

The role of tocotrienols in liver health and disease

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ABSTRACT

Tocotrienols are part of the vitamin E family and are largely found in palm oil, rice bran and annatto. However, tocotrienols are less popular and not as comprehensively studied in comparison to tocopherols, which is the other isomer of vitamin E. Tocotrienols are similar to tocopherols in which alpha, beta, gamma and delta subtypes of both forms of vitamin E exist naturally. Tocotrienols are unsaturated and contain isoprenoid side chains, which differentiates them from tocopherols. Previous studies indicated that tocotrienols have superior antioxidant and biological effects against chronic diseases compared to tocopherols. The liver is the major powerhouse organ involved in metabolism and thus very susceptible to injury caused by xenobiotics, harmful chemicals and toxic metabolites. Uncontrolled levels of these reactive molecules leads to increased oxidative stress in the liver environment, which is thought to be the major aetiology of liver toxicity and liver cancer. This review will discuss the antioxidant activity of tocotrienols and the potential molecular targets of tocotrienols in the liver. The role of tocotrienols in the prevention of liver toxicity and liver cancer will also be discussed.

KEYWORDS: tocotrienols, vitamin E, antioxidant, oxidative stress, cancer prevention, molecular targets, liver toxicity, drug-induced liver injury, liver cancer.

1. Introduction

Tocotrienols are hydrophobic fat-soluble compounds that belong to the vitamin E family and have been reported to possess powerful antioxidant activity [1]. Their beneficial health properties towards various body systems have been clearly elucidated [2-4]. Vitamin E contains two major homologous subgroups identified as tocopherols and tocotrienols. Tocopherols and tocotrienols are identical compounds in which they both possess a chromanol nucleus (which is the site for their strong antioxidant activities); however, the difference lies in the tails of these molecules in terms of the characteristics of their side-chains [5]. Tocopherols have saturated side chains, while tocotrienols' side chains possess double bonds at the 3', 7', and 11' positions [6, 7]. Both are further subdivided into alpha (α), beta (β), gamma (γ) and delta (δ) tocopherols and alpha (α), beta (β), gamma (γ) and delta (δ) tocotrienols, depending on the numbering and location of methyl substitutions on the chromanol ring [8]. Tocotrienols are distributed throughout the body *via* the bloodstream and tend to accumulate in various parts of the body e.g. adipose tissue, heart, and skin. Vitamin E absorption depends on the individual's lipid intake, bile and esterase secretion [9]. In order to facilitate the absorption of fat-soluble vitamins from the gastrointestinal tract, they must first be emulsified by bile and packaged into micelles for transport into the circulation. Tocotrienols' absorption is reduced in individuals who fast than those who are fully fed, while tocotrienols' biliary excretion depend on the levels and types of dietary fats

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consumed [10]. Increased palm oil consumption has been shown to facilitate bile excretion and micelle formation, both of which enhance tocotrienols' absorption due to the high fatty acid composition of palm oil [11]. All isomers of vitamin E are metabolized through omega oxidation, before undergoing beta oxidation. Omega oxidation is mediated by phase I xenobiotic metabolizing enzymes i.e. cytochrome P450s (CYP450s) and is predominantly regulated by their substrates [12]. The metabolism rate of vitamin E is the highest for tocotrienols and lowest for tocopherols, further suggesting on the stronger biological effects of tocotrienols [13].

