



Photoprotective response of novel compound against UVB induced skin damage in animal model

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Abstract

The present research focuses to prepare novel bioactive compound from two different plant sources using classical biochemical Techniques and formulate a topical product with therapeutic efficiency against UV B induced skin damage. Catechin was purified from Green tea and impregnated in Aloe vera gel and used for topical application to evaluate its efficiency to protect skin against radiation. In the study, these two different bioactive substances were formulated as a single product which could be topically applied to treat skin damage induced by UVB radiation in animal model. The results revealed phytochemicals possess anti-oxidant activity that exerts free radical scavenger role to reduce toxic effect thereby promoting regeneration of epidermal cell growth. Antioxidant activities were decreased in the control batch but were increased in product treated experimental animals indicated Antioxidant activities such as GSH, catalase, SOD, GP, GR showed defense against oxidative stress. Results suggested beneficial effects of topical application of formulated herbal product in UVB induced skin damages. Based on these promising findings, the addition of tea polyphenols into topical products such as sunscreens and creams may enhance protection against the development of UVB-induced skin cancer by reducing the medical and economic burdens of this disease.

Keywords: aloe vera, anti-oxidant, catechin, skin damage, UVB.

Introduction

Environmental health entails grasping the effects of environment and human-made vulnerabilities/ hazards and insulation of human health and environmental systems from these hazards. The sun and stars send a constant stream of cosmic ^[1] radiation to Earth, much like a steady drizzle of rain. Differences in elevation, atmospheric conditions, and the Earth's magnetic field can change the amount (or dose) of cosmic radiations earth received. The stratospheric ozone layer is vitally important to life because it absorbs the biologically harmful UV radiation emitted from the sun. Ultraviolet radiation is part of the non-ionizing region of the electromagnetic spectrum which comprises approximately 8-9% of the total solar radiation.

UV-B (280-320 nm) radiation in sunlight is the major environmental factor causing skin cancer development. Skin cancer is one of the most common and widespread cancers despite technological and medicinal advancements and demands persistent attention. Skin cancer is basically damaged skin cells which keep on growing abnormally. Upon every exposure to UV radiation, hazardous material and corrosive chemicals, there is a fair chance of structural and functional alterations in skin cells, which in turn leads to skin cancer. With the passage of time, these damages become more serious and worse. Repetitive exposures further increase the cancer risk. At any age, many sun protection products help in prevention of skin cancer and melanoma ^[2]. Skin cancer is named after the cell type in which the cancer expands. There are three major types of skin cancer: Basal cell carcinoma, Squamous cell carcinoma, Melanoma. Basal and squamous cell carcinoma are non-melanoma cancers, but melanoma is most serious type of

skin cancer.

Camellia sinensis is the species of plant whose leaves and leaf buds are used to produce green tea has a reputed role in cancer prevention as tea catechins have been shown to inhibit tumour cell proliferation as well as destruction of leukaemia cells ^[3]. Green tea has a reputed role in cancer prevention as tea catechins have been shown to inhibit tumour cell proliferation as well as destruction of leukaemia cells. Laboratory studies on cultures of tumour cells and mice given carcinogenic chemicals, showed green tea's potential to inhibit cancer hemorrhage ^[4]. Component or ingredients which are derived from *Aloe barbadensis* leaf extract act as functional role primarily as skin-conditioning agents and are included in cosmetics only at low concentrations also used in a wide variety of cosmetic product. *Aloe vera* boosts immune function and destroys tumours ^[5].

The present work focused to study combination both of green tea catechins and Aloin purified from *Aloe barbadensis* leaf extract resulted semisolid paste can be considered a formulated efficient promising agent as therapeutic skin protective medicine after primarily experimental trial with UV-B induced skin damage on animal model.

Materials and Methods

Plant material

Aloe barbadensis miller plants were collected from in and around cumbum belonging to the district Theni of Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India. *Camellia sinensis* is an evergreen shrub,

the green tea plant belongs to the Theaceae family. Green tea was purchased from AVT Carady Goody Estate, Karadikuzhy, Idukki District.

