Chemoprevention of photocarcinogenesis by selected dietary botanicals

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Epidemiological, clinical and laboratory studies have implicated solar ultraviolet (UV) radiation as a tumor initiator, tumor promoter and complete carcinogen, and their excessive exposure can lead to the development of various skin disorders including melanoma and nonmelanoma skin cancers. Sunscreens are useful, but their protection is not adequate to prevent the risk of UV-induced skin cancer. It may be because of inadequate use, incomplete spectral protection and toxicity. Therefore new chemopreventive methods are necessary to protect the skin from photodamaging effects of solar UV radiation. Chemoprevention refers to the use of agents that can inhibit, reverse or retard the process of skin carcinogenesis. In recent years, considerable interest has been focused on identifying naturally occurring botanicals, specifically dietary, for the prevention of photocarcinogenesis. A wide variety of botanicals, mostly dietary flavonoids or phenolic substances, have been reported to possess substantial anticarcinogenic and antimutagenic activities because of their antioxidant and antiinflammatory properties. This review summarizes chemopreventive effects of some selected botanicals, such as apigenin, curcumin, grape seed proanthocyanidins, resveratrol, silymarin, and green tea polyphenols, against photocarcinogenesis in *in vitro* and *in vivo* systems. Attention has also been focused on highlighting the mechanism of chemopreventive action of these dietary botanicals. We suggest that in addition to the use of these botanicals as dietary supplements for the protection of photocarcinogenesis, these botanicals may favorably supplement sunscreens protection and may provide additional antiphotocarcinogenic protection including the protection against other skin disorders caused by solar UV radiation.

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Introduction

Solar ultraviolet radiation, skin and photodamage

The skin is the largest organ of the body and comprising a surface area of approximately 1.5–2.0 m^2 which protects the internal organs of the body by acting as an effective barrier against

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the detrimental effects of environmental and xenobiotic agents. Among many environmental and xenobiotic factors, the exposure of solar ultraviolet (UV) radiation is the key factor in the initiation of several skin disorders, such as wrinkling, scaling, dryness, mottled pigment abnormalities consisting of hypopigmentation and hyperpigmentation and skin cancer.[1–3] Solar UV radiations are mainly divided in to three categories based on their wavelengths and adverse biological effects:[1–3]

(i) UVC (200–280 nm). UVC radiation largely absorbed by the atmospheric ozone layer and does not reach to the surface of the earth. These wavelengths have enormous energy and mutagenic in nature.

(ii) UVB (280–320 nm). UVB radiation constitutes approximately 5% of the total solar UV radiation and mainly responsible for variety of skin diseases including the nonmelanoma and melanoma skin cancers. UVB radiation can penetrate inside epidermis of the skin and can induce both direct and indirect adverse biological effects including induction of oxidative stress, DNA damage, cancer and premature aging of the skin.[1–3] Excessive exposure of UVB radiation decreases the levels of antioxidant defense enzymes in the skin, impairing their ability to protect from harmful effects.[4,5]

(iii) UVA (320–400 nm). UVA comprises the largest spectrum of solar UV radiation (90–95%) and is considered as the "aging ray". UVA penetrates deeper into the epidermis and dermis of the skin and has recently been shown that extensive UVA exposure can lead to benign tumor formation as well as malignant cancers.[6,7] Its exposure induces the generation of singlet oxygen and hydroxyl free radicals, which can cause damage to cellular macromolecules, like proteins, lipids and DNA.[8] In contrast to UVC or UVB, UVA is hardly able to excite the DNA molecule directly and produces only a small number of pyrimidine dimers in the skin, therefore it is presumed that much of the mutagenic and carcinogenic action of UVA radiation appears to be mediated through reactive oxygen species,[9,10] however, it is still a matter of debate. It has been suggested that bipyrimidine photoproducts rather than oxidative lesions are the main type of DNA damage involved in the genotoxic effect of solar UVA radiation.[11] UVA is a significant source of oxidative stress in human skin and causes photoaging in the form of skin sagging rather than wrinkling[12] and can suppress some immunological functions.[13]

Statistical analysis indicates that the average annual UV dose that an average American typically receives in a year is about 2500–3300 mJ cm^{-2}. Further, an average female is exposed to 2200 mJ cm^{-2} and males 2800 mJ cm^{-2} each year with an additional exposure of about 800 mJ cm^{-2} of solar UVB radiation during a conservative vacation.[14,15]

Characteristics of UV radiation

Solar UV radiation, particularly UVB (290–320 nm), possesses suppressive effects on the immune system,[16] and can act as a tumor initiator,[17] tumor promoter[18] and co-carcinogen.[19,20] Skin exposure to UVB radiation induces a variety of biological effects including inflammation, sunburn cell formation, immunologic alterations and induction of oxidative stress which play an important role in the generation and maintenance of UV-induced neoplasms.[21–23] Although skin possesses an elaborate defense system consisting of enzymatic and non-enzymatic components to protect the skin from adverse biological effects however, excessive exposure to UV radiation overwhelms and depletes the cutaneous defense system and leads to the development of various skin disorders including the risk of skin cancer.[18,23–25]

