Modulation of NFκB signalling pathway by tocotrienol: A systematic review

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ABSTRACT

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 several regular proteins and genes as well 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).[1] Evans and Bishop (1922) termed it tocopherol, Greek word that translates into childbirth and to produce, as its function was 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.[2] Both analogues of vitamin E have four isomers and include alpha (α), beta (β), gamma (γ), and delta (δ). These isomers differ in their position and number of methyl groups.[3] The main structural difference between tocopherol and tocotrienol is in the isoprenoid tail. Tocotrienol possesses three double trans bonds compared to tocopherol which have single trans bond in the isoprenoid side chain.[4] 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.[5]

Tocotrienol and tocopherol have been widely recognized as potent antioxidant agents, which provide resistance to oxidative damage induced disease progression in human body.[6,7] Phenolic bonds found in their structure attack the free radicals and neutralize them thus diminishing the formation of oxidative species.[8] In comparison to tocopherol, tocotrienol possesses higher antioxidant activity,[9] and higher therapeutic potentials.[10,11] Other biological

properties of tocotrienols include anti-cancer,[12] radioprotective,[13] cardioprotective,[14] cholesterol-lowering,[15] anti-diabetic,16 and anti-inflammatory.[17]

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.[18] The main function of NFκB is to regulate inflammatory response and balance cell survival and cell death.[19] NFκB was discovered as a nuclear transcription factor back in 1986 by Sen and Baltimore.[20] 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 pathway. In canonical pathway, which is commonly triggered in response to infections or pr-inflammatory cytokines, IκBα is phosphorylated by multi-subunit IκB kinase (IKK) complex. IKKα is mainly associated with canonical pathway. This phosphorylation process triggers IκBα 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 IκBα 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 NFκB 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 NFκB signalling pathway and the potential mechanisms involved in various pathological conditions.

MATERIALS AND METHODS

Search strategy

Selection of the articles was based on two electronic databases (PubMed and Scopus) according to the following keywords: tocotrienol AND NFκB 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 NFκB 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 of tocotrienol treatment period and outcome parameters showing the effect of tocotrienol on NFκB 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 NFκB, 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 NFκB and/or its phosphorylated protein expression using Westen blot technique. Besides Western blot, some studies also measured NFκB expression using immunofluorescence, immunohistochemistry and ELISA. The DNA binding activity of NFκB was mainly measured using electrophoretic mobility shift assay (EMSA). Only one study measured NFκB activation indirectly by observing IκBα expression,[21] as IκBα is an important protein that binds with NFκB 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,[22-26] and was extrapolated to the activity of NFκB signalling pathway.

Effect of tocotrienol on NFκB modulation in cancer

Among the included articles, 29 presented the data showing the effect of tocotrienol on NFκB expression in various experimental models of cancer (Table 1). Most of the studies targeted breast cancer,[27-36] followed by pancreatic cancer,38-40 lung cancer,[34,41,42] and colorectal cancer.[43-45] Largely, γ-, δ-tocotrienol and tocotrienol-rich fraction (TRF) were found to downregulate NFκB expression and activation in various models of cancer, but not α-, β-tocotrienol. Some of the studies investigated the mechanism underlying the NFκB modulation by tocotrienol.[28,30,32,33,41,45-50] These mechanisms included; upregulation of tumor suppressor proteins,[41-47] inhibition of regulator protein involved in carcinogenesis,[33,48-50] as well as inhibition of several signalling pathways such as Ras/Raf/ERK, PI3K/Akt, EGF-R dependent signalling and HGF-dependent mitogenic signalling.[28,30,32,46] It was observed that by modulating NFκB expression, tocotrienol enhances cancer cell apoptosis via suppression of inflammatory reaction, cell invasion, angiogenesis, and metastasis.

Effect of tocorienol on NFκB modulation 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 was using human subjects.[56] In the various studies that used inflammatory disease models, γ-, δ-tocotrienol and tocotrienol-rich fraction (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,[57-59] inhibition of several signalling pathways such as AMPK and MAPK,[21,60,61] as well as inhibition of peroxisome proliferator-activated receptor gamma (PPARγ),[62] and toll-like receptors (TLRs).[56]

Effect of tocorienol on NFκB modulation in bone disease model

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 receptor activator of nuclear factor κB ligand (RANKL). RANKL plays an important role in osteoclastogenesis. Reduction of 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[26] failed to show reduction in RANKL expression by tocotrienol mixture, although bone calcium level was maintained.

