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Review Article

Tocotrienols as Potential Therapeutic Supplement

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Abstract

Natural products have historically been a rich source of biologically active compounds for drug discovery (Schiff et al,1979). Tocotrienols are vitamin isomers which are "essential" meaning they cannot be manufactured by the human body. Hence they have to be obtained from food and supplements. In nature they occur in four different forms: α, β, γ, and δ Tocotrienols. Palm oil, one of the richest sources of Tocotrienols, is an ideal source since it contains more of the, γ and δ than α, β- Tocotrienols. Taken orally, tocotrienols are bio available to all vital organs. Tocotrienols have wide ranging clinical effects with anticancer, radioprotective, anti hypercholesterolemic, antiangiogenic, antioxidant, anti-inflammatory, antineurodegenration and antimicrobial properties. A safe and effective therapy for radiation injuries as well as cancer prevention is the need of the hour. Analytical and clinical study on Tocotrienols may prove vital for life. The main objective of this review study is to highlight clinical potential significance of the tocotrienols in health and diseases as a therapeutic supplement.

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Introduction

Tocotrienols are members of the vitamin E family. They are an "essential" meaning they cannot be manufactured by the human body. Hence, they have to be obtained from food and supplements [1]. Vitamin E is made up of four tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ). Chemical structure of Tocotrienols consist of three double bonds in hydrocarbon tail. Tocotrienols thus exists in an unsaturated form [2]. Tocotrienols chiefly present in palm and annatto oil, barley and rice bran . This variant of vitamin E typically only occurs at very low levels in nature [3].

Tocopherols constitute a series of related benzopyranols (or methyl tocols) that occur in plant tissues and vegetable oils and are powerful lipid-soluble antioxidants. In the tocopherols, the C16 side chain is saturated, and in tocotrienols it contains three trans double bonds.Togather, these two groups are termed the tocochromanols [4].The biosynthesis of tocopherols and tocotrienols share common pathways, by the condensation of homogentisate, derived from the shikimate pathway, and phytyl pyrophosphate, derived from the non-mevalonate, through the action of the homogentisate prenyltransferase (HPT) [5] .

All the tocotrienols and tocopherol isomers have the antioxidant activity due to the ability to donate a hydrogen atom (a proton plus electron) from the hydroxyl group on the chromanol ring, to a free radical in the body. This process inactivates (quenches) the

free radical by effectively donating a single unpaired electron (which comes with the hydrogen atom) to radical [6].Tocotrienols show wide ranging effects, possess unsaturated side-chains which help in their rapid transport into cell membranes[7].They prevent lipid peroxidation and DNA damage. Moreover, they protect the cell membranes by lipid repair and replacement [1].

In animal cells, Tocotrienols inhibit production of cholesterol by liver cells by suppressing 3-hydroxy-3-methyl glutaryl CoA reductase enzyme (HMGR), a key enzyme in the sterologenic pathway [8]. Tocotrienols have found to possess anti-proliferative and apoptotic activities on normal and cancerous human cells. Tocotrienols also inhibit vascularization, reducing cell proliferation and the result increase in HMGR activity. γ & δ Tocotrienols have been found to have stronger anti-cancer activity than similar compounds and their efficacy is due to multiple mechanism [9].

The actions of Tocotrienols are mediated by interacting directly or indirectly with several molecular targets. The molecular targets fall under categories such as apoptosis regulators, cytokines, adhesion molecules, enzymes, growth factors, kinases, receptors, transcription factors and several others.

Acute radiation syndrome or ARS is characterized by the response of the body's vital organ systems to radiation exposure. It is understood that radiation injury is caused due to lysis of the aqueous cellular components, their interaction with one another and oxygen. Ionizing radiation predominantly causes formation of reactive oxygen species (ROS) by hydrolysis of water. Externally supplied agents, such as Tocotrienols, can quench these ROS offering protection to cellular components from dangerous effect of radiation exposure [10].

On a concentration basis, the neuroprotective effects of Nm Tocotrienol represent the most potent biological function of all natural forms of vitamin E. Glutamate- toxicity is a major contributor to neurodegenration. It includes excitotoxicity and an oxidative stress component also known as oxytosis [11, 12]. Tocotrienol-dependent neuroprotection includes a significant antioxidant-independent mechanism has been now established [13].The neuroprotective property of Tocotrienols not only in response to glutamate challenge but also in response to other insults such as homocysteic acid, glutathione deficiency, and linoleic acid induced stress [13, 14]. It is now evident that at micromolar concentrations Tocotrienol protects neural cells by virtue of its antioxidant property. At nanomolar concentrations, however, Tocotrienols regulate specific neurodegenerative signaling processes.

Tocotrienols are better able than tocopherols at combating oxidative stress of skin that had been exposed to U. V rays of the sunlight [15]. Since 2000, scientists have suggested tocotrienols are better antioxidants than tocopherols at preventing cardiovascular diseases [16]. Prostate cancer is the most common type of cancer in developed countries. It is responsible for more male deaths than any other cancers, except for lung and bronchial cancer [17]. Tocotrienol-rich fraction has demonstrated anti-proliferative effect on prostate cancer cells. ã fraction of Tocotrienol suppresses prostate cancer cell proliferation and invasion through multiple-signaling pathways [18]. A study was conducted by the Malaysian Palm Board to assess the Tocotrienol concentrations in malignant and benign adipose tissuses of breast cancer patients. 65% higher concentrations of Tocotrienols, were found in the adipose tissues of patients with benign breast lumps, compared with those of patients with malignant tumors [19]. In this way it is supposed that Tocotrienol has wide ranging clinical effects with anticancer, anti-dyslipidemic, anti neurodegenrative, anti angiogenic, anti-inflammatory, antioxidant, antimicrobial, and radio protective properties. Its elaborative clinical research study may be blissful for human health.

