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POLYPODIUM LEUCOTOMOS EXTRACT: A NUTRACEUTICAL WITH PHOTOPROTECTIVE PROPERTIES

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Summary

Ultraviolet (UV) irradiation causes multifaceted damage to the skin and adjacent tissue layers, and is one of the leading causes of premature skin aging, immunosuppression and carcinogenesis. Photoprotection can be achieved by the use of sunscreens and also by systemically administered compounds that fight the deleterious biological effects of UV exposure, or preferably both. In this review, we summarize the current knowledge on the tissue, cellular and molecular mechanisms underlying the photoprotective effect of *Polypodium leucotomos* fern extract. *P. leucotomos* blocked the deleterious effect of UV irradiation both *in vivo* and *in vitro*. The molecular basis of photoprotection relies on its ability to inhibit free radical generation, prevent photodecomposition of both endogenous photoprotective molecules and DNA, and prevent UV-induced cell death. Its complete loss of toxicity combined with its multifactor protection makes it a valuable tool not only for direct photoprotection, but also as an efficacious adjuvant to phototherapy of various skin diseases.

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Introduction

The search for natural compounds with beneficial properties is as old as research itself. This search was focused on mitigating or preventing the deleterious effects of different diseases or external aggressions. Among these, sunburn is one of the

most obvious manifestations of aggression from natural sources. Sunburn causes inflammation, pain and itching, and has long-term deleterious effects, including skin cancer.

Indigenous tribes from Central and South America have used different plant extracts as part of their folk medicine to alleviate the symptoms of inflammatory diseases of the skin. Among these, *Polypodium leucotomos* (from the Greek *poly* meaning *many* and *podia* meaning *little feet*, based on the foot-like appearance of the rhizome and branches) is a tropical fern plant of the *Polypodiaceae* family (1). It is native to the plateaus of the Andes range in South America and elevated (700–2,500 meters) areas of Central America, including Honduras and Guatemala. It was first introduced in Europe after the return of the botanical expedition of Ruiz, organized and funded by the Spanish Crown (2). *P. leucotomos* has long been known to possess beneficial properties for the skin (3). However, direct application of the plant itself in cases of psoriasis and atopic dermatitis yields scarcely significant results, probably due to a low concentration of the active principle(s) involved in its beneficial effect. More recently, a purified hydrophilic extract of the leaves of *P. leucotomos* has been shown to reduce skin sensitivity to damaging UV radiation and to possess a wide array of beneficial effects in the prevention of sunburn and related processes.

In this review, we summarize our current knowledge on the composition, pharmacological properties and reported effects of *P. leucotomos*, focusing on the molecular mechanisms involved in photoprotection from UV light.

The need for photoprotection: Effect of UV radiation on skin

Ultraviolet (UV) radiation forms part of the electromagnetic spectrum, including wavelengths between 200 nm and 400 nm (4). It is usually divided into three further components, UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm). UVC, the most energetic of the UV light spectrum, is also the most harmful for the skin, but it is completely absorbed by ozone in the stratosphere. In fact, no wavelengths under 290 nm reach the Earth's surface (5). UV exposure of the skin has a number of biological effects, most of them detrimental (Table I). These include the following:

i. Local generation of reactive oxygen species (ROS) and other deleterious metabolites. Exposure of the skin to UV radiation results in generation of reactive oxygen species (6, 7). ROS are normally removed by cellular antioxidant systems, which prevent the deleterious effects of ROS and establish a pro-oxidant/antioxidant balance. Chronic exposure to elevated levels of UV light causes accumulation of an excess of free radicals that results in a cascade of events leading to deterioration of cellular integrity and function due to DNA damage and aberrant gene expression (see below), which leads to tumor formation and premature aging. ROS damages cell membranes by peroxidation of fatty acids that constitute the inner structure of the membrane, and it generates lipid peroxide radicals, lipid hydroperoxides and other byproducts that amplify and perpetuate oxida-

Table I: Deleterious effects of UV light on the skin.

