ABSTRACT: UV radiation is a very potent initiator of photochemical reactions. In biological systems, the absorbed light can interact with endogenous molecules (lipids, proteins and DNA) so producing, directly or indirectly, deleterious cytotoxic and genotoxic effects. Chronic exposure of mammalian skin to UV radiation induces a number of biological responses (including edema, hyperplasia, immune suppression, DNA damage, photoaging, etc.), which may be involved in the development of skin cancer. Thus, it is imperative to protect the skin from UV light-induced damage. Recent researches have been carried out to investigate the scientific basis for photoprotection by naturally occurring antioxidants to be topically or systemically employed for minimizing the harmful effect of sun exposure. Several plant compounds have gained considerable attention as chemopreventers effective against skin cancer. In particular, flavonoids represent an interesting class of active compounds in the protection of UV light-induced skin damage due to their wide spectrum of activities including anti-inflammatory, antioxidant, anti-mutagenic, anti-carcinogenic and capability to modulate enzyme activities involved in cell response. Herein the state of art of flavonoids in the protection against UV-induced skin cancer will be reviewed, with particular regard for issues relating to their topical application or oral administration.

KEY WORDS: Botanical antioxidant, Chemoprevention, Flavonoid, Skin cancer, UV.

Corresponding Author: Prof. Francesco Cimino, University of Messina, Department Farmaco-Biologico, School of pharmacy, Viale Annunziata, 98168 Messina, Italy; Fax: +39-090-3533142; E-mail: fcimino@pharma.unime.it

FLAVONOIDS

Flavonoids consist of a large group of benzo-γ-pyrone derivatives endowed with diverse chemical structures. This class of compounds includes low-molecular weight polyphenols and high-molecular weight polyphenols; the latter ones, known as tannins, are polymers of catechines or epicatechines (condensed tannins) and polymers of gallic or ellagic acids (hydrolysable tannins) (King and Young, 1999). Flavonoids exhibit a wide variety of beneficial biological activities in mammals, including antiviral, antibacterial, immune-stimulating, anti-allergic, anti-hypertensive, anti-ischemic, anti-arthritic, anti-thrombotic, hypocholesterolemic, anti-lipperoxidant, hepatoprotective, anti-inflammatory, and anti-carcinogenic actions. They are powerful antioxidants, due to their capability to scavenge reactive oxygen species (ROS), reactive nitrogen species (RNS) and free radicals (such as superoxide anions, singlet oxygen, hydroxyl radicals, and lipid peroxyl radicals) (Rice-Evans et al., 2000; Torel and Cillard, 1986), to chelate transition metal ions (such as iron and copper, which play a key role in triggering oxidative stress reactions) (Van Acker et al., 1998), and to inhibit the activities of several enzymes (including lipoxygenase, cyclooxygenase, monoxygenase, xantinoxidase, mitochondrial succinate dehydrogenase and NADH-oxidase, phospholipase A2 and protein kinases) (Cao et al., 1997). Furthermore flavonoids have proven to be able of acting in redox-sensitive signalling cascades to inhibit DNA damage (Rice-Evans et al., 2000). Interestingly, many flavonoids such as quercetin, luteolin and catechins are better antioxidants than the well-known antioxidant nutrients vitamin C, vitamin E and β-carotene (Cao et al., 1997).

Therefore, flavonoids may be beneficial in preventing and treating pathological and physiopathological conditions related to oxidative stress. Since overexposure to ultraviolet (UV) radiation from sun can induce generation of free radical, oxidation of cellular macromolecules and decrease of endogenous antioxidants in the skin, so inducing pathological states such as photoaging and skin cancer, flavonoids may provide protection against cutaneous photodamage.

However, several reports indicate the necessity to deeply investigate possible toxic effects of flavonoids before hypothesizing or approving their use for human health protection (Morton et al., 2000). In fact some flavonoids have also been found in vitro to be mutagenic; such a harmful effect is very likely due to the prooxidant action (rather than antioxidant properties) of these compounds.

Flavonoids and protection against UV light in plants

Higher plants are naturally exposed to solar radiation and therefore they are subjected to relatively high dose of UV
radiation. UV-B radiation (which comprises about 5% of the whole UV light) can induce severe damage to plants via direct and indirect effects on cellular macromolecules and structures. In particular the photosynthetic apparatus of higher plants is very susceptible to UV-B induced damage. The concept of UV resistance in plants would explain the ability of plants to adapt to increasing amounts of UV that might reach the ground. As a defence against UV-irradiation, higher plants have developed a number of protective mechanisms, including the capability to absorb UV radiation by plant superficial layers. In fact accumulation of phenolic compounds selectively absorbing UV radiation in cuticle and epidermis represents a very efficacious strategy to protect plants against continuous exposure to high doses of sunlight (Harborne and Williams, 2000). One well-known example is represented by apple fruit, whose skin contains large amounts of phenolic substances and especially flavonoids (Solovchenko and Schmitz-Eiberger, 2003).

Flavonoids, which are almost universally present in green leaves, generally absorb in the 280-315 nm region and thus are capable of acting as UV filters, thereby protecting the underlying photosynthetic tissues from damage. The flavonoids most frequently cited as being UV-protective are flavone or flavonol synthesis in epidermal cells and occasionally also in epicuticular waxes. Furthermore, the exposure to high sunlight generally induces a marked increase in metabolism of flavonoids, but not of phenolic acid (Awad and De Jager, 2000).

UV DAMAGE AND SKIN CANCER
UV radiation and the skin
UV radiation forms a part of the electromagnetic spectrum with wavelengths between 200 nm and 400 nm. It is divided into three categories with different wavelengths: UVC (200-290 nm), UVB (290-320 nm) and UVA (320-400 nm). UV light is constituted by electromagnetic radiation, occurring in the form of continuous waves or as a series of photons. Photons of UV light are highly energetic and can initiate photochemical reactions in biomolecules (Freeman et al, 1989). Absorbing the energy of the photons by the biological molecules changes the distribution of electrons in the molecule and creates the excited singlet state. The molecule in the excited singlet state can emit fluorescence, lose energy as heat (internal conversion), form photoproducts, or change into a triplet-excited state (Hussein, 2005). In this last state, the molecule emits phosphorescence, photochemically reacts or returns to the ground state (Kanoisky and Sima, 1991). Human skin is exposed daily to UV radiation from sun; because situated at the interface between the body and its environment, it directly suffers from the deleterious effects of UV radiation.

