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## Review Article

# Botanicals as sunscreens: Their role in the prevention of photoaging and skin cancer

**K P Balakrishnan*[1] & Nithya Narayanaswamy[1]**
Personal Care Division, ITC R&D Centre, Peenya Industrial Area, Bangalore-560 058, India.
E mail: kp.balakrishnan@itc.in

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**Abstract**

Botanicals are gaining importance in recent times as active ingredients for cosmetic formulations due to their dermal protective effect against the harmful substances from endogenous and exogenous sources. The probability of exposure of the skin to UV radiation and other environmental factors are more since it is present in the outer layer of body as the primary defense mechanism. The exposure of skin to UV radiation poses erythema, the production of inflammatory mediators, the alteration of vascular responses and immunosuppression. The generation of free radicals from the exogenous sources like UV radiation targets the various biomolecules like lipids, proteins and nucleic acids present in the skin. As a result, the structure and function of the cell is lost due to the oxidation of biomolecules. The free radical attack is also associated with the deterioration of antioxidant status of the cell. The regulation pathways of skin are severely affected by the imbalance in the antioxidant level leading to photoaging and the development of skin cancer. The possible strategy for preventing the photoaging and skin cancer is the application of plant extracts with potential UV absorbing capacity (sunscreen or photoprotective) and antioxidant activity. This review gives an overview of photoaging, its corresponding changes in the ultrastructure of skin after UV exposure and the various approaches for protecting the skin from sunlight.

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**Key words**: Botanicals, erythema, photoaging, skin cancer, sunscreen.

## 1. Introduction

Skin is the outermost and largest organ of the body hence it is most prone to photodamage as it is directly exposed to sun light. In recent years, the incidences of ultraviolet radiation related diseases and disorders are continuously growing. When the mammalian skin is exposed long term to ultraviolet radiation, it induces the oxidative stress by generating the reactive oxygen species. These substances further trigger the development of skin cancer in individuals [1, 2]. The various other biological responses occur in the skin due to UV exposure include the development of erythema, edema, sunburn cell formation, hyperplasia, immunosuppression, DNA damage, photoaging and melanogenesis. Melanin pigmentation of the skin absorbs UV light and thus protects skin cells from the detrimental effects of UV exposure. But in certain circumstances, the amount of melanin produced is not sufficient enough to protect the skin. Hence, the protection of skin from photo-damage by some other means is an urgent concern. One strategy for safeguarding the skin from UV radiation is the use of sunscreens to counteract the reactive oxygen species by blocking the UV radiation exposed on the epidermis.

The use of sunscreen is a most common practice now a days that provides protection against the adverse effects of UV radiation [3]. Many synthetic sunscreens are available in the market but they pose possible adverse side effects. Thus, the use of botanicals as sunscreen has been gaining attention in recent times. Natural substances extracted from herbs acts as the potential photoprotective resources owing to their UV absorbing property in the UV region [4]. In addition, they exhibit antioxidant property [5], antimutagenic property, anti-inflammatory property and anticarcinogenic activity. So the use of botanicals is an approach to reduce the UV generated ROS-mediated photodamage, immune-suppression and skin cancer in patients. For example, polyphenols from green tea exhibits

UV blocking capacity, antioxidant property and antimalignant property. The following section deals with the source of UV radiation, its types, adverse effects on the skin and its protection by botanical sunscreen.

**2. Sun light as a carrier of UV radiation**

The main exogenous source of the UV radiation is sun light. It is composed of various wavelengths ranging from ultraviolet light through infrared to visible light. Among all, ultraviolet light is the most harmful to the skin. Ultraviolet radiation from the sun can be further divided into three categories based on the wavelength, long wave UVA (320-400 nm), medium wave UVB (280-320 nm) and short wave UVC (200-280nm) [6].The substantial damage to the protective ozone layer in the sky resulted in an increased amount of UV radiation reaching the earth's surface [7]. The harmful effects of UV radiation in the skin can be divided into acute (sun burn or erythema, phototoxic reactions, photoallergy and photosensitivity) and chronic (Photoaging, skin cancer and immunosuppression) [8].

More than 90% of solar radiation that reaches the earth is UVA which penetrates deep into the epidermis and dermis of the skin (Fig.1). It is about 1000 times more effective in producing an immediate tanning effect when compared to UV B. Long term exposure to UVA can burn sensitive skin and damage the underlying structures in the dermis and cause premature photoaging of the skin. It causes skin sagging and suppress some immunological functions. It also triggers the oxidative changes in exposed individuals which generate singlet oxygen, hydrogen peroxide and hydroxyl free radicals. These can cause damage to cellular proteins, lipids and saccharides. UV injury also tends to cause necrosis of endothelial cells, thus damaging the dermal blood vessels. It can produce structural changes in DNA and impair the immune system which inturn results in cancerous condition. The contribution of UVA radiation for malignant melanoma is 67% [9].

UV B radiation makes up 4 to 5 % of UV light and it is the most active constituent of solar light. UV B is 1000 times more capable of causing sun burn than UVA so it is called as burning ray. It is more genotoxic than UVA and acts predominantly in the epidermal basal layer of the skin. It induces direct and indirect adverse biological effects which include the formation of pyrimidine photoproducts, isomerisation of trans to cis-urocanic acid, stimulation of DNA synthesis, free radical production in the skin, cell cycle growth arrest, photoaging and photocarcinogenesis. It is capable of lowering the skin's immune system [10]. It decreases the antioxidant defense of the cell against free radicals. It is also considered to be responsible for skin cancer. Fig. 2 summarizes the effects of UV radiation on the skin.

