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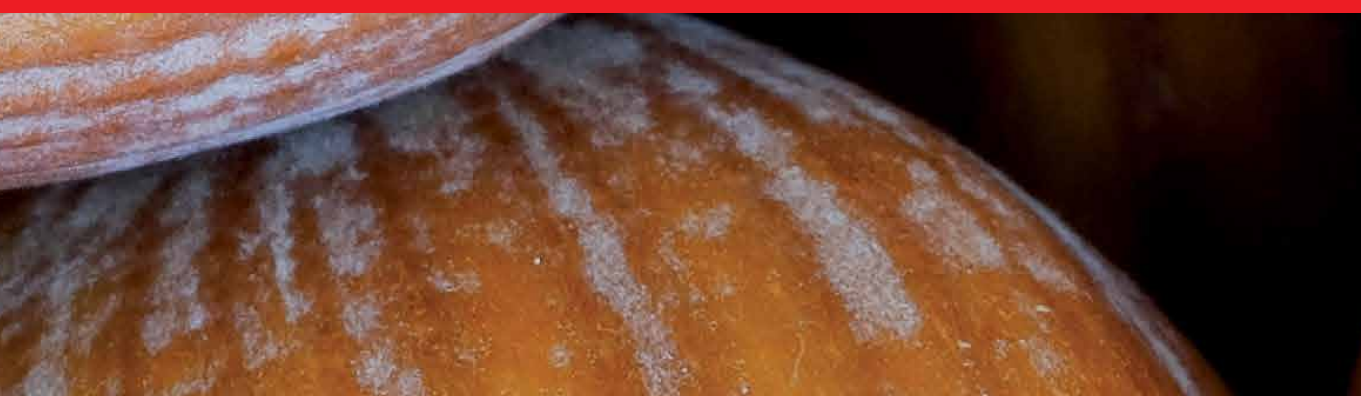
Biochemistry, Volume 22

Vitamin E in Health and Disease

Interactions, Diseases and Health Aspects

Edited by Pınar Erkekoglu and Júlia Scherer Santos



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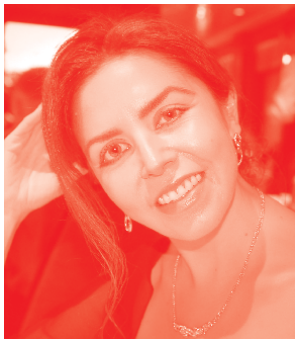

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Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of the life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, co-enzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation, etc. More recently, biochemistry embraced the 'big data' omics systems.

Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) "Don't waste clean thinking on dirty enzymes." Today, however, biochemistry is becoming more agglomerative and comprehensive, setting out to integrate and describe fully a particular biological system. The 'big data' metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., the bovine rumen. This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Contents

Preface

Vitamin E is a group of fat-soluble antioxidant compounds found in a wide variety of foods. Naturally occurring vitamin E exists in eight chemical forms that have varying levels of biological activity. Good sources of vitamin E include plant oils, nuts and seeds, and cereals and cereal products. Vitamin E deficiency is rare and a balanced diet consisting of different foods ensures that humans get as much vitamin E as their daily needs. Taking high-dose vitamin E supplements is more harmful to human health than beneficial. Much scientific research has been published on vitamin E's potential to promote health, as well as prevent and treat disease. The mechanisms by which vitamin E might provide this protection include its function as an antioxidant and its roles in anti-inflammatory processes, inhibition of platelet aggregation, and immune enhancement. It has been suggested that vitamin E supplementation could be beneficial against coronary artery disease, eye disorders, cognitive decline, and cancer. Moreover, vitamin E is essential for promoting skin health as it is suggested to provide skin-aging reduction, lower the production of pyrimidine dimers, improve melasma, and reduce melanoma progression. This book is mainly focused on vitamin E in health and disease. The readers will receive information on the diverse functions of vitamin E, importance of vitamin E status, and the interaction between vitamin E and several pathological conditions. We believe that readers will gain qualified scientific knowledge and a general overview of the importance of vitamin E in health and different pathological conditions from this book.

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Section 1

Vitamin E Applications in Pathological Conditions

Chapter 1

Vitamin E in Human Skin: Functionality and Topical Products

Claudineia Aparecida Sales de Oliveira Pinto, Tércio Elyan Azevedo Martins, Renata Miliani Martinez, Thamires Batello Freire, Maria Valéria Robles Velasco and André Rolim Baby

Abstract

Vitamins are part of the antioxidant system of human skin, and are detectable in different layers, so the topical application can be an alternative to maintain the functionality of the system. The capacity of the antioxidant gradient of keratinocytes is associated with attenuation of the action of related free radicals in both esthetics and health. These problems arise from extrinsic aging and are related to the risk of cancer. Vitamin E has been proven to have antioxidant and moisturizing properties in the skin and can protect against the damage of UVB radiation, with emphasis on the reduction of acute erythema and photoaging. The choice for the use of topical vitamin E, compared to the oral is given by the safety as mild irritation and it has potential for multifunctional topical formulations. The purpose of the chapter is to review the topical use of formulations with vitamin E, addressing the development, safe use and evaluation of effectiveness.

Keywords: vitamin E, skin, protection, antioxidant, cosmetology

1. Introduction

Vitamin E is the most well-known fat-soluble non-enzymatic antioxidant, mainly for its ability to inhibit the activity of pro-oxidant agents generated by reactive oxygen species (ROS). Vitamin E can eliminate free radicals induced by endogenous and/or exogenous agents such as ultraviolet radiation, drugs and pollution agents, avoiding their deleterious effects. The antioxidant activity of vitamin E is directly linked to its ability to inhibit the lipid peroxidation in unsaturated fatty acids, incorporating itself into cell membranes, which effectively inhibits lipid peroxidation [1, 2].

The antioxidant activity of vitamin E (alpha-tocopherol) has its property due to its ability to react mainly with the peroxyl radical ($HOH^{\bullet}$) and singlet oxygen ($^1O_2$), which favors lipid peroxidation. The free radical scavenging reaction occurs through the formation of a stable, low-energy radical, tocopheroxyl, which does not have the capacity to react with the free radical-forming agent [3]. Alpha-tocopherol

is the main agent capable of removing peroxyl radicals from lipid membranes, such as membranes or low-density lipoproteins (LDL) [4].

It is a classic dermatological ingredient used alone in its purified form, alpha-tocopherol or by its derivatives. However, the conversion to the purified (isolated) form is required in skin to obtain the desired effects. Topical applications are designed for treating melasma, protecting against ultraviolet radiation (UVR) and improving aging damages [5, 6], The association of vitamin E with other antioxidants increase the effects in skin [7].

Some studies suggest that a poor diet of vitamin E could be related with skin disorders. Oral supplementation of vitamin E is recommended in many skins' therapies, such as: yellow nail syndrome, epidermolysis bullosa, cutaneous ulcers, pressure ulcers and burns, sub corneal pustular dermatosis, scleroderma, morphea, calcinosis cutis, Raynaud's phenomenon, and inflammatory diseases. The oral supplementation of vitamin E could reduce the pigmentation in melasma and contact dermatitis lesions, too demonstrated remission of atopic dermatitis, prevention of sunburn reaction as well as the subsequent chronic skin damage [5]. Vitamin E combined with other antioxidants has shown positive results topically in the photoprotection, as well as delay the growth of the melanoma by promoting the apoptosis of tumor cells and inhibiting VEGF-mediated angiogenesis. Other results with alpha-tocopherol: improvement in periorbital fine lines, roughness, radiance, skin tone, elasticity, density, collagen production and overall appearance by clinical evaluations of skin. Topical application of tocopherol acetate significantly reduces the severity of erythema, edema and skin sensitivity associated with sunburn by UVB [8]. It is difficult to determine the *in vivo* antioxidative activity of vitamin E because it is naturally present in the skin, but future studies with the isolated form and its derivatives can be explored in topical products [9].

2. Topical products (cosmetics x medicines)

Skin products can be classified as medicines, cosmetics and cosmeceuticals, however, the teaching line between the categories is tenuous, being widely discussed by dermatologists, pharmacists and beauticians. Medicines and cosmetics are already widely discussed and accepted by world regulatory agencies; however, the term cosmeceutical is used as a marketing appeal and is not recognized as an official legal category. Skin products considered to be cosmetics are generally defined as products to clean, beautify, promote attractiveness, or change appearance, while medicines are intended for the diagnosis, cure, mitigation, treatment or prevention of diseases, which can affect the structure or any function of the skin. Regulatory agencies in different countries seek to organize the offer of products to ensure the safety for users.

Topical products that contain vitamin E can be classified as medicines or cosmetics, depending on their purpose. If the product is intended to lubricate the skin, it will be considered cosmetic and if it has therapeutic use as a healing agent, it will be a medicine. There are legal limits on the daily consumption of vitamin E as a supplement, however, for most international regulatory agencies, such as the NHS, FDA, Health Canada the limits for topical use are not described [10].

3. Types of vitamin E for topical applications

The antioxidant alpha-tocopherol acetate is the most common form of vitamin E in skin care products. In 2001, the Scientific Committee on Cosmetic and Non-Food

Products for Consumers (SCCNFP) presented its opinion during the 18th Plenary Meeting. At the time, SCCNFP believed that alpha-tocopherol acetate did not pose a threat to consumer health and therefore did not propose any restrictions or use conditions [11].

The Cosmetic Ingredients Review Panel (CIR) in 2002 has assessed the safety of 14 tocopherols and tocotrienols and concluded that these ingredients are safe when used in cosmetics. The Panel further reviewed data from clinical and animal studies to determine the safety of tocopherols and tocotrienol ingredients and considered it appropriate to extrapolate existing information to conclude on the safety of all tocopherols and tocotrienols [12].

Since vitamin E can absorb ultraviolet light to produce free radicals, there is a possibility that strong exposure to sunlight after topical application may cause skin reactions. However, vitamin E concentrations between 0.1–1.0% are generally considered to be safe and effective for increasing vitamin E levels in the skin, but higher levels of α-tocopherol have been used with no apparent side effects [8]. Vitamin E as alpha-tocopherol or tocopherol acetate is used in over-the-counter products in concentrations ranging from 1.0 to 5.0% [13–15].

Vitamin E is the main fat-soluble antioxidant in the body with biological activity and it is the collective name for the eight mains naturally occurring substances such four tocopherols and four tocotrienols. The eight analogues of vitamin E share similar chemical antioxidant activity, however, they are distinguished by their individual physico-chemical and biological effects at the molecular level in humans and higher animals. Alpha tocopherol is the most active, being considered an important asset in protecting cell membranes from lipid peroxidation promoted by free radicals [13–15].

Alpha-tocopherol is practically insoluble in water and this characteristic can difficult the development of topical products with high water content. In addition, this molecule is easily oxidized by atmospheric oxygen. Vitamin E acid acetate and succinate esters are applicable for clinical use due to their high oxidation stability but require the use of surfactants to improve the water solubility. Alpha-tocopherol is solubilized by large amounts of surfactants, but the hydrolysis of acetate is the limiting step in terms of its concentration during bioavailability [15].

The antioxidant properties of vitamin E are attributed to its free aromatic hydroxyl group; thus, the esters of vitamin E need to be hydrolyzed during absorption by the skin to exhibit this activity. In the biologically inactive esterified form, vitamin E acetate is more used because of its greater stability, acting as a prodrug, being hydrolyzed in active free vitamin E (alpha-tocopherol) after penetration into the skin. The bioconversion of vitamin E into the active form can be influenced by the technology involved in the development of formulations, by the target layer of the skin and exposure to ultraviolet rays. The stratum corneum seems to have less efficiency in the bioconversion of esters of vitamin E when compared to the nucleated epidermal layers. Thus, alpha-tocopherol should provide more efficient antioxidant protection for skin surface lipids and skin barrier constituents than vitamin E esters. However, in the nucleated epidermis the bioconversion of vitamin E acetate to active free form occurs at a much higher rate. In this sense, the choice of which vitamin E molecule to be used must consider the target layer of the skin and include product development strategies so that the activity of vitamin E is fully utilized [15, 16].

4. Vitamin E in skin damage

Vitamin E, more specifically alpha tocopherol, is considered one of the main fat-soluble and non-enzymatic antioxidant agents of natural origin, due to its

advantages in terms of the protective activity against physical and chemical damage promoted by free radicals (FR). Vitamin E is an antioxidant capable of binding to the membrane in various tissues [17, 18]. Therefore, it is involved in several oxidative mechanisms in epidermis and dermis, catalyzed by ultraviolet radiation (UVR) and pollutants (**Figure 1**).

4.1 Oxidative stress: lipid peroxidation and free radicals' formation

The first studies related to the damage caused by the formation of FR on the skin, promoting lipid peroxidation, date from the 1950s and 1960s. To avoid the damages, the use of natural and synthetic substances was suggested in order to prevent the formation of FR [19, 20].

Reactive oxygen species (ROS) such as superoxide ($O_2^{-\bullet}$), hydroxyl radicals ($OH^{\bullet}$), peroxyl ($HOH^{\bullet}$) and singlet oxygen ($^1O_2$), can be formed by endogenous (physiological) processes such as inflammation, physical activity in excess, nutritional disorder, hereditary issues, neoplasms, and even, by processes related to exogenous sources such as UVR and pollution agents. In the skin, the main damage related to lipid peroxidation generated by FR from exogenous sources is the activation of melanogenesis and damage to collagen fibers [19, 21, 22].

The lipid peroxidation of the epidermis cells occurs through the action of the ROS, which has the ability to bind to the unsaturated bonds present in the polyunsaturated fatty acids of the cell membrane phospholipids [22, 23]. The process starts between polyunsaturated fatty acids (PUFA) and the oxygen radical, obtaining a lipid radical, which causes a rearrangement process in the presence of molecular oxygen, becoming a peroxy lipid radical. The lipid peroxyl radical is also capable of attacking unsaturated lipids, generating new radicals, such as the lipid radical as in the first stage of the reaction and the lipid hydro peroxide radical, thus promoting a cyclic reaction. Thus, it is necessary to use substances capable of interacting with cell membranes and to extinguish the free radicals formed, such as vitamin E [24, 25].

In a more detailed way, the mechanism involved in the lipid peroxidation process occurs through a chain reaction of the polyunsaturated fatty acids (PUFA) of biological membranes, which due to the large amount of unsaturation, become extremely susceptible to attack by free radicals. The process begins with the activity

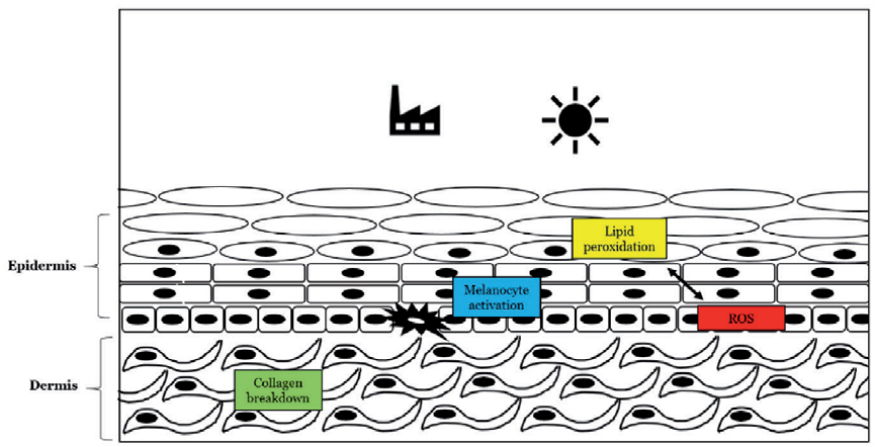

Figure 1.
Oxidative mechanisms involving vitamin E in human skin exposed to ultraviolet radiation and pollution. ROS, reactive oxygen species.

of the free radical like OH*, which extracts H from PUFA resulting in the radical PUFA*. After the molecular rearrangement of a conjugated diene, the molecule is susceptible to attack by O_2, resulting in a peroxyl radical (PUFAOO*). PUFAOO* can extract H from the adjacent PUFA, thus propagating a chain reaction. Self-oxidation occurs continuously, which can seriously affect the functionality of the tissue [26].

The action of pollutants and UV radiation (UVR) on the skin has already been studied, but the mechanisms involved are still uncertain, knowing that the damage is initially related to the composition of the skin's sebum and the quality of the stratum corneum, which may lead to the formation of wrinkles, hyperchromies (spots), wrinkles and accelerated extrinsic aging and dermatological diseases such as atopic dermatitis, related to lipid peroxidation [27–29].

The chronic and acute damage to the skin caused by UVR (UVB and UVA) are related to the direct absorption of rays and indirectly through photosensitization reactions. Mostly (> 95%), UVA radiation, more specifically UVAI (340–400 nm), has the major ability to penetrate the skin and it causes deeper damage. The aggression of UVA radiation targets collagen and supporting fibers, in addition to cellular DNA. The DNA damage is related to the mutagenic power of UVA radiation, which can act directly or indirectly through photosensitization reactions [30].

Studies prove the mutagenic power of UVA through direct oxidation reactions of DNA nucleic acids with ROS, which can lead to simple disruptions of the DNA strands or to disruptions in symmetrical positions in the two strands. Several studies (*in vivo* and *in vitro*) have evaluated the damage to DNA bases caused by oxidative stress, such as the oxidation of purine and guanine [31, 32].

As the UVR, polluting agents have harmful effects on the skin by increasing the oxidative stress and decreasing the physiological enzymatic and non-enzymatic antioxidant capacity. With the formation of FR and ROS, an interaction occurs with the lipid layer membrane, initiating the cascade reactions of lipid peroxidation and the release of pro-inflammatory mediators, which result in the accumulation of neutrophils and phagocytic cells, that also generate radicals free, thus promoting a cyclical reaction. Oxidative stress initiates a series of quite complex biological processes that result in DNA damage, activation of transcription factors such as activating protein 1 (AP1) and the nuclear factor Kappa-B (NF-KB) and even some pathways of signaling involved in cell growth and differentiation and degradation of dermal connective tissue. Pollutants are also capable of inducing functional changes in lipids, DNA, skin proteins, favoring the acceleration of skin aging, inflammatory processes and dermatological pathologies [33, 34].

4.2 Free radicals and the activation of melanogenesis

Melanogenesis can be considered as the first skin defense, being directly influenced by the skin phototype and, consequently, by the amount and type of melanin present. Melanocytes are particularly vulnerable to excessive oxidative stress from ROS due to their pro-oxidant state and the melanin synthesis involves oxidation reactions and generation of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), promoting oxidative stress. The initiation of melanin synthesis occurs by a single route, with the conversion of tyrosine to dopa by the catalytic activity of the enzyme tyrosinase, releasing $O2^-$, which also oxidizes dopa to dopaquinone with the release of $O2^-$. From the obtaining of dopaquinin, a specific orthoquinone, capable of reacting with nucleophilic compounds, the synthesis follows two distinct pathways, eumelanogenesis and pheomelanogenesis, which respectively produce the darkest and lightest melanin monomers (red-yellow) [35, 36].

The homeostasis of human melanocytes in the epidermis is maintained mainly through a complex paracrine network, involving growth factors and cytokines synthesized by epidermal keratinocytes and dermal fibroblasts and modulated by UV radiation. Keratinocyte-derived endothelin-1 is a potent mitogen and a melanogenic factor capable of reducing H_2O_2 generation and apoptosis in human UV-irradiated melanocytes [37]. The α-MSH melanocortin and adrenocorticotropic hormone (ACTH) are synthesized by keratinocytes and melanocytes and stimulate the synthesis of eumelanin, as well as the survival and proliferation of melanocytes by binding and activating the melanocortin 1 receptor (MC1R). The MC1R is a receptor located on the surface of melanocytes with the ability to bind to protein G. Studies show that the treatment of human melanocytes in culture with α-MSH, results in a decrease in the generation of H_2O_2, due to exposure to UV rays [35].

With the production of ROS, oxidative stress formed can interrupt melanocyte homeostasis, compromising their survival or even leading to malignant pathogens. Thus, the balance between the pro and antioxidant properties of melanin in the skin is mainly determined by the proportions of eumelanin and pheomelanin, the levels of melanin intermediates and the concentrations of reactive metals in the melanosome microenvironment. The generation of H_2O_2 in response to the action of UV radiation is inversely proportional to the constitutive pigmentation, suggesting a natural antioxidant effect of melanin [35, 38]. The inhibition of melanogenesis occurs in several stages, such as the inhibition of the enzyme tyrosinase that acts in several phases of the melanin production cascade, and also influences the post-transcriptional concentration of tyrosinase and other enzymes related to melanogenesis, such as tyrosinase-related protein 1 (TRP1) and DOPA chrome tautomerase (TRP2) [39].

4.3 Role of vitamin E in skin's oxidative stress

The mechanism of action of vitamin E (**Figure 2**) regarding the antioxidant activity in the skin is directly related to the chemical mediation of the phenolic hydroxyl (OH) of its structure, capable of donating H to the peroxyl radical (PUFAOO*), resulting in the formation of a stable lipid species (PUFAOOH). Thus, when donating the hydroxyl H, vitamin E becomes a relatively non-reactive free

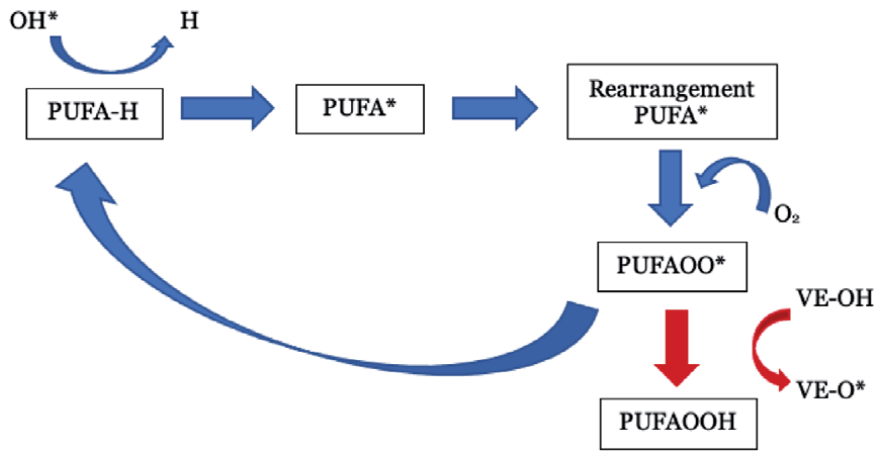

Figure 2.
Mechanism of lipid peroxidation and vitamin E in cells. PUFA, polyunsaturated fatty acids; PUFA, lipid radical; PUFAOO*, Peroxy lipid radical; OH*, oxygen radical - hydroxyl; O_2, oxygen; VE-OH, Vitamin E, alpha-tocopherol; VE-O*, radical tocopheroxyl.*

radical, as the unpaired electron moves to the aromatic ring. Thus, with electronic displacement, incorporation occurs in biological membranes, being located awfully close to the polyunsaturated fatty acids of the cell membrane phospholipids, interrupting the chain reaction. Vitamin E stops the reaction by the ability to donate hydrogen from the OH group to the unsaturated lipid or to the lipid peroxyl radical (PUFOO *), forming the low-energy tocopheroxyl stable radical (VE-O *), which in turn does not present the ability to act as a free radical forming agent [40, 41]. Vitamin E also has antioxidant activity involving other lipid radicals, acting directly on the radical's singlet oxygen and superoxide anion [19].

Studies have shown the activity of vitamin E in the modulation of damage caused by FR mediated by the action of UVR on the skin, such as lipid peroxidation, photoaging, immunosuppression and photocarcinogenesis [42]. Vitamin E is able to reduce the inflammatory reactions of the skin, attenuating the production of prostaglandin involved in the process, pro-inflammatory cytokines, cyclooxygenase-2 (COX-2) and NADPH oxidase [43–45].

In addition to its anti-inflammatory capacity, vitamin E is also able to modulate the protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3-K) signaling pathways and to reduce the increase in collagenase expression. PKC modulation may be representative in terms of cell growth control, however, the interaction between vitamin E (alpha-tocopherol) and PKC protein does not occur directly, assuming that it occurs preventively to its action at the cellular level [45, 46].

Vitamin E has the ability to significantly suppress collagen degradation by inhibiting metalloprotein 1 (MMP-1), involved in the initial process of collagen hydrolysis [44]. It can be identified in deeper layers of the skin, supposing its activity to minimize the photocarcinogenesis process, being considered as one of the main antioxidants of the human epidermis. Another characteristic of vitamin E is its use as an early and very sensitive marker for oxidative damage promoted by the environment [47, 48]. Thus, vitamin E prevents the lipoperoxidation of cell membranes and the degradation of fatty acids that are essential for the proper functioning of the body and skin [8, 49, 50].

Vitamin E can eliminate FR induced by UVA radiation, protect endogenous antioxidants from degrading processes, prevent lipid peroxidation and reduce immunosuppression caused by UVR. To increase protection against erythema and sunburn, the association of vitamins E and C is indicated, presenting potential against skin aging and skin cancer. Another activity of vitamin E on the skin is its application before sun exposure, avoiding the formation of the cyclobutane pyrimidine dimer (CPD) induced by UVB [46].

In general, exposure excessive to pollution and ultraviolet radiation promotes a greater production of free radicals, thus requiring an oral and/or topical supplementation of antioxidant substances, such as vitamin E, thus, the endogenous mechanism is not sufficient to prevent deleterious skin damage [51].

5. Topical treatments with vitamin E

After vitamin E depletion, oral intake is the best way to replenish the stock of this antioxidant in skin. In fact, oral supplementation brings cosmetic effects after 8–12 weeks. For alpha-tocopherol alone, a photoprotection effect by reduction of human skin malondialdehyde concentration was observed [52]. The combination of vitamin E with other antioxidants is very beneficial for skin treatments. Alpha-tocopherol in combination with ascorbic acid increased UVB photoprotection in the human epidermis [53, 54]. The same combination showed a reduction in UV-induced inflammation [55]. Good outcomes for treating chloasma were seen

with the same mixture during double blinded clinical trials [56]. When more antioxidants act together, strong outcomes are seen, such as reduction of UVB-induced wrinkle and increased collagen synthesis [57] and treatments of melasma [58, 59]. Despite the benefits to skin appearance, oral intake is not considered cosmetic treatment for its systemic effects.

Topical delivery also plays an important role in restock vitamin E. It is widely used in its purified forms or indirectly using vegetable seed oils [60]. It is a classical ingredient in dermatology and still used in cosmetics worldwide in a recent growth tendency. Cosmetics containing vitamin E are most valuable in the USA, UK and France. The top cosmetic claims used in labels and the categories are in **Figure 3** [61].

The lipophilic nature of vitamin E requires an oily or alcoholic phase at the topical formulation. In cosmetics, the main drivers capable of delivering this type of molecule are serums, tonics, oils and especially emulsions. For vitamin E alone, hydro-alcoholic solution of alpha-tocopherol showed a reduction of UV-induced erythema in the epidermis [62] and the reduction on the number of epidermal sunburn cells. While O/W and W/O emulsions containing alpha-tocopherol acetate increased skin hydration and water-binding capacity in the stratum corneum [63]. Vitamin E is also used as coadjutant in other topical products to improve physical–chemical characteristics or to donate different effects. One of the most studied associations is with vitamin C, due its primary replenisher of vitamin E mechanism in skin. Vitamin C regenerates the oxidized form of vitamin E to its reduced form [64, 65]. A similar mechanism is expected using other antioxidants. **Table 1** shows examples of associations of vitamin E and other molecules. The type of molecule and/or type of formulation is chosen depending on the target to address.

The metabolization of derivatives into the active form of vitamin E (alpha-tocopherol) occurs at a far extend in the nucleated epidermis [6]. Therefore, the conversion is highly dependent on the delivery system of cosmetic preparations into controlling skin permeation [14]. To address this issue, several innovations on cosmetic formulations have appeared during the last decade.

The use of chemical permeation enhancers (e.g. alcohol, surfactants, terpenoids) is a good strategy to change stratum corneum polarity and fluidity. Likewise, the use of devices that create micron-scale pores in skin (e.g. iontophoresis, microneedles) is also available in clinics [78]. The benefits of using those techniques is to maintain the original formulation. However, adaptations may be needed to maintain stability in the case of adding chemical agents. Another strategy is to change the formulation completely by using new delivery systems to encapsulate vitamin E.

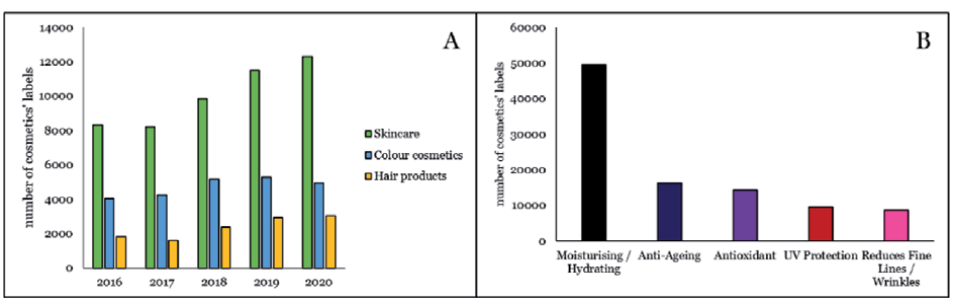

Figure 3.
Evolution of the most explored categories using tocopherol in cosmetics' labels between 2016 and 2020 (A). Claims used in cosmetics' labels containing tocopherol between 2016 and 2020 (B).

Vitamin E	Combined molecule	Effect	Model	Reference
Alpha-tocopherol (1%)	L-ascorbic acid (15%)	Synergic protection against erythema and sunburn cell formation	Aqueous solution applied to pig skin	[66]
Tocopheryl acetate (1%)	L-ascorbic acid (20%) + *Rubus idaeus* leaf cell culture (0.0005%)	Improved the appearance of aging skin (Skin color, elasticity, radiance, smoothness, scaliness and wrinkles)	Commercial serum applied *in vivo*	[67]
Tocopheryl acetate	Bioflavanoids from *Gingko biloba* + ascorbyl tetraisopalmitate + retinyl palmitate	Protected the skin from UV damage by reduction on the number of sunburn cells	Emulsion containing 5% of the mixture *in vivo*	[68]
Vitamin E	*L. plantarum*	A good antibacterial activity against *S. aureus* and *P. aeruginosa* with a sustained release of probiotic cells over 24 h	Dressing	[69]
Vitamin E 5 IU	vitamin A (10 000 IU) + vitamin D (1000 IU) + vitamin B1 (50 mg) + vitamin B2 (12.7 mg) + vitamin B6 (15 mg) + vitamin C (500 mg) + nicotinamide (100 mg) + dexpanthenol (vitamin B5) (25 mg)	Reduction of age spots and melasma	*In vivo* application of the multivitamin by iontophoresis	[70]
Vitamin E	Resveratrol + Baicalin	Improvement on the periorbital fine lines, roughness, radiance, skin tone, elasticity, density, and overall appearance	*In vivo*	[71]
Vitamin E	Photostable filters (octyl methoxycinnamate, avobenzone and 4-methylbenzilidene camphor) + vitamins A (1,700,000 UI/g) and C [2% (w/w) ascorbyl tetraisopalmitate]	The formulation with filters showed better stability comparing with the vitamins alone	*In vivo* In mouse skin	[72]
Vitamin E	Amniotic membrane mesenchymal stem cell	Decreased the diameter of lesions	In chronic leprosy	[73]
Vitamin E	Ferulic acid + Vitamin C	Suggests preventing skin cancer	Topically solution in the skin of white Yorkshire pigs	[74]
Vitamin E	Vitamin C	Prevention of inflammation due lipid peroxidation caused by *Propionibacterium acnes* leakage through follicles and sebaceous glands in *acne vulgaris*	*In vivo*	[5]

Vitamin E	Combined molecule	Effect	Model	Reference
Alpha-tocopherol	Ferulic acid	Inhibition of melanization	*In vitro* application of alpha-tocopheryl ferulate	[75, 76]
Delta-tocopherol glucoside (0.05%)	Retinaldehyde (0.05%) + glycylglycine oleamide (0.1%)	Improvement on the elastin fiber production and a protection effect of the elastin and fibrillin fiber network against UV-induced alterations	*In vitro* and *ex vivo*	[77]

Table 1.
Examples of association between vitamin E and other active molecules.

Micro and nanoemulsions are strong candidates for its permeation capacity. Nevertheless, reduced sizes may have systemic effects, which is not allowed for cosmetics. Regulatory issues must be address in controlling the particle size. Vitamin E microemulsions (256 nm) reach dermis, however with the aid of surfactants [79]. More recently, bigels are a viable cosmetic formulation. These biphasic systems formed by hydrogels and organogels show good spreadability and emollient and moisturizing effect, besides its transdermal capacity [80]. However, bigels containing alpha-tocopherol showed no difference against regular emulsions for hyperpigmentation and inflammatory markers in in vivo tests [81]. More tests are required to evaluate the benefits and safety of new cosmetic formulations.

6. Safety and efficacy of topical products with vitamin E

Many products in the cosmetic market have vitamin E in its composition. The definition of optimal dosage of vitamin E in cosmetics products depends on the derivative molecule and the type of formulation.

6.1 Safety of Vitamin E

Studies with animals to evaluate safety is common in many countries, especially in oral products. In animal experiments, 200 mg/kg was administered orally to frogs, rabbits, cats, dogs, and monkeys, with repeated application to mice over a period of 10–61 days. In food of rats, 4.000 mg/kg of Vitamin E was added and, in these experiments, was not mutagenic, teratogenic nor carcinogenic properties [82].

The toxicity of vitamin E is very low, because in clinical studies, a daily dosage of 100–300 mg of vitamin E was considered harmless, even when their use extends over a long period of time. Double-blind studies demonstrated that large oral doses of up to 3,200 USP-Units/day led to no consistent adverse effects. They mentioned that the optimal human plasma concentration of vitamin E is between 1.0 and 1.5 mg/dl [82].

Numerous genotoxicity studies were conducted with tocopherol, tocopheryl acetate, tocopheryl phosphate (MTP), and tocopheryl succinate. The only remarkable result was tocopheryl succinate with only a weak positive in a sister chromatid exchange assay in the presence of metabolic activation [12].

Tocopherol and tocopheryl acetate are generally recognized as safe food ingredients [12]. According to Brigelius-Flohe et al. [83], vitamin E supplements for pregnancy usually contain only small doses of vitamin E, although adverse effects have not been observed at higher doses. The original report on tocopherols indicated that tocopheryl succinate, up to 75 mg/d in the diet did not have reproductive or developmental effects in rats. In relation to tocopheryl acetate, 1.6 g/kg/d, generally did not have any reproductive or developmental effects in rabbits, hamsters, rats, or mice [84]. There is no published report documenting adverse fetal effects due to use of topical vitamin products. Topical application of vitamin E can rarely cause contact dermatitis, erythema multiforme, and xanthoma [5].

Vitamin E and its derivatives are widely used in many cosmetic and dermatologic products, in general, papers with side effects such as allergic or irritant skin reactions are rare. In clinical studies, tocopherol and tocopherol acetate were found to be safe for use in topical skin formulation since irritant or sensitizing reactions were found only in very small percentages [85]. Tocopheryl acetate was not irritating to rabbit eyes in one study, but it produced weak-to-moderate conjunctival irritation in another study [86]. Positive patch test results of alfa-tocopherol are rare and need to be critically reviewed. However, the derivative (alpha-tocopheryl linoleate), demonstrated allergic popular and follicular contact dermatitis in 1000 cases, reported in Switzerland by a line cosmetic in 1992. This compound was easily oxidized under the storage condition [8]. According to Baumann and Spencer [87], 33% of the patients studied developed a contact dermatitis to the vitamin E. The ingredients considered safe to use in cosmetics were Ascorbyl tocopheryl acetate, Ascorbyl tocopheryl maleate, Dioleyl tocopheryl methylsilanol, Potassium ascorbyl tocopheryl phosphate, Sodium tocopheryl phosphate, Tocopherol, Tocophersolan, Tocopheryl acetate, Tocopheryl linoleate, Tocopheryl linoleate/oleate, Tocopheryl nicotinate, Tocopheryl phosphate, Tocopheryl succinate and Tocotrienols. remembering that the concentrations and conditions of use in the safety tests must be observed [12].

Tocopheryl acetate, 0.2 mL applied under an occlusive patch for 24 hours prior to irradiation, was not phototoxic in a study in 11 participants [84]. According to ECHA [86], animal and clinical testing concluded that tocopheryl acetate was not photoallergenic or phototoxic. The dermal LD50 of tocopheryl acetate is >3 g/kg bw in albino rats. Five animals per group were dosed with 1 or 3 g/kg bw undiluted tocopheryl acetate in vegetable oil under an occlusive patch for 24 hours. Slight erythema was observed 24 to 48 hours after exposure. Slight abrasion was observed in one low dose female, two high-dose females, and two high-dose males [86]. The acute dermal toxicity of mixed tocopheryl phosphates (MTPs) was determined in New Zeal and rabbits; the dermal LD50 was greater than 1,130 mg/kg bw MTP in female rabbits [88].

An aqueous gel containing 1,130 mg/kg bw MTP (918 mg/kg bw a-tocopherol equivalents) was applied to the clipped dorsal skin of 5 male and 5 female rabbits for 24 hours using surgical gauze. At 24 hours, slight-to-well-defined erythema was observed in 4 of 5 males and all females, and slight-to-moderate edema was observed in 2 of 5 males and all females. Signs of irritation were not observed at days 7 and 14 [12].

6.2 Efficacy of Vitamin E for topical formulations

According to Costa [89], vitamin E has a wetting action and in an *in vitro* study, it was found that if it was applied on living skin equivalent cultures also reduced the Transepidermal Water Loss (TEWL), so improving barrier function [72]. Lin *et al* [66], reported that a stable aqueous solution of 15% vitamin C (L-ascorbic

acid) and 1% vitamin E (alpha-tocopherol) when applied topically to pig skin, daily for 4 days, could provide quadruples photoprotection for skin. This was observed by skin biopsy specimens processed for routine histology. The entire 8 mm center section of the histologic ribbon was analyzed, and the results expressed as sunburn cells/mm.

An *in vivo* study with resveratrol, baicalin and Vitamin E topic formulation demonstrated activation of endogenous antioxidants with ROS scavenging, simultaneously. This was observed by percutaneous absorption, biopsies, and biomarkers. A significant improvement was observed in periorbital fine lines, roughness, radiance, skin tone, elasticity, density, and overall appearance by clinical evaluations that were performed by an expert clinical grader at baseline and for 8–12 weeks. In addition to the increase in collagen production (18.9%) in dermal thickness detected by ultrasound measurements [71].

Topical application of tocopherol acetate significantly reduces the severity of erythema, edema and skin sensitivity associated with sunburn by UVB. Magnetic resonance images showed that there was no increase in skin thickness associated with edema. However, the cytotoxic effects of UV exposure as measured in Chinese hamster embryo cells can also be reversed by the presence of other antioxidants as well as a-tocopherol, ascorbic acid, butylated hydroxytoluene (BHT) and GSH. However, before exposure UV these components do not protect against cytotoxicity. In this study, it was observed that high dietary levels of vitamin E can restore the level of incorporation of thymidine dimers into DNA, in UV-exposed epidermal cells in relation to control non irradiated cells. The DNA was isolated and determined by the method of Gendominico Record et al. 1991 [90].

Gaspar and Campos [72], evaluated photoprotective formulations with a combination of photostable (octyl methoxycinnamate, benzophenone-3 and octocrylene or photoinstable filters (octyl methoxycinnamate, avobenzone and 4-methylbenzilidene camphor), both in addition to A, C and E. vitamins The combination of photostable filters showed a better response compared to the others. The filter components and vitamins were quantified by HPLC analysis and spectrophotometry. The formulation containing only vitamins, showed irritation and hairless in mouse skin, this was observed by histopathology.

Ferulic acid, by protecting vitamins C and E, can prevent UV-induced thymine dimer formation when applied topically to skin evaluated by fluorescence microscope coupled with a camera. Studies mentioned a presence of mutations in thymine dimer in keratoses and squamous cell carcinomas of skin, so this result requires that this combination can prevent skin cancer [74]. The photoprotective actions demonstrated by the topical application of alpha-tocopherol in mice may not be restricted to the action of itself [91]. It is likely that the dimers formed from UVB photo-oxidation of alpha-tocopherol, and perhaps the trimers as well, may themselves confer photoprotection, this was observed by similarities in the UV absorbance spectrum. Vitamin E slowed melanoma growth by promoting tumor cell apoptosis and inhibiting VEGF-mediated angiogenesis. The mechanism of the *in vivo* antitumor effect of VES was determined by immunohistochemical detection of proliferation and apoptosis [8].

6.3 Skin disorders

Some studies suggest that a poor diet of vitamin E could be related with skin disorders. Oral supplementation of vitamin E is recommended in therapy of yellow nail syndrome in a dosage of 1000 IU once a day for a period of 6 months; epidermolysis bullosa (300–600 IU/day); in cutaneous ulcers with treatment of pressure sores in doses of 800 IU/L gradually increasing to 1600 IU/L; in wound healing with zinc and

vitamin C for pressure ulcers and burns; in subcorneal pustular dermatosis (d-alpha-tocopheryl acetate) 100 IU/day, gradually increasing to 400 IU/day for 4 weeks; in scleroderma, morphea, calcinosis cutis, and Raynaud's phenomenon respond to vitamin E in a range from 200 to 1200 IU per day; in Hailey–Hailey disease with derivative of vitamin E *d-* alpha-tocopheryl acetate in doses of 800–1200 IU/L by clinically evaluation [5].

Vitamin E has been reported to be effective in inflammatory diseases with attenuation of pro-inflammatory cytokine TNF, evaluated by a section of skin mice by quantitative ELISA kit [64]. In a combination of oral vitamins, A, C, and E with or not proanthocyanidin there was a significant reduction of pigmentation in melasma and pigmented contact dermatitis lesions in two randomized clinical double-bind study [5, 92]. Oral vitamin E (400 IE/day) for 8 months, improvement and near remission of atopic dermatitis and a 62% decrease in serum IgE levels [93]. In oral combination with carotenoids (ß-carotene and lycopene), vitamins C and E, selenium and proanthocyanidins there was decreases the UV-induced expression of Metalloproteinases 1 and 9, that means prevention of sunburn reaction as well as subsequent chronic skin damage, evaluated by clinical trials [94].

In chronic leprosy a topical combination of vitamin E and with an amniotic membrane mesenchymal stem cell decreased the diameter of these lesions, evaluated by randomized controlled trial and monitored weekly [73]. In a dressing based on the association of Vitamin E and *L. plantarum* showed a good antibacterial activity against *S. aureus* and *P. aeruginosa* and guaranteed a sustained release of probiotic cells over 24 h, suggesting a successful and ecologically sustainable alternative to the cotton in wound care [69].

Chung *et al*. [85], demonstrated that a topical occlusive pretreatment with 5% vitamin E for 24 h protected against UV-induced upregulation of human macrophage metalloelastase in human skin *in vivo*. There was improving photoprotection of sunscreens against free radical formation in viable epidermal layers in the cultured human dermal fibroblasts. In a topical tocoretinate, hybrid compound of retinoic acid and tocopherol was reduced the clinical symptoms of lichen and macular amyloidosis [5].

Studies show that there was an improvement in the healing of wounds in diabetic rats by topical vitamin E [95]. According to Kuriyama et al. [9], some animal's studies even suggest that topical vitamin E at a concentration of 20% suppressed allergic and irritant contact dermatitis, exerting a comparable effect to 0.5% prednisone ointment. Those skin conditions are generally self-reported as dry skin [96].

With the onset of xerosis, several inappropriate situations can arise, such as the release of inflammatory mediators, hyperproliferation of keratinocytes and interruption of epidermal differentiation, in addition to changes in lipid structure and enzymatic activity [97]. Skin aging, specifically after age 65, presents several constitutional and functional changes in all layers of the skin, such as cellular senescence, decreased proliferative capacity, decreased ability to repair cellular DNA, abnormalities related to chromosomes, loss of telomeres, DNA extranuclear related mutations, oxidative stress and genetic mutations, promoting the formation of wrinkles, loss of elasticity and dryness of the skin [98, 99]. According to Rhie et al., 2001, the alpha-tocopherol concentration in the epidermis is negatively affected with aging and especially with photoaging, in this case the levels found are even lower when compared to young skin [100].

Over the years, the main histological changes occur with the basal cells, which suffer from dyscrasia, presenting an increase in volume and size, which can be accentuated by the action of UV radiation. As for the functionality of the basal cells, there is a decrease in mitotic activity and an increase in the cell cycle time and

the cell migration time, which can promote changes in the outermost layer of the skin. The horny extract does not change its thickness, however, the replacement of lipids happens slowly, which significantly affects the function of barrier, protection and maintenance of natural hydration [101, 102].

Thus, the topical use of vitamin E is adequate for its recognized antioxidant and protective activities, favoring the improvement of the skin barrier due to its lipophilic character and also, effectively avoiding lipid peroxidation by protecting cell membranes from the action of free radicals [103]. Gehring et al. [63] evaluated the hydration capacity of the stratum corneum by the use of vitamin E (5%) in water/oil and oil/water emulsions, demonstrating moisturizing activity in the stratum corneum, in addition to providing indications that indicate retention of water in the stratum corneum. Gonullu and collaborators [104] also report that the topical use of vitamin E for a period of two to four weeks can improve the ability of water to retain in the skin, favoring hydration.

While aging decreases keratinocyte proliferation, the abnormal hyperproliferation those cells are seen in psoriasis. Psoriasis is a chronic inflammatory process of the skin which affects 1–2% of the population and can affect the quality of life. It is most characterized by the presence of erythematous plaques with silvery scale on various regions of the body including the scalp, extensor regions of the extremities, and intertriginous areas of the skin [19, 105, 106]. Studies on the influence of vitamin E on psoriasis include oral and topical treatment evaluation of the vitamins combination, minerals, among others (vitamins A, C, D, E, B1, B2, B3, B5, B6, B12, magnesium, zinc, selenium, folic acid, copper, lysine and proline), that act on oxidative stress, energy metabolism, the immune system and optimized collagen formation. The consumption of olive oil, a vitamin E source, is also associated with improvement in psoriasis symptoms, acting positively in the suppression of serum levels of metalloproteinase-3 (MMP-3), protein of the cartilage olimeric matrix (COMP) as well as the levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-17) [107]. Topical treatments include the application of products added with plant extracts containing vitamin E and other derivatives, the results are representative in relieving the symptoms of psoriasis induced in the mouse model, suppressing the levels of Interleukin-22 involved in extensive proliferation of keratinocytes and pathogenesis of psoriasis. The critical importance of the interleukin axis for the pathogenesis of psoriatic disease has resulted in new biological treatments targeting these cytokines, indicating that vitamin E is a component of interest in the treatment of psoriasis [106–109].

7. Discussion

Antioxidants consumed orally or topically may impact several organs in the body and among them, the skin. Systemic or centralized effects of these molecules can be modulated by the administration pathway and cellular machinery involved in their metabolization. When compared with other natural bioactive compounds, vitamin E has a specific mechanism of activation in skin due its lipophilicity. Alpha-tocopherol is the active molecule, but several derivatives are available in the market to address solubility, cost and pharmacotechnical necessities. The acetate and succinate esters exhibit better oxidation stability and are often associated with surfactants to improve water-solubility. The hydrolyzation of those molecules is mandatory to achieve biological effects and is mainly driven by enzymatic complexes in skin. The application of derivatives is an interesting alternative for slow delivery of this vitamin since the necessity of activation may lead to accumulation and a reservoir effect [110].

The natural lipophilicity of vitamin E impacts also its biological effects in skin. Vitamin E structure forms complexes with lipids in the cellular membrane and therefore acts promptly against the ROS formation due UV or pollution exposure [111]. The reduction/blockage of oxidative stress' cascade protects skin against several damages visible as wrinkles, melasma and cancer. Besides the lipid peroxidation, vitamin E has an important role in DNA integrity and epigenetic gene modulation [112]. As a natural component of the healthy tissue, vitamin E is associated with impaired skin treatments, such as psoriasis, dry skin, atopic dermatitis and other skin disorders related to oxidative stress and inflammation [5].

The presence of vitamin E is expected in skin, which makes permeation experiments, bioavailability studies and quantification analysis more challenging. Raman confocal spectroscopy showed good sensibility to evaluate the health benefits and safety of vitamin E in human skin *in vivo* [113]. This technique can elucidate the extend of vitamin E overcoming skin barrier and achieving the nucleated epidermis for bioconversion. The complex mechanism of action of vitamin E depends on other bioactive molecules, especially vitamin C. As the primary replenisher of vitamin E in skin, the benefits of using those vitamins cannot be investigated separately and a wider look of the literature is required to make a fair comparison [114]. Further investigation using Raman could bring more data about the mechanisms and contribution of each ingredient. Nevertheless, it is very common that topical products present a combination of molecules acting synergistically to achieve better results.

The cosmetic market is always releasing innovative products despite vitamin E is considered a very classic dermatological active. New delivery systems focused on better absorption, deeper permeation or simpler hydrolysis are the R&D main targets. Since vitamin E is generally recognized as safe (GRASA) food ingredient and used in over-the-counter products with broader concentration range (1.0 to 5.0%), there is little regulatory concern about the exploration of this molecule in cosmetics and supplements. The focus of this chapter is topical applications and therefore, the oral toxicity data was not extensively covered. Safety assessment of alpha-tocopherol and its most used esters showed no phototoxicity, no genotoxicity and no ocular and dermal sensibilization [12]. The use of vitamin E in topical applications is a safe, effective and well accepted worldwide, especially in association with other antioxidants.

8. Conclusions

Vitamin E, more specifically alpha-tocopherol, can be considered a substance with antioxidant activity with the ability to protect long-chain unsaturated fatty acids. It is also capable of playing an important role in a wide variety of physiological and biochemical functions, mediated by the antioxidant function or by its stabilizing effect on cell membranes, breaking down the peroxyl chain propagation reactions and eliminating the efficient lipid peroxyl radicals. Is has been used for decades and is still a very good a widespread ingredient for dermatological products and formulations, especially when associated with other antioxidants such as vitamin C. However, it is important to emphasize the need for more in-depth studies on the use of its derivatives and associations, regarding the conversion speed and the converted amount of vitamin E in skin. There are few studies related to the topical safety and efficacy of vitamin E in the literature, although it is widely used in cosmetics and dermatologic products. A low incidence of contact dermatitis has been reported. However, more studies would be needed for a conclusive answer regarding its topical safety. The definition of optimal dosage in cosmetics depends on the derivative molecule and the type of cosmetic formulation.

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Chapter 2

Pharmaceutical Applications of Vitamin E TPGS

Adnan Mansour Jasim and Mohammed Jasim Jawad

Abstract

D-tocopheryl polyethylene glycol succinate (Vitamin E TPGS) has been approved as a safe pharmaceutical adjuvant by FDA, and several drug delivery systems (DDS) based on TPGS have been developed. TPGS properties as a P-gp inhibitor, solubilizer/absorption and permeation enhancer in drug delivery and TPGS-related formulations such as nanocrystals, nanosuspensions, tablets/solid dispersions, vaccine system adjuvant, nutritional supplement, film plasticizer, anticancer reagent, and so on, are discussed in this review. Consequenly, TPGS can inhibit ATP-dependent P-glycoprotein activity and act as a potent excipient that promotes the efficiency of delivery and the therapeutic effect of drugs. Inhibition of P-gp occurs through mitochondria-dependent inhibition of the P-gp pump. Many of the latest studies address the use of TPGS for many poorly water-soluble or permeable drugs in the manufacture of nanodrugs or other formulations. In addition, it has been reported that TPGS shows a robust improvement in chylomicron secretion at low concentrations and improves intestinal lymphatic transport, which would also boost the potential of drug absorption. It also indicates that there are still many problems facing clinical translation of TPGS-based nanomedicines, requiring a more deep evaluation of TPGS properties and a future-based delivery method.

Keywords: TPGS, Bioavailability, Cancer cell, Prodrugs, Malaria and Osteoartharitis

1. Introduction

An alternative to PEG, D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS), is an amphiphilic macromolecule, a water-soluble natural vitamin E derivative. It is a powerful nanotechnological emulsifier for biomedical applications. TPGS co-administration can enhance solubility, cellular internalization, inhibit the multi-drug efflux transport mechanism mediated by P-glycoprotein, which increase the oral bioavailability of different anticancer drugsVitamin E TPGS is a water-soluble derivative of natural vitamin E derived from vitamin E succinate esterification with polyethylene glycol (PEG) 1000 [1]. Because of its superior water solubility and biocompatibility, PEG is the most widely applied hydrophilic segment. In order for the molecular weight to be higher than the hydrophobic core, the micellar shell is usually chosen to shape the molecule. In micelles, these findings have critical micellar concentrations in the micromolar range, and are often smaller than 100 nm. Vitamin E TPGS is a nonionic surfactant with a molecular weight rate of 1513 $g.mol^{-1}$ and a lipophilic alkyl tail and hydrophilic polar head amphiphilic frame that is fully soluble in water. It is constant, range of pH 4.6–7.6 less than 12 percent break down when kept for three months in neutral aqueous buffer. The

Vitamin E TPGS safety has been notify at the oral LD50 is >7 000 mg/kg for adult male rats [2, 3]. In addition, a variety of compounds such as cyclosporines, taxans, hormones and antibiotics, both water-soluble and water-insoluble, can be solubilized by vitamin E TPGS [3]. Vitamin E TPGS could act as a P-gp inhibitor. It has the ability to inhibit the action of P-gp, stronger than other non-ionic surfactants such as Tween 80, Pluronics and Cremophor. It has been used in various formulations/applications, such as producing nano suspensions [4], self-microemulsifying [3], nutrition supplement formulates nanoparticles, dependent prodrug, and strong dispersion/tablet, vaccine system adjuvant [5–8].

Vitamin E TPGS is widely used, with several functions, such as: hydrophobic drug vehicle, to amelorate ocular permeability and provide ocular retention. TPGS is used as a vitamin E accessory or to treat vitamin E insufficiency in people who are unable to consume lipids due to specific illness [9]. Tocofersolan oral solution has been confirmed by the European Medicines Agency in the treatment of vitamin E deficiency due to digestive malabsorption in pediatric patients, inborn misery or hereditary chronic cholestasis [10]. Tocofersolan is also used as an antioxidant and anti-inflammatory in cosmetics and pharmaceutical products. In different parts of health, especially in neuroprotection, dermal, cardiovascular, and bone health, these crucial benefits of vitamin E are valuable. In nanoformulations involving solid-lipid nanoparticles, nanoemulsions, nanostructured lipid carriers, and polymeric nanoparticles, several TPGS formalizations have recently shown favorable results in improving the efficacy and bioavailability of many drugs [11]. Vitamin E has a prospective ban on metabolic syndrome and cardiovascular diseases (CVDs) [12]. These influences are mediated via inhibition of the HMG-CoA reductase enzyme thus antioxidant, anti-inflammatory activity, and block expression of adhesion molecules. Diabetic rat studies have confirmed that supplementation with TPGS decreases fasting blood glucose, oxidative stress and strengthens the integrity of vascular walls that help to resolve atherosclerotic lesions [13, 14]. Additionally, in dermatology, vitamin E is often used as a protective antioxidant and ultraviolet (UV) radiation that suits photoprotection and retards skin aging through its ability to improve collagen synthesis and avoid collagen dissolution [15, 16]. In addition to adding, TPGS has beneficial anti-cancer properties, such as preventing cancer cell proliferation, prohibiting angiogenesis, altering growth factors, encouraging cell cycle arrest, and inducing apoptosis [17]. The physicochemical and biological properties of TPGS relevant to drug delivery applications are generally described in **Figure 1** as well as, the role of TPGS in enhancing the bioavailability and targetability of anticancer drugs has been highlighted in this review.

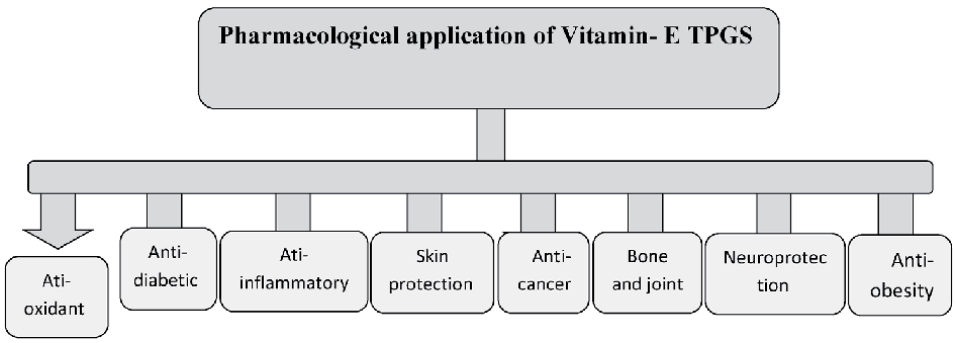

Figure 1.
Various pharmacological properties of vitamin E.

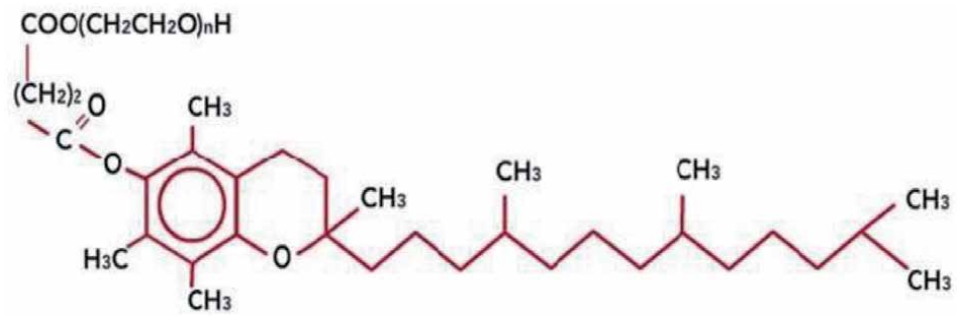

Figure 2.
Chemical structure of vitamin E TPGS. The lipophile to hydrophile equilibrium of the TPGS is an unique amphiphilic structure. Consequently, it is a waxy solid with an air-stable melting point of about 41° C, ranging from yellow to light brown in color. In nature, it is bulky and has a broader surface area, so it is considered the ideal emulsifier and strong solubilizer [19]. The chemical structure of TPGS used in many formulations/ applications shown in ***Figure 2****, such as: 1. Improving drug bioavailability, 2. Properties of surfactants which improve the solubilization of drugs poorly water soluble, 3. Stabilizer of amorphous drug forms 4. Inhibiting the efflux of P-glycoprotein which improves drug permeability, 5. Emulsion vehicle, 6. The active ingredient in self-emulsifying formulations, 7. Minimize drug damage to dermal tissues, 8. Carrier for wound care and therapy, 9. Vitamin E water-soluble source, 10. Fabrication nanosuspensions, 11. Self-microemulsifying and solid tablet/dispersion 12. Vaccine device Adjuvant, 13. Boost of nutrition, 14. Nano-particles formulation, 15. Micelles, 16. Liposomes, 17. Based prodrug [20].*

1.1 Vitamin E TPGS, an amphiphilic polymer

E TPGS is a water-miscible form of vitamin E, which approved by the FDA and commonly used in drug delivery systems as a safe adjuvant. TPGS's biological and physicochemical properties provide several advantages for its drug delivery applications such as high biocompatibility, drug solubility enhancement, drug permeation improvement, and selective antitumor activity [18].

1.2 Structure and properties

Vitamin E TPGS (d-alpha-tocopheryl polyethylene glycol 1000 succinate or TPGS) is a water-soluble derivative of natural vitamin E, formed together with polyethylene glycol 1000 by esterification of d-alpha-tocopheryl polyethylene glycol succinate. Furthermore, TPGS is a macromolecule consisting of a lipophilic alkyl tail and a hydrophilic polar head which has amphiphilic properties (see **Figure 2**).

Vitamin E TPGS is an active solubilizer of various compounds that are water-soluble and insoluble in water, such as steroids, antibiotics, cyclosporins, taxanes, etc. [21]. TPGS vitamin E could function as a P-gp inhibitor with a higher capacity than other non-ionic surfactants, such as Tween 80, Pluronics and Cremophor EL, to inhibit P-gp activity [21].

2. Absorption/bioavailability enhancer

Several studies indicated that the increased bioavailability was due to micelle formation improving solubility, while others showed that P-glycoprotein (P-gp) inhibition contributes to increased permeability support [22, 23]. Although several instances of TPGS use are poorly water-soluble drugs, there are also examples of the use of TPGS with water-soluble poorly permeable drugs. Many studies have been done to evaluate the mechanism by which TPGS improve bioavailability, many of these suggestions to micelle formation and through enhancing permeability across cell membranes by inhibition of multidrug efflux pump P-with regard to oral

deliveryBy beneficially emulsifying and solubilizing the medication in the finished dosage type and by considering a self-emulsifying drug delivery mechanism in the stomach that may be due to TPGS, TPGS increases the permeability of a drug across cell membranes by inhibiting P-glycoprotein and thus facilitates the absorption of a drug over the intestinal wall and into the cell membranes. Furthermore, TPGS is a more potent P-gp inhibitor than many associated excipients with surfactant properties, such as Pluronic P85 cremophor EL, Tween 80, and PEG 300, **Figure 3**. Yu *et al* [24] The solubility of amprenavir was amended in the existence of vitamin E-TPGS out of micelle solubilization. Vitamin E-TPGS prevent the efflux system and boost the permeability of amprenavir [24]. Chiefly, vitamin E-TPGS promotes the absorption flux of the drug by increasing its solubility and permeability.

2.1 TPGS properties in drug delivery systems

The water-miscible type of vitamin E, TPGS, consists of a hydrophilic chain of PEG connected to the hydrophobic portion of vitamin E. According to a particular amphiphilic structure, it shows wonderful drug delivery capability. Further research has shown that TPGS has great potential for P-gp inhibition and selective anticancer outcomes to resolve MDR tumors [25]. TPGS can be readily conjugated with polymers or therapeutic agents to form TPGS based polymers **Figure 4**. It is possible to further self-assemble the resulting composition into nanoparticles, in order to form nanoformulations, unmodified TPGS can also participate with other active compounds. After cell internalization, in response to the unique intracellular environment (e.g. pH, GSH and ROS), the nanoparticles can be degraded to release the therapeutic agents and TPGS [26]. The drugs can be easily pumped out into the extracellular environment without P-gp inhibition [27]. Dissociated TPGS can bind to mitochondrial respiratory complex II and cause mitochondrial dysfunction, resulting in decreased potential for mitochondrial membranes and increased generation of ROS cell apoptosis with decreased activation a level of P-gp ATP [18]. Besides, to further resolve MDR, TPGS can also inhibit the substrate-induced activity of ATPase. The intracellular concentration of therapeutic drugs can be greatly improved with P-gp inhibition. Meanwhile to enhance cell apoptosis, TPGS can inhibit Bcl-2 and Survivin (**Figure 1**) [28].

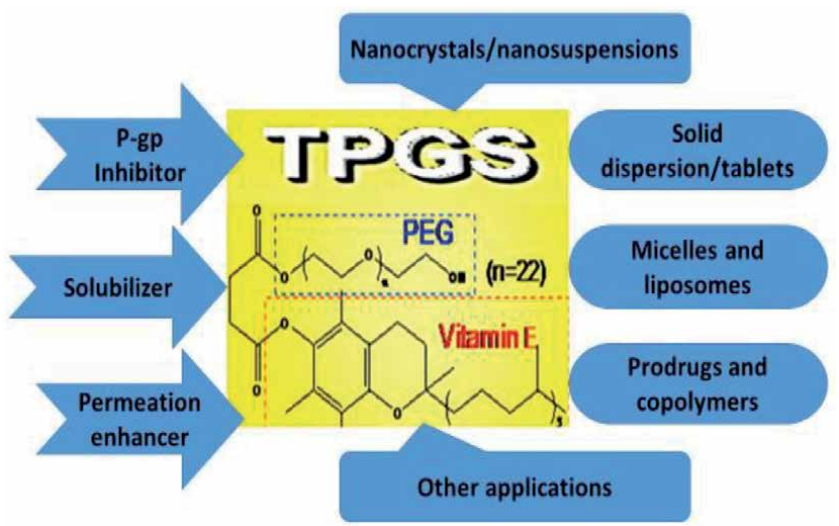

Figure 3.
The applications of vitamin E TPGS in drug delivery [20].Polyethyeleneglycol (PEG).

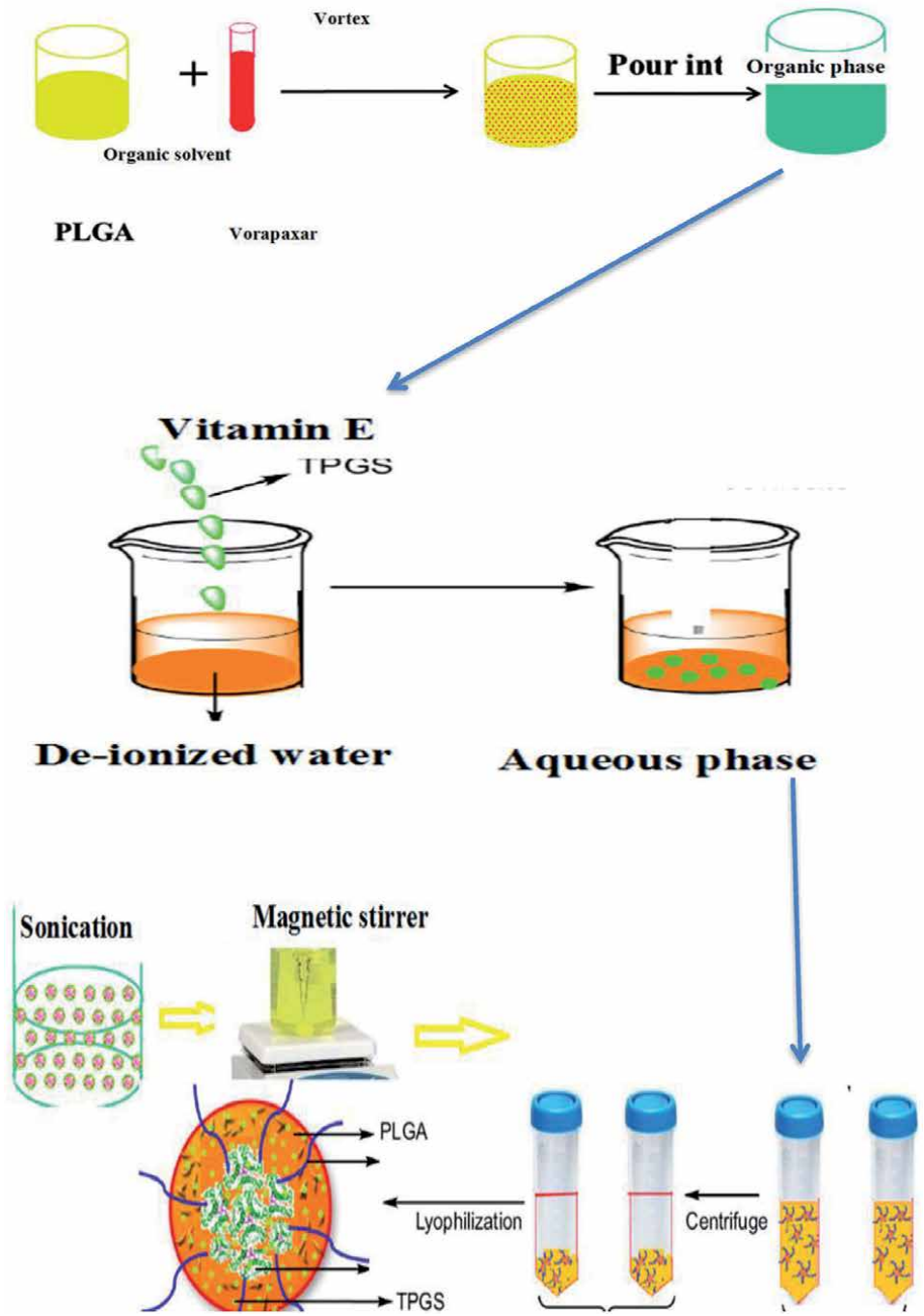

Figure 4.
Nanoprecipation methoed for preparing TPGS coated PLGA polymer nanoparticles. PLGA (Polylactic co glycolic acid).

