



## Mode of action of tocotrienol as anticancer

Widiyasri Lodu<sup>1\*</sup>, Ferry Fredy Karwur<sup>1,2\*</sup>

<sup>1</sup>Graduate Student of Master of Biology Program, Satya Wacana Christian University. <sup>2</sup>Lecturer Faculty of Medicine and Health Science, Satya Wacana Christian University. Jl. Diponegoro 52-60 Salatiga, Jawa Tengah 50711

### ABSTRACT

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Tocotrienols are fat soluble substances members of the vitamin E family with the main properties of antioxidant. They are composed of the chiral center of chromanol ring with polyprenyl side chains. Research suggests that tocotrienols have a number of health benefit, and one of them as an anticancer agent. As an anticancer, tocotrienols could perform in different processes of the multi stages of cancer development and interact with other anticancer medicines and other bioactive substances, suchs geneistein, lovastin, hydroxychavicol, 6- gingerol and sesamin. The anticancer activities occur through their roles as antioxidant, coenzyme, gene expression regulator, and preventing cholesterol synthesis by inhibiting the expression of HMGCR enzyme after transcription, through this method, tocotrienols could control the cholesterol level of cancer cells and prevent the growth of the cancer. Tocotrienols target several signalling pathways at cellular and molecular levels.

### ABSTRAK

Tocotrienol adalah zat larut lemak anggota keluarga vitamin E dengan sifat utama antioksidan. Zat tersebut terdiri dari pusat kiral cincin kromanol dengan rantai samping polypreniyl. Penelitian menunjukkan bahwa tokotrienol memiliki sejumlah manfaat kesehatan, dan salah satunya sebagai agen antikanker. Sebagai antikanker, tokotrienol dapat bekerja dalam proses yang berbeda dari berbagai tahap perkembangan kanker dan berinteraksi dengan obat antikanker dan zat bioaktif lainnya, seperti genistein, lovastin, hidrosikaviol, 6-gingerol, dan sesamin. Aktivitas antikanker terjadi melalui peran mereka sebagai antioksidan, koenzim, pengatur ekspresi gen, dan mencegah sintesis kolesterol dengan menghambat ekspresi enzim HMGCR setelah transkripsi, melalui metode ini, tocotrienol dapat mengontrol kadar kolesterol dari sel kanker dan mencegah pertumbuhan sel-sel kanker. Pada tingkat seluler dan molekuler, Tocotrienol menargetkan beberapa jalur pensinyalan pada tingkatan selular dan molekul.

### Keywords:

anticancer  
antioxidant  
bioactive substances  
gene expression  
HMGCR enzyme  
tocotrienol

## INTRODUCTION

Tocotrienol, one of vitamin E groups, is fat soluble antioxidant which are composed of chromanol core ring and polyprenyl side chain. Tocotrienol differs from other vitamin E groups such as tocopherol. Tocotrienols have polyprenyl side chains and have 3 unsaturated bonds at 3', 7' and 11' positions which are connected to carbon number 2 of the chiral center (FIGURE. 1). This side chain

gives non-polar properties to the whole molecule, and in the context of the cell, facilitates access of the fatty layer on the cell membrane.<sup>1</sup> Like tocopherol, tocotrienol has four different isomers, based on the number and position of the methyl groups on the chromanol ring. They are  $\alpha$ -tocotrienol 5,7,8-trimethyl;  $\beta$ -tocotrienol 5, 8-dimethyl;  $\gamma$ -tocotrienol 7, 8-dimethyl and  $\delta$ -tocotrienol 8-monomethyl.<sup>2</sup>

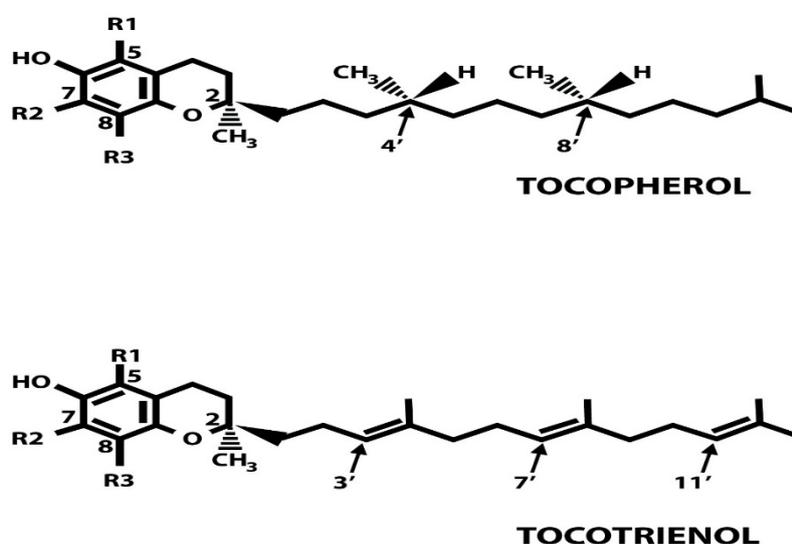


FIGURE 1. Chemical Structure of Tocopherol and Tocotrienol adapted from Kasparek (1980).<sup>2</sup>