UVC is the very dangerous radiation to all forms of life. The short exposure to UVC radiation poses extensive damage to the skin. But UV C radiation from the sun is completely absorbed by molecular oxygen and ozone in the earth's atmosphere and no solar radiation of wavelengths below 290 nm reaches the surface of the earth [11]. The

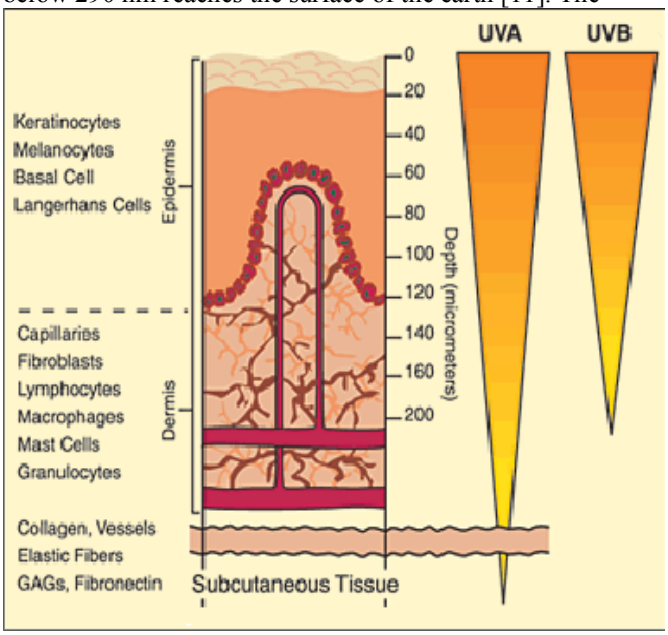


**Fig.1** shows the penetration capacity of UV radiation on the skin. UVA radiation penetrates deep until subcutaneous tissue whereas the penetrating ability of UVB shows that it pose cutaneous damage very frequently.

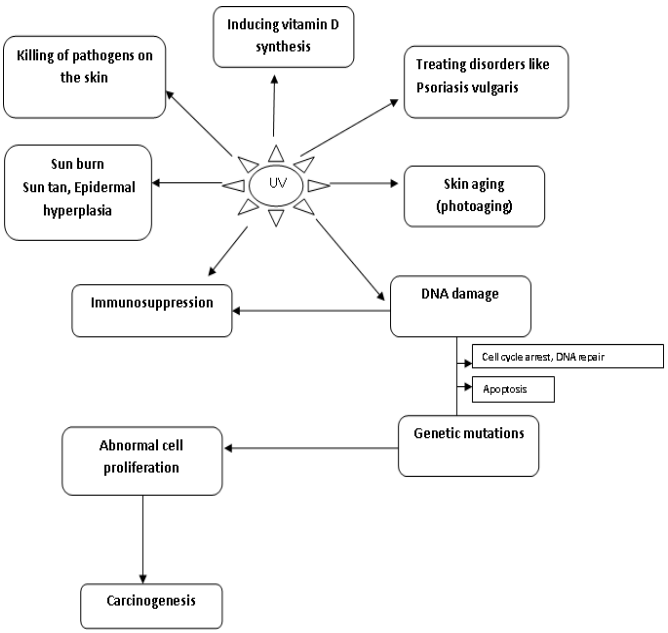


**Fig.2** summarizes the effects of UV radiation on the skin. UV radiation imparts both beneficial and harmful effects to the skin. It facilitates the synthesis of vitamin D, killing of pathogens and treating the disorders like psoriasis. On the other hand, they cause photoaging and skin cancer by making alterations in the cellular levels.

following section deals with effect of UV radiation on the skin and its cellular components.

### 3. Effect of UV radiation on the skin

The exposure of skin to UV radiation results in the generation of reactive oxygen species [12, 13]. The reactive oxygen species comprise a number of active metabolites including hydroxyl radical, superoxide anion, nitric oxide, peroxyl radical and their active precursors namely singlet oxygen, hydrogen peroxide and ozone. The constantly generated free radicals in keratinocytes and fibroblasts are rapidly removed by non-enzymic and enzymatic antioxidant mechanism (Fig 3). Hence, they prevent the living system from the harmful effects of free radicals by maintaining a proxidant/antioxidant balance which in turn results in the stabilization of cell structure. Excess of free radicals in the skin results in a cascade of events mediating progressive deterioration of cellular structure and function leading to loss of cellular integrity and function by the modification of biomolecules.