3. TPGS as a surfactant

Poor water solubility and/or poor permeability remain the great snag for maximum activity of therapeutic drugs [3, 29, 30]. In drug delivery, TPGS can be used as a solubilizer, permeation enhancer, diffusion, and emulsifier as well as as

a surface stabilizer. It has been commonly used for many poorly water-soluble or permeable drugs in the manufacture of nanodrugs or other formulations, mainly for class II and IV Biopharmaceutical Classification System (BCS) drugs [31]. In addition, it has been clearly documented that TPGS shows a good boost in low concentration chylomicron secretion and promotes intestinal lymphatic transport [32], which would further improve the drug absorption potential. As a surfactant, TPGS shows a remarkable ability to surpass drug absorption through different biological barriers. For example, TPGS is used to generate repaglinide nanocrystals to increase the saturation solubility of the oral bioavailability reference drug from 15-to 25.7-fold [33]. In addition, TPGS can increase the penetration of drugs in colonic tissue [34]. Importantly, in the production of nanoparticles with small particle size, high drug encapsulation strength, and quick drug release, TPGS may also act as a pore-forming agent [35]. Furthermore, TPGS can be used as an emulsifier or surface stabilizer for drug formulations since the hydrophobic portion can trap hydrophobic drugs and the formulations can be stabilized by the hydrophilic portion.

4. Role of TPGS to control cancer cell

TPGS acts selectively as an anticancer TPGS by synergistic antitumor action, can induce apoptosis and demonstrate selective cytotoxic activity against in vitro cancer cells that can be combined or loaded with chemotherapeutic drugs to resolve adverse effects and potentiate therapeutic efficiency. The response of cancer cells and normal immortalized breast cells after TPGS therapy is of significant value. Via activating the apoptotic signaling pathways, TPGS can induce G1/S cell cycle arrest in breast cancer cell culture [28]. Jurkat clone E6–1 cells will induce apoptosis on T cell acute lymphocytic leukemia. Apoptosis has been demonstrated by encouraging cell cycle arrest, accelerating nuclear DNA fragmentation, and reducing the possible mitochondrial membrane after treatment with TPGS [36]. The selective processes for TPGS-mediated apoptosis cancer cells are sophisticated and can be described as follows:-

4.1 α-Ractive oxygen species stimulation

Alpha-tocopheryl succinate (alpha-TOS), through the eradication and suppression of mitochondrial respiratory complex II, would induce cancer cell apoptosis [37]. ROS formation can be activated by the subsidiary electron transfer chain defect. The increase of intracellular ROS, an apoptosis mediator, can induce protein, lipid, and enzyme oxidation and DNA damage that leads to cell destruction [38]. This mechanism is also associated with the selective activity of anticancer, as tumor cells may be more sensitive than healthy cells to ROS. Anti-apoptotic protein downregulation TPGS could inhibit the phosphorylation of protein kinase B and then downregulate Survivin, which represents anti-apoptotic proteins, and Bcl-2, which can induce caspase-3 and caspase-7 potential for programmed cell death dependent on caspase [39]. At the same time, caspase-independent programmed cell death and G1/S phase cell cycle arrest also happened. In general, TPGS also appears to be harmful to malignant cells, such as lung adenocarcinoma and breast cancer, through mitochondria-associated apoptosis and ROS [40], generation [41]. Recently reported that TPGS induces OS apoptosis in acute lymphoblastic leukemia involving a cell death signaling pathway. However, no information is ready to limit whether TPGS might eliminate Neuroblastoma tumor cells [42].

4.2 DNA damage

TPGS can induce both caspase-dependent caspase and -independent DNA damage [43]. The ability of vitamin E to trigger caspase-independent programmed cell death could indeed be effective in prostate cancer chemotherapy as it can block tumor resistance usually associated with the use of classical chemotherapeutic drugs that trigger programmed cell death dependent on caspase [44].

Prodrug	Payload	Tumor oodel	Application	Dose	References
TPGS-DOX	DOX	Resistant breast cancer, hepatoma melanoma,	95-fold lower IC50 in MCF-7/ADR vs. free drug, MCF-7/ADR, B16F10, H22 tumor growth/metastasis inhibition	Dox-TPGS-LPs at concentrations equivalent to 5 μg/ml Dox	[47]
TPGS-DOX	DOX	Glioma, Breast cancer	High cellular uptake and cytotoxicity	5.86 μg mL – 1 of DOX	[48]
TPGS-PTX	PTX	Reluctant ovarian cancer, hepatoma	PTX accumulation in A2780/T, cytotoxicity against A2780 and A2780/T, S180 tumor inhibition	Dox-TPGS-LPs at concentrations equivalent to 5 μg/ml Dox	[47]
TPGS-cisplatin	Cisplatin	Hepatoma	High cell uptake and cytotoxicity, significant neuroprotective effects	At dose 25, 2.5, 0.25, 0.025 g/mL of cisplatin	[49]
TPGS-cisplatin	Cisplatin, DTX, Herceptn	Breast cancer	Enhanced cytotoxicity against SK-BR-3 cells with overexpression of HER2	Dose at concentrations of 0.5, 0.05 and 0.005 μg/mL	[50]
TPGS-mitoxantrone	5-FU, PTX	Resistant epidermal carcinoma	P-gp, β-tubulin, and p53 protein extracted from KB-8-5 cells, tumor growth inhibition in KB-3-1 and KB-8-5 tumor model	PTX at dose 5 mg/kg)	[51]
TPGS-gemcitabine	Mitoxantrone	Resistant breast cancer	Cell cytotoxicity against MCF7 and MCF7/ADR cells	25 mg/kg	[49]
TPGS-cantharidin	Gemcitabine	Pancreatic cancer	Improved cytotoxicity against pancreatic cancer BxPC-3	At concentration 15.6 mg /ml	[52, 53]

Doxorubicin (DOX), Paclitaxel (PTX), docetaxel (DTX), 5-fluorouracil (5-FU), lipopolysaccharide (LPS).

Table 1.
The P-gp inhibition effect of MDR in the drug delivery system is mainly discussed in this section.

4.3 TPGS based prodrugs

A prodrug is a drug class with minimal to no therapeutic activity and can be submitted to a set of in vivo metabolism to generate parental drugs [45]. It is designed to improve the concentration of pharmacokinetic (PK), pharmaceutical and pharmacodynamic (PD) products, such as boosting drug solubility, safety, bioavailability, permeability, efficiency of treatment and reducing adverse effects. The prodrug can be classified purely into prodrug and precursor prodrug carrier-setup. The carrier-based prodrug, which is synthesized by a temporal connector merely conjugating polymer with the drug, can easily collect itself into nanoformulation as well as provide great potential for clinical recruitment [46]. The data summerized in **Table 1** explain the role of TPGS and prodrug payload on several types of tumor model with their application.

Over one natural system, stimulus-responsive prodrugs based on TPGS can be prepared to recognize optimum cancer therapy [54].

5. Effect of TPGS on malaria

Malaria is one of the main worldwide infectious diseases. In 2015 only, 212 million cases of malaria and 430,000 malaria deaths were reported [55]. *Plasmodium falciparum* and *P. vivax* respect the majority of the etiology of malaria and the vast majority of deaths are due to *P. falciparum* malaria [56]. *P. falciparum* infections are most likely to develop into severe symptoms such as intense anemia, difficult respiration and cerebral malaria (CM) among human-adapted Plasmodium spp. infections [57]. Several studies show that alpha-TOS inhibits the mitochondrial complex II in ROS generation, which induces selective apoptosis in several types of malignant cells, although it is mainly non-toxic to healthy cells [44, 58]. In addition, cells that lack the potency of the mitochondrial respiratory chain are resistant to alpha-TOS toxicity. Nevertheless the mechanism for alpha-TOS to remain obscure is selectively effective on cancer cells. The effect of plasmodium parasites that are highly susceptible to oxidative stress is doubtful for alpha-TOS. Alpha-Tocopheryl succinate-inhibits the development of cerebral malaria in mice [59].

TPGS is a suitable candidate for safe new anti-malarial drug, This research has shown that TPGS therapy of malaria, survival rates in mice infected with two parasites have been significantly elevated. Similarly, the severity of Evans blue staining on the brains taken from mice treated with TPGS was lower than the remedy not received by mice. This indicates that TPGS should prohibit the collapse of the BBB and the development of cerebral malaria. These data suggest that the potential candidate for malaria treatment drugs could be vitamin E-TPGS. Higher levels have been found after TPGS administration particularly in mitochondria, plasma membranes, and hepatocyte nuclei [60]. The majority of alpha-TOS in hepatocytes that can hydrolyze the esterified forms of vitamin E which may sooner or later hydrolyzed into alptocopherol [61]. In addition, the amount of alpha-tocopherol is comparatively lower in erythrocytes than in other organs such as the liver, kidney, or heart. Although the amount of TPGS is 10 times greater in well-vascularized normal organs such as the liver and kidney than that found in tumors, Alpha-TOS damages tumor cells but not normal cells, indicating that selective anti-tumor activity of alpha-TOS is not correlated with differences in levels in tissue accumulation [62]. Artemisinin and its derivatives that interact with iron to create free radicals are well known as anti-malarial drugs which reported that have growth inhibitory effects on cancer cells and non-toxicity to normal cells in both *in vitro* and *in vivo* studies. Cancer cells typically contain higher free iron levels than normal cells In the

form of heme molecules, plasmodium parasites often contain a high amount of Fe2+ [63]. A time-dependent stimulation of mitochondrial hydrogen peroxide development was triggered by the adding of alpha-TOS to cultured cancer cells.

6. TPGS based polymers in drug delivery

TPGS-based polymers are extensively used in the drug delivery system, which can enhance the drug's encapsulation efficiency, intracellular cell uptake and therapeutic efficacy in vitro and in vivo [64]. The first synthesized PLA-TPGS drug delivery copolymer which produces significant antitumor efficiency. A set of TPGS-based polymers including poly(lactic-co-glycolic acid) (PLGA)-TPGS. [40] hyaluronic acid (HA)-TPGS, poly(beta-amino ester) (PBAE)-TPGS, polycaprolactone (PCL)-TPGS and chitosan-TPGS have obtained significant benefits and have been synthesized for medical applications [65, 66]. PLGA, a biocompatible polymer, is non-immunogenic and can be metabolized in nature to non-toxic products. PLGA is however, hydrophobic and can be quickly filtered and captured by the reticuloendothelial system in the liver. With the assistance of the TPGS, these shortages could be masterfully prevented. As a polymeric matrix for nanoparticles, the PLGA-TPGS polymer can be used to deliver therapeutic agents that can achieve high drug encapsulation performance, sustained-release action, and improved therapeutic effects [67].

To enhance the pharmacological effects, PLGA-TPGS nanoparticles can be prepared to encapsulate these. Emodin, Tanshinone was loaded through qurecetin-loading nanoparticles of PLGA-TPGS, resulting in improved antitumor activity for liver cancer [68, 69]. Gao et al. combined separately loaded heparin sodium and oleanolic acid with PLGA-TPGS nanoparticles indicating synergistic antitumor activity in the HCa-F liver cancer cells [70]. Star-shaped polymer-based drug carriers have lower hydrodynamic radius, minimize solution viscosity, increase drug loading content and increase drug encapsulation performance in comparison to the linear polymers of the same molar mass [71], in comparison to linear PLGA-b-TPGS copolymer-based nanoparticles, doxitaxil-loaded-PLGA-b-TPGS block copolymer nanoparticles present perfect cellular uptake efficiency and sufficient antitumor efficacy. **Table 2** showed that effect of polymers types and drugs loading on tumer model with their application.

6.1 TPGS based formulations to improve drug oral bioavailability

Oral administration is an appealing drug delivery way owing to the simplicity, convenience, high patient compliance, perfect for chronic therapy, and minimize costs for industry and physicians [77]. In addition various inherent challenges, such as reduced permeability through the gastrointestinal tract, low water solubility, enzyme hydrolysis and first-pass elimination, which lead to lower absorption and bioavailability, continue to limit effective drug delivery [78]. P-gp and CYP3A4 substrates are the majority of Class IV biopharmaceutical classification system drugs, resulting in low permeability and existing class metabolism [79]. TPGS-based formulations have several advantages in enhancing the bioavailability of orally administered drugsIn addition, for the sake of a nonionic surfactant, TPGS can improve drug solubility. On the other hand, due to the P-gp inhibition effect, TPGS can enhance drug permeability [72]. Furthermore, the ability to boost drug stability by inhibiting CYP3A4 and CYP2C9-mediated metabolism was confirmed by TPGS [80]. TPGS has shown little inhibition effect on CYP3A activity in other studies [81], which may be linked to dosage [82]. Nanocrystals, nanosuspensions, the

Polymer	Payload	Tumor model	Application	Ref
Chitosan-g-TPGS	DOX	Hepatoma	2.4-fold AUC, 2.0-fold MRT vs. free drug after oral administration	[72]
iRGD-TPGS	PTX	Resistant lung cancer	Significant drug accumulation, downregulation of Survivin expression, and tumor apoptosis	[73]
PLA-PGS, Ce6-TPGS, tLyp-1-TPGS	DOX, Ce6	DOX-resistant breast cancer	In vivo near-infrared imaging of tumor-bearing mice and enhanced antitumor efficiency in MCF-7/ADR	[47, 74]
Transferrin conjugated TPGS	DTX, gold clusters	Breast cancer	In vivo imaging and antitumor efficacy	[75]
4-arm-PEG-TPGS	PTX	Hepatoma	Significant in vivo antitumor effect on S180 sarcoma-bearing mice	[66]
PBAE-g-TPGS	PTX	Resistant breast cancer	Stimuli-responsive release of PTX, targeted drug delivery to tumor, and remarkable MCF-7/ADR tumor inhibition	[66]
PLGA-TPGS	Zontivity	Atherosclerosis atheroma	Reduce the therapeutic dose and remove DNA damage.	[76]

Table 2.
Inspired by the use of PLA-TPGS copolymer loading antitumer and their effects.

self-emulsifying/micro emulsifying drug delivery mechanism (SEDDS/SMEDDS), solid dispersions/tablet, solid lipid nanoparticles (SLNs), liposomes and micelles and emulsified TPGS nanoparticles are included in the TPGS formulations.

6.2 TPGS based formulations to improve drug oral bioavailability

Oral administration is an appealing drug delivery way owing to the simplicity, convenience, high patient compliance, perfect for chronic therapy and minimize costs for industry and physicians [83, 84]. Furthermore, there are still different inherent challenges hampering the effective delivery of drugs, such as limited permeability through the gastrointestinal tract, low water solubility, hydrolysis by enzymes and first pass elimination, which lead to lower absorption and bioavailability [85]. In fact, a majority of biopharmaceutics classification system class IV drugs are substrates of P-gp and CYP3A4, result in poor permeability and extensive pre-systemic metabolism [86]. TPGS-based formulations have numerous advantages to improve bioavailability of orally administered drugs. In addition to, as TPGS has ability to increase drug solubility due to a nonionic surfactant. On the other aspect, TPGS can enhance drug permeation due to the P-gp inhibition effect. Furthermore, TPGS has been confirmed with the ability to improve drug stability by inhibiting the CYP3A4 and CYP2C9-mediated metabolism [87]. In other studies, TPGS showed little inhibition effect on CYP3A activity [88, 89], which may be related to the dosage [90]. The TPGS formulations involve nanocrystals, nanosupensions, self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS), solid dispersions/tablet, solid lipid nanoparticles (SLNs), liposomes and micelles, TPGS emulsified nanoparticles and so on.

Vitamin E (TPGS) is a lipid-soluble organic compound and usually present in the cell membranes. This vitamin has robust antioxidant properties and inhibits the

lipid peroxidation formd by the free hydroxyl and superoxide radicals [91]. This vitamin save the cell membrane of sperm cells from damages of ROS. In vitro studies have demonstrated that the utilize of vitamin E -TPGS amilorate the availability, motility and fertilizing capacity of sperm in the egg penetration of animals. Likwise, in vivo research, supplementation of vitamin E was found to be effective in reducing the number and motility of sperms caused by reactive oxygen species (ROS) [92]. The administration of this vitamin during oral route has significant advantages influnce on the motility of sperms through the depression synthesis of malondialdehyde (MDA), which is known as the final part of lipid peroxidation [93].

The deficiency of vitamin E may spoil the reproductive organs like harm in the spermatogenesis, testicular dysfunction and seminiferous tubules shrinkage. The utilize of this vitamin boost the functions of testes in the form of excess in the weight ofepididymis and testes. In addition, the antioxidants properties of TPGS, endogenous, antioxidant enzymes like superoxide dismutase (SOD), and glutathione peroxidase are augmented due to the use of this vitamin [94]. This imbalance between the endogenous antioxidants and oxidative stress results in a situation of infertility in males. Antioxidants play an essential role in eliminating these free radicals. Vitamin E is one of the better antioxidants for the sweep of oxidative stress in the male reproductive system. Its use raise functions of the reproductive system and its effecacy. The lack of TPGS result in the declination of germinal epithelium and Leydig cells in seminiferous tubules.

The use of selenium and vitamin E posses synergistic effects on the male reproductive system. More than 25% of males defeat to output functional sperms for effective insemination [94]. The over production of ROS is the main cause of infertility by damaging the genetic material and enzymes activities of [13]. ROS can be harmful or benefit according to their site as well as level of production [95]. The sperm have the capability to move after the transit stay in epididymis. They demand some physiological processes such as capacitation takes place during the female reproductive tract to fertilize the egg, through this physiological event the suffcint amount of ROS is produced [96]. In the ROS, superoxide is count as the most harmful agent. The male germ cells are susceptible to ROS due to a higher amount of polyunsaturated fatty acids within the cell membrane and cytoplasm [97].

Vitamin E is considered an essential portion of antioxidants in sperm [38] and acts as a substantial protection to minimize the production of reactive oxygen species [30]. Spermatozoa demand the ROS for natural functions like acrosome functions, capacitation, and incorporation of spermatozoa through the operation of fertilization [43]. But the production of an excess quantity of ROS leads to lipid peroxidation in the membrane of sperm [43, 44]. Vitamin E prevents the production of ROS in the sperm membrane during the various motility processes due to lipid-soluble [43]. In addition to scavenging of ROS, this vitamin has the capacity to conserve the primary reproductive organs and accessory reproductive organs in males. Feeding of vitamin E sbsquent metabolism and absorption of vitamin reduction of ROS in blood result in Increase in semen quality parameters and testosterone rise in antioxidants enzymes (Superoxide dismutase, glutathion peroxidase). Glutathion peroxidase (GPx) is considered as the asignificant antioxidant and minimize the amount of lipid peroxidation. This enzyme act potentiating to vitamin E as a oppsite agent for hydrogen peroxide [98].

7. Osteoartharitis

Osteoarthritis (OA) of the knee is a major reason of chronic, incompetence in elderly people, the pathogenesis of this disease until now not clear understood [60].

Recent guide demonstrates that oxidative stress, the event wherein oxidant levels overtake those of antioxidative agents, is one of the motives factors of OA [99–101]. ROS including oxidants that are generated under the physiological situation in the human body and controlled by cellular antioxidants, lead to functional and structural damage of cartilage cells. Several report studies of the relationship between oxidative stress and OA have been undertaken. The elevation nitrite, a stable deterioration biochemical marker of the being of nitric oxide, has been confirmed in the plasma and synovial fluid of patients with OA [102]. Vitamin E, a dietary antioxidant capable of augmenting the total cellular antioxidant ability, reportedly has a positive influence on the symptomatic therapy of arthritis [103, 104]. However, there is very little proof from high quality trials that vitamin E modifies oxidative markers and clinical signs in people with knee osteoarthritis [28, 105]. This is the primary randomized controlled trial that converge on the influence of vitamin E in end stage knee OA and entirely estimate clinical symptoms, biochemistry and histology results. We hypothesized that a sustained peroid of vitamin E administration will reduce the oxidative stress, inflammatory process and ameloriate symptoms in patients with end stage knee OA.

8. Conclusions

In this chapter, we summarized the feature and recent advancements of TPGS in drug delivery and pharmaceutical application. TPGS has been approved by FDA as a secure pharmaceutical adjuvant with top biocompatibility. In addition to TPGS can serve as an effective P-gp inhibitor for overcoming MDR. TPGS oneself can be active as an anticancer agent with selective toxicity to tumor cells. TPGS can be easily combined with nanotechnology to develop nanomedicines, which has been shown as a promising strategy in cancer treatment with increased solubility and stability of therapeutic agents, improved PK/PD, enhanced treatment efficiency, and minimize side effects. Furthermore, the impact of TPGS on the immune system, TPGS can be used as an adjuvant in vaccine development. As to TPGS based formulations, the limitations to realize the precise stimuli-responsive property and deep penetration of nanoformulations in the tumor microenvironment still remain as obstacles for the widespread application of these nanomedicines. However, the production of TPGS nanomedicines is yet on a laboratory scale and the progress in developing novel nanomedicines is comparatively slow, which hinders the effective clinical translation of TPGS based nanomedicines.

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Chapter 3

Vitamin E in Chronic Myeloid Leukemia (CML) Prevention

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Abstract

The resistance to inhibitors of tyrosine kinase necessitates novel approaches to the therapy of chronic myeloid leukemia (CML). The progression of CML to blast crisis is associated with down-regulation of C/EBP-alpha being involved in the differentiation block in leukemic blast cells. Moreover, lowered C/EBP-alpha expression correlates with resistance to imatinib in CML. We have demonstrated that vitamin E up-regulates expression of C/EBP-alpha and down-regulates expression of Snail transcription factor in K562 cells in vitro contributing to the putative recovery of myeloid differentiation potential. In parallel with increased CEBP alpha expression, Vitamin E treatment results in the decreasing expression of placental-like alkaline phosphatase and increasing expression of tissue non-specific alkaline phosphatase. We suggest that vitamin E could be used as the plausible biological modulator to prevent the progression to blast crisis and to overcome drug resistance of leukemic cells in CML.

Keywords: chronic myeloid leukemia, vitamin E, C/EBP-alpha, Snail, K562 cells, drug resistance

1. Introduction

Chronic myelogenous leukemia (CML) is a clonal hematopoietic stem cell disorder associated with the activity of *Bcr-Abl* fusion oncogene that arises from the translocation of chromosomes 9 and 22 as t (9:22) (q34;q11) [1, 2]. The BCR/ABL fusion protein with elevated ABL tyrosine kinase activity is crucial for transformation of hematopoietic stem cells (HSCs) [3]. The constitutively active P210 BCR-ABL tyrosine kinase is considered as a key player in the molecular pathogenesis of CML [3, 4]. The disease begins with an indolent chronic phase that can last for several years. If untreated, it then progresses into accelerated phase and within a year into blast crisis phase. The survival of patients in blast crisis is less than one year. Because the preeminent rearrangement driving CML is *Bcr-Abl*, only BCR-ABL tyrosine kinase inhibitors such as imatinib (or nilotinib and dasatinib) are a known curative therapy of CML with extraordinarily successful 5-year survival rates greater than 90% [5–8]. Nevertheless, the secondary mutations finally contribute to the therapy resistance and blast crisis of the disease. The search for the novel compounds for the effective control of CML progression is now in the spotlight.

The free radical scavengers like alpha-tocopherol may be effective against cancer-associated oxidative stress. The mean serum vitamin E level significantly

decreased in CML patients that seems to be quite in agreement with free radical involvement in CML progression [9]. In contrast to antioxidant function of vitamin E in CML, we suggest new modulation mechanisms of vitamin E that could be operative in prevention of CML progression. In particular, we analyzed the modulation function of vitamin E for molecular unblocking of myeloid differentiation potential in CML cells via vitamin E-dependent induction of pivotal transcription factor CEBP alpha (CCAAT/enhancer-binding protein) as myeloid master regulator of myelopoiesis/granulopoiesis and consequently G-CSFR (granulocyte-colony stimulation factor receptor) [10]. Moreover, we have found that vitamin E could be involved in targeting epithelial-mesenchymal transition (EMT) mechanism in CML cells via SNAIL as EMT inducer [11, 12]. Therefore, we propose that vitamin E could be a therapeutic option when CML progresses in setting of imatinib therapy. Finally, since alkaline phosphatase is considered as a marker of stem cells [13], we studied the aberrant expression of placental-like alkaline phosphatase (PLAP) and discovered the potential of vitamin E in remodeling of CML-associated aberrant expression of this enzyme [14]. Vitamin E-dependent induction of tissue non-specific alkaline phosphatase (TNAP) is paralleled by restored CEBP alpha expression as myeloid master regulator in CML cells [14].

Taken together, these findings suggest that vitamin E shows ability of remodeling leukemic stem cell (LSC) phenotype in CML cells to hematopoietic stem cell (HSC) phenotype with myeloid differentiation potential development.

2. Vitamin E activates expression of C/EBP alpha transcription factor and G-CSF receptor in CML blast crisis leukemic K562 cells

C/EBPα is mainly involved in cell fate decisions for myeloid differentiation [15]. The progression of CML to blast crisis is correlated with down-modulation of C/EBP-alpha contributing to the differentiation block, enhanced proliferation, and development of acute myelogenous leukemia [16, 17]. The level of C/EBPα expression is significantly declined in CML patients [18]. Currently, the deregulation of C/EBP alpha is considered as a paradigm of leukemogenesis [19]. Therefore, C/EBPα is a critical regulator of myeloid development guiding granulocyte and monocyte differentiation.

We have studied the modulating potential of vitamin E as the possible inducer of C/EBP-alpha expression in BCR-ABL-positive CML K562 cells. K562 cell line originated from a CML patient in blast crisis progression is recognized as a model for leukemia research. We studied the effects of vitamin E in K562 cells in comparison with valproic acid with known differentiation properties towards myeloid cells [20–22].

Valproic acid in a concentration of 4 mM for 48 h reduced the growth rate and cell viability and induced apoptosis in a fraction of K562 cells (up to 30%). As to vitamin E, in the series of our preliminary experiments, no evidence of toxicity has been demonstrated when K562 cells were cultured with vitamin E in a concentration of 100 μM for 48 h. These concentrations were further used in the experiments for assaying the expression of C/EBP-alpha and G-CSFR mRNA. **Figure 1A** demonstrates that valproic acid did not change significantly the level of mRNA C/EBP expression in K562 cells. On the contrary, vitamin E proved to be an effective inducer of mRNA C/EBP with about 10-fold increase in expression as compared with non-treated K562 cells. When mRNA G-CSFR expression in K562 cells was assessed, both valproic acid and vitamin E induced mRNA of this receptor, with effect of vitamin E surpassed that of VA (**Figure 1A** and **Table 1**).

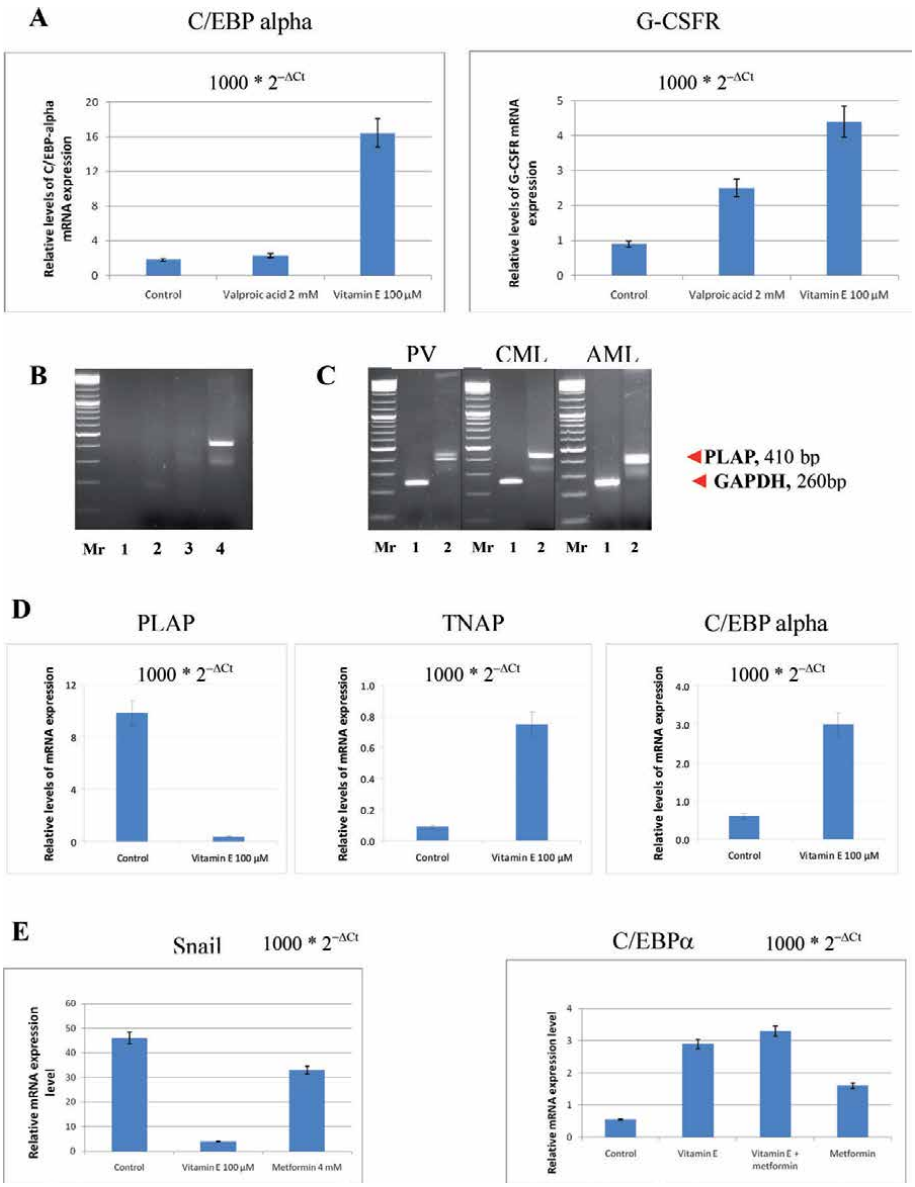

Figure 1.
mRNA expression in CML cells modified by vitamin E. (A) The relative levels of mRNA C/EBP-alpha and G-CSFR expression in K562 cells exposed to valproic acid (2 mM) or vitamin E (100 μM) for 48 h. (B) The aberrant AP mRNA detected by qRT-PCR in leukemic cells of the patient with CML blast crisis: 1 – control without primers; 2 – primers to PAP; 3 – primers to TNAP; 4 – primers to IAP. (C) Ectopic gene expression of embryonic PLAP mRNA in peripheral blood cells of the patient with CML, acute myeloid leukemia (AML), and polycytemia vera (PV): 1 – GAPDH, reference gene; 2 – aberrant PLAP. (D) The relative levels of mRNA expression of PLAP, TNAP, and CCAAT-enhancer binding protein alpha (C/EBPα) in K562 cells exposed to vitamin E (100 μM) for 48 h by real time RT-PCR $2^{-(\Delta Ct)}$ method. (E) Relative mRNA expression level of transcription factor Snail and transcription factor CEBPα in CML blast crisis K562 cells exposed to vitamin E (100 μM) and metformin (4 mM) for 48 h. The relative levels of mRNA expression were analyzed by qRT-PCR and calculated by $2^{-(\Delta Ct)}$ method.

Thease findings are quite confirmed by Tavor et al. [23] have first shown that the restoration of C/EBP-alpha expression in BCR-ABL-positive KCL22 blast cell line provided by transfection with C/EBPα plasmid vector caused a block in the G_2/M phase of the cell cycle with gradual increase in apoptosis suggesting that C/EBP-alpha may be considered as a putative target in differentiation therapies in acute myeloid

N (n = 3)	C/EBP alpha		G-CSFR	
	Fold increase	Standard Deviation, σ	Fold increase	Standard Deviation, σ
1	8.395 ± 1.481	1.219	3.930 ± 1.843	1.988
2	9.854 ± 0.023		4.626 ± 0.853	
3	11.381 ± 1.506		5.134 ± 1.357	

Table 1.
Fold increase C/EBP alpha and G-CSFR mRNA expression (analyzed in triplicates) in gene expression in K562 cells line culture under 48-h vitamin E exposure (100 μM) calculated by $2^{-(\Delta\Delta Ct)}$ *method. Note:* $\sigma = \sqrt{1/n \sum(x_i - x^-)^2}$.

leukemia. C/EBPα directly activates G-CSFR transcription in lineage committing activation of common myeloid progenitor [24–26]. Therefore, C/EBPα loss is causally connected with early block in myeloid maturation suggesting that C/EBPα is a master regulator of hematopoietic differentiation. The transcription factor C/EBPα is known as a critical regulator of myeloid development, directing granulocyte, and monocyte differentiation [27].

Our findings gave evidence of C/EBP alpha-dependent activation to granulocytic differentiation via targeted increase in G-CSFR expression in vitamin E treated K562 cells. It should be further elucidated whether such effects of vitamin E on myeloid transcription factor C/EBP-alpha are direct or mediated indirectly due to the antioxidant properties of vitamin E. Nevertheless, our data suggest vitamin E-associated hematopoietic differentiation-like potential associated with C/EBPα and G-CSFR up-regulation. Our findings might be very important for future studies of imatinib resistance in CML clinical setting taking into account the recent report by S. Kagita et al. demonstrating correlation of C/EBPα expression with response and resistance to imatinib in CML [28].

3. Aberrant expression of placental-like alkaline phosphatase in chronic myeloid leukemia cells in vitro and its modulation by vitamin E

LSCs in CML do not depend on BCR-ABL signaling for their survival [29, 30], and their persistence remains a major obstacle to curing CML [31, 32]. The search for new biological markers of LSC phenotype is still relevant today. Placental-like alkaline phosphatase (PLAP) is expressed by many tumors. Its aberrant expression has been considered to be potentially useful as tumor marker [33]. However, the biological background of the role of this aberrant alkaline phosphatase (AP) in cancer is still unclear. The AP activity in blood serum known as nonspecific marker of bone metastasis [33] is also of potential significance for the identification of stem cell phenotype [13, 34]. Moreover, AP activity is a widely accepted marker of stem cells associated with embryonic stem cell pluripotency [35]. The expression of various forms of AP in CML cells has not yet been studied. Therefore, we aimed to analyze the expression patterns of various AP forms in cells originated from CML patients in blast crisis and to modify their expression by vitamin E (100 μM) in K562 cells. We used the primers to three known tissue AP, namely placental AP (PAP), non-specific AP (TNAP) (expressed in bone, kidney, liver) and intestinal AP (IAP) [36] to analyze the mRNA expression of these APs in CML cells by qRT-PCR. We have observed the aberrant expression of mRNA IAP in cells of CML patient in blast crisis (**Figure 1B**) that upon sequencing (data not shown) demonstrated the significant alignment with known cancer-associated PLAP sequence, while no gene homology with tissue PAP was detected. This fact gave reason to

consider revealed PLAP as embryonic-like placental AP (ELAP), to be more precise, the aberrant PLAP in blast cells of CML patients (**Figure 1C**). Indeed, such PLAP is expressed in early embryo pre-implantation period as was detected in studying mouse embryonic cell development, while tissue TNAP begins to express in post-implantation period [35]. We have not detected TNAP in cells of CML patients. Only the embryonic-like PLAP was detected, which expression also increased in acute myeloid leukemia (**Figure 1C**). Recently, TNAP recognized ultimately as mesenchymal stromal cell antigen-1 (MSCA-1) [13] was described as a biomarker associated with normal hematopoiesis as well as with terminal myeloid differentiation [37]. The decreased TNAP synthesis is a classical feature of CML used as one of diagnostic cytochemical markers in differential diagnosis [2]. We have observed vitamin E targeted decrease in aberrant embryonic-like PLAP expression at mRNA level with increased TNAP mRNA expression. Moreover, along with down-regulation of aberrant PLAP the up-regulation of C/EBP alpha mRNA expression was restored by vitamin E in exposed K562 cells as we founded (**Figure 1D** and **Table 2**).

Taken together, we have concluded that the loss of TNAP and CEBP alpha in CML may contribute to pathogenesis of this disease whereas aberrant embryonic-like PLAP may be considered as a new CML biomarker of LSC pluripotent phenotype in CML progression. Therefore, aberrant embryonic-like PLAP may be considered as a putative target in differentiation therapies in myeloid neoplasms. Our findings suggest the biomodulation role of vitamin E as the available inducer of differentiation potential of CML leukemic cells. The ectopic PLAP expression in leukemic cells of different myeloid neoplasms suggests its importance in biology of these malignancies.

Conclusivelly, to analyze whether ectopic PLAP expression in CMLcells *in vitro* may be modulated, we studied PLAP and TNAP expression in CML blast crisis K562 leukemic cells incubated with vitamin E for 48 h. In fact, vitamin E treatment affects expression of PLAP and TNAP in opposite ways. Namely, PLAP expression decreased significantly while TNAP expression increased. The increase in TNAP expression was paralleled with increased CEBP alpha expression (**Figure 1D** and **Table 2**). **Figure 1**, D demonstrates that vitamin E targeted aberrant PLAP expression is closely related to the restoration of CEBP alpha and TNAP expression. These key regulators tightly contribute to potential reactivation of myeloid differential as we studied in K562 leukemic cells. Therefore, we point out that vitamin E could be able to affect leukemic blast stem cell phenotype remodeling.

To sum up, we have demonstrated increased aberrant PLAP expression in leukemic cells of myeloid origin (CML) in the setting of the decreased TNAP expression. The aberrant expression of embryonic PLAP may be considered as

N(n=3)	Fold decreasing	Standard deviation, σ	Fold increasing	Standard deviation, σ	Fold increasing	Standard deviation, σ
	PLAP (M ± m)		CEBPα (M ± m)		TNAP (M ± m)	
1	4.088 ± 0.322	1.42	2.972 ± 1.594	1.35	6.023 ± 2.809	1.98
2	6.292 ± 1.883		4.377 ± 0.117		1.690 ± 1.524	
3	2.848 ± 1.561		6.207 ± 1.713		1.931 ± 1.283	

Table 2.
The relative fold decreasing PLAP corresponding to fold increasing CEBP α and TNAP mRNA expression (analyzed in triplicates) in gene expression in K562 cells line culture under 48-h vitamin E exposure (100 μM) calculated by $2^{-(\Delta\Delta Ct)}$ method. Note: $\sigma = \sqrt{1/n \sum (x_i - x^-)^2}$.

one of the putative markers of myeloid cell undifferentiated state. On the other hand, potential of PLAP as one of the possible target for controlling LSC phenotype should be further explored. More attention is needed to explore the potential of the bioactive molecules such as vitamin E that may induce granulopoiesis reprogramming.

4. Vitamin E suppresses EMT-SNAIL transcription factor and restores CEBP alpha transcription factor as master regulator of myelopoiesis in K562 cells

The persistence of LSC remains a major obstacle to cure CML [38, 39]. Epithelial mesenchymal transition (EMT) mechanism is known to contribute to tumor stem cell progression [40, 41]. Although EMT has been studied in relation to epithelium-derived tumors, there is increasing evidence implicating the involvement of EMT activators in hematopoietic malignancies [42, 43]. The expression of some EMT modulators has been demonstrated in Ph + leukemia cells [44]. EMT inducer Snail if of most important role in maintaining stemness properties in tumor progression [45, 46]. It was shown that Snail also drives LSC phenotype in leukemia progression [44, 47]. Earlier, we revealed that alpha-tocopherol might be an effective inducer of mRNA CEBP alpha in K562 cells *in vitro* [10]. The loss of C/EBPα contributes to leukemogenesis [16, 19] and CEBP alpha expression prevents from appearance of EMT phenotype [48].

We have determined the relationship between EMT-Snail suppression and restored CEBP alpha myeloid differentiation potential in CML blast crisis K562 cells exposed to vitamin E. Metformin as known substance mediating EMT reversal [49] was used to compare EMT suppression effect of vitamin E in K562 cells.

We have found highly detectable Snail1 mRNA expression and down-regulated CEBP alpha in K562 cells (**Figure 1E**). Vitamin E suppressed EMT-Snail mRNA expression and up-regulated myeloid master regulator CEBP alpha mRNA expression (**Figure 1E** and **Tables 3**, **4**). Such reactivation of CEBP alpha is enhanced by metformin pointing to the possible synergistic effect with alpha-tocopherol. We observed that vitamin E is a modulator of gene expression that affects Snail1 and CEBP alpha mRNA expression in K562 cells in opposite directions. One could suggest the causal relationship between EMT-Snail1 suppression and restoration of CEBP alpha expression that seems to contribute to recover myeloid differentiation potential of CML blast cells. As seen in **Figure 1E**, myelopoietic master regulator C/EBPα is also restored upon metformin treatment, although the effect of vitamin E is more pronounced (**Table 4**).

Taken together, schematic model of the Vitamin E modulation effects in CML blast crisis progression with Snail-EMT phenotype is presented (**Figure 2**).

N(n=3)	Fold decreasing	Standard deviation, σ	Fold decreasing	Standard deviation, σ
	Vitamin E/Snail M ± m		Metformin/Snail M ± m	
1	14.160 ± 0.437	0.408	1.579 ± 0.110	0.086
2	13.176 ± 0.547		1.366 ± 0.103	
3	13.833 ± 0.110		1.464 ± 0.005	

Table 3.
Fold increase EMT-inducer transcription factor SNAIL mRNA expression (analyzed in triplicates) in gene expression in K562 cells line culture under 48-h vitamin E exposure (100 μM) calculated by $2^{-(\Delta\Delta Ct)}$ method. Note: $\sigma = \sqrt{1/n \sum (x_i - x^-)^2}$.

N(n=3)	Fold increasing	Standard deviation, σ	Fold increasing	Standard deviation, σ	Fold increasing	Standard deviation, σ
	Vitamin E M ± m		Vitamin E + Metformin M ± m		Metformin M ± m	
1	5.156 ± 0.328	0.232	5.564 ± 0.075	0.371	2.841 ± 0.007	0.272
2	4.634 ± 0.194		5.00 ± 0.489		3.164 ± 0.330	
3	4.696 ± 0.132		5.902 ± 0.413		2.498 ± 0.336	

Table 4.
The fold increase of relative levels of the transcription factor CEBP alpha mRNA expression compared with metformin (4 mM) under decreasing of relative levels of the transcription factor Snail mRNA expression by vitamin E (analyzed in triplicates) in K562 cells line culture under 48-h vitamin E exposure (100 μM) calculated by $2^{-(\Delta\Delta Ct)}$ method. Note: $\sigma = \sqrt{1/n \sum (x_i - x^-)^2}$.

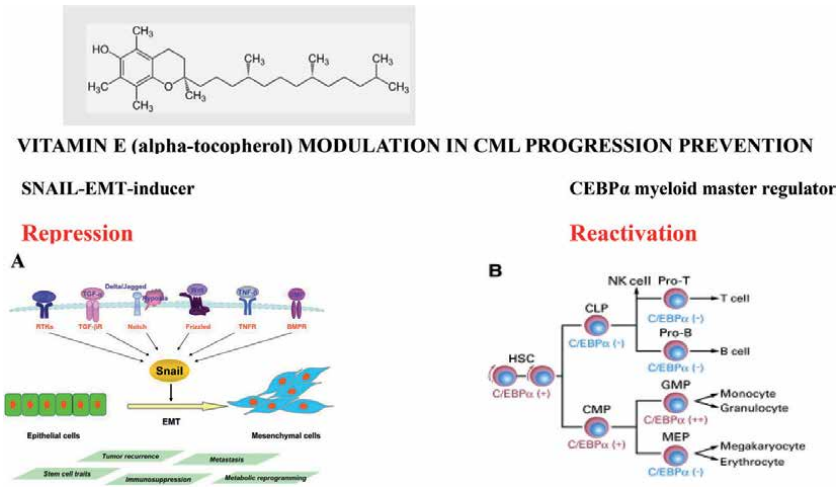

Figure 2.
Schematic model of the vitamin E modulation in CML progression with EMT phenotype.

5. Discussion

CML is characterized by an accelerated and unregulated proliferation of predominantly myeloid cells in the bone marrow with their accumulation in the blood. CML develops as a result of malignant transformation and clonal proliferation of pluripotent hematopoietic stem cells (HSCs), leading to overproduction of immature myeloid progenitor cells that results in blast cell crisis. The CML blast crisis resembles acute leukemia. Because the preeminent mutation driving CML is Bcr-ABL thyrosine kinase oncogene, the use of Bcr-Abl kinase inhibitors (TKIs), such as imatinib, dasatinib, and nilotinib, significantly improves treatment outcomes and extends the life expectancy of CML patients. However, imatinib resistance drives blast crisis progression. The persistence of LSCs remains a major obstacle to cure CML. The clinical CML blast crisis progression with LSC phenotype is practically incurable. Therefore, the blocking of terminal myeloid differentiation and LSC phenotype development defines a putatively new strategy for CML prevention.

The potential of vitamin E in regulation of these interdependent mechanisms in CML progression was hinted by several observations that were reported earlier. Sangodkar et al. [50] showed that vitamin E activates PP2A phosphatase resulting in Bcr-Abl thyrosine kinase inhibition and re-activation of myeloid differentiation

pathway. In BCR/ABL transformed cells and CML blast crisis hematopoietic progenitors, the PP2A activity is strongly inhibited, while the pharmacological activation of PP2A suppresses BCL/ABL activity and induces BCR/ABL degradation [51]. The pharmacological modulation of PP2A activity is becoming an attractive strategy for cancer treatment. The substances of several different classes are known as PP2A activating compounds, vitamin E (α-tocopherol) and its analogues having been reported among such compounds [50, 52].

Nevertheless, the effects of vitamin E on differentiation pathways in cells of blast crisis CML, in particular those involving restoration of the expression of CCAAT-enhancer binding protein alpha (C/EBPα) and granulocyte colony-stimulating factor receptor (G-CSFR) have not been yet studied. The expression of these proteins decreases drastically in chronic phase and blast crisis of CML [53, 54]. In this regard, we evaluated the effect of vitamin E on RNA expression of crucial factors of myeloid differentiation, C/EBRα and G-CSFR, in BCR-ABL-positive CML blast crisis K562 cells. Our data demonstrate that vitamin E restores the expression of C/EBPα and concequently G-CSFR. Our results are consistent with Tavor et al. [23] who demonstrated that the restoration of C/EBPα expression in BCR-ABL-positive KCL22 blast cell line triggered a proliferative arrest, a block in the G2/M phase of the cell cycle and a gradual increase in apoptosis suggesting the activation of differentiation. Therefore, C/EBPα stimulated by vitamin E may be considered as a putative target in differentiation therapies in myeloid leukemias.

The second effect of vitamin E potentially useful for CML treatment was reported by Nieborowska-Skorska et al. [55] who demonstrated that vitamin E prevents accumulation of imatinib-resistant BCR-ABL1 kinase mutations in mice CML xenografts. The authors stressed anti-oxidant function of vitamin E in this processes. We use vitamin E as modulating factor in CML that involves vitamin E-dependent EMT mechanism of repression taking into account the pivotal role of EMT in the development of LSC phenotype. In this connection, we observed Snail1 overexpression suggesting some features of EMT phenotype in K562 cells seemingly contributing to CML pathogenesis. Furthermore, we have determined down-regulation of CEBP alpha transcription factor representing the master regulator of myelopoiesis in CML cells coinciding with Snail1 overexpression. Our findings are quite consistent with the recent report by Lourenço et al. [43] who suggest that C/EBP α is crucial determinant of epithelial maintenance by preventing EMT. Indeed, we have found that CEBP alpha is repressed by overexpression of EMT-inducer Snail in CML blast crisis K562 cells. Consequently, the reactivation of CEBP alpha by vitamin E is paralleled by suppression of Snail.

Therefore, our findings make deeper understanding of the role of vitamin E in suppression of CML LSC phenotype. In addition, we have revealed a new marker – aberrant placental-like alkaline phosphatase (PLAP) that expressed ectopically in CML progression. Moreover, its suppression by vitamin E consequently re-activates CEBP alpha and TNAP as myeloid differentiation factors. Taken together, our findings presented in this Chapter stress the role of vitamin E in modifying expression profile of CML cells towards restoration of myeloid differentiation potential.

6. Conclusion

Vitamin E is a complex group of lipid-soluble antioxidants comprising four tocopherols and four tocotrienols. It prevents production of reactive oxygen species (ROS) that are elevated in majority of tumor cells leading to lipid peroxydation, changing signaling pathways that control cell proliferation and apoptosis, expression of several transcription factors, epigenetic modulators, resistance to treatment,

etc. We have suggested the causal relationship between EMT-Snail1 suppression and restoration of CEBP-alpha myeloid master regulator expression that seems to contribute to recover myeloid differentiation potential of CML blast cells by vitamin E. We first observed that vitamin E is an effective modulator of down-regulation of transcription factor Snail EMT-inducer and up-regulation of pivotal myelopoietic transcription factor CEBP alpha resulting in restoration of TNAP expression. Taken into account the data of literature and our findings, we can postulate that vitamin E might be used as a potential pharmacopoeian biological modulator capable of preventing the onset of blast crisis development, ameliorating disease progression and possibly overcoming drug resistance of leukemic cells in CML patients.

Additional information

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Chapter 4

Vitamin E and Derivatives in Skin Health Promotion

Júlia Scherer Santos, Guilherme Diniz Tavares and Thaís Nogueira Barradas

Abstract

Vitamin E is fundamental for a proper function of human cells. Mostly obtained from vegetable oils, it has antioxidant and non-antioxidant actions. At times, its oral intake or skin application are employed. Oral intake is recommended in some cases. Differently, the topical application is a part of daily skin routine. Both in oral or in topical formulations, it is employed in its isoforms or derivatives. Tocopherols and tocotrienols are isoforms while derivatives are synthetic forms. In pharmaceutical and cosmetic formulations, vitamin E and its derivatives are widely used due to its antioxidant and photoprotective properties. However, the clinical success treatment is often impaired by its low skin penetration, high lipophilicity, and chemical instability. A rational formulation design in the development of novel vitamin E dosage forms is required. In this chapter, the most successful and innovative approaches towards Vitamin E and its derivatives loaded in formulations for skin health promotion are reviewed. Conventional and nanoparticle-based formulations enable vitamin E chemical stabilization, and they are suitable vehicles for its release on the skin. Further, nano-sized carriers can increase vitamin E content in formulations as well as favor its skin penetration.

Keywords: antioxidant, tocopherols, tocotrienols, skin, health

1. Introduction

Neurodegenerative and metabolic diseases progression is related to oxidative stress [1, 2], a condition where there is a lower ability of endogenous antioxidants to scavenge free radicals [3] resulting in free radicals increase. Most frequent free radicals are the reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide and hydroperoxide. ROS are formed endogenously [4] and its production is raised by some environmental factors [3]. Major internal sources are mitochondrial oxidative reactions, phagocytosis by macrophages and xenobiotics metabolization [4]. Environmental factors include pollution, ultraviolet radiation and smoking [3]. Free radicals damage DNA, protein and lipids [4] and their increase is involved in diabetes progression [1] and in Alzheimer and Parkinson's diseases onset [2]. In addition, cystic fibrosis patients are more prone to oxidative stress owing to vitamin E deficiency [3].

Some endogenous antioxidants are glutathione peroxidase, vitamin C and vitamin E [4]. Vitamin E is a non-enzymatic endogenous antioxidant [4] preventing atherosclerosis due to reduction of low density lipoprotein (LDL) oxidation. Beyond from antioxidant, it has a fundamental role in neurological and immune system function [4]. Accordingly, oral intake of vitamin E would be an interesting

alternative treatment to oxidative related diseases to improve patients quality of life [5, 6]. Apart from oral intake, natural sources of this vitamin are the vegetable oils. Wheat germ oil, sunflower oil, rice bran oil, canola oil and palm oil are some representants rich in vitamin E. Nuts and fresh foods contain vitamin E, but in smaller amounts [4].

1.1 Vitamin E isoforms and derivatives

A sum of 4 tocopherols isomers and 4 tocotrienols isomers compose vitamin E. Isomers are named as alpha, beta, gamma and delta and their chemical structures are shown in **Figure 1**. Tocopherols and tocotrienols differ only in their side chain. Tocotrienols have an unsaturation on its side chain. In respect to isomers, the nomenclature is due to substitutions in R_1 and R_2 positions. Alpha isomers have a methyl group both at R_1 and R_2 while delta isomers do not have any methyl group. Instead, beta and gamma isomers have one single methyl group, in R_1 or in R_2. Regardless of the source, vegetables contain a mixture of isoforms and one of them is predominant [7, 8]. Isoforms are obtained through extraction from vitamin E- rich vegetables such as wheat (shown in **Figure 1**) whose principal isoform is alpha-tocopherol [7]. Chemical synthesis is employed to obtain alpha-tocopherol [7, 8].

Commercially, vitamin E is available mainly as alpha-tocopherol [9, 10] or tocopheryl acetate [11–13] which are used above all to oral [14, 15] and skin [10, 12, 16] applications, respectively. Among vitamin E derivatives are tocopheryl acetate, tocopheryl glucoside and tocopheryl phosphate. Tocopheryl acetate is the most used vitamin E derivative [17] also named as tocopherol acetate or vitamin E acetate [18]. It is obtained through tocopherol modification to improve stability since tocopherol is a labile form. However, tocopheryl acetate is biologically inactive and it must be converted to tocopherol in skin and intestine. Often, there is no mention about tocopheryl acetate isomer as alpha-tocopheryl acetate is the most used [8].

Regarding human use, there is no standardization about vitamin E dose neither in oral intake [14, 15] nor in skin formulations [10, 19, 20]. Although its deficiency in adults is unusual [4], its oral intake may be recommended in cystic fibrosis

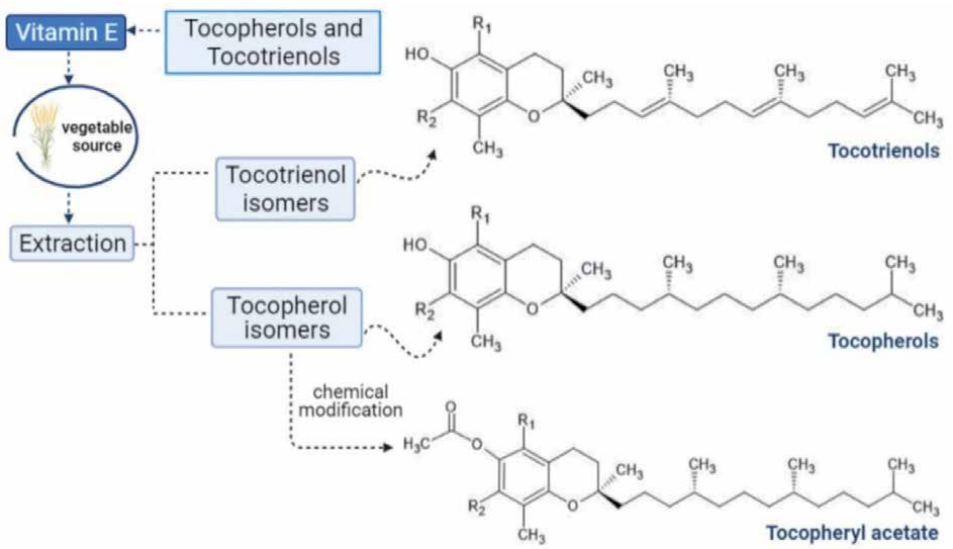

Figure 1.
Extraction of vitamin E isoforms, chemical structure of Tocopherols, Tocotrienols and Tocopheryl acetate. Created in BioRender and ACD/ChemSketch.

Skin effect	Mechanism of action	References
Photoprotection	Lipid peroxidation reduction	[23–25]
	Endogenous antioxidants protection	[24–26]
	Erythema decrease	[27, 28]
	Inflammation reduction	[29]
Cancer prevention	Pyrimidine dimers reduction	[30]
Reduction of melanoma progression	Apoptosis induction	[31, 32]
	Cell cycle arrest	[32]
Improvement of melasma	Reduction of tyrosinase activity	[33, 34]
	Down-regulation of TYRP-2 expression	[34]
	Down-regulation of TYR, TYRP-1, TYRP-2*	[35]
Reduction of Skin Aging	Increased collagen expression	[36, 37]
	Decrease metalloproteinases expression	[37]

**TYR: Tyrosinase, TYRP-1: tyrosinase-related protein-1, TYRP-2: tyrosinase-related protein- 2.*

Table 1.
General skin effects and mechanisms of vitamin E isoforms and derivatives.

patients [21]. Oral supplementation is equally used to reduce ultraviolet damage to skin [14, 15]. Furthermore, its combination with other antioxidants is a common approach. Vitamin C is the most used one [15, 19, 20] because it regenerates oxidized vitamin E [22]. Oxidized vitamin E if not properly regenerated may promote lipid peroxidation instead of preventing it [4]. The association of several antioxidants is then extremely important to reduce oxidative stress.

1.2 Benefits on skin health and dermatological diseases

The knowledge about vitamin E effects is essential to guide its use in dermatological treatments. **Table 1** shows some skin effects and mechanisms of action to vitamin E isoforms and derivatives. Photoprotection was approached mostly in earlier studies [23, 27, 28] while current ones approach mostly skin diseases [31, 35]. The antioxidant activity accounts for many skin effects including photoprotection [23–25], skin aging reduction [36, 37] and pyrimidine dimers reduction. The latter effect is important to prevent cancer onset [30]. Moreover, as reactive oxygen species are involved in the pathogenesis of psoriasis and atopic dermatitis [38–40], the topical application of vitamin E isoforms would be likewise beneficial in these diseases.

In relation to isomers, earlier researches were directed mainly to alpha-tocopherol whose action is lipid peroxidation reduction [24]. Nowadays, research is focused on tocotrienols [31] and tocotrienol-rich fraction [35–37] which are able to reduce melanoma progression [31, 32], melanogenesis [35] and skin aging [36, 37]. Tocotrienol-rich fraction (TRF) is a mixture of tocotrienols and alpha-tocopherol [41] allowing to combine the pharmacological benefits of several isomers. Further studies over tocotrienols and TRF are required to prove their efficacy in skin diseases treatments.

2. Vitamin E in skin care formulations

Conventional formulations [42] and nanotechnological-based formulations [16, 43] have been used to deliver vitamin E and its derivatives into the skin due

to its moisturizing, photoprotective, antioxidant [44, 45] and anticancer properties [46]. Some formulations applied to skin care are summarized in **Table 2**. Mainly sunscreens and anti-aging commercial products contain this vitamin [42]. Additionally, some cosmetic brands have explored the "anti-pollution" claim in their labels. As pollution triggers oxidative stress, the "anti-pollution" effect prevents skin damage induced by pollutants [19].

Nevertheless, several limitations impact vitamin E isoforms and derivatives bioavailability. Their bioactivity in different target sites, such as the skin is affected. Vitamin E is an unstable molecule because it undergoes oxidation, especially the light-triggered phenomena [60]. In this sense, novel drug delivery systems have been extensively investigated to improve vitamin E bioavailability, solubility, stability and biodistribution. Consequently, a better skin penetration can be accomplished [61, 62].

2.1 Conventional formulations

Emulsions and hydroalcoholic gel are the most common conventional formulations bearing either tocopherol, tocopheryl acetate or other esters (succinate, nicotinate, linoleate, and phosphate). The isoform α-tocopherol is the one with the best cost–benefit ratio [42]. One single α-tocopherol molecule is capable of neutralizing 2 peroxidil radicals which is responsible for lipid oxidation initiation. Then, a delay in the development of several oxidation-based disorders could be achieved [38]. Despite being less effective than tocopherol, tocopheryl acetate is widely used in formulations intended to skin delivery [42].

In sunscreens formulations, vitamin E and its derivatives increase the sun protection factor [47] and contribute to the photostabilization of chemical filters [49]. After skin permeation, they can minimize the oxidative stress harmful effects

Skin formulation	Skin care application	Vitamin E isoform or derivative	Reference
Conventional formulations	Photoprotection	α-tocopherol	[10, 47]
		Tocopheryl acetate	[48, 49]
		Tricotrienol-rich fraction	[25]
	Melasma	Tocopheryl acetate	[50]
	Anti-pollution	α-tocopherol	[19]
	Skin aging	Tocopheryl acetate	[51]
	Acne vulgaris	Tocopheryl phosphate	[52]
Nanotechnology-based systems	Photoprotection	Tocopheryl acetate	[43, 53]
		α-tocopherol	[54, 55]
	Wound healing	Tocopheryl acetate	[56, 57]
	Dermatitis	α-tocopherol	[58, 59]
		γ-tocotrienol	[59]
	Skin aging	α-tocopherol	[9]
	Moisturization	Tocopheryl acetate	[16]
		α-tocopherol	[9]

Table 2.
Vitamin E isoforms and derivatives in conventional forms and nanotechnology-based systems.

caused by UV radiation [48, 63]. In the latter case, an adequate vehicle is important since it can influence its permeation. In this regard, especially o/w (oil- in-water) emulsions have been used as the vehicle of choice [64]. From this perspective, a report showed that o/w emulsion containing vitamin E prevented erythema induction and reduced inflammatory damage caused by UV exposure in healthy volunteers [48].

In anti-aging formulations, vitamin E and its derivatives act as antioxidants, scavenging free radicals, the principal accelerators of skin aging [65]. As regards to α-tocopherol, it decreased expression lines, wrinkles, and freckles induced by photoaging in a study performed *in vivo* [66]. In addition, α-tocopherol smooths the skin, increases the stratum corneum ability to maintain its humidity and accelerates the epithelialization process [67]. For these purposes of use, most commercially available formulations are emulsions o/w, both in creams and lotions.

Furthermore, the association of vitamin E and its derivatives with other ingredients increased the effectiveness of different dermocosmetic treatments [45]. In this sense, the application of a lotion combining α-tocopherol phosphate, ascorbyl 2-phosphate 6-palmitate, and glyceryl-octyl-ascorbic acid reduced the complications of acne vulgaris [52]. On the other hand, in a randomized controlled trial, a cream containing hydroquinone, buffered glycolic acid, vitamins C and E, and sunscreen was safe and effective in melasma treatment [50]. Recently, a serum containing vitamin C, tocopheryl acetate and raspberry leaf cell culture extract had anti-aging and brightening effects on the skin, with significant improvement of skin color, elasticity, and radiance. The smoothness, scaliness, and wrinkles were also improved by topical use of the product once a day, during eight weeks [51].

2.2 Nanotechnology-based formulations

Bioactives molecules and lipophilic vitamins release on or into the skin by topical products comprise a challenging task owing to the characteristics of the stratum corneum barrier. Thereby, the drug accumulates on the skin surface. Besides, vitamin E low stability by its direct exposure to UV radiation can limit conventional formulations effectiveness [58]. Therefore, when it comes to topical administration, nanostructured drug vehicles have shown advantages over conventional delivery systems. The most investigated nanostructured carriers for vitamin E comprise liposomes, nanoemulsions, polymer nanoparticles and lipid-based nanoparticles [54].

Liposomes are self-assembled vesicles composed by one or more hydrophobic bilayers constituted by amphiphilic phospholipids which originate an aqueous core domain. Phospholipids contain phosphorus in their composition [68]. Diversely, nanoemulsions are thermodynamically unstable surfactant-stabilized systems composed of nano-sized micelles bearing an oily nucleus [69]. Polymer nanoparticles, whether nanocapsules or nanospheres, are colloidal artificially prepared spherical carriers surrounded by a polymer membrane. Nanocapsules contain an oily core and nanospheres contain a polymeric matrix [70]. Besides, chitosan obtained from shrimp and crab shells [71] is employed to form polymeric nanoparticles. In these nanoparticles, there is a matrix formed by chitosan and tripolyphosphate. The latter is used as a crosslinking agent [58]. Elseways, lipid nanoparticles either solid lipid nanoparticles (SLN) or nanostructured lipid carriers (NLC) have a lipophilic bioactive entrapped. SLN are formed by a solid lipid-based core while NLC are formed by a mixture of solid and liquid lipids [72]. **Figure 2** shows the general structures of some nanocarriers used to deliver vitamin E into skin.

Concerning liposomes, an optimized composition [73], a proper selection of preparation methods and a suitable particle size range [74] are essential as skin

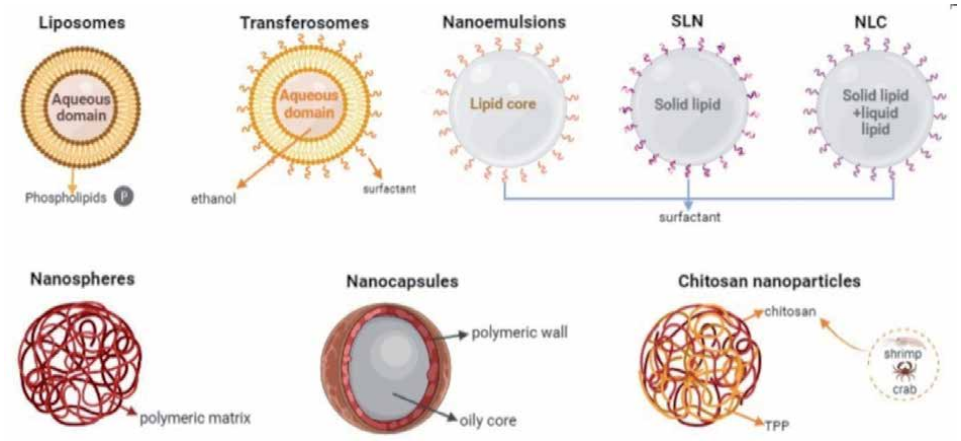

Figure 2.
Structure of some vitamin E nanocarriers. SLN: Solid lipid nanoparticles. NLC: Nanostructured lipid carriers. TPP: Tripolyphosphate. Created in BioRender.

penetration will be affected by these factors. Regarding biological activity, vitamin E-loaded liposomes inhibited lipid peroxidation more effectively than free vitamin E [74]. Lately, tocopherol acetate-loaded transferosomes optimized wound healing process [56]. As transferosomes are elastic liposome-like ultra-deformable vesicles, a higher diffusion across the stratum corneum can be accomplished [75, 76]. Topical administration of vitamin E-loaded liposomes are also interesting to enable a high drug penetration and transdermal release into skin tumors [68].

Lipid nanoparticles ability to increase sunscreens efficacy was previously shown [53, 55]. Tocopherol acetate-loaded SLN increased sunscreen UV-blocking effect [53]. Moreover, alpha-tocopherol and sunscreens loaded in NLC and SLN increased vitamin E photostability. Additionally, nanoencapsulated vitamin E promoted a better photoprotection than nanoparticle-based formulation without Vitamin E [55]. Besides, tocopheryl acetate and idebenone loaded in NLC provided a skin hydration increase because lipids have occlusive properties. Vitamin E loaded in NLC reduced skin pigmentation which was attributed to the photoprotective effect of Vitamin E [43].

An innovative nanocomposite dressing for burn wound healing containing vitamin E- loaded polymer nanoparticles allowed a vitamin controlled release [57]. In another report, α-tocopherol loaded to nanospheres was crosslinked to cellulose fiber to obtain a novel cosmetic fabric with potential application to atopic dermatitis patients [58]. As to nanoemulsions, tocopherol-loaded nanoemulsions increased skin delivery *in vitro* and they protected vitamin E from UV-triggered degradation [54]. More recently, α-tocopherol and γ-tocotrienol were loaded in nanoemulsions to treat dermatitis as an attempt to avoid the use of steroid anti-inflammatory drugs. This nanotechnological formulation could be in the future an alternative to dermatitis patients [59].

Lastly, clinical trials are essential to complement in *vitro assays*. According to human experiments, different nanosystems could be employed to ensure a more immediate or a more prolonged skin hydration [16]. Beyond skin moisturization, lipid nanoparticles improved human skin elasticity and firmness [9]. Importantly, a protocol clinical trial proposes the use of a formulation containing vitamin E-loaded NLC to reduce radiodermatitis in breast cancer patients. Since radiodermatitis is a recurrent radiotherapy side effect, the use of this topical formulation could improve cancer treatment as there would be lower patients quitting radiotherapy treatment [77].