Effect of tocorienol on NFκB modulation in Other Disease Models

Three studies investigated the effect of tocotrienols on the expression of adhesion molecules that play a role in atherosclerosis.[77-79] All 3 studies showed that tocotrienols reduces NFκB expression. Reduction of NFκB expression resulted in reduced expression of adhesion molecules such as ICAM-1, VCAM-1 and e-selectin, as well as the inflammatory markers such as IL-6 and TNF- α, which play a major role in atherosclerotic plaque formation. In one of the studies, Nasir et al[80] studied the effect of tocotrienol on NFκB modulation and associated nitro-oxidative stress in rat model of diabetic cataract. It was observed that tocotrienol causes reduction of NFκB and iNOS expression, which led to reduced nitrosative stress in diabetic cataractous lenses. All of these studies did not investigate the mechanism of NFκB modulation by tocotrienols.

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 and conditions with underlying inflammatory reactions.[14,81,82]

Several studies included in this review showed that suppression of NFκB signalling pathway by tocotrienols reduces cell growth and invasion, angiogenesis and induces cell apoptosis. Tocotrienol was shown to modulate several tumor suppressor proteins associated with NFκB activity in various experimental models of cancer used in these studies. Sun et al[47] observed that pre-treatment of γ-tocotrienol increased PP2A expression in a time and dose-dependent manner in gastric cancer cell line. Higher PP2A by expression by γ-tocotrienol correlated with significantly reduced phosphorylation of ataxia-telangiectasia mutated (ATM) protein, which was induced with okadaic acid (OA) in this study. Activated ATM is known to activate NFκB nuclear translocation.[83] Rajasinghe et al[41] reported involvement of another tumor 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 δ-tocotrienol. This effect of tocotrienol was associated with reduced expression of Notch-1 pathway proteins, uPA and matrix-degrading metalloproteinase (MMP)-9 expression, which may directly or indirectly affect the NFκB pathway. Other than tumor suppresoor 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 NFκB activation when treated with tocotrienols.[33,48,50,57] 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 NFκB,[84] which was also observed by Rajendran et al.[48] Notably, γ-tocotrienol also inhibited STAT3, which has also been reported to modulate NFκB nuclear translocation in cancer cells.[85] Id1 was suggested to be suppressed through epidermal growth factor receptor (EGFR) by γ-tocotrienol in prostate cancer and melanoma cells.[49,50] Yap et al[33] observed that γ-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 pahway PI3K/Akt and Ras/Raf/ERK involved in tumorigenesis.[28,30,32,59] These pathways were shown to be inhibited in breast cancer cells when there was suppression of EGF-dependent ErbB/HER receptor,[28,30,32,65] and Met receptor,[28] by γ-tocotrienol. This eventually reduced nuclear translocation of NFκB. Other mechanism that has been suggested to underlie inhibition of NFκB activation through PI3K/Akt and Ras/Raf/ERK pathways was proposed to involve inhibition of farnesyl prenyl transferase (FPTase).[46] Ras protein can be activated through post-translational modification of the farnesyl group by FPTase.[86] Therefore, inhibition of FPTase, which catalyzes the farnelysation process, inhibited the mutation of Ras protein, and reduced its activation.[87,88]

Several studies involved in this review also showed that tocotrienols modulated expression and activation of NFKB in experimental models of inflammatory diseases via different mechanisms. One of the mechanisms suggested is the upregulation of A20 molecule and cylidromatosis (CYLD) gene, which are the negative regulator of NFκB activation.[57,58,65] A20 terminates NFκB activation by its role in ubiquitin-editing activity, which helps to catalyze de-ubiquitination of adaptor proteins needed for NFκB activation.[89] A20 is also involved in attenuating TLR signalling in inflammatory condition.[90] As δ- tocotrienol suppressed TLR signalling along with TNF receptor associated factor (TRAF) in hepatitis C patients,[56] this effect of tocotrienol is likley to involve upregulation of A20. Upregulation of A20 by δ- and γ-tocotrienol in macrophages and embyronic fibroblast cells was also associated with upregulation of intracellular dihydroceramides (dhCer) that is important in autophagic process and endoplasmic reticulum stress.[65] The suppression of NFκB activation through upregulation of A20 alone, however, was not adequate to fully suppress priming of NLRP3 inflammasome and IL-1β.[58] Therefore, other mechanisms may contribute to enhance suppression of NFκB and its downstream signalling molecules. Crosstalk between MAPK and PPAR contribute significantly towards activation of inflammatory process.[91,92] Phosphorylation of MAPK suppress PPAR activity, whereas, PPARα and PPARγ inhibit activation of NFκB.[93] NFκB is suppressed by PPAR through several mechanisms, namely, increase expression of IκBα, PTEN, and increased activity of SIRT1, SOD and catalase.[91] Matsunaga et al.[62] observed that γ-tocotrienol upregulated PPARγ expression and resulted in inhibition of NFκB activation in TNFα-treated adipocytes. Similar effects of δ- tocotrienol on PPARγ were reported in LPS-induced macrophages with additional observations that it inhibits MAPK activation and upregulates PPARα expression.[61] Interestingly, deacetylation of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α is associated with upregulation of PPARγ expression.[94] In hyperglycemic environment, tocotrienol-rich fraction was reported to increase