Lipid-rich plants and vegetable oils are the main sources of vitamin E. Tocopherols can be found in the leaves and seeds of common crop plants such as corn, olive, peanut and sunflower. Tocotrienols are harder to extract and were initially detected in the husks and brans of cereal grains [14, 15]. It is noted that the ratio of tocopherols to tocotrienols in rice bran, palm oil and annatto are 50:50, 25:75 and 0.1:99.9, respectively [16]. Tocotrienols are the predominant vitamin E found in palm oil, rice bran and barley [17]. Approximately 70% of the vitamin E content in palm oil consists of tocotrienol isomers, while 30% are α -tocopherol [18]. Palm oil is rich in tocotrienols in which unadulterated palm oil obtained from the fruits of palm trees (*Elaeis guineensis*) essentially contains considerable quantities of tocotrienols (up to 800 mg/kg, mainly α - and γ -tocotrienols) [14]. In plants, tocotrienols are present in several non-photosynthetic and photosynthetically active tissues [19]. A standardized tocotrienol-rich fraction (TRF) that consists largely of 68% mixture of α , γ , δ -tocotrienols and 32% α -tocopherols are obtainable from palm oil after phases of esterification and subsequently, phases of distillation, crystallization, and chromatography [20].

2. The liver and oxidative stress

Liver is the most metabolically active organ in the body and physiologically it is responsible for optimising the levels of endogenous and nutritional compounds *in vivo*. It is the major target organ of chemically induced injuries, especially because most drugs and herbal preparations are given orally and are absorbed into the hepatic portal system through the gastrointestinal tract. Moreover,

the liver is the essential storage of metabolic enzymes, resulting in vigorous metabolism of many xenobiotics, with the potential production of reactive metabolites that can cause liver toxicity. The liver is also responsible for the clearance and transport of xenobiotics and therefore prevents the accumulation and build-up of toxic compounds in the body [21]. In this sense, liver is very likely to be afflicted by drug-induced liver injury (DILI). DILI is the most common aetiology of acute liver failure in the United States, which can be categorised as a major healthcare burden [22]. The commonest drug responsible for DILI is acetaminophen (paracetamol) due to its wide availability over-the-counter. The main factor which is implicated in DILI is oxidative stress which generates from the reactive metabolites resulting from drug biotransformation process in the liver. Other factors include reduction in the level of *in vivo* antioxidants, elevation of drug redox recycling and mitochondrial dysfunction due to the presence of toxic metabolites [23].

Overproduction of reactive metabolites could potentially disrupt mitochondrial function, which further leads to uninhibited oxidative stress in the liver. These reactive metabolites are able to react with various cellular components such as the endogenous antioxidant enzymes, DNA, RNA and structural lipid membranes. If unchecked, the excessive production of reactive metabolites could potentially cause massive damage to hepatocytes, which leads to cell death and necrosis. These reactive metabolites could also deplete the liver's glutathione content. Glutathione is the most important endogenous antioxidant, and individuals with low levels of glutathione are further susceptible to the effects of uninhibited oxidative stress. Reactive metabolites had also been found to inhibit the activity of liver transporter proteins, and the accumulation of toxic metabolites could potentially worsen liver injury and subsequently increase the level of oxidative stress in the liver [24]. The use of natural or synthetic chemical agents to reverse, suppress or prevent the dangerous effects of harmful metabolites, perhaps by increasing the expression of metabolizing enzymes, could be important in preventing liver stress and dysregulation, and this concept is called chemoprevention. Numerous mechanisms have been

reported to account for the antioxidative actions of dietary phytochemicals; however, much emphasis has been focused on intracellular signaling cascades as important molecular targets for many chemopreventive phytochemicals [25]. This approach can potentially nullify the use of conventional chemical therapeutics in combatting liver stress, in which these chemical therapeutics might pose unwanted side effects to the body.

