

## Original Article

# Modulation of NFκB signalling pathway by tocotrienol: A systematic review

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Tocotrienols have been reported to exert anticancer, anti-inflammatory, antioxidant, cardio-protective and bone-protective effects through modulation of NFκB signalling pathway. The objective of this systematic review is to evaluate available literature showing the effect of tocotrienols on NFκB signalling pathway and identify the potential mechanisms involved. A comprehensive search was conducted using PubMed and SCOPUS databases using the keywords “tocotrienol” and “NFκB” or “nuclear factor kappa b”. Main inclusion criteria were English language original articles showing the effect of tocotrienol on NFκB signalling pathway. Fifty-nine articles were selected from the total of 117 articles initially retrieved from the literature search. Modulation of regulatory proteins and genes such as inhibition of farnesyl prenyl transferase were found to be the mechanisms underlying the tocotrienol-induced suppression of NFκB activation.

**Key Words:** NFκB signalling, tocotrienol, inflammation, cancer, antioxidant

## INTRODUCTION

Vitamin E, one of the vital micronutrients, was discovered in the early 19th century by Evans and Bishop (1922).<sup>1</sup> Evans and Bishop (1922) termed it tocopherol, a Greek word that translates into childbirth and to produce, as its functions were thought to be vital for reproduction and fertility. Forty years later, Pennock et al (1964) discovered another analogue of vitamin E, which was named as tocotrienol.<sup>2</sup> Both analogues of vitamin E have four isomers that include alpha (α), beta (β), gamma (γ), and delta (δ). These isomers differ in their position and number of methyl groups.<sup>3</sup> The main structural difference between tocopherol and tocotrienol is in the isoprenoid tail. Tocotrienol possesses three double trans bonds compared to tocopherol which has single trans bond in the isoprenoid side chain.<sup>4</sup> Both tocopherol and tocotrienol are found in edible oils, nuts and cereal grains. Edible oils from palm, annatto seeds and rice bran consist more of tocotrienol whereas wheat germ and sunflower consist more of tocopherol.<sup>5</sup>

Tocotrienol and tocopherol have been widely recognized as potent antioxidant agents, which provide resistance to oxidative damage induced disease progression in human body.<sup>6,7</sup> Phenolic bonds found in their structure attack the free radicals and neutralize them thus diminishing the formation of oxidative species.<sup>8</sup> In comparison to tocopherol, tocotrienol possesses higher antioxidant activity,<sup>9</sup> and higher therapeutic potential.<sup>10,11</sup> Other biological properties of tocotrienols include anti-cancer,<sup>12</sup> radioprotective,<sup>13</sup> cardioprotective,<sup>14</sup> cholesterol-lowering,<sup>15</sup> anti-diabetic,<sup>16</sup> and anti-inflammatory.<sup>17</sup>

Modulation of NFκB signalling pathway is thought to play a significant role in some of the tocotrienols' biological properties such as the anti-inflammatory and anti-cancer activities.<sup>18</sup> The main function of NFκB is to regulate inflammatory response and balance cell survival and cell death.<sup>19</sup> NFκB was discovered as a nuclear transcription factor back in 1986 by Sen and Baltimore.<sup>20</sup> It is a heterodimer protein complex that consist of any of these five NFκB monomers; RelA (p65), RelB, c-Rel, p50 and p52. They all share a similar Rel homology domain (RHD) structure which binds to DNA. However, p50 and p52 do not contain transcriptional activation domains (TADs), and originate from their precursor proteins p105 and p100, respectively. RelA, RelB and c-Rel, however, are transformed into mature proteins by TADs. NFκB protein complex is bound to inhibitory kappa B (IκB) in the cytoplasm, which renders NFκB inactive. One of the IκB family member widely studied is IκBα.

NFκB is activated through two different pathways, canonical and non-canonical pathways. In canonical pathway, which is commonly triggered in response to infections or pro-inflammatory cytokines, IκBα is phosphory-

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lated by multi-subunit I $\kappa$ B kinase (IKK) complex. IKK $\alpha$  is mainly associated with canonical pathway. This phosphorylation process triggers I $\kappa$ B $\alpha$  degradation by proteasome, therefore, making the p50/RelA or p50/c-Rel dimers active and ready to translocate to nucleus and activate gene transcription. Meanwhile, non-canonical pathway is not associated with I $\kappa$ B $\alpha$  degradation, but it induces ubiquitination and processing of p100. The p100 processing eventually results in nuclear translocation of p52/RelB dimers. Non-canonical pathway is mainly involved in regulating adaptive immune cell signalling.

Although the role of tocotrienols in modulating NF $\kappa$ B signalling pathway has been reported in several studies, the precise mechanisms of action of tocotrienols have not been fully elucidated. In this review, we aim to provide up-to-date insights into the literature on the effect of tocotrienol on NF $\kappa$ B signalling pathway and the potential mechanisms involved in various pathological conditions.

## METHODS

### Search strategy

Selection of the articles was based on two electronic databases (PubMed and Scopus) according to the following keywords: tocotrienol AND NF $\kappa$ B OR nuclear factor kappa b. The search for articles was made from January 2020 to July 2020. The search using these two databases yielded a total of 117 articles.

### Study selection

All the retrieved articles were screened by two independent reviewers (NAAN and MZS) based on the inclusion criteria. In case of discrepancy for selection of the articles, a third reviewer (RA) was called for consensus.

### Inclusion criteria

Inclusion criteria included English language original articles with independent data showing the effect of tocotrienol on NF $\kappa$ B activity along with its signalling pathway involving human subjects or in an experimental set up using animals, tissue or cells.

### Exclusion criteria

This review excluded abstract-only, narrative review, systematic review, meta-analysis or systematic review with meta-analysis articles. This review did not include articles written in languages other than English. Studies in which experimental groups were treated with tocotrienol in combination with other compounds without any tocotrienol only treatment group were also excluded.

### Data items extraction

All papers collected from the two electronic databases were subjected to initial screening. Duplicates were removed. The abstract and content of each paper were screened to assess if the inclusion criteria were met. Several study characteristics were extracted from the selected articles and tabulated. These characteristics included year of study, type of tissue or cells or animals used, source of tocotrienol, dose(s) of tocotrienol used, duration of tocotrienol treatment period and outcome parameters showing the effect of tocotrienol on NF $\kappa$ B activity.

## RESULTS

### Literature search result

By using the keywords listed above, a total of 136 articles were retrieved. Among these, 83 articles were retrieved from PubMed and 53 from Scopus. A total of 34 review articles were excluded, of which 20 were from PubMed and 14 from Scopus databases. Further 9 articles (5 from PubMed and 4 from Scopus database) did not present data showing the effect of tocotrienol towards NF $\kappa$ B, hence did not meet the inclusion criteria and were excluded. Thus, a total of 58 articles from PubMed and 35 articles from Scopus satisfied the inclusion criteria; however, 34 of them were duplicated and were excluded leaving 59 articles to be included in this study (Figure 1).