Tocotrienol was first reported by Bunyan (1961),<sup>3</sup> when he wanted to know the biological properties of  $\alpha$  and  $\beta$  tocopherol, which in fact found vitamin E with side chain was unsaturated, ie (2-methyl-2-(4', 8', 12'-trimethyltrideca-3', 7', 11'-trienyl) chroman-6-ol. Then he gave a new name to the compound, namely 'tocotrienol'. Bunyan also corrected vitamin E in wheat, known as  $\epsilon$ -tocopherol, whereas it is 5,8-dimethyl tocotrienol. Research on tocotrienol continued with the discovery of the  $\delta$ -tocotrienol isomer isolated from rubber latex,<sup>4</sup> and started to receive

attention in the early 1980s when it was first reported that tocotrienols were able to lower cholesterol levels *in vitro* in hepatocytes (liver parenchymal cells) chicken, mouse and in both experimental animals *in vivo*.<sup>5</sup> In the early 1990s, the anti-cancer properties of tocotrienols were reported.<sup>6,7</sup>

Tocotrienols are exclusively synthesized only in photosynthetic organisms. The presence, distribution and concentrations of tocotrienols have been widely reported, primarily in palm oil, rice bran, wheat germ oil, coconut oil and annatto seeds. Palm oil

contains 0.66%  $\alpha$ -tocotrienol, 0.019%  $\beta$ -tocotrienol, 0.71%  $\gamma$ -tocotrienol, 0.31%  $\delta$ -tocotrienol. Rice bran contains 0.43%  $\alpha$ -tocotrienol, 0.08%  $\beta$ -tocotrienol, 0.63%  $\gamma$ -tocotrienol, 0.04%  $\delta$ -tocotrienol. Wheat germ oil 1.94%  $\alpha$ -tocotrienol and 0.05%  $\delta$ -tocotrienol, whereas in coconut oil there is 0.2%  $\alpha$ -tocotrienol, 0.04%  $\gamma$ -tocotrienol, 0.76%  $\delta$ -tocotrienol.<sup>8,9</sup> In annatto seeds, most of the tocotrienol is  $\delta$ -tocotrienol with a content ranging from 140-147 mg/100 g of dry seed.<sup>10</sup> The data above conclude that both the isomeric composition and the total content of tocotrienol vary greatly from one source to another. Palm oil, rice bran and annatto seeds are the main sources of tocotrienols.

Tocotrienols have a wide range of biological and health effects as a vitamin with antioxidant capacity. Tocotrienols exhibit an inhibitory effect of aging,<sup>11</sup> cardioprotective effects,<sup>12,13</sup> anti-atherosclerosis,<sup>14</sup> specifically protect the blood vessel wall in diabetics.<sup>15</sup> Tocotrienol also provides a protective effect on bone damage caused by ferric nitrilotriacetate free radicals in mouse model animals.<sup>16</sup> The bone-protective effect is consistent with Norazlina's findings that  $\gamma$ -tocotrienol intake in experimental animals fed with foods lacking of tocotrienols was capable of maintaining vitamin D metabolism under normal and homeostatic conditions of calcium. This effect was not happen to the experiment treated with  $\alpha$ -tocopherol.<sup>17</sup> Tocotrienol has also been reported as liver antitoxin<sup>18</sup> and gastroprotective, i.e preventing changes in acidity of the stomach due to the increase of gastrin.<sup>19</sup> In combination with ascorbic acid, tocotrienol increases neutrophil and lymphocyte leukocytes that are part of the body's defense system.<sup>20</sup> It has also been tested for its ability to reduce joint inflammation in patients with rheumatoid arthritis.<sup>21</sup> Radioprotection of tocotrienols through its effect in apoptosis and increased

hematopoiesis after radiation have been reported by Satyamitra.<sup>22</sup> The role of tocotrienol in cancer prevention and therapy is to inhibit the secretion of angiogenesis factors from cancer cells,<sup>22,23</sup> as adjuvant drugs to enhance anti-cancer effectiveness through their role as immunomodulators.<sup>24,25</sup> Tocotrienol, particularly the delta isomer, can be an alternative chemotherapy agents to treat lung cancer and brain cancer.<sup>26</sup>

Research on the interactive effects of natural ingredients with each other in relation to the biological and health functions of cancer patients, both in terms of prevention and treatment, is still an enormously questionable area, not to mention tocotrienols with other potential bioactive compounds. Therefore, the next section will examine the role of tocotrienols in cancer prevention and treatment, in inhibiting cancer cell growth, range of action and the synergistic interactions of tocotrienols and other bioactive substances, which were reported to have anticancer potential.

