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REVIEWS

The potential effect of *Polypodium leucotomos* extract on ultraviolet- and visible light-induced photoaging

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Abstract

Photoaging induced by both ultraviolet and visible light has been shown to lead to increased inflammation and dysregulation of the extracellular matrix. Standardized extract of the *Polypodium leucotomos* fern, PLE, possesses anti-inflammatory and antioxidant properties, and has been shown to potentially mitigate photoaging through various mechanisms. This comprehensive review presents the data available on the effects of *P. leucotomos* extract on UV and VL-induced photoaging in vitro as well as in vivo in murine and human models.

Keywords *Polypodium leucotomos* · Photoaging · Visible light · Ultraviolet light

Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts
AP1	Activator protein 1
COX2	Cyclooxygenase 2
CPD	Cyclobutane pyrimidine dimer
FRAP	Ferric reducing antioxidant power
H2AX	H2A histone family member X
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
HSP	Heat shock protein
i-NOS	Inducible nitric oxide synthase
IL	Interleukin
LDH	Lactate dehydrogenase
MART-1	Melanoma antigen recognized by T cells 1
MED	Minimal erythema dose
MMP	Matrix metalloproteinase
NF-kB	Nuclear factor KB
NRF2	Nuclear factor erythroid 2-related factor 2
NO	Nitric oxide
TGF-β	Tumor growth factor-β
TIMP	Tissue inhibitor of matrix metalloproteinase
TiO_2	Titanium dioxide
TNF-α	Tumor necrosis factor
UCA	Urocanic acid

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1 Introduction

Electromagnetic radiation emitted by sunlight as it reaches the surface of the earth consists of ultraviolet A (320–400 nm) and ultraviolet B (290–320 nm), visible (400–700 nm), and infrared (> 700 nm) spectra [1]. It is established that UV radiation (UVR) contributes to photoaging, which encompasses the clinical and histological changes from chronic solar radiation exposure due to increased inflammation and oxidative burden. Although not as extensively studied as UVR, visible light (VL) has also been reported to induce photoaging changes [2–6].

The structural integrity of the skin relies on the extracellular matrix (ECM) and its fibrillary proteins, collagen and elastin, which are secreted by fibroblasts [4]. UVR-induced cell membrane lipid oxidation by reactive oxygen species (ROS) leads to the generation of arachidonic acid, which is converted by cyclooxygenase (COX) enzymes into eicosanoids, leading to inflammation [7, 8]. UVR-induced expression of the transcription factor activator protein (AP)-1 leads to ECM damage through over expression of matrix metalloproteinases (MMPs) which degrade collagen and elastin [4, 8–10]. AP-1 also blocks the effect of tumor growth factor (TGF)-β which is involved in collagen I and III gene expression and

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synthesis [11]. Photoaging also involves elastase activity, reduced expression of tissue inhibitors of matrix metalloproteinases (TIMPs), fibrillin, and altered cellular stress response proteins, such as heat shock proteins [12]. Histologic changes of photoaging include increased inflammatory cells, elastotic material, glycosaminoglycans and disordered collagen which manifest clinically as wrinkles, and disordered skin remodeling and wound healing [2, 13, 14]. While much of the information on photoaging has been studied in relation to UVR, VL has also been shown to induce ROS and MMP expression [6].

Although non-tinted topical sunscreens can prevent actinic damage from UVR, they are not protective against VL-induced changes [15]. Traditional sunscreens containing organic ultraviolet (UV) filters have also increasingly been scrutinized for their effects on the environment [16]. These limitations have garnered an increased interest in environmentally safe topical sunscreens and oral polyphenol compounds, the latter to provide systemic protection against a broad wavelengths of solar radiation through increasing plasma and skin antioxidant levels and mitigating the detrimental effects of ROS [17]. The concept of oral antioxidants used as photoprotection, as compared to commonly utilized topical agents, has recently become seriously proposed in the field of photoprotection.