3. Antioxidant activity of tocotrienols

Vitamin E is widely established as having robust antioxidant activity and the most important lipid-soluble antioxidant in humans [26, 27]. Scientists have proposed that tocotrienols have superior antioxidant and biological effects compared to tocopherols, particularly in the prevention of cardiovascular disease and cancer [27-29]. There is a growing body of evidence regarding the manner in which tocotrienols mediate their antioxidant activities. Experimental studies both *in vitro* and *in vivo* have reported that tocotrienols demonstrated enhanced antioxidant activities compared to tocopherols [27]. Results from earlier studies suggested that d- α -tocotrienol has 40-60 times higher antioxidant potency than the conventional d- α -tocopherol, even though its absorption and distribution after oral intake is not as comprehensive as α -tocopherol [30, 31]. Two factors should be looked into when comparing the effectiveness of various vitamin E homologues i.e. the substituents on the chromanol nucleus as well as the side chain properties. The higher antioxidant efficiency of d- α -tocotrienol can be explained by the combined effects of three activities exerted by d- α -tocotrienol in contrast to d- α -tocopherol, and the differences include; (1) its higher recycling efficiency from chromanoxyl radicals, (2) its more constant distribution in the membrane lipid bilayer, and (3) its more effective interaction of the chromanol ring with lipid radicals. These effects render chromanols' interaction with lipid radicals more efficient [32, 33]. In a previous report, tocotrienols were shown to be remarkably more efficient than tocopherols against lipid peroxidation and protein oxidation in rat brain mitochondria [34]. As the oxidative lipid hydroperoxides are induced, the hydroxyl group of α -tocopherol responds with lipid peroxy radical, forming lipid hydroperoxide

and α -tocopheroxyl radicals, which can be recycled to their active reduced forms through the reduction by other antioxidants. The lipid peroxy radicals interestingly interact with vitamin E more swiftly than they do with polyunsaturated fatty acids, thus averting the auto-oxidation of lipids and the subsequent propagation of free radicals [35].

Apart from its established role as a chain-breaking antioxidant, the activation and induction of antioxidant enzymes such as superoxide dismutase (SOD), NADPH: quinone oxidoreductase (NQO) and glutathione peroxidase (GPx) is thought to be one of the main mechanisms in which tocotrienols exert their antioxidant effect [36-39]. The antioxidant effects of tocotrienols have been widely investigated in animal models. Chronic oral administration of tocotrienols managed to increase liver antioxidant defense and decreased liver enzymes involved in liver damage in rats treated with 2-acetylaminofluorene (AAF) [40]. In another study which also involved rats treated chronically with AAF, co-administration of tocotrienols increased the activities of phase II xenobiotic metabolizing enzymes, e.g. glutathione S-transferases (GSTs), glutathione reductase (GR) and glutathione peroxidase (GPx) in the liver and kidney [41]. Chronic gammatocotrienol (γ T) supplementation in rats with liver cancer due to diethylnitrosamine (DEN) induction was able to reduce the cancerous lesions and tumour marker enzyme activities [42]. Tocotrienol-rich fraction (TRF) supplementation in exercise-induced rats significantly increased antioxidant/phase II enzyme, e.g. superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels and significantly reduced blood lactate, plasma and liver thiobarbituric acid reactive substances (TBARS), and liver and muscle protein carbonyl, indicating that TRF was able to counter the effects of exercise-induced oxidative stress [43].

The induction of phase II enzymes protects against free radical damage and reduces the incidence of radical-derived degenerative diseases like cancer [5]. Furthermore, researchers have associated the antioxidant activities of tocotrienols with nuclear factor E2-related factor-2 (Nrf2), a member of the Cap 'n' collar (CNC) family of basic region-leucine zipper transcription factors [44]. The binding of

Nrf2 to a *cis*-acting DNA promoter sequence, more familiarly known as the antioxidant response element (ARE), permits the transactivation of a variety of cytoprotective genes. Under normal condition, Nrf2 is bound in cytoplasm with a repressor protein i.e. Kelch-like ECH-associated protein-1 (Keap1) and degraded regularly by the proteasome enzyme. Nevertheless, under oxidative stress status, the interaction between Nrf2 and Keap1 is disrupted, causing Nrf2 to be dissociated from Keap1 and accumulated in the nucleus where it activates genes with an ARE sequence within their promoters, resulting in the induction of many genes linked with the classical phase II detoxification enzymes and antioxidants [45].