Below UVC wavelength value, all radiations from the sun are absorbed from ozone layer. Between 1970 and 1987, substantial damage to the protective ozone layer resulted in an increased amount of UV radiation reaching the earth’s surface. UVC radiations are completely absorbed by the earth’s atmosphere and thus, although highly mutagenic and toxic to the cells, are not biologically relevant. UVB radiation is only partly absorbed by the atmospheric ozone layer, and UVA radiation is not absorbed at all (De Laat et al., 1996). Both UVC and UVB are absorbed by the proteins and nucleic acids and cause skin erythema on moderate exposure, while UVA is not strongly absorbed by the proteins and nucleic acids and does not produce erythema on moderate exposure (Hussein, 2005).

Also at the skin level, UV photons should be transmitted through the skin layers, absorbed by the biomolecules, and initiate series of biological reactions. The effects of UV light on the skin are dependent upon the epidermal thickness, the radiation dose and the distribution of UV absorbing biomolecules (called chromophores), such as melanin, DNA, amino acids, carotenes, and urocanic acids (Anderson and Parrish, 1981). In the skin, DNA is the most critical chromophore for UVB-induced biological response. Furthermore, large amounts of UVB radiation are absorbed by aromatic amino acids in the stratum corneum before reaching DNA molecules in the underlying viable cells (Kiss et al., 1991).
More than 90% of solar radiation that reaches us is UVA. This longer wavelength penetrates deep into the epidermis and dermis of the skin. Compared to UVB, UVA is about 1000 times more effective in the production of an immediate tanning effect, which is caused by darkening of the melanin in the epidermis. Intense or extensive exposure to UVA can burn sensitive skin, and if prolonged, it can cause premature photoaging of the skin, impair the immune system, and lead to cancer (Trautinger, 2001). UVA-induced responses in cells happen mainly because of oxidative processes initiated by ROS, which can cause damage to cellular macromolecules (Afaq and Mukhtar, 2002).

UVB is a minor (making up 4 to 5 % of UV light) but the most active constituent of solar light. UVB is 1000 times more capable of causing sunburn than UVA, and is also more genotoxic than UVA. UVB acts mainly in the epidermal basal cell layer of the skin, inducing direct and indirect adverse biological effects, in particular formation of pyrimidine photoproducts, isomerization of trans- to cis- urocanic acid, induction of ornithine decarboxylase activity, stimulation of DNA synthesis, free radical production, cell cycle growth arrest, photoaging, photocarcinogenesis, immunodepression, and impairment of skin antioxidant pool (De Grujić, 2002).

**Acute effects of UV irradiation**

Acute UVB irradiation induces primarily cyclobutane-type pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts, which are formed exclusively in runs of tandemly located pyrimidine residues, and thus gives rise predominantly to C→T and CC→TT transitions at the dipyrimidine sequences (now recognized as the hallmark, or hot spots, of UV-induced mutagenesis) (Mitchell and Nairn, 1989).

Numerous studies have shown that acute UV irradiation activates the p53 protein. The p53 tumor suppressor gene codes for a 53-kDa phosphoprotein that modulates the cell cycle via transcriptional control of regulatory genes (Vogelstein and Kinzler, 1992). High levels of this protein lead to cell cycle arrest, allowing DNA repair before cellular mitosis; furthermore, they induce apoptosis in cells with excessive DNA damage, in particular directly upregulating the expression of proapoptotic genes, that trigger cytochrome c release from mitochondria (Harris, 1996).

Thus, resistance to cell death due to acquired p53 mutations is a key event in photocarcinogenesis and appears to be an early genetic event in the development of UV-induced skin cancers. Mutations in the p53 gene have been detected in 50% of all human cancers and in almost all skin carcinomas (Melnikova and Ananthaswamy, 2005). Conversely, elimination of cells containing excessive UV-induced DNA damage is a key step in protecting against skin cancer development. Simultaneously, acute UV irradiation triggers cell survival and proliferation mechanisms, as evident in epidermal hyperplasia, by activating receptors to various growth factors (e.g. erbB receptors and epidermal growth factor receptors [EGFR]) and cytokines (Bender et al., 1997).

**Chronic UV irradiation and skin cancer**

UV radiation is considered as the most prevalent environmental carcinogen acting as tumor initiator, tumor promoter and as a complete carcinogen. The incidence of skin cancer has been increasing dramatically, and this increase is expected to continue as the population ages and larger amounts of UV radiation reach the surface of the Earth because of depletion of the ozone layer. Furthermore changes in lifestyle over the past several decades have allowed individuals to spend outdoors much greater amounts of time for recreational activities. As a result there has been alarming increase in the incidence of solar UV radiation induced skin disorders. In fact, while acute UV overexposure causes sunburn, more chronic exposure of UV can lead to skin photoaging and immune suppression, basal and squamous cell carcinoma and melanoma (Mukhtar and Elmets, 1996) (Figure 1).

Studies have shown that UV radiation to the skin results in the formation of ROS that interact with proteins, lipids and DNA so altering cellular functions. The epidermis is composed mainly of keratinocytes, which are rich in ROS detoxifying enzymes, such as superoxide dismutase, catalase, thioredoxin reductase, and glutathione peroxidase, and in low-molecular-mass antioxidant molecules, such as tocopherol, glutathione and ascorbic acid, and thus provides some natural protection against ROS (Afaq and Mukhtar, 2001). Skin spontaneously responds to increased ROS levels; however, this response may not be sufficient to prevent the progression of skin cancer, because the increased generation of ROS can overwhelm antioxidant defences. Furthermore, oxidative stress elicited by UV irradiation activates redox sensitive transcription factors, including nuclear factor kappa B (NF-kB), and members of the activator protein-1 (AP-1) complex, such as c-fos and c-jun (Salìou et al., 1999; Melnikova and Ananthaswamy, 2005).