	Mediator	Short-term effects	Long-term effects
Generation of free radicals	ROS, RNS		Photoaging, cancer, immunosuppression
Generation of other deleterious metabolites	UCA photoisomerization, *t*-photodecomposition		Photoaging, immunosuppression
Inflammation	Cell death, apoptosis	Sunburn erythema, pain, itching	Photoaging, cancer
DNA damage	Pyrimidine dimers (mainly thymine-thymine) and pyrimidine-pyrimidone		Tumorigenesis, cancer
Immunosuppression	Depletion of eLC	Susceptibility to infection	Chronic inflammatory diseases, cancer

ROS = reactive oxygen species; RNS = reactive nitrogen species; UCA = urocanic acid; eLC = epidermal Langerhans cells.

tive damage (6). Other deleterious metabolites induced by UV light include *cis*-urocanic acid (*c*-UCA), which appears as a product of UV-induced photoisomerization of *trans*-urocanic acid (*t*-UCA). *t*-UCA is produced by deamination of the amino acid histidine and is a photoprotective molecule occurring naturally in the skin, due to its ability to absorb UV photons (8). *c*-UCA has been involved in immunosuppression due, at least partially, to its effect on epidermal Langerhans cells (9), and also induces abnormal mast-cell degranulation (10).

ii. Inflammation. UV-induced erythema is a process, due to increased blood flow and vasodilation (11), in which nitric oxide and prostaglandins are key players (12, 13). UV irradiation also induces sunburn of keratinocytes and underlying fibroblasts, which is actually the result of UV-induced apoptosis (14). Other cells of the skin with critical functions in the homeostasis of the skin, such as epidermal Langerhans cells, are very sensitive to UV-induced apoptosis (15). In addition, inflammation causes neutrophil infiltration, which contributes to physical damage due to secretion of ROS and pro-inflammatory cytokines (16).

iii. Ultraviolet damage to DNA. The heterocyclic bases of the DNA make up the major UVB-absorber chromophore present in skin. Such absorption results in DNA damage due to the formation of pyrimidine dimers (mainly thymine-thymine) and pyrimidine-pyrimidone photoproducts (17, 18). These products have been proposed as initial steps in mutagenesis and tumor formation (19). In addition, DNA damage has been linked to systemic immunosuppression (20).

iv. Immunosuppression. UV light causes a wide array of immunosuppressive responses, including the transition from immunologically competent to tolerogenic Langerhans cells, which results in clonal anergy of Th1 cells (21).

In summary, UV irradiation on the skin results in immediate burns, inflammation and local immunosuppression, as well as long-term effects such as premature aging and tumor development. These facts reinforce the need for the development of photoprotective measures. Such investigations are currently following two routes: the development of progressively more efficient sunscreens and of nontoxic substances that can fight the deleterious effects of UV irradiation. *P. leucotomos* lies in both categories, providing sunscreen protection through the presence of efficient UV blockers in its formulation, and also inhibiting some of the molecular mechanisms that cause UV-induced damage in skin.

P. leucotomos: Molecular composition and pharmacology

The fact that *P. leucotomos* is a natural extract from fern leaves prevents the analysis of its minute composition. However, mass spectrometric analysis revealed that its main constituents are monosaccharides (mainly fructose and glucose); quinic, shikimic, glucuronic and malic acids; and a high proportion of phenolics (22). Most of these phenolics belong to the family of benzoates and cinnamates. These nonflavonoid catecholic compounds have anti-inflammatory, antimutagenic and anticarcinogenic properties (23–26). These effects rely on their intrinsic antioxidant capability (27). Some of them (caffeic and ferulic acids) are naturally conjugated to the saccharidic moiety of *P. leucotomos*, which provides additional stability (28). Caffeic and ferulic acids prevent UV-mediated peroxidation by inhibiting propagation of the lipid peroxidative chain reaction, and also react with nitrogen oxides (29). In addition, ferulic acid is a strong UV photon absorber (30). Both caffeic and ferulic acids are effective in protecting human skin from UVB-induced erythema and are thus employed in the chemical formulation of different skin lotions and sunscreens (31).

Dose-response and kinetics experiments revealed that these phenolics were readily absorbed and completely metabolized 24 hours postingestion. Absorption of most of these compounds is very efficient (70–100%). We found that coumaric, ferulic and vanillic acids were metabolized efficiently (half-life ~4–6 hours) by CYP450-dependent mono-oxygenases and partially conjugated to glucuronic acid and sulfate (32, 33). Such conjugates appear in serum and thus are active reporters of metabolization and absorption of orally ingested phenolics through the intestinal barrier (34). Due to the readiness of *P. leucotomos* absorption through the skin, it is also used topically with good results (35).