A previous study had shown that tocotrienol treatment induced Nrf2 expression, as indicated by an analogical decrease in Keap1 levels in estrogen receptor-negative MDA-MB-231 cells [44]. Tocotrienols were also found to be successful in regulating Nrf2-related phase II enzymes such as UDP-glucuronyl transferase (UDP-GT), γ -glutamyl transferase (GGT) and glutathione S-transferase (GST), altogether suggesting that tocotrienols might contain some regulatory effects on Nrf2/Keap1 system which could determine the level of expression of several phase II enzymes involved in cancer chemoprevention [40, 46, 47]. The exact mechanism by which tocotrienols exert these effects on several phase II enzymes has yet to be thoroughly understood. However, in light of the knowledge that tocotrienols are the major chain-breaking antioxidant, a suggestion may be given whereby tocotrienols can break oxidative stress-induced protein carbonyl adducts thereby dissociating the Nrf2/Keap1 complex, allowing Nrf2 to translocate to the nucleus and ultimately activate diverse cytoprotective genes [48, 49]. Extensive biochemical assays are essential for such interactions to be confirmed.

4. Potential molecular targets of tocotrienols in the liver

Tocotrienols have become more prominent lately, not just as the second-degree forms of vitamin E, but also as distinctive nutritional compounds with equally distinctive antioxidant properties. These properties are thought to be mediated by the indirect modulation of several targets at the

transcriptional, translational, and post-translational levels, or by direct interactions with cellular targets [27]. The consumption of vitamin E for the prevention and treatment of human diseases has been well documented. It has been suggested that tocotrienols possess chemopreventive activity. As an example, palm oil tocotrienols suppressed the proliferation of human breast cancer cell lines *in vitro* [50]. Tocotrienols have been established to carry out various specific activities, such as antioxidant, anti-proliferative, anti-inflammatory, anti-angiogenic and anti-apoptotic activities [51]. The molecular mechanisms that have been supporting these beneficial effects are thought to be diverse, but their interplay is still not very well understood. It is mentioned in earlier works that the antioxidant effects of tocotrienols are exerted through up-regulating several antioxidant enzymes, thus safeguarding the cells from detrimental xenobiotics and oxidants [5]. Additionally, the up-regulation of these enzymes by tocotrienols has been associated with the activation of nuclear factor E2-related factor-2 (Nrf2), a member of the Cap 'n' collar (CNC) family of basic region-leucine zipper transcription factors [44]. Tocotrienols was able to raise the level of several phase II enzymes such as UDP-glucuronyltransferase (UDP-GT), γ -glutamyltransferase (GGT) and glutathione S-transferase (GST), undeniably important in the cellular defence against chemical and oxidative stress [51].

Tocotrienols inhibit proliferation and growth of many cancer cells both *in vivo* and *in vitro* [52]. Tocotrienols' anti-proliferative effect is thought to be mediated by means of interfering with signal transduction events at physiologically attainable concentrations. Tocotrienol-mediated growth suppression is ascribed to cell cycle arrest, mostly at the G1 phase of cell cycle, and apoptosis. Signalling activities connected to the enhancement of the cell cycle growth and survival, including vascular endothelial growth factor (VEGF), mitogen-activated protein kinases (MAPK) such as p38 MAPK, ERK and c-Jun N-terminal protein kinases (JNK), c-Jun, c-myc, FLICE-like inhibitory protein (FLIP), cyclin-dependent kinases (CDK2, CDK4, CDK6), protein kinase C, phosphatidylinositol kinase (PIK), protein kinase B (PKB), I κ B (inhibitor of kappa B) kinase, nuclear factor κ -B (NF- κ B), telomerase, B-cell lymphoma-2 (Bcl-2),

B-cell lymphoma extra-large (Bcl-xL), cyclooxygenase-2 (COX-2), and matrix metalloproteinases (MMP), are suppressed by tocotrienols. Conversely, signalling pathways that promote cell growth arrest and apoptosis, including transforming growth factor- β (TGF- β), cyclin-dependent kinases inhibitors such as p21, p27 and p53, activation of caspase-8, apoptotic protease activating factor 1, Fas protein, up-regulation of BCL2-associated X protein (Bax), cleavage of BH3 interacting-domain (Bid), caspases, DNA fragmentation, and release of cytochrome C, are activated by tocotrienols [51, 53-55].

