EVALUATION OF THE THERAPEUTIC EFFICACY AND SAFETY OF TOPICAL APPLICATION DERIVED FROM TOCOTRIENOL RICH FRACTIONS

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PENILAIAN KESAN EFFIKASI TERAPEUTIK DAN KESELAMATAN FRAKSI SEBATIAN TOKOTRIENOL SEBAGAI BAHAN TOPIKAL

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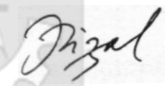
DECLARATION

I hereby declare that the work done in this thesis is my own except for quotations and summaries, which have been duly acknowledged.



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ABSTRACT

The therapeutic effects and safety of topical Tocotrienols Rich Fractions (TRF) in high concentration were studied using animal and human skin. Non-invasive methods were applied on human subjects using various skin bioengineering instruments. Undiluted TRF causes slight inflammation to the skin relative to sodium lauryl sulphate, which causes severe inflammation. Although the undiluted TRF is indicative of weak irritant, diluted TRF of 5% and below did not invoke any cutaneous reactions on human skin and 21 days of repeated exposure did not show any significant skin irritations. The 5% TRF preparation was found to be the maximum concentration with no cutaneous reactions. At 5% concentration it significantly increased 43% of skin hydration after single application and the skin hydration level was maintained for 90 minutes. No significant difference in transepidermal water loss was observed between SLS-damaged skin treated with 5% TRF and untreated area. The 5% TRF application did not significantly improve the recovery of a damaged-skin barrier. However, it significantly reduced the UVB-induced skin erythema of 1 Minimal Erythema Dose on area pre-treated with TRF. The protective effect percentage of 5% TRF was 36% against the SPF15 sunscreen which had protective percentage of 44%. Combination of 5% TRF with 1% Ascorbic Acid significantly reduce 43% of the skin erythema. The TRF preparation was also effective in recovering UVB-induced skin erythema. An immediate post-treated of 5% TRF on test area pre-exposed to UVB, may inhibit 28% of the skin erythema compared to positive control of 1% Indomethacin which inhibit 17% of the skin erythema. However, combination of 1% Ascorbic Acid and 5% TRF inhibited 31% of the erythema which is indicative of synergistic effects of the antioxidants. These findings showed that topical application of TRF at 5% did not induce adverse skin reactions but significantly increase the skin hydration and protects the skin against UVB induce skin damage. The TRF efficacies are much enhanced with the addition of other antioxidant.

ABSTRAK

Terdapat banyak jenis kesan tindakbalas kulit yang mungkin terjadi akibat kulit yang terdedah kepada bahan kimia, bahan cemar atau radiasi. Tindakbalas yang paling ketara ialah ekzema atau dermatitis sentuh yang hanya merupakan inflamasi kulit atau edema antara sel dalam lapisan epidermis yang disebabkan oleh interaksi sesuatu bahan kimia dan kulit. Terdapat dua cara dimana respon dermatitis sentuh boleh berlaku iaitu secara iritasi atau alahan. Iritasi dermatitis sentuh dan alahan dermatitis sentuh adalah dua masalah utama dalam bidang dermatologi. Tindakbalas alahan adalah khusus kepada agen tertentu, perlukan proses rangsangan terdahulu dan berlaku dalam populasi yang mampu dirangsang terlebih dahulu oleh sesuatu antigen. Manakala, tindakbalas iritasi adalah tidak khusus dan tidak memerlukan rangsangan terlebih dahulu. Kebanyakan bahan iritasi di persekitaran menghasilkan tindakbalas iritasi lewat atau secara kumulatif dan selalunya akan berakhir dengan pembentukan ekzema. Tokotrienol adalah vitamin larut lemak yang berkait rapat dengan tokoferol (Vitamin E). Kedua-dua tokoferol dan tokotrienol terkenal dengan ciri-ciri antioksidaan dan telah lama digunakan dalam persiapan topikal. Penggunaan tokotrienol pada peratusan yang tinggi mungkin akan meningkatkan kesan effikasinya tetapi ianya juga mungkin akan meningkatkan risiko mendapat dermatitis. Kajian keupayaan iritasi TRF dan penentuan peratus maksimum TRF tanpa tindakbalas kulit adalah penting bagi membolehkan penggunaan TRF secara selamat. Sebatian tokotrienol pada kepekatan 50% didapati memberi sedikit kesan inflamasi berbanding bahan rengsa kulit iaitu Sodium Lauryl Sulphate yang memberikan kesan inflamasi teruk pada kulit. Walaupun tokotrienol tersebut menunjukkan ciri bahan rengsa yang lemah, tokotrienol pada kepekatan yang kurang dan sama dengan 5% tidak mengaruhkan sebarang kesan rengsa kulit dan penggunaan berulang bahan ini juga tidak mendatangkan sebarang iritasi kulit. Tokotrienol pada kepekatan 5% adalah kepekatan maksimum dengan kesan rengsa kulit yang minima. Dari segi kesan effikasi, penggunaan kepekatan 5% tokotrienol telah menyebabkan peningkatan sebanyak 43% kelembapan kulit selepas sekali penggunaannya dan kesan ini kekal selama 90 minit. Penggunaan tokotrienol pada kepekatan 5% sebelum aruhan cahaya ultralembayung B, mampu mengurangkan kesan inflamasi kulit. Peratus perlindungan tokotrienol adalah 36% berbanding kawalan positif iaitu krim pelindung cahaya matahari SPF15 dimana peratus perlindungannya adalah sebanyak 44%. Kombinasi tokotrienol 5% dengan asid askorbik 1% mampu mengurangkan 43% inflamasi kulit. Tokotrienol 5% juga berupaya memulihkan kesan kemerahan kulit akibat aruhan cahaya ultralembayung B. Penggunaan tokotrienol 5% selepas aruhan cahaya ultralembayung B mampu memulihkan kemerahan kulit sebanyak 28% berbanding Indomethacin 1% yang merencatkan 17% kemerahan kulit. Walaubagaimanapun, penambahan asid askorbik 1% ke dalam tokotrienol mampu merencatkan 31% kesan tersebut. Penggunaan TRF secara topikal pada kepekatan 5% tidak mengaruhkan sebarang inflamasi kulit tetapi mampu meningkatkan kelembapan kulit dan melindung kulit dari aruhan bahaya cahaya ultralembayung matahari.

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LIST OF SYMBOLS & ABBREVIATIONS

α	Alpha
δ	Delta
γ	Gamma
%	Percentage
MCT	Medium Chain Triglyceride
O/W	Oil-in-Water
PII	Primary Irritation Index
W/O	Water-in-Oil
SC	Stratum Corneum
TRF	Tocotrienol Rich Fractions
SLS	Sodium Lauryl Sulphate or Sodium Dodecyl Sulphate
UV	Ultraviolet
UVB	Ultraviolet B
a.u	Arbitrary Units
s.d	Standard Deviation
N.S	Not Significant
S	Significant
ANOVA	Analysis of Variance
RPM	Revolutions Per Minutes
ASTM	American Society for Testing and Materials

CHAPTER I

INTRODUCTION

1.1 THE SKIN

Human skin is relatively a complex structure with adapted barrier to the environment. Administration of chemical agents to the skin surface has long been practiced, whether for healing or purely decorative or cosmetic purposes. Historically, the skin was thought to be totally impervious to exogenous chemicals. Topical administration such as drug was therefore restricted to those situations where skin lesions were apparent. However, it was then realized that the skin was really a semi permeable membrane rather than a totally exclusive barrier and this open new possibilities for local treatment of the skin.