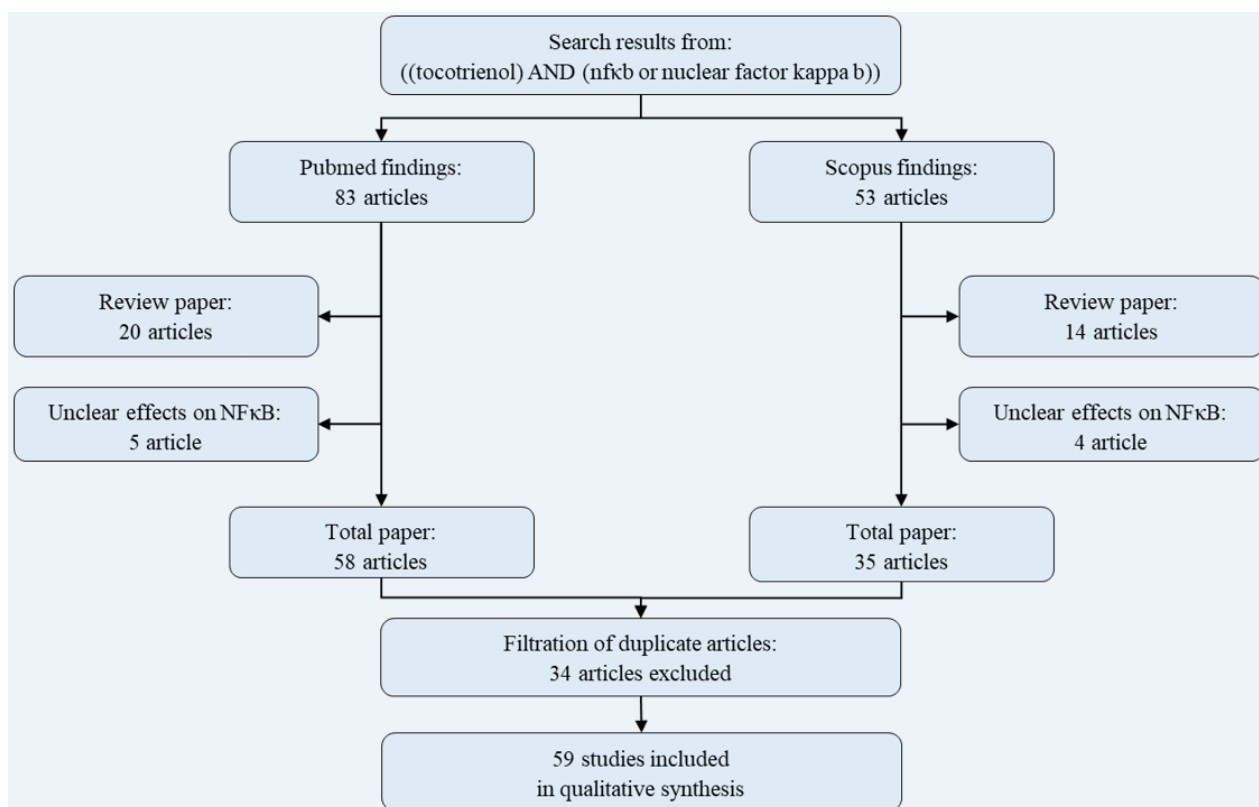
### Characteristics of included articles

Articles included in this review were original articles that have been published between year 2002 and 2020. From the articles gathered, one study was on human subject and the remaining 52 used *in vitro*, *in vivo* or combination of *in vitro* and *in vivo* experimental designs. The articles were grouped into several categories based on the pathological condition targeted. These categories included cancer, inflammation and others. Accordingly, the results are presented category-wise.

Majority of included articles showed determination of NF $\kappa$ B and/or its phosphorylated protein expression using Western blot technique. Besides Western blot, some studies also measured NF $\kappa$ B expression using immunofluorescence, immunohistochemistry and ELISA. The DNA binding activity of NF $\kappa$ B was mainly measured using electrophoretic mobility shift assay (EMSA). Only one study measured NF $\kappa$ B activation indirectly by observing I $\kappa$ B $\alpha$  expression,<sup>21</sup> as I $\kappa$ B $\alpha$  is an important protein that binds with NF $\kappa$ B in cytoplasm retaining it in inactive form. In five of the included articles, receptor activator of nuclear factor kappa-B ligand (RANKL) expression was measured,<sup>22-26</sup> and was extrapolated to the activity of NF $\kappa$ B signalling pathway.

### Modulation of NF $\kappa$ B by tocotrienol in cancer

Among the included articles, 29 presented the data showing the effect of tocotrienol on NF $\kappa$ B expression in various experimental models of cancer (Table 1). Most of the studies targeted breast cancer,<sup>27-36</sup> followed by pancreatic cancer,<sup>38-40</sup> lung cancer,<sup>34,41,42</sup> and colorectal cancer.<sup>43-45</sup> Largely,  $\gamma$ -tocotrienol,  $\delta$ -tocotrienol and tocotrienol-rich fraction (TRF) were found to downregulate NF $\kappa$ B expression and activation in various models of cancer, but not  $\alpha$ -, and  $\beta$ -tocotrienols. Some of the studies investigated the mechanisms underlying the NF $\kappa$ B modulation by tocotrienol.<sup>28,30,32,33,41,45-50</sup> These mechanisms included upregulation of tumour suppressor proteins,<sup>41-47</sup> inhibition of regulatory proteins involved in carcinogenesis,<sup>33,48-50</sup> as well as inhibition of several signalling pathways such as rat sarcoma virus/rapidly accelerated fibrosarcoma/extracellular signal-regulated kinase (Ras/Raf/ERK), phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), epidermal growth factor receptor (EGFR) dependent signalling and hepatocyte growth factor (HGF)-dependent mitogenic signalling.<sup>28,30,32,46</sup> It was observed that by modulating NF $\kappa$ B expression, tocotri-



**Figure 1.** Flow chart showing the article selection process and the number of articles retrieved for this study.

enol enhances cancer cell apoptosis via suppression of inflammatory reaction, cell invasion, angiogenesis, and metastasis.

#### **Modulation of NFκB by tocotrienol in inflammatory diseases**

Among the included articles, a total of 21 presented data showing the effect of tocotrienol on NFκB modulation in various inflammatory disease models (Table 2). Only one study involved human subjects.<sup>56</sup> In various studies that used inflammatory disease models,  $\gamma$ -, and  $\delta$ -tocotrienols and TRF were shown to downregulate NFκB expression and activation. Several studies investigated the mechanism underlying NFκB modulation by tocotrienols and these included; upregulation of A20 anti-inflammatory molecules,<sup>57-59</sup> inhibition of several signalling pathways such as AMP-activated protein kinase (AMPK) and microtubule associated protein kinase (MAPK),<sup>21,60,61</sup> as well as inhibition of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ),<sup>62</sup> and toll-like receptors (TLRs).<sup>56</sup>

#### **Modulation of NFκB by tocotrienol in bone diseases**

Five studies investigated the effect of tocotrienol on NFκB expression and activity in experimental models of bone diseases (Table 3). These studies mainly investigated effect of tocotrienols on NFκB activation through RANKL. RANKL plays an important role in osteoclastogenesis. Reduced RANKL expression by tocotrienols was shown to cause inhibition of NFκB activation, which resulted in inhibition of osteoclast formation, improvement of osteoblast production and reduction of inflammatory cytokines. However, study by Norazlina et al<sup>26</sup> failed to

show reduction in RANKL expression by tocotrienol mixture, although bone calcium level was maintained.

#### **Modulation of NFκB by tocotrienol in other disease**

Three studies investigated the effect of tocotrienols on the expression of adhesion molecules that play a role in atherosclerosis.<sup>77-79</sup> All 3 studies showed that tocotrienols reduces NFκB expression. Reduction of NFκB expression resulted in reduced expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and e-selectin, as well as the inflammatory markers such as interleukin (IL)-6 and tumour necrosis factor (TNF)- $\alpha$ , which play a major role in atherosclerotic plaque formation. In one of the studies, Nasir et al<sup>80</sup> showed the effect of tocotrienol on NFκB expression and associated nitro-oxidative stress in rat model of diabetic cataract. It was observed that tocotrienol causes reduction of NFκB and inducible nitric oxide synthase (iNOS) expression, which led to reduced nitrosative stress in diabetic cataractous lenses. All of these studies did not investigate the mechanisms causing NFκB modulation by tocotrienols (Table 4).

#### **DISCUSSION**

Tocotrienols have been reported to inhibit NFκB expression and/or activation in various diseases. The studies that were included in this review mainly investigated the anti-cancer and anti-inflammatory effects of tocotrienols and the potential underlying mechanisms. This reflects the trend of tocotrienols-related research which is largely targeted to discover its potential in the treatment of cancer. Several studies included in this review showed that suppression of NFκB signalling pathway by tocotrienols