3. Conclusion

Reactive oxygen species are implicated in systemic and skin diseases pathogenesis. Hence, topical use as well as oral intake of antioxidants should be encouraged to reduce stress oxidative effects. Vitamin E isomers and derivatives are widely known for their antioxidant activity. Tocopherols and tocotrienols isomers are found in vegetable oils. Elseways, vitamin E derivatives are synthetic forms obtained from natural isomers. Endogenously, alpha-tocopherol scavanges reactive oxygen species and owed to this effect, the oral supplementation of vitamin E is beneficial to prevent the appearance and progression of diseases. In relation to cutaneous effects, both oral and topical formulations provide a photoprotection against harmful ultraviolet radiation. Moreover, despite tocotrienols potential application in melanoma treatment, their skin effects are not fully understood.

Majority of skin care formulations contain alpha-tocopherol isoform or tocopherol acetate derivative whose effects are mainly due to their scavenging ROS ability. Therefore, the reduction of skin aging, melasma and cancer prevention can be achieved by different vitamin E pathways on the skin. As conventional forms and nanotechnology-based systems bearing vitamin E are useful in skin diseases treatment, their use is essential to skin health promotion and maintenance. Nevertheless, its therapeutic effectiveness is limited. Vitamin E loaded in nanostructured delivery systems can significantly increase antioxidant-based therapy effectiveness. In the future, there will be a need for well-designed controlled trials to support the benefits of nanotechnology-based products containing this vitamin.

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Chapter 5

Role of Vitamin E in Boosting the Immunity from Neonates to Elderly

Mariyappan Kowsalya, Mohan Prasanna Rajeshkumar, Thangavel Velmurugan, Kattakgounder Govindaraj Sudha and Saheb Ali

Abstract

The vitamin E is a fat-soluble vitamin which occurs as a tocopherol component abundant in humans. The vitamin E supplements in humans and animals have provided numerous health benefits. The vitamin E is rich in antioxidants which slow the aging process and reduce the free radical damage. Vitamin E isoforms play an important role in respiratory health. It is also important in health and well-being of preterm neonates. Vitamin E deficiency in new born includes hemolytic anemia, disease of retina, bronchopulmonary dysplasia. Further, in vitro studies, vitamin E has increased the oxidative resistance and prevents the atherosclerotic plaque. The consumption of vitamin E rich foods reduces coronary heart diseases. This chapter focuses on the treatment of vitamin E deficiency in preterm babies and the role of vitamin E in preventing coronary heart diseases.

Keywords: Vitamin E, neonates, immunity, preterm infants, α-tocopherol

1. Introduction

Vitamins are defined as "organic compounds required in diet in little quantity to execute specific biological functions for normal protection of ideal growth and health of the organism" [1]. In 1922, vitamin E was discovered by Evans and Bishop named it as "X-factor" and got its name after the classification of other vitamins [2]. Vitamin E is generally used as a common word for four tocopherols (α, β, γ and δ) and tocotrienols (α, β, γ and δ) present in food. The main component of the group of compounds in vitamin-E is α-tocopherols. The tocopherols are products of 6-hydroxy chromane (tocol) ring with isoprenoid side chain. Vitamin E with the aid of selenium prevents the non-enzymatic oxidation of cellular components and free radical formation. Vitamin is lipophilic in nature and present on association with derivatives of lipids and cell membranes [1]. The synthetic α- tocopherol is called as *all-rac*- α-tocopherol and naturally available form is RRR- α-tocopherol [3]. The key characteristics of vitamin-E was identified as scavenger of free radical and it is the foremost fat-soluble vitamin responsible for protecting cell membranes against peroxidation [4]. In early stages of life, vitamins are extremely important. Vitamin -E supplies the essential antioxidant protection and stimulates the development of the

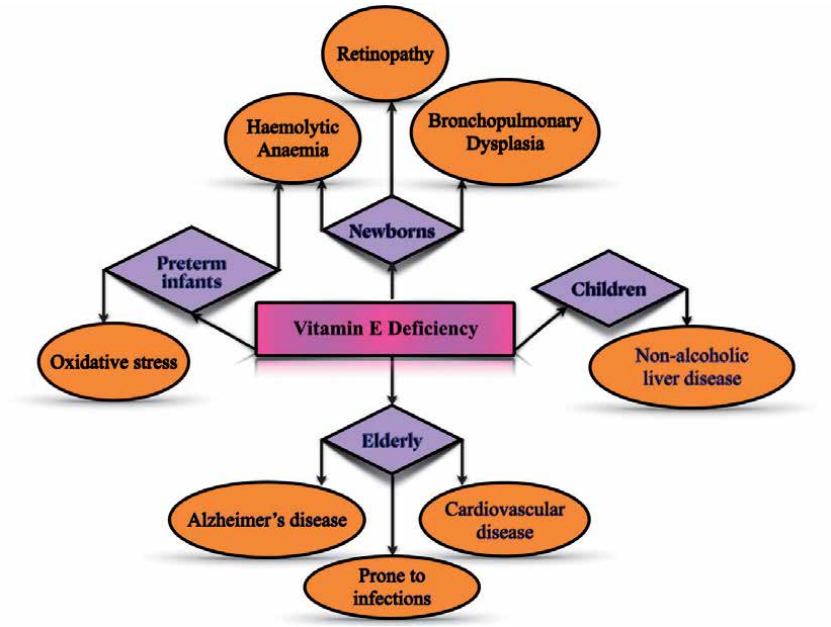

Figure 1.
The low level of serum α-tocopherols was observed in certain disease was mentioned which cause high rate of morbidity in neonates. And also, elderly patients have diseases associated with low level of vitamin E and other micronutrients in their diet.

immune system in new-borns [5]. Our human system does not synthesize Vitamin E and it is obtained through dietary intake. Vitamin E rich foods are wheat germ oil, sunflower oils, soybean oil, sunflower seeds, cotton seed, walnut, hazel nuts, peanut butter, corn, palm, spinach, broccoli, kiwi fruit, mango, raspberries, blackberries, blackcurrant, avocado and tomato [6–8]. The vitamin E deficiency was known as the cause of foetal death. Early high dose of vitamin E either through intravenous or intramuscular route decreases the chance of hemorrhage, bronchopulmonary dysplasia, haemolytic anemia, retrolental fibroplasia and retinopathy of prematurity [9]. The low level of vitamin E in serum was observed in certain diseases which was depicted in **Figure 1**.

The antioxidant potential of vitamin E could protect the polyunsaturated fatty acids in the membrane from oxidation, regulating the production of reactive oxygen species, reactive nitrogen species and modulates signal transduction [10]. The effect of antioxidant activity of vitamin E was based on the number of methyl group in its chromane ring. The *α*-tocopherol have three methyl group whereas the *δ*- tocopherol has only one methyl group in it [2]. Vitamin E also has anti-cancer potential by stimulating the p^{53} gene, down regulation of mutant p^{53} gene and activates the heat shock proteins. Production of PKC and collagenase was inhibited by *α*-tocopherol. In milieu, *γ*- tocopherol has effective anti-cancer activity than the *α*-tocopherol [5]. Intrauterine growth restriction (IUGR) is one of the major causes of neonatal morbidity and mortality. Some studies shows that *α*-tocopherol aids in the intrauterine development of foetus. 15 million babies are born premature every year and they need special care just to stay alive [11]. During gestational period, the maternal oral intake of vitamin E increases the weight of foetus [12]. The oral administration of vitamin E was given to children to combat malabsorption disorders. Some of the compounds used as vitamin E therapy in newborn and preterm infants are *α*-tocopherol, tocofersolan, dl- *α*-tocopherol, dl-*α*-tocopheryl acetate [13]. In 2000, the institute of medicine (IOM) chose the hydrogen peroxide induced

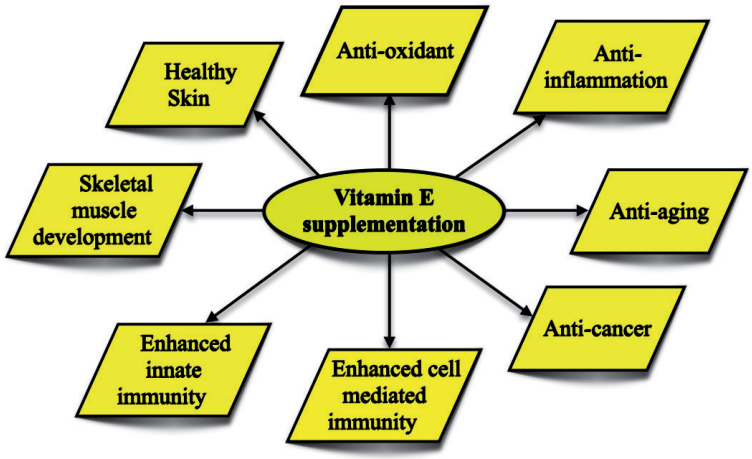

Figure 2.
Vitamin E supplementation has many biological activities and it enhances the immune response.

erythrocyte haemolysis test as a marker to determine the vitamin E status. Because, erythrocyte fragility and anemia with higher erythrocyte was observed in children. In adults, vitamin E deficiency occurs due to genetic disorders in α-tocopherol transfer protein (TTP) which causes ataxia. Further, fat malabsorption also leads to vitamin E deficiency due to genetic disorder in TG transfer protein [14]. The people with ataxia are provided with 800-1200 mg of vitamin E as a supplement to prevent the progression of disease [6]. Orally, vitamin E was given in the form of dl-*α*-tocopherol acetate. Supplementation of *α*-tocopherol increases insulin sensitivity and decreases the oxidative stress [15]. Oxidative stress was due to the disturbance in balance between generation of free radicals and their exclusion by free radical scavenging activity of the system [16]. Vitamin E supplementation has enhanced the host defense system by heightening the humoral and cell mediated immune system. In elderly people, vitamin E has boosted the resistance against viral diseases and also stimulates immune response to distinctive antigen [5]. Immunostimulatory property of Vitamin E provides enhanced resistance to many pathogens [9]. Earlier studies show that vitamin E possess neuroprotective activity in both foetus and adult rats [17]. The vitamin E supplementation has numerous biological activity which was depicted in the **Figure 2**.

2. Immunological functions of vitamin E in different stages of life

2.1 Preterm infants

Preterm birth was defined as parturition prior to 37 weeks of gestation, the leading cause of mortality and morbidity in neonates. The rate of prematurity complications increases with decreased gestational age and birth weight [10]. Newborns are one of the risk groups of vitamin E deficiency. The limited transfer of *α*-tocopherol through the placenta which results in low level of vitamin-E in tissues and serum at the time of birth in premature infants than in full term infants [18]. The preterm infants' blood vessels are exposed to high oxygen tension due to the deficient amount of vitamin E [19]. Preterm infants are born with low body weight and have less stored fat-soluble vitamins. The level of *α*-tocopherol is low at their time of birth was shown by the elevated erythrocyte haemolysis in the presence

of hydrogen peroxide. The enteral dose of vitamin E is given at 50 IU/kg within 4 hours of birth and it increases the serum α-tocopherol level [20]. The preterm infants those less than 1000 g in NICU were given 1.5 ml of vitamin E per day through infuvite pediatric as a portion of parental nutrition. And infants in 1000 g to 3000 g obtain 3.5 ml of vitamin E per day. Each vial (1 ml) of infuvite encompasses 7 mg of α-tocopheryl acetate [3].

The preterm infants administering the oral dose of vitamin E helps in treating the vitamin E deficiency syndromes like anemia, retinopathy, thrombocytosis and congenital malformation [21]. The retrolental fibroplasia occurs often in preterm infants because the last portion of the retinal blood vessel continues to develop till the end of eight month of gestation. The blood vessels of the preterm infants might not be developed well and they need an appropriate environment with arterial oxygen tension, blood supply, nutrient, disposal of wastes and exposure to visible light [18]. The haemolytic anemia was frequently observed in preterm infants, where their intake of fortified iron is higher along with linolenic acids. Nowadays, the formula for preterm babies had increased the vitamin E and reduces their iron content so that this helps to prevent the development of haemolytic anemia [22]. The vitamin E supplements in preterm infants increases the hemoglobin level after 8–10 weeks of birth [23]. Previous studies reported that preterm infants born below 2000 g, when supplemented with 16.5 mg of α-tocopherol acetate per day for about ten days has improved the hemoglobin level and decreases the reticulocyte count. Supplementing 16.5 mg concentration of vitamin E is higher in their study compared to regular dose of 1.5 mg/day but it shows betterment of disease condition, however their consequences have to be studied [24].

The bronchopulmonary dysplasia (BPD) was observed among preterm infants due to free radicals which injures the lung cells. It was generally noticed in preterm infants particularly born before 28 weeks of gestation. Oral supplementation of vitamin E is tolerable in pregnancy and infancy. They are present routinely in the total parenteral nutrition (TPN) which is used in the treatment of BPD. And also, the α-tocopherol plays a key role as anti-inflammatory factor in neonatal lungs [15]. A study with 133 preterm infants were grouped into 68 preterm infants with confirmed case of BPD, 65 preterm infants without BDP. It was observed that as the condition of BPD increases with vitamin E deficiency. The involvement of vitamin E in BDP was confirmed and their supplementation would become a therapy to BPD [25].

2.2 Neonates

The adequate intake of Vitamin E is essential in neonates as they are born with low stores of it. The routine recommended intake of vitamin E is 2.8 IU/kg per day (Maximum 7 IU/kg per day) [8]. The term babies gestational age was between 37 and 41 weeks and their weight at the time of birth was 2500 g -3700 g [26]. It was observed that very low birth weight in infants were deficient in Vitamin E. Hence, vitamin E supplementation is required to enhance the weight gain in infants [27]. The yellow coloration of the skin and sclera of new born babies are referred to as neonatal jaundice which is the result of bilirubin deposition. Vitamin -E is supplemented along with phototherapy of the full-term neonates [28]. In an extrauterine environment, vitamin E acts as defense against oxygen toxicity and their placental transfer to the foetus is limited during gestational period. Hence, maternal milk is important to supply this vitamin to the neonates in their initial period and during lactation, thus, protecting it from haemolytic anemia, bronchopulmonary dysplasia, neurological dysfunction and increased neonatal mortality. The studies show that vitamin E in maternal milk decreases with increase in lactation period i.e., colostrum (40.5 ± 15 μmol/L) provides the highest content of vitamin E to infants.

Whereas it gradually decreases in transitional (13.9 ± 5.2 μmol/L) and mature (8.0 ± 3.8 μmol/L) milk of lactation. The higher concentration of alpha-tocopherol in colostrum acts as complementary mechanism [29]. Hyperbilirubinemia was observed in neonates which was treated with phototherapy and vitamin E supplementation [30]. The world health organization WHO has issued a global public health recommendation that infants should exclusively breastfed for 6 months to attain optimal growth and development as the maternal milk was the infant's best source of nutrients. The average amount of vitamin E in maternal milk is 2.5–2.9 mg it contributes to prevention of free radical propagation in numerous lipid structures within the system [31]. The Dietary Reference Intake (DRI) recommends the intake of 4 mg/day of vitamin E for children between 0 and 6 months. The vitamin E supplementation in children is essential to the development of the immune system, lungs, extracellular matrix of the vascular system and mental development [29].

2.3 Children

Vitamin E enhances the immune responses of the children. Cardiomyopathy was observed as a symptom in children with critical deficiency of vitamin E. The prescribed amount of vitamin E supplementation in children is 1000 mg/d [13]. Low level of maternal plasma α-tocopherol increase the chance of asthma in children within their first ten years [32]. The antioxidant intake of vitamin E during pregnancy and their effect on development of wheezing and eczema in children is needed to be confirmed with the follow up investigation on the children's growth and their dietary intake [33]. In previous report, the association of vitamin E intake during pregnancy and chance of asthma in children was observed in 2000 pregnant women. The α-tocopherol level in plasma was examined during gestation and cord blood after delivery. Their intake of anti-oxidant vitamins are also examined. Then follow up studies was performed up to five-year age of children. The symptoms of asthma, wheezing and dietary intake was collected for 1253 children and 797 children were participated in hospital evaluation. They have concluded that low level of serum vitamin E during pregnancy was observed among the children with phenotypic symptoms of asthma. Though, vitamin E does not directly improve the conditions of asthma patients their deficiency in early stage of life was perceived in children be inflicted with asthma [34].

The children suffering from chronic kidney disease are at high risk of micronutrient deficiency. The concentration of vitamin A and vitamin B12 was within the range of reference while vitamin E shows major changes as the kidney disease worsens hence advance studies are essential to determine the required concentration of vitamin supplements in infants and children with this disease [35]. In children, respiratory tract infection is common cause of the morbidity. The vitamin E supplementation helps to treat this disease. A substantial amount of plasma vitamin E improves the hemolytic uremic syndrome in children. Which was due to the defect in production of prostacyclin and thus preventing the mortality and morbidity rate [36]. Supplementation of vitamin E (100 IU/ day) has increased the glutathione level and decreased the lipid peroxidation and concentration of HBA1C in the erythrocytes of type 1 diabetics children [37]. The childhood obesity was growing and it may be due to imbalance in the oxidant and antioxidant level. The obese children were observed with increased level of oxidants like isoprostanes and decreased level of antioxidant like vitamin E. Oxidative stress markers were decreased by vitamin E supplementation in obesed children with liver steatosis. Hence, early implementation of vitamin E will reduce the risk of cardiovascular and metabolic changes connected with non-alcoholic fatty liver disease in children and adolescence [38].

2.4 Adults

The reactive oxygen species include (ROS) like hydrogen peroxide, hydroxyl radicals, and superoxide were studied exclusively. The formation of ROS during electron transport chain was presumed due to increased ingestion of oxygen by mitochondria in the cell. Some recent studies reported that exercises increased the ROS production. The studies revealed that vitamin-E supplementation in athlete improved the muscle performance and protects the cell membrane from damage through oxidative stress. This study augment that vitamin E would strengthen the skeletal muscle of the older people [39].

In the previous research, Women with the possibility of pre-eclampsia during pregnancy was provided with 400 IU of vitamin E and 1 g of vitamin C per day. As a result, very low birth weight infants were born to supplemented group compared to placebo group. Hence, the vitamin E concentration should not be exceeded above the required dietary allowances [40]. Furthermore, the greater the concentration of serum vitamin E during pregnancy then there is a chance of macrosomia [41]. Recently, a study with 897 girls in their adolescence found that vitamin E aids in the inflammatory response and oxidative stress during menstrual cycle [42].

Cystic fibrosis was one the genetic disorder which leads to deterioration of pulmonary functions in children and adults, partially due to oxidative stress. The vitamin E supplementation greater than required dietary allowances has enhanced the serum α-tocopherol [43]. In USA, the higher acceptable level of vitamin E per day was 1000 mg/day [44].

2.5 Elderly

Vitamin E group has tocopherol and tocotrienol groups each with identical isomers but with alteration in hydrophobic tridecyl chain saturation point. The tocotrienol was recently discovered to be more effective than tocopherol in the treatment of age-related cardiovascular diseases [45]. The low intake of vitamin E in childhood and adulthood leads possibly to hypertension than those who have taken an adequate amount of vitamin E. The cardiovascular disease risk can be decreased by - α-tocopherol which inhibits LDL oxidation and reduces the inflammatory responses [46]. The intake of antioxidants could prevent the lipid peroxidation [47]. The epidemiological study with 27271 men who were smokers and by no records of myocardial infraction in the age group 50–69 was divided into group and supplemented with vitamin E, placebo and beta-carotene. The groups were keenly monitored to detect the possible effect of vitamin E supplementation in cardiovascular disorder. The results revealed that 50 mg/day supplementation of vitamin E has prevented the occurrence of nonfatal myocardial infraction by 4% and fatal coronary heart disease by 8%. The vitamin E (α-tocopherol) supplemented group has lessened the chance of coronary heart disorder in human trails followed up to six years related to the placebo group. The beta-carotene supplementation has no effect on non-fatal myocardial infraction disease [48].

Immunological function especially cell-mediated immune response will decrease with age and hence elders are prone to infectious diseases. The 200-800 mg supplementation of vitamin E enhances the production of antibody to a primary immunization [49]. In the in vivo study, healthy elderly people in the age group greater than 60 years are supplemented with 800 mg of vitamin E per day and the fasting blood sample was collected for the duration of 6 months and this study has evidenced the increase of α-tocopherol level in serum and increase in delayed

type hypersensitivity was observed [50]. In four month of clinical trials, vitamin E supplementation greater than RDA in elderly people had enhanced production of antibodies against various diseases like tetanus, hepatitis B and DTH vaccines [51].

The oxidative stress was one of the reasons for neurodegenerative diseases like Alzheimer's disease, Parkinson's disease and processes in cells related to aging. The brain cells were easily damaged by free radicals due to its consumption of high amount of oxygen along with certainly peroxidable lipid membranes and comparatively lesser enzymes with antioxidant potential. The vitamin E and vitamin C were the antioxidant rich supplements which will scavenge the reactive oxygen species and helps in regeneration of neurons [52]. Vitamin E also protects the nervous system with aging [13]. Neurological abnormalities are observed in children and adults when the serum α-tocopherol level is less than 0.5 mg. Vitamin E deficiency manifests as neuropathic and myopathic disorders. Spinocerebellar syndrome, ataxia, hyporeflexia, vibratory sensation was commonly observed clinically. Moreover, skeletal myopathy and pigmented retinopathy also occurs due to the vitamin E deficiency [6]. Further, Vitamin E supplementation has upgraded the cognitive function and vascular dementia in elderly [53]. The higher level of α-tocopherol in the brain generates an anti-inflammatory milieu to decrease the density of microglial cells. It also protects from the Alzheimer's disease [54]. A study group involved 39,876 women in US, in which 6377 women were greater than 65 years. It was evidenced that vitamin E supplementation in 600 IU alternative days with the follow up for 4 years had enhanced cognitive development. Because the oxidative stress was the major cause for dementia pathogenesis and numerous reports proved that vitamin E has decreased the lipid peroxidation in brain and also prevent the occurrence of Alzheimer's disease [55].

Previous studies reported that intervention of Vitamin E improves the lymphocyte proliferation and delayed type hypersensitivity was enhanced with higher production of Il-2 and lesser production of Il-6 [9]. An intervention of 200 mg of vitamin E has enhanced immune response of elderly people [56]. When the older mice supplemented with vitamin E has enhanced the cell mediated immune response, production of IL-2 and delayed type hyper sensitivity. In elder human subjects also vitamin E supplementation progresses both the innate and adaptive immunity. The phagocytic capability of leucocyte was improved but declined the bactericidal activity which might be due to the antioxidant potential of vitamin and lesser production of hydrogen peroxide. Further, the optimal concentration of vitamin E supplementation is 200 IU per day than the 60 and 800 IU per day of vitamin E supplementation has increased the T-cell proliferation in elder people [57].

In vivo studies of both animals and human have evident that immunity decreases with aging process. It was observed by decreased antibody response, delayed-type hypersensitivity, proliferation of T cell in response to mitogens. Research has proved that antioxidants have helped to improve the immunity in aging process [50]. Aging is a gradual and typical loss of the physiological system along with its immune response. The elderly people are prone to infections and other diseases like cancer due to the age related decrease of immunity. One of the widely consented reason for aging was oxidative stress. The previous study suggested that supplementation of both vitamin C (500 mg/day) and vitamin E (200 mg/day) to the elderly people has improved their immune function by enhancing the humoral and cell- mediated immune response. The α-tocopherol acts as immunomodulator and enhances the cytokine levels in older population of group. Level of INF- γ has increased in tested groups of older people supplemented with vitamin E [58].

S. No	Groups	No. of Participants	Age	Disease Condition	Dosage form of Vitamin E	Quantity	Outcome	Reference
1	Preterm Infants	12	28–30 week gestation	Very low Birth weight	dl-alpha-tocopheryl-acetate	3.5 mg/dl	Reduce the risk of Retinopathy	[62]
2		215	28–30 week gestation	Very low Birth weight	Soybean oil-based lipid emulsion	—	Reduce cholestasis	[63]
3		34	≤ 35 week gestation	Very Low Birth Weight	alpha-tocopheryl acetate	16.5 mg/ day	Increases Hemoglobin concentration	[64]
4		168	≤ 30 week gestation	Very Low Birth Weight	Vitamin E	3.3 mg/day	Prevent retrolental fibroplasia	[65]
5		25	< 30 week gestation	Very Low Birth Weight	dl-alpha-tocopheryl acetate	25 mg Ephynal	Enhances serum alpha-tocopherol	[66]
6		36	25.5 week gestation	Very Low Birth Weight	Mixed vitamins in parenteral solution	3.17 mg/ day	Increased serum alpha-tocopherol	[67]
7		151	< 35	Very Low Birth Weight	alpha- tocopherol	50 mg/kg	Decreases the bilirubin level	[33]
8	Neonates	77	37–42 week gestation	—	RRR-alpha- tocopheryl acetate; *all*-rac-alpha-tocopheryl acetate	20 IU; 13.5 IU	Infants discriminates natural and synthetic vitamin E	[15]
9		80	—	Hyperbilirubinemia	Phototherapy & Vitamin E	4 mg/day	Faster Recovery	[23]

Table 1.
Role of vitamin E in improving immune system in various disease condition of preterm infants and neonates.

S. No	Groups	No. of participants	Age	Disease Condition	Dosage form of Vitamin E	Quantity	Outcome	Reference
1	Children	2372	≤ 5 years	Kwashiorkor	Vitamin E, riboflavin, Se	—	No data on morbidity	[68]
2		16	—	Chronic Cholestasis	d- alpha -tocopheryl polyethylene glycol 1000 succinate (TPGS)	20–25 IU	Improves neurological development	[69]
3		61	≤10 years	Acute pyelonephritis	Vitamin E	—	Reduces Renal scarring	[70]
4		141	≤ 14.5 years	Cystic fibrosis	Vitamin E	—	Enhances vitamin E level	[71]
5	Adults	716	Gestation period	HIV infected	alpha -tocopherol acetate	30 mg/ day	Improves vitamin status in infants	[72]
6		23	—	Oxidative stress	Vitamin E	400 IU	Prevents exercise induced oxidative stress	[73]
7	Elders	184	—	Cardiovascular disease	alpha -tocopheryl acetate	400 IU	Reduces lipid per oxidation	[74]
8		29133	50–69	Pneumoniae	alpha -tocopherol	50 mg/ day	Decrease the risk	[75]

Table 2.
Role of vitamin E in improving immune system in various disease conditions of children, infants and elderly.

3. Immunological functions of vitamin E in infections

3.1 Pneumoniae

The in vivo studies on older mice affected with pneumonia was supplemented with vitamin E at the concentration of 500 mg per kg for four weeks and found that it has help to improve the lung functions. It was observed that migration of neutrophil and production of inflammatory cytokines was reduced with the intake of vitamin E. In human subjects, the older people supplemented with 200 IU of vitamin E per day has subsided the necessary to re-hospitalization of older people with pneumoniae up to 63% and it aids in faster recovery [57].

3.2 Human immunodeficiency virus

The vitamin E possess the anti-inflammatory property and people infected with HIV was found be lack of it and weakened immune system. Vitamin E supplementation in 400 IU per day has reinstate the delayed type hypersensitivity, production of IL-2 and T_H cells. The higher level of α-tocopherol in serum had blocked the advancement of infection. The murine model with HIV infection supplemented with increase in fifteen-fold of dietary vitamin E regularize the distorted immune system caused by infection to normal state [5]. Further, there was greater incidence of non-alcoholic steatohepatitis in HIV infected patients. The previous studies with 27 HIV patients with NASH reported that vitamin E supplementation was a most effective treatment as it has enhanced the ALT level, ck-18 and CAP score. Moreover, it does not cause any adverse effects in participated individuals [59].

3.3 Influenza virus

Influenza virus cause severe damage to lungs and also inflammation leads to greater oxidative stress. The vitamin E acts as an effective antioxidant therapy in influenza diseases. Thus, vitamin E supplementation protects the respiratory system and prevents the occurrence of oxidative damage due to influenza [60].

3.4 Others

The mouse models were infected with coxsackievirus which induces myocarditis. And in vitamin E deficient group the virulence of this virus is greater. In human studies, the coxsackie virus was isolated from infected individual, which was called as keshan an endemic disease commonly observed among children and women. The affected individuals were deficient in vitamin E and Se hence their supplementation in diet can prevent the viral infection [61]. The role of vitamin E in boosting immune system in various diseases in infants, children and elderly are depicted in **Tables 1** and **2**.

4. Discussion

The vitamin E was present in many natural foods which should be taken in adequate amount because the fortified or supplementation of vitamin E does not provide greater health benefits. The natural form of vitamin E rich foods should be incorporated into the diet. The healthy foods should be added to the diet for the efficient action of antioxidants in the human system [47]. The mother's milk

especially colostrum contains higher concentration of vitamin E which prevents the infants from oxidative damage and develops their immune system. Since, very less amount of vitamin E is transferred through the placenta, maternal milk plays a crucial role in enhancing the serum α-tocopherol level in new born [76]. The babies born with lesser gestational age was highly prone to oxidative stress and the maternal milk is the best source of antioxidants than the formulas to protect the new born infants [77]. Still today, α-tocopherol level in the serum was frequently measured through HPLC analysis. A novel method should be developed for their measurements.

The preterm infants were susceptible to oxygen radical disease and it can treat with antioxidant therapy in which the well-known antioxidant nutrient vitamin E can be used to treat this condition in preterm infants [78]. The formula fed preterm infants have the risk of developing high oxidative stress but in the study with 31 healthy preterm infants shows that long chain poly unsaturated fatty acid supplemented group does not affect the solubility of α and γ- tocopherol [79]. Another research also suggest that both the breast fed and formula fed preterm infants possess the ability to tolerate oxidative stress. It was confirmed by the presence of malonaldehyde (MDA) in the urine which was measured by HPLC analysis [80]. The vitamin E supplementation is required in preterm infants to improve certain disease conditions like haemolytic anemia, retrolental fibroplasia, bronchopulmonary dysplasia but their long-term high dosage leads to sepsis, necro colitis and in some cases even death of premature infants. Hence the ideal dosage of vitamin E is prerequisite to treat the disease in preterm infants on the other hand to ensure the safety and longer healthy life to the preterm babies [81].

In children, vitamin E deficiency results in the development of chronic cholestasis. In this case, the children have normal serum α-tocopherol level but decreased ratio to the total lipid content [82]. The vitamin E deficiency could be combated by intaking fortified foods can enhance the vitamin E and it was one of the best methods to reach daily requirements of vitamin E [83]. Vitamin E supplementation along with other micronutrients and trace elements increases our defense barrier system and prevents the development of infection [84].

The oxidative stress in the human body was mainly originated from the free radicals produced by mitochondria and other cellular components. The external factor for oxidative stress includes UV light rays from sun, ozone, pollutants, smoke from cigarette and smog. These factors contribute to the aging process. The antioxidant rich foods would help to balance the oxidant- antioxidant level in human body. The foods enriched with vitamin E are a good choice to delay the aging process and to have healthy and youthful glowing skin in later stages of life. [85].

Further, vitamin E supplementation also improves the cognitive function when their dietary intake is higher at the earlier stage of Alzheimer's disease. And also in the later stages of this disease their admission to centre is greatly reduced but it does not improve any cognitive functions [52]. The vitamin E is an effective antioxidant which shows beneficial report in preventing the progression of Alzheimer's disease, Parkinson's disease and dementia [55]. Though, there was no significant cognitive improvement in healthy individuals supplemented with vitamin E, it helped in the diseased condition We suggest that vitamin E has positive impact in improving the brain disorders associated with oxidative damage of brain cells.

Vitamin E was one of the efficacious nutrients which could modulate the immune system. The concentration of vitamin E is higher in immune cells than other cells in the blood. It has been observed that deficiency of vitamin E worsens the immune system in both animals and humans. Thus, vitamin E supplementation exceeding the required dietary recommendation has contributed to enhance the immune system. It has intensifies the differentiation and proliferation of T cell,

production of IL2, activity of T_H cells, macrophages and phagocytic cells [57]. The vitamin E supplementation in elderly has improved their immune response towards improved antibody response, delayed type hypersensitivity and also T-cell was proliferated was stimulated in response to mitogens. And Vitamin E in combination with vitamin C has also shows an enhanced immune response in elderly group than with the study group administered with vitamin E alone [50]. The higher level of vitamin E (α-tocopherol) in serum the greater the ability to resist viral infection in elder population [5]. Furthermore, the dietary intake of vitamin E enhances the immunity in elders and immunocompromised persons [86].

The vitamin E supplementation has modulated the immune system in both direct and indirect way. Directly it has maintained the cellular integrity and protected the cells from damage caused by oxidative stress. While indirectly it has aid the modulation of inflammatory intermediaries like proinflammatory cytokines and prostaglandin E2. The studies in mice suggests that inflammatory lung disease can be treated with the combination of probiotic strain *Bifidobacterium lactis* and vitamin E, C to lessen the lung inflammation due to air polluting agents [87]. In animal models, the α-tocopherol possess the anti-inflammatory and γ- tocopherol with pro-inflammatory property it could be used in the treatment of asthma [88].

The elderly people in the age group of 65 years and above was investigated for the association of vitamin E supplementation and respiratory tract infection. The study results shows that the vitamin E supplantation does not show any statistical difference among the lower respiratory infection among the supplemented and placebo group. However, the vitamin E supplementation group has a remarkable result in preventing the upper respiratory tract infection and against common cold [89]. Acute and chronic lung injuries was observed in new-borns due to oxidative damage. Usually, surfactant lipids protect the type II alveolar cells of lungs from air-borne pathogens. The vitamin E supplementation enhance surfactants and prevent the development of lung diseases like bronchopulmonary dysplasia [90]. Thus, based on these results vitamin E supplementation in our diet would prevent the upper respiratory tract infection and strengthens the lungs alveolar cells which might help us to combat the Covid-19 infection and help us to build a stronger immunity in current pandemic situation.

5. Conclusion

In past decades, vitamin E deficiency was frequently observed in preterm infants and neonates leading to various diseases like bronchopulmonary dysplasia, retrolental fibroplasia, hyperbilirubinemia, haemolytic anemia. Most often, the very low birth weight infants are at the high risk of developing vitamin E deficiency. The adequate amount of vitamin E supplementation has prevented the development of these disease conditions. The alpha tocopherol and alpha tocopheryl acetate are the most common form of vitamin E supplemented to preterm infants and new born infants. Vitamin E supplementation has prevented the development of asthma in children but not in patients with chronic severe asthma. Hence, the mechanism of vitamin E involved in preventing the disease in juvenile stage in children is need to be investigated. Vitamin E also possess numerous health benefits along with its antioxidant property. The aging process decreases the immunological response and increases the chances of infection in elder population. Thus, vitamin E supplementation has improved the T-cell mediated immune response and prevented the progression of Parkinson's disease and dementia in older people. Further, vitamin E is an important micronutrient which plays a crucial role in the early stage of our life to lead a healthy life. Many reports proved that vitamin E supplementation

has enhanced the immunity and prevented many diseases in infants. However, the optimal dosage amount, isoform and duration of vitamin E supplementation in each disease conditions in preterm infants and newborn is still need to be validated. Indeed, the mechanism behind the vitamin E in curing the infectious diseases and improving the immune response is little-known and future research will bring to light the unknown mechanism of vitamin E in boosting immune response in infants.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 6

Role of Vitamin E in Pregnancy

Mohd Aftab Siddiqui, Usama Ahmad, Asad Ali, Farogh Ahsan and Md. Faheem Haider

Abstract

Vitamins play important roles in female health. They are essential for many functions, including menstruation and ovulation, oocyte (egg) quality and maturation. Vitamin E was first discovered in 1922 as a substance necessary for reproduction. It has become widely known as a powerful lipid-soluble antioxidant. There are various reports on the benefits of vitamin E on health in general. Vitamin E helps your body create and maintain red blood cells, healthy skin, eyes and strengthens your natural immune system. However, despite it being initially discovered as a vitamin necessary for reproduction, to date studies relating to its effects in this area are lacking. Vitamin E supplementation may help reduce the risk of pregnancy complications involving oxidative stress, such as pre-eclampsia. This chapter is written to provide a review of the known roles of vitamin E in pregnancy.

Keywords: Vitamin E, Pregnancy, Oxidative stress, Tocopherol

1. Introduction

Vitamin E is an important micronutrient in the human body. Vitamin E maintains various body functions. It plays a very important role in maternal health and child development [1]. Vitamin E is an essential fat-soluble micronutrient for higher mammals and functions as an antioxidant for lipids [2]. American scientists Herbert McLean Evans and Katherine Scott Bishop discovered vitamin E in 1922. Vitamin E is an essential lipid-soluble vitamin. It was initially denoted as an "anti-sterility factor X" that was necessary for reproduction. The vital role of vitamin E in reproduction was first investigated 80 years ago [3]. It was named according to a consecutive alphabetical order preceded by the discovery of vitamins A to D. Later vitamin E was called alpha-tocopherol, according to the Greek term "tokos" childbirth, "phero" to bear, and -ol indicating alcohol. Vitamin E is also called the "protecting vitamin" [4]. The amount of vitamin E is determined by age. For adults, the safest dose of vitamin E supplements is 1,500 IU/day for natural forms and 1,000 IU/day for man-made (synthetic) forms. **Table 1** shows the average daily prescribed doses as determined by the Food and Nutrition Board of the Institute of Medicine [5–7].

Some vitamin E containing foods include wheat, rice bran, barley, oat, coconut, palm, and annatto [8–9]. Other sources include rye, amaranth, walnut, hazelnut, poppy, sunflower, maize and the seeds of grape and pumpkins [10]. The richest sources are nuts, spinach, whole grains, olive oil, and sunflower oil [11]. Vitamin E now refers to eight different isoforms that belong to two categories, four saturated analogues (α, β, γ, and δ) called tocopherols and four unsaturated analogues

Life stage	Recommended Amount
Birth to 6 months	4 mg/day
Infants 7 to 12 months	5 mg/day
Children 1 to 3 years	6 mg/day
Children 4 to 8 years	7 mg/day
Children 9 to 13 years	11 mg/day
Teens 14–18 years	15 mg/day
Adults	15 mg/day
Pregnant women	15 mg/day
Breastfeeding women	19 mg/day

Table 1.
Recommended Dietary Allowances (RDAs) for Vitamin E.

(α, β, γ, and δ) referred to as tocotrienols [12]. α-, β-, γ- and δ-homologues contain three, two, two and one methyl groups, respectively. These structural differences and isomerism determine the biological activity [13]. Tocotrienols differ in the presence of 3 double bonds in their side chain from tocopherols. The position of the methyl groups on the chromanol ring varies between the tocopherol and tocotrienol isomers. Tocopherols can form 8 stereoisomers due to the presence of 3 asymmetrical carbons in their side chains (RRR, RRS, RSR, RSS, SRR, SRS, SSR, SSS) [14]. Among these isomers, α-tocopherol (**Figure 1**) has the highest biologically active form [15]. α-tocopherol is the most abundant in plasma, cell membranes, other human tissues, and nutritional supplements, whereas γ-tocopherol is the primary form found in the human diet [16]. Tocopherols and tocotrienols, collectively known as tocols, are phenolic compounds. Although phenolic and polyphenolic compounds such as phenolic acids, flavonoids, anthocyanins, proanthocyanidins, and ellagitannins have received much attention due to their antioxidant activities and potential health benefits [17, 18].

Natural and synthetic forms of the tocopherols and tocotrienols are equally absorbed from the intestinal lumen in the form of mixed micelles. After the passage of the micelles into the intestinal mucosa, chylomicrons are synthesized to transport vitamin E from the intestinal mucosa through the lymphatic system to the circulatory system [19]. In plasma, alpha-tocopherol is found in all lipoprotein fractions but mostly is associated with apo B-containing lipoproteins. Via the action of lipoprotein lipase (LPL), extrahepatic tissues pick up parts of the tocopherols transported in chylomicrons, and the remaining chylomicrons transport the remaining tocopherols to the liver. Here, a large proportion of alpha-tocopherol is incorporated into nascent very-low-density lipoproteins by the operation of the "alpha-tocopherol transfer protein" (VLDL), whereas the excess of alpha-tocopherol plus the other forms of vitamin E is excreted in bile. When VLDL is secreted into circulation, the action of LPL transforms VLDL into IDL and LDL, and the excess surface components, including alpha-tocopherol, are transferred to HDL. In addition to the LPL action, alpha-tocopherol is transmitted to tissues via the absorption of lipoproteins by different tissues through their corresponding receptors [20–24].

Metabolism of vitamin E begins with one cycle of CYP4F2/CYP3A4-dependent ω-hydroxylation followed by five cycles of subsequent β-oxidation and forms the water-soluble end-product carboxyethyl hydroxy chroman. α-Tocopherol can be oxidized to the tocopheroxyl radical. Further oxidation of the tocopheroxyl radical forms tocopheryl quinone. Other Metabolites of vitamin E include

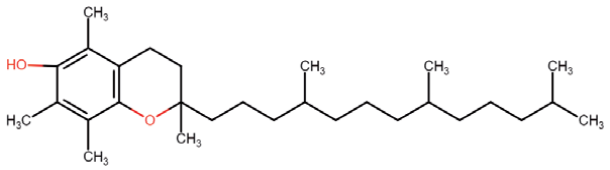

Figure 1.
Chemical Structure of alpha-Tocopherol.

2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (α-CEHC) derived from α-tocopherol and 2,7,8-trimethyl-2- (2′-carboxyethyl)-6-hydroxychroman (γ-CEHC) derived from γ-tocopherol. There are two primary pathways for the excretion of vitamin E. Bile, which is then excreted in the urine, is the primary path of excretion. The second path is in the urine to make it more water-soluble after vitamin E is chain-shortened in a process similar to beta-oxidation. The major route of excretion of ingested vitamin E is fecal elimination because of its relatively low intestinal absorption [25, 26].

Because of its antioxidant function, it has several significant functions within the body. Numerous potential complications and disorders, including cancer, diabetes, arthritis and cataracts, have been related to oxidation; vitamin E is beneficial against these conditions. Vitamin E may also prevent platelet hyper aggregation, which can lead to atherosclerosis; it also helps to reduce the development of prostaglandins, such as thromboxane, that cause platelet clumping [27].

2. Role of vitamin E in pregnancy

Vitamin E supplementation may help reduce the risk of pregnancy complications involving oxidative stress. There is a need to evaluate the efficacy and safety of vitamin E supplementation in pregnancy [28]. A lack of vitamin E can lead to female infertility, miscarriage, premature delivery, eclampsia, fetal intrauterine growth restriction and other diseases associated with pregnancy [29–31]. Here some conditions in which vitamin E role are being described:

2.1 Infertility

Due to excessive production of ROS and/or insufficient consumption of antioxidants, oxidative stress arises. When the generation of reactive oxygen species (ROS) and other radical species exceeds then scavenging ability of antioxidants fail, injury to cells can occur. The mitochondrial respiratory chain produces the majority of ROS, although they may also be generated through exogenous exposures such as alcohol, cigarette smoke, and environmental pollutants. Antioxidants (such as vitamins C and E) and antioxidant cofactors (such as selenium, zinc, and copper) can dispose of, scavenge, or suppress the formation of reactive oxygen species (ROS). Reduced sperm motility, sperm number, and sperm–oocyte fusion have all been linked to oxidative stress in male infertility. In women, several animal and in-vitro studies suggest that oxidative stress may affect female fertility. Adequate intake of vitamin E protects from free radical generation [32].

According to Cooper et al., vitamin E deficiency impaired both male and female rats' germ cells. Vitamin E deficiency has a significant impact on secondary spermatocytes and spermatids [33]. According to several studies, vitamin E deficiency has been linked to reduced fertility in both humans and lab animals. Rengaraj et al.

discovered that a moderate amount of vitamin E in poultry diet preserves semen/sperm quality in male birds and egg quality in female birds by reducing lipid peroxidation in semen/sperms and eggs [34]. The effects of vitamin E on sperm motility were studied by Suleiman et al. A total of 11 out of 52 treated patients (21%) were pregnant, and 31 subjects experienced increased sperm motility [35]. In a systematic review of the effect of oral antioxidants (vitamins C and E, zinc, Se, carnitine) on male infertility by Ross et al., 17 randomized trials, including a total of 1665 men, were identified. Of the 17 trials, 14(82%) showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy [36]. Cicek et al., studied the impact of vitamin E on the treatment results of women who were going through intrauterine insemination and controlled ovarian stimulation and had an unknown cause of infertility. Two groups A and B had 53 and 50 volunteers respectively. Group A received 400 IU/day of vitamin E and clomiphene citrate. This combination was used for producing controlled ovarian stimulations. Group B (control) also received controlled ovarian stimulation but without vitamin E. Outcome of the study demonstrated that both the groups had a significant difference in the thickness of endometrium on the day which human chorionic gonadotropin (hCG) was administered. Nevertheless, implantation and pregnancy rates had no connection with the administration of vitamin E. Based on the study it can be concluded that vitamin E possesses antioxidant effect and its administration could enhance the response of endometrium in females with unknown cause of infertility [37]. Das et al. performed a study in which female rats (30 days age) were maintained on a vitamin E-deficient diet for 70 days. At 100 days of age, the vitamin E-deficient and control animals were sacrificed. A group of animals was supplemented with a normal diet for the last 25 days following a 45-day deficient diet, or vice versa. The most notable findings were (i) a significant decrease in uterine weight in the deficient group, (ii) a significant decrease in estrogen, LH, and estrogen-induced uterine enzymes alkaline phosphatase and peroxidase, and (iii) ovarian dysfunction as shown by degenerating graffian follicles [38].

2.2 Endometriosis

Endometriosis is a condition characterized by the presence of endometrial tissue outside the uterine cavity [39]. Endometriosis is a condition that affects mostly women of reproductive age. The peak incidence is between 35 and 45 years old [40]. Endometriosis is found in 25 to 40% of women with infertility and 40–87% of women with chronic pelvic pain have endometriosis [41–43]. Endometriosis is associated with oxidative stress, even though the pathogenesis of the condition is currently unknown. Patient with endometriosis have an altered balance of prooxidant and antioxidant molecules [44–46].

Santanam et al. performed a randomized, placebo-controlled trial of antioxidant vitamins (vitamin E and C) in women with pelvic pain and endometriosis. This study included 59 women between the ages of 19 and 41 who had pelvic pain and had a history of endometriosis or infertility. Before surgery, patients were randomly assigned to one of two groups: vitamin E (1200 IU) and vitamin C (1000 mg) or placebo for eight weeks. Results indicated that after treatment with antioxidants, chronic pain ("everyday pain") improved in 43 percent of patients in the antioxidant treatment group ($P = 0.0055$) compared with the placebo group. The results of this clinical trial show that administration of antioxidants reduces chronic pelvic pain in women with endometriosis and inflammatory markers [47]. East-Powell et al. performed a randomized, placebo-controlled trial of antioxidant vitamins (vitamin E and C) in women with pelvic pain and endometriosis and/or infertility. A total of 59 women were included in the trial. Patients were randomly assigned to

2 groups: vitamin E 1200 IU (3 capsules of 400 mg each) and vitamin C 1000 mg (2 tablets of 500 mg each) daily for eight weeks before surgery. The results of this clinical trial show that administration of antioxidants (vitamin E and C) reduces chronic pelvic pain in women with endometriosis and inflammatory markers [48]. Hashemi et al. performed a randomized clinical trial in 40 women with implantation failure aged 18–37 years old. Participants were randomly divided into two groups: group A received 400-IU vitamin E supplements and group B received a placebo for 12 weeks. Vitamin E supplements were shown to dramatically improve serum vitamin E levels and endometrial thickness in women with implantation failure [49]. Kavtaradze et al. performed a clinical trial in 59 patients age 19–41 years with pelvic pain and history of endometriosis and/or infertility. Patients were randomly assigned to 2 groups: vitamin E (1200 IU) and vitamin C (1000 mg) combination or placebo daily for two months before surgery. This clinical trial's preliminary findings indicate that antioxidants (vitamins E and C) improve pelvic pain in women with endometriosis. According to this report, antioxidant vitamins are effective in reducing chronic pelvic pain in women with endometriosis. This research supports the development of a new class of medicines for the treatment of endometriosis-related pelvic pain. This information further supports our overall conclusion that endometriosis is an oxidative stress-related condition [50].

2.3 Miscarriage

Miscarriage is a serious pregnancy complication that can be brought about by a variety of causes. Vitamin deficiency has been linked to an increased risk of miscarriage, so supplementing women with vitamins before or during pregnancy can help prevent miscarriage [51]. Vitamin E deficiency's effects on human health have yet to be thoroughly reported and investigated. Low plasma vitamin E, on the other hand, has been linked to miscarriage in the first trimester of a woman's pregnancy. Furthermore, vitamin E supplementation in the diet reduced the rate of miscarriage in pregnant women by around 50% [52].

Pregnant women have quicker metabolism, increased production of free radicals, and increased lipid peroxidation. Thus, low levels of vitamin E can lead to the production of excessive free radicals, leading to placental aging, endothelial vascular damage, which increases the incidence of high-risk infections in pregnancy [53, 54]. It can also damage the lining of the fetal cell membranes, increasing the risk of premature rupture of the embryo [55]. Increased reactive oxygen species and decreased antioxidant levels in men are associated with recurrent miscarriage (RM). Antioxidant therapy has recently been recognized as a way to improve sperm parameters. Pourmasumi et al. evaluate the effect of paternal factor and antioxidant therapy on sperm parameters in couples with RM. Sixty samples with RM patients were analyzed before and after 3 months of vitamin E and selenium therapy. Results of this study show that antioxidants can improve sperm parameters and chromatin condensation in recurrent miscarriage male partners [56].

Vitamin E has anticoagulant activity; excessive vitamin E can have an impact on blood clotting in the fetus, increasing the risks of high levels of bilirubin and nuclear jaundice for newborn babies. Also, excessive vitamin E has an antagonistic effect on other fat-soluble vitamins in the blood of pregnant women, preventing the absorption and functions of other vitamins. As a result, clinicians should pay careful attention to changes in vitamin E levels during pregnancy and offer appropriate dietary advice, with an emphasis on reasonable vitamin E supplementation [57].

Kurmacheva et al. conduct a pharmacoeconomic analysis of two schemes of vitamin-mineral drugs in the peri-gestation period in women. In two classes of women, the cost-effectiveness of vitamin-mineral formulations was calculated. Patients in

the first group (n = 60) were given a vitamin-mineral complex before and during pregnancy that included metafolin, other B vitamins, vitamins C, E, PP, and iodine (150 mcg) in physiological doses, as well as 200 mg of docosahexaenoic acid in a capsule intended for use from the 13th week until the end of pregnancy. During pregravid preparation and the gestational period, women in the second group (n = 54) took high doses of synthetic folic acid, vitamins B6 and B12 as part of two vitamin and mineral preparations. The use of vitamin-mineral complex containing physiological dosages of vitamins of group B, vitamins C, E, PP and iodine in the peri-gestation period in women with habitual miscarriages has tangible clinical and economic advantages in comparison with the administration of high doses of synthetic folic acid, vitamins B6 and B12 [58].

Shamim et al. studied the contribution of deficiencies of vitamin E to human pregnancy loss (pregnancy losses <24 wk. of gestation) in rural Bangladesh. A trial was done in 1605 pregnant Bangladeshi women, gestational age: 8–13 weeks. Of the 1,605 women in the study, 141, or 8.8%, miscarried. About 5.2% of women with adequate alpha-tocopherol miscarried in the first or second trimester, compared with 10.2 percent of women with low levels. It was found that low plasma α-tocopherol was associated with an increased risk of miscarriage. Maternal vitamin E status in the first trimester may influence the risk of early pregnancy loss [59].

Junovich et al. investigate the fertility properties of Vitamin E. Pregnant females from CBA/J × DBA/2 miscarriage model (creates an immune type miscarriage) were orally supplemented with Vitamin E (15 mg/day). It was found that Vitamin E has able to decrease the miscarriage rate [60]. Şimşek et al. investigated plasma levels of vitamin E in 40 women with habitual abortion (HA) at the Department of Obstetrics and Gynaecology, Medical Faculty of Firat University, ElazigÏ, Turkey. The mean age of the patients was 28.5 years (21 ± 38 years). The levels of vitamin E were significantly lower in women with HA than in controls. According to the results of this study, it was found that a level of vitamin E was significantly decreased ($P < 0.01$) in HA. The decrease of this antioxidant may play a significant role in women with habitual abortion [61]. Vural et al. performed a clinical trial to determine the relationship between changes in some parameters of the antioxidant system like vitamin E and recurrent abortion. For the study 120 women with recurrent abortions, 25 non-pregnant healthy women in the productive era and 25 normotensive pregnant women within their first trimester were taken into the study in Istanbul Medical Faculty, Gynecology and Obstetric Department. According to the etiology, women with chronic miscarriage were classified into four subgroups: autoimmune, luteal phase defect, anatomical disease, and unexplained. Vitamin E levels in the autoimmune, unexplained, and luteal phase defect subgroups were slightly lower than in the two control groups and the anatomical defect group. It was found that decreased concentrations of plasma vitamin E reflect the increased oxidative stress. In a conclusion, recurrent miscarriages may also result in oxidative stress and depletion and weakness of antioxidant defence [62].

Von Mandach et al. studied that whether there is an association between reduced vitamin E levels and abnormal pregnancy. Abnormal pregnancies were compared with normals. In normal pregnancies, mean vitamin E levels rose from 12.9 +/− 1.1 micrograms/ml in early pregnancy to 22.5 +/− 1.5 micrograms/ml at term ($p < 0.05$, n = 11). In pregnancies with fetal complications or maternal risks, vitamin E levels were lower than in normal at corresponding gestational age. The results show lower maternal levels of vitamin E in abnormal pregnancies [63].

Oladimeji et al. performed a clinical trial to examine the relationship between serum vitamin E levels and unexplained infertility and recurrent miscarriages. Eighty-two healthy Nigerian Women volunteers were recruited for this study.

The mean serum vitamin E concentration in pregnant women was found to be insignificantly higher (10.36 ± 3.52 mg/ml) than the reported values in women with unexplained infertility and persistent miscarriage (8.97 ± 3.56 mg/ml). It was concluded from this study that there is no relationship between recurrent miscarriages and unexplained infertility and vitamin E levels [64].

Miscarriage risk is significantly reduced by taking supplementary vitamin E (at least 200 IU and perhaps 400 IU daily). There were already results of medical reports confirming this by the end of WW II [65].

2.4 Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome is a common birth defect in women during childbearing age. According to the Endocrine Society released training strategies for PCOS, in adolescents with PCOS, metformin and hormonal contraceptives are the treatment options [66].

Vitamin E is not a hormone but acts as a hormone. It works by impersonating the effects of progesterone on the body, as well as by reducing the side effects of high levels of androgens (testosterone and estrogen). In a study after 12 weeks, the amount of serum testosterone decreased which provides evidence against it. The study also found that lifestyle changes and the use of supplements including omega-3 and/or Vitamin E will improve inflammation and insulin sensitivity thus remaining an effective treatment approach for PCOS subjects. Vitamin E may serve as a necessary supplement included in the current treatment guide to improve PCOS parameters, which will also improve the quality of life in PCOS and reduce overall medical costs, often unaffordable for most people with PCOS in India [67]. Vitamin E reacts often with lipid peroxyl radicals which leads to the elimination of peroxidation chain reactions and thus reduces oxidative damage. The serum concentration of vitamin E in the study was significantly lower in PCOS patients compared with controls. Similar reports of reduced vitamin E concentration in PCOS patients were stated in various studies [68]. The prospect of a fast response of vitamin E to cellular oxygen and free radicals may be the cause of a significant decrease in vitamin E concentration. Therefore, it is proposed that vitamin E through its natural scavenging method protects polyunsaturated fatty acids from peroxidation reactions [69]. Mohan et al. 2009 estimated plasma vitamin E level in fifty-six Polycystic Ovary Syndrome patients. It was observed that there was a significant decrease in plasma vitamin E levels in patients with polycystic ovary syndrome when compared to controls [70]. Hamad et al. studied the effect of vitamin E and selenium on ovulation in PCOS patients. The participants in this sample included 25 PCOS patients who were untreated and 26 PCOS patients who were treated, as well as 42 healthy controls. From the results of this study, it was found that vitamin E has a significant role in the ovulation of PCOS patients [71]. Angiogenesis disturbances are common in women with polycystic ovary syndrome (PCOS). Shirazi et al. performed a randomized, double-blind, placebo-controlled trial on 43 women, ages 20–40 years, with PCOS to evaluate antiangiogenic properties of Vitamin E. Patients were randomly assigned into two groups: group A received vitamin E 400 IU/day and group B received placebo for 8 weeks. At the start and end of the analysis, anthropometric and angiogenic parameters such as body weight, fat mass, and fat-free mass, vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) were calculated using standard methods. Vitamin E supplementation for eight weeks had beneficial effects on body weight, Ang-1, Ang-1/Ang-2 ratio, and VEGF level in PCOS women [72]. Carotid intima-media thickness (CIMT) artery in women with PCOS was significantly higher than healthy women. CIMT has been widely

used as a surrogate index of atherosclerosis and CVD events. Talari et al. performed a randomized, double-blind, placebo-controlled trial in 60 women with PCOS to evaluate the beneficial effects of omega-3 and vitamin E co-supplementation on carotid intima-media thickness (CIMT). Participants were randomly assigned into two groups and assigned to take either 1000 mg omega-3 plus 400 IU vitamin E supplements or a placebo for 12 weeks. It was found a significant reduction in maximum and mean levels of the left and right CIMT in patients with PCOS compared with placebo. Antioxidant and anti-inflammatory effects of Vitamin E may improve CIMT [73].

PCOS is a heterogeneous syndrome characterized by hyperandrogenism symptoms. Izadi et al. performed a randomized, double-blind, placebo-controlled clinical trial to evaluate the effects of CoQ10 and/or vitamin E on glucose homeostasis parameters and reproductive hormones in women with PCOS. In this study, it was found that CoQ10 with or without vitamin E supplementation for 8 weeks among patients with PCOS significantly decreased serum total testosterone levels ($P < 0.001$) compared with those of the placebo group. CoQ10 supplementation in combination with vitamin E significantly improved sex hormone-binding globulin (SHBG) levels compared with other groups ($P = 0.008$) [74].

2.5 Embryonic development

Human embryonic development refers to the development and formation of the human embryo. It is characterized by the processes of cell division and cellular differentiation of the embryo that occurs during the early stages of development [75].

ROS are highly reactive molecules, their accumulation can lead to damage and breakage of DNA strands. Many pieces of evidence have been found that ROS compromises embryo development in many species [76–78].

Selenium and Vitamin E are the important antioxidants that protect mammalian cells against lipid peroxidation. Tsujii et al. conducted a study to investigate whether Selenium or Vitamin E and Selenium + Vitamin E overcome the undesirable oxidative stress produced by hydrogen peroxide (H_2O_2) and enhance the development of pre implanted mice embryo. Co-incubating the embryos with 60 nM Selenium and/or 100 nM Vitamin E were increased ($P < 0.05$) the blastocyst development rate. The addition of H_2O_2 reduced the development of mouse embryos, but the addition of Vitamin E, Se and Selenium+Vitamin E reduced the detrimental effect of H_2O_2 and influenced the higher rate of development to blastocysts, compared to CZB alone ($P < 0.05$). The incorporation and oxidation of 14C-glucose in the blastocysts developed by the medium supplemented with Se and/or Vitamin E in the presence or absence of H_2O_2 were significantly higher ($P < 0.05$) than that of the control. Moreover, Vitamin E is more effective than Selenium and Selenium + Vitamin E in reversing ROS-induced mouse embryotoxicity [79]. McDougall et al. studied the long-term effects of Vitamin E deficiency on embryonic development and improvement effect after feeding Vitamin E-adequate diets by using a zebrafish model. Adult zebrafish maintained on Vitamin E-deficient (E-) or sufficient (E+) diets up to 12 days post-fertilization (dpf) to obtained E- and E+ embryos. The E- group suffered significantly increased morbidity and mortality as well as altered DNA methylation status through 5 dpf when compared to E+ larvae, but upon feeding with a Vitamin E -adequate diet from 5 to 12 dpf both the E- and E+ groups survived and grew normally; the DNA methylation profile also was similar between groups by 12 dpf. However, 12 dpf E- larvae still had behavioral defects. Outcomes suggest that embryonic Vitamin E deficiency causes behavioral impairments due to persistent lipid peroxidation and metabolic perturbations that are not resolved via later dietary vitamin E supplementation [80].

2.6 Pre-mature delivery

Every year, about 15 million babies (1 in 10) are delivered prematurely around the world. Prematurity is the second leading cause of death among newborns after pneumonia. Pre-mature babies struggle with visual, auditory and learning disabilities. Over 60% of pre-mature delivery worldwide occurs in Sub-Saharan Africa and South Asia [81].

Cruz et al. studied the effect of vitamin E supplementation on mothers with threatened premature delivery and premature infants. It was found that maternal vitamin E treatment did not prevent either erythrocyte hemolysis or lipid peroxide formation in premature infants after birth. On the other hand, intramuscular vitamins E to infants after birth prevent erythrocyte hemolysis and low lipid peroxide formation when serum vitamin E increased above 2 mg/100 ml [82]. Hittner et al. performed a double-blind study in 101 preterm infants to evaluate the efficacy of oral vitamin E in preventing the development of retrolental fibroplasia. 50 infants received vitamin E 100 mg/Kg/ day and 51 infants received 5 mg mg/Kg/ day (controls). The severity of retrolental fibroplasia was found to be significantly reduced in infants given 100 mg of vitamin E ($P = 0.012$) [83]. The link between vitamin E deficiency and hemolytic anemia in small premature infants prompted researchers to look into vitamin E absorption in infants of different gestational and developmental ages. Premature infants' capacity to sustain vitamin E sufficiency during the first three months of life was shown to be directly related to their gestational age; infants with the lowest gestational age were the least likely to attain vitamin E sufficiency, even when given a vitamin E supplement. In infants with a gestational age of fewer than 32 weeks, there was a gradual increase in vitamin absorption in the intestine. Oral iron administration has been linked to a reduction in vitamin E absorption in the intestine. Since maintenance of vitamin E sufficiency appears to be nutritionally important in the premature infant, the efficacy of other routes of administration of the vitamin should be explored [84]. Vitamin E has been linked to several positive outcomes in premature newborn infants. Vitamin E deficiency is believed to be at least partially responsible for the anemia that happens often 4 to 6 weeks after premature birth, and regular vitamin E supplementation is often recommended. However, a review of published controlled trials of vitamin E supplementation shows that the extent, if any, of this preventive effect against anemia is debated. According to research, the dietary ratio of alpha-tocopherol to polyunsaturated fatty acids is normally sufficient to avoid symptoms of vitamin E deficiency without the use of supplements. Premature infants exposed to oxygen-rich conditions and artificial ventilation are protected from the risks of retrolental fibroplasia and bronchopulmonary dysplasia by receiving large parenteral doses of vitamin E. However, subsequent research has yet to verify these positive early findings of preventive effects. At this time, there does not seem to be any clear evidence that supplementing a premature infant's usual vitamin E intake is essential [85].

2.7 Uterine fibroids

Uterine fibroids or myomas are benign tumours of the human uterus. The main symptoms are prolonged or heavy menstrual bleeding, pelvic pressure or pain, and reproductive dysfunction [86]. Fibroids have an effect on a woman's pregnancy as well as her quality of life. Fibroids affect approximately 35–77% of reproductive-age women. Fibroids may cause infertility by obstructing the fallopian tubes and impairing gamete transport [87].

A total of 49 patients were enrolled in a double-blind, randomized, placebo-controlled trial conducted by Harrison et al. in 2003. For six months, all patients were

given either vitamins E (1000 IU) and C (1000 mg) or a placebo. Results show that a statistically significant improvement in fibrosis score ($p = 0.002$) [88]. Fruscella et al. studied the effect of vitamin E (300 mg per day) in a group of 25 women, aged between 25 and 41 years old, suffering from uterine myomas in pregnancy. All the pregnancies continued to term. The neonatal outcome was satisfactory in all cases and no collateral effects were observed in either mothers or fetuses [89]. Tocopherol can stop cancer cells from growing in culture by trapping free radicals and other mechanisms [90]. Young et al. discovered that vitamin E succinate (a vitamin E analogue) decreased the number of UF cells and caused cell death [91]. In addition, Zhang et al. discovered that vitamin E succinate ester could suppress steroid hormone signalling [92].

2.8 Preeclampsia

Preeclampsia is a major cause of both maternal and fetal neonatal morbidity. Endothelial damage in the arteries is believed to play a role. The simple clinical definition [gestational hypertension (>90 mmHg diastolic) occurring after the 20th week of gestation with superimposed proteinuria (>300 mg/day) belies the complexity of preeclampsia, which is often accompanied by multi-organ dysfunction. Free radical-mediated lipid peroxidation may be involved in endothelial damage in preeclampsia. Complications such as endothelial cell dysfunction of blood vessels in women with preeclampsia and other hypertensive conditions are linked with oxidative stress and lipid peroxidation.

Antioxidants may be essential for lipid peroxidation prevention and, hypothetically, pre-eclampsia prevention. Vitamin E, which is a free radical scavenger and thus inhibits the development of lipid peroxides, opposes the toxic acts of lipid peroxides. It acts as in-vivo antioxidant that protects tissue lipids from free radical attack and thus stabilizes cell membranes. Compared to non-pregnancy, maternal levels of vitamin E are elevated in pregnancy, which is consistent with previous studies. In women with preeclampsia, the antioxidant function is decreased relative to women who have normal pregnancies. Antioxidant activity increases throughout normal gestation, but not with preeclampsia. It has been suggested that evidence of vitamin E consumption is an alerting mechanism for the development of preeclampsia [93, 94]. Some preclinical studies show that vitamin E plays a role in preeclampsia [95]. But some clinical studies suggest that there is no role of vitamin E in preeclampsia [96, 97]. A study has found that level of vitamin E in preeclampsia was low, but no preventive role was found in preeclampsia [98].

2.9 Intrauterine growth restriction

Intrauterine growth restriction (IUGR) is the inability of fetuses to achieve their genetically defined growth rate resulting in offspring with low birth weight (LBW) and is a problem for both human and veterinary medicine. IUGR has significant consequences for the mortality and morbidity of LBW newborns and has long-term effects on their development and health. The presence of IUGR is directly linked to an insufficient supply of nutrients and oxygen to the fetus due to maternal malnutrition and/or placental insufficiency [99, 100].

In the study, Sales et al. found that combined maternal administration of vitamin C and E in sheep was associated with increased levels of both vitamins in the fetal cord, enhanced antioxidant status, and increased fetal development in singleton and twin pregnancies, but with a greater impact on twin pregnancies. These findings demonstrate the ability of supplementation of vitamin C and E to reduce the effects of IUGRR [101]. Since the transplacental transmission of

alpha-tocopherol is minimal, newborns are considered an at-risk category for vitamin E deficiency. Low serum levels of alpha-tocopherol are associated with the development of edemas, thrombocytosis, and hemolytic anemia, which can result in cardiomyopathy and the possible consequence of this vitamin deficiency is its restriction on the intrauterine growth of fetuses [102].

Atherosclerosis is one of the main factor of intrauterine growth restriction. Busso et al. [103] studied LDL KO mice diet-induced maternal hypercholesterolemia and atherosclerosis during pregnancy can negatively impact fetal growth. Vitamin E dietary supplementation has a beneficial effect, preventing growth restriction in a significant proportion of fetuses from HC-fed mice [103].

2.10 Premature rupture of membranes

Preterm, premature rupture of the membranes (PPROM) is defined as membrane rupture before 37 weeks' gestation in the absence of labour.

PPROM occurs in 1–2% of all deliveries and results in a major portion of preterm deliveries with the regular mortality rate in neonates [104]. Infection, cigarette smoke, and inflammation have all been linked to preterm premature rupture of fetal membranes. Since hypochlorous acid (a reactive oxygen species) is essential to the body's reaction to infection, it may cause tissue damage when destroying pathogens. Plessinger et al. found that antioxidant therapy (vitamins C and E) has a protective effect against hypochlorous acid-induced damage [105].