## DISCUSSION

### Tocotrienol as an anticancer

As well as many historical discoveries of nutrients and nutraceutical compounds, the discovery of the role of tocotrienols in cancer inhibition does not begin with a direct test of the effects of tocotrienols on inhibition of cancer cells, but through studies of the indirect effects of vegetable oils (palm oil and corn oil) to mice triggered by DMBA carcinogens (or 7,12-dimethylbenz [a] anthracene).<sup>27</sup> Testing of palm oil rich in tocotrienol fractions (TRF, tocotrienol rich fraction) and  $\alpha$ -tocopherol towards the proliferation of breast cancer cells of the positive MDA-MB-435, strains of estrogen receptor was the first experiment to prove that the tocotrienol fraction. It is responsible for inhibiting

proliferation cancer cells.<sup>28</sup> Subsequent research proves that tocotrienol works as an anticancer in almost every important stage of cancer development.

By the nature of antioxidants, antiproliferative and pro-apoptotic, tocotrienol is considered capable of preventing the occurrence of cancer. However, whether the cellular signaling system influenced by tocotrienols has an antimutagenic effect has not been reported, but the role of tocotrienols and their application in ionizing radiation through several pathways, among others, DNA aberration protection,<sup>29</sup> DNA damage reduction<sup>30</sup> or by mobilization of progenitor cells,<sup>31</sup> provides an opportunity for tocotrienol involvement as an antimutagen or ionizing radiation barrier that prevents the occurrence of genetic degeneration, which is a prerequisite for the emergence and development of cancer. This has been argued by Punvittayagul *et al.*<sup>32</sup> for the antimutagenic effect of lipophilic extracts rich in tocotrienol from cv. Kum Doisaket purple rice husks from northern Thailand.

At the promotional stage of cancer development,  $\gamma$ -tocotrienol was reported to inhibit cell proliferation and accelerate apoptosis of prostate cancer cells. The anti-proliferative effect of  $\gamma$ -tocotrienol acts via a NF- $\kappa$ B signaling pathway, in which  $\gamma$ -tocotrienol suppresses the activity of cancer pro-survival signal of NF- $\kappa$ B through the phosphorylation and accumulation I  $\kappa$ B $\alpha/\beta$  (inhibitor of kappa B). In the same study reported that  $\gamma$ -tocotrienol induces apoptosis by activating pro-caspase. Caspase activation by  $\gamma$ -tocotrienol is associated with down-regulation of Id1, Id3 (Id of inhibitor of differentiation) and the EGF-R. ID1 and ID3 play a role in cell growth while EGF-R is involved in the development and progression of cancer cells.<sup>33,34</sup>

Studies of the effects of  $\gamma$ -tocotrienols on breast cancer cells by Yap *et al.* showed

that  $\gamma$ -tocotrienols induced apoptotic cell death as evidenced by the activation of procaspases, the accumulation of cells in G1 stages and DNA fragmentation.<sup>35</sup> This death is related to suppression of the activity of the Id1 and the NF- $\kappa$ B genes. The role of  $\gamma$ -tocotrienols in this case is in modulating the upstream regulators of Id1 and NF- $\kappa$ B (ie, Src, SMAD 1/5/8, Fak, and LOX). In the promotion stage, tocotrienol is also reported to suppress the activation of the receptor tyrosine kinases family genes (*ErbB3*, *ErbB4*, *ErbB2* *ErbB2*) which are members of the epidermal growth factor receptor that are cancer-triggering genes.<sup>36</sup>

In the stage of cancer growth which occurs rapidly or is in the progression stage, combination treatment of the isomers of  $\gamma$ -tocotrienol and  $\delta$ -tocotrienol was found effectively inhibiting the cells proliferation in HeLa cervical cancer through increased expression of proinflammatory central cytokines-6 (IL-6) also back in turn decreases the cyclin expression of *D3*, *p16*, and *CDK6* in the cell cycle signaling path.<sup>37</sup> Both cyclin *D3* and *p16* are suppressor activity of *CDK6* gene, which is a positive regulator of cell cycle.

