Herbal Photoprotective Formulations and their Evaluation

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Abstract: Photochemoprevention has become an important armamentarium in the fight against ultraviolet radiation induced damage to the skin. UV irradiation to skin results in erythema, edema, sunburn cells, hyperplasia, immunosuppression, photoaging and photocarcinogenesis. Various synthetic agents have been used as photoprotectives but they have limited use because of their potential toxicity in humans and their ability to interfere only in selected pathways of multistage process of carcinogenesis. Several botanical compounds have been shown to be antimutagenic, anticarcinogenic and non-toxic and have ability to exert striking inhibitory effects on a plethora of cellular events at various stages of carcinogenesis. Since multiple pathways are involved in photocarcinogenesis so mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreens could also be an effective approach for reducing photoaging and skin cancer in humans. The performance of sunscreen substances could be improved by modification of their chemical, physical and technological properties or by the use of novel carriers like liposomes, nanoparticles, phytosomes, transferosomes, nanospheres etc. The application of novel approaches can also improve its efficacy regarding continuous action of herbs on the human body. This review will focus on list of herbs, formulations and evaluation parameters of photoprotective herbs.

Keywords: Photoprotectives, evaluation, herbs, photoaging, herbal formulations.

INTRODUCTION

Long exposure of UV radiation increases the risk of skin cancer including basal cell and squamous cell carcinoma as well as malignant melanoma. There are also many specific diseases like phototoxic or photoallergic reactions, autoimmune diseases including lupus erythematous, idiopathic photodermatosis and varieties of skin cancers which are triggered or exacerbated by UV radiation exposure. Solar UVR is divided into three categories UV-C (200-280 nm), UV-B (280-320 nm) and UV-A (320-400 nm). UV-C is most biologically damaging but it is filtered out by ozone layer. Currently UV-B radiation and to a lesser extent UV-A are responsible for inducing skin cancer [1]. Among many UV-R damages, skin cancer is of greatest concern as its rates have been increasing. Ultraviolet radiation increases oxidative stress in skin cells by causing excessive generation of reactive oxygen species (ROS), leading to cancer initiation and promotion. The main histological features of photoaging include dermal damage with marked elastotic degenerative change, loss of collagen, reduction in number and size of fibroblasts, an increase in proteoglycans and a moderate mononuclear inflammatory cell infiltrate. Irregular epidermal thickening progressing in the later stages leads to dysplasia and cytological atypia [2]. Development of novel strategies to reduce the occurrence of skin cancer and delay the process of photoaging are highly desirable goals. Photoprotection could be achieved by the use of sunscreens, moisturizers, keratolytics and antioxidants.

There are so many synthetic sunscreen agents (Octabenzone, Octyl methoxyciinnan~ate, Benzophenone-3, Provatene, 2-Ethoxy Ethyl P-Methoxycinnamate, Sulisobenzone, Mexenone, Avobenzone, Dioxybenzone, 4-Dimethyl Amino Benzoic acid etc.) are available in form of photoprotectives with certain limitation, which restrict their use at cellular level. They have limited use because of their potential toxicity in humans and their ability to interfere only in selected pathways of the multistage process of carcinogenesis [3]. Botanical agents may work in various ways by stimulating the immune response, by inducing gene suppression, by detoxifying carcinogens, by blocking oxidative damage to DNA, by initiating selected signaling pathways or by other mechanisms. Thus many of these agents play multiple roles in ameliorating the process of carcinogenesis [3]. (Fig. **1**) Few examples include tea polyphenols, curcumin, silymarin, garlic compounds, apigenin, resveratrol, ginkgo biloba, beta- carotenoids, ascorbic acid etc. Compounds that can protect against UV- A and UV- B radiations both could be ideal photochemoprotective agents [3].

The active synthetic molecules are being used since years, but the research shows that these active molecules adversely affects on human skin *via* self inducing reactive oxygen species. Thus, to overcome such serious side effects of synthetic molecules, the research is now diverted towards natural biomaterials [4]. Naturally occurring antioxidants like alpha carotene, ascorbic acids, flavones, flavanone, have ability to donate electrons and stops free radical chain reactions and also showed broad spectrum UV absorption [5,6] It was reported that association of botanical extracts (bioflavonoids) and vitamins produce synergistic photoprotective effects in preventing increased erythema, transepidermal water loss and sunburn cell formation [7].