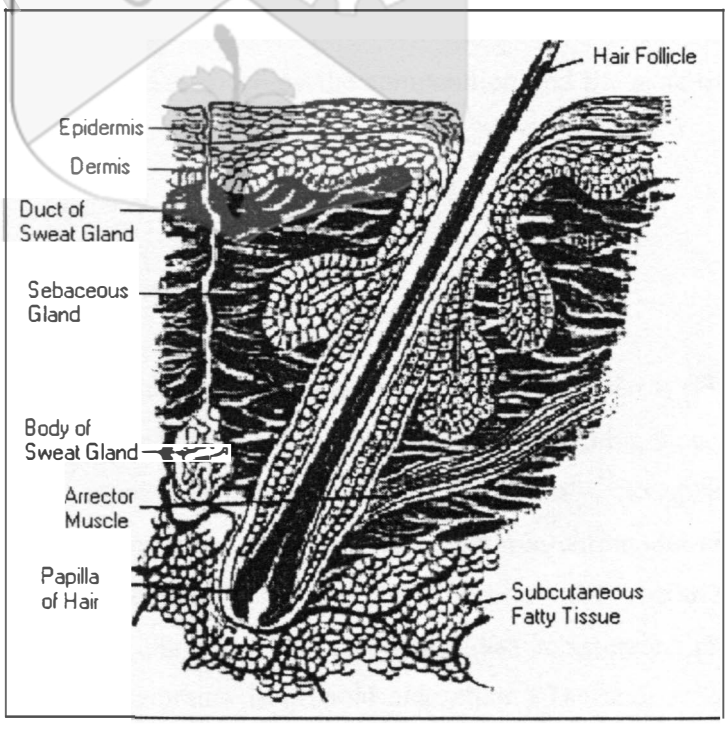

FIGURE 1.1 The Human Skin (Cross section)

The skin is the largest organ of the body with many functions – a multipurpose, nonhomogenous membrane with complex structure. It contains and protects the internal body organs and fluids from the surroundings and also control over the body temperature and humidity. The multilayered nature of human skin can be resolved into three distinct layers: epidermis, dermis and subcutaneous (Figure 1.1).

The cells of the basal layer divide and differentiate as they migrate upwards through the different layers of the epidermis to produce the Stratum Corneum (SC), the outer most layer of the skin. The SC or horny layer consists of several layers of flattened and keratinized dead cells (corneocytes), embedded in a lipid matrix. Terminal differentiation of keratinocytes is accompanied by the synthesis of specific lipids, which are organized in multilamellar bilayers (Elias & Menon, 1991). Most of these lipids are derived from precursors lipids upon their extrusion from the lamellar bodies into the intercellular spaces in the stratum compactum (Downing, 1992). The main barrier lipids in the stratum corneum are ceramides, cholesterol and free fatty acids. It is acknowledged that both the qualitative and quantitative composition of these barrier lipids, together with the hydrolipidic mixture partly covering the skin surface, play a role in the modulation of the transepidermal water loss (Schürer & Elias, 1991). Skin penetration of xenobiotics and the process of physiological skin desquamation are also being influenced by the composition and the state of the lipid lamellae.

1.2 TOCOTRIENOLS

Tocotrienols are fat-soluble vitamins related to the family of tocopherols (Figure 1.2). The term vitamin E is now considered to be a generic name describing bioactivities of both tocopherols and tocotrienols derivatives. Structurally, tocopherols and tocotrienols share some resemblance consisting of a common chromanol head and a side chain at the C-2 position. However, tocopherols and tocotrienols are distinguished by their side chains. While tocopherol has a saturated phytyl tail, tocotrienol possesses an unsaturated isoprenoid side chain (Theriault *et al.*, 1999).

Tocopherols and tocotrienols are further separated into individual compounds assigned by the Greek letter prefixes (α,β,δ and γ) depending on the number and position of methyl substitution on the chromanol ring.

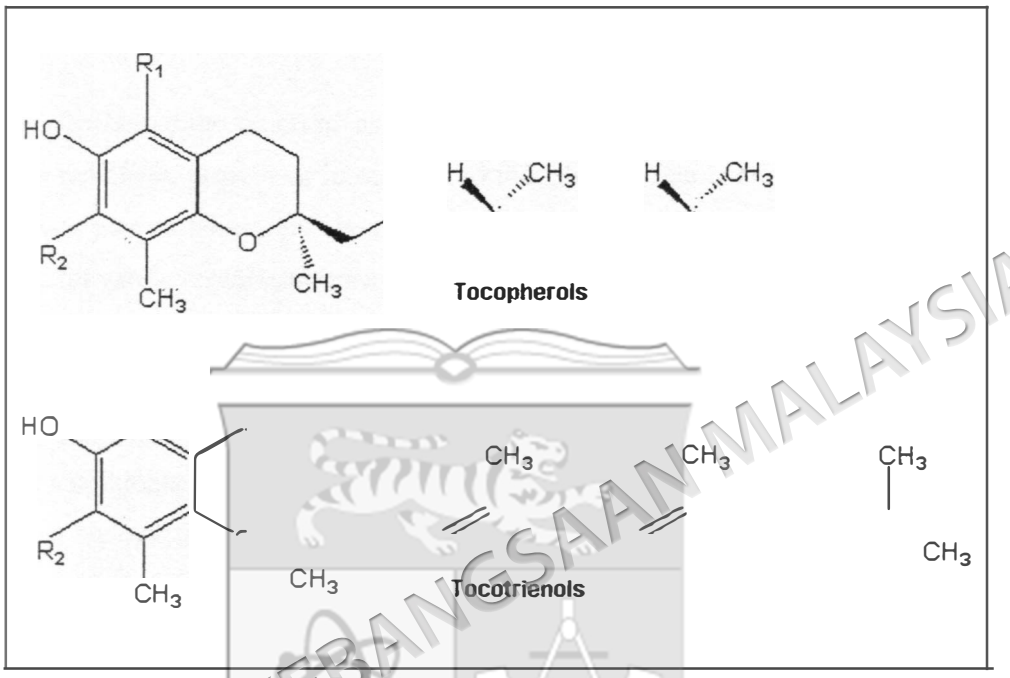

FIGURE 1.2. Vitamin E Structures: α, R_1 and R_2 = CH_3; β, R1 = CH3, R2 = H; γ, R1 = H, R2 = CH3; δ, R1 and R2 = H.

Vegetable oils, especially the seed oils, are rich sources of tocopherols. Vitamin E has traditionally been extracted from the residues of the soybean refining industry. Tocotrienols, on the other hand, are predominantly found in palm oil and in cereal oils such as barley and rice bran oils (Ong, 1995). With the emergence of palm oil as the second largest edible oil in the world markets, technological advances have been made enabling the extraction of tocotrienols from palm oil that is currently available commercially. The vitamin E content in crude palm oil ranges between 600 - 1000 parts per million (ppm) and is a mixture of tocopherols (18 - 22%) and tocotrienols (78 - 82%). The major tocotrienols occurring in palm oil are α-tocotrienol (22%), γ-tocotrienol (46%) and δ-tocotrienol (12%).

Both tocopherol and tocotrienol are well recognized for their antioxidant effect (Kamal-Eldin & Appleqvist, 1996). In general, antioxidants are suggested to reduce cardiovascular disease and cancer by inhibiting free radical damage. While tocopherols are generally present in nuts and common vegetable oil, tocotrienols are concentrated in cereal grains and certain vegetable oils (i.e. palm oil and rice bran oil).