**Table 1.** Summary of studies showing the effect of tocotrienol on NFκB in cancer

| Reference | <i>In vivo</i> model               | <i>In vitro</i> model   | Source of tocotrienol  | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)  |
|-----------|------------------------------------|---|--|---|---|
| 43        | Female Fisher 344 rats             | Human colon cancer cells lines HCT-116, HT-29 and SW480 and SW620.<br><br>Immortalized normal colonic mucosal cells NCM460. | α-, β-, γ- and δ-tocotrienol from Davos Life Ltd (Helios, Singapore) | <i>In vitro</i> method:<br>Cells were treated with α-, β-, γ- and δ-tocotrienol (50 μM) for 72 hours.<br><br><i>In vivo</i> method:<br>Rats were given oral sulindac (20 mg/kg) or δ-tocotrienol (200 mg/kg, twice a day) for 20 weeks.   | δ-tocotrienol inhibited NFκB activity in both the <i>in vivo</i> and <i>in vitro</i> setting, which reduced inflammatory process and colorectal cancer genesis.   |
| 47        | BALB/c nu/nu female mice           | Human gastric cancer SGC-7901 and MGC-803 cells   | γ-tocotrienol from Hygeia Industries, Inc. (USA)                     | <i>In vitro</i> method:<br>Cells were treated with γ-tocotrienol (30 μM) for 2, 4, 6, 12, and 24 hours.<br><br><i>In vivo</i> method:<br>Rats were given γ-tocotrienol (25 mg/kg) daily for 1 week  | γ-tocotrienol increased the activity of PP2A, a tumour suppressor protein, and reduced phosphorylated ATM, an important protein that enables IKK to activate the NFκB, which lead to inhibition of NFκB activity (by PP2A-dependent mechanism).   |
| 40        | -                                  | Human lung cancer cells A549 and H1299.   | δ-tocotrienol from American River Nutrition (USA)                    | <i>In vitro</i> method:<br>Cells were treated with δ-tocotrienol (10, 20 and 30 μM) for 72 hours.   | δ-tocotrienol increased expression miR-451, tumour suppressor protein, along with suppression of Notch-1 signalling pathway. This led to inhibition of MMP-9 expression and NFκB binding activity, which inhibited cancer cell invasion and migration.  |
| 44        | 4-week-old male athymic nu/nu mice | Human colorectal cancer (CRC) cell lines: HCT 116, HT-29, and Caco-2.   | Palm oil-derived γ-tocotrienol from Davos Life Science, (Singapore)  | <i>In vitro</i> method:<br>Cells were treated with either capecitabine or different doses of γ-tocotrienol for 1, 3, and 5 days.<br><br><i>In vivo</i> method:<br>Mice were treated with either oral capecitabine (60 mg/kg), γ-tocotrienol (100 mg/kg), or combination of capecitabine (60 mg/kg) and γ-tocotrienol (100 mg/kg) for 2 weeks. | γ-tocotrienol suppressed NFκB activation greater than capecitabine alone. Suppression of NFκB activation downregulated proteins involved in inflammation, tumour invasion, angiogenesis and metastasis, which led to reduction in tumour growth and size.   |
| 46        | -                                  | Human leukemia HL-60 cells  | γ-tocotrienol from Sigma-Aldrich (USA)                               | <i>In vitro</i> method:<br>Cells were treated with 10 to 30 μM for 4 hours.   | γ-tocotrienol inhibited HMG-CoA reductase and the subsequent Ras/Raf/ERK and Ras/PI3K/Akt pathways, which led to inhibition of NFκB activation. The inhibition of NFκB activation further led to suppression of GLO1, which is an important enzyme in cell growth. Therefore, through these inhibitions, cancer cell apoptosis was enhanced |
| 51        | 12- to 14-week-old CD2F1 male mice | Human primary hematopoietic CD34+ cells   | δ-tocotrienol from Yasoo Health Inc. (Johnson City, Tennessee)       | <i>In vitro</i> method:<br>Cells were treated with 2 μM δ-tocotrienol for 24 hours<br><br><i>In vivo</i> method:<br>Mice were treated with single dose of subcutaneous δ-tocotrienol (75 mg/kg)   | δ-tocotrienol suppressed radiation-induced pro-inflammatory protein expression and enhanced apoptotic cell death by neutralizing IL-1β activation, which then inhibited NFκB activation.  |

**Table 1.** Summary of studies showing the effect of tocotrienol on NFκB in cancer (cont.)

| Reference | <i>In vivo</i> model  | <i>In vitro</i> model  | Source of tocotrienol   | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)  |
|-----------|---|--|---|---|---|
| 52        | -   | Breast cell lines: MCF-7 & triple negative MDA-MB-231 cell line and primary mouse embryonic fibroblast (NIH/3T3) cells | Tocotrienol-rich fraction (TRF) (Golden Hope Plantations, Selangor, Malaysia)   | <i>In vitro</i> method:<br>Cells were treated with 0-20 µg/ml of TRF for 24, 48 or 72 hours.  | TRF suppressed cell growth by inhibiting NFκB activation, which in turn induced PARP cleavage to increase apoptosis.  |
| 29        | Female BALB/c mice  | Estrogen receptor-independent +SA mouse mammary epithelial cells   | Pure δ- and γ-tocotrienol (First Tech International Ltd. (Hong Kong)).<br>Oxazine derivatives of δ- and γ-tocotrienols derived from the pure compounds. | <i>In vitro</i> method:<br>Cells were treated with 0 to 5 µM α-tocopherol, γ-tocotrienol, δ-tocotrienol, γ-tocotrienol oxazine derivatives (compounds 26, 31, and 39), or δ-tocotrienol oxazine derivatives (compound 40 and 44) for 4 days.<br><i>In vivo</i> method:<br>Mice were treated with either α-tocopherol, γ-tocotrienol, δ-tocotrienol, γ-tocotrienol oxazine derivatives (compounds 26, 31, and 39), or δ-tocotrienol oxazine derivatives (compound 40 and 44) by intralesional injection at a concentration of 120 µg. Treatment was given every other day for 11 days. | γ- and δ-tocotrienol oxazine derivatives suppressed NFκB transcription factor better than the parent compounds, which in turn, reduced proteins and protein kinases involved in cell survival and growth and led to reduction of growth of mammary cells and tumour size. |
| 53        | -   | Adult T-cell leukemia (ATL) cell line: ED-40515 cells  | α-,β-,γ- and δ-tocotrienol from Eizai Food & Chemical (Tokyo, Japan)  | <i>In vitro</i> method:<br>Cells were treated with 0-50 µM of all tocotrienols isomers (α-,β-,γ- and δ-tocotrienol) for 6, 12 and 24 hours.   | δ-tocotrienol enhanced apoptosis through intrinsic pathway by modulation of NFκB signalling. δ-tocotrienol also induced apoptosis through suppressing squalene synthesis in the mevalonic acid pathway.   |
| 28        | -   | Highly malignant +SA mouse mammary epithelial cells  | γ-tocotrienol from First Tech International Ltd. (Hong Kong)  | <i>In vitro</i> method:<br>Cells were treated with either γ-tocotrienol or SU11274 (a specific Met inhibitor), alone or in combination for 3 days.  | γ-tocotrienol in combination with SU11274 suppressed HGF-dependent mitogenic signalling, which partly involved NFκB activation. The suppression of this signalling pathway contributed to the anti-proliferative effect of γ-tocotrienol.                                 |
| 37        | <i>LSL-Kras<sup>G12D</sup>; PDX-1-Cre</i> mice (pancreatic intraepithelial neoplasms (PanINs) genetically engineered mouse model) | -  | δ-tocotrienol from Davos Life Science Ltd (Helios, Singapore).  | <i>In vivo</i> method:<br>The mice were treated with vehicle (ethanol-extracted olive oil, 1.0 mL/kg twice daily) or δ-tocotrienol (200 mg/kg twice daily). The treatment period was 12 months.   | δ-tocotrienol inhibited Kras-induced pancreatic carcinogenesis, possibly by modulation of Raf-MEK-ERK signalling pathway, AKT and NFκB activation, which in turn, increased the cell-cycle progression and pro-apoptotic markers, respectively.                           |