A recent study demonstrates that the involvement of tocotrienols in cancer growth occurs through retardation of cell cycle progression in G1 phase coupled with apoptotic death, as demonstrated in bladder cancer cells. Interestingly, not all tocotrienol isomers give inhibitory and death effects.  $\delta$ - and  $\gamma$ -tocotrienol have an anticancer effect, whereas  $\alpha$ -tocotrienol has no effect. Furthermore, the role of cell cycle rate retardation occurs through increased levels of cell cycle inhibitor expression (p21, p27) and inhibition of cyclin D1 cell protein cycle expression. Induction of apoptosis through the expression of bax proapoptosis protein and vice versa emphasize the expression of antiapoptotic proteins Bcl-2, Bcl-xL, and Mcl-1.<sup>38</sup>

Tocotrienol is also reported to

inhibit the growth of malignant cells through induction of apoptosis in breast epithelial cells + neoplastic SA (Neoplastic + SA mammary epithelial cells).<sup>39</sup> The induction of death occurs through the activation of caspase-8 and caspase-3, a trajectory of apoptotic death mediated by receptors. However, it has been shown that tocotrienols in the apoptotic induction of cancer cells take place through both trajectories of death receptors; trajectory of mitochondrial stress (with trajectory of Bcl-2 antiapoptosis and procemesis of Bad and Bax) via caspase-9; trajectory of the endoplasmic reticulum with the involvement of PARP components or JNK components. Which pathway is affected, depends on the type of cancer cell.<sup>40</sup>

Intratumor vascularization (angiogenesis) is essential for rapid cancer growth and angiogenic growth factors produced by cancer cells play an important role.<sup>41,42</sup> In this case, tocotrienol acts as an antiangiogenic factor.<sup>23,24</sup> It is hypothesized that tocotrienols are involved in inhibition of tumor angiogenesis by the inhibitory expression of VEGF (vascular endothelial growth factor), HIF-1 $\alpha$  (hypoxia-inducible factor- $\alpha$ ), interleukin-8 (IL-8), and COX-2 (cyclooxygenase 2). These factors play a critical role in cancer neovascularization. Using 2 cancer cell lines (ie DLD-1 and HepG2) Shibata *et al.*<sup>24</sup> showed that at 2  $\mu$ M concentrations, tocotrienols inhibited the hypoxic-induced VEGF secretion and IL-8 from the DLD-1 cancer cell strain. Such inhibition occurs at the level of mRNA and protein by the  $\delta$ -tocotrienol isomers. They even show that the mechanism of inhibition by these isomers by lowering the expression of HIF-1 $\alpha$  protein or increasing its degradation. Interestingly,  $\delta$ -tocotrienol does not affect the expression of COX-2 mRNA induced by hypoxia but precisely at the level of its protein expression. Thus, tocotrienol inhibits the secretion

of angiogenic factors from cancer cells.

Migration of cancer cells out of the initial tissue where cancer cells grow and develop is one of the critical phases in terms of clinical pathology of the cancer. At this stage, or the stage of cell migration and invasion,  $\gamma$ -tocotrienol is reportedly capable of inhibiting migration and invasion of the SGC-7901 gastric adenocarcinoma cell strain, by acting to decrease the transcriptional activity of metalloproteinase matrices MMP-2 and MMP-9 and by increasing the expression of tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2.<sup>43</sup> MMP-2 and MMP-9 are two important proteins that driving cancer cells to be invasive and metastatic,<sup>44</sup> while TIMPs are important to inhibit MMP activities.<sup>45</sup> Furthermore, in the process of invasion of prostate and melanoma cancer cells,  $\gamma$ -tocotrienol is reported to inhibit the invasion process by increasing the expression of E-cadherin and protein  $\gamma$  catenin. Treatment with  $\gamma$ -tocotrienol in PC-3 prostate cancer cells and G361 strain melanoma cancer cells decreased the expression of vimentin as mesenchymal markers,  $\alpha$ -SMA ( $\alpha$ -Smooth muscle actin), and twist transcription factor involved in regulating the development of mesenchymal epithelium.<sup>36,46</sup>

The development of cancer into metastatic stages begins with epithelial-to-mesenchymal transition (EMT) epithelial transition cells. In EMT, epithelial cells lose their cellular polarity and obtain mesenchymal cell-cell mobility that allows them to invade surrounding tissue. The action of tocotrienols in cancer cells that end in anticarcinogenic properties can take place through a variety of cellular signaling types, namely (a) NF- $\kappa$ B-mediated pathways, (b) phosphatidylinositol-2 kinase (PI3K) / phosphoinositide-dependent / Akt, (c) the Raf / Erk track, and (d) the associated JNK path.<sup>47-50</sup>

