



**PLANT AND ANIMAL DERIVED AGENTS AS ORAL
PHOTOPROTECTION: A SYSTEMATIC REVIEW OF
CLINICAL STUDIES**

BY

MISS CHAVANATDA CHANYASAK

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN DERMATOLOGY
CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE
THAMMASAT UNIVERSITY
ACADEMIC YEAR 2016
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THESIS

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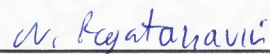
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PLANT AND ANIMAL DERIVED AS ORAL PHOTOPROTECTION: A
SYSTEMATIC REVIEW OF CLINICAL STUDIES

was approved as partial fulfillment of the requirements for
the degree of Master of Science (Dermatology)

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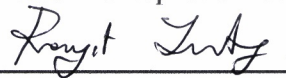
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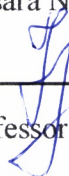
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ABSTRACT

Background: To be effective in protecting against the harmful effects of ultraviolet radiation (UVR), many photoprotective strategies have been used. Inadequate physical protection, amount of topical application and allergic reactions to topical agents are limitations associate with current photoprotective strategies. Systemic agents are an emerging alternative, providing promising protection against solar radiation.

Objective: Our primary objective is to systematically review photoprotective outcomes of oral plants and animals derived agents from available clinical studies by using evidence-based method and our secondary objective is to summarize the possible mechanisms of individual plants and animals derived agents as oral photoprotection from available clinical studies.

Methods: PubMed EBSCO and Cochrane databases were systematically searched for randomized controlled trials (RCTs), evaluating outcomes of the systemic natural product-based agents.

Results: Total 22 clinical trials with 800 participants were identified and organized into two categories. Plant-derived agents include golden serpent fern, green tea, cocoa bean, tomato, beta-carotene and vitamin E, while animal-derived products consist of nicotinamide, and omega-3 polyunsaturated fatty acids.

Conclusion: Systemic plant and animal-derived photoprotective agents may be a promising alternative in addition to conventional photoprotection. To better determine its effectiveness and roles, further extensive clinical trials are required.

Keywords: Ultraviolet rays, systemic photoprotection, oral sunscreen, systematic review



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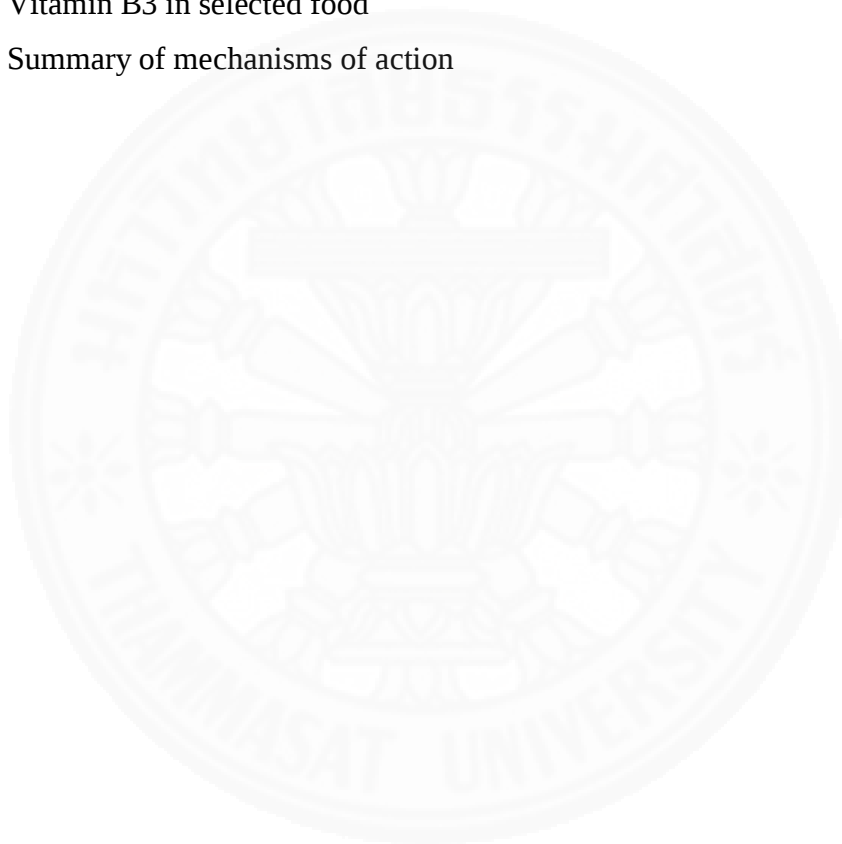
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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
α -Tocopherol	Alpha-tocopherol
β -carotene	Beta-carotene
8OHdG	8-hydroxy-2'-deoxyguanine
ALA	Alpha-linolenic acid
ATP	Adenosine triphosphate
BC	Benzylidene camphor
BCC	Basal cell carcinoma
BMDBM	Butyl methoxydibenzoylmethane
BP	Benzophenone
CPDs	Cyclobutane pyrimidine dimers
c-UCA	Cis-urocanic acid
DHA	Docosahexaenoic acid
DTS	Drometrizole trisiloxane
EHMC	Ethylhexyl methoxycinnamate
EC	(-)-epicatechin
ECG	(-)-epicatechin-3-gallate
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin-3-gallate
eLC	Epidermal Langerhans cells
EM	Electromagnetic
EPA	Eicosapentaenoic acid
EPR	Electron paramagnetic resonance
GTC	Green tea catechins
GTE	Green tea extract
H ₂ O ₂	Hydrogen peroxide
HaCaT	Human keratinocyte
HFC	High flavanol cocoa

IPD	Immediate Pigment Darkening
IR	Infrared
KC	Keratinocyte cancer
LC	Langerhans cell
LFC	Low flavanol cocoa
LXR	Liver X receptors
MAPK	Mitogen-activated protein kinase
MBBT	Methylene bisbenzotriazolyl tetramethylbutylphenol
MBC	Methylbenzylidene camphor
MED	Minimal erythema dose
MeSH	Medical section Heading
MIE	Mantoux-induced erythema
MMP	Matrix metalloproteinase
MPPD	Minimal Persistent Pigment Darkening
mtDNA	Mitochondrial DNA
n-3 PUFA	Omega-3 polyunsaturated fatty acids
NAD	Nicotinamide adenine dinucleotide
NHEK	Normal human epidermal keratinocytes
$^1\text{O}_2$	Singlet oxygen
$\cdot\text{O}_2$	Superoxide anion
OA	Oleic acid
OCR	Octocrylene
OH	Hydroxyl radicals
OMC	Octyl methoxycinnamate
PA	Protection grade of Ultraviolet A
PABA	Para-aminobenzoic acid
PARP-1	Poly-ADP-ribose polymerase 1
PDT	Photodynamic therapy
PGE2	Prostaglandin E2
PL	Polypodium leucotomos
PLE	Polypodium leucotomos extract

PMMA	Polymethylmethacrylate
PUVA	Psoralens+UVA
PPAR	Peroxisome proliferator-activated receptors
PPD	Persistent Pigment Darkening
PTFE	Teflon
RF	Radical formation ratio
ROS	Reactive Oxygen Species
RXR	Retinoid X receptors
SCC	Squamous cell carcinoma
SDH	Succinate dehydrogenase
SPF	Sun Protection Factors
SREBP	Sterol regulatory element-binding proteins
TDSA	Terephthalylidene dicamphor sulphonic acid
TIMP	Tissue inhibitor of metalloproteinase
TiO ₂	Titanium dioxide
TNF- α	Tumor necrosis factor alpha
<i>t</i> -UCA	Trans-urocanic acid
UCA	Urocanic acid
UIE	UV-induced erythema
USPF	Universal Sun Protection Factor
UVA	Ultraviolet A
UVAPF	Ultraviolet A Protection Factor
UVB	Ultraviolet B
UVC	Ultraviolet C
UVR	Ultraviolet Radiation
VL	Visible Light
ZnO	Zinc oxide

CHAPTER 1

INTRODUCTION

As interest in physical fitness and outdoor recreational activities rises, exposure to sunlight is unavoidable. Vitamin D3 photosynthesis and treatment of skin conditions are main beneficial effects of exposure to sunlight. However, many harmful effects are present as well. Acute effects include delayed tanning and sunburn. Chronic effects include photoaging and squamous cell carcinoma. Avoiding sun exposure altogether is unnecessary and impractical to the general public. Instead, seeking shelters and shades, avoiding ultraviolet B (UVB) during peak hours of 10.00 – 14.00 and using photoprotective measures such as clothings, hats, sunglasses, umbrellas, sunscreens and oral supplements are more suitable. There are continuous improvements in photoprotective measures as people seek a protective method that will better suit their lifestyle with less limitation.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Spectrum of Ultraviolet Radiation (UVR)

Electromagnetic radiation can be divided into different types including gamma-rays, X-rays, ultraviolet light, infrared radiation, microwaves, radio waves. Originating from the sun, UV light is invisible to the eye, allowing for black light glows. UVR cause sunburns and skin tans, with excessive exposure resulting in damages to living tissues.

The transmission of electromagnetic radiation occurs at different frequencies and wavelengths through waves or particles in a broad range generally known as electromagnetic (EM) spectrum. In order of increasing energy and frequency, and decreasing wavelengths, EM spectrum is commonly divided into seven regions. Radio waves, microwaves, infrared (IR), visible light (VL), ultraviolet (UV), X-rays and gamma-rays are common designations.

Falling in the EM spectrum range, UV light is in between VL and X-rays. UV is commonly categorized into three different types, UVA, or near UV (320–400 nm); UVB, or middle UV (280–320 nm); and UVC, or far UV (180–280 nm). “Vacuum or extreme UV” are often referred to radiations with wavelengths in the range of 10 nm to 180 nm (1), being blocked by air and only disseminate in vacuum.

UVR has many sources both naturally and artificially. Natural sources include the sun and artificial sources include black lights, halogen lights, high-intensity discharge lamps, curing lamps, mercury vapor lamps, tanning booths, fluorescent, incandescent sources and various type of lasers, including nitrogen laser, excimer lasers. Depending on the range of the wavelength and sources, unique hazards applies to the emitted UVR.

UVC (180-280 nm) is fully absorbed by the atmosphere, similarly to Far UV and Vacuum UV and is almost never seen in nature. Through its abilities to kill bacteria, UVC is artificially emitted using germicidal lamps. UVC is absorbed in the outer dead layers of the epidermis in humans.

UVB (280-320 nm) is usually regarded as one of the harmful UV radiation due to its energy causing photochemical damage on a cellular DNA level but not enough for the atmosphere to completely absorb it. UVB is essential in the process of vitamin D synthesis for humans. Nonetheless, UVB has many harmful effects including, erythema (sunburn), cataracts and skin cancers. Outdoor workers are at a higher risk of being affected by UVB. On the other hand, UVB does not pass through glass, deeming a lower risk for indoor workers. As most UVB are absorbed by the ozone layers of the atmosphere, the depleting ozone layers post new concerns for the prevalence of skin cancer.

UVA (320-400 nm) is the form of UV light that is generally most encountered upon. It has been recognized and sub divided into two different ranges for the UVA, UVA2 (320-340 nm) and UVA1 (340-400 nm). Effects of UVA exposure include pigment-darkening, and erythema. The ozone layers of the atmosphere absorbs very little in this range of UV spectrum. Epidermal hyperplasia, cataract formation, suppression of the immune system are some of the effects as a result of overexposure to UVA. Another designation for UVA light is black light. Artificial UVA are usually in the form of UVA lamps used for phototherapy and in tanning booths. Outdoor and indoor workers face an equal risk of UVA effects as UVA penetrates through glass. **(Figure 2.1) (Table 2.1)**

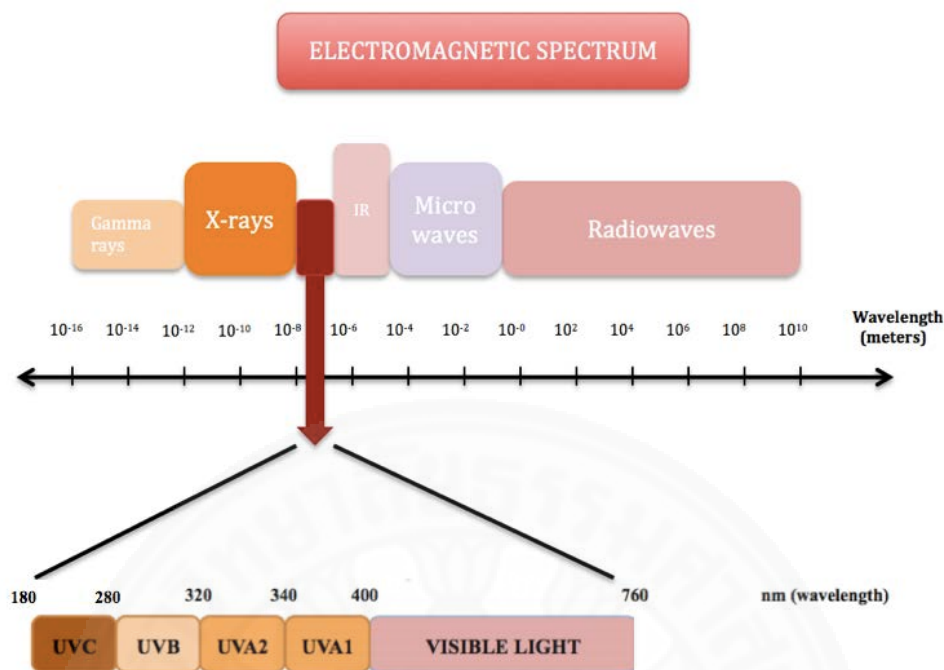


Figure 2.1. Spectrum of ultraviolet radiation

Table 2.1. Types of Ultraviolet Radiation and their attributes

Types of UVR	General attributes
Ultraviolet A radiation (UVA) 320-400 nm	-90–99% reaches the earth's surface^[1] -Not filtered by the stratospheric ozone layer in the atmosphere -Long wavelength & low energy penetrate deeper into dermis -Harmful through excessive and long-term exposure -Aging of the skin; induces immediate and persistent pigmentation -Passes through glass^[1]
Ultraviolet B radiation (UVB) 280-320 nm	-1–10% reaches the earth's surface^[1] -Filtered by stratospheric ozone layer in the atmosphere^[1] -Short wavelength & high energy penetrate the upper layers of the epidermis^[1] -Responsible for sunburns, tanning, wrinkling, photo aging and skin cancer -Carcinogenic and a thousand times more effective causing sunburns than UVA -Does not pass through glass^[1]
Ultraviolet C radiation (UVC) ^[1] 180-280 nm	-Filtered by the stratospheric ozone layer in the atmosphere before reaching earth -Major artificial sources, germicidal lamps^[1] -Burns the skin and causes skin cancer

2.2 Consequences of ultraviolet radiation exposure

To comprehensively discuss the harm caused by UVR, two key points should be mentioned, the acute effects of UVR and the chronic effects of UVR. Acute effects include erythema, pigment darkening, delayed tanning, epidermal hyperplasia, free radical formation, vitamin D synthesis and immunosuppression. Chronic effects include photo-aging and photo-carcinogenesis. **(Figure 2.2)**

Through the use of visual assessment of the minimal erythema dose (MED), it is possible to measure the personal UVR sensitivity, the minimal amount of UVR in which induce a perceptible erythema after exposure (usually 24 hours). Generally, increase in MED coincides with Fitzpatrick skin type. However, there are many skin types overlap, causing inconsistency for MED as a skin type predictive measure (2). MED is widely adopted for the use of calculation of the sun-protection factor (SPF), experimental photobiology and phototherapy.

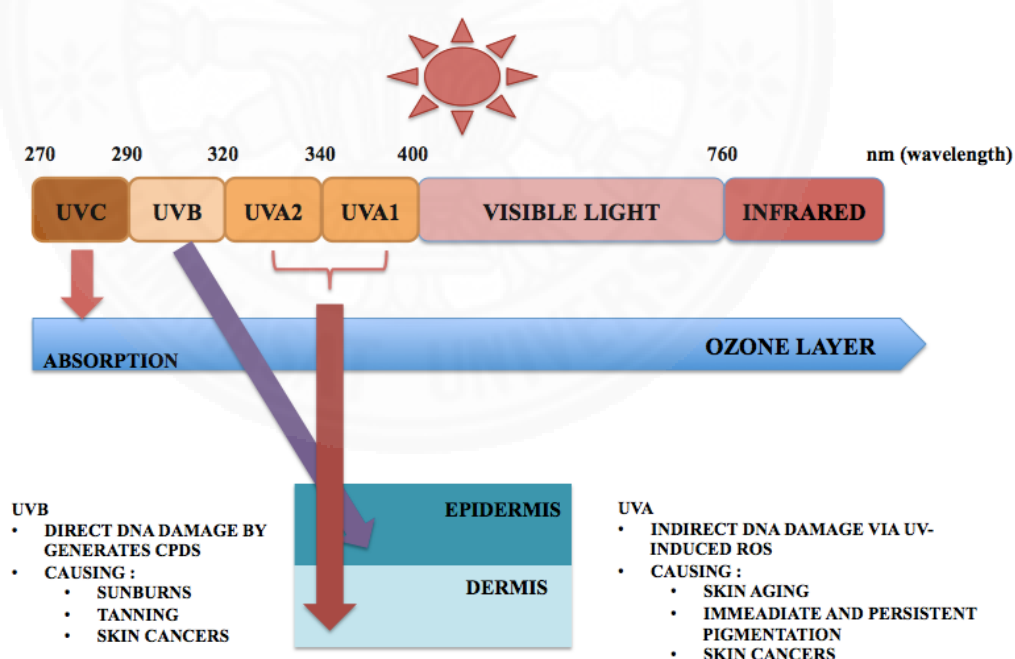


Figure 2.2 Acute and chronic effects of ultraviolet radiation

2.2.1 Acute consequences of ultraviolet radiation exposure

The acute and foremost response of human skin exposure to UVR include, but not only subject to, erythema, pigment darkening, delayed tanning, epidermal hyperplasia, free radical formation, vitamin D synthesis and immunosuppression. (3, 4)

On a molecular level, initiation of the effects of UVR involves epidermal and dermal chromophores, absorbing visible radiation or UV, with an absorption spectrum characteristic of its own. When photo energy is absorbed by the chromophore, energy increases to a higher and excited state, becoming unstable. This will either result in a structural change, binding of molecules which defines as a “direct effect” or formation of reactive oxygen species (ROS), damaging adjacent biomolecules such as proteins and DNA, behaving as a sensitizer, “indirect effects”. During the excited, higher energy state, short-term and long-term photobiological responses are initiated by chromophores (5, 6). Cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine are the most common and frequent photolesions, resulting in a damage to the DNA helix structure, creating inhibition to the DNA replication and transcription (7).

Erythema

Erythema is mainly induced by exposure to UVB. It is the most recognized acute effect of UVR on human skin. Depending on different skin types, dose and wavelengths, the duration and intensity of UVB induced erythema differs. Erythema that is induced by UVB exposure will reach its peak during 6 to 24 hours. Similarly, an immediate erythema reaction that lasts from 48 to 72 hours after exposure is a result of high-dose UVA exposure (4).

Pigment darkening

UVR exposure can also lead to pigmentary alteration causing both an immediate and persistent pigment darkening. Immediate pigment darkening (IPD) is mainly caused by UVA light and is most time categorized as grey in color. This usually occurs almost immediately or within a very short time frame of minutes of irradiation and subsides within hours. Persistent pigment darkening (PPD) develops in

sequence after IPD and is characterized by a discoloration of brownish color occurring 2 hours after exposure and lasts up to 24 hours. Photo-oxidation and the redistribution of existing melanin in epidermal melanocytes where melanin is not synthesized, is the cause for IPD and PPD (8). **(Figure 2.3)** It has also been observed that particularly in darker skinned individuals, visible light (VL) can lead to both IPD and PPD responses (9).

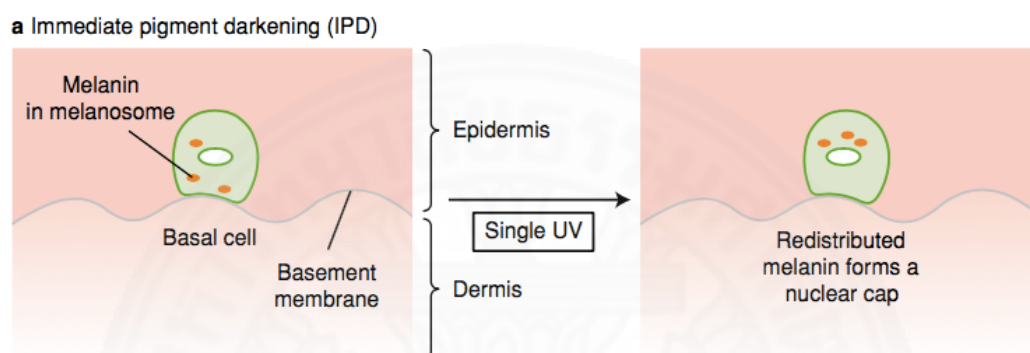


Figure 2.3 Mechanism of IPD after UVR irradiation(10)

Delayed tanning

Different from IPD and PPD, delayed tanning is a result of increase in the number of melanocytes and its activity. The activity of melanocytes increases if it is a single UV exposure, however, repeated UV exposure will lead to an increase in the number of melanocytes, the size and number of melanosomes and melanocyte dendrites elongate and branch. Furthermore, it increases melanin transfer to keratinocytes resulting in a large deposit in melanin granules in the epidermis (10). **(Figure 2.4)**

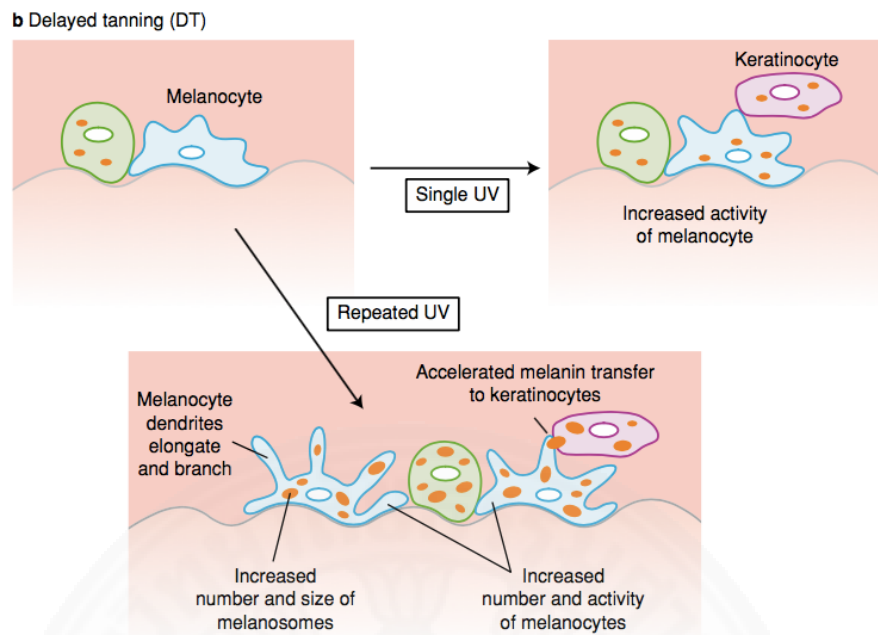


Figure 2.4 Mechanism of delay tanning after UVR irradiation (10)

Epidermal hyperplasia

UVR also leads to an increase in epidermal thickness, termed hyperkeratosis (4). By causing cell injury, UV induces damage response pathways in keratinocytes. Several hours after UV exposure, epidermal keratinocytes proliferate vigorously (11), mediated by a variety of epidermal growth factors. Increased keratinocyte cell division after UV exposure leads to accumulation of epidermal keratinocytes, which increases epidermal thickness. Epidermal hyperplasia protects the skin better against UV penetration (12). Differently than delayed tanning, epidermal hyperplasia is induced mostly by UVB and rarely by UVA.

Free radical formation

UV-induced ROS most likely are singlet oxygen, hydrogen peroxide and superoxide radicals (13). Nucleotides are highly prone to free radical injury, causing mutagenesis through nucleotide bases oxidation (14). The production of 8-hydroxy-2'-deoxyguanine (8-OHdG) through oxidation of the guanine at the 8th position is a well-characterized mutation as a result of ROS, an example of guanine to

thymine conversion (15, 16). The mutation of G/C pair into an A/T pair occurs because of oxidative change, 8-OHdG pairing with an adenine rather than cytosine. As these mutations can be found in tumors in isolation from the skin, oxidative injury may be carcinogenic (17). The foundation of DNA mutagenesis are believed to have stem from ROS induced damages to the DNA, cellular membranes and proteins. VL and UVB light on the other hand may be the reason for ROS induced damages (18). Increased melanin synthesis and cell membrane lipid peroxidation is a result of UVA induced ROS and in the end will lead to inflammation (19). The degradation of skin structural integrity is the result of matrix metalloproteinase (MMP) activation that is triggered by ROS and the growth factors that changes both elastin and collagen in the extracellular matrix and also the release of pro-inflammatory cytokines (20).

Vitamin D synthesis

Most positive effects of solar radiation are seen in the context of UVB-induced production of vitamin D3 in the skin (21, 22). There are only three ways in which vitamin D synthesis can happen, one is through diet, secondly through vitamin D supplements and finally through sunlight exposure. By incident sunlight, 7-dehydrocholesterol, present in the plasma membranes of epidermal keratinocytes and dermal fibroblasts, is converted into previtamin D3. By the rearrangement of double bonds (thermal isomerization), stable vitamin D3 is formed and ejected into the extracellular space where it binds to the vitamin D-binding protein and thus enters the circulatory system. With protein binding, vitamin D3 is converted into 25-hydroxyvitamin D3 (25(OH) D). After being transported to the kidney, 25(OH)D is metabolized to 1,25-dihydroxyvitamin D3 (1,25(OH)2D), its biologically active form (23). **(Figure 2.5)**

Vitamin D3 is essential to the process of intestinal absorption of phosphorus and calcium, an important attribute to healthy bone growth. Only 60% of phosphorus and as low as 10 – 15% of calcium are absorbed through diet without vitamin D3 (24, 25). Sunlight exposure provides approximately 90% of the vitamin D requirement for humans, as a result, deficiencies only affect a small group of people including elderly individual staying indoors, heavily veiled women and high melanin pigmented individuals (26-28).

