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Teaser Vitamin E is a lipid-soluble, radical buster existing in two different isoforms: tocotrienols and tocopherols, and has been used continuously because of its many biological activities, which are pharmacologically capable of tackling a wide array of diseases, thus improving health issues occurring every day.

Tocotrienols: the unsaturated sidekick shifting new paradigms in vitamin E therapeutics

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Vitamin E family members: tocotrienols and tocopherols are widely known for their health benefits. Decades of research on tocotrienols have shown they have diverse biological activities such as antioxidant, anti-inflammatory, anticancer, neuroprotective and skin protection benefits, as well as improved cognition, bone health, longevity and reduction of cholesterol levels in plasma. Tocotrienols also modulate several intracellular molecular targets and, most importantly, have been shown to improve lipid profiles, reduce total cholesterol and reduce the volume of white matter lesions in human clinical trials. This review provides a comprehensive update on the little-known therapeutic potentials of tocotrienols, which tocopherols lack in a variety of inflammation-driven diseases.

Introduction

Tocotrienols and tocopherols are natural forms of the vitamin E family, discovered in 1922 by Evans and Bishop [1] and consisting of eight distinct isomers named: α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol (Fig. 1) [2]. Tocotrienols acts as effective lipid cellular antioxidants as well as anti-inflammatory agents that are devoutly notified in curing age-associated diseases [3]. Even though tocopherols (saturated) have been largely reported to be found in vegetable oils (canola, wheat germ, sunflower), tocotrienols are abundant in rice bran oil, palm oil and annatto seeds [4]. Tocotrienols are also found in cereal grains such as barley, oats, rice, rye and wheat, and are often in higher ratios compared with tocopherols. There are subtle differences between the vitamin E group of isomers that reflect their chemical structure as well as their activity. These differences

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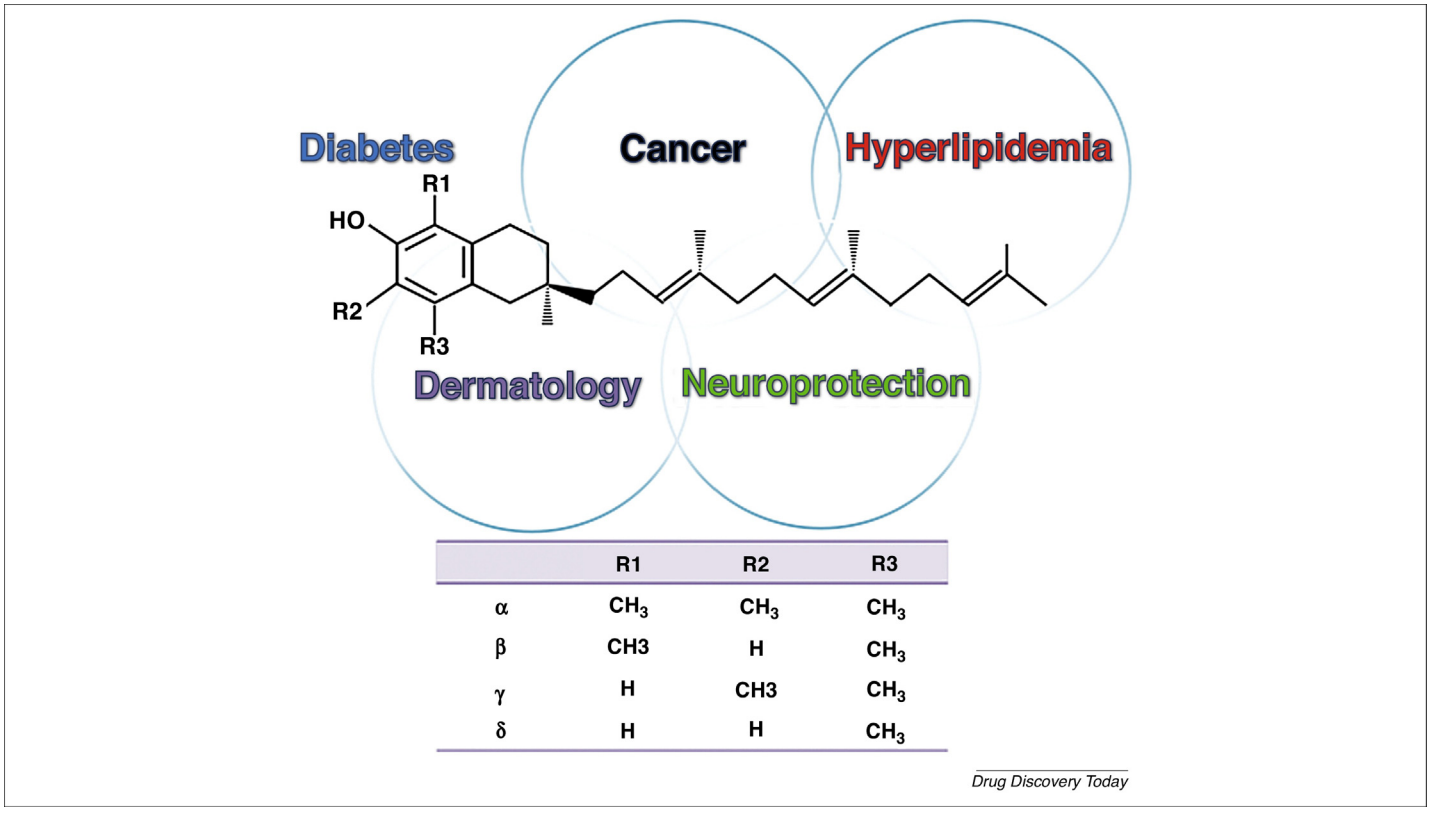

	R1	R2	R3
α	CH_3	CH_3	CH_3
β	CH3	H	CH_3
γ	H	CH3	CH_3
δ	H	H	CH_3

FIGURE 1

Chemical structure of tocotrienol isomers and their protective effects against chronic diseases.

have driven researchers to focus on creating new interventions in the field of nutrigenomics. Interestingly, the usage of tocotrienols as a diet supplement in Asian populations is significantly higher when compared with developed countries. Thus, nutrigenomics seems to play a vital part in reducing the risk of development of inflammatory age-related diseases [3].

Tocotrienols and tocopherols exist as monophenols, mostly differing in their structure by the methyl group distribution. Structurally, tocopherols vary in having a long tail, saturated side-chain with hardly no double bonds, thus showing lower anti-oxidative properties, inhibiting the functional effects in humans. Tocotrienols have an unsaturated, short tail with three double bonds, increasing the antioxidative properties and enhancing efficacy – the varied unique molecular structure helps to integrate into the cell to show its activity and govern the immune system [5,6]. Even though there are many beneficial effects of vitamin E, its occurrence is found to be existing naturally in tocotrienols in foods such as wheatgerm, barley, oats, rice and bran at low concentrations [3]. Tocopherols are synthesised and used vastly in as an ingredients in cosmetic industries in manufacturing soaps, creams, and ointments as well [7,8].

In addition, the importance of vitamin E deficiencies plays a crucial role to health of the individual. For example, deficiencies of vitamin E leads to degenerative diseases such as ataxia, leading to muscular weakness, infertility and blindness, cardiac arrhythmia, cystic fibrosis, short bowel syndrome and malabsorption leading to brain deficits like dementia [9] (Table 1). Vitamin E analogs, tocotrienols and tocopherols are known to interact with several cellular molecular targets to manifest the physiological properties to protect from diseases or malfunctioning. These execution of antioxidant function by tocotrienols are a little bit known to occur in distinct cellular pathways interacting with binding partners directly or indirectly [10]. These modulators targeting the cell in the form of growth factors, cytokines, receptors, adhesion molecules, kinases, transcription factors, apoptotic regulators and others [8] Table 2.

There has been a plethora of *in vivo* applications of tocotrienol, with several positive studies showing different kinds of validations related to healthcare benefits [11]. Most evidently, tocotrienols, among the other isoforms of vitamin E, are characterized based on their anticancer, anti-inflammatory and/or neuroprotective properties compared to tocopherols. Most predominantly, the anti-inflammatory pathways are targeted by vitamin E analogs [12]. However, the effective dose of tocotrienols for the treatment of various human diseases is still an unknown quantity. Several clinical trials on tocotrienols have shown that the average dose of tocotrienols varies, depending upon disease type such as neuroprotection (100–200 mg/day), kidney protection (200 mg/day), anti-inflammatory (200 mg/day), cardiovascular complications (100–960 mg/day) and anti-ageing (100–200 mg/day) [13] (Table 3). Tocotrienols have also been shown to suppresses the inflammatory biomarker C-reactive protein (CRP) and nitro-tyrosine in diabetic patients [14]. This review provides a compilation of vitamin E therapeutic benefits, protective effects and its prominent mechanism(s)-of-action *in vitro* and *in vivo*.