TABLE 1. Cancer drugs reported to be synergistic with tocotrienol

Type of medicine	Type of cancer cell	Isomer	Mechanism
Tamoxifen	Breast cancer cells	$\alpha$ , $\gamma$ and $\delta$	Inhibition of cancer cell proliferation <sup>6</sup>
Erlotinib	Breast cancer cells	$\alpha$ and $\gamma$	Inhibits ErbB receptors <sup>37</sup>
Statins	Various type of cancer cells	$\gamma$ - and $\delta$ -isomers	Suppression of proliferation through the induction of G1 arrest and/or apoptosis <sup>51</sup>
Gemcitabine	Pancreatic cancer cells	( $\alpha$ -, $\beta$ -, $\delta$ -, and $\gamma$ -) $\delta$ -Tocotrienol (the most effective)	Inhibition of proliferation and induction of apoptosis <sup>52</sup>
Celoxib	Breast cancer cells	$\gamma$ -Tocotrienol	Suppression of PGE <sub>2</sub> levels and reduction in ErbB2-4 receptor levels <sup>53</sup>
PPAR $\gamma$ antagonists (GW9662 and T007907)	Breast cancer cells	$\gamma$ -Tocotrienol	Downregulation of COX-2, PGDS, and PGD <sub>2</sub> synthesis <sup>54</sup>

### Synergistic interaction of tocotrienol and anti-cancer drugs

The survey proved that the combination of tocotrienol with anti-tumor drug can produce significant synergistic response in inhibiting cancer. The reported types of medicine synergizes with tocotrienol in the inhibition of cancer growth can be seen in TABLE 1.

The interactive effects of tocotrienol with anti-cancer drugs such as tamoxifen show a particularly significant inhibition of the growth of estrogen receptor-positive (MCF-7) breast cancer cells and estrogen receptor negative (MDA-MB-435) breast cancer cells.<sup>6</sup> Similarly, the combination of  $\gamma$ -tocotrienols with other anti-cancer drugs such as the tyrosine kinase inhibitor group (erlotinib or gefitinib) with the ErbB inhibitory receptor shows a more effective effect in reducing the growth of mammary

tumor cells. Targeting ErbB receptors with combination therapy in mammary cancer patients with erlotinib dose (0.25  $\mu$ M) or gefitinib (0.5  $\mu$ M) and  $\gamma$ -tocotrienol (0.5-3.0  $\mu$ M) significantly inhibited the growth of mammary cancer.<sup>37</sup> Other cancer drugs such as statins also show synergies with a combination of  $\gamma$ -tocotrienol as an anti-cancer agent, as shown in the synergistic suppression of proliferation through the induction of G1 arrest and/or apoptosis.<sup>51</sup> Another isomer  $\delta$ -tocotrienol is reported to synergize with gemcitabine in inhibiting the growth of pancreatic cancer cells *in vitro* and *in vivo* by suppressing NF- $\kappa$ B activity and the expression of target transcription by either inhibiting the proliferation or induction of apoptosis.<sup>52</sup> Furthermore, low doses of  $\gamma$ -tocotrienol in combination with celecoxib (selective COX-2 inhibitor) have been shown to be effective in suppressing breast cancer cell proliferation.<sup>53</sup>

### **The interaction of tocotrienol with other bioactive compounds as an anticancer**

Bioactive compounds are chemicals that have physiological and positive health effects. The role of the bioactive compound in the body is obtained when the compound reaches its site of action and by its presence and in its interaction with certain cellular components causes certain physiological effects as well. In this case, the effects of bioactive compounds are often studied as molecules that act independently with specific targets as well as their physiological effects. This perspective is certainly not wrong, but the physiological effects of bioactive compounds are often more critically evaluated in their interactive effects with other compounds. In this context, tocotrienol isomers have a great opportunity to work through their interactions with other bioactives, and this has been little reported, although still mechanically far from clarity. The next section deals with studies to demonstrate the interactions of tocotrienol with other biofuels that have links to anticancer effects.