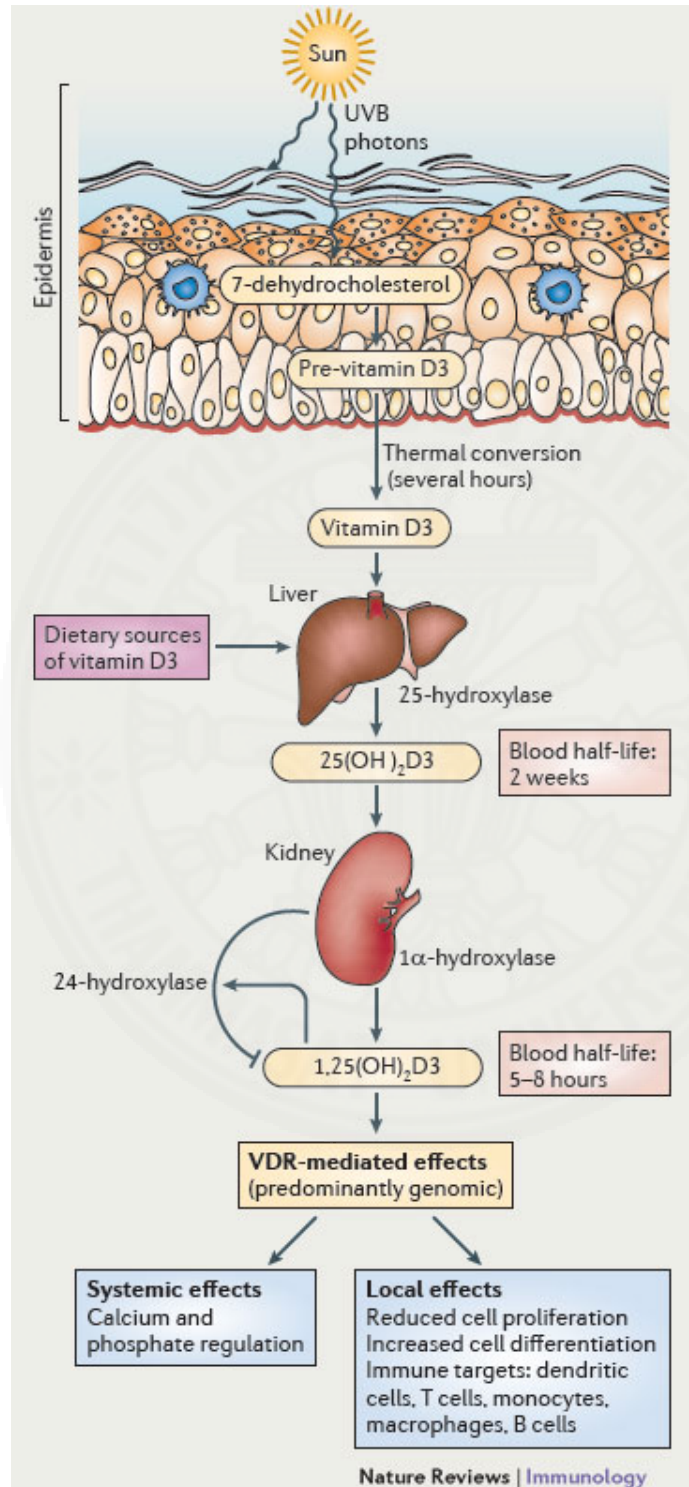


Figure 2.5 Vitamin D synthesis (29)

Immunosuppression

There are several ways in which the immune system is suppressed, one of them is UVR, particularly in the UVB range. The apoptosis of leukocytes is caused by the release of immunosuppressive cytokines, a result induced by UVB inhibiting antigen presentation. Apoptotic death of Langerhans cells (LCs) is induced through higher dose of UVR. Keratinocytes are considered a rich source for various soluble mediators including immunosuppressive, immunostimulatory and pro-inflammatory cytokines. Keratinocytes are effectively induced by UVR to release cytokines. IL-10, a major soluble mediator involved with systemic UV-induced immunosuppression (30), abrogate the ability of LCs to present antigens to Th1 clones and further anergy them. Tumor necrosis factor alpha (TNF- α) (31, 32) and Prostaglandin E2 (PGE2) (33) are some of the other soluble mediators related with UV-induced immunosuppression.

Recognized as another chromophore within the epidermis, Urocanic acid (UCA) is involved in UV-induced immunosuppression (34), a metabolic product of the essential amino acid histidine. The inhibition of the presentation of antigens by LCs happens through UVR conversion of *trans*- into *cis*- UCA (35).

Immunosuppression by UVR can be considered as a positive method in which UVR is used to treat inflammatory skin disorders through alteration of Langerhans cell migration, altering cutaneous cytokine profile and producing suppressor T lymphocytes, cutaneous irradiation suppresses cell-mediated immunity (36). **(Figure 2.6)**

2.2.2 Chronic consequences of ultraviolet radiation exposure

Ultraviolet light exposure on human skin can lead to a few chronic consequences. We will discuss a few including, photo-carcinogenesis and photo-aging.

Photo-carcinogenesis

Erythema and concomitant immunosuppression and transition mutations for example C (cytosine) / T (thymine) or even CC / TT may lead to keratinocyte cancers (KCs) is a result of CPDs induce cytokine-mediated

inflammation. Little is known about the relationship between CPDs and melanoma, although intense UVR exposure is significantly related to melanoma risk (37). 65% of melanoma cases and as high as 90% of KC cases are related to UVR exposure (38).

The risk of skin cancer formation in adulthood increases when there are unprotected UV exposures and sunburns during childhood and adolescence. The risk of subsequent melanoma formation doubles as anamneses of five or more sunburn are present (39, 40).

Even though UVR is considered a major risk factor in the formation of melanoma, its role through UVA and UVB radiation are still controversial (41, 42). Nonetheless, it is commonly accepted that both wavelength range plays different roles in the development of the tumor (43, 44).

Photo-aging

Both UVA and UVB radiation contribute towards skin photoaging. It is generally assumed that UVA radiation leads to a balance shift towards the collagen-degrading matrix metalloproteinases (MMPs) and a simultaneous downregulation of their tissue-specific inhibitors (45, 46). The consequences are an accelerated and increasing degradation of collagen fibers with the concurrent inhibition of the formation of collagen and hyaluronic acid. This leads to an increased formation of deep skin folds and wrinkles (47, 48).

Clear and convincing evidences are provided and present through *in vivo* studies in regards to clinical signs of photoaging and dermal structure deterioration in the long run being a result of UVA radiation. It is accountable for mottled/patchy pigmentation, laxity, wrinkling, sagging, dryness and various other early appearance signs of photoaging. The most significant mechanism of action of UVA radiation is the generation of oxidative stress, a notable factor in the pathogenesis of photoaging (49).

Exploration of biological effects for repeated suberythemal UVA exposure has been studied *in vivo* (50-52). Example in clinical study reported 14 volunteers were exposed to increasing doses of UVA radiation for a total period of 13 weeks, at 3 times per week (52). Various biological markers associate to skin photoaging were studied including ferritin. A significant marker of antioxidant

activity, as a result, increase in ferritin demonstrates that UVA radiation induced oxidative stress.

Heterogeneous to UVB, UVA radiation is able to penetrate deeper, reaching the dermal compartment *in vitro*. Through ROS generation, UVA radiation has demonstrated an induction of major alterations in the dermal compartment in a reconstructed skin model. Additionally, increase in MMP-1 post UV exposure was observed in reconstructed skin model (53, 54).

Acceleration of photoaging has been demonstrated through low-dose UVB exposure, including significant phenotypes such as sagging and wrinkles (55). Upregulation of the expression of MMPs through low-dose UVB irradiation results in elasticity loss and wrinkle formation increased in dermal tissue (56).

Low-dose UVB irradiation demonstrates notable upregulation of MMP-1 expression in human keratinocyte HaCaT cells through a pathway dependent on BLT2 (a receptor for LTB₄ and 12(S)-HETE) (57). It has also been shown that in UVB-irradiated HaCaT cells, mediation of MMP-1 upregulation through ROS production dependent signaling pathway is a result of BLT2 and the subsequent stimulation of ERK, two notable characterized mediators of UVB-induced MMP-1 expression. This suggests possible contributions to skin photoaging.

Correlation is presence between photo-aging and increasing formation of mutated mitochondrial DNA, induced by ROS formation, for example, hydrogen peroxide or hydroxyl radical and superoxide radical (58, 59). Resulting in a dose-dependent summation of mitochondrial genome damages, the mutagenesis affects mostly the dermis, the non-proliferating skin part, therefore, UV-damaged skin cells are not susceptible to elimination by the endogenous repair mechanisms (60, 61). Nevertheless, the aging process is not solely limited to the dermis but rather extends to the epidermis.

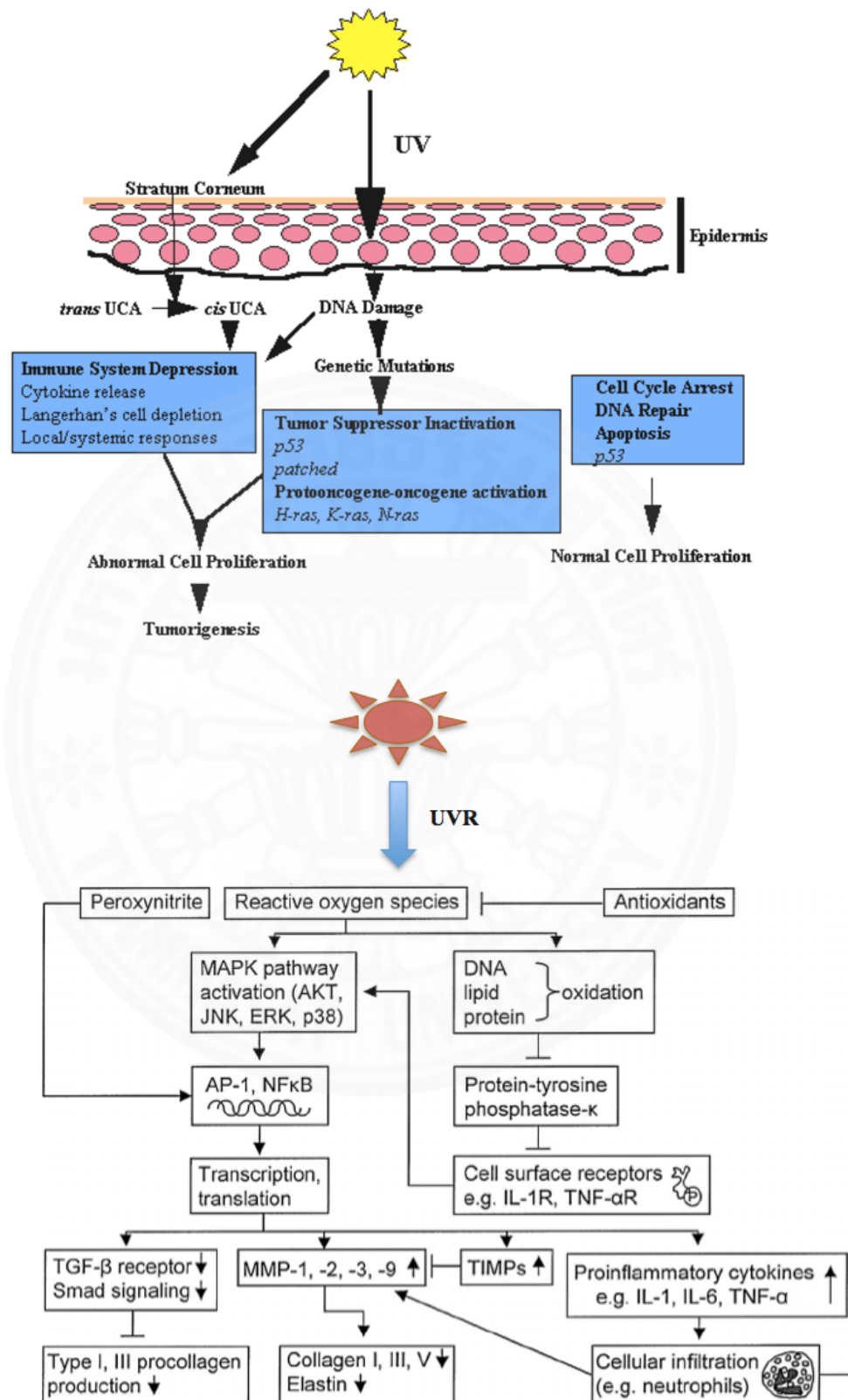


Figure 2.6 Effects of ultraviolet radiation (modified from (62, 63))

2.3 Visible Light and Infrared Radiation

UV light and its effect on the skin are well documented. Nevertheless, evidence from Asia, Europe and United States during the past decade have suggested that wavelengths existing beyond the UV spectrum could also play a role in photoaging and solar damage. Emerging researches specifically in the area of wavelengths inside visible light and infrared radiation spectrums are widely present, including photoprotection covering these spectrums and not solely identifying photodamage correlation.

Electromagnetic radiation with a wavelength of 400–700 nm that is visible to human eyes is referred to as VL. Compared to UVA and UVB, VL has a longer wavelength. In skin type IV–VI, pigmentation is induced through both VL and UVA1, however, pigmentation was darker and sustained for a longer period when induced by VL. Pigmentation was absent for skin type II, both quantity and quality of pigmentation induced by UVA1 and VL shows differences. Utilizing a simulator distributing radiation with wavelengths of 385–690 nm (VL) to varying skin types resulted in an immediate pigmentation and erythema within 24 hours (4).

VL causes skin erythema at high doses. It has been demonstrated that different skin type result in different intensity and the timing of erythema when exposed to an artificial light source emitting 98.3% VL, 1.5% IR and 0.19% UVA1 (4). A halo of erythema is induced by VL, surrounding IPD response and resolving within 2 hours for skin types IV–VI. As the dose of VL is increased, the degree of erythema also increases for these skin types. Nevertheless, in skin type II, even at a dose of 480 J cm^{-2} , erythema could not be induced. This may result from the reaction within the chromophores generating heat induced by VL and a greater heat production is a result of increasing concentrations of melanin in darker skin types, leading to the appearance of erythema through vasodilation (9). Another study using an artificial light source emitting 385–690 nm reveals that in skin types II–IV, there is an immediate erythema which subsides with 1 day (4). Interestingly, unlike the first study, the light source used in this study had a greater UVA1 output. The study suggested that the erythema response may have been a representation of thermal effects, similarly to the first study (9).

A polychromatic light source ranging from 390–1700 nm was used in 1984 to demonstrate that in the absence of high level of UVR, pigmentation could still be induced by VL. In addition, pigmentation lasting up to 10 weeks could be induced by VL with doses greater than 720 J cm^{-2} (4).

In a recent study situated in Bangalore, India, skin types IV–V are subjected to mid-day sunlight with filters, evaluating the cutaneous effect of electromagnetic radiation with wavelengths less than 400 nm and greater than 420 nm. It has been reported that VL induced IPD is similar to and not significantly different to UVR induced IPD, with similar shapes of action spectra IPD induced both by VL and UV (64). UV is however 25 times more efficient and effective in inducing pigmentation than VL (64).

VL demonstrates a possible role as a causation of photo-induced pigmentary disorders such as post inflammatory hyperpigmentation or melasma through pathogenesis especially in darker skin, a clinical relevance for its ability of inducing pigmentation. As sunscreens protect against UVR and not VL, these conditions may progress even when sunscreen are applied, especially in darker skin types where conditions are even more pronounced. It has been recently proposed that, in melasma, even VL present indoors can sufficiently worsen hyperpigmentation conditions through interaction with potential photoallergens (65).

Further implication on photoaging process has shown correlation with IR, wavelength ranging from 770 nm–1 mm. MMP is induced through ROS activation by IR, degrading collagen. IR further reduces type 1 collagen expression through inhibition of procollagen–1 production and stimulation of TGF- β 1, TGF- β 2 and TGF- β 3 in human skin. Increased in number of skin mast cells have been linked to IR, a histological feature of photoaging. At present, there are no existing chemical UV filters capable of infrared range absorption .

IR represents roughly 40% of the solar radiation reaching the ground at sea level, dividing itself into three different bands; IR-A (760–1400 nm), IR-B (1400–3000 nm) and IR-C (3000 nm–1 mm). Subjected to different wavelengths range being studied, IR radiation penetrates the epidermis, dermis and subcutaneous tissue at different extents. IR exposure are most often perceived as heat (66).

With minor temperature increments, studies have demonstrated increasing ROS production and collagen degradation. The effects of water-filtered infrared-A (wIRA) have been studied in natural viable skin with inflammation, content with antioxidant enzyme, inducible free radicals and heating on viability or convective cooling (67). At an irradiance of 190 mW/cm^2 and a temperature maintained at 37 degrees Celsius through convective cooling air ventilation, wIRA is applied onto the skin for over 30 minutes showing no significant effect on the cell viability, free radical content, inflammatory status or the skin and its antioxidant defense systems. On the other hand, free radical formation has shown to almost doubled and there was a reduction of antioxidant power of about 50% after convective heating to roughly 45 degrees Celsius. They may possibly be the result of temperature dependent polymer photodegradation demonstrating a radiation dose with linear increase.

Collagen degradation is observed even through a simple non-IR heating pad applied for 15 minutes at 43 degrees Celsius (68). The dorsal skin of hairless mice has been exposed to heat for a total period of 6 weeks, 3 times per week in this experiment. Through sustained and chronicle exposure of the skin to the heat, skin wrinkling is evident through matrix metalloproteinase 13 (MMP-13) expression and reduction in antioxidant enzyme activity with further oxidative damage. MMP-13 shows promotion of skin wounds closure (46). In another study, chicken embryonic gastrocnemius tendon has been used at 37 degrees Celsius and 43 degrees Celsius and demonstrated an increased in mRNAs representing various collagen regulators, heat shock protein 47 (Hsp47), transforming growth factor beta (TGF- β) and connective tissue growth factor (CTGF) at 43 degrees Celsius (69).

To further investigate the relevance to an *in vivo* application for these observations, a study of normal buttock skin of 23 healthy human volunteers has been implemented (70). The source of radiation for all the volunteers are the same, an artificial source (Hydrosun 500) provided a single dose of either 360 or 720 J/cm^2 radiation of IR-A and thereafter assessed for MMP-1 expression (mRNA by RT-PCR or immunohistochemistry or protein by Western blotting). The skin was exposed to an irradiance measured at 105 mW/cm^2 . There was an upregulation of MMP-1 post IR-A irradiation in the study (71). This particular dose was mentioned as it depicted a similar type of radiation for a few hours outside on a summer day in Central Europe.

However, similar radiation dose in the summer of areas further south in the tropics would mean an exposure to the sun from 6 am to 6 pm. In reality, solar IR-A peak irradiance is at 40 mW/cm² with an average at roughly 20 mW/cm² during the day (72).

The molecular effect in relation to VL and IR is yet to be clarified to the full extent, igniting interests in understanding the potential cause of skin damage. It is only until then that photoprotective measures can be developed and is fully effective in protecting the skin.

2.4 Mechanism of photoaging

Skin aging is at the pinnacle of environmental and genetic factors, significantly influenced through the collective damages of UVR exposure.

Exposure to UVR can cause a significant damage to the skin. Generation of ROS is known to be one of the mechanisms involved. Resulting in a breakdown of collagen through oxidative damage to cells, ROS in an excessive amount are harmful to the skin. Being implied as an indirect cause of ROS generation, UVA radiation leads to DNA strand breakage. On the contrary, UVB radiation leads to DNA mutagenesis and cyclobutane dimers and 6-4 photoproducts formation through the instigation of DNA damage through epidermal cytokines interactions.

Substantial amount of studies have been carried out over the past two decades in regards to the role of mitogen-activated protein kinase (MAPK) towards photoaging. A key role in cell growth regulation and formation of procollagen I, MAPK is unique. Commonly known, the upregulation of MAPK signal transduction pathway is caused by UVR. The process is a result of previously mentioned cell surface cytokines, causing downstream transduction of signal. Respectively, transcription factors c-Fos and c-Jun undergoes AP-1 manifests formation and heterodimerization.

Metalloproteinase expression increases as a result, inducing dermal extracellular matrix degradation, explicitly procollagens I and III. Collagen synthesis reduction occurs simultaneously, demonstrated through post UV exposure TGF- β receptor type II photoaged skin inhibition. This leads to compromised dermis integrity

and defective reparative response. Although with continued UV exposure result in the production of fresh compensatory collagen in response to UV insult, wrinkles develop overtime through the development of solar scar. The collective summation of oxidative damage to the skin resulting from excess ROS is a result of advancing age. This is validated through studies that investigate the production of collagen pertinent regulators and degradation that shows increasing AP-1 levels and in aged skin, matrix metalloproteinase (MMP) activity is presence in contrast to young skin (73). **(Figure 2.7)**

Acute and chronic states of angiogenesis as a result of UV exposure to skin demonstrate self-contradictory effects. Angiogenesis is induced by UVR through the activation of vascular endothelial growth factor and thrombospondin-1 downregulation, a potent angiogenesis inhibitor (74). However, these new vessels are hyperpermeable and cause a cutaneous inflammation due to escaping inflammatory mediators, for example, IL8. As a result, this further contributes to photoaging through accelerating degradation of extracellular matrix and reducing dermal vasculature in the chronic setting (74).

Additionally to photoaging, immunosuppression can lead to skin cancer and stimulate various infectious diseases such as oral herpes simplex through intricate mechanism of action directly caused by UVR. It allows for inhibition of antigen presentation, stimulating immunosuppressive cytokines release, for example, IL4 an induces leukocytes apoptosis.

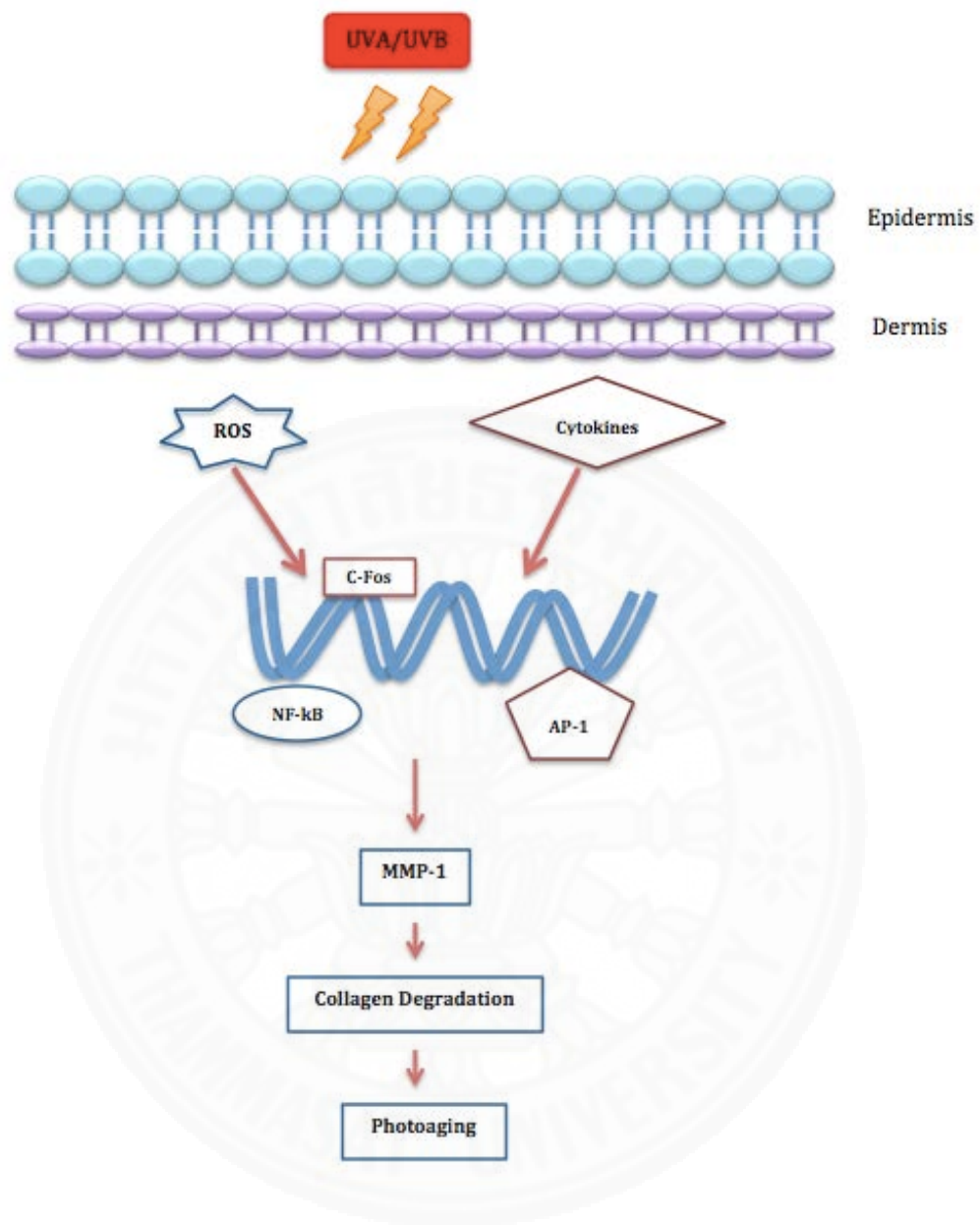


Figure 2.7 Pathway of photoaging showing changes in molecular level (20)

2.5 History of photoprotection

Light skin has been considered as more attractive compared to darker skin in times of ancient Egypt. Through translations of hieroglyphics from Egyptian tombs, ingredients such as jasmine, rice bran extract and lupine extract have been used to reduce the likelihood of tan or sunburn. Oryzanol, rice bran extract, have been found to possess UV-absorbing properties (75).

Across Africa, African clays are used for various purposes ranging from ceremonial purposes to social signaling and photoprotection. Particles size of the clay ranges from 1000 to 2000 nm. This allows for densely packed clay components, allowing for photoprotective properties. Studies have determined these clay to have a low sun protection factor (SPF) but a rather broad spectrum coverage (75).

Prior to modern era of photoprotection, heat was believed to be the cause of sunburns rather than ultraviolet light. In 1801, ultraviolet rays was discovered by Johann Wilhelm Ritter. Only until 1820 that the notion that heat led to sunburns was dismissed. Everard Home of England observed that when one of his pale hands was covered with dark color clothing and the other was fully exposed to sunlight, the covered hand was significantly warmer had a much lesser degree of sunburn compared to the exposed hand that was cooler in temperature. He realized that heat did not cause sunburn and skin pigment protects against sunburn, concluding that melanin, the dark pigment in the skin protects the skin from UVR-induced damage.

In 1922, wavelengths in the range of 280-320 nm were discovered to induce sunburns. Through developments of filters that target this range of wavelengths, the first sunscreens were developed containing para-aminobenzoic acid, benzyl cinnamate and benzyl salicylate. During World War II, sunscreens were used to protect the soldiers from sunburns and early sunscreens only targeted the UVB range of solar spectrum. Today, sunscreens have been developed to protect against a wider range of solar spectrum and advancement in research and technology have allowed for other photoprotective methods for example through systemic photoprotective agents.

2.6 Photo-protective strategies

There are many types of photo-protective strategies including naturally occurring protective agents, physical protective agents, topical protective agents and systemic protective agents. Naturally occurring protective agents include environmental-atmospheric agents and skin agents. The degree of protection relies solely on what is given. Physical protective agents include different types of glasses, sunglasses, clothing, make-up and etc. Topical protective agent is all the variation of sunscreens and is the most widely used protective agent, providing protection only to the areas in which the agents are applied upon. Topical agents block the penetration of UVB into the skin, preventing skin erythema. However, the protection is only limited to the few hours subsequent to their application. In addition, topical protective agents may cause an allergic reaction on some individuals. Systemic protective agents include plant and animal derived agents in the form of antioxidant supplementation, preventing free radicals formation and its harmful effects. With the objective of providing photoprotection, diet and oral supplementation of non-toxic substances can help to aid protection for the skin through rescuing cutaneous endogenous antioxidant defense.

2.6.1 Naturally Occurring Photo-protective Agents

There are various types of natural occurring photo-protective agents that exist with our environment and within our existences. It can be as broad as the geographic and environmental variation that occur within our world or as specific as the chromophores within our skin that helps to protect the negative effects of UVR.

Atmospheric and environmental agents

There are several agents within the atmosphere and the environment surrounding us. The atmosphere itself filters much of the UVB that enters the earth's atmosphere, letting in only about 4% of its radiation, while UVA accounts for more than 95% of the radiation that reaches the earth's surface (76). The natural filtration of the radiation is a result of nitrogen and oxygen molecules and the absorption of the ozone layers within our atmosphere. However, in the current environmental climate,

the deterioration of the earth's protective ozone layer has allowed for higher UVB radiation at higher altitudes due to the chlorofluorocarbons substances. With this in mind, different societies have joined together to reduce the greenhouse gasses and substances in which contributes to harm the ozone layers. In recent years, the ozone columns have started to stabilized and will continue to slowly recover in the following decades (77).