**Table 1.** Summary of studies showing the effect of tocotrienol on NFκB in cancer (cont.)

| Reference | <i>In vivo</i> model      | <i>In vitro</i> model   | Source of tocotrienol   | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)  |
|-----------|---------------------------|---|---|---|---|
| 27        | -                         | Breast cell lines: MCF-7 & triple negative MDA-MB-231 cells                 | TRF from Golden Hope Plantations (Selangor, Malaysia), tocotrienol fraction free from α-tocopherol (TEF) from Davos Life Sciences Ptd Ltd (Singapore) and pure α-, γ- and δ-tocotrienol from Eisai Food & Chemicals Co. Ltd. (Tokyo, Japan) | <i>In vitro</i> method:<br>Cells were treated with 0-20 μg/ml of TRF for 24, 48 or 72 hours.  | Tocotrienols suppressed cell growth by inhibiting NFκB activation, which in turn induced PARP cleavage to promote apoptosis.  |
| 54        | -                         | Metastatic human oral cancer cell line: B88 cells                           | γ-tocotrienol from Eizai Food & Chemical Co. (Tokyo, Japan)   | <i>In vitro</i> method:<br>Cells were treated with 0, 75, or 100 μM γ-Tocotrienol for 6 days.   | γ-tocotrienol inhibited constitutively active and inducible NFκB activation, which led to reduction of NFκB-regulated gene products expression, such as survival proteins. This, in turn, led to induction of PARP cleavage and activation of apoptotic cascades.                 |
| 30        | -                         | Mouse +SA mammary epithelial cell lines                                     | γ-tocotrienol from First Tech International Ltd.  | <i>In vitro</i> method:<br>Cells were treated with either 3 μM γ-tocotrienol or 20 μM sesamin or 2 μM gefitinib for 4 days.   | Combination of γ-tocotrienol at subeffective dose with sesamin inhibited ErbB2-4 activation, which in turn downregulated Ras/ERK, PI3K/Akt, and Jak/Stat pathways. NFκB is one of transcription factors involved and its inhibition contributed toward inhibition of cell growth. |
| 55        | Athymic nu/nu female mice | Gastric cancer cell lines: SNU-5, SNU-16 and MKN45 cells                    | γ-tocotrienol from Davos Life Science Ltd (Helios, Singapore).  | <i>In vitro</i> method:<br>Cells were treated with 10 μM γ-tocotrienol for 4 hours or 10 μM capecitabine for 24 hours.<br><i>In vivo</i> method:<br>Mice received either (i) vehicle (ii) γ-tocotrienol (100 mg/kg, intraperitoneal (i.p.)) (iii) capecitabine (60 mg/kg, oral gavage); and (iv) combination of γ-tocotrienol (dose as in (ii)) and capecitabine (dose as in (iii)). Treatment was given for 4 weeks. | γ-tocotrienol inhibited cell proliferation and tumour growth through suppression of NFκB expression, along with its gene products that are involved in cell proliferation, survival, angiogenesis and metastasis.   |
| 38        | Female NIH SCID nude mice | Human pancreatic ductal epithelial cells (HPDE6 C7) and HPDE6 C7-KRas cells | α-, β-, γ- and δ-tocotrienol from Davos Life Science (Singapore)  | <i>In vitro</i> method:<br>Cells were treated with either 50 μM of α-, β-, γ- and δ-tocotrienol or 20 μM gemcitabine or combination δ-tocotrienol and gemcitabine for 72 hours.<br><i>In vivo</i> method:<br><u>Study 1</u><br>Mice received either vehicle or α-, β-, γ- and δ-tocotrienol (200 mg/kg, twice daily) via oral gavage. Treatment was given for 4 weeks.  | γ- and δ-tocotrienol inhibited NFκB activation, and subsequently, downregulated Bcl-X <sub>L</sub> , survivin, and XIAP (pro-survival factors in carcinogenesis)  |

**Table 1.** Summary of studies showing the effect of tocotrienol on NFκB in cancer (cont.)

| Reference | <i>In vivo</i> model                           | <i>In vitro</i> model   | Source of tocotrienol   | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)  |
|-----------|--|---|---|---|---|
| 42        | -  | Human non-small cell lung cancer cells (NSCLC) cell lines: A549 and H1299 cells | δ-tocotrienol from American River Nutrition, Inc (USA)                | <u>Study 2</u><br>Mice received either (i) vehicle, (ii) δ-tocotrienol (200 mg/kg, orally, twice daily) (iii) gemcitabine (100 mg/kg, intraperitoneally, twice a week) or (iv) combination δ-tocotrienol and gemcitabine. Treatment was given for 4 weeks.<br><br><i>In vitro</i> method:<br>Cells were treated with 10, 20 or 30 μM of δ-tocotrienol for 72 hours.   | δ-tocotrienol inhibited NFκB activation and its downstream proteins which are involved in cell proliferation, invasion and apoptosis.   |
| 48        | -  | Human HCC cell lines: HepG2, Hep3B, C3A, SNU-387, and PLC/PRF5 cells            | γ-tocotrienol from Davos Life Science (Singapore)                     | <i>In vitro</i> method:<br>Cells were incubated with 5, 10, 25 or 50 μM of γ-tocotrienol for 1, 2, 4 or 6 hours.  | γ-tocotrienol inhibited NFκB activation, which was attributed to suppression of JAK2 activation. This may have contributed to the anti-apoptotic effect of γ-tocotrienol.   |
| 56        | BALB/c, C57BL/6, and Swiss albino strains mice | CD4 <sup>+</sup> T cells and primary spleen lymphocyte cells                    | α- and γ-tocotrienol from Kyoto Prefectural University (Kyoto, Japan) | <i>In vitro</i> method:<br>Cells were incubated with 10 to 50 μM α- and γ-tocotrienol for 4 or 12 hours.<br><br><i>In vivo</i> method:<br><u>Lymphopenia induction</u><br>Mice were treated with intraperitoneal injection of γ-tocotrienol (200 mg/kg) for 24 hours.<br><u>Homeostatic proliferation study</u><br>Mice were given purified CD4 <sup>+</sup> T cells and treated with 50 μM γ-tocotrienol.<br><u>Graft-versus-host disease (GVHD) induction</u><br>Mice were given 50 μM γ-tocotrienol-treated splenocytes. | Immunosuppressive effects of γ-tocotrienol (suppression of T cells, cell proliferation and cytokine production) were suggested to be due to inhibition of NFκB and AP-1 activation. This was followed by inhibition of expression of their gene products. However, with transient exposure to γ-tocotrienol, immunostimulatory effect involving NFκB and AP-1 activation leading to activation of prosurvival molecules was observed. |
| 32        | -  | Mouse +SA mammary epithelial cell lines   | γ-tocotrienol from First Tech International Ltd.                      | <i>In vitro</i> method:<br>Cells were incubated with either (i) 3.5 μM γ-tocotrienol (ii) 2.5 μM celecoxib (iii) 20 μM celecoxib or (iv) combination of 0.25 μM γ-tocotrienol and 2.5 μM celecoxib for 72 hours.  | Combination of γ-tocotrienol at subeffective dose with celecoxib inhibited EGF-dependent ErbB receptor activation and subsequently inhibited NFκB activation, which inhibited cancer cell growth.   |
| 39        | Four weeks old male athymic nu/nu mice         | Pancreatic cancer cell lines: BxPC-3, MIA PaCa-2, PANC-1 w and MPanc-96.        | γ-tocotrienol from Davos Life Science Ltd (Helios, Singapore).        | <i>In vitro</i> method:<br>Cells were treated with either 10 or 50 μmol/L γ-tocotrienol for 2, 4 and 6 days.<br><br><i>In vivo</i> method:<br>Mice were treated with either (i) vehicle (ii) γ-tocotrienol (400 mg/kg once daily, oral gavage) (iii) gemcitabine (25 mg/kg twice weekly, i.p); and (iv) combination of γ-tocotrienol and gemcitabine (dose and route as in (ii) and (iii) respectively). Treatment was given for 4 weeks.   | γ-tocotrienol inhibited constitutive NFκB activation and proteins associated with inflammation, proliferation, invasion, and angiogenesis in pancreatic cancer cells.   |