### **The interaction of $\gamma$ -tocotrienol with dietary polyphenols resveratrol and epigallocatechin gallate**

The combination of  $\gamma$ -tocotrienols with resveratrol and epigallocatechin gallate (ECGG) were reported suppresses oncogene cyclin gene expression D1 and bcl-2. It is functionally inhibit the proliferation of MCF-7 breast cancer cells through the induction of the plasma membrane redox enzyme NQO1.<sup>55</sup>

### **The synergistic interactions of $\gamma$ -tocotrienol and sesamin**

$\gamma$ -Tocotrienol and a lipid soluble lignan extracted from sesame seed oil show anticancer activities with

multiple-targets. Harikumar *et al.*<sup>56</sup> see previous section for the tocotrienol. A question arise if the two compound work synergistically or independently. First indication of synergistic interaction between the two was shown by the research that a diet containing sesame seed oil and tocotrienol rich fraction increases the concentration of tocotrienol in skin and adipose tissues of rat significantly.<sup>57</sup> Considering the effect of sesamin on tocopherol catabolism,<sup>58</sup> and their catabolic pathway similarities with different kinetics, Parker *et al.* and Birringer *et al.*<sup>58,59</sup> were suggested that the sesamin inhibit degradation of  $\gamma$ -tocotrienol in the tissues causing the accumulation of the  $\gamma$ -tocotrienol and its bioavailability. Akl *et al.*<sup>60</sup> showed that the increase in bioavailability of  $\gamma$ -tocotrienol by the addition of sesamin enhances their synergistic anticancer activity. Further studies to examine interactions of  $\gamma$ -tocotrienol and sesamin beyond its bioavailability, they found the two compounds show synergistic growth inhibition of mice +SA mammary cancer cell proliferation. They identified molecular components affected by the interactions between  $\gamma$ -tocotrienol and sesamin are the EGF-dependent pathway, since they synergistically prevent the activation of ErbB receptor and its downstream signalling molecules. Other study by Akl *et al.*<sup>61</sup> showed that in mouse and human mammary cancer cells, interaction effects between  $\gamma$ -tocotrienol and sesamin are through G1 cell cycle arrest by affecting cell cycle regulators of the G1/S phase transition.

### **The interaction of $\gamma$ -tocotrienol and hydroxyl-chavicol**

A major phenolic compound in *Piper betle* leaves, hydroxyl-chavicol (4-allyl-catechol, 1-allyl-3,4-dihydroxybenzene) (EC), has been reported to possess anti-carcinogenic properties by modulating different cellular signaling events.<sup>62</sup>

Combined studies of  $\gamma$ -tocotrienol and EC show synergies in inhibiting the proliferation of human glioma cells of strain 1321N1, SW1783, LN18,  $\gamma$ -tocotrienol combinations with doses of 42-100 mg/mL and hydroxyl-chavicol 75-119 mg/mL increases apoptosis through the activation of caspase-3 signaling, in the cultured cell strain 1321N1 the compound index (IC) is 0, 55 and SW1783 0.54. While in cell LN18 0,73. Thus the combination of  $\gamma$ -tocotrienol and hydroxyl-chavicol showed synergies in inhibiting cell proliferation through the induction of human glioma cell apoptosis.<sup>63</sup>

### The interaction of $\gamma$ -tocotrienol and 6-gingerol

6-Gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone), a pungent ingredient of ginger (*Zingiber officinale* Roscoe) has been known to possess anticancer of various cancer types.<sup>64-67</sup> Synergistic interactions between 6-gingerol and tocotrienol as anticancer were reported although still limited. It was proven that  $\gamma$ -tocotrienol and 6-gingerol when used in combination act synergistically increasing cytotoxicity and apoptosis in cancer cells. Yusof *et al.*<sup>68</sup> found that the combination of  $\gamma$ -tocotrienol (0-150 mg/mL) and 6-gingerol (0-300 mg/mL) for 24 hours showed significant anti-proliferative and cytotoxicity effects, by increasing apoptosis of 21.2% in colorectal cancer cells of HT-29 and 55.4% in SW837. Further studies are needed to examine their mechanistic interactions as anticancer in various cancer cell types and *in vivo*.

### The interaction of $\gamma$ -tocotrienol and indole alkaloid, jerantinine

Jerantinines are related group of indole alkaloids from leaf extract of the plant species of *Tabernaemontana*