The development of skin cancer is a complex multistage phenomenon involving three-distinct steps: initiation, promotion and progression; each of these stages is mediated via various cellular, biochemical, and molecular mechanisms (Hussein, 2005). Initiation is essentially an irreversible step in which genetic alterations occur in epidermis basal stem cells. UVB irradiation to the skin has direct effects on biomolecules; for example, the formation of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photodimers, photoisomerization of trans- to cis- urocanic acid, DNA strand break, DNA crosslinks, DNA-protein crosslinks and generation of ROS. Mammalian cells have efficient mechanisms to preserve genomic stability; furthermore, if the damage is too severe after a high dose of UV irradiation, cells have a mechanism to trigger apoptosis or programmed cell death to prevent the propagation of the damaged DNA. However, formation of cyclobutane pyrimidine dimers, if not repaired through nucleotide excision repair, leads to signature mutations.

Tumor promotion, related to alterations in signal transduction pathways, is characterized by clonal expansion of initiated cells leading to pre-malignant and malignant lesions. During the third step, or tumor progression, pre-malignant
and malignant lesions are converted into invasive and metastatic malignant tumor. Main biochemical mechanisms involved in skin cancer development include: stimulation of DNA synthesis and DNA damage, depletion of antioxidant defences, impairment of signal transduction pathways, cell cycle dysregulation, induction of cyclooxygenase (COX) and increase in prostaglandin synthesis, inflammation, induction of ornithine decarboxylase (ODC), immunosuppression, cell proliferation and epidermal hyperplasia (Matsumura and Ananthaswamy, 2004).

Chronic UV irradiation results in dysregulation of apoptosis leading to abnormal proliferation of keratinocytes containing DNA damage, acquisition of p53 mutations and loss of Fas-Fas ligand interaction, all events contributing to the onset of skin cancer. Long-term exposure to UV irradiation significantly changes the expression of the Fas receptor and its ligand in the epidermis, resulting in induction and accumulation of more p53 mutations, dramatic decrease in apoptotic cells and suppression of host immunity (Melnikova and Ananthaswamy, 2005).

Several studies have demonstrated that in some kinds of skin cancer increased expression of cyclin D1 occurs and may contribute to the tumor phenotype even in the presence of elevated p53 levels; on the contrary decreased expression of cyclin D1 leads to reduce skin carcinogenesis (Afaq et al., 2005a).

Recent studies have shown that UVB irradiation activates Akt pathway in primary human keratinocytes. Akt, a serine/threonine kinase also known as protein kinase B, acts as a key protein mediator for a wide range of cellular processes and may be activated by various stimuli following phosphorylation of both Ser473 and Thr308 residues through a phosphatidylinositol 3-kinase (PI3K)-dependent mechanism (Nicholson and Anderson, 2002). Activation of the Akt signalling pathway reduces apoptosis in many cell types. UVB radiation results in the activation of EGFR that triggers the phosphorylation of the protein kinase B (PKB/Akt). Furthermore UV radiation generates H$_2$O$_2$ that in turn phosphorylates Akt. Finally, Akt blocks UVB-induced apoptosis in human keratinocytes by blocking mitochondrial cytochrome-c release and preventing caspase activation (Nicholson and Anderson, 2002).

NF-kB is a ubiquitously expressed transcription factor belonging to the Rel family; it regulates genes involved in inflammation, immunity, cell cycle progression, apoptosis, and oncogenesis and can be activated by a wide range of stimuli including UV light. These stimuli activate directly or indirectly IKB kinases, which on turn phosphorylate the inhibitory kB proteins for degradation by proteasomes; the released heterodimer then moves from the cytoplasm to the nucleus, where both p50 and p65 contribute to NF-kB DNA binding and stimulate transcription of target genes (Yamamoto and Gaynor, 2004).

The transcription factor AP-1 is a protein dimer that consists of either heterodimers between fos and jun family gene products or homodimers of jun family gene. AP-1 proteins regulate the expression and function of a number of cell cycle regulatory proteins. Several studies have demonstrated that UVB irradiation produces strong induction of c-jun and c-fos transcripts and AP-1 activation in several cells including human primary keratinocytes (Afaq et al., 2005a).

Mitogen-activated protein kinases include a large number of serine/threonine kinases involved in regulating a wide array of cellular processes. They are divided into three multimember subfamilies: the extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases (JNK), and the p38 kinases, all activated in response to oxidant injury. Interestingly, both p38 and ERK are required for UVB induced c-fos expression in human keratinocytes, and their inhibition may influence basal and UV-induced c-fos transcription (Afaq et al., 2005a).

UV irradiation of animal and human skin can induce expression of COX-2, the cyclooxygenase primarily considered as an inducible immediate-early gene product. In fact prostaglandin production in the skin following UV irradiation occurs as a result of an increased release of arachidonic acid by phospholipases and also of the induction of COX-2. Elevated levels of prostaglandin E2 (PGE2) have been linked with the
carcinogenic process by contributing to the uncontrolled proliferation of damaged cells. COX-2 overexpression and elevated PGE2 levels have been observed in both pre-malignant and cancerous skin lesions. In addition, levels of COX-2 activity seem to be predictive of invasive potential and seriousness of skin tumors (Einspahr et al., 2002).

Enzymatic decarboxylation of the dibasic amino acid, ornithine, by ODC is the first and generally regarded as the rate-limiting step in the biosynthesis of the polyamines: putrescine, spermidine, and spermin. Aberrations in ODC regulation and subsequent polyamine accumulation appear to be associated with neoplastic transformation (Afaq et al., 2005a).