UVB radiation has multiple effects on the immune system.[26,27] There is ample clinical and experimental evidence to suggest that immune factors contribute to the pathogenesis of solar UV-induced skin cancer in mice and probably in humans as well.[28,29] Chronically immunosuppressed patients living in regions of intense sun exposure experience an exceptionally high rate of skin cancer.[30] This observation is consistent with the hypothesis that immune surveillance is an important mechanism designed to prevent the generation and maintenance of neoplastic cells. Further, the incidence of skin cancers, especially squamous cell carcinoma (SCC), is also increased among organ transplant recipients.[31–33] The increased frequencies of SCC, especially in transplant patients, is presumably attributable to a long-term immunosuppressive therapy,[34] however nonimmune mechanisms may also play a role.[35] These studies provide evidence in support of the concept that UV-induced immune suppression promotes skin cancer risk.
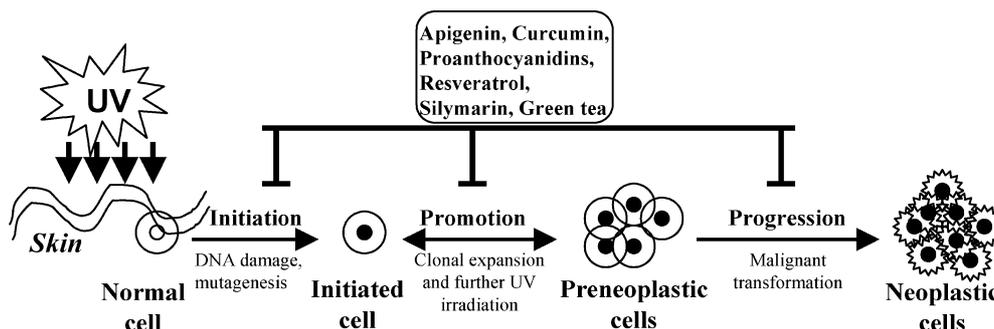
Photocarcinogenesis

Solar UV radiation-induced skin cancer or photocarcinogenesis is a complex process that involves a series of individual steps. UV-induced tumor development has been generally considered to consist of three distinct stages: (i) tumor initiation which consists of genotoxic effects in normal cells, (ii) tumor promotion, consists of clonal expansion of initiated cells and this stage is considered to be reversible, and (iii) tumor progression which consists of malignant transformation of papillomas to carcinomas and requires further genotoxic stimulus. A schematic representation of these stages is shown in Fig. 1.

Nonmelanoma skin cancer, comprising of SCC and basal cell carcinoma (BCC), represent the most common malignant neoplasms in humans particularly in Caucasians. Extensive exposure to UV radiation has been considered as a well-recognized etiological agent for both nonmelanoma and melanoma skin cancers, which accounts for approximately 1.3 million new cases of skin cancers each year in the USA alone.[36] These numbers are probably underestimates as many skin cancers are treated or removed in clinics without being reported to cancer registries. Thus, UV-induced skin cancer is a major burden on public health and healthcare expenditures. Moreover, it is expected that the dramatic recent increase in the incidence of skin cancer will be sustained due to the ageing of the population, the greater amounts of UV radiation reaching the surface of the earth because of depletion of ozone layer[28,37,38] and the extensive use of sun tanning devices for cosmetic purposes. Therefore, the development of effective chemopreventive agents that can reduce or control the risk of UV-induced skin cancer is required to address this public health issue.

Sunscreens inadequately protect against UV carcinogenesis

Sunscreens are widely advocated as a means of reducing skin cancer risk. This advice is largely based on extrapolation from

Fig. 1 Schematic representation of UV radiation-induced multi-stage (initiation, promotion and progression) skin carcinogenesis. UV radiation acts as a tumor initiator, tumor promoter and as a complete carcinogen. Multiple UV irradiation is required for tumor promotion and progression. Chemopreventive agents can block, reverse or slow down the process of multi-stage carcinogenesis at one or at all the stages depending on their efficacy and/or anticarcinogenicity.

animal studies, as it is difficult to evaluate long-term protection in humans. Limited data indicate that sunscreens can inhibit actinic keratoses that are regarded as precursors of squamous cell carcinoma.[39,40] A study conducted in Australia has shown that daily use of a SPF 16 sunscreen, over a period of 4.5 years, reduced the total number of squamous cell carcinoma by 40% but not the number of people with the tumor. No protective effect was seen for basal cell carcinoma.[41] Some studies even show that sunscreen use is associated with an increased risk of melanoma.[42] Haywood et al.[43] also showed that sunscreens inadequately protect against UV-induced free radicals in skin which are implicated in skin aging and melanoma. Moreover, it is difficult to find an effective sunscreen which can provide full spectral protection against ultraviolet light. In addition, sunscreen ingredients may become free radicals themselves when activated by ultraviolet irradiation,[44] and sunscreen chemicals may be absorbed into skin[45] to potentially cause harm.