The latitude and altitude can also play a significant role in UV radiation. At the equator and at any high altitudes, UVR exposure will be higher. This is due to the fact that the UVR travels a shorter distance from the Sun to our planet. At the equator, the UVR will travel the shortest distance, but at higher and lower latitude, the UVR will have to travel a longer distance. Similarly with altitudes, the higher you are from the sea level, the shorter the distance will be for the UVR level to reach you. As the latitude increase or decrease by a degree, the amount of UVR exposure will decrease by 3%. A 1000-foot increase in elevation will also see an increase of 4% to 10% of UVB light. As for the amount of radiation, the transmission increases exponentially with increasing altitudes due to less UVR-absorbing atmosphere (78).

The time of the day and the season also plays a role in UVR exposure. The time range in which the UVB light is strongest is between 10 AM to 2 PM. As the earth spins around on its axis, when the sun and a certain point is directly facing each other, the solar zenith will allow for the shortest path of UVR travel from the sun to the ozone layer (79). This is when the radiation is at its peak. Differently with UVA, UVA has a long-spectrum, therefore, allowing for a constant light throughout the day. The amount of cloud coverage also plays a minimal role in affecting UVA. The different season affects the UVR due to the elliptical orbit of the sun, radiations peaks during the summer.

Clouds can affect different areas of the solar transmission. First and foremost, it will reduce the amount of VL we can see, the intensity of UVR and the heat we feel due to the infrared radiation (IR). Because clouds also decrease the IR transmitting through to us, on cloudy days, we will feel less heat, when actually the amount of UVR, especially UVA, is still very much present. This allow for an increasing risk of UVR overexposure (80).

Pollutions or the pollutants in our environment can also affect the amount of radiation we are exposed to. Pollutants include the ozone, sulfur dioxide, nitrogen dioxide, and particles in the cloud within the troposphere, affecting shorter wavelengths UVR versus longer wavelengths UVR (80). It has also been observed that in polluted areas, UVR significantly reduces compared to areas with no pollutions (81). Naturally occurring pollutants such as dust, wild fire aerosols and volcanic ash can significantly reduce the amount of UVB radiation (82). At the same time, metal, glass, sand, ice and snow can reflect UVB light by up to 85%.

Staying in the shade is another way most people believe will reduce the amount of UVR. However, 50% of UVA exposure occurs within the shade itself (83). A tree, depending on the density of the branches and leaves and the distance to the perimeter of the shade, will only provide a Sun Protection Factors (SPFs) of 4 with a maximum of 50 (84).

Skin agents

Endogenous skin chromophores include DNA, melanins, and their precursors, urocanic acid, aromatic amino acids, flavins, and porphyrins. The human skin is the final barrier in which helps to protect our body from UVR harm. UVR does not penetrate further into our bodies and will only reach our skin. Different factors and variances between each person's skins will influence the effects of UVR. Factors include, skin type, absorption spectra of different skin chromophores and the depth of cutaneous penetration of specific wavelengths (4). As the final barrier of our human body, human skins is able to absorbs UVB radiation and at the same time diminish most VL and reflects 5 to 10% of all solar radiation within the range of 250 to 3000 nm (85). The epidermis absorbs most of the UVB light and the dermis is reached by UVA light. Skin color also plays a significant role in protecting our bodies from UVR harms. Lighter skin types have increased UVR penetration compared to darker skin types primarily because of decreased melanin (86). UVA and UVB light on average, penetrate the epidermis of white skin 5 times more than black skin. Different body sites also allow for different levels of UVR penetration due to the differences in thickness of the epidermis (87, 88).

Epidermal melanin is a vast obscure particle that extinguishes ROS and diminishes UVR and VL entrance into the skin by disseminating and physically blocking UVR and changing over the ingested vitality into warmth. The degree of photo-protection provided by melanin relates with skin thickness and level of constitutive skin pigmentation. Thinner facial skin creates erythema more effortlessly than thicker skin, and obscurely pigmented skin is less prone to the impacts of UVR than delicately pigmented skin.

2.6.2 Physical Photo-protective Agents

Physical protective agents are things that exist within our environment through the process of manufacturing. This can include different types of glasses, clothing, hats, makeup and many more. It goes to show that even windows in typical residential and commercial buildings can block the transmission of UVB light while advances in technology leads to laminated, tinted glass and films which can protect us from the UVA light up to 380 nm.

Glass

The average person spends most of their daily lives in vehicles or indoor, this is when UVR exposure through windows is most often neglected. At the present time, most educational campaigns and photo-protection strategies are aimed towards outdoor activities. This allows for a chance for us to increase the awareness of UVR exposure associated with building windows and glasses, motorized vehicle glasses and sunglasses to help us better diminish the risk of being exposed to UVR.

Windows whether residential or commercial buildings, blocks the transmission of UVB. Newer technologies has allowed for some windows to be able to block off transmission of UVA light and VL as well (89). Evidence suggests that UVR exposure via glass-filtered sunlight is apparent and is proven through studies which show significant increase in the distribution of BCCs, SCCs, Merkel cell carcinomas and malignant melanoma (90, 91).

Motor vehicle glass

Laminated glass is mandatory for all vehicles window shields as an attempt to decrease motor vehicle injuries and also to promote safety. This allows for the blockage of UVB and most of the UVA radiation. However, side, rear and overhead windows are not imposed by the safety regulation to be laminated glass but rather tempered glass. This reduces the amount of filtration from UVA radiation. On a good note, with new government standard further pushing forward safety, laminated glass is soon to be mandatory for side windows as an attempt to decrease injuries or death. This indirectly helps to reduce the amount of UVR.

Films and tinted windows provide an extra protection, decreasing the transmission of UVA compared to non-tinted windows (92, 93). They also provide an opportunity to decrease the transmission of VL and IR. As most films are made up of multiple layers of materials, they are able to filter out up to 99% of UVR as deep as 380 nm. As new regulations are being imposed regarding vehicle's fuel economy, laminated windows are further coated with reflective properties to reduce heat within the cabin, requiring less air conditioning and in turn using less fuel. This then could further help to reduce the harmful effect that UVR has on our skin regarding skin aging as a result of IR (94).

Sunglasses

Ocular protection from UVR is provided by sunglasses as there are consequences of UV light on ocular tissue. Ocular complications affect both short and long term. In the short term, problems include self-limited photokeratoconjunctivitis and in the long run, cause climatic droplet keratopathy, pterygium, pinguecula, cortical cataract formation (95-99). However, UVR cannot reach our retina otherwise (100). Children should then always wear sunglasses as their clear ocular lenses transmit more VL, further increasing the chance of developing macular degeneration (101, 102). The time of day that there is the highest amount of UVR to our skin is at exact mid-day when the sun is directly above us but strangely it is not when our eyes are most at risk to UVR. UVR exposure peaks out when our eyes and the solar radiation is parallel to each other. This occurs during the early morning hours and late afternoon. This however varies according to the seasons and latitude. Different types

of sunglasses also give different levels of photo-protection. It is determined by the size, coverage area and distance from the face. Usually a wraparound style with side shields gives the highest level of protection. The type of coating on the lenses allows for different levels of VL reaching the eye but is not indicative to the amount of UVR exposure. A good lens with tinted coating and UV protection properties can eliminate most of the UVR. However, dark lenses with no UV protection can allow for more exposures to UVR because when less VL comes through, you pupils will dilate and is more subjectable to UVR.

Fabric

Types of fabric, its manufacturing process and after use care affect the level of UVR protection it provides. For example, a thick fabric that is manufactured through the process of tightly weaving fibers that has dark colors, made from wool and polyester, shrinking after laundry, using additives such as Tinosorb FD, will have an increased level of UVR protection. On another hand, the stretching of fabric and chemical processing methods promote the process of bleaching, de-sizing and removing starch before weaving will cause a decreased in protection. Hydration can also lead to both increase and decrease in protection depending on the type of fabric (103).

Hats

Hats are a part of the overall clothing that we wear in our lives to look good and also to protect us from the IR heat of the sun and as well as the UVR. Similarly to sunglasses, different sizes and styles will also affect the level of protection. Variable protection is a result of brim width, weaving, materials. Varying according to brim size and the area of the face covered, hats can have an SPF rating of up to SPF 7. SPF rating of SPF 5 for the neck, SPF 2 for the chin, SPF 3 for the cheeks and SPF 7 for the nose is possible with a hat having its brim greater than 7.5 centimeters (104). The best protection is the wide-brimmed hats compared to a narrow one.

Makeup

Although the primary purpose of makeup is to enhance the looks and appearances, pigments in the colored of cosmetic products can help to enhance photo-protection. Normal foundations can give up to SPF 2 to 6, as for foundations with organic plus inorganic filters, it can provide up to SPF 15 (8). Today, more and more cosmetic products contain sunscreen ingredients which further helps to provide better UVR protection.

2.6.3 Topical Photo-protective Agents

The most commonly used mode of protection against UVR by the public when engaged in outdoor activities is through the use of sunscreen (105, 106), regardless of the existence of other ways of protection that provides superior photoprotection such as clothing, hats and seeking shades. Physicians and the cosmetic industry and its marketing efforts may have a direct effect on this matter (107, 108). In the beginning, only UVB absorbers compounds were available, making UVB protection the sole initial goal for sunscreen development. Through the years, UVA absorbing compounds became available in the 1990s. Erythema, an endpoint used to determine the SPF can easily be reduced through the usage of sunscreen, making sunscreen an effective prevention for erythema. Repeat and regular use of sunscreen has shown to ease the photoaging process, subsequently reducing the probability for skin cancers. Sunscreen is also highly potent in reducing the number of squamous cell cancers (SCCs) (109), and actinic keratosis (110). However, its protective benefits for basal cell carcinomas (BCCs) through routine use of sunscreen is yet to be conclusive as there is no notable reduction of BCCs is observed. An overall trend however demonstrates a reduction in incidences of BCCs (109, 111). Other cosmetic and medical benefits are also apparent through the use of sunscreens. Regular usage of sunscreen can slow down the deteriorating process of aging, appearance of wrinkles, formation of pigmentation, blood vessels dilation and collagen loss (112). Sunscreen is also often used as the first mean of protection for photodermatoses. Different compounds are used in the formulation of sunscreens to increase the absorption spectrum. Sunscreen active compounds are divided into two groups, inorganic and organic sunscreens.

To prevent sunburns and protect the skin from serious damage, sunscreens must meet certain criteria. They should be photostable, dissipate the absorbed light energy through photophysical and photochemical pathways without the formation of harmful reactive intermediates, be water resistant and well tolerated; in addition, they should not penetrate the skin (113, 114).

In animal studies, using UVB-induced “sunburn cells” (apoptotic keratinocytes) as an end point for reference, SPF-12 sunscreen has demonstrated protection against epidermal cell damage (115). UVR-induced mutations in the p53 tumor suppressor gene were nearly eradicated through the application of SPF-15 sunscreen on to the skin of the mouse (116).

However, in comparison to the manufacturers’ test, people usually apply sunscreens much inadequately and less thoroughly. Typical application suggests a much lower dose (less than 2 mg / cm^2) compared to the SPF testing process (117-124). In reality, most users only achieve a mean value in between 20% and 50% of the SPF labeled (34, 125, 126). Sunscreens with SPF rating greater than SPF 15 would be deemed unnecessary if sunscreens are applied adequately and properly (127). As a result, the protection factor stated by the manufacturers is not reached (123, 127) and in turn causing a sense of false impression for the degree of protection, leading on to more time spent outdoors (128). In addition, an alarming proportion of a person’s annual UVR exposure happens during regular routines (non-holiday period), when topical sunscreens are not typically applied (80, 129).

2.6.3.1 Organic UV filter

The group of organic UV filters includes different substance classes, which can be divided into UVA and UVB filters due to their specific absorption characteristics. Most organic UV filters contain aromatic compounds conjugated with carbonyl groups. The excitation of electrons in the respective benzene ring leads to absorption in the UV range. The absorption range and strength of the different UV filter classes are additionally affected by further substituents (130). **(Figure 2.8)**

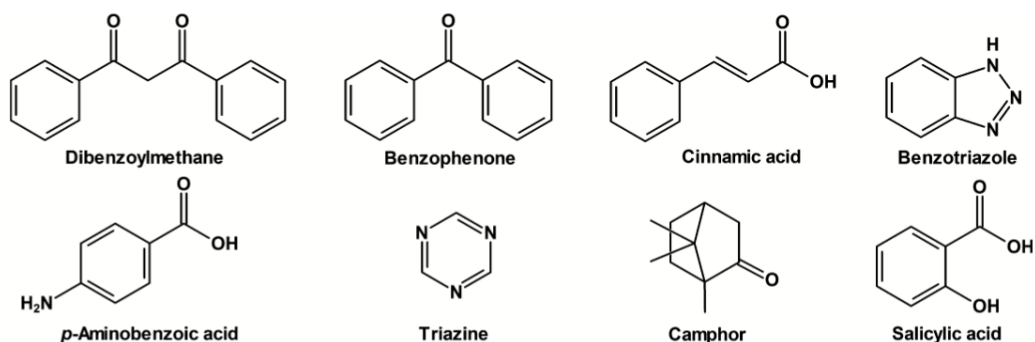


Figure 2.8 Basic chemical structures of UV filters (131)

Dibenzoylmethane derivatives

The most common UVA filter in cosmetic products is butyl methoxydibenzoylmethane (BMDBM, Avobenzone). Ranging from 310 to 400 nm is its absorption profile. The molecule is divided into two structural isomers, each having its own absorption peak. The average absorption is around 360 nm. It is the most potent organic filter, extending its protection through to the long-range UVA1 rays. Despite its effectiveness and wide spectrum range, BMDBM is essentially photolabile. After one hour of UV exposure, there is a 50 to 90% molecules loss and a significant photodegradation. In addition, BMDBM and octinoxate, the most potent UVB filter, should not be mixed together. This is due to the fact that after UV exposure, BMDBM molecules becomes excited and unstable, reacting to octinoxate, losing both UVA and UVB protection (132). By adding other molecules to accelerate and facilitate the changing state of BMDBM through excited to unstable to stable state, the photostability increases. Non-UV filter such as diethylhexyl 2,6-naphthalate and UV absorber such as octocrylene are some of the stabilizers.

Benzophenone derivatives

A group of aromatic ketones commonly used by sunscreens producers, benzophenones, has a broad spectrum UV coverage. Oxybenzone (benzophenone-3) is a part of this class of UVA filters. It was affirmed in the mid 1980s and has an assimilation profile spreading across from 270 to 350 nm. The two retention pinnacles are at 288 and 325 nm. As an UVA filter, it for the most part

creates a protection for the UVA2 rays. In spite of the fact that oxybenzone is generally found in sunscreens, it still has various weaknesses. To start off, it has been viewed as the most allergic agent and has been involved with photoallergic dermatitis (133, 134). Secondly, the compound has systemic assimilation and has been identified in urine and circulation system (135). Developing concerns raised by non-profit organizations have proposed the endocrine and carcinogenic effect of this compound, despite the fact that current studies have not bolster these concerns (135). In conclusion, oxybenzone is not photostable and can produce oxygen radicals upon UV radiation (136).

Para-aminobenzoate derivatives

The first widely adopted UVB filter within the United States was para-aminobenzoic acid (PABA) (137). The absorption wavelength peaks out at 283 nm. PABA has been known for its water-resistant properties due to its insoluble characteristics, holding together tightly through hydrogen bond to the keratinocytes, perfect for producing water-resistant sunscreens. Nevertheless, due to the fact that its leave yellow stains on clothing, consumers have been drawn away and PABA deemed undesirable (138). Moreover, 4% of the population has reported photoallergic reaction to the PABA (139) and concerns regarding carcinogenic potential are high (140).

Salicylate derivatives

Another existing group of available UVB filters are salicylates. At present, octisalate, homosalate and trolamine salicylate are approved and readily used. Homosalate has peak absorption at 306 nm, octisalate at 307 nm and a group UV radiation absorption range from 290 to 315 nm. At this range, salicylates are considered to be a weak UVB absorbers and a high dosage of compounds are required to meet certain SPF prerequisite (141). Nevertheless, salicylates are considered to be useful, as it does not penetrate the stratum corneum, making it an ideal alternative for a low tolerance to sensitivity. Other advantages include the ability to retain its effectiveness after being introduced to water and perspiration for octisalate and homosalate, due to its water insolubility. Trolamine salicylate on the other hand is water soluble and has been used in the mixture for creating hair products

(19). Photodegradation of sunscreens ingredients like avobenzone and oxybenzone can also be stabilized and prevented to a minor degree by these compounds.

Camphor derivatives

Camphor derivatives 3-benzylidene camphor (3-BC) and 4-methylbenzylidene camphor (4-MBC) were popular UVB filters for an extended period of time due to its competitive photostability properties (142). In 1994, 30% of all sun protection products contained 4-MBC and during 2004 to 2006, 4-MBC was still considered a typical ingredient for sunscreens (143). A camphor derivative extension, terephthalylidene dicamphor sulphonic acid (TDSA) is considered as an effective UVA filter. Negative effects of UVA such as epidermal hyperplasia, skin pigmentation, decrease skin hydration and elasticity were greatly reduced through TDSA (52, 144, 145). In addition, TDSA shows no percutaneous absorption tendencies and is photostable (146).

Cinnamate derivatives

Cinnamates offers two members within this class of compounds, cinoxate (2-ethoxyethyl-p-methoxycinnamate) and (octyl methoxycinnamate [OMC]). The latter provides the most effective UVB absorber used worldwide. The absorption profiles range from 270 to 328 nm with a maximum absorption at 320 nm. Furthermore, when OMC is encapsulated in polymethylmethacrylate microsphere, its efficacy enhanced (147). OMC is also water resistant and at the same time provides a low irritation potential to the skin, making it an ideal choice for sunscreens manufacturers (148). Its only downside is its incapability to be paired with the most commonly used UVA filter, avobenzone. OMC and avebenzone creates a photolabile formulation and therefore undermine the overall UV protection (149).

Octocrylene (2-ethylhexyl-2cyano-3,3-dephenylarylate) has an absorption range of 290 to 360 nm and a peak absorption at 307 nm. This compound provides an competitive safety profile with low photoallergic potential, irritation and phototoxicity (148). However, it loses its efficacy when exposed to water and perspiration. Presently, the compound is considered to be the best at

providing photostabilization for avobenzone, increasing its popularity amongst sunscreen formulators, regardless of its formulation difficulties and high cost.

Triazones

UV filters with a molecular weight exceeding 500 Da has recently been in development, reducing skin penetration. This is due to the increased safety and efficiency concern for each substances. As a result of the extension and chromophoric groups multiplication, the UVB filter ethylhexyl triazone (EHT), the UVB filter diethylhexyl butamido triazone (DEBT, iscotrizinol, Uvasorb HEB) and the broad-spectrum UV filter bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT, bemotrizinol, Tinosorb S) have a molecular weight exceeding 500 Da. These UV filters demonstrate relatively high absorption coefficients, remarkable anti-inflammatory effects and are highly photostable and greatly efficient (132, 150, 151). Additionally, other UV filters substances benefit from improved photostability through Tinosorb S (132). As a result of the positive properties, more and more sun protection and skincare products incorporate triazones.

Benzotriazoles

UV filters drometrizole trisiloxane (DTS, Mexoryl XL) and methylene bisbenzotriazolyl tetramethylbutylphenol (MBBT, Bisotrizole, Tinosorb M) falls in the weight category of the 500 Da. As a result, skin penetration down to a minimum and there are little photoallergic reactions (152, 153). Offering protection for the whole range of UVB and UVA, Mexoryl XL was considered the first photostable UV filter. Combining Mexoryl SX and XL, their protection potential further increases (144).

Being dispersible in the water phase of sunscreen, Tinosorb M is produced in a microfine particles organic form. As a result, it is able to combine both organic and inorganic UV filters' properties, scattering, reflecting, and absorbing UVR. Tinosorb M demonstrates excellent photostability over the whole UVB, UVA1 and UVA2 broad-spectrum range (154, 155).

2.6.3.2 Inorganic UV filter

Through absorbing and scattering UV rays, inorganic sunscreen does its magic. The inorganic sunscreens are titanium dioxide (TiO_2) and zinc oxide (ZnO). In comparison to organic actives, TiO_2 and ZnO are superior in a number of ways. The inorganic actives are photostable, allowing for a production of sunscreen that we can anticipate and predict the amount of photoprotection it provides after UV exposure. On the other hand, organic actives such as avobenzone, if not stabilized properly, after 1 hour of UV exposure, it could lose up to 50% of its photoprotective properties. Low rates of sensitization and low allergenic potential are also competitive traits of both TiO_2 and ZnO . ZnO is also able to extend its photoprotection to the UVA1 (reaching up to 380 nm) range (156), however, its enormity of UV protection is less significant in comparison to other organic UV filters.

Regardless of its advantages, early generations of inorganic sunscreens are not particularly welcomed by the general public due to its undesirable whitening appearance, a result of high refractive indices (TiO_2 refractive index = 2.6 and ZnO refractive index = 1.9) and its large particle size. TiO_2 products due to its higher refractive index, makes it appear whiter when applied on the skin, in contrast to ZnO products. Put aside all the mentioned shortcomings, gritty sensations are observable when the sunscreens dry due to subpar dispersion and large particles size.

In the past decades, successful attempts have been made in reducing the size of the particle of both TiO_2 and ZnO . The improvement led to reduced visible light scattering and enhanced cosmetic appearances. Nevertheless, in an effort to micronize the particles, trends are developing toward incorporating nano-sized TiO_2 and ZnO into the sunscreens. Nanoparticles are characterized as being less than 100 nm in size. Compared to normal sized counterparts, nanomaterials possesses emerging mechanical, optical and electrical properties, to a certain extent, a result of a tremendous increase in surface area to volume ratio. Unprecedented outcomes when dealing with biological tissues are sometimes a result of these emerging properties. This in turn creates an increasing concern towards incorporating nanomaterials into personal care products as it tampers with its safety profile.

When encompassed into sunscreens, white residues are less obvious and UV protection is supercilious due to the nano-sized ZnO and TiO₂. Optimal range for UV absorption and scattering properties is in the range of 20 to 30 nm for the size of titanium dioxide particles. On the other hand, to get the optimal UV protection from zinc oxide particles, it needs to be in the range of 60 to 120 nm for its size. Due to its popularity, in excess of thousand tons of these nanoparticles are produced each year for the formulation of sunscreens. Consequently, in addition, increase in scrutiny from nonprofit organizations, governmental regulatory agencies and nonprofit organization also increases as well, focusing on the issues concerning toxicity profiles and skin penetration.

In regards to skin penetration, the main concern is that the nanoparticles will effortlessly seep through the skin barriers and therefore interacting with the living cells situated in the lower part of the epidermis. Despite all the assumptions, actual *in vitro* and *in vivo* studies suggests that there are no penetration of the nano-sized ZnO and TiO₂ into the lower part of the epidermis but rather stayed at the stratum corneum level when using porcine, murine and human skin (157-166). There are no differences in the penetration level in comparison to its counterpart, macro-sized particles. However, in some cases where minor penetrations of nanoparticles were presence, only the superficial portion and the pilosebaceous openings of the follicles were protruded. There are various factors why nanoparticles usually stay in the corneum level. Through transdermal drug delivery research, various factors, for example; size and concentration, affect the molecule penetration of the stratum corneum. Minute compounds with molecular size ranging from 0.8 to 1.6 nm (molecular weight ranging from 163 to 357 Da) can easily penetrate the skin barrier. The final formulation of sunscreen usually results in a larger agglomeration of nanoparticles. These agglomerations are 10 to 100 times larger than the molecule size and molecule weight range that can easily penetrate through the stratum corneum. Studies are also mostly conducted on subjects with healthy skin with the stratum corneum being intact. Increase penetration is possible through subjects with skin diseases; however, there are no conclusive evidences that support this hypothesis (167). On the other hand, skin diseases; for example, hyperkeratosis and psoriasis, result in a reduction of penetration.

Depending on the amount of exposure and the exposure levels to the living cells, also taking into consideration the intrinsic toxicity of TiO_2 and ZnO , the toxicity profile of nanoparticles is then determined. Through earlier discussions, the use of topical sunscreens with nanoparticles shows no evidence of increased penetration into the living cells compared to topical sunscreens without nanoparticles. The profile of toxicity is minimal for TiO_2 and ZnO . For decades, both of the compounds have been in existence with minimal to no track record of systemic effects or adverse skin. In addition, health benefits and essential nutrients are provided through Zinc and food additives are known to incorporate Titanium. A study demonstrated TiO_2 being photogenotoxic in Chinese hamster lung cells and mouse lymphoma, instigating concerns towards TiO_2 and ZnO (168). After being exposed to UV, there is a formation of hydroxyl radicals from TiO_2 , resulting in the breakage of DNA strand, the mechanism behind photogenotoxicity of TiO_2 .

A reviewed for the preparation of nano-sized TiO_2 and ZnO sunscreens was initiated by the European Commission to investigate the toxicity issue. It has been concluded that after topical application, the particles does not create sensitization, photosensitization, irritation or toxicity.

In conclusion, both nano-sized and micro-sized TiO_2 and ZnO provide superior photoprotection and is also able to enhance the cosmetic appearance. Present studies support its safety profile, showing no increase penetration.

2.6.3.3 Sunless Tanning Agents

Naturally, skin pigmentation enhancing agents have demonstrated some photoprotection (169). Through the oxidation effect that changes skin color to orange-brown, Dihydroxy acetone (DHA) or the active ingredient in sunless tanning preparations, protects against the low end of visible region and UVA (170). As the color chemically binds to the stratum corneum, it is not washed off easily, remaining intact to the stratum corneum and does not affect normal skin function (171). Nevertheless, DHA induced tanning only results in a SPF 2 rating.

The popularity of DHA spray has increased over the past few years but there are no current data regarding the safety of aero-solized DHA partiles inhalation.

2.6.3.4 Limitations of Topical UV filters

Naturally, our skin provides protection against the harmful effects of UVR for a momentarily period of time, depending on the skin type, varying between 10 to 40 minutes in exposure to sunlight. To be able to stay in the sun for a longer period of time, extra protection measures are required, for example, direct sunlight avoidance at midday, wearing protective clothing (long sleeve shirt, sunglasses) and using sun protection products such as sunscreens. In order to prevent the skin from serious damages and sunburns, certain criteria and qualification must be met by the sunscreens used for protection. Sunscreens should be photostable (unaffected by heat and UVR); the agents within the sunscreen formulation should not affect each other, causing the finished product to unstable. Through photochemical and photophysical pathways without harmful reactive intermediates formation, sunscreens should dissipate the light energy absorbed. It should be water resistant and should not penetrate into the skin (113). Sunscreens generally contain both organic and inorganic UV filters in combination, ensuring protection for the whole UVA and UVB range. Active ingredients provide protection through either absorption or reflection of UVR.

Tolerability and performance are very important factors in sunscreen consideration as high concentrations are used over the surface of the whole body. In accordance to the European Cosmetics Regulation No. 1223/2009 as a safety assessment, qualified dermatological tests are mandatory. Even though UV filter agents are tested for their photosensitization and photoirritative potential, allergic potential may show up years after a widespread usage of various products. VL is currently not protected through UV filters, requiring opaque filters including physical filters such as clothing, ZnO or TiO₂ (172).

According to approximately 55 to 80% of positive photopatch test results, various organic UV filters are found to trigger photoallergic and allergic reactions (173, 174). Due to their small molecular size and in addition their lipophilic character, sufficient skin penetration is presence, initiating allergic responses.

