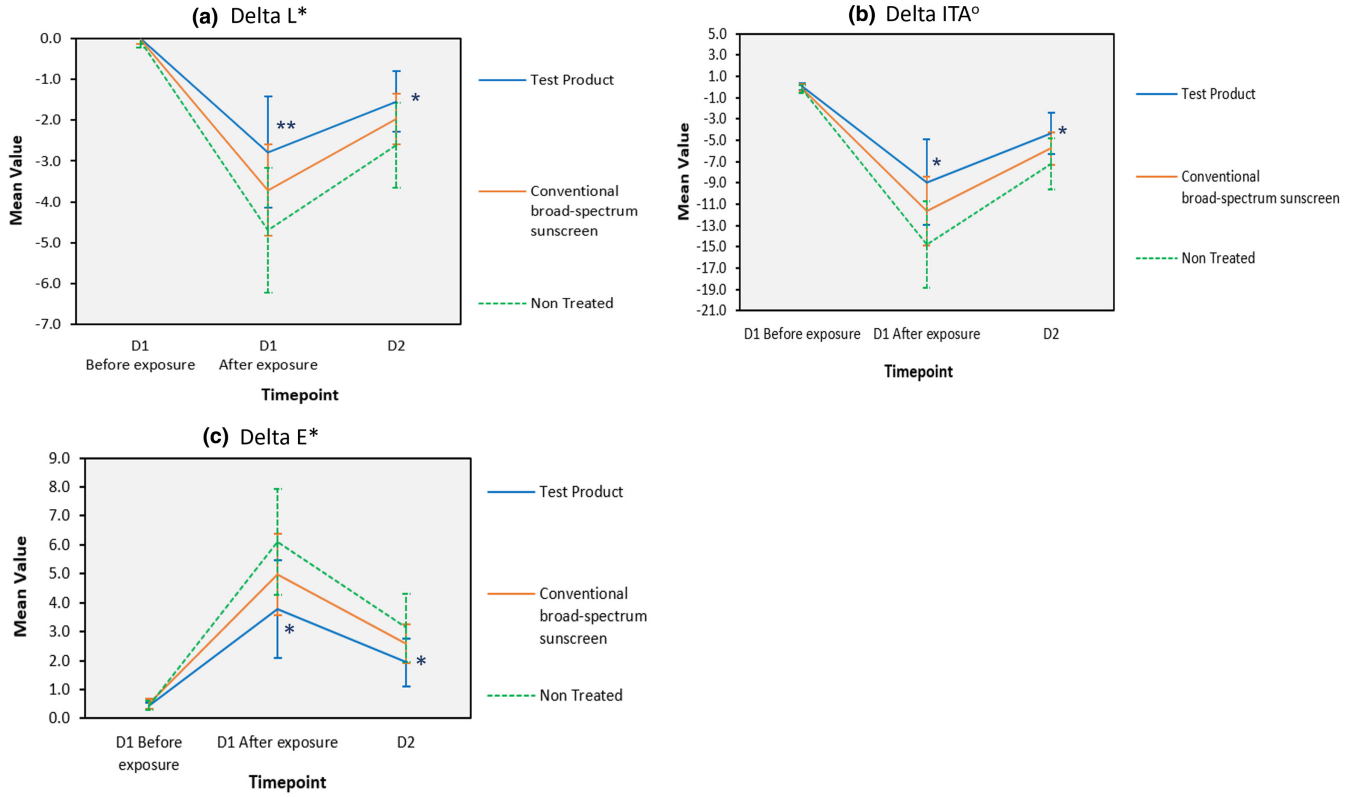

FIGURE 4 Immediate pigmentation—colorimetric measurement of the treated and non-treated zone immediately after blue light exposure (D1) and 24 h after blue light exposure (D2). (a) Delta L* by zone; (b) Delta ITA° by zone; (c) Delta E* by zone. All figures show error bars: 95% CI. Statistical analysis was performed by Student's paired t-test or the Wilcoxon signed rank test; $*p < 0.05$, $**p < 0.01$

hexyllactone crosspolymer at a concentration of 1%. PMSQ is a hybrid polymer with good thermal stability due to its organic nature and was used due to its good biocompatibility, non-toxicity, and chemical stability [20]. HDI/Trimethylol hexyllactone crosspolymer was used as a sensory enhancer to create an aesthetically appealing formulation to improve the spread on the skin, absorb oil, reduce tack and greasiness [21]. In combination, these ingredients are capable of absorbing the wavelength of blue light while reflecting the longer wavelengths of visible light such as red light (Data not shown). On the other hand, the conventional broad-spectrum sunscreen does not contain any blue light blocking agent.