AMPK/SIRT1 expression, which promote deacetylation of PGC1α and lead to inhibition of NFκB activation.[60]

Receptor activator of nuclear factor–kappa B (RANK) and its ligand, RANKL play important roles in bone metabolism.[95] Binding of RANK to RANKL activates NFκB canonical and non-canonical signalling pathway in the osteoclasts and their precursors.[96] Tocotrienols were shown to reduce RANKL expression,[23] and activity of osteoclasts and its precursors cells,[22,25] 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.[22-25] However, Norazlina et al[26] observed prevention of nicotine-induced bone loss in tocotrienols-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, anti-oxidant and anti-adhesion effects.[14] These effects are partly associated with termination of NFκB activation. Adhesion molecules such as ICAM and VCAM are known to be upregulated in human coronary atherosclerotic plaques.[97] Tocotrienols were observed to downregulate adhesion molecules expression in HUVEC cells by suppressing NFκB activation.[77-79]

Findings by Nasir et al[80], correlate the tocotrienol's induced suppression of NFκB 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 NFκB activation. Low 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 NFκB activation. By lowering the level of oxidants, the vicious cycle that activates NFκB is interrupted.

Conclusion

The modulation of NFκB 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 NFκB activation under various pathological conditions (Figure 2). Overall, it can be concluded that tocotrienols suppress NFκB activation through several mechanisms which include modulation of several regulator proteins and genes. Suppression of NFκB signalling pathway, in turn, increases expression of pro-apoptotic molecules and reduce 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 NFκB activation. Importantly, we could find only one study involving human subjects. More data from human studies is likely to support the potential therapeutic applications of tocotrienols.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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REFERENCES

1. Evans HM, Bishop K. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science. 1922;1:650-1.
2. Pennock JF, Hemming FW, Kerr JD. A reassessment of tocopherol chemistry. Biochem Biophys Res Commun. 1964;17:542-8.
3. Caraffa AL, Varvara G, Spinas E, Kritas SK, Lessiani G, Ronconi G et al. Is vitamin E an anti-allergic compound? J Biol Regul Homeost Agents. 2016;30:11-5.
4. Suzuki YJ, Tsuchiya M, Wassall SR, Choo YM, Govil G, Kagan VE, Packer L. Structural and dynamic membrane properties of alpha.-tocopherol and alpha.-tocotrienol: implication to the molecular mechanism of their antioxidant potency. Biochemistry. 1993;32:10692-9.
5. Aggarwal BB, Sundaram C, Prasad S, Kannappan R. Tocotrienols, the vitamin E of the 21st century: its potential against cancer and other chronic diseases. Biochem Pharmacol. 2010;80:1613-31.
6. Azman NHEN, Goon JA, Ghani SMA, Hamid Z, Ngah WZW. Comparing palm oil, tocotrienol-rich fraction and α-tocopherol supplementation on the antioxidant levels of older adults. Antioxidants. 2018;7:74. doi: 10.3390/antiox7060074.
7. Seppanen CM, Song Q, Saari Csallany A. The antioxidant functions of tocopherol and tocotrienol homologues in oils, fats, and food systems. J Am Oil Chem Soc. 2010;87:469-81.
8. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-26.
9. Shahidi F, De Camargo AC. Tocopherols and tocotrienols in common and emerging dietary sources: Occurrence, applications, and health benefits. Int J Mol Sci. 2016;17:1745. doi: 10.3390/ijms17101745
10. Fu JY, Maniam G, Wong FS, Tan DMY, Meganathan P, Chuah LH. Tocotrienols: From Bench to Bedside. In: Niki E. (ed.) Vitamin E: Chemistry and Nutritional Benefits. The Royal Society of Chemistry; 2019. pp. 12-31.
11. Sen CK, Khanna S, Roy S. Tocotrienols: Vitamin E beyond tocopherols. Life Sci. 2006;78):2088-98.