Preterm infants have a higher risk of oxidative stress and free-radical-mediated diseases, which is partially due to their low antioxidant levels. Bolisetty et al. studied the effect of maternal supplementation of antioxidant vitamins before delivery to reduce the oxidative stress in the mothers and their infants. Five mothers between the ages of 30 and 36 weeks who were at risk of preterm delivery were given a daily oral dosage of betacarotene 20 mg, vitamin E 167.8 mg, and vitamin C 1000 mg until delivery. There was a trend of lower plasma MDA and higher vitamin E at birth in infants born to supplemented mothers. Finally, it has been concluded that short supplementation of antioxidant vitamins to preterm pregnant women reduced the oxidative stress at delivery in mothers and probably in their neonates [106].

The fetal membranes (amnion and chorion) derive their strength principally from collagen. Collagen provides fetal membranes with both tensile strength and elasticity. Reactive oxygen species (ROS) generated by the body's response to infection, cigarette smoking, bleeding, or cocaine use can activate collagen lytic enzymes and impair fetal membrane integrity. Vitamin E, a lipid-soluble antioxidant, inhibits membrane-damaging effects of reactive oxygen species induced lipid peroxidation [107]. Hauth et al., studied that maternal supplementation with vitamin C and E did not reduce the occurrence of spontaneous preterm birth [108].

3. Discussion

Several risk factors contributing to reproductive- and pregnancy-related disorders have been reported. Environmental and lifestyle factors are the two main types factors. Examples of major environmental pollutants include hazardous man-made chemicals, industrial discharge, agricultural run-off, human and animal waste, municipal and domestic effluents, and spillage of vessels and oil spills [109]. Lifestyle factors represent another category of major risk factors for reproductive and pregnancy-related disorders. Unhealthy lifestyle behaviors, including cigarette smoking, alcohol consumption, and/or drug abuse,

have negative impacts, particularly on female fertility [110, 111]. Furthermore, exposure to multiple environmental pollutants may also result in reactive oxygen species (ROS)-induced oxidative stress (OS). High levels of OS can be linked to a variety of pregnancy-related issues, including embryonic death, early spontaneous abortion, IUGR, fetal death, preterm births, and low birth weight [112, 113]. ROS are highly reactive and unstable. They acquire electrons from nucleic acids, lipids, proteins, carbohydrates, or any other nearby molecule causing a string of chain reactions to become stable. These chain reactions result in cellular damage and diseases [114]. The human body produces reactive compounds known as free radicals which exert a positive as well as a negative impact on the body. To minimize the harmful effect, a complex protection system is required which is known as the antioxidant system. When there is an imbalance between the production of free radicals and the defense mechanism of the antioxidant system it leads to a condition known as oxidative stress [115]. Oxidative stress affects the interaction of gametes and their quality. Spermatozoa, embryos, and oocyte and their environment are affected by free radicals such as reactive oxygen species (ROS). The quality of sperm-mediated oocyte activation, early embryo development, implantation, sperm oocyte interaction is dependent on the microenvironment associated with peritoneal fluid, follicular fluid, and hydrosalpingeal fluid. Implantation and early embryo development are adversely affected by oxidative stress leading to a negative effect on the rate of pregnancy. One of the causative factors for infertility and endometriosis is oxidative stress [116]. ROS or prooxidant or free radicals production have a connection with aerobic metabolism [117, 118], and also the hormones, cytokines, and other stressors are associated with its production. Hydroxyl radical, superoxide anion and hydrogen peroxide are examples of reactive oxygen species (ROS) and they act by modulating the gene expression and transcription factor. A broad range of antioxidants is available which hold the capability to repair cell damage caused by ROS and can neutralize them as well [119, 120].

Some studies suggested that there is a direct relationship between the outcome of the pregnancy and the level of ROS. The placental mitochondrion is the place that has been identified for the production of superoxide in a great amount [121, 122]. Antioxidants such as vitamin C and vitamin E have been reported to be efficient, and their uses in reproductive- and pregnancy-related disorders have been the subject of significant clinical trials [123]. Vitamin E is a chain-breaking antioxidant that helps to inhibit lipid peroxidation [124, 125].

All of these studies show that vitamin E is essential for a natural and stable pregnancy and that vitamin E supplementation has no negative effects on pregnancy outcomes. As discussed above, vitamin E has been proven to be beneficial in pregnancy and neonatal health.

4. Conclusion

Vitamin E has antioxidant properties that may support pregnancy, but more research is needed to determine its effectiveness. There is a need to evaluate the efficacy and safety of vitamin E supplementation in pregnancy. More research is needed to accurately quantify antioxidant-like vitamin E levels in pregnancy and how they change throughout the pregnancy. It is necessary to investigate the effects of antioxidants on maternal, fetal, and placental health. Prenatal advice should be clear to ensure that women and physicians understand the dietary conditions during pregnancy and how a balanced diet contains antioxidant micronutrients like vitamin E will help avoid pregnancy-related diseases.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Impact of Vitamins and Minerals Enriched Flora in the Management of Calciphytoliths: A Special Focus on Vitamin E

Ramu Govindan, Tilak Meenakshisundaram, Navanita Sivaramakumar, Podila Naresh, Duraiswamy Basavan and Dhanabal Palanisamy

Abstract

Calciphytoliths (calcium oxalate calculi) have a great influence on human health and are a disease with a high likelihood of recurrence at a rate of more than 10% within a year. Plant flavonoids, saponins, and tannins are reported to be Litholytic by inhibiting calcium oxalate crystals or by their calcium channel blocking activity. Vitamins and minerals containing flora completely prevent deposition of oxalate by preventing pre-oxidation injury and restoring renal tissue antioxidants. So vitamin therapy also might protect against oxalate calculi deposition in the human kidneys. The present chapter discusses the impact of vitamins especially vitamin E, calcium, and low oxalate-containing plants for the management of various urinary or kidney disorders.

Keywords: calciphytoliths, prevention, vitamin E, vitamin C, calcium supplement, medicinal plants

1. Introduction

1.1 Urinary stones - kidney stones

1.1.1 Definition

Urinary stones are solid masses composed of a collection of small crystals which are formed and present in the urinary tract, due to the agglomeration of some components in the urine under certain physicochemical conditions. The urinary stone disease has a great influence on human health and is a disease with a high likelihood of recurrence (or recurrent at a rate of more than 10% within a year).

Most urinary stones are formed in kidneys (80%), then migrate in the urine stream to other places of the urinary tract. When a stone appears at any part of the urinary system, it means a urinary stone disease. Thus, urinary stones include kidney stones, ureteral stones, bladder stones, and urethral stones.

According to research, among cases of urinary stones on average, kidney stones (**Figure 1**) account for the highest proportion (40%), ureteral stones account for 28%, bladder stones account for 26% and urethral stones account for 4% (**Figure 2**).

1.1.2 Epidemiological characteristics

Urinary stone is a common disease in the world. The Urolithiasis prevalence in countries varies from 2–12% of the population, particularly with higher data as in the study of Albuquerque (Brazil), the rate of urinary stones is up to 14% and in 2014, nearly 4–15% of the population has urinary stone problems globally.

The incidence of urinary stones is related to age, gender, race, geographical environment, and eating habits [1].

Regarding gender, epidemiological studies showed that kidney stones are less common in women (6%) than in men (12%). The sex ratio ranges from 5: 1 in Japan to 15: 1 in Iran, but the likelihood of developing kidney stones is increasing in both

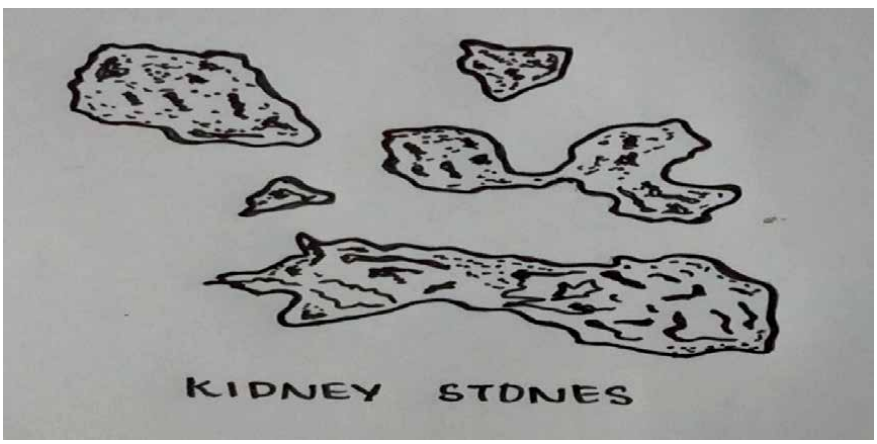

Figure 1.
Kidney stones.

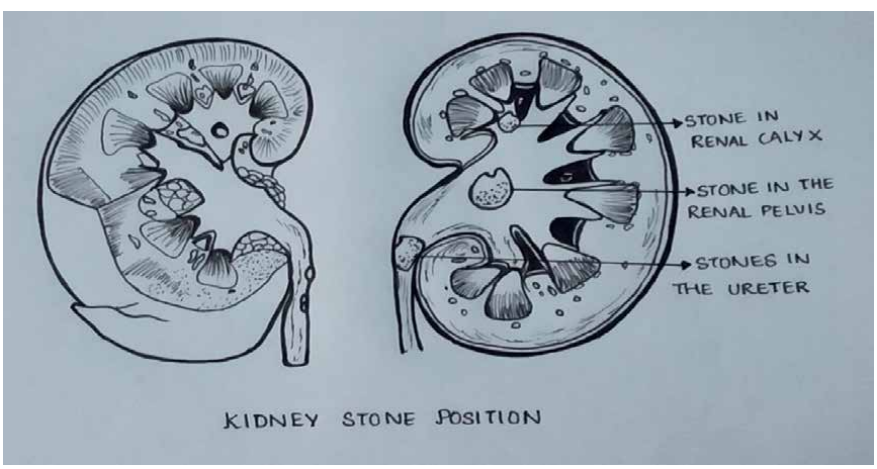

Figure 2.
Kidney stone position.

genders [2]. The risk of kidney stones increases in men in their 40s and continues to increase until the age of 60s. Several studies show that white-skin people and men are at higher risk of urinary stones than other ethnic groups.

The incidence of Urolithiasis is usually low among black-skin Americans but higher in Asian countries, typically in Thailand and India [3]. The regions with the highest percentage of kidney stones are called the "Stone Belt" in Humberger and Higgins maps. Vietnam is a country located in the "Stone Belt" of the world with a high incidence (40–60% of the total number of patients treated in the Urology department and has its special characteristics of stone disease including coming late to the hospital when the stones have enlarged; there are many serious complications such as urinary tract infections or kidney failure.

In India, about 12% of the population has Urolithiasis, of which 50% may suffer kidney loss or kidney damage. The incidence of Urolithiasis is prevalent in the North of India (nearly 15% of the population), while this disease is less commonly found in the South [4].

People who live in rocky areas, where the climate is hot and dry, are more likely to get urinary stones [5]. The incidence of urinary stones has been increasing globally. Dietary changes, as well as the effects of global warming, can be the major drivers of this trend [6].

1.1.3 Classification of urinary stones

Based on the composition of stones, kidney stones are classified into 2 groups with 6 common types including inorganic stones (Calcium, Oxalate, Phosphate) and organic stones (Cystine, Struvite, and Uric acid). Each type has different causes as well as different methods of treatment. Stones often exist as a mixture of chemical components.

A collaborative study in France involving 51,747 stones, in mid-January 2001 and December 2004 by many laboratories showed that Calcium oxalate (CaOx) is the most common ingredient accounting for 71.8% of stones. Calcium phosphate accounts for 13.6% and Uric acid accounts for 10.8% of the total stones.

Among the types of kidney stones, CaOx is the most common type. Calcium oxalate stones have two types including calcium oxalate monohydrate (whewellite) and calcium oxalate dehydrates (weddellite). The incidence of whewellite is 78% while that of weddellite is 43% [7]. Whewellite is highly capable of agglomerating, tightly binds to renal tubular epithelial cells, and retained forming stones. Whewellite accounts for 39% in men and 37.4% in women. Weddellite does not agglomerate into stable masses, does not attach to renal tubular epithelial cells and is easily swept with urine, and difficult to form urinary stones.

In Vietnam, all stone samples analyzed have two or more components, of which the most common component is calcium oxalate (90.7% of incidence), followed by calcium phosphate, struvite, ammonium urate, and cysteine.

In India, calcium oxalate stones are found to be the most important component of Urolithiasis. Calcium oxalate stones make up 80% of the analyzed stones. Calcium phosphate stones account for 15–25%, while 10–15% is mixed stones. The others are struvite with 15–30%, cystine with 6–10%, and uric acid stones with 2–10%.

It was observed that urinary stones in England, Scotland, and Sudan are mainly composed of a mixture of calcium oxalate and calcium phosphate. Meanwhile, uric acid stones in the upper urinary tract are quite common in Israel. In Saudi Arabia, calcium stones make up the majority (84.6%), uric acid stones account for 12.8% [8].

1.1.4 Causes and mechanism of formation of kidney stones

1.1.4.1 Causes

The generation and formation of urinary stones come from different sources that are not only caused by any group of reasons but due to a combination of factors including diet, diseases in the body, and genetic factors which together form urinary stones. Factors leading to the risk of kidney stones include

1. **Not drinking enough water** (<1,200 ml/day) makes the urine more concentrated with minerals such as calcium and phosphorus leading to the risk of kidney stones.

2. **Eating more animal protein in the diet**

High animal protein in food causes acidosis of urine, creating conditions for crystals as uric acid to crystallize to stones. Eating a lot of sodium (salty foods) causes an increase in the renal tubular sodium and this will reduce the reabsorption of calcium there. Also, studies by scientists from Ahvaz University of Medical Sciences, Iran showed that there is an intimate relationship between the consumption of purines, calcium oxalate foods, and the formation of stones [9].

3. **Genetics**

Families with a regular incidence of developing stones (an abnormal amount of calcium filtered into the urinary tract) are at higher risk of kidney stones than other families.

4. Chronic enterocolitis leads to kidney stone formation during pregnancy.

5. The increase in parathyroid activity is another reason. Hyperparathyroidism can be either a primary or secondary method, increasing calcium reabsorption from bone and leading to hypercalciuria.

6. **Improper calcium supplementation**

Pregnant women often need a supplement with a lot of vitamins and minerals, including calcium. However, calcium excess causes kidney stones. Moreover, calcium content is usually absorbed better during pregnancy. Therefore, both of these factors increase the risks. Pregnant women with urinary tract infections should discuss with their doctor for appropriate examination and treatment.

7. **Occupation**

Urinary stones are closely related to the profession of the patients. People who work in a hot environment like metallurgists, construction workers, sailors, stressed-minded workers like doctors, officers, etc. are more likely to be in the disease than those who are unskilled laborers.

8. Another reason causing kidney stones is long-term treatment with certain medication. It is believed that 1–2% of kidney stones are drug-induced. Certain medications including the antiviral drug Indinavir, Triamterene (diuretic), Sulfadiazine (an antibiotic) as well as Ephedrine and Guaifenesin are known as a cause of kidney stones [10].

Also, different reasons for stone formation purely depend on stone type such as

1. **Calcium stones**

The main reason is due to the state of oversaturated urine with calcium salts causing by the increase of calcium absorption in the intestine or the increase of calcium reabsorption in the renal tubular region. Causes that increase the concentration of calcium in the urine are hyperparathyroidism, major bone fractures, and immobilization for a long time, taking lots of Vitamin D and Corticoid, metastases of cancer through the bone, causing bone destruction. However in many cases not able to find the reason for calcium increase in urine. High level is not a determinant of urinary stone formation but a favorable factor.

2. **Oxalate stone**

This type of stone account for a high proportion in tropical countries like Vietnam and India. Oxalate is often combined with calcium to form calcium oxalate stones and 2/3 of stones in oxalate stone are due to the insoluble calcium oxalate salt. When the urine is saturated with oxalate, by eating foods and vegetables which are high in oxalate and poor in calcium, these molecules will pass through the digestive tract and be excreted as waste. When passing through the intestine, oxalate can combine with calcium to form calcium oxalate and be excreted in the waste. Having too much oxalate in the kidneys can lead to kidney stones. According to Prien, lack of vitamin B6 in the body is a cause of oxalate stones. Experiments in rats showed that vitamin B6-deficient foods produce in the renal tubule and thorn the Randall-like lesions in kidneys and oxalates are crystallized. In contrast, vitamin B6 will reduce the excretion of oxalate in urine.

3. **Phosphate stones**

A common type of phosphate stone is ammonium-magnate-phosphate (struvite stone), accounting for 5–15% of the number of stones. This type of stone has a large size, coral shape, and is in contrast.

Stones are formed as a result of a bacterial infection, especially proteus bacteria in the urinary system. This bacterium releases the urease enzyme, which breaks down urea into ammonia, making urine alkaline, resulting in a reduction in struvite solubility which facilitates stone formation.

4. **Uric acid stones**

With the increase of uric acid concentration in blood, in addition to deposition in cartilage, mucous membranes, muscle skin, the concentration of uric acid in the kidneys also increase. Uric acid deposition in kidneys is a major cause of uric acid stones, which is more common in gout patients (the result of nucleic acid metabolism disorders). Possible causes of purine metabolism increase include the intake of purine-rich foods such as pig intestine, dried fish, meat, and mushrooms. It is noted that uric acid is soluble in an alkaline environment and crystallizes in an acidic environment when urine pH is below 6. Accordingly, acidified urine is a good environment for forming stones.

5. **Cystine stones**

Stones are formed when cystine is excreted to the kidneys but less soluble making it easily be deposited. The stone is often congenital with the disorders of cystine reuptake transport and some other amino acids such as lysine and arginine in the

renal tubules and intestinal mucosa. Cystine stones are usually simple and rarely combine with other stones [11].

1.1.4.2 Mechanism and development of stones

The formation of stones in the body is a complex process, which is a result of a series of physicochemical processes including five main stages: (i) oversaturation of substances dissolved in urine (ii) nuclear formation (iii) nuclear proliferation (iv) crystallization and (v) crystals attachment to epithelial cells of renal tubules [12].

Oversaturation of substances that are capable to crystallize in urine is a phenomenon of concentration of a substance being able to crystallize in urine exceeds its solubility. There are many causes of urine oversaturation, including a change in urine pH, decrease in urine volume or metabolic disorder that increases the elimination of one or several substances through kidneys, imbalances in urine calcium excretion, hypercalciuria which is familiar or idiopathic, imbalance in oxalate excretion, reduction in urinary citrate and hyperuricemia due to purine-rich foods. It not only depends on the concentration of the stone formation substances but also depends on other factors such as urine pH, the ions representing inhibitors of the stone formation process such as citrate, pyrophosphate, magnesium, ribonucleic acid, and glycosaminoglycan.

1.1.4.2.1 Crystal nucleus formation

In oversaturated urine, free ions tend to coalesce into very small particles. The result of this process is the formation of a crystal nucleus which may be single-component or multi-component. In urine, a crystal nucleus can be formed on the structure of cellular debris and urinary crystals. The majority of urinary stones is a mixture of more than two substances. When there is a saturation of calcium or oxalate by urine when passing through the renal tubules, it will form a nucleus making COD form (which is common in the urine of healthy people) and COM form (mostly common in urinary stones).

1.1.4.2.2 Nuclear proliferation

It is the process of a nucleus which is made up of very small size continues to grow during the movement in the urinary tract, through the transferring of free ions in the solution into the crystals. This process does not cause any problems if the crystals are easily removed (as stones that are less than 5 mm in diameter). Larger stones are difficult to be excreted causing the blockage, pain, injury, and bleeding during urinary tract movement. However, this process takes a long time. Therefore in the process of moving through the nephron (5–7 minutes), the growth of crystals will not reach a large size which is enough to obstruct the renal tubules. The growth of the crystal is then explained by the aggregation of small crystals or the creation of secondary nuclei on the initial crystal surface.

1.1.4.2.3 Crystal aggregation

It is the process of linking small crystals together by chemical or electrostatic forces to form large crystals. For oxalate stones, the formation of urinary stones from COD crystals is difficult because COD crystals do not coalesce into stable masses, do not attach to the tubular epithelial cells and so they have easily swept away with urine. COM crystals are highly agglomerated and can bind tightly to tubular epithelial cells and be trapped facilitating the formation of stones.

1.1.4.2.4 Crystal attachment to kidney cells

The mechanism of this process is still unclear and explained by many different theories. The first theory is that stone crystals are formed in the kidney's lumen, where they aggregate, grow to a size large enough to block the renal tubules, and be trapped there. The second theory also states that stone crystals are formed in the renal tubules but suggests that crystals are formed by the development of apatite plaques and attached to a certain location at the surface of the tubular epithelial cells and structures present in the renal papillae also known as Randall's plaque. Various studies have confirmed the role of microscopic lesions in kidneys in the formation of kidney stones. These lesions were detected in causing urinary stones by a model that causes an increase of urinary oxalate.

1.1.5 Harmful effects

After forming, if the stone is small, it usually passes through the urine and is expelled. But if the stone is trapped somewhere in the urinary tract, it will grow larger obstructing the flow of urine, leading to stagnation and swelling above the blockage and causing many symptoms such as obstruction, infection, additional stones, and gradual destruction of kidney structure.

If urolithiasis is not monitored and treated promptly, it can lead to many serious complications such as urinary tract infections, kidney failure even death.

1.1.5.1 Common and dangerous complications

1.1.5.1.1 Obstruction is a severe acute complication

If ureters are completely obstructed, the renal pelvis dilates, and after 6 weeks the renal parenchyma may not recover. The consequence of water retention is structural damage leading to functional damage.

1.1.5.1.2 Acute renal failure

It may be occurred due to a severe obstruction (completely or near completely) on both sides of the ureters. Kidney failure occurs in the case of ureteral stones on one side but creating vasoconstriction on both sides causing anuria. Clinical manifestations are anuria, urea test, creatinine, a rapid increase of potassium (K+) in blood, and metabolic acidosis.

1.1.5.1.3 Chronic renal failure

Chronic pyelonephritis is the most severe consequence of kidney and urinary stones because it is no longer able to recover due to gradual renal fibrosis.

1.1.6 Symptoms

Symptoms of kidney stones can range from asymptomatic to the frequency of mild and uncomfortable urination, then severe colic in the abdomen, hips, and lower back. When a stone passes through the ureter, it can cause hematuria, severe pain, nausea, vomiting, diarrhea, sweating, and a fast heartbeat. In severe cases, kidney stones can obstruct the urinary tract, scar, kidney infections, and kidney damage.

It was identified that pain in the lumbar region is the main symptom of ureteral stones; renal colic occurs when stones move and cause edematous inflammation and acute ureteral obstruction.

1.1.7 Treatments

The choice of treatment method depends on many factors such as the size of stones, the severity of symptoms, the degree of obstruction, kidney function, the location of stones, and whether or not the infection is present.

2. Applications of medicinal plants in the management of kidney stones

2.1 Some effects of natural medicines in the treatment of kidney stones

The use of drugs which are from herbal plants in the treatment of kidney stones is increasingly common but its majority comes from folk remedies. In recent years, there have been many studies of scientists on the treatment effects of medicinal plants for kidney stones.

Proven studies have shown the action mechanism of herbal extracts on the treatment and the relapse prevention of kidney stones, including

1. Help to erode and reduce stone size naturally.

2. An alkaline urine and inhibit the process of crystallization of stones on the urinary tract.

3. Inhibit stone formation by preventing calcium oxalate nucleation and the growth of calcium oxalate crystals, inhibiting synthesis and accumulation of the crystals.

4. Reduce the deposition of crystals on tissue and in the lumen of kidneys.

5. Enhance the concentration of inhibitors of stone formation in the kidneys to increase urine citrate excretion, reduce calcium and oxalate excretion in urine.

6. Reduce the concentration of calcium in kidney tissue and improve the state of saturation.

7. Support diuretic, analgesic, and anti-inflammatory activities.

8. Balance electrolytes and minerals and regulate oxalate metabolism helping to reduce the recurrence of kidney stones.

The antioxidant activity of medicinal plants also helps to prevent urinary cell damage. Some promising plants containing vitamin E, vitamin C, and calcium showing their antioxidant and anti-urolithic activity demonstrated through *in vivo* and *in vitro* studies are discussed below.

2.1.1 Flax

Flax (*Linum usitatissimum* L) in the genus Linum, which belongs to the Linaceae family, is an annual herb with green flowers, produces different small flat seeds

ranging from yellow to reddish-brown color. The plant is 40–70 cm tall, smooth, with only branches in the upper part. Leaves are staggered, spear-shaped, 1–3 cm long, and 0.3 cm wide with three ribs. Flowers often grow individually with 5 blue petals; petals are three times longer than sepals. The capsule has smooth walls, divided into 10 cells containing one grain in each. The grain is oval, long, pointed, glossy, and brown.

Since ancient times, flax has been grown for fiber or seeds using for medicinal purposes and as nutritional products [13]. Currently, flax is grown in more than 50 countries, mainly in the Northern Hemisphere. Canada is the world's largest producer and exporter of flaxseeds [14]. Important developing countries for flaxseed include India, China, United States, and Ethiopia [15, 16]. India ranks first among the top flaxseed producing countries in terms of area, accounting for 23.8% of total production and third in production, contributing to 10.2% of world production [16]. In India, flaxseeds are mainly grown in Madhya Pradesh, Maharashtra, Chattisgarh, and Bihar. The bark of the plant has good fiber quality and high durability, so it is used to weave cloth.

Flaxseeds, also known as Linseed, have a crunchy texture and chestnut flavor [17, 18]. Seeds with oil after refining are used for edible purposes [16]. People have been consuming flaxseeds since ancient times. In India, flaxseeds are still being consumed as food and also for medicinal purposes [19]. It has an important position among oilseeds because of its versatility. It is considered an attractive nutritional food because of its exceptionally high content of alpha-linolenic acid (ALA), high-quality fiber and protein, lignans, vitamin E, phenolic compounds, and phytoestrogen.

2.1.1.1 Chemical composition

Flaxseeds contain about 55% of alpha-linolenic acid (ALA), 30% of protein and 35% of fiber [18, 20, 21]. Flaxseed oil has alpha-linolenic fatty acid, which is an essential acid (ALA). From these facts, the body will convert to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Flaxseeds are increasingly considered by nutritionists and medical researchers due to their potential health benefits associated with bioactive components, lignan-secoisolariciresinol glycoside (SDG), and dietary fiber [22]. The composition of flaxseeds is presented in **Figure 3** [17, 23, 24]. Flaxseed oil has alpha-linolenic fatty acid, which is an essential acid (ALA). From these facts, the body will convert to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

The chemical composition of flaxseed depends on its growing environment, genetics, and processing conditions [17]. The lipid content of flaxseeds varies from 37 to 45 g/100 g of seeds as reported by various scientists [17, 20, 24]. Cotyledons are the main oil storage tissues containing 75% of seed oil [16, 18, 25].

Flaxseed oil makes up 98% of triacylglycerol, phospholipids, and 0.1% of free fatty acids [26]. On average it contains 21% protein. The majority of protein is concentrated in cotyledons [21]. The main protein segments are globulin (26–58%) and albumin (20–42%). The nutritional value and amino acid content of flaxseeds are equivalent to that of soy-bean protein [27, 28].

Flaxseed protein is rich in arginine, aspartic acid, and glutamic acid, while lysine is restricted [16, 25, 29]. A high content of cysteine and methionine improves antioxidant content, thereby reducing the risk of cancer [14]. Treatment, de-heat, and defatting conditions affect protein content. Defatted and de-heat meals are high in protein [15, 30]. Flaxseed protein exhibits antifungal properties against *Alternaria solani, Candida albicans,* and *Aspergillus flavus* [31, 32].

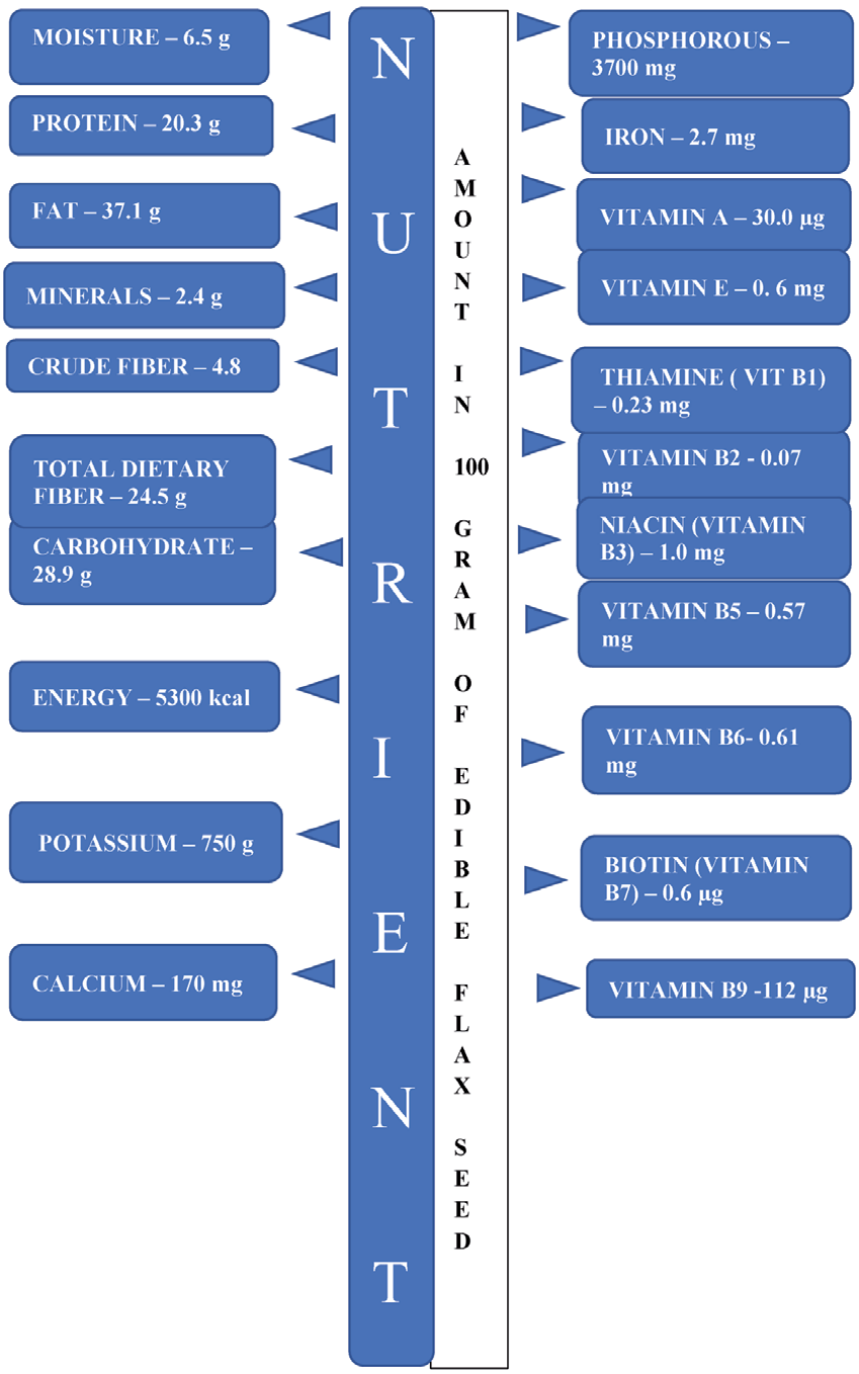

Figure 3.
Nutrient composition of flaxseed.

2.1.1.2 Pharmacological effects and uses

Scientists are interested in flaxseed for its health benefits due to its high content of α-linolenic acid and lignans. Natural treatment agents which have the effects on lipid-lowering, antioxidant, anti-inflammatory, and antihypertensive are expected to have a protective effect on the kidneys.

For kidney stones, flaxseeds may help reduce the amount of calcium in the urine. Daily intake of organic, unrefined, and cold-pressed flaxseed oil has been shown to have good results for patients with kidney stones.

William F. Clark, Anwar Parbtani (1995) studied the impact of flaxseed on immune and renal damage models. ALA in flax constituents has been shown to have anti-inflammatory and anti-thrombotic properties, while flaxseed lignans have been reported as platelet-activating factor receptor antagonists (PAF). PAF contributes to the inflammatory response in progressive glomerulonephritis. The authors conclude that results from animal and human studies indicated that flaxseeds provide significant benefits in kidney function as well as important atherogenic and inflammatory mechanisms in the pathogenesis of lupus nephritis. Thereby, the authors recommend long-term studies with flaxseeds as a potential treatment for lupus nephritis and other forms of progressive kidney diseases [33].

According to research by the University of Manitoba and the Manitoba Children's Health Institute, Winnipeg, Man., Canada (2007), late intervention with soy protein and flaxseed oil in the diet has reduced the number of diseases related to multipurpose kidney diseases which were present in the rat. The potential benefits of antioxidant and anti-inflammatory effects on kidney disease results are continuing to be studied [34].

According to the report on "medical and dietary therapies for the prevention of kidney stones" by Zeyneplrul and Manoj Monga (2014), the main source of polyunsaturated fats in the Western diet is arachidonic acid (AA) an n-6 fatty acid found in vegetable oils and animal fats. N-6 fatty acids are involved in stone formation. The breakdown of AA leads to the formation of precursors including prostaglandin E2 (PGE2) [35].

PGE2 causes hypercalciuria because it increases intestinal calcium absorption, reduces renal tubular reabsorption, and increases bone resorption. Eicosapentaenoic acid (EPA) is an n-3 fatty acid and is a component of fish oil, as well as is found in flaxseeds. EPA undergoes the metabolism similar to n-6 fatty acids. Therefore, increasing EPA and reducing n-6 fatty acid metabolites, especially PGE2. Lower PGE2 concentration not only reduces calcium excretion through urine but also leads to the activation of nephron Na/K/2Ca transporters, leading to the increase of renal calcium reabsorption. The clinical experiment showed that consuming 1,200 mg/day of flax oil is associated with a significant decrease of calcium and oxalate concentration and an increase of citrate concentration in urine.

Research Institute for Pharmaceutical Education, Narsapur, Medak, Telangana, India (2015) conducted a study to assess *in vitro* anti-inflammatory activity of flax. Ethanolic extract showed its maximum effectiveness in dissolving calcium oxalate crystals, thereby clearly showing that ethanolic extract of flaxseed was quite promising for further research on the treatment of kidney stones [36].

Several clinical studies have realized the great potential of n-3 polyunsaturated fatty acids (eicosapentaenoic acid - EPA) found in flaxseeds, which not only work against inflammatory mediators (such as prostate E2, leukotriene B4, TNF-α, interleukin, and cytokine) but are also very helpful in reducing the risk of calcium stone formation for kidney stones. According to a study by the authors of the Department of Urology, Nagoya City University of Medicine, Japan, to determine the effect of EPA on Urolithiasis, the authors conducted a clinical study in which a high-purity EPA product is provided (at a dose of 1,800 mg/day) for 88 patients with urinary stones for 3 months (short term) and 18 months (long term). The results suggest that EPA has reduced the amount of calcium in the urine which well affects the urine composition in a way that can reduce the risk of calcium stone formation.

The Japanese authors when doing clinical research "Preventive effects for the recurrence of kidney stones", implemented high-purity EPA preparation at

1,800 mg EPA/day for 29 patients in 36.4 ± 22.0 months after treatment of kidney stones. By observing the recurrence of Urolithiasis in these patients during 8 years (before, during, and after taking the drug) and studying the preventive effects for the recurrence of kidney stones, the study results showed that prevalence of nephrolithiasis (times/year) before, during and after taking EPA are respectively 0.22283, 0.0693 and 0.11742. The incidence of Nephrolithiasis while taking EPA was significantly lower than the findings before and after using ($p < 0.05$). Thus, the results suggest that EPA may reduce the risk of calcium stone formation.

2.1.2 Plantago major

Plantago major is also known as broadleaf plantain, white man's foot, or greater plantain, which belongs to the Plantaginaceae family. It is a kind of plant that is used both as food and as a medicinal plant. *Plantago major* is native to most of Europe and northern and central Asia. It is one of the most abundant and widely distributed medicinal crops in the world.

Plantago major is a herbaceous perennial plant with a rosette of leaves. Each leaf is oval-shaped. The leaf blade is 12 cm long and 8 cm broad. There are five to seven parallel veins that diverge in the wider part of the leaf. The inflorescences are borne on stalks. Flowers are always bisexual. They are small, greenish-brown with purple stamens, produced in a dense spike 5–15 cm long on top of a stem, 13–15 cm tall, and rarely to 70 cm tall. *Plantago major* propagates primarily by seeds, which are held on the long, narrow spikes which rise well above the foliage. *Plantago major* fruits are canned fruits, containing many glossy dark brown seeds. Each fruit has 8–13 seeds. The outer shell of the seed becomes mucous in water. Each plant part of *Plantago major* has been used in many traditional medicines for the treatment of cough, diarrhea, dysentery, urinary stones. The seeds of *Plantago major* have long been used in the treatment of urinary retention and urination.

2.1.2.1 Chemical composition

Plantago major leaves contain iridoid, aucubosid, catalpol), phenolic acid, and phenylpropanoid esters of glycosides, majorosides. Leaves also contain mucus with a content of 20%. *Plantago* contains mucus which is rich in D-galactose, L-arabinose, and has about 40% uronic acid, fatty oils including 9-hydroxy-cis-11-octadecenoic acid. Besides, *Plantago major* is also rich in flavonoids including apigenin, quercetin, scutellarin, baicalein, hispidulin (5,7,4′-trihydroxy-6-methoxy-flavon), luteolin –7-glucoside, luteolin-7-glucuronide, homoplantaginin (= 7- O-D-glucopyranosyl-5,6,3', 4'-trihydroxyflavon). Besides, *Plantago major* contains many other substances such as coumaric acid, ferulic acid, caffeic acid, chlorogenic acid, carotene, vitamin K and vitamin C.

2.1.2.2 Pharmacological effects and uses

In the document of medicinal plants, Prof. Dr. Do Tat Loi also mentioned the effect of increased excretion of urea, uric acid, and salt (the components that make up urinary stones in urine) of *Plantago major* seeds. This activity was confirmed again by research done at Vietnam - Sweden hospital. The scientists found that the extract of *Plantago major* seed contains the active ingredient aucubin - 1 iridoid glycoside that works to increase the amount of urine, thereby stimulating more urine excretion, helping to enhance bacterial excretion and stones out of the urinary tract for people who have a urinary tract infection or have urinary stones.

In Japanese traditional medicine, the decoction of *Plantago major* helps to treat cough, urinary tract infection, and inflammation. In Thailand, whole plants or leaves are used for the effects of diuretic, antipyretic, laxative, anti-inflammatory, and flatulence. In Korea, *Plantago major* is used to treating liver diseases. In Haiti, *Plantago major* is used to treating nerve stutter and eye pain.

Scientists at Malaysia Kebangsaan University (2003) also found that ethanol extract of whole *Plantago major* plant significantly reduces the size of calcium oxalate crystals better than allopurinol and potassium citrate drugs, at the same time inhibits newly formed stones. Malaysian scientists (2012) conducted a study to determine the inhibitory effect of *Plantago major*'s terpenoid extract on *in vitro* crystalline calcium oxalate and compared the effects of *Plantago major* with drugs which are clinically used as zyloric and potassium. The results showed that terpenoid extract of *Plantago major* inhibited the size of calcium oxalate crystals much better than zyloric and potassium citrate in the treatment of urinary stones.

According to research by Istanbul University and Kafkas University (Turkey), *Plantago major* seed extract works against most bacteria such as *Bacillus cereus*, *Staphylococcus aureus,* and especially *Escherichia coli* (E.coli)-the main agent of most urinary infections.

Research by scientists from Mashhad University of Medical Sciences, Mashhad, Iran showed that *Plantago major* extract can improve kidney function as well as oxidative stress in cisplatin-induced kidney toxicity in rats.

In the study of *Plantago major* activity for urinary tract-related infectious diseases, scientists from Kaohsiung Medical Institute, Taiwan found that *Plantago major* extract is in high concentration (> 50 micrograms/ml) can inhibit inflammatory mediators such as leukotrienes, nitric oxide, prostaglandins, etc.

2.1.3 Spinach

Spinach has the scientific name of *Basella alba* L. (*Basella rubra* L.), belongs to the family of Spinach - *Basellaceae*, also known as Malabar spinach, is an ancient tropical species, often grown as a vegetable. This plant is native to South Asian countries, spreads and grows wildly in many tropical Asian countries. It is grown in Asia, Africa, South America, and also in temperate regions of Asia and Europe. Its common distribution is in Africa, the Angels, Brazil, and Asia, Japan, China, Thailand, Laos, Cambodia, and Vietnam. In Vietnam, Malabar spinach grows wildly and is planted everywhere.

Malabar spinach is a herbaceous climbing plant with wrapped, fat and viscous, and reddish stems. Plants live annually or for two years. Leaves are staggered with raw and succulent leaf-blade. Flowers are arranged in flower form and are light purple. Fruits are oval-shaped or egg-shaped. Clump roots grow deep in the soil and are suitable for loose soil. Inflorescence in flower-shape growing in spaces between leaves which are white or pale reddish-purple. Berries of Malabar spinach are small, sphere-shaped, or egg-shaped, about 5–6 mm long. They are green and turn dark purple when being ripe. Malabar spinach grows fast. Its stem can grow up to 10 m long.

2.1.3.1 Varieties of spinach

There are 3 varieties of Malabar spinach. They are

1. White Malabar spinach (also known as Green Malabar spinach)

Leaf-blade is small with a slender stem. Malabar spinach stems and leaves are pale green. The most commonly grown species is white Malabar spinach with a small leaf blade and slender stems. Its stems and leaf are pale green.

2. Purple Malabar spinach

Purple Malabar spinach has small leaves, reddish-purple stems, and veins. This is a wrapped climbing plant. The stem is fat and viscous with a 2–3 year lifespan. Leaves of purple Malabar spinach are thick, heart-shaped, and intertwined. The inflorescence is flower-shaped and white or pale reddish-purple. Berries of purple Malabar spinach are small, sphere-shaped, or egg-shaped, about 5–6 mm long. They are green and turn dark purple when being ripe. According to some Chinese medicine documents, the whole stem of Malabar spinach has a light sweet taste, a cold property which has the effects of heat dissipation, cool blood, diuretic, detoxification, and pain relief. Leaves and young stem buds of Malabar spinach are often used to stir-fry, boil, or cook soup as a cool and laxative food.

3. Large leaf Malabar spinach

Large leaf Malabar spinach is very similar to the green Malabar spinach but has different characteristics, with a larger leaf blade, the leaves are thicker, the color is darker green and the stems are fatter.

Spinach contains high amounts of vitamin A3, vitamin B3, vitamin E, saponin, and iron which are essential for general health and well-being Spinach is a wild-growing variety so it is not picky, easy to grow and take care of and grow fast with an over 10 m length of the stem.

2.1.3.2 Chemical composition

According to the documents of the United States Department of Agriculture (USDA, 2016), 100 g of edible Malabar spinach portion contains 93 g of water; 79 kJ (19 kcal) of energy; 1.4% glucid; 2.5% fiber; 0.9% ash; 1.8 g of protein; 0.3 g of fat; 109 mg Ca; 52 mg of P; 1.2 mg of Fe; 8000 IU of vitamin A; 0.05 mg of thiamin, 0.16 mg of riboflavin; 0.50 mg of niacin; 140 mg of folate; 102 mg of ascorbic acid. Besides, spinach leaves also contain aminoglycosides, several oleanane triterpenes, including *basella* saponins, betavulgaroside I, spinacoside C, and momordins. The spinach seeds contain 2 antifungal peptides and ribosome-inactivated proteins, with antiviral activity isolated from seeds.

Spinach contains many bioactive compounds such as carbohydrates, proteins, enzymes, fats and oils, vitamins, alkaloids, quinine, terpenoids, flavonoids, carotenoids, sterols, simple phenol glycosides, tannins, saponins, polyphenols, etc. It also contains Vitamin A, Vitamin E, Vitamin K, flavonoids, saponins, and β Carotene [37].

2.1.3.3 Pharmacological effects and uses

The whole plant of Malabar spinach is used to treat dysentery, esoteric defecation, cystitis, and appendicitis. Externally used for bone fractures, injuries (outside of the body), outside hemorrhage, burns. In India, people use leaves in the treatment of gonorrhea and glansitis. Leaf fluid is used to treat urticaria and constipation, especially in children and pregnant women. In Thailand, the leaves are used to treat round spots, flowers used to treat tinea diseases, roots used for laxative effect, and externally used to treat discoloration of the skin of hands, feet, and dandruff; berries used as food dyes. In Vietnam, Malabar spinach is recorded as having cold property, sour taste, heat dissipation, blood cooling, diuretic, urinary retention, dysuria, detoxification, and pain relief.

Many scientific reports indicate that sponges can be used to treat laxatives, inflammation, rubefacient, skin diseases, burns, ulcers, diarrhea, diuretics, and cancer.

Spinach leaves contain several active ingredients including flavonoids with antioxidant and anti-inflammatory properties. Spinach extract has been shown to have numerous effects such as chemotherapy, protection of the central nervous system, anti-cancer, anti-aging functions, and hypoglycemia.

For kidney stones, the Indian Institute of Pharmaceutical Sciences and Research (2020) conducted a study to evaluate the diuretic [38] and anti-diuretic activities of ethanolic extract of Malabar spinach leaves in rats that had been induced to cause kidney stones. The study results showed that ethanolic extract from Malabar spinach leaves has a significant diuretic activity by increasing the total amount of urine and the excretion of sodium, potassium, chloride, and bicarbonate, and also has significant anti-thrombotic activity by reducing high concentrations of oxalate, calcium, and phosphate in the urine and adding calcium, creatinine and uric acid in the serum. The extract of the seed's peel of Malabar spinach has properties that enhance the reducing properties of the calcium oxalate crystals. The results obtained from the studies show the potential benefits of the extract of Malabar spinach in treating and preventing the recurrence of kidney stones.

In Ayurveda, used for hemorrhages, skin diseases, sexual weakness, ulcers, and as a laxative in children. Leaves are applied to the head for half an hour before bathing to help bring about good refreshing sleep. The sap is applied to acne eruptions to reduce inflammation. Decoction of leaves used for a mild laxative effect. Pulped leaves applied to boils and ulcers to hasten suppuration. Leaf juice mixed with butter applied to burns and scalds for a soothing and cooling effect. Leaves and stems have been used as anticancer for melanoma, leukemia, and oral cancer. Roots and leaves are used for stomach pains and increase milk production. Used orally for anal prolapse and hernia. In Nigeria, used for hypertension and also used for fertility enhancement in women. In Nepal, leaf juice is used to treat dysentery, catarrh, and applied externally to boils. In Thai traditional medicine, mucilage is used as an application for bruises, ringworm, and laboring. Stem and leaves are used as a mild laxative, diuretic and antipyretic. In Cameroon, used for malaria. Herbal healers use plant extracts to enhance libido and as a remedy for infertility. In the Antilles, leaves are considered good maturative as a cataplasm. In Thai traditional medicine, mucilage is used as a topical medicine for skin irritation, bruises, ringworm, and laboring.

A study of leaves extract of *Basella alba* showed an admirable dissolving capacity of calcium oxalate crystals in both *in vitro* and *in vivo* studies. Although Malabar spinach has a rich nutrient (1/2 cup of spinach after cooking provides 190% of vitamin A and 20% of iron which the body needs), it should not be abused. Eating too much spinach makes the body absorb less because it contains a high content of oxalic acid. This is a chemical that binds calcium and iron, making it difficult for the body to absorb other important nutrients. Therefore, when eating spinach, it is better to eat with foods that are rich in vitamin C such as oranges, lemons, tomatoes, star fruit, or consuming oxalate removed spinach is much safer and useful for preventing kidney stones. Especially eating spinach cooked with star fruit will be very good for the body. Because of the content of oxalic acid and purine, eating lots of spinach converting to uric acid will increase the concentration of calcium oxalate in urine and accumulate in the body, easily causing gout or kidney stones.

2.1.4 Fenugreek

Fenugreek, also known as *Trigonella foenum-graecum* L., belongs to the family Fabaceae. The plant is about 60–90 cm tall and green. The flowers are small and white. Its seed is small and yellow-brown. Because of its preventive and curative

properties, fenugreek is used as a herb. It is native to the Middle and Near East and is widely used in the Indian subcontinent. There is even evidence that ancient Egyptians understood the benefits of Fenugreek because its seeds were found in tombs, especially Tutankhamen. The plant is grown in countries across the globe as a semi-arid crop, but most are grown and consumed in India.

Fenugreek is used as a medicinal plant (leaves) as well as a spice (seeds). Parts that are used as a medicine of Fenugreek include stems, leaves, and seeds. Fenugreek seeds are yellow or amber and generally used for dipping food, dried and pasty curry powders, commonly found in Indian cuisine. Young leaves and buds of fenugreek are also used as a vegetable, while fresh or dried leaves are used to flavor other dishes.

2.1.4.1 Chemical composition

According to the study documents, fenugreek seeds contain 26.2% of protein, 5.8% of lipids, 3% of minerals including iron, calcium, phosphorus, magnesium, potassium, sodium, zinc, copper, manganese, vitamin C, folic acid, vitamins E, B1, B2, B3, 3% of fiber and 44.2% of powdered sugar. Besides, fenugreek seeds contain saponins, oil substances, flavonoids and mucus, 4-hydroxy isoleucine (amino acids that stimulate insulin secretion), and galactomannan that slow down the absorption of blood glucose.

According to a study in 2014 published in the Journal of Nutrition, fenugreek contains soluble fiber, which can reduce the glucose absorption of cells, helping to low down blood sugar. Besides, it also contains trigonelline a compound that increases insulin sensitivity, and 4-hydroxy isoleucine -an amino acid that helps stimulate insulin release in pancreatic cells, helping to control blood sugar levels automatically.

Fenugreek contains polyphenols and flavonoids that have antioxidant effects, which reduce the amount of cholesterol and triglycerides in the body. The galactomannan forms a layer of mucus in the intestines limiting the absorption of lipids and glucose.

2.1.4.2 Pharmacological effects and uses

Fenugreek seeds are commonly used in Northern Africa to prevent and treat kidney stones. In a study on an animal, it was found that fenugreek seeds significantly reduce kidney calcification and the total calcium content of kidney tissue in rats, helping to prevent kidney stones.

According to a study of King Saud University, Riyadh, Saudi Arabia on the effects of fenugreek seeds and *Ammi majus* [39] on calcium oxalate urinary stones in rats, when treated daily by oral route with fenugreek extract, it significantly reduced the amount of calcium oxalate deposited in the kidneys while the inhibitory effects obtained from *Ammi majus* grass were negligible.

In 2014, the Science University of Salahaddin, Kurdistan - Iraq region conducted a study on the effects of fenugreek in preventing the formation of kidney stones. The study result has proven its potential effects of antioxidants and Urolithiasis prevention, thus making a beneficial effect to prevent the formation of kidney stones and related free-radical complications in kidney tissues.

According to a study result on the effects of some medicinal plants used in the treatment of urinary stones of Abulcocation-Rabat University, Morocco, the extract of fenugreek seed has a good effect on dissolving cystine and carbapatite stones, probably due to the complex formation between stones and polyphenols or flavonoids in the extract [40].

3. Role of vitamins and minerals in the management of calciphytoliths

3.1 Calcium oxalate in plants

Calcium oxalate deposits in numerous plants and animal cells. Neither it excretes in urine nor retained in the form of urinary calculi. Calcium oxalate in plant sources was first described by "Leeuwenhoek" using a Simple microscope. Oxalate ranges in plants from 3 to 80%. Non-accumulating oxalate plants have less oxalic acid in them. In plants, oxalic acid is obtained from glycolate conversion. Oxidation takes place where glycolic acid is oxidase and glyoxylic acid is intermediate. Glyoxylic acid as intermediate is obtained by cleavage of isocitric acid and oxaloacetate separates in attaining oxalate and acetate.

It is present in numerous parts of plants and is considered the strongest acid present in plants. Oxalic acid as a chelating agent reacts with cations and results in the end product being oxalates. Oxalic acid is considered as the end product but in some cases, it converts to oxalate by several conditions as the alternate change in oxalate concentration. Oxalic acid acts as an "ionic balance" in plants and the formation of soluble or insoluble compounds. Oxalate in plants promotes antioxidant property. Content of oxalate more in plants results in attaining uncomfort taste but promotes plant protection from insects and animals and oxalic acid content fruits have high superoxide dismutase activity. This plays a major role in systematic resistance, programmed cell death, redox homeostasis, and anti-senescence effect in harvested fruits.

Calcium oxalate crystals form by oxalic acid interiorly present in plants and calcium obtained from the environment. Calcium oxalate occurs in many plant regions except pollen. The highest oxalic acid concentration commonly occurs in leaves and it is lowest in all other parts. It exerts its effects by binding calcium, magnesium, and other trace minerals like iron making them unavailable for assimilation. The calcium ions bind with free oxalic acid or oxalate and precipitates as insoluble crystals of calcium oxalate which may lead to hypocalcemia and urolithiasis. In the human being, <0.5% soluble oxalate in a diet may be acceptable. Plants accumulate oxalate in high proportion only during the young stage of growth and the content decreases with maturity and drying of the plant. Matured plant organs of the selected plants were discussed here which contain considerable calcium and very low amounts of soluble oxalate and also were used traditionally in treating kidney stones.

Generally oxalic acid is present in many plant families but commonly occurs in Amaranthaceae, Polygonaceae, Chenopodiaceae, Oxiladaceae, Convolvulaceae, etc. General natural products that contain oxalic acids are Sugarbeet, Spinach, Saltbush, Goosefoot, Buckwheat, Rhubarb, Mangold, Green cabbages, Tea, Chocolate, Almonds, White beans, Soyabean, Sweet potato, Ipomoea, Okra, Cocoa, Drumstick leaves, Coriander leaves, Radish leaves, etc.

3.2 Vitamins and minerals

Oxalic acid easily combines with cations to form oxalate crystals which are then excreted in urine as minute crystals. So calcium supplements given along with foods containing oxalic acid can cause calcium oxalate to precipitate out in the gut and reduce the levels of oxalate absorbed by the body by 97%. Calcium supplement like milk also has characteristics of digestive and metabolic utilization of minerals such as phosphorus, magnesium, and iron. It's calcium enrichment does not interfere in the bioavailability of the minerals but interferes with iron. So iron may not be utilized when milk is used which is an important aspect in the management of kidney stones.

A low oxalate diet is recommended for the prevention of CaOx stones, however, a recent study proved dietary oxalate had little effect on urinary oxalate excretion although vitamin C was highly correlated with urinary oxalate excretion. But high vitamin C intake can be a risk for stone formation by increasing endogenous oxalate.

A high vitamin D and protein content regimen increase hypercalciuria which lowers the pH of urine and increases uric acid level, which increases kidney stone. So consumption of deproteinized drugs is much better. All the plants discussed here contain considerable amounts of vitamin E but do not contain much vitamin D and anti-nutrient like phytates. The absorption of mineral nutrients is adversely affected by the presence of inhibitors like phytates. It is a higher calcium diet (1200 mg/day) associated with lower kidney stone formation because the higher calcium intake will bind oxalate in the gut. So calcium can bind with dietary oxalate and thus it is not absorbed. Potassium will improve hyperoxaluria and also it is a good source in the control of diuretic and hypertensive implications. Magnesium also forms a complex with oxalate and decreases oxalate in the urine, which can reduce the risk of stone formation. Hypomagnesium is not a risk factor for stone formation. Magnesium also binds with oxalate in gastrointestinal tract to reduce oxalate absorption. Citrate supplementation is one of the effective pharmacological options for preventing the recurrence of kidney stones. Calcium oxalate or calcium phosphate crystallization is antagonized by the citrate in the urine due to which recurrence of kidney stone is prevented by increased urinary citrate excretion. Potassium citrate along with thiazides are prescribed for kidney stone treatment at the same time it impaired by poor long term compliance, gastrointestinal upset and unpalatable taste. The remedy for this issue is taking potassium citrate with magnesium citrate combination. Magnesium can bind with oxalate due to which both diminishing and absorption of oxalate takes place thereby acting as a stone preventive. The binding of oxalate in urine is reduced by the binding of magnesium which results in the decrease in the recurrence of kidney stones. Patients who have diabetes, hypertension, and high blood cholesterol are often instructed to consume high oxalate foods such as fruits and vegetables. In this case, it is better to consume less and only selected fruits and vegetables to prevent kidney stones. The vitamins and mineral composition of the selected plants were given in **Figures 4** and **5** [41–56]. The plants discussed here contain all the nutrients in considerable quantities required for preventing and managing calculi forming oxalates.

3.3 Impact of vitamin E in the management of disease and calciphytoliths

3.3.1 Renal insufficiency due to vitamin E deficiciency

In rats, a combination of fat-soluble vitamin deficiency and Se or glutathione deficiency causes extreme and progressive aerophilic damage to the urinary organ's structure and function [57–59]. Furthermore, urinary organ dysfunction caused by ischemia–reperfusion damage causes 50% mortality in dietary E- and selenium-deficient rats compared to controls [60], and a-tocopherol administration increases the fatality rate by 46% after 120 minutes of heat anemia [61]. During a marked increase in the creatinine/molar creatol magnitude relationship, aerophilic urinary organ injury in nutrients E-deficient rats is depicted.

Conversion is directly mediated by chemical group radicals [62]. The living nephrons enhanced the absorption of the element and the square oxide levels multiplied within the residual cortex of rats. Dietary fat-soluble vitamin administration 11 to 16 weeks after urinary tubule reduction has been shown to attenuate glomerulosclerosis by more than 500 weeks [63]. The fat-soluble vitamin deficiencies are never seen in humans and have been delineated by sensory neurological disorders.

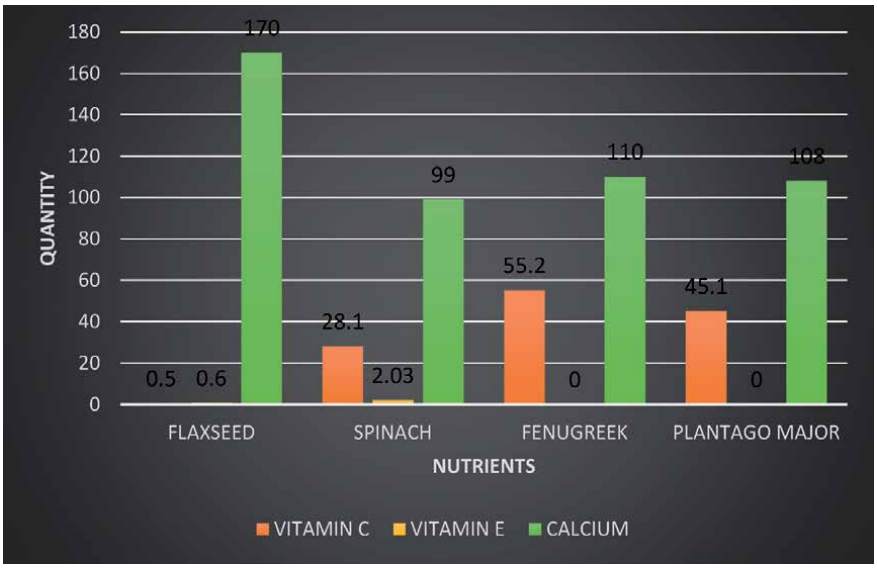

Figure 4.
Vitamins and calcium content in the selected plants (mg/100 g).

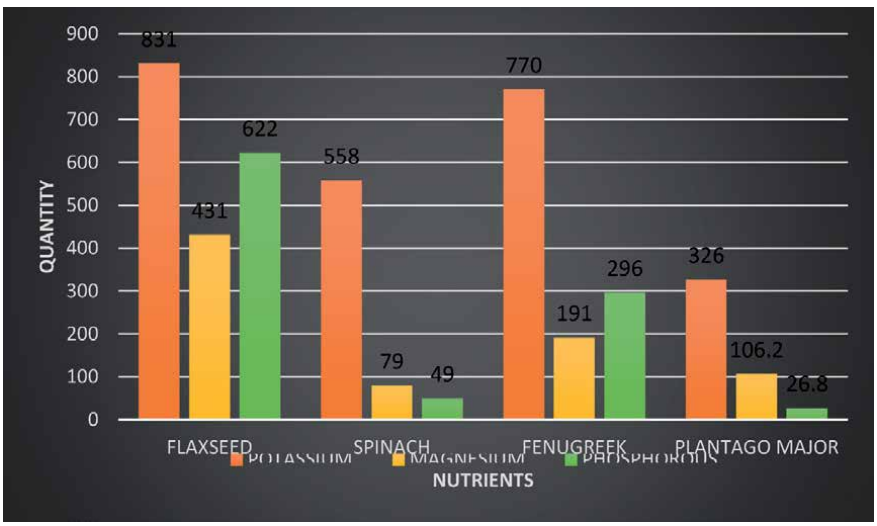

Figure 5.
Mineral content in the selected plants (mg/100 g).

It is due to diseases that hinder the streamlined absorption of fat (such as chronic disorders or abetalipoproteinemia) or, in rare highly contagious conditions, Deficit in the family of confined fat-soluble vitamins (FIVE) due to mutations in the a-tocopherol enzyme that export fat-soluble vitamins from the liver to blood [64]. If patients with permanent and high fat-soluble vitamin deficiency have urinary organ dysfunction, this is not recognized.

3.3.2 Inflammatory kidney disease

Immune globulin nephrosis (formerly known as Berger's disease) is now confirmed to be the end-stage urinary organ dysfunction in twenty-five percent of affected patients over a 25-year follow-up period [65], despite being initially

thought to be mild. It is the most common form of nephrosis on the planet. The mechanism of harm is unknown, but related medical specialty insults to the kidneys seem to be introduced as a result of immune globulin accumulation, which induces chronic oxidative stress. There are currently no lucky drugs available that may bleed down the path of the disorder, with the exception of dominant cardiovascular disease and decreased dietary macromolecule use.

The associated experimental model of early immune globulin nephrosis in rats, mild oxidant-mediated inflammation and associated structure–functional changes in glomeruli have been shown to attenuate dietary fat-soluble vitamin levels from thirty to one hundred IU/kg of a biological attack, leading to a five-fold increase in humor-soluble vitamins. Nutrition E-supplemented rats had a four-fold reduced rate of symptom, the five-hundredth decline in albuminuria, eluted urinary organ tissue lipoid peroxidation, Stabilized urinary organ plasma flow, decreased expression of fibrinogenic protein transmission protein b1 (TGFb1), and had less extreme capillary hypertrophy than control animals [66]. During the additional analysis, supported by these initial observations, Chan et al. [67] indisputably argued that a two-and-a-half to five-fold increase in fat-soluble vitamin chow supplements (i.e. 250–500 IU/kg) may further reduce plasma and urinary organ lipoid peroxidation and albuminuria, but no further change in TGFb1 RNA has been identified. The use of fat-soluble vitamin 800 IU/day is expected to be useful in patients with immune globulin nephrosis and albuminuria but one g/day and a long-term regulated, double-blind trial are currently underway to explore this risk. Twenty-nine urinary organ response injuries may also be the product of advanced immune deposition directed to the capillary basement membrane. Anti-glomerular basement membrane (anti-GBM) nephrosis is concretely caused by anti-GBM antibodies in rats. 2 stages of capillary injury square measure are obvious. The first acute (heterologous) strategy involves the attachment of antibodies to the basement membrane amid neutrophils invasion and chronic capillary inflammation. Capillary vascular square measures blocked by microthrombus that reduces the rate of filtration and plasma flow [68].

The second delayed reaction is due to the target host (autologous phase) which ends up with symptomatic, multiplied urinary macromolecule excretion, capillary cardiovascular disease, capillary wall integrity defects, protein chemical action, and macrophages aggression. Convergence of urinary organ activity and improved maintenance of the capillary structure is detected in nephrosis-controlled rats with 5 mg/100 g fat-soluble vitamin weight 3 days before management of anti-GBM protein rather than in untreated controls [69]. The amendments were marked within the capillary filtration quantity, While chronic inflammation of immune globulin {nephropathy|renal disease|nephrosis|uropathy} and anti-GBM nephrosis is not the first event of the diseased part, it is crucial for aerophilic urinary organ injury seen in these situations. Fat-soluble vitamin decreases this injury by combining its medicinal and anti-coagulant effects and its ability to strengthen membranes. Potential for therapeutic action with fat-soluble vitamins may also arise during a host of alternative urinary organ abnormalities create through medical specialty insults and chronic inflammation.

Insufficiency and extreme albuminuria are two common examples. Fx1A [70, 71] PHN coupled with lipoid peroxidation was discovered in Passive Heymann nephrosis (PHN), a gnawing animal model for human membranous nephrosis resulting from sub-animal tissue immune absorption in capillary walls following injection of heterologous protein guided against a crude, autologous annular material. Wegener's granulomatosis, for example, is caused by the expansion of anti-neutrophil living antibodies (ANCAs) against proteins expressed on the surface of activated polymorph nuclear neutrophils. Subsequent degranulation releases toxic cell-degrading enzymes, oxygen-free radicals, and pro-inflammatory substances, a method that coincides with necrotizing crescentic capillary nephrosis.

3.3.3 Aging kidneys

The age-related improvements in the excretory organ function and the anatomy area are correlated with lipid peroxidation and oxidative stress. Excretory organ aging is characterized by gradual glomerulosclerosis, capillary filtration, and constriction. Old (13-month old) rats on an impact diet containing fifty IU/kg E have a three-fold increase in F2 isoprostane agent, a 60-percent decrease in capillary vessel filtration, and an increase in advanced glycosylation end product (AGE) compared to young animals (3–4 months of age) on a basic diet. Supplementing the old with a high E (5000 IU/kg) diet for an additional nine months, the liquid body substance level of the inhibitor increased by thirty percent and specifically improved the capillary vascular filtration rate by fifty percent, suppressed the initiation of F2 isoprostanes and amplified each glomerulosclerosis and AGE receptor expression, as well as decreasing the activation of excreting. It has been envisaged that a 40% decline in albuminuria seen in aged nutrients E-treated animals at twenty-two months compared to old controls may well be related to higher retention of capillary vessel permeability, a parameter that reduces with age [72].

3.3.4 Oxidative stress in kidney failure

There is a significant amount of evidence to support the involvement of oxidative stress in progressive urinary organ impairment. Mononucleate leukocytes in patients with chronic renal disease (CRF) area are additionally prone to lipid peroxidation of the membrane compared to those confined to healthy controls [73] and therefore red cells in patients with E-depleted quality area unit nutrient analysis [74–77]. Bochev et al. [78] stated that the development of nephropathy from chronic insufficiency to discontinued azotemia in the qualitative analysis was related to a lower blood inhibitor capacity; An increase in polymorphic nuclear blood cells due to aerophilic activity was correlated with an abnormally high level of lipid peroxidation. Penchant et al. [79] reported that in patients with advanced CRF, a very low macromolecule diet (0.3 g/kg per day) accompanied by essential amino acids and keto acids and vitamins, as well as E (a-tocopheryl acetate, 5 mg/day), A (7.5 mg/day) and A (7.5 mg/day) was found. Sixty-one of the E levels in this patient cluster ranged from thirty-eighth to fifty-six of the management values [79] suggesting that although the sweetening was achieved, and a key red blood cell E deficiency persisted. Accumulated lipid peroxidation of red cell membranes and associated E deficiency contribute to their shortened half-life in circulation and the next anemia associated with pre-dialysis pathology seen in CRF. Thanks to dietary restrictions, CRF patients are also deficient in inhibitor vitamins and micronutrients. This is often markedly true for immediate post-hemodialysis in that the full inhibitor capacity falls from a pre-dialysis level of 1.54 to 1.38 mmol/L post-dialysis [80]. These rapid, degraded, standing inhibitor episodes could lead to windows that are usually vulnerable to general lipid peroxidation and subsequent aerophilic changes in transmitted lipoproteins, atherogenicity, and therefore distinctive upset and high blood pressure associated with CRF. In healthy plasma, salt and albumen account for seventy-five percent of full inhibitor activity in vitro, while ascorbate (vitamin C) and E account for 100 percent of full inhibitory action (a-tocopherol) [81].