TABLE 1

Biological activities of tocotrienols

Biological activities of tocotrienols	Refs
α-T3 is shown to exhibit pro- and antioxidant activity in a comparative study conducted with T3	[143]
δ-T3 is shown to be an effective inhibitor of angiogenesis and telomerase activity in HUVEC cells	[144]
δ-T3 inhibitor of angiogenesis and eukaryotic polymerase lambda activity	[145]
γ-T3 is shown to have increasing antioxidant synergistic effects	[146]
γ-T3 is shown to support the bone formation in normal rats as well as in myocardial ischemia	[89,147]
γ-T3 has been reported to have a 30-fold increased inhibitory activity towards cholesterol biosynthesis when compared with α-T3 in HepG2 cells	[148]
α-T3 protects from ischemic brain damage in *in vivo* mouse study	[123]
δ-T3 has higher efficiency in suppressing carcinogenesis (lung and liver) *in vivo* and antiproliferative effect *in vitro*	[148,149]
γ-T3 and δ-T3 can inhibit ubiquitination and block the cholesterol mechanism *in vivo*	[150]
γ-T3 protects from oxidative damage in liver microsomes *in vivo*	[151]
γ-T3 is most potent in inhibiting lipid peroxidation and protein oxidation in rat brain mitochondria when compared with α-T3 and δ-T3	[152]
α-T3 and γ-T3 are effective in abrogating cell proliferation by inhibition of cell cycle signaling in HeLa cells	[153]

Role of inflammatory mediators in the development of diseases

Inflammation is a worldwide disability that can be either acute or chronic in nature. Whereas acute inflammation has been associated with therapeutic implications, the inadequate resolution of persistent inflammatory responses can lead to various human diseases such as arthritis, obesity and cancers. Epidemiological, preclinical and clinical studies have indicated that the process of chronic inflammation plays a vital part in the initiation and development of carcinogenesis [15]. Chronic inflammation mediates various key steps involved in tumorigenesis, including cellular transformation, survival, proliferation, invasion, angiogenesis and metastasis. Within the tumor microenvironment, reactive oxygen species (ROS), prostaglandins, leukotrienes and cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 deregulate the process of inflammation and promote the development of genomic aberrations as well as the initiation of carcinogenesis [15]. Derivatives of vitamin E are capable of controlling ROS and reactive nitrogen species generation, preventing monocutaneous chronic DNA virus infections and reducing the recurrence of skin infections [16].

Role of tocotrienols and tocopherols in inflammation in vitro

An inflammatory reaction is the manifestation of multitude reactions of physiological, cellular and immunological events. The main effector cells in the inflammatory process are leukocytes, neutrophils, eosinophils, basophils, monocytes, macrophages, mast cells, dendritic cells, T cells, B cells and natural killer (NK) cells. Activation of these cells by environmental allergens, stress or endogenous proinflammatory molecules leads to a cascade of events activating diverse biological and immune responses [17]. Chronic inflammation is a manifestation of the constant release of proinflammatory cytokines that are a product of autocrine and paracrine activation of immune cells, thus sustaining the inflammatory condition [18]. Inflammatory signals often activate prosurvival cell signaling processes such as NF-κB, signal transducer and activator of transcription (STAT)3, antiapoptosis, cyclooxygenase (COX)-2 and angiogenic pathways [18]. Progressive stages of inflammation are often observed in cardiovascular diseases (CVDs), atherosclerosis, rheumatoid arthritis, skin diseases and cancers [19].

Tocotrienols, especially the α-tocotrienol isoform, are reported to be predominant for their potent antioxidant and anticancerous activity. The reaction rates between various vitamin E isoforms are dependent upon the number of methyl groups on the chromanol ring. The antioxidant activity of α-, β-, γ-isoforms of both tocotrienols and tocopherols is similar, but the γ-isoform showed weaker activity when tested in pyrogallolsulfonphthalein and 2,7-dichlorodihydrofluorescein diacetate methods [2]. Indeed, different forms of vitamin E derivatives suppress COX-2 involvement in prostaglandin (PGD2) and PGE2 formation in lipopolysaccharide (LPS)-activated macrophages (RAW264.7). In lung epithelial cells, γ-tocotrienol showed effective results when compared to other isoforms of tocopherols [20]. According to the preference of activity, vitamin E analogs tend to inhibit the production of leukotrienes, especially LTB4, in the inflammatory microenvironment [21]. Tocotrienols well known as antioxidant able to inhibit proliferation of breast cancer cells [22]. In contrast vitamin E is developed as a new method to develop as a drug delivery system with polyethylene glycol in C6 glioma cells [23]. Another study has demonstrated that γ-tocotrienol is a potent anti-osteoporosis agent. Using murine RAW264.7 bone-marrow-derived macrophages, γ-tocotrienol upregulated genes related to osteoblastogenesis and regulated A20, an inhibitor of NF-κB, to prevent osteoclastogenesis [24]. Combinational therapy with resveratrol, quercetin and δ-tocotrienol produced significant inhibition of nitric oxide production and CRP, demonstrating better efficacy in the prevention of age-associated diseases [25]. Muscadine grape seed oil, a novel source of tocotrienols, abrogates adipose inflammation in human adipocytes by reducing peroxisome proliferator-activated receptor (PPAR)γ and adaptor protein (AP)-2 expression at mRNA and protein levels [26]. However, comparative studies with tocotrienol-rich fraction (TRF), α-tocopherol and α-tocopheryl acetate in mouse peritoneal macrophages showed the TRF protective effects were better compared with α-tocopherol and α-tocopheryl acetate against nitric oxide and proinflammatory cytokines (TNF-α, IL-1β and IL-6) [27].

The distribution of tocopherols varies between different parts of the plant. α-Tocopherol is abundant in the green leafy portions of the plant while γ-tocopherol is abundant in the non-green parts of the plant such as seeds [2]. Commonly used cooking oils such as corn, peanut and soybean oil contain large amounts of α- and

TABLE 2

Molecular modulators of tocotrienols *in vitro*

Molecular targets	Refs	Type of cancer
Transcription factors		
c-myc↓	[51,154]	Pancreatic, hepatocellular, colorectal carcinoma
HIF-1α↓	[60,155]	Colorectal, prostate cancer
NF-κB↓	[51,96,153]	Breast and colon cancer, human monocytic cells
STAT3↓	[59,156]	Breast and malignant mesothelioma cancer
STAT5↓	[156]	Malignant mesothelioma cancer
EBP-α↓	[157]	
PPAR-γ↑	[158,159]	Breast cancer
Growth factors		
HER2/neu↓	[75]	Breast cancer
TGF-β↑	[96]	Breast cancer
VEGF↓	[155]	Prostate cancer
Adhesion molecules		
VCAM↓	[160,161]	
Cytokines		
IL-1↓	[162]	Mammary cancers
IL-8↓	[155]	Colorectal adenocarcinoma
IL-12↓	[162]	Mammary cancers
Enzymes		
hTERT↓	[154]	Human colorectal adenocarcinoma
MAO-A↑	[163]	TNBC
MMP-2↓	[164]	Gastric adenocarcinoma
MMP-9↓	[51,164]	Breast and gastric adenocarcinoma
PARP↑	[165]	Gastric cancer
TIMP-1↑	[164]	Gastric adenocarcinoma
TIMP-2↑	[164]	Gastric adenocarcinoma
Telomerase↓	[154]	Human colorectal adenocarcinoma
HMGCR↓	[166–168]	Lowering cholesterol
Caspase-3↑	[96,168,169]	Gastric cancer
Caspase-8↑	[76,170–172]	Hepatocellular carcinoma
Caspase-9↑	[76,171,173]	Hepatocellular carcinoma
GST↓	[174]	Hepato carcinogenesis
Kinases		
JNK↑	[169]	Gastric cancer
MAPK↑	[175]	Breast cancer
IKKα,β↓	[51]	Breast cancer
VEGFR	[144,176,177]	Hepatocellular carcinoma
CDK-2↓	[178]	Breast cancer
CDK-4↓	[178]	Mammary tumor cells
CDK-6↓	[178]	Mammary tumor cells
ER-α↓	[179,180]	Breast cancer cells
ER-β↑	[179]	Breast cancer cells
Apoptotic genes		
Bcl-2↓	[51,168,169]	Human gastric and colon carcinoma
Bcl-xl↓	[51,181]	Human lung adenocarcinoma
Bax↑	[171,182]	Hepatocellular carcinoma
IAP-1↓	[51]	
IAP-2↓	[51]	
Survivin↓	[51]	
XIAP↓	[51]	
Ras↓	[183]	
Raf ↓	[169]	Adenocarcinoma, colon carcinoma
Cyclin D1↓	[71,178,184]	Mammary, breast and prostate cancer
Cyclin D3↓	[71,153]	Prostate cancer
Cyclin E↓	[71]	Prostate cancer

Abbreviations;: CDK, cyclin-dependent kinases; ER-α, estrogen receptor alpha; ER-β, estrogen receptor beta; GST, glutathione S-transferase; hTERT, human telomerase reverse transcriptase; HMGCR, 3-hydroxy-3-methylglutaryl co-enzyme A reductase; HER2/neu, human epidermal growth factor receptor-2; IAP, inhibitor of apoptosis; JNK, c-jun N-terminal kinase; MAO-A, monoamine oxidase A; MMP, matrix metalloproteinase; MAPK, mitogen-activated pathway kinase; PARP, poly(ADP-ribose) polymerase; PPAR, peroxisome proliferator-activated receptor; STAT, signal transducer and activator protein; TIMP, tissue inhibitor of metalloproteinases; TGF, tissue growth factor; VEGF, vascular endothelial growth factor receptor; VCAM, vascular cell adhesion molecule; XIAP, X-linked inhibitor of apoptosis protein.