The herbs as biological additives in form of extracts are utilizing since long period of time in the cosmetic formulation and now scientific evidences proven that many plant

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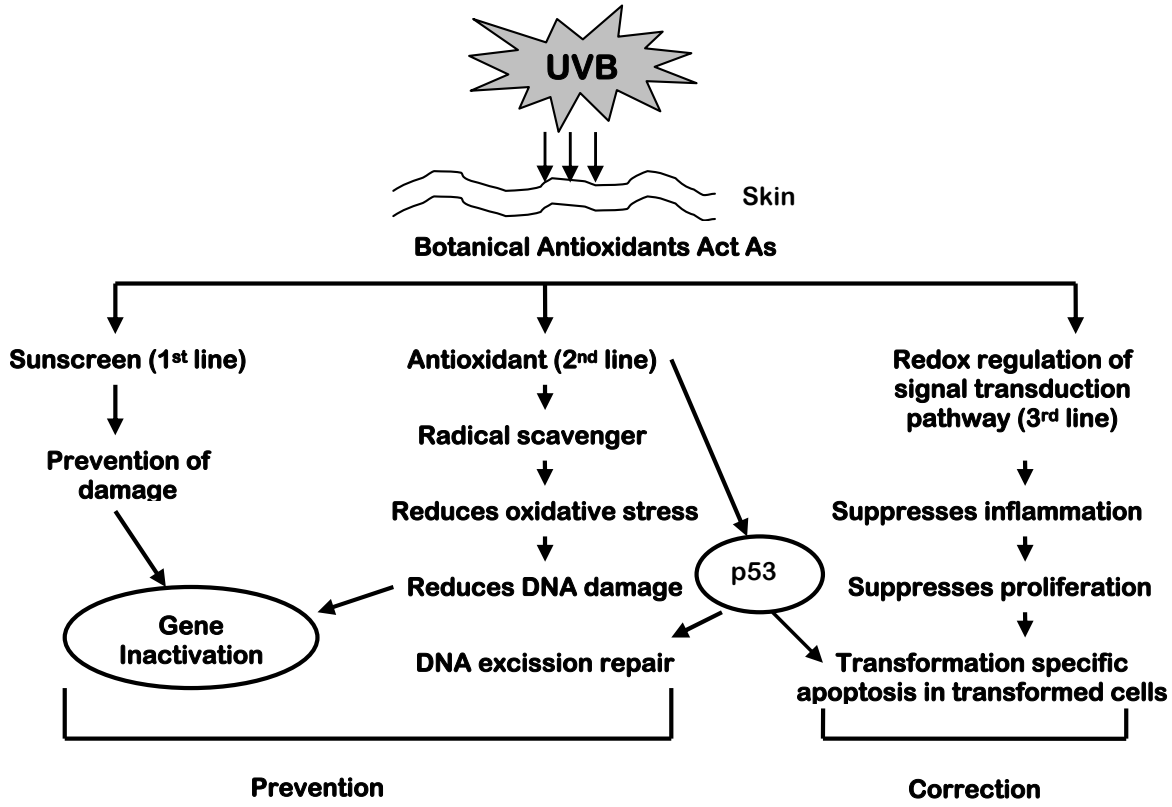

Fig. (1). Multiple pathway mechanism of botanical photoprotectives [3].

extracts showed their photo protective activity with significant improvement in enzymes like superoxidedismutase, catalase and total protein and ascorbic acid level [8]. Extracts of many plants, citrus fruits and leafy vegetables as source of ascorbic acid, vitamin E and phenolics compounds and enzymes possess the ability to reduce the oxidative damage. These oxidative damages ultimately reduce the protective enzyme level and imbalances the level of total protein and ascorbic acid and other antioxidants level in cells. The creams comprises such extracts could be utilized for the protection of photo induced intrinsic oxidative stress as well as structural alteration in skin [9].

In the series of natural photo protective agents, enzymes like superoxide dismutase (SOD), peroxidase and proteolytic enzymes have opened up new avenues to photoprotective reaction occurring within the cell. These chemical entities were first discovered in 19th century, where as use of enzymes in cosmetics have been advocated for many years [10]. Proteolytic enzymes like bromelaine, papain etc. has been used for skin peeling and smoothing [11]. These are utilized as efficient tool to reduce UV-induced erythema, which can also be thought of as free radical scavenging ability [12].