Besides their function as antioxidant, tocotrienol has been shown to have unique functional properties in contrast to tocopherol. Tocotrienols have been shown to reduce plasma cholesterol levels, as well as other lipid and non-lipid related risk factors for cardiovascular diseases (Hood, 1996). It was also reported that tocotrienol possesses hypocholesterolemic activity (Qureshi *et al.*, 1991). The compound was also shown to display better anti-tumor activity than tocopherol (Carroll *et al.*, 1996). Contrary to popular believe, tocotrienol was observed in vitro to possess a remarkably higher antioxidant activity against lipid peroxidation than tocopherol (Serbinova *et al.*, 1991) in biological systems.

1.2.1 Antioxidant Systems in Skin

The stratum corneum is frequently exposed to a prooxidative environment including air pollutants, UV solar light, chemical oxidants and microorganisms. The skin counteracts the oxidative injury of the structural lipids and proteins by having enzymatic and nonenzymatic antioxidants. There are a variety of antioxidant mechanisms, which form an "antioxidative network" of interlinked components. Humans can synthesize antioxidants such as glutathione or ubiquinol-10 while other antioxidants have to be supplied by intake (e.g. vitamin C and E, and trace metals). The skin, as the outermost organ of the body is frequently and directly exposed to exogenous (e.g. UV exposure) and endogenous (e.g. inflammatory disorders) of reactive oxygen species (ROS) (Thiele *et al.*, 2000). The skin antioxidants can intervene these oxidative processes by:

(i) Scavenging free radicals
(ii) Scavenging lipid peroxyl radicals
(iii) Binding metal ions
(iv) Removing oxidatively damaged biomolecules

Excessive exposure to endogenous or exogenous ROS may disrupt the level of antioxidants. Such a disturbances of the prooxidant/antioxidant balance may results in oxidative damage to the biomolecules such as lipids, proteins and DNA.

Vitamin E is among the early recognized biological antioxidants, and its redox and free radical chemistry are well documented (Traber & Sies, 1996). Vitamin E acts as an antioxidant by scavenging free radicals. The major antioxidant role of vitamin E is generally considered to be the arrest of chain propagation by scavenging lipid peroxyl radicals. The initial oxidation product of tocopherol is the metastable vitamin E (tocopherol) chromanoxyl radical (Figure 2a), which can be reduced to tocopherol by co-antioxidants. The presence of several hydrophilic co-antioxidants such as ascorbate and glutathione can regenerate vitamin E from the tocopheroxyl radical and thus enhance the antioxidant capacity of vitamin E.

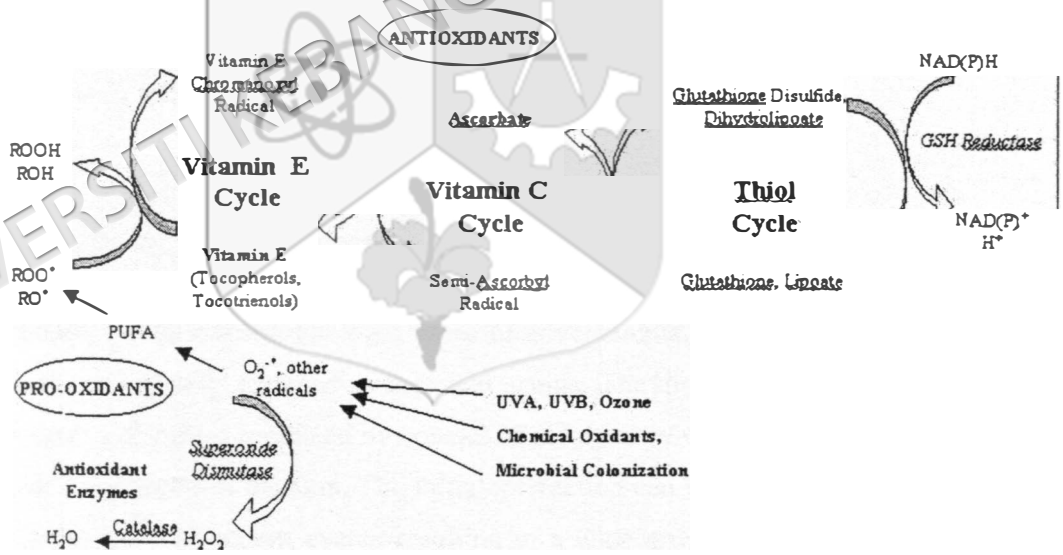

FIGURE 1.2.1. The antioxidant network in the skin: predominant role of tocopherol and tocotrienol and its recycling by co-antioxidants

In Figure 1.2.1, the oxidation of ascorbate results in the formation of dehydroascorbate via the ascorbyl radicals, which can be recycled back to ascorbate in the presence of thiols or reversibly decomposes to the unstable diketogulonic acid.

Although ascorbate is not able to scavenge lipophilic radicals directly, in the presence of vitamin E it is synergistically reduces lipid peroxy radicals by reacting with tocopheroxyl radicals. This leads to the generation of active tocopherol and tocotrienols was also reported to have similar actions (Thiele *et al.*, 2001).

Vitamin E in the stratum corneum plays a vital role in protection against UV light. It was reported that a single suberythemogenic dose of solar simulated UV (0.75 minimal erythema dose, MED), depleted human stratum corneum α-Tocopherol by almost 50% and murine stratum corneum α-Tocopherol by 85% (Thiele *et al.,* 1998). It was hypothesized that the high susceptibility of stratum corneum vitamin E to solar simulated UV due to a lack of co-antioxidants in the stratum corneum. In solar simulated UV-irradiated murine skin homogenates, ascorbate, the major hydrophilic co-antioxidant, can recycle photooxidized α-Tocopherol (Haratake *et al.,* 1997). However, in murine and human stratum corneum, the levels of ascorbate were very low as compared to epidermal and dermal tissue, which is not surprising since the stratum corneum is a very hydrophobic tissue. Thus, topical application of vitamin E (tocopherols and Tocotrienols) may offer an alternative way in providing the stratum corneum with antioxidants.

1.3 SAFETY ASSESSMENT

A number of different morphologic types of adverse cutaneous reactions may occur when skin is topically exposed to chemical agents. The diverse reactions may be due to disease or disorders produced by contact of the agent with any of the cells and non-cellular components of the skin. The initial interaction can lead to a variety of cell and biological agent dependent events resulting in a wide array of cutaneous responses. These include localized or generalized urticaria with and without anaphylaxis, which is mediated by mast cell activation; acneiform eruptions; alteration of melanocyte biology resulting in hypopigmentation or hyperpigmentation; interaction of the chemical agent with non-ionizing radiation to induce photosensitization of various forms and effects on dermal cells, supporting structures and dermal vessels that result in atrophy or purpura. However, the most common pathologic response pattern

resulting from skin contact with a chemical agent is eczema or contact dermatitis. Contact dermatitis is simply inflammation of the skin with spongiosis or intercellular edema of the epidermis that results from the interaction of a chemical and skin (Mark & DeLeo, 1991).

The response pattern of contact dermatitis is produced through one of two major pathways; irritant or allergic. Irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) are two of the most common problems in dermatology. An allergic reaction is agent specific, requires sensitization, and by definition occurs only in a genetically determined segment of the population capable of being sensitized to a given antigen. Conversely, irritant reactions are nonspecific and do not require sensitization. In clinical practice the two responses may be difficult to distinguish. Very potent irritants like acids can result in severe skin necrosis. Other, moderately strong irritants produce acute burning and stinging, with vesicular or bullous reactions. The vast majority of irritants in the environment, however produce delayed or cumulative irritant reactions that becomes evident in the form of eczema, which resembles the morphologic appearance of the eczematous allergic response.