Total post-dialysis inhibitors fall significantly, leading to a loss of soluble ascorbate and salt. Ha et al. [80] advised that water-soluble vitamin supplements up to one g/day should be routinely administered in qualitative analysis to reduce this temporary lack of soluble inhibitor capability. Although E is the major lipid-soluble inhibitor in plasma, the amount of nutritional inhibitor carotenoids (lutein, lycopene, a-and b-carotene) is also crucial for the effectiveness of lipoproteins from

aerophilic changes, and there are also inequalities within the traditional level of this category of antioxidants throughout CRF. Of these compounds, carotenoid is the most impacted at physiological levels and its plasma concentration is significantly reduced in post-dialysis CRF patients (0.17 mmol/L) compared to healthy controls (0.44 mmol/L). The carotenoid deficiencies may additionally contribute to the associated obstructed plasma defense inhibitor system and make up one for dietary therapeutic intervention in addition to E.

3.3.5 Effective dose of vitamin E in renal failure treatment

Normalizing the standing inhibitor of CRF patients with dietary E victimization would be a cost-effective and straightforward therapeutic target. In healthy individuals, the common daily allowance of E is 8 mg (12 IU) for girls and 10 mg (15 IU) for men (1 mg = 1.5 IU) supported common levels needed to prevent symptoms of deficiency [82]. The Plasma pool-turnover is fast (1.4 ± 0.6 pools/day) [83] and therefore the traditional transmitted plasma varies among eleven. 5 and 35.0 m [84] and giant oral doses (1500–3200 IU/day) occur together to be safe, with minor and well-tolerated duct appearance effects [85] although current levels will only be increased by 2 to 4 times the standard quantity. (The higher limit is limited by the viscus E enzyme, which preserves the plasma levels within the slime, with excess tocopherols being excreted in the digestive juice.) [86]. Vitamin E has been tested in humans with some success in the treatment of system diseases involving oxygen-free radicals and aerophilic stress in their clinical expression, as well as Friedreich's neurological disease (400 IU/day) [87]. Alzheimer's disease (2000 IU/day) [88]. Parkinson's disease (3200 IU/day) [89]. the first stages of Huntington's disease (3000 IU/day) [90] and dyskinesia (800–160 IU/day) [91–93].

The best dose of E for preventing human failure is also between 300 and 700 IU per day. (This is a healthy 'therapeutic variance,' meaning that the nutrient levels needed to prevent long-term muscular disease caused by aerophilic stress are below the edge for aspect effects.) Suha et al. [77] discovered that giving chronic dialysis patients 300 mg/day (450 IU/day) E for one month resulted in critical dialysis. Low doses of E, such as 100 mg/day (150 IU/day), have been shown to provide critical defense against the risk of upset, which is a major complication of CRF [94]. Dietary E supplements that boost plasma levels could be beneficial for sluggish people. E medical care may also be cost-effective in alleviating chronic kidney disease and standing pathophysiology.

Impaired plasma inhibitor arms are characterized by chronic kidney disease and standing pathology. Besides, E medical care is considered to be a method of correcting the position of plasma antioxidants and attenuating disorders related to kidney disease. In conclusion, the mixture of E and antioxidants protects against HLP-induced salt uropathy. Calculus disease has been known to be the leading disease of life in every human and animal. Calculus could destroy the excretory organ of hollow epithelial tissue, leading to impaired excretory organ function. Aerophilic stress in chronic inflammatory conditions, anti-glomerular basement membrane kidney disease, focal segmental glomerulosclerosis, rhabdomyolysis (myoglobin acute excretory organ failure), diabetic uropathy, and toxic poisoning compounds such as transition metals, weed killers, and medicines such as cyclosporine A and cisplatin all aggravate the progression to failure. E inhibitor membrane (5–007-tocopherol) is considered to be the possible therapeutic intervention that will make it easier to weigh reduce the level of decrease in excretory organ activity under these conditions. The impaired plasma inhibitor arm is indicative of chronic kidney disease and standing pathology. E medical care is also thought of as a method of correcting the status of plasma antioxidants.

4. Discussion and conclusion

Kidney stone disease has been identified together as a major disease in every human and animal. Calculus (stone) may harm the excretory hollow epithelial tissue of the organ, resulting in the impaired excretory activity of the organ. The most important of human excretory organ stones is the metallic element salt, which relates to the metallic element and the metabolism of salt in the tissue and urinary tract system [95–97]. CaOx stone also had a high incidence and recurrence rate in animals and iatrogenic aerobic stress [98]. Several previous studies have shown that increased aerobic stress has been associated with the formation of CaOx crystal and stone. As a result, intrusion with aerobic damage could also play a key role in stopping the continuous formation of stones and the resulting excretory organ pathology. Antioxidant supplement (Vit E) reduces metabolic stress, salt excretion, and crystal formation of CaOx in hyperoxaluric rats receiving antifreeze (EG) [99]. In hypertensive and hyperoxaluric patients, oral supplementation of Vit E at four hundred mg/day for 9 months may normalize the organic chemistry and kinetic properties of Tamm-Horsfall supermolecules that impede CaOx crystal aggregation [100].

Also, overproduction of antioxidants avoided metallic element salt accumulation by preventing peroxidant injury and preserving excretory organ tissue antioxidants and glutathione oxidation–reduction balance. Antioxidant medical aid may therefore protect against the accumulation of metallic element salt stones within the human circulatory organ [101].

It is reportedly stated that oxygen-free radicals square measure essential for the pathology of multiple chronic disorders as well as renal failure. Enhanced element radical generation and/or compromised inhibitor weaponry leads to chronic oxidative stress that markedly worsens several things. Three excellent concerns were jointly endorsed by the likelihood of delaying the regression to kidney disease through inhibitor action. Restoration of capillary membrane integrity is important for urinary organ activity, and the biomembrane square measure is protected from aerophilic deterioration by the fat-soluble vitamin lipotropic inhibitor, primarily within the species of a-tocopherol [102].

In summary, although the use of herbal medicine in the treatment and prevention of urinary stones recurrence has been proven and promising, further studies are needed to understand disease physiology, the action mechanism of herbal medicine to develop an effective and safe lithophytic agent. At the same time, it is necessary to identify the mechanism of action for the discussed vitamins containing plants, thereby assessing the dosage, controlling herbal quality, and investigating their interactions and side effects. Vitamin E plants may completely prevent deposition of oxalate by preventing pre-oxidation injury and restoring renal tissue antioxidants. So vitamin E therapy also might protect against oxalate calculi deposition in the human kidneys.

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Section 2

General Information on Vitamin E

Chapter 8

Vitamin E: Natural Antioxidant in the Mediterranean Diet

Samia Ben Mansour-Gueddes and Dhouha Saidana-Naija

Abstract

Oxidation has been related to several diseases in humans. Indeed, to protect the body from high free radical damages, organism requires natural resources of antioxidant compounds, such as phenols, tocopherols (α, β, γ, and σ) which have important roles in the cell antioxidant defense system. In Mediterranean areas, olive oils and pepper fruits are considered among the best foods in a diet, which keeps on attracting the interest of scientists due to the health benefits linked with its consumption. The Olive oil and pepper fruits are among the most consumed nutrients in the Mediterranean diet; their richness in naturally powerful antioxidants, such as alpha-tocopherols, polyphenols, carotenoïds, and capsaicinoïds (specific of capsicum species), and monounsaturated fatty acids in olive and seed pepper oils, constitutes good health protection against oxidative damages and inflammation. Also, these phytochemicals shield and prevent the human body from many diseases such as cardiovascular, coronary, Alzheimer's diseases, and cancers.

Keywords: tocopherols, antioxidants, *Olea europaea*, *Capsicum* sp., fruits, oils, Mediterranean areas

1. Introduction

In recent years, oxidation constitutes a major problem in human health. Oxygen is considered a vital element, but its instability has deleterious effects on the human body. At high concentration, free radicals cannot gradually be destroyed, their accumulation in the organism generate oxidative stress. This process can damage all cell structures as lipids, proteins, and DNA and trigger many human diseases, such as cancer, arteriosclerosis, and rheumatoid arthritis. Moreover, it may play a role in neurodegenerative diseases and aging processes [1]. Hence, to protect human organisms against reactive oxygen species (ROS) a request for external nutritional intake rich in antioxidants can assist in coping with this oxidative stress. Many studies accorded that dietary vitamin antioxidants and polyphenols have been explored extensively as an exogenous mechanism of defense against oxidative stress [1, 2].

The antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a fairly 'stable radical'. The human body produces an insufficient level of antioxidants which are essential for preventing oxidative stress. To protect against oxidant radicals, organism requires natural resources of antioxidant compounds from nutrients of different origins. These bioactive molecules play an important role in helping endogenous antioxidants for the neutralization of free radicals. Nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies [3].

It has been demonstrated that many vegetables, fruits, medicinal plants, and other foods contain compounds with bioactivity against oxidative stress. This activity has been attributed to vitamin C, vitamin E, α-tocopherol, β-carotene, and polyphenolic compounds [4, 5]. Therefore, research regarding natural antioxidants from foods and plants, particularly from folk medicinal plants, is receiving increasing attention around the world.

In Mediterranean areas, nutrition is specific to each country. The Mediterranean diet takes into account the various religious and cultural traditions, as well as the various national identities, the current needs of Mediterranean populations, respecting regional dietary variations. The Mediterranean model, qualified as a healthy lifestyle [6]. It is considered as a model of sustainable nutrition [7] due to its richness in vegetables, fruits, in a quantity moderate fish, dairy, and meat products, condiments, and spices [8]. In fact, in Mediterranean countries, especially in North Africa, as Tunisia, the diet is based on olive oils, olive derivatives, cereals, Solanaceae species (pepper, tomatoes, potatoes), green vegetables, legumes, fresh and dried fruits. These sources of aliment are rich in macro and micro-nutrient such as fibers, antioxidants (vitamins, polyphenols, carotenoids), oligo-elements. In general, the Mediterranean diet is low in animal fat and fast sugar, but it is rich in fiber, omega 3, and antioxidants. The abundance of fresh fruits and vegetables and the use of olive oil instead of hard fats are key factors for which the Mediterranean diets are renowned. So many researches showed that Mediterranean eating habits appeared to meet all the criteria for a healthy and prudent diet [6, 9, 10].

In addition to macronutrients, humans need vitamins and minerals which are micronutrients required by the body to carry out a range of normal functions. However, these micronutrients are not produced in our bodies and must be derived from the food we eat. Vitamins, such as Vitamin A, B, C, E... are crucial for normal development. These micronutrients protect the organism against many diseases by their antioxidant property. In this context, this work aims to evaluate the richness in antioxidants, especially vitamin E, of the main Mediterranean nutriment olive oils, and pepper and their role in preventing many diseases.

2. Properties of vitamin E

2.1 Chemical properties

Vitamin E is composed of eight naturally isoforms, four tocopherols with the same molecular formula C28H48O2 (α-, β-, γ-, and σ-tocopherols), and four tocotrienols with a molecular formula C26H38O2 (α-, β-, γ-, and σ-tocotrienols) (**Figure 1**). These molecules are synthesized by photosynthetic organisms including plants, algae, and cyanobacteria, from homogentisic acid and phytyl-diphosphate or farnesyl-diphosphate reaction in plastid membranes [11]. All homologs are derivatives of 6-chromanol and differ in the number and position of methyl groups on the ring structure. The four tocopherol homologs have a saturated 16-carbon phytyl side chain. Whereas, the tocotrienols homologs have three double bonds on the side chain. The tocopherols and tocotrienols have the same basic chemical structure; the main difference is in the saturation of the aliphatic side chain attached to the chromanol ring [12, 13] (**Figure 1**). The various isoforms are not interchangeable and only α-tocopherol meets the human vitamin E requirements [14].

The reaction of aromatic chromanol ring with phytyl-diphosphate or farnesyl-diphosphate, in plastid membranes, synthesize different isomers of tocopherol or tocotrienol.

Vitamin E is a fat-soluble vitamin that exists in different chemical forms. The most active compound is alpha-tocopherol which existed in natural and synthetic forms.

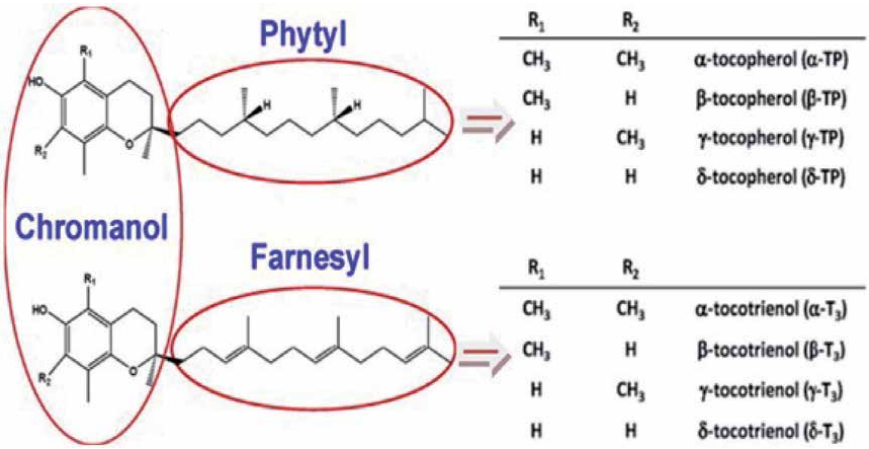

Figure 1.
Chemical structure of different isoforms of vitamin E [13].

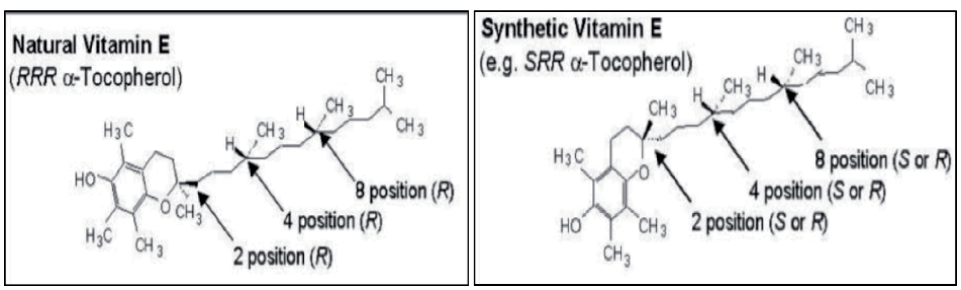

Figure 2.
Natural and synthetic Sterio-isoforms of vitamin E [14].

The natural vitamin E (RRR-α-tocopherol or D-α-tocopherol) consists of a single stereoisomer. While, synthetic vitamin E is a mixture of eight stereoisomers (RRR, RSR, RSS, RRS, SRR, SRS, SSR, SSS) distributed equally (**Figure 2**). Only one of them (1/8th) has a molecular structure identical to that of the natural vitamin. According to many studies, the natural vitamin E is twice as powerful and fixed twice as good as the synthetic version. This means that natural vitamin E reaches the blood and organs at least twice as good as synthetic ones [13, 14].

The stereoisomer S and R are the spatial arrangements of the alpha-tocopherol; The RRR refers to R at the 2,4 and 8 positions, hence RRR-α-tocopherol which correspond to Natural vitamin E. The synthetic Vitamin E agree to eight stereoisomers S and/or R at positions 2, 4 and 8.

2.2 Biological properties of vitamin E in the human body

Vitamin E is considered a natural phytochemical that is frequently associated with human health [15]. This vitamin is an example of a phenolic antioxidant; it serves as an antioxidant and protects membrane lipids from oxidative degeneration [16]. Therefore, both lipophilicity and membrane localization of vitamin E explain its antioxidant property. In this context, the incorporation of vitamin E into the cell membrane explained their major biologic role to protect polyunsaturated fatty acids (PUFAs) and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Via their localization, within the phospholipid bilayer of cell membranes; It is particularly effective in preventing lipid peroxidation, a series of chemical reactions involving the oxidative deterioration of PUFAs [14].

Vitamin E under the term α-tocopherol is a powerful biological antioxidant. It is the major lipid-soluble component in the cell antioxidant defense system and is exclusively obtained from the diet. Among the eight isomers, The RRR-α-tocopherol is the most isoform of vitamin E that is essential for humans and is preferentially retained within the organism [14, 17, 18]. This is explained in part by the specific selection of RRR-α-tocopherol by the α-tocopherol transfer protein and in part by its low rate of degradation and elimination compared with the other vitamers, especially tocotrienols, which are rapidly metabolized and excreted similarly as other xenobiotics [14]. Also, [19] mentioned that γ-Tocopherol is slightly less efficient than α-tocopherol as a scavenger of oxygen radicals, but it is an efficient scavenger of reactive nitrogen species due to the unsubstituted 5-position on the chromanol ring. This isomer of Tocopherol is present in significant amounts in the human diet especially in several widely consumed vegetable oils [14].

This form is considered the most important fat-soluble antioxidant in humans metabolizing peroxyl radicals [19]. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, which then become unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalized into the aromatic ring structure thereby increasing its stability [14].

The potent lipid-soluble antioxidant property of α-tocopherol is to maintain the integrity of long-chain polyunsaturated fatty acids in the membranes of cells and thus maintain their bioactivity [20]. The α-tocopherol protects the peroxidation of unsaturated fatty acids of the cell membrane. When peroxyl radicals ($ROO^{\bullet}$) are formed, these react 1000-times faster with vitamin E (Vit E-OH) than with polyunsaturated fatty acids (PUFA: ROOH) [21]. The hydroxyl (OH) group in the chromanol head of α-tocopherol can donate hydrogen to scavenge lipid peroxyl radicals ($ROO^{\bullet}$) generated from the peroxidation of the lipids to form the corresponding lipid hydroperoxide and the tocopheryl radical (Vit E-$O^{\bullet}$). The tocopheryl radical (Vit E-$O^{\bullet}$) reacts with vitamin C, thereby oxidizing the latter and returning

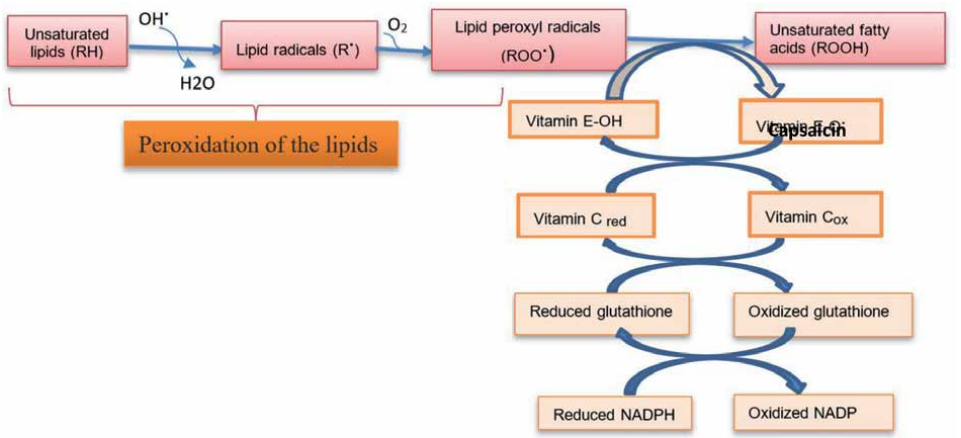

Figure 3.
The antioxidant property of Vitamin E and its regeneration by other antioxidants "Vitamin E recycling". Vitamin E-OH: alpha-tocopherol; Vitamin E-O.: tocopheryl radical; NADP: nicotinamide adenine diphosphate; NADPH: reduced NADP. The peroxidation of unsaturated lipids leads to forming lipid peroxyl radicals (ROO·). α-tocopherol easily diffuses into cell membranes, due to its lipophilic nature, and scavenges rapidly, with a hydroxyl group, the lipid peroxyl radicals and protects polyunsaturated fatty acids from lipid peroxidation. The redox reaction between tocopherol and harmful lipid peroxide radicals leads to forming neutral lipid hydroperoxide and an unreactive vitamin E radical (Vitamin E-O.). The presence of other antioxidants, such as vitamin C, is required to regenerate the antioxidant capacity of α-tocopherol.

vitamin E to its reduced state. The presence of other antioxidants such as vitamin C is required to regenerate the antioxidant capacity of α-tocopherol (**Figure 3**).

The interaction of vitamins E and C has led to the idea of "vitamin E recycling", where the antioxidant function of oxidized vitamin E is continuously restored by other antioxidants (**Figure 3**).

3. Role of vitamin E against diseases

The Mediterranean diet (MedDi) is characterized by a high content of bioactive phytochemicals especially the antioxidants such as polyphenols, carotenoids, vitamins C and E which are key components of many plant foods. These bioactive compounds in (MedDi) have a particular interest in the prevention of many diseases such as cancer, cardiovascular diseases, etc. [22]. Also, the major dietary sources of vitamin E are fruits, vegetables, nuts, and oils. Vitamin E is known to inhibit lipid peroxidation eventually protecting DNA from damage involved in the pathogenesis of cancer [23].

Antioxidants from our diet play an important role in helping endogenous antioxidants for neutralizing free radical species. Thus, free radicals are involved in some diseases including tumor inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, neurodegenerative diseases, etc. [24, 25].

By its natural antioxidant property, vitamin E plays a key role in maintaining health and preventing many chronic and degenerative diseases [26]. In fact, vitamin E could help avoid or delay coronary heart disease, it could also prevent atherosclerosis by inhibits or reduces the oxidation of low-density lipoprotein (LDL) cholesterol which is associated with the development of atherosclerosis [27]. Also, the supplement of this nutrient plays a cardioprotective role and decreases cardiovascular events [28, 29], and avoids the formation of blood clots that could lead to a heart attack or venous thromboembolism [30]. Nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies [1].

Among the different forms of vitamin E, alpha-tocopherol constitutes the most biologically active form and is preferentially absorbed and retained in the body. It has anti-inflammatory, antiplatelet, and vasodilator properties with which vitamin E enhances the immune system presents the capacity to promote health, prevents and treats many diseases [29, 31]. Also, due to its natural properties to be fat-soluble and to incorporate into biological membranes, alpha-tocopherol prevents protein oxidation and inhibits lipid peroxidation, thereby maintaining cell membrane integrity and protecting the cell against damage [32]. Alpha-tocopherol also modulates the expression of various genes, plays a key role in neurological function, inhibits platelet aggregation, and enhances vasodilatation. Many researches showed that the supplementation of vitamin E (200–400 mg/day) may be suitable to moderate some aspects of degenerative diseases such as Parkinson's disease, reduce tissue injury arising from ischaemia and reperfusion during surgery, delay cataract development, and improve mobility in arthritis sufferers [33].

4. Vitamin E in olive trees

4.1 Importance of vitamin E in olive oil

Olive fruits and olive oils are considered excellent Mediterranean nutriments. Their consumption in the Mediterranean diet (MedDi) constitutes the cause of

many health-promoting effects. The olive oils are characterized by their richness in oleic acid, vitamin E, polyphenols, and some other minor components some of which are known to be anti-inflammatory, make it the model functional food [34]. Olive oils, virgin, and extra virgin are a symbol of the Mediterranean Diet. Alpha-tocopherol was the most abundant tool and detected in all the studied olive oil samples [35]. Many research proved that the levels of tocopherols in olive oils are high variety and geographic areas-dependent.

The alpha-tocopherol is more active than others $\beta > \gamma > \delta$ against free radicals. It protects free fatty acids from peroxidation. The tocopherol radicals are resonance stabilized within the chromanol ring and do not propagate the chain reactions or are rapidly recycled back to the corresponding tocopherol, allowing each tocopherol to participate in many peroxidation chain-breaking events. One tocopherol molecule can protect about 103–108 polyunsaturated fatty acids at low peroxide values [36]. According to [37], α-tocopherol represented almost 95% of total tocopherols and their contribution is greater than the rest of tocopherols; their content in virgin olive oils varies from 97 to 785 mg/kg. In fact, α-tocopherol concentration ranges from 170 to 485 mg/kg in Spanish varieties [37]; 160 to 428 mg/kg in the

Countries	**Varieties**	**Total Tocopherol (mg/kg)**	**α- tocopherol (mg/kg)**	**References**
Spain	Arbequina	371 ± 16	373 ± 16	[37]
	Morisca	501 ± 26	485 ± 27	
	Picual	355 ± 19	346 ± 20	
	Manzanilla-cacerena	336 ± 31	333 ± 31	
	Corniche	366 ± 31	366 ± 31	
	Verdial de Badajoz	292 ± 23	311 ± 22	
	Carrasquena	310 ± 58	280 ± 56	
Greece	Koroneiki	121 ± 22	117 ± 21	[42]
	Kolovi	123 ± 28	110 ± 15	
Turkey	Ayvalik	183.27 ± 15.5	180.43 ± 15.17	[35]
	Domat	160.78 ± 18.7	106.8 ± 18.27	
	Gemlik	114.87 ± 9.73	112.59 ± 9.49	
Italy	Leccino	455.25 ± 4.4	405.6 ± 4.6	[43]
	Frantoio	270.7 ± 1.7	230.0 ± 1.6	
Tunisia	Chetoui	405.65 ± 4.17	385.35 ± 2.48	[44]
	Chemlali	199	184	[44]
	Chemlali Sfax	467	425	[45]
	Chemlali Zarzis	400	374	[45]
	Oueslati	204	185	[45]
	Sayali	282	264	[45]
	Zalmati	351	336	[45]
	Zarrazi	208	193	[46]
	Meski	-	74.6 ± 4.6	[47]
	Neb Jmal	-	232.29 ± 2.00	[47]
	El Hor	-	335.27 ± 1.16	[47]
	Jdallou	-	364.23 ± 3.30	[47]
Maroc	Picholine marocaine	311 ± 11.4	272.0 ± 8.0	[48]
Algeria	Chemlal Bordj	202.35	193.55	[49]
	Arima	188.55	179.72	
	Chemlal Zenata	240.1	228.11	
	Chemlal (SBA)	215.6	202.9	
	Sigoise Sebra			

Table 1.
Total tocopherols and α-tocopherol composition in olive oils of different varieties from Mediterranean countries.

Argentinean oils [38]; 98–370 mg/kg in the Greek oils [37], 97–403 mg/kg in oils from Turkey [39], 120–478 mg/kg in oils from Tunisia [40], and 138–298 mg/kg in the Portuguese oils [41] (**Table 1**).

4.2 Effect of olive oils in many diseases

Olive oil is the main source of fats in Mediterranean diets. This type of diet has often been associated with improving the resistance to certain diseases, including cardiovascular disease and illness degenerative. Many scientific studies have focused on the nutritional aspect of olive oil to understand the mechanisms of this phenomenon. The first explanation is its specific fatty acid composition. The proportion of saturated fatty acids is very low (14%); while the majority of monounsaturated fatty acids (MUFA) is oleic acid. Essential polyunsaturated fatty acids (PUFA) are also present in interesting proportions in the oil. MUFA supplement allows to increase the resistance of LDL to oxidation [50], thus reducing one of the factors that can cause coronary heart disease [9].

The Extra Virgin Olive Oil (EVOO) is one of the most important health-protective foods in the Mediterranean diet [51]. The high-quality EVOO is considered as a true pharm-food. This oil contains a relevant concentration of efficient chemopreventive molecules, including Tyrosol, hydroxytyrosol, tocopherols (vitamin E), β-carotene, and phenolic compounds [51, 52]. [53] showed the ability of VOO phenolic compounds to shield lipoproteins from oxidation and to reduce systolic blood pressure in hypertensive individuals. These antioxidants compounds are thought to be beneficial to protect against neurodegenerative diseases and cardiovascular diseases [54]. Also, [27] suggested that the antioxidants compounds in EVOO can prevent and treat cancers, diabetes, neurodegenerative diseases, inflammation, and aging. They have an antimicrobial property and also play an important role in strengthening the immune system and protecting certain tissues and organs from damage. The presence of phenolic compounds and tocopherols in Extra Virgin Olive Oil (EVOO) protects the unsaturated fatty acids from peroxidation, thus contributes to the stability of cellular brain structures [51, 55] and it has beneficial effects on learning and memory [55]. The phenolic compound and tocopherols have often been linked to reducing the risk of cognitive decline and are essential for proper brain function.

5. Impact of pepper composition in human health

5.1 Importance of pepper in Mediterranean diet

Pepper is a very important vegetable worldwide and has economic and agro-food importance in many countries. In the Mediterranean, pepper is cultivated in the warm regions particularly in Tunisia where its cultivation has spread due to its strong uses in Tunisian cuisine. Pepper fruits were appreciated and consumed mostly as fresh food or dried as a spice. The nutritional contribution due to the presence of beneficial healthy-related compounds, Pepper is among the most fascinating and consumed spice foods, largely appreciated for its flavor, high nutritional and health contribution to human diets [56].

Pepper is a usual part of a traditional Mediterranean diet. Hot peppers are intensively used as food additives for their pungency, aroma, and color [57]. Their consumption is nutritionally valuable and also contains ingredients that promote health. The presence of phytochemicals and antioxidants in fruits increases its importance in controlling diseases to protect the human body from the harmful

effects of free radicals [58]. Therefore, integrating a pepper-rich diet in our daily meals can prevent cardiovascular diseases, could help in fighting blood cholesterol levels, and can have, by capsaicin, an antidiabetic activity [58, 59].

5.2 Antioxidants in pepper fruits

Pepper fruits are recognized for their richness in phytochemicals and antioxidants with high nutritional value. The fruits are considered an excellent source of macro and micro-nutrients such as provitamin A, vitamins C and E, carotenoïds, capsaicinoïds, minerals, polyphenols, phytosterol, metabolites with famous antioxidant properties that positively affect human health [60–62]. These phytochemicals are influenced by a variety of peppers and environmental factors. The analysis of 23 accessions of peppers, collected from multiple Peruvian locations, showed that the tocopherols varied strongly from 0.23 to 29.1 mg/100 g, the total polyphenols between 0.97 and 2.77 g gallic acid equivalents (GAE)/100 g and the concentrations of capsaicinoïds range from 1.0 mg/100 g to 1515.5 mg/100 g (GAE)/100 g [63]. So, the consumption of pepper fruits and integrating a pepper-rich diet into our daily meals can be helpful in the continuing quest to combating micronutrient deficiency [64].

Peppers are considered one of the best sources of natural vitamin E and C. Many studies showed that the level of α-tocopherol in dry red pepper powder is similar to those in spinach and asparagus and four-fold higher than that in dry tomatoes [64]. The recommended daily intake of vitamin E was 15 mg/day of α-tocopherol for both women and men. Pepper fruits can supply above 100% α-tocopherol per 100 g serving depending on the cultivar [61]. Also, the red pepper seed oils showed a high antioxidant capacity due to their richness in bioactive phytochemical compounds such as polyphenols, carotenoids, tocopherols,

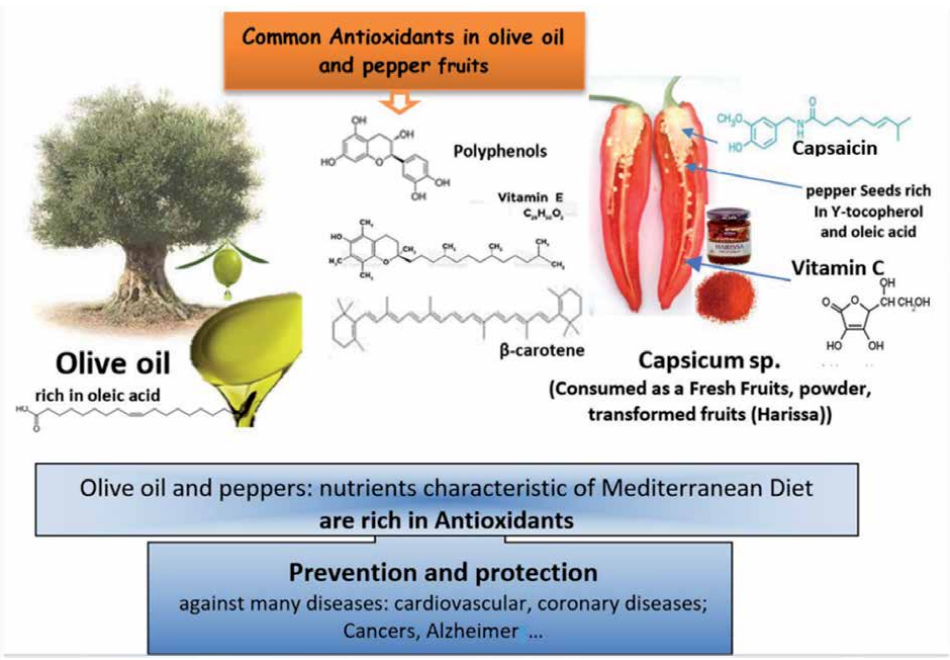

Figure 4.
Graphical abstract of the main antioxidants commonly present in olive oils and pepper fruits, characteristic of the Mediterranean diet, such as phenolic compounds, carotenoids, tocopherols and oleic acid. The capsaicin is specific to capsicum species, which have anti-inflammatory properties.

phytosterols, and unsaturated fatty acids especially the linoleic acids which are higher than those of oleaginous seed oils [62]. It has been reported that polyphenols and tocopherol were the predominant antioxidant compounds in red pepper seed oils; Which γ-tocopherol was the main tocopherol at 278.65 mg/100 g seed oil, followed by alpha-tocopherol and delta-tocopherol [62]. The ratio between α- and γ-tocopherol depends on the number of seeds in the chili powder. As a matter of fact, the amount of α-tocopherol in the pericarp is higher, however, γ-tocopherol is more dominating in the seeds [63].

Hot pepper fruits are rich in capsaicinoïds, unique compounds of Capsicum species, which are responsible for the pungency. Capsaicin is widely influenced by the variety and maturity stages and by environmental factors [65]. These alcaloïds have antioxidant, anti-inflammatory, analgesic properties and are characterized by great medical and pharmacological values [58, 66]. These molecules also have a therapeutic effect as a neuropharmacological tool. Their effect in the treatment of painful conditions has been evaluated, such as rheumatic diseases [66]. Many recent studies have shown the effective treatment of capsaicinoïds for several sensory nerve fiber disorders, including arthritis and human immunodeficiency virus [67]. In this context, the proposed diet rich in pepper fruits can be considered an excellent strategy to improve the nutritional value of the population due to its high antioxidants and phenolic compound content.

The pepper fruits and olive oil constitute an excellent nutrient, for their richness in antioxidants (**Figure 4**). Since these Mediterranean foods have an important effect on human health, it is encouraged, around the world, to consume them.

6. Conclusion

The Mediterranean diet is rich in nutrients that have antioxidant properties. Particularly olive oil and pepper fruits constitute the most abundant and consumed vegetable nutriments in MedDi areas. Their richness in polyphenols, tocopherols, carotenoids, chlorophylls, unsaturated fatty acids, olive oils constitute a health treasure. These minor components are known to prevent and protect the human organism against many diseases such as cardiovascular, coronary diseases; also, some of these antioxidants have anti-inflammatory action, make it the model functional food. Pepper fruits are mostly consumed by the Mediterranean population as traditional spices and food products. Fruits are characterized by a means of antioxidants such as vitamin A alpha and gamma tocopherols, vitamin C, capsaicinoïds, polyphenols. The consumption of both nutriments rich in natural powerful antioxidants, such as tocopherols and polyphenols, constitutes a good strategy for reducing oxidative damage and to improve the health state of the human body, and preventing it from diseases.

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Conflict of interest

The authors declare no conflict of interest for this chapter.

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Chapter 9

Vitamin E: Recommended Intake

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Abstract

Data of vitamin E intake and status are controversial. Vitamin E is an essential micronutrient for humans and achieving an optimal status is assumed to produce beneficial health outcomes. Dietary intake recommendations for vitamin E vary considerably by different countries and organizations. It appears to be still a challenge to define these despite the wealth of data published. Vitamin E requirements have been proposed to depend on other nutritional factors, such as the intake of polyunsaturated fatty acids (PUFA). Although several foods contain naturally occurring sources of vitamin E, it is frequently the case that the intake recommendations are not achieved. Several other dietary factors affect the need for vitamin E. In this regard, significant challenges to be considered include the efficiency of other tocopherol variants and their properties that could affect the revision of the nutritional recommendations for vitamin E. Particularly, an ever-increasing evidence indicates that other vitamin E homologs may potentially present with a higher biological activity. Low dietary consumption of vitamin E, coupled with compelling evidence that increased intake of vitamin E above current recommendations for the general population may benefit older individuals.

Keywords: vitamin E recommendations, tocopherols, tocotrienols, nutrition, requirement, dietary intake, food source

1. Introduction

Vitamin E was first described in 1922 by Evans and Bishop as a dietary factor essential to prevent fetal reabsorption in rats [1], vitamin E was soon after identified as an antioxidant of polyunsaturated lipids [2]. Evans and Bishop (1922) reported the discovery of a molecule that was lacking in rats on a diet based on lard, and which resulted in an impaired fertility [3]. This deficiency was reversed by the administration of lipid extract prepared from cereals, which was defined as the "antisterility factor" [4]. Finally, vitamin E was officially recognized as the 5th vitamin in 1925. Subsequently, the name "tocopherol" originating from the Greek words "toc" (child) and "phero" (to bring forth) was conceived to characterize the roles of vitamin E as an essential dietary compound for a normal fetal development and childhood.

Vitamin E is a fat-soluble compound. The name represents a collective title for 4 tocopherols (α-, β-, γ-, and δ-tocopherol) and 4 tocotrienols (α-, β-, γ-, and δ-tocotrienols) that are present in food and exhibit antioxidant properties, however which cannot be interconverted, and only α-tocopherol meets the requirements for the daily intake of vitamin E in humans [5].

As an antioxidant vitamin E protects cell membranes from oxidation and destruction [4]. Oxidative processes are normal cellular events, but uncontrolled oxidation, particularly of membrane lipids and lipoproteins, has been implicated in a variety of degenerative conditions, including cancer, rheumatoid arthritis, drug-associated toxicity, coronary heart disease, diabetes, and acute clinical conditions, such as ischemia–reperfusion injury [6–9]. According to the widespread consensus, vitamin E is a powerful antioxidant molecule, which may be found in lipid compartments such as cell membranes because of its hydrophobic chemistry. Its primary function lies in the prevention of lipid peroxidation [10], leading to the preservation of the membrane stability. Vitamin E is also essential in the stabilization of erythrocytes and in the nerve conductivity of the central as well as peripheral nervous system [11, 12]. The molecule prevents hemolytic anemia and neurological dysfunction associated with its deficiency, such as ataxia, neuro-, myo- or retinopathy. The vitamin is also highly efficient in the prevention or stabilization of a variety of health complications because of its antioxidant, anti-inflammatory, antiaggregant and immune-enhancing properties [13]. However, the beneficial effects of vitamin E in human health may also be due to the ability of its phosphorylated metabolite to modulate signal transduction and gene expression in numerous conditions, including inflammation and immune system disorders [14]. This chapter aims to provide a brief overview of the current strategies that are employed to define the intake recommendation for vitamin E. Furthermore, we wish to evaluate the available evidence on the fundamental biological roles of vitamin E in the human body to in order to establish intake requirements for vitamin E to exhibit its antioxidant properties by protecting the polyunsaturated fatty acids (PUFAs) from being oxidized in human tissues. The question which will be addressed in this chapter is how the current vitamin E status (as measured by vitamin E intakes and serum levels) of populations in various countries differs. Special attention is also given to the vitamin E food sources.

2. Vitamin E intake recommendations

Vitamin E is defined as an essential micronutrient for humans, and its beneficial health outcomes are dependent upon reaching its optimal nutritional status. A variety of dietary intake recommendations for vitamin E have been established around the world, all of which point out to its ability to act as a chain-breaking antioxidant and thus to protect the stability of the cell membrane [1].

2.1 Determination vitamin E intake recommendations

In Europe, the **European Food Safety Authority (EFSA)** recently concluded that the recommended daily allowance (RDA), average requirements (ARs) and population reference intakes (PRIs) for vitamin E (in the form of α-tocopherol) cannot be established for adults, children and infants equally. As such, EFSA determined adequate intakes (AIs), which are based on intakes that had been observed in a supposedly healthy population that presents with no apparent α-tocopherol insufficiency in the EU [15].

The EFSA Panel on Dietetic Products, Nutrition and Allergies proposed RDA to replaced by a newly defined adequate intake (AI), depending on age, as follows: men, 13 mg/d; women, 11 mg/d (**Table 1**); and infants/children, 5–13 mg/d [15].

The US **Institute of Medicine (IOM)** define vitamin E recommendations for generally healthy population as intake level of 12 mg/d and above (**Table 1**).

		Age (years)	Men (mg/d)	Women (mg/d)
EFSA	(2015)[(a)]	≥18	13	11
IOM	(2000)[(b)(c)]	≥19–50	15	15
D-A-CH	(2013)[(a)]	≥19	13-15	12
WHO/FAO	(2004)[(d)]	≥19	10	7.5
AFSSA	(2001)	20–74	12	12
NCM	(2014)[(a)(b)]	≥18	10	8
SCF	(1993)[(e)(f)]	≥18	0.4	0.4
NL	(1992)[(c)(f)]	≥18	0.67	0.67
DH	(1999)[(g)]	>18	>4	>3

DRVs in α-tocopherol equivalents is defined by the biological activity of 1 mg natural α-tocopherol in the resorption-gestation test.
EFSA European Food Safety Authority; IOM US Institute of Medicine of the National Academy of Science; D-A-CH Deutschland (Germany)-Austria-Confoederatio Helvetica (Switzerland); WHO/FAO World Health Organization/ Food and Agriculture Organization of the United Nations; AFSSA Agence Française de Sécurité Sanitaire des Aliments; NCM Nordic Council of Ministers; SCF Scientific Committee on Food, NL Netherlands Food and Nutrition Council; DH UK Department of Health.
[(a)] Adequate Intake.
[(b)] Applicable to RRR-, RSR-, RRS- and RSS-isomers of α-tocopherol only.
[(c)] PRI – Population Reference Intake.
[(d)] Data were insufficient to set PRIs; the indicated figures represent the 'best estimates of requirements' (WHO/FAO, 2004)
[(e)] 'vitamin E requirement'.
[(f)] mg α-TE/g PUFA.
[(g)] 'Safe' intakes.

Table 1.
Vitamin E dietary reference values (DRVs) for adults - overview [15].

This value has been characterized as the Estimated Average Requirement (EAR) and is defined as the amount needed to meet the requirements of 50% healthy people, and it became the foundation to determine RDA, which is predicted to meet the dietary demands of 97.5% healthy individuals. In the case of the USA, the RDA for vitamin E is established to be 15 mg/d α-tocopherol for men as well as women older than 14 years of age [16].

The current intake recommendations for vitamin E vary between 3 and 15 mg/d in different countries and depending on the age and gender of the person.

The current adult Dietary Reference Values (DRVs) for vitamin E in UK is determined >3 mg/d, in German-speaking countries 15 mg/d for adult. The French Food Safety Agency (AFSSA) derived a separate reference value of 20–50 mg/d for adults aged 75 years and over [17].

The IOM [16, 18] recommends Vitamin E dietary reference doses based on a previous extensive research. The IOM recommendations are based on a premise that there is no scientific basis to assume variations in the demand for vitamin E between men and women, or that aging could impair its absorption or secretion. As such, the IOM recommendations do not discriminate between sex or age in adults.

On the other hand, DACH reference values [18, 19] for Germany, Austria and Switzerland published in the same year and based on a similar methodology, does distinguish between age and/or sex.

In the meantime, IOM and DACH applied two different methodological approaches to estimate the recommendations for dietary vitamin E intake. On one hand, IOM is based on the prevention of deficiency symptoms, particularly the sensitivity of erythrocytes to hemolysis. Available human data reveal that subjects with plasma concentrations of at least 12 μmol/L α-tocopherol present with a low degree of haemolysis.

On the other hand, the DACH recommendations, supported by EFSA, regard dietary intake of PUFA to estimate the demands for vitamin E. The basic vitamin E demand of 4 mg/d and a ratio of 0.4 mg α-tocopherol/g of dietary linoleic acid were used to compute vitamin E requirements [18]. Dietary vitamin E demands were estimated at levels of 12 and 15 mg/d based on a general dietary PUFA consumption, which differs between women and men due to the differences in energy intake.

It is nevertheless intriguing to observe, that despite two different methods, both approaches will result in reference values ranging between 10 and 30 mg/d.

It is recommended that a baseline α-tocopherol requirement should be estimated to which extra vitamin E compensating for the dietary PUFA intake can be added to finally obtain an appropriate balance of dietary fatty acids with vitamin E. Nevertheless, the optimum demand for vitamin E is also directly correlated to the amount and degree of dietary PUFA unsaturation. In order to assess the precise vitamin E prerequisites in infants, children, adolescent, adult men and women, an extended observation is imperative to deplete the body's vitamin E storage in order to describe anypotential long-term adverse or harmful consequences that are often complicated to be diagnosed at an early stage. Currently however it is not possible to carry out any long-term follow-up or depletion studies due to ethical reasons [18, 20].

2.2 Vitamin E – other nutritional factors

It has been suggested that vitamin E requirements depend on a variety of other nutritional factors, primarily on the ingestion of polyunsaturated fatty acids (PUFAs). Based on this factor, an increased demand for vitamin E should be regarded for RDA calculation, which has been estimated to oscillate between 15 and 25 mg/d or more [18, 19].

Various reports revealed that the nutritional demand for vitamin E requirement is associated with the dietary intake of PUFAs, which is why in order to calculate the actual vitamin E requirement, a basal vitamin E demand as well as an additional requirement for dietary PUFAs may be taken into consideration. Preclinical as well as human data point out to the fact that a minimal basal need for 4–5 mg/d of RRR-α-tocopherol is necessary even if the diet is lacking PUFAs [20]. Nevertheless, no consensus exists on the precise vitamin E/PUFA ratio in order to establish the vitamin requirement since a strictly pre-determined vitamin E/PUFA proportion may not be relevant to all diets or health conditions. On the other hand, the demand for vitamin E increases proportionally to the PUFA consumption and to the level of the PUFA unsaturation in the diet. As such, currently available human data hypothesize that the additional dietary need for vitamin E fluctuates between 0.4 and 0.6 mg RRR-α-tocopherol/g of PUFA, particularly in the case of a diet containing an average amount of PUFAs with linoleic acid being the predominant dietary PUFA [18]. What is more, animal experiments reveal that in case of fatty acids presenting with a higher degree of unsaturation, the demand for vitamin E increases almost in a linear fashion correspondingly to the extent of PUFA unsaturation in the relative proportions of 0.3, 2, 3, 4, 5, and 6 for, or alternatively, mono-, di-, tri-, tetra-, penta-, and hexaenoic fatty acids. Summarizing evidence from human as well as animal studies, it may be suggested that in the case of a standard ingestion of PUFAs, the estimated dietary need for vitamin E oscillates between 12 and 20 mg/d [20].

Generaly, the recommended intake of vitamin E should correlate with the amount of polyunsaturated fatty acids (PUFA) in food: 1 g of diene fatty acid or rather diene equivalent requires an intake of 0.5 mg RRR-α-TOH.

An optimal daily intake of vitamin E may be broken down into two categories: a required daily intake that provides enough vitamin E for the molecule to exhibit its basic biological effects, as well as a second one, which is determined by a higher

ingestion range that promotes its additional beneficial properties that may assist in the disease prevention. The most favorable intake of vitamin E in healthy subjects, defined as the actual dose that is associated with its major positive attributes in the absence of possible adverse effects, remains to be determined in appropriately designed and executed trials, which represents a considerable hurdle to be overcome in the definition process of appropriate nutritional vitamin E recommendations [1].

As the data come from different countries, it is important to take into account the differences in dietary behaviors in a comprehensive assessment of vitamin E intake. Nevertheless, a substantial number of countries are still not represented. Furthermore, the studies applied various scientific strategies to assess the intake of dietary vitamin E. If just one single 24-hour dietary anamnesis was performed per person, this might not necessarily reflect on an everyday nutrient ingestion, based on a day-to-day fluctuation. Other reports were based on 3-day food records, providing a better notion of the dietary routine [21]. Other research strategies may include food frequency questionnaires or a dietary history.

2.3 Vitamin E – dietary intake

Diet, nutritional status, lifestyle and environmental factors are among the most complicated issues to be investigated with respect to chronic diseases [22]. Large multicentre nutritional studies are accompanied by additional challenges to assess, correlate, and understand dietary exposure in a comparable way across countries, as well as to conclude evidence and recommendations.

Generally speaking, the intake of vitamin E is low and very similar across regions all around the world. According to a recent systematic review, dietary ingestion of α-tocopherol and other vitamin E derivates is well below the RDA for the majority of the population, or even lower than the EAR of 12 mg/d, which is applicable equally for developing as well as industrialized countries [23]. The biggest investigation focused on the vitamin E intake is the pan-European EPIC study involving 36 000 participants recruited across 10 European countries and followed-up for as long as 15 years. Details on the dietary patterns, lifestyle characteristics, anthropometric measurements, and medical history were collected in the EPIC study at recruitment (1992–1999). While the overall mean consumption of vitamin E was 11.9 mg/d, an intriguing regional difference was observed: the intake was higher in the southern countries in comparison to the northern ones [24]. This revelation may be explained by the differences in the food preferences, particularly in the case of vegetable oils, which are more popular in the south.

The NHANES study showed a mean intake of α-tocopherol of 7.2, 6.8, 6.1, 6.0 mg/d in men aged 19–50 years, >50 years, women aged 19–50 years, and > 50 years, respectively [25]. Vitamin E RDA (15 mg/d) was recorded only in 4% women and 5% men, while EAR (12 mg/d) was observed in 7–8% women and 10–11% men.

The prevalence of inadequate vitamin E intake was reported to be 92.5% in the total Brasil adolescent population, 91.6% in boys, and 93.5% in girls ($p = 0.358$). Brasil adolescents aged 10 to 13 years showed a less inadequate ingestion ($p < 0.001$) when compared to those aged 14 to 19 years: 87.7% and 95.1%, respectively [26] (**Table 2**). Jordão et al. [26] identified a high prevalence of vitamin E inadequacy, verified by a low intake of the nutrient, and the observation that ultra-processed foods, such as cookies, packaged snacks, and margarine, provided for almost 33% of the vitamin E content ingested by adolescents in Campinas. Furthermore, healthy foods considered as critical dietary sources of vitamin E did not contribute significantly when extrapolated to the total nutrient intake.

State/city/years/ [Ref.]	Subjects (n: M/F)	Intake of vitamin E (estimation methods)	Plasma/serum concentration
Europe pan-European EPIC study recruitment participants in (1992–1999) Jenab et al. [24]	n = 36000	11.9 mg α-TE/day	
West Europe ZENITH study (2002–2005) Polito et al. [27]	older European adults aged 55-70y and 70-85y (n = 387; 195 M/192F) Clermont-Ferrand (France) n = 95 Grenoble (France) n = 106 Coleraine (Northem Ireland) n = 95 Roma (Italy) n = 96	4-day recall-record method (2 week and 2 weekend days) mean ± SD; (dietary adequacy as % of RDA); [% of subjects at dietary risk] middle-aged (55-70y): C-Ferrand (France) M: 11.3 ± 6.3 mg/d; (141 ± 79%); [8%] F: 9.5 ± 4.6 mg/d; (118 ± 57%); [15%] older aged (70-85y) Grenoble (France) M: 7.1 ± 3.0 mg/d; (89 ± 37%); [26%] F: 7.1 ± 4.8 mg/d; (91 ± 59%); [33%] Roma (Italy) M: 13.7 ± 3.3 mg/d; (172 ± 41%); [0%] F: 12.3 ± 2.6 mg/d; (154 ± 32%); [0%]	α-TOH (HPLC) mean ± SD middle-aged (55-70y): C-Ferrand (France) M: 28.2 ± 5.2 μmol/L F: 28.8 ± 5.4 μmol/L Coleraine (N. Ireland) M: 28.4 ± 6.0 μmol/L F: 29.0 ± 4.9 μmol/L older aged (70-85y) Grenoble (France) M: 29.7 ± 5.4 μmol/L F: 32.5 ± 5.5 μmol/L Roma (Italy) M: 29.3 ± 5.8 μmol/L F: 29.4 ± 6.2 μmol/L
Ireland/Europe (2008–2010) The National Adult Nutrition Survey (NANS) Zhao et al. [28]	healthy Irish adult population aged 18 years and above; mean 40.3 ± 15.9 years BMI 25.9 ± 3.9 kg/ m^2 (n = 601; 305 M/296F)	(record over four consecutive survey days) (dietary + supplemental vitE) α-tocopherol equivalent (α-TE) vitamin E intake quartiles: Q1: 6.0 ± 1.1 mg/d Q2: 9.0 ± 0.7 mg/d Q3: 11.9 ± 1.0 mg/d Q1: 20.5 ± 8.5 mg/d	plasma α-TOH (HPLC) vitamin E intake quartiles: Q1: 24.0[b] ± 5.9 μmol/L Q2: 25.8[a] ± 7.4 μmol/L Q3: 25.4[a] ± 6.3 μmol/L Q1: 25.7[a] ± 7.1 μmol/L
US/North America (NHANES) (2001–2002) Gao et al. [25]	US population aged >18 years M: 19-50y n = 1141 >50y n = 997 F: 19-50y n = 1196 >50y n = 1017	(dietary + supplemental vitE) α-tocopherol equivalent (α-TE) M: 19-50y 7.2 ± 0.1 mg/d M: >50y 6.8 ± 0.2 mg/d F: 19-50y 6.1 ± 0.1 mg/d F: >50y 6.0 ± 0.2 mg/d	

State/city/years/ [Ref.]	Subjects (n: M/F)	Intake of vitamin E (estimation methods)	Plasma/serum concentration
Brazil/South America/ city Campinas ISACamp (2014–2015) ISACamp-Nutri (2015–2016) Jordão et al. [26]	Brazilian adolescents aged 10–19 years (n = 891; 463 M/428F) M: 10-13y n = 169 14-19y n = 294 F: 10-13y n = 143 14-19y n = 285	food consumption assessment questionnaire that contained the 24-hour recall mean (95%CI) M: 10-13y 2.8 mg/d (2.5–3.1) 14-19y 3.4 mg/d (3.0–3.7) F: 10-13y 3.5 mg/d (2.8–4.2) 14-19y 3.6 mg/d (2.8–4.4)	
South Africa/ Sharpeville - periphery of city Johannesburg Oldewage-Theron et al. [29]	(n = 235; 39 M/196F) mean age 73.4 ± 7.0y (60-93y) BMI M: 25.7 ± 4.6 kg/ m^2 (64.1% normal BMI) F: 29.9 ± 6.4 kg/ m^2 (20.9% normal BMI; 31.1% overweight and 47.4% obese)	two 24-h recall (DRI 12 mg/d) M: (n = 26) 5.4 ± 5.2 mg/d 88%DRI F: (n = 113) 4.0 ± 0.5 mg/d 96% DRI total: (n = 139) 4.3 ± 5.8 mg/d 95% DRI	by HPLC mean ± SD (95%CI) deficient <1.2 mg/L) marginal 1.2–1.6 mg/L M: n = 39 2.01 ± 1.11 mg/L (1.65–2-37) deficient 8 (20.5%) marginal 9 (23.1%) F: n = 196 2.07 ± 1.12 mg/L (1.92–2.23) deficient 41 (20.9%) marginal 29 (14.8%) total: n = 235 2.07 ± 1.11 mg/L (1.92–2.21) deficient 49 (20.9%) marginal 38 (16.2%)
Korea/Soul (2009–2010) Kim & Cho [30]	20-59y old health adults (n = 106; 33 M/73F)	(3 consecutive 24-h food recalls) dietary α-TE/day: 17.68 ± 14.34 and total α-TE/day: 19.55 ± 15.78 mg (dietary + supplemental vitE) α-tocopherol equivalent (α-TE) daily α-TOH 3.07 ± 2.27 mg daily γ-TOH 5.98 ± 3.74 mg • 12.3% consumed vitamin E less than the AIs for vitamin E	plasma α-TOH M: 15.45 ± 10.16 μmol/L F: 15.00 ± 4.54 μmol/L • 23% < 12 μmol/L indicating a biochemical deficiency of vitE • 89.6% < 20 μmol/L

Table 2.
Selection of surveys/studies regarding intake vitamin E and serum (α-tocopherol) concentrations.

In Germany infants and children up to age twelve commonly do not reach the recommended levels of vitamin E intake [31], as shown in a number of studies including the VELS investigation and the EsKiMo study, a follow-up of the KiGGS study.

Although the recommended amount of vitamin E is higher for men than for women, Dutch women consume less vitamin E more often compared to Dutch men [32].

Numerous research groups analyzing compliance to the vitamin E intake recommendations in Americans have found that a significant number of individuals consume insufficient amount [33, 34]. Data by Traber [35] suggest that more than 90% of United States Americans consume insufficient amounts of vitamin E from natural sources. Bjelakovic et al. [36] claims that when combined with the dietary ingestion, the total intake of vitamin E of antioxidant supplement users in the United States exceeds 700% of the estimated average requirement.

A systematic review (2000–2012) by Péter et al. [37] focused on vitamin E intake levels and serum concentrations in order to obtain a global overview of α-tocopherol status. The authors state that only 17 studies (12.9%) included both intake data as well as vitamin E status measured in blood. Most of the studies (132) were conducted in Europe (47.7%), followed by North America (24.2%), and the Western Pacific region (14.8%). Worldwide, 82% of the population had a vitamin E intake below 15 mg/d, 91% in North and South America, 80% in Europe and 79% in the Asian-Pacific region.

Nutrient intake in children and adolescents in Slovakia was studied in 1991–1994 and 1995–1999. Apart from these surveys, no nationally representative data were found for Slovakia, which would be carried out since 2000, which is why a comprehensive information on vitamin E intake and status in all age groups of the population are missing. The Slovak surveys were not indeed nationally representative but were nationwide and designed to "recruit a diverse sample of entities of different ages and socio-economic backgrounds" [38].

The situation is similar in other countries, particularly in the case of Central and Eastern Europe, Africa, Asia (India), and South America [37].

Evidence in the literature that vitamin E intake does not correlate with plasma levels of α-tocopherol is inconclusive [28, 35]. Previous studies have shown that total α-tocopherol intake was positively associated with the plasma α-tocopherol levels [28], while the main indicator of plasma α-tocopherol concentration was the intake of vitamin E supplements [28]. However, other studies have shown that plasma concentrations of α-tocopherol correlated weakly with dietary vitamin E ingestion [16]. The reasons for the lack of a conclusive correlation may lie in the variations of the activity of the α-tocopherol transfer protein [35], genes involved in lipid metabolism [28, 39] and micronutrients with a synergistic effect, such as vitamin C [28, 40]. Niki et al. [41] revealed that lipid peroxidation and oxidative damage may lead to decline in the levels of plasma and tissue α-tocopherol, which may be another plausible argument for different relationships observed between the vitamin E intake and plasma α-tocopherol levels.

Many scientists believe that it is difficult for an individual to consume more than 15 mg/d α-tocopherol form food (RRR-α-tocopherol) alone, without increasing fat intake above recommended levels [42].

3. Vitamin E status

Unlike vitamins A and D, vitamin E does not have a specific carrier protein in the plasma. Instead, it is rapidly transferred from chylomicra to plasma lipoproteins, to which it binds nonspecifically. The metabolism of circulating chylomicra can result in tocopherols being transferred directly to tissues by partitioning into their plasma membranes, or indirectly by transfer to and between circulating lipoproteins [43]. 90% of the tocopherol is transported by the lymph, the rest by the

portal circulation. It is stored 65% in LDL-c, 8% in VLDL and about 24% in HDL-c. There is a close correlation between tocopherol concentration and total plasma lipid content [44]. These transport processes can be disrupted under dyslipidemic conditions. Patients with hypercholesterolemia and/or hypertriglyceridemia show reduced plasma uptake of newly absorbed vitamin E [43]. Vitamin E is present in all tissues where it has a universal protective effect (**Table 3**).

All tocopherols and tocotrienols belonging to the vitamin E family are absorbed from the intestine to a comparable extent and are subsequently transported via chylomicrons and HDL-c to the liver. Within liver, α-tocopherol is sorted out and is distributed to the bloodstream via VLDL and HDL-c [45]. Consequently, among all vitamin E varieties α-tocopherol is present at the highest proportion in the body, followed by γ-tocopherol. Inversely, tocotrienols are usually not found in tissues [46]. That postprandial levels of tocopherols exceed those of tocotrienols reflects the more rapid metabolic degradation of the latter [43]. In the meantime, only minimal concentrations of β- and δ-tocopherols are found in the blood plasma. An advantageous distribution of α-tocopherol in comparison to other vitamin E forms comes from a faster metabolic rate of the other tocopherols as well as from the α-tocopherol transfer protein (α-TTP). Because of this affinity, α-tocopherol is largely excreted through the urine, while most of the absorbed β-, γ- and δ-tocopherol will be secreted into the bile and subsequently excreted in the feces [13]. Nevertheless, as each class of lipoproteins derives its tocopherols ultimately from chylomicra, α-tocopherol transport by the latter is the major source of interindividual variation in response to ingested vitamin E [43].

Until today and nearly a century after the discovery of vitamin E [1], the molecular mechanisms controlling cellular sorting and preferential retention of one of the eight vitamin E congeners, α-tocopherol, are still incompletely understood.

As with other serum nutrients, vitamin E concentrations are affected primarily by age and a variety of lifestyle factors (obesity, smoking, alcohol consumption, etc.) [47].

Differences in the serum and tissue levels of vitamin E have been studied on different occasions. According to Campbell et al. [48] vitamin E decreased in

Tissue	**α-tocopherol**	
	μg/g Tissue	**μg/g Lipid**
Adipose	150	0.2
Adrenal	132	0.7
Hypophysis	40	1.2
Testis	40	1.0
Platelets	30	1.3
Heart	20	0.7
Muscle	19	0.4
Liver	13	0.3
Ovary	11	0.6
Plasma	9.5	1.4
Uterus	9	0.7
Kidney	7	0.3
Erythrocytes	2.3	0.5

Table 3.
Concentration of α-tocopherol in human tisseues [43].

participants aged over 80 years, which may be associated with a generally reduced food intake in elderly people. Inversely, hepatic levels of vitamin E have been reported to be unaffected by age [49]. According to other reports, increased serum concentrations of vitamin E were found in people older than 60 years [47, 50, 51], which may be explained by an age-dependent rise in the levels of serum cholesterol and lipoproteins [50]. Arguabely, this phenomenon may exhibit protective effects against extensive lipid peroxidation occuring as a side effect of aging [49, 52]. In the meantime, Succari et al. [51] hypothesize that lifestyle and age-associated changes independent of serum cholesterol/lipoprotein levels could be responsible for an increased vitamin E level observed in elderly French women [53].

With respect to smoking as an important factor contributing to the fluctuations of vitamin E, Al-Azemi et al. [54] and Shah et al. [55] reported that smokers presented with lower serum concetrations of α-tocopherol in comparison to non-smokers (**Table 4**).

The presence of 5-nitro-gamma-tocopherol in the blood plasma of smokers indicates that vitamin E can be nitrated by reactive nitrogen species heavely overproduced during smoking, coupled with inflammatory processes frequently observed in smokers. This process will then enhance the turnover of tocopherols and lead to a reduction of carboxyethyl-chromanyl metabolites in smokers [56].

Alcoholism could also contribute to decreased serum levels of α-tocopherol, partially due to malnutrition [57]. *In vivo* studies have also revealed that chronic consumption of alcohol is associated with lower hepatic levels of α-tocopherol, which may be caused by decreased amounts of α-tocopherol in the hepatic mitochondria [57–59].

3.1 Assessment of vitamin E status

Vitamin E status is often assessed by determining the concentration of α-tocopherol in blood plasma or serum [60].

Human studies published in the 1950s and 1960s aimed to address vitamin E levels that could prevent peroxide-induced hemolysis as well as a reduction in the cell survival in subjects on a vitamin E-deprived diet over a period of six years [1]. It was found that ingestion of 12 mg α-tocopherol/day was sufficient to reach a threshold level of 12 μmol/L serum α-tocopherol exhibiting protective effects on the organism. This conclusion was then extrapolated to the definition of the Estimated Average Requirement (EAR) which became the theoretical ground for RDA calculated. Even though this approach has been heavely criticized, currently there is no alternative for the RDA calculation that has been agreed upon. Accordingly, the American Institute of Medicine (IOM) defined the levels of serum α-tocopherol as deficient, if these are to be found below 12 μmol/L [16].

Metabolite	Non-smokers (n = 19)	Smokers (n = 15)
α-tocopherol (μmol/L)	16.0 ± 4.0	15.9 ± 5.0
γ-tocopherol (μmol/L)	1.76 ± 0.98	1.70 ± 0.69
5-nitro-γ-tocopherol (nmol/L)	4.03 ± 3.10*	8.02 ± 8.33

*Note: *P < 0.05.*

Table 4.
Plasma α- and γ-tocopherol in smokers and non-smokers [56].

To evaluate tocopherol levels in the human body, the serum concentration is commonly analyzed after 12–24 h of fasting.

Tocopherol exchanges rapidly between the lipoproteins mediated by the phospholipid transfer protein [43], and between lipoproteins and erythrocyte (about one-quarter of total erythrocyte vitamin E turns over every hour); thus, the level of vitamin E and the concentration of erythrocytes are strongly correlated (as red blood cells carry 15–25% of total vitamin E found in the blood) [43]. As vitamin E is a membrane-protective molecule, tocopherol levels found in the plasma are inversely correlated to the sensitivity toward oxidative hemolysis. This association makes the plasma levels of alpha-tocopherol a suitable indicator of vitamin E status. In the healthy population concentrations above 0.5 mg/dL (12 μmol/L) are associated with hemolysis prevention and are accepted as indicators of nutritional adequacy [43].

For adults, an amount of 0.5–2 mg tocopherols/100 mL plasma (12–46 μmol/L) is recommended according to D-A-CH association [61].

As noted by Traber [35], circulating α-TOH concetrations are not necessarily a reliable marker for an adequate vitamin status in humans.

Serum concentrations of vitamin E are significantly affected by the levels of lipids, which is why they do not reflect its tissue levels in a consistent manner [62]. Thus, more accurate vitamin E levels may be assessed as follows:

$$\text{effective serum vitamin E level} = \alpha - \text{tocopherol} / (\text{cholesterol} + \text{triglycerides})$$

Based on the equation, a ratio above 0.8 mg α-tocopherol/g total lipids is considered to be normal. In the case of individuals with normal levels of serum lipids, the concentration of serum α-tocopherol levels serve as an adequate estimate of vitamin E sufficiency. Any cocentration of alpha-tocopherol lower than 0.5 mg/dL or 5 μg/mL, or 11.5 μmol/L is considered deficient [4].

Supplementation of α-TOH, for example increased amount of α-CEHC in urine. Hence, α-CEHC in urine can be used as a marker for α-TOH status in healthy humans [63] or at a minimum as a marker for an adequate level of α-TOH [64].

Péter et al. [37] determined ranges of α-tocopherol concentrations based on a systematic review of the global state of alpha-tocopherol as the concentration in functional deficiency range (≤12 μmol/L), concentration between functional deficiency and desirable threshold (13–29 μmol/L), and finally concentration in desirable range (≥30 μmol/L). Alpha-tocopherol levels less than 20 μmol/L, is yet a more conservative cut-off marker, because of the apparent increased risk for cardiovascular diseases below this limit [24].

More attention should be given to further explore of measuring vitamin E serum levels as they may be a much more useful marker to assess vitamin E status rather than relying on dietary intake reports. Determining the right analytical parameters for evaluating vitamin E status is critically important; however it is also crucial that new analytical parameters and procedures be validated, optimized and standardized for ensuring optimal diagnosis and comparability [59].

3.2 Vitamin E status in the population

Plasma α-tocopherol concentrations in humans range from 11 to 37 μmol/L, whereas γ-tocopherol concentrations are roughly 2–5 μmol/L, and tocotrienol concentrations are less than 1 μmol/L in non-supplemented humans [65].

In an US national survey, the 5th percentile for vitamin E serum levels was 0.62 mg/dL or 14.3 μmol/L, and the 25th percentile was 0.79 mg/dL or 18.5 μmol/L [4].