Sample extraction

Aloe vera (*Aloe barbadensis* miller) leaves were collected and washed then peeled thick green epidermis layer to expose white pulp cut into small pieces weighed for about 100g extracted as follows: 100g of sample (Pulp) was homogenized with electric blender in 10mg calcium bicarbonate and 70-80% warm ethanol and filtered using whatmann no.1 filter paper and the recovered extracts were centrifuged at 5000 rpm for 10 minutes and the supernatant was discarded and the residues were preserved in refrigerated condition till further uses. Crude samples were subjected for phytochemical screening according to the standard methods as described by Trease and Evans. *Camellia sinensis* dried leaves were subjected for the application of heat during extraction process also causes the oxidation of water-soluble flavonols, which contribute to the yellow color of green tea infusions showed influence taste and aroma profiles. These extracts were used for further analysis.

UVB Irradiation

The source of irradiation was a lamp of 312 nm wavelength, 15 watts; VILBER-LOURMAT-FRANCE. Mice from both groups (exposure and treatment groups) were exposed to UVB light as per experimental design. This was done after making a window by shaving the mouse's back skin (cm). UV exposed experimental animals were treated with formulated product [combination of *Aloe vera* gel and green tea paste (10mg). Treatment was started from 8th day continued for another 10 days (18th day of the experiment) for the batch 2, 3, 4&5 subsequently maintained batch 1 as normal/ control. Body weight of the animal was noted daily in all groups during treatment period. Animal samples were collected and used for further analysis after completion of treatment period as per experimental scheduled.

Preparation of catechin

Camellia sinensis dried leaves extract were homogenized with electric blender then carried out centrifugation at 12000 rpm for 5 minutes. The upper liquid content was discarded further residue was washed and resuspension in 10mM Tris buffer (pH-8.0) resulting in crude extract. This extract was further centrifuged (10,000 for 10 min) to generating the soluble extract. A glass column tube was packed to a bed size of 2.5 by 18 cm with Sephadex G 25 and was equilibrated with 0.1M-phosphate buffer (pH-7.0). The sample was applied on the column subsequently the elution was done with same buffer. The fractions were monitored for the catechin estimation and the fractions corresponding to catechins were concentrated then the pooled fractions used for ammonium sulphate precipitation and were used for next purification step. In the same way soluble extract was loaded and the fractions were eluted by phosphate buffer (0.1M, pH 7.0) by using sephdex G50 column Chromatography [varnika pap 2]. In the first step of purification, solid ammonium sulfate was added to the

Extract at various concentrations and the mixture was stirred for 2 h and centrifuged at 10,000 rpm for 30 min. The eluted fraction with the highest activity of catechin was pooled and subjected on 15% SDS –PAGE (6) and stained with coomassive brilliant blue. The molecular size of the catechin was determined with known molecular standard catechin for confirmation of purified molecules as catechin in the present experiment.

Animal Model

After obtaining the approval from the ethical committee/College of thirty adult albino mice, BALB/c strain (15 males and 15 females) were used in this experiment, each of which weighing 25–35g. The animals were fed with standard pellet diet (Pico Lab) and provided with water ad libitum. Animals were housed in the animal house in Department of Biology, Madurai Kamaraj university, Madurai, under a controlled room temperature about 25°C and photo-periodicity of 12 hours light/dark system. Animals were assigned into five groups:

Experimental scheduled for UVB treatment

Batch 1 animals were not exposed to UVB and were used as control (n=5)

Batch 2 animals were exposed to UVB and not treated with formulated product

Batch 3 animals were exposed to UVB and not treated with standard drug

Batch 4 animals were exposed to UVB for 15 min / alternate days / for 10days and treated with formulated product

Batch 5 animals were exposed to UVB for 30 min / alternate days / for 10 days and treated with formulated product