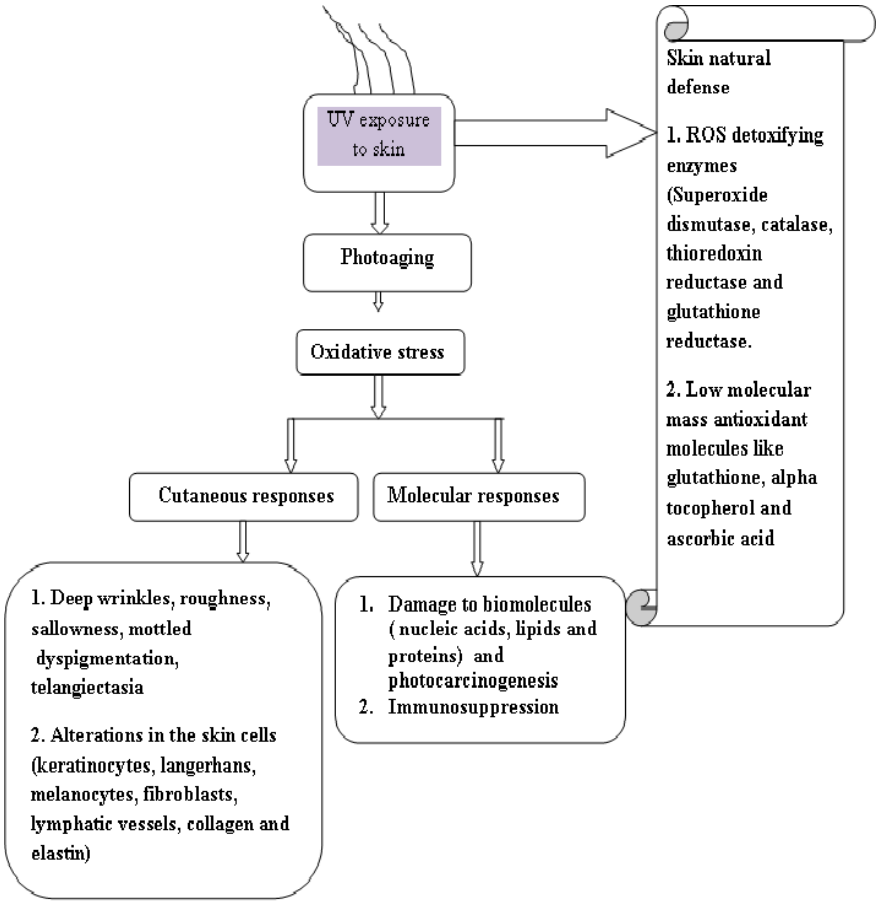


**Fig.3** represents the effects of UV radiation on the skin and its cellular components. The dermal tissue is usually protected by the antioxidant defense molecules. If the balance is not maintained between prooxidants and antioxidants, the living structure sensitizes the oxidative stress. The exposure of UV radiation on the skin generates oxidative insults resulting in structural and functional changes in the epidermis and biomolecules present within the cell.

### 4. Photoaging

Photoaging also called as extrinsic, premature or accelerated aging. It is the superposition of chronic ultraviolet radiation on the skin and accounts for most age related changes. The exposure of individuals to UV radiation results in cutaneous alterations which includes deep wrinkles, roughness, sallowness, mottled dyspigmentation, telangiectasia and a variety of benign and malignant neoplasms. The histological and ultrastructural changes that occur during photoaging are reviewed in the following section.

#### *4.1. Histological changes in photoaging*

Photoaged skin has a variable but characteristic histological appearance which differs from sun-protected skin of the same individual. The epidermal cells will get flattened and the number of keratinocytes in epidermal layer is also

reduced drastically. The thickness of the basal membrane is increased, possibly reflecting damage to basal keratinocytes. The distribution of melanocytes along the basal membrane is irregular and these cells vary widely in size, dendricity and pigmentation [14, 15]. The number of langerhans cells and melanocytes were also reduced in the epidermis; meanwhile the hyperactivity of melanocytes in the skin causes blotches of hyperpigmentation.

The middle layer of skin, dermis has a vertical gradient of damage consistent with progressive attenuation of UV exposure. The most prominent histological feature of photoaging in the dermis is elastosis [16]. The number of fibroblasts in the dermal layer is lowered and the lymphatic channels in the dermis dilate. Altered elastic fibers can span a varying portion of the dermal compartment and it is not observed in chronologically aged skin. Another prominent feature of photoaged skin is the replacement of mature collagen fibers by collagen with a distinct basophilic appearance. This is called basophilic degeneration. The extracellular matrix components (collagen and elastin) in the dermis were also decreased. [17].

The ultra structural changes in the skin were also observed after UV irradiation [18]. The outermost epidermal cells are damaged as early as 2 hours after UV irradiation. The initial indicator of damage is decrease in the number of keratinosomes, which results in the formation of dyskeratotic cells. After 16-18 hours of UV exposure, intracellular edema can be seen. Prolonged exposure to UV radiation induces the development of intracellular edema around the damaged keratinocytes resulting in the formation of sunburn cells for apoptosis. UV-induced apoptotic cells are rapidly phagocytised by the surrounding keratinocytes. After UV treatment, the number of phagocytic cells (macrophages) in the skin also increases dramatically. The degenerative changes that occur in the keratinocytes include mitochondrial swelling and rupture, condensation of the cytoplasm and the appearance of pyknotic nuclei. The observed cellular damage is maximal at 48-72 hours for the total UV range.

### *4.2. Activation of Matrix Metalloproteinase (MMPs) in photoaging*

When the mammalian skin is exposed to UV B radiation in the sun light, the epidermis primarily comprising keratinocytes absorbs the radiation. As a result, the transcription factors such as Activation Protein-1(AP-1) and Nuclear Factor kappa- B (NF-*k*B) in the epidermis is induced [19,20]. These factors in turn induce the expression of matrix metalloproteinases. When the UV light reaches the dermis layer, it is absorbed by fibroblasts. UVA generated free radicals affects the signalling cascades which in turn results in the induction of enzymes namely heme oxygenase-1 (HO-1) and matrix metalloproteinases (MMPs) in the skin. Increased level of HO-1 elevates cellular levels of iron that can promote further free radical generation. The induction of MMPs by both UVA and UVB enhances the degradation of extracellular matrix proteins that favour wrinkle formation and metastases (Fig 4). Reactive oxygen species also triggers the oxidation of fatty acids within the phospholipid structure of the membrane. During this process, lipid peroxide radicals, lipid hydroperoxides and other fragmentation products initiate the chain reaction further that enhances the oxidation of the cell membrane. The extensive damage to the skin results in various skin related disorders like melasoma and skin cancer (Basal cell carcinoma and squamous cell carcinoma).