UV exposure has been shown to suppress a wide variety of immune reactions (Ullrich, 2005), and several studies support the hypothesis that UV exposure-induced immune suppression plays a critical role in skin cancer induction. The dendritic cell network constituted by Langerhans cells in the epidermis functions to capture and ingest microorganisms and to process their antigens; matured Langerhans cells arrive at the draining lymph nodes and present the microbial antigen to T cells. UV-exposure alters the function of Langerhans cells; being UV-induced DNA damage in Langerhans cells the initiating event, which leads to local immune suppression (Ullrich, 2005). In fact cyclobutane pyrimidine dimers are primarily produced in keratinocytes and Langerhans cells following UVB exposure, and also in dendritic cells in lymph nodes draining the irradiated sites. Multiple mechanisms are involved in the generation of immune suppression and tolerance by UV-irradiated Langerhans cells (Simon et al., 1990). There are two types of CD4+ and CD8+ T cells, which may be distinguished for the immune reactions they participate in and for the types of cytokines they secrete. Type 1 cells (Th1 cells) secrete gamma-interferon (IFN-γ) but not IL-4, and are generally associated with inflammatory immune reactions; type 2 cells (Th2 cells) produce IL-4 and not IFN-γ, and are generally associated with allergic immune reactions. Normal Langerhans cells present equally well to Th1 or Th2 cells, but UV-irradiated Langerhans cells efficiently present antigen only to Th2 cells, failing to stimulate Th1 cell proliferation and rendering the Th1 cells tolerant.

CHEMOPREVENTION OF SKIN CANCER

Primary prevention of UV-related cutaneous disease and in particular of skin cancer (that is a significant problem associated with mortality and morbidity), is focused on education about the harmful effects of UV radiation present in the sunlight, the need to avoid its excessive exposure by wearing protective clothing, and the use of sunscreens; for many reasons these primary prevention approaches have had limited success. Therefore, additional efforts are needed to prevent serious pathological consequences of UV exposure.

One approach for controlling the occurrence of skin cancer is through chemoprevention, that is a means of cancer control in which the occurrence of the disease can be entirely prevented, slowed or reversed by topical or oral administration of naturally occurring compounds (Afaq et al., 2002; Lambert et al., 2005; Sies and Stahl, 2004). Chemopreventive agents are known to be anti-mutagenic, anticarcinogenic and non-toxic, have the ability to exert striking inhibitory effects on diverse cellular events associated with multistage carcinogenesis, and can be targeted for intervention at the initiation, promotion, or progression stage of the multistage carcinogenesis process. Also chemoprevention of skin cancer is based especially on naturally occurring novel mechanism-based effective antitumor-promoting agents (Afaq et al., 2002).

One of the excitements of chemoprevention is that agents can be targeted for intervention at the initiation, promotion, or progression stage of the multistage carcinogenesis process. However, strategies to prevent initiation process appear to be less appropriate and practical than interventions of cancer promotion and progression stages.

The main reason is that the long latency period between the tumor initiation and promotion stages and the large number of biochemical mechanisms involved in tumor promotion step offers a large range of opportunities for intervention before cancer development. In fact, tumor initiation is a rapid and irreversible process characterized by a cascade of events modifying gene expression profiles, while tumor promotion is considered a relatively lengthy process, reversible at least in the early stages and requiring repeated and prolonged exposure to promoting agents (Afaq et al., 2005a).

The use of herbal remedies is receiving considerable interest by researchers, industry and consumers as a complementary and alternative medicine for various skin disorders, in particular for the protection of human skin from the damaging effects of external environmental stimuli, such as solar UV radiation. As demonstrated by a large number of studies carried out in vivo and in vitro by means of experimental models related to skin protection against UV-induced damage, the most important herbal drugs as regards of their skin photoprotective effects are those possessing antioxidant, antiinflammatory and immunomodulatory properties, such as vitamins E and C, green tea, garlic, ginger, silymarin, proanthocyanidins, lutein, pycnogenol, etc. (Afaq et al., 2002; Sime and Reeve, 2004). Furthermore, intake of plant-derived antioxidants has been suggested as an important preventive strategy against the toxic effects of mutagenic and carcinogenic agents. Since oxidants play an important role in several skin disorders including the initiation and promotion stages of multistage skin carcinogenesis, may be supposed that botanical antioxidants, both uptaken by dietary supplementation and topically applied on the skin, can be targeted also for intervention at the different stages of multistage skin carcinogenesis (Afaq et al., 2005a) (Figure 2). Sunscreens are widely claimed as the best strategy to reduce skin cancer risk by solar UV radiation. However, this is based on findings from animal studies, but data obtained by trials on humans, especially concerning long-term protection, are limited and often inconsistent, sometimes evidencing a limited sunscreen efficacy and also a potential to increase risks of skin damage and cancer and of systemic adverse reactions.
In fact, only few sunscreens can provide full spectral protection against ultraviolet light; furthermore sunscreen chemicals may become free radicals themselves when activated by UV light and may be absorbed through the skin. Thus the use of formulations containing both sunscreens and plant-derived antioxidants/immunomodulators may be highly beneficial for a more efficacious prevention of skin cancer.

**FLAVONOIDS AND SKIN CANCER**

**Silymarin**

Milk thistle (_Silybum marianum_), from which silymarin is isolated, belongs to the aster family (Asteraceae or Compositae) (Pepping, 1999) and has been used in folk medicine to treat disorders of the spleen, liver, and gall bladder. Silymarin consists of a mixture of 3 flavonoids present in the fruit, seeds, and leaves of the plant: silybin (silibinin), silydianin and silychristine (Wagner _et al._, 1974; Pepping, 1999), being silybin the most abundant (70%-80%) and biologically active component. Nowadays silymarin is used in humans for the treatment of liver diseases of different etiologies (Saller _et al._, 2001).

In cancer-prone SENCAR mice low doses of topically applied silymarin were shown to almost completely inhibit the effect of 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter, from inducing ODC activity (Agarwal _et al._, 1994). Topical silymarin has proven also to reduce the number and incidence of UVB light induced tumors and to inhibit UVB-induced sunburn cell formation and apoptosis in the skin of hairless mice. Furthermore silymarin appears to induce a protective effect against all the stages of UV-induced carcinogenesis (Katiyar, 2005).

Singh _et al._ (2002) have demonstrated that, also when given orally, silymarin can effectively inhibit skin tumor growth after 7,12-dimethylbenz[a]anthracene (DMBA) initiation and TPA promotion, and cause regression of established tumors. Interestingly, dietary intake of silymarin results in its distribution in several important organs of the body; in particular silybin may be detected also in skin tumor samples. More recently, Gu and co-workers (2005a) have demonstrated that dietary feeding with silybin can prevent early biomarkers of UV-induced carcinogenesis in epidermis of SKH-1 hairless mice.