**Table 1.** Summary of studies showing the effect of tocotrienol on NFκB in cancer (cont.)

| Reference | <i>In vivo</i> model | <i>In vitro</i> model   | Source of tocotrienol  | Groupings, treatment dose and duration   | Outcomes (in relation to NFκB)   |
|-----------|----------------------|---|--|--|--|
| 31        | -                    | Mouse +SA mammary epithelial cell lines   | γ-tocotrienol from Carotech Bhd. (Malaysia).                   | <i>In vitro</i> method:<br>Cells were incubated with either (i) 3.5 μM γ-tocotrienol (ii) 2.5 μM celecoxib (iii) 20 μM celecoxib or (iv) combination of 0.25 μM γ-tocotrienol and 2.5 μM celecoxib for 72 hours. | Combination of γ-tocotrienol at subeffective dose with celecoxib inhibited NFκB activation, which is involved in cancer cell proliferation and survival.   |
| 33        | -                    | Human estrogen-dependent BCa cells (MCF-7), human estrogen-independent BCa cells (MDA-MB-231), androgen-independent prostate cancer cells (PC-3) and immortalized human non-tumorigenic breast epithelial cell line (MCF-10A) | γ-tocotrienol from Davos Life Science (Singapore)              | <i>In vitro</i> method:<br>Cells were incubated with either 20, 40, or 80 μM γ-tocotrienol or combination of 80 μM γ-tocotrienol and 20 μM of SP600125 (JNK inhibitor) for 24 hours.                             | γ-tocotrienol inhibited ID1 through suppression of ID1 upstream regulator proteins. Inactivation of ID1 inhibited NFκB activation, which in turn induced apoptosis through cleavage of the caspase proteins and PARP.  |
| 49        | -                    | Amelanotic (C32) and melanotic (G361) malignant melanoma cells  | α-,β-,γ- and δ-tocotrienol from Davos Life Science (Singapore) | <i>In vitro</i> method:<br>Cells were treated with 20, 40 or 60 μM of tocotrienol isomers for 24 hours.  | γ-tocotrienol inhibited cancer cell progression by modulating prosurvival signalling pathways which involved suppression of NFκB activation. Inhibition of NFκB activation was associated with down-regulation of EGF-R, which then inhibited ID-1 expression and induced apoptosis. |
| 45        | -                    | Human colon carcinoma cell line: HT-29 cells  | γ-tocotrienol from Davos Life Science (Singapore)              | <i>In vitro</i> method:<br>Cells were treated with 15, 30, 45 or 60 μM of γ-tocotrienol for 24, 48, 72 or 96 hours.  | γ-tocotrienol inhibited NFκB expression which led to activation of apoptotic pathway and inhibition of cell proliferation and growth.  |
| 50        | -                    | Human androgen-dependent PCa cells (LNCaP), human androgen-independent PCa cells (PC-3), immortalised human prostate epithelial cells (PZ-HPV-7)  | γ-tocotrienol from Davos Life Science (Singapore)              | <i>In vitro</i> method:<br>Cells were treated with either 10, 20 or 40 μM γ-tocotrienol or combination of 80 μM γ-tocotrienol and 20 μM of SP600125 (JNK inhibitor) for 24 hours.                                | γ-tocotrienol inhibited NFκB activation through suppressing EGF-R, which in turn induced apoptosis through PARP and caspase cleavage as well as inducing MKK/JNK pathways.   |
| 34        | -                    | Human myeloid KBM-5 cells, human lung adenocarcinoma H1299 cells, human embryonic kidney A293 cells, Human breast cancer MCF-7, multiple myeloma U266 cells, and Human squamous cell carcinoma SCC-4 cells.                   | γ-tocotrienol from Carotech, Inc., Edison, NJ                  | <i>In vitro</i> method:<br>Cells were treated with 5 μM γ-tocotrienol for 12 hours.  | γ-tocotrienol induced apoptosis and inhibited cell proliferation in cancer cells through suppression of NFκB signalling pathway.   |
| 35        | -                    | Mammary epithelial (highly malignant +SA) cell line from adenocarcinoma in BALB/c female mouse  | γ-tocotrienol from Malaysian Palm Oil Board (Malaysia)         | <i>In vitro</i> method:<br>Cells were treated with a range of 1 to 20 μM of γ-tocotrienol for a treatment period of 1 hour to 5 days.  | γ-tocotrienol inhibited NFκB signalling pathway which led to inhibition of cell growth-  |
| 36        | -                    | Mammary epithelial (highly malignant +SA) cell line from adenocarcinoma in BALB/c female mouse  | γ-tocotrienol from Malaysian Palm Oil Board (Malaysia)         | <i>In vitro</i> method:<br>Cells were incubated with 1 to 8 μM of γ-tocotrienol for 1 to 3 days.   | γ-tocotrienol inhibited NFκB signalling pathway which led to suppression of tumour cell mitogenesis.   |



**Table 2.** Summary of studies showing the effect of tocotrienol on NFκB in inflammatory diseases

| Reference | <i>In vivo</i> model  | <i>In vitro</i> model   | Source of tocotrienol  | Groupings, treatment dose and duration   | Outcomes (in relation to NFκB)   |
|-----------|---|---|--|--|--|
| 58        | -   | RAW 264.7 macrophages and A20 <sup>-/-</sup> and A20 <sup>+/+</sup> mouse embryonic fibroblasts | δ-, γ-tocotrienol from American River Nutrition DeltaGold  | <i>In vitro</i> method:<br><u>Study 1</u><br>Cells were treated with 5, 10 or 20 μM of δ- or γ-tocotrienol for 4, 8 or 16 hours<br><br><u>Study 2</u><br>Cells were treated with 20 μM of δ-tocotrienol for 4 hours.   | δ-tocotrienol exerted anti-inflammatory effect through modulation of sphingolipid metabolism, which led to A20 upregulation and then, inhibition of NFκB signalling pathway.   |
| 61        | Male C57BL/6J mice  | -   | TRF from Palm- Eisai Food & Chemical   | <i>In vivo</i> method:<br>Mice were treated with oral TRF at concentrations of 100 or 300 mg/kg five times per week for 12 weeks.  | TRF supplementation improved hyperglycaemia-induced skeletal muscle injury by regulating AMPK/SIRT1 pathway, which is involved in insulin signalling pathway. This regulation led to reduction in skeletal metabolic demand, oxidative stress, inflammation, and apoptosis which was partly attributed to NFκB activation. |
| 57        | Human study: Chronic hepatitis C patients   | -   | δ-tocotrienols from American River Nutrition, Inc. (USA)   | Patients were given annatto tocotrienols (500 mg/day) for 6 weeks.   | δ-tocotrienol downregulated toll-like receptors, which inhibited several downstream signalling molecules including NFκB pathway. Inhibition of NFκB pathway was involved in the expression of pro-inflammatory cytokines.  |
| 62        | -   | RAW 264.7 macrophages   | δ-tocotrienol from Chromadex, Inc. (Irvine, CA, USA) and rice-bran δ-tocotrienol extract from Hunan Jinjian Cereals Industry Co., Ltd. (Changde, China). | <i>In vitro</i> method:<br>Cells were treated with 5, 10, 20, 40 or 80 μM of δ-tocotrienol for 2 hours   | δ-tocotrienol inhibited MAPKs/AP-1 and PPARs/AP-1 pathways, which led to inhibition of c-Jun and NFκB activity and resulted in decrease in pro-inflammatory marker expression.   |
| 64        | BALB/c mice   | -   | γ-tocotrienol from Davos Life Science (Singapore)  | <i>In vivo</i> method:<br>Mice were treated with oral γ-tocotrienol (30, 100 and 250 mg/kg) for 3 and 15 days.   | γ-tocotrienol inhibited STAT3 and NFκB activation which reduced pro-inflammatory mediators expression in cigarette smoke exposed mice.   |
| 59        | Male BKS.Cg- <i>Dock7</i> <sup>m+/+</sup> <i>Lepr</i> <sup>db/j</sup> ( <i>db/db</i> ) mice | Primary bone marrow-derived macrophages (BMDM) and iJ774 macrophages                            | γ-tocotrienol from Carotech (Edison, NJ, USA).   | <i>In vitro</i> method:<br>Cells were treated with 0 to 5 μM of γ-tocotrienol for 24 hours.<br><br><i>In vivo</i> method:<br>Hyperglycemic mice were treated with oral 0.1%(w/w) γ-tocotrienol, which was incorporated in the AIN93G diet. Treatment was given once daily for 8 weeks. | γ-tocotrienol inhibited NLRP3-inflammasome through suppression of TRAF6/IKK/NFκB signalling pathway by reducing the A20 induction.   |
| 41        | BALB/c mice   | -   | α-, δ- and γ-tocotrienol from Davos Life Science (Singapore)   | <i>In vivo</i> method:<br>Mice were treated with oral γ-tocotrienol (250 mg/kg) for 6 days.  | γ-tocotrienol inhibited NFκB activation which reduced pro-inflammatory mediators expression in HDM-induced mice.   |