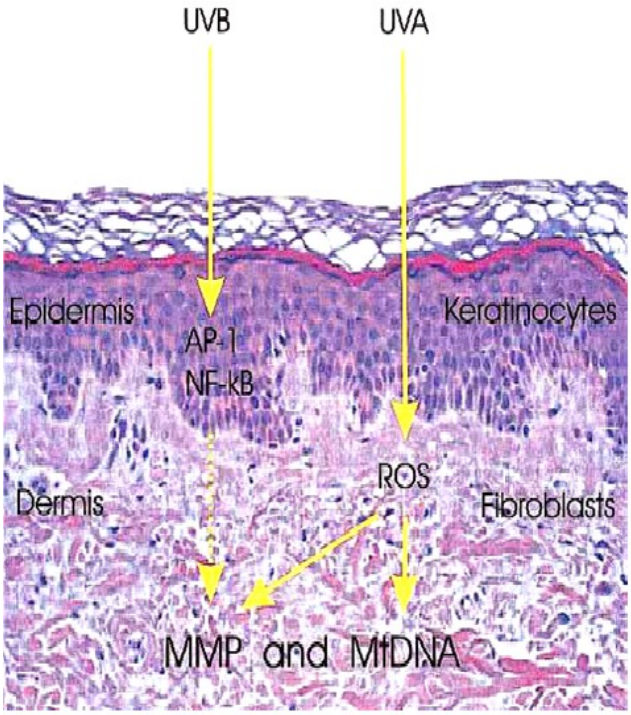


**Fig.4** illustrates the photoaging of human skin. When the epidermis of the skin absorbs the UVB radiation from the sunlight, it results in the induction of transcription factors Activation protein-1 and Nuclear Factor-κB (NF- κB) which in turn results in the induction of matrix metalloproteinases (MMPs). The penetration of UVA deeper in dermis also induces the MMPs. As a result, the proteins present in the extracellular matrix are degraded that favours the wrinkle formation.

### *4.3. Mitochondrial DNA mutations in photoaging*

Mitochondria are the main energy producing organelles of the cell. It generates energy for the cell in the inner mitochondrial membrane by a multistep process called oxidative phosphorylation or electron-transport-chain. The inner mitochondrial membrane comprises of five multi-protein complexes which generate an electrochemical proton gradient used in the last step of the process to turn ADP and organophosphate into ATP. This ultimate step in this process is not completely error free and this leads to the generation of

reactive oxygen species, making the mitochondrion as the highest ROS turnover site. The mitochondrion's own genetic material (mtDNA) lies closer proximity to this site. So it is easily susceptible to free radical damage. Besides, mitochondria do not contain any repair mechanism to remove bulky DNA lesions. Although they do contain base excision repair mechanisms and repair mechanisms against oxidative damage [21], they are insufficient to prevent the mtDNA from oxidative damage. Hence, the mutation frequency of mtDNA is approximately 50-fold higher than nuclear DNA [22]. The mutations in mtDNA impair the energy production of the cell. In addition, the mutations of mtDNA have also been found to play a causative role in degenerative diseases such as Alzheimer's disease.

The large-scale deletions of mtDNA in photoaged skin were reported [23]. The appearance of the common deletion was paralleled by a reduction in cellular oxygen consumption and mitochondrial membrane potential which are markers for mitochondrial function. Assessment of the underlying photobiological mechanisms has revealed that, similar to UVA-induced MMP-induction, the generation of mtDNA mutations is due to production of singlet oxygen. This indicates that substances with ROS-quenching potential may be employed to prevent photoaging of human skin.

### *4.4. Photooxidation of lipids*

UV radiation induces the formation of reactive oxygen species resulting in damage to various components of skin like lipids. The unsaturated free fatty acids and cholesterol present in the cell membrane are particularly susceptible to oxidative attacks. If $O_2$ is present long term, chain lipid peroxides may be formed by a rapid free radical chain reaction causing disruption of cell membrane functions.

### *4.5. Photo-oxidation of proteins*

Proteins can be affected by UV induced oxidative damage and photodamaged skin shows accumulation of damaged upper dermal proteins [24]. The amino acids which are most prone to oxidative attack include cysteine, methionine, tyrosine, proline, arginine and threonine. The modifications that proteins encounter during oxidation are the formation of side-chain aldehydes and ketones (protein carbonyls), tyrosine cross-links, amino acid interconversion (e.g. histidine to aspartic acid), amino acid oxidation, adducts and peptide bond cleavage [25]. UV can also cross-link proteins such as dermal collagens and elastin. Oxidative protein damage may result in loss or gain of activity (i.e. enzymes), loss of structural protein function and increased /decreased susceptibility to degradation. The epidermal proteins are routinely degraded by epidermal methionine sulfoxide reductases, whereas the oxidized dermal proteins persist longer [26]. Besides, the accumulation of oxidised proteins in the cell inhibits the ability of the cell to successfully degrade additional damaged proteins through proteosomal mechanism [27]. In vitro studies suggest that UVA is a major contributor to protein oxidation in the skin.

### *4.6. DNA damage and Photocarcinogenesis*

The long term exposure of individual to UV radiation causes photocarcinogenesis. The biological effect of UV radiation is seen when it is absorbed by the UV cellular chromophores. Nucleic acids and proteins (tyrosine and tryptophan) are the major cellular chromophores absorbing radiation in the UV B wavelength range which transform the energy into biochemical signal. Subsequent photobiochemcial