The evaluation for irritant or allergic contact dermatitis can easily be carried out via common patch testing on human subjects. However, for all new topical raw materials, assessment of their irritation potential must be carried out via animal study prior to any biocompatibility study on human skin. The toxicity level of the compound must be determined before further study on human can be carried out. One of the most important animal studies is the Primary Skin Irritation, which determines the compound irritation potential on rabbit skin.

1.4 HYDRATION OF THE STRATUM CORNEUM

The primary function of epidermis is to produce the stratum corneum that protects the body from desiccation and invasion of chemicals or microbes. The present of adequate water in the stratum corneum is important for a well-moisturized skin. When our skin dehydrates, it loses its flexibility, suppleness and healthy appearance. Normal skin

contains 70% water of its total mass. In the stratum corneum this percentage is 10 to 15% and dehydration occurs when the water content of the stratum corneum drops below 10% (Batt & Fairhurst, 1986). The stratum corneum is therefore unique in comparison with other epithelial tissues and hydration depends on the balance between the supply and loss of water. The delicate balance between stratum corneum hydration and the relative humidity of the atmosphere is determined by three factors:

1. The present of natural moisturizing factors (NMFs), which are capable of attracting and retaining water molecules.
2. The water flux, coming from the deeper layers of the epidermis of the stratum corneum, which is known as transepidermal water loss (TEWL).
3. Evaporation of water from the stratum corneum surface due to climatological conditions.

Consequently, any disruption of the balance between loss and influx of water will immediately affect the appearance of the outer epidermis. Although the exact mechanism by which skin moisturization works at molecular level is yet to be revealed, it is generally accepted that the skin preserves water through intercellular occlusion (water permeability barrier) and cellular humectancy (natural moisturizing factors) (Rawlings *et al.*, 1994).

Lipids such as beeswax, lanolin and various oils may exert the moisturizing effects on the skin by forming an inert, epicutaneous occlusive membrane that prevent TEWL and evaporation of water from the stratum corneum. Topically applied lipids may also penetrate to the living cells of normal epidermis and enter into metabolism and significantly modify endogenous epidermal lipids (Downing, 1992).

Alpha-tocopherol was also shown to improve the moisturizing potential of a cosmetic emulsion when present in a concentration of 5% w/w. The short-term moisturizing study revealed no significant difference between vitamin E-enriched emulsion and the placebo treatment. The long-term moisturizing study (nine weeks) detected a significant increase in the performance of vitamin E treatment compared to placebo (Tamburic *et al.*, 1999).

1.5 SKIN BARRIER

The skin protects the body from excessive water loss and at the same time hinders the entrance of chemical substances and microorganisms. These exposures, including solvents, water and detergent eventually damages the stratum corneum. Exposure of normal skin to organic solvents or detergents will remove the lipids and affect the barrier integrity (Grubauer *et al.*, 1989a). Besides the depletion of lipids, the multiple lamellar structures between the corneocytes are disrupted (Man *et al.*, 1993). Studies have shown that topically application of lipids may restore the barrier function of damaged skin. Topically applied fish oil, borage oil, sunflower seed oil, canola oil, shea butter and unsaponifiable lipids from canola oil and shea butter have been used to treat skin irritations. However, only canola oil and its sterol-enriched fraction resulted in effective reduction of surfactant-irritated skin (Loden & Anderson, 1996).

Tocotrienol is known to inhibit free radical processes in cells, especially in against lipid peroxidation. Tocotrienol, both as TRF and purified γ-Tocotrienol was reported to reduce the synthesis of an eicosanoid, namely thromboxane B2 by inhibiting activated phospholipase A2 and also postulated to inhibit the transformation of arachidonic acid to prostaglandin by inactivating the cyclooxygenase gene (Theriault *et al.*, 1999). Based on these facts, it is interesting to determine the effects of TRF on sodium lauryl sulfate-induced skin irritation. Sodium Lauryl sulfate (SLS) is a known anionic surfactant used in many pharmaceutical vehicles, cosmetics and foods. It is the most widely utilized model for studying acute and cumulative irritation (Lee & Maibach, 1995).

In the case of irritant contact dermatitis, autooxidative tissue damage may occur and the role of free radicals and reactive oxygen species is important. The present study was undertaken to examine the efficacy of Tocotrienol Rich Fraction in recovering skin barrier function on SLS-induced irritated skin.

1.6 PHOTOPROTECTION

Ultraviolet irradiation from the sun has deleterious effects in human skin, including sunburn, immune suppression, cancer and premature aging (photoaging). Sunburn and immune suppression occur acutely in response to excessive exposure to the sun, whereas skin cancer and photoaging result from accumulated damage caused by repeated exposures. Photoaged skin is characterized by wrinkles, laxity, uneven pigmentation, brown spots and leathery appearance (Gilchrest & Yaar, 1992). Histologic and ultrastructural studies have shown that multiple exposures to ultraviolet irradiation may also lead to degradation of skin collagen and alteration of the connective tissue (Fisher *et al.,* 1997) and lipid abnormalities (Meguro *et al.*, 1999).

1.6.1 Photoprotective effects of vitamin E

Tocopherol, which is one of the biologically active components of vitamin E fraction, is known to play an essential role as antioxidant in animal cell membranes and also in the defense of eye and skin cells against light-induced skin diseases. Vitamin E absorbs strongly in the UV-B region (280–320nm) of sunlight and therefore can act as a photoprotective compound in skin cell membranes against UV-induced erythema (Jurkiewicz *et.al.*, 1995). It has been shown that topically applied tocopherol and its derivative reduce the severity of erythema, edema and skin sensitivity associated with UV-B sunburn (Trevithick *et al.*, 1992). *In vitro* studies with Chinese hamster V-79 cells also showed that tocopherol protects the cells from UV-B induced cytotoxicity, possibly through its ability to scavenge free radicals (Sugiyama *et al.*, 1992).

It is well known that topically applied Tocopherol or Vitamin E inhibits UVB photodamage. However, it was also reported that Tocopherol undergoes rapid photooxidation by UVB in vitro. The major product fraction formed in UVB irradiated mice treated topically with Tocopherol contained a Tocopherol dihydroxy dimer. Thus, topically applied Tocopherol is extensively oxidized in skin and suggests that Tocopherol photoproducts may involve in the observed effects of topically applied vitamin E in UVB irradiated skin (Kramer-Stickland *et al.*, 1999). This problem may be avoided by addition of a more photostable derivative of Tocopherol, i.e. Tocopherol-acetate. Although it is biologically inactive, this derivative is slowly

metabolized to Tocopherol in the skin (Henegouwen *et al.*, 1995; Norkus *et al.*, 1993). Several other Tocopherol derivatives have been shown to have the same activities, e.g. Tocopheryl Sorbate (Jurkiewicz *et al.*, 1995). The resulting small amount of extra Tocopherol may exert its photoprotective activity. However, it was also shown that only minute amount of Tocopherol acetate was converted to its active form and the bioconversion was very slow (Henegouwen *et al.*, 1995).

The skin possesses a wide range of interlinked antioxidant defense mechanisms to protect itself from damage by UV-induced reactive oxygen species. However, the capacity of these systems is not unlimited, and they can be overwhelmed by excessive exposure to UV. An interesting strategy to provide photoprotection to the skin would be to support or enhance these endogenous systems. Supplementation of non-enzymatic antioxidants such as glutathione, tocopherol, ascorbate and β-carotene was also found to be very effective in photoprotection (Muizzuddin *et al.*, 1999; Steenvoorden & Henegouwen, 1997). Many of these supplements were topical applications and not oral supplementation. It was found that oral supplements of Vitamin E to be ineffective. No clinical or histologic difference in the response to UVB could be detected between the placebo and vitamin E-supplemented groups where healthy human subjects were supplemented with their usual diet daily with 400 IU of oral vitamin E (Werninghaus *et al.*, 1994). Topically application in combinations of vitamins or of melatonins with vitamins was also found to enhance the photoprotective response. Vitamin E (Tocopherol), Vitamin C (Ascorbic Acid) and melatonin (N-acetyl-5-methoxytyptamine) were topically applied, alone or in combinations, 30 minutes before ultraviolet-irradiation of the skin (Dreher *et al.*, 1998).