12. Constantinou C, Charalambous C, Kanakis D. Vitamin E and cancer: an update on the emerging role of γ and δ tocotrienols. Eur J Nutr. 2020;59:845-57.
13. Ahmed MM, Montaser SA, Mohamed MR, Said ZS. Radioprotective role of α-tocotrienol against oxidative damage in γ-irradiated human blood cultures. Biosci J. 2018;34:1000-9.
14. Ramanathan N, Tan E, Loh LJ, Soh BS, Yap WN. Tocotrienol is a cardioprotective agent against ageing-associated cardiovascular disease and its associated morbidities. Nutr Metab. 2018;15:6. doi: 10.1186/s12986-018-0244-4.
15. Wong SK, Chin KY, Suhaimi FH, Ahmad F, Ima-Nirwana S. The effects of palm tocotrienol on metabolic syndrome and bone loss in male rats induced by high-carbohydrate high-fat diet. J Funct Foods. 2018;44:246–54.
16. Tan SMQ, Chiew Y, Ahmad B, Kadir KA. Tocotrienol-rich vitamin E from palm oil (Tocovid) and its effects in diabetes and diabetic nephropathy: A pilot phase II clinical trial. Nutrients. 2018;10:1315. doi: 10.3390/nu10091315.
17. Lewis ED, Meydani SN, Wu D. Regulatory role of vitamin E in the immune system and inflammation. IUBMB Life. 2019;71:487-94.
18. Kabir I, Rahman M, Rahman K, Islam T. A brief journey of tocotrienols as anticancer agents. J Silico Vitr Pharmacol. 2017;3:19. doi: 10.21767/2469-6692.100019
19. Luo JL, Kamata H, Karin M. IKK/NF-κB signaling: Balancing life and death - A new approach to cancer therapy. J Clin Invest. 2005;115:2625-32.
20. Sen R, Baltimore D. Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. Cell. 1986;47:921-8.
21. Zhao L, Kang I, Fang X, Wang W, Lee MA, Hollins RR et al. Gamma-tocotrienol attenuates high-fat diet-induced obesity and insulin resistance by inhibiting adipose inflammation and M1 macrophage recruitment. Int J Obes. 2015;39:438-46.
22. Kim KW, Kim BM, Won JY, Min HK, Lee SJ, Lee SH et al. Tocotrienol regulates osteoclastogenesis in rheumatoid arthritis. Korean J Intern Med. 2021;36(Suppl 1):S273-82.
23. Wong SK, Chin KY, Ima-Nirwana S. The effects of tocotrienol on bone peptides in a rat model of osteoporosis induced by metabolic syndrome: The possible communication between bone cells. Int J Environ Res Public Health. 2019;16:3313. doi: 10.3390/ijerph16183313.
24. Deng L, Peng Y, Wu Y, Yang M, Ding Y, Chen Q et al. Tissue distribution of emulsified γ-tocotrienol and its long-term biological effects after subcutaneous administration. Lipids Health Dis. 2014;13:66. doi: 10.1186/1476-511X-13-66.
25. Ha H, Lee JH, Kim HN, Lee ZH. α-Tocotrienol inhibits osteoclastic bone resorption by suppressing RANKL expression and signaling and bone resorbing activity. Biochem Biophys Res Commun. 2011;406:546-51.
26. Norazlina M, Maizatul-Neza J, Azarina A, Nazrun AS, Norliza M, Ima-Nirwana S. Effects of vitamin E on receptor activator of nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) in rats treated with nicotine. Med J Malaysia. 2010;65:14-7.