The inhibition of the phosphatidylinositol-3-kinase (PI3K)/AKT pathway by tocotrienols could well abolish the mitogen-dependent growth and survival in the many different types of cancer cells. Previous studies have also shown the ability of tocotrienols to inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, thus suppressing tumour growth. In addition, inhibition of the expression of cell survival proteins e.g. XIAP, IAP-1, IAP-2, bcl-2, bcl-xl, c-FLIP, TRAF-1, and down-regulation of telomerase, c-myc, and raf-ERK signaling pathways by tocotrienols, could partly explain its ability to obstruct the survival and growth of tumour cells [51, 53-55].

In both *in vitro* and *in vivo* experimental systems, tocotrienols exhibit anti-angiogenic activity. Angiogenesis plays an important role in the progression of cancer, and finding effective anti-angiogenic agents is a focal part of cancer research. Tocotrienols suppress angiogenesis by inhibiting processes of proliferation, migration and tube formation of endothelial cells *in vitro*. Tocotrienols promoted the inhibition of VEGF and VEGF receptor signaling, and therefore it is proposed that tocotrienols inhibit angiogenesis through the regulation of growth factor receptor on the cellular surface. Furthermore, the inhibition of fibroblast growth factors (FGF), interleukin-8 (IL-8), tumour necrosis factor-alpha (TNF- α), matrix metalloproteinase (MMP)-9 gene and angiopoietin-1 are also connected with the tocotrienols' angiogenesis-suppressive activity [51, 53-55].

Previous studies have suggested that tocotrienols exhibit strong anti-inflammatory activities, mainly

through the suppression of transcription factors NF- κ B and STAT3. NF- κ B and STAT3 suppression inhibits the proliferation and invasion of tumours and therefore, the inhibition of these pro-inflammatory pathways may open doors for both cancer prevention and treatment. Furthermore, suppressing the expression of hypoxia-induced factor-1 (HIF-1), inducible nitric oxide synthase (iNOS), COX-2, prostaglandin E2, TNF, IL-1, IL-6, and IL-8 by tocotrienols also offers contribution to the anti-inflammatory activity of this compound. As a matter of fact, tocotrienols have an impending development as both cancer chemopreventive and chemotherapeutic agents [51, 53-55].

5. The role of tocotrienols in the prevention of liver toxicity

Liver is one of the largest organs of the body and the major powerhouse of metabolism in the body. It is involved in the detoxification of xenobiotics, chemicals and drugs that are introduced to the body. Therefore, liver is prone to be affected by diseases that are brought about by toxic and harmful metabolites. Dietary antioxidants such as tocotrienols are therefore important in preventing liver damage due to the harmful chemicals. As mentioned previously, the activation of antioxidant enzymes such as superoxide dismutase (SOD), NADPH: quinone oxidoreductase (NQO) and glutathione peroxidase (GPx) due to tocotrienols' supplementation is thought to be one of the main mechanisms in which tocotrienols exert their hepatoprotective effect [36-38]. Oral supplementation of tocotrienols managed to increase liver antioxidant defence and decreased liver enzymes involved in liver damage in rats treated with 2-acetylaminofluorene (AAF) [40]. In another study which also involved rats treated chronically with AAF, administration of tocotrienols increased the activities of hepatoprotective enzymes such as glutathione S-transferases (GSTs), glutathione reductase (GR) and glutathione peroxidase (GPx) in the liver [41]. Gamma-tocotrienol (γ T) supplementation in rats afflicted with liver cancer due to a carcinogenic chemical induction (diethylnitrosamine) was shown to be able to significantly lessen the cancerous lesions and tumour marker enzyme activities [42].