History of Tocotrienols

The discovery of tocotrienols was first reported by Pennock and Whittle in 1964,describing the isolation of tocotrienols from rubber [6].The biological significance of tocotrienols was clearly delineated in the early 1980s, when its ability to lower cholesterol was first reported by Qureshi and Elson in the journal of medicinal chemistry [20]. During the 1990s, the anti-cancer properties of tocotrienols began to be delineated [21].

The current commercial sources of Tocotrienol are annatto,palm and rice. Annatto is rich in delta and gamma fraction of tocotrienols. Other natural tocotrienols sources include rice bran oil, coconut oil, cocoa butter, barley, and wheat germ [22, 23]. In number of clinical trials, doses of tocotrienols as low as 42 mg/day have shown to reduce blood cholesterol by 5-35% [24]. Tocotrienols are safe and human studies show no adverse effects with consumption of 240mg/ day for 48 months [25]. Tocotrienol rich fractions (TRF) from rice, palm, or annatto are used in nutritional supplements, functional foods, and anti- aging cosmetics etc. For

the utility and quality it is desired for the absolute Tocotrienol concentration to be highest, tocopherol to be lowest, and the process used to be solvent- free.

The Chemistry and Antioxidant Properties of Tocotrienols

Lipid Peroxidation is a degradative, free radical mediated process responsible for the development of objectionable odors and flavors in oils, fats, and food containing them [26–30]. Moreover, oxidation of polyunsaturated fatty acids of the bio membranes causes functional abnormalities and pathological changes [31, 32]. Although the mechanism responsible for lipid peroxidation has been extensively studied, they are still not fully understood. Both the rates and pathways of lipid peroxidation are dramatically affected by other chemical species in the reaction medium as well as by the physical conditions of the reactions.

Vitamin E compounds (tocotrienols and tocopherols) are well recognized for their effective inhibition of lipid peroxidation in food and biological systems [33]. Since vitamin E only synthesized by plants, it is very important dietary nutrient for humans and animals [34].

Vitamin E consists of a group of 8 molecules belonging to 2 classes designated as tocopherols and tocotrienols. Both tocopherols and tocotrienols possess 4 structurally similar forms designated α, β, γ and δ. Tocotrienol differs from tocopherol by having 3 double bonds in the carbon side chain of the molecule [35]. This unique structure makes this tocotrienol a potent antioxidant with many health benefits. The tocotrienol molecule looks like a tadpole, with a head and a tail. The head with polar chromanol ring and tail with a long isoprenoid side chain. Tocopherols containing a phytyl chain while tocotrienols with geranyl farnesyl chain. The head of T_3 is designated as α, β, γ and δ depending on the amount of substituted methyl groups. Tocotrienols (T_3) have only a single chiral centre, which exist at the 2' chromanol ring carbon, at the point where the isoprenoid tail joins the ring. Tocotrienols extracted from natural sources always consist of dextrorotatory enantiomers only. These naturally occurring dextrorotatory stereoisomers are generally abbreviated as the "d" forms, for example "d – tocotrienol" or "d alpha – tocotrienol" [6].

All the tocotrienols and tocopherol isomers have antioxidant activity due to the ability to donate a hydrogen atom (a proton plus electron) from the hydroxyl group on the chromanol ring, to a free radical in the body. This process inactivates (quenches) the free radical by effectively donating a single unpaired electron (which comes with the hydrogen atom) to radical [6].

The biosynthetic pathway of tocotrienols only differs in one step from the biosynthesis of tocopherols. The first committed step of the pathway catalysed by the homogentisate geranyl geranyl transferase (HGGT) is characterized by the high acceptance of geranyl geranyl diphosphate over the similar phytyl diphosphate which leads the way in to tocopherol biosynthesis (Cahoon 03, Yangii).All other enzymes after this step,i.e. tocopherol cyclases and tocopherol methyltransferases can work either one of the precursors and produce either tocotrienols, tocopherols or mixture of both (Albermann08).