The *Polypodium leucotomos* fern from the family of *Polypodiaceae* is found in Central and South America [18]. Standardized extract of *P. leucotomos*, herein referred to as PLE, is an antioxidant available as an over the counter oral supplement; Polypodium extract as a photoprotectant was patented by **Pathak et al. in 1995 (Patent number: US5614197) and the combination of phenolics and other ingredients observed in this controlled extract were subsequently patented in 2008 and registered as Fernblock® (Cantabria Labs, Madrid, Spain) [19]. Studies have shown that PLE decreases UV-induced erythema, DNA damage, epidermal hyperproliferation, and acute PUVA (psoralen + UVA) induced phototoxic reactions [9, 20–23]. It has been shown to down-regulate the exacerbation of polymorphous light eruption [24, 25]. The photoprotective properties of oral PLE are thought to be due to its antioxidative and anti-inflammatory capacity [17, 26].

This comprehensive, in-depth review aims to explore the data available on the effects of *P. leucotomos* on UV and VL-induced photoaging. This is an update of a previous review published in 2016 [27]. The effects on UV-induced changes will be discussed first, followed by those induced by VL. Each section is will be subdivided into in vitro studies, then animal and human studies. Alterations in molecular photoaging biomarkers as a result of UV or VL irradiation in the skin will be emphasized. It should be noted that except as noted, all studies used PLE preparation of Fernblock® or its precursor.

2 Studies involving ultraviolet radiation and PLE

PLE's effects on UV-induced photoaging has been investigated in vitro in human dermal fibroblasts and keratinocytes, and in vivo in murine models and humans (Table 1).

2.1 In vitro

In a study by Philips et al., the effects of post-irradiation incubation with PLE on cell membrane damage as measured by LDH, lipid peroxidation, matrix metalloprotease (MMP)-1 and elastin were assessed in UVR (UVA 1.8 J/cm^2 or UVB 2.5 mJ/cm^2) irradiated human dermal fibroblasts and human epidermal keratinocytes [10]. PLE was shown to significantly inhibit cellular LDH release from fibroblasts but not from keratinocytes. PLE also significantly inhibited UVR-induced lipid peroxidation and decreased UVR-induced MMP-1 generation from fibroblasts and keratinocytes. Following UVR exposure, an increase generation of elastin was noted from fibroblasts and keratinocytes, regardless of treatment with PLE; however, the PLE-related increase was a reflection of well-formed elastin fiber deposition. The authors postulated that PLE may prevent photoaging by preserving membrane integrity and inhibiting MMP-1 synthesis while reversing intrinsic aging-associated loss of normal elastic fibers [10]. In another study by Philips et al., the effect of various PLE concentrations on collagen and TGF-β expression of UV-irradiated (UVA 1.8 J/cm^2 or UVB 2.5 mJ/cm^2) human neonatal dermal fibroblasts. Cells were pretreated for 24 h with PLE doses from 0 to1%. PLE treatment resulted in increased TGF-β, type I collagen, and type V collagen levels in the cell media of irradiated fibroblasts. No significant changes were noted in type III collagen expression. These effects on the ECM were thought to be due to PLE's antioxidative properties and possible cell-specific up-regulation of TGF-β expression [28].

Philips et al. also evaluated the effect of various PLE concentrations (0–0.1%) on generation of elastase, tissue inhibitors of matrix metalloproteinases (TIMPs), fibrillin, TGF-β, and heat shock proteins (HSP) by keratinocytes after UVR (7.5 mJ/cm^2 UVA or 7.5 mJ/cm^2 UVB). HSP are induced by cellular stress and aid in stabilizing proteins. HSP-27 is linked to epidermal differentiation and HSP-70 is anti-inflammatory and prevents UVB radiation induced epidermal damage [29, 30]. PLE inhibited elastase activity, stimulated TIMP-1, TIMP-2, fibrillin-1, fibrillin-2 and TGF-β expression and stimulated synthesis of HSP-27 and HSP-70 by irradiated keratinocytes, thereby strengthening ECM integrity [12].