**Table 2.** Summary of studies showing the effect of tocotrienol on NFκB in inflammatory diseases (cont.)

| Reference | <i>In vivo</i> model | <i>In vitro</i> model   | Source of tocotrienol  | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)   |
|-----------|----------------------|---|--|---|--|
| 65        | -                    | Murine RAW 264.7 macrophages and primary bone marrow-derived macrophages (BMDM) | γ-tocotrienol from BASF (Germany)  | <i>In vitro</i> method:<br>Cells were incubated with either 10, 20 or 40 μM γ-tocotrienol for 8, 14 or 16 hours.  | γ-tocotrienol inhibited TNF-α stimulated activation of NFκB through modulation of de novo synthesis of sphingolipids, which led to higher endoplasmic reticulum stress. This resulted in increased expression of A20 (negative regulator of NFκB) and/or Cezanne, which inhibited the cytokine-stimulated activation of inflammatory pathways through NFκB, JNK, and TAK1. |
| 21        | Male C57BL/6J mice   | Primary bone marrow cells from 6-week-old C57/BL/6 mice                         | γ-tocotrienol from Carotech (USA)  | <i>In vitro</i> method:<br>Cells were treated with either vehicle or 5 mM γ-tocotrienol for 24 hours.<br><i>In vivo</i> method:<br>Mice were treated with either vehicle or oral γ-tocotrienol (50 mg/kg) once daily for 4 weeks. | γ-tocotrienol reduced MAPK activation and IκBα degradation, which may correlate to NFκB inactivation. The inactivation of NFκB, reduced the monocyte attraction to adipocytes.   |
| 66        | -                    | Murine RAW264.7 macrophages & bone marrow-derived macrophages (BMDM).           | γ-tocotrienol from BASF (Germany).   | <i>In vitro</i> method:<br>Cells were incubated with either 10, 20 or 40 μM γ-tocotrienol for 8, 14 or 16 hours.  | γ-tocotrienol inhibited IL-6 production through inhibition of NFκB activation and C/EBPβ expression.   |
| 63        | -                    | 3T3-L1 adipocyte cells  | γ-tocotrienol from Cayman Chemical (USA)                                     | <i>In vitro</i> method:<br>Cells were treated with 0.024 to 2.4 μM γ-tocotrienol for 6 hours.   | γ-tocotrienol reduced adipokines production through modulation of PPARγ. PPARγ also inhibited NFκB activation.   |
| 67        | New Zealand rabbit   | -   | Tocotrienol-enriched vitamin E from Sime Darby Bioganic Sdn. Bhd. (Malaysia) | <i>In vivo</i> method:<br>Rabbits were treated with either placebo or oral tocotrienol (15 mg/kg) for 8 weeks.  | Tocotrienol reduced NFκB expression, which led to reduction in adhesion molecules and inflammatory markers.  |
| 68        | -                    | Primary peritoneal macrophages.   | Palm oil-derived TRF from Carotech Ltd. (Malaysia)                           | <i>In vitro</i> method:<br>Cells were treated with either 5, 10 or 30 μg/mL of TRF for 24 hours.  | TRF inhibited pro-inflammatory cytokines expression through reduction of PGE <sub>2</sub> and NO production, which may be due to inhibition of NFκB activation.  |
| 69        | Wistar rat           | -   | Tocotrienol mixture from Golden-Hope   | <i>In vivo</i> method:<br>Rat were treated with either placebo or oral tocotrienol (50 or 100 mg/kg) for 3 weeks.   | Tocotrienol reduced cognitive deficits in ethanol-treated rat pups by modulating NFκB signalling pathway which reduced cerebral cortex and hippocampal nitro-oxidative stress, apoptosis and inflammatory status.  |
| 70        | Male C57BL/6 mice    | -   | Annatto δ-tocotrienol fraction from American River Nutrition (USA)           | <i>In vivo</i> method:<br>Mice were treated with either oral δ-tocotrienol (200 mg) or quercetin (200 mg) or dexamethasone (20 mg) for 4 weeks.   | δ-tocotrienol inhibited gene expression of NFκB, which is associated with ageing and pro-inflammatory process.   |

**Table 2.** Summary of studies showing the effect of tocotrienol on NFκB in inflammatory diseases (cont.)

| Reference | <i>In vivo</i> model         | <i>In vitro</i> model  | Source of tocotrienol  | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)  |
|-----------|------------------------------|--|--|---|---|
| 71        | -                            | RAW 264.7 cells, primary peritoneal macrophages from C57BL/6, BALB/c, double knockout LMP7/MECL-1 <sup>-/-</sup> , and PPAR-α <sup>-/-</sup> knockout mice | Annatto δ-tocotrienol fraction from American River Nutrition (USA)                   | <i>In vitro</i> method:<br>Cells were treated with either quercetin, riboflavin or δ-tocotrienol (5, 10, 20, or 40 μM) for 1 hour.  | δ-tocotrienol inhibited NFκB translocation, which then suppressed production of inflammatory markers (TNF-α and NO)   |
| 72        | White Leghorn female chicken | -  | δ-tocotrienol from American River Nutrition, Inc. (USA)                              | <i>In vivo</i> method:<br>Chickens were treated with either (i) placebo (ii) δ-tocotrienol (125 μM/kg), (iii) quercetin, (iv) riboflavin (v) Corey lactone (vi) amiloride (vii) dexamethasone, (viii) δ-tocotrienol & quercetin (ix) δ-tocotrienol & riboflavin (x) δ-tocotrienol & Corey lactone, (xi) δ-tocotrienol & amiloride (xii) δ-tocotrienol & dexamethasone.<br>Treatment was given for four weeks. | δ-tocotrienol blocked NFκB activation, which resulted in reduced expression of inflammatory markers in age-associated diseases.   |
| 73        | -                            | HaCaT keratinocyte cells   | γ-tocotrienol from Chromadex (USA)   | <i>In vitro</i> method:<br>Cells were treated with γ-tocotrienol (0.1 or 1.0 μM) for 3 or 24 hours.   | γ-tocotrienol reduced ROS production as well as NFκB activation, which led to reduction in the expression of inflammatory mediators in SQ-OOH-induced keratinocyte cells.                         |
| 74        | Male Wistar rats             | -  | Tocotrienol mixture from Golden-Hope Bioganic, Malaysia.                             | <i>In vivo</i> method:<br>Rats received either vehicle or tocotrienol mixture (25, 50 and 100 mg/kg daily) via oral gavage for 3 weeks.   | Tocotrienol reduced diabetic nephropathy progression in diabetic rats by modulating NFκB signalling pathway which reduced renal oxidative–nitrosative stress and inflammatory status.             |
| 75        | Male Wistar rats             | -  | Tocotrienol mixture from Golden-Hope Bioganic (Malaysia)                             | <i>In vivo</i> method:<br>Rats received either vehicle or tocotrienol mixture (25, 50 and 100 mg/kg daily) via oral gavage for 10 weeks.  | Tocotrienol reduced cognitive deficits in diabetic rats by modulating NFκB signalling pathway which reduced cerebral cortex and hippocampal oxidative–nitrosative stress and inflammatory status. |
| 76        | -                            | THP-1 human monocytic cells  | Palm oil-derived tocotrienol rich fraction (TRF) from Carotech Ltd. (Ipoh, Malaysia) | <i>In vitro</i> method:<br>Cells were treated either with TRF (0.5, 1.0, and 5.0 μg/mL) or LPS for 24 hours.  | TRF suppressed NFκB activation, which led to reduction in the expression of its gene products and pro-inflammatory cytokines. This protected monocytic cells against LPS-induced cytotoxicity.    |

**Table 3.** Summary of studies showing the effect of tocotrienol on NFκB in bone diseases models

| Reference | <i>In vivo</i> model | <i>In vitro</i> model   | Source of tocotrienol   | Groupings, treatment dose and duration   | Outcomes (in relation to NFκB)  |
|-----------|----------------------|---|---|--|---|
| 22        | -                    | Primary rheumatoid arthritis fibroblast-like synoviocytes and peripheral blood mononuclear cells. | Tocotrienol from Sigma Chemical Co. (USA)   | <i>In vitro</i> method:<br>Cells were treated with tocotrienol at concentration ranging from 0.2 μM to 5 μM for 72 hours.  | Tocotrienol decreased RANKL expression, which led to inactivation of mTOR/AMPK/JNK/ERK/IκB-α signalling pathway and resulted in inhibition of osteoclast differentiation.   |
| 23        | Wistar rats          | -   | Annatto tocotrienol from American River Nutrition Inc. (USA) and Palm tocotrienol extracted from Excelvite Sdn. Bhd. (Malaysia) | <i>In vivo</i> method:<br>Rats were treated with either (i) vehicle, (ii) 60 mg/kg annatto tocotrienol, (iii) 100 mg/kg annatto tocotrienol, (iv) 60 mg/kg palm tocotrienol, or (v) 100 mg/kg palm tocotrienol for 12 weeks. | Both annatto and palm tocotrienol decreased sRANKL expression which inhibited NFκB activation and production of inflammatory cytokines. The inhibition of NFκB activation promoted osteoblastogenesis and improves bone resorption. |
| 24        | C57BL/6 female mice  | -   | γ-Tocotrienol from Yasoo Health Inc. (USA)  | <i>In vivo</i> method:<br>Mice were treated with single dose of γ-tocotrienol (100 mg/kg, subcutaneous injection).   | γ-tocotrienol reduced RANKL expression and preserved OPG levels, which indicated inhibition of osteoclast formation (prevent bone loss).  |
| 25        | -                    | Mouse bone marrow-derived macrophages, primary osteoblasts and bone marrow cells.                 | α-tocotrienol from Calbiochem (USA)   | <i>In vitro</i> method:<br>Cells were treated with either vehicle, 50 μM α-tocopherol or 50 μM α-tocotrienol for 12 hours.   | α-tocotrienol inhibited RANKL-induced delayed NFκB activation, which reduced the osteoclastogenesis.  |
| 26        | Sprague-Dawley rats  | -   | Tocotrienol mixture from Malaysian Palm Oil Board   | <i>In vivo</i> method:<br>Rats were treated with either (i) vehicle, (ii) 60 mg/kg tocotrienol mixture, or (iii) 60 mg/kg α-tocopherol for 8 weeks.  | Tocotrienol mixture increased RANKL expression, however, the bone loss was prevented in nicotine-treated rats.  |