*corymbosa*. They consist of 7 molecules species (jerantine A-G) and most of them displayed pronounced *in vitro* cytotoxicity against human KB cells ( $IC_{50} < 1 \mu\text{g/mL}$ ).<sup>69</sup> A study reported that jerantine A possess cytotoxic activity against vincristine-resistant nasopharyngeal carcinoma cells and it works through apoptosis and mitotic arrest.<sup>70</sup> The other study have reported the effect of jerantine B to inhibit human cancer cell lines. It significantly arrested cells at the G2/M. It also provoked significant increases in reactive oxygen species and apoptotic induction.<sup>71</sup> Interestingly that both jerantine A and B has shown synergistic effects with tocotrienol as anticancer. Abubakar *et al.*<sup>72</sup> demonstrated that low dose of jerantine B in combination with low-dose of  $\delta$ -tocotrienol induces a synergistic apoptosis in human glioblastoma and colorectal adenocarcinoma cancer cells. Furthermore, a combination study between low dose of  $\gamma$ -tocotrienol (0-24  $\mu\text{g/mL}$ ) and fixed low dose of concentration of jerantine A ( $IC_{20} = 0.16 \mu\text{g/mL}$ ), in order to reduce toxic effect on normal cells of the jerantine A, induced a potent antiproliferative effect on U87MG cells and led to a reduction on the new half maximal inhibitory concentration of  $\gamma$ -tocotrienol (i.e.  $IC_{50} = 1.29 \mu\text{g/mL}$ ) as compared to that of individual  $\gamma$ -tocotrienol (i.e.  $IC_{50} = 3.17 \mu\text{g/mL}$ )

### The interaction of tocotrienol and garcinol

One of tocotrienols' action mechanisms in inhibiting cancer is through the NF- $\kappa$ B pathway, which is a transcription factor that serves to stimulate the expression of the regulated gene (NF- $\kappa$ B responsive genes). Tocotrienol suppresses the activity of iD1 and NF- $\kappa$ B.<sup>33-35</sup> It is interesting that garcinol from the *Garcinia indica* plant is able to inhibit cancer by a similar

mechanism, suppressing the expression of I  $\kappa$ B $\alpha$  and the antiapoptosis protein (Bcl-xL Bcl-2).<sup>73</sup> NF- $\kappa$ B is a transcription factor of anti-apoptotic genes such as Bcl-2 and Bcl-XL. It also regulates the antiproliferative Id member. The two bioactive compounds (garcinol and tocotrienol, especially  $\gamma$ -tocotrienol ) are also involved in STAT-3 signalling pathways as antiproliferative, pro-apoptotic and chemosensitizing agents.<sup>74,75</sup> Thus, if tocotrienol and garcinol show the inhibitory effect of antiapoptosis and antiproliferative on cancer cells through suppression of NF- $\kappa$  activation, and STAT-3 signalling, combinatorial research between the two bioactive substances are interesting to further investigate.

### **Tocotrienol involvement in cancer control through inhibition of hmg-coa enzymes**

Secondary metabolites in terpenoid groups (monoterpene, sesquiterpene, carotenoids and tocotrienols) are able to inhibit the synthesis of modestly cholesterol-and therefore lower LDL cholesterol.<sup>76</sup> Tocotrienol rich fraction (200g) combined with corn oil (300g) was reported to reduce cholesterol levels by 15% of total serum cholesterol of 6.21-8.02 mM identified in humans hypercholesterol, while  $\gamma$ -tocotrienol (200g) cholesterol 31% of total serum cholesterol of 7.84 mM,<sup>77</sup> then tocotrienols were reported to affect mevalonate pathways in mammalian cells by suppressing the expression of 3-hydroxyl-3-methylglutaryl coenzyme A reductase (HMGCR) post-transcription, specifically modulate the mechanism intracellular by stimulating the degradation of HMGCR.<sup>78</sup> The inhibitory activity is caused by terpenoid group compound which can inhibit the activity of HMGCR post-transcription.

Inhibition of tocotrienols against

HMGCR can be associated with mevalonateformationpathwaysresulting in a reduction in cellular substrate capability required for isoprenylation and as a final result is inhibition of the pro-carcinogenic pathway, so that the synthesis of mevalonate inhibited by the interaction of isoprenoids leads to a drastic reduction of two products in the tissues tumors: farnesyl pyrophosphate and geranylgeranyl pyrophosphate are important in growth control-associated proteins.

### **Tocotrienol, gene expression and cancer control**

The fundamental question of tocotrienol's role in a number of health problems, including its role as anticancer has not been clearly explained by its mechanism of action. However, number of studies provide evidence of tocotrienol involvement in these issues through the mechanism of gene expression. The involvement of tocotrienols in various levels of gene expression in transcriptional and translational mechanisms in the aging process of fibroblasts, mioblasts, suppression of HMG CoA reductase and radiation-induced damage will be discussed more specifically to the mechanisms of gene expression and cancer control.