The protective effect of the sunscreen was further confirmed in healthy subjects with darker skin tones through clinical studies where the immediate and persistent pigmentation were monitored by colorimetric measurement. The parameters evaluated comprised of ΔL* which monitors lightness of the skin; ΔITA° which monitors the degree of skin pigmentation; and ΔE* which characterizes total colour difference and is calculated based on delta L*, a*, b* [22]. A decrease in L* and ITA° characterize a darkening of the skin and an increase in skin pigmentation, respectively.

Two sources of blue light, namely, 415 nm and 460 nm were used. We engineered two monochromatic sources of those two wavelengths of blue light (415 nm and 460 nm) which faithfully mimic the irradiance of natural sunlight and the screen of electronic devices, respectively. A broadband thermopile detector was used to control and finetune the dosage of the blue light radiation on the skin. Both immediately and 24 h after exposure, TDF® Blu Voile Sunscreen offered significant protection. A single dose of 60 J/cm^2 of blue light which corresponded to an hour of sun at midday on a clear summer day was used to assess the pigmenting effect immediately after exposure [5]. However, it is interesting to note that the single-dose exposure during the clinical evaluation induced a darkening of the skin which recovered rapidly 24 h after exposure but did not return to the baseline. Similar findings were reported in a previous study whereby exposure of the skin of human subjects to blue light 450 nm generated darkened skin that did not recover fully to the baseline [5]. In our other study, when the healthy subjects were exposed to a higher dose of blue light of 87.5 J/cm^2 corresponding to 2 h and 45 mins of sun exposure in the summer [12] for four consecutive days, the skin darkening was even more prominent and long-lasting. This was in line with previous