Recent studies with hairless mice that were fed with diet enriched with tocotrienol rich fraction indicated that tocotrienol might be more effective than tocopherol against UV light-induced oxidative stress (Traber *et al.*, 1997). It is not possible to enrich the tissues to a large extent with tocotrienols by dietary means, probably due to preferential enrichment of the plasma with tocopherol. Therefore, topical application of tocotrienol may provide an efficient way of enriching the skin with vitamin E that has better antioxidative activity than tocopherol. In another study

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The skin possesses a wide range of interlinked antioxidant defense mechanisms to protect itself from damage by UV-induced reactive oxygen species. However, the capacity of these systems is not unlimited, and they can be overwhelmed by excessive exposure to UV. An interesting strategy to provide photoprotection to the skin would be to support or enhance these endogenous systems. Supplementation of non-enzymatic antioxidants such as glutathione, tocopherol, ascorbate and β-carotene was also found to be very effective in photoprotection (Muizzuddin *et al.*, 1999; Steenvoorden & Henegouwen, 1997). Many of these supplements were topical applications and not oral supplementation. It was found that oral supplements of Vitamin E to be ineffective. No clinical or histologic difference in the response to UVB could be detected between the placebo and vitamin E-supplemented groups where healthy human subjects were supplemented with their usual diet daily with 400 IU of oral vitamin E (Werninghaus *et al.*, 1994). Topically application in combinations of vitamins or of melatonins with vitamins was also found to enhance the photoprotective response. Vitamin E (Tocopherol), Vitamin C (Ascorbic Acid) and melatonin (N-acetyl-5-methoxytyptamine) were topically applied, alone or in combinations, 30 minutes before ultraviolet-irradiation of the skin (Dreher *et al.*, 1998).

Recent studies with hairless mice that were fed with diet enriched with tocotrienol rich fraction indicated that tocotrienol might be more effective than tocopherol against UV light-induced oxidative stress (Traber *et al.*, 1997). It is not possible to enrich the tissues to a large extent with tocotrienols by dietary means, probably due to preferential enrichment of the plasma with tocopherol. Therefore, topical application of tocotrienol may provide an efficient way of enriching the skin with vitamin E that has better antioxidative activity than tocopherol. In another study

with hairless mice skin, indicated that topically applied tocotrienol rich fraction readily penetrate the skin and present in high levels far above baseline and are consumed during UV-light induced oxidative stress (Weber *et al.*, 1997). Numerous topical studies demonstrated significantly reduced acute skin responses when vitamin E was applied before ultraviolet irradiation, such as erythema and edema, sunburn cell formation, lipid peroxidation, DNA-adduct formation, and immunosuppression. However, most studies used animal models while only few studies exist demonstrating photoprotection by topical application of vitamin E in humans.

It is well known that the untreated skin will react upon exposure to UVB irradiation by developing erythema on the respective site. However the exact mechanism is still under investigation. It is also known that UVB erythema reactions resulted in an increased in local prostaglandin formation (Black *et al.*, 1978). Prostaglandin have profound physiological effects at extremely low concentrations where they mediate the inflammatory response, production of pain and fever, regulation of blood pressure, induction of blood clotting, control of several reproductive functions such as induction of labor and regulation of sleep/wake cycle (Voet & Voet, 1990). In human, the most important precursor is arachidonic acid. Metabolism of arachidonic acid resulted in the formation of prostaglandins, prostacyclins and thromboxanes. The biosynthesis of these eicosanoids was regulated by the enzyme synthase. The first step in the metabolism of arachidonic acid was catalyzed by Prostaglandin Endoperoxide Synthase. The activity of this enzyme was found to be suppressed or inhibited by topical Non-Steroidal Anti-Inflammatory Drugs (NSAID) e.g. Indomethacin (Black *et al.*, 1978) resulting in reduced UVB-induced skin erythema. The ability of indomethacin to inhibit erythema in response to UVB irradiation suggested the involvement of prostaglandin activity in the observed reactions. In this study, topical application of TRF after UVB irradiation and its subsequent reactions may indicate whether or not TRF can reduce the UVB-induced erythema and to compare its efficacy against Indomethacin.

1.7 NON-INVASIVE SKIN BIOENGINEERING TECHNIQUES

1.7.1 Hydration State of the Stratum Corneum: Capacitance Measurement

In this study, skin hydration was determined by the Corneometer CM820 (*Courage + Khazaka Electronic GmbH, Köln, Germany*). The instrument measures the electrical capacitance of the SC. The measuring probe works as a condenser, which is influenced by a change of any dielectric constant of any material with which it comes into contact. Since water has a high dielectric constant (81) compared to other materials (mainly > 7), an increase in the water content will raise capacitance values. These values are displayed by the instrument in non-dimensional arbitrary units (a.u) that are not linearly correlated with the hydration state of the skin (Courage, 1994; Berardesca, 1997). In addition, readings may be affected by the presence of salts and ions on the skin (Barel & Clarys, 1995). It should be noted that the instrument only provides an indirect measure of SC hydration rather than absolute values of weight water per volume SC.

1.7.2 Transepidermal water loss (TEWL) measurement

Through the upper layers of the epidermis, a passive diffusion of water coming from the deeper skin layers to the outside, takes place (Kompaore *et al.*, 1991). Below the thermal sweating threshold, this diffusion is different from the water excretion of the sweat glands and is called the transepidermal water loss (TEWL) or skin surface vapour loss (Batt & Fairhurst, 1986; Rogiers, 2001). TEWL is a sensitive indicator of an altered stratum corneum barrier function and is elevated when the barrier is perturbed by physical removal (tape stripping) of the stratum corneum or by chemical treatment with organics or aggressive surfactants (Kompaore *et al.*, 1991). Even subtle changes in barrier integrity can be detected by this non-invasive measuring technique offering the possibility to quantify skin responses following exposure to irritants (Kompaore *et al.*, 1991). TEWL is even more sensitive indicator of skin damage than the visual assessment of irritation (Rogiers *et al.*, 1999).

The method currently used to measure TEWL is based on the estimation of the water gradient through an open chamber. This open chamber method provides continuous measurements in ambient air, with little alterations of the microclimate

overlying the skin surface. Once the probe is applied onto the skin surface, the water vapour pressure gradient is measured by two pairs of a combined hygrosensors, present at two different heights above the skin surface. This gradient is approximately constant and proportional to the amount of water vapour passing through this zone per unit time and area by the evaporation from skin surface, i.e. TEWL ($g/m^2.h$) (Pinnagoda & Tupker, 1995). A commercially available device working according this gradient principle is the Tewameter TM210 (*Courage+Khazaka electronic GmbH, Koln, Germany*), which has been used during our experimental work. Important factors to consider during TEWL measurements are air convection, room temperature and ambient humidity. In a relaxed state, the subjects need to rest for at least 15-30 minutes in a temperature-controlled room with a relative humidity of 45-55% and a room temperature of 20-22°C, the latter should not being higher in order to avoid sweating. TEWL measurements are registered over a given amount of time (usually 30 minutes), in order to have average values and not single readings. Mean values are recorded.