Antioxidant Enzymes assays

Blood samples were obtained by cardiac puncture into sterile vacuum tubes with and without anticoagulant (EDTA). Serum was separated by centrifuge and stored at –20°C until use. Antioxidant enzymes of serum were measured according to the method of mention below. The procedure to estimate the reduced glutathione (GSH) level followed the method described by Ellman ^[7]. Catalase activity was determined spectrophotometrically by the method of Koroliuk *et al.* ^[8] SOD activity was determined at room temperature according to the method of Misra and Fridovich ^[9]. The assay of Glutathione peroxides was determined by the method of Flohe and Gunzler ^[10]. GST activity was measured in the supernatant fractions according to the method of Habig *et al.* ^[11].

Results and Discussion

In the study, plant based natural products are formulated with the combination of both *Camellia sinensis* and *Aloe vera* constituents for its therapeutic implications in health maintenance through the modulation of biological activities. Topical application of herbal product offered protection against detrimental effects of UVB on cutaneous immunity. Green tea Catechin impregnated in *Aloe vera* gel expressed beneficial effect against skin damage induced UVB exposure. Topical application of formulated herbal paste showed protective response against radiation.

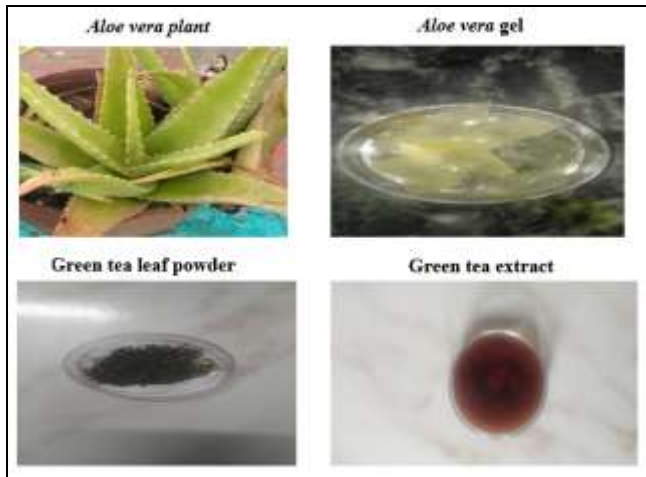


Fig 1: Preparation of herbal product from *Camellia sinensis* and *Aloe vera*

Catechins were extracted from Green tea then purified by using classical biochemical techniques. The separation of Catechin by column chromatography had yield high purity of product which were separated on SDS PAGE in order to get single compound. The column was activated to obtain a better separation results. Separation of catechins with the stationary phase in column chromatography was done by polyamide with 10% ethanoleluent and 95% ethanol. Column chromatographyse parathion with a poly amide stationary phase can separate analyzed compound based on the polarity. About 10% ethanol-water elution was used for separating catechins with a fairly high polarity, such as (+)-catechin(C),(-)-EC,(-)-EGC, and(+)-gallocatechin(GC). Whereas, elution with 95% ethanol was for desorption of EGCG and ECG from polyamide. The fractions were collected separately and analyzed catechin concentration in all the eluted samples. The estimated quanties has represented in figure 2 results revealed Maximum (2.51 microgram/ ml) amount found in 15 the eluted fraction subsequent reduction in the concentration were observed. The product was considered combination of both green tea and Aloe vera gel expressed it's efficiency to protect skin from radiation damage in animal trial experiments. The herbal paste might be used as photo protective agents to treat various skin disorder that in conjunction with established product of sun protection.