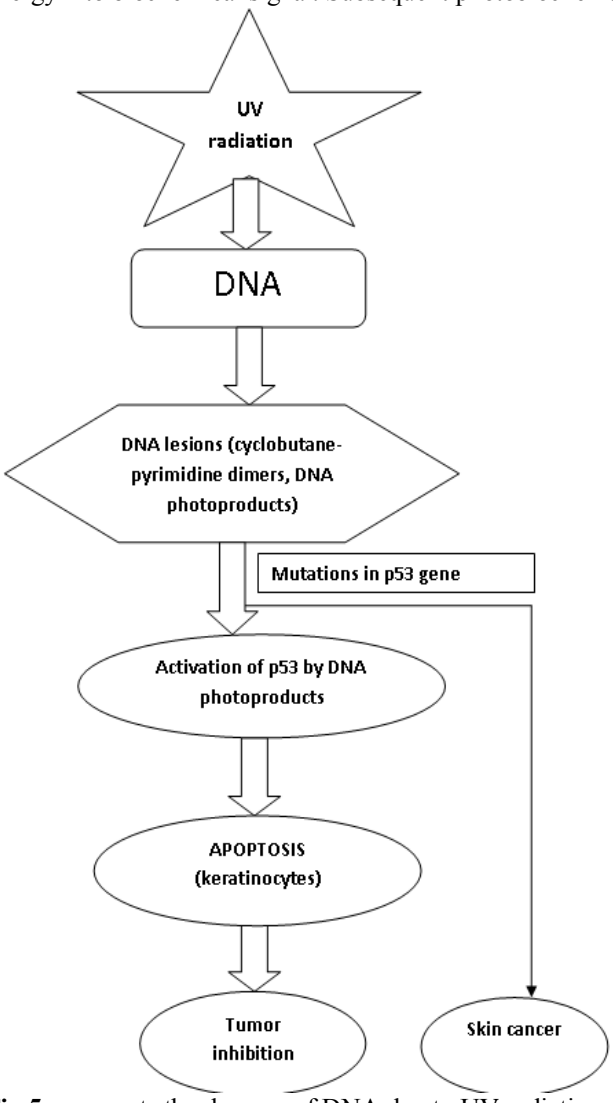


**Fig.5** represents the damage of DNA due to UV radiation and the development of cancer. The DNA lesions are produced after long term exposure to UV radiation. The tumor suppressor gene (p53) recognises the DNA lesions and it undergoes programmed cell death in normal cells. But the mutations in p53 gene will promote the development of skin cancer.

reactions provoke changes in the cell structure which in turn results in increased photoaging and the occurrence of skin cancer.

The absorption of photons by the DNA from the UV region of the spectrum induces genotoxic effects in the skin. DNA lesions (adducts) are the main indicator of DNA damage in the cells. The approximate estimate of DNA adducts formed during free radical insult was found to be 35 and one of the key markers of oxidative DNA damage is 8-hydroxy -2-deoxy-guanosine (8-OHdG) which induces the change of guanine to thymine in DNA [28]. The main lesions induced by UVB are cyclobutane-pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts [29, 30]. It also produces breaks in DNA, DNA-DNA cross links and DNA-protein cross links [31, 32].The DNA photoproduct blocks the RNA transcription leading to the activation of p53 gene that induces apoptosis of irradiated keratinocytes. The keratinocytes will lose their ability to undergo apoptosis when mutations occur in the p53 gene as a result of high dose UV exposure. Such long term exposure results in the development of skin cancer (Fig 5).

The development of skin cancer is a complex multistage phenomenon. Since UV radiation is a complete carcinogen it induces three stages of carcinogenesis. Initiation, promotion and progression. The initiation is the first event in the process of photocarcinogenesis. It is an indispensable and irreversible step in which DNA damage altered gene expression of epidermal cells [32]. Tumor promotion is the process that involves clonal expansion of initiated cells that produce the premalignant and malignant lesions by alterations in signalling pathways. The promotion of damaged cells to the state of preneoplastic lasts about 10 years. Finally, the progression of carcinoma in situ and the conversion of the lesion into an invasive and potentially metastatic malignant tumor [33, 34].

### *4.7. Immunosuppression*

UV radiation also causes an immunosuppressive effect in individuals exposed chronically to ultraviolet radiation. The skin primarily comprising keratinocytes produces trans-urocanic acid (trans UCA) which participates in the differentiation of the stratum corneum and contributes to the homeostasis of the upper layers of the skin maintaining the pH. The UV induced DNA damage in keratinocytes of epidermis induces the isomerisation of trans-UCA to the cis-UCA [8]. This cis-UCA has an immunosuppressive effect by altering the activity of antigen presenting cells, possibly through the secretion of interleukin -10 (IL-10) which in turn alters the local and systemic immune response. Normally, the hypersensitivity to a large variety of antigens is observed in systemic immune response. Upon exposure to UV radiation, the hypersensitivity of individual to foreign antigen is decreased. This situation favours the growth of microorganisms in the skin [28, 35]. The adverse effects caused by the UV radiation need to be blocked for safeguarding the immune system. This can be achieved by using the photoprotectives or sunscreens. The various approaches for the prevention of photoaging are reviewed in the following section.

## 5. Strategies in the prevention of photoaging

### *5.1. Melanin as the natural photoprotective*

Photoaging of the skin is mainly due to the generation of free radicals from UV radiation. The melanin produced in the skin as a result of UV exposure protects the skin from free radical insults naturally. Studies carried-out by researchers indicated that oligonucleotides that contain thymine dinucleotides (pTpT) induce tanning of the skin [36]. It also provides protective effects against photocarcinogenesis and photoaging as a natural sun protective.