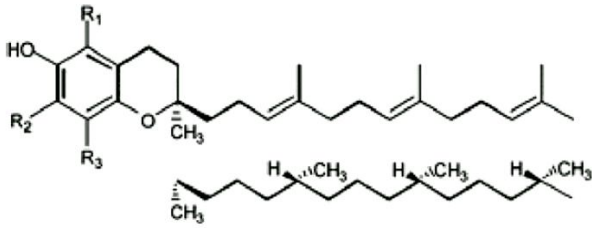

Fig. 1: Chemical structure of tocotrienol

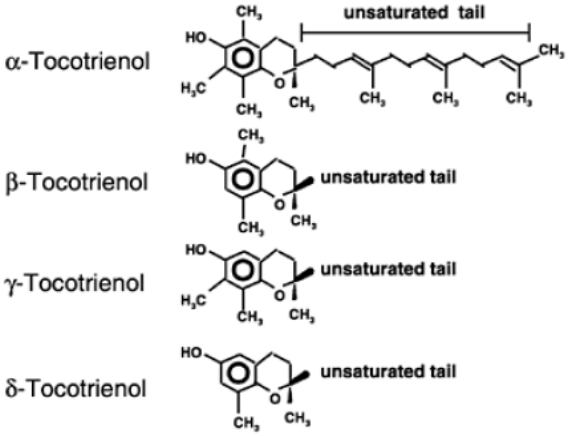

Fig. 2: Four different forms of tocotrienol

Natural Sources of Tocotrienols

Tocotrienols are the primary form of vitamin E in the seed endosperm of most monocots, including agronomically important cereal grains such as wheat, rice and barley. Palm oil contains significant quantities of tocotrienol [36]. Crude palm oil extracted from the fruits of Elaeis guineesis particularly contains a high amount of tocotrienols (upto 800mg/kg), mainly consisting of γ– tocotrienol and α – tocotrienol. Compared to tocopherols, tocotrienols are considerably less widespread in the plant kingdom [37]. The identification of α-tocotrienol as a cholesterogenesis-inhibitory factor derived from barley (Hordeum vulgare L.) represents a landmark

early discovery highlighting the unique significance of tocotrienols in health and disease [38]. Palm oil represents one of the most abundant natural sources of tocotrienols [39]. The distribution of vitamin E in palm oil is 30% tocopherols and 70% tocotrienols [36].

α- Tocotrienol is the predominant form of tocotrienol in oat (*Avena sativa L.*) and barley (56 and 40 mg/kg of dry weight, respectively). β-Tocotrienol is the major form of tocotrienol found in hulled and dehulled wheats (from 33 to 43 mg/kg of dry weight) [40].