1.7.3 Colorimetric measurement

Since the assessment of colour is mainly a matter of perception and subjective interpretation, a colour-control system based on objective measurements and numerical values, is necessary, e.g. reflectance colorimetry (Fullerton *et al*., 1996). In particular, the Minolta Chroma meter (*Minolta, Osaka, Japan*) became quite popular during the last years. The principle is based on the tristimulus analysis of reflected flash light and expresses colours in a three dimentional system with green-red (a*), blue-yellow (b*) and brightness (L*) axes. Furthermore, this technique allows the gathering of information otherwise not detectable by the eye (Berardesca & Distante, 1998).

There are many instrumental evaluation of erythema due to irritants. The use of reflectance spectroscopy instrument, i.e. Chromameter by several workers had correlated the degree of erythema (a* value) with the concentration of the irritant applied from 0.125 to 3% sodium lauryl Sulphate. Differences in red color reflectance and change in total skin color were highly significantly correlated with the applied dose of SLS ($p < 0.0001$) and to visual scores (Wilhelm & Maibach, 1989). Other

researchers had also used reflectance spectroscopy instrument to determine the minimal erythema dose in UV-exposed skin (Lock-Andersen *et al.*, 1999).

In this study, the Chromameter CR300 is used. The light is illuminated from all directions at an almost constant luminance and only the light reflected perpendicularly to the skin surface is accepted for measurement. In the colorimetric assessment in this thesis, the parameter chroma a* from the L*a*b* colour space is used which reflects the green-red axis. The numerical values are expressed in arbitrary units (a.u) and the measurement conditions remain the same, guaranteeing accurate and reproducible measurements. Before every measurement session, calibration is performed using a standard white plate provided by the manufacturer.

1.8 OBJECTIVES

1.8.1 General Objective

To determine a maximum TRF concentration in topical preparation that exhibits minimal cutaneous reactions and evaluates the therapeutic efficacies. Study 1 to study 3 are safety assessments done to determine the acceptable concentration of TRF that will be used in study 4 to study 6 which aims at determining therapeutic effects of the acceptable concentration of TRF.

1.8.2 Specific Objectives

1.8.2.1 Study 1

To determine the potential skin irritation reaction of undiluted Tocotrienol Rich Fractions on rabbits' skin

1.8.2.2 Study 2

To determine the maximum concentration of TRF in topical preparation that does not show evidence of skin irritations in human subjects.

1.8.2.3 Study 3

To determine the cumulative irritation potential of TRF concentrations that showed no indication of skin irritation on human subjects.

1.8.2.4 Study 4

To evaluate the moisturizing effects of topical TRF preparations on skin against vehicle-treated and untreated skin.

1.8.2.5 Study 5

To evaluate the barrier recovery of SLS-irritated skin treated with topical TRF preparations against skin treated with base emulsion and untreated skin.

1.8.2.6 Study 6

To evaluate the photoprotective effects of skin pre-treated with topical TRF against ultraviolet B irradiation against skin treated with base emulsion and untreated skin.

1.8.2.7 Study 7

To evaluate the inhibition of photodamage on skin pre-treated with topical TRF preceeding to UVB exposure against skin pre-treated with vehicle.

1.9 NULL HYPOTHESES

1.9.1 Null Hypothesis 1

Undiluted Tocotrienol Rich Fraction does not induce any skin irritation reaction.

1.9.2 Null Hypothesis 2

There are no safe concentrations of TRF in topical preparations that would not cause any cutaneous reaction.

1.9.3 Null Hypothesis 3

Lower TRF concentrations showed cumulative irritation effects on human subjects.

1.9.4 Null Hypothesis 4

Topical application of TRF preparation on skin does not elevate the skin moisture.

1.9.5 Null Hypothesis 5

Topical application of TRF preparation on SLS-irritated skin does not improve the skin barrier.

1.9.6 Null Hypothesis 6

Topical application of TRF preparation on skin does not protect the skin from UVB damage.

1.9.7 Null Hypothesis 7

Topical application of TRF preparation on skin does not improve the skin photodamage.

REFERENCES

Aeling, J.L., Panagotacos, P.J. & Andreozzi, R.J. 1973. Allergic contact dermatitis to vitamin E aerosol deodorant. *Arch. Dermatol.,* **108**: 579-580.

Andersen, P. H. 1997 Reflectance spectroscopic analysis of selected experimental dermatological models with emphasis on cutaneous vascular reactions. *Skin Res. Technol.* **3** (1): 23-27.

A.S.T.M. 1998 *Standard Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation.* A.S.T.M Designation F 719 – 81. Reapproved 1996. pp. 178-179. Philadelphia: ASTM Intl.

Barel, A. O. & P. Clarys. 1995. Comparison of methods for measurement of transepidermal water loss. In. Serup J. & Jemec G.B.E. (eds.). *Handbook of Non-Invasive Methods and the Skin.* pp. 179-184. Boca Raton: CRC Press Inc.

Basketteer, D. A. 1999. *Intensive Course of Dermatocosmetic Sciences.* pp. 381-402. Brussel: Vrije University Publisher.

Batt, M. & Fairhurst E. 1986. Hydration of the stratum corneum. *Int. J. Cosmet. Sci.* **8**: 253-264.

Berardesca, E. 1997. EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. *Skin Res. Technol.*, **3**: 126-132

Berardesca, E. & Distante F. 1998. Bioengineering of the skin. *SÖFW J.* **124**: 630-632.

Berger, R. S. & Bowman, J. P. 1982. A reappraisal of the 21-day cumulative irritation test in man, *J. Toxicology Cut. and Ocular Toxicol.* **2**: 109-115.

Bisset, D. L., Chatterjee R. & Hannon D. P. 1990. Photoprotective effect of superoxide scavenging antioxidants against ultraviolet radiation induced chronic skin damage in the hairless mouse. *Photodermatol. Photoimmunol. Photomed.* 7: 52-62.

Black, A. K., Greaves M. W., Hensby C. N. & Plummer N. A. 1978. Increased prostaglandins E_2 and $F_{2\alpha}$ in human skin at 6 and 24 H after ultraviolet B irradiation (290-320nm). *Br. J. Clin. Pharma.*,**5**: 431-436.

Black, A. K., Greaves M. W., Hensby C. N., Plummer N. A. & Warin A. P. 1978. The effects of Indomethacin on arachidonic acid and prostaglandin E_2 and $F_{2\alpha}$ levels in human skin 24 H after UVB and UVC irradiation. *Br. J. Clin. Pharma.*, **6**: 261-266.

Black, A. K., Greaves M. W., Hensby C. N., Plummer N. A. & Eady R. A. J. 1977. A new method for recovery of exudates from normal and inflamed human skin. *Clin. Exp. Dermatol.*, **2**: 209-216.

Bonina, F., Saija A., Tomaino A., Locascio R., Rapisarda P. & Dederen J. C. 1998. *In vitro* and *in vivo* photoprotective effect of a red orange extract. *Int. J. Cosm. Sci.* **20**: 331-342.

Carroll K.K., Cuthrie N., Nesaratnam K., Gapor A., & Chambers A. F. 1996. Anti-cancer properties of Tocotrienols from palm oil. In. Ong, A.S.H., Niki E. & Packer L. (eds.). *Nutrition, Lipids, Health and disease*.pp. 117-121. Champaign, IL: AOCS Press.

Courage, W. 1994. Hardware and measuring principles: Corneometer. In. Elsner, P., Berardesca, E. & Maibach, H. I. (eds.) *Bioengineering of the Skin: Water and the Stratum Corneum.* pp. 171-175. Boca Raton: CRC Press Inc.