Botanical antioxidants and photoprotection

In recent years, there has been a considerable interest among the human population for the use of naturally occurring botanicals for the prevention of UV-induced photodamage including skin cancer risk. Botanical supplements, specifically dietary botanicals, possessing antiinflammatory, immunomodulatory and antioxidant properties are among the most promising group of compounds that can be exploited as ideal chemopreventive agents for a variety of skin disorders in general and skin cancer in particular. Recent advances in our understanding at the cellular and molecular levels of carcinogenesis have led to the development of promising strategies for the prevention of cancer or so-called 'chemoprevention'. Chemoprevention is a means of cancer control by the use of specific natural or synthetic chemical substances which can suppress, retard or reverse the process of carcinogenesis. In this respect, chemoprevention offers a realistic promise or strategy for controlling the risk of cancer. Furthermore, chemopreventive approach appears to have practical implications in reducing skin cancer risk because unlike the carcinogenic environmental factors that are difficult to control, individuals can modify their dietary habits and lifestyle in combination with a careful use of skin care products to prevent photodamage and photocarcinogenesis. Studies from our laboratory have shown the efficacy of

naturally occurring botanical antioxidants, such as green tea polyphenols, silymarin and grape seed proanthocyanidins (GSP), against UV radiation-induced inflammation, oxidative stress and photocarcinogenesis.[46–48] Here, we will briefly summarize and discuss the photoprotective potential of some dietary botanicals. A summary of molecular targets or mechanism of action of each dietary agent against photocarcinogenesis is shown in Table 1.

Apigenin

Apigenin is flavonoid (5,7,4′-trihydroxyflavone) in nature and widely present in herbs (endives, cloves), fruits (apples, cherries, grapes), vegetables[49] (beans, broccoli, celery, leeks, onions, barley, parsley, tomatoes) and beverages[50] (tea, wine). It is nontoxic, antimutagen, antioxidant, free radical scavenger, antiinflammatory and anticarcinogenic in nature.[49,51,52] Birt et al.[53] reported that topical application of apigenin prior to UV irradiation prevents UV-induced tumorigenesis in mice. In this study they also observed that apigenin treatment to mouse skin resulted in inhibition of UV-induced increase of ornithine decarboxylase activity, which is considered as a biomarker of tumor promotion, and reduced tumor incidence as well as increased tumor-free survival in mice. Studies have provided further evidence that apigenin prevents UV-induced skin tumorigenesis by inhibiting the cell cycle machinery and cyclin-dependent kinases (cdk).[54] Apigenin treatment to mouse keratinocytes induced G2/M cell cycle arrest, accumulation of the p53 tumor suppressor protein and expression of a cdk inhibitor protein p21/WAF1, a downstream effector of p53 tumor suppresser protein.[52,54] The cell cycle arrest was also accompanied with the inhibition of p34/cdk2 kinase protein level and activity, which was found independent of p21/WAF1 protein.[54] These molecular mechanisms support the evidence that apigenin has the ability to protect the skin from UV-induced harmful effects.

Curcumin

Curcumin is one of the most extensively investigated phytochemicals with regard to its medicinal value. Curcumin is a yellow pigment obtained from the turmeric rhizome (*Curcuma longa*, Linn), and is known for its medicinal values since ancient times. Its medicinal values have been described in 'Ayurveda' (an Indian

Table 1 Photoprotective effects of selected dietary botanicals on some biological parameters altered after exposure to UV radiation in *in vitro* and *in vivo* systems

Botanical	Source	Molecular targets/mechanisms	Refs.
Apigenin	Apples, cherries, grapes, broccoli, Onions, parsley, tomatoes, tea, wines, Barley, celery	ODC	52
		p53	51
		Cell cycle regulatory proteins,	53
Curcumin	Rhizome (*Curcuma longa*, Linn)	ODC, ROS scavenger,	63–66
		Inhibits superoxide anions, H_2O_2	66
		Inhibits activation of AP-1, NF-κB	67
		Induces p53, p21Waf1, Gadd45	68
		Metalloproteins	64
Proanthocyanidins	Grape seeds (*Vitis vinifera*)	Interleukins,	24,75
		Inhibits LPO	24
		p53, proteins of Bcl-2 family	77
Resveratrol	Grape skin, peanuts, red wine, mulberries	Inhibition of H_2O_2, LPO	82,83
		COX-2, ODC	82,83
		NF-κB, IKKα	85
		MAPK proteins	84
		Cell cycle regulatory proteins, surviving	83,84
Silymarin	Milk thistle (*Silybum marianum*, Linn)	Inhibits H_2O_2, LPO, NO, iNOS, MPO	89
		COX-2, PGs, ODC	17,88
		NF-κB, IKKα, AP-1, MAPK proteins	90,91
		DNA, PCNA, cell cycle proteins	88,91,92
Green tea	Leaves and bud (*Camellia sinensis*, Linn)	Inhibits, H_2O_2, NO, iNOS, LPO, MPO	5,22,23,108–110,115
		COX-2, PGs, Interleukins, Immune system	107–109
		p53, cell cycle regulatory proteins,	111,
		NF-κB, IKKα, AP-1, MAPK proteins,	117,118
		Enhance antioxidant defense enzymes	5,106
		Inhibition of DNA damage	111,122,124
		MMP	120