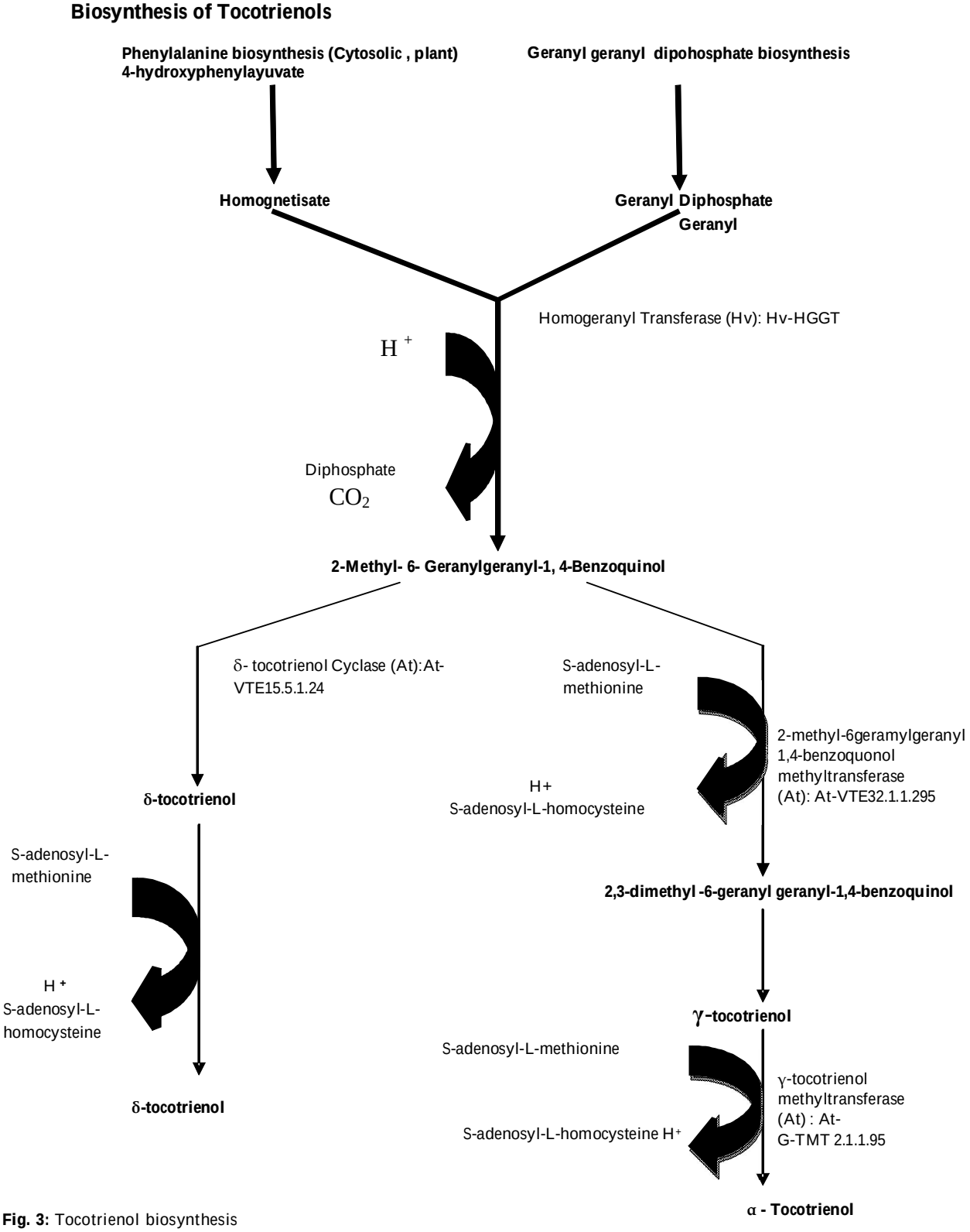

Fig. 3: Tocotrienol biosynthesis

Table 1: Natural sources of tocotrienols

Natural Source	Tocotrienol Quantity (Mg/Kg)
Palm Oil	*940*
Barley	910
Rice Bran	465
Grape Fruit seed oil	380
Oat	210
Hazelnut	209
Maize	200
Wheat germ oil	189
Olive oil	180
Buckthorn berry	130
Rye	92
Flaxseed oil	25.1
Poppy seed oil	20.5
Safflower oil	11.8

Although tocotrienols are present in edible natural products, it is questionable whether these dietary sources could provide sufficient amounts of tocotrienol to humans. For example, the processing of 1000 kg of crude palm oil is necessary to derive 1 kg of the commercial product Tocomin®50% (Carotech, NJ) [41].

Bioavailability and Metabolic Fate of Tocotrienols

During the last two decades, efforts to understand how dietary vitamin E is transported to the tissues have focused on α-tocopherol transport [42 – 45]. α-Tocopherol transfer protein (TPP) has been identified to mediate α- tocopherol secretion in to the plasma while other tocopherol binding proteins seems to play a less important role [43]. Tocopherol transfer protein (TPP) has to ability to bind to both tocotrienol and α- tocopherol and TTP is known to bind to Tocotrienol with 8.5- fold lower affinity than that for α- tocopherol [46 – 47]. It has not been clear whether, or to what extent, the delivery of orally supplemented α-Tocotrienol to vital organs is dependent on TPP. Previously it has been reported that TPP- deficient female mice are infertile presumably because of vitamin E deficiency [48] but recently it has been noted that oral supplementation of female mice α-tocotrienol restored fertility of TPP knok-out mice suggesting that Tocotrienol was successfully delivered to the relevant tissues and Tocotrienol supported reproductive function under conditions of α-tocopherol deficiency [47]. It is clear, however, that natural isomers of vitamin E do get transported to vital organs even in the absence of TTP. Current findings that support that oral Tocotrienol (Carotech Inc., NJ) not only reaches the brain [43, 49] it does so in amounts sufficient to protect against stroke [43]. Long term lack of Tocotrienol in the diet may repress any putative Tocotrienol transport mechanism in vivo.

Once ingested, the T3 and α-T form chylomicrons in the intestine when they are released and mixed with human gastrointestinal fluid to enter the circulation from the intestine. The conversion of chylomicrons to remnant particles results in the distribution of newly absorbed T3 and α-T to all of the circulating lipoproteins and ultimately to tissues [45]. In the liver, newly absorbed dietary lipids are incorporated into nascent very low-density-lipoproteins (VLDL). The liver is responsible for the control and release of α-T into blood plasma. The data indicate that tissue delivery of α-T3 is likely to be compromised when α-T and α-T3 are co-supplemented [47]. However, a study of Khanna et al. (2005a) with the TTP-deficient mice indicates the existence of a TTP-independent mechanism for the tissue delivery of oral α-T3.

Recently it has been demonstrated that γ-T is more rapidly metabolized in women than in men [50]. In rat studies, delivery of oral α-T3 to the heart of females was more efficient compared to that of males [51, 52]. The accumulation of α-T3 in various organs over time was eliminated in 7 wk when the sub-group of rats was subjected to a vitamin E-deficient diet.

Health Effects of Tocotrienols

Tocotrienols and Cancer

Pure and mixed isoprenoids are known to possess potent anti-cancer activity [53]. Tocotrienols are isoprenoids but tocopherols are not. Unlike in the case of neuroprotection where α-tocotrienol has emerged to be the most potent isoform [13, 51, 54, 55], there seems to somewhat of a consensus that γ- and δ -tocotrienols are the most potent anti-cancer isoform of all natural existing tocotrienols. One of the first studies addressing the role of tocotrienols in neoplastic disorders was reported in 1989 [56]. The effects of intraperitoneally injected α- and γ-tocotrienol, as well as that of α-tocopherol, were examined. Both tocotrienols were effective against sarcoma 180, Ehrlich carcinoma, and invasive mammary carcinoma. γ-Tocotrienol showed a slight life-prolonging effect in mice with Meth A fibrosarcoma, but the tocotrienols had no antitumor activity against P388 leukemia at doses of 5–40 mg / kg / d [56]. Compared to tocotrienols, α-tocopherol was not as effective. The antitumor activity of γ–tocotrienol was higher than that of α-tocotrienol. In contrast to α-tocopherol, tocotrienols caused growth-inhibition of human and mouse tumor cells when the cells were exposed to these agents for 72 h in vitro [56]. In an independent study published in the year 1989 the anti-carcinogenic properties of palm oil, a