Darr, D. & Fridovich I. 1994. Free radicals in cutaneous biology. *J. Invest. Dermatol.* **102**: 671-675.

De Boer, E. M & Bruynzeel D. P. 1995. Irritancy. In. Berardesca, E., Elsner, P. & Maibach, H. I. (eds.). *Bioengineering of the skin: Cutaneous Blood Flow and Erythema.* pp. 199-215. Boca Raton: CRC Press Inc.

De Paepe, K., Derde M. P., Roseeuw D. & Rogiers V. 2000. Claim substantiation and efficiency of hydrating body lotions and protective creams. *Contact Dermatitis* **42**: 227-234.

Downing, D. T. 1992 Lipid and protein structures in the permeability barrier of mammalian epidermis. *J. Lipid Res.* **33**: 301-313.

Downing, D. T., Wertz P. W. & Stewart M. E. 1986. The role of sebum and epidermal lipids in the cosmetic properties of the skin. *Int. J. Cosmet. Sci.* **8**: 115-123.

Dreher, F., Gabard, B., Schwindt, D. A. & Maibach H. I. 1998. Topical melatonin in combination with vitamins E and C protects skin from ultraviolet-induced erythema: a human study in vivo. *Br. J. Dermatol.* **139**: 332-339.

Elias, P. M. & Menon G. K. 1991. Structural and lipid biochemical correlates of the epidermal permeability barrier. *Adv. Lipid Res.* **24**: 1-26.

Fisher G. J., Wang Z., Datta S. C., Varani J., Kang S. & Voorhees J. J. 1997. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med.*, **337**: 1419-1428.

Friberg, S.E., Kayali I., Rhein L. D., Simion F. A. & Cagan R. H. 1990. The important of lipids for water uptake in stratum corneum. *Intl. J. Cosmet. Sci.* **12**: 5-12.

Frosch, P. J. & Pilz B. 1995. Irritant Patch test technmiques. In. Serup. J. & Jemec G. B. E. (eds.). *Handbook of Non-Invasive methods and the skin.* pp. 587-591. Boca Raton: CRC Press Inc.

Fryer, M. J. 1993. Evidence for the photoprotective effects of vitamin E. *Photochem. Photobiol.* **58** (2): 304-312.

Fullerton, A., T. Fischer, A Lahti, K. –P. Wilhelm, H. Takiwaki & Serup J. 1996. Guidelines for measurement of skin colour and erythema. A report from the standardization group of the European Society of Contact Dermatitis. *Contact Dermatitis* **33**: 1-10.

Goldman, M. P. & Rapaport M. 1986. Contact dermatitis to vitamin E oil. *J. Am. Acad. Dermatol.* **14**: 133.

Gilchrest B.A. & Yaar M. 1992. Ageing and photoageing of the skin: observations at cellular and molecular level. *Br. J. Dermatol.* **127** (41): 25-30.

Grubauer G., Elias P.M. & Feingold K. R. 1989a. Transepidermal water loss: the signal for recovery of barrier structure and function. *J. Lipid Res.* **30**: 323-333.

Grubauer G., Feingold K R., Harris R. M. & Elias P. M. 1989b. Lipid content and lipid type as determinants of epidermal permeability barrier. *J. Lipid Res.* **30**: 89-96.

Haratake A., Uchida Y., Mimura K., Elias P. M. & Holleran W. M. 1997. Intrinsically aged epidermis displays diminished UVB-induced alterations in barrier function associated with decreased proliferation. *J. Invest. Dermatol.* **108**: 319-323.

Hartel, B. 1999. Molecular and cellular mechanisms of intrinsic aging and photoaging (cellular stressors, defense mechanisms). *SOFW J.* **125**: 33-42.

Henegouwen, G. M. J. B., Junginger H. E. & de Vries H. 1995. Hydrolysis of RRR-α-tocopheryl acetate (vitamin E acetate) in the skin and its UV protecting activity (an in vivo study with the rat). *J. Photochem Photobiol. B: Biology*. **29**: 45-51.

Hood R. L. 1996. Tocotrienols and Cholesterol Metabolism. In. Ong, A.S.H., Niki E. & Packer L. (eds.). *Nutrition, Lipids, Health and disease*. pp. 96-103. Champaign, IL: AOCS Press.

Jaas, H. E. & Elias P. M. 1991. The living stratum corneum: implications for cosmetic formulation. *Cosmet. Toiletries* **106**: 47-53..

Jurkiewicz, B. A., D. L. Bissett & Buettner G. R. 1995. Effect of topically applied tocopherol on ultraviolet radiation - mediated free radical damage in skin. *J. Invest. Dermatol*., **103**(4): 484-488.

Katsarou, A., E. Davoy, K. Xenos, M. Armenaka & Theoharides T. C. 2000. Effect of an antioxidant (quercetin) on sodium-lauryl-sulfate-induced skin irritation. *Contact Dermatitis* **42**: 85-89.

Kamal-Edlin A. & Appelqvist L. A. 1996. The chemistry and antioxidant properties of tocopherols and Tocotrienols. *Lipids* **31**: 671-701.

Kompaore, F., Dupont, C. & Marty J. P. 1991. *In vivo* evaluation in man by two non-invasive methods of the stratum corneum barrier function after physical and chemical modification. *Int. J. Cosmet. Sci.* **13**: 293-302.

Kramer-Stickland K., Krol E. S. & Liebler D. C. 1999. UVB-induced photooxidation of vitamin E in mouse skin. Chem Res. Toxicol. **12**(2): 187-191

Lee, C. H. & Maibach H. I. 1995. The Sodium Lauryl Sulphate Model: An Overview. *Contact Dermatitis* **33**: 1-7.

Leung, H. –W., Vang M. J. & Mavis R. D. 1981. The cooperative interaction between vitamin E and Vitamin C in suppression of peroxidation of membrane phospholipids. *Biochim. Biophys. Acta*. **664**: 266-272.

Leveque, J. L., de Rigal J., Saint-Leger. D. & Bolly D. 1993. How does sodium lauryl Sulphate alter the skin barrier function in man? A mutiparametric approach. *Skin Pharmacol.* **6**: 111-115.

Lock-Andersen, J., Olivarius F. de F., Haedersdal M., Poulsen T. & Wulf H. C. 1999. Minimal erythema dose in UV-shielded and UV-exposed skin predicted by skin reflectance measured pigmentation. *Skin Res. Technol*. **5**: 88-95.

Loden M. & Anderson A. -C. 1996. Effect of topically applied lipids on surfactant-irritated skin. *Brit. J. Dermatol*. **134**: 215-220.

Lopez-Torres, M., Thiele J. J., Shindo Y., Han D. & Packer L. 1998. Topical application of α-Tocopherol modulates the antioxidant network and diminishes ultraviolet-induced oxidative damage in murine skin. *Br. J. Dermatol*. **138**: 207-215.

Man M-Q., Feingold K R. & Elias P. M. 1993. Exogenous lipids influence permeability barrier recovery in acetone-treated murine skin. *Arch. Dermatol*. **129**: 728-738.

Mark, J. G. & DeLeo V. A. 1991. Photoallergens. *Contact and Occupational Dermatology*. pp. 200-208. Missouri: Mosby-Year Book, Inc.

Meguro S., Arai Y., Masukawa K., Uie K. & Tokimitsu I. 1999. Stratum corneum lipid abnormalities in UVB-Irradiated skin. *Photochem. Photobiol*. **69**(3): 317-321.

Muizzuddin N., Shakoori, A. R. & Marenus K. D. 1999. Effect of antioxidants and free radical scavengers on protection of human skin against UVB, UVA and IR irradiation. *Skin Res. Technol*. 5: 260-265.