Table 1 Effects of *Polypodium leucotomos* extract on ultraviolet radiation-induced photoaging in vitro and in vivo

References	Cell types/substrates	Effect of PLE following UV exposure
In vitro studies		
Philips et al. [10]	Fibroblasts, keratinocytes	↓LDH release (fibroblasts only) ↓MMP-1 ↑Elastin
Philips et al. [28]	Fibroblasts	↑TGF-β ↑Type I collagen ↑Type V collagen
Philips et al. [12]	Keratinocytes	↓Elastase ↑TIMP-1 ↑TIMP-2 ↑Fibrillin-1 ↑Fibrillin-2 ↑TGF-β ↑HSP-27 and HSP-70
Alonso-Lebrero et al. [31]	Fibroblasts, keratinocytes	↑Cell survival (fibroblasts and keratinocytes) ↑Cell proliferation (fibroblasts and keratinocytes) ↓Cytoskeletal disorganization (fibroblasts only)
Capote et al. [32]	Trans-urocanic acid, Fibroblasts	↓Cis-UCA (± H_2O_2) ↓Trans-UCA photodecomposition (in the presence of TiO_2) ↑Fibroblast survival
Janczyk et al. [35]	Keratinocytes	↓TNF-α ↓NO ↓i-NOS ↓NF-kB ↓AP1 ↓Loss of cell–cell contacts ↓Abnormalities in cell morphology ↓Cell death ↓Annexin V binding
Delgado-Wicke et al. [36]	Keratinocytes	↑Cell viability ↑Transcription of gene targets of the (NRF2) pathway ↓IL6 and IL8 ↓Induction of melanin production
Gonzales et al. [38]	Fibroblasts Keratinocytes	↑ Cell survival ↓DNA damage (H2AX and CPD)
References	**Model**	**Effect of PLE following UV exposure**
In vivo studies		
Mulero et al. [39]	Rat	↑Epidermal catalase ↑Epidermal glutathione peroxidase activity ↑Langerhans cells
Alcaraz et al. [26]	Mouse	↓Mast cells ↓Number of capillary loops ↓Dermal elastosis
Zattra et al. [40]	Mouse	↓Inflammatory infiltrates ↓Neutrophil infiltration ↓Macrophages ↓COX-2
Kohli et al. [22]	Human	↓COX-2 ↓Erythema
Middelkamp-Hup et al. [20]	Human	↓Dermal mast cell infiltration
Villa et al. [21]	Human	↓Mitochondrial common deletion (trend)
Granger et al. [44][a]	Human	↓Mean FRAP ↓Lipid peroxidation ↑Radiance, moisture, elasticity and skin lightness

AP1 activator protein 1, *COX2* cyclooxygenase 2, *CPD* cyclobutane pyrimidine dimer, *FRAP* ferric reducing antioxidant power, *H2AX* H2A histone family member X, H_2O_2 hydrogen peroxide, *HSP* heat shock protein, *IL* interleukin, *i-NOS* inducible nitric oxide synthase, *LDH* lactate dehydrogenase, *NF-kB* nuclear factor KB, *NRF2* nuclear factor erythroid 2-related factor 2, *NO* nitric oxide, *MMP* matrix metalloproteinase, *TGF-β* tumor growth factor-β, *TIMP* tissue inhibitor of matrix metalloproteinase, TiO_2 titanium dioxide, *TNF-α* tumor necrosis factor, *UCA* urocanic acid

[a]Antioxidant food supplement containing a non-Fernblock extract of *P. leucotomos*