Naturally occurring herbal compounds such as phenolic acids, flavonoids and high molecular weight polyphenols are very useful for prevention of the adverse effects of UV radiation on the skin and evaluation of their clinical efficacy is awaited [13]. Adequate UV A protection and an appropriate testing method of UV A efficacy are the most pressing unmet needs [14]. Most commonly used herbal photoprotectives are listed in Table **1**, this table will be helpful in the selection of herbal constituent for formulation of photoprotective dosage form.

HISTOPATHOLOGICAL CHANGES OF PHOTOAGING

In skin aging two independent processes occur simultaneously (i) Chronological or intrinsic aging which affects the skin by slow and irreversible tissue degeneration and (ii) extrinsic aging or photoaging, which results from exposure to environmental factors including primarily ultraviolet (UV) radiation. In chronological aging skin becomes thin, dry, pale and exhibits fine wrinkles while, photoaging leads to marked cutaneous alterations characterized clinically by deep wrinkles, roughness, sallowness, mottled dyspigmentation, telangiectasia and a variety of benign and malignant neoplasms. In chronologically aged skin, the different characteristic steps of epidermal differentiation are preserved, however, based on a reduced keratinocyte proliferation, epidermal thinning is observed histologically. The dermis is also thin in aged skin, resulting from a reduction in the amount and organization of connective tissue. The extracellular matrix in the dermis is composed primarily of type I collagen, associated with type III collagen, elastin, proteoglycans and fibronectin. Sun-exposed sites usually display a reduction in mature type I collagen and an increase in collagen III-tocollagen I ratio. The histopathological hall-mark of photoaging is a massive accumulation of elastotic material in the upper and mid dermis also known as solar elastosis. Elastosis is due to accumulation of damaged elastin, the main component of elastic fibers, and is associated with degeneration of the surrounding collagen meshwork [15]. The histological effects can be well studied by Table **2**, it helps to decide the evaluation parameter (Table **3**).

Table 1. Herbal Constituents Used as Photoprotectives [2, 13]

Natural Photoprotective Agents	Source/Family	Components	Action/Uses
Tea	Green, black and oolong teas	Catechin, gallocatechin, gallic acid, kaempferol, myricintin	Potent antioxidant and can scavenge ROS
Curcumin	Root of *Curcuma longa* Zingiberacea	Curcumin (diferuloylmethane)	Antioxidant, anti-inflammatory
Silymarin	Milk thistle(*Silybum marianum)*	Silybin, silibinin, silidianin, silychristin, isosylibin	Antioxidant and anticarcinogenic
Genistein	Soy, red clover, ginkgo biloba, Greek oregano and Greek sage	Genistein	Antioxidant and anticarcinogenic
Garlic compounds	*Allium sativum*	Garlic sulphur compounds	Antioxidant and photochemopreventive
Apigenin	Vascular plants	5,7,4' – trihydroxyflavone	Anticarcinogenic
Resveratrol	Grapes, nuts, fruits	Trans-3'4'5'-trihydroxystilbine	Potent antioxidant, anti-inflammatory and antiproliferative.
Ginkgo biloba	*Ginkgoacea*	Quercetin,epicatechin, rutin,apigenin	Antioxidant, anti-inflammatory and anticarcinogenic
Carotenoids	Green plants, carrots,tomatoes etc.	β – carotene, lycopenes	Photoprotective
α- tocopherol	Plant oils	α- tocopherol	Photochemoprotective
L-ascorbic acid	Most fruits and vegetables	L-ascorbic acid	Antioxidant
Caffeic and ferulic acids	Vegetables, olives, olive oil	Caffeic and ferulic acids	Photochemoprotective

Table 2. Histopathological Effects of Photoaging [16]

Parameters	Changes in Photoaged Skin
Number of keratinocytes	Reduces
Number of fibroblasts	Reduces
Epidermal cells	Get flattened
Number of Langerhans cells and melanocytes	Decreases
Lymphatic channels of dermis	Dilates
Collagen and Elastic fibers	Reduces
Fat content	Loses and redistributes
Pigmented cells	Overactive causing blotches of hyperpigmentation