27. Loganathan R, Selvaduray KR, Nesaretnam K, Radhakrishnan AK. Tocotrienols promote apoptosis in human breast cancer cells by inducing poly(ADP-ribose) polymerase cleavage and inhibiting nuclear factor kappa-B activity. Cell Prolif. 2013;46:203-13. doi: 10.1111/cpr.12014.
28. Ayoub NM, Akl MR, Sylvester PW. Combined γ-tocotrienol and Met inhibitor treatment suppresses mammary cancer cell proliferation, epithelial-to-mesenchymal transition and migration. Cell Prolif. 2013;46:538-53. doi: 10.1111/cpr.12059.
29. Ananthula S, Parajuli P, Behery FA, Alayoubi AY, El Sayed KA, Nazzal S et al. Oxazine derivatives of γ-and δ-tocotrienol display enhanced anticancer activity in vivo. Anticancer Res. 2014;34:2715-26.
30. Akl MR, Ayoub NM, Sylvester PW. Mechanisms mediating the synergistic anticancer effects of combined γ-tocotrienol and sesamin treatment. Planta Med. 2012;78:1731-9.
31. Shirode AB, Sylvester PW. Synergistic anticancer effects of combined γ-tocotrienol and celecoxib treatment are associated with suppression in Akt and NFκB signaling. Biomed Pharmacother. 2010;64:327-32.
32. Shirode AB, Sylvester PW. Mechanisms mediating the synergistic anticancer effects of combined γ-tocotrienol and celecoxib treatment. J Bioanal Biomed. 2011;10;3:1-7.
33. Yap WN, Zaiden N, Tan YL, Ngoh CP, Zhang XW, Wong YC et al. Id1, inhibitor of differentiation, is a key protein mediating anti-tumor responses of gamma-tocotrienol in breast cancer cells. Cancer Lett. 2010;291:187-99.
34. Ahn KS, Sethi G, Krishnan K, Aggarwal BB. γ-Tocotrienol inhibits nuclear factor-κB signaling pathway through inhibition of receptor-interacting protein and TAK1 leading to suppression of antiapoptotic gene products and potentiation of apoptosis. J Biol Chem. 2007;282:809-20.
35. Sylvester PW, Shah SJ, Samant G V. Intracellular signaling mechanisms mediating the antiproliferative and apoptotic effects of γ-tocotrienol in neoplastic mammary epithelial cells. J Plant Physiol. 2005;162:803-10.
36. Shah SJ, Sylvester PW. γ-tocotrienol inhibits neoplastic mammary epithelial cell proliferation by decreasing Akt and nuclear factor κB activity. Exp Biol Med. 2005;230:235-41.
37. Loganathan R, Radhakrishnan AK, Selvaduray KR, Nesaretnam K. Selective anti-cancer effects of palm phytonutrients on human breast cancer cells. RSC Adv. 2015;5:1745-53.
38. Husain K, Centeno BA, Chen D-T, Fulp WJ, Perez M, Zhang Lee G et al. Prolonged survival and delayed progression of pancreatic intraepithelial neoplasia in LSL-Kras G12D/+; Pdx-1-Cre mice by vitamin E δ-tocotrienol. Carcinogenesis. 2013;34:858-63.
39. Husain K, Francois RA, Yamauchi T, Perez M, Sebti SM, Malafa MP. Vitamin E δ-tocotrienol augments the antitumor activity of gemcitabine and suppresses constitutive NF-κB activation in pancreatic cancer. Mol Cancer Ther. 2011;10:2363-72.
40. Kunnumakkara AB, Sung B, Ravindran J, Diagaradjane P, Deorukhkar A, Dey S et al. γ-Tocotrienol inhibits pancreatic tumors and sensitizes them to gemcitabine treatment by modulating the inflammatory microenvironment. Cancer Res. 2010;70:8695-705.

41. Rajasinghe LD, Pindiprolu RH, Gupta SV. Delta-tocotrienol inhibits non-small-cell lung cancer cell invasion via the inhibition of NF-κB, uPA activator, and MMP-9. Onco Targets Ther. 2018; 24;11:4301-14.
42. Ji X, Wang Z, Geamanu A, Sarkar FH, Gupta S V. Inhibition of cell growth and induction of apoptosis in non-small cell lung cancer cells by delta-tocotrienol is associated with notch-1 down-regulation. J Cell Biochem. 2011;112:2773-83.
43. Husain K, Zhang A, Shivers S, Davis-Yadley A, Coppola D, Yang CS et al. Chemoprevention of azoxymethane-induced colon carcinogenesis by delta-tocotrienol. Cancer Prev Res. 2019;12:357-66.
44. Prasad S, Gupta SC, Tyagi AK, Aggarwal BB. γ-Tocotrienol suppresses growth and sensitises human colorectal tumours to capecitabine in a nude mouse xenograft model by down-regulating multiple molecules. Br J Cancer. 2016;115:814-24.
45. Xu WL, Liu JR, Liu HK, Qi GY, Sun XR, Sun WG et al. Inhibition of proliferation and induction of apoptosis by γ-tocotrienol in human colon carcinoma HT-29 cells. Nutrition. 2009;25:555-66.
46. Chen CC, Liu TY, Huang SP, Ho CT, Huang TC. Differentiation and apoptosis induction by lovastatin and γ-tocotrienol in HL-60 cells via Ras/ERK/NF-κB and Ras/Akt/NF-κB signaling dependent down-regulation of glyoxalase 1 and HMG-CoA reductase. Cell Signal. 2015;27:2182-90.
47. Sun WG, Song RP, Wang Y, Zhang YH, Wang HX, Ge S et al. γ-Tocotrienol-inhibited cell proliferation of human gastric cancer by regulation of nuclear factor-κB activity. J Agric Food Chem. 2019;67:441-51
48. Rajendran P, Li F, Manu KA, Shanmugam MK, Loo SY, Kumar AP et al. γ-Tocotrienol is a novel inhibitor of constitutive and inducible STAT3 signalling pathway in human hepatocellular carcinoma: Potential role as an antiproliferative, pro-apoptotic and chemosensitizing agent. Br J Pharmacol. 2011; 163:283-98.
49. Chang PN, Yap WN, Wing Lee DT, Ling MT, Wong YC, Yap YL. Evidence of -tocotrienol as an apoptosis-inducing, invasion-suppressing, and chemotherapy drug-sensitizing agent in human melanoma cells. Nutr Cancer. 2009;61:357-66.
50. Yap WN, Chang PN, Han HY, Lee DTW, Ling MT, Wong YC et al. γ-Tocotrienol suppresses prostate cancer cell proliferation and invasion through multiple-signalling pathways. Br J Cancer. 2008;99:1832-41.
51. Li XH, Ha CT, Fu D, Landauer MR, Ghosh SP, Xiao M. Delta-tocotrienol suppresses radiation-induced MicroRNA-30 and protects mice and human CD34+ Cells from radiation injury. PLoS One. 2015;10:e0122258. doi: 10.1371/journal.pone.0122258.
52. Yamasaki M, Nishimura M, Sakakibara Y, Suiko M, Morishita K, Nishiyama K. Delta-tocotrienol induces apoptotic cell death via depletion of intracellular squalene in ED40515 cells. Food Funct. 2014; 5:2842-9.
53. Kani K, Momota Y, Harada M, Yamamura Y, Aota K, Yamanoi T et al. γ-tocotrienol enhances the chemosensitivity of human oral cancer cells to docetaxel through the downregulation of the expression of NF-κB-regulated anti-apoptotic gene products. Int J Oncol. 2013;42:75-82.