TABLE 3

Protective effects of tocotrienols in clinical studies

Serial	Study	Number of patients	Health conditions	Dose	Outcome	Refs
1	Randomized, double-blind placebo-controlled trial	53	Healthy volunteers of aged 20–50 years	200 mg/day of either α-tocotrienol or TRF	No significant difference is observed in immune parameters (IL-4) between control group and treatment group	[139]
2	Subjects are divided into two groups: young and old individual groups	32 (young) and 52 (old)	Subjects from each group are divided into two age groups	TRF supplementation of 150 mg/day for 6 months	Significant increase in plasma levels of tocopherols and tocotrienols in old group than young individual group. Apolipoprotein A-1 precursor and c-protein precursor	[185]
3	Randomized double-blind, placebo-controlled, parallel trial conducted	81	Dietary intervention study conducted on chronic hemodialysis patients developing atherosclerosis developed by dyslipidemia, inflammation	Daily with vitamin E TRF (180 mg of tocotrienol) and placebo (0.48 mg tocotrienols, 0.88 mg tocopherols)	Supplementation of TRF improved lipid profiles in hemodialysis patients	[44]
4	A randomized placebo study	121	121 volunteers aged >35 years	Supplemented with 200 mg of mixed tocotrienol twice a day for 2 years	Mixed tocotrienols help to reduce volume of white matter lesions observed by MRI compared with baseline	[186]
5	Therapeutic study			Tocopherol and tocopherol supplementation to access the antioxidant activity in mediating airway modification in asthma	γ-Tocotrienol played therapeutic role in modification of airways by inhibiting cell proliferation and migration in airway smooth muscle.	[187]
6	Beneficial therapeutic role	40	Infertile men	Carni-Q-Nol® softules (CoQ + α-tocopherol) were supplemented daily 2–3 times daily for 3–6 months	Blood plasma and seminal fluid showed improved sperm density and improvement in oxidant stress marker	[188]
7	A randomized controlled trial			Vitamin E used in the treatment of NAFLD	Intake of vitamin E significantly improved liver function	[92]
8	Randomized control group and tocotrienol treatment group	87	Treatment of nonalcoholic fatty liver disease	Control group of $n = 44$ and tocotrienols group $n = 43$ were treated with 200 mg/ twice daily for 1 year	Significant response toward hepatic echogenic response in NAFLD is observed	[92]
9	Subjects were divided in to four and treated with placebo or drug	84	32 young and 52 old	Supplementation of TRF (78% tocotrienols and 22% tocopherols) 150 mg/day for 6 months	Change of plasma protein levels helps to use as a biomarker for the study	[185]
10	Human clinical trial	80	Efficacy against stroke and end-stage liver diseases in humans	Supplementation of tocotrienols or tocopherols 200 mg for mean duration 20 week; range: 1–96 weeks	Vital transportation of tocotrienol to vital organs	[189]
11	Efficacy study compared with retinol and vehicle control	30	30 patients with photoprotective effect on topical application	Topical application of tocopherols 10% and 0.3% tocotrienols	High protection against photosensitivity to irradiation compared to vehicle control	[106]
12	Randomized, double-blind placebo-controlled trial	53	53 healthy patients with 20–50 years were recruited based on study inclusion and exclusion criteria	Supplementation with 200 mg/day with TRF	Protection against the immunomodulation and change in plasma vitamin E levels	[139]
13	Randomized, placebo-controlled clinical trial	Meta-analysis data		Supplementation of vitamin E provides health benefits	Curing the consequences of cardiovascular disease	[190]
14			In Friedreich's ataxia patients are supplemented with tocotrienols	Supplementation of tocotrienol 7 mg/kg bodyweight per day	Helps in recovering Friedreich's ataxia when compared to control	[191]
15	Randomized controlled study	50	Supplementation of dietary rich consumption food with nuts, high carbohydrate, protein, green tea and red wine	Supplementation for 4 weeks	Dietary supplementation helps to increase the lipid profiles	[192]

TABLE 3 (*Continued*)

Serial	Study	Number of patients	Health conditions	Dose	Outcome	Refs
16	Randomized, double-blind trial	122	Healing surgical scars	Application of 5% topical tocotrienols	Topical application of 5% tocotrienols or placebo group after the 2 weeks of surgery until 6 weeks. Assessment of the scars at 0, 2, 6, 16 weeks showed promising results	[193]
17	Double-blind placebo-controlled clinical trial		Healthy female volunteers	400 mg of TRF over 2-month period	Supplementation of TRF increases immune stimulatory effects with response to vaccines	[194]
18	Placebo, with combination group	64		(i) 500 mg of vitamin C (ii) 200 mg Tocovid™ (tocotrienol) (iii) combination of 500 mg vitamin C and 200 mg Tocovid™ compared to placebo intake for 8 weeks	Reduce the oxidative stress	[195]
19	Randomized, placebo-controlled, double-blind study	36	Healthy male volunteers	50, 100, 200 mg daily for 2 months	Improvement in arterial compliance	[196]
20	Randomized, double-blind placebo-controlled study	64	64 subjects, 37–78 years old related to treat aging process in free radical theory of aging	Daily dosage of 160 mg of Tri E tocotrienol supplements for 6 months	Reduces DNA damage with a strong correlation with urinary (8-OHdG)	[140]
21	Questionnaire based upon intake of food frequency and food chemistry analyses	29 133	29 133 male Finish smokers aged 50–69 with occurrence of prostate cancer	50 mg daily for 5–8 years	Lowering incidence risk of prostate cancer with occurrence particularly in progressing cancer	[197]
22	Randomized, blinded endpoint, placebo controlled	36	36 healthy male subjects	80 mg, 160 mg or 320 mg for 2 months	320 mg group showed significant effect on total antioxidant status	[198]

Abbreviations: TRF, tocotrienol-rich fraction; IL-4, interleukin-4; NAFLD, nonalcoholic fatty liver disease; LDL, low-density lipoprotein; ApoB, apolipoprotein B; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

γ-tocopherol [2]. Preclinical animal studies have provided evidence of health benefits of γ-tocopherol and other forms of vitamin E derivatives in inflammatory and oxidative stress conditions and protection against lung injury. Interestingly, α- and γ-tocopherol present in tomato ketchup inhibited proinflammatory molecules and chemotaxis in human umbilical vein cells [28]. Tocopherol's potent antioxidant and photoprotective effects of tocopherols were observed in solar simulator-irradiated human skin fibroblasts when treated in combination with luteolin and ubiquinone [29]. Nishio *et al*. showed that α- and γ-tocopherol suppressed LPS-induced intracellular ROS formation, lipid peroxidation, induction of inflammatory cytokines and induction of cell death in human lung carcinoma A549 cells [30].

Role of tocotrienols and tocopherols in inflammation in vivo

Some of the scientifically proven mechanistic studies *in vivo* have shown that long-chain carboxychromanols have a better anti-inflammatory and potent free scavenger activity than unmetabolized forms of vitamin E derivatives. *In silico* docking (autodock4.0) showed that tocotrienols could directly bind to antioxidant enzymes, thus augmenting its protective role [31]. In another study, Syrian hamsters were administered naturally occurring tocotrienol (tocomin) 10 mg daily for 10 days prior challenging with LPS, zymosan or turpentine (compounds that mimic acute localized and systemic inflammation and produce enormous amounts of free radicals and oxidative stress). Tocomin suppressed antiperoxidase enzymes such as glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) and glutathione enzymes (GPx) in the following order: GR > CAT > SOD > GST > GPx [31]. In another study by the same group, it was demonstrated that tocomin (10 mg/kg) administered orally for 10 days significantly reduced the levels of plasma and lipoprotein lipids, cholesterol, Apo-B, small dense (sd) LDL as well as LDL in hyperlipidemia-induced hamsters [32]. In two xenobiotic-induced liver injury models, using acetaminophen and/or hydrogen peroxide, α-tocopherol and α-tocotrienol exerted cytoprotective effects, whereas a lower concentration of γ-tocotrienol was effective in inhibiting the toxicant-induced injury by inhibiting free radical generation and oxidative stress [33]. Uniquely, γ-tocotrienol was reported to be a potent radioprotectant in addition to being a strong antioxidant. When mice were exposed to 9 Gy γ-irradiation, γ-tocotrienol treatment protected 85% of the mice, with significant hematopoietic recovery [34]. In rats, δ-tocotrienol and γ-tocotrienol treatment showed a significant reduction in organ inflammation compared with α-tocotrienol and α-tocopherol, where only a minimal reduction was observed [35]. In another study, obese Zucker rats and control lean Zucker rats aged 8 weeks were daily fed an enriched diet with either 1% or 5% water-soluble rice bran enzymatic extract rich in polyphenols, tocotrienols or γ-oryzanol for 20 weeks, demonstrating suppressed obesity-related proinflammatory responses [36]. Tocotrienols co-administered with biodegradable epirubicin nanoparticles greatly reduced oxidative stress, inflammation, apoptosis and angiogenesis in hepatocellular carcinoma (HCC)

[37]. Numerous preclinical studies are currently evaluating several distinct forms of vitamin E analogs of tocopherols and tocotrienols. Several lines of evidence have highlighted the importance of tocopherols as being biologically active and bioavailable *in vivo*. In one study, α- and γ-tocopherol, administered at either 50 ppm or 500 ppm to C57BL/6 mice for 4 weeks, resulted in the modulation of inflammation and T cell activation [38]. Regardless of the health benefits as a diet supplement, tocopherols were shown to suppress expression of the proinflammatory transcription factor NF-κB pathway in thioacetamide-induced liver injury in male Sprague–Dawley rats [39]. Furthermore, tocopherols are approved as supplements to augment the endogenous antioxidant defense system in athletes and in negating acute and chronic oxidative stress [40].