PHOTOPROTECTIVE FORMULATIONS

Commonly used cosmetics have low affinity to the skin and have little percutaneous absorption. The conventional cosmetics show little efficiency as a cosmetic. Novel technology has shown great potential for improving the effectiveness and efficiency of delivery of nutraceuticals and bioactive compounds. Recent advances in nanotechnology show their promise as potential cosmetics for poorly soluble, poorly absorbed and labile herbal extracts and phytochemicals. An innovative approach can improve both the aesthetics and performance of a cosmetic product. The application of novel approaches can also improve its efficacy regarding continuous action of herbs on the human body [17]. The formulation and selection of approach to be used for herbal cosmetics will depend upon purpose of preparation that is for topical or systemic effect, inherent properties of drug or herb extract such as hydrophilic or hydrophobic; surface characteristics of a system like permeability and charges; degree of biodegradability, biocompatibility, toxicity; release profile, size of the product required and antigenicity of the final product [18].

The novel approaches that could be used include Microemulsions [19-21], multiple emulsions [22-24], liposomes [25, 26], phytosomes [27-32], transfersomes [33-39] noe-

mulsions [40] multiple nanoemulsions [41], nanocrystals [42], cubosomes [43] and transdermal Delivery System [44].

EVALUATION PARAMETERS OF PHOTOPROTECTIVES

Erythema Determination

Erythema can be determined by investigating the histological , ultrastructural, biochemical and immunological effects of UV radiation on skin and its relationship to photodamage and skin cancer.

Method

Hairless mouse model could be used for photo protection studies. Prior to experimentation four hairless mice are anesthetized (using ketamine) and a rectangular area approximately 2.5 cm x 4 cm is marked off on the dorsal area of each animal. Then sunscreen formulations are applied and one mice is left as control mice which is untreated. After 15 min drying period, ultraviolet radiation are produced by a planar array of two UV- A 340 fluorescent lamps which stimulate UV radiation present in sunlight from wavelength 295 nm to 365 nm. Irradiance could be measured using Erythema UV A and UV B Intensity meter and minimal erythemal dose (MED) is calculated. One MED is defined as the amount of UV radiation necessary to cause a slight reddening of the skin 24 hr after exposure. For hairless mouse one MED is approximately 140 m J / cm ^{2} [45].

Lipid Damage Determination

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

Method

Immediately following UV exposure, mice are sacrificed by carbondioxide asphyxiation and full thickness, dorsal skin is removed by blunt dissection. The epidermis is separated from the full thickness skin by placing the skin dermis side down on filter paper saturated with 2.5 % (w/v) trypsin solution. After storage at 37 °C for 4 hr, epidermal sheets are gently lifted from the skin using forceps and then covered with fresh trypsin solution and stored at 37 °C for 1 hr. Remove trypsin from epidermal sheets with gentle rinsing using deionized water. Epidermal samples are stored at room temperature and 75 % relative humidity overnight. Then 10 mg samples of epidermis are sealed in aluminium pans and analyzed using Differential scanning colorimeter. Scan the samples at a heating rate of 5 °C per min from 10 °C to 80 °C with a temperature modulation of 0.759 °C per min and determine the lipid melting temperatures [45].

Edema Determination

Inflammation is acute biological response to UV radiation. The vasodilatation of cutaneous blood vessels results in erythema (reddening) and edema (swelling). The treatment reduces this edema thus it is also an evaluation parameter for photoprotectives.

Method

Immediately prior to irradiation, the thickness of skin folds, at the back of the neck of two sedated mice are measured using a spring loaded pocket thickness gauge. Three measurements are taken for each mouse and these data are used as the baseline. Twenty four hours post- UV exposure, mice are sacrificed by carbondioxide asphyxiation and skin fold thickness is measured. Edema is calculated by the difference in skin fold thickness between the baseline and post-UV exposure data [45].

Sunburn Cell Count

Objective

Sunburn cells are apoprotic keratinocytes that have absorbed a lethal dose of UV radiation and have been observed in humans, mice, rabbits and guinea pigs. They are indication of acute photodamage.