Regardless of technological advancements, sunscreen protection will always rely on proper usage. Repetitive daily application is the most promising way of protection against UVR. Putting compliance and frequency of

application aside, the quantity or usage amount is the most essential factor in regards to the efficacy of sunscreen (19). In studies assessing the SPF of sunscreens, an amount of $2\text{mg}/\text{cm}^2$ is applied versus 0.5 to $1.5/\text{cm}^2$ (175). A linear relationship is not applicable to the amount of sunscreen applied against SPF, therefore, sunscreens lose their protective value quickly at small amount (125, 176). To put it in perspective, application of $1\text{mg}/\text{cm}^2$ of SPF 52 sunscreen provide a result of SPF 7 protection (177). Reapplication of sunscreens further adds to issues previously stated as guidelines generally state that individuals should apply sunscreens approximately 30 minutes before sun exposure and thereafter, reapplying every 2 to 3 hours (178).

Inevitably, addition protection method is needed to better provide protection against UVR as many limitations plague current existing protection methods.

2.6.3.5 Sunscreen Measurement

UVA protection measurement

The sunburn action spectrum is largely weighed and attributed towards UVB range with only a small portion contributed by UVA (320 – 400 nm). As a result, the effectiveness of sunscreens against UVA cannot be measured using the sun-protection factor method. When a broad UVA source is used, end points including IPD, PPD and UVA erythema, demonstrates sensitivity across the entire UVA spectrum (179). Such reactions are used as *in vivo* measurement for UVA attenuation by photoprotective materials.

The method mentioned above has been modified to provide a new 'range' of UVA protection encompassing a test method called 'critical wavelength' (180). The absorbance of the thin film of the sunscreen is integrated (summed) from 290 nm across the UV wavelengths until the sum reaches 90% of the total absorbance of the sunscreen in the ultraviolet region (290-400 nm). The wavelength at which the summed absorbance reaches 90% of total absorbance is defined as the 'critical wavelength' and is considered to be a measure of the breadth of sunscreen protection. Filters are then classified as 'broad spectrum', having a significant part of their absorbance in the UVA, when the critical wavelength is longer than 370 nm.

Protection Grade of UVA (PA) measurement is a system invented by the Japanese, a different version of the UVA measurement for sunscreens, converting UVA score from standard PPD test. Different countries and regions have different UVA measurement rating systems. Currently, there are five countries with their own UVA measurement rating system with Japan being one of them.

In order to find the PA rating, a specific test protocol is followed. A minimum of 10 subjects is recruited for the study with additional test subjects if deemed necessarily. An application of 2 mg/cm² is applied to the subject. A series of UVA light exposures, 150 watt Xenon Arc Solar Simulator equipped with an Ultraviolet (UV) reflecting dichroic mirror, 3mm thick Schott WG-335 filter together with a 1mm thick Schott UG-11 filter to produce simulation of the UVA solar spectrum are administered 15 minutes after application at 25% increments. At each site, the PPD threshold is determined. UVA – Protection factor (UVAPF) is the lowest dose of UVA that stimulate the first clearly perceptible PPD response with well-defined borders appearing all over across the area exposed to UVA. UVAPF is calculated as minimal PPD (MPPD) (seconds) of the protected skin / MPPD (seconds) of the unprotected skin.

PA rating = PA+ for UVAPF = 2 – less than 4

PA rating = PA++ for UVAPF = 4 – less than 8

PA rating = PA+++ for UVAPF = 8 – less than 16

PA rating = PA++++ for UVAPF = 16 or more

PPD ratings are the ratings used for assessing sunscreens and its UVA protection potential. Currently, there are five countries employing some kind of UVA testing including the US, Germany, United Kingdom, Australia and Japan.

PPD on its own does not indicate the level of UVA protection but is rather converted into a score used by different regions or specifically to certain countries. Japan use their own conversion system to covert PPD score to a “Protection of UVA” score, PA. PA is written followed by plus symbols (+), either one, two, three or four pluses, varying upon the PPD score from UVA testing for that particular

sunscreens. The designation is then required to be placed on the label of the sunscreen (181). PPD score is obtained through a certain test protocol.

UVB protection measurement

At the moment, an accepted international standard for the evaluation of sunscreen efficacy is called SPF (1-4). SPF is based upon the erythema formation, calculated as a ratio of the minimal erythema dose on the skin protected by the application of sunscreen in the amount of 2 mg/cm² to the minimal erythema dose on the skin without sunscreen application. However, through a single efficacy indicator, employing only erythema formation, consequences of radiation in volunteer testing is omitted and a single process of biology, UVB radiation induced, is primarily focused upon (182, 183). Development of new efficacy indicators is crucial as current indicators insufficiently account for damages induced by UVA, IR and VL radiation through SPF application (184-186).

An *in vitro* SPF testing is a quicker and cheaper means and ethical issues associated to *in vivo* testing would be avoided altogether. Various techniques are developed for *in vitro* studies but a commonly accepted method has yet to be formed. A common *in vitro* approach usually involves a film layer of sunscreen being applied to an artificial test substrate and using a spectrophotometer to analyze and monitor the amount of UVR penetrating through the film layer of sunscreen. Spectral transmission is measured pre and post exposure to UV source, applying sunscreen to the substrate, in the case of transmission spectroscopy. There are several variables influencing the spectrophotometric analysis including, different compositions of filters, types of artificial substrate, quality of spectrophotometer, amount of sunscreen application and spreading method.

Through facilitation of artificial UVR source, solar simulator, photostability test is viable through repeat transmission measurements post exposing sunscreen application upon artificial substrate to this source. One of the most widely adopted simulators is a Xenon Arc solar simulator. Light spectra meeting the specifications for SPF testing set by the European Cosmetic Toiletry and Perfumery Association (COLIPA) can be produced by the simulator and is regularly used for *in vivo* SPF testing. The Xenon Arc solar simulator is able to cut off radiation at around

380 nm, omitting IR and VL radiation. Laboratory that utilizes a solar simulator such as the ORIEL 300 W full spectrum solar simulator can use filters accessories to discard selected wavelengths.

Various artificial substrates are utilized in this type of analysis. The test substrate should be able to replicate as much as possible the characteristics of the physical skin. Common substrates include Transpore, Roughened Quartz Plate, Vitro-Skin, PTFE (Teflon) and polymethylmethacrylate (PMMA) plates. Transpore is commonly known as a readily available and inexpensive surgical tape currently uncommon in usage. Roughened Quartz is mostly used due to its high UV transmittance properties but its main disadvantage is cost. In order to reuse it, certain cleaning procedures are mandatory and can be costly. Vitro-Skin is a synthetic skin substrate, requiring exact hydration procedure to be followed before usage. Vitro-Skin has shown to demonstrate exceeding performances in sunscreen tests against some its short comings including hydration requirements, short lifetime and a relatively high cost. PTFE has been used extensively due to its decent transmittance in UV application and its Lambertian properties. PMMA plates are currently most widely adopted in the industry for *in vitro* testing of UVA. This is due to its easy to handle properties and reproducible roughness. Biological-derived substrates have also been used in tests but the outcomes are not reproducible (187).

New methods for measuring sunscreen efficacy

Several photobiologists proposed new methods in determining a more complete and comprehensive manner of sunscreen efficacy through different mechanisms (188). This includes the universal SPF (USPF) (189) and the radical formation ratio (RF), based upon measurements of electron paramagnetic resonance (EPR) (190).

The USPF allows for the entire UV range to be objectively quantified by the protective efficacy of sunscreen products. Based upon the sum transmission spectrum, this method employs noninvasive tape stripping procedure *in vivo*, determined spectroscopically. The basis of new protection factor calculation is the result of sum transmission quantifying the reduction in UVR intensity through the product.

$$\text{USPF} = 100 / \text{average UV sum transmission in percent}$$

RF determined via EPR spectroscopy uses a different approach. Based on the findings, free radicals are found to be produced not only in the UV range but also up to 50% in the VL and IR ranges (184). Using the underlying biological response of RF, this method determines sun protection efficacy, therefore providing possible determination of the efficacy of protection in the UV, VL and IR ranges.

$$\text{RF} = \text{EPR signal before irradiation} / \text{EPR signal after irradiation}$$

2.6.4 Systemic Photo-protective Agents

There are various methods to which we can protect ourselves from the harmful UVR. Topical sun protection is the first type of protection that spurs to mind. It is considered to be the protector for UVR induced damages. On the other hand, there is a systemic way, preventing the UVR damage to happen in the first place underneath our skin. Topical system is a protector and systemic system is a preventer.

Relations between skin cancers and foods and interventional experiments have realized UV skin damage mechanisms through epidemiological identification. Oral substances have been identified through these approaches as being photoprotective in humans. An energy crisis result from decreasing of adenosine triphosphate (ATP) production through UV inhibition prevents DNA repair and optimal skin immunity. Supplementation of oral nicotinamide allows for ATP production enhancement; reducing skin cancer in humans, enhancing DNA repair and protecting against UV immunosuppression. Photodamage is also a result of ROS. Oral supplements or even nontoxic substances consumed regularly in our diet can help to provide protection through various potential mechanisms and can influence cutaneous responses to UV. Some of the substances include polyphenols in fruits and vegetables, wine, tea and caffeinated foods. Contribution to photodamage also includes UV-induced PGE₂. Through partial reduction of PGE₂, fish oils are photoprotective.

In summary, systemic protective agents can be grouped into plant and animal derived agents. There are many systemic photoprotection studies for example, experiment in both humans and animals.

Deriving from a tropical fern extract, polypodium leucotomos extract (PLE) is driven primarily by caffeic and ferulic acid, reducing UV-induced erythema, inflammatory mechanisms and promoting other cellular responses. Prevention of acute sunburn and minimization of photoaging has been described as beneficial effect of PLE (191, 192). The inhibition of ROS formation induced by UV light (193) and anti-inflammatory properties has also been reported through PLE (194). *In vitro* study demonstrates the effect of PLE through its preservation of the proliferative ability of UV-treated cells and protection of the cells against UVA-induced cytoskeletal changes. Preservation of human fibroblast survivability and restoration of their proliferative capability was efficiently achieved through PLE, when the cells were exposed to UVA light.

Found in various different plants, polyphenols provide many health benefits from its antioxidant, anti-inflammatory and immunomodulating properties. Consumed as a part of our daily diets through fruits and vegetables, various examples of these compounds provide protection against photodamage (195, 196).

Through the mechanism of reducing PGE₂ and inflammatory cytokines and enhancing DNA damage repair, UV-induced photocarcinogenesis is a result of green tea polyphenols added to the drinking water for mice (197).

Lycopene is carotenoid present in tomatoes at high concentrations. When orally administered in human, lycopene is able to provide protection against UV-induced erythema, acting as an effective quencher of singlet oxygen (198). In a randomized controlled study, diet of women that has been supplemented with tomato paste demonstrates a reduction in UV-induced MMP-1 production and mitochondrial DNA damage (199).

Diets consisting of fish oils that contain omega-3 polyunsaturated fatty acids have been shown to reduce UV-induced inflammation in humans. This may be a result of fish oils reducing PGE₂ production (200). In the same group, a double-blinded, randomized controlled study was conducted showing a reduction in UV-induced immunosuppression in humans through supplementation of omega-3

fatty acids (201). Partially due to a decreased inflammatory response, high levels of fat fish oil diet fed to mice increased latency prior to UVB-induced skin cancers development and a reduction in incidence of tumors (202).

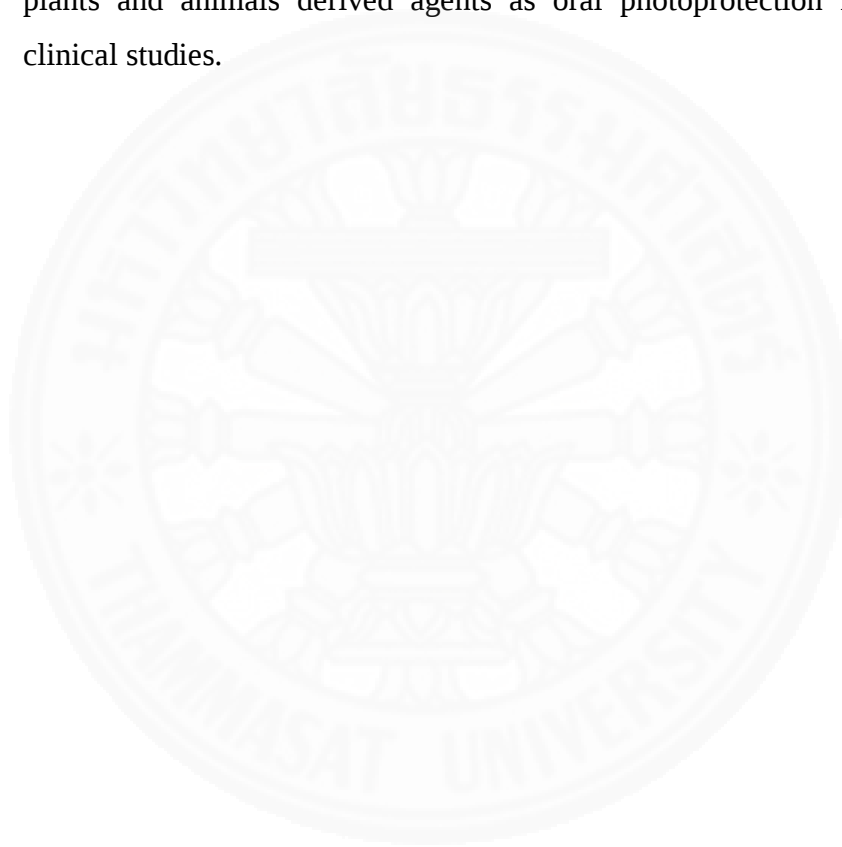
Oral systemic supplements, drugs and food substances have shown considerable evidences indicating an additional level of photoprotection. As a result, systemic photoprotection could be an alternative method used solely or in adjuvant with other photoprotective strategies in providing protection from UVR.



CHAPTER 3

OBJECTIVE

- Primary objective: to systematically review photoprotective outcomes of oral plants and animals derived agents from available clinical studies in healthy human subjects by using evidence-based method.
- Secondary objective: to summarize the possible mechanisms of individual plants and animals derived agents as oral photoprotection from available clinical studies.



CHAPTER 4

SIGNIFICANCE OF THE RESEARCH

Topical sunscreens that filter UVR or reflect sunlight have solely long been used in protecting the damaging harmful effects of sunlight. Sun avoidance, including, wearing clothes, sunglasses, hats and other shading accessories in direct sunlight or avoiding sunlight as a whole and staying in shades are another way to avoid the harmful UVR. However, there are limitations such as frequency of topical application and allergic reaction to topical agents. A significant amount of evidence suggests that systemic or oral drugs, supplements and food substances can supply extra levels of photo-protection.

The mechanism of actions, illustrating the pathways in which sunlight damages the skin has been brought to light by experimental studies leading to the discovery of new and safe substances that can be orally consumed to provide better protection from sunlight. These findings bring great advantages as oral supplements can reduce and suppress damages by sunlight. Millions of people are subjected to the harmful effects of UVR each day and yet does not have adequate protection against it. With a better understanding and the development of promising systemic agents as an oral supplement, more people will be able to avoid sunlight related skin diseases.

CHAPTER 5

RESEARCH METHODOLOGY

Our systematic review is based on the PRISMA protocol. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines is an international group of experts has created a guideline to improve the transparency, accuracy, completeness, and frequency of documented systematic review and meta-analysis protocols—PRISMA-P (for protocols) 2015. The PRISMA-P checklist contains 17 items considered to be essential and minimum components of a systematic review or meta-analysis protocol (203).

According to PRISMA-P, systematic review attempts to collate all relevant evidence that fits pre-specified eligibility criteria to answer a specific research question. It uses explicit, systematic methods to minimize bias in the identification, selection, synthesis, and summary of studies. When done well, this provides reliable findings from which conclusions can be drawn and decisions made (204, 205). The key characteristics of a systematic review are:

- (a) a clearly stated set of objectives with an explicit, reproducible methodology
- (b) a systematic search that attempts to identify all studies that would meet the eligibility criteria
- (c) an assessment of the validity of the findings of the included studies (such as assessment of risk of bias and confidence in cumulative estimates)
- (d) systematic presentation, and synthesis, of the characteristics and findings of the included studies

5.1 Eligibility criteria

Studies will be selected according to the criteria below.

5.1.1 Study designs

The systematic review was completed using electronic databases, PubMed, EBSCO and Cochrane database and the first relevant result was from the year 1986 up until 2016. Inclusion criteria include clinical trials and English full-texted journals in RCTs. Exclusion criteria include animal studies, topical photoprotective agent, combination therapies and Photodermatoses or Photosensitive subjects (**Table 5.2**)

Table 5.2 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Oral photoprotective agents (natural agents included plant and animal-derived agent after irradiation)	• Animal studies
• Studies in healthy human subjects	• Topical photoprotective agents
• Full-text journal	• Non-English publications
• RCTs	• Combination therapies
	• Photodermatoses or Photosensitive subjects

PubMed, EBSCO and Cochrane was searched using MeSH (Medical section Heading) terms: “sunscreening agents” OR “sun protection factor” OR “sunburn” OR “ultraviolet rays” OR “free radical scavengers” OR “infrared” OR “visible light” NOT “animal experimentation” NOT “administration, topical” AND “Clinical Trial”; “Full text”; “Humans”; “English”

Relevant studies were additionally identified through manual reviewing of reference lists. Two dermatologist reviewers reviewed the title and abstract for suitability of the article through inclusion and exclusion criteria. Duplicate studies were removed. (**Figure 5.7**)

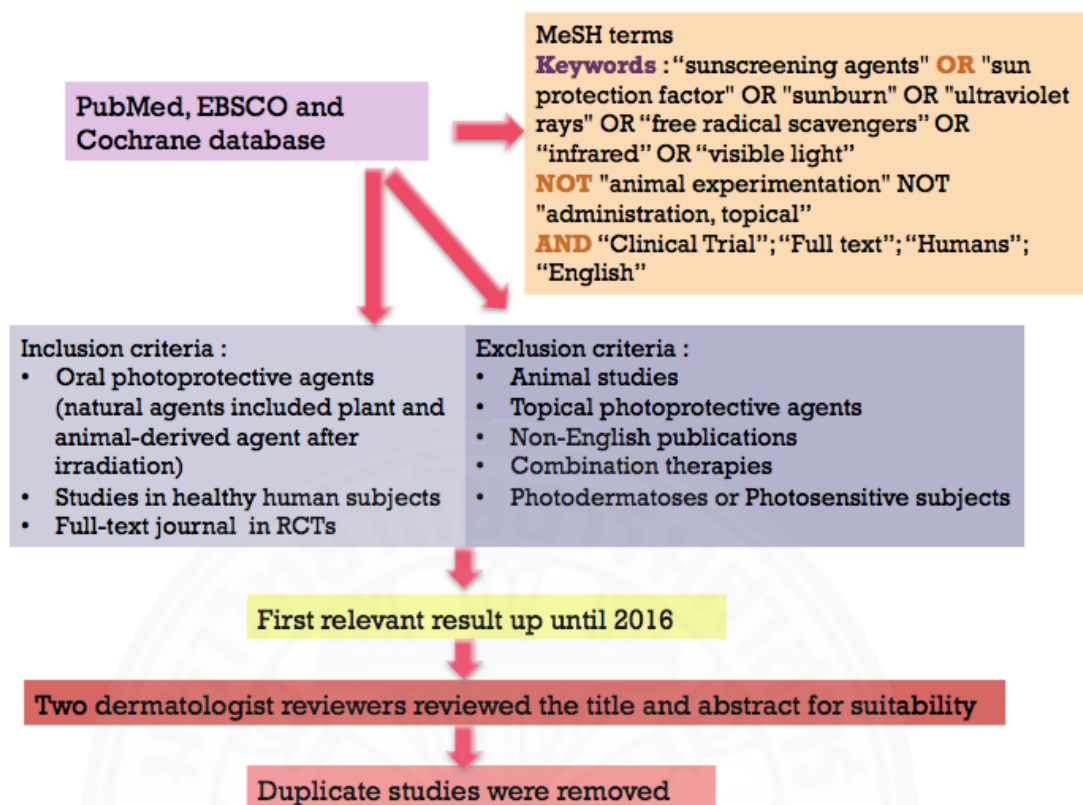


Figure 5.7 Diagram of literature search

5.1.2 Participants

Participants are general adult human population, healthy adult humans (18 years or older) with skin phototype I-VI, but will otherwise exclude patient exposed to excessive natural or an artificial UVR source in at least 1 month prior to recruitment, pregnant and breast feeding patients.

5.1.3 Interventions

In an individual study, supplement or specific diet had been administered within the specified period of time.

5.1.4 Comparisons

Pre-post outcomes, placebo comparison and dose difference are compared in each study.

5.1.5 Outcomes

The primary outcomes are clinical outcomes measured through MED and erythema intensity. The secondary outcomes are histology or biomarker outcomes measured through number of sunburn cells, number of LCs, number of mast cells, level of PGE2 and level of p53.

We graded level of evidence according to Centre for Evidence-Based Medicine, Oxford. (**Table 5.3**)

Table 5.3 Class, Level of evidence and Recommendation From the Centre for Evidence-Based Medicine, Oxford

Therapy/Prevention/Etiology/Harm:

1A :	Systematic reviews (with homogeneity) of randomized controlled trials
1B :	Individual randomized controlled trials (with narrow confidence interval)
1C :	All or none randomized controlled trials
2A :	Systematic reviews (with homogeneity) of cohort studies
2B :	Individual cohort study or low quality randomized controlled trials (e.g. <80% follow-up)
2C :	"Outcomes" Research; ecological studies
3A :	Systematic review (with homogeneity) of case-control studies
3B :	Individual case-control study
4 :	Case-series (and poor quality cohort and case-control studies)
5 :	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"

CHAPTER 6

RESULTS

6.1 Study characteristics

Total 3,529 articles were initially identified through PubMed, EBSCO and Cochrane databases. After removing duplicates, 111 articles were left, in which 18 studies met the inclusion criteria with 4 additional studies from other sources selected for full text article review. All studies included adult healthy participants aged 18 years and older. MED or changes in UV induced erythema intensity was assessed in most of the studies providing measurement for clinical outcomes. Histopathology was identified as biomarker outcomes. Two main groups were categorized regarding to their natural sources. (Figure 6.8)

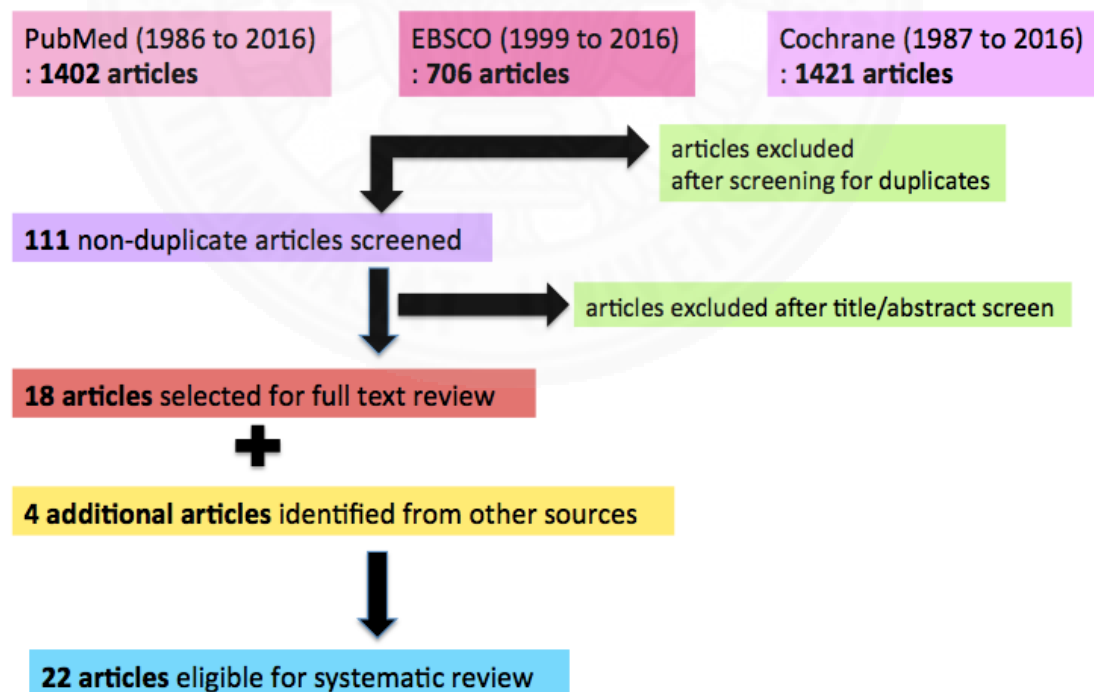


Figure 6.8 Diagram of literature search and study selection

6.2 Summary of Outcomes

Table 6.4 Summary of clinical studies

PLANT-DERIVED PHOTOPROTECTIVE AGENTS						
<u>Interventions</u>	<u>Subjects</u>	<u>Comparison</u>	<u>Measurements</u>	<u>Clinical outcomes</u>	<u>Histology/Biomarker outcomes</u>	<u>Level of evidence</u>
Beta-carotene						
β -carotene 24mg daily vs mixed carotenoids (β -carotene 8mg, lutein 8mg and lycopene 8mg) daily for 12 weeks (206)	N=12 in each group (total N=24)	Placebo	MED, UV-induced erythema	Significantly decreased UV-induced erythema intensity in both groups ($p<0.001$)	N/A	IB
Total carotenoids 25mg daily vs carotenoids 25mg with α -Toc 500 IU daily for 12 weeks (207)	N=8	Carotenoids with vitamin E group	MED, UV-induced erythema	Significantly decreased UV-induced erythema intensity in combination of carotenoids and α -Toc ($p<0.01$) and in carotenoids alone ($p<0.05$)	N/A	IB
β -carotene 180mg daily for 10 weeks (208)	N=18	Placebo	MED, degree of sunburn	Significantly increased MED ($p<0.05$), less pigmentary changes after sun-exposure ($p<0.05$)	N/A	IB

<i>Solanum lycopersicum</i> (Tomato)						
Tomato paste (16 mg lycopene) vs lycopene capsule (16 mg lycopene) daily for 10 weeks (212)	N=10	Lycopene capsule group	MED, erythema	Significantly decreased UV-induced erythema in both groups (p=0.054). No significant changes of MED in both groups	N/A	IB
Tomato paste 55g (16 mg lycopene) in olive oil vs olive oil alone daily for 12 weeks (199)	N=10	Placebo	MED, histology	No statistically significant difference of MED between both groups	Significantly reduced UV-induced MMP-1 (p=0.04), decreased mtDNA 3895-bp deletion (p=0.01)	IB
Tomato extract (9.8 mg lycopene) vs Drink containing solubilized tomato extract (8.2 mg lycopene) vs Synthetic lycopene (10.2 mg lycopene) daily for 12 weeks (213)	N=12 in each group (total N=36)	Placebo	MED, UV-induced erythema	Significantly decreased erythema intensity of both tomato extract (p<0.001) and drink containing tomato extract (p<0.001) but not in synthetic lycopene group	N/A	IB
Tomato paste 40 g (16 mg lycopene) in olive oil vs olive oil alone daily for 10 weeks (214)	N=9	Placebo	MED, UV-induced erythema	Significantly decreased erythema intensity (p=0.02)	N/A	IB

<i>Camellia sinensis</i> (Green tea)						
GTCs 540mg with 50 mg vitamin C, twice daily for 12 weeks (209)	N=25	Placebo	MED, histology	No statistically significant changes of MED	No statistically significant difference in leukocyte infiltration	IB
Total catechins 1402 mg daily for 12 weeks (210)	N=30	Placebo	MED, UV induced erythema	Significantly decreased UV induced erythema (p<0.05)	N/A	IB
EGCG 800 mg daily vs EGCG 400 mg twice daily vs EGCG as Polyphenon E 800 mg daily vs EGCG as Polyphenon E 400 mg twice daily for 4 weeks (211)	N=8 in each group (total N=32)	Placebo	MED	No statistically significant changes of MED	N/A	IB
<i>Polypodium leucotomos</i> (Golden serpent fern)						
P.leucotomos extract 1080 mg (240 mg*3 times a day before exposure and additional 360 mg 3 hours before exposure) (191)	N=13	Placebo	MED, IPD, histology	Significantly increased MED (p<0.001), IPD (p<0.01)	Preservation of CD1a-expressing epidermal LCs in treated group	IIB

<i>Theobroma cacao</i> (Cocoa bean)						
HFC 600 mg vs LFC <30 mg daily for 12 weeks (215)	N=33 for HFC	LFC	MED	Increased MED in both groups; however, no significant difference between HFC and LFC group	N/A	IB
HFC 600 mg vs LFC <30 mg daily for 12 weeks (216)	N=15 for HFC	LFC	MED	Significantly increased MED in HFC group ($p=0.005$) but not in LFC Significant difference between HFC and LFC group ($p\leq 0.05$)	N/A	IB
HFC 329 mg vs LFC 27 mg daily for 12 weeks (217)	N=12 for HFC	LFC	MED, UV induced erythema	Significantly decreased UV-induced erythema ($p<0.05$) in HFC group but not in LFC group Significant difference between HFC and LFC group ($p<0.05$)	N/A	IB

Tocopherol (Vitamin E)						
α -Toc 400 IU daily vs β -carotene 15mg daily for 8 weeks (218)	N=8 for α -Toc	β -carotene	MED, histology	No significant changes of MED in both groups	Significantly decreased skin malondialdehyde concentration only in α -Toc group (p<0.05)	IB
α -Toc 1,200 IU daily vs Asc 2 g daily vs α -Toc 1,200 IU with Asc 2 g daily for 1 week (219)	N=14 for α -Toc	Asc and α -Toc with Asc	MED	Significantly increased MED in α -Toc alone (p=0.002) and in Asc with α -Toc (p=0.0001) No significant changes in Asc alone group	N/A	IB
α -Toc 2 g daily vs Asc 3 g daily vs α -Toc 2 g with Asc 3 g daily vs placebo for 50 days (220)	N=10 in each group (total N=30)	Placebo	MED	Significantly increased MED only in α -Toc with Asc group (p<0.005)	N/A	IB
α -Toc 400 IU daily for 6 months (221)	N=6	Placebo	MED, histology	No significant change of MED	No significant difference in sunburn cells between two groups	IB

ANIMAL-DERIVED PHOTOPROTECTIVE AGENTS						
Nicotinamide						
Nicotinamide 500 mg twice daily for 1 week (222)	N=30 for Mantoux-positive subjects	Mantoux-positive subjects (crossover)	MIE, Mantoux diameter	Significantly reduced MIE ($p<0.0001$)	Significantly reduced Mantoux diameter ($p<0.0001$)	IB
Nicotinamide 500 mg three times daily (high dose) vs once daily (low dose) vs placebo for 1 week (223)	N=61 for Mantoux-positive subjects	Mantoux-positive subjects (crossover)	MED, Mantoux diameter	No significant changes of MED	Significantly reduced Mantoux diameter ($p<0.001$) in both groups	IB

Omega 3 polyunsaturated fatty acids						
95% EPA 4 g vs 95% Oleic acid 4 g daily for 12 weeks (224)	N=21 for EPA	Oleic acid	MED, p53, CPD, comet assay	Significantly increased MED (p<0.01) in EPA but not in OA group	Significantly reduced in UV-induced p53 expression (p<0.01) in EPA but not in OA group No statistical difference in CPDs changes	IB
Fish oil (280 mg EPA+120 mg DCHA) daily for 4 weeks (225)	N=10	Placebo	MED, PGE2	Significantly increased MED (p<0.02)	No significant changes of PGE2	IB

6.3 Plant-derived Photo-protective Agents

6.3.1 β -carotene and Lycopene

Most of the carotenoids in our diet are from fruits and vegetables that we eat and adapting to the continuous supply by utilizing some of them as precursors for vitamin A, crucial to cell signaling and vision. Carotenoid pigments aid in the protection of photo-synthetic apparatus in plants scattering excess energy. Various evidences suggest that carotenoids protect the human skin from UV-induced lesions.