Tocotrienol is reportedly worked in the process of transcription to translation in the process of gene expression. Associated with gene expression,  $\gamma$ -tocotrienol is reported to prevent cellular aging in human diploid fibroblasts characterized by modulating cell cycle in the aging process by increasing the expression of genes encoding elastin and collagen fibrillers (ELN, COL1A) and decreasing the expression of cyclin D1 (CCND1), retinoblastoma RB1), metalloprotenase (MMP1) and interleukin (IL6).<sup>79</sup> Research using microarray analysis,  $\gamma$ - tocotrienol was reported to increase the expression

of 100 differential genes, furthermore, using the NES (normalized enrichment score) analysis  $\gamma$ -tocotrienol was reported to modulate expression genes in extensive cellular functions such as inflammation, protein transport, apoptosis and homeostasis.<sup>80</sup>

At the post-transcription stage tocotrienol is reported to prevent the aging process of fibroblast diploid in humans via miRNAs modulation and target gene expression.<sup>81</sup> Tocotrienol also regulates the production of cholesterol in mammalian cells through post transcription by suppressing HMG CoA reductase and specifically modulating intracellular mechanisms to control the degradation of protein reductase. Control of this enzyme activity has implications for the control of cholesterol synthesis and mevalonant line products.<sup>82</sup>

Tocotrienol is involved in the inhibition of aging of human fibroblast cells. Giving a TRF will reduce the activity of  $\beta$ -galactosidase. It is involved in the acceleration of cell cycle by reducing the amount in G0/G1 phase and increasing the cell in phase S. The TRF is observed to restore telomere elongation and activity and reduce DNA damage.<sup>54</sup>

Tocotrienol is reportedly involved not only in the process of gene transcription but also involved in the translational process. It was proved that  $\delta$ -tocotrienol protects the bone marrow in mice and CD34<sup>+</sup> in humans from radiation damage through activation of extracellular signal kinases related to rapamycin pathway signaling target in mammals. The increased target of rapamycin and 4EBP-1 downstream effect or phosphorylation of mammals were associated with the translation activity of the eIF4E mRNA regulator and the S6 ribosome protein responsible for growth and survival.

The involvement of tocotrienols in gene expression levels is not only limited to transcriptional and translational mechanisms but some studies have reported that tocotrienols act on the level

of gene expression as a cancer control. Studies in animal model have shown that the excessive expression of PPAR $\gamma$  that plays a role in the transcription process is associated with increased growth of mammary tumors, whereas suppression of PPAR $\gamma$  expression significantly inhibits the development of mammary tumors. Therefore, PPAR $\gamma$  can be an effective means of inhibiting expression of breast cancer growth by modifying tocotrienols that have anticancer effects. Malaviya & Sylvester<sup>54</sup> confirmed that the combined treatment of  $\gamma$ -tocotrienol with PPAR $\gamma$  synergizes agonistically in inhibiting cell growth of MCF-7 and MDA-MB-231. These findings suggest that excessive PPAR $\gamma$  expression correlates with breast enhancement, growth and viability of the breast and treatment with  $\gamma$ -tocotrienol is a significant treatment in decreasing PPAR expression. The combination of  $\gamma$ -tocotrienols each with epigallocatechin gallate (EGCG) and resveratrol in the MCF-7 breast cancer cell strain showed significant effects in reducing expression of Bcl-2 and cyclin D1, subsequently reported that  $\alpha$ - $\gamma$ -tocotrienol may increase catalase activity in strains cell MCF-7 and  $\alpha$ -,  $\delta$ -tocotrienol can increase the activity of glutathione peroxidase in MDA-MB-231 cell strain, tocotrienol isomer inhibits cancer by altering NRF2/KEAPI transcription control which is a mediator of oxidative stress response regulating genes of more than 100 antioxidants.<sup>83</sup>

## CONCLUSION

Tocotrienol is one of vitamin E groups with the main property is fat soluble antioxidants and plays an important role as antioxidants and free radical control. One of the prominent roles of tocotrienol is as an anti-cancer agent, which is involved in various stages of cancer progress from initiation stage to invasion and metastasis stage. Tocotrienol works through various cell

signaling systems and causes slowing of cell cycle progress, suppressing cell cycle rate, apoptotic death, maintaining and/or restoring genome integrity. This bioactive synergizes with other bioactive substances with a wide range of chemicals, including hydrochavicol, farnesol, genisten, pomegranate extract and with some anticancer drugs (a.l. gemcitabine and erlotinib). The anticancer properties of tocotrienols are also involved through inhibition of cholesterol synthesis, one of which suppresses activation of HMG-CoA enzymes in cancer cells. The key lies in the fact that cancer cells in their growth require large amounts of cholesterol. The need for a mechanistic explanation of how tocotrienols, with their divergent effects for each isomers and in combination, acts as an anticancer through a wide range mode of actions. This provides an advanced research space to bring about a more thorough understanding in order to develop an anticancer system that involves more specific and effective bioactive compounds.

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