### *5.2. Antioxidants as sun protectives*

Another protective strategy is the use of antioxidants to neutralize the free radicals that plays a major role in the induction of photoaging. A large number of antioxidants have been found to exhibit protective effects against the different ROS involved in photoaging. The detrimental effects of sun exposure can be decreased by the application of antioxidants. For example, all-trans retinoic acid (derivative of vitamin A) reduces the effects of UV exposure. *In vitro* and *in vivo* studies done by scientists have recently demonstrated that all-*trans* retinoic acid exhibits the property of blocking the photoaging-involved transcription factor namely Activation Protein (AP-1) [37]. They act by attenuating the induction of AP-1 and MMPs when applied before UVB irradiation. They have also studied that ultraviolet radiation causes a functional vitamin A deficiency and photoaging of human skin. This deficiency could be overcome by pretreating the skin with all-trans retinoic acid. Thus, this work not only provided a mechanistic model for the process of photoaging but also a rationale for the efficacy of all- trans retinoic acid in the repair of photoaged skin. Typical examples of other antioxidants used in photoaging are tocopherol acetate, retinoids, resveratrol (trans-3, 4', 5-trihydroxystilbene) etc.,

### *5.3. Application of DNA damage repair enzymes in photoaging*

The utilisation of DNA damage repair enzymes is a new fascinating technology in photoaging. The cyclobutane pyrimidine dimers (CPDs) are the common photolesions generated during DNA damage. The enzyme T4 endonuclease V derived from a bacteriophage recognizes the above lesions and initiates repair by enhancing their cleavage. Encapsulating the enzyme in liposomes facilitates its delivery into the skin. Likely via enhancing DNA damage repair, T4 endonuclease also decreases the synthesis and release of immunosuppressive cytokines like TNF-α and IL-10 that is speculated for skin cancer risk [38, 39].

Another enzyme, photolyase, which is most prevalent in plants, bacteria, reptiles, amphibians, marsupials

and even mammalian placentae, absorbs visible light and utilizes the energy to break up the cyclobutane pyrimidine dimers through the mechanism called photoreactivation. The effect of liposome encapsulated photolyase was investigated through *in vivo* and *in vitro* studies. The experimental studies demonstrated that reduced UV-induced apoptosis, decreased CPD levels, less inhibition of contact hypersensitivity, decreased erythema and diminished apoptotic cell death *in vitro* was observed after treatment [40, 41]. Previous studies had demonstrated that immunosuppression of UV-irradiated skin is caused by generation of DNA damage in immune cells of the skin. In a recent study, the application of the repair enzyme photolyase restored the skin's immune responsiveness; this was shown to be due to the removal of DNA damage [42].

The enzyme oxoguanine glycosylase 1 (OGG1) removes the oxidatively damaged guanine bases namely 8-oxo-guanine (8oG) from the DNA. Studies supported that plant-derived liposome-encapsulated OGG1 enhances 8oG removal from human epidermal keratinocytes exposed to oxidative damage [43].

### *5.4. Chemical substances as photoprotectives*

Sunscreens are the first line of defense against UV irradiation. Topically applied sunscreens protect by absorbing or reflecting radiation at the skin surface. UV filters can be grouped into two broad categories based on their mechanism of action: chemical and physical UV blockers. Chemical sunscreens are generally (not inclusive) aromatic compounds conjugated with carbonyl group. This general structure allows the molecule to absorb high-energy ultraviolet rays and release the energy as lower energy rays and also, exposure of chemicals to UV light does not allow it to undergo significant structural change. This property makes the chemical substances to retain the UV absorbing potency without significant photodegradation, thereby preventing the skin from damaging effects of ultraviolet radiation. Typical example for chemical sun screen includes Oxybenzone, sulisobenzone, Octyl methoxy cinnamate etc., Chemical sunscreens are usually 'invisible' and hence cosmetically appealing, but UV absorption may activate them and they may in turn interact with cutaneous molecules, causing unwanted reactions.

Physical sunscreens contain particles that reflect photons away from the skin. Because they reflect visible as well as UV photons, they are often visible on the skin surface and therefore cosmetically undesirable for many users. It contains 10–100-nm inert particles such as zinc oxide or titanium dioxide [44] and they protect against both UVA and UVB irradiation. Their merit is that they are chemically inert and hence do not cause allergic sensitization.

Chemical UV blockers used in sunscreen formulation cause adverse effects in cases. For example the chemical blockers namely aminobenzoic acid and its esters, cinnamates and oxybenzone can cause contact dermatitis or photosensitivity reactions [45]. Thus, the photoprotectives from naturally occurring substances has gained considerable attention in recent years.

## 6. Botanicals as photoprotectives

The use of active photoprotectives from natural origin is very beneficial in combating the deleterious effects of UV rays. The important group of compounds acts as the UV blockers include phenolic acids, flavonoids and high molecular weight polyphenols [46, 47]. Naturally occurring phenolic acids include hydroxycinnamic acid and hydroxyl benzoic acid. High molecular weight polyphenols include condensed polymers of catechins or epicatechins and hydrolysable polymers of gallic or ellagic acids. Many flavonoids such as quercetin, luteolin and catechins are found to be better antioxidants as well as good UV blocker. The following section reviews the use of certain botanicals as sun screen against photoaging and prevention of skin cancer.

### *6.1. Camellia sinensis*

*Camellia sinensis* or green tea belongs to the family of theaceae and the most popular beverage consumed by people. It contains four major types of polyphenols: (-))-epicatechin (EC), (-))-epicatechin gallate (ECG), (-))-epigallocatechin (EGC), and (-))-epigallocatechin-3-gallate (EGCG). These polyphenolic compounds act as the potent antioxidants thereby scavenging the free radicals such as lipid-free radicals, superoxide radicals, hydroxyl radicals, hydrogen peroxide, and singlet oxygen. EGCG, which is the most abundant polyphenol present in green tea constituting approximately 40% of the total polyphenolic mixture and it is mainly responsible for its antioxidant activity.

Green tea polyphenols is capable of scavenging the reactive oxygen species generated by UV irradiation and prevent single stranded breaks in DNA [48]. Topical application of green tea polyphenols prior to the UV exposure resulted in reduced production of cyclobutane pyrimidine dimers (CPD) in the epidermis and dermis of human volunteers [49].This reduction in CPD formation is probably due to the protection of the DNA repair enzymes from inactivation of ROS and by the absorption of UV energy by the antioxidant EGCG ($\lambda_{max}$ 270-273 nm).