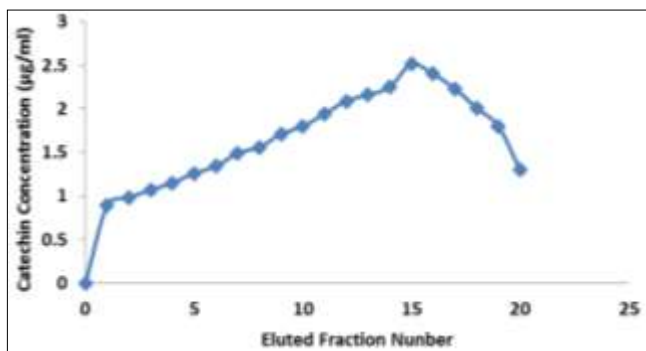


Fig 2: Estimation of Catechin in Green tea extract

Legends: Concentration of catechin found in green tea extract was mentioned in figure 2. X - Indicated different no fractions collected with eluted sample; Y -indicated content of catechin in each eluted sample in terms of µg / ml. Green tea catechins are polyphenols which are believed to

provide health benefits also marketed as health supplements and are studied for their potential effects on a variety of medical conditions. The polyphenol prepared from Green tea was separated to derive high yield of group known as catechins. The SDSPAGE analysis also showed a time dependent change in the mobility of the band corresponding to band in the lane, although that band moved down ward as shown in Figure3.

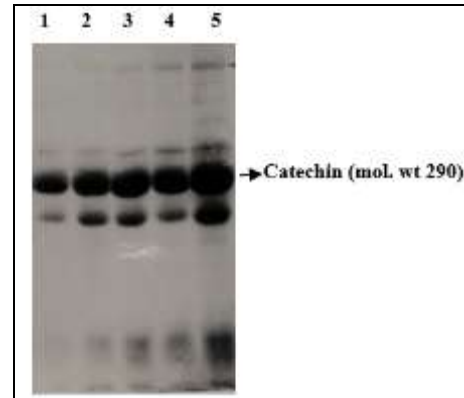


Fig 3: Separation of Catechin on 15% SDS PAGE

Legends: Separation of components from green tea extract was carried out on electrophoresis process according to size. 1 - 5 indicated no of Lanes in which samples loaded with various step involved in purification technical approach. Lane 1 showed separated band's from crude extracts; Lane 2 explained ammonium sulphate precipitated sample bands; Lane 3 indicated C18 passed sample bands; Lane 4 showed the samples purified through sephadex G 50 column and Lane 5 showed sample loaded after purification of column sephadex 25.

Each lane marked presences of Green tea composition and appeared gradually thin remarkable band at 5th lane as catechison gel. The increased mobility of the complex relative to that of BSA can be explained by the negative charge of the polyphenol moieties in the complexes. The formation of complexes were separated by stepwise purification process and analyzed by SDSPAGE. The catechin band alone eluted from gel then removed all gel based particles there covered fine yield of catechin as herbal based products could be considered as novel compound for drug formulation.



Fig 4: Formulated herbal product

Legends: Processed Aloe Vera gel 100mg mixed with 10 µl of catechin were agitated for 5 min then applied as topical paste/ Herbal drug for UVB exposed animal model in clinical trial.

The efficiency of cutaneous absorption is concerned with size of molecule. Consider topical treatment. The molecular weight of a compound must be fewer than 500 Dalton to allow skin absorption and that larger molecules cannot pass the corneal layer. The most commonly used pharmacological agents applied in topical dermatology are all under 500 Dalton; all known topical drugs used in transdermal drug-delivery systems are under 500 Dalton. However, formulated product used as topical drug compound has considered lesser than targeted size so that novel product applied to the skin surface penetrates through the follicular appendages, as well as through the unbroken stratum corneum enhances the therapeutic effect of phototherapy, showed well tolerated and can be widely used in cosmetic and dermatological practices.