Regarding the toxicity profile of *P. leucotomos*, it has been shown that: i) *P. leucotomos* is not mutagenic, as shown by employment of the Ames test (*in vitro* reverse mutation assay) (36); ii) oral acute toxicity as defined in the Organisation for Economic Co-operation and Development guide-

lines (37) yielded negligible results even at high doses of *P. leucotomos* in both rats and mice (LD_{50} >2 g/kg weight); and ii) repetitive treatment (28 and 90 days) has no long-term toxicity (Escario *et al.,* unpublished results).

Evidence for the photoprotective effect of *P. leucotomos*

Classic studies on this and similar fern extracts attributed antitumoral properties to them (38, 39), although their mechanism of action was never properly investigated. Follow-up studies focused on their anti-inflammatory properties, especially regarding skin-related diseases such as psoriasis (40–42), vitiligo (43) and atopic dermatitis (44). The molecular basis for this anti-inflammatory effect relies at least partially on the immunomodulatory capability of these extracts (see next section).

However, classic references pointed out that the native population from Central and South America also used extracts from these ferns to prevent skin inflammation (2). This suggested that these extracts might exert a photoprotective effect. Proof of *P. leucotomos*–induced photoprotection was reported in 1996, when guinea pigs were exposed to UVB light in the presence of placebo or topically applied *P. leucotomos*, resulting in almost complete photoprotection. *P. leucotomos* was also efficacious when a smaller group of human volunteers was exposed to limited UVA light in the presence of *P. leucotomos* (45). These results encouraged the authors to extend their findings. A second study with a larger group of human volunteers demonstrated the efficacy of *P. leucotomos* in the prevention of acute sunburn (35). In this study *P. leucotomos* also reduced significantly the phototoxic effects of exposure to sunlight after oral ingestion of psoralens. This would provide a critical advantage in phototherapy protocols used for the treatment of various skin disorders, *i.e.,* psoriasis. Psoralens-UVA therapy is a very successful treatment for psoriasis, but the fact that it can induce skin cancer limits its application, especially in the cases of subjects with skin phototypes I and II, which are prone to redness, irritation and increased photoaging (46–48). In addition, this treatment causes hyperpigmentation, which makes it necessary to increase the dose of UVA light, and thus exacerbates phototoxicity and photoaging (49). Preliminary data suggested that *P. leucotomos* was efficacious in reducing phototoxicity induced by exposure to UV light and coadministration of psoralens (35) (Fig. 1). This was confirmed

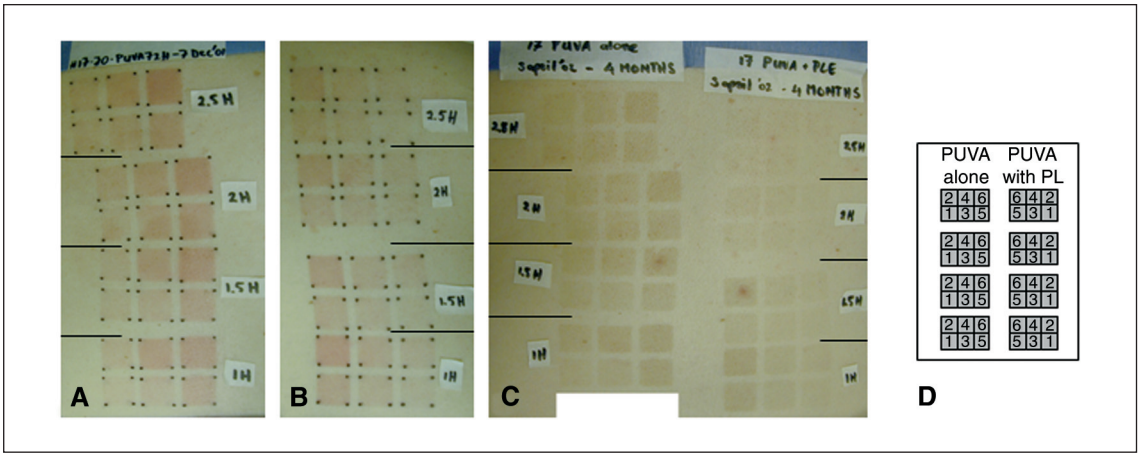

Fig. 1. Pictures of one subject. A: Phototoxic reaction 48 hours after exposure to psoralens-UVA (PUVA) alone. B: Phototoxic reaction after 48 hours of psoralens-UVA with *Polypodium leucotomos*. C: Pigmentation reaction four months after exposure, showing skin sites exposed to psoralens-UVA alone on the left side and skin sites exposed to psoralens-UVA with *P. leucotomos* on the right side. Pictures show decreased erythema and pigmentation in *P. Leucotomos*–treated skin, indicating that *P. leucotomos* decreases the phototoxic and pigmentary response. D: Schematic representation of skin sites in the pictures. Numbers in squares indicate relative UVA intensities of each site (1 = lowest UVA intensity, 6 = highest UVA intensity). Sites exposed to psoralens-UVA without and with *P. leucotomos* are mirrored. Reproduced from ref. 51 with permission from Elsevier, Inc. © 2004.