medicine system), and very commonly used as a spice in Indian cooking. In general, curcumin possesses antitumoral, antiinflammatory and antiinfectious activities.[55–59] Several studies carried out in the past two decades have conclusively suggested that curcumin has antimutagenic activity, inhibits the radiation- as well as chemical carcinogen-induced neoplastic lesions in many tumor models including skin, probably *via* an antioxidant mechanism.[60–62] Turmeric has been used since time immemorial in the Indian traditional medical system for wound healing, as an antiinflammatory agent and for treatment of various skin disorders and infections. Because of the antiinflammatory and antioxidant effect of curcumin, curcumin supplemented cosmetics and skin care products/lotions are available in several parts of the world. Curcumin protects cultured human cells from radiation-induced DNA damage and this effect may be due to its strong antioxidant properties.[63] Ishizaki *et al.*[64] observed that topical application of curcumin significantly inhibited TPA- and UVA-induced ornithine decarboxylase activity in mouse epidermis. Oguro and Yoshida[65] have also confirmed this investigation and reported that topical application of curcumin inhibits TPA- and UVA-induced gene expression of metalloprotein in the mouse skin and postulate that these chemopreventive effects may be due to its role as an ROS scavenger. Chan *et al.*[66] have observed that UV-induced increase in intracellular oxidative stress was reduced by curcumin in human epidermoid carcinoma A431 cells. Curcumin has also been shown to decrease superoxide radical formation in normal human keratinocytes leading to lower levels of cytotoxic hydrogen peroxide, which could be proposed as an explanation for the photoprotective effects from UV radiation.[67] Curcumin is a potent inhibitor of AP-1 and NF-κB activation, and also inhibits c-Jun N terminal kinase activation by UVC radiation, which may explain its antiinflammatory and anticarcinogenic effects.[68] Jee *et al.*[69] reported that curcumin induces apoptosis in human basal cell carcinoma by increasing the expressions of p53, p21Waf1 and Gadd45 proteins. Chen *et al.*[70] have found that treatment of HL-60 cells with curcumin increased the expression of the mismatch repair proteins, hMSH2 and hMLH1, when they were exposed to UV radiation. These studies suggest the chemopreventive effect of curcumin against the deleterious effects of UV radiation. Further studies are warranted to examine its effects in the human system.

Grape seed proanthocyanidins

Grape seeds are byproducts of grapes (*Vitis vinifera*) and separated during the industrial production of grape juice and wine. Seeds are potent source of proanthocyanidins or procyanidins, which are mainly composed of dimers, trimers and highly polymerized oligomers of monomeric catechins.[71,72] Grape seed proanthocyanidins (GSP) are potent and better antioxidants and free radical scavengers than ascorbic acid and vitamin E.[73,74] Bouhamidi *et al.*[75] have observed that GSP treatment inhibits the UVC-induced peroxidation of polyunsaturated fatty acids *in vitro* at very low physiologically attainable concentrations (2 mg L^{-1}) and that this effect was better than that of epigallocatechin and epigallocatechin gallate monomers at equivalent levels. The first evidence for the prevention of UV-induced skin cancer by GSP came from our laboratory.[25] We showed that dietary feeding of GSP (0.2 and 0.5%, w/w) inhibits UVB-induced photocarcinogenesis in SKH-1 hairless mice in terms of tumor incidence (20 and 35% inhibition), tumor multiplicity (46 and 65% inhibition) and tumor growth/size (66 and 78% in terms of total tumor volume/group) following

different photocarcinogenesis protocols. Dietary GSP (0.5%, w/w) also resulted in prevention of malignant transformation of UVB-induced papillomas to carcinomas in mice in terms of carcinoma incidence (45% inhibition), carcinoma multiplicity (61% inhibition) and carcinoma size (75% inhibition) compared with non-GSP treated mice following UVB-induced skin carcinogenesis protocol.[25] Recently the studies from our laboratory have shown that dietary GSP prevent UVB-induced suppression of immune responses in mice which was associated with the induction of immunoregulatory cytokine IL-12.[76] Yamakoshi et al.[77] have shown that administration of GSP was also effective in lightening the UV-induced pigmentation in guinea pig skin. Recently, we observed that treatment of JB6 C141 cells (a well-developed cell culture model for studying tumor promotion in keratinocytes) with GSP resulted in a dose-dependent induction of apoptosis.[78] The induction of apoptosis by GSP was p53-dependent because it occurred mainly in cells expressing wild type p53 to a much greater extent than in p53-deficient cells.[78] This study also suggested the involvement of Bax/Bcl-2 proteins and caspase 3 activation in the induction of apoptosis by GSP treatment. Thus, these in vitro and in vivo observations indicate the photoprotective or antiphotocarcinogenic potential of grape seed proanthocyanidins.