Recently, a combination study of tocotrienols and polymethoxylated flavones (PMF) showed reduction in subclinical inflammation in hypercholesterolemic patients when treated with a lower dosage of 27 mg/day of tocotrienols and 32 mg/day of PMF in humans [41]. In a randomized, double-blind, placebo-controlled clinical trial in patients with type-2 diabetes, 200 mg of tocotrienols added to canola oil and taken for a period of 8 weeks protected patients against kidney inflammation and nitrosative stress [42]. A double-blind, randomized, placebo-controlled clinical trial revealed that the combination of natural product compounds such as resveratrol, pterostilbene, quercetin, nicotinic acid and γ-tocotrienol showed significant activity by delaying the progression of age-associated diseases with deregulated immune function and altered redox fluctuations *in vivo* [43]. In another randomized, double-blind, placebo-controlled, parallel clinical trial, 81 patients were given TRF (180 mg tocotrienols + 40 mg tocopherols) (TRF) or placebo (0.48 mg tocotrienols + 0.88 mg tocopherols) for 12 or 16 weeks. The TRF-supplemented group showed improvements in lipid profiles after 12 and 16 weeks of intervention when compared with placebo at the respective time points [44]. Clinically based studies investigating tocopherol as part of a natural diet supplement of nuts and legume seeds have found a higher rate of decrease in inflammatory and oxidative stress markers in premenopausal women [45,46]. The effect of α- or γ-tocopherol supplementation on serum CRP levels was analyzed in 12 trials with 246 patients and showed a significant decrease in the levels of CRP when compared to the control group [47]. However, in one long-term observation of a population cohort after 12 years, the antioxidant nutrient levels and CRP levels were found to be elevated [48].

Role of vitamin E analogs in regulating proinflammatory signaling pathways

Isoforms or derivatives of vitamin E family members were shown to be potent anti-inflammatory compounds that inhibit molecules that are actively involved in inflammation such as eicosanoids, COX-2 and proinflammatory pathways such as NF-κB and STAT3 (Fig. 2) [49]. Several lines of evidence show that tocotrienols reduce

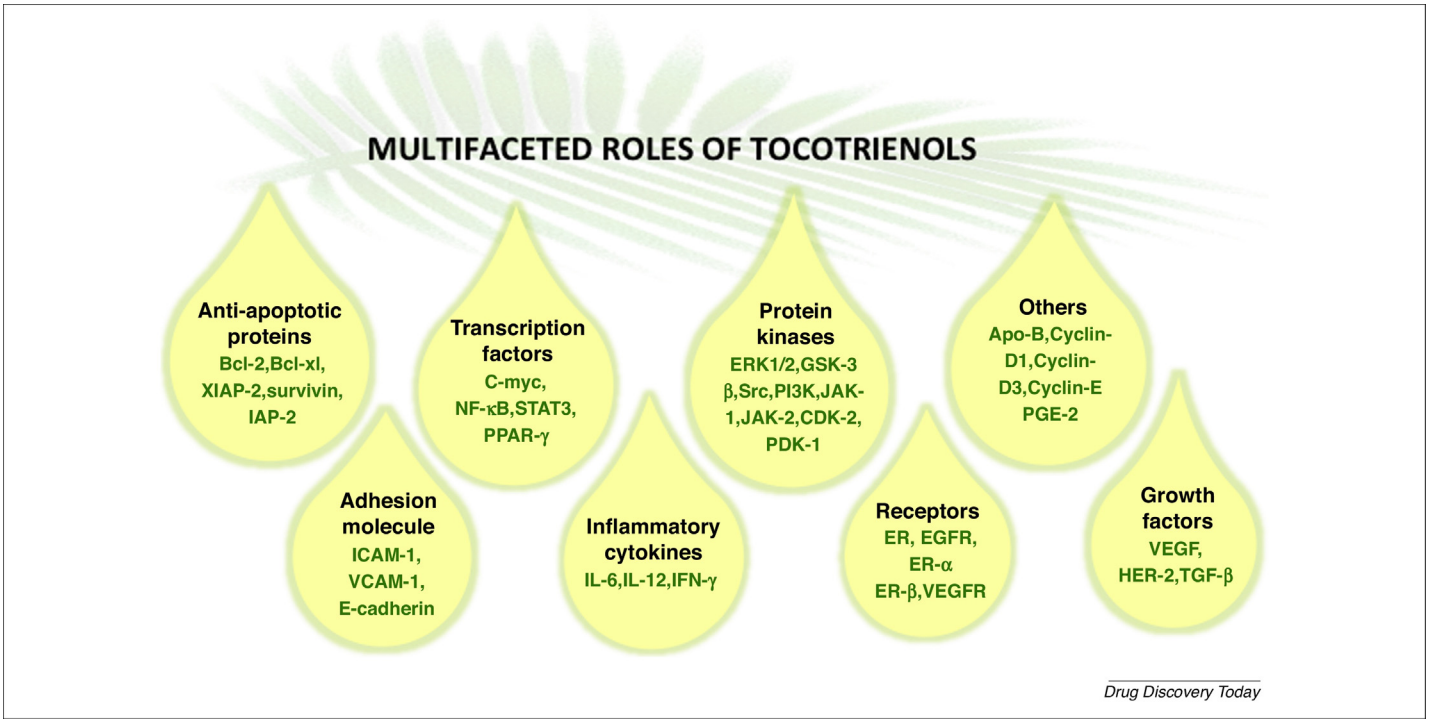

FIGURE 2

Multifaceted roles of tocotrienols. Pharmacological roles of tocotrienols involved in proinflammatory signaling pathways. Abbreviations: ApoB, apolipoprotein B; Bcl-2, B-cell lymphoma 2; Bcl-xl, B-cell lymphoma extra large; C-myc, myelocytomatosis cellular oncogene; CDK-2, cyclin-dependent kinase 2; ERK, epidermal growth factor; ER, estrogen receptor; EGFR, epidermal growth factor receptor; ER-α, estrogen receptor alpha; ER-β, estrogen receptor beta; GSK-3, glycogen synthase kinase 3; HER-2, human epidermal growth factor receptor 2; IAP-2, inhibitor of apoptosis; ICAM-1, intracellular adhesion molecule 1; IL-6, interleukin-6; IL-12, interleukin-12; IFN-γ, interferon-gamma; JAK-1, Janus kinase 1; JAK-2, Janus kinase 2; NF-κB, nuclear factor κB; PI3K, phosphoinositide-3-kinase; PPAR-γ, peroxisome proliferator-activated receptor gamma; PGE-2, prostaglandin E2; PDK-1, pyruvate dehydrogenase kinase isoform 2; STAT3, signal transducer and activator of transcription 3; Src, Src-non receptor tyrosine kinase; TGF-β, transforming growth factor β; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; XIAP-2, X-linked inhibitor of apoptosis 2.

the severity of the inflammatory process in cardiovascular diseases such as atherosclerosis [18].

NF-κB signaling pathway

NF-κB is a pro-survival transcription factor that has an important function in regulating cell proliferation and apoptosis. NF-κB comprises five family members: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB and c-Rel [50]. All family members share a highly conserved Rel homology domain (300 amino acids) that is responsible for DNA binding and heterodimerization with inhibitory κB (IκB), which are the cellular inhibitor of NF-κB (Fig. 3). The most predominant heterodimer involving p50 and p52 has a transactivation domain that participates in the regulation of cellular functions. γ-Tocotrienol abrogates TNF-α-induced activation of NF-κB through the downregulation of transforming growth factor (TGF)β-activated kinase 1 (TAK1), TNF receptor associated factor (TRAF)-2 and IκB kinase (IKK) phosphorylation and downregulates genes involved in antiapoptosis, proliferation, invasion and angiogenesis [51]. Moreover, the concentration of the other isomer of the vitamin E analog: α-tocopherol, is lower in apoE4 compared with apoE3 mice. Several studies conducted *in vitro*, in transgenic mice and in human volunteers, indicate that an upregulated proinflammatory state is associated with the apoE4 allele. In apoE4 macrophages, a higher level of constitutive activation of NF-κB was associated with a higher production of proinflammatory molecules [52]. In another study, α-tocopherol downregulated the activation of mitogen-activated protein kinase (MAPK), p38, intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 in human alveolar type II and bronchial epithelial cells stimulated with TNF-α [53]. Tocopherols and tocotrienols present in virgin olive oil demonstrated antioxidant, anti-inflammatory and hypolipidemic properties, thereby reducing the development of atherosclerosis [54]. Supplementation of tocopherols brings about a change in cytokine biology by controlling the release of cytokines and suppressing NF-κB activation [55].