Tocotrienols are also able to increase the expression of several phase I and phase II enzymes involved in drug metabolism including many subtypes of cytochrome P450 (CYP450) enzymes, UDP-glucuronosyltransferase 1A1 (UGT1A1) and multidrug resistance protein-1 (MDR1). The increased expression of these enzymes is mediated through the stimulation of nuclear receptors such as the pregnane-X receptor (PXR) and steroid and xenobiotic receptor (SXR). These enzymes increase the clearance of drugs from the body, thus limiting the exposure to harmful chemicals [56, 57]. Tocotrienol supplementation in exercise-induced rats was found to increase the levels of glycogen, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the liver which indicated the rats' increased resistance towards oxidative stress [43]. In humans, oral tocotrienols' supplementation resulted in significant distribution of tocotrienols to the liver. Oral intake of tocotrienols in patients before undergoing liver transplantation also lowered the model for end-stage liver disease (MELD) score in 50% of these tocotrienol-supplemented patients [58]. The findings from both animal and human studies suggested that tocotrienols are worthy to be explored further to be used therapeutically in combatting liver toxicity.

6. Tocotrienols and its potential role in liver cancer prevention

Reputable studies have suggested that the anti-carcinogenic effect of tocotrienols against hepatocellular carcinoma (HCC) was through the interaction with several important cellular pathways, which resulted in upregulation of Prx4, Bax, caspase-8 and caspase-9 and downregulation of Bcl-2, Bcl-xL, survivin, cyclin D1, Mcl-1 and VEGF [16, 59-62]. Also, as mentioned previously, administration of tocotrienols inhibited hepatocarcinogenesis induced by diethylnitrosamine (DEN)/2-acetylaminofluorene (AAF) in rats [40, 63]. The presence of tocotrienols was only detected in tumour tissues and not in normal tissues, which indicated tocotrienols' preferential distribution to tissues afflicted by tumour [60]. In animal models of HCC, tocotrienols were also discovered to reduce lipid levels *in vivo* via its effect on several

lipogenic enzymes and upstream regulators of lipid homeostasis genes, which might halt the progression of the disease [64, 65]. A recent *in vitro* study suggested that δ -tocotrienol is a very potent proteasome inhibitor that is able to induce the apoptosis of liver cancer cell lines. The downregulated expression of proteasomal subunits and TNF- α after δ -tocotrienol treatment was thought to be responsible for the apoptosis of liver cancer cells [66]. These findings revealed the potential benefits of tocotrienols in halting liver carcinogenesis and also as adjuvant oncogenic therapeutics in combatting liver cancer.

7. Conclusion

Even though there are very limited *in vivo* studies using animal and human models on the effectiveness of tocotrienols in the prevention and treatment of liver toxicity and liver cancer, the potential benefits of tocotrienols should not be underestimated. Numerous cellular and animal studies have indicated its potential effectiveness in combatting chronic ailments such as cardiovascular and neurological diseases. The use of tocotrienols in preventing liver toxicity and liver cancer could provide a safer and more natural alternative as opposed to treatment using conventional chemical drugs which are not free from untargeted side-effects. Recent mechanistic studies have unveiled the molecular targets and signaling pathways affected by tocotrienols that could be involved in the prevention of liver toxicity and cancer. Out of all the signalling pathways affected by tocotrienols, the effect of tocotrienols in inducing phase II enzymes levels and activation by means of the Nrf2/Keap1 pathway in the liver *in vivo* should be explored further and should include the involvement of more human studies. If the positive results of cellular and animal studies in terms of tocotrienols inducing phase II enzymes in the liver through the Nrf2/Keap1 pathway could be translated more conclusively in humans, tocotrienols could then be marketed as the inexpensive, safe and natural liver tonic that could potentially prevent drug-induced liver disease, liver toxicity and liver cancer. Therefore, further research and clinical trials are needed to conclusively confirm the enormous potential benefits of tocotrienols in promoting liver health and disease in humans.

ACKNOWLEDGEMENT

We thank Universiti Kebangsaan Malaysia for financial assistance through the endowment of research grants GGP-2017-079 and FF-176-2013.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest, financial or otherwise.

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