54. Manu KA, Shanmugam MK, Ramachandran L, Li F, Fong CW, Kumar AP et al. First evidence that γ-tocotrienol inhibits the growth of human gastric cancer and chemosensitizes it to capecitabine in a xenograft mouse model through the modulation of NF-κB pathway. Clin Cancer Res. 2012;18:2220-9.
55. Wilankar C, Sharma D, Checker R, Khan NM, Patwardhan R, Patil A et al. Role of immunoregulatory transcription factors in differential immunomodulatory effects of tocotrienols. Free Radic Biol Med. 2011;51:129-43.
56. Qureshi AA, Khan DA, Mushtaq S, Ye SQ, Xiong M, Qureshi N. δ-Tocotrienol feeding modulates gene expression of EIF2, mTOR, protein ubiquitination through multiple-signaling pathways in chronic hepatitis C patients. Lipids Health Dis. 2018;17:167. doi: 10.1186/s12944-018-0804-7.
57. Yang C, Jiang Q. Vitamin E δ-tocotrienol inhibits TNF-α-stimulated NF-κB activation by up-regulation of anti-inflammatory A20 via modulation of sphingolipid including elevation of intracellular dihydroceramides. J Nutr Biochem. 2019;64:101-9.
58. Kim Y, Wang W, Okla M, Kang I, Moreau R, Chung S. Suppression of NLRP3 inflammasome by γ -tocotrienol ameliorates type 2 diabetes. J Lipid Res. 2016;57(1):66–76.
59. Wang C, Husain K, Zhang A, Centeno BA, Chen DT, Tong Z et al. EGR-1/Bax pathway plays a role in vitamin E δ-tocotrienol-induced apoptosis in pancreatic cancer cells. J Nutr Biochem. 2015;26:797-807.
60. Lee H, Lim Y. Tocotrienol-rich fraction supplementation reduces hyperglycemia-induced skeletal muscle damage through regulation of insulin signaling and oxidative stress in type 2 diabetic mice. J Nutr Biochem. 2018;57:77-85.
61. Shen J, Yang T, Xu Y, Luo Y, Zhong X, Shi L et al. δ-Tocotrienol, isolated from rice bran, exerts an anti-inflammatory effect via MAPKs and PPARs signaling pathways in lipopolysaccharide-stimulated macrophages. Int J Mol Sci. 2018;19:3022. doi: 10.3390/ijms19103022.
62. Matsunaga T, Shoji A, Gu N, Joo E, Li S, Adachi T, et al. γ-tocotrienol attenuates TNF-α-induced changes in secretion and gene expression of MCP-1, IL-6 and adiponectin in 3T3-L1 adipocytes. Mol Med Rep. 2012;5:905-9.
63. Peh HY, Tan WSD, Chan TKK, Pow CW, Foster PS, Wong WSF. Vitamin E isoform γ-tocotrienol protects against emphysema in cigarette smoke-induced COPD. Free Radic Biol Med. 2017;110:332-44.
64. Peh HY, Ho WE, Cheng C, Chan TK, Seow ACG, Lim AYH et al. Vitamin E isoform γ-tocotrienol downregulates house dust mite–induced asthma. J Immunol. 2015;195:437-44.
65. Wang Y, Park N-Y, Jang Y, Ma A, Jiang Q. Vitamin E γ-Tocotrienol inhibits cytokine-stimulated NF-κB activation by induction of anti-inflammatory A20 via stress adaptive response due to modulation of sphingolipids. J Immunol. 2015;195:126-33.
66. Wang Y, Jiang Q. γ-Tocotrienol inhibits lipopolysaccharide-induced interlukin-6 and granulocyte colony-stimulating factor by suppressing C/EBPβ and NF-κB in macrophages. J Nutr Biochem. 2013;24:1146-52.
67. Muhammad NA, Omar E, Nawawi HM. Reduced in-vivo C-reactive protein aortic tissue expression following tocotrienol-enriched mixed fraction supplementation in cholesterol-induced atherosclerosis.