UVB radiation from sunlight induces minimal erythemal dose. This dose normally induces erythema, an influx of inflammatory cells, prostaglandin synthesis, myeloperoxidase activity, production of hydrogen peroxide and nitric oxide, both in epidermis and dermis, IL-10 positive cells, depletion of langerhans cells, alteration in endogenous antioxidant levels and induction of lipid peroxidation [50, 51]. Topical application of green tea polyphenols protects the skin against minimal erythema dose of UVB radiation.

The preventive effect of green tea against the photoaging and photocarcinogenesis was studied [32].They have topically applied or orally fed the polyphenolic fraction of green tea to mice for a shorter period of time that prevents

photocarcinogenesis. Long term oral feeding of green tea polyphenols to mice exposed chronically to UV radiation resulted in lowering the tumor burden in these animals compared with their control [52].

### *6.2. Silybum marianum*

*Silybum marianum* is also called as milk thistle which contains the phytocompounds namely silymarin, silybin, silidianin, silychristin and isosilybin. UV irradiation poses the depletion of catalase, induction of cyclooxygenase and ornithine decarboxylase in mouse models [53]. When silymarin is applied topically, it restores the normal conditions. The antioxidant and anti-inflammatory activity of this plant is conferred by the active principle Silybin. Besides, topical application of silymarin protects the skin against UV B induced formation of CPD in mouse skin [54] and infiltration of inflammatory leukocytes which are responsible for the generation of oxidative stress. The protective effect of silymarin in skin cancer was demonstrated [55]. Treatment with silymarin significantly reduces the number of hydrogen peroxide producing cells and inducible nitric oxide synthase expressing cells upon UV exposure. Silymarin exerts a dose dependant protective effect against UVB induced damage in human keratinocytes.

### *6.3. Curcuma longa*

The rhizome of turmeric has been extensively investigated for its cancer chemopreventive potential in many tumor model systems [56]. The active principle responsible for its biological activity is curcumin which possess anti-inflammatory and antioxidant properties [57, 58]. The active constituent of this plant inhibits chemically induced neoplastic lesions in skin probably via an antioxidant mechanism [59].

The beneficial effects of curcumin against the oxidation of lipids in the mouse skin were studied [60]. Curcumin inhibits the chain reaction of lipid peroxidation and arachidonic acid metabolism in mouse skin. They have observed the enhanced levels of non-enzymic antioxidant glutathione and the activity of enzyme glutathione-S-transferase in mouse skin after the topical application of curcumin.

The preventive effect of curcumin on human basal cell carcinoma was studied [61]. The exposure of human skin to UV radiation induces basal cell carcinoma. They have shown that curcumin induces apoptosis in human basal cell carcinoma cells in a dose and time dependant manner where curcumin mediates the programmed cell death. These studies suggest that curcumin may impart beneficial effect against the reponses of UV radiation in skin.

### *6.4. Vitis vinifera*

Grapes are the most commonly consumed fruits in the world and the richest source of polyphenols (60%-70%) present in grape seeds. The polyphenols located in grape seeds include flavan-3-ol derivatives, catechin, epicatechin and oligomeric proanthocyanidins [62]. Besides, the skin and seeds of grapes also contains the polyphenolic phytoalexin namely resveratrol (trans-3, 5, 4'-trihydroxystilbene). It is an excellent antioxidant with strong anti-inflammatory and antiproliferative activity.

The skin edema induced by UVB radiation was significantly reduced by the topical application of resveratrol caused a decrease in UVB-induced generation of hydrogen peroxide and infiltration of leukocytes [6]. The enzyme, ornithine decarboxylase responsible for tumor promotion was elevated after UVB radiation. The gene expression of this enzyme was significantly reduced by the topical application of resveratrol.

### *6.5. Spathodea campanulata*

It is an ornamental plant along the roadsides of tropical Africa. The plant stem bark was previously reported to have anti-hyperglycemic, antimalarial, antioxidant as well as wound healing properties [63]. The flowers of this plant have been known for its anti-solar activity [64]. They have found qualitatively that the presence of flavonoids in this plant absorbs the UV radiation strongly at 205 nm and 252 nm. Besides, the compounds showed moderate absorption in the range of 280-330 nm. The results showed that the strong to moderate absorption of this plant makes it as a better and safe alternative to harmful chemical sunscreens.

### *6.6. Caffeic and ferulic acids*

Caffeic acid (3, 4-dihydroxycinnamic acid) and ferulic acid (4-hydroxy-3-methoxycinnamic acid) are largely present in grains, fruits and vegetables where they are conjugated with sugars [65]. These two acids have been demonstrated to protect the phospholipid bilayer from UV mediated peroxidation and to react with nitrogen oxides [66]. Ferulic acid is more effective in protecting the human skin from UVB –induced erythema as compared to caffeic acid.

Ferulic acid is a potent ubiquitous plant antioxidant. The effect of ferulic acid in solutions of vitamin C and Vitamin E have been examined [67]. The incorporation of ferulic acid into topical solution of 15% L-ascorbic acid and 1% α-tocopherol improved the chemical stability of both the vitamins and doubled the photoprotection to solar radiation. The photoprotection was increased from 4-fold to 8-fold as measured by erythema and sunburn cell formation. They have also found that this antioxidant formulation efficiently reduced thymine dimer formation. So, this type of combination provides synergistic protection against oxidative stress in the skin.