Results on vitamin status presented in a review from Valtuena et al. [66] and Böhm et al. [60] were published between 2001 and 2011. Besides data from the United States and India, several studies were conducted in Europe (Austria, France, Germany, Greece, Slovakia - only children and adolescents, and Sweden). Intake surveys as well as the assessment of vitamin E concentrations in the blood plasma/serum of children and teenagers were performed in a number of countries. While the intake oscillated between 2.1 and 12.2 mg/dL, the plasma or serum levels of vitamin E ranged between 16.9 and 29.2 μmol/L.

In the case of rural Nepal, about 33% of pregnant females were affected by a severe vitamin E deficiency (less than 10 μmol α-tocopherol/L serum) [67]. Vitamin E status was found to be even worse in Bangladesh, where almost 65% of women in early pregnancy presented with a more severe vitamin E deficiency (< 9.3 μmol α-tocopherol/L serum). On the other hand, a recent study has reported that more than 65% of South Korean adults presented with suboptimal blood vitamin E levels (12–30 μmol α-tocopherol/L serum; lower than the threshold set at 30 μmol/L, as per recommendations by the German Federal Ministry of Health Consensus Statement [68]), while 25% of the participants were vitamin E deficient (less than 12 μmol α-tocopherol/L serum) [46]. Zhao et al. [28] have demonstrated a positive relationship between vitamin E intake and plasma α-tocopherol concentration and plasma n-3 PUFA profile (**Table 2**).

Malik et al. [69] collated for the purpose of review limited available data from 31 studies on vitamin E status in healthy people from Asia, the most populated continent.

Despite a substantial quantity of reports focused on the evaluation of the vitamin E status, data on the extent of vitamin E deficiency from Asian countries, such as India, are lacking. In this sense, information on validated biomarkers for vitamin E status are missing, and no consensus exists on cut off values to define a possible vitamin E deficiency. As such, a possible interpretation of the collected data is complicated.

With respect to a threshold concentration of 20 μmol/L as recommended by various nutritionists [35], data collected from previous studies reveal that 27% Americans, 80% Middle Eastern/Africans, 62% Asians, and 19% Europeans presented with serum levels below this value. Average blood serum concentrations of 20 μmol/L α-tocopherol can be acheived in normal adults on a balanced diet, which includes nuts, seeds and whole grains. Inversely, only 21% of the total data revised in global review [17] indicated a desirable serum concentration of α-tocopherol equal to or above 30 μmol/L. Furthermore, 66% of all subentries ranged between 12 and 30 μmol/L.

Several prospective observational studies (**Table 5**) suggested that a serum α-tocopherol concentration of 30 μmol/L or above has beneficial effects on human health, with alleged applications including prevention of cardiovascular disease and different types of cancer, higher baseline serum concentrations of α-tocopherol were associated with lower total and cause-specific mortality; the lowest total mortality was observed at 30 μmol/L serum α-tocopherol concentrations.

On the other hand, results from the large SELECT trial reveal that vitamin E supplements (400 IU/day [180 mg/d] as *dl*-alpha-tocopheryl acetate) may harm adult men in the general population by increasing their risk for prostate cancer [76]. Follow-up studies are assessing whether the cancer risk was associated with baseline blood amounts of vitamin E and selenium prior to the consumption of supplements as well as whether changes in one or more genes might increase a man's risk to develop prostate cancer while consuming supplemental vitamin E.

3.3 Deficiency of vitamin E (hypovitaminosis)

There exists a subtle difference in definitions describing levels of vitamin intake [59]. Whereas vitamin deficiency is caused by diseases, metabolic disorders [31], or

Source	Aim of the study	Treatment	Results and conclusion of the study
Mangialasche et al. [70]	To examined the relation of all plasma vitamin E forms and markers of vitamin E damage to mild cognitive impairment (MCI) and Alzheimer's disease (AD)	plasma tocopherols, tocotrienols, α-tocopherylquinone, and 5-nitro-γ-tocopherol were assessed in 168 AD cases, 166 MCI, and 187 cognitively normal (CN) people	• Low plasma tocopherols and tocotrienols levels are associated with increased odds of MCI and AD.
Wright et al. [71]	To examine whether baseline serum α-tocopherol concentrations are associated with total and cause-specific mortality	A prospective cohort study Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study 29 092 Finnish male smokers aged 50–69 y who participated in the study Fasting serum α-TOH was measured at baseline by using HPLC	• Higher circulating concentrations of α-tocopherol within the normal range are associated with significantly lower total and cause-specific mortality in older male smokers
Meydani et al. [72] Meydani et al. [73]	The effect of vitamin E supplementation and in vivo immune response in healthy elderly subjects	Elderly (n = 88); age ≥ 65 60, 200, 800 mg/d for 235 days	• ⇑DTH and antibody titer to hepatitis B and tetanus • with 200 and 800 mg • Subjects in the upper tertile of serum α-TOH concentration (>48.4 μmol/L [2.08 mg/dL]) after supplementation • dose of 200 IU vitamin E was shown to be most effective in improving T cell–mediated functions, compared with 60- or 800-IU/d doses
Pallast et al. [74]	The effect of 50- and 100-mg vitamin E supplements on cellular immune function in noninstitutionalized elderly persons	Elderly (n = 161); age 65–80 50, 100 mg/d for 6 months baseline plasma α-TOH 29.4 ± 6.9 μmol/L	• ⇑Number of positive DTH response with 100 mg • ⇑Diameter of induration of DTH response in a • ⇔ IL-2 production • significant trend toward increased postintervention plasma α-TOH • 50 mg supll: ⇑ by 10.1 ± 5.0 μmol/L • 100 mg supll: ⇑ by 15.8 ± 7.4 μmol/L

Source	Aim of the study	Treatment	Results and conclusion of the study
Ohrvall et al. [75]	To determine the tocopherol concentrations in serum after two diets with identical nutrient content but with different fat quality	20 moderately hyperlipidemic, healthy subjects (6 females and 14 males) double-blind cross-over study two isoenergetic diets in a randomized order during two 3-week periods, wash-out period of 3–4 weeks	• ⇑lipid-corrected serum concentrations of α- and γ-TOH during the diet rich in rapeseed oil (by 7 and 23%, respectively, $P < 0.001$) compared with on the baseline diet, while these concentrations ⇓ (by 5 and 37%, respectively, $P < 0.01$) during the diet rich in saturated fat • ⇓ ratio between α- and γ-TOH significantly during the rapeseed oil diet (−23%, $P < 0.01$) and ⇑ (+46%, $P < 0.001$) during the butter diet

Table 5.
Selection of prospective observational studies regarding serum α-tocopherol concentrations.

impaired absorption of the vitamin. Vitamin undersupply is characterized as an intake issue and they can result from insufficient dietary intake, which does not achieve reference values [31]. Because of an abundance of tocopherols in the human diet, its deficiency is rare except in individuals with pancreatic insufficiency or other conditions causing substantial fat malabsorption, or protein-energy malnutrition and may be caused by rare genetic defects affecting vitamin E metabolism or transport [4].

Vitamin E can be mobilized from adipose tissue for a relatively long time [77], so that the symptoms of slightly vitamin E deficiency may manifest following many years, even decades [59].

Nevertheless, a severe vitamin E deficiency may reveal itself almost immediately in acute symptoms such as neuro- and myopathy, as vitamin E is essential for an optimal development and condition of the central nervous system [78]. Insufficient vitamin E saturation can occur in intestinal resection, in severe liver disease (e.g., biliary cirrhosis) and in cystic fibrosis (less frequently). In the absence of vitamin E, the accumulation of radicals with lipoperoxidation in humans leads to various defects in membrane function, muscle metabolism and the nervous system [1]. These reactions should be considered if vitamin E is not absorbed or cannot be used.

Next to dietary habits, hereditary disorders are known to cause primary and secondary vitamin E deficiencies or inadequate vitamin E bioavailability [59].

Although several foods contain naturally occurring sources of vitamin E, it is frequently the case that the intake recommendations are not achieved. Several other dietary factors affect the need for vitamin E. Two are most important in this regard: selenium and PUFAs.

Selenium spares the need for vitamin E. In contrast, the dietary intake of PUFAs directly affects the need for vitamin E. Previous studies have established values neccessary for the incremental impact of dietary PUFAs on the nutritional demand for vitamin E in the range of 0.18–0.60 mg α-tocopherol/g PUFA. Even though the upper limit of the established range is often noted as a guideline to estimate the needs for vitamin E, it must be said that there is no consensus with respect to the quantitation of this certainly critical relationship [43].

3.4 Excess intake of vitamin E (hypervitaminosis)

Vitamin E has been viewed as one of the least toxic of the vitamins. No syndrome of acute vitamin E toxicity has been described. Both animals and humans appear to be able to tolerate rather high levels [43].

When obtained from food sources alone, vitamin E has no documented evidence of toxicity. However, evidence of pro-oxidant damage has been found to be associated with supplements, but usulally only at very high doses (for example at >1000 mg/d) [13, 79]. In the case of humans, daily doses as high as 400 IU are recognized to be nontoxic, while high oral dosages reaching up to 3200 IU, have not been revealed to have any persistent adverse effects [43].

These opinions were questioned a few years ago by a meta-analysis comprising 19 trials, and hypothesizing that supplemental vitamin E (≥400 IU/day) could contribute to an all-cause mortality [43]. Nevertheless, a recently published meta-analysis which comprised even a larger set (57) of trial data, suggested that vitamin E supplements do not have an impact on the all-cause mortality even at doses up to 5500 IU/day [80]. In premature infants, high-dose vitamin E treatment was associated with increased risk for sepsis. Chronic intake of supplements in excess of 400 IU daily has been associated with increased risk of hemorrhage and all-cause mortality [4].

Factors, they could influence the interpretation of data from studies focused on intake of vitamin E, are several: e.g., the NHANES study [25] reported the most data on serum concentrations, differentiated by gender, age group, and race; the EPIC study [81] focused on intake levels, differentiated by country, gender, and age categories, whereas race was not differentiated. No distinction has been made between representative and nonrepresentative studies. No consideration could be given to the quality of the dietary assessment data or to the standardization of blood assays in different studies, and supplement use was not always sufficiently reported [17].

Higher vitamin E doses than the RDA seem to significantly increase the general mortality. In a meta-analysis by Bjelakovic et al. [36] vitamin E at a dose above the RDA (> 15 mg) significantly increased the mortality of the subjects (RR 1.03, 95% CI 1.00 to 1.05, I2 = 0%). The effects of vitamin E on the mortality seemed neutral when administered in doses within the RDAs, however the available data are sparse.

In observational studies, high α-tocopherol intake was reported to be associated with a lower risk of cardiovascular disease, type 2 diabetes, hypertension, cancer, loss of cognitive function, and Alzheimer's disease [82]. Nevertheless, randomized, placebo-controlled intervention trials did not support these observations [25]. Recent studies speculate about possible adverse effects of high dose vitamin E supplements [25]. To avoid risks associated with high-dose nutritional supplements, emphasis on an optimal food intake of vitamin E within the range of the DRI is crucial.

4. Conclusions

Dietary intake recommendations for vitamin E are set in many countrie, however there is an ongoing need to review, establish, and harmonize dietary vitamin E requirements and daily allowance across populations. It has become clear that despite a major scientific progress, new understanding on a molecular level, as well as a broad variety of animal and human studies generating valuable data, the challenge to agree upon general and uniform dietary intake recommendations for vitamin E remains persistent. The key element in defining the recommended dietary recommendations for essential vitamin E is, of course, the biomarker chosen, and all agencies and science authorithis are trying to agree on a suitable biomarker. In future, more dietary intake data as well as status data are needed for specific

subgroups to adjust recommendations for vitamin E intake. However, well-founded recommendations are a reflection of current nutritional science and certainly not a definitive opinion. We are aware that research in the field of nutrition will bring new knowledge and conclusions, and that the nutritional recommendations for vitamin E within the European area will also have their dynamism and development. At present, only expertly based and transparently compiled recommendations can succeed and be applied in practical life. We believe that this emerging knowledge is worth of consideration to improve nutritional recommendations and the criteria to design the next generation of prevention trials on age-related and chronic diseases. More research is needed to understand the molecular action of metabolites and/or their targets in order to further develop therapeutic strategies and improve nutritional recommendations on vitamin E.

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Conflict of interest

The autors declare no conflict of interest.

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Chapter 10

Biosynthesis Pathways of Vitamin E and Its Derivatives in Plants

Makhlouf Chaalal and Siham Ydjedd

Abstract

Naturally occurring vitamin E, comprised of four forms each of tocopherols and tocotrienols, are synthesized solely by photosynthetic organisms and function primarily as antioxidants. The structural motifs of the vitamin E family and specifically the chroman moiety, are amenable to various modifications in order to improve their bioactivities towards numerous therapeutic targets. Tocopherols are lipophilic antioxidants and together with tocotrienols belong to the vitamin-E family. These lipid-soluble compounds are potent antioxidants that protect polyunsaturated fatty acids from lipid peroxidation. Biosynthetic pathways of plants producing a diverse array of natural products that are important for plant function, agriculture, and human nutrition. Edible plant-derived products, notably seed oils, are the main sources of vitamin E in the human diet. The biosynthesis of tocopherols takes place mainly in plastids of higher plants from precursors derived from two metabolic pathways: homogentisic acid, an intermediate of degradation of aromatic amino acids, and phytyldiphosphate, which arises from methylerythritol phosphate pathway. Tocopherols and tocotrienols play an important roles in the oxidative stability of vegetable oils and in the nutritional quality of crop plants for human and livestock diets. Here, we review major biosynthetic pathways, including common precursors and competitive pathways of the vitamin E and its derivatives in plants.

Keywords: Vitamin E, Biosynthetic pathways, Tocopherols, Tocochromanol, Shikimate pathway, Methylerythritol pathway

1. Introduction

Under biotic and abiotic stresses conditions, including pathogens, temperature, drought, salt, and high light, the reactive oxygen species (ROS) resulting the oxidation of cellular components, as proteins, chlorophyll, and lipids [1]. To defend against oxidative stress, the plants have developed two general protective mechanisms, enzymatic and non-enzymatic detoxification, of which the latter involves vitamin E [2].

Plants are a major source of vitamins in the human diet. Due to their significance for human health and development, research has been initiated to understand the biosynthesis of vitamins in plants [3]. Vitamin E is thought to be involved in many essential processes in animals and plants. The function of vitamin E in plants is far from being clear. Likewise, in animal cells, the vitamin E acts as an antioxidant, thus it protects the plant from oxygen toxicity.

Four different forms of tocopherols and tocotrienols occur in nature and differ by the numbers and positions of methyl groups on the aromatic portion of the chromanol head group (**Figure 1**).

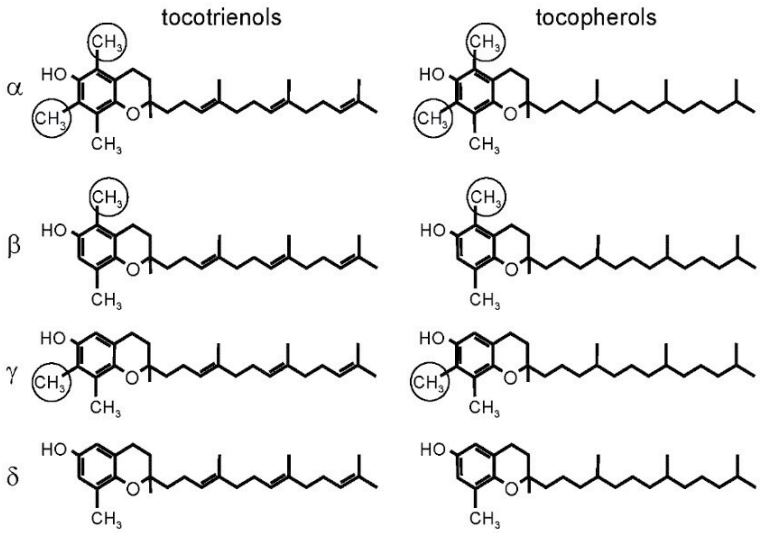

Figure 1.
The eight forms of naturally occurring vitamin E (or tocochromanols) [4].

Sources	Plant organs	Usable Products	Vitamin E contents (g/kg)
Wheat	Kerne	Germ	1500
Sunflower	Seed	Oil	610
Sunflower	Seed	Kernel	351
Almond	Kernel	Oil	392
Safflower	Kernel	Oil	450
Canola	Seed	Oil	270
Walnut	Fruit	Oil	200
Peanut	Seed	Edible nut	172
Palm	Kernel	Oil	150
Olive	Seed	Oil	120
Soybean	Kernel	Oil	116
Maize	Seed	Entire grain	20
Oat	Seed	Kernel	15
Coconut	Seed/fruit	Oil	10
Asparagus	Shoot Young	shoot	15
Spinach	Leaf	Raw leaf	20
Spinach	Leaf	Cooked leaf	21
Tomato	Fruit	Raw fruit	9
Carrot	Root	Taproot	6
Tobacco	Leaf	Young leaf	57
Tobacco	Leaf	Old leaf	180

Table 1.
Vitamin E content in different cultivated plant species (reported by Has).

Only plants and some cyanobacteria are able to synthesise vitamin E. α-Tocopherol is the predominant form of vitamin E green parts of higher plants, and is synthesized and localised mainly in plastids, whereas generally in non-photosynthetic tissues, γ-tocopherol is the major form [5].

The accumulation of vitamin E was varied in a number of plant species and in different plant parts. Generally, their content was ranged between 100 and 500 mg/kg fresh weight of normal plants with some exceptions. Oil-yielding plants present a higher vitamin E amount. Likewise, the seeds showed a highest total vitamin E content compared to other plant parts. **Table 1** indicates the amount of α-tocopherol in different plant species. In seeds, the vitamins were localized in plastids; however, in some cases it was also observed in cytoplasmic lipid bodies [6]. Commonly, α-Tocopherol was the major form of vitamin E in leaves, while many plants seeds contain γ-Tocopherol. Heowever, β-tocophenrol and δ-Tocophenrol are uncommon in plants [1, 7]. Thus, this work complements highlighted the biosynthetic origins of vitamin E biosynthetic precursors in plants.

2. Biosynthesis of vitamins in plants

The biosynthesis of different vitamins in plants has been carried out generally by bacterial pathways, except in the case of vitamin C, which is synthesized exclusively by eukaryotes. The biosynthesis of some vitamins is limited to the compartment as carotenoids (pro-vitamin A), vitamins E and K and water-soluble riboflavin are produced in the plastids of plants [8, 9]. However, some enzymes of phylloquinone biosynthesis have been found in peroxisomes [10] and riboflavin is further converted to flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) in the cytosol, plastids or mitochondria [11]. Furthermore, the biosynthesis of the water soluble vitamins is split between different compartments, including the mitochondria [12] (**Figure 2**).

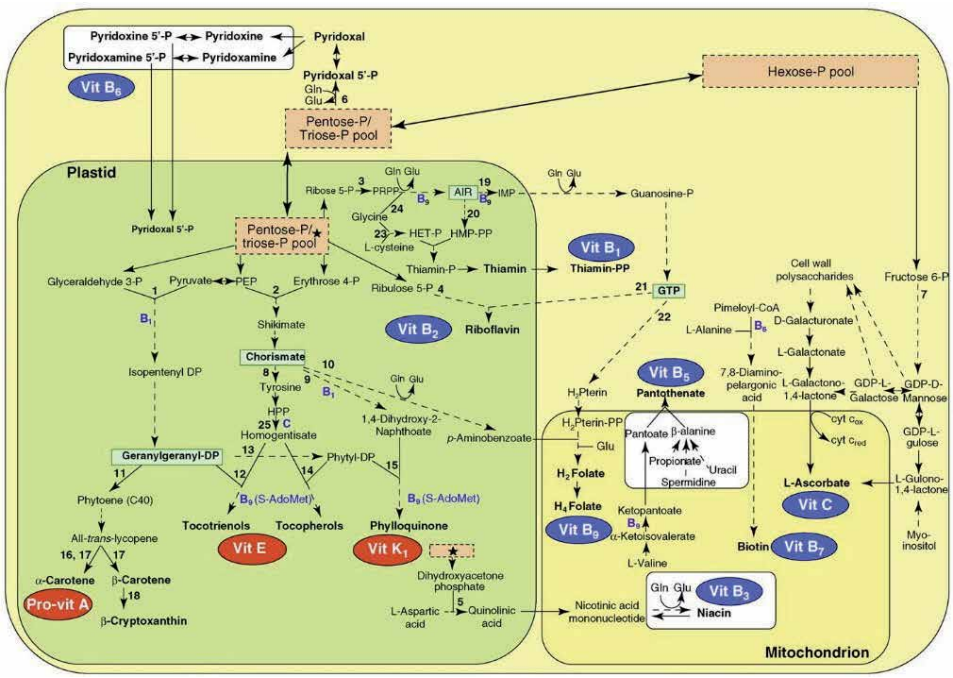

Figure 2.
Cross-points on the biosynthetic pathways of vitamins in plants [13].

The vitamins precursors were coming from carbohydrate metabolism, which regulates the pools of hexoses, pentoses and trioses in the plastids and the cytosol. The pentose and triose pool in the plastids provides: (a) erythrose-P and phosphoenolpyruvate for the synthesis of chorismate, the common intermediary in the biosynthesis of tocochromanols [14, 15]; (b) glyceraldehyde 3-P and pyruvate (from phosphoenolpyruvate), which are required for the synthesis of geranylgeranyl-PP, a key shared precursor of lipid-soluble vitamins [8, 14].

3. Vitamin E structures and biosynthesis

Plants synthesize eight different molecules with vitamin E antioxidant activity, including α-, β-, γ-, and δ-tocopherols and the corresponding four tocotrienols. These forms were different with respect the number and position of the methyl groups on their chromanol ring. The tocotrienols have an unsaturated tail containing three double bonds, while the four tocopherols have a phytyl tail.

Two main pathways of vitamin E biosynthesis are occurs at the inner envelope of plastids. The shikimate pathway gives rise to the chromanol ring from homogentisate (HGA). While, the methylerytrithol phosphate (MEP) pathway provides the prenyl tail from geranylgeranyl diphosphate (GGDP) for the synthesis of tocotrienol and phytyl diphosphate (phytyl-DP) for the synthesis of tocopherol (**Figure 3**). Furthermore, an additional pathway for phytyl-DP production from chlorophyll degradation, also known as the phytol recycling pathway (**Figure 4**). Seeds and leaves showed 80% and 65% reductions in total tocopherol content, respectively, compared to other plant parts. Chlorophyll synthase and geranylgeranyl diphosphate reductase (GGDR) are also involved in

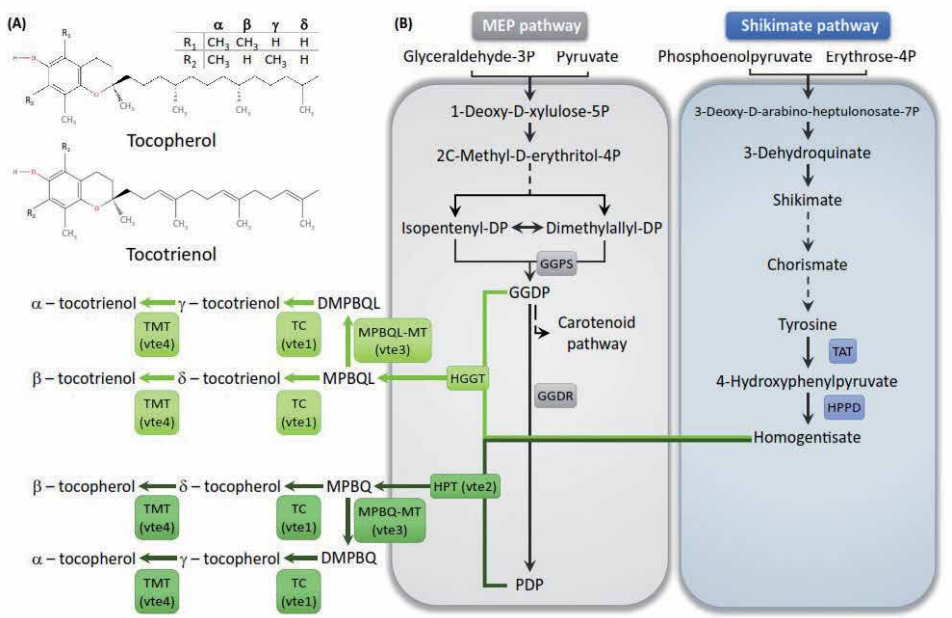

Figure 3.
Vitamin E Chemical Structure and Biosynthesis in Plants [16]. (A) Vitamin E chemical structure. A chromanol head and a prenyl tail constitute the chemical structure of tocopherols and tocotrienols. While tocopherols have a saturated tail, tocochromonals have three unsaturations (orange lines), at 3', 7', and 11'. (B) Biosynthesis of tocopherols and tocotrienols in plants. Tocopherols and tocotrienols are formed from the combination of the methylerythritol phosphate and shikimate pathways.

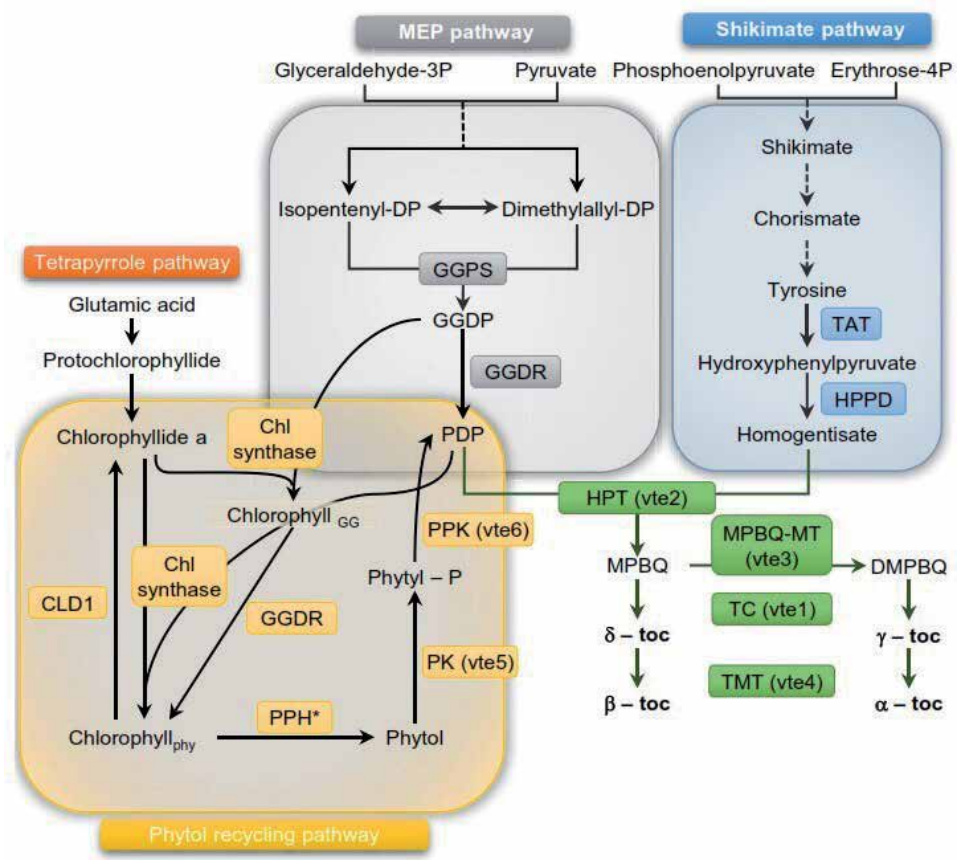

Figure 4.
Tocopherol Biosynthesis with Chlorophyll Degradation in Plants [16].

vitamin E biosynthesis [17]. The identity of the enzymes involved in chlorophyll dephytylation is less clear and the hydrolases such as CLD1 may allow phytol remobilization during fruit ripening and seed maturation [18, 19].

4. Tocopherols biosynthetic pathway

Tocopherols are found in higher plants, in algae, and in some nonphotosynthetic plants, such as yeasts and mushrooms [20]. Tocopherol biosynthesis was carried out via the condensation of homogentisate, derived from the shikimate pathway, and phytyl pyrophosphate (phytyl-PP), derived from the non-mevalonate pathway, through the action of the homogentisate prenyltransferase (HPT) (**Figure 5**). Subsequent ring cyclization and methylation reactions result in the formation of the four major tocopherol derivatives. The final methylation reaction resulting inα - and β-tocopherol, respectively, is expected to be catalysed by the same methyltransferase (γ-TMT) [21]. Theγ -TMT gene was isolated from the putative 10-gene tocopherol biosynthetic operon in *Synechocystis* sp.

4.1 Shikimate pathway

The shikimate pathway has been found in plants and in some microorganisms serves as a biosynthetic way of aromatic amino acids (phenylalanine (Phe),

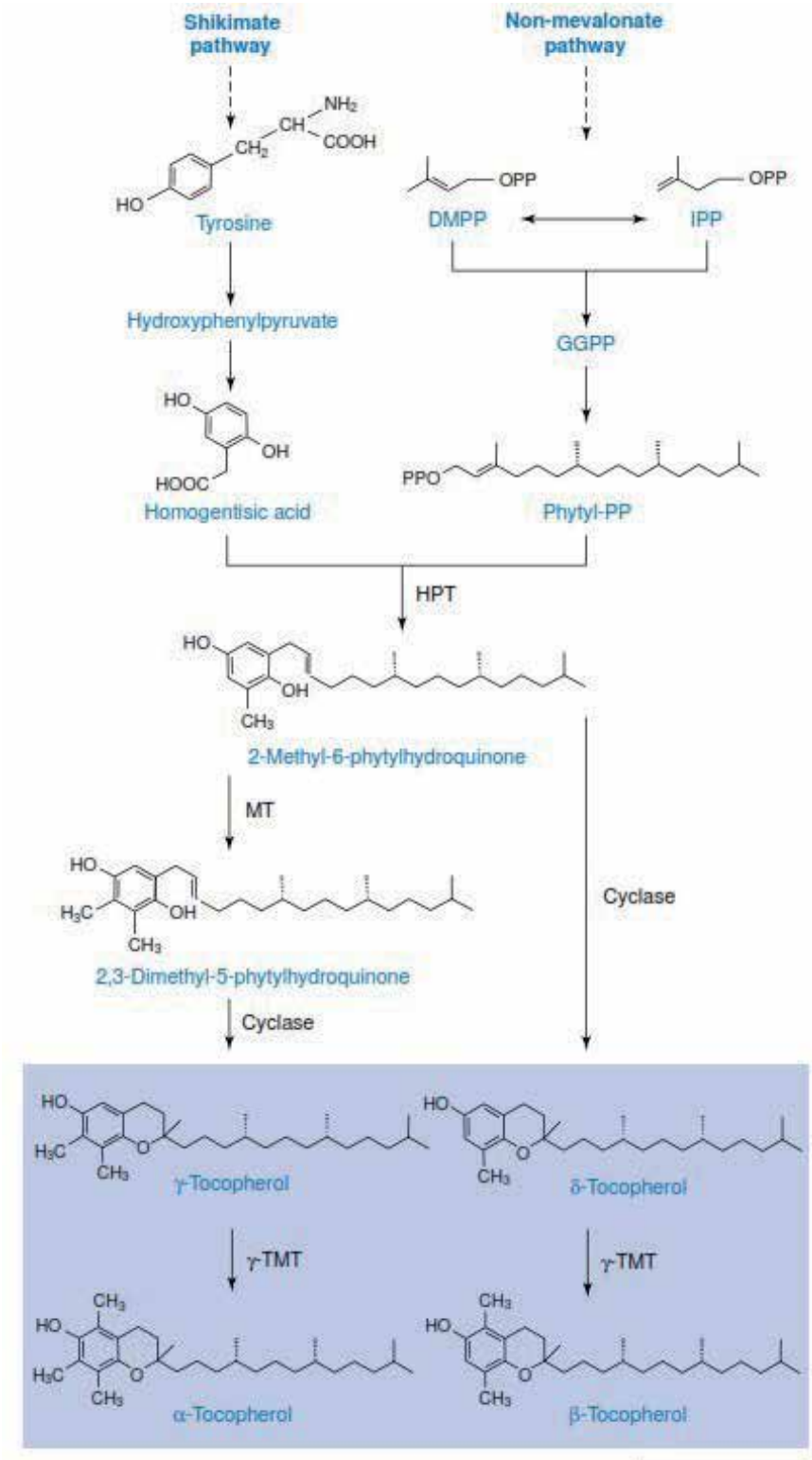

Figure 5.
Vitamin E biosynthetic pathway [21]. The blue box highlights the four naturally occurring tocopherol derivatives in plants.

tyrosine (Tyr) and tryptophan (Trp)), and as precursors for many secondary metabolites, such as pigments, vitamins, etc. [22]. It consists of seven steps where the glycolytic intermediate phosphoenol pyruvate and the pentose phosphate pathway intermediate erythrose-4-phosphate are converted in chorismate (**Figure 6**). Numerous synthases, dehydratases and kinases are involved in this pathway, but their participation in tocopherols biosynthesis is not clear. The limitation step in the shikimate pathway are the reversible formation of

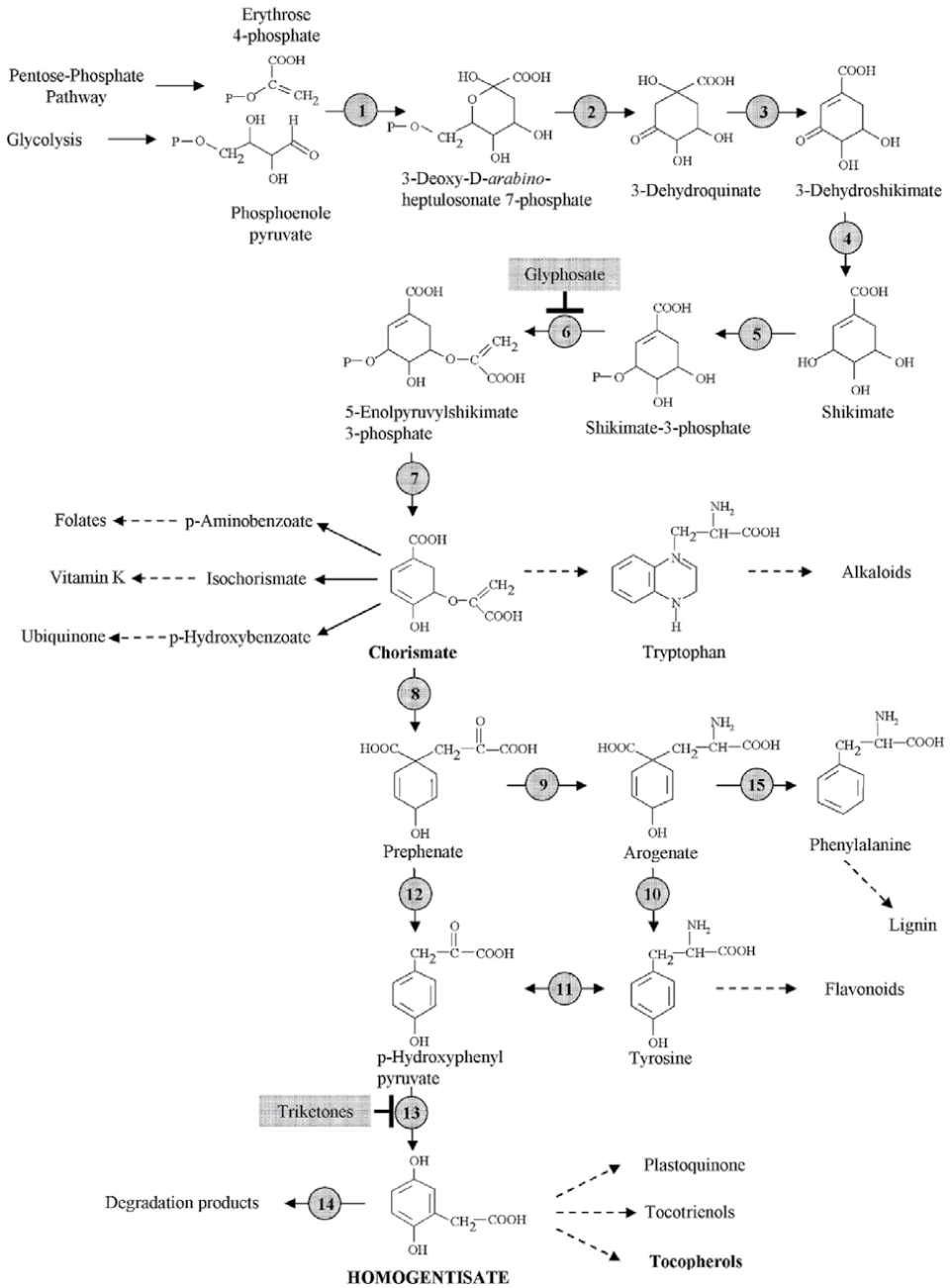

Figure 6.
The shikimate pathway of homogentisate biosynthesis in photosynthetic organisms [23].

5-enolpyruvylshikimate 3-phosphate (EPSP) and inorganic phosphate from shikimate 3-phosphate and phosphoenolpyruvate. Likewise, the reaction is catalyzed by EPSP synthase (EC 2.5.1.19), which is the unique target for herbicide glyphosate (N-phosphonomethylglycine) [24]. Glyphosate interacts with the binding site of phosphoenolpyruvate and forms a stable ternary complex with the enzyme and shikimate 3-phosphate Likewise, Chorismate is the end product of the shikimate pathway and, at the same time, is a precursor for many primary and secondary metabolites, such as vitamin-K, folates, alkaloids,

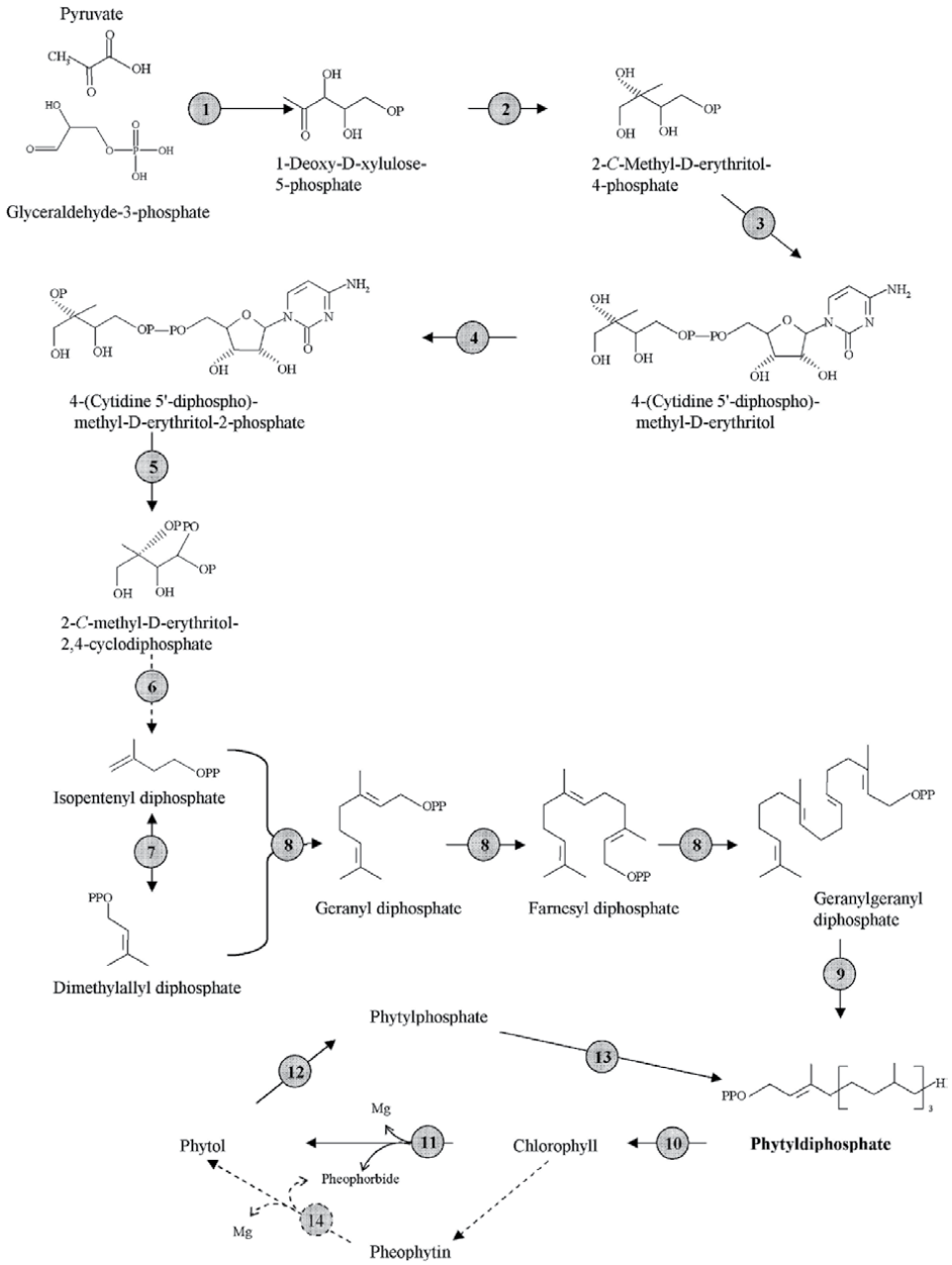

Figure 7.
Methylerythritol pathway of phytyl diphosphate biosynthesis in the plastids of higher plants [23].

quinones, tocopherols and three aromatic amino acids (Phe, Tyr and Trp) [23]. *p*-Hydroxyphenylpyruvate (HPP) is the first intermediate in tocopherol biosynthesis. Different ways of HPP synthesis exist in photosynthetic organisms. In higher plants, it is formed from prephenate via arogenate and tyrosine. A portion of fixed carbon is incorporated into Tyr used for biosynthesis of HPP and homogentisate, a tocochromanol (tocopherols and tocotrienols) precursor [25].

The formation of homogentisate from HPP occurs in the reaction catalyzed by HPPD. Homogentisate may either enter the prenylquinone biosynthesis pathway or be metabolized by homogentisate dioxygenase (EC 1.13.11.5) to yield maleylacetoacetate, which further is catabolized to fumarate and acetyl-CoA [23].

4.2 Methyl erythritol phosphate (MEP) synthesis

The plastidic 2C-methyl-D-erythritol 4-phosphate (MEP) pathway produces isopentenyl diphosphate (IPP) that is used for the biosynthesis of isoprenes, monoterpenes (C_{10}), diterpenes (C_{20}), carotenoids, plastoquinones, and phytol conjugates such as chlorophylls and tocopherols.

The first step in the MEP pathway involves a transketolase-type condensation reaction of pyruvate and glyceraldehyde 3-phosphate to form 1-deoxy-D-xylulose-5-phosphate (DOXP) (**Figure 7**), which is also an intermediate in the biosynthesis of thiamin and pyridoxol [26–28]. The formed isopentenyl diphosphate is further isomerized to DMAPP. However, the IPP is suggested to be the final production of the MEP pathway in higher plants [26]. The chlorophyll-derived phytol may be a precursor for the biosynthesis of tocopherols, because, the accumulation of tocopherol negatively correlated with chlorophyll content in some plant species during leaf senescence [29].

5. Tocochromanol biosynthetic pathway

According to the degree of methylation of the chromanol ring, four different forms of tocochromanol was obtained (**Figure 8**). Four forms of tocopherol, tocotrienol, and tocomonoenol have been identified in wild-type plant extracts, only the solanesyl-derived tocochromanol PC-8form is exists in the nature [30].

It has been assumed for a long time that tocochromanol biosynthesis was the exclusive appanage of plants, algae, and some cyanobacteria that are all photosynthetic organisms. Tocochromanol biosynthesis is initiated by the condensation of the polar aromatic head HGA with various lipophilic polyprenyl pyrophosphates that determine the type of tocochromanol. The condensation reaction is catalyzed by three types of HGA prenyltransferases that possess each their substrate specificities. Tocopherol synthesis is initiated by HGA phytyltransferases (HPTs) that condense HGA and PPP. The condensation between HGA and polyprenyl pyrophosphates produces 2-methyl-6-phytyl-1,4-benzoquinol (MPBQ), 2-methyl-6-geranylgeranyl- 1,4-benzoquinol (MGGBQ), 2-methyl-6-solanesyl-1,4-benzoquinol (MSBQ), and 2-methyl-6-tetrahydrogeranylgeranyl-1,4-benzoquinol (MTHGGBQ) for tocopherols, tocotrienols, PC-8, and for tocomonoenols, respectively (**Figure 8**). Finally, tocochromanol biosynthesis consists of the methylation of γ- and δ-tocochromanols intoα - and β-tocochromanols, respectively [31, 32]. In Arabidopsis leaves and seeds, VTE4 converts γ anjdδ -tocopherols intoα - and β-tocopherol, respectively [32].

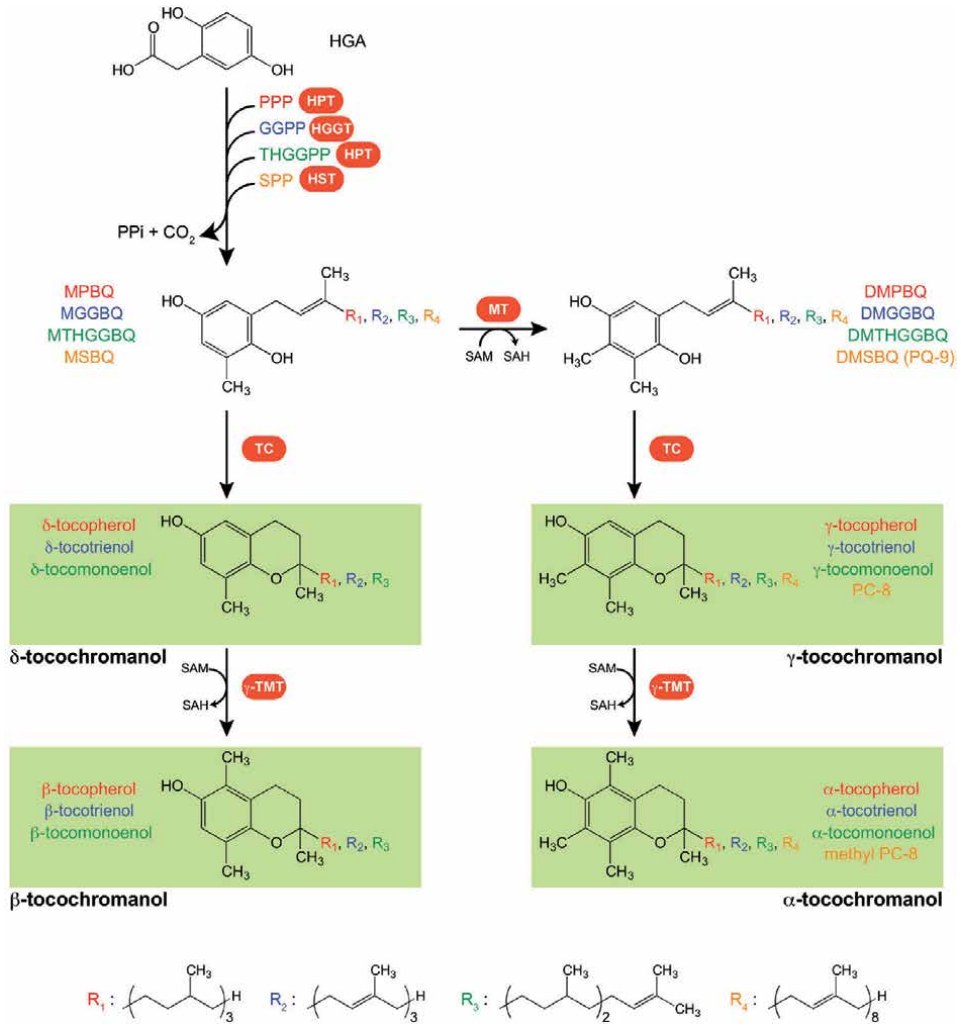

Figure 8.
Tocochromanol (tocopherol, tocotrienol, tocomonoenol, and methyl PC-8) biosynthetic pathways in plants [30].

In addition, transgenic Arabidopsis lines overexpressing the barley HGGT gene notably produceα -tocotrienol [33].

6. Conclusion

Vitamin E biosynthesis mobilizes two distinct biosynthetic pathways, the shikimate pathway and the MEP pathway. Indeed, the shikimate pathway gives rise to the chromanol ring from homogentisate (HGA). While, the methylerytrithol phosphate (MEP) pathway provides the prenyl tail from geranylgeranyl diphosphate (GGDP) and phytyl diphosphate (phytyl-DP) for the synthesis of tocotrienol and tocopherol, respectively. An additional pathway for phytyl-DP production from chlorophyll degradation, known as the phytol recycling pathway. Understanding the regulation of vitamin E biosynthesis will imply that we take up the challenges to understand the regulation of each of these numerous events. The fundamental role of this vitamin in human reproduction and its benefit in current widespread diseases such as high cholesterol and neurodegenerative pathologies makes it a candidate of choice to improve human health.

Acronyms and abbreviations

AIR	5-aminoimidazole ribonucleotide
CLD1	chlorophyll dephytylase 1
DMGGBQ	dimethylgeranylgeranylbenzoquinol
DMPBQ	dimethylphytylbenzoquinol
DMPP	dimethylallyl pyro-phosphate
GGDP	geranylgeranyl diphosphate
GGDR	geranylgeranyl diphosphate reductase
GGPP	geranylgeranyl pyrophosphate
GGPS	geranylgeranyl diphosphate synthase
HET-P	4-methyl-5-b-hydroxyethyl thiazole phosphate
HGGT	homogentisate geranylgeranyl transferase
HMP-PP	2-methyl-4-amino-5-hydroxymethylpyrimidine diphosphate
HPP	hydroxyphenylpyruvate
HPPD	hydroxyphenylpyruvate dioxygenase
HPT	homogentisate phytyl transferase
IMP	inosine monophosphate
IPP	isopentenyl pyrophosphate
MEP	methylerythritol phosphate
MGGBQ	methylgeranylgeranylbenzoquinol
MPBQ	methylphytylbenzoquinol
MPBQ-MT	MPBQ methyltransferase
MT	2-methyl-6-phytylhydroquinone methyltransferase
PDP	phytyl diphosphate
phytyl-PP	phytyl pyrophosphate
PPRP	5-phosphoribosyl-1pyrophosphate
S-AdoMet	S-adenosylmethionine.
TAT	tyrosine aminotransferase
TC	tocopherol cyclase
TMT	tocopherol methyltransferase.

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Chapter 11

Tocotrienol: An Underrated Isomer of Vitamin E in Health and Diseases

Ahmad Farouk Musa

Abstract

Vitamin E was first discovered as a fertility factor in 1922 in the laboratory of Herbert McLean Evans, a scientist and anatomist. Following this discovery, it was extensively researched and found to possess a potent antioxidant property. It soon dawned that the family of vitamin E has eight members: four tocopherols, namely α-, β-, δ- and γ-tocopherol; and four tocotrienols in the form of α-, β-, δ- and γ-tocotrienols. This chapter discusses this rather unknown and underrated isomer of vitamin E with unsurpassed health benefits: tocotrienols. Until recently, tocotrienols rarely figured in vitamin E research in spite of their relative superiority to tocopherol coupled with their abundant presence in palm oil. In fact, since palm oil contains about 70% of all tocotrienol homologues, it would be no exaggeration to call it nature's best kept secret, if not the most promising natural substance in influencing health and disease. While highlighting the wonders of tocotrienols as a safe and efficacious product, this chapter offers a panoramic view of recent research into tocotrienols that demonstrates their undeniable benefits in conferring protection against cancer as well as a whole litany of ailments including cardiovascular, metabolic, autoimmune, bone and neurological diseases. Admittedly, many of these researches were conducted in the laboratory, with some preclinical trials translated into clinical trials. Nonetheless, it is hoped that more randomised clinical trials will be carried out on a global scale in the near future. From the vessels in the heart to the neurons in the brain, tocotrienols have the extraordinary potential to be the future of vitamin E research.

Keywords: vitamin E, tocotrienols, health benefits

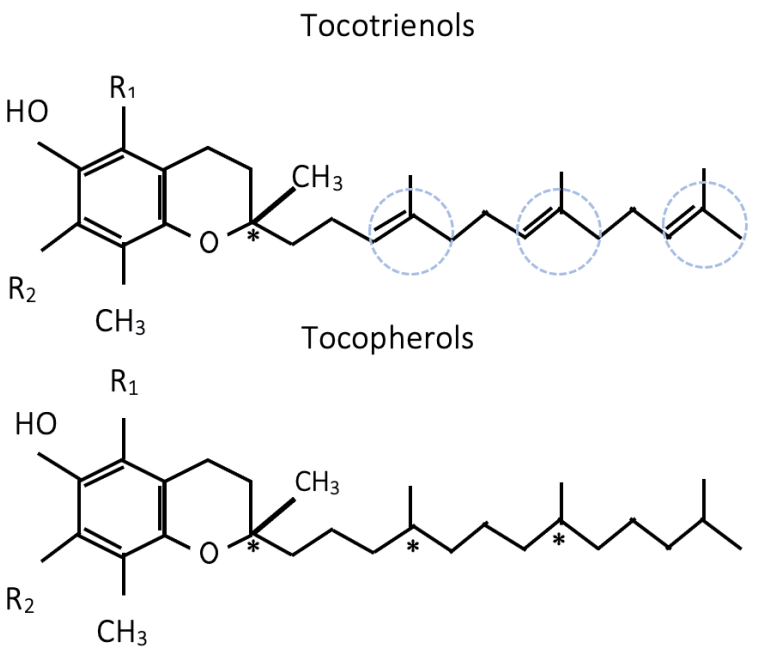
1. Introduction

Vitamin E, an important nutrient in the human diet that is readily available in lipid-rich plant products, is well known for its antioxidant properties with multiple health benefits. Historically, drug discovery researches have focused on natural products that abound in biological compounds with pharmacologic properties [1]. The discovery of vitamin E began in 1922 when Herbert Evans and Katherine Bishop [2] isolated an uncharacterised fat-soluble compound (which they termed 'substance X') from green leafy vegetables that they imagined might play a role in fertility. When the compound was finally identified in 1924, it was named tocopherol — derived from the Greek word tokos meaning childbirth and pheros which means to

bring forth [3]. Vitamin E was rediscovered as 'factor 2 antioxidant' in 1965 [4]. Not surprisingly, as the major isoform of Vitamin E ever identified, α-tocopherol was thrust into the limelight while its sisters were ignored. Tocopherol is regarded as the most biologically active and potent antioxidant currently in existence. However, recent studies have shown that tocotrienols may have superior anti-oxidant [5], anti-inflammatory [6], anti-cholesterolaemic, [7–11] anti-cancer [12, 13], anti-diabetic [14–16], anti-atherogenic [17, 18], blood pressure lowering, [19, 20] and neuroprotective effects [21–23]. Unfortunately, despite the recent interest in tocotrienols, it comprises only 3% of all vitamin E research papers listed in PubMed [24].

2. Biochemical properties of vitamin E isoforms

Generally, vitamin E is classified into tocopherols and tocotrienols, and there are eight isoforms altogether: α-, β-, γ-, and δ-tocopherol, and α-, β-, γ-, and δ-tocotrienol. Structurally, they are very similar and possess a chromanol head formed by phenolic and heterocyclic rings, and a phenyl tail [25]. Designation as α-, β-, γ- or δ-tocopherol or tocotrienols is dependent on the methyl substitutions on



Isomer	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
σ	H	H

Figure 1.
The different structures between tocopherols and tocotrienols.

the phenolic ring [26]. The main difference between the two groups is that tocopherols have a long-saturated carbon side-chain with chiral centres, whereas tocotrienols possess three unsaturated bonds in the carbon side-chain with one chiral centre [27]. This unique property somehow increases the efficiency of tocotrienols in its metabolic function; it allows a better penetration of saturated fatty layers by tocotrienols as compared to tocopherols. **Figure 1** above illustrates the difference between the structures of tocopherols and tocotrienols [28].

3. Sources of vitamin E

Vitamin E occurs naturally in vegetables, plants and plant oils. With regard to tocopherols, α-tocopherol is generally found in green leafy plants while γ-tocopherol occurs in the non-green parts of the plants, notably seeds and fruits [29]. Based on the United States Department of Agriculture nutrient database, α-tocopherol is commonly found in almonds, avocados, hazelnuts, peanuts and sunflower seeds; β-tocopherol in oregano and poppy seeds; γ-tocopherol in pecans, pistachios, sesame seeds and walnuts; and δ-tocopherol in edamame and raspberries. Both α-tocopherol and γ-tocopherol are present in food oils such as corn, peanut and soybean oil. Conversely, tocotrienols are rarely found in food sources or vegetable oils, with rice bran and palm oil being the only known exceptions. This explains the scarcity of scientific literature on tocotrienols compared to tocopherols as a form of vitamin E that is widely accepted by the public. In fact, most people are unaware that up to 70% of vitamin E from crude palm oil consists of tocotrienols [30, 31]. Studies have proven that the extraction of crude palm oil (scientifically known as *Elaeis guineensis*) can yield up to 800 mg/kg of tocotrienols in the form of α-tocotrienol and γ-tocotrienol [32]. Moreover, cereal grains such as barley, oat, rice, rye and wheat contain a higher concentration of tocotrienols than tocopherol in a ratio of 79:21, 77:23, 67:55, 51:49 and 55:45 respectively [24]. However, in food supplements, tocotrienols are often prepared in soft-gel capsules. Even at a dose of 1000 mg daily, this is equivalent to a daily intake of 16.7 mg of tocotrienol/kg/day for a 60-kg person, or about seven times below the level where no adverse effects were observed in rats [33]. Hence, the usual dosage for any experimental use in humans has always been 400 mg daily in two divided doses which is a safe dosage with no observable adverse effects on any study patients to date.

4. Bioavailability and pharmacokinetics of vitamin E

Compelling evidence from recent research prove that tocotrienols are detected at appreciable levels in the plasma after supplementation, whether this was done on a short-term or long-term basis [34]. While both compounds are basically bioavailable, tocotrienol has a shorter plasma half-life [33]. One study showed that the half-life of α-, γ- and δ-tocotrienols in human plasma was estimated to be 2.3, 4.4 and 4.3 hours respectively [35]. However, the half-life of α-tocopherol and γ-tocopherol was 57 and 13 hours respectively [36]. Given that tocotrienols are essentially oil-based compounds, and that emulsions are known to increase the absorption of oil-based compounds based on the new system of self-emulsifying drug delivery system [37], new products such as Tocovid Suprabio™ were developed following this technique. This new product led to a threefold increase in the peak plasma concentration of α-tocotrienol in humans compared to a previous study [38]. It has also been shown that the bioavailability of tocotrienols is dependent on several

factors, food status being one of them. The mean apparent volume of tocotrienol distribution values is lower in the fed state, which means that the absorption is much better than in the fasting state [35]. Undoubtedly, tocotrienols have a very different pharmacokinetics from tocopherols which remain longer in the blood-stream. However, the biodistribution of tocotrienols pointed to the accumulation of the compound in the vital organs [34]. Therefore, tocotrienols would score high in terms of therapeutic efficacy since this requires not only bioavailability but also presence in the target organs.

5. Vitamin E as an anti-oxidant

One of the most well-known effects of vitamin E is its anti-oxidant capability in inhibiting the peroxidation of lipids after its incorporation into the cellular membranes [39]. It is well documented that tocotrienol scavenges the chain propagating the per-oxyl radicals [39]. Indeed, α-tocotrienol has a much stronger anti-oxidant effect than α-tocopherol. This superiority is due to a more even distribution of α-tocotrienol in the plasma membrane as a result of a more efficient collision of α-tocotrienol with the radicals. Compared to tocopherols, tocotrienols also have a higher recycling efficiency thanks to their chromanoxyl radicals [39]. These anti-oxidant properties of tocotrienol have a lasting impact on health in general. For instance, patients with hyperlipidae-mia and carotid stenosis have been shown to demonstrate a significant reduction in thiobarbituric-acid-reactive substances that were related to platelet peroxidation. It is also proven that tocotrienols have the ability to scavenge free radicals that cause DNA damage, hence providing protection especially to the older age group [40].

6. Preventing cardiovascular diseases

One of the most feared ailments is cardiovascular disease, and with 17.9 million deaths every year, the World Health Organization (WHO) considers it the main cause of death globally [41]. WHO estimated that out of five mortalities from cardiovascular diseases, four were caused by heart attacks and strokes, with almost a third of these deaths occurring in people less than 70 years of age [41]. Among the modifiable risk factors of atherosclerosis that cause heart attacks are hyperlipidae-mia, hypertension, diabetes mellitus and thrombosis. Given its ability as a lipid-lowering, blood pressure-lowering, anti-diabetic and anti-thrombogenic agent, the effects of vitamin E – especially tocotrienol – deserve a thorough investigation.

6.1 Prevention of atherosclerosis

Atherosclerosis is considered the most important event that leads to heart attack and stroke as a result of abnormal deposits of lipids, cholesterol and plaque build-up. Animal studies have been conducted in rabbits to look at the microscopic development of atherosclerosis and lipid peroxidation. After ten weeks of treatment with tocotrienol-rich fraction (TRF) which normally consists of 80% tocotrienol and 20% tocopherol, the researchers [42] found that the cholesterol-fed rabbits had a lower content of malondialdehyde (MDA) or modified low density lipoprotein – a diagnostic biochemical marker for atherosclerosis. The rabbits also had less intimal thickening, while their internal elastic lamina was better preserved compared to those fed on a normal diet. Another study [43] showed that atherosclerosis is prevented through the modulation of the peroxisome proliferator-activated recep-tors (PPAR) when TRF is administered. A different study [44] found that, after

being given TRF, subjects undergoing chronic haemodialysis showed improvement in their high-density lipoprotein (HDL), triglycerides and total plasma cholesterol as compared to placebo. All these studies prove that TRF intake essentially improves the lipid profiles, thereby preventing atherosclerosis.

6.2 Protection against reperfusion injury

Another important issue is related to myocardial ischaemia reperfusion injury. This happens when blood flow is restored to an infarcted myocardium either via percutaneous transluminal coronary angioplasty or bypass surgery. Restoring the blood flow, a process known as reperfusion, could also cause injury to the heart muscles and is therefore termed as myocardial ischaemia reperfusion injury. Reperfusion injury could account for almost 50% of the final size of myocardial infarction [45]. Almost all isoforms of tocotrienol have been shown to have cardioprotective effect. Nonetheless, γ-tocotrienol is demonstrably the most potent in myocardial ischaemic injury model. This particular study [46] shows that the interaction between mitogen-activated protein kinase (MAPK) with caveolin and proteasome plays an important role in the cardioprotective effect of tocotrienol that is achieved by altering the availability of pro-survival and anti-survival proteins.

6.3 Reduction of thromboembolic events

The discussion on this topic would be incomplete without any mention of thromboembolic events. In one animal study on dogs [47], an injection of intravenous tocotrienols and tocopherols was administered; it was noted that the cyclic flow that measures the acute platelet-mediated thrombus formation and collagen-induced platelet aggregation was significantly reduced in those receiving tocotrienol compared to those injected with tocopherol.

All these data suggest that tocotrienols provide better cardioprotective effect than tocopherols insofar as myocardial infarction, stroke or thrombosis is concerned.

7. Lipid-lowering effect

Studies on the hypercholesterolaemic properties of tocotrienols have gained traction after it was shown that the addition of tocotrienols significantly lowered the cholesterol level [48]. This effect was mediated by the inhibition of HMG-CoA reductase by post-transcriptional suppression of the enzyme itself by tocotrienols [49]. Indeed, γ-tocotrienol has been observed to have a dramatic 30-fold activity in inhibiting HMG-CoA reductase [50]. A later study further indicated that the American Heart Association Step 1 diet and TRF25 (25–200 mg/day) from rice bran could reduce the total cholesterol, LDL, triglycerides, and also apolipoprotein B in hypercholesterolaemic patients [51]. Another study demonstrated that when 30 mg tocotrienols are mixed with 270 mg flavonoids, the total serum cholesterol level, LDL, triglycerides and apolipoprotein B are also reduced in hypercholesterolaemic patients [52]. Furthermore, hypercholesterolaemic patients with non-alcoholic fatty liver disease who were treated with mixed-tocotrienols showed a higher percentage of normal liver echogenic response [53]. A study on atherogenesis using human monocyte-macrophages showed that α-tocotrienol, like the new compound FeAOX-6 which combines both the anti-oxidant structural features of tocopherols and carotenoids, reduced the cholesterol accumulation in the cells, with α-tocotrienol having a more potent effect [54].

8. Anti-diabetic effect

Diabetes mellitus – which has risen dramatically in all countries irrespective of their income levels – is a chronic metabolic disease characterised by elevated blood sugar level that could affect the eyes, kidneys and nerves in the long run. While Type II diabetes develops when the body becomes resistant to insulin, Type I diabetes arises when the pancreas produces less or no insulin at all. According to current WHO estimates, approximately 422 million people worldwide suffer from diabetes [55]. Indeed, about 1.6 million deaths are attributed to diabetes on a yearly basis [55]. Alarmingly, these dismal numbers have been growing steadily in the last few decades.

Studies on the antidiabetic effects of vitamin E were conducted as early as the 1990s to determine any possible association between vitamin E and diabetic risks [56–58] as well as the correlation between the dietary intake of vitamin E and insulin action [59, 60]. In a 2004 study with a very long follow-up, it was demonstrated that the intake of vitamin E reduces the risk of Type II diabetes onset [61]. It was also found that TRF reduces the total cholesterol level, low-density lipoprotein (LDL) and total plasma lipid in diabetic patients [62]. Patients who were given canola oil enriched with tocotrienol also showed a significant reduction in their urine microalbumin and the serum C-reactive protein (CRP) known for its protective effect on the kidney and against nitrosative stress [63]. In an animal model, it was observed that both TRF and α-tocopherol improved the vascular endothelial function in streptozotocin-induced diabetic rats through their sparing effect on endothelium derived nitric oxide bioavailability [64]. Another study determining the effects of TRF on erythrocyte membranes and leukocyte deoxyribonucleic acid (DNA) damage in streptozotocin-induced diabetic rats revealed that daily supplementation of tocotrienol for four weeks could inhibit lipid peroxidation while increasing the level of antioxidant markers [65]. In an animal study on the cognitive function and neuroinflammatory cascade in streptozotocin-induced diabetes, it was shown that the administration of tocotrienol significantly prevented behavioural, biochemical and molecular changes associated with diabetes. This points to the potential benefit of tocotrienol in preventing diabetic encephalopathy [66].

8.1 Preventing diabetic nephropathy

Diabetic nephropathy is a common complication of both Type I and Type II diabetes. Diabetic nephropathy (also called clinical nephropathy, proteinuria or microalbuminuria) is defined by the presence of protein of >0.5 g/24 h in the urine [67] and it increases the risk of death. In an animal study [68] designed to investigate the impact of tocotrienol in streptozotocin-induced diabetes in terms of renal function and reno-inflammatory cascade, it was found that tocotrienol has a more profound effect than tocopherol in preventing biochemical and molecular changes associated with diabetes [68]. It was concluded from the study [68] that tocotrienol modulates the release of pro-fibrolytic cytokines, apoptosis, the ongoing inflammation, and the associated oxidative stress, which confers a renoprotective effect on the kidneys. Another study [69] was designed to determine whether TRF from palm oil (PO) or rice bran oil (RBO) could improve the renal function of rats as a result of their hypoglycaemic and anti-oxidant effect. The results analysed the fasting blood glucose, glycosylated haemoglobin, renal function biological markers, and oxidative stress in the serum and urine of the rats. It was revealed that both palm-oil TRF (PO-TRF) and rice bran oil TRF (RBO-TRF) significantly improved renal function and glycaemic status, although PO-TRF conferred a better efficacy than RBO-TRF [68]. Hence, it was concluded that PO-TRF was more effective as a neuroprotective

and hypoglycaemic agent compared to RBO-TRF [69]. Another study [70] revealed that TRF ameliorated lipid induced nephropathy in type-II diabetes by modulating the TGF-β – besides leveraging on its hypoglycaemic, hypolipidaemic and antioxidant properties – in order to prevent the increased expression of collagen type IV and fibrinogen. A recent prospective, randomized double-blind study [71] that was conducted to assess the effect of tocotrienol-rich vitamin E on diabetic nephropathy found that it attenuates the progression of diabetic nephropathy. It was also observed that a 12-week supplementation with tocotrienol-rich vitamin E led to a statistically significant improvement in renal function despite having no effect on glycaemia [71].