rich source of tocotrienols, was reported [57]. In this study, young female Sprague-Dawley rats were treated with a single dose of 5 mg of 7, 12-dimethylbenz (a) anthracene intragastrically. Three days after carcinogen treatment, the rats were put on semisynthetic diets containing 20% by weight of corn oil, soybean oil, crude palm oil, refined, bleached, deodorized palm oil and metabisulfite-treated palm oil for 5 months. During the course of experiments, rats fed on different dietary fats had similar rate of growth. Rats fed 20% corn oil or soybean oil diet had marginally higher tumor incidence than rats fed on palm oil diets. At autopsy, rats fed on high corn oil or soybean oil diets had significantly more tumors than rats fed on the three palm oil diets. Palm oil is different from corn oil and soybean oil in many ways. In addition to possessing higher levels of tocotrienol, palm oil has a contrasting fatty acid profile and also much higher levels of tocopherol and carotenes. As such, the favorable anti-carcinogenic effects noted in this study cannot be directly associated with tocotrienols [57]. The antioxidant or redox property of tocotrienol is not responsible for its anti-cancer property. Results in support of this hypothesis show that a redox-silent analogue of α-tocotrienol, 6-O-carboxypropyl-α- tocotrienol is cytotoxic against A549 cells, a human lung adenocarcinoma cell line [58]. Although the phenolic antioxidant group in tocotrienol may not be implicated in its anticancer property, it is apparent that the side-chain has some antioxidant property which prevents against carcinogenesis [59].

Tocotrienols inhibited proliferation of breast cancer cell lines by 50% at concentration of 180 µgm / mL and also reduced their plating efficiency. Moreover, these inhibitory effects were seen in both estrogen – receptor positive and negative breast cancer cells. Combination of tocotrienols with tamoxifen further enhanced their anti proliferative abilities. Tocotrienols inhibit proliferation of preneoplastic mammary epithelial cells by inhibiting early post – receptor events involved in cAMP production upstream from EGF – dependent mitogen – activated protein kinase and phosphoinositide 3- kinase / Akt mitogenic signaling [19, 60, 61].

Tocotrienols were more effective as anticancer agents than similar compounds. Treatment with tocotrienols decreased mammry carcinogenesis in female rats and was effective against sarcoma, Ehrlich and invasive mammary carcinoma [7].

In liver cancer cells, tocotrienols reduced cell viability and proliferation through DNA fragmentation and S phase arrest. In stomach cancer cells, tocotrienols suppressed cell migration and invasion through down regulation of matrix metalloproteinase (MMP). In Prostate cancer cells, tocotrienol inhibited cell growth, proliferation, invasion and accelerated apoptotic events through multiple signaling pathways. Treatment with tocotrienol inhibited non – small cell lung cancer (NSCLC) cell growth, decreased survival and invasion capacity of lung cancer cells and enhanced apoptosis [7].

In breast cancer cells, treatment with γ–Tocotrienol exhibited synergism with statins, phytochemicals, celecoxib and erlotinib / gefitinib in suppressing proliferation of tumour cells. Both γ–Tocotrienol and δ – Tocotrienol showed synergistic inhibition of colon cancer cells with atorvastatin. Additionally, the triple combination of γ– Tocotrienol, atorvastatin and celecoxib also induced cell cycle arrest and apoptosis in colon cancer cells. In lung cancer, tocotrienol treatment increased cisplastin – induced cytotoxicity in mesothelioma cells [7, 62].

In a 2009 study at the Li Ka Shing Faculty of Medicine, The University of Hong Kong, scientists found reduction in skin cancer cells when treated with *gamma*-tocotrienol with chemotherapy drugs. For the first time, researchers recorded the anti-invasion and chemonsensitization effect of *gamma*-tocotrienol against human malignant melanoma cells[63]. In cell line and animal studies, δ- and γ- tocotrienols have been shown to suppress the growth of melanoma [64, 65].

Tocotrienol is effective in suppressing mevalonate synthesis. By doing so, T3 can deplete tumor tissues of two intermediate products, farnesyl pyrophosphate and geranyl geranyl Pyrophosphate [66].

Tocotrienols as Radiation Countermeasures

Radiation countermeasures are mainly divided into radioprotectors (agents administred before radiation exposure), radiation mitigators (agents given shortly after radiation exposure to accelerate repair) and radiation therapeutics (agents given after symptoms appear to stimulate repair and regeneration of damaged tissues / organ systems). The magnitude of protection against radiation damage is expressed as the dose reduction factor (DRF) or dose modification factor (DMF) [10].

The 3 main body systems affected by radiation are hematopoetic, gastrointestinal and neuro / cerebrovascular. The severity of the effect is dependent upon radiation exposures total dose, dose rate and duration of exposure [10].

It is now understood that radiation injury is caused due to lysis of the aqueous cellular components, their

interaction with one another and oxygen. Ionizing radiation predominantly causes formation of reactive oxygen species (ROS) by hydrolysis of water. Examples of ROS are superoxide, hydrogen peroxide and hydroxyl radicals. ROS can cause oxidative cellular injury, activate intracellular signaling pathways and stimulate cytochrome C release from mitochondria leading to apoptosis. Externally supplied agents, such as Tocotrienols, can quench these ROS offering protection to cellular components from dangerous effects of radiation exposure [10].

Following exposure to gamma radiation, hematopoietic stem cells (HSCs) in the bone marrow, which are important for producing blood cells, rapidly undergoapoptosis (cell death). There are no known treatments for this acute effect of radiation[67]. Two studies conducted by researchers at the U.S. Armed Forces Radiobiology Research Institute (AFRRI) found that treatment with γ-tocotrienol or δ-tocotrienol significantly enhanced survival of hematopoietic stem cells, which are essential for renewing the body's supply of blood cells [67, 68].