in an open-label study aimed to determine the beneficial effect of the coadministration of *P. leucotomos* in subjects undergoing psoralens-UVA therapy (50). Orally administered *P. leucotomos* greatly decreased sunburn and mast cell infiltration in the skin, and reduced the loss of epithelial Langerhans cells of the skin associated to these treatments (51). These results together with the lack of toxic effects strongly support the use of *P. leucotomos* as a cotreatment during psoralens-UVA therapy and other phototherapy protocols.

The clinical value of *P. leucotomos* correlates with its histological effect on skin. *P. leucotomos* consistently decreased UV-induced histological damage, characterized by maturation disarray, microvesiculation and keratinocyte vacuolization. Compared to control (UV-irradiated skin), *P. leucotomos*–treated and irradiated skin showed fewer sunburn cells, decreased levels of cyclobutane pyrimidine dimers (an indicator of UV-induced DNA damage) and epidermal cell proliferation, as well as decreased numbers of infiltrating mast cells and neutrophils (52, 53) (Fig. 2).

Other research has aimed to study the photoprotective effect of *P. leucotomos in vitro*. In one of these studies, *P. leucotomos* efficiently blocked UVA-induced fibroblast and keratinocyte cell death, and restored proliferation. At the cellular level, *P. leucotomos* inhibited the disorganization of the actin cytoskeleton and adhesive cell–cell and cell–matrix contacts induced by UV light (54). *P. leucotomos* also inhibited protease secretion induced by UV irradiation, and improved the integrity of the membranes of irradiated cells (55). Together, these *in vitro* studies complement the *in vivo* and clinical findings, and demonstrate that the photoprotective effect of *P. leucotomos* occurs at both cellular and systemic levels.

It seems that the photoprotective effects of *P. leucotomos* and similar extracts are related to their anticarcinogenic properties. At the molecular level, *P. leucotomos* has been shown to inhibit UV-induced damage to DNA (53), which is a long-term risk factor in skin cancer (56). Consistent with this, *P. leucotomos* inhibited the appearance of skin tumors after irradiation with UVB light in hairless albino mice (57).

Together, these reports strongly support the photoprotective role of *P. leucotomos* and justify its employment in sunscreen lotions, ointments and even as a complementary oral supplement, as well as its use as a cotreatment in phototherapy. The oral commercial formulation of *P. leucotomos* (*Heliocare*®; IFC S.A., Madrid, Spain, and Baker Cummins Dermatologicals, Miami, FL, USA) has been recently approved for distribution in the United States, and whereas it is not a substitute for sunscreens, it prevents sunburns and limits the damage caused by exposure to UV radiation, adventing a new generation of oral compounds aimed to complement the use of sunscreen formulations. As such, it has been welcomed by both the scientific community and the general public.

Molecular basis of photoprotection

As seen in the previous section, the photoprotective effects of *P. leucotomos* are well documented in different *in vivo* and *in vitro* systems. In this section, we outline the mechanisms by which *P. leucotomos* induces photoprotection and prevents photoaging (Table II).

i. Direct antioxidant activity. *P. leucotomos* contains a moiety of nonflavonoid phenolics with proven antioxidant activity, especially members of the hydroxycinnamic acid family (32). These prevent UV-mediated peroxidation by inhibiting propagation of the lipid peroxidative chain reaction (29). In addition, *P. leucotomos* can scavenge ROS, a causal agent of aging and various diseases, including cancer, heart disease and autoimmune disorders (58–61). In a free radical scavenging study, *P. leucotomos* was found to efficiently scavenge different ROS, including superoxide anion ($^{\bullet}O_2$), hydroxyl radicals ($^{\bullet}OH$), singlet oxygen ($^1O_2$) and H_2O_2, however to a lesser extent (62). This is an important feature of *P. leucotomos*. ROS are invariably produced in aerobic environments through different mechanisms, such as electron "leakage" during biological oxidation, the action of flavin dehydrogenases and membrane-associated secretions, and by UV-induced activation of oxygen. Thus, ROS scavenging prevents the deleterious effects of local accumulation of ROS.