The antioxidant and anti-inflammatory properties of silymarin may be the possible mechanism of its skin cancer chemopreventive effect, as demonstrated by results obtained in several skin carcinogenesis models (Afaq and Mukhtar, 2002).

Silymarin inhibits TPA-induced depletion of antioxidant enzyme activities, such as superoxide dismutase, catalase and glutathione peroxidase (Zhao _et al._, 1999), and TPA-induced lipid peroxidation in mouse skin (Chatterjee _et al._, 1999), thus supporting its _in vivo_ antioxidant activity. In skin cell systems Saliou _et al._ (1999) demonstrated that silymarin inhibits cellular signal transduction and suppresses activation of redox-regulated NF-kB following UV-irradiation; furthermore silymarin at low doses inhibits mitogenic signalling molecules, resulting in growth inhibition and apoptosis (Bhatia _et al._, 2001), but at higher doses causes apoptotic cell death (Zi and Agarwal, 1999). Interestingly, Dhanalakshmi and co-workers (2004a) demonstrated, in HaCaT keratinocytes, a

---

**Figure 2:** Possible opportunities for intervention of flavonoids in UV-induced skin cancer development.
dual efficacy of silibinin in protecting or enhancing UVB-caused apoptosis and suggested that silibinin possibly works as a UVB damage sensor to exert its biological action. The same authors (Dhanalakshmi et al., 2005) demonstrated that in mouse epithelial JB6 cells silibinin preferentially activates the DNA-PK-p53 pathway for apoptosis in response to UVB-induced DNA damage, and that this could be a predominant mechanism of silibinin efficacy against UVB-induced skin cancer. In vivo experiments on SKH-1 hairless mice have shown topical application of silibinin before or immediately after UVB exposure or its dietary feeding resulted in a strong protection against photocarcinogenesis via a decrease in thymine dimer positive cells, an up-regulation of p53-p21/Cip1, an inhibition of MAPK and AKT signalling and caspase-3 activation in epidermis (Dhanalakshmi et al., 2004b; Gu et al., 2005a,b; Mallikarjuna et al., 2004). Finally the capability of silibinin to modulate mitochondrial apoptotic machinery and MAPK signalling cascade has been confirmed also in UV-irradiated human epidermoid carcinoma A431 cells (Mohan et al., 2004).

In SENCAR mice topical pre-treatment with silymarin resulted in inhibition of TPA-induced skin myeloperoxidase activity (Zhao et al., 1999); these findings suggest that silymarin can inhibit UV-induced infiltration of inflammatory leukocytes, and thus elicit an anti-inflammatory effect. Furthermore, in the same experimental model, silymarin reduced also TPA-induced epidermal lipoxygenase, interleukin-1 and cyclooxygenase-2 expression, that are biochemical pathways implicated in inflammatory processes and tumor promotion (Zhao et al., 1999).

**Genistein**

Epidemiologic studies have suggested a possible role for soy isoflavones in the lower risk of cardiovascular disease and breast cancer in Asian populations consuming larger amounts of soy (Glazier and Bowman, 2001) in comparison with North-American populations (Barnes et al., 1995).

Two types of estrogen receptors (ERs) α and β have been identified and both are present in skin (Brandenberger et al., 1997). Isoflavones are weak estrogens working by coupling with ERs. Phytoestrogens may potentially block the receptor and this receptor occupancy leads to antiestrogenic effects. Estradiol has 700-fold more ERα and 45-fold more ERβ activity than genistein, the most active isoflavone. However, circulating levels of phytoestrogens may be high, and thus induce a strong biologic effect.

Topical genistein has a protective effect against skin cancer development in mice initiated with DMBA and promoted with TPA (Wei et al., 1998), and substantially reduces tumor incidence and multiplicity in a complete photocarcinogenesis study on mice chronically exposed to UVB (Wei et al., 2003). Furthermore genistein significantly blocks the subacute and chronic UVB-induced cutaneous damage and histological alterations related to photoaging (Wei et al., 2003) and decreases PUVA (psoralen plus UVA) induced skin thickening, edema, cutaneous erythema and ulceration (Shyong et al., 2002).

Genistein is known to possess good bioavailability in human and rodents (Ju et al., 2001). As shown by Wei and co-workers (2003), genistein is able to inhibit UVB induced skin carcinogenesis also when orally administrated, although the photoprotective effect has appeared less than that observed following topical application. Consistently with these findings, a chemical carcinogenesis study in mice has demonstrated that dietary soy can inhibit skin tumor formation and growth (Limtrakul et al., 1993). Finally oral genistein can enhance the activities of antioxidant enzymes in the skin (Cai and Wei, 1996). Furthermore, one has to point out that soy isoflavones are present in soy as glycosides, which are converted in the gut to the free isoflavones (Bingham et al., 1998). However the glycosides are not estrogienically active, which may have implications for topical use of soy active principles (Miksicek, 1995).

The mechanisms involved in the anticarcinogenic effect of genistein, although unclear, seem to be primarily associated with antioxidative and antiinflammatory pathways, downregulation of protein tyrosine kinase (PTK) activities, and expression of cell proliferation-associated proto-oncogenes. In vitro and in vivo experiments have demonstrated that genistein is able to inhibit tyrosine kinase and is a potent antioxidant/free radical scavenger (Hwang et al., 2000; Wiseman et al., 2000). In particular genistein is reported to inhibit in vitro UV-induced DNA oxidation (Wei et al., 1996), PGE2 synthesis and COX-2 expression (Miller et al. 1994), and apoptotic changes, such as caspase-3, p21-activated kinase 2 and phosphokinase C (Chan and Yu, 2000; Fukunaga et al., 2001), in epidermal cells.

Shyong et al. (2002) report that topically applied genistein is able to reduce PUVA-induced erythema and inflammation in mouse skin, and PUVA-induced cleavage of poly(ADP-ribose) polymerase (PARP) and pro-caspase-3 in skin of SKH-1 female mice. Furthermore, topical genistein significantly inhibits TPA-induced proto-oncogene expression (c-fos) in mouse skin apparently by tyrosine kinase inhibition (Wang et al., 1998). Finally, topical application of soy isoflavones prior to the application of TPA prevented the induction of ODC activity and DNA synthesis mediated by TPA in mice (Sharma and Sultana, 2004).