Alonso-Lebrero et al. assessed the effects of PLE on UVA-induced survival and proliferation of human dermal fibroblasts and keratinocytes [31]. Fibroblast morphology was also investigated under these conditions. Cells were irradiated with either UVA or UVB and PLE doses of 0.1–2 mg/ml were used. Fibroblast survival with PLE was compared to other antioxidants including N-acetylcysteine (NAC), Trolox (a synthetic analog of α-tocopherol) or ascorbic acid. PLE induced a noticeable, dose dependent increase in fibroblast survival after irradiation with UVA at 1 J/cm^2 when compared to untreated controls. The other antioxidants preserved fibroblast survival above the untreated controls, but were not as efficient as PLE. PLE also improved the ability of fibroblasts to proliferate after exposure to UVA (1 J/cm^2). Compared to untreated, irradiated fibroblasts, PLE provided complete photoprotection up to 1 J/cm^2 of UVA; the degree of photoprotection declined at doses higher than 1 J/cm^2. PLE protected against UVA-induced (1 J/cm^2) cytoskeletal disorganization and abnormalities in adhesion molecule distribution patterns. PLE with UVA doses of 10 J/cm^2 and 15 J/cm^2 and UVB at 10 mJ/cm^2 demonstrated preserved keratinocyte survival. PLE also demonstrated a protective effect on keratinocyte proliferation at UVA doses of 10 J/cm^2 and 15 J/cm^2. The authors noted that the photoprotection shown for both keratinocytes and fibroblasts suggests that the specificity of PLE is not limited by cell lineage. PLE's ability to block UV-induced cytoskeletal rearrangements also suggests that its photoprotective effect may not only be due to regulation of DNA synthesis, but also cytoskeletal mechanisms [31].

Capote et al. evaluated the effect of various doses of PLE on UVA and UVB-induced photoisomerization of *trans*-urocanic acid (*t*-UCA; 0.5 mg/ml), a photoreceptor located in the stratum corneum [32]. The *cis* form of urocanic acid is immunosuppressive and not photoprotective [33]. PLE significantly inhibited UVB (4.8 J/cm^2) and UVA-induced (6.48 J/cm^2) formation of *cis*-UCA. *Trans*-UCA plus H_2O_2 irradiated with UVB (4.8 J/cm^2) in the presence of increasing concentrations of PLE (0–30 μg/ml) demonstrated a dose-dependent decrease in *cis*-UCA formation. H_2O_2 was used to simulate the presence of endogenous hydroxyl radicals formed by UVB irradiation [34]. TiO_2 is known to generate reactive oxygen species (ROS) upon exposure to UV. In the presence of TiO_2, PLE significantly decreased levels of UVA (6.48 J/cm^2) induced *t*-UCA photodecomposition. UVA-irradiated (1 J/cm^2) fibroblasts in the presence of PLE demonstrated increased cell survival when compared to untreated irradiated cells. The results demonstrated that PLE may be able inhibit UV-induced TiO_2-dependent ROS production and photodecomposition of endogenous photoprotective molecules, such as *t*-UCA [32].

Janczyk et al. evaluated the effects of PLE 1 mg/ml on levels of TNF-α, nitric oxide (NO), inducible nitric oxide synthase (i-NOS), cell membrane damage and cell death, and PLE 0.5–2 mg/ml on NF-kB and AP1 levels in keratinocytes pre-treated with PLE and irradiated with different doses of UVR. Doses of UVR varied depending on the experiment (UVA 6 J/cm^2 + UVB 0.5 J/cm^2, UVA 11 J/cm^2 + UVB 1 J/cm^2 UVA = 2.4 J/cm^2 + UVB = 0.2 J/cm^2) [35]. PLE pretreatment significantly decreased the up-regulation of TNF-α, nitric oxide and inducible nitric oxide synthase levels. Comparison of pre and post treatment with PLE demonstrated a significant inhibition of UVR-induced activation of NF-kB, AP1, and partial mitigation of loss of cell–cell contacts, abnormalities in cell morphology, and cell death. In PLE pretreated keratinocytes, a partial counteraction of UV-induced annexin V binding, a marker related to apoptosis, was noted. These findings demonstrated the anti-inflammatory and cell protective effects of PLE against UVR [35].

Delgado-Wicke et al. evaluated the effect of PLE (7.8–62.5 μg/ml) on keratinocytes pre-treated with PLE and subsequently irradiated with UVB (90 mJ/cm^2) or exposed to $PM_{2.5}$ (50 μg/ml), an experimental model of fine pollutant particles [36]. PLE was found to reduce the decrease in UVB-induced viability of keratinocytes and to induce the transcription of gene targets of the nuclear factor erythroid 2-related factor 2 (NRF2) pathway, which counteracts oxidative stress [37]. UVB-induced interleukin (IL)6 and IL8, as well as induction of melanin production were all decreased by PLE. Similarly, PLE partially reverted $PM_{2.5}$-induced toxicity of keratinocytes and upregulated NFR2 transcription factor and some of its downstream antioxidant targets. This study demonstrated how PLE protects against not only photooxidative stress but also other environmental pollutants.