### *6.7. Capparis spinosa extract*

It is used in traditional system of medicine for their diuretic, antihypertensive tonic effects [68] and in certain pathological conditions related to uncontrolled lipid peroxidation [69]. The whole extracts of the floral buttons of

this plant is reported to possess hydrating properties on dry, aged and undernourished skin [70]. The major constituents of lyophylised extract of *C.spinosa* have been identified as kaempferol, quercetin derivatives, caffeic, ferulic, *p*-cumaric and cinnamic acids through HPLC analysis. The lyophilized extract of *C.spinosa* shows significant antioxidant effect [71]. Topical application of this extract reduces UVB induced skin erythema in healthy human volunteers. Percentage inhibition of erythema by the *C.spinosa* and tocopherol acetate was compared. The results showed that *C.spinosa* (59.60%) is more effective than tocopherol acetate (22%). Thus, it could have a potential application as an ingredient in sunscreen formulations.

### *6.8. Ocimum basilicum*

It is native through the subtropics, especially throughout the Mediterranean region. It is widely used in India, since the Ayurveda and Unani medicinal systems use it for the treatment of several ailmets. The essential oil of basil is reported to have various properties like antioxidant [72] and anti-inflammatory activities [73]. The study was done on sunscreen activity of basil oil by the researchers [73]. They have prepared a sunscreen cream incorporated with basil oil and then analysed for Sun Protection Factor (SPF). The SPF value for sunscreen above 2 is considered as having good sunscreen activity. The sunscreen cream incorporated with basil oil was found near the range of good sunscreen activity and hence, *Ocimum basilicum* essential oil may be considered as a good candidate for sunscreen formulations.

### *6.9. Prunus amygdalus*

It is commercially known as almonds whose seeds are rich in polyphenolic compounds especially flavonoids and phenolic acids [75, 76]. The UVB protective property of this plant's skin extract was tested [77]. The mice was exposed to UVB radiation and analysed for changes in lipid peroxidation and glutathione levels. Topical application of formulated cream to mice after irradiation and 2 hrs prior to irradiation showed the decreased levels of lipid peroxidation and increased levels of glutathione. The significant change in the moisture content was observed in the formulation treated mice as compared to UV irradiated control. The results showed that topical application of cream formulation has significant antioxidant and antiphotoaging properties.

### *7.0. Pongamia pinnata*

It is used in the Ayurveda and siddha traditional medicine systems for the treatment of clinical lesions of skin. The bioflavonoids present in the flowers were reported for treating diabetes, various skin diseases and renal disorders [78]. The sunscreen activity of various solvent (Aq, methanol and acetone) extracts of leaves of this plant was compared with the standard sunscreen drug *p*-aminobenzoic acid [79]. The absorption spectra of various solvent extracts of this plant were measured using UV-visible spectrophotometer. The aqueous and methanol extracts were found to be highly effective in UVB region and moderately effective in UVA region. Acetone extract was found to greatly absorb exclusively in the UVA region. The extracts of leaves of this plant are showing good absorbance throughout the UV region including UVA region. Hence, *P.pinnata* extract can be used to formulate highly effective sunscreen preparations.

## 8.0. Evaluation parameters for photoprotectives

The sunscreen activity of plant actives can be measured by various biochemical parameters as discussed below.

### *8.1. Erythema determination*

Erythema is the slight redding of skin after 24 hours exposure to UV radiation. It is determined by investigating the histological, ultrastructural, biochemical and immunological effects of UV radiation on the skin and its relationship to photodamage and skin cancer.

### *8.2. Lipid damage determination*

UV radiation induces the formation of reactive oxygen species resulting in damage to various components of skin like lipids which results in degradation of free fatty acids and cholesterol. It is observed that UV exposure decreases lipid melting temperature of the mouse skin and that application of sunscreen prior to UV radiation would reduce this epidermal damage.

### *8.3. Sunburn cell count*

Sunburn cells are apoptotic keratinocytes observed in humans, mice, rabbits and guinea pigs. They absorb the lethal dose of UV radiation and acts as the indicator of acute photodamage.

### *8.4. SPF determination*

The sun protective activity of sunscreens was measured as Sun Protection Factor (SPF). This *in vitro* method measures the reduction of the irradiation by measuring the transmittance after passing through a film of product [80].

### *8.5. Quantification of UV induced DNA damage*

Exposure of UV radiation in the skin results in the generation DNA lesions. The DNA damage caused by UV radiation is estimated before and after the application of test formulation and analyse whether it has any protective effect on DNA lesions.

### *8.6. Skin viscoelasticity determination*

To determine the effects of treatment with the test formulation on skin firmness.

### *8.7. Wrinkle volume determination*

To determine the decrease in wrinkle volume after treatment with test formulation.

### *8.8. Epidermal cell turnover determination*

The decrease in corneocyte size is correlated with accelerated epidermal turnover.

## 9.0. Conclusion

The skin is constantly exposed to UV radiation resulting in the generation of free radicals posing damage to various cellular molecules. From long back, the use of chemical sunscreens as photoprotectives in the formulation is a common practice. Owing to their harmful effects, they are less desirable now a days. So the research was much focused in finding out the alternative source for synthetic sunscreens. The use of botanicals as photoprotectives has been gaining significant attention of researchers due to their safety, multiple biological actions on the skin and cost effectiveness. In addition, the plant actives are preferred over the chemical sunscreens due to the broad spectrum of UV absorption, protective effect against oxidative stress, inflammation and cancer. The additive properties exerted by the phytoconstituents of plant actives make them as the most suitable ingredient for sunscreen formulations. Concerted focus is now needed in the immediate future for exploring the herbals with potent anti-solar activity. Besides, the synergistic combination of plant actives is to be tested for obtaining the very good antisolar compounds for cosmetic formulations.


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