In: CHUSER 2012 - 2012 IEEE Colloquium on Humanities, Science and Engineering Research. IEEE; 2012. pp. 148-53.

68. Ng LT, Ko HJ. Comparative effects of tocotrienol-rich fraction, α-tocopherol and α-tocopheryl acetate on inflammatory mediators and nuclear factor kappa B expression in mouse peritoneal macrophages. Food Chem. 2012;134:920-5.
69. Tiwari V, Arora V, Chopra K. Attenuation of NF-κβ mediated apoptotic signaling by tocotrienol ameliorates cognitive deficits in rats postnatally exposed to ethanol. Neurochem Int. 2012;61:310-20.
70. Qureshi AA, Tan X, Reis JC, Badr MZ, Papasian CJ, Morrison DC et al. Inhibition of nitric oxide in LPS-stimulated macrophages of young and senescent mice by δ-tocotrienol and quercetin. Lipids Health Dis. 2011;10:239. doi: 10.1186/1476-511X-10-239.
71. Qureshi AA, Tan X, Reis JC, Badr MZ, Papasian CJ, Morrison DC et al. Suppression of nitric oxide induction and pro-inflammatory cytokines by novel proteasome inhibitors in various experimental models. Lipids Health Dis. 2011;10:177. doi: 10.1186/1476-511X-10-177.
72. Qureshi AA, Reis JC, Qureshi N, Papasian CJ, Morrison DC, Schaefer DM. δ-Tocotrienol and quercetin reduce serum levels of nitric oxide and lipid parameters in female chickens. Lipids Health Dis. 2011;10:39. doi: 10.1186/1476-511X-10-39.
73. Nakagawa K, Shibata A, Maruko T, Sookwong P, Tsuduki T, Kawakami K et al. γ-tocotrienol reduces squalene hydroperoxide-induced inflammatory responses in HaCaT keratinocytes. Lipids. 2010;45:833-41.
74. Kuhad A, Chopra K. Attenuation of diabetic nephropathy by tocotrienol: Involvement of NFkB signaling pathway. Life Sci. 2009;84:296-301.
75. Kuhad A, Chopra K. Tocotrienol attenuates oxidative-nitrosative stress and inflammatory cascade in experimental model of diabetic neuropathy. Neuropharmacology. 2009;57:456-62.
76. Wu SJ, Liu PL, Ng LT. Tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. Mol Nutr Food Res. 2008;52:921-9.
77. Muid S, Froemming GRA, Rahman TH, Ali AM, Nawawi H. Tocotrienols reduce monocytes adhesion to stimulated human endothelial cells. Int Food Res J. 2017;24:2580-5.
78. Muid S, Anisah Froemming GR, Rahman T, Manaf Ali A, Nawawi HM. Delta- and gamma-tocotrienol isomers are potent in inhibiting inflammation and endothelial activation in stimulated human endothelial cells. Food Nutr Res. 2016;60:31526. doi: 10.3402/fnr.v60.31526.
79. Theriault A, Chao J-T, Gapor A. Tocotrienol is the most effective vitamin E for reducing endothelial expression of adhesion molecules and adhesion to monocytes. Atherosclerosis. 2002;160:21-30.
80. Nasir NAA, Agarwal R, Kadir SHSA, Vasudevan S, Tripathy M, Iezhitsa I et al. Reduction of oxidative-nitrosative stress underlies anticataract effect of topically applied tocotrienol in streptozotocin-induced diabetic rats. PLoS One. 2017;12:e0174542. doi: 10.1371/journal.pone.0174542.