8.2 Preventing diabetic retinopathy

One of the most common complications of diabetes mellitus is diabetic retinopathy which could lead to blindness in severe cases [72]. It is estimated that the prevalence of diabetic retinopathy worldwide is about 35%, with approximately 10% of the world population afflicted with a vision-threatening disease [73, 74]. A strong correlation has been established between chronic hyperglycaemia and poor diabetic control with diabetic retinopathy [75]. Indeed, with the incidence of diabetes mellitus rising worldwide [76], a concomitant increase in diabetic retinopathy is to be expected [77]. A characteristic feature of diabetic retinopathy is retinal microvascular changes accompanied by an earlier neurodegeneration [78]. Oxidative stress, which induces hyperglycaemia, is considered as one of the main factors responsible for microvascular complication in diabetes mellitus [79]. Hyperglycaemia triggers cellular events resulting in inflammatory cytokines reactions that in turn accelerate microvascular changes [80]. Another important event is angiogenesis, an over-expression of vascular endothelial growth factor (VEGF) associated with neurodegeneration and diabetes-induced oxidative stress [81]. As mentioned earlier, antioxidants confer their benefit in oxidative stress-induced diseases, including diabetic retinopathy [82, 83], by scavenging free radicals through the hydrogen atom situated at the chromanol ring [84]. Indeed, a recent study [85] on streptozotocin-induced diabetic retinopathy in rats alluded to the beneficial effect of tocotrienol in preventing retinal neurodegenerative changes; it was shown that TRF prevented diabetic-induced changes in retinal layer thickness, retinal cell count, retinal cell apoptosis and retinal expression of VEGF.

8.3 Preventing diabetic neuropathy

One of the complications faced by almost 26% to 53% of diabetic patients worldwide is diabetic peripheral neuropathy [86, 87] that significantly impairs their quality of life [86]. The total cost of diabetic care [88] is around 4.2-fold higher among diabetic patients with neuropathic pain [89]. The mainstay treatment for managing diabetic neuropathy surely lies in glycaemic control and pain management [88]. To that end, various pharmacological agents [90, 91] have been used, but they are all limited either by their adverse effects or by having no effect at all on the pathway of the neuropathic pain [92]. It is believed that oxidative stress plays a role in the pathogenesis of peripheral neuropathy [93]. One animal study with diabetic rats has shown that neuropathic pain is reversed by tocotrienols via the modulation of oxidative-nitrosative stress, caspase-3, and inflammatory cytokine release [94]. Another prospective study [95] on human subjects was aimed at evaluating the protective effect of mixed tocotrienol on the white matter lesion (WML) that reflects neurodegenerative changes; it was shown that subjects who received 200 mg of mixed tocotrienols twice daily for two years have attenuated progression of WMLs compared to placebo [95]. However, a recent study by the investigators of the

Vitamin E in Neuroprotective Study (VENUS) [96] found that the supplementation of oral mixed tocotrienols of 400 mg daily on diabetic patients with neuropathic pain did not show any remarkable improvement in alleviating the neuropathic symptoms. Nonetheless, the researchers qualified their statement by saying that their observation on the lancinating pain among the subsets of patients studied would require further exploration. More optimistically, a more recent randomized-controlled study [97] on the effects of tocotrienol on diabetic neuropathy showed that supplementation of tocotrienol-rich vitamin E of 200 mg twice daily led to a higher serum nerve growth factor (NGF) and improved the nerve conduction velocity for all nerves tested after eight weeks of supplementation. The researchers concluded that TRF could be a disease-modifying agent that targets the NGF in improving nerve conduction velocity [97].

9. Preventing neurological diseases

Neurodegenerative diseases have been widely believed to be caused by oxidative damage due to reactive oxygen species [98]. Indeed, the increased levels of oxidative stress have been associated with numerous pathophysiological conditions together with derangements in mitochondrial complex I activity [99, 100]. Since vitamin E is a potent anti-oxidative agent, it is hypothesised that the neuroprotective effects of vitamin E is mediated via its anti-oxidative property [101]. A growing body of evidence supports the view that tocotrienol is a potent neuroprotective agent against Alzheimer's disease [102]. However, as it stands today, the pathogenesis of Alzheimer's disease remains unclear with a few different hypotheses [103]. Nonetheless, the ability of tocotrienol in reducing oxidative stress and promoting cellular repair contributes to its positive and beneficial effect in protecting the neurons. Admittedly, no clinical trials are available to support the hypothesis that tocotrienol could prevent Alzheimer's disease, with available data based on only four human epidemiological studies [104–107]. With more studies in the future, this research gap could certainly be narrowed. **Figure 2** below summarizes the possible pathways for the neuroprotective actions of tocotrienol.

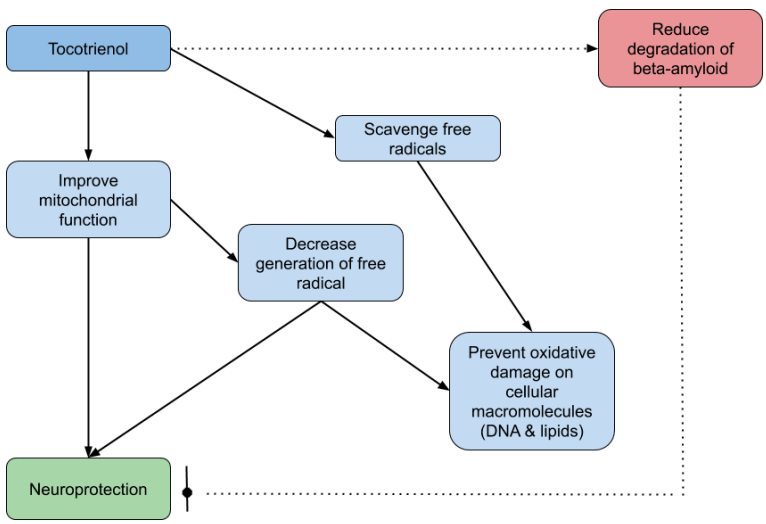


Figure 2.
A summary of the current in vitro evidence of neuroprotective actions of tocotrienol. Legend: Solid line represents beneficial effects of tocotrienol on neurons. Dotted line represents potential adverse effects of tocotrienol on neurons.

10. Preventing bone diseases

Osteoporosis, a metabolic bone disease requiring extensive healthcare, is common in both men and women, though women suffer fragility fracture from osteoporosis at a ratio of 6:1 to men [108]. Osteoporosis is caused by an imbalance in bone remodelling, where the rate of bone resorption is faster than bone formation [109]. While it is known that menopause in women leads to oestrogen deficiency, in men, however, it is due to late-onset testosterone deficiency [110, 111]. The existing therapies for osteoporosis include bisphosphonates, teriparatide and strontium ranelate, all of which increase bone mineral density [112]. Recent studies have tried to explore the use of natural products to cure osteoporosis. With its inherent antioxidative and anti-inflammatory properties that are implicated in the pathogenesis of osteoporosis, tocotrienol is an agent of choice for such studies [113, 114]. Oxidative stress is known to damage osteoblasts by affecting both its differentiation and survival [115]. Oxidative stress also affects the signalling of osteoclasts and promotes the differentiation process [116]. Similarly, proinflammatory cytokines such as tumour necrosis factor (TNF), interleukin 1 (IL-1), and interleukin-6 (IL-6) promote osteoclasts differentiation [117]. The expression of proinflammatory cytokines is also suppressed by tocotrienol [118]. Hence, it is reasonable to assume that by reducing both oxidative stress and inflammation, the process of osteoporosis could be mitigated, if not prevented, by tocotrienol. A study on bone histomorphometry [119] that describes the bone volume and trabecular number, thickness and separation showed that palm tocotrienol preserved the trabecular bone structure, volume, and trabecular separation in rats with ovarian deficiency from ovariectomy. It was also demonstrated in another study [120] that in the bone loss model of rats, palm tocotrienol decreased the eroded surface and increased the osteoblast number, osteoid surface and osteoid volume in the supplemented study animal as compared to the other arm of the study. In another experiment on ovariectomised rats [121], the group that was treated with palm vitamin E showed significantly higher bone mineral density at the femur and vertebrae compared to the untreated group. The bone calcium level at the femur and vertebra of orchidectomised and ovariectomised was also found to be restored with palm vitamin E supplementation [122, 123]. Nonetheless, while tocotrienol has been proven to improve bone density and microarchitecture, and enhance bone biomechanical strength, the study [124] done was not convincing enough to show any statistical difference. The effects of tocotrienol on the bone are summarised below in **Figure 3** [125].

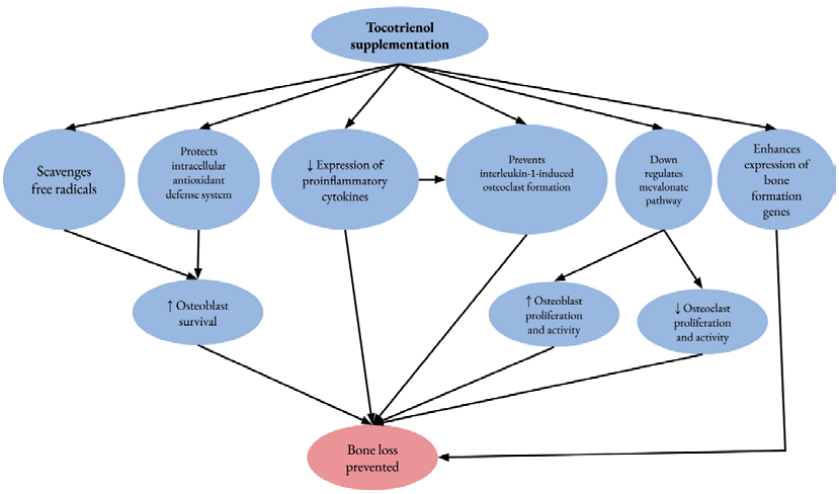


Figure 3.
The effects of tocotrienol on bone histomorphometry, bone mineral and bone calcium content.

Let us now look at the mechanism of actions of tocotrienol in the prevention of osteoporosis. Studies have revealed that oxidative stress plays a major role in the development of osteoporosis [126, 127]. Any increase in the oxidative stress process would lead to a decrease in differentiation of osteoclasts [128] as well as the bone resorption activity [129], which would subsequently impair the musculoskeletal system. Some in vivo studies [124, 130] which supplemented the study rats with tocotrienol showed a reduction in oxidative stress and anti-oxidant enzyme activities such as malondialdehyde. Additionally, an in vitro study [131] showed that γ-tocotrienol homologue decreased the oxidative damage on primary osteoblast culture. Tocotrienol exerts its effect by preserving the antioxidant enzyme activities in bone cells affected with oxidative stress [132]. Another effect is via the mevalonate pathway which is known to regulate osteoblastogenesis and osteoclastogenesis [133]. Tocotrienol suppresses the mevalonate pathway via the hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that is also involved in cholesterol synthesis [134]. Another study [135] further revealed that tocotrienol, in combination with statins, enhances bone static histomorphometry and remodeling markers in the ovariectomised rats although it could not be confirmed whether this was via the mevalonate pathway alone or if it involved some other pathways as well. The anti-inflammatory effect of tocotrienol in preventing proinflammatory cytokines such as IL-1 and IL-6 has also been shown to preserve bone health in rats [120, 136, 137]. It is worth noting that the differentiation and activity of osteoclasts and osteoblast are governed by some genes [138] and that supplementation of palm vitamin E has been shown to significantly enhance the gene expression [139]. Another study [140] demonstrated that tocotrienol could enhance the gene expression related to bone formation and osteoblast activity. All the above-mentioned studies confirmed that tocotrienol possesses some promising bone-protective effect: it increases the osteoblast number, mineral deposition and bone formation; and it reduces the osteoclast number, thereby preventing the bone resorption, erosion, and degeneration of bone mineral density and microarchitecture. The summary of the whole mechanism is illustrated in **Figure 4** below.

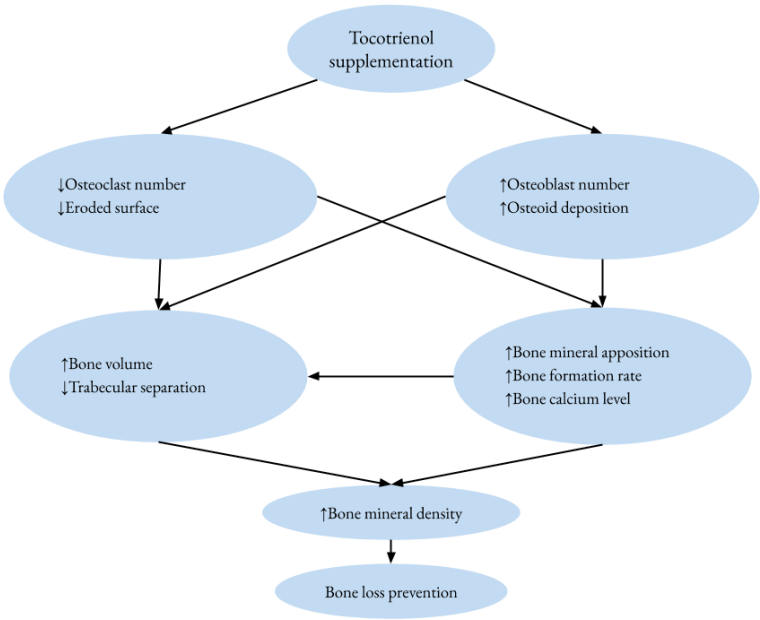


Figure 4.
The bone protective mechanism of Tocotrienol.

11. Preventing autoimmune diseases

One of the most common autoimmune diseases is rheumatoid arthritis. With a prevalence of about 0.5 to 1.0% worldwide [141], it is presented as a typical systemic autoimmune disease of unknown aetiology that affects many joints [142]. The joint inflammation is characterised by some marked changes in the cartilage from the effects of proinflammatory mediators such as cytokines and C-reactive protein [143]. These include destruction of cartilage [144], leukocytes infiltration [145], and bone erosion [146]. The proinflammatory cytokines such as tumour necrosis factor-α (TNF-α), Interleukin-1α (IL-1α) and IL-1β [147] play a role in modulating inflammatory responses in the affected joints [148]. These cytokines have been shown to be involved in the pathogenesis of rheumatoid arthritis in animal studies. Since such studies closely mimic human disease [149], the development of biological agents that have the potential to modulate the cytokine mediators could yield an effective prevention against rheumatoid arthritis [150]. It has been demonstrated in an animal study [151] that palm γ-tocotrienol exerts an effect against both oxidative stress and joint pathology. In another study [152], it was discovered that palm δ-tocotrienol somehow reduced inflammation in arthritic rats. This should not be surprising given that palm tocotrienol has been shown to downregulate proinflammatory cytokines such as TNF-α, IL-1α, IL-β, IL-6 and IL-8 [153]. In another recent study [154] on the temporomandibular joint (TMJ) of a rat model, it was observed that in the group fed with TRF, the bone mineral density was notably increased. The researchers concluded that the concomitant decrease of plasma level of inflammatory cytokines with the increased bone density is sufficient evidence that TRF could be used in the management of TMJ rheumatoid arthritis.

It is also pertinent to look at another common ailment: bronchiole asthma. This chronic respiratory problem with a female preponderance afflicts more than 339 million people worldwide, according to a WHO estimate [155]. An increase in antinuclear antibodies and autoantibodies against bronchial epithelial antigens or endothelial antigens suggest that asthma is an autoimmune disease [156]. As the first experiment to demonstrate the effectiveness of tocotrienol in preventing asthma, a study [157] on rats showed that γ-tocotrienol possesses better free radical-neutralizing activity in vitro; reduces the eosinophil and neutrophil counts in vivo; and promotes lung-endogenous antioxidant activity. Another study investigated the effect of tocotrienol on airway remodelling [158], undoubtedly one of the characteristic features of asthma. It was shown that several inflammatory mediators were involved in airway remodelling [159, 160] and the most important among them is transforming growth factor beta1 TGF-β1 [161, 162]. The researchers convincingly proved the effect of γ-tocotrienol on the TGF-β1 induced differentiation of human airway smooth muscle and the extracellular deposition and the down-signaling of the airway smooth muscle cells activated by TGF-β1 [158]. This study suggested that γ-tocotrienol could play a therapeutic role in regulating airway remodelling in asthmatic patients.

12. Gastroprotective effect

Non-steroidal anti-inflammatory drugs (NSAIDs) are probably the most frequently used therapeutic agents in the world [163] for the treatment of pain, arthritis and trauma, besides many other indications. Achieving more than 73 million prescriptions per year [164], NSAIDs have been notoriously associated with gastrointestinal bleeding [165]. In an earlier study on a rat model of three different study groups [166], it was found that both TRF and tocopherol were equally

effective in preventing aspirin-induced gastric ulcer. In another recent study [167] on a rat model comparing control to a group fed with omeprazole and another group with tocotrienol, it was discovered that while both groups were effective against gastric ulcer, the tocotrienol group displayed various modes in its protective effect – via the nitric oxide (NO) pathway and superoxide dismutase (SOD) activity – and in reducing TNF-α activity.

13. Radioprotective effect

With the increasing adoption of radiation in both clinical and non-clinical applications [168], human exposure to radiation is set for an exponential increase. Radiation toxicity is manifested in oxidative stress and DNA damage [169], inflammatory changes [170] and cell apoptosis [171]. Studies were carried out to examine the potential benefits of naturally occurring products such as vitamin E, a potent anti-oxidant with the capacity to neutralize free radicals from radiation exposure by donating H atoms [172]. It was shown that exposure to ionizing radiation yields reactive oxygen species (ROS) and nitrogen species (RNS), hydroxyl radical, superoxide, peroxynitrite and hydrogen peroxide. These reactive species of ROS and RNS with radiation-induced radicals damage proteins, DNA and lipids, besides activating intracellular signalling pathways that release cytochrome C from the mitochondria, eventually leading to cell apoptosis [173–175]. Thanks to its potent anti-oxidant properties, tocotrienol has been a subject of several studies and has been reported to be radioprotective [176–178]. Studies on a rat model [176, 179] have showed the protective effect of γ-tocotrienol against radiation-induced DNA damage through the activation of haematopoietic progenitors, red and white blood cells including platelets, and also through the inhibition of 3-hydroxy-3-methylglutaryl-CoA Reductase (HMG-CoA Reductase) – a protein-coding gene-mediated nitrosative stress [180]. It is also proven that γ-tocotrienol increases serum IL-6 and granulocyte colony stimulating factor (G-CSF), both of which induce haematopoiesis and are protective against radiation-induced neutropenia and thrombocytopenia in mice [181].

14. Anti-cancer effect

Cancer (also known as malignant tumours or neoplasms) is the second leading cause of mortality globally according to WHO [182], with an estimated 9.6 million deaths in 2018. The cancer burden keeps on growing inexorably, exerting its pressure emotionally and financially on individuals and families, not to mention the community and health system. While chemotherapy has been the mainstay of treatment, it is limited by a few factors such as tumour immune evasion [183, 184], drug toxicity and resistance, and inappropriate cancer metabolism [185], all of which lead to possible metastases and recurrence. Hence the search for a more effective and potent anti-cancer agent. Buoyed by the earlier success in extracting anti-cancer agents from plants, the search has been on for a natural product. Tocotrienol became the choice of study due to its multitargeted actions in destroying cancer cells, promoting cancer cells apoptosis and inhibiting angiogenesis and metastases [186–188]. It is certainly beyond the scope of this writing to discuss all the research conducted on the effects of tocotrienol on cancer. Notable among the most recent research papers on this subject are "Tocotrienols and Cancer: From the State of Art to Promising Novel Patents" [189] and "Tocotrienols Modulate a Life or

Death Decision in Cancers" [190]. For our purpose, it suffices to understand how tocotrienol exerts its anti-cancer effect. The mechanism of action is illustrated in **Figure 5**. Indeed, the effect of tocotrienol in suppressing the growth of different form of malignancies, including that of the uterine, ovary, prostate, liver, gastric, breast and brain, is well documented [191, 192]. **Figure 5** below depicts the possible mechanism of actions of tocotrienols in exerting its anti-cancer effect [193].

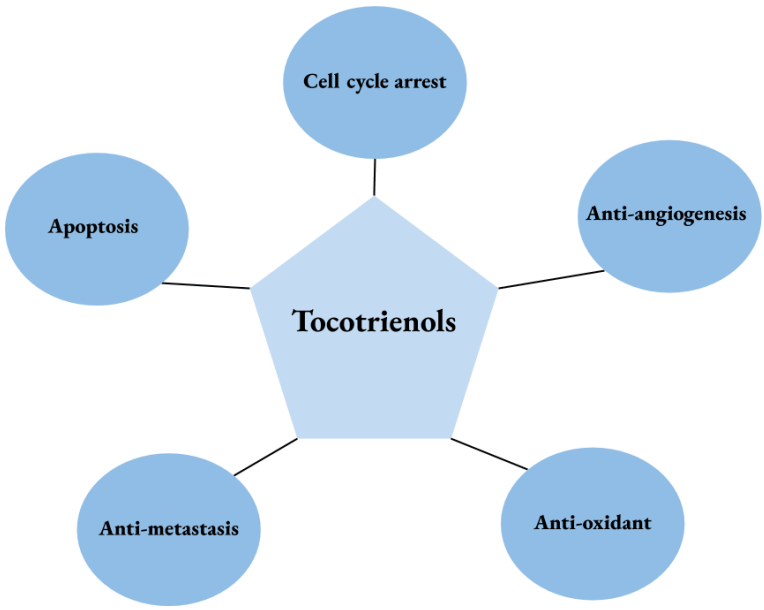


Figure 5.
Anti-cancer mechanism of actions of tocotrienol.

14.1 Apoptosis

As an innate defence mechanism against cancer, apoptosis is considered critical [194, 195]. Natural molecules have the potential to exert their apoptosis-inducing quality [196–199] and tocotrienols are one of those compounds that could exert the anti-neoplastic activity via this apoptosis mechanism [200]. One study [201] demonstrated that γ-tocotrienol caused substantial apoptosis in tumour cells by down-regulating several oncogenic gene products' expression. It also displayed chemosensitisation and anti-invasive properties against prostate cells [202], and induced apoptosis in gastric cancer cells [203]. In another study [204], both α-tocopherol and γ-tocotrienol showed anti-proliferative activities and apoptosis on both the cervical carcinoma and hepatoma cell lines. Tocotrienols were also found to induce apoptosis in breast cancer cell lines [205] and effected both apoptosis and antiangiogenic activity of murine mammary cancer cells in mice [206]. A different study revealed that δ-tocotrienol is more efficacious than both α- and γ-tocotrienol in exerting its apoptosis effect on both human lung adenocarcinoma and glioblastoma [207]. In a study [208] on human bladder cancer cells, δ-tocotrienol was observed to have effectively induced apoptosis and chemosensitization, in addition to arresting the growth of human bladder cells. A study conducted on human chronic myeloid leukaemia cells [209] found that γ-tocotrienol was an effective inducer of apoptosis in the myeloid leukaemia cells. TRF mixture is also found to prevent cell proliferation, migration, and tumour cell invasiveness by inducing apoptosis in non-small cell lung cancer

cells (NSCLC) [210]. Furthermore, γ-tocotrienol exerted its anti-proliferative effect and induced apoptosis in human cervical cancer cells [211].

14.2 Cell cycle arrest

Cell cycle has its checkpoints from one phase to another, and any aberrant activation may lead to the proliferation of tumour cells. Hence, it is imperative to target these checkpoints in cancer therapy [212]. The cell cycle and its checkpoints are illustrated in **Figure 6** below [213].

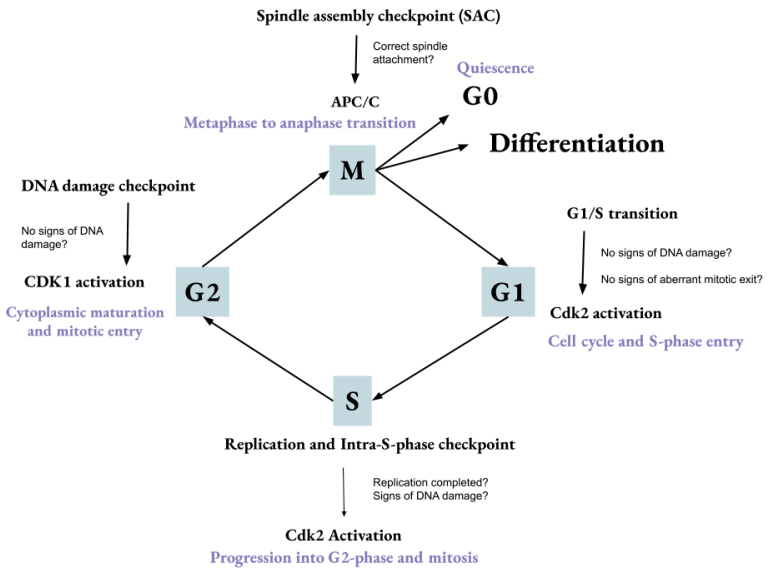


Figure 6.
The cell cycle and its checkpoints. There are four phases in the cell cycle – G1 phase, S-phase, G2-phase, and M-phase. The checkpoints control the progression of the cell cycle which is unidirectional in nature.

It is worthy of note that γ-tocotrienol had an effect on the G2/M arrest and apoptosis of breast cancer cells, with the potential to reverse multi-drug resistance [214]. Another study on brain cancer cells [215] documented the anti-proliferative effect of γ-tocotrienol in combination with another agent, jerantinine (an indole alkaloid obtained from leaves extract) which led to G0/G1 cell cycle arrest. The combination effect of γ-tocopherol and δ-tocotrienol was also cited in successfully arresting the G1 phase and G2/M phase in the cell cycle of prostate cancer cells [216], besides inhibiting prostate cancer cell growth. A synergistic effect was observed between δ-tocotrienol and geranylgeraniol (a compound synthesised endogenously in the human body via mevalonate pathway) in arresting G1 phase activity in prostate carcinoma cells [217]. With the addition of γ-tocotrienol, the cell cycle at G0/G1 phase was also arrested while the S phase was reduced in cervical cancer HeLa cells [211]. In short, tocotrienols, whether alone or in combination with other agents, are capable of exerting their inhibitory effects through the checkpoints in the cell cycle. This promising evidence supports their future development as therapeutic agents in modulating the checkpoints of the cell cycle.

14.3 Anti-angiogenesis

Angiogenesis, defined as the formation of new blood vessels, is an important process for tumour growth and metastases [218] triggered by chemical signals from tumour

cells. Researchers have identified more than a dozen angiogenic activators, including vascular endothelial growth factor (VEGF) which is a powerful angiogenic factor in neoplasms as well as normal tissue [219]. Therefore, targeting these angiogenic factors seemed to be the most rational intervention to combat tumour growth [220, 221]. Several trials [222–224] have shown the effectiveness of tocotrienol in inhibiting angiogenesis in various cancers, with the process illustrated in **Figure 7** below [225].

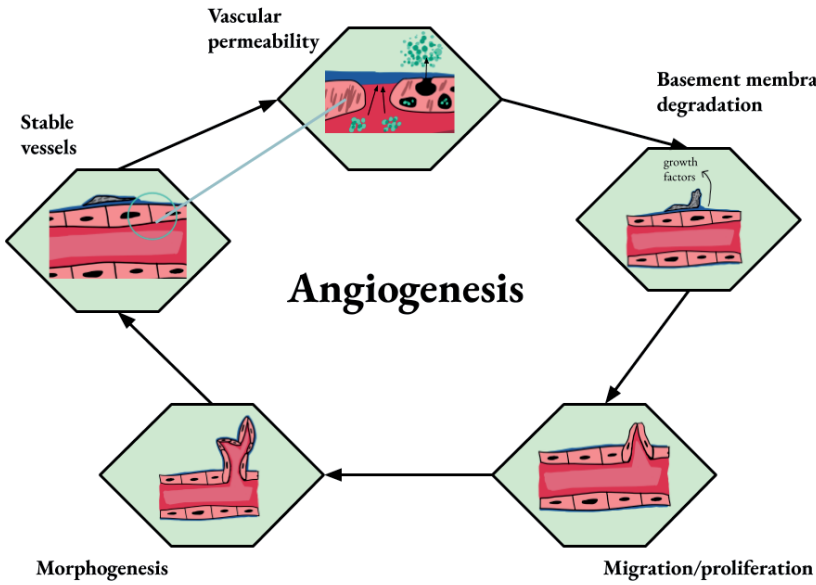


Figure 7.
The angiogenic cascade. (A) During the process of angiogenesis, stable vessels undergo (B) a vascular permeability increase which allows extravasation of plasma proteins. (C) Degradation of the ECM by matrix metalloproteases (MMPs) relieves pericyte-endothelial cells (EC) contacts and liberates extracellular matrix (ECM)-sequestered growth factors. (D) ECs then proliferate and migrate to their final destination and (E) assemble as lumen-bearing cords.

A study has shown that both δ- and γ-tocotrienol inhibit angiogenesis and proliferation in human hepatocellular carcinoma cells [226]. In another study, it was demonstrated that δ-tocotrienol inhibited tumour angiogenesis via VEGF and MMP-9 in pancreatic cancer cells; it also decreased the expression of cell surface markers in cancer stem cells [227]. Another study revealed that δ-tocotrienol exhibited potential against both melanoma and its associated stem cells [228, 229], while displaying suppressive action on prostate cancer stem-like cells [230]. Recent findings also indicated that tocotrienols displayed antiangiogenic protein expression of VEGF in colorectal cancer [231], malignant mesothelioma [232], breast cancer [233], ovarian carcinoma [234], and head and neck squamous cell carcinoma [235]. All these studies provide ample evidence of the role of tocotrienol in arresting tumour growth by inhibiting angiogenesis.

14.4 Anti-metastasis

The morbidity and mortality from cancer are mainly caused by cancer metastases; in fact, almost 90% mortality is thought to be due to metastases [236]. Cancer metastasis starts at the primary tumour with the detachment of metastatic cells which then travel to different parts of the body either through the bloodstream or lymphatic drainage, and thereafter settle and start growing at the distal site [237]. To put it simply, the process of metastasis involves four essential steps: detachment, migration, invasion and adhesion. This is illustrated in **Figure 8** below [238].

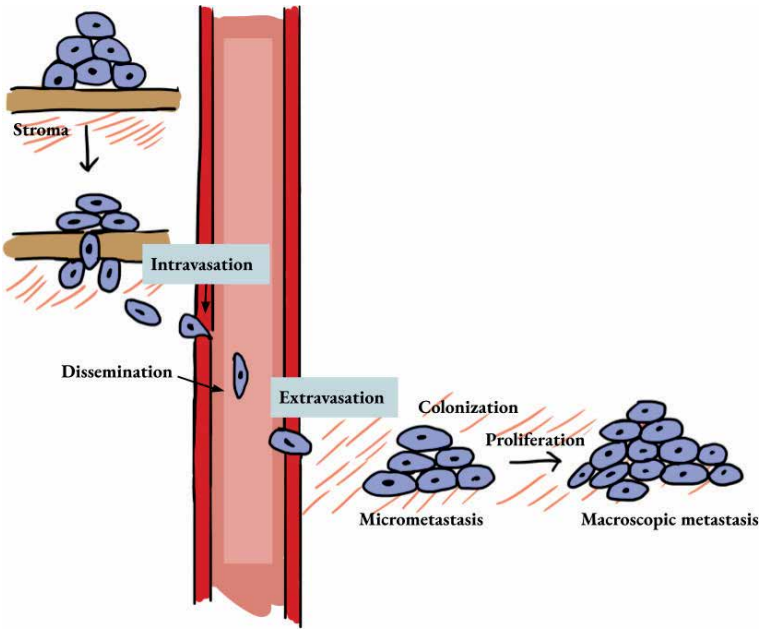


Figure 8.
A schematic representation of the four stages of metastatic dissemination of cancer cells from the primary tumour into the blood circulation, involving detachment, migration, invasion and adhesion.

Cancer survival rate has improved significantly over the years from early diagnosis and inhibition of cancer growth. Nevertheless, the mainstay of cancer treatment for metastasis remains chemotherapy or radiotherapy. Tocotrienols have gained prominence in the last several years due to their anti-proliferative, anti-angiogenic, anti-migratory and anti-metastatic properties as exhibited in vivo and in vitro data [239]. Indeed, for metastasis to occur, cancer cells need to detach and migrate to a distant target organ, a process followed by adhesion and local invasion [240]. The ability of tocotrienol in halting cell migration has been demonstrated in several studies [224, 241–243]. In one study on human umbilical vein endothelial cells (HUVEC), treatment with δ-tocotrienol suppressed VEGF-induced migration by 50% [224]. Another study proved a dose-dependent inhibition of non-small cell lung cancer (NCSLC) cells migration [241], while a different one demonstrated the ability of γ-tocotrienol in inhibiting in-vitro human gastric cells migration [242]. In another study on VEGF-stimulated HUVEC migration essay, it was found that γ-tocotrienol suppressed the migratory potential of the HUVEC cells [243]. After the cancer cells migration, the subsequent event in the process of metastases is cell adhesion and invasion; this is preventable by suppressing the tumour cell invasion after adhesion [242]. An in vitro study has shown that after being treated with δ-tocotrienol, a pancreatic cancer mouse model no longer displayed any signs of invasive cancer [244]. A previous study has also showcased the ability of γ-tocotrienol in halting the invasion of the prostatic cancer cells in the control group [202], thereby suppressing the main process in the perpetuation of metastasis.

14.5 Anti-oxidant

Oxidative stress refers to an imbalance of free radicals or reactive oxygen species (ROS) and antioxidants in the body [245]. This imbalance has been linked to a litany of chronic conditions including neurodegenerative disease, cardiovascular disease, diabetes mellitus, and many other pathologies such as cancer [246]. A variety of

deleterious modifications of macromolecular components such as DNA, lipids and proteins were due to this chronic oxidative stress [247]. There is also a possibility that ROS mediates an indirect attack on DNA, resulting in secondary reactive intermediates that would couple with the DNA bases to form DNA adducts [248]. This formation is central to what is known as carcinogenesis [249]. Oxidative lesions have been implicated in the aetiology of cancer due to the oxidative DNA damage [250–254]. It is now clear how carcinogenesis is perpetuated by this oxidative stress process, as illustrated in **Figure 9** below [255].

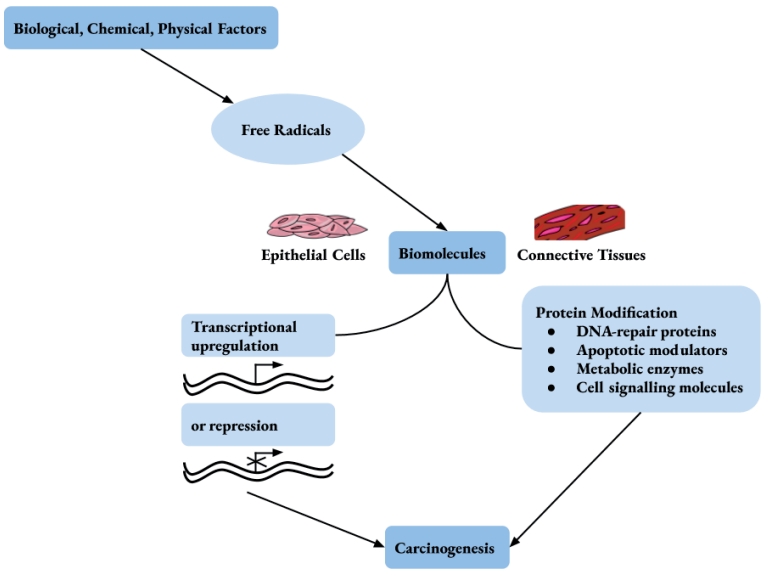


Figure 9.
Oxidative stress mediating cancer development. Biological, chemical and physical factors mediated free radicals (ROS) which damage the biomolecules that initiate the neoplastic cells through the up-regulation of transcriptional factors and inactivation of tumour suppressor genes. They also alter the functions of DNA repair proteins, apoptotic modulators, metabolic enzymes and signalling pathways that induce the neoplastic condition.

Evidence from clinical and laboratory studies have showed that the elevated level of ROS contributed to both cancer initiation and cancer progression. Consequently, the most rational, if not preventive, approach is to use antioxidants for combating ROS [256]. Although the results regarding the use of dietary antioxidants were promising, research on this topic is still inconclusive and controversial [257]. Moreover, while studies have indicated that anti-oxidant supplementation resulted in an increase in survival rates and tumour response, with fewer toxicities than controls, a systematic review previously done on this topic showed no evidence of interference by antioxidants on chemotherapy mechanisms that conclusively proves that anti-oxidants (such as vitamin E) improve tumour response rate or the patients' survival [258]. Despite promising results on improving the side effects from chemotherapy or radiotherapy treatment of cancer, further research into anti-oxidants [259], especially vitamin E in general and tocotrienol in particular, is highly warranted.

15. Discussion

Long ignored despite being close yet superior to its related isomer tocopherol, tocotrienol is increasingly becoming a subject of interest in vitamin E research

among the scientific community. One of the main reasons why it was understudied could be due to its abundant presence in palm oil, itself a much maligned product that had to bear the full brunt of a damaging smear campaign for decades. In fact, palm oil contains about 70% of all tocotrienol homologues namely α-, β-, δ- and γ-tocotrienols. Consequently, it would be no exaggeration to say that palm oil is nature's best kept secret, if not the most promising natural substance in influencing health and disease.

Growing interest in tocotrienols has led to research exploring the molecular basis of their action in health. This chapter has highlighted the recent advances in this rapidly developing field of study. Indeed, recent studies have shown that tocotrienols may have superior chemopreventive or chemotherapeutic effects when used either alone or in combination with tocopherols. Indeed, tocotrienols are well adapted for their biochemical function. Thanks to their organic structure featuring a long-saturated carbon side-chain, they are able to penetrate more efficiently in the lipid membrane and in the intermembrane of tissues containing saturated fatty layers. This ability contributes immensely to their therapeutic efficacy.

Without doubt, the beneficial health effects of tocotrienols are partly related to their anti-oxidant activity. Though both tocotrienol and tocopherol have the ability to scavenge the free radicals directly by donating the phenolic hydrogen of their chromanol ring, tocotrienol is considered a better anti-oxidant due to its generally uniform distribution in the membrane bilayer coupled with a stronger disordering of its membrane lipid structure. Vitamin E, in particular tocotrienol, was shown to play a vital role in maintaining the integrity of the central nervous system through its anti-oxidant property. Indeed, as an organ with very high metabolic needs in terms of oxygen consumption, the brain is extremely susceptible to any forms of oxidative stress. However, current evidence is largely focused on Alzheimer's disease – an age-related inflammatory neurodegenerative disease characterised by the presence of pathognomonic amyloid plaques and neurofibrillary tangles. It is postulated that the main mechanism of action of tocotrienol in attenuating the neurodegenerative changes is via its anti-oxidant action, either by inhibiting the production of ROS or by reducing the lipid peroxidation by-products. Admittedly, a gap still persists in this area insofar as other neurodegenerative conditions (such as Parkinson's disease) are concerned. Notwithstanding the fact that some recent studies have reported contradicting outcomes on the relationship between tocotrienol and Parkinson's disease, it is hoped that future studies will shed more light in this area.

Given the potential of tocotrienol in preventing auto-immune diseases, especially through its anti-inflammatory properties, the evidence available warrants further investigation into its molecular action. That would enable the development of drug targets to combat inflammatory diseases. Nevertheless, the therapeutic potential of tocotrienol as an anti-inflammatory agent cannot be denied. On the one hand, δ-tocotrienol somehow lessened joint inflammation in arthritic rats by reducing the level of proinflammatory cytokines. On the other hand, in a human study, γ-tocotrienol improved airway remodelling that characterises bronchiole asthma which is essentially an inflammatory disorder. Another rat model also showed that tocotrienol is effective against gastric ulcer. The gastroprotective effect of tocotrienol was mainly modulated through a reduction in inflammatory response, besides its anti-oxidative properties. Though this protective effect was witnessed in various animal models of gastric ulcer, clinical studies on the use of tocotrienol in patients with peptic ulcer disease or even gastritis are yet to be conducted.

As one of its health benefits, tocotrienol – through its ability to improve the lipid profiles – has been shown to confer a cardioprotective effect, at least with respect to atherosclerosis, myocardial infarction and thrombosis. There is sufficient evidence

to prove that tocotrienol, especially in the form of γ-tocotrienol, is anti-cholesterolaemic; this is achieved by inhibiting the mevalonate pathway responsible for the synthesis of cholesterol and other isoprenoids. Overall, the potential of tocotrienol as a potential hypocholesterolaemic agent is evidenced by in-vitro, in-vivo and human clinical trials. Thus, tocotrienol supplementation is highly recommended for patients suffering from hypercholesterolaemia.

In fact, human studies on the effects of tocotrienol on cardiovascular disease have been limited to its anti-hypercholesterolaemic property. The only exception is an ongoing clinical study at the National Heart Institute of Kuala Lumpur, Malaysia. Conducted by this author, it investigates the ability of tocotrienol in preventing atrial fibrillation in post-coronary artery bypass grafting surgery. Indeed, while tocotrienol has been shown to be protective against cardiovascular disease in animal models, its direct effects on humans are inconsistent. Our current evidence serves as a basis for further clinical trials aimed at validating the positive effects of tocotrienol especially among patients susceptible to cardiovascular complications.

The potency of α-tocotrienol as an anti-atherogenic agent, besides being a bulwark against cerebrovascular disease, is well documented. Several animal models have demonstrated that tocotrienol protects against ischaemic stroke by attenuating brain lesion volume. A similar scenario was observed during clinical trials where it was shown to attenuate the progression of brain white matter lesion. Consequently, it could be safely concluded that tocotrienol protects against cerebrovascular disorders.

Tocotrinol-rich vitamin E (TRF) has been observed to ameliorate diabetes in animal studies through its superior antioxidant, anti-hyperglycaemic and anti-inflammatory properties. A recent clinical trial also showed that TRF significantly reduced serum creatinine level, and therefore has the potential to be used as a supplement in the treatment of diabetic nephropathy. Moreover, the anti-diabetic properties of tocotrienol in preventing nephropathy, retinopathy and neuropathy have been proven in several other studies.

Studies conducted on animal models have demonstrated that tocotrienol can mitigate, if not prevent, osteoporosis in rats by reducing oxidative stress and inflammation. Indeed, tocotrienol has been proposed to counter osteoporosis which leads to fragility fracture, a leading cause of morbidity and mortality worldwide. It is postulated that tocotrienol mediated bone protection via its anti-oxidant, anti-inflammatory, mevalonate suppression and gene-modulating properties. Despite strong evidence in animal models showing improved bone structure and strength after tocotrienol supplementation, limited human clinical trials on the effects of tocotrienol on bone health has been a serious impediment to its clinical use.

The role of γ-tocotrienol in protecting against radiation toxicity has been a subject of numerous animal studies, and the results are very promising. With the widespread use of ionising radiation in various non-clinical applications such as construction, sterilization of food products and engineering, exposure to radiation – whether intentional or unintentional – is very high. Studies have shown that γ-tocotrienol has a protective effect against radiation injury by increasing haematopoietic progenitors, neutrophils, platelets, white blood cells and reticulocytes. It has also been demonstrated that γ-tocotrienol protects against vascular injury by inhibiting HMG-CoA reductase. Since tocotrienols accumulate in the small intestine and colon at a higher level than tocopherols, they could protect the gastrointestinal tract from injury.

Last, but certainly not least, with cancer being one of the leading causes of death worldwide, the role of tocotrienol as an anti-cancer agent cannot be underestimated. Tocotrienol has been shown to modulate intracellular signalling pathways; it induces apoptosis and cell cycle arrest, and inhibits angiogenesis, cell proliferation

and metastases. Compared to its isoform tocopherol, tocotrienol displayed superior activities in anti-cancer studies. Indeed, in a structural-activity relationship study, the chromanol ring and phytyl carbon tail played a major role in inducing cancer cell apoptosis. However, despite the abundance of cell and animal studies investigating the role of tocotrienol, evidence regarding its preventive effects on cancer remain inconclusive, with most trials still at the preliminary stage. Nonetheless, our improved understanding of the mechanism of actions of tocotrienol in the suppression of cancer cell growth by inhibiting proliferation, migration, and invasion should not be discounted; it will inform more targeted research into cancer therapy in the future.

16. Conclusion and future direction

This chapter has highlighted the wonders of tocotrienols which, thanks to their efficacy and safety profile, are attracting increased attention. Examining the latest research into tocotrienols, it has demonstrated the undeniable benefits of tocotrienols in conferring protection against cancer as well as a whole litany of diseases including cardiovascular, metabolic, autoimmune, bone and neurological diseases. Admittedly, many of the researches were conducted in the laboratory, with some preclinical trials translated into clinical trials. Nonetheless, it is hoped that more randomised clinical trials will be carried out on a global scale in the near future. From the vessels in the heart to neurons in the brain, tocotrienols have the extraordinary potential to be the future of vitamin E research.

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Section 3

Vitamin E and Oxidative Stress

Chapter 12

Vitamin E in Human Health and Oxidative Stress Related Diseases

Israel Ehizuelen Ebhohimen, Taiwo Stephen Okanlawon, Augustine Ododo Osagie and Owen Norma Izevbigie

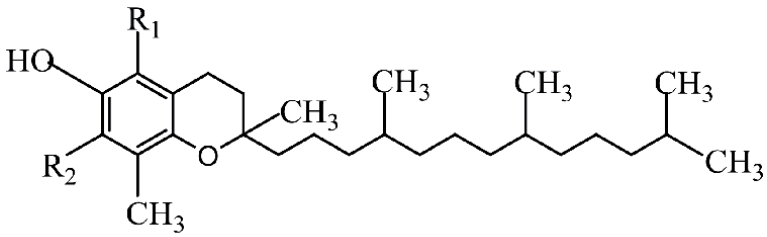
Abstract

Oxidative stress characterized by an imbalance in the production and degradation of radical species has been implicated in the onset and progression of several diseases. The efficacy of antioxidants acting via the inhibition of radical chain reactions, scavenging of free radicals, direct donation of electrons to radical species and chelation of metal ions have been reported to attenuate the oxidative process. Vitamin E is an effective antioxidant and its hydrophobic nature and membrane permeability offer some benefits to application and bioavailability. This chapter highlights the following; structural differences in the vitamin family, biosynthesis in plants and the native biological role, antioxidant mechanisms of vitamin E, an overview of the prophylactic action of vitamin E as well as the effect on the oxidative process in some diseases.

Keywords: vitamin E, antioxidant, bioactivity, tocopherol, tocotrienol

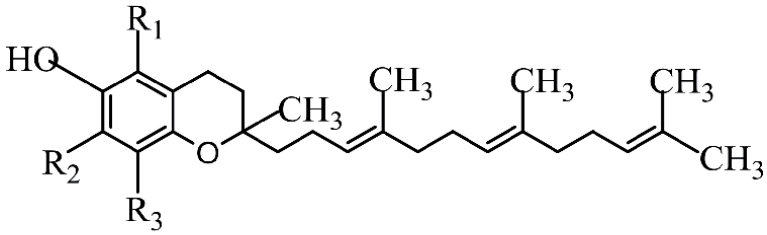
1. Introduction

The tocochromanols generally called the vitamin E family are amphipathic organic molecules with antioxidant capacity. They are categorized as tocopherols, tocotrienols and plastochromanol-8 (PC-8) [1]. All groups have a similar chemical structure comprising a polar chromanol head linked with a hydrophobic prenyl tail. The positive effect of tocopherols and tocotrienols on reproduction in animals called research attention to this family of organic compounds. Vitamin E was first described by Evans and Bishop in 1922 [2] as substance 'X' [3, 4]. The class of compounds was later called 'tocopherol' coined from the Greek words 'to'kos' meaning 'child birth', and 'phe'rein' meaning 'to bring forth'. The suffix '-ol' was included due to the presence of an alcohol functional group. The tocopherols have a saturated prenyl tail while the tocotrienols have unsaturated tails with carbon–carbon double bonds at 3′,7′ and 11′ positions. Each group comprises four molecular forms (α, β, γ and δ) that are differentiated by the methyl group substitutions in the chromanol head group (**Figures 1** and **2**) which strongly influence their antioxidant activity in various systems [3–5]. Plastochromanol-8 (PC-8), is also a natural component of plant tissues and was first discovered in the leaves of the rubber tree (*Hevea brasiliensis*), where its concentration exceeded that of α-tocopherol and plastoquinone [6]. Structural studies revealed that the compounds were identical to those of synthetic PC-8, a γ-tocotrienol homolog but had longer side chains [1].



$R_1 = R_2 = CH_3$ α-Tocopherol
$R_1 = CH_3, R_2 = H$ β-Tocopherol
$R_1 = H, R_2 = CH_3$ γ-Tocopherol
$R_1 = R_2 = H$ δ-Tocopherol

Figure 1.
Tocopherol.



$R_1 = R_2 = R_3 = H$ Tocotrienol
$R_1 = R_2 = R_3 = CH_3$ α-Tocotrienol
$R_1 = R_3 = CH_3, R_2 = H$ β-Tocopherol
$R_1 = H, R_2 = R_3 = CH_3$ γ-Tocopherol
$R_1 = R_2 = H, R_3 = CH_3$ δ-Tocotrienol

Figure 2.
Tocotrienol.

The human body tends to accumulate α-tocopherol due to the activity of the liver α-tocopherol transfer protein (α-TTP), which enriches plasma with a-tocopherol [7]. Besides α-TTP, which resides only in the liver, a system of tocopherol-binding proteins (TBPs) cause the localization of tocopherols in various human tissues where they are required [8].

The main dietary sources of vitamin E compounds include vegetable oils, nuts and seeds. The vitamin E family has been studied extensively due to their diverse biological functions and α-tocopherol is reported to have the highest biological activity [3]. Although α-tocopherol is universally distributed in the plant kingdom and is the predominant vitamin E form in photosynthetic tissues, γ-tocopherol and tocotrienols predominate in the seeds of several dicots and monocots [5].

1.1 Biological functions of vitamin E

The most notable biological function of this lipid-soluble is their antioxidant capacity. All vitamin E compounds meet the definition of an antioxidant moiety with the capacity to inhibit oxidative reactions *in vitro* [3]. Vitamin E is widely accepted as one of the most potent antioxidant in nature and the antioxidant property is based on the capacity to rapidly transfer its phenolic hydrogen atom to

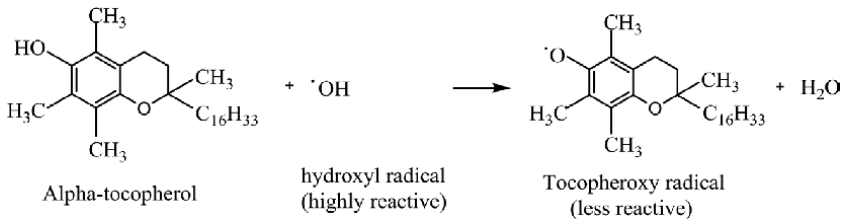


Figure 3.
Reaction of alpha-tocopherol with hydroxyl radical.

neutralize free radicals (**Figure 3**). The regeneration mechanism, mostly by vitamin C is essential for maintaining viability [9, 10].

The antioxidant capacity of α, β and γ isoforms of both tocopherol and tocotrienol are similar and the δ isoforms have weaker activity [11, 12]. As the main chain-breaking antioxidant in body tissues, the vitamin E isoforms inhibit lipid peroxidation especially in polyunsaturated fatty acid component of cell membranes. The in vivo antioxidant capacity of vitamin E is not completely clear. The suggested in vivo activity is based on the reported in vitro activity [3]. Based on the capacity to inhibit oxidation, vitamin E may help ameliorate or suppress the progression of oxidative stress related diseases [13].

Other reported biological functions of vitamin E include; regulation of inflammatory response, gene expression, cell proliferation, as well as modulation of cellular signaling and activity of membrane bound enzymes.

1.2 Biosynthesis of vitamin E

Tocochromanols are only synthesized by photosynthetic organisms. In plants, tocochromanol biosynthesis utilizes cytosolic aromatic amino acid pathway for head group synthesis while the tail is synthesized by the plastidic deoxyxylulose-5-phosphate pathway.

The formation of homogentisic acid (HGA) from p-hydroxyphenylpyruvic acid (HPP) by p-hydroxyphenylpyruvic acid dioxygenase (HPPD) is the rate-limiting step in the synthesis of the head group (**Figure 4**).

The biosynthesis of tocopherols and the tocotrienols follow the same pathway, each class requiring specific substrates and enzymes. HGA is prenylated with either phytyl-diphosphate (PDP) or geranylgeranyl diphosphate (GGDP) to yield the committed intermediates 2-methyl-6-phytylplastoquinol (MPBQ) and 2-methyl-6-geranylgeranylplastoquinol (MGGBQ) in tocopherol and tocotrienol synthesis respectively.

MPBQ methyltransferase (MPBQ MT) transfers a second methyl group to MPBQ to form 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ) and to MGGBQ to form 2,3-dimethyl-5-geranylgeranyl-1,4-benzoquinone (DMGGBQ) respctively. Tocopherol cyclase converts MPBQ and DMPBQ to δ- and γ-tocopherols, respectively, and the corresponding geranylgeranylated intermediates to δ- and γ-tocotrienols. Finally, γ-tocopherol methyltransferase (γ-TMT) adds a methyl group to C-6 of the chromanol ring, converting δ- and γ-tocopherols and tocotrienols to β- and α-tocopherols and tocotrienols, respectively [14].

Among the vitamin E family present in foods, α-tocopherol is the most important to human health [15]. Although all tocopherols are absorbed equally during digestion, only α-tocopherol is preferentially retained and distributed throughout the body [16]. The concentration of γ-tocopherol is far higher than that of α-tocopherol in oil seeds though the former is the biosynthetic precursor of the

latter. This suggests that the γ-tocopherol methyltansferase reaction is limited. One approach to increase α-tocopherol yield in these seeds is to increase the expression of γ-tocopherol methyltansferase gene [17].

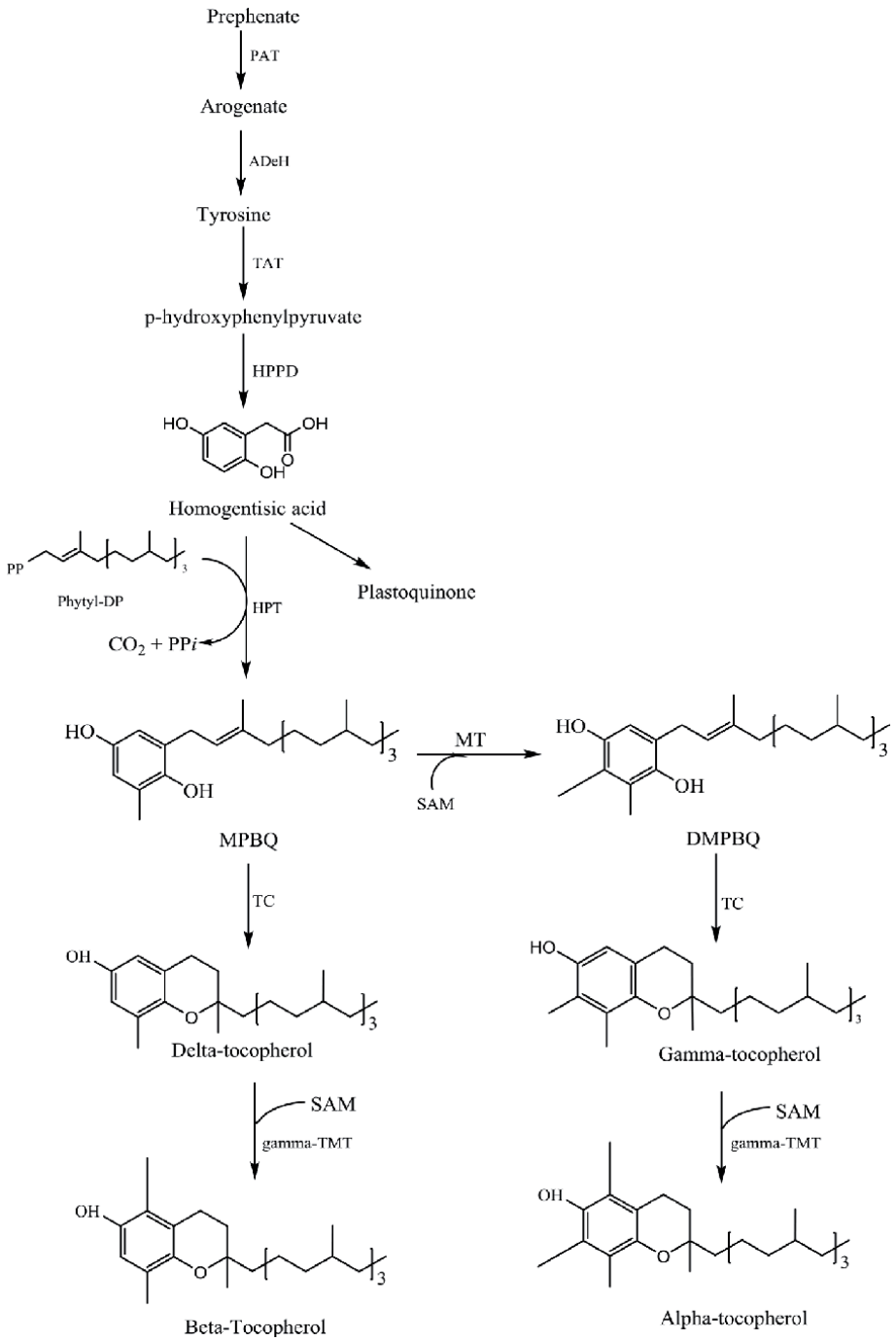


Figure 4.
Tocopherol biosynthesis [14]. Abbreviations: PAT, prephenate amino transferase; AdeH, arogenate dehydrogenase; TAT, tyrosine amino transferase; HPPD, p-hydroxyphenylpyruvate dioxygenase; HPT, homogentisate phytyltransferase; phytyl-DP, phytyl-diphosphate; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinone; MT, methyltransferase; SAM, S-adenosyl methionine; TC, tocopherol cyclase; MPBQ MT, MPBQ methyltransferase; gamma-TMT, gamma-tocopherol methyltransferase.

2. Oxidative stress

The reactive oxygen species (ROS) represents the most important class of radical species generated in living systems formed from the partial reduction of molecular oxygen. Notable members of this family of radicals include superoxide anion (O_2^-), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) which are generated by the respiratory chain in mitochondria, enzymatic reactions, exposure to UV light, ionizing radiation and heavy metal ions. The mitochondrial electron transport chain generates superoxide radicals through the single-electron leak at respiratory complexes I and III of the oxidative phosphorylation pathway. The flavin-dependent enzymes in the mitochondrial matrix also produce a considerable amount of reactive oxygen species.

The superoxide radical is readily dismutated to hydrogen peroxide. The reactivity of hydrogen peroxide as a molecule is low but it can penetrate cell membranes and generate hydroxyl radical via the Fenton's reaction [18].

The first step involves the reduction of ferric to ferrous ion:

$$Fe^{3+} + O_{2^{\bullet -}} \rightarrow Fe^{2+} + O_2 \quad (1)$$

The second step is the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\bullet} \quad (2)$$

Haber and Weiss reaction:

$$HO^{\bullet} + H_2O_2 \rightarrow H_2O + O_2^{\bullet -} + H^+ \quad (3)$$

$$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + HO^- + HO^{\bullet} \quad (4)$$

The chain termination reaction:

$$Fe^{2+} + HO^{\bullet} + H^+ \rightarrow Fe^{3+} + H_2O \quad (5)$$

The hydroxyl radical is regarded as the most reactive oxygen radical and can cause oxidative damage to cells by attacking biomolecules located a few nanometers from the site of generation [19].

Low levels of ROS production are required for important physiological functions, including proliferation, host defense, signal transduction, and gene expression [20]. There is a cellular balance between ROS generation and clearance in eukaryotic cells. This is achieved by the activity of several antioxidative defense mechanisms that comprise enzymes and antioxidants. The five main types of primary intracellular antioxidant enzymes are Cu/Zn-superoxide dismutase (Cu/Zn-SOD, SOD1) in the cytosol, manganese superoxide dismutase (Mn-SOD, SOD2) in the mitochondrial matrix, catalase, glutathione peroxidase (GPx), and glutathione reductase (GR). Small molecular weight and non-enzymatic antioxidants are also involved in the protection of the intracellular

components against the reactive oxygen species. However, when cellular production of ROS overwhelms these antioxidative mechanisms, oxidative stress occurs [18].

The use of the term 'oxidative stress' became frequent in the 1970s, but its origin dates back to the 1950s when researchers were studying the toxic effects of ionizing radiation and free radicals. Oxidative stress refers to a pathological state that arises from an imbalance between the production of free radicals and the ability to neutralize them by antioxidants. When the antioxidant capacity is reduced, prooxidants can react with surrounding biomolecules and the extent of the reaction is dependent on the susceptibility of the biomolecules [20–22].

2.1 Free radical reaction with biomolecules

Biological molecules, notably DNA, proteins and lipids, can be affected by free radicals. The reaction of reactive oxygen species (ROS) with these macromolecules if not checked generates additional free radicals thereby causing more damage. The incorporation of modified bases into a growing DNA molecule has serious phenotypic consequences [23]. Mitochondrial DNA is mainly vulnerable, because of its closeness to the site of metabolic ROS generation [24]. Telomeres are also vulnerable to ROS attack. ROS-accelerated reduction in telomere length hasten cell senescence [25].

Oxidation of proteins induce the formation of irreversible disulphide bridges, changes in secondary and tertiary structure and ultimately impaired function. The degree of the damage depends on the location of the proteins, their composition and structure [26]. Some amino acids (tryptophan, tyrosine, histidine and cysteine) are more susceptible to oxidation than others [24].

Damage to lipids is also of great significance because of the negative impact on membrane structure and function. The composition of biological membranes is very important to the membrane function but also influence susceptibility to oxidative damage. Polyunsaturated fatty acids (PUFAs) are much more prone to peroxidation than monounsaturated or saturated fatty acid acids. Oxidation of lipids can generate a wide range of reactive intermediates which can trigger complex chain reactions with widespread effects.

2.1.1 DNA oxidation

Technological advancement in analytical chemistry have provided sensitive and specific methods for identifying and quantifying DNA adducts. Application of these techniques to the analysis of nuclear DNA from human tissues has made it clear that the notion "human genome is pristine if there is no exposure to environmental carcinogens" is incorrect. Much damage is done to DNA molecules endogenously by intermediates of oxygen reduction that either attack the nitrogenous bases or the deoxyribosyl backbone of DNA (**Figure 5**) [28].

Hydroxyl radical ($HO^{\bullet}$) is a provable candidate in DNA oxidation because it is extremely reactive. Hydroxyl radicals cannot diffuse beyond two molecular diameters because of their high reactivity [29, 30]. It can add to DNA bases or abstract hydrogen atoms to produce DNA adducts in no specific order [28]. The effect on nuclear DNA can only be possible if H_2O_2 generate $HO^{\bullet}$ on reaction with a metal ion in the vicinity of a DNA molecule [31, 32].

Peroxinitrite, a product of the coupling of nitric oxide and superoxide ion (O_2-) has also been identified as an extremely strong DNA oxidant. Apart from its ability to generate $HO^{\bullet}$, its protonated form (peroxinitrous acid, ONOOH) is an extremely reactive oxidant [28].

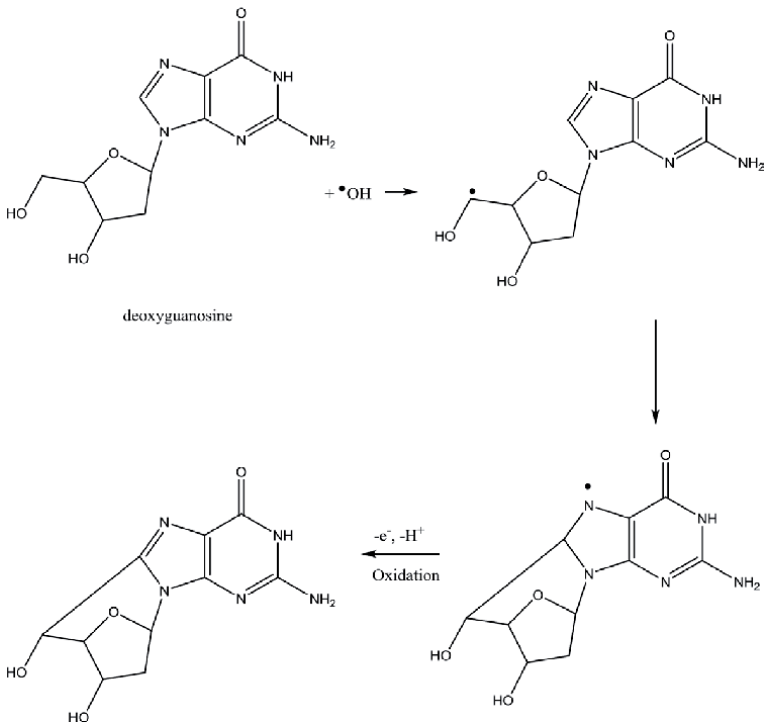


Figure 5.
Oxidation of deoxyribose in purines [27].

Secondary products of lipid peroxidation reactions are also culpably involved in DNA oxidation. Malondialdehyde and 4-hydroxylnonenal can oxidize DNA thereby generating several DNA adducts [30].

2.1.1.1 Mechanism of DNA oxidation

When a H-atom is abstracted from C5' carbon atom in the sugar moiety, the C5′-centered radical generated binds to the C8-position of the purine base in the same nucleoside. The products of this intramolecular cyclization are 8,5'-cyclopurine-2′-deoxynucleosides (**Figure 5**). The reactions of carbon-centered sugar radicals result in the DNA strand breaks [27].

The reaction of HO• with purines in DNA produces a C-8-hydroxy-adduct radical of the purine base which can be converted to the 2,6-diamino-4-hydroxy-5-formamidopyrimidine by reduction that ultimately lead to ring opening. The oxidation of the C-8-hydroxy-adduct radical of purines yield 8-hydroxypurine HO• can react also with the heterocyclic part of the pyrimidine bases to yield several base adducts. For example, the reaction of HO• with cytosine and thymine at C5- and C6-positions, yields C5–OH and C6–OH adducts respectively. Further oxidation of these adducts by water and concomitant deprotonation results in the formation of the respective glycols.

2.1.2 Proteins

The interest in the study of protein oxidation started in the early 1990s with the aim to explain oxidative damage to specific purified enzymes or in oxidative stress processes involving proteins. The process of protein oxidation by free radicals is an important biochemical event in living cells and is implicated in a number of human diseases.

2.1.2.1 Oxidation of the protein backbone

The removal of one hydrogen atom from an amino acid residue in a polypeptide molecule by a free radical generates a carbon-centered radical. The reaction of the carbon-centered radical with O_2 forms an alkylperoxyl radical intermediate which can be converted to an alkylperoxide. The resulting alkoxyl radical may be converted to a hydroxyl protein. Steps in this pathway are mediated by interactions with $HO_2^{\bullet}$ and are catalyzed by Fe^{2+}. The radical intermediates generated can further react with other amino acid residues within the same or in different protein molecules thereby generating a new carbon-centered radical that can undergo similar reactions. However, when oxygen is absent, two carbon-centered radicals may react with each other to form a protein–protein cross-link (**Figure 6**) [33].