Lycopene are mostly derived from tomato products, accounting for more than 80% of the lycopene consumed, with the remaining deriving from various fruits including watermelon, pink grapefruit, guava, apricots and papaya. Depending on the type of tomato and its state of ripening, lycopene contents can vary significantly. Lycopene levels can reach as high as 50 mg kg⁻¹ in the reddest strains of tomatoes and on the other hand, can reach as low as 5 mg kg⁻¹ in the yellow strains. Through thermal processing and dietary lipids coingestion, the bioavailability of lycopene from dietary sources increases tangibly (226, 227). The accessibility of the lipophilic compound in regards to the formation of lipid micelles in conjunction with dietary bile acids and lipids improves through process of food processing, releasing lycopene from the food matrix. As the bioavailability of carotenoids increases through cooking and food processing process, an ingestion of tomato paste (processed tomatoes) yield a higher lycopene uptake versus ingestion of fresh tomatoes (228).

6.3.1.1 Molecular Composition and Pharmacology

Carotenoids are a class consisting in excess of 600 naturally occurring pigments synthesized by photosynthetic bacteria, algae and plants. The origins of the vibrant colors, yellow, orange and red found in many plants are from these full bodied colored molecules. Most of the carotenoids supplemented into our diets are from fruits and vegetables. The most common dietary carotenoids are α -Carotene, β -carotene, β -cryptoxanthin, lutein, lycopene and zeaxanthin. Carotenoid that can be converted by the body to retinol or pro-vitamin A carotenoids consist of α -Carotene, β -carotene, β -cryptoxanthin. On the other hand, lutein, lycopene and

zeaxanthin are unable to convert to retinol suggesting that no vitamin A activity exist. Carotenoids are usually classified into two broad classes, carotenes (α -carotene, β -carotene, and lycopene) and xanthophylls (β -cryptoxanthin, lutein, and zeaxanthin).

β -carotene, α -carotene, lycopene, xanthophylls lutein, zeaxanthin, α -cryptoxanthin and β -cryptoxanthin are the most copious carotenoids in human organism (229, 230). Ranging from 0.2 to 0.6 nmol (g wet tissue) is the level of carotenoid in human skin, nevertheless, significant differences are present in regards to the level of single carotenoids and carotenoids distributed in different areas of the skin (231). (Figure 6.9)

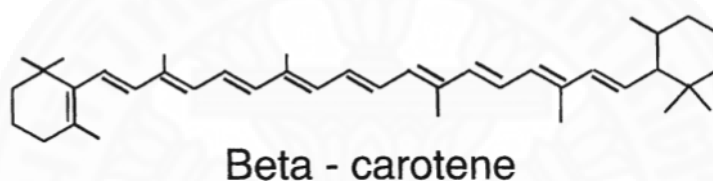


Figure 6.9 β -carotene molecular structure

6.3.1.2 Photoprotective Outcomes

β -carotene

Three RCTs of beta-carotene reported significant decrease UIE intensity and increased MED after 10-12 weeks of supplementation (206-208). The major source of β -carotene in two studies was from the alga *Dunaliella salina* (206, 207). β -carotene 24 mg was given daily versus mixed carotenoids (β -carotene 8 mg, lutein 8 mg, lycopene 8 mg) for 12 weeks in 36 healthy adults with skin type II in each group (206). The result suggests that is a significant decreased in UV-induced erythema intensity in both group ($p < 0.001$). In another study, carotenoid supplementation (25 mg total carotenoid was given daily for 12 weeks versus combination of carotenoid supplement (25 mg) with vitamin E (500 IU) daily in a group of 17 (207). Result shows a significant decrease in erythema intensity in combination of carotenoids and α -tocopherol ($p < 0.01$) and in carotenoids alone ($p < 0.05$). This suggests that supplementation of lycopene 8-25 mg helps to increase

the basal protection of skin against erythema. Nevertheless, there was no study evaluating histological changes.

Another study involve high dose β -carotene (180 mg) daily on 30 healthy adults for 10 weeks (208). The result shows a significant increase in MED ($p<0.05$) and less pigmentary change post sun exposure ($p<0.05$).

Lycopene

Four RCTs of lycopene from tomato paste or tomato extract showed similar trends of photoprotection. After 10-12 weeks of lycopene ingestion at dose ranging between 8.2 to 16 mg daily, a significant decrease in UIE intensity or an increase of MED in lycopene group was noticed (199, 212-214).

An evaluation of the photoprotective effect between supplementation of 16 mg lycopene in the form of tomato paste and capsule was initiated in 20 patients, skin type II to III for a period of 10 weeks (212). Results suggest a decrease in UV-induced erythema in both group ($p=0.054$).

A similar study with 16 mg lycopene supplementation for 12 weeks in 20 healthy adults with skin type I to II, showed no statistically differences in MED (199). However, histology shows a significant decrease in MMP1 and the 3,895-bp deletion in mitochondrial DNA following UV exposure. This suggests that tomato paste containing lycopene may play a role in decreasing DNA mutagenesis, providing protection against acute and long term photodamages.

Similarly to the previous studies, tomato paste (16 mg lycopene) was administered to 9 healthy adults with skin type II for 10 weeks compared to placebo ($n=10$) (214). A significant reduction in erythema intensity was observed ($p=0.02$).

Another study evaluated the photoprotective effects of tomato extract and synthetic lycopene through supplementation of tomato extract (9.8 mg lycopene) versus drink containing solubilized tomato extract (8.2 mg lycopene) versus synthetic lycopene (10.2 mg lycopene) daily for 12 weeks through groups of 48 healthy adults, skin type II (213). The group receiving synthetic lycopene had no other carotenoid present in the preparation. On the other hand, other supplements were derived from tomato-based products. Carotenoids phytofluene and phytoene,

both lycopene precursors were present in addition to lycopene. The result suggests a significant decrease in erythema intensity of both tomato extract ($p < 0.001$) and drink containing tomato extract ($p < 0.001$), but not in synthetic lycopene group.

6.3.1.3 Mechanisms of action

(1) Systemic Antioxidant Properties

Crucial to the antioxidant properties of carotenoids is the extended system of conjugated double bonds (232-234). Considered to be amongst the most efficient natural scavengers of singlet molecular oxygen is carotenoids (232, 233, 235). It has been observed that signaling pathways in relation to singlet oxygen formation is associated to UVA dependent skin aging, and the effects of β -carotene on signaling are partly associated with singlet oxygen quenching properties (236). The process of lipid peroxidation is inhibited through carotenoids scavenging peroxy radicals when oxygen tension is low (237). **(Figure 6.10)**

Lycopene and its health beneficial properties are a result of its ability to defend and protect cells against oxidative damages. Compared to other carotenoids, lycopene has been given less attention and researched focused, yet *in vitro* studies suggests that lycopene is a potent quencher of singlet oxygen and a efficient scavenger of oxygen radicals (235, 238, 239).

Further studies on the latter parameters in addition to erythema response has been conducted on a group healthy premenopausal women ingesting lycopene sourced from tomato paste for a period of 12 weeks (199). Compared to previous studies, the protective effect against UV-induced erythema of lycopene is comparable. Post tomato paste supplementation shows a lower UV-induced expression of MMP-1, degrading interstitial collagen. Playing an important part in the mechanisms of photoaging, including ROS generation related to damaging reactions, mtDNA is a result of UV exposure. Accordingly, tomato supplementation and its preventive effects on UV-induced mtDNA deletion are a result of lycopene acting as scavengers of ROS. **(Figure 6.10)**

Further research is required upon the concept of using dietary constituents such as carotenoids for endogenous photoprotection. Current studies involve small numbers of participants and usually a specific group of the population

such as specific skin type, younger individuals and women. In treatment of light sensitivity disorders, doses that are beyond physiological range of β -carotene are applied. Carotenoids have been successful for over 30 years in ameliorating skin disorders related to photosensitivity, for example, patients suffering erythropoietic protoporphyria (240-242). It is commonly known that lesions are caused by singlet molecular oxygen, together with molecules or separately, being excited in a triplet state and that β -carotene is an effective quencher of these molecules.

Through human intervention study, skin texture and structure affected by antioxidant supplements have been investigated through mixture with β -carotene as a part of the ingredient (243). Nevertheless, there are no evident proofs regarding the effect being unambiguously attributed to β -carotene.

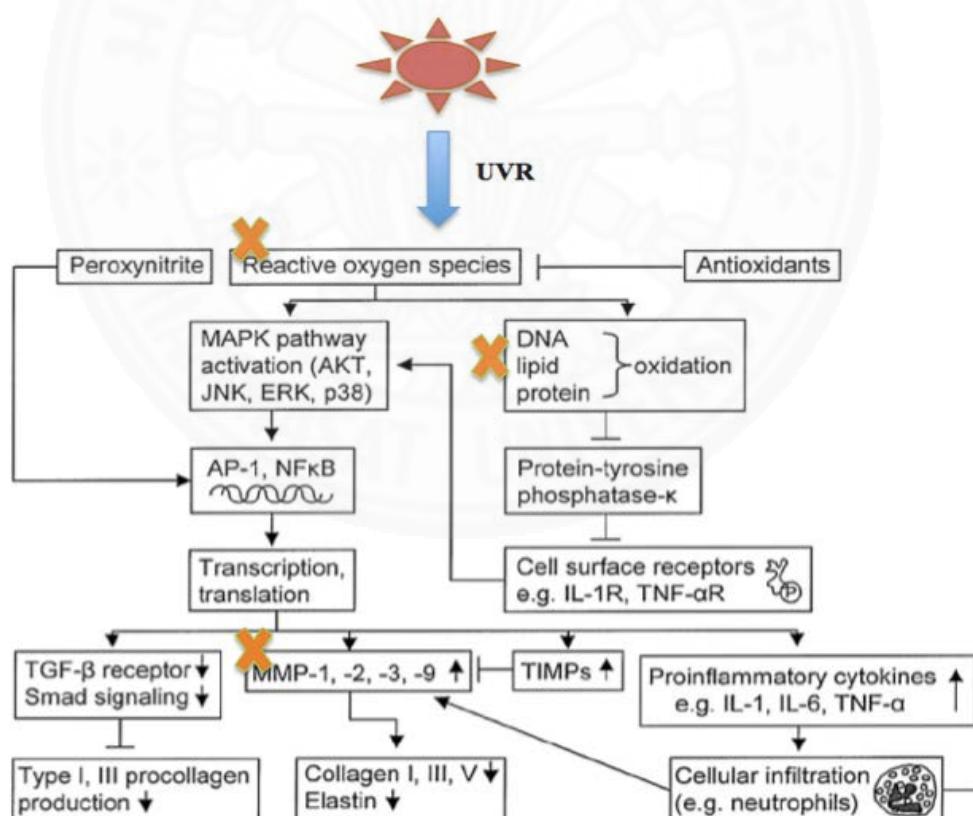


Figure 6.10 β -carotene and lycopene mechanisms of action (modified from (62))

6.3.2 *Camellia sinensis*

Tea is one of the most consumed beverages in the entire world, especially in Asian countries. The consumption of tea may be the reason behind lesser amount of pathological conditions incidences including, diabetes, obesity, cardiovascular disease and cancer. The fresh leaves of the plant *Camellia sinensis* is then used to manufacture Green tea (244). The plant's leaves are fermented, preventing polymerization and oxidation of the plant's polyphenols for the most part through the entire process. Four major polyphenols are contained within green tea including, (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG). Other agents are also contain in green tea including, phenolic acids, caffeine, flavandiols, flavonoids as well as theophylline and alkaloids theobromine (245). It has been found that polyphenols which possesses in green tea is the most effective at suppressing the carcinogenic activity of UVR. Some of the damaging effects of UVR that green tea is able to protect against are UV-induced sunburn response, UV-induced immunosuppression and photo-aging. The photo-protective effects are utilized through several cellular, molecular and biochemical mechanisms in *in vivo* and *in vitro* systems. Therefore, green tea polyphenols possess the potential when used together with topical sunscreens to further enhance the photo-protective properties against the adverse effects of UVR.

6.3.2.1 Molecular Composition and Pharmacology

The fundamental anti-oxidative ingredient in the green tea extract is green tea catechins (GTC), which consist of four major epicatechin derivatives including, (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG) (**Figure 6.11**). Of the four polyphenols, EGCG accounts for more than 40% of the total content and is the most potent at suppressing the carcinogenic effect of UV radiation. Other constituents include three kinds of flavonoids, known as kaempferol, quercetin, and myricetin. A significantly higher level of myricetin is perceived in tea and its extracts compared to many other plants, and this high concentration of myricetin may be the reason behind the bioactivity of tea and its extracts.

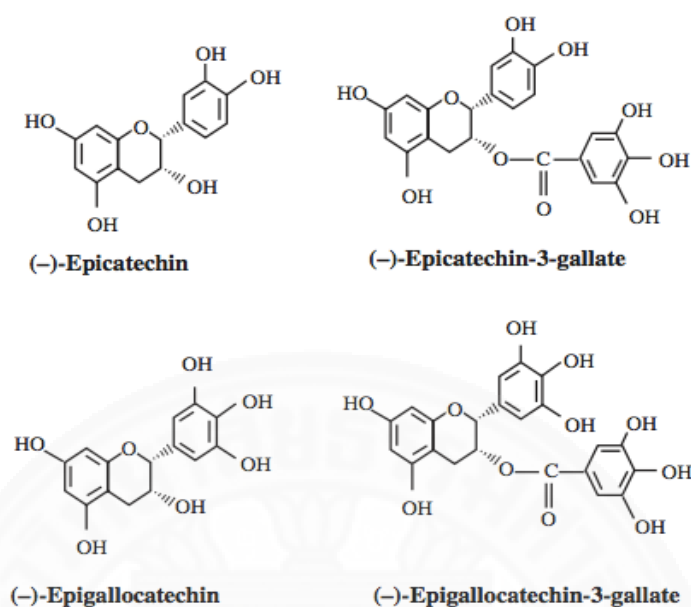


Figure 6.11 Molecular structure of green tea

6.3.2.2 Photoprotective Outcomes

In mice, both topical and systemic administration of green tea polyphenols and EGCG has been found to be potent in protecting against UV-induced immunosuppression, UV-induced sunburn, and photo-aging of the skin (246-248). Supportive results have been observed in respect to sunburn in human skins where crude extract of green tea or with EGCG has been applied or pretreated.

From a total of 3 studies, MED was measured as a clinical determination without any other measurements of biomarkers outcomes. Among three studies, two RCTs at low dose did not show significant changes of MED after supplementation (209, 211).

540 mg of GTC was administered twice daily for 12 weeks on 25 healthy adults with skin type I to II (209). Through daily consumption of 1,080 mg of green tea extract (GTE) (436 mg EGCG, 75 mg EC and 156 ECG), equivalent to 5 cups of green tea, there was no significant differences in visual threshold erythema and in MED median among study group ($p=0.047$). Histology shows no statistically

significant differences in leukocyte infiltration or the cutaneous production of proinflammatory metabolites. From this trial, it may be concluded that this study did not have a statically effect on the skin basal inflammatory status or the response to acute proinflammatory UVR challenge based on histologic sunburn cells and proinflammatory mediator.

Supplementation of EGCG or Polyphenon E (decaffeinated green tea polyphenol mixture) at a dose of 800 mg was administered per day for 4 weeks on 30 healthy adults with skin type II or III (211). Results suggest that there are no significant changes in MED and did not provide protection against UV-induced erythema. The supplementation is equivalent to EGCG content in 8-16 cups of green tea (depending on cup size) and can be concluded as safe for healthy individuals to consume once a day or in divided doses twice a days for 4 weeks.

Only one double-blinded RCT exhibited a significant reduction of UV-induced erythema (UIE) ($p < 0.05$) following daily administration of total catechin 1,402 mg for three months on 30 healthy adults (210). Skin elasticity, roughness, wrinkles, hydration and cutaneous blood flow were also improved. Nonetheless, this study contained a higher proportion of EGCG compared to previous studies (980 mg EGCG, 100 mg EC and 238 mg ECG), possibly the most biologically active GTC.

6.3.2.3 Mechanisms of Action

(1) Systemic Antioxidant Properties

Exposure to UVR at an excessive level inundates the natural anti-oxidant defense mechanisms of the body resulting in an increase of ROS and exhaustion in endogenous anti-oxidant enzymes (249, 250). ROS have also been implicated in photo-carcinogenesis, photo-aging and sunburn. Green tea polyphenols have substantial evidence in indicating its abilities to protect the skin against UV-induced oxidative injury (246, 249-253). **(Figure 6.12)** The observations in this regard started off with *in vitro* studies utilizing mouse epidermal microsomes as a substrate. By using green tea polyphenols as a pretreatment, UV-induced lipid peroxidation in the organelles was inhibited (254). Cultured keratinocytes were treated with EGCG prior to UVB exposure in another *in vitro* study in which UVB-

induced intracellular release of hydrogen was decreased by EGCG, furthermore, EGCG inhibited the phosphorylation of MAPK proteins, a hydrogen peroxide dependent reaction (251).

MAPK including the extracellular signal regulated kinase (ERK, c-Jun N-terminal kinase stress-activated protein kinases (JNK/SAPK) and p38 proteins are a significant regulators of the NF- κ B transcription factors and AP-1 (255). Studies have demonstrated that treatment of normal human epidermal keratinocytes (NHEK) with EGCG result in an inhibition of UV-induced phosphorylation of the MAPK family proteins through oxidative stress inhibition, given that generation of oxidative stress mediated most of the UVR-induced adverse biological effects (251).

One proposed mechanism of protection against oxidative damage to DNA through the ability of EGCG is utilizing the direct scavenging of ROS (256). It has also been demonstrated that low concentrations of green tea polyphenols result in a reduction of hydroxyl radical-induced base damage and through the mechanism of electron transfer on the DNA from catechins to radical sites, a single-strand breaks in DNA.

Adding on to their *in vitro* effects, inhibition of UVB-induced markers of oxidative stress *in vivo* in animal models is a result of green tea polyphenols. If before UVB radiation, pretreatment with EGCG or green tea polyphenols are applied topically or given orally to SKH-1 hairless mice, there will be a protection against the anti-oxidant enzymes glutathione peroxidase, exhaustion of glutathione, reduction of UV-induced lipid peroxidation and restrains UVB-induced protein oxidation (246, 249, 250). EGCG also protect against UV-induced oxidative stress in humans. Before exposure to a four MED of UVB radiation, application to the skin of volunteers significantly reduced the lipid peroxidation in the dermis and epidermis as well as the production of hydrogen peroxide and nitric oxide (249). **(Figure 6.12)**

(2) Prevention of DNA damage

Various studies have shown the photo-protective properties of green tea polyphenols and its abilities in preventing UV-induced DNA damage. In

an *in vivo* study, it has been proven through application of green tea polyphenols to the skin that it is an effective method in protecting the skin against UV-induced DNA damage.

As a counter measure to DNA damage, levels of p53 protein increase resulting in an arrest of the cell cycle. Eventually, UVB-induced DNA damage can lead to the inactivation of p53, impeding this series of events and allowing the duplication of damaged DNA to happen (257). Oral administration of green tea result in a stimulation of UV-induced increase in p53 in regular mouse skin suggesting that the photo-protective properties of green tea are linked to the enhancement of the UV-induced increase in p53-positive cells (257). **(Figure 6.12)**

(3) Anti-inflammatory activities

Epidermal keratinocytes that are going through apoptosis, sunburn cells, are reduced by green tea and its polyphenolic constituents. As anticipated, direct examination of topical EGCG pre-treated UV-irradiated skin *in vivo* resulted in a decrease in the amount of apoptotic keratinocytes as identified by TUNEL staining (258). There are supportive evidences for *in vivo* observations through *in vitro* studies where there was UVB radiation *in vitro* exposure to cultured normal human keratinocytes (258, 259). Further investigation suggested that an EGCG-induced reduction in the pro-apoptotic protein Bax and increase in the expression of the anti-apoptotic molecule Bcl-2 caused the anti-apoptotic effect *in vitro* (258).

The influx of neutrophils and macrophages into the UV-irradiated skin site make up for the development of a profound inflammatory response, a major significant of *in vivo* exposure to UVB radiation. The contribution of inflammatory response led to the pathogenesis of the sunburn reaction, photo-aging of the skin, photo-carcinogenesis and UV immune suppression. Skin application of green tea polyphenols and also orally administer green tea in the drinking water notably decrease the inflammatory response caused by UV radiation (252). An effect, much the same, has been identified in humans (260).

(4) Immunoregulation Properties

The immunosuppressive effects of UV have been shown to reverse through usage of murine-allergic contact hypersensitivity model when green tea polyphenols are topically applied both before and after being exposed to UVR (247, 248).

The balance between IL-10 and IL-12 has also been shown to alter due to EGCG resulting in a reduction of IL-10 production and an increasing IL-12. Further analysis suggests that EGCG-induced IL-12 adds to and increase the synthesis of enzymes that repair UV-induced DNA damage, in addition to promoting cell-mediated immune responses (261). **(Figure 6.12)**

(5) Anti-photoaging Properties

The polyphenolic components of green tea help to protect us from the damaging effects of UVR. In mice, both topical and systemic administration of green tea polyphenols and EGCG has been found to be potent in protecting against UV-induced immunosuppression, UV-induced sunburn, and photo-aging of the skin (246-248). Supportive results have been observed in respect to sunburn in human skins where crude extract of green tea or with EGCG has been applied or pretreated.

In animal studies, there has been a beneficial effect from green tea polyphenols on photo-aging (262). A noticeable reduction in the skin wrinkling amount has been observed in UVA-irradiated SKH-1 hairless mice. A reduction of UV-induced production of MMP-2, -3, -7 and -9, known to deteriorate collagen leading to photo-damage, has been observed through topical application of EGCG. (246) In addition, it is affiliated with a reduction in protein oxidation in the skin associating to photo-aging skin (246).