Gonzales et al. evaluated the antioxidant capacity of various extracts of *Polypodiun leucotomas,* and their effects (1 mg/ml) on UV-induced cell viability and DNA damage in human fibroblasts and keratinocytes irradiated with UVR (2.2 J/cm^2), though the spectral output was not stated in the article [38]. A total of six extracts of *Polypodium leucotomas* (3 from the leaves and 3 from the rhizome portion) were used in this study. The antioxidant moieties protocatechuic, vanillic, caffeic, and ferulic acid were also evaluated by high performance liquid chromatography (HPLC). All samples from the leaves and one sample from the rhizome were found to have significant antioxidant activity as determined by 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and ferric reducing antioxidant power (FRAP) assays. Samples 1 and 2, both from leaves, were found to contain detectable amounts of these antioxidant moieties. Samples 1 and 2 were also the most effective in terms of improving cell survival and decreasing DNA damage (decrease in γ-H2A histone family member X

(H2AX) and cyclobutane pyrimidine dimers (CPD)), with sample 2 (Fernblock®) being most effective [38].

2.2 In vivo (murine)

Mulero et al. assessed the levels of antioxidants and Langerhans cells in the epidermis of hairless rats given oral PLE at a concentration of 30 mg/kg in drinking water for 7 days, and subsequently irradiated with UVR (UVB/UVA-ratio of 0.9; peak at 312 nm;7 J/cm^2) [39]. Dorsal skin biopsies taken 48 h after irradiation demonstrated a significant increase in epidermal catalase and glutathione peroxidase activity when compared to irradiated animals without PLE, and higher numbers of Langerhans cells compared with the vehicle-treated, irradiated group and the control, non-irradiated group. These findings demonstrated the efficacy of PLE as an oral antioxidant and photoimmunoprotective agent [39].

The effect of topical PLE on inflammation and histologic parameters of photoaging in UVB-irradiated hairless albino mice was studied by Alcaraz et al. [26] Mice were divided into control non-irradiated, UVB-irradiated animals without PLE and animals that received topical application of PLE either before (PLE 100 ml of 0.02%) or immediately after (PLE 100 ml of 0.2%) each UVB exposure (cumulative 1.9 J/cm^2 over 12 weeks). Mice treated with PLE before UVB irradiation showed lower levels of mast cells than those of irradiated control mice. PLE treated mice had a lower number of capillary loops per square millimeter of dermal area indicating decreased inflammation. Inhibition of UV-induced dermal elastosis was also observed in mice receiving topical PLE before and after each UVB irradiation session. These findings demonstrated how PLE ameliorated and partially inhibited some of the histologic damage associated with photoaging [26]. Zattra et al. evaluated the effect of PLE on UVB-induced COX-2 expression and inflammation in the skin of hairless mice. Mice fed with PLE 300 mg/kg for 10 days and exposed to UVB (25 mJ/cm^2) experienced a decrease in UVB-induced inflammatory infiltrates, neutrophil infiltration, macrophages and COX-2 [40].