STAT3 signaling pathway

The STAT family of transcription factors was first reported by Darnell *et al.* [56]. Among the STAT family of transcription factors, STAT3 is the most active acute-phase response factor that is associated with inflammation, cellular transformation, proliferation, survival, metastasis, invasion and angiogenesis. Similar to NF-κB, STAT3 can be activated by environmental factors, proinflammatory cytokines, growth factors and viruses [57]. In a HCC cell line and multiple myeloma cells, γ-tocotrienol abrogated cell proliferation and downregulated STAT3-regulated gene products such as cyclin D1, survivin, Mcl-1 and vascular endothelial growth factor (VEGF) (Fig. 3) [58]. *In vivo* use of γ-tocopherol has been shown to suppress STAT3 signaling by downregulating Janus-activated kinase (JAK) and Src, and upregulating protein tyrosine phosphatase enzyme (SHP-1), thereby inhibiting tumor cell proliferation and downregulating the expression of antiapoptotic and angiogenic genes [12]. γ-Tocotrienol (3 μM) in combination with

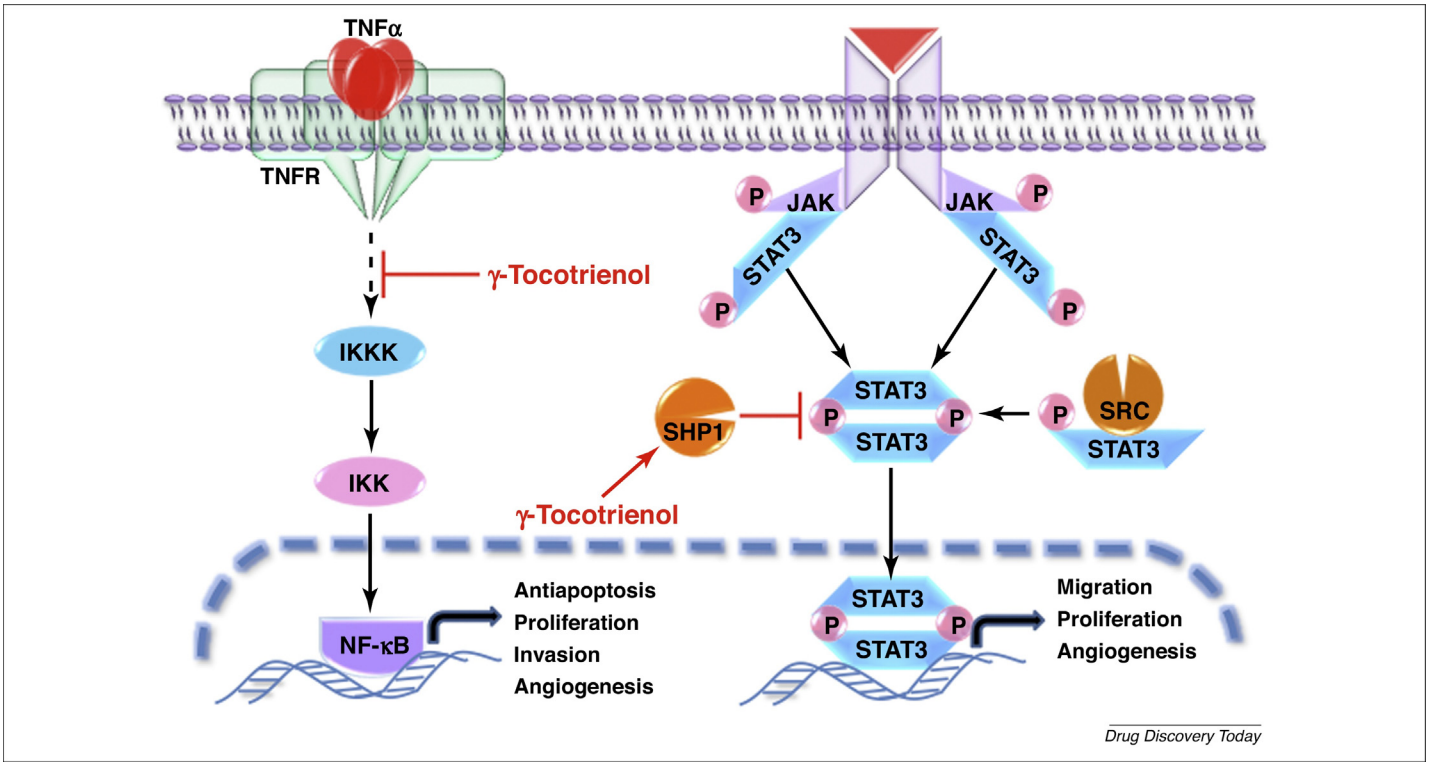

FIGURE 3

Role of γ-tocotrienol in NF-κB and STAT3 signaling pathways. γ-Tocotrienol abrogates TNF-α-induced activation of NF-kB and STAT3 signaling. γ-Tocotrienol can hence prevent proliferation, invasion and angiogenesis, properties that are highly desirable in cancer therapy. Abbreviations: IKK, I kappa B kinase; JAK, Janus kinase; NF-κB, nuclear factor κB; SHP1, Src homology 2-containing protein tyrosine kinase 1; STAT3, signal transducer and activator of transcription; SRC, src-non receptor tyrosine kinase; TNF-α, tumor necrosis factor α; TNFR, tumor necrosis factor receptor.

tyrosine kinase inhibitors such as erlotinib (0.25 μM) and/or gefitinib (0.5 μM) suppresses cyclin D1, pyruvate dehydrogenase kinase (PDK)-1, protein kinase B (AKT) and STAT3 in murine mammary tumor cells [59]. Bi *et al.* reported another important significant finding that tocotrienols abrogated cobalt(II)-chloride-induced expression of VEGF in SGC-7901 human gastric adenocarcinoma cells [60]. Apart from all these effects, tocotrienols have been shown to inhibit IL-6-induced JAK activation (JAK1 and JAK2) and subsequent phosphorylation of STAT3 cell signaling in hepatocellular carcinomal [59]. Several *in vitro* and *in vivo* studies have reported that tocotrienols modulate numerous pathways linked with tumorigenesis such as NF-κB, STAT3, death receptor (DR), apoptosis, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), hypoxia-inducible factor (HIF)-1, growth factor receptor kinases and angiogenic pathways. Therefore, tocotrienols have potential in the prevention and the treatment of cancer [18].

Role of tocotrienols and tocopherols in cancer

Cancer remains a major health issue and is the second leading cause of mortality worldwide with an incidence rate of 2.6 million cases per year [61]. Natural products present as important sources for bioactive compounds in the anticancer drug discovery process by serving as important leads for the development of novel therapies [62]. These natural compounds exhibit multitargeted effects and can affect diverse oncogenic targets including transcription factors, cytokines, chemokines, adhesion molecules, growth factor receptors and inflammatory enzymes [63]. While γ-tocotrienol plays a major role in curing multiple diseases in humans such as inflammation, it has also been shown to lower prostaglandin synthesis upon lipopolysaccharide stimulation in macrophages (RAW264.7) as well as human epithelial cells (A549) treated with IL-1β [199]. In human colorectal carcinogenesis, there are elevated levels of PGE2 and increased COX-2 activity; hence, γ-tocopherol might be useful in cancer chemoprevention. In another study, using a human prostate cancer cell line *in vitro*, γ-tocopherol was found to be superior to α-tocopherol in terms of cell inhibition [64]. Bellezza *et al.* showed that pretreatment with α-tocopheryl succinate, one of the most effective analogs of vitamin E, produced a synergistic cytotoxic effect in combination with docetaxel [65]. In human-androgen-independent prostate PC3 cells, γ-tocopherol increased activity of transglutaminase (TG)2, reduced DNA synthesis and significantly decreased the levels of cyclin D1 and cyclin E [66]. A combination study using methane seleninic acid and γ-tocopherol at low dose showed antiproliferative effects against prostate cancer in an *in vivo* xenograft mouse model [67]. Gysin *et al.* and colleagues demonstrated the superior effects of γ-tocopherol compared with α-tocopherol on the suppression of cell proliferation, cell cycle and DNA synthesis in various cancer cell lines (colon, prostate and osteosarcoma). γ-Tocopherol manifests its effect by causing G1 to S phase transition delay and by down-regulation of the cell cycle proteins such as cyclin D1 and cyclin E. In breast, colon, lung and prostate cancer cell lines, γ-tocopherol was shown to be more effective at suppressing cell growth than α-tocopherol [68–70]. In MCF-7 breast cancer cells, treatment with γ-TmT, γ- and δ-tocopherol inhibited cell proliferation in a dose-dependent manner, unlike α-tocopherol [71]. Additionally, γ-tocopherol has been found to induce apoptosis in breast, colon and prostate cancer cells *in vitro* and in *in vivo* xenograft tumors [70,72]. Dietary supplementation with annatto-tocotrienols (T3) (90% δ-T3 and 10% γ-T3) delayed the development of mammary tumors and reduced the number and size of mammary tumor masses and lung metastases in HER-2/neu transgenic mice [73]. However, the effects of tocotrienols are more proficient compared to tocopherols in reducing the activity of DNA polymerase and protein kinases in cancer cells [8]. In TRF, the combination of tocotrienols and α-tocopherol was shown to inhibit the proliferation of cancer cells up to 50% in MDA-MB-435, HeLa and p388 cells (breast, cervical and murine leukemia, respectively). In *in vivo* experiments, tocotrienols suppress subcutaneous lymphoma in hairless mice [12]. Esters of tocotrienols have been shown to reduce proliferation and migration in metastatic breast cancer MDA-MB-231 cells, indicating their anticancerous properties [74]. Several comparative studies have demonstrated the anticancer effects of tocotrienols and α-TOS (synthetic derivative) in human and mouse breast cancer cells with and without HER-2/neu oncogene overexpression [75]. Furthermore, the combination of tocotrienols with caspase inhibitors could block the growth of malignant mammary epithelial cells and induced apoptosis [76]. Sesamin synergistically potentiates the anticancer effects of γ-tocotrienol in mammary cancer cell lines [77].