**Tea flavonoids and epigallocatechin-3-gallate**

Tea (Camellia sinensis) contains a large amount of polyphenolic compounds represented for almost 50% by flavonoids. Green, oolong, and black teas all come from the leaves of the Camellia sinensis plant, but differ for the way they are processed. Green tea is dried for a shorter time, and is heated sooner to prevent fermentation; it contains predominantly monomeric catechins including epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG), (+)-gallocatechin and (-)-catechin. Conversely, black tea and oolong tea are made from fermented leaves, that results in green tea catechins being oxidized and converted into oligomeric flavanols, such as theaflavins and thearubigins (Lin and Liang, 2000).
Tea flavonoids, and especially EGCG, are claimed as promising chemopreventive agents. They appear to be effective in animal models of skin cancer, although their protective effect in humans is supported by epidemiologic studies concerning only skin squamous cell carcinoma (Katiyar and Mukhtar, 1996). In particular, a statistically significant inverse association was observed between skin cancer incidence and black tea consumption (Hakim et al., 2000). While the skin chemopreventive properties of green tea polyphenols have been widely demonstrated, only little information regarding major polymeric polyphenols in black tea is available. Recently, Krishnan and Maru (2005) demonstrated that topical pretreatment of mice with polymeric black tea polyphenol fractions results in a significant decrease in the levels of single topical B(a)P-induced DNA adducts in epidermal DNA.

In vitro EGCG and its derivatives have been observed to decrease the growth of human melanoma cell lines (Valcic et al., 1996), and to inhibit TPA- and EGF-induced transformation of JB6 mouse epidermal cells (Dong, 2000). Despite the possible employment of bioflavonoids as chemopreventive agents is often discouraged due to their poor selectivity, EGCG can induce a specific cytotoxic effect on neoplastic cells; in fact, it was shown to cause significant induction of cell cycle arrest and apoptosis of melanoma cells, but not of normal melanocytes, through modulations in the cki-cyclin-cdk network and Bcl2 family proteins (Nihal et al. 2005). Furthermore, elevated levels of polyamines in skin tumor cells sensitize them to EGCG-induced apoptosis, as demonstrated by oral administration of EGCG to ODC/Ras double transgenic mice, which develop spontaneous skin tumors due to over-expression of ODC and a v-Ha-ras transgene (Paul et al., 2005).

Topically applied EGCG has been shown to inhibit the development of UV-induced skin squamous cell cancer in mice (Gansler et al., 1996), and to decrease tumor incidence and volume and to selectively increase apoptosis in UVB-induced skin tumors in SKH-1 hairless mice (Lu et al., 2002). However, as demonstrated by several authors (Fang et al., 2005; Mittal et al., 2003a; Proniuk et al., 2002), skin and tumor deposition of tea polyphenols following topical application, and thus their effectiveness in protecting against UV-induced skin damage, may be strongly influenced not only by the chemical features of active principles but also by composition of the employed formulations.

Few data are reported about the effect of dietary intake of green tea and EGCG in UV-induced skin carcinogenesis, and unfortunately they are often inconsistent. Oral green tea is reported to decrease chemically- and UV-induced skin tumors (Wang et al., 1989; 1991), inhibit growth of established skin tumors (Wang et al., 1992) and prevent conversion of benign skin tumors to squamous cell carcinoma (Katiyar et al., 1993). Conversely, no effect of orally administered EGCG on UV-induced skin tumor incidence was observed by Gansler and co-workers (1996). However Morley and co-workers (2005) have demonstrated that green tea and/or some constituents can offer protection against UV-induced DNA damage not only to cultured skin fibroblasts and keratinocytes but also to peripheral leucocytes isolated from human volunteers before and after green tea consumption, so indirectly demonstrating the good bioavailability of green tea constituents after oral administration.

As to the mechanisms involved in the anticarcinogenic effect of green tea flavonoids, these compounds are endowed with strong antioxidant, free radical scavenger and anti-inflammatory properties, which depend on their chemical features. Recently Katiyar and co-workers (2001a) have reported that EGCG inhibits UVB-induced release of intracellular hydrogen peroxide from normal human epidermal keratinocytes with concomitant inhibition in the phosphorylation of mitogen-activated protein kinases; furthermore topical application of EGCG in humans restores UV-induced decrease in GSH levels preserving the antioxidant enzyme glutathione peroxidase (Katiyar et al. 2001b), and protects against UV-A- and UVB-induced skin damage and lipid peroxidation in guinea pigs (Kim et al., 2001).

Topical treatment with EGCG results in inhibition of UVB-caused inflammatory response, such as leukocyte infiltration, myeloperoxidase activity, erythema development and microsomial PGE2 formation, in human skin (Katiyar et al., 1999a; Katiyar and Mukhtar, 2001), and elicits a protective effect against UVB-induced immune suppression in mice (Katiyar et al., 1999b). Furthermore, topical green tea flavonoids reduces UV-induced erythema and sunburn cell formation in human skin (Elmets et al., 2001), and EGCG and, at a lower degree, theaflavins can inhibit TPA-induced skin edema in mice (Liang et al., 2002). Finally, Katiyar and Mukhtar (2001) have shown that topical application of EGCG protects against UVB radiation-induced local as well as systemic suppression of contact hypersensitivity in C3H/HeN mice.

As to molecular targets of tea flavonoids in skin chemoprevention, EGCG inhibits UVB-induced mitogen-activated protein kinase cell signalling pathways, ERK 1/2, JNK and p38 kinase in human keratinocytes (Katiyar et al., 2001a). Furthermore EGCG inhibits TPA-induced NF-κB activity in a dose-dependent manner by blocking phosphorylation of IκBα in JB6 mouse epidermal cells (Nomura et al., 2000).

Procyanidins

Procyanidins are a particularly interesting type of flavonoids consisting of oligomers of flavan-3-ols, and are known to be potent free radical scavengers, antioxidants, anti-inflammatory and antiproliferative agents (Cos et al., 2004).