Gene expression profiling conducted on human umbilical vein endothelial cells (HUVEC) cells showed that γ– tocotrienol was involved in the expression of 898 genes which contribute to its potent radioprotective effects. In addition to genes regulating cell dealth, it also interacted with several genes / gene groups related to radioprotection such as those governing response to oxidative stress and DNA damage stimuli, cell cycle phase, regulation of cell proliferation and inflammatory response, hematopoiesis, blood vessel development and leukocyte migration. This fine tuning of gene expression by γ– tocotrienol makes it a formidable radioprotective agent [67].

Tocotrienols and Cholesterol Reduction (Bpmishra et al, 2014)

Hypercholesterolemia is a recognized risk factor for atherosclerotic disease [69]. Studies have demonstrated that with very few expectations, populations that consume large quantities of saturated fat and cholesterol have relatively high concentration of serum cholesterol and correspondingly high mortality rates from coronary heart disease [70].

Purification of an oily, non-polar fraction of high protein barley flour by high pressure liquid chromatography yielded ten major components. Two of these components were identified as potent inhibitors of cholesterogenesis both in vivo as well as in vitro. Addition of the purified inhibitor I (2.5–20 ppm) to chick diets significantly decreased hepatic cholesterogenesis and serum total and low density lipoprotein cholesterol and concomitantly increased lipogenic activity. The high resolution mass spectrometric analysis and measurement of different peaks of inhibitor I gave a molecular ion at m/e 424 ($C_{29}H_{44}O_2$) and main peaks at m/e 205, 203, and 165 corresponding to $C_{13}H_{17}O_2$, $C_{13}H_{15}O_2$, and $C_{10}H_{13}O_2$ moieties, respectively. Based on these results, d-α-tocotrienol was identified as the active principle. This identification was confirmed against synthetic samples [38]. That the α-tocotrienol form of natural vitamin E, not tocopherol, may have significant cholesterol - lowering properties represents one of the early findings describing the unique biological properties of tocotrienol that was reported two decades ago [38]. The endoplasmic reticulum enzyme 3-hydroxy-3-methylglutaryl (HMG-CoA) CoA reductase produces mevalonate, which is converted to sterols and other products. It is proposed that tocotrienols are effective in lowering serum total and LDL-cholesterol levels by inhibiting the hepatic enzymic activity of HMG-Co A reductase through a post-transcriptional mechanism. α-Tocopherol, however, had an opposite effect (induces) on this enzyme activity [71]. This contrast is of outstanding significance and requires further characterization. Evidence that the tocotrienol-rich fraction (TRF) of palm oil may indeed lower plasma cholesterol in mammals came from a study of normolipemic and genetically hypercholesterolemic pigs of defined lipoprotein genotype [72]. The pigs were fed a standard diet supplemented with 50 micrograms/g TRF isolated from palm oil. Hypercholesterolemic pigs fed the TRF supplement showed a 44% decrease in total serum cholesterol, a 60% decrease in LDL-cholesterol, and significant decreases in levels of apolipoprotein B (26%), thromboxane-B2 (41%), and platelet factor 4 (PF4; 29%). It was also noted that TRF had a marked protective effect on the endothelium and platelet aggregation. The effect of the lipid-lowering diet persisted only in the hypercholesterolemic swine after 8 week feeding of the control diet [72]. These interesting observations were quickly put to test in humans by means of a double-blind, crossover, 8-week study [73]. The goal was to compare effects of the tocotrienol-enriched fraction of palm oil (200 mg palmvitee capsules / day) with those of 300 mg corn oil / d on serum lipids of hypercholesterolemic human subjects (serum cholesterol 6.21–8.02 mmol / L). Concentrations of serum total cholesterol (-15%), LDL cholesterol (-8%), Apo B (-10%), thromboxane (-25%), platelet factor 4 (-16%), and glucose (-12%) decreased

significantly only in the 15 subjects given palmvitee during the initial four weeks. Results from the crossover study established that the noted beneficial effects were indeed caused by palmvitee. A carry-over effect of palmvitee was also reported. Serum cholesterol concentrations of seven hypercholesterolemic subjects (>7.84 mmol/L) decreased 31% during a four-week period in which they were given 200 mg γ-tocotrienol/d. These results suggest that γ-tocotrienol could be the active principle cholesterol inhibitor in palmvitee capsules [73]. Experimental data from the study of hamsters are in agreement [74]. What added to the interest in tocotrienol as a cholesterol-lowering nutrient in humans was a concurrent independent study reporting the hypocholesterolemic effects of palmvitee [24]. Each palmvitee capsule contained approximately 18, 42, and 240 mg of tocopherols, tocotrienols, and palm olein, respectively. All volunteers took one palmvitee capsule per day for 30 consecutive days. Overnight fasting blood was recorded from each volunteer before and after the experiment. Palmvitee lowered both serum total cholesterol and low-density-lipoprotein cholesterol concentrations in all subjects. The magnitude of reduction of serum total cholesterol ranged from 5.0% to 35.9% whereas the reduction of low-density-lipoprotein cholesterol values ranged from 0.9% to 37.0% when compared with their respective baseline values [24].