Peptide bond cleavage occurs by either the diamide or α-amidation pathways. ROS attack on glutamyl, aspartyl and prolyl side chains of polypeptide residues lead to peptide bond cleavage via a different pathway. Oxalic acid is formed and the N-terminal amino acid of the peptide derived from the C-terminal portion of the protein will exist as an N-pyruvyl derivative. The involvement of proline oxidation is linked to the observation that the number of peptides formed during radiolysis of proteins is approximately equal to the number of prolyl residues. It was thus proposed that oxidation of prolyl residues would lead to peptide bond cleavage. This was verified by Uchida et al. [34] who showed that the oxidation of proline residues leads to the formation of 2-pyrrolidone and concomitant peptide bond cleavage. The hydrolysis of 2-pyrrolidone by an acid yields 4-aminobutyric acid. The observation of 4-aminobutyric acid in protein hydrolysates serve as reasonable evidence of the involvement of proline oxidation in peptide bond cleavage.

2.1.3 Lipid peroxidation

The adverse effects of lipid peroxidation in biological systems gained attention in the 1960's and it is now known that this reaction is a relevant event in biology and medicine [35].

Lipid peroxidation is a chain reaction, catalyzed by transition metals ultimately resulting in the breakdown of membrane phospholipids that contain polyunsaturated fatty acids (PUFAs). The severity of the resulting damage depends on the nature and concentration of the oxidant and it may range from reductions in membrane fluidity to full disruption of bilayer integrity [30]. The two most dominant ROS that can affect lipids profoundly are; hydroxyl radical ($^{\bullet}OH$) and hydroperoxyl radical ($HO_2\bullet$), a protonated form of O_2^{-}. The hydroxyl radical ($^{\bullet}OH$) attack biomolecules located a few nanometers from the site of generation [36]. H_2O_2 generated from $HO_2\bullet$ can react with redox active metals to generate $^{\bullet}OH$ through Fenton or Haber-Weiss reactions. The $HO_2\bullet$ can also directly initiate the oxidation of polyunsaturated phospholipids in cell membrane [37].

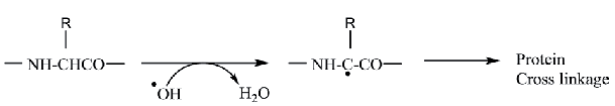


Figure 6.
Formation of carbon-centered radicals during protein oxidation [33].

2.1.3.1 Lipid peroxidation process

Lipid peroxidation reactions involve the abstraction of hydrogen from a carbon in a lipid molecule followed by the insertion of oxygen to form lipid peroxyl radicals and hydroperoxides. Glycolipids, phospholipids, and cholesterol are susceptible to these damaging and possibly lethal peroxidative alterations. The enzymes; lipoxygenase, cyclooxygenase and cytochrome P450 can also oxidize lipids.

Lipid peroxidation reactions are categorized into three phases: initiation, propagation, and termination. In the initiation step, pro-oxidants like hydroxyl radical removes an allylic hydrogen forming a carbon-centered lipid radical (L•). During the propagation phase, the lipid radical (L•) rapidly reacts with oxygen to form a lipid peroxyl radical (LOO•). The LOO• can react with neighboring lipid molecules form a new L• and lipid hydroperoxide (LOOH) (**Figure 7**).

The reaction process can be terminated by antioxidants that donate hydrogen atom(s) to the lipid peroxyl radical species resulting in the formation of non-radical products. For example, vitamin E donate hydrogen atom to the LOO• species. The resulting 'oxidized' vitamin E radical reacts with another LOO• forming non-radical products. The chain reaction continues in the absence of antioxidants [38].

2.1.3.2 Lipid peroxidation products

Lipid peroxidation produces a number of oxidation products categorized as primary and secondary products. Lipid hydroperoxides (LOOH) are the main primary products of lipid peroxidation. Several aldehydes are formed as secondary products from the hydroperoxides including; malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) [39, 40]. 4-HNE and MDA have been reported to be the most toxic and most mutagenic product of lipid peroxidation respectively [41].

The decomposition of arachidonic acid (AA) and larger PUFAs as well as enzymatic processes during the biosynthesis of thromboxane A_2 (TXA_2) and 12-l-hydroxy-5,8,10-heptadecatrienoic acid (HHT), or the non-enzymatic processes

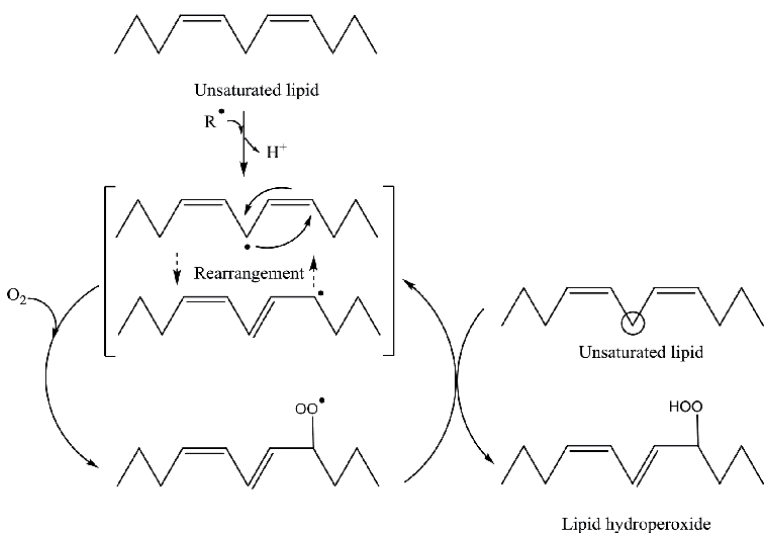


Figure 7.
Lipid peroxidation process [30].

by bicyclic endoperoxides produced during lipid peroxidation, can generate MDA in vivo. MDA generated by these processes can be enzymatically metabolized or form adducts with biomolecules [30].

2.2 Oxidative stress and human diseases

Oxidative stress has been implicated in the onset and progression of pathological conditions such as cancer, cardiovascular disease, neurological disorders, diabetes as well as aging [42–44]. The negative impact of oxidative stress is due to the damaging consequences of free radicals on important biological molecules [45]. Free radical mediated oxidative stress increases with age and may overwhelm natural repair systems [46]. A review of the mechanism of human diseases resulting from oxidative stress was published by Rahman [47]. The influence of oxidative stress on aging is now established and the associated diseases include; cardiovascular diseases, Huntington's disease, Alzheimer's disease, stroke, Parkinson's disease and cancer [47, 48].

The use of oxidative stress biomarkers for the diagnosis of acute and chronic diseases indicate the involvement of oxidative stress in such pathological conditions. This is confirmed by the difference in the concentration of these biomarkers in healthy and ill subjects evaluated for long periods. Representative biomarkers of oxidative damage associated with some human diseases were summarized by Valko et al. [20] and are presented in **Table 1**.

3. Roles of vitamin E in human health and disease

Natural antioxidants including vitamin E have gained relevance in combating oxidative stress [49]. The vitamin E required by humans is solely acquired from diet. The lipohilic nature of vitamin E support the theory of high antioxidant capacity in cell membranes [47]. The capacity to be replenished by other antioxidants such as ascorbic acid is another important factor [9, 10]. Vitamin E has been reported to slow down the progression of oxidative assaults on biomolecules thus suppressing diseases [13]. The remaining sections of this chapter shall focus on the roles of vitamin E in the management of some illnesses and human wellbeing.

Oxidation products used as biomarkers for oxidative stress	Disease
MDA, GSH/GSSG ratio, NO_2-Tyr, 8-OH-dG	Cancer
HNE, GSH/GSSG ratio, acrolein, NO_2-Tyr, F2-isoprostanes	Cardiovascular disease
F_2-isoprostanes, GSH/GSSG ratio	Rheumatoid arthritis
MDA, HNE, GSH/GSSG ratio, F_2-isoprostanes, NO_2-Tyr, AGE	Alzheimer's disease
HNE, GSH/GSSG ratio, carbonylated proteins, Fe-level	Parkinson's disease
F_2-isoprostanes, GSH/GSSG ratio	Ischemia and reperfusion
MDA, HNE, Acrolein, NO_2-Tyr, F_2-isoprostanes	Atherosclerosis
MDA, GSH/GSSG ratio, F_2-isoprostanes, NO_2-Tyr, AGE, S-gluathionylated proteins	Diabetes mellitus

Abbreviations: MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; AGE, advanced glycation end products; 8-OH-dG, 8-hydroxy-20-deoxyguanosine; GSH, reduced glutathione; GSSG, oxidized glutathione; NO_2-Tyr, 3-nitro-tyrosine.

Table 1.
Oxidative stress biomarkers associated with some human diseases [20].

3.1 Vitamin E in disease prophylaxis

Epidemiological studies and observational surveys link populations that consume a high amount of vitamin E to a reduced incidence of chronic diseases. This disease preventing capacity is largely linked to the antioxidant capacity of the vitamin E family. The antioxidant property is based on the capacity of vitamin E to donate hydrogen to free radicals and its lipid membrane solubility. The resulting tocopheroxyl radical is far less reactive compared to free radicals so does not propagate the oxidative chain reaction. The tocopheroxyl radical can be reduced by ascorbic acid or react with another tocopheroxyl radical to form stable products [50].

Vitamin E is an immune booster which may play an essential role in the observed prophylactic action. The vitamin E family have been reported to regulate cell growth and induce apoptosis in tumor cells. Several other anticarcinogenic mechanisms have been reported for the vitamin E family including the stimulation of the migration of macrophages and lymphocytes that contain tumor necrosis factor to tumor sites [51] and modulation of the expression of oncogenes. Additional studies have revealed that vitamin E succinate, a modified product can specifically induce apoptosis in tumor and cancer cells but not normal epithelial cells in mammary and prostatic glands [50, 52].

The hypothesis that atherosclerosis may be prevented by blocking the oxidative modification of LDL cholesterol, a key process in the onset and progression of atherosclerosis renewed interest in vitamin E. Research outcomes have documented beneficial effects of vitamin E on several stages of the atherosclerotic process [53].

3.2 The role of vitamin E in cardiovascular disease

The development of atherosclerosis depends on the pro- and anti-inflammatory as well as pro- and anti-oxidant balance [54]. The oxidation of LDL is a principal component of atherosclerosis and is implicated in the onset of cardiovascular diseases via several mechanisms. At low concentrations, oxidized LDL can stimulate the production of inflammatory markers such as cell adhesion molecules and macrophage colony stimulating factor by endothelial cells. The resulting endothelial dysfunction can result in either cell growth or apoptotic cell death that can cause vasoconstriction.

At high concentrations, oxidized LDL are recognized by scavenger receptors and they are phagocytosed by macrophages resulting in the formation of lipid-laden foam cells. The cytotoxic property of oxidized LDL in cultured endothelial cells, the ability to inhibit macrophage motility and the inhibitory effect on nitric oxide-induced vasodilation are other potential atherogenic possibilities. Experimental evidence also back the involvement of free radicals in congestive heart failure (CHF), vascular injury and organ dysfunction [55, 56].

The vitamin E family has received considerable attention in atherosclerosis research based on the capacity to inhibit LDL oxidation and decrease uptake of oxidized LDL by macrophages in human arterial lesions. The vitamin E compounds are reported to be favorable modulators of the atherogenic process at the molecular and cellular levels [57, 58]. Other potential mechanisms of action include; reduction of endothelial injury, reduction in the expression of adhesion molecules, reduction in endothelial cell adhesion, inhibition of inflammatory cytokines and chemokines synthesis, inhibition of smooth muscle cell proliferation, inhibition of platelet aggregation, increased NO production and arterial dilation.

An in vitro study on the effect of vitamin E on LDL oxidation in the blood plasma of healthy volunteers revealed that enrichment with vitamin E increased resistance to LDL-oxidation in a dose-dependent manner [59] and decreased uptake

of oxidized LDL by macrophages in human arterial lesions. In another study, vitamin E enrichment increased LDL vitamin E concentration by approximately 2.5 folds and the susceptibility to oxidation was reduced by 30–40% [60].

The results obtained from in vitro studies have not been replicated exactly in clinical trials.

3.3 The role of vitamin E in cancer

Cancer is a complicated disease condition characterized by the inability to control cell growth. Carcinogenesis has been categorized into three stages; initiation, promotion, progression and the action of free radicals have been implicated in all stages due to their capacity to react with all components of DNA. The concentration of oxidized DNA adducts is directly linked to the size of benign tumors and can directly affect the transformation to malignancy [47].

The vitamin E compounds are powerful antioxidants thus can inhibit DNA oxidation. The natural forms of vitamin E have been reported as effective agents for cancer therapy [61]. The result of the selenium and vitamin E cancer prevention trial (SELECT) revealed that dietary supplementation of α-tocopherol at 400 IU/d increased the risk of prostate cancer [62]. The anticancer properties of α-tocopherol have been studied the most among the vitamin E compounds and available results reveal that the anticancer property of the compound is not very promising.

However, the vitamin E forms; γ-tocopherol, δ-tocopherol, γ-tocotrienol and δ-tocotrienol have been reported to have higher anticancer property compared to α-tocopherol. These vitamin E forms are able to inhibit multiple cancer promoting pathways by inhibiting the formation of eicosanoids. In conjunction with their metabolic product; 13′-carboxycromanol, they inhibit the cyclooxygenases (COX-1 and -2) and 5-lipoxgenase (5-LOX). Gamma- and δ-tocotrienols also suppress the activation of *nuclear factor kappa B* (NF-kB) and signal transducer and activator of transcription factor 3 (STAT3). These activities neutralize pro-inflammatory tumor microenvironments that favor cancer development, invasiveness, and resistance to treatment. These vitamin E compounds also target cancer cells and cancer stem cells by promoting apoptosis, antiangiogenesis, and antiproliferation partially via modulating epigenetic events and other signaling pathways. The modulatory effect of tocotrienols on immunity may also contribute to cancer prevention [61].

The anti-inflammatory mechanism of vitamin E compounds and their metabolites is based on their capacity to inhibit the cycloxogenases and the lipoxygenase involved in eicosanoid synthesis. Reduced synthesis of prostaglandins and leukotrienes have been reported to slow down tumorigenesis, angiogenesis and metastasis. The activity of COX-2 and 5-LOX as well as the concentration of Prostaglandin E_2 (PGE_2) are increased in tumor cells. These events promote angiogenesis and resistance to apoptosis via PGE_2 receptor-mediated signaling in cancer cells [61, 63, 64].

The non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit inflammation via the inhibition of COX and 5-LOX have been shown to inhibit tumor development in various cancer models [65, 66]. Studies have revealed that γ-tocopherol, δ-tocopherol and γ-tocotrienol as well as their metabolites can inhibit COX- and 5-LOX at physiological concentrations [61].

Pro-inflammatory cytokines secreted by macrophages associated with tumor and cancer cells also promote tumor growth and invasiveness. The cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6)) and *tumor necrosis factor alpha* (TNF-α), activate NF-kB and STAT3 in cancer cells. The activation of NF-kB and STAT3 increase the expression of genes that promote cell survival, proliferation, angiogenesis and

invasiveness [67]. The inhibition of NF-kB and STAT3 as well as their regulatory cytokines is a potent target to suppress tumor development and progression.

Vitamin E compounds have been shown to block NF-kB and STAT3 activation and their regulated genes in macrophages and cancer cells [68]. The inability to express survival genes following the inhibition of NF-kB and STAT3 sensitizes the cancer cells to therapeutic drugs. γ- and δ-Tocotrienols have been reported to be active inhibitors of NF-kB and STAT3 [69].

The immune system play an important role in the defense against cancer by detecting and killing tumor cells [68]. A combination of tocotrienols as supplements have been reported to modulate immune response and has a higher anticancer activity than α-tocopherol alone [70]. Tocotrienol supplementation enhanced lymphocyte proliferation without affecting major cytokines in old mice but not young ones, suggesting an age-dependent immune modulatory function [71]. The supplementation with the vitamin E compounds increase interferon gamma (IFN-γ) and interleukin 4 (IL-4), thus enhancing antibody production while suppressing TNF-α in stimulated splenocytes. This activity observed in response to tetanus toxoid vaccination suggest anticancer activity via immune modulation [68].

The vitamin E forms have been reported to directly target cancer cells. γ-Tocopherol, δ-tocopherol, γ-totrienol, δ-tocotrienol and 13′-carboxychromanol have been reported to induce the arrest of cancer cell growth, apoptosis and autophagy in several types of cancer cells [72]. The preferential accumulation of γ-totrienol, δ-tocotrienol and 13′-carboxychromanol in cancer cells may be responsible for the observed higher anticancer activity compared to the tocopherol counterparts [73].

The capacity of these vitamin E forms to induce pathways associated with antiproliferation [74], elevation of mitochondria apoptotic proteins [73], autophagy marker LC3II and endoplasmic reticulum stress markers such as c-Jun N-Terminal kinase (JNK) phosphorylation and death receptor-5 (DR5) pro-apoptotic pathway [75] may contribute to the reported anticancer activity. The anticancer activity has also been linked with the capacity of the vitamin E forms and 13′-carboxylchromanol to modulate sphingolipid metabolism. At elevated concentrations, sphingolipids such as dihydroceramide, dihydroshpingosine and ceramides induce stress and apoptosis as well as inhibit cell growth [76]. This has been shown in prostate, colon, pancreatic and breast cancer cells were an elevation of the sphingolipids precede or happen simultaneously with cell death [74]. Suppressing de novo synthesis of sphingolipids reverses the anticancer activity of the vitamin E forms. Research is still ongoing to completely unravel the interactions and effect of sphingolipid modulation in vivo [61].

3.4 The role of vitamin E in cataracts

Cataracts are one of the commonest reasons for critical vision distress in adult humans. They essentially happen because of the aggregation of proteins oxidized by free radicals. A few observational examinations have uncovered a likely connection between vitamin E supplements and the danger of cataracts development. Leske et al. [77] reported that lens clarity was higher in individuals receiving vitamin E supplements and those with higher plasma concentrations of the vitamin. In another investigation, vitamin E supplementation was related with reduced opacification of the lens. However, in a randomized Age-Related Eye Illness Study (AREDS), vitamin E had no clear impact [78]. Like in other disease conditions, the exact mechanism of the observed positive effects of vitamin in the reduction of cataract formation in vivo is in progress [79].

3.5 Roles of vitamin E in other diseases

The role of vitamin E gas been studied in several other diseases conditions linked to oxidative stress. For example, stroke has been linked with free radical reactions arising from xanthine oxidase, cyclooxygenase and inflammation [80]. These free radical reactions can cause neuronal death [81]. The oxidative assault is increased by the biochemical processes associated with stroke.

Oxidative stress is also implicated in the onset and progression of the neurological disorders; Alzheimer's disease and Parkinson's disease. In both conditions, logarithmic age-dependent increase in the oxidation of proteins, lipids and DNA as well as decreased in vivo antioxidant activity has been reported [47]. In Alzheimer's disease, oxidation induce protein cross linking and aggregation of β-amyloid protein which in turn induce the oxidation of carbohydrate side chains of membrane lipids leading to neuronal membrane breakdown [82]. Oxidation of lipids also accompany the process and has been quantitatively assessed by increased concentration of 4-hydroxyl-2-nonenal-glutathione conjugates in the brain [83].

In diabetes, free radical-induced OS has been reported to play a significant role in the development of insulin resistance, β-cell dysfunction and impaired glucose tolerance. Hyperglycemia worsens the oxidative burden following the formation of advanced glycation end products (AGEs).

The biochemical importance of oxidative stress in the onset and progression of disease conditions underscores the relevance of vitamin E in prevention and management.

In HIV infection, it is not exactly clear if vitamin E supplementation has the same effect at all stages of the disease. However, high serum concentration of vitamins A and E have been reported to affect disease progression. Vitamin E concentrations higher than 23.5 μM reduced the risk of progression of HIV-1. Vitamin E has also been shown to normalize immune system parameters in murine acquired immunodeficiency syndrome as well as protect against bone marrow toxicity of azidothymidine. Protection against azidothymidine toxicity was confirmed in stage IV HIV patients on alpha-tocopherol supplementation. High doses have also been reported to restore delayed skin hypersensitivity, stimulate interleukin-2 production and T-helper (CD4 T-cell) proliferation [79].

4. Discussion

The in vitro antioxidant activity of vitamin E compounds are well documented. However in vivo studies on prevention and treatment of oxidative stress-related diseases have been disappointing. Till date, there is no approval for the clinical use of vitamin E as a drug even though vitamin E consumption has been reported to boost immunity, improve skin health and vision. Vitamin E remains a popular supplement and is generally regarded as safe by the FDA.

Vitamin E is the main lipid soluble antioxidant [79] in the cell membrane thus will always attract research attention considering the relevance of membrane lipid oxidation [84]. The bioactivity of vitamin E in the cell membrane may be a direct approach to limit oxidation of other biomolecules that can form conjugates with oxidized lipids.

Emerging research outputs are shedding light on the disparity between in vitro and in vivo antioxidant capacity of vitamin E [85]. In macrophages, the oxidation of lipids occur in the lysosomes. Alboaklah and Leake [85] conducted an experiment on LDL oxidation at lysosomal pH (about 4.5). In their experiment, LDL enriched with vitamin E was oxidized by Cu^{2+} more slowly compared to control LDL. At

pH 4.5, the enriched LDL was not protected against oxidation by low concentrations of Cu^{2+} or Fe^{3+}. They observed that the enriched LDL reduced the Cu^{2+} and Fe^{3+} to the more pro-oxidant Cu^{+} and Fe^{2+} at a faster rate than control LDL at lysosomal pH. This may partly be responsible for the observed reduction in the bioactivity of vitamin E compounds in vivo.

The bioavailability of vitamin E has been another complication in clinical applications. Recent data indicate that the absorption of vitamin E is far more complex than previously thought. Details about the digestion, absorption and transport of vitamin E are presented in a review by Reboul [86]. The author concluded that the process is only partly understood and suggested further studies to decipher the molecular mechanisms [86].

The poor water solubility of vitamin E has been implicated in the low oral bioavailability [87]. Recently, nanoformulations such as nanovesicles, solid-lipid nanoparticles, nanostructured lipid carriers, nanoemulsions, and polymeric nanoparticles have shown promising outcomes in improving the efficacy and bioavailability of vitamin E.

Lipid-based nanovesicles such as niosomes and liposomes are highly promising for the delivery of lipophilic drugs and active compounds. Although niosomes and liposomes have similar physicochemical properties, niosomes have a higher permeability to small solutes and ions than liposomes. The application of these nanovesicles as drug delivery vehicles is suitable because they are non-toxic and stable.

In a study to enhance the tumor-suppressing effect of tocotrienols in vivo, Fu et al. [88] first developed a D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)-based noisome [88, 89]. This system appeared to significantly increase tocotrienol uptake in vitro using A431, B16F10 and T98G cell lines and hence improved the therapeutic efficacy. TPGS can be functionalized as an excellent solubilizer, emulsifier, permeation and bioavailability enhancer for hydrophobic drugs [90]. TPGS has demonstrated capacity to selectively induce apoptogenic activity against many cancer types by targeting the activation of mitochondrial mediators of apoptosis [91]. Another 6-*O*-palmitoyl-ascorbic acid (PA) based niosomes (comprising AP, TPGS, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (DSPE-PEG (2000)-carboxylic acid)) was developed by Tan et al. [92] targeting transferrin receptors for intravenous administration of γ-tocotrienol aimed at treating breast cancer. Both in vitro and in vivo studies have proven that tumor-targeted niosomes significantly improve the therapeutic efficacy of γ-tocotrienol. These studies suggests that nanovesicles can be suitable carriers for improved delivery and enhanced efficacy of vitamin E [93].

5. Conclusion

Although the antioxidant and antiproliferative properties of vitamin E against oxidative stress related diseases have been reported, there is currently no approval for clinical application. A modified product, TPGS has been approved by the FDA as a safe pharmaceutical adjuvant with high biocompatibility. Despite the reported bioactivity of the tocotrienols, studies so far are inconclusive. The reported in vitro biochemical properties of the vitamin E family will continually call the attention of researchers since they are natural and can play essential roles in ameliorating the impact of oxidative assault in biological membranes. To maximize the prophylactic and curative properties of vitamin E, further research on absorption, cellular uptake, solubility, and stability is required to improve bioavailabilty and efficacy in vivo.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 13

Male Infertility, Oxidative Stress and Antioxidants

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Abstract

Within the male reproductive system, oxidative stress (OS) has been identified as prevailing etiology of male infertility. The effects of reactive oxygen species (ROS) on male fertility depend on the dimensions, "modus operandi" of the ROS and the oxido-reduction potential (ORP) of the male reproductive tract. Hereupon, for an adequate response to OS, the cells of our body are endowed with a well-sophisticated system of defense in order to be protected. Various antioxidant enzymes and small molecular free radical scavengers, maintain the delicate balance between oxidants and reductants (antioxidants), crucial to cellular function and fertility. Therapeutic use of antioxidants is an optimal and coherent option in terms of mitigating OS and improving semen parameters. Therefore, recognizing and managing OS through either decreasing ROS levels or by increasing antioxidant force, appear to be a requesting approach in the management of male infertility. However, a clear defined attitude of the experts about the clinical efficacy of antioxidant therapy is still deprived. Prominently, antioxidant such as coenzyme Q10, vitamin C and E, lycopene, carnitine, zinc and selenium have been found useful in controlling the balance between ROS production and scavenging activities. In spite of that, healthy lifestyle, without smoke and alcohol, everyday exercise, reduction of psychological stress and quality well-designed meals, are habits that can overturn male infertility.

Keywords: Male infertility, reactive oxygen species, oxidative stress, antioxidants, sperm parameters

1. Introduction

The World Health Organization (WHO) defines infertility as the inability (failure) to attain clinical pregnancy after one year or more of regular unprotected sexual intercourse [1]. Since infertility presents a certain disability (impaired reproductive function), medical assessment and treatment falls under the umbrella of the United Nations Convention on the Rights of Persons with Disabilities – UNCRPD, which is formally accepted by many countries. The article 1 of this Convention summarizes the overall objective as: "to promote, protect and ensure the full and equal enjoyment of all human rights and fundamental freedoms by all persons with disabilities, and to promote respect for their inherent dignity" [2]. Due to its health, cultural and socio-economic impact, infertility is a major globally underestimated public health concern [3, 4]. Therefore, proper evaluation of male

infertility is a substantial stride in qualifying, quantifying and configuring necessary laboratory assessment, credential treatment strategies as well as policies to diminish the burden of this global sensitive health issue.

There are approximately 186 million infertile people [5] or 15% of couples globally, 50% due to male factor infertility which experience problems in conceiving [6, 7]. In male dominated societies, generally, the female partner is blamed for barrenness, even though ancient Greeks were aware that male factor is a contributor to the reproductive success [8].

In fertile couples, spontaneous conception is most likely to occur in 30% of cases during the first month, 75% after 6 months, 90% after 12 months and 95% between 18 to 24 months [9]. Also, there are studies which consider that 80% of couples having unprotected sexual intercourse will achieve pregnancy in the 6-month [10] or 12-month interval [11].

In addition, male fertility reaches its maximum potential at ages of about 25 to 30 years and declines sharply in the beginning of fifties [12], however, there are men reported to give life to offspring into their eighties [13]. Paternal age of >40 years is associated with more than 20% higher chance of congenital defects in the offspring [14]. Over the past decades, an age-related decline in semen quality resulting in declined fertility was observed [15].

Oxidative stress (OS) has been identified as one of the major contributors affecting male fertility potential [16] and has thus been extensively studied in the last three decades. Although cells of the human body have efficient mechanisms to cope with factors disturbing the normal cell homeostasis, OS may arise due to an imbalance between generation of oxidants and antioxidants mechanisms, resulting in cell damage.

Reactive oxygen species (ROS) are important mediators of OS status, because of their capacity to oxidize proteins, lipids, and DNA, resulting in cellular dysfunction [17]. ROS are oxygen-based molecules that have unpaired electrons on their most outlier spin-orbit, derived from the reaction of carbon-centered radical with oxygen (except hydrogen peroxide), which makes them highly reactive [18]. The most common ROS are hydroxyl radical (OH•), hydrogen peroxide (H_2O_2) and the superoxide anion (O_2•-). ROS are generated not only by leukocytes (neutrophils and macrophages mostly) [19], but also by any aerobe living cell including spermatozoa [20]. Moreover, another subclass of free radicals deriving from nitrogen-based molecules are called reactive nitrogen species (RNS) [21, 22]. At physiologic amount, RNS are important for various functions within the male reproductive tract such as: (1) signal transduction, (2) regulation and assembly of tight junction within the blood-testis barrier, (3) mediation of cytotoxic and pathological events, (4) production of hormones, (5) inflammation and (6) other important physiological changes of spermatozoa [23].

Some of the most common ROS and RNS are listed in **Table 1**. Effects, consequences, mode of formation and action of these molecules are presented in details in **Table 2**.

Under physiological conditions, high levels of ROS are counterbalanced by antioxidants, which competently maintain a delicate redox balance by donating their electrons to the ROS and thus interrupting further intake of electrons from surrounding compartments [37]. The seminal antioxidant system comprises a network of enzymatic and non-enzymatic molecules, dispersed mostly within seminal plasma and spermatozoa [38]. The three major antioxidant enzymes are glutathione peroxidase (GPx), catalase (CAT) and the superoxide dismutase (SOD) [39].

With an increasing knowledge on the role of OS in the clinical manifestation of male infertility, antioxidant prescription and its implementation in treating male infertility may be helpful. Several antioxidant compounds are currently prescribed

Reactive oxygen species				**Reactive nitrogen Species**	
Radicals		**Non-radicals**			
Lipid peroxyl	**LOO•**	*Lipid hydroperoxide*	**LOOH**	*Nitryl chloride*	**NO_2Cl**
Thyl	**RS•**	*Ozone*	**O_3**	*Nitrous acid*	**HNO_2**
Peroxyl	**RO_2•**	*Singlet oxygen*	**$^{-1}O_2$**	*Nitrogen dioxide*	**NO_2**
Nitric oxide	**NO•**	*Hydrogen peroxide*	**H_2O_2**	*Dinitrogen trioxide*	**N_2O_3**
Superoxide	**O_2•—**	*Hypochloric acid*	**HOCl**	*Nitroxyl anion*	**NO^-**
Hydroxyl	**OH•**	*Peroxynitrite*	**ONOO—**	*Nitroxyl cation*	**NO^+**

Table 1.
Most common ROS and RNS.

Hydrogen peroxide (H_2O_2)	**Ref.**
Hydrogen peroxide is not a free radical, because it does not contain an unpaired electron, but it is classified as ROS because it participates in the generation of highly reactive hydroxyl free radicals through interactions with iron and copper, based on the Fenton reaction.	[22,24–26]
Superoxide (O_2•—)	Ref.
It is generated by electron transport leaks from several reaction in cytosol. It does not spread easily and faraway its origin. It is responsible for cell injury, by deconstructing iron–sulphur clusters in proteins through the inactivation of iron regulatory protein-1. Superoxide is insoluble for the cell membrane.	[27–29]
Hydroxyl (•OH)	Ref.
This represents the neutral form of the hydroxide ion, deriving from the reaction between Fe^{2+} and H_2O_2 (Fenton reaction). It is the most reactive free radical. The hydroxyl radicals and hydroxide ions can be generated also by the reaction of H_2O_2 and O_2•- catalyzed by iron (Haber-Weiss reaction). The hydroxyl radical has the potential of reacting fast and nonspecifically.	[30, 31]
Peroxynitrite (ONOO—)	Ref.
It is generated during reaction of nitric oxide (NO) with O_2—, it can react with thio groups of structural proteins, resulting in the formation of nitrosotioles, which can disunite metal-protein interactions and result in the formation of metal-derived free radicals.	[32]
Peroxyl radical (ROO•)	Ref.
Peroxyl radicals remove electrons from lipids during the process of lipid peroxidation. During this process, intermediates are generated that participate in further reactions with oxygen to form lipid peroxyl (LOO•) and lipid hydroperoxide (LOOH) which are responsible for sperm DNA and protein damage.	[33–35].
Hypochloric acid (HOCl)	Ref.
Hypochloric acid is produced by macrophages and neutrophils during respiratory burning that accompanies phagocytosis. This radical is generated in the reaction between H_2O_2 and chloride ion (Cl^-).	[36]

Table 2.
The mode of formation of the biologically active ROS responsible for the major consequences of oxidative stress.

without any scientific rationale, ensuing neither semen parameters improvement, nor fertilization outcomes. Contrary, some other studies even showed a worsening of semen parameters [40–42], because an excess intake of antioxidants can contribute in the establishment of reductive stress (RS), a condition which has been reported being as harmful as OS [43]. Therefore, there still lack of conclusive consensus regarding the clinical advantages of antioxidants - based therapy in male infertility.

2. Oxidative stress and male infertility

OS is a condition characterized by an elevated generation of ROS and a reduced response of biological mechanisms to promptly neutralize the reactive intermediates or to repair the damage [44]. An increased quantity of ROS and RNS has now been established with strict evidence to be a prominent attribute of many acute and chronic pathologies [45].

Nearly eight decades after the Macleods discovery in 1943, highlighting ROS as key players in cell physiology and sperm motility [46], scientists all over the world turned their attention toward the association between free radicals and the male infertility.

2.1 Sources of ROS

Semen comprises a variety of cells including spermatozoa, germ cells, leukocytes and epithelial cells [47], whereby leukocytes produce about 1000-times more ROS than immature sperm cells [48].

ROS originate from a different countless endogenous and exogenous sources.

Endogenous sources of ROS can be generated extracellularly and intracellularly. Intracellular ROS include $O_2^{\cdot -}$, H_2O_2 and OH^-, generated mainly in the mitochondria [49]. In the mitochondria, about 5% of the consumed oxygen is physiologically converted into ROS. The ROS production is increased when the electron transporting chain (ETC) derails as a result of mitochondrial dysfunction [50].

Exogenous sources of ROS include smoking, alcohol and drugs abuse, environmental pollutants, heavy metals, ionizing radiation, diets rich in energy-yielding nutrients like carbohydrates, saturated fats and proteins [51].

2.2 Mechanism of ROS production within human sperm

ROS are generated in two pathways: the extrinsic and the intrinsic pathway, described in **Figure 1**.

Leukocytes are responsible for the extrinsic pathway of generating ROS, while spermatozoa for the intrinsic pathway of ROS generation [52]. Granulocytes are the white blood cells (WBC) in seminal fluid which are predominantly responsible for demolishing pathogens by ROS production [53, 54].

An association between OS and the elevated leukocyte numbers has been found [19]. On the other hand, the relationship between the seminal leukocyte concentration and male infertility is not clear. In fact, leukocytospermia, i.e. the presence of more than 1×10^6 WBC/mL, is not predictive of male infertility [55, 56]. However, the significance of WBC activation in ROS generation and its impact on elevated OS levels cannot be left unnoticed. Various studies reported high levels of proinflammatory chemokines in human semen along with high ROS quantity [57, 58]. Recently, in the seminal plasma of oligozoospermic and azoospermic men it was observed a negative correlation between levels of interleukin-6 (IL-6), interferon alpha (IFN-α) and interferon gamma (IFN-γ) and sperm parameters such as concentration, motility and morphology [59, 60].

Among spermatozoa, it has been shown that morphologically abnormal spermatozoa are the main source of ROS generation [61]. Excess residual cytoplasm (ERC) around the mid-piece of spermatozoa (observed in teratozoospermic sperm) contains high levels of cytoplasmic enzymes responsible for generating ROS [62].

ERC has a considerable amount of enzymes to regulate glucose metabolism, specifically glucose-6-phosphate dehydrogenase (G6PD) [63], which induces

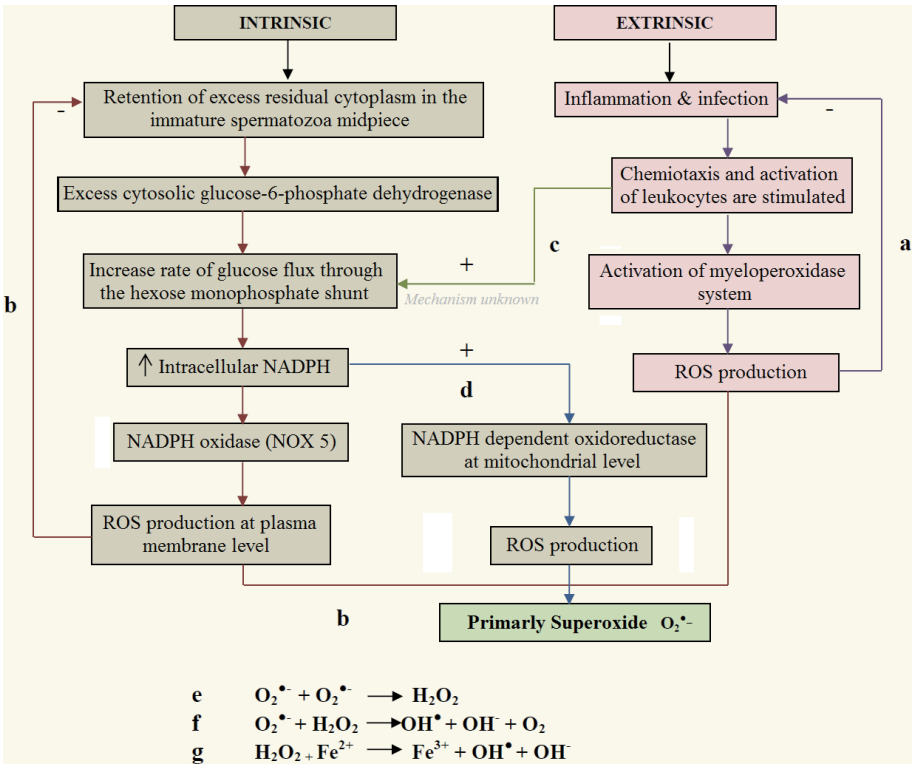

Figure 1.
Mechanism of free radical production within semen. (a) The intrinsic and extrinsic pathway contribute in the formation of $O_2^{\bullet-}$. (b) Superoxide is transformed directly and indirectly to secondary (e, f, g) ROS. Adapted from reference [28]. (mathematical symbols + and - stand for positive and negative feedback).

increased ROS levels by activating (1) the NADPH - nicotinamide adenine dinucleotide phosphate located in the plasma membrane of spermatozoa, and (2) NADPH – dependent oxide-reductase, known as diphorase, detected in the middle piece of mitochondrial level [64–66]. In a study by Sabeur et al., calcium-dependent NADPH oxidase 5 (NOX5) of spermatozoa plays a considerable role in ROS generation [67]. However, there is a difference between NOX5 found in spermatozoa, which does not require protein kinase C for expressing its activity, and in leukocytes, where protein kinase C is essential [68].

2.3 Physiological role of ROS

ROS are very important molecules as they act as cellular mediators essential for (1) normal spermatogenesis, (2) activation of steroidogenic pathway, (3) modulation of mitochondrial and death receptor-apoptotic pathways. These fundamental cascades are required for the process of: maturation, hyperactivation, capacitation, acrosome reaction as well as sperm-oocyte fusion, crucial for the fertilization process, all presented in **Figure 2**.

2.3.1 Maturation

After spermiation, spermatozoa are transported into the epididymis where they undergo a maturation process, leading to significant chemical and physiological modifications including recombination of cell-surface proteins, and enzymatic and

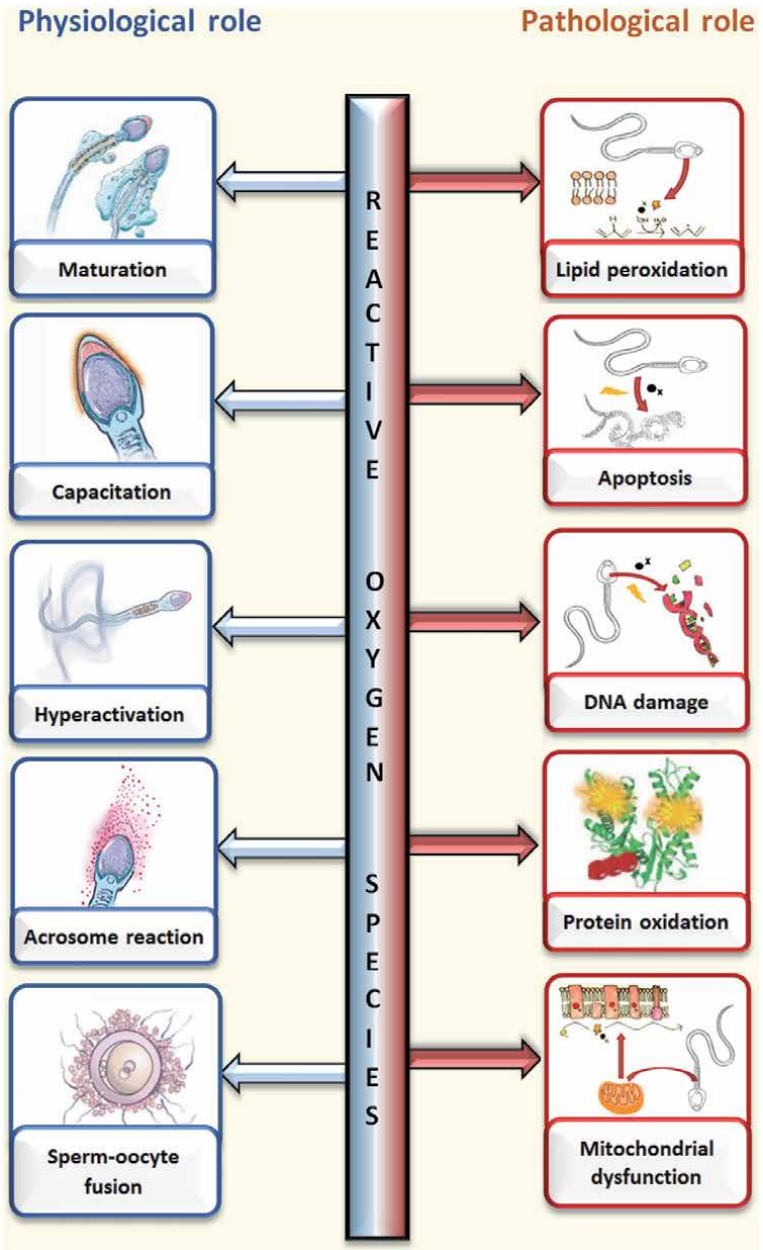

Figure 2.
Physiological and pathological consequences of ROS. ROS dose is a critical parameter in determining the ultimate cellular response, low (necessary) dose for physiological processes and high (toxicity) dose expressing their pathological effects.

nuclear modifications [69, 70]. These result in the assembly of the signal transduction machinery that is crucial for the sperm capacity to undergo hyperactivation and capacitation [69, 71]. The nuclear DNA of mammalian spermatozoa is densely packed, as histones are substituted by smaller-sized (arginine-rich) protamine [72]. Protamines substitute histones during spermiogenesis [73] and compact DNA tightly through inter/intramolecular disulphide bonds between cysteine residues [74]. The oxidizing process of thiol groups on protamines and the formation of disulfide bonds increase chromatin stability and DNA protection from any physical or chemical damage [75], which is fundamental because human spermatozoa have

limited capability to repair DNA damage [76]. Protamination occurs when spermatozoa pass through the caput and caudal part of the epididymis [77].

Another important event is the formation of "mitochondrial capsule" made by a complex protein material, which is necessary to abolish proteolytic degradation [78].

2.3.2 Hyperactivation

Hyperactivation is a particular state of sperm motility characterized by vigorous, large asymmetric flagellar (whiplash-type) beat and head sperm shifting (large lateral head displacement) [79]. Hyperactivation is reported to facilitate the capacitation process and is indispensable for successful accomplishment of acrosomal reaction, sperm-egg fusion, and fecundation [74].

Undoubtedly, ROS play an inclusive role in the regulation of these processes, by triggering hyperactivation and capacitation. This occurs by induction of Ca^{2+} and HCO_3^- influx, probably through the deactivation of the enzyme Ca^{2+}-ATPase and further basification of the cytosol [80]. ROS (especially O_2•-) upregulate the Ca^{2+} mediate adenylate cyclase (AC) enzymatic activity, increasing cAMP (cyclic adenosine monophosphate) generation by activating protein kinase A (PKA). Further, this triggers NADPH oxidase activation and thereby promotes the upregulation of ROS production [81]. PKA-mediated phosphorylation leads to protein tyrosine kinase (PTK) activation, phosphorylating consecutive tyrosine residues in the axonemal fibrous sheath and the cytoskeleton of sperm tail [69, 82].

2.3.3 Capacitation

Capacitation has been documented in 1951 by Austin and Chang [83, 84]. Capacitation involves cholesterol outflow from the sperm membrane and a global intensification of tyrosine phosphorylation [85]. The signal transduction pathway is guided by the cAMP and modulated by the oxido-reductive state [86]. During capacitation, spermatozoa undergo molecular modifications such as alkalization of inner cell pH, activation of cAMP-dependent pathways, cholesterol efflux from cell-membrane and phosphorylation of surface proteins by cAMP-dependent kinase [87]. Researchers have emphasized the impact of free radicals in modulating the cAMP pathway, which involves PKA activation and phosphorylation of its substrates [88]. A correlation between elevated protein phosphorylation rate, increased presence of the second messengers and ROS synthesis have been observed during capacitation [69]. The cholesterol oxidation and its consequent discharge from the sperm membrane is necessary in tuning-up spermatozoa for the next step, resulting in greater bicarbonate and Ca^{2+} ion permeability via activation of sodium/bicarbonate cotransporter (NBC) and ion channels [89].

2.3.4 Acrosome reaction (AR)

The hyperactivated spermatozoon tends to penetrate over the cumulus-oocyte-complex and attach to the zona pellucida of the egg, whereas acrosome reaction (AR) is a well-regulated exocytotic reaction in response to coordinated stimuli [90]. These changes are triggered by tyrosine phosphorylation of sperm-membrane proteins regulated by ROS signaling [63, 88]. NO is implicated in AR by activating the second messenger cyclic guanosine-mono-phosphate (cGMP), PKC and protein kinase G (PKG) [91]. Physiological levels of H_2O_2, O_2^- and NO are needed for AR [88]. In the oocyte, the release of Ca^{2+} is followed by cleavage of phosphatydilinositol-4,5-bisphosphonate (PIP_2) into inositol tri-phosphate (IP_3) and

diacylglycerol (DAG), which are responsible for acrosomal exocytosis and activation of PKC. This further results in Ca^{2+} inflow and activation of PLA2 (phospholipase A2), which play a key role in the cleavage of secondary fatty and consequently increasing the membrane fluidity, necessary sperm-oocyte fusion [92].

2.3.5 Sperm-oocyte fusion

ROS are also necessary in the finalization of the fertilization process. This final step is due to enhanced membrane fluidity, which is controlled and directed by ROS in inhibiting the protein tyrosine phosphatase activity, which prevents deactivation of PLA_2, a necessary step for accomplishing sperm-oocyte fusion [93]. When the spermatozoon penetrates the zona pellucida and the corona radiata, the oocyte changes the composition of the vitelline layer [24]. This envelope is catalyzed by ovoperoxidase making o,o-dityrosine crosslinks to prevent polyspermy [94].

2.4 Pathological repercussions of oxidative stress

High levels of ROS have the potential to damage cellular components by mediating lipid peroxidation, apoptosis, DNA damage, mitochondrial dysfunction and protein oxidation.

2.4.1 Lipid peroxidation (LPO)

Sperm membranes are mostly constituted by poly-unsaturated fatty acid (PUFAs), which represents a disadvantage in terms of OS susceptibility [95].

Lipid peroxidation (LPO) is as a chemical reaction by which oxidants assault carbon double bond(s) in lipid compounds, especially PUFAs, by detaching hydrogen and adding oxygen to carbon, by generating LOO• and LOOH [96]. *In vitro* research highlighted a negative correlation between malondialdehyde (MDA - end product of LPO) concentration, and sperm morphology and motility [97–99]. LPO is a self-propagating process passing through three phases: (1) initiation; (2) propagation; (3) termination. Through all three phases free radicals enter in a radical-chain reaction [32].

The propagation of the oxidative wave can also result in DNA fragmentation and protein damage, affecting particularly sperm motility, morphology and fertilizing capacity.

2.4.2 Apoptosis

The programmed cell death, known as apoptosis, is a physiological phenomenon. In the male reproductive tract, apoptosis is responsible for supervising the excess production of male gametes, a process being regulated by extrinsic and intrinsic stimuli [80]. The intrinsic stimuli include apoptosis-including genes like p53, Bax and Fas, but also Bcl-2 and c-kit genes which act as apoptosis suppressors [100], while extrinsic stimuli consist of varicocele, infection, heat stress, environmental toxins, advanced male age lifestyle factors, ionizing and nonionizing radiations, defective protamination and idiopathic causes [101, 102] . During the process of spermatogenesis, spontaneous germ cell apoptosis in all developing stages of spermatozoa has been seen in the testis of normozoospermic and non-obstructive azoospermic men [20]. This guarantees that only functionally and genetically competent germ cells become mature spermatozoa [103]. Prolactin and insulin are considered as pro-survival hormones which bind to specific receptors on sperm membrane [104]. The inhibition of this cascade will result in increased ROS

generation by mitochondria, followed by the release of cytochrome C, which in turn activates the apoptotic caspases, triggering the apoptosis [74, 82, 105]. High levels of cytochrome C have been found in seminal plasma of infertile men [82, 106].

2.4.3 DNA damage

It is reported that infertile males with high seminal OS levels present high fragmentation of sperm DNA [107]. Numerous contributors can include lifestyle factors, radiation, advanced male age, varicocele, infection and idiopathic causes [108, 109]. Guanine base (G) is the most common DNA's organic base exposed to OS assault and converts into 8-hydroxy-deoxyguanosine (8-OHdG) by free radicals [110]. Mechanisms by which OS cause DNA damage involve warping single and double-stranded DNA crosslinks, direct oxidation of DNA bases and DNA mutations [111]. Comparing to nuclear DNA, mitochondrial DNA is more susceptible to DNA damage, due to the lack of histones and protamines, and nucleotide excision repair mechanisms [112].

In addition, mitochondrial damage affects the interior mitochondrial membrane, causing electron outflow from the transporting chain, inducing a further increase of OS status [113].

2.4.4 Mitochondrial dysfunction

Mitochondria represent the most important place in generating ATPs, which serves as a fuel for sperm to move. This is why its proper function represents a fundamental key point in the mosaic of male infertility problems. Defects in the pathway for ATP production correlate with low sperm motility, known as asthenozoospermia [114]. There is an inactivation of genes which encode constituting proteins of the electron transport chain, mainly those that are involved in ATP formation [115]. When the extent of such injury overwhelms DNA repair capacity mechanisms, the subsequent alterations in mitochondrial biology stimulate the activation of the genes responsible for stress–response, hereby inducing apoptosis [116].

2.4.5 Protein oxidation

Formation of radical amino acids is of the result of protein oxidation (PO), especially of the alpha-central carbon, causing disintegration of peptide skeletons [117]. Moreover, the SH-rich lateral chains of methionine and cysteine are inclined to be oxidised with propagation of methionine sulphoxide and disulphides, respectively [87]. Similarly, arginine, proline, threonine and lysine are oxidised, resulting in the formation of carbonylated proteins (aldehyde and ketones), markers of PO status [117]. These alterations impact the protein morphology and physiology, with a wide impact on spermatogenesis and fertility potential.

3. Antioxidants in male infertility treatment

Antioxidants are defined as chemicals compounds with the ability to donate electrons and thereby neutralize an excessive production of ROS [118]. Humans possess a well-sophisticated antioxidant system to shelter the body's cells and tissues against oxidation [119].

As a physiological response to OS, seminal plasma is endowed with various scavengers acting enzymes indexed as total antioxidant capacity (TAC) measured to be 10x higher comparing to blood plasma [120].

The anti-oxidant defense system implicates a co-action of different endo/exogenous players to scavenge the potential oxidative damage of ROS [121]. These consist of CAT, SOD, glutathione peroxidase (GPx), peroxiredoxins and glutathione-S-transferase [122], and water-soluble and fat-soluble vitamins [123]. The role and effect of endogenous and exogenous antioxidants are discussed below.

3.1 Endogenous antioxidants

The major endogenous antioxidant enzymes are: (1) CAT, (2) SOD and (3) GPx. Studies about their efficacy in clinical trials are presented in **Table 3**.

3.1.1 Catalase

Activity of catalase (tetrameric protein) is consisted in dissolving hydrogen peroxide into water and oxygen, through the oxidation of hydrogen ion donors, such as methanol (CH_3OH), ethanol (CH_3CH_2OH), with the consumption of 1 mol of H_2O_2 [128]. In addition, CAT has an important role in terms of physiological effects during sperm capacitation, inducing NO activity and the removal of ROS [129].

3.1.2 Superoxide dismutase (SOD)

SOD is known as metallo-enzyme, as it has the catalytic metal in the active site [130]. The SOD enzyme consists of three different classes existing in both extra- and intracellular compartments. SOD-1 or CuZnSOD is the first intracellular enzyme, with Cu and Zn in the active center; it is usually localized in the cytosol [131]. SOD-2 or MnSOD is the second intracellular isoform, localizing in mitochondria and showing Mn in the active center [132]. The extracellular form of SOD (EC-SOD or SOD-3) is a glycosylated homotetramer mainly secreted into the extracellular area. It is upregulated by cytokines, downregulated by TNF-α, and anchored to the extracellular matrix [133]. CuZnSOD is highly active (75%) in comparison with SOD-3 (25%) [119, 130].

3.1.3 Glutathione peroxidase (GPX)

GPx is a cytosolic antioxidant seleno-enzyme mainly expressed in the epididymis and testis [134]. GPx catalyzes the reduction of detrimental hydroperoxides with thiol cofactors [119]. A "catalytic triad" is formed by the selenocysteine in the active site with tryptophan and glutamine: this activates the selenium portion and

Enzyme	Study findings	Ref.
CAT	• ↑ CAT activity in the group that received antioxidant therapy, comparing to control samples that received placebo. • Positive correlation between levels of CAT and fertilization rates. • Studies are limited in this field.	[124, 125]
SOD	• Its levels are positively associated with sperm concentration ($p<0.001$) and motility ($p=0.008$). • Negative relationship was found with DNA fragmentation ($p=0.014$).	[126]
GPx	• 10x greater GPx activity in the fertile group comparing with the GPx activity in infertile men. • Statistically significant ($p<0.001$).	[127]

Table 3.
The role of endogenous antioxidants enzymes.

neutralizes peroxides [135]. It is mainly expressed in the mitochondrial sperm matrix, while nuclear isoform of GPx has been correlated with sperm DNA preservation from oxidative detrimental impact and chromatin condensation [136]. GPx reduces fat hydroperoxides into alcohols and free H_2O_2 to H_2O, it is fundamental for protecting lipid integrity and maintaining sperm viability and membrane integrity [134].

3.2 Exogenous antioxidants

Most common exogenous antioxidants refer to carnitines, α-tocopherol, ascorbic acid, carotenoids, zinc and selenium. Spermatozoa carry with them minimal endogenous antioxidant amounts, thus during the entire process of spermatogenesis, sperm rely on exogenous antioxidants [137]. Studies about their efficacy in clinical trials are presented in **Table 4**.

3.2.1 Carnitines

L-carnitine (LC) and L-acetyl carnitine (LAC), a water-soluble antioxidant, are implicated in sperm metabolism, motility and viability [147]. It helps in preventing lipid peroxidation, sperm DNA protection and apoptosis [148]. The highest concentration of carnitine is found in the epididymis and spermatozoa [132]. Studies of the semen samples of infertile men, especially oligoasthenoteratozoospermic (OAT) men, have shown lower carnitine levels compared to fertile men [133].

3.2.2 Vitamin C (L-ascorbic acid)

This is a water-soluble vitamin. Humans and other vertebrates lack the enzyme L-glucono-gamma lactone oxidase (LGGLO), which is essential for *in vivo* synthesis. Hence, its intake with diet or as a supplement is fundamental. Vitamin C concentration is 10-times higher in seminal plasma comparing to serum [149]. It nullifies the activity of •OH, O_2•- and H_2O_2 radicals, thereby protecting against oxidative damage [150].

3.2.3 Carotenoids

Carotenoids can be found naturally in fruits and vegetables. Carotenoid cannot be synthesized by humans, by introduced by the diet. Lycopene, a fat-soluble aromatic carotenoid, is reported to be strong neutralizer of $^{-1}O_2$, but a combination of carotenoids seem to be more effective [151]. It can alter the levels of antioxidant enzymes by modification of the levels of ROS, making great contribution to the human antioxidant system [43, 119]. There are studies on fertile men that show high concentration of Lycopene, and reduced levels in seminal plasma of infertile men [152].

3.2.4 Coenzyme Q-10 (CoQ10)

CoQ10 is an intermediate of the mitochondrial electron transport chain [153, 154]. Low seminal plasma/sperm concentrations of CoQ10 have been associated with reduced sperm motility [155].

3.2.5 Zinc (Zn)

Zn is one of the most abundant elements in human [156]. It acts as metalloprotein cofactor in the metabolism of nucleic acids transcription, signal transduction, protein synthesis and cell death regulation [157]. Moreover, Zn is fundamental

Antioxidants	Study findings	Ref.
LC & LAC	• Analyzed in certain systematic reviews and meta-analysis. • Intake (two times daily, not more than 30 weeks) is associated with a remarkable increase in sperm motility and morphology.	[138, 139]
Vit. C	• Studies suggest positive association between levels of ascorbic acid in seminal plasma and sperm morphology and viability. • Very effective in controlling sperm agglutination. • Kobori et al. treated 169 males for 6 months with vitamin C, E and CoQ10, and reported a noteworthy improvement of sperm concentration and sperm motility.	[140, 141]
Carotenoids	• In a randomized clinical trial, Nouri et al. included 44 patients with oligozoospermia. • Tretament with 25 mg lycopene resulted in increased sperm count, concentration, total motility and TAC.	[142]
CoQ10	• Alahmar et al., study treated 65 oligoasthenozoospermic men and 40 fertile control groupwith 200 mg/day CoQ10 for 3 months. • Authors observed a significant improvement in total sperm motility, sperm concentration, TAC, and GPx levels as well as reduced SDF.	[143]
Zn	• Randomized cross-sectional study and case study, combined antioxidant formula. • Significantly correlated with sperm density ($r = 0.341$, $p < 0.0001$), motility ($r = 0.253$, $p < 0.0001$) and viability ($r = 0.286$, $p < 0.0001$) • Decrease levels of MDA, enhancing sperm motility and concentration ($p < 0.001$). • No significant change of Protein Carbonyl (PC) ($p=0.554$).	[144, 145]
Se	• Longitudinal study by Mossa et al. • Included 12 males, treated twice daily with 50 microgram in 3 months period. • Significantly increase in sperm count (39.24 ± 27.4–58.1 ± 21.6; $p<0.01$), motility (22.14 ± 12.9–50.7 ± 17.6; $p<0.01$) and morphology (68 ± 5.7–82.1 ± 6.4; $p<0.01$).	[146]

Table 4.
The role and effect of exogenous antioxidants enzymes.

for optimal sustain of spermatogenesis and adequate function of the male reproductive organs [158]. It also plays a key role in preventing LPO and preserves sperm structure, by reducing generation of H_2O_2 and •OH, through separating active redox transition metals, such as Fe and Cu [144].

3.2.6 Selenium (Se)

Se is an important trace mineral, implicated in many biological processes. Se is the constituent of enzymes such as GPx and seleno-proteins, it shows a major impact in redox defense system, spermatogenesis and increased fertility capacity in both males and females [159]. It protects sperm DNA against OS damage, although the mechanism is still unclear [160].

3.2.7 Role and effect of vitamin E in male reproduction

Vitamin E is the major lipophilic antioxidant [156] and it has been recognized as an essential nutrient for reproduction since its discovery in 1922 [161]. It neutralizes •OH and O_2•- by lessening lipid per-oxidation commenced by ROS, thus protecting cell membranes from oxidation [160]. Vitamin E ameliorates other scavenging oxidants manners and helps maintaining sperm morphology and motility (which

depends on the integrity of the mitochondrial sheath) [162]. Effects and the roles of vitamin E are presented in **Figure 3**.

Various vitamin e isoforms have been found, but their role and importance remains enigmatic, and of the eight naturally occurring forms, only α-tocopherol is maintained in the plasma [163]. Therefore, vitamin E is crucial in maintaining all the necessary functions of healthy sperm and protecting it from detrimental effects of OS. Studies show lower levels of vitamin E in infertile men compared to fertile men [135], allowing somehow to increase concentration of the peroxidation by-product MDA in the seminal fluid [164]. It is mainly used in combination with other vitamins and minerals. In vitro and in vivo studies which show improvements exclusively in the sperm motility and other semen parameters, successful pregnancies and mitigation of oxidative stress markers, presented in **Table 5**.

Vitamin E intake and its dosage should exclusively be determined by a healthcare professional because of adverse events due to vitamin toxicity. The recommended daily dose of vitamin E is 15 mg (30 IU) for adults [173], a dose of 200–800 mg/day may cause gastrointestinal distress, while a daily dose greater than 1000 mg (1500 IU) is associated with increased risk of hemorrhage (antiplatelet effects), thrombophlebitis,, elevated creatinine, gonadal dysfunction and death [163, 174].

Infertile patients which want to increase concentrations vitamin E, its sources can be found in nuts, seeds, vegetable oils, leafy vegetables and fortified cereals.

It needs proper and critical analysis for establishing the correct dosage and duration of antioxidants administration. In case of raised OS status, remedy must be administered at least for 12 weeks, according to the proper minimal period for spermatogonia (72 ± 3 days), or for three to six months [175, 176].

Referring to the studies analyzed above, vitamin E consumption has its obvious beneficial effects. But, the question here is whether vitamin E is more effective solely or in a combination? If used solely, is the efficacy more accentuated in *in vivo*

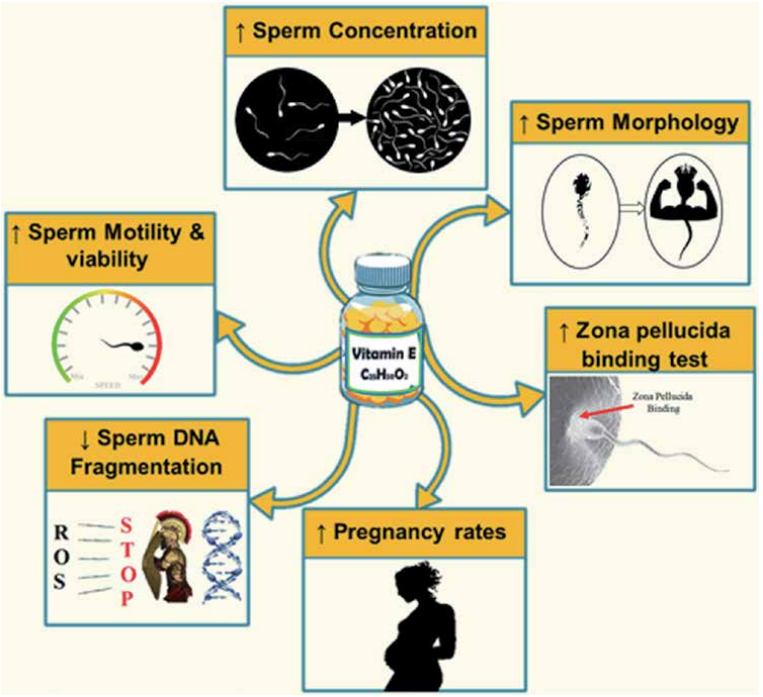

Figure 3.
Effects of vitamin E in male reproduction physiology.

Study design	Number of study subjects/abnormality	Dose/duration	Results	Ref.
Vitamin E *in vivo* studies				
Double-blind, placebo-controlled, randomized study	101 couples (50 in the vitamin E group and 51 in the placebo group)	400 mg/daily p.o	↑motility in the vitamin E group; Morphology was better in the placebo group; Statistically significant higher live-birth rate per transfer in the vitamin e group.	[165]
Randomized placebo-controlled double-blind trial	87 asthenospermic men (52 treated with vitamin E; 35 placebo treatment)	100 mg s.3x1 p.o./ 6 months	↑motility in the vitamin E group, comparing to placebo group ($p<0.001$); ↑Pregnancy (81% with a live birth); ↓ MDA levels (sperm LPO).	[162]
Randomized controlled study	45 infertile men after varicocelectomy, n=22 receiving vitamin E and n=23 control group without supplementation.	300 mg s.2x1 p.o./ 12 months	No significant differences were found in terms of sperm count, sperm motility and pregnancy rates comparing to control group.	[166]
Vitamin E *in vitro* studies				
Double-blind randomized placebo cross-over controlled trial	30 healthy men with high levels of ROS in semen.	300 mg s.2x1 p.o./ 3 months	Improvement of the performance of the spermatozoa in the zona pellucida binding test ($p=0.004$); No significant effect was demonstrated in the conventional semen parameters and levels of ROS;	[167]
Evaluation study	43 subjects, normal (n=23) and abnormal (n=20).	100 or 200 µmol Vitamin E to cryopres-ervation medium	↑ post-thaw motility ($p=0.041$); No improvements in sperm vitality and the degree of DNA fragmentation.	[168]
Experimental study	50 asthenoterato-zoospermic men	2 mM (milli-molar) vitamin E.	Significantly higher total sperm motility ($p<0.001$), progressive motility ($p<0.001$) and viability ($p<0.001$) compared with control group after 2, 4 and 6 hours of incubation; MDA levels were decreased significantly after 6 hours ($p<0.001$).	[169]
Vitamin E in combination with one or more vitamins				
Randomized controlled trial	54 voluntary infertile men	Vit. E 100 mg s.2x2/3 months Selenium 35 µg s.3x2/3 months	Significant improve in sperm motility ($p<0.05$), without significant effects on other parameters; Significant decrease in the MDA concentration.	[170]
Comparative prospective randomized trial	90 idiopathic oligoastheno-zoospermic men	Vit. E 400 mg s.1x1/6 months Clomiphene	Significant increase in sperm concentration ($p=0.001$); Improvement in the mean	[171]

Study design	Number of study subjects/abnormality	Dose/duration	Results	Ref.
		citrate 25 mg s.1x1/6 months	total sperm motility ($p<0.001$).	
Randomized controlled trial	60 asthenozoospermic men	Vit. E 400 mg s.1x1/2 months Vit. C 1000 mg s.1x1/2 months	Increased sperm total motility ($p\leq0.05$); No significant effect on other parameters.	[172]

Table 5.
The role and effect of vitamin E solely and in combination.

or *in vitro* studies? Data presented above from different studies demonstrate the complexity and the unpredictability of vitamin E or antioxidant supplementation, even though there are studies that suggest improvements in sperm parameters, decrease of oxidative stress status, improvements in zona pellucida binding test and higher pregnancy rates.

Vitamin E doesn't work only as an antioxidant, but it is also involved in the modulation of cellular responses by modulating enzymes or by regulating the activity of specific transcription factors [173, 177].

4. Conclusion

ROS are very important in certain physiological processes; however they can be very dangerous for male fertility potential if the levels overcome a physiological threshold.

Therefore, normal fine redox equilibrium between ROS and antioxidants is extremely important. The understanding of this fine balance will facilitate steps towards proper diagnosis and treatment in ideal dosages of antioxidant treatment.

The most widely utilized antioxidants either as single therapy or combined are: vitamin C, E, NAC, carnitines, CoQ10, zinc, selenium, and lycopene.

According to current literature we can conclude that vitamin E used alone is more effective when used for *in vitro* procedures, and very effective used in a dual, triple or more combinations in terms of sperm parameters and oxidative stress status.

Further augmentative clinical trials are needful to ascertain the right and effective antioxidant combination, for reliable and appropriate guiding of this sensitive medical issue.

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Vitamin E is a group of fat-soluble compounds found in a wide variety of foods. Daily requirements of vitamin E can be met with a balanced diet. High-dose supplementation may be hazardous rather than beneficial. Vitamin E serves as an antioxidant, participates in anti-inflammatory processes, inhibits platelet aggregation, and enhances immunity. Vitamin E supplementation can be beneficial against coronary artery disease, eye disorders, cognitive decline, cancer, and skin aging. This book will mainly focus on the diverse functions of vitamin E, importance of vitamin E status to provide a healthy lifespan, and the interaction between vitamin E and several pathological conditions. Readers will receive a general overview of the importance of vitamin E in health and different pathological conditions.

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