**Table 4.** Summary of studies showing the effect of tocotrienol on NFκB in other disease models

| Reference | <i>In vivo</i> model            | <i>In vitro</i> model                          | Source of tocotrienol   | Groupings, treatment dose and duration  | Outcomes (in relation to NFκB)  |
|-----------|---------------------------------|--|---|---|---|
| 78        | -                               | Human umbilical vein endothelial cells         | Tocotrienol-tocopherol mixed fraction (TTFM) from Golden Hope Jomalina Sdn. Bhd (Malaysia) $\alpha$ -, $\beta$ -, $\gamma$ - and $\delta$ -tocotrienol from Davos Life Science (Singapore). | <i>In vitro</i> method:<br>Cells were treated either with TTFM or tocotrienol isomers at concentration ranging from 0.3 $\mu$ M to 10 $\mu$ M for 16 hours.   | Tocotrienol isomers, especially $\delta$ -tocotrienol, reduced monocytes adhesion by reducing NFκB(p50) and adhesion molecules expression.  |
| 80        | Male <i>Sprague Dawley</i> rats | -  | Annatto tocotrienol from American River Nutrition, Inc. (USA)   | <i>In vivo</i> method:<br>Rats were treated with either vehicle tocotrienol (0.03%), topically to ocular surface. Treatment was given twice daily for 8 weeks | Tocotrienol reduced NFκB activation in lenses of diabetic rats, which reduced iNOS expression. This, in turn led to reduced nitrosative stress produced by peroxynitrite. Overall, tocotrienol reduced oxidative-nitrosative stress in the pathogenesis of diabetic cataract. |
| 77        | -                               | Human umbilical vein endothelial cells         | $\alpha$ -, $\beta$ -, $\gamma$ - and $\delta$ -tocotrienol from Davos Life Science (Singapore)   | <i>In vitro</i> method:<br>Cells were treated with the tocotrienol isomers at concentration ranging from 0.3 $\mu$ M to 10 $\mu$ M for 16 hours.              | Tocotrienol isomers, especially $\delta$ - followed by $\gamma$ -tocotrienol, inhibited expression of adhesion molecules and inflammation through downregulation of NFκB(p50) expression.   |
| 79        | -                               | Human umbilical vein endothelial cells (HUVEC) | $\alpha$ -tocotrienol from Malaysian Palm Oil Board (Malaysia)  | <i>In vitro</i> method:<br>Cells were treated with 5 to 50 $\mu$ M of $\alpha$ -tocotrienol for 20 hours.   | $\alpha$ -tocotrienol reduced NFκB activation (contains promoter binding sites for adhesion molecules) which led to downregulation of adhesion molecules expression.  |

reduces cell growth and invasion, angiogenesis and induces cell apoptosis. Tocotrienol was shown to modulate several tumor suppressor proteins associated with NF $\kappa$ B activity in various experimental models of cancer used in these studies. Sun et al<sup>47</sup> observed that pre-treatment with  $\gamma$ -tocotrienol increased PP2A expression in a time and dose-dependent manner in gastric cancer cell line. Greater PP2A expression by  $\gamma$ -tocotrienol correlated with significantly reduced phosphorylation of ataxia-telangiectasia mutated (ATM) protein, which was induced with okadaic acid in this study. Activated ATM is known to activate NF $\kappa$ B nuclear translocation.<sup>83</sup> Rajasinghe et al<sup>41</sup> reported involvement of another tumour suppressor protein, miR-451, to be associated with anti-cancer effect of tocotrienol. Increased miR-451 expression was noted after non-small-cell lung cancer cells were pre-treated with  $\delta$ -tocotrienol. This effect of tocotrienol was associated with reduced expression of Notch-1 pathway proteins, urokinase plasminogen activator (uPA) and matrix-degrading metalloproteinase 9, which may directly or indirectly affect the NF $\kappa$ B pathway. Other than tumour suppressor proteins, several other regulatory proteins involved in carcinogenesis, including janus kinase 2 (JAK2) and inhibitor of differentiation/DNA binding 1 (ID1), were found to reduce NF $\kappa$ B activation when treated with tocotrienols.<sup>33,48,50,57</sup> All the above-mentioned regulatory proteins are known to regulate the cell proliferation, differentiation and apoptosis in cancer cells. Phosphorylation of JAK has been commonly associated with nuclear translocation of STAT family of transcription factors, but it has also been reported to activate other transcription factors such as NF $\kappa$ B,<sup>84</sup> which was also observed by Rajendran et al.<sup>48</sup> Notably,  $\gamma$ -tocotrienol also inhibited signal transducer and activator of transcription 3 (STAT3), which has been reported to modulate NF $\kappa$ B nuclear translocation in cancer cells.<sup>85</sup> ID1 was suggested to be suppressed through epidermal growth factor receptor (EGFR)-by  $\gamma$ -tocotrienol in prostate cancer and melanoma cells.<sup>49,50</sup> Yap et al<sup>33</sup> observed that  $\gamma$ -tocotrienol also reduces the expression of Src, Smad1/5/8 and lysyl oxidase (LOX), the upstream regulators responsible for ID1 activation in breast cancer cells. Tocotrienols were also shown to inhibit several signalling pathways which crosstalk and inhibit the main signalling pathway PI3K/Akt and Ras/Raf/ERK involved in tumorigenesis.<sup>28,30,32,59</sup> These pathways were shown to be inhibited in breast cancer cells when there was suppression of epidermal growth factor (EGF)-dependent ErbB/HER receptor,<sup>28,30,32,65</sup> and Met receptor,<sup>28</sup> by  $\gamma$ -tocotrienol. This eventually reduced nuclear translocation of NF $\kappa$ B. Other mechanism that has been suggested to underlie inhibition of NF $\kappa$ B activation through PI3K/Akt and Ras/Raf/ERK pathways was proposed to involve inhibition of farnesyl prenyl transferase (FPTase).<sup>46</sup> Ras protein can be activated through post-translational modification of the farnesyl group by FPTase.<sup>86</sup> Therefore, inhibition of FPTase, which catalyses the farnesylation process, inhibited the mutation of Ras protein, and reduced its activation.<sup>87,88</sup>

Several studies involved in this review also showed that tocotrienols modulated expression and activation of NF $\kappa$ B in experimental models of inflammatory diseases via different mechanisms. One of the mechanisms sug-