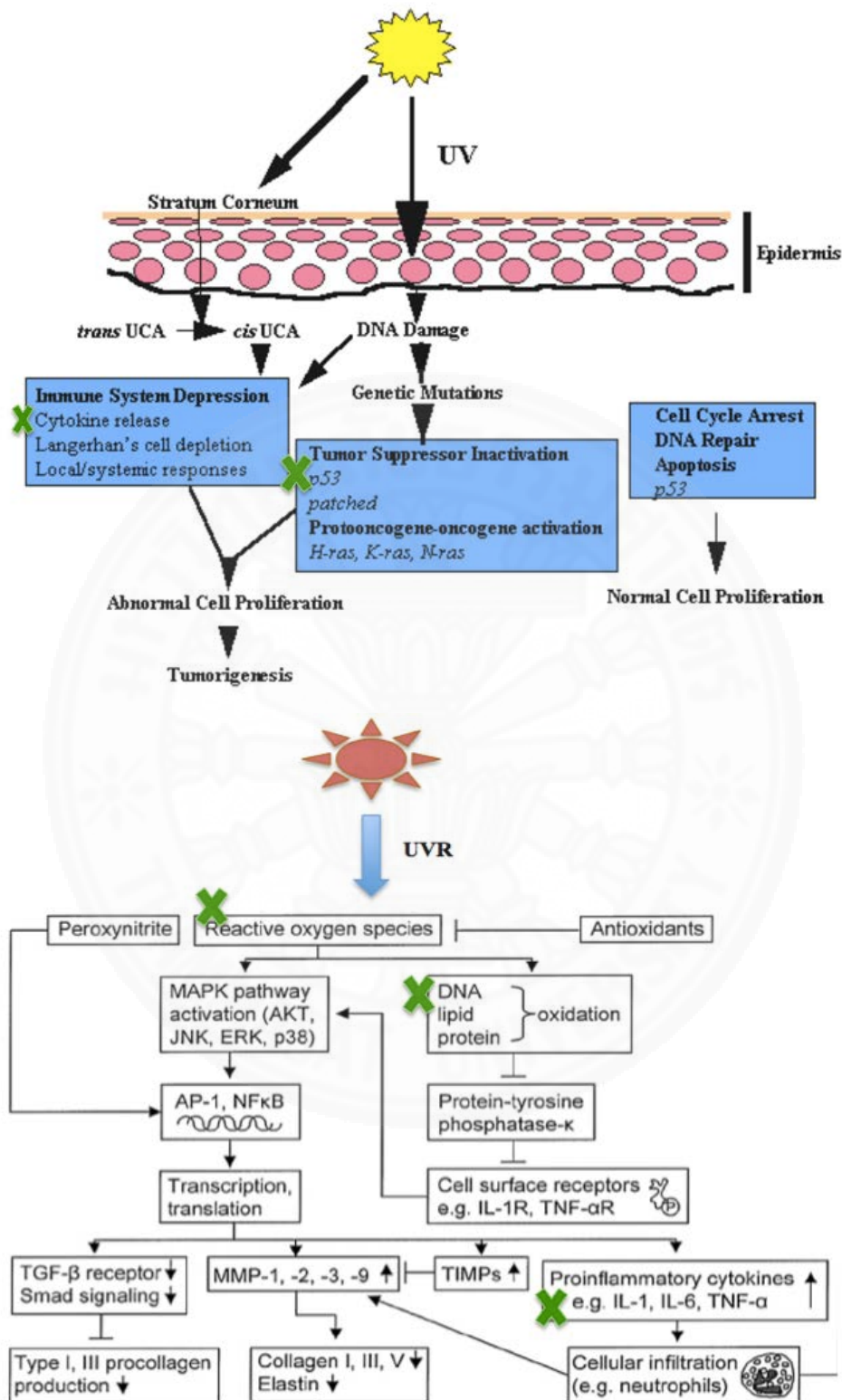


Figure 6.12 Green tea mechanisms of action (modified from (62, 63))

6.3.3 *Polypodium leucotomos*

Polypodium leucotomos (PL) is a tropical fern plant found in Central America and also in some parts of South America. It thrives and flourishes, growing in the rain forest and has been used by the indigenous people of Honduras for ages as an herbal ingested medicine to cure various ailments. Other cultures have also been using the fern for various applications. The Mayans drank it as a tea and during the time of ancient Europe, it was used for different medical purposes. Since the 1970s, commercial extracts of PL have been readily available.

6.3.3.1 Molecular Composition and Pharmacology

The entire fern plant can be used for medical purposes. The leaf and the stem that grows underground (rhizomes) have been used for medicine. Various researches and studies have concluded that the composition of the leaf extract of PL contains a variety of active ingredients and a lot of these active ingredients are antioxidant polyphenols. These polyphenols include the phenolic acids p-coumaric, ferulic, fumaric, vanillic (3-methoxy-4-hydroxybenzoic), quinic (4-hydroxycinnamoyl-quinic), caffeic (4-hydroxycinnamic), chlorogenic (3-caffeoylquinic), 3,4-dihydroxybenzoic, and 4-hydroxybenzoic, along with five phenolic chlorogenic acid isomers. Not only this, PL leaf extract has abundance in monosaccharides and flavonoids. **(Figure 6.13)**

PL contains high level of phenolics, much of this are, benzoates and cinnamates, similar to caffeic acid and its derivative ferulic acid (263). The molecular basis of PL's anti-inflammatory, anti-mutagenic and anticarcinogenic properties are based on the non-flavonoid catecholic compounds that encompass antioxidant properties (264-266). On one hand, Caffeic acid restrain UV-mediated peroxidation by restraining propagation of the lipid peroxidative chain reaction and also restrain nitric oxide production (267). On another hand, ferulic acid is a UV photon acceptor (268). Skin lotions and sunscreens often incorporate these two acids as they restrain UVB induced skin erythema (269).

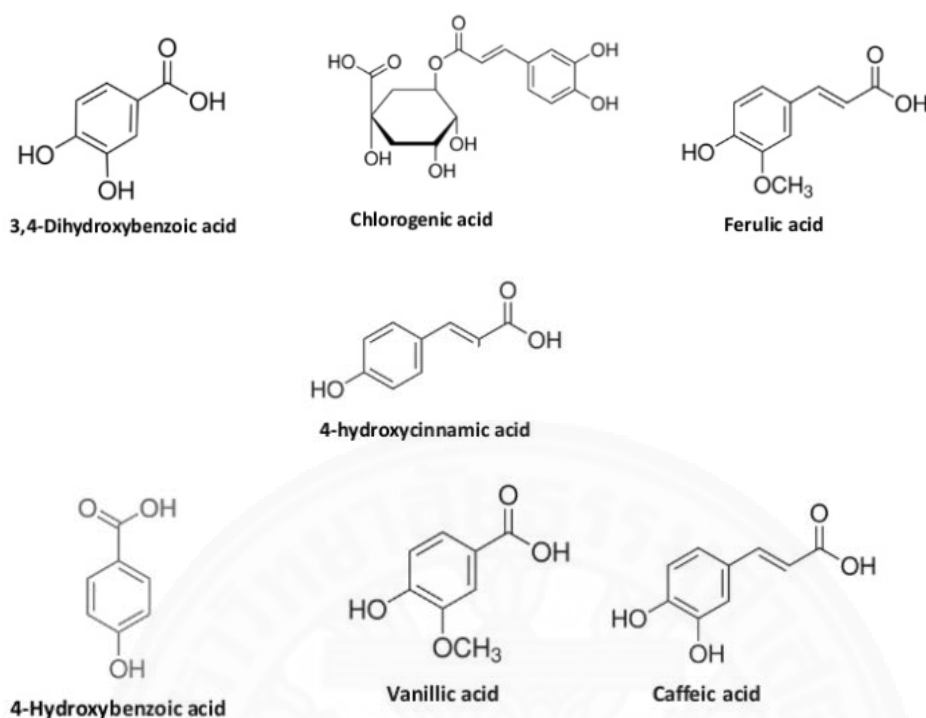


Figure 6.13 Fundamental phenolic compounds of *Polypodium leucotomos*

6.3.3.2 Photoprotective Outcomes

There was only one study with a daily intake of 1,080 mg of PL for 1 day (191). The studies showed a significant increase of MED and the result was confirmed by the reduction of sunburn cells in histology.

The study of total of 1080 mg of PL extract was administered at a dose of 240 mg x 3 times per day, 1 day before exposure and additional 360 mg was administered 3 hours prior to exposure to 13 healthy adults with skin types III to IV, compared to administration of placebo. The result demonstrates significant increase in MED ($p < 0.001$) and IPD ($p < 0.01$) after oral administration. A preservation of immunohistochemical assessment of CD1a-expressing epidermal cells were present in treated group. Through immunohistochemical study, photoprotection of eLC is present through oral administration of PL.

6.3.3.3 Mechanisms of action

(1) Systemic Antioxidant Properties

The phenolic compound of PL attributes it with antioxidant activity (270, 271). By restraining propagation of the lipid peroxidative chain reaction, PL is able to avert UV-mediated peroxidation. **(Figure 6.14)** ROS including H_2O_2 , hydroxyl radicals ($\cdot\text{OH}$), singlet oxygen ($^1\text{O}_2$) and superoxide anion ($\cdot\text{O}_2$) are rummaged by PL (272). Not restricted to ROS, the effect of PL extends to preventing NO synthesis by preventing iNOS expression (273). **(Figure 6.14)** PL is able to successfully reduced glutathione oxidation both in blood and epidermis in a hairless rat model. This indicates that PL has a formidable systemic antioxidant effect, restraining UV-mediated eLC depletion and hindering UV-induced immunosuppression (274). PUVA therapy is another area in which the antioxidant properties of PL can play a major role in helping to eradicate some of its adverse effects. As a result of PUVA therapy, ROS and free radicals are produced, subsequently causing erythema, edema and pain. This in turn is the factor in which limits the use of PUVA therapy as a mean to treat skin disorders due to the acute photo-toxicity and succeeding development of hyperpigmentation. The potential of PL to extinguish oxidative stress has been amplified through a study in which shows that there is a statistically lower grade of edema and erythema on sites treated with PL versus sites exposed to PUVA alone (191, 275).

(2) Prevention of DNA Damage

Crucial studies have undergone to determine the effects of PL on the genome level, one particularly, a hairless mice model, examined the UV-induced COX-2 expression (276). In nominated pathways of mutagenesis, cumulative UV light exposure and COX-2 is upregulated leading to the production of prostaglandin E, which in turn, prompt epidermal hyperplasia with reduced differentiation (277). Symptoms of photo-damage and possible progression to actinic keratosis and SCC are believed to be clinically correlated to the atypical keratinocytes proliferating (276, 277).

An objective was set by the investigators of this study to investigate whether *Polypodium leucotomos* Extract (PLE) and its photo-protective

effects' potential can be justified by the assay (276). The study was designed involving feeding PLE 300 mg/kg or placebo for a period of 10 days to hairless Xpc^{+/-} mice and these mice were then UV irradiated for a single time. As a supplement to determine the diminishing of the over expression of COX-2, p53 expression was assayed by the investigators, as well as the reduction of cyclobutane pyrimidine photoproducts to examine for defects in DNA repair due to UVB exposure, accompanied by the environment of UV-induced oxidative DNA damage (276). Lastly, the investigator appraised the responsibility of PLE in removing cells that expressed 8-hydroxy-2'-deoxyguanosine, a familiar biomarker for carcinogenesis and oxidative stress (278). 8-hydroxy-2'-deoxyuanosine has its duty in linking to the effect of endogenous oxidative damage to DNA and also being a component of commencement and advocacy of carcinogenesis (278). It has been observed that there were 79% fewer 8-hydroxy-2'-deoxyuanosine-positive cells in PLE treated mice 24 hours after feeding in contrast with the placebo group ($p < 0.03$), in concurrent with a 5-fold reduction in COX-2 levels induced by UV ($p < 0.05$), and notable reduction of mutations induced by UV recognized 2 weeks prior to UV exposure. The findings shed lights upon the fact that PLE decreases COX-2 levels induced by UV through the encouragement of p53 expression, associating with a diminishment in prostaglandin-mediated inflammation that is correlated to photo-aging. However, the anti-inflammatory and photo-protective effects have not yet been performed in patients (277).

Damage to UV-induced DNA is specific to wavelength because UVA is associated to oxidative damage to guanine residues and a usual eradication of mutation of mitochondrial DNA (mtDNA). At the same time, the formation of CPDs and pyrimidine-pyrimidine (6-4) photo-products, a process of UVB, creates direct damage to DNA. Hyperplasia, erythema and epidermal can be visually observed on the skin as a result of these events (275, 277, 279).

The inhibition of formation of thymine dimers mediated by UV in humans (280) and in a Xeroderma pigmentosum rodent model (XPC) is a result of oral systemic administration of PL (276). These animals show an aggravation of inflammatory response to UV irradiation and a worsen capability for DNA repair. They are then susceptible to develop skin cancer as a result. When PL is

supplemented into the diet, the level of basal oxidative stress decreases (number quantification of (8-ox-dG)-positive cells), proposing that a reduction of constitutive DNA damage is a result of PL (276). **(Figure 6.14)**

A study demonstrated a notable decrease in the levels of CPD in a skin treated by PL in contrast to healthy volunteers being untreated ($n=9$, $P<0.001$), suggesting the prevention of UV accumulation of CPDs by PL. PL antioxidant properties could be the cause of CPD reduction through enhancing repair enzyme function, keeping in mind that DNA repair enzymes are prone to oxidative stress (280). Further on, a study conducted on mice suggested that $54 \pm 5\%$ CPDs is still presence in vehicle-fed mice compared to $31 \pm 5\%$ in PL-fed mice ($P<0.003$). Decrease oxidative stress and clearance of CPDs are promoted by the increase in p53 activity (281).

(3) Anti-inflammatory Properties

The suppression of UV-induced expression of COX-2 is a crucial anti-inflammatory mechanism (282). It has been demonstrated in a study that PL fed mice ($P<0.05$) were four to five times lower in UV-induced Cox-2 levels (276). These effects are responsible for decreased leukocyte extravasation and in the irradiated area, limit mast cell infiltration, as confirmed and supported by reports of a 50% decrease in macrophages ($P < 0.02$) and a 60% decrease in neutrophil infiltration $P < 0.001$) (276).

In addition, PL is able to inhibit the expression of pro-inflammatory markers including inducible NO synthase and TNF- α , also hindering the transcriptional activation of AP-1 and NF κ B. **(Figure 6.14)** Apoptosis prevented by NO through inhibition of caspase-3 activity and/or instigating expression of Bcl-2, anti-apoptotic factor. However, additional damage may result from the exacerbated production of NO, it is mandatory for regulatory mechanism. Following irradiation, it has been reported through various studies that there is an increase in NO as a result of inducible NO synthase enzyme being upregulated. (273, 283) PL pre-treatment diminished upregulation causing up to 60% reduction of NO production (273). **(Figure 6.14)**

(4) Inhibition of Photoisomerization of *t*-UCA

Various chromophores normally existing in the skin absorb UVR, including DNA, RNA, melanin, lipids, proteins, aromatic amino acids such as tryptophan or tyrosine or trans-urocanic acid (*t*-UCA). *t*-UCA is the main outcome of histidine metabolism, possessing properties to scavenger ROS and also photo-protective properties. As a way of avoiding damage to other cellular structures, isomerization to *c*-UCA is induced through photons absorption. PL restrains *t*-UCA photo-isomerisation causing a dose-dependent reduction in the levels of *c*-UCA through the existence of hydrogen peroxide. **(Figure 6.14)** In addition, UV photons result in the creation of hydroxyl and other oxygen radicals when a catalyzer, TiO₂ and ROS are present. They are the cause of inactivation of various enzymatic systems, a requirement for skin homeostasis. PL hinders the degradation of *t*-UCA and when incubated together under UVR with TiO₂, improves peroxidase inactivation. These results take in to consideration the efficacy of PL as an inhibitor of *t*-UCA photo-isomerisation and recommending it for the prevention of oxidative metabolites' creation, catalyzed by TiO₂.

(5) Immunoregulation Properties

Langerhans cells (LC) are bone marrow-derived antigen-presenting cells that inhabit in the epidermis for extended periods of time. The activation of cells that act as effector cells for cell-mediated immunologic processes is through LC. LCs are remarkably vulnerable to UV injury and damage to these cells has a significance in the development of UV-induced immune suppression.

PL inhibits the reduction of eLC, antigen-presenting cells, therefore protecting the skin's immune surveillance. **(Figure 6.14)** Under UVR exposure, LCs goes through apoptosis and changes in morphology. LC depletion together with infiltration of macrophages activates suppressor T cells causing antigen tolerance and the susceptibility of skin to UV-induced cancer to increase. In addition, UV-induced immunosuppression is promoted through the inhibition of adhesion molecules allowing for LC migration. Preservation of LCs in pretreated irradiated rats as opposed to vehicle pretreated irradiated rats has been reported in an experimental study. PL also blocked the changes in morphology of LCs (274).

(6) Anti-photoaging Properties

Not only subjected to its anti-oxidant activity, its effects on extracellular matrix remodeling make it a robust agent in prevention and treatment of photo-aging (284). Expression and activity of different MMPs are restrained by PL and the expression of tissue inhibitor of metalloproteinase (TIMP), TGF β , elastin and various types of collagen are encouraged (285). In conclusion, PL restrains the expression and properties of molecules concerning matrix degradation, therefore advocating the revitalization and indemnification for the detrimental effects of irradiation that justifies photo-aging (284).



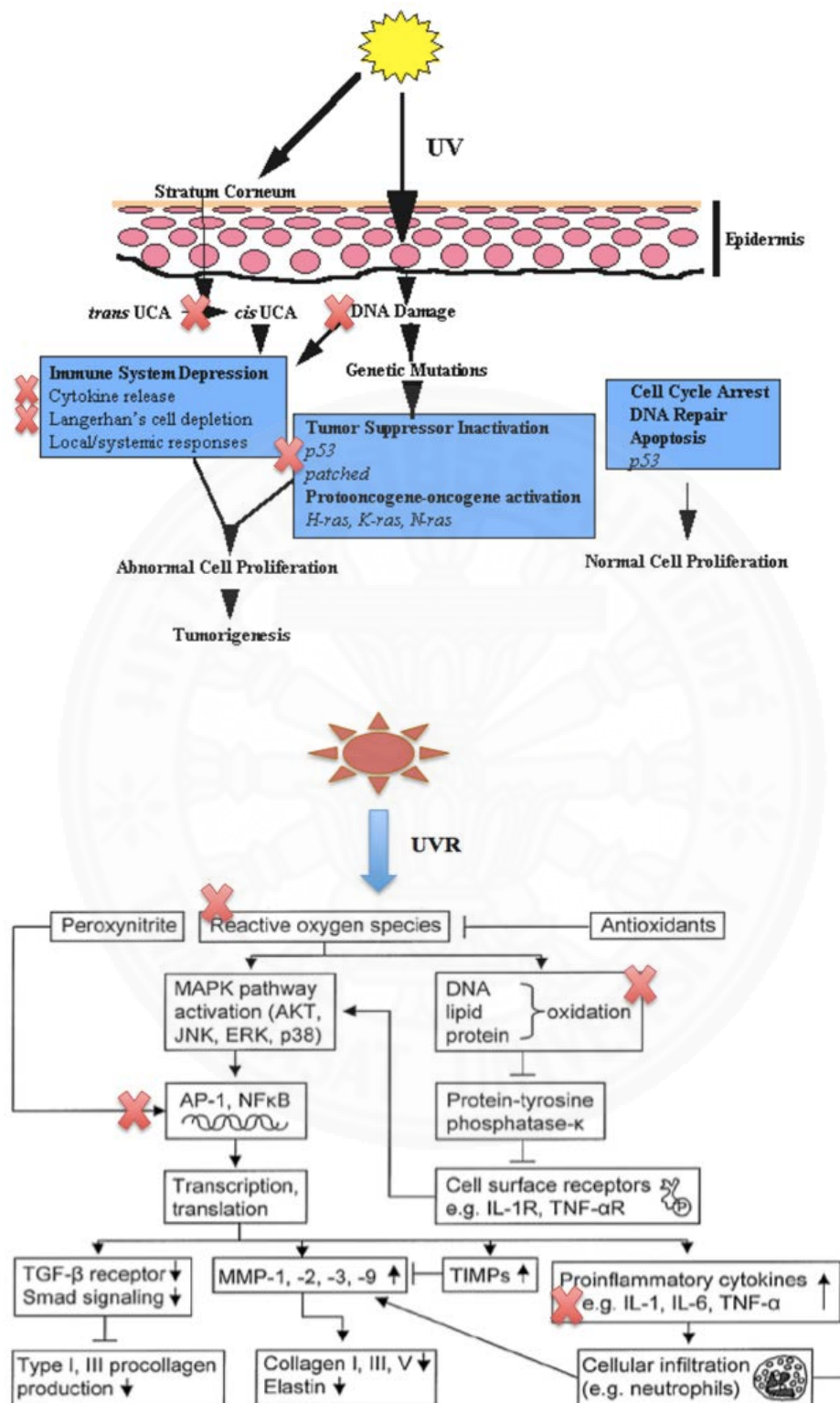


Figure 6.14 PL mechanisms of action (modified from (62, 63))

6.3.4 *Theobroma cacao*

Cocoa existence can be traced back to BC 4000 years ago and being first cultivated since BC 2000 years throughout the central region of Mexico. In Greek language, Cocoa or Theobroma translates into “Food for God”. It is believed since ancient times that for one to preserve a healthy life and longevity, cocoa are the precious food that will yield the result. One observable characteristic of cocoa plant is the production of flowers and fruits on the stem of the plant. The fruit of cocoa is in a shape of a rugby ball with an approximate length of 20 cm. Inside, the flesh of cocoa is embedded with cocoa seeds and white in color (286).

6.3.4.1 Molecular Composition and Pharmacology

The characteristics of the flavor and nutrient profile of the end products is a result of the time and conditions in regards to temperature during the roasting process (287). Representing the underlying aspect of palatability and organoleptic characteristics of chocolate, the bitterness is mainly attributed to the high levels of flavanols (288). More significantly, wide arrays of health benefits and healing properties may also be attributed to flavanols. As an example, improvement in dermal blood flow and increased effects of photoprotection, contributing to the maintenance of the skin is a result of flavanol-rich cocoa consumption (217, 289).

Cocoa extract is a mixture of bitter chocolate taste. It is made up of xanthine molecules (caffeine and theobromine) and procyanidins. The bio-active compounds presence in cocoa products is what we refer to as cocoa extract. Such compounds include procyanidins, flavanols and epicatechin. Other plant products also contain these molecules, however cocoa extract particularly, has a high level of epicatechin compared to other plant products. Supplementation of cocoa extract and oral consumption of dark chocolate coincide with improved insulin sensitivity and better blood flow.

6.3.4.2 Photoprotective Outcomes

In three studies, daily intake of high flavanol cocoa (HFC, more than 329 mg) and low flavanol cocoa (LFC, less than 30 mg) for 3 months were compared. All studies revealed increasing MED or decreasing UIE following HFC

ingestion (215-217). Only one research reported increasing MED in LFC administration (215). Skin elasticity and hydration were improved after HFC administration (215, 217).

The first study compared HFC 600 mg (n=33) against LFC less than 30 mg (n=41) in skin types I to II (215). Both studies show a similar increase in MED at 12 weeks but no significant differences between HFC and LFC group. The result was affected by the different season of participation in the 2 groups where more participants in the LFC group entered the trial during winter and finishing during the spring season. As a result, the longer solar exposure attributed to physiologically-increased MED during this period.

The second study enrolled 22 women and 8 men with skin types II or III randomly divided into HFC 600 mg and LFC less than 30 mg group (216). After 12 weeks, mean MED remained constant in the LFC group in comparison to baseline. In HFC group, mean MED increased significantly (within-group comparison) ($p=0.005$). Nonetheless, between-group comparisons were not reported, both men and women were included in the trial and the characteristics of participants were not clarified.

In another study, 24 women with skin type II were enrolled to determine the effect of HFC 329 mg against LFC 27 mg intake daily in regards to MED in solar simulation radiation (217). Women designated to the HFC group showed significant increase in MED compared to women in LFC group ($p<0.05$). It is important to take into consideration that the characteristics of the women in each group, the information regarding trial profile (loss-to-follow-up, intent-to-treat analysis) and MED was not available.

6.3.4.3 Mechanisms of Action

(1) Systemic Antioxidant Properties

Flavanols and carotenoids contribute to photo-protection in plants through their efficient anti-oxidants properties (290). Singlet molecular oxygen or peroxy radicals are an example of primary or secondary reaction products of photo-oxidation scavenged. As most polyphenols absorb UV light, it provides photo-protection through this shielding process. Biological properties are exhibit by dietary

anti-oxidants; for example, affecting intracellular and intercellular signaling. The association between inflammatory event and complex biochemical processes of this tissue response is a synonym for UV-induced erythema. Particular flavonoids are effective inhibitors of prostaglandins production and impinge with key enzymes involved in prostaglandin biosynthesis (291). **(Figure 6.15)**

(2) Anti-inflammatory Properties

Studies have also been carried out in regards to cocoa flavanols and its regulatory effects on NF- κ B activation. NF- κ B activation inhibition is a result of (-)-Epicatechin, (+)-catechin and their dimeric forms, demonstrating a definite reduction in NF- κ B-DNA binding activity, leading to IL-2 production reduction. Supplementation of cocoa polyphenols at a dose of 40 or 200 mg/kg for 30 minutes is significantly reduce the levels of COX-2 expression induced in mouse skin after 4 hour of topical 12-O-tetradecanoylphorbol-13-acetate (TPA) exposure (10 nmol) (292). **(Figure 6.15)** Recent studies have suggested that effective inhibition of TNF- α -induced vascular endothelial growth factor (VEGF) expression in mouse epidermal cells is a result of cocoa polyphenol extract (293). The ability of cocoa polyphenols to obstruct TNF- α -induced activation of the nuclear transcription factors, AP-1 and NF- κ β , decreasing photoaging of the skin is related to the aforementioned effect. **(Figure 6.15)**

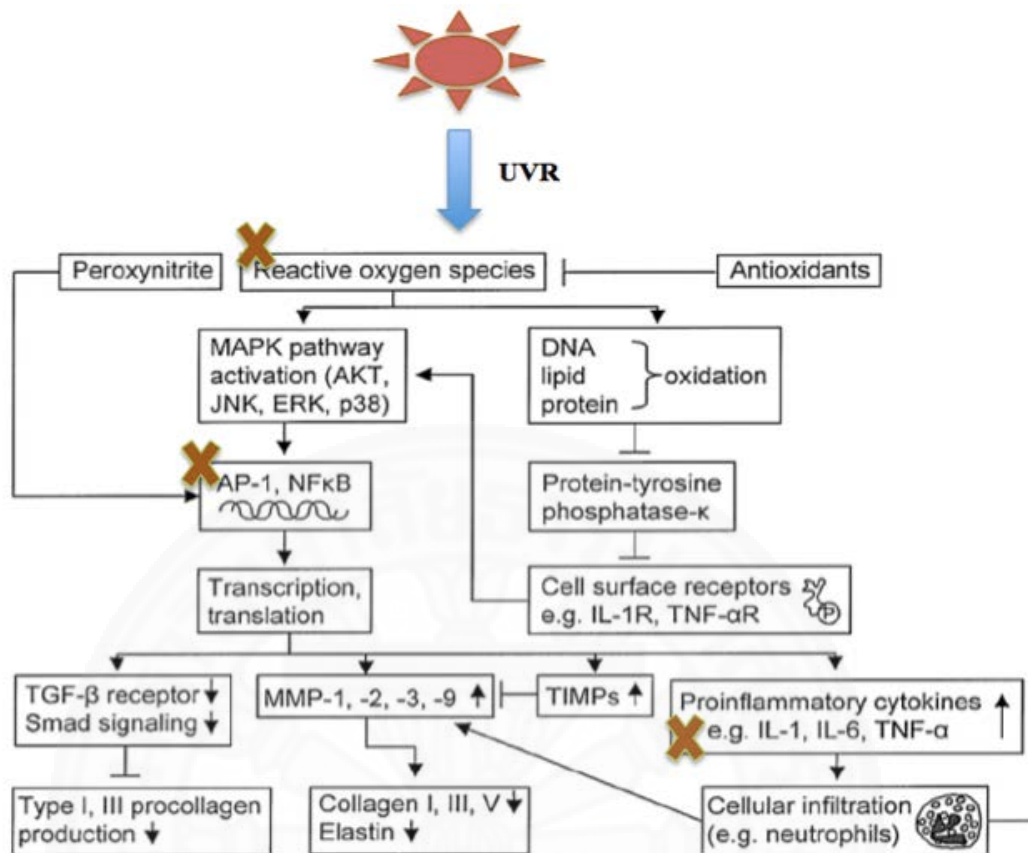


Figure 6.15 Cacao mechanisms of action (modified from (62))

6.3.5 Vitamin E

Regardless of the ever-constant innovations and increasing skincare alternative methods, vitamin has proven itself as a potent skincare ingredient, standing through the test of time. For over half a century, photoaging prevention, scar tissue repairs to free radical control, have been thoroughly documented in various studies in the areas of skin health as beneficial effects of Vitamin E.

Various antioxidants are a result of natural synthetization of the human body; however, vitamin E in the skin relies on the daily consumption of vegetables, vegetables oils, nuts and vitamin E supplementation. It has been demonstrated in numerous studies that vitamin E is the most important antioxidant within the human skin. Vitamin E plays a crucial role in our natural defense system against damages caused by UV exposure such as free radical cell damage. It also protects and prevents processes such as inflammation from occurring within our own bodies.