Tocotrienol, not only of palm oil origin, but also isolated from rice bran show cholesterol lowering properties [75, 76]. Amaranth oil, containing tocotrienol, possesses hypocholesterolemic properties as well [77]. A human study with 28 hypercholesterolemic subjects has been executed in 5 phases of 35 days each. The goal was to check the efficacy of a TRF preparation from rice bran alone and in combination with lovastatin. After placing subjects on the American Heart Association (AHA) Step-1 diet (phase II), the subjects were divided into two groups, A and B. The AHA Step-1 diet was continued in combination with other treatments during phases III to V. Group A subjects were given 10 mg lovastatin, 10 mg lovastatin plus 50 mg TRF, 10 mg lovastatin plus 50 mg α-tocopherol per day, in the third, fourth, and fifth phases, respectively. Group B subjects were treated exactly according to the same protocol except that in the third phase, they were given 50 mg TRF instead of lovastatin. The TRF or lovastatin plus AHA Step-1 diet effectively lowered serum total cholesterol (14%, 13%) and LDL-cholesterol (18%, 15%), respectively. The combination of TRF and lovastatin plus AHA Step-1 diet significantly reduced the lipid parameters by 20–25%. Especially significant were the increase in the HDL/LDL ratio to 46% in group A and 53% in group B. None of the subjects reported any side-effects throughout the study of 25 weeks [78]. Consistent results were obtained using rice-bran derived TRF in another human study [71]. A dose of 100 mg/day of TRF decreased the level of serum total cholesterol, LDL-cholesterol, apolipoprotein B and triglycerides compared with the baseline values. The work led to the suggestion that a dose of 100 mg/day TRF plus AHA Step-1 diet could control the risk of coronary heart disease in hypercholesterolemic humans [71].

Tocotrienols and Neuroprotection

On a concentration basis, the neuroprotective effects of nM tocotrienol represent the most potent biological function of all natural forms of vitamin E. Glutamate-toxicity is a major contributor to neurodegeneration. It includes excitotoxicity and an oxidative stress component also known as oxytosis [11, 12]. Murine HT hippocampal neuronal cells, lacking intrinsic excitotoxicity-pathway, have been used as a standard model to characterize the oxidant-dependent component of glutamate toxicity. In 1999, it was conducted a side by side comparison of all eight forms of natural vitamin E in a model of glutamate-induced neurodegeneration of HT neural cells. In subsequent experiments it was observed that the neuroprotective property of tocotrienol applies not only to neural cell lines but also to primary cortical neurons. This line of experimentation led to an observation that eventually turned out to be the most potent function of any natural form of vitamin E on a concentration basis reported. Tocotrienol-dependent neuroprotection includes a significant antioxidant independent mechanism has been now established [13]. The neuroprotective property of tocotrienol holds good not only in response to glutamate challenge but also in response to other insults such as homocysteic acid, glutathione deficiency, and linoleic acid induced oxidative stress [13, 14]. It is now evident that at micromolar concentrations tocotrienol protects neural cells by virtue of its antioxidant property. At nanomolar concentrations, however, tocotrienol regulates specific neurodegenerative signaling processes.

GSH is the major cellular thiol present in mammalian cells and is critical for maintenance of redox homeostasis [79]. GSH is a key survival factor in cells of the nervous system and lowered [GSH]i is one of the early markers of neurotoxicity induced by a variety of agonists [80, 81]. We observed that α-tocotrienol clearly protects primary cortical neurons against a number of GSH-lowering neurotoxins [82].

Of interest, the neurons survived even in the face of GSH loss. These observations led to the hypothesis that loss of [GSH] alone is not lethal [82]. Given that pro-GSH agents are known to be neuroprotective in a variety of scenarios [80, 83, 84] it becomes reasonable to hypothesize that glutamate-induced lowering of [GSH]i triggers downstream responses that execute cell death. These works led to the identification of 12-lipoxygenase (12-Lox) as a key tocotrienol-sensitive mediator of neurodegeneration [82]. Specific inhibition of 12-Lox by BL15 protected neurons from glutamate-induced degeneration although [GSH]i is compromised by 80%. Similar protective effects of BL15 were noted when BSO, a specific inhibitor of GSH synthesis, was used as the agonist. Importantly, neurons isolated from mice lacking the 12-lipoxygenase gene were observed to be resistant to glutamate-induced loss of viability [82]. This key piece of evidence established that indeed 12-Lox represents a critical checkpoint in glutamate-induced neurodegeneration.