gested is the upregulation of A20 molecule and cyldromatosis (CYLD) gene, which are the negative regulator of NF $\kappa$ B activation.<sup>57,58,65</sup> A20 terminates NF $\kappa$ B activation by its role in ubiquitin-editing activity, which helps to catalyse de-ubiquitination of adaptor proteins needed for NF $\kappa$ B activation.<sup>89</sup> A20 is also involved in attenuating TLR signalling in inflammatory condition.<sup>90</sup> As  $\delta$ -tocotrienol suppressed TLR signalling along with TNF receptor associated factor (TRAF) in hepatitis C patients,<sup>56</sup> this effect of tocotrienol is likely to involve upregulation of A20. Upregulation of A20 by  $\delta$ - and  $\gamma$ -tocotrienols in macrophages and embryonic fibroblast cells was also associated with upregulation of intracellular dihydroceramides (dhCer) that is important in autophagic process and endoplasmic reticulum stress.<sup>65</sup> The suppression of NF $\kappa$ B activation through upregulation of A20 alone, however, was not adequate to fully suppress priming of NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome and IL-1 $\beta$ .<sup>58</sup> Therefore, other mechanisms may contribute to enhance suppression of NF $\kappa$ B and its downstream signalling molecules. Crosstalk between MAPK and PPAR contribute significantly towards activation of inflammatory process.<sup>91,92</sup> Phosphorylation of proteins in MAPK pathway suppresses PPAR activity, whereas, PPAR $\alpha$  and PPAR $\gamma$  inhibit activation of NF $\kappa$ B.<sup>93</sup> NF $\kappa$ B is suppressed by PPAR through several mechanisms, namely, increased expression of I $\kappa$ B $\alpha$ , phosphatase and tensin homologue (PTEN), and increased activity of sirtuin 1 (SIRT1), superoxide dismutase (SOD) and catalase.<sup>91</sup> Matsunaga et al<sup>62</sup> observed that  $\gamma$ -tocotrienol upregulated PPAR $\gamma$  expression and resulted in inhibition of NF $\kappa$ B activation in TNF $\alpha$ -treated adipocytes. Similar effects of  $\delta$ -tocotrienol on PPAR $\gamma$  were reported in lipopolysaccharide (LPS)-induced macrophages with additional observations that it inhibits MAPK activation and upregulates PPAR $\alpha$  expression.<sup>61</sup> Interestingly, deacetylation of peroxisome proliferator-activated receptor- $\gamma$  coactivator (PGC)-1 $\alpha$  is associated with upregulation of PPAR $\gamma$  expression.<sup>94</sup> In hyperglycaemic environment, tocotrienol-rich fraction was reported to increase AMPK/SIRT1 expression, which promoted deacetylation of PGC-1 $\alpha$  and lead to inhibition of NF $\kappa$ B activation.<sup>60</sup>

Receptor activator of nuclear factor- $\kappa$ B (RANK) and its ligand, RANKL play important roles in bone metabolism.<sup>95</sup> Binding of RANK to RANKL activates NF $\kappa$ B canonical and non-canonical signalling pathway in the osteoclasts and their precursors.<sup>96</sup> Tocotrienols were shown to reduce RANKL expression,<sup>23</sup> and activity of osteoclasts and its precursors cells,<sup>22,25</sup> in rat model of osteoporosis. The reduction of RANKL expression was associated with reduction of interleukins production, which is important in osteoclastogenesis or bone resorption process.<sup>22-25</sup> However, Norazlina et al<sup>26</sup> observed prevention of nicotine-induced bone loss in tocotrienol-treated group despite high RANKL expression, which suggested that other pathways might be involved in the tocotrienol-induced protection against bone resorption.

Tocotrienol also possesses cardioprotective properties because of its direct anti-inflammatory and immunomodulatory effects and indirect cholesterol-lowering, antioxidant and anti-adhesion effects.<sup>14</sup> These effects are partly

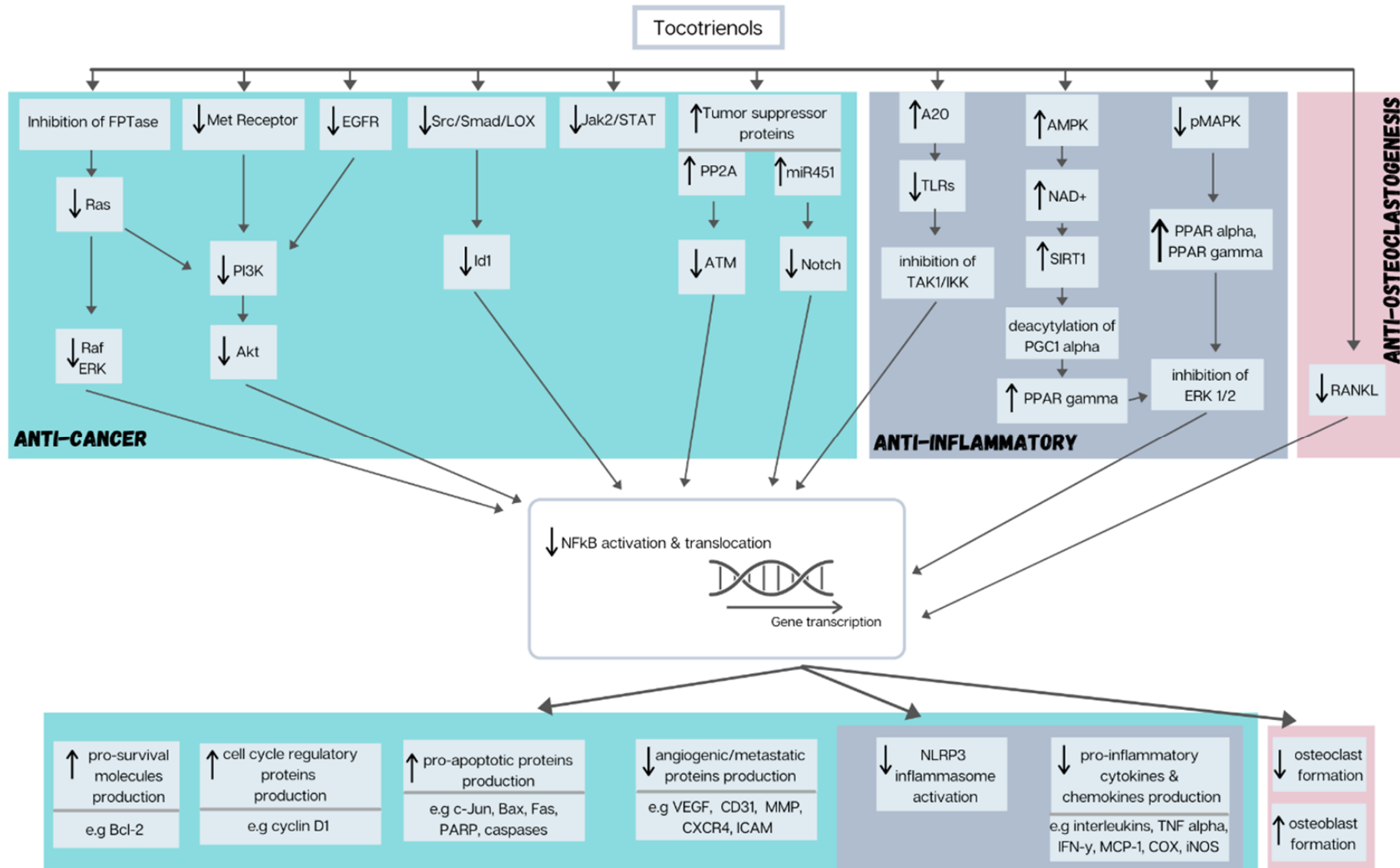


Figure 2. Modulation of NFκB signalling pathways by tocotrienol.

associated with termination of NF $\kappa$ B activation. Adhesion molecules such as ICAM and VCAM are known to be upregulated in human coronary atherosclerotic plaques.<sup>97</sup> Tocotrienols were observed to downregulate adhesion molecules expression in human umbilical vein endothelial (HUVEC) cells by suppressing NF $\kappa$ B activation.<sup>77-79</sup>

Findings by Nasir et al,<sup>80</sup> correlate the tocotrienol induced suppression of NF $\kappa$ B activation with lowering of nitrosative stress in the diabetes-induced cataractous lens in rats. The nitrosative stress was reduced through reduction of iNOS gene expression, which is dependent on NF $\kappa$ B activation. Reduced iNOS expression leads to low level of nitric oxide production, therefore, reducing the production of peroxynitrite, a powerful oxidant. Free radicals and oxidants are known as the stimulators of NF $\kappa$ B activation. By lowering the level of oxidants, the vicious cycle that activates NF $\kappa$ B is interrupted.

### Conclusion

The modulation of NF $\kappa$ B signalling pathway is important in the regulation of inflammation, immunity, proliferation, differentiation, apoptosis and survival. This systematic review summarizes the mechanisms and effects of tocotrienols towards NF $\kappa$ B activation under various pathological conditions (Figure 2). Overall, it can be concluded that tocotrienols suppress NF $\kappa$ B activation through several mechanisms which include modulation of several regulatory proteins and genes. Suppression of NF $\kappa$ B signalling pathway, in turn, increases expression of pro-apoptotic molecules and reduces proliferative molecules in cancer cells. It also reduces production of pro-inflammatory cytokines which have wide ranging effects on a spectrum of diseases. Nevertheless, more studies are required to fully understand the mechanism underlying the effects of tocotrienol on NF $\kappa$ B activation. Importantly, for this review, we included only one study involving human subjects. More data from human studies is likely to support the potential therapeutic applications of tocotrienols.

### AUTHOR DISCLOSURES

The authors declare no conflict of interest.

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