Resveratrol

Resveratrol is a polyphenol in nature and chemically known as trans-3,5,4′-trihydroxy-trans-stilbene. It is a phytoalexin and has been identified in more than 70 plant species including grapes, peanuts, fruits, red wine and mulberries. However, grape skin is a particularly good source of resveratrol as the fresh skin contains about 50–100 micrograms of resveratrol per gram, while in red wine its concentration ranges from 1.5 to 3.0 milligrams per liter.[79] Studies have shown that resveratrol is a potent antimutagen, antioxidant, antiinflammatory, antiproliferative, inducer of phase II drug-metabolizing enzymes, and inhibitor of cyclooxygenase and hydroperoxidase in diverse experimental systems.[80,81] Resveratrol inhibits diverse cellular events associated with tumor initiation, promotion and progression of skin cancer and cancers of other organs.[80–82]

Topical application of resveratrol before UVB irradiation resulted in significant inhibition of UVB-induced increase in bi-fold skin thickness (marker of edema), hyperplastic response, leukocyte infiltration, generation of hydrogen peroxide, lipid peroxidation and activities of COX-2 and ornithine decarboxylase in SKH-1 hairless mouse skin.[83,84] At the molecular level, treatment of resveratrol inhibits UV-mediated increase in proliferating cell nuclear antigen, cyclin-dependent kinases (cdk 2, 4 and 6), cyclins (D1 and D2), the mitogen-activated protein kinase kinase (MAPKK) and mitogen-activated protein kinase (MAPK) in SKH-1 hairless mice.[85] In in vitro studies, the treatment of HaCaT cells with resveratrol inhibits UVB-induced activation of NF-κB in a dose and time dependent manner.[86] Resveratrol treatment also resulted in significant inhibition of UVB-induced increases in cellular proliferations, survivin (a biomarker of tumor promotion) and phosphorylation of survivin.[84] To corroborate these findings, treatment of resveratrol reversed the UVB-mediated decrease in levels of Smac/DIABLO, a promoter of caspase-9 activation, in mouse skin.[84] These results demonstrate the usefulness of resveratrol against solar UV-induced damage in the skin.

Silymarin

Silymarin, a flavonoid, extracted from the seeds of milk thistle or Mary thistle (Silybum marianum L. Gaertn), is composed of mainly silibinin (∼90%) with small amounts of other silibinin stereoisomers (i.e. silydianin, isosilybin and silychristin).[87] Silymarin has been shown to possess antiinflammatory, antioxidative and anticarcinogenic properties in in vivo animal models.[88] For the first time, Katiyar et al.[18] showed that topical application of silymarin to SKH-1 hairless mice inhibits UVB-induced skin carcinogenesis in terms of tumor incidence (75%), tumor multiplicity (90%) and tumor volume/mouse by 97%. Topical application of silymarin also provides substantial protection against UVB induced DNA damage in mouse skin.[89] In short-term experiments, silymarin treatment inhibited UVB-induced sunburn cell formation, edema, apoptotic cell death, COX and ornithine decarboxylase activities and ornithine decarboxylase mRNA expression.[18,89] We showed that topical treatment of silymarin to C3H/HeN mice inhibits UVB-induced suppression of contact hypersensitivity response/immune responses to a contact sensitizer, dinitrofluorobenzene.[90] UV-induced suppression of immune response has been considered as a risk factor for nonmelanoma skin cancer.[26,29] UVB-induced suppression of immune responses is also associated with the infiltration of leukocytes, particularly CD11b+ cells, at the UVB irradiated site. Interestingly, topical treatment of silymarin inhibited UVB-induced infiltration of CD11b+ cell type and myeloperoxidase activity in the skin.[90] Additionally, reversal of UVB-induced immunosuppression by silymarin was also associated with the significant reduction in immunosuppressive cytokine interleukin-10 at UV irradiated skin sites and draining lymph nodes. In the same animal model, treatment of silymarin inhibited UVB-induced several biomarkers of oxidative stress, such as expression of inducible nitric oxide synthase, and the production of H_2O_2 and nitric oxide.[90] Silymarin inhibits UVB-induced activation of the transcription factors, nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) in HaCaT keratinocytes.[91] NF-κB is a pleotropic transcription factor involved in the transcription of genes related to inflammation and cancer while the AP-1 is a dimer composed of proteins from the fos and jun families and is inducible by UV radiation via ras pathway. These results indicate that silymarin can efficiently modulate the cellular response to UV through their selective action on NF-κB activation.

Series of chemopreventive studies were also conducted and repeated with silibinin, a major component of silymarin, in in vitro and in vivo systems, and it was found that photoprotective effects of silibinin and silymarin are similar.[88] In vivo studies with SKH-1 hairless have shown that topical application of silibinin, before or immediately after UVB exposure or its dietary feeding resulted in protection against photocarcinogenesis in terms of tumor multiplicity, tumor volume per mouse following cell cycle regulatory mechanisms and MAPK pathways.[92] Silibinin inhibits UV-induced thymine dimer formation in mouse skin which is a marker of UV induced DNA damage and initiation of photocarcinogenesis, and inhibits UV induced apoptosis and levels of proliferating cell nuclear antigen.[93] During the cell cycle progression, silibinin decreases the levels of cyclin-dependent kinase 2 and cyclin-dependent kinase 4 and associated cyclins A, E and D1, together with an up-regulation of Cip1/p21 and p53 in UV exposed skin.[92,93]