Mitochondrial-targeting vitamin E succinate (MitoVES) was shown to inhibit mtDNA transcription, thus abrogating the mitochondrial respiration, mitochondrial membrane potential and increased generation of ROS in breast cancer cells *in vitro* and *in vivo* in a HER2-overexpressing breast cancer mouse model [78]. γ-Tocotrienol inhibited HCC cell proliferation, induced apoptosis and downregulated the expression of STAT3-regulated gene products, including cyclin D1, Bcl-2, Bcl-xL, survivin, Mcl-1 and VEGF, and significantly potentiated the apoptotic effects of chemotherapeutic drugs (paclitaxel and doxorubicin) [58].

A mixed tocopherol diet rich in γ- and δ-tocopherol was shown to reduce *N*-methyl-*N*-nitrosourea-induced mammary tumor growth in *in vivo* rat models [68]. In a breast cancer xenograft model, mice fed orally with 1 mg/day of TRF for 20 weeks demonstrated a reduction in tumor size compared with control mice [79]. Using an immature mouse uterine bioassay, a combination therapy of tocotrienols with anti-estrogen was shown to be effective in inhibiting the growth of ZR-75-1 cells in the presence of tamoxifen and estradiol [80]. Tocopherols were shown to abrogate prostate cancer cell proliferation by increasing the uptake of transglutaminase and reducing cell cycle progression through cyclin D1 and cyclin E [66]. γ-Tocotrienol was shown to suppress NF-κB and chemosensitize gastric cancer cells to capecitabine, and it potently inhibited the growth of gastric cancer in a xenograft mouse model [81]. In a recent study by Siveen *et al.*, γ-tocotrienol was shown to inhibit the pro-survival AKT/mTOR pathway in an HCC orthotopic mouse model [82].

Role of tocotrienols and tocopherols in other diseases

Cardiovascular diseases

CVDs are the most common cause of death throughout the world. Hence, the role of tocotrienols could have significant clinical implications when it comes to preventing CVD [83]. The cardioprotective effects of tocotrienols are mediated through their antioxidant mechanisms and their ability to suppress inflammation, inhibit HMG-CoA reductase (a rate-limiting enzyme in cholesterol

biosynthesis) and reduce the expression of adhesion molecules and monocyte–endothelial cell adhesion [84]. Tocotrienols were found to be more effective than α-tocopherol in attenuating age-related increases in systolic blood pressure of hypertensive rats [85]. Moreover, tocotrienols significantly alleviated atherosclerotic iliac artery stenosis induced by a high cholesterol diet. It significantly lowered aortic contents of malondialdehyde, reduced intimal thickening and maintained the internal elastic lamina in rabbits [86]. Tocotrienols also significantly alleviated ischemia-reperfusion injury, and reduced infarct size in the ischemic region of myocardial tissue [87]. Studies have determined the effects of tocotrienols on lipid peroxidation and total antioxidant status of spontaneously hypertensive rats. Treatment with tocotrienols exhibited a dose-dependent hypotensive effect on the systolic blood pressure of spontaneously hypertensive rats. They also caused a significant drop in the mean arterial pressure in a dose-dependent manner, decreased lipid peroxidation and increased the activity of antioxidant enzymes in hearts of rats [88]. Myocardial ischemic injury results from severe impairment of coronary blood supply. Tocotrienols significantly reduced coronary perfusion pressure and heart rate. They exerted protection against myocardial injury by mitigating cardiac dysfunction and oxidative injury in rats and by the differential interaction of MAPK with caveolin, together with proteasome stabilization. This possibly altered the availability of pro-survival and antisurvival proteins, hence mitigating myocardial injury [89].

Role of tocotrienols and tocopherols in hyperlipidemia

Hyperlipidemia is a group of disorders in which a person has elevated levels of lipids in the bloodstream. These lipids include cholesterol, phospholipids, triglycerides and cholesteryl esters. Lipids are insoluble in an aqueous medium, hence they are often circulated in body fluids as soluble protein complexes called lipoproteins. Hyperlipidemia can lead to several metabolic diseases such as cardiovascular dysfunction and atherosclerosis. Oxidative stress is a crucial risk factor for hyperlipidemia occurrence. Tocotrienols have been extensively used for reducing blood lipid levels because of their intrinsic antioxidant activity [84]. Tocotrienols are thought to have more-potent antioxidant properties than α-tocopherol. This is because of the unsaturated side-chain of tocotrienols, which enables more-efficient penetration into tissues that have saturated fatty layers, such as the brain and liver. Experimental research examining the antioxidant, free-radical scavenging effects between tocopherols and tocotrienols revealed that tocotrienols appear to be superior as a result of their better distribution in the fatty layers of the cell membrane [90]. Several reasons have been suggested for the increased antioxidant activity of tocotrienols versus α-tocopherol, primarily focusing on the differences in the tail structure. The chromanoxyl radical of tocotrienols (α-tocotrienoxyl) has been shown to be more rapidly recycled in membranes and lipoproteins compared with the corresponding α-tocopheroxyl radical. NMR studies have indicated that tocotrienols are located closer to the membrane surface, which could facilitate the more rapid recycling. Furthermore, tocotrienols have a stronger disordering effect on membranes than α-tocopherol and are distributed more uniformly within the membrane. This results in increased antioxidant activity, which would prevent against hyperlipidemia [90].

Oral administration of tocotrienols to pigs expressing hereditary hypercholesterolemia significantly reduced serum total cholesterol (TC) and LDL, ApoB, platelet factor 4, thromboxane B(2), glucose, triglycerides and glucagon [91]. Tocotrienol administration also lowered the hepatic HMG-CoA reductase activity and fatty acid levels in various other tissues. This particular cholesterol-lowering effect was mediated by suppressing HMG-CoA reductase activity via a post-translational modification [43]. Magosso *et al.* conducted a first clinical trial that demonstrated mixed palm tocotrienols exhibit significant hepatoprotective effects in hypercholesterolemic adults [92]. Furthermore, the study showed that tocotrienols could reduce serum levels of TC, LDL cholesterol and triglycerides compared to baseline levels. In patients with hyperlipidemia and carotid stenosis, long-term treatment with palm oil (a rich source of tocotrienols) resulted in significant lowering of oxidative modification of LDL, and this in turn prevented the initiation and propagation of atherosclerosis, which is a chronic disease [93].

Role of tocotrienols in the management of diabetes

Dietary antioxidants have been reported to exert a significant effect on controlling diabetic manifestations, and some of them have been experimentally evaluated. Tocotrienols (0.1 g/kg) significantly prevented oxidative damage in the streptozotocin (STZ)-induced, diabetic osteogenic disorder Shionogi rat [94]. They also effectively blocked the increase in serum advanced glycosylation end-products and malondialdehyde, and caused a reduction in blood glucose and glycated hemoglobin in diabetic rats [95]. Diabetes is associated with several secondary complications such as neuropathy, retinopathy, nephropathy, lower limb amputations, etc. Studies evaluated the impact of tocotrienols on cognitive function and the neuroinflammatory cascade in STZ-induced diabetes. STZ-induced diabetic rats were treated with tocotrienol for 10 weeks. The rats demonstrated a major increase in transfer latency, which was coupled with enhanced acetylcholinesterase activity, increased oxidative-nitrosative stress, TNF-α, IL-1β and caspase-3 activity, and active p65 subunit of NF-κB in different regions of the diabetic rat brain. Co-administration of tocotrienols significantly attenuated stereotypical behavioral, biochemical and molecular changes associated with diabetes. Moreover, diabetic rats treated with an insulin–tocotrienol combination exhibited a more pronounced effect on molecular parameters as compared with their control groups [96]. Tocotrienols also prevented diabetic neuropathy in rat models [97,98]. Oral administration of tocotrienols also significantly reduced the fasting serum glucose level in STZ-induced diabetic rats via increased glucose metabolism. Tocotrienols also promoted an oxidative-stress-reducing property in diabetic rats, which is believed to be a pathogenic factor in the development of diabetic complications [97]. Oral administration of tocotrienols also decreased the levels of HbA1c, plasma glucose, lipids, peroxylipid (malonedialdehyde), albuminuria, proteinemia and uremia, and improved insulin sensitivity in various animal models. It also prevents the incidence of long-term complications in diabetic nephropathy [96].