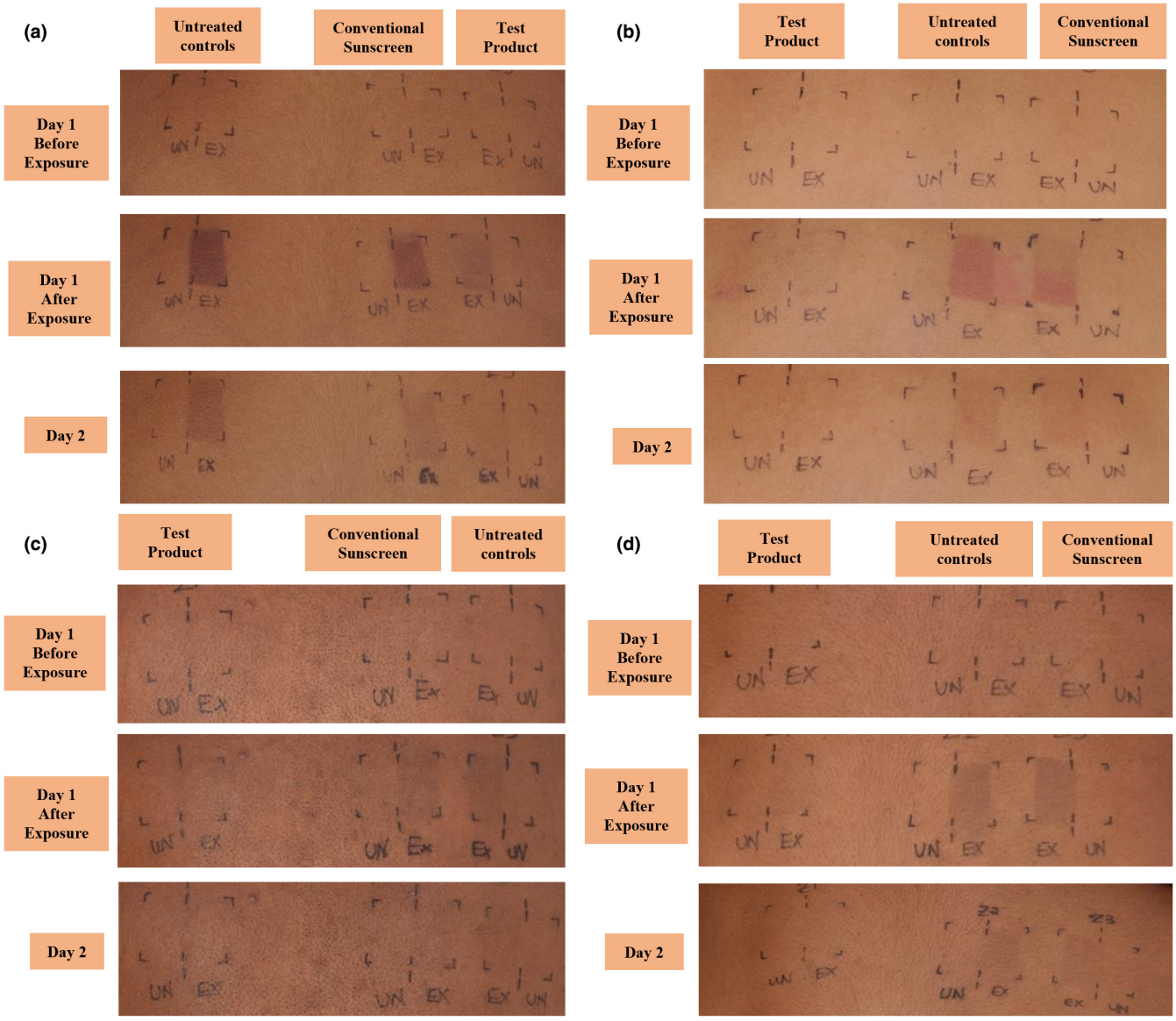

FIGURE 5 Illustrative photographs of (a) subject 5; (b) subject 6; (c) subject 12; (d) subject 13 at day 1 before blue light exposure, day 1 after blue light exposure and day 2. Ambient lighting was maintained throughout the study. UN stands for unexposed zones to blue light; EX refers to exposed zones to blue light

investigations which demonstrated that blue light was capable of inducing persistent pigment darkening similar to UV-A. Mahmoud et al. reported that visible light of wavelength 400–700 nm is a significant contributor of cutaneous photoaging in vitro, ex vivo, and in vivo [4]. It was further reported that visible light induced a more sustained pigmentation of the skin compared with pigmentation induced by UV-A radiation [6]. An increase in the a* parameter immediately after the irradiation phase was visually perceived. Indeed, this was attributed to the influx of haemoglobin and oxygen saturation which gave the skin a bluish to reddish tint following exposure [6, 23].

When comparing the zones treated with TDF® Blu Voile Sunscreen and a conventional broad-spectrum sunscreen, it was obvious that not all sunscreens offer the same degree of protection against blue light-induced pigmentation. Many previous studies have investigated the effects of immediate and persistent pigmentation on the skin induced by UVR [11, 12, 24]. As a result of these niche studies, conventionally available sunscreens contain mostly UV-A and UV-B filters and are not formulated to shield against the harmful effects of high-energy visible lights such as blue light. A rather recent study conducted by Dumbaya et al. investigated the efficacy of sunscreen formulations containing a mineral oxide in stopping pigmentation induced by visible light. It was reported that formulations containing mineral oxide shielded the skin against pigmentation in subjects. As solar radiation causes more acute skin darkening and worsens dyschromia in individuals with darker skin phototypes, including physical