6.3.5.1 Molecular Composition and Pharmacology

Vitamin E is a group of compounds including both tocopherols and tocotrienols (294). γ -Tocopherol is considered to be the most common form of vitamin E, widely found in our diets through soybean oil, corn oil, margarine and dressings (294, 295). Another form of vitamin E that is most common in our diet is α -tocopherol. It is the most biologically active form of vitamin E, found most abundantly in safflower, sunflower and wheat germ oils (294, 296).

Compounds that have 6-chromanol ring with isoprenoid side chain and a biological activity of α -tocopherol is called vitamin E in generic term. **(Figure 6.16)** Completely soluble in fat, fat solvents and oils, α -tocopherol is insoluble in water. Oral intake vitamin E has an absorption rate of 50–70% through the intestines but is notably lower through higher doses intakes. Varying in parallel to lipid concentration of the plasma, the levels of plasma α -tocopherol is carried by lipoproteins. Free α -tocopherol, plasma membrane and those in conjunction to the mitochondria and endoplasmic reticulum are concentrated within the membranes of the cells.

In therapeutic studies, doses range between 100 mg to 1000 mg for α -tocopherol per day. It is apparent through data summarization of a large number of studies that the amount of dosage require for a systemic photoprotective effect requires a much higher dosage, several hundred milligrams daily, compared to a dose for correcting dietary deficiency. Patients receiving α -tocopherol dosage of 400 IU, equivalent to 33 times the dosage of the recommended daily allowance, shows no notable difference in the levels of α -tocopherol in the skin, implying that a higher dosage is required for a therapeutic effect (221). Supplementation of α -tocopherol at doses up to 800 mg/day for periods of up to years shows no apparent harm. However, dosage in excess of 1000 mg/day supplemented in a long-term could lead to fatigue, muscle weakness, nausea and diarrhea. Vitamin E is assessable and widely available across the world as an over-the-counter product. α -Tocopherol on the other hand can be assayed in skin (297) samples and plasma (298) through chromatography in high-performance liquid.

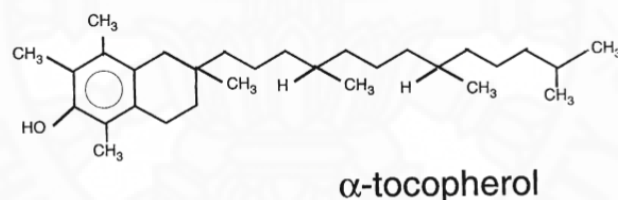


Figure 6.16 Tocopherol molecular structures

6.3.5.2 Photoprotective Outcomes

Four RCTs with daily supplement of α -tocopherol at dosage between 400 to 1,200 IU were found (218-221). The results were controversial.

The study of McArdle 2014 examined the ability of α -tocopherol 400 IU and β -carotene 15 mg, to reduce markers of oxidative stress and UVR-induced erythema in human skin. A total of 16 healthy adults were randomly assigned to take either α -tocopherol (n=8) or β -carotene (n=8) for 8 weeks. The results suggest no significant change of MED in both groups. However, there is a significant reduction of skin malondialdehyde concentration which is an index of lipid

peroxidation. Nonetheless, neither α -tocopherol nor β -carotene significantly affects other oxidative measures in UVR-exposed skin.

There are 2 more studies that compare α -tocopherol to ascorbic acid. The first study involved 45 healthy volunteers divided into three groups. The first group was supplemented with α -tocopherol 1,200 IU daily, the second group was supplemented with ascorbic acid 2 g daily and the third group was supplemented with ascorbic 2 g plus α -tocopherol 1,200 IU for a total period of 1 weeks (219). Results show a significant increase in MED in α -tocopherol group ($p=0.002$) and α -tocopherol plus ascorbic acid ($p=0.0001$). There are no significant changes in the sole ascorbic group.

There is a comparison of 3 groups in another study. Group 1 was given α -tocopherol 2 g daily, group 2 was given ascorbic acid 3 g daily and group 3 was given α -tocopherol 2 g with ascorbic acids 3 g daily for 50 days using a total number of 40 participants with skin type II (220). The results suggest a significant increase in MED only in the combined group ($p<0.005$).

The following study involves solely with α -tocopherol and its photoprotective role of antioxidants, supplementing 12 healthy humans with 400 IU of α -tocopherol or placebo for a period of 6 months (221). The results suggest no significant changes in MED and significant differences in sunburn cells between the 2 groups.

6.3.5.3 Mechanisms of Action

(1) Systemic Antioxidant Properties

Sharing the ability to quench singlet oxygen, α -tocopherol shares some properties with β -carotene (299). A single molecule of α -tocopherol is able to quench up to 120 singlet oxygen molecules before degradation. In addition, α -tocopherol is efficient in scavenging chemicals, resulting in irreversible oxidation to α -tocopherol quinone (300). According to *in vitro* studies, α -tocopherol has also been shown to scavenge hydroxyl radical, perhydroxyl radical and superoxide anion radical (301), yet it is still undetermined whether it will act accordingly *in vivo*. Acting as a chain-breaking antioxidant during lipid peroxidation is considered the main effect of α -tocopherol *in vivo* (302), and in the process, vitamin C supplementing through the

important role of subsequent regeneration of α -tocopherol (303). It has been shown that a single α -tocopherol molecule can protect up to 220 polyunsaturated fatty acid molecules before degradation (304). Being one of the integrated pathways responsible for generating and maintaining adequate amount of cellular reducing power and interacting with enzymatic and nonenzymatic pathways of other antioxidant systems, α -tocopherol is considered a dynamic molecule. As a result, α -tocopherol and its photoprotective properties is not a sole product of itself but rather through the involvement of other antioxidants, ascorbic acid in the vitamin E cycle as an example (305).

A large number of animal and human studies have shown a reduction in photoaging (306, 307), lipid peroxidation (308), immunosuppression (309-311) and photocarcinogenesis (311, 312) post vitamin E application. On a molecular basis, topical application of α -tocopherol reduces the transcription levels of MMP-1 and causes thymine dimer formation inhibition, as a result, the process of collagen breakdown and mutagenesis slows down respectively (313, 314). It has been suggested that the protection against the formation of dimer is a result of antioxidant interplaying with ROS and not the UVB-absorbing effect of sunscreen (315). **(Figure 6.17)**

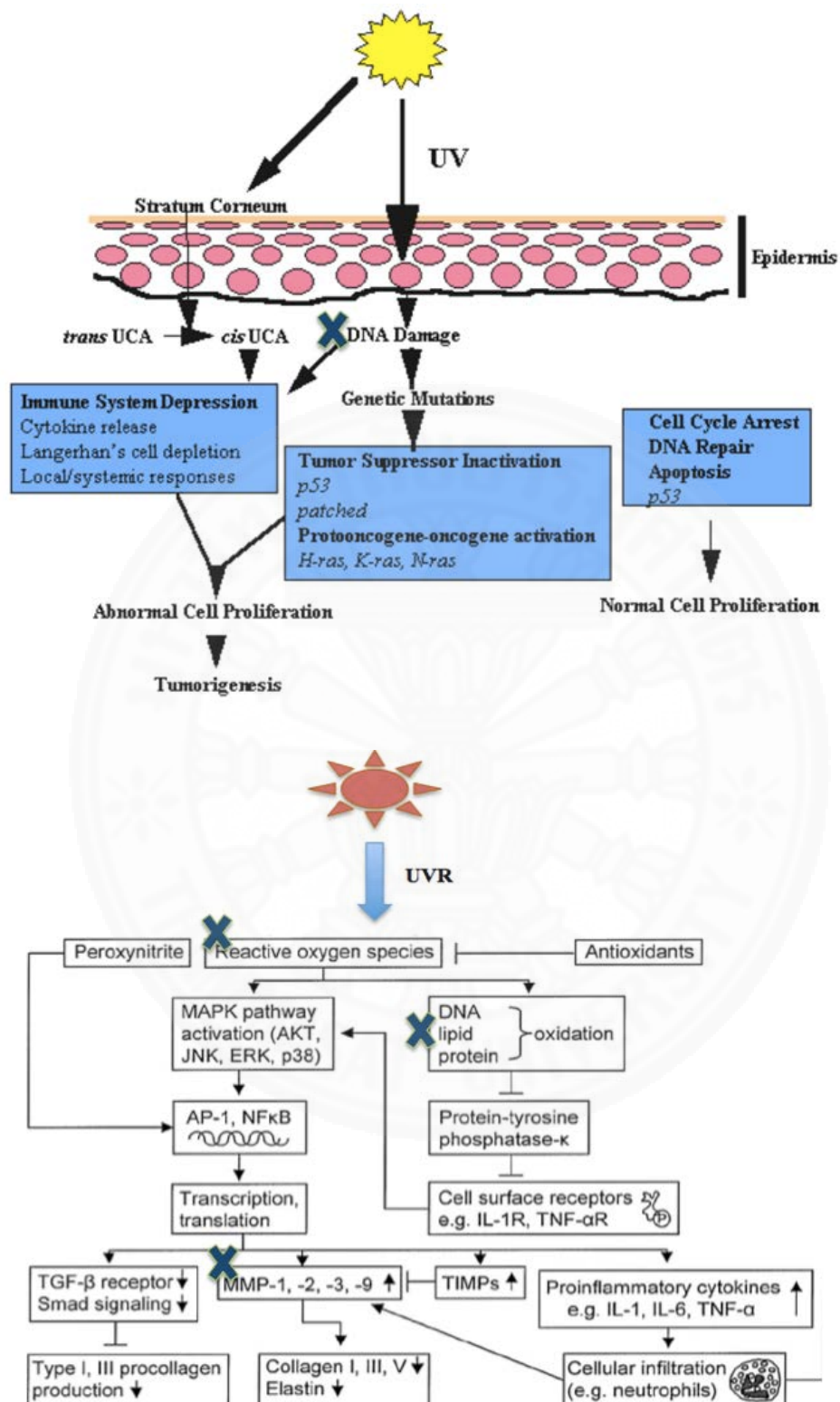


Figure 6.17 α -tocopherol mechanisms of action (modified from (62, 63))

6.4 Animal-derived Photo-protective Agents

6.4.1 Nicotinamide

Vitamin B3 has many different forms with its amide form being Nicotinamide (niacinamide). Other forms include niacin (nicotinic acid), various esters and greater complexion amides; for example, inositol hexanicotinate. An essential water soluble vitamin, Vitamin B3 however is not stored in the body. Maintenance is only possible through dietary consumption of vitamin B3 and tryptophan. An essential amino acid, tryptophan is commonly found in most variations of protein and make up approximately 1% of total dietary protein. Even though the conversion to niacin is possible through the liver, it is however relatively inefficient, requiring 60 mg of tryptophan to produce 1 mg of niacin. **(Figure 6.18)** Vitamin B3 through its form of niacin or nicotinamide can be found in many different types of food including beef, pork, chicken, fish, legumes, mushrooms, nuts, grain products, yeast extracts and coffee. **(Table 6.5)** The recommended dosage for the intake of vitamin B3 for men in niacin equivalent is 16 mg and 14 mg for women, 17 mg for lactating women and 18 mg for pregnant women. Nicotinamide also possesses photo-protective and anti-carcinogenesis effects in mice and in humans (223, 316-319). Relatively cheap, well-tolerated and patent-free, nicotinamide is widely available in health food stores and pharmacies in the form of a vitamin supplement.

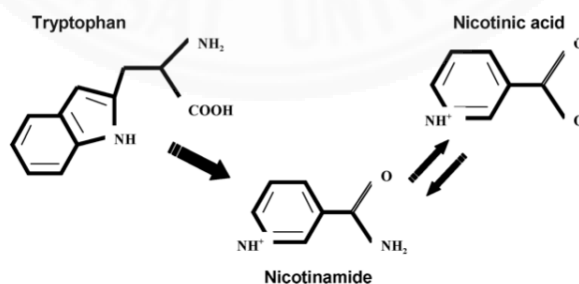


Figure 6.18 Nicotinamide molecular composition

(Nicotinamide can also derive from endogenous conversion of nicotinic acid (niacin) or the amino acid tryptophan. Conversion of nicotinamide to nicotinic acid can also result from nicotinamidase, produced by intestinal bacteria)

Food (100 g)	Niacin equivalent (mg)
Meat	
Chicken breast, grilled	11.32
Lamb, cooked	9.41
Bacon, grilled	8.32
Beef, cooked	3.51
Seafood	
Tuna, grilled	18.91
Salmon, grilled	7.72
King prawns, cooked	5.13
Barramundi, steamed	3.81
Fruit	
Bananas	0.60
Apples	0.13
Vegetables	
Mushrooms	3.72
New potatoes, baked	2.62
Carrots	0.90
Brown onions	0.71
Cos lettuce	0.47
Tomatoes	0.17
Nuts and seeds	
Peanuts, roasted	22.71
Sunflower seeds	18.98
Almonds	7.74
Walnuts	5.04
Macadamia nuts	3.27
Legumes	
Soya beans, boiled	4.25
Lentils, boiled	1.53
Bread and rice	
Wholemeal bread	7.56
White bread	6.52
Brown rice, boiled	2.35
White rice, boiled	0.56
Dairy and eggs	
Cheddar cheese	5.10
Mozzarella cheese	4.96
Eggs, boiled	2.71
Full cream milk	1.02
Skim milk	0.85
Beverages	
Espresso coffee	5.23
Ale	0.51
Red wine	0.43
Yeast	
Vegemite	130.17

Table 6.5 Vitamin B3 in selected food (320)

6.4.1.1 Molecular Composition and Pharmacology

Nicotinamide, nicotinic acid and niacin, through conversion becomes nicotinamide *in vivo*, are readily available in foods; for example, meats, nuts, grains, legumes, coffee and tea, and also niacin-fortified cereals (321-323). A precursor of nicotinamide adenine dinucleotide (NAD), Nicotinamide is an essential coenzyme in the production of adenosine triphosphate (ATP). The cellular energy currency responsible for the transportation of chemical energy within cells is so-called ATP. As a result, nicotinamide supplementation boosts cellular energy and elevate DNA repair, an energy-dependent cellular processes (324). A sole substrate and a nuclear enzyme poly-ADP-ribose polymerase 1 (PARP-1) inhibitor, nicotinamide is activated by UVR (325). DNA repair and genomic stability, including the regulation of several transcription factors, specifically with relation to the expression of chemokines, inflammatory mediators, adhesion and inflammatory cytokines are various essential cellular properties of PARP-1 (326). Nonetheless, cellular dysfunction or necrosis could result from over consumption of NAD through PARP-1 overexpression (326). Possession of sufficient cellular energy and appropriately functioning PARP-1 is crucial for several skin conditions, for which beneficial effects may result from nicotinamide (327).

6.4.1.2 Photoprotective Outcomes

The objective of the study was to determine the effects of oral nicotinamide on photodynamic therapy (PDT) induced immunosuppression in human. Using a Mantoux delayed-type hypersensitivity model, oral nicotinamide was administered at a dosage 500 mg, twice daily for 1 week on 30 healthy Mantoux-positive volunteers (222). The results suggest that oral nicotinamide reduced immunosuppression, reducing Mantoux-induced erythema (MIE) by 66% ($p < 0.0001$). Immunosuppression can also be assessed as the change in Mantoux diameter. The result significantly reduce the Mantoux diameter by 28% ($p < 0.0001$). A similar study was conducted in 2009 utilizing the Mantoux model of skin immunity in 122 healthy Mantoux positive subjects with skin type I to IV and supplemented with 500 mg nicotinamide daily versus 500 mg 3 times per day for 1 week. The two groups were divided equally into groups of 61 subjects (223). The result demonstrated no

significant change in MED in both groups, however, significant reduction of Mantoux diameter was observed in both groups ($p < 0.001$).

6.4.1.3 Mechanisms of Action

(1) Prevention of DNA Damages

The mechanisms of nicotinamide in regards to *in vivo* immune protective effects are most likely related to its responsibility in cellular energy metabolism and post-irradiation DNA repair, an energy-dependent cellular process. The production of several immunoregulatory cytokines, for example, IL-1b, IL-6, IL-8, TNF (328) and IFN-g (329) are known to be regulated by nicotinamide, through mechanisms which are not depended upon its actions as a PARP inhibitor.

A total of 6 healthy volunteers was recruited and irradiated on the lower back at single dose of solar stimulating UV at a sub-erythral level. Immediate treatment of vehicle and 5% nicotinamide lotion is applied upon different areas after irradiation and after 24 hours upon the four sites (no UV, UV, and each with either vehicle or nicotinamide), 3 mm punch biopsies were taken. Using Gene set enrichment analysis (GSEA), a microarray study regarding these particular biopsies pinpointed an UV-induced down-regulation of genes associated with energy metabolism, immune and anti-apoptotic pathways in skin treated with vehicle not skin treated with nicotinamide (330).

Further investigation is carried out in regards to the mechanisms of action of nicotinamide using human keratinocyte (HaCaT) cell line. Cells were incubated for a period of 24 hours either with or without 50 mM nicotinamide after radiation through 4 J cm^{-2} solar stimulating UV. Result suggests that succinate dehydrogenase (SDH), a crucial enzyme producing energy in both electron transport chain and Krebs cycle, at both transcriptional (mRNA) and post-transcriptional levels, for example, enzyme activity, was upregulated in UV and nicotinamide treated cells, however, results suggest otherwise for un-irradiated cells treated with nicotinamide or irradiated cells treated with vehicle (317). When tumor protein p53 expression was measured, similar results were observed. Several cellular responses to irradiation are modulated by p53 and in regards to UV protection, p53 upregulation has been previously observed (331). These findings suggest that UV and

nicotinamide cooperatively work together in tumor protein p53 message upregulation and SDH message and activity. **(Figure 6.19)**

Nicotinamide has been demonstrated to replenish ATP in UV-irradiated cultured human keratinocytes and unblock glycolysis (324). Activation through UVR, nicotinamide also regulates PARP-1, an essential DNA repair enzyme (325). DNA repair enhancement through nicotinamide has been demonstrated in studies utilizing cultured human keratinocytes and *ex vivo* human skin (332). Nicotinamide elevated and increased the rate of repair in cultured human keratinocytes and the proportion of cells going through excision repair. Nicotinamide has been found to reduce the formation of CPDs, post UVB exposure generated photo lesions; and also UVA-induced oxidative DNA lesions, 8-oxo-7, 8-dihydro-2'-deoxyguanosine, in cultured human keratinocytes and *ex vivo* human skin alike. **(Figure 6.19)**

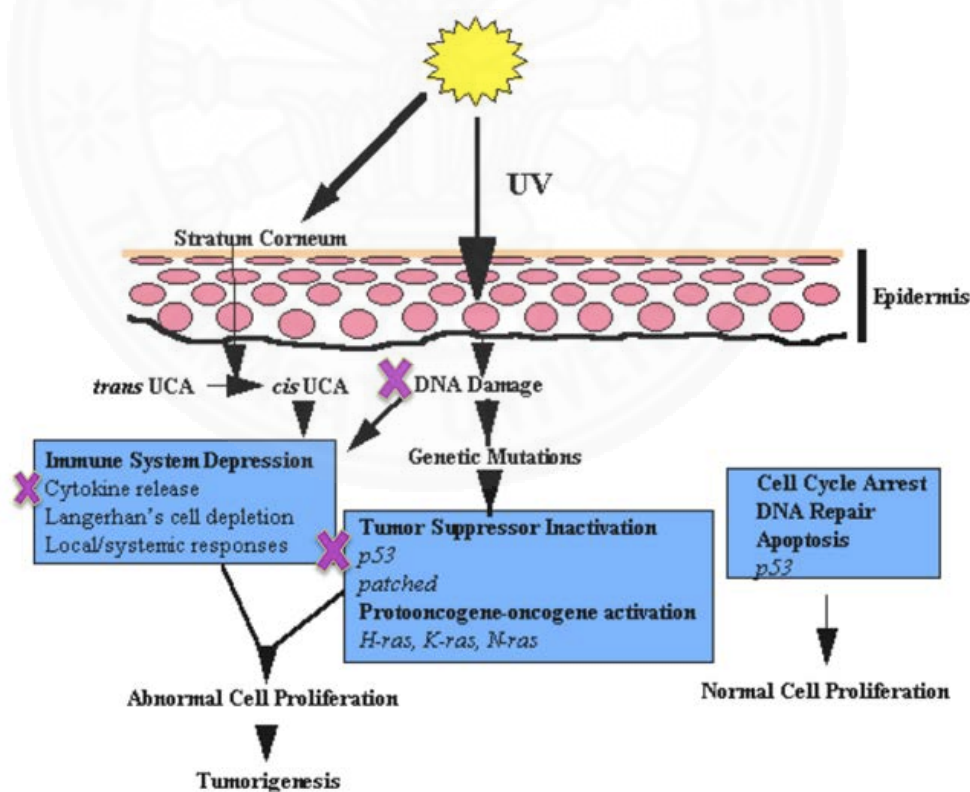


Figure 6.19 Nicotinamide mechanisms of action (modified from (62, 63))

6.4.2 Omega-3 Polyunsaturated Fatty Acids

Most types of fat that the body needs can either be made from other fats or raw materials; however, this is not the case with omega-3 fatty acids (also known as n-3 fats and omega-3 fats). Omega-3 are essential fats that the body cannot produce but rather derived from food consumption. Food with abundance of omega-3 includes fish, nuts (particularly walnuts), flaxseed oil, vegetable oils, flax seeds and leafy vegetables.

Omega-3 fats are an integral part of cell membranes throughout the whole body and in these membranes; the cell receptors and its functions are affected. The production of hormones that regulate blood clotting, relaxation and contraction of artery walls and inflammation use Omega-3 as a starting point. Omega-3 also binds to receptors in cells in which regulate genetic function. These effects may be the reason behind omega-3 and its prevention of heart disease and stroke, lupus control, rheumatoid arthritis and eczema and could possibly provide protection in cancer and other conditions such as photo-protection properties.

6.4.2.1 Molecular Composition and Pharmacology

Increasing attention has risen towards macronutrients such as omega-3 polyunsaturated fatty acids (n-3 PUFA) as agents potentially for skin health maintenance, skin disorders treatment, especially those mediated by UVR. These include photo-aging, photo-sensitivity, sunburn and cancer. There has been an increase in awareness that most prominent signs of skin aging are caused by UVR. Protection of the skin against harmful effects of UVR, reduction in UVR-induced inflammation and indicators of photo-carcinogenesis and photo-aging are a result of Eicosapentaenoic acid (EPA, 20:5n-3), a long-chain (LC) n-3 PUFA (200, 224, 333-337).

The predominant structural constituent of the human brain, cerebral cortex, testicles, sperm, skin, retina is omega-3 fatty acids, Docosahexaenoic acid (DHA). It is either acquired directly from maternal milk (breast milk), algae oil or fish oil or it can be synthesized from alpha-linolenic acid (ALA).

6.4.2.2 Photoprotective Outcomes

In the study of Rhodes 2003, a range of indicators of UVR-induced DNA damage in humans was examined in regards to the effects that supplementation had on them and also the effect on basal and after UVR oxidative status.

In this study, daily intake of 4 g purified ω -3 PUFAs, either 95% EPA or monounsaturated, 95% oleic acid (OA) for 3 months on 42 healthy subjects with skin type II to III. The result shows increased MED and UIE threshold after supplementation only in EPA group ($p < 0.01$). In addition, immunohistochemically was assessed 24 hour post UVR exposure, UVR-induced p53 expression, shows a significant reduction ($p < 0.01$). There were no changes in regards to basal DNA strand-break or CPDs in both groups. Following OA supplementation, there was no significant change in any of the parameters.

Another study involved the administration of fish oils containing 280 mg EPA together with 120 mg DHA in 20 healthy adults with skin type III for 4 weeks compared with placebo. Results suggest statistically increased MED ($p < 0.02$) and no significant change of PGE₂ level in fish oils group. There was no change in any of the parameters in the placebo group.

6.4.2.3 Mechanisms of Action

(1) Anti-inflammatory Properties

Photo-sensitivity disorders where patients display abnormal reactions to UVR could be protected through the use of n-3 PUFA. 13 patients with polymorphic light eruption photo-sensitivity disorder in an open study was given a daily supplementation of 3g mixed n-3 PUFA (1.8g EPA+1.2g DHA) for a period of 3 months, a significant result was achieved through the reduction in sensitivity to broadband UVA papule provocation, moreover, there was an increase in MED to UVB as well (200). Even though the reduction in UVB-induced PGE₂ in the PLE study conveys that the n-3 PUFA may be the cause of reduction of pro-inflammatory milieu, the protective effects and its underlying mechanisms are yet to be determined. In photo-sensitivity, there are no reports of controlled trials evaluating protection from ambient UVR.

Photo-aging is clinically defined as deep wrinkles, uneven pigmentation and decreased elasticity. The reduction of c-Jun phosphorylation and decreased MMP expression is a result of inhibition of ERK and JNK activation through the addition of EPA to irradiated dermal fibroblasts *in vitro* (338). **(Figure 6.20)**

Physiological effects examined as a result of changes in the composition of membrane LC-PUFA have been comprehended to be because of altered membrane fluidity and n-3/n-6 PUFA-derived eicosanoid ratios. Nonetheless, LC-PUFA also regulate nuclear transcription factors, for example, NF- κ B, AP-1 and sterol regulatory element-binding proteins (SREBP) and receptors including peroxisome proliferator-activated receptors (PPAR), liver X receptors (LXR) and retinoid X receptors (RXR), the sum of which modulate genes dealing with lipid metabolism and inflammation (339, 340). According to date, and, to tissue, the influence to which n-3 PUFA has on their activity differs, a handful of studies have examine the impact concerning these fatty acids on UVR induced transcriptional regulation in human skin.

An essential transcription factor dealing with epidermal homoeostasis and is upregulated in response to oxidative stress and UVR is NF- κ B (341, 342). Pro-inflammatory cytokines such as TNF- α , IL-1 α , IL-1 β , IL-6 and IL-8 and the expression of various genes dealing with inflammation are regulated by it (343). It has been reported that there is an increased in NF- κ B activity after UVB irradiation in hairless mouse skin (344). LC n-3-PUFA, eicosatrienoic acid was found to be capable of creating a reduction in UV-induced NF- κ B activation and at the same time a reduction in IL-1 β , MMP-13 and COX-2 (the crucial collagenase in the murine system), indicating that n-3 PUFA could subdue the express of these proteins through NF- κ B signaling pathway regulation (344). **(Figure 6.20)**

It has been reported that DHA and EPA, not including AA, through AP-1 activity reduction, effective at cellular transformation inhibition in a mouse epidermal cell line (345). Additionally, AP-1 is implicated in photo-aging (338). **(Figure 6.20)**

Sunburn response, an inflammatory reaction clinically resulting in an erythema and edema resulting in stratum corneum thickening, dermal

leucocytic infiltration and apoptotic epidermal cells (sunburn cells) caused by acute overexposure to UVR (346-348). UVR causes the release of PUFA from the cell membrane and triggers upregulation of inducible COX-2 and PGE₂, resulting in the regulation of vasodilatation and leucocyte, respectively (349-351). Following the supplementation of fish oil (1.8g EPA+1.2g DHA) in human studies, the anti-inflammatory effects are evident, significantly reducing UV erythema sensitivity in conjunction with a reduction in UV-induced skin PGE₂ levels greater than 60% and an increase in cutaneous n-3/n-6 PUFA ratio (200, 225, 337). There are still a limited number of studies regarding the potential of orally administered n-3 PUFA in UVR protection.