ii. Inhibition of photoisomerization and photodecomposition of *t*-UCA. *t*-UCA is the main byproduct of histidine metabolism, and it is endowed with photoprotective properties and ROS-scavenging capability (63). Absorption of UV photons induces its isomerization to *c*-UCA, thus UV photons do not damage other structures. *P. leucotomos* was shown to inhibit *t*-UCA photoisomerization and the appearance of *c*-UCA in a dose-dependent fashion in the pres-

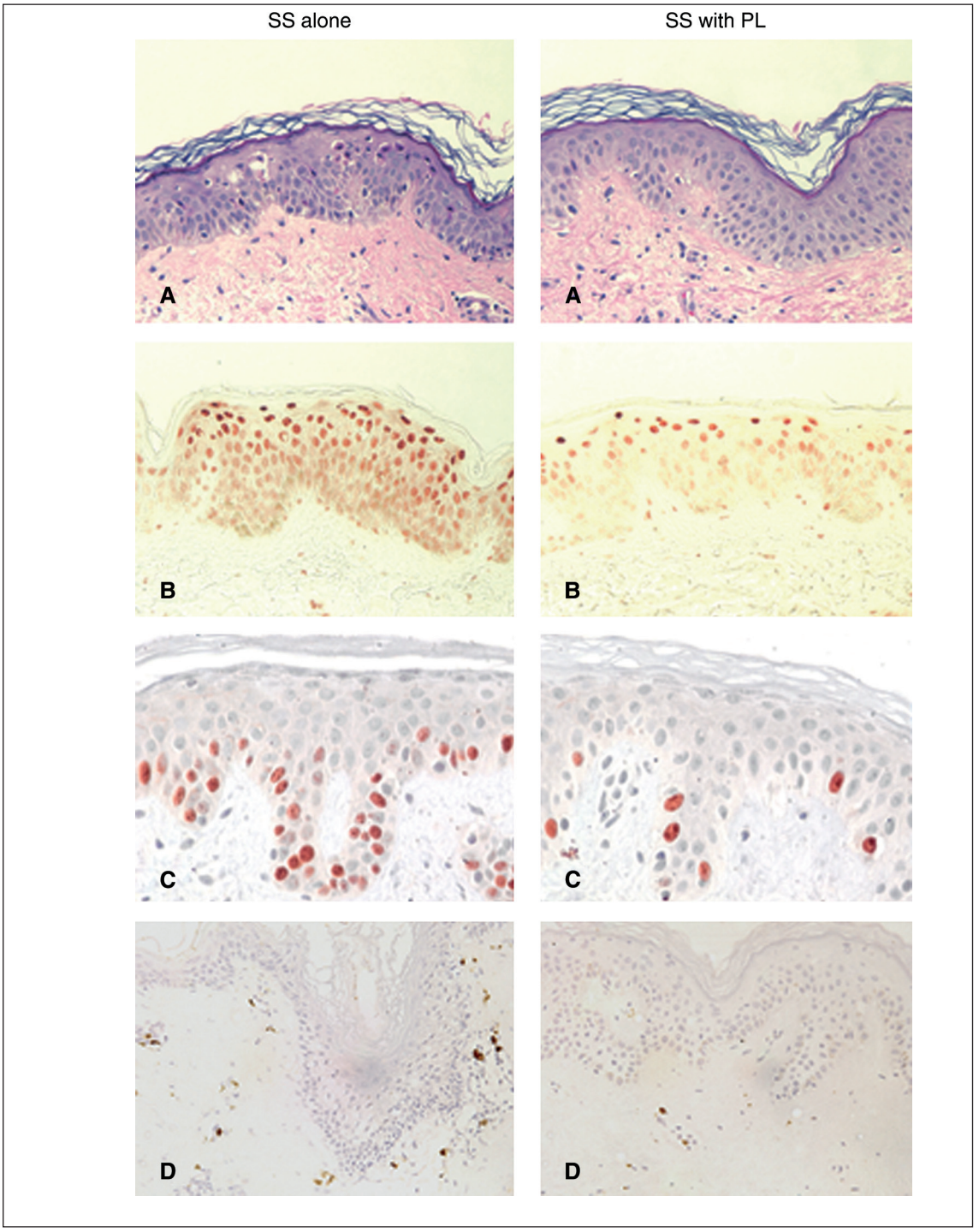

Fig. 2. Histology from paired biopsies of skin treated with ultraviolet radiation alone (left columns) and with *P. leucotomos* (right columns). *P. Leucotomos*–treated skin shows the following: fewer sunburn cells, maturation disarray, microvesiculation and vacuolization (A); fewer cyclobutane pyrimidine dimers (B); less epidermal proliferation (C); and less dermal mast cell infiltration (D). Reproduced from ref. 53 with permission from Elsevier, Inc. © 2004.