Pycnogenol® is the registered trade name of a standardized extract of the bark of the French maritime pine (Pinus pinaster Ait.), consisting of naturally occurring phenolic and polyphenolic flavonoids (the major components being monomeric and oligomeric procyanidins) and phenolic acids (in the free or glucuronidated form). The extract has multiple biological effects, including antioxidant, anti-inflammatory and anticarcinogenic properties (Packer et al., 1999; Virgili et al., 1998), and is now marketed as a nutritional supplement.
and phytochemical remedy for various disease states associated with oxidative stress (Rohdewald, 2002). When topically applied, as well as orally administered, Pycnogenol® elicits an evident protective effect against UV-induced skin carcinogenesis in hairless mice (Kyrizzi et al., 2005; Sime and Reeve, 2004). Previous studies have shown that long-term oral supplementation with Pycnogenol® prevents solar UV-induced skin erythema in humans (Saliou et al., 2001), and that it can protect against UV-induced skin edema and inflammation in rodents (Blazso et al., 1997). The mechanisms involved in chemopreventive effect of Pycnogenol® have not been deeply investigated. One may suggest that Pycnogenol® acts through its antioxidant, immunoprotective and anti-inflammatory properties (Sime and Reeve, 2004); furthermore it can modulate NF-kB-dependent gene expression in UV-irradiated keratinocytes (Saliou et al., 2001).

Interestingly, also proanthocyanidins from other sources exert beneficial effects against UV-induced skin damage (Mittal et al., 2003b; Greul et al., 2002; Yamakoshi et al., 2003, Spagna et al., 2002). In particular dietary feeding of grape seed extracts, rich in procyanidins, to hairless mice resulted in prevention of UVB-induced photocarcinogenesis in terms of reduced tumor incidence, multiplicity and size (Mittal et al., 2003b).

Apigenin

Besides the compounds mentioned above, several other flavonoids and botanical drugs rich in flavonoids have proven to be efficient photoprotective agents, as demonstrated in biological in vitro and in vivo models and in chemical systems (Bonina et al., 2002; Spagna et al., 2002; Bonina et al., 2000, 1998; Tarozzi et al., 2005; Afaq et al., 2005b), and thus, although such a hypothesis is merely speculative, might be good candidates as skin chemopreventers against UV carcinogenesis. Furthermore, their effectiveness in protecting against UV-induced skin damage may be significantly ameliorated in different ways, e.g. optimization of bioavailability by means of suitable formulations, semisynthetic reactions finalized to improve the structure-activity relationship, concomitant employment of other therapeutic strategies, etc. (Paliwal et al., 2005; Saija et al., 2003, 1998).

In particular a relatively large number of studies is focussed on apigenin (5,7,4′-tri-hydroxyflavone), a flavonoid present in several fruits and vegetables, such as apples, endive, beans, broccoli, celery, cherries, cloves, grapes, leeks, onions, barley, parsley and tomatoes (Lepley et al., 1996), and in some plant-derived beverages, including tea and wine (Janssen et al., 1998). Several authors have suggested that apigenin may be a useful chemopreventive agent against skin cancers. For example, topical pre-treatment with apigenin is reported to decrease skin cancer incidence and increase tumor-free survival in UV light irradiated mice, very likely by inhibiting UV-induced induction of ODC activity in the skin (Birt et al., 1997). Furthermore topical application of apigenin in mice decreases the number and size of chemically induced skin tumors (Wei et al., 1990). However, there are no studies about the effect of dietary intake of apigenin against skin cancer, and also bioavailability of this flavonoid has been poorly reported.

Concerning the molecular mechanisms underlying the observed anticarcinogenic effect, apigenin treatment results in G1 cell-cycle arrest, inhibition of cyclin-dependent kinase 2 (CDK2) activity and accumulation of the hypophosphorylated form of retinoblastoma protein in human fibroblasts (Lepley and Pelling, 1997); furthermore apigenin treatment induces stabilization of p53 protein and G2/M phase arrest in mouse skin keratinocytes (McVean M, et al., 2002).

CONCLUSIONS

UV radiation is considered as the most prevalent environmental carcinogen and the incidence of skin cancer has been increasing dramatically. Primary prevention of UV-related skin cancer is focussed on the use of sunscreens and on education about the harmful effects of UV radiation present in the sunlight, but additional efforts are needed to prevent serious pathological consequences of UV exposure. One approach for controlling the occurrence of skin cancer is through topical or oral administration of plant-derived chemopreventive agents possessing antioxidant, antiinflammatory and immunomodulatory properties. In particular there is an increasing interest in flavonoids, compounds employed by plants for their protection against excess light. A number of carefully designed, mechanism-based studies in cellular systems and in animals have demonstrated that mainly EGCG, genistein, sylimarin and proanthocyanidins can act on specific and multiple cellular and molecular targets in the skin, so giving a rational basis to understand the potential health benefits of these compounds (Table 1). Unfortunately there is no sufficient evidence to prove the protective effect of flavonoids against UV-related skin cancer in humans. Furthermore, a deeper evaluation of the real availability of flavonoids (especially when orally administered) at the skin cells, target of UV-dangerous effects, is awaited. Finally, several reports indicate the necessity to deeply investigate possible toxic effects of flavonoids before approving their use for skin health protection in humans.

ACKNOWLEDGEMENTS: This work was supported by Italian Government (MiPAF, Prog. Ricerche e Sperimentazione nel Settore dell’Agrumicoltura Italiana, Azione n.4).

REFERENCES


Table 1: Flavonoids shown to possess protective effect against UV-induced skin cancer and their target mechanisms, as demonstrated in different experimental models.