Method

After measurement of postexposure edema , UV exposed dorsal skin of each mouse is removed and fixed in 10 % formalin solution. two nonsequential sections of the skin are removed and mounted to a slide and Hematoxylin and Eosin stained. The stained samples are then subjected to microscopic examination (600X) and the number of sunburn cells per linear centimeter are calculated. Counts are done on 1.5 cm sections of interfollicular epidermis [45].

SPF Determination

SPF by definition is determined *in vivo* as the increase in exposure time required to induce erythema. Ratio of UV doses protected to unprotected gives the SPF. The *in vitro* method measures the reduction of the irradiation by measuring the transmittance after passing through a film of product [46].

Method

The most common *invitro technique* involves measuring the spectral transmittance at UV wavelengths from 280 nm to 400 nm. The *invitro* SPF is calculated as follows:

$$SPF = \frac{\int_{200\,nm}^{400\,nm} E_\lambda . S_\lambda . d_\lambda}{\int_{200\,nm}^{400\,nm} E_\lambda . S_\lambda . T_\lambda \, d_\lambda}$$

where,

E = CIE erythemal spectral effectiveness

S = solar spectral iradiance

T = spectral transmittance of the sample

Quantification of UV Induced DNA Damage

To estimate whether the application of the test formulation exhibits a protective effect on DNA lesions.

Table 3. Bioengineering Methods of Evaluation [47]

S.No.	Parameter of Evaluation	Instrument Used
1	Skin hydration	Corneometer
2	Skin color	Chromameter
3	Skin Elasticity	Cutometer
4	Barrier Function Assessment	Evaporimeter
5	Skin surface lipid measurement	Sebumeter
6	Skin roughness and wrinkle	Skin visiometer
7	Transepidermal water loss (TEWL)	Tewameter
8	Erythema index	Reflectance Spectrophotometry using Mexameter

Method

The test formulations are applied to volunteers twice daily. After a two week treatment three test areas are established on the inner forearms of every volunteer and marked with a stencil. Five and 24 hr prior to isolation of suction blister epidermis, two areas on every forearm are irradiated with 1.2 minimal erythemal dose solar simulated radiation. The third area is not irradiated and used as a control. One forearm is treated as described above; the colateral forearm is left untreated. To assess DNA damage by single-cell gel electrophoresis (Comet assay), epidermal keratinocytes are isolated from suction blister epidermis and embedded in low gelling agarose gels [47].

Skin Viscoelasticity Determination

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

Method

Two test areas are marked on the forearms of the volunteers. One test area is treated twice daily with the test formulation; the second is left untreated and used as a control. At the baseline visit and after 1, 2, and 4 weeks of regular use, skin viscoelasticity is determined using a cutometer. As an additional means of control for volunteer compliance, skin moisture is examined using a corneometer [47, 48].

Wrinkle Volume Determination

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

Method

Volunteers treated the assigned side of the face for 4 weeks twice daily with the test formulation. At the baseline visit and after 4 weeks of treatment, facial wrinkles in the crow's feet area are evaluated by means of *in vivo* topometry using phase shift rapid *in vivo* measuring of human skin phase induction PRIMOS. The PRIMOS system represents an established and widely used method to quantify effects on skin wrinkles [47].

Epidermal Cell Turnover Determination

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

Method

Three test areas are established on the inner forearms of each volunteer, and a stencil is used to mark these test areas. Two areas are treated with the test formulations, and one area is left untreated and served as additional control. Volunteers apply the test formulations twice daily (morning and evening) for 6 weeks. The size of single corneocytes is determined at the baseline visit and after 4, 5, and 6 weeks of treatment. Ten to 20 h after application of test samples, measurements are performed. The determination is carried out by image analysis of D-Squame® sheets [47].

CONCLUSION

The photoprotective formulation must be such that it is stable pharmaceutically and is able to reverse some of the characteristic signs of the chronological aging and photoaging in both skin compartments after topical applications. Various chemical photoprotectives are available but researches are going for use of more herbal constituents as photoprotectives and there is need of better evaluation parameters for those formulations. We have studied above evaluation parameters but more researches have to be carried out to validate simple, economic and rapid methods to carry out efficacy studies of herbal photoprotective formulations. According to the present scenario there is need for adoption of new alternative methods to ensure the safety of cosmetic preparations without or minimum use of animals.

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