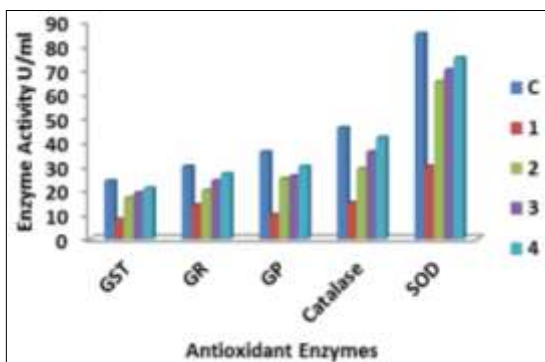


Fig 5: Antioxidant activities of serum sample

Legend: Measurement of antioxidants level in experimental animals. C -indicated control, 1- indicated animals were exposed to UVB and not treated with formulated product, 2 -indicated animals were exposed to UVB and not treated with standard drug (Fluorouracil), 3 - animals were exposed to UVB for 15 min and treated with formulated product, 4- indicated animals were exposed to UVB for 30 min and treated with formulated product.

Results in Figure: 5 explained remarkable increased antioxidant enzymes activities, such as total antioxidant capacity, catalase, GSH and SOD level in blood of experimentally UVB exposed herbal paste topically treated animals compared to control. ROS play an important role in the process of apoptosis, and include the superoxide, hydrogen peroxide, and hydroxyl radicals, which damage cell components including DNA, ultimately leading to cell death [12]. GSH has an important role in normal cell metabolism, i.e., inhibiting production of reactive oxygen species (ROS) and free radicals. Antioxidants are considered to be the first line of defense against oxidative stress, which suggests their usefulness in reducing the risk of oxidative damage during carcinogenesis. SOD and catalase are antioxidative enzymes that protect against ROS. Experiments have shown that green tea polyphenols (GTPs) enhanced intracellular antioxidant defense and promoted the down regulation of proapoptotic genes, and it could be used to fight against the dysfunction induced by UV radiation. These search advances in anti-inflammatory, antioxidant and anti-carcinogenic effects of GTPs against UVB irradiation were studied (data not discussed in this paper). Results suggested that the anticancer activity of novel herbal paste may be due to its antioxidant and antigenotoxic properties. Reactive oxygen species (ROS) have been shown to be responsible for inducing DNA damage after ultraviolet

exposure radiation (UVB). Combination of Aloe vera gel and catechin extracted from green tea, applied topically to the skin, delayed the onset of UV-induced skin cancer in mice. Since these formulations are reported to have a potent antioxidative effect on topical use *in vivo* studies. Antioxidant Potential of these two herbal products reduces the sign and delays the onset of UV-induced skin cancer in mice. Investigation revealed that topical application immediately after UVB radiation significantly delayed the onset and reduced the inflammation on skin also, regular application either retarded or reduced skin cancer formation in UV-irradiated mice. The epidermis of the skin is a keratinized and stratified squamous epithelium composed mainly of keratinocytes, cells whose proliferation and differentiation must be tightly regulated and coordinate [13]. Keratinocytes first attach to the basal membrane of the epidermis as undifferentiated precursor cells. The precursors migrate toward the surface of the epidermis and subsequently form its outer most layer. Both the normal development of keratinocytes and the onset of tumors involving these cells require complex proliferative/apoptotic events. The biochemical pathway underlying many of these events have been delineated through the use of transgenic and knockout mice. However, the molecular mechanisms underlying hair morphogenesis are not well understood.

Conclusion

The present finding confirmed the photoprotective efficiency of two different bioactive substances combination as a topical product in order to heal/protect skin infections in animal model exposed to ultraviolet radiations. Green tea catechin and Aloe vera gel are rich source of phytochemicals has potential health benefits. Experimental studies explained UV-induced production of reactive oxygen species and the resultant oxidative stress exposure plays an important role in photo carcinogenesis caused by UV, and reactive oxygen species are scavenged due to the application of herbal based product would be involved in inflammatory skin disorders, skin cancer formation, photo toxicity, and skin aging. Aloe Vera gel paste mixed with catechin formulates new products are photo protective in nature, and can be used as pharmacological agents for the prevention of solar UVB light induced skin disorders.

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