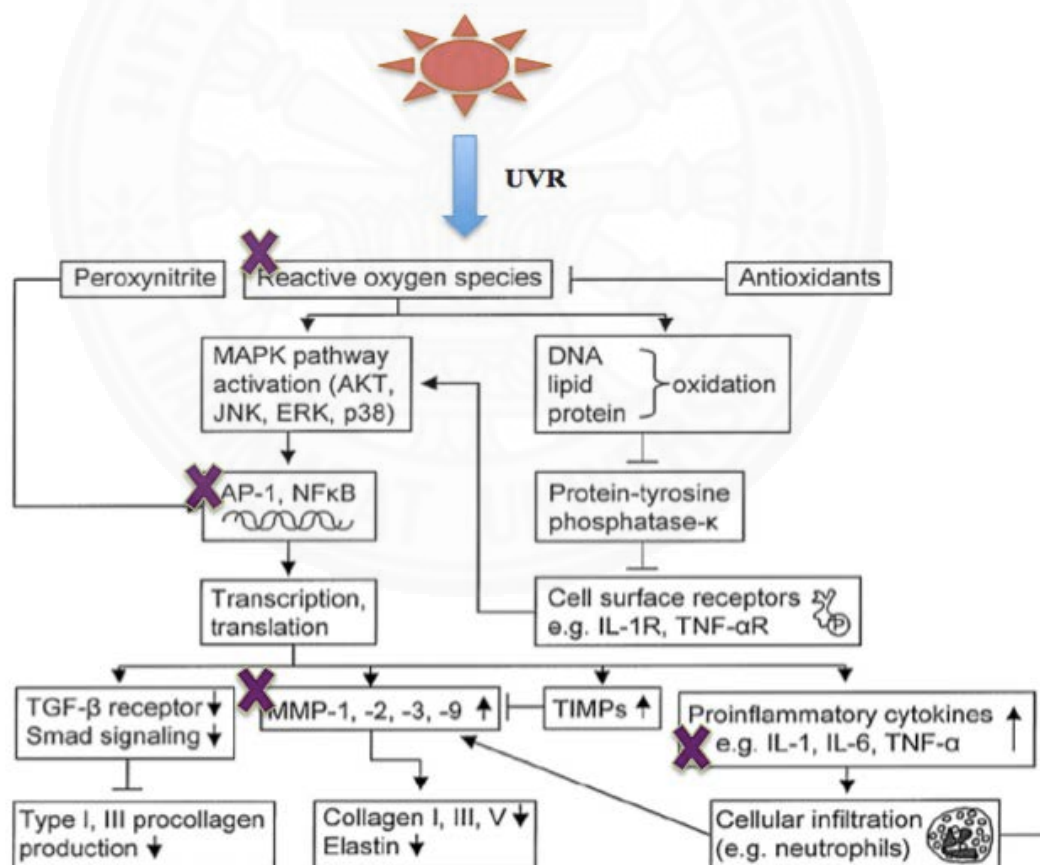


Figure 6.20 n-3 PUFA mechanisms of action (modified from (62))

6.5 Risk of Bias

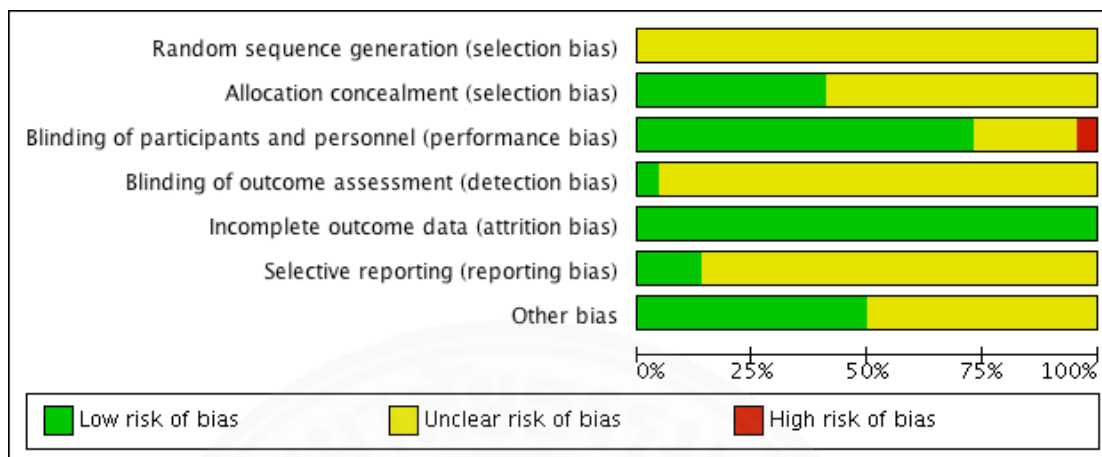


Figure 6.21 Risk of Bias Graph

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Aust 2005	?	?	+	?	+	?	+
Chow 2003	?	?	?	?	+	?	+
Farrar 2015	?	+	+	?	+	?	+
Fuchs 1998	?	?	-	?	+	?	+
Gonzalez 1997	?	?	?	?	+	?	?
Heinrich 2003	?	?	+	?	+	+	+
Heinrich 2006	?	?	+	?	+	?	+
Heinrich 2011	?	?	+	?	+	?	+
Mathews-Roth 1972	?	+	+	?	+	?	+
McArdle 2004	?	+	?	?	+	?	+
Mireles-Rocha 2002	?	?	+	?	+	?	+
Mogollon 2014	?	+	+	?	+	+	+
Orengo 1992	?	?	?	?	+	?	?
Rhodes 2003	?	+	+	?	+	?	?
Rizwan 2011	?	+	+	+	+	+	?
Sokoloski 2015	?	+	+	?	+	?	?
Stahl 2000	?	?	+	?	+	?	?
Stahl 2001	?	?	?	?	+	?	?
Thanos 2012	?	+	+	?	+	?	?
Werninghaus 1994	?	?	+	?	+	?	?
Williams 2009	?	?	+	?	+	?	?
Yiasemides 2009	?	+	+	?	+	?	?

Figure 6.22 Risk of Bias Summary

CHAPTER 7

DISCUSSION

Most clinical studies in oral photoprotective products were uncontrolled trials which had no comparison; then, they were not included into this study. As a result, this study reviewed only the RCTs comparing to either placebo or different agents.

Photoprotective effects of systemic products can be clinically observed through increased MED and decreased UIE intensity. Some studies also investigated physiological changes in skin, histology and immunohistochemistry. These may help to explain their mechanism of actions.

Total six plant-derived agents, including β -carotene, green tea, golden serpent fern, tomato, cocoa bean, and vitamin E demonstrated the evidences of photoprotection. Polyphenols are a large group of plant products occurring naturally. It prevents damages affecting cellular lipids, protein, DNA, and premature aging of the skin from photooxidative damage. Based on chemical structures, polyphenols can be classified into flavonoids, stilbenes, lignans and phenolic acids (352). Golden serpent fern, green tea and cocoa bean have polyphenol structures and they exhibited the same mechanisms of action in ROS suppression and anti-inflammation (352).

Carotenoids, naturally fat-soluble pigments, are synthesized by plants and algae. Most of the carotenoids are from food with yellow to orange hues such as carrots, plums and apricots. Carotenoids are usually classified into two broad classes; carotenes (lycopene, α -carotene and β -carotene) and xanthophylls (β -cryptoxanthin, lutein, and zeaxanthin). Major biological effects of carotenoids include provitamin A activity, cellular signaling and antioxidation (353). All clinical studies in carotenoids show evidences of photoprotection. Lycopene in tomato paste or tomato extract contributed a total of four RCTs reporting positive effects on UV protection (199, 212-214). It also reduces mitochondrial DNA deletion and UV-induced MMP-1 which may improve mitochondrial function, reducing oxidative stress as a result of UVR-generated ROS stimulating both MMP-1 induction and mtDNA deletion, representing its role in mutagenic suppression (199). The study associates with tomato

extract and drink containing tomato extract shows a reduction in erythema intensity but not in synthetic lycopene. The efficacy differs between tomato products in comparison to synthetic lycopene may be a result of phytofluene and phytoene being present.

Previous study reported an increase in the ability to tolerate sunlight among photosensitive subjects, especially in erythropoietic porphyria after high dose administration of beta-carotene (354). However, there were only three RCTs in healthy subjects showing reduction of UIE intensity following an administration for three months (206-208). β -carotene was mainly derived from *Dunaliella salina*, a unicellular biflagellate green alga, exhibiting potent protection from UV-induced oxidative damage in animal models due to the increase of antioxidative activity and the inhibition of lipid peroxidation (355). From the studies, supplementation of β -carotene ranging from 8-25 mg for 10 weeks or more lead to an increase in basal protection of the skin against erythema. An alternative for sun protection is possible through usage of carotenoid mixture at a lower dosage instead of a higher dose of a single carotenoid (β -carotene). β -carotene at a high dosage of 180 mg is normally used to treat patients with erythropoietic protoporphyria against photosensitivity to visible light, moreover, it also helps to protect photosensitivity reactions to UVR.

Camellia sinensis leaves that have not undergone the withering and oxidation process are commonly known as green tea. Four major polyphenols in green tea include epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate (EGCG), the most potent antioxidant. In animal models, green tea polyphenols and EGCG demonstrated the protection against the UV-induced sunburn and photoaging of the skin (356). Green tea, a notable antioxidant product, has an extensive *in vitro* studies of its properties. Interestingly, there was only one randomized, double-blind, placebo-controlled trial indicating an increase of photosensitivity threshold and improving skin physiology following a daily intake of 1,402 mg of catechins for 3 months (210). The amount of supplementation was interestingly the highest amount in all studies with a dosage of 980 mg EGCG, 100 mg EC and 238 mg ECG, equivalent to more than 8-16 cups of green tea (depending on cup sizes) (211). This further provides suggestion that it can protect the skin from harmful UVR but the pathway of mechanism is not provided within the study.

Though PL extract is one of the most well known systemic photoprotective agents, there were only one RCTs comparing PL and placebo in healthy subjects. PL commonly known as golden serpent fern, is a tropical fern plant found in Central America. Its active ingredients are polyphenols including phenolic acids p-coumaric, ferulic, fumaric, vanillic, quinic, caffeic chlorogenic, 3,4-dihydroxybenzoic, and 4-hydroxybenzoic, along with five phenolic chlorogenic acid isomers. The photoprotective properties of PL may link to its antioxidant activities, scavenging ROS and possessing anti-inflammation. However, only high dose of PL at 1080 mg could prevent acute sunburn (191). Immunomodulating effect of PL was found by the preservation of CD1a-expressing epidermal LCs in human skin (191).

Cocoa beans harvested freshly are polyphenols abundance. Flavanoids are the main phenolic phytochemical structure. However, during the production process of conventional chocolate, much of the antioxidant capacity of fresh cocoa beans is greatly diminished (357). The mechanism of action is most likely its antioxidant activity of cocoa flavanols and its anti-inflammatory properties. All three RCTs in cocoa bean demonstrated similar trends. Daily ingestion of chocolate with high flavanol cocoa for three months exhibited higher photosensitivity threshold (215-217). In studies where there are no statistically differences when measured by MED in both HFC and LFC, the extrinsic effect of season of participation and longer solar exposure may affect the MED of LFC. Similarly, participation at different seasons may play a role towards bias results as natural increase in MED could make the photoprotective effects of chocolate less apparent. However, there are no measurements of histology and biomarkers outcomes in any of the studies, therefore, it is not possible to conclude and understand the mechanism of actions of cacao and its photoprotective effects against UVR.

Vitamin E, α -tocopherol, can be found most abundantly in wheat germ oil, sunflower and safflower oils. It affects oxidative stress by interrupting the free radical formation. For daily supplement of vitamin E, only high dosage at 1000 IU and above exhibited an increase of MED (219-221). The mechanisms of vitamin E may involve lipid peroxidation (218). Within the McArdle 2004 study of vitamin E and β -carotene supplementation, there are no effects on skin sensitivity to UVR as a result of no change in MED (218). Although skin malondialdehyde concentration is

significantly reduced through supplementation of vitamin E, other measures of UVR-induced oxidative stress in human skin is unaffected by neither of the supplement, suggesting that the supplementation provides no photoprotection.

Results show a significant increase in MED in groups administered with α -tocopherol 1,200 IU or with a combination of α -tocopherol 1,200 IU and ascorbic acid 2 g with an increase being even greater with the combined administration (219). In another study that uses the same combination of α -tocopherol 2 g combined with ascorbic 3 g demonstrates a similar result (220). The result is due to the synergic action occurring between both vitamins, demonstrated in both *in vitro* and *in vivo* studies. It is known that the regeneration of antioxidant form of vitamin E is a result of the ascorbic acid and that in lipid peroxidation inhibition (358, 359), this interaction is present. The dose used in this study is 1,200 IU which is considered to be a large amount compared to other studies and may be excessive for different patients and there are no guarantees for the safety of long term supplementation at this level. The study duration was only for 1 week and the side effects are too soon to determined. From the study using α -tocopherol 400 IU as a monotherapy for 6 months, there are no significant changes in both MED and histology indicating that α -tocopherol less than 1,000 IU or low dose does not provide photoprotective effects.

It should be taken into consideration that even though α -tocopherol has photoprotective activity, most of the studies are topically administered and there are a limited numbers of studies in regards to oral administration in human, with varying dose and ununiformed results.

Animal-derived photoprotective agents include nicotinamide and ω -3 PUFAs. Nicotinamide, an amide form of vitamin B3, is the precursor of NAD, an essential coenzyme for ATP production. It has been demonstrated that nicotinamide prevents UV-induced ATP depletion, enhances UV-induced DNA repair, regulates inflammatory cytokines and mediators in human keratinocytes (332) and prevent photocarcinogenesis in animal studies (325). In human studies, topical and oral nicotinamide administration could reduce the number of actinic keratosis (360, 361).

Foods that are abundant in nicotinamide include meats, eggs and legumes. Nicotinamide ranging from 500 to 1500 mg daily reduced UV-induced immunosuppression of the skin (222, 223). Nevertheless, there was no significant

changes of MED (223). Depending on the dose and the responsibility of molecular mechanisms on immune suppression, the mechanisms of nicotinamide immune protection are still unclear. Nonetheless, it should involve the ability of nicotinamide as an NAD precursor, replenishing diminished cellular ATP levels as seen in cultured keratinocytes post UV exposure (324). A key trigger for photoimmunosuppression is DNA damage and DNA repair is a process highly depended on energy. After PDT, nicotinamide may help to replenish cellular ATP levels and in return, support DNA repair. Even at high doses of up to 3.5 g daily, oral nicotinamide is well tolerated with no known side-effects (362). Oral nicotinamide looks to be fairly safe and inexpensive promising supplementation for reducing immunosuppressive effects of UVR.

Dietary ω -3 PUFAs are extracted mostly from oily fish such as mackarels, sardines and salmons. EPA and DHA are major components. ω -3 PUFAs modulate NF-kB and AP-1, which control genes associated with inflammation and lipid metabolism. Dietary ω -3 PUFAs have been previously reported to reduce UVR-induced prostaglandin E₂, a mediator of UV immunosuppression, in both animals and human skins (200). Clinical studies also indicated an increase of UIE threshold (224, 225) and a reduction in UV-induced p53 expression indicating its effect on skin cancer reduction or DNA repair was observed in histology (224). Through the reduction of early markers such as sunburn sensitivity, rising UIE threshold and UVR-induced p53 in the skin, dietary EPA indicates protection against acute DNA mutagenesis. However, there are only 2 studies in regards to ω -3 PUFAs, as a result it is still questionable about its abilities to provide photoprotection.

Mechanisms of action

After reviewing various RCTs studies in regards to β -carotene, cacao and GTC there are no human histology or biomarker measurements supporting evidences. As a result, the mechanism of actions is not clearly elucidated.

From the study that we reviewed in regards to PL, histology suggests shows a preservation of CD1a-expressing epidermal LCs in human skin, suggesting the immunomodulating effects of PLE. (191). **(Table 7.6) (Figure 7.23)**

Lycopene has shown to reduce mitochondrial DNA deletion and UV-induced MMP-1 which may improve mitochondrial function, reducing oxidative stress as a result of UVR-generated ROS stimulating both MMP-1 induction and mtDNA deletion, representing its role in mutagenic suppression (199). This suggests a close relation between the induction of MMP-1 and mtDNA damage, increasing oxidative stress leading to increasing MMP-1 induction, potentially a result of mtDNA damage. **(Table 7.6) (Figure 7.23)**

Vitamin E has shown significant decrease in skin malondialdehyde concentration, an index of lipid peroxidation, suggesting antioxidant properties (218).

In both of the studies regarding supplementation of nicotinamide, there is a significant reduction in Mantoux diameter which is possibly relate to the reduction in UV-induced immunosuppression (222, 223). **(Table 7.6) (Figure 7.23)**

ω -3 PUFAs demonstrates a significant reduction in UV-induced p53 expression in EPA treated group. This indicates that there is a protection against acute DNA mutagenesis. Due to greater protection for p53 induction than erythema, p53 is the more sensitive indicator for skin damage. These effects may indicate free-radical mediated mutagenesis protection from the ω -3 fatty acid (224). **(Table 7.6) (Figure 7.23)**

In the end, there are still limited numbers of studies that show histology and biomarker outcomes, therefore, the mechanism of actions cannot be conclusively identified.

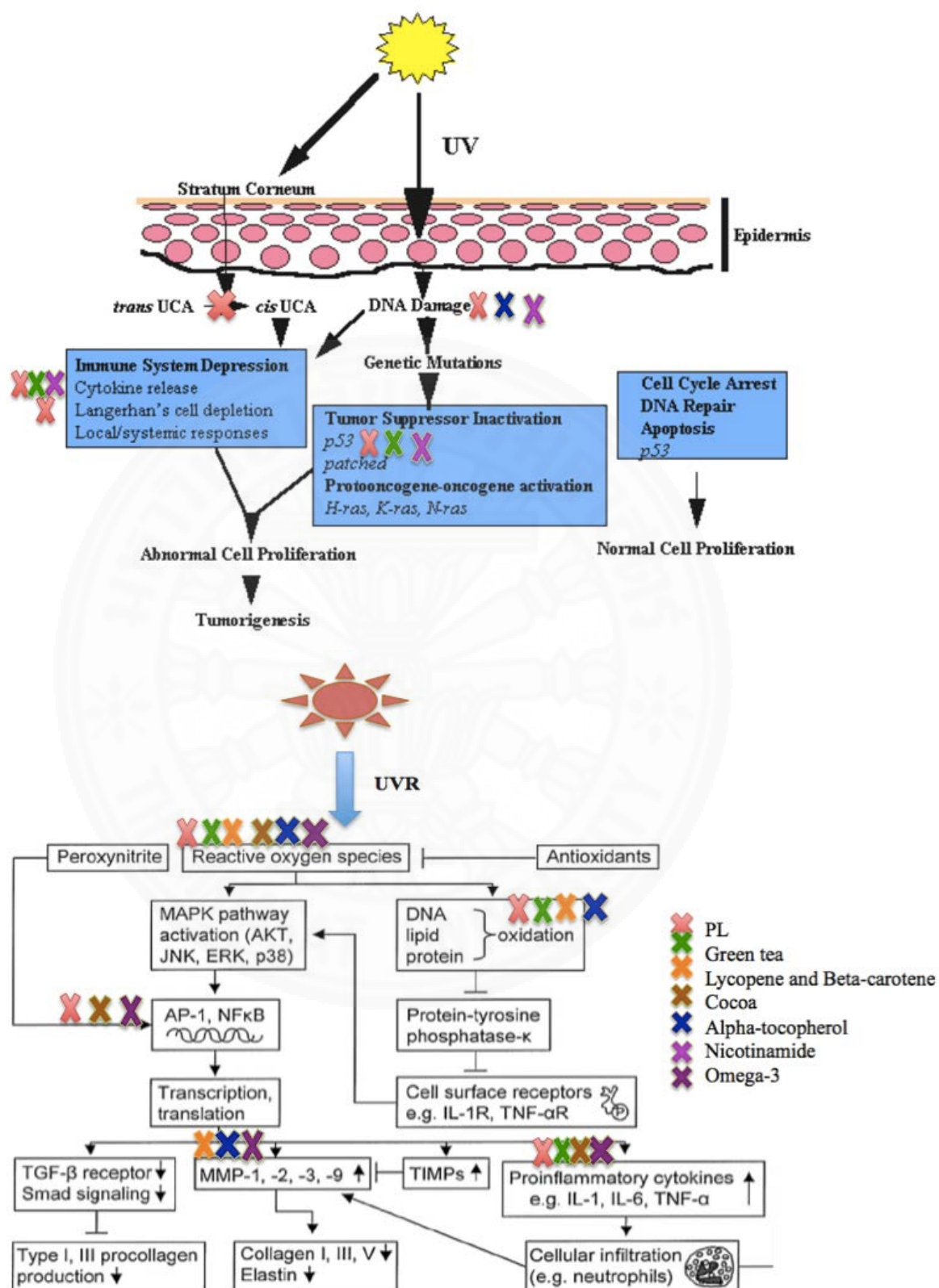


Figure 7.23 Mechanisms of action of all agents (modified from (62, 63))

Table 7.6 Summary mechanisms of action

Scientific name/Compound	Common name	Mechanisms of action
<i>Polypodium leucotomos</i> (191)	Golden serpent fern	Immunomodulating effects (191)
<i>Solanum lycopersicum</i> / Lycopene (199),(212-214)	Tomato	-Anti-photo aging effects -Decrease DNA mutagenesis (199)
Alpha-tocopherol (218-221)	Vitamin E	Antioxidant activities (218)
Nicotinamide (222-223)	Vitamin B ₃	Immunomodulating effects (222-223)
EPA (224-225)	Omega-3	Decrease DNA mutagenesis (224-225)

CHAPTER 8

CONCLUSIONS

Drawing a conclusion from the whole systematic review, carotenoid is the most promising agent as a mean of protection against erythema or UVR. However, more histology evidences are required for a precise conclusion of UVR protection pathway. The extent of protection of all agents are not comparable to the application of sunscreen with high SPF, nevertheless, these systemic agents can provide a basal protection, contributing to a permanent defense against skin damages induced by UVR.

Conventionally, topical sunscreens, physical protection and sun avoidance are recommended as standard photoprotection. However, several limitations including inadequate amount, reapplication needs, granting only UV protection and photoallergic reactions limit their use in clinical practice. Evidences of available natural product-based are promising as an addition for photoprotection by free radical scavenger. Although, there are numerous in vitro and animal studies of the agents, most of their clinical studies are conducted in small sample size of the subjects. To get better understanding of the efficacy, mechanism of actions and role of a natural product for photoprotection, further high-quality controlled trials with higher number of participants are needed.

In the studies with the same agents, the dosage and duration varies significantly and further study and investigation is needed to find the optimal dosage and to further analyze its effect and side effects in individuals.

Oral agents efficacy may not be precisely evaluated due to unavailable effective measurement.

In order to further enhance research and attract more participants, more effective and favorable measurement of histology and biomarker outcomes such as blood test should be used as an alternative or an adjuvant to skin biopsy. A follow up should also be implemented long enough after supplementation in order to investigate any side effects and evaluate whether the dosage is effective.

8.1 Systematic Review Advantages

A systematic review consists of well-defined inclusion and exclusion criteria, as a result, the information and data are more credible than other types of review. Due to this fact, the amount of bias is significantly reduced as studies that do not meet the well-defined inclusion criteria will be dismissed.

8.2 Systematic Review Limitations

There are limitations in regards to this systematic review due to various factors. This systematic review is based upon reviewing manuscripts which may include the author's bias, possibly omitting information and data from the original full paper. As a result, our findings from information and data that contains bias might not be fully accurate. Another limitation from reviewing manuscripts and not the original full paper is not knowing whether the number of participants in each study is statistically adequate or not. As all of the studies involve different participants, variations in lifestyles such as different amount of exposure to UVR and different daily diets may cause inaccurate results and findings. In regards to the plants and animals derived agents used as supplementations in the different manuscripts reviewed, there are limited numbers of studies to support these agents and additional studies are required to support their usage and a clear guidance for the dosage and duration of use for each agent. Moreover, long term effects for each intervention and the quality of life data are not included, not being able to determine the safety of use for each intervention. As current evidences are based upon limitations, better and improved methodological quality is required such as longer intervention period of 6 months or more and at least a 12-month period for follow up in order to better assess maintenance.

Due to the nature of an *in vivo* control trial study and a limited number of studies, it is difficult to determine the mechanism of actions for each supplemented agent. Moreover, histology and biomarker outcomes are additionally required.

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The seal of Thammasat University is a circular emblem. It features a central five-tiered umbrella (parasol) with a lotus flower at its base. The lotus has eight petals. Radiating from the lotus are eight stylized human figures, each holding a torch. The entire emblem is enclosed within a circular border. The top half of the border contains the university's name in Thai script, and the bottom half contains the name in English, "THAMMASAT UNIVERSITY".

APPENDICES

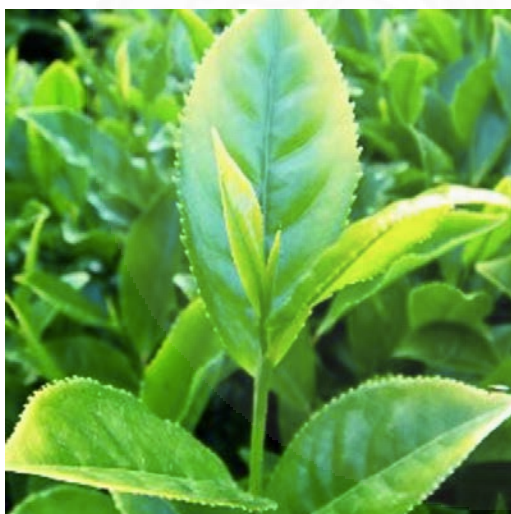
APPENDIX A

PICTURE OF PLANTS

Polypodium leucotomos (363)



Camellia sinensis (364)



Solanum lycopersium (365)



β -carotene (366)

Source of β -carotene

β -carotene are commonly found in orange and yellow color fruits and vegetables.



Theobroma cacao (367)

Theobroma cacao tree



Theobroma cacao (368)

The cacao beans (seeds) which is found inside the cocoa pod is the source of chocolate.



α -Tocopherol (369)

Natural Vitamin E Food Sources



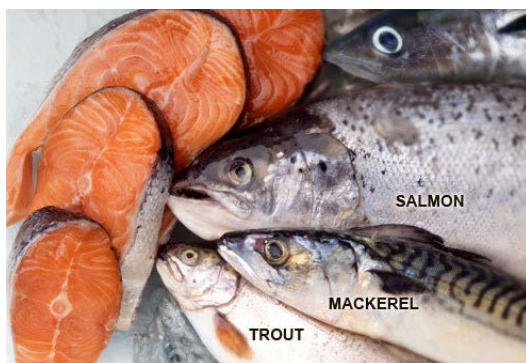
Nicotinamide (320)

Vitamin B3 can be found in poultry, red meat, eggs, and dairy products.



Omega-3 Polynsaturated Fatty Acid (370)

The best source of omega-3 fatty acids DHA and EPA is fish. Top choices are salmon, mackerel, herring, lake trout, sardines, anchovies, and tuna.



APPENDIX B

PRISMA-P 2015 CHECKLIST

PRISMA-P (preferred reporting items for systematic review and meta-analysis protocols) 2015 checklist : recommended items to list in a systematic review protocol (203)

Section and topic	Item No	Checklist item
Administrative information		
Title:		
Identification	1a	Identify the report as a protocol of a systematic review
Update	1b	If the protocol is for an update of a previous systematic review, identify as such
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number
Authors:		
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments
Support:		
Sources	5a	Indicate sources of financial or other support for the review
Sponsor	5b	Provide name for the review funder and/or sponsor
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol
Introduction		
Rationale	6	Describe the rationale for the review in the context of what is already known
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)
Methods		
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated
Study records:		
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2 , Kendall's τ)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)

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