<table>
<thead>
<tr>
<th>Botanical drug</th>
<th>Source</th>
<th>Target/mechanism(s)</th>
<th>Experimental model</th>
<th>Administration route</th>
<th>REF.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silymarin</td>
<td>Milk thistle</td>
<td>Inhibits activation of redox-regulated NF-kB</td>
<td>Human keratinocytes</td>
<td>Oral</td>
<td>Salin et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activates the DNA-PK-p53 pathway</td>
<td>Mouse epithelial JB6 cells</td>
<td></td>
<td>Dharmalalshani et al., 2004b, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits ODC activity</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Agarwal et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits depletion of antioxidant enzymes</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Zhao et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits lipid peroxidation</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Chatterjee et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits epidermal lipooxygenase and IL-1 and COX-2 expression</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Zhao et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits leukocyte infiltration and COX-2 overexpression</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Zhao et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against tumor promotion</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Chatterjee et al., 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits autocrine signalling molecules</td>
<td>SENCAR mice / human epidermoid carcinoma A431</td>
<td>Topical/oral</td>
<td>Singh et al. 2002; Bhatia et al., 2001; Gu et al., 2005b; Mallikarjuna et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits apoptosis</td>
<td>SKH-1 hairless mice</td>
<td>Topical/oral</td>
<td>Katiyar et al., 1997; Katiyar, 2005; Gu et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits growth and causes regression of established skin tumors</td>
<td>SENCAR mice</td>
<td>Oral</td>
<td>Singh et al., 2002</td>
</tr>
<tr>
<td>Genistein</td>
<td>Soy, red clover, ginkgo</td>
<td>Inhibits DNA oxidation and lipid peroxidation</td>
<td>Epidermal cells</td>
<td>Topical</td>
<td>Wei et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits PGE2 synthesis and COX-2 expression</td>
<td>Epidermal cells</td>
<td>Topical</td>
<td>Miller et al. 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits caspase-3, p21-activated kinase 2 and phosphokinase C activation</td>
<td>Epidermal cells</td>
<td>Topical</td>
<td>Chan and Yu, 2000; Fukunaga et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces c-fos and c-jun expression</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Wang et al., 1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces PUVA-induced erythema and inflammation</td>
<td>SENCAR mice</td>
<td>Topical</td>
<td>Shyong et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits ODC activity</td>
<td>Mice</td>
<td>Topical</td>
<td>Sharma and Sultana, 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces PUVA-induced cleavage of PARP and pro-caspase-3</td>
<td>SKH-1 mice</td>
<td>Topical</td>
<td>Shyong et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhances the activities of antioxidant enzymes in the skin</td>
<td>Mice</td>
<td>Topical/oral</td>
<td>Cai and Wei, 1996; Sharma and Sultana, 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits skin tumor formation and growth</td>
<td>Mice</td>
<td>Oral</td>
<td>Limtrakul, 1993</td>
</tr>
<tr>
<td>Tea</td>
<td>Green and black tea</td>
<td>Inhibits release of intracellular ROS</td>
<td>Epidermal cells</td>
<td>Oral</td>
<td>Katiyar et al., 2001a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits phosphorylation of MAPKs</td>
<td>Epidermal cells</td>
<td>Oral</td>
<td>Katiyar et al., 2001a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Modulates NF-kB pathway</td>
<td>Epidermal cells</td>
<td>Oral</td>
<td>Nomura et al., 2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits MAPKs, ERK 1/2, JNK and p38</td>
<td>Fibroblasts / keratinocytes</td>
<td>Oral</td>
<td>Katiyar et al., 2001a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against DNA damage</td>
<td>Human skin</td>
<td>Oral</td>
<td>Morley et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restores decrease of the antioxidant enzymes</td>
<td>Human skin</td>
<td>Oral</td>
<td>Katiyar et al., 2001b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces erythema and sunburn cell formation</td>
<td>Human skin</td>
<td>Oral</td>
<td>Ehrnsperger et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits inflammatory responses and leukocyte infiltration</td>
<td>Human skin</td>
<td>Oral</td>
<td>Katiyar et al., 1999a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases apoptosis</td>
<td>SKH-1 hairless mice / ODC/Ras double transgenic mice</td>
<td>Topical</td>
<td>Lu et al., 2002; Paul et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits lipid peroxidation</td>
<td>Guinea pigs / hairless mice</td>
<td>Topical</td>
<td>Kim et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against immune suppression</td>
<td>Mice</td>
<td>Topical</td>
<td>Katiyar et al., 1999b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against suppression of contact hypersensitivity</td>
<td>C57/HeN mice</td>
<td>Topical</td>
<td>Katiyar and Mukhtar, 2001</td>
</tr>
<tr>
<td>Apigenin</td>
<td>Vascular plants, fruits, vegetables, tea, wine</td>
<td>Induces stabilization of p53 protein</td>
<td>Mouse keratinocyte</td>
<td>Oral</td>
<td>McVean et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induces G1 cell-cycle arrest</td>
<td>Human fibroblasts</td>
<td>Oral</td>
<td>Lepley and Pelling, 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits CDB2 activity</td>
<td>Human fibroblasts</td>
<td>Oral</td>
<td>Lepley and Pelling, 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibits ODC activity</td>
<td>Mice</td>
<td>Topical</td>
<td>Birt et al., 1997</td>
</tr>
<tr>
<td>Procyanidin</td>
<td>French maritime pine, cocoa, coffee, green and black tea, grape seed</td>
<td>Modulates NF-kB-dependent gene expression</td>
<td>Epidermal cells</td>
<td>Topical</td>
<td>Salin et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduce immunosuppression</td>
<td>Hairless mice</td>
<td>Oral</td>
<td>Sine and Reeve, 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protect against skin edema and inflammation</td>
<td>Rodents</td>
<td>Oral/Topical</td>
<td>Blazou et al., 1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevent against initiation and promotion stages of photocarcinogenesis</td>
<td>Hairless mice</td>
<td>Oral</td>
<td>Kyrnazi et al., 2003; Mittal et al., 2003b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prevent skin erythema</td>
<td>Human skin</td>
<td>Oral</td>
<td>Salin et al., 2001</td>
</tr>
</tbody>
</table>


Dhanalakshmi, S., Mallikarjuna, G.U., Singh, R.P., Agarwal, R. (2004a) Dual efficacy of silibinin in protecting or enhancing...


Photodermatology, Photoimmunology & Photomedicine 21, 15-22.


Rice-Evans, C., Spencer, J.E., Schroeter, H., Rechner, A.R.