Norkus, E. P., Bryce G. F. & Bhagavan H. N. 1993. Uptake and bioconversion of α-Tocopheryl acetate to α-Tocopherol in skin of hairless mice. *Photochem. Photobiol*. 57 (4): 613-615.

Olson, C. L. 1987. Statistics. *Making Sense of Data*. pp. 163-170. Maryland: Allyn Bacon, Inc..

Ong, A. S. H. 1995. Natural sources of Tocotrienols. In. Packer L. & Fuch J. (eds.). *Vitamin E in health and disease*. pp. 3-8. New York: Marcel Dekker Inc.

Packer, L. 1995. Nutrition and biochemistry of the lipophilic antioxidants Vitamin E and Carotenoids. In. Ong, A.S.H., Niki E. & Packer L. (eds.). *Nutrition, Lipids, Health and disease*. pp. 8-35. Champaign, Il: AOCS Press.

Pinnagoda, J. & Tupker R. A. 1995. Measurement of Transepidermal Water Loss. In. Serup, J. & Jemec G.B.E. (eds.). *Handbook of Non-Invasive Methods and the Skin*. pp. 173-178. Florida, U.S.: CRC Press Inc.

Qureshi A. A., Qureshi N. & Wright J. J. K. 1991. Lowering of serum cholesterol in hypocholesterolemic humans by Tocotrienols (palm vitee). *Am J Clin Nutr*. **53**: 1021S-6S.

Rawlings, A. V., I. R. Scott, C. R. Harding & Bowser P. A. 1994. Stratum corneum moisturization at molecular level. *J. Invest. Dermatol* **103**: 731-740.

Rieche, L., C. Willis, J. Wilkinson, S. Shaw & Lacharriere O. De. 1998. Clinical morphology of sodium lauryl sulfate (SLS) and nonionic acid (NAA) irritant patch test reactions at 48h and 96h in 152 subjects. *Contact Dermatitis* **39**: 240-243.

Ritter, E. F., Axelrod M., Minn K W., Eades E., Rudner A. M., Serafin D. & Klitzman B. 1996. Modulation of ultraviolet light –induced epidermal gamage: beneficial effects of Tocopherol. *Plast. Reconstr. Surg*. **100**: 973-980.

Roed, P.J. & Hjorth N. 1975. Patch test sensitization from d,l-α-tocopherol (vitamin E). *Contact Dermatitis,* **1**: 391

Rogiers, V. 2001. EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacol. Appl. Skin Physiol*. **14**: 117-128.

Rogiers, V., Balls, M., Basketter, D., Berardesca, E., Edward, C., Elsner, P., Ennen, J., Leveque, J.L., Loden, M., Masson, Ph., Parra, J., Paye, M., Pierard, G.E., Rodrigues, L., Schaefer, H., Salter, D. & Zuang V. 1999. The potential use of non-invasive methods in the safety assessment of cosmetic product. *Atla* **27**:515-537.

Rogiers, V., Derde M. P., Verleye G. & Roseeuw D. 1990. Standardised conditions need for skin surface hydration measurements. *Cosmet. Toiletries*. **105**: 73-82.

Sayama, T. 1993. The E factor. *SPC* **66**: 33.

Schürer, N. Y. & Elias P. M. 1991. The biochemistry and function of stratum corneum lipids. *Adv. Lipid Res*. **24**: 27-56.

Serbinova E., Kagan V., Han D. & Packer L. 1991. Free radical recycling and intramembrane mobility in the antioxidant properties of α-tocopherol and α-tocotrienol. *Free Radic Biol Med*. **10**: 263-275.

Steenvoorden, D. P. T., & Henegouwen G. M. J. B. 1997. The use of endogenous antioxidants to improve photoprotection. *J. Photochem Photobiol. B: Biology* **41**: 1-10.

Sugiyama, M., K. Tsuzuki, K. Matsumoto & Ogura R. 1992. Effect of vitamin E on cytotoxicity, DNA single strand breaks chromosomal aberrations and mutation in Chinese hamster V-79 cells exposed to ultraviolet-B light. *Photochem. Photobiol*. **56** (1): 31-34.

Tamburic S., G. Abamba & Ryan J. 1999. Moisturizing potential of d-α-tocopherol. *Cosm. Toil*. **114** (5): 73-82.

Theriault, A., Chao J-T., Wang Q., Gapor A. & Adeli K. 1999. Tocotrienol: A review of its therapeutic potential. *Clinical Biochemistry* **32** (5): 309-319.

Thiele, J. J., Dreher, F. & Packer L. 2000. Antioxidant Defense Systems in Skin. In. Elsner, P. & Maibach H. I. (eds.). *Cosmeceuticals: Drugs vs. Cosmetics*. pp. 145-187. New York: Marcel Dekker Inc.

Thiele, J. J., Schroeter, C., Hsieh, S. N., Podda, M. & Packer L. 2001. The antioxidant Network of the Stratum Corneum. *Curr. Prob. Dermatol.* **29**: 26-42.

Thiele, J. J., Traber M. G. & Packer L. 1998. Depletion of human stratum corneum vitamin E: an early and sensitive *in vivo* marker of UV induced photo-oxidation. *J. Invest. Dermatol.* **110**: 756-761.

Traber, M. G. & Sies H. 1996. Vitamin E in humans – demand and delivery. *Ann. Rev. Nutr.* **16**: 321-347.

Traber, M. G., M. Podda, C. Weber, J. Thiele, M. Rallis & Packer L. 1997. Diet-derived and topically applied tocotrienols accumulate in skin and protect the tissue against ultraviolet light-induced oxidative stress. *Asia Pacific J. Clin. Nutr*. **6** (1): 63-67.

Trevithick, J. R., H. Xiong, S. Lee, D. T. Shun, S. E. Sanford, S. J. Karlik, C. Norley & Dilworth G. R. 1992. Topical tocopherol acetate reduces post-UVB, sunburn-associated erythema, edema and skin sensivity in hairless mice. *Arch. Biochem. Biophys*. **296**: 575-582.

Tupker, R. A., C. Willis, E. Berardesca, C. H. Lee, M. Fartasch, T. Agner & Serup J. 1997. Guidelines on Sodium Lauryl Sulphate (SLS) exposure tests. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* **37**: 53-69.

Voet, D. & Voet J. G. 1990. Arachidonate metabolism: prostaglandins, prostacyclins, thromboxanes and leukotrienes. *Biochemistry*. pp. 658-667. New York: John Wiley & Sons.

Weber, C., M. Podda., M. Rallis, J. J. Thiele, M. G. Traber & Packer L. 1997. Efficacy of topically applied tocopherols and tocotrienols in protection of murine skin from oxidative damage induced by UV-irradiation. *Free Rad. Biol. Med.* **22** (5): 761-769.

Welzel, J., K. P. Wilhelm & Wolff H. H. 1995. Occlusion does not influence the repair of the permeability barrier in human skin. In. Elsner P. & Maibach H. I. (eds.). Irritant Dermatitis. New clinical and experimental aspects. *Curr. Probl. Dermatol.* **23**: 180-186.

Werninghaus K., Meydani M., Bhagwan J., Margolis R., Blumberg J. B. & Gilchrest B. A. 1994. Evaluation of the Photoprotective Effect of Oral Vitamin E Supplementation. *Arch. Dermatol.* **130**: 1257-1261.

Wilhelm, K. & Maibach H. I. 1989. Skin color reflectance measurements for objective quantification of erythema in human beings. *J. Am. Acad. Dermatol.*, **21**: 1306