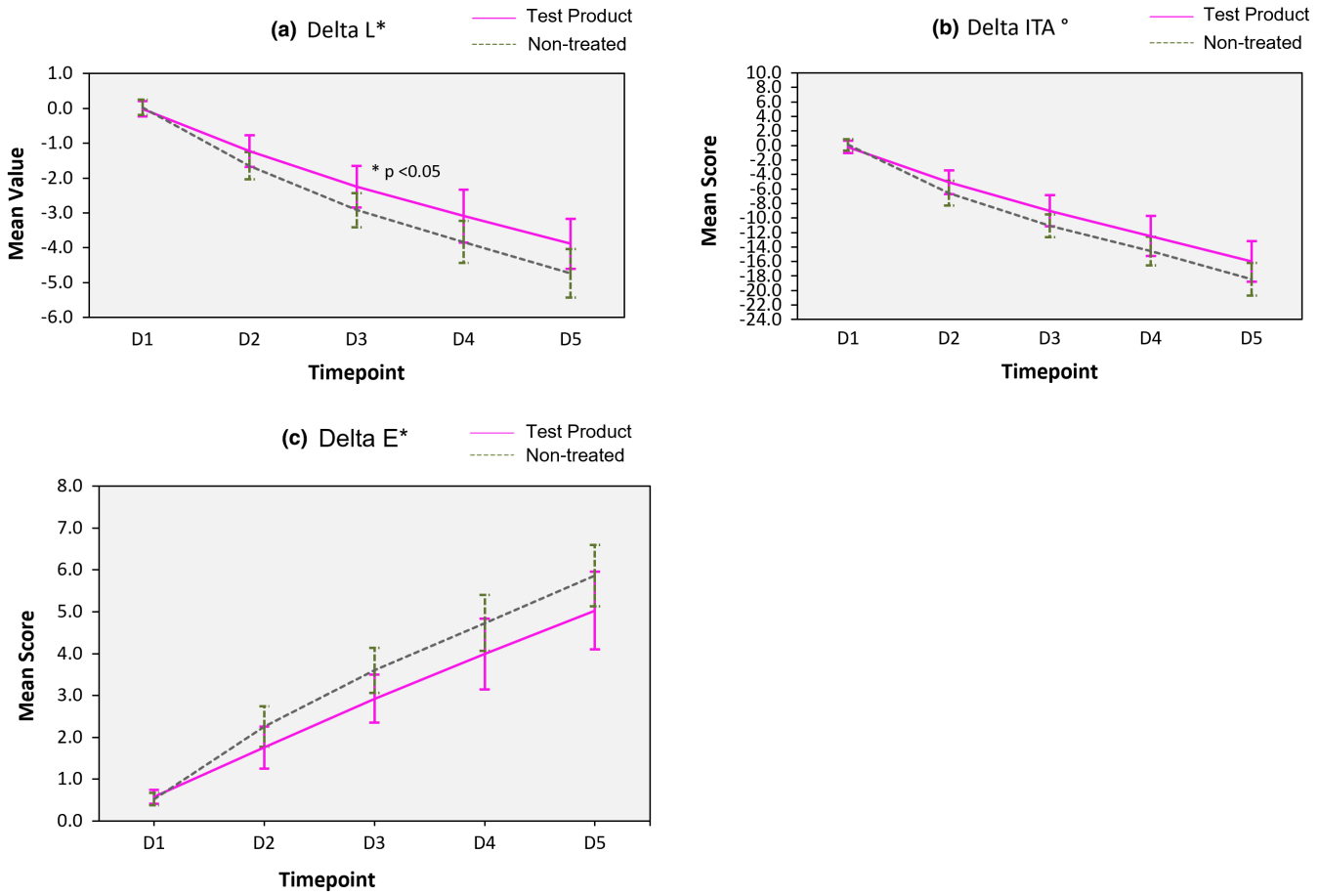

FIGURE 6 Persistent pigmentation—colorimetric measurement of the treated and non-treated zone exposed to consecutive days of blue light. (a) Delta L* by zone; (b) Delta ITA° by zone; (c) Delta E* by zone. All figures show error bars: 95% CI. Statistical analysis used was performed by area under curve analysis; *$p < 0.05$

barriers in sunscreen formulations was reported to protect the skin from solar radiation-induced hyperpigmentation [25].

CONCLUSION

We described in this study, the property of a novel sunscreen in protecting against the detrimental effect of blue light on human skin ex vivo and in vivo. This study utilized ex vivo models and irradiation with blue light to assess its efficacy against blue light-induced protein oxidative damage. Ensuing in vivo trials were then carried out to confirm the immediate and persistent efficacy of the sunscreen on randomized subjects. Findings presented in this study suggest that TDF® Blu Voile Sunscreen resulted in lower levels of carbonylated proteins accumulation in human skin explants. This significant protection was confirmed by an absolute quantification of the carbonylated proteins. It also showed better protective efficacy in vivo for both immediate and persistent pigmentation induced by blue spectrum of solar light. In comparison to conventional broad-spectrum sunscreen, TDF® Blu Voile Sunscreen has been formulated to offer a more thorough and complete protection against both UV-A/UV-B and blue light.

AUTHOR CONTRIBUTIONS

All authors discussed the results, contributed equally, and approved the final manuscript.

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CONFLICT OF INTEREST

Authors would like to declare no conflict of interest.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study is according to the guidelines of the Declaration of Helsinki and OECD Good laboratory Practices (GLP). The study was conducted within the national guidelines of Mauritius and obtained a favourable opinion by the

Fortis-Darné Clinique Independent Ethics Committee (IEC).

INFORMED CONSENT STATEMENT

Informed consent was acquired from all patients involved in this study.

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