Table II: Beneficial effects of P. leucotomos.

On...	Molecular effects	Short-term effects	Long-term effects	References
Generation of free radicals	Inhibits ROS generation		Relieves photoaging, prevents tumor formation	(32, 62, 75)
Generation of other deleterious metabolites	Inhibits *t*-UCA photoisomerization, photodecomposition		(Predicted) Relieves immuno-suppression and tumor formation	(64)
Inflammation	Inhibits cell death and apoptosis	Reduces erythema, sunburn*	Relieves photoaging, prevents tumor formation	(35, 45, 50, 51, 53)
DNA damage	Decreases the formation of pyrimidine dimers	Reduces SOS response	(Predicted) Inhibits tumorigenesis	(53)
Immunosuppression	Prevents depletion of eLC	(Predicted) Reduces recurrent infections	(Predicted) Inhibits chronic inflammation and cancer	(51, 53, 73, 74)

*Oral administration of *P. leucotomos* (1,080 mg) increases skin tolerance to sunlight by a factor of 2.81 (48). ROS = reactive oxygen species; UCA = urocanic acid; SOS = the SOS response: the depression of multiple genes encoding DNA repair proteins, leading to more rapid DNA repair and enhanced bacterial survival; eLC = epidermal Langerhans cells.

ence of H_2O_2 (64). In addition, UV photons in the presence of ROS and a catalyst such as TiO_2 lead to the formation of hydroxyl radicals and other ROS, which are responsible for the inactivation of enzymatic systems required for homeostasis of the skin (65–68). This process also generates oxidative breakdown metabolites of *t*-UCA that participate in skin immunosuppression (8, 63, 69, 70). This may be an important side effect of many sunscreen lotions, since TiO_2 is used in formulations. *P. leucotomos* efficiently inhibited the breakdown of *t*-UCA and horseradish peroxidase inactivation when coincubated with TiO_2 and irradiated with UV light (64). Together, these results demonstrate the efficacy of *P. leucotomos* as an inhibitor of UV-induced *t*-UCA photoisomerization and photodecomposition, and also in the prevention of the generation of oxidative metabolites catalyzed by TiO_2.

iii. Immunoregulation. The immunoregulatory effect of *P. leucotomos* and other fern extracts has been thoroughly characterized. *P. leucotomos* modulates inflammation through the control of Th1/Th2 responses and prevents immunosuppression caused by depletion of costimulatory cells on the skin. *P. leucotomos* blocks the depletion of Langerhans cells in UV-irradiated skin, a major cause of UV-dependent immunosuppression (51, 53). Depletion of epidermal Langerhans cells by UV irradiation is induced by the combination of direct apoptosis, inflammation, induction of an aberrant morphology (9) and inhibition of the expression of adhesion molecules required for the migration of epidermal Langerhans cells to the skin (71, 72). *P. leucotomos* not only prevented Langerhans cell depletion but also abolished UV-dependent changes in the morphology of Langerhans cells (63). Similar results were obtained using blood dendritic cells irradiated using a solar simulator. In this system, *P. leucotomos* inhibited dendritic cell apoptosis and restored the secretion of anti-inflammatory cytokines by irradiated dendritic cells (73). In addition, expression of IL-10 and TGF-β induced by *P. leucotomos* may account for limitation of the response of other accessory cells, such as macrophages (74).

Conclusions

Originally described as a natural alternative for the treatment of inflammatory skin disorders such as psoriasis, *P. leucotomos* is a more powerful tool than previously thought. It combines extremely low toxicity with proven beneficial effects, even with oral administration. These include antioxidant properties, the capability to inhibit *t*-UCA photoisomerization (which makes it a very valuable additive in sunscreen formulations), and the ability to block UV-induced apoptosis and DNA photodamage and immunosuppression. It seems clear that *P. leucotomos* is not an alternative to traditional sunscreen use, but it provides a very effective complement for sensitive skin phototypes and adds extra protec-

tion in cases in which exposure to UV radiation cannot be avoided, such as those in UVB phototherapy and psoralens-UVA treatments.

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