Neuroprotective properties of tocotrienols and tocopherols

In one study, HT4 hippocampal neuronal cells were studied to compare the efficacy of tocopherols and tocotrienols to protect

against glutamate-induced death. Tocotrienols were significantly more effective than α-tocopherol in preventing glutamate-induced death. It was proposed that tocotrienols might have protected cells via an antioxidant-independent mechanism [99]. Examination of relevant signal transduction and cellular pathways revealed that protein tyrosine phosphorylation processes played a central part in the execution of glutamate-induced death. Activation of pp60 (c-Src) kinase and phosphorylation of ERK were observed in response to glutamate treatment. Nanomolar amounts of tocotrienols, but not tocopherols, blocked glutamate-induced death by suppressing glutamate-induced early activation of c-Src kinase. Overexpression of kinase-active c-Src made cells more vulnerable to glutamate-induced death; however, tocotrienol treatment prevented death of Src-overexpressing cells treated with glutamate. Current developments in tocotrienol research demonstrate neuroprotective properties for this lipid-soluble vitamin in brain tissue that is rich in polyunsaturated fatty acids (PUFAs). Arachidonic acid (AA), one of the most prominent PUFAs of the central nervous system (CNS), is vulnerable to oxidative metabolism under pathologic conditions. PUFA is cleaved from the membrane phospholipid bilayer by cytosolic phospholipase A2 and is metabolized by enzymatic and nonenzymatic pathways. Several neurodegenerative conditions in the human brain are associated with disrupted PUFA metabolism of AA, including acute ischemic stroke [100]. Tocotrienols at nanomolar concentrations have been shown to reduce enzymatic and nonenzymatic mediators of AA metabolism and neurodegeneration. To date, the neuroprotective qualities of tocotrienols in neurodegenerative disorders of the CNS are well characterized, with specific molecular targets (cPLA2, 12-LOX and c-Src) and mechanisms of action identified [101]. More studies address a novel molecular mechanism via which tocotrienols can be protective against stroke *in vivo*. Elevation of intracellular oxidized glutathione (GSSG) triggers neural cell death. Multidrug-resistance-associated protein (MRP)1, a key mediator of intracellular oxidized glutathione efflux from neural cells, might possess neuroprotective functions. Stroke-dependent brain-tissue damage was evaluated in MRP1-deficient mice and tocotrienol-supplemented mice. Elevated MRP1 expression was observed in glutamate-challenged primary cortical neuronal cells and in stroke-affected brain tissue. MRP1-deficient mice displayed larger stroke-induced lesions, exemplifying a protective role of MRP1. *In vitro*, protection against glutamate-induced neurotoxicity by tocotrienols was reduced under conditions of MRP1 knockdown; this suggests a role for MRP1 in tocotrienol-dependent neuroprotection. MRP1 expression was increased in the stroke-affected cortical tissue region of tocotrienol-supplemented mice [102]. Elucidation of the underlying mechanism identified MRP1 as a target of a microRNA (miRNA). In tocotrienol-supplemented mice, the miRNA was downregulated in stroke-affected brain tissue. Furthermore, research contributes a new perspective to the current understanding of the molecular basis of tocotrienol-based neuroprotection in two ways: by identifying MRP1 as a tocotrienol-sensitive target and by unveiling the general prospect that oral tocotrienols might regulate miRNA expression in stroke-affected brain tissue [102]. Neuroprotective, as well as hypocholesterolemic, properties of tocotrienols make them good candidates for nutrition-based intervention in people at high risk of stroke. Transient ischemic attack, or mini-stroke, serves as a crucial warning sign for high-risk stroke patients. Prophylactic stroke therapy therefore provides an opportunity for intervention in patients with transient ischemic attack before undergoing a major stroke event. Furthermore, tocotrienols are a nutrient certified by the FDA to be generally safe and not drugs with potential side-effects. Thus, tocotrienols should be considered as a preventive nutritional supplement for people at high risk of stroke.

In order to determine whether the neuroprotective activity of tocotrienols is antioxidant-independent or –dependent, a study was conducted using two different triggers of neurotoxicity: homocysteic acid (HCA) and linoleic acid [102]. HCA and linoleic acid caused neurotoxicity with comparable features, such as increased ratio of oxidized:reduced glutathione GSSG:GSH, raised intracellular calcium concentration and compromised mitochondrial membrane potential. Mechanisms underlying HCA-induced neurodegeneration were comparable to those in the path implicated in glutamate-induced neurotoxicity. Inducible activation of c-Src and 12-lipoxygenase (12-Lox) represented early events in that pathway. Overexpression of active c-Src or 12-Lox sensitized HT4 neural cells and primary cortical neurons to HCA-induced death. Knockdown of c-Src or 12-Lox attenuated HCA-induced neurotoxicity. Oxidative stress represented a late event in HCA-induced death [103]. Another study investigated whether phospholipase A2 (PLA2) activity is sensitive to glutamate and mobilizes AA, a substrate for 12-Lox. Furthermore, the researchers examined whether tocotrienols regulate glutamate-inducible PLA2 activity in neural cells. Glutamate challenge induced the release of AA from HT4 neural cells [103]. Such a response was decreased by calcium chelators, ethylene glycol tetraacetic acid (EGTA) and 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), cytosolic PLA2 (cPLA2)-specific inhibitor arachidonyltrifluoromethyl ketone (AACOCF3), as well as tocotrienols at 250 nM. Glutamate also caused the elevation of free polyunsaturated fatty acid (AA and docosahexaenoic acid) levels and disappearance of phospholipid-esterified AA in neural cells. Furthermore, glutamate induced a time-dependent translocation and enhanced serine phosphorylation of cPLA2 in the cells. The effect of glutamate on fatty acid levels and on cPLA2 was significantly decreased by tocotrienols. The observations that AACOCF3, transient knockdown of cPLA2 and TCT significantly protected against the glutamate-induced death of neural cells implicate cPLA2 as a tocotrienol-sensitive mediator of glutamate-induced neural cell death. The study suggested that tocotrienols provided neuroprotection through glutamate-induced changes in cPLA2. Tocotrienols have also been found to possess neuroprotective activity in animal models of diabetic neuropathy and alcoholic neuropathy [84]. In addition, tocotrienols have been reported to suppress the proinflammatory pathways in diabetes and chronic alcoholism, which in turn prevented the animals from cognitive impairment and oxidative–nitrosative stress [84].

Role of tocotrienols and tocopherols in dermatology

For centuries, people have attempted to present a healthy and youthful appearance to their skin, and researchers and physicians are keen to discover more oral natural extracts or topical applicants to keep skin youthful [104]. Vitamin E and its analogs are well

known in the field of dermatology as 'caretakers', used for more than 50 years for their lipophilic antioxidant and ultraviolet-radiation-protective properties in clinics as well as in experimental dermatology. The role of vitamin E analogs in the area of dermatology in maintaining skin integrity has driven research to introduce different formulations that bring about improved photoprotection [105]. Pretreatment with vitamin E formulations was found to give better protection against photoinduced skin damage [106]. In melanocytes, tocotrienols increase the enhancement of melanosome (which helps in degradation of endosome fusion through docking) in B16F10 mouse melanoma cells, and act as a melanogenesis inhibitors and abrogate tyrosinase activity [107]. α-Tocopherol, the most active form of vitamin E, has been shown to have several benefits, when used in combination with other dietary supplements such as lipoic acid, resveratrol, selenium, coenzyme Q10 and krill oil, for the prevention of skin disorders. *In vitro* studies using human keratinocytes showed reduced levels of ROS generation as well as inflammatory cytokine secretion. These findings indicate that α-tocopherol can be used to treat chronic skin inflammation by inhibiting the NF-κB pathway [108]. Although there are listed benefits such as being antioxidative, antiaging and anti-photo-damage, tocopherols reduce UVA- and UVB-induced redox alterations, apoptosis and cell damage by up to 40% in HaCaT cells and in human melanocytes without exhibiting any toxicity [109,110]. Besides the beneficial effects for skin treatment, recent findings have shown that α-tocopherol helps in a remarkable moisturizing way and minimizes transepidermal water loss associated with antioxidant and anti-inflammatory effects [111].