2.3 In vivo (human)

Kohli et al.evaluated the effect of oral PLE on COX-2 in UV irradiated skin of humans [22]. Twenty-two subjects with Fitzpatrick skin phototype I to III were irradiated with UVB (using 308-nm excimer laser) with doses ranging from 100 to 350 mJ/cm^2. Pre-PLE minimal erythema dose (MED)/trace erythema assessment and biopsy were performed 24 h after irradiation. On day 3 subjects took PLE 240 mg 2 h and 1 h (480 mg total) before irradiation was repeated as on day 1. On day 4 a biopsy was taken from the site of MED/trace erythema. A statistically significant decrease of COX-2 was found post-PLE consumption ($p < 0.0005$), indicating PLE's anti-inflammatory effect. A significant improvement in erythema was noted after PLE administration. Overall, the results showed that PLE was able to lessen the negative photobiologic effects of UVB [22]. Middelkamp-Hup et al. studied the effects of PLE on dermal mast cell infiltration after UV irradiation [20]. The back skin of nine participants with Fitzpatrick skin phototype II and III were exposed to UVR (305–400 nm) doses ranging up to 2–3 times the MED dose, before and after taking PLE at different timepoints [20]. After receiving an initial dose of UVR, initial biopsies were taken from pre-PLE irradiated sites from 5 participants after 24 h and from 2 participants 72 h after irradiation. The evening before the second exposure of UVR, the first dose of PLE (7.5 mg/kg) was given to participants. The next day, a second dose of PLE was administered and participants were exposed again at the same UVR fluences 30 min, 1 h, 1.5 h, 2 h and 3 h after the second dose of PLE. A second biopsy was taken from the same participants who had an initial biopsy done from the site at the timepoint showing maximal photoprotection (5 participants after 24 h and 2 participants 72 h after irradiation.) Compared to pre-PLE irradiated sites, a reduction in mast cells of the papillary dermis was noted from biopsies taken from post-PLE irradiated sites at either 24 or 72 h after irradiation [20]. Mast cell products are thought to play a role in photoaging by induction of fibroblast elastin production. The ability of PLE to down-regulate UV-induced mast cell responses suggested that PLE might play a role in reducing changes of photoaging [20, 41].

Villa et al. evaluated the effect of PLE on a photoaging-associated mitochondrial common deletion (CD) after UVA irradiation (320–400 nm with a peak of 350 nm) [21]. In this randomized, investigator-blinded, controlled trial, ten participants with Fitzpatrick skin phototype II and III were irradiated with UVA two to three times the MED (using doses of 10–35 J/cm^2) either having taken a single dose oral PLE 480 mg before exposure or no PLE. Forearm biopsies taken 24 h after irradiation demonstrated that at two times the MED, the average CD values decreased by 84% in the group taking PLE, compared to the 217% increase over baseline in the non-PLE treated group, trending toward significance ($p = 0.06$). The CD is a specific mitochondrial DNA deletion induced by chronic UVA radiation in fibroblasts and keratinocytes, and this study's findings suggested that PLE's effect on photodamage may be through preventing UVA-dependent mitochondrial DNA damage [21, 42, 43].

More recently, Granger et al. conducted a clinical trial investigating the effects of an antioxidant food supplement containing a non-Fernblock extract of *P. leucotomos* on the antioxidant capacity and lipid peroxidation of the skin after UVA irradiation. Thirty participants with Fitzpatrick skin phototype I to III consumed 480 mg daily for 12 weeks and UV irradiation was performed on the

back skin at various timepoints over 12 weeks to achieve one MED, with doses ranging from 38 to 41 mJ/cm^2. The mean Ferric Reducing Antioxidant Power (FRAP) measurement significantly decreased. Lipid peroxidation was also measured via malondialdehyde (MDA) 4 h and 24 h after UVA irradiation (5 J/cm^2) at each visit, and was found to decrease with the supplement. Radiance, moisture, elasticity and skin lightness were also significantly increased after supplementation. The authors concluded that this supplement improved photoprotection, enhanced the antioxidative status of the skin and improved general skin condition [44].

3 Studies involving visible light and PLE

While most of the studies related to PLE and photoaging have been done following exposure to UVR, PLE's effects on VL-induced photoaging has also been assessed in vitro in human dermal fibroblasts and in vivo in humans (Table 2).