Neurons and the brain are rich in arachidonic acid (AA; 20:4ω-6). Massive amounts of AA are released from the membranes in response to brain ischemia or trauma [85 – 89]. Subsequent work has established that AA and its metabolites may be neurotoxic. There are three major pathways of AA metabolism: lipoxygenases, cycloxygenases and cytochrome P450. The cycloxygenase pathway has been preliminarily ruled out from being a contributor to neurodegeneration [90]. In the lipoxygenase pathway, metabolites of 12-Lox seem to be the major metabolite of arachidonic acid in the brain [91, 92] as well as in cultured cortical neurons [93–95]. Lipoxygenases, mainly 5-, 12- and 15-Lox, are named for their ability to insert molecular oxygen at the 5, 12, or 15-carbon atom of arachidonic acid forming a distinct hydroperoxy-eicosatetraenoic (HPETE) acid [96]. 12-Lox produces 12(S)- HPETE which is further metabolized into four distinct products: an alcohol [12(S)-HETE], a ketone (12-keto-eicosatetraenoic acid), or two epoxy alcohols (hepoxilin A3 and B3). Immuno histochemical studies revealed the occurrence of 12-Lox in neurons; particularly in hippocampus, striatum, olivary nucleus, as well as in glial and in cerebral endothelial cells [97, 98]. Using immature cortical neurons and HT cells, it has been shown that a decrease in [GSH]i triggers the activation of neuronal 12-Lox, which leads to the production of peroxides, the influx of Ca^{2+}, and ultimately to cell death [12, 99]. The 12-Lox metabolite 12-HPETE proved to be capable of causing cell death [100]. Inhibition of 12-Lox protected cortical neurons from β- amyloid induced toxicity [101]. Intracellular calcium chelation delayed cell death by lipoxygenase-mediated free radicals in mouse cortical cultures [102]. In sum, 12-Lox poses clear threat to neuronal survival especially under GSH-deficient conditions.

Lipoxygenase activity is sensitive to vitamin E. α-Tocopherol strongly inhibits purified 5-Lox with a IC50 of 5 μM. The inhibition is independent of the antioxidant property of tocopherol. Tryptic digestion and peptide mapping of the 5-Lox-tocopherol complex indicated that tocopherol binds strongly to a single peptide [103]. Another study reported inhibition of 15-Lox by tocopherol *via* specific interaction with the enzyme protein [104]. Of interest, inhibitors specific for cycloxygenase or 5-Lox are not effective in protecting neuronal cells against glutamate-induced death suggesting a specific role of 12-Lox in glutamate-induced death [51, 82]. These studies addressing the effect of α-tocotrienol on pure 12-Lox indicate that α- tocotrienol directly interacts with the enzyme to suppress arachidonic acid metabolism.

Tocotrienols and Diabetes Mellitus

According to the World Health Organization (WHO), 170 million people were affected by diabetes in the year 2002, and this number is likely to increase to 366 million by the year 2030 [105]. Diabetes Mellitus (DM) is a complex, progressive disease, which is accompanied by multiple complications. It has been recognized as the sole independent risk factor for the development of any cardiovascular disease [106]. Cardiovascular complications such as stroke and heart attack are increasingly causing death in diabetic patients. Alarmingly, literature statistics indicate that atherosclerosis accounts for about 8 to 10% of all diabetic deaths [107].

Hyperglycemia has been accepted as an essential factor in the development of diabetic complications [108]. Oxidative stress is significantly increased in DM, due to prolonged exposure to hyperglycemia. Oxidative stress is a common feature of DM, in which the activity of the antioxidant system is overwhelmed by excessive ROS production [109]. Chronic hyperglycemia increases the generation of free radicals through non enzymatic glycosylation, glucose autoxidation and increased activity in polyol pathway [110]. Disturbances in capacities of the antioxidant defense system include scavenging enzymes such as superoxide dismutase (SOD) and glutathione reductase and deficiencies of antioxidants such as vitamin C and E [111, 112].

Recent studies are showing that vitamin E intake

significantly reduced risk of type 2 diabetes. The relative risk (RR) of type 2 diabetes between the extreme quartiles of the intake was 0.69 (95% CI 0.51-0.94, P for trend=0.003). Intakes of α-tocopherol, γ-tocopherol, δ-tocopherol, and β-tocotrienol were inversely related to a risk of type 2 diabetes. While correlation does not imply causation, these data suggest the possibility that the development of type 2 diabetes might be modified by the intake of antioxidants in the diet [113].

In 2009, animal trials carried out in India and Malaysia revealed palm tocotrienols improved blood glucose, dyslipidemia and oxidative stress in diabetic rats. It is able to prevent the progression of vascular wall changes occurring in DM [114, 115]. Moreover, tocotrienols alone and in mixture with alpha-tocopherol had the capability to enhance lymphocyte proliferation among streptozotocin-induced diabetic rats [116].

Conclusion

Members of the natural vitamin E family possess overlapping as well as unique functional properties. Among the natural vitamin E molecules, d– α– tocopherol has the highest bioavailability and is the standard against which all the others are compared. Interestingly symptoms caused by α– tocopherol deficiency can be alleviated by tocotrienols. Study concluded that tocotrienol has wide ranging effects regarding to their mode of action and clinical properties. It has multiple effects such as free radical scavenger, anti proliferative, anti angiogenesis, anti inflammatory, pro – apoptotic, anti tumor (inhibiting survival of tumor cell), anticancer, radiation countermeasures etc. A safe and effective therapy for radiation injuries as well as cancer prevention is the need of the hour. Tocotrienols, especially ä and ã isomers present maximally in palmoil have proven radioprotectant and anticancer properties. Tocotrienols also exhibit chemosensitizing properties in hormone refractory prostate cancer and assist in prevention of cancer. Apart from therapeutic use of tocotrienol as anticancer and radiation countermeasure it may prove to be beneficial in diabetes, cardiovascular and neurodegenerative diseases. Recently it has been suggested that the safe dose of various tocotrienols for human consumption is 200 – 1000mg / day (Yu et al. 2006). In this way tocotrienol represents one of the most fascinating natural resources that have the potential influence a broad range mechanism underlying human health and disease.

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