Tea/Green tea

Tea (*Camellia sinensis*) is consumed worldwide as a beverage because of its characteristic aroma, flavor and health benefits.[94] Of the total commercial tea production worldwide, about 80% is consumed in the form of black tea, mainly in western countries and some Asian countries, and 20% in the form of green tea. Green tea is primarily consumed in some Asian countries such as Japan, China, Korea, parts of India and a few countries in North Africa and the Middle East.[46] Oolong tea, a partially fermented tea, is consumed in some parts of South-eastern China.[46] The basic steps of manufacturing different tea varieties are more or less similar except to protect and develop their aroma during fermentation process, which also controls the oxidation status of the individual catechin/epicatechin derivatives present in fresh tea leaves.[94] The characteristic aroma and health benefits of green tea are associated with the presence of catechin/epicatechin derivatives, which are commonly termed as 'polyphenols'. The major polyphenolic constituents present in green tea are (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin-3-gallate and (−)-epigallocatechin-3-gallate (EGCG).[46,95] In addition to small amount of catechins, black tea contains thearubigins and theaflavins which are the polymerized forms of catechin monomers and are the major component formed during enzymatic oxidation and the fermentation process.[95] Experimental evidences indicate that the polyphenols present in green tea are better chemopreventive agents than the polyphenols present in black tea. Therefore, most of the chemopreventive investigations have been conducted with the green tea polyphenols. The details of the antiphotocarcinogenic effect and mechanism of actions of green tea polyphenols are discussed below:

Several *in vitro* and *in vivo* animal models have been used to examine the anticarcinogenic effects of green tea. Subsequently, it has been found that oral administration of polyphenolic fractions isolated from green tea or green tea polyphenols (GTPs) to laboratory animals resulted in significant protection against UV-induced skin carcinogenesis in terms of tumor incidence, tumor multiplicity and tumor size compared to those animals which were not given GTPs in drinking water (reviewed in refs. 46, 95 and 96). The animals which were given a crude water extract of green tea as a sole source of drinking water and UVB irradiated following UVB radiation-induced tumor initiation and tumor promotion protocols had developed a lesser number of tumors compared to those mice which were not given water extract of green tea.[97,98] Administration of GTPs in drinking water or topical application of EGCG also induced partial regression or inhibition of tumor growth of established skin papillomas (46–89% inhibition) in mice.[99] Oral administration or topical application of GTPs and EGCG did not show any sign of visible toxicity. The effects of green tea or black tea, or decaffeinated black or green tea on UV-induced skin carcinogenesis have been studied in detail.[100–103] Oral administration of green tea or black tea (1.25%, w/v) as the sole source of drinking water for 18–23 weeks resulted in a decreased number of papillomas (>80%), keratoacanthomas (53%) and SCC (32%) induced by UVB irradiation (60 mJ cm^{-2}) for 30 weeks. Topical application of EGCG on mouse skin also inhibits photocarcinogenesis.[104] Our laboratory developed a hydrophilic ointment based formulation for the topical treatment of green tea polyphenols.[105] Topical treatment of GTPs or EGCG in hydrophilic ointment to SKH-1 hairless mice resulted in exceptionally high protection from photocarcinogenesis which was not observed before without this kind of topical formulation. The protection was determined in terms of tumor incidence (60% inhibition), tumor multiplicity (86% inhibition) and tumor growth in terms of total tumor volume per group (95% inhibition).[105] These results indicated that the use of EGCG or GTPs with hydrophilic ointment might increase their penetration or absorption capacity inside the skin layers and the presence of higher concentration may be responsible for the higher protection. Treatment of EGCG also increased the latency period of tumor appearance by several weeks during complete photocarcinogenesis (tumor initiation + tumor promotion) protocol. Record and Dreosti[106] have shown that green tea can protect against both UVA and UVB radiation-induced skin cancer in mice. Experimental studies conducted in *in vitro* and *in vivo* models indicate that GTPs or EGCG prevent photocarcinogenesis following several mechanisms which involve various molecular targets. Some of these are summarized below:

Solar UV radiation-induced inflammatory responses which include erythema, edema and hyperplasia are considered to play a crucial role in skin tumor promotion.[3] Oral feeding of GTPs in drinking water to SKH-1 hairless mice protects from UV-induced cutaneous edema, erythema, and depletion of antioxidant-defense enzymes, such as glutathione peroxidase, catalase and endogenous antioxidant glutathione in the epidermis.[5,107] Treatment of GTPs also inhibits UVB-induced expression of cycloxygenase-2 enzyme and the production of its prostaglandin metabolites, which have been implicated in skin carcinogenesis.[108] UVB-induced increase in cyclooxygenase-2 expression was abrogated both in murine and human nonmelanoma skin cancer models by topical application of GTPs.[108] Topical treatment of GTPs to mouse skin before UV exposure decreased UV-induced myeloperoxidase activity and number of infiltrating inflammatory leukocytes in the skin.[23,109] Studies from our laboratory showed that topical application of GTPs before UV irradiation on the backs of human individuals resulted in significantly reduced erythema development as compared to those individuals who did not receive GTPs treatment.[110,111] Topical treatment of GTPs or EGCG (<1 mg cm^{-2} skin area) to human skin also reduced UVB-induced erythema,[110] myeloperoxidase activity and infiltration of inflammatory leukocytes.[110] Increase in myeloperoxidase activity is considered as a marker of tissue infiltration. In the same study, EGCG treatment inhibited UVB-induced production of prostaglandin metabolites, such as PGE$_2$, PGF$_{2α}$ and PGD$_2$, which contribute in inflammatory disorders and in tumor promotion. Zhao *et al.*[112] demonstrated that oral administration of green tea extract prevents multiple treatments of psoralen plus UVA-induced erythema, edema, hyperplasia and hyperkeratosis in mouse skin. Treatment of green tea extract to EpiDerm, a reconstituted human skin equivalent, also inhibited psoralen plus UVA-induced 8-methoxypsoralen–DNA adduct formation and p53 protein accumulation.[112] These *in vitro* and *in vivo* observations provide possible mechanisms of photoprotective effect of green tea polyphenols.