3.1 In vitro

Zamarron et al. assessed various photoaging markers in human dermal fibroblasts pretreated with PLE (0.5–1 mg/ml) and irradiated with VL [45]. Cells exposed to VL (247.3 J/cm^2) without PLE immediately showed signs of cellular stress, such as cytoplasmic retraction and cellular stretching. Cells exposed to VL in the presence of PLE

Table 2 Effects of *Polypodium leucotomos* extract on visible light-induced photoaging in vitro and in vivo

References	Cell types/substrates	Effect of PLE following VL exposure
In vitro studies		
Zamarron et al. [45]	Fibroblasts	Delayed morphologic abnormalities ↓MMP-1 ↓Cell death rates ↓Cathepsin-K (trend) ↑Fibrillins ↑Elastin
References	**Model**	**Effect of PLE following VL exposure**
In vivo studies		
Mohammad et al. [47]	Human	↓COX-2 ↓MMP-2 (trend) ↓MART-1 (trend) ↓MMP-1 (trend) ↓MMP-9 (trend) ↓Persistent pigment darkening ↓Delayed tanning
Truchuelo et al. [48]	Human	↓MMP-1

COX2 cyclooxygenase 2, *MART-1* melanoma antigen recognized by T cells 1, *MMP* matrix metalloproteinase

showed similar morphological findings; however, these were not observed until 24 h after irradiation. PLE also decreased MMP-1 expression compared to that seen in non-PLE treated cells, decreased cell death rates to levels of non-irradiated control cells, and trended toward a decrease in expression of cathepsin-K, a lysosomal cysteine protease that degrades collagen [46]. Fibrillins, structural components of extracellular microfibrils, generally increased with PLE treatment after VL exposure. When compared to control non-irradiated fibroblasts, a significant increase in elastin expression was noted in irradiated fibroblasts, PLE-treated non-irradiated fibroblasts and PLE-treated irradiated fibroblasts. Elastin expression was highest in the highest PLE treatment group. These results indicated that PLE helps prevent VL-induced fibroblast damage, reduces VL-induced ECM degradation enzyme expression and minimizes alterations in VL-induced ECM protein expression [45].

3.2 In vivo (human)

Mohammad et al. evaluated the effects of oral PLE on VL-induced photoaging markers. In this prospective clinical trial, 22 volunteers with Fitzpatrick skin types IV through VI were irradiated on their backs with VL doses up to 480 J/cm^2 after administration of 480 mg of PLE per day for 28 days. A statistically significant decrease in COX-2 was reported in PLE treatment skin compared to that irradiated without PLE ($p=0.027$). A strong trend towards reduction in MMP-2 was noted, along with a weak trend towards reduction in melanoma antigen recognized by T cells 1 (MART-1), a melanocytic marker used to assess pigmentation, MMP-1, and MMP-9. Spectroscopic assessments demonstrated a decrease in post-PLE persistent pigment darkening and delayed tanning. These findings reflected the ability of PLE to mitigate VL-induced inflammation, structural integrity and pigmentation [47].

Truchuelo et al. investigated the effect of oral PLE on MMP-1 after irradiation with VL and infrared (IR). In this prospective clinical trial of 7 volunteers, gluteal biopsies were performed on skin irradiated with a single dose of VL (200 J/cm^2) + IR (600 J/cm^2) before and after taking a total oral dose of 960 mg per day of PLE for 21 days. A statistically significant global reduction of MMP-1 expression was noted by 52%, reflecting PLE's ability to mitigate photoaging-related ECM degradation [48].

4 Discussion

Many of the photoprotective effects of PLE in the studies described above are related to its ability to prevent UV and VL-induced ECM degradation by regulating factors involved in ECM remodeling. PLE has been shown to stimulate tissue inhibitor of metalloproteinases (TIMPs), TGF-β and Type I, III and V collagen expression in fibroblasts [28] In addition to decreasing MMP-1 expression in keratinocytes and MMP-2 in fibroblasts, PLE also inhibits levels of MMP-1, 3, and 9 enzymes themselves [10, 28]. PLE-induced reinforcement of the dermal elastic fiber network in mouse skin and increased elastin expression in VL-irradiated fibroblasts support PLE's potential photoprotective role in counteracting photo-induced elastosis [45, 49]. PLE is well-known for its antioxidative properties which likely also contribute to ECM homeostasis. Rich in polyphenols, such as ferulic and caffeic acid, *P. leucotomos* has been found to scavenge ROS and inhibit UV radiation-induced ROS [50]. In vivo, PLE increases catalase and glutathione peroxidase activity in mice skin [39]. PLE also increases the levels of plasma antioxidants, such as superoxide dismutase (SOD), glutathione S-transferase (GST), and GSH peroxidase (GPx) which likely provides a reservoir of antioxidants to the skin [49, 51, 52].