Tocotrienols, identified as antioxidants and also being superior to tocopherols or other vitamin E analogs, have been well known in the field of dermatology for 50 years for their protection against photosensitivity [105]. Topical application of α-tocopherol acetate (ATA) for 3 weeks before administration of a single dose of ultraviolet light radiation (UVB) (0.9 j/cm2) to guinea pigs decreased acute and chronic skin disorders [112]. In another study, pretreatment with α-tocopherol prevented lipid peroxidation induced by UVA in hairless mice [113]. The existence of vitamin E analogs in the human skin was analyzed by HPLC, and the epidermis was found to contain 1% α-tocotrienol, 3% γ-tocotrienol, 87% α-tocopherol and 9% γ-tocopherol. Even though the tocotrienols are at minimal levels, they help as a protective physiological barrier and in modulating the growth factors of skin [114]. Some studies have shown that other analogs of vitamin E isoforms play a part in attenuating photosensitivity in humans [114]. In humans, skin is the most exposed area to environmental factors such as stress, ROS, UV radiation and chemicals, which are some of the extrinsic factors that bring about photo-ageing and skin ageing [115]. To diminish the consequences of these irritants toward photo-ageing, direct topical application or dietary supplementation of the low molecular weight tocopherols and vitamin C has been indicated to protect against oxidative stress *in vivo* and *in vitro* [116]. The importance of vitamin E derivatives was demonstrated when continuous dietary supplementation of α-tocopherol-acetate, for a period of 2–3 weeks, maintained sebaceous gland secretion and also reduced blister formation [117,118]. Furthermore, in 14 patients with nonlesional atopic dermatitis, α-tocopherol supplementation (16.1 ± 2.2 mol/g) reduced reactive oxidant intermediates released during oxidative stress [119]. In a 21-year-old female student administered with α- and δ-tocopherol, 100 mg 3-times/day for 1 week, followed by 100 mg/day in combination with ascorbic acid (200 m/day) for 25 days, reduction of allergic contact dermatitis was reported, which was caused by suppression of systemic oxidative stress [120]. In a randomized, double-blind, placebo-controlled clinical study with 30 patients with a history of polymorphous light eruption (PLE), a new topical formula was tested consisting of 0.25% α-glucosylrutin (AGR) (a natural, modified flavonoid), 1% tocopheryl acetate (vitamin E) and a broad-spectrum, highly UVA-protective sunscreen (SPF 15) in a hydrodispersion gel vehicle. This formulation was compared with a sunscreen-only gel and vehicle over a period of 4 days. The combination of a potent antioxidant with a highly UVA-protective sunscreen was very effective in preventing PLE when compared with sunscreen alone or placebo [121].

Pharmacology and drug delivery systems

The route of administration of vitamin E and its analogs is one of the pharmacological limitations for its uptake and complete dissociation to the tissue or organ of interest. Numerous studies have been undertaken to observe the pharmacokinetics of tocotrienols and tocopherols. The bioavailability of different forms of tocotrienols was assessed in a group of healthy volunteers taking a single dose of α-, γ- or δ-tocotrienol under fasting conditions as an oral supplement along with food. The study concluded that oral supplementation gives a higher rate of bioavailability [122]. Another *in vivo* study revealed that different routes of administration had no significant effect on α-tocotrienol levels; however, α-tocotrienol was shown to have higher bioavailability than γ- and δ-tocotrienol, which is the result of the methyl groups in the chromanol ring in their structures [2]. However, a study using an *in vivo* mouse model showed that intravenous injection of α-tocotrienol could decrease cerebral artery occlusion, and its adsorption was found to be more active than γ-tocotrienol and γ-tocopherol [123]. A double-blind, placebo *in vivo* study demonstrated the increased bioavailability of α-tocotrienol when compared with the other forms of tocotrienols by analyzing the effect of a 250 mg/kg dose for 8 weeks on plasma levels, which replicated the effects seen *in vitro* [124]. Further studies in human healthy volunteers analyzed the absorption and metabolism of tocotrienols and tocopherols, which are excreted into urine in minute quantities, using HPLC. The study was conducted for a total of 4 weeks, in which the subjects received either 125 mg or 500 mg γ-tocotrienyl acetate for the first and second week, followed by alternate doses for the third and fourth week of the study. The researchers found 1–2% of α-tocotrienol and 4–6% of γ-tocotrienol in the urine in the form of CEHC metabolites [2,7,8-trimethyl-2-(b-carboxyethyl)-6-hydroxychroman] [125]. Vastly improved systems of drug delivery in anticancer therapy are in the form of nanoparticles, in which liposomes and micelles are formulated with a polymeric d-α-tocopheryl polyethylene glycol 1000 succinate core. These have been used to envelop the drug to increase its efficiency and maintain its structure until it reaches the cell where it is internalized. This technique has been successfully applied *in vitro* to the MDA-MB-231 breast cancer cell line and in *in vivo* xenograft mouse models where it induced apoptosis [126–128].

TABLE 4

Completed and ongoing anticancer clinical trials of tocotrienols

Drug	Type of cancer	Phase	Status (year)	Identification number for clinical trial obtained from http://clinicaltrials.gov/
Tocotrienol-rich factor	Breast cancer	Pilot trial	Completed (2010)	NCT01157026
γ-Tocotrienol and tocotrienol-rich factor	Metastatic breast cancer	I	Completed (2014)	NCT01571921
γ-Tocotrienol	Pancreatic cancer	II	Ongoing (2015)	NCT01450046 NCT01446952 NCT00985777
Tocotrienols	Ovarian cancer	II	Ongoing (2015)	NCT02399592

There has been a rapid increase in developing different formulations to improve the bioavailability of α-tocopheryl succinate (α-TOS), one of which utilized acetic acid for liposomal delivery and showed significant activity at inducing apoptosis in preclinical models [129]. In another study, dendrimer-entrapped gold nanoparticles (altered with RGD peptide and α-tocopheryl succinate) successfully targeted cancer cells at pH 5–8 and a temperature range of 4–50 °C [130]. An alternative study fabricated a nanoemulsion-based delivery system of vitamin E that was accomplished using natural biopolymers [131]. Another study demonstrated that topical application of a gel formulation in a rat model could reduce peripheral inflammatory pain without any side-effects at the site of application [132]. Furthermore, it has been demonstrated that vitamin E helps to stabilize the emulsification of various drug formulations [133]. Emerging branches of biomedical science are devising novel drug delivery systems to target tumors directly and have shown a significant increase in antitumor efficacy in a xenograft mouse model by the PEGylation of TPGS/DTX-M and by adding vitamin-E–TPGS formulated with Genexol®-PM, which replicated the activity of the formulated drug complex *in vitro* [134,135]. These pharmacokinetics studies help to develop a new approach toward drug delivery to target the site of interest as well as to improve safety and efficacy *in vivo* and *in vitro* [136]. Recent technical improvements have permitted biomedical delivery of peptides with aqueous formulations in the form of encapsulated nanostructures formulated with vitamin-E–TPGS for *in vitro* application to increase their potential activity [137].

Human clinical studies with tocotrienols and tocopherols

Markedly, there are several clinical studies that are still ongoing and some that have completed successfully upon supplementation of vitamin E for its antioxidant activity used as a therapeutic remedy for treatment of divergent disorders to cancers. Recent surveillance studies conducted on a placebo-controlled, double-blind basis did not show much difference, owing to epidemiological investigations and shorter duration of treatment and optimizing the dosage used. Instead, some studies do not reach the therapeutic targets, but none of the side-effects and mortalities were recorded upon the studies conducted on dementia and mild cognitive impairment [138]. Focusing more toward the clinical investigations conducted on healthy volunteers, a study was conducted with a TRF-rich fraction in randomized, placebo-controlled studies in healthy Asian volunteers (20–50 years) supplemented with 20 mg/day. Blood drawn from these volunteers was subjected to checks for the immunogenic parameters when they could not find any difference [139]. Another interesting study was conducted in older healthy adults who were subjected to checks for the reduction of DNA damage in healthy volunteers upon the supplementation of triE tocotrienols of 160 mg for a period of 6 months revealing protection against DNA damage; also a pronounced decrease in DNA damage biomarkers such as urinary 8-OhdG was observed [140]. Alternatively, there are promising efficacy studies conducted with vitamin E formulations bringing about the protection against photosensitivity and skin damage [141]. Accordingly, several interesting studies were conducted on tocotrienols and tocopherols covering potential treatment for the wide range of neurodegenerative disorders such as Alzheimer's disease and dementia through to cancer (Table 3).

A pilot study was conducted for a double-blind, placebo-controlled trial with a combination of TRF and tamoxifen for a period of 5 years. It was shown to be potent in reducing breast cancer with improved survival rate [142]. Furthermore, ongoing studies conducted on an intake of mixed tocotrienols showed recovery of end-stage liver disease occurred during pancreatic cancer, cardiovascular stroke and neuroprotective effects [84] (Table 4).

Concluding remarks

Of all the vitamin E analogs, tocotrienols have superior benefits in various diseases when compared with tocopherols. The many distinctive roles of tocotrienols in cancer, inflammation, neuroprotection and metabolic syndromes exemplify that tocotrienols have significant implications for clinical use. Comprehensive studies performed in cardiovascular, nonalcoholic fatty liver disease, cancer and photosensitization models have shown significant benefits at all levels including cellular, molecular and disease outcomes, highlighting their ability to inhibit several inflammatory processes and skirting the development of inflammation-driven diseases. Several *in vitro* and *in vivo* research and clinical trials conducted over the past few decades substantiated the potential usefulness of tocotrienols and tocopherols as anticancer agents. Clinical trials with tocotrienols and tocopherols indicate safety, tolerability, nontoxicity and efficacy. These studies provide an excellent data source for more well-controlled studies in larger cohorts as well as opening avenues for future drug development. However, vitamin E analogs are limited by their poor bioavailability. The development of formulations of vitamin E in the form of nanoparticles, liposomes, micelles or phospholipid complexes to enhance bioavailability and efficacy are still in the early stages. Nevertheless, vitamin E analogs have established themselves as safe and promising molecules for the prevention and therapy of not only cancer but also other inflammation-driven diseases.

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