Solar UVB radiations (290–320 nm) are absorbed by the skin and result in inflammation and oxidative stress, which may lead to the initiation of skin cancer.[113–115] Though, the skin possesses an antioxidant defense system to deal with UV-induced oxidative

stress, however, excessive and chronic exposure to UV radiation can overwhelm the cutaneous antioxidant defense capacity and result in skin damage, disorders, immune suppression and premature aging of the skin. Our laboratory has reported that *in vitro* treatment of epicatechin derivatives to mouse epidermal microsomes inhibits photo-induced lipid peroxidation.[116] Topical application of EGCG before UV exposure to mouse and human skin reduces UVB-induced nitric oxide and hydrogen peroxide production.[109–111] The infiltrating leukocytes are the major source of oxidative stress, and it has been shown that UV-induced infiltrating cells enhanced the tumor growth in UV-irradiated skin.[117] EGCG treatment inhibits UVB (4× MED)-induced leukocyte infiltration in mouse as well as in human skin, thus decreases UVB-induced oxidative stress in the skin.[24,109–111] Additionally in human skin, EGCG treatment also inhibited UV-induced epidermal lipid peroxidation and protected antioxidant defense enzymes.[24] Inhibition of UV-induced lipid peroxidation by EGCG is a characteristic feature, which may prevent human skin from solar UV light-induced basal cell and squamous cell carcinoma and premature aging of the skin. Kim *et al.*[118] observed that EGCG treatment to guinea pig skin inhibits UVB-induced lipid peroxidation and skin photodamage. This study also reveals that treatment of human fibroblasts in culture with EGCG blocked the UV-induced increase of collagen secretion and collagenase mRNA level, and also inhibited UV-induced nuclear transcription factors NF-κB and AP-1 binding activities.[118] These investigations suggest that green tea has a potential to decrease UV-induced oxidative stress and oxidative stress-mediated skin diseases in humans.

Human epidermal keratinocytes were also used to evaluate the chemopreventive effect of EGCG against UVB-induced oxidative stress-mediated cell signaling events which contribute in the tumor promotion stage of photocarcinogenesis. In these studies, treatment of EGCG to normal human epidermal keratinocytes was found to inhibit UVB-induced intracellular release of hydrogen peroxide concomitant with the inhibition of phosphorylation of cell signaling proteins, such as epidermal growth factor receptor and ERK1/2, JNK and p38 proteins of the mitogen-activated protein kinase family.[119] The inhibition of phosphorylation of these proteins indicates that EGCG has the ability to inhibit oxidative stress-mediated cellular signaling responses, which are essentially involved in various skin disorders. The treatment of EGCG or GTPs with topical formulation resulted in an exceptionally high photoprotective effect against short term and long-term adverse biological effects of UV radiation. Topical EGCG and GTPs treatment protect against acute and chronic UVB radiation-induced depletion of endogenous antioxidant defense enzymes, glutathione peroxidase, catalase and the level of glutathione content.[5] A photoprotective effect on these antioxidant defense enzymes was also observed when GTPs were given in drinking water to animals. Oxidation of some amino acids such as lysine, arginine and proline leads to the formation of carbonyl derivatives that affects the nature and function of native protein molecules.[120] The presence of carbonyl groups in protein molecules is a measure of oxidative damage of proteins under conditions of oxidative stress. Chronic exposure of UV radiation to the mouse skin resulted in a several-fold increase in the level of protein carbonyls in comparison to mice which were not exposed to UVB radiation. Topical treatment of EGCG or GTPs inhibits acute or chronic

UVB radiation-induced protein oxidation in mice.[5] Additionally, oral administration of GTPs in drinking water prevents chronic UVB irradiation-induced protein oxidation as well as expression of matrix metalloproteinases in mouse skin which may contribute to the prevention of photocarcinogenesis.[121] The inhibition of UVB-induced protein oxidation may also contribute in the prevention of premature aging of the skin.