PLE's anti-inflammatory properties are demonstrated through its inhibition of UV-induced immunosuppression and inflammatory cells, likely leading to further inhibition of ROS and generation of prostaglandin metabolites, such as COX-2 [22, 26, 32]. The decrease in UVR-induced TNF-α, NO, iNOS, NF-kB and AP1 in keratinocytes by PLE are a reflection of its anti-inflammatory anti-apoptotic effects [35, 53, 54]. PLE may also exert its photoprotective effects via the prevention of UV-induced cytoskeletal derangements [31]. In addition, the effect of PLE on phototoxicity and environmental pollutants via the NFR2 antioxidant pathway evidence a possible mechanism by which PLE may protect against other environmental insults that can cause skin aging [36].

Oral PLE is generally well tolerated with minimal adverse effects [51, 52]. The ability of PLE to inhibit the deleterious effect of UV-exposed TiO_2 on *t*-UCA and UVA/TiO_2-induced fibroblast cell death mediated by ROS generation may make it an appropriate adjunct to use in sunscreen formulations [32]. While the studies reviewed above demonstrate several photoprotective properties of PLE, most were done in vitro or with animals. The amount and modes of delivery of PLE as well as doses and forms of radiation differed among studies. Very few studies have been done related to PLE and VL-induced photoaging. Clearly, future large-scale studies assessing the photoprotective effects of oral and topical PLE in humans using natural sunlight over longer periods of time are warranted.

5 Conclusion

P. leucotomos may protect against UV and VL-induced photoaging of the skin through regulation of enzymes involved in ECM remodeling, upregulation of ECM

proteins, downregulation of inflammation and ROS due to its anti-inflammatory and antioxidant properties and cytoprotective effects on fibroblasts and keratinocytes. *P. leucotomos* extract is a well-tolerated and safe agent with photoprotective properties, making it potentially a suitable adjunct in combating UVR and VL-induced skin aging.

Author contributions All authors whose names appear on the submission: 1. Made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; 2. Drafted the work or revised it critically for important intellectual content; 3. Approved the version to be published; and; 4. Agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Code availability Not applicable.

Declarations

Conflict of interest Henry W. Lim has served as investigator/co-investigator for research studies sponsored by Incyte, L'Oréal, Pfizer, and PCORI; he has served as a consultant for Pierre Fabre, ISDIN, Ferndale, Galderma, La Roche-Posay, and Beiersdorf; he has been a speaker on general education sessions for La Roche-Posay, and Cantabria Labs. Iltefat Hamzavi has served as an advisory board member for AbbVie; a consultant for Incyte, Pfizer, and UCB; a principal investigator for AbbVie, Allergan, Bayer, Clinuvel Pharmaceuticals, Este Lauder, Ferndale Laboratories, Galderma Laboratories LP, GE Healthcare, Incyte, Janssen, Janssen Biotech, Johnson & Johnson, Lenicura, LEO Pharma, Pfizer, and Unigen; a subinvestigator for Amgen, Bristol-Myers Squibb, Foamix Pharmaceuticals, and Janssen; president of the HS Foundation; and co-chair of the Global Vitiligo Foundation. Indermeet Kohli is an Investigator for Ferndale, Estee Lauder, L'Oreal, Unigen, Johnson and Johnson, Allergan and Bayer with grant received by the institution and is a Consultant for Pfizer, Johnson and Johnson, and Bayer with fee and equipment received by the institution. Aunna Pourang is a subinvestigator for Pfizer, Biofrontera and L'Oreal. Nneamaka Ezekwe is a subinvestigator for Pfizer, Biofrontera and L'Oreal. Mohsen Dourra has no conflicts of interest or disclosures.

Ethical approval Not applicable.

Humans and animals rights Not applicable.

Consent to participate Not applicable.

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