Solar UV radiation also damages DNA molecules and thus initiates carcinogenesis. The exposure of skin to UV radiation results in formation of cyclobutane pyrimidine dimers (CPD).[122] Most of the UV-induced CPDs were found in the epidermis but a significant number of CPDs were also observed in the dermis.[122] The presence of CPD in the dermal layer of the skin indicates the penetrating depth of UV radiation inside the skin. UVA (320–400 nm) radiation has the ability to penetrate inside the dermal compartment whereas maximum fraction of UVB (290–320 nm) spectrum is absorbed by the epidermal layers. Therefore, the penetration of UV light inside the skin is reflected by the distribution pattern of CPD in the skin. In previous studies, we reported that UV exposure at less than one minimal erythema dose is sufficient to damage DNA in the skin.[122] Higher UV doses induced severe DNA damage in the skin when determined in the form of CPD. Topical treatment of GTPs to human skin resulted in a dose-dependent inhibition of CPD formation at the UVB irradiated skin site.[123] Pharmacokinetic studies reveal that UV-induced DNA damage in the form of CPD in human skin declines after three to four days of UV exposure. This may occur because cells with damaged DNA undergo apoptosis or the damaged DNA has been repaired. Histological observations of CPD indicate that topical treatment of GTPs to human skin resulted in reduction of UV-induced DNA damage compared to those skin sites which were not treated with GTPs.[123] CPDs have been implicated in UV-induced immune suppression and initiation of carcinogenesis.[124] The inhibition of UV-induced CPD formation by GTPs may represent a possible mechanism of prevention of UV-induced immune suppression and photocarcinogenesis. Wei *et al.*[125] have shown that water extract of green tea scavenges H_2O_2 and inhibits UV-induced oxidative DNA damage in an *in vitro* system. Zhao *et al.*[112] demonstrated that application of green tea extract to a reconstituted human skin equivalent also inhibited psoralen-UVA-8-methoxypsoralen–DNA adducts formation.

It has been recognized that UVB exposure to laboratory animals suppresses the immune system,[16,29,126] and alterations in immunologic responses have been considered as a risk factor for the induction of skin cancer.[16,27,29] Katiyar *et al.*[127] have shown that topical application of GTPs on C3H/HeN mouse skin results in protection from UVB-induced suppression of immune responses both in local and systemic models of contact hypersensitivity (CHS). Topical treatment of EGCG followed by a single exposure of UVB irradiation to C3H/HeN mice almost completely prevented UVB-induced suppression of CHS to sensitizer, 2,4-dinitrofluorobenzene, and partially inhibited tolerance induction.[109] Several mechanisms have been proposed to be involved in UV-induced immune suppression. Some studies defined the role of IL-12 in the induction and elicitation of CHS. CHS appears to be a Th1-mediated immune response.[128] Epidermal Langerhans cells, which are critical antigen presenting cells in the induction phase of CHS, have been described

as a source for IL-12 production.[129] We were interested to determine whether green tea has immunomodulatory effects in UVB irradiated skin. Since UVB-induced infiltrating leukocytes (CD11b+ cells) play a crucial role in UVB-induced suppression of immune responses, we investigated the effect of EGCG on UVB-induced infiltration of CD11b+ cells, and it was found that treatment of EGCG results in prevention of UVB-induced immune suppression and tolerance induction which was associated with reduction in the number of infiltrating CD11b+ cells at UVB-irradiated sites.[109] Hammerberg *et al.*[130,131] demonstrated that blocking of infiltrating leukocytes using anti-CD11b antibody or treatment with soluble complement receptor type-1 blocked UV-induced immune suppression and tolerance induction in C3H/HeN mice. These results indicate that UV-induced infiltrating CD11b+ cells are critical in suppressing the CHS response. EGCG treatment also inhibits the secretion of immunosuppressive cytokine IL-10 at UV irradiated sites as well as in draining lymph nodes compared to those mice which were not treated with EGCG but exposed to UVB radiation.[109] This observation suggests a possible mechanism by which EGCG prevents UVB-induced suppression of the immune system in mice.[109] Simultaneously, IL-12 is another immunoregulatory cytokine and augments the immune responses and possesses antitumor activity. In the same study, application of EGCG in combination with UVB synergistically increased IL-12 production in regional lymph nodes.[109] Enhanced IL-12 may contribute to protect the immune system and enhance the antitumor activity in *in vivo* systems. It has been shown that treatment of EGCG inhibits UVB-induced apoptosis in normal human keratinocytes, and significantly induced IL-12 production by monocytes.[132] Findings from this study speculated that EGCG might reduce UVB-induced apoptosis *via* induction of IL-12 and subsequent up-regulation of DNA repair enzymes or mechanism. Thus, it can be another photoprotective mechanism by which EGCG inhibits photocarcinogenesis in animals.

Conclusion

The dietary botanicals discussed in this review article possess striking antioxidant and antiinflammatory properties through which they contribute to their antiphotocarcinogenic effects and to abrogate various biochemical processes induced or mediated by the solar UV radiation. Based on laboratory and epidemiological evidences we suggest that routine consumption of these dietary botanicals may provide efficient protection against harmful effects of solar ultraviolet radiation. Moreover, these botanicals in combination with sunscreens or skin care lotions may provide an effective strategy for reducing nonmelanoma and melanoma skin cancers and other skin disorders caused by excessive UV exposure.

Acknowledgements

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