81. Ahsan H, Ahad A, Iqbal J, Siddiqui WA. Pharmacological potential of tocotrienols: A review. Nutr Metab. 2014;11:1-22.
82. Montagnani Marelli M, Marzagalli M, Fontana F, Raimondi M, Moretti RM, Limonta P. Anticancer properties of tocotrienols: A review of cellular mechanisms and molecular targets. J Cell Physiol. 2019;234:1147-64.
83. Miyamoto S. Nuclear initiated NF-κB signaling: NEMO and ATM take center stage. Cell Res. 2011;21:116-30.
84. Huang F, Kao C-Y, Wachi S, Thai P, Ryu J, Wu R. Requirement for Both JAK-Mediated PI3K signaling and ACT1/TRAF6/TAK1-dependent NF-κB activation by IL-17A in enhancing cytokine expression in human airway epithelial cells. J Immunol. 2007;179:6504-13.
85. Lee H, Herrmann A, Deng JH, Kujawski M, Niu G, Li Z et al. Persistently activated stat3 maintains constitutive NF-κB activity in tumors. Cancer Cell. 2009;15:283-93.
86. Leonard DM. Ras farnesyltransferase: a new therapeutic target. J Med Chem. 1997;40:2971-90.
87. Rowinsky EK, Windle JJ, Von Hoff DD. Ras protein farnesyltransferase: A strategic target for anticancer therapeutic development. J Clin Oncol. 1999;17:3631-52.
88. Xiang S, Bai W, Bepler G, Zhang X. Activation of Ras by post-translational modifications. In: Azmi AS. (ed). Conquering RAS: From Biology to Cancer Therapy. Elsevier; 2017. pp. 97-118.
89. Wertz IE, O'rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. Nature. 2004;430:694-9.
90. Oshima N, Ishihara S, Rumi MAK, Aziz MM, Mishima Y, Kadota C et al. A20 is an early responding negative regulator of Toll-like receptor 5 signalling in intestinal epithelial cells during inflammation. Clin Exp Immunol. 2010;159:185-98.
91. Korbecki J, Bobiński R, Dutka M. Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. Inflamm Res. 2019;68:443-58.
92. Roberts RA. Evidence for cross talk between PPARα and p38 MAP kinase. Toxicol Sci. 2002;68:270-4.
93. Diradourian C, Le May C, Caüzac M, Girard J, Burnol AF, Pégorier JP. Involvement of ZIP/p62 in the regulation of PPARα transcriptional activity by p38-MAPK. Biochim Biophys Acta - Mol Cell Biol Lipids. 2008;1781:239-44.
94. Rius-Pérez S, Torres-Cuevas I, Millán I, Ortega ÁL, Pérez S, Sandhu MA. PGC-1 α, inflammation, and oxidative stress: an integrative view in metabolism. Oxid Med Cell Longev. 2020;2020:1452696. doi: 10.1155/2020/1452696.
95. Ikebuchi Y, Aoki S, Honma M, Hayashi M, Sugamori Y, Khan M et al. Coupling of bone resorption and formation by RANKL reverse signalling. Nature. 2018;561:195-200.
96. Boyce BF, Xiu Y, Li J, Xing L, Yao Z. NF-κB-mediated regulation of osteoclastogenesis. Endocrinol Metab. 2015;30:35-44.
97. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol. 2007;27:2292-301.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
43	Female Fisher 344 rats	Human colon cancer cells lines HCT-116, HT-29 and SW480 and SW620. Immortalized normal colonic mucosal cells NCM460.	α-,β-,γ- and δ-tocotrienol from Davos Life Ltd (Helios, Singapore)	*In vitro* method: Cells were treated with α-,β-,γ- and δ-tocotrienol (50 μM) for 72 hours. *In vivo* method: Rats were given oral sulindac (20 mg/kg) or δ-tocotrienol (200 mg/kg, twice a day) for 20 weeks.	δ-tocotrienol inhibited NFkB activity in both the *in vivo* and *in vitro* 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)	*In vitro* method: Cells were treated with γ-tocotrienol (30 μM) for 2, 4, 6, 12, and 24 hours. *In vivo* method: Rats were given γ-tocotrienol (25 mg/kg) daily for 1 week	γ-tocotrienol increased the activity of PP2A, a tumor suppressor protein, and reduced phosphorylated ATM, an important protein that enables Iκκ 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)	*In vitro* method: Cells were treated with δ -tocotrienol (10, 20 and 30 μM) for 72 hours.	δ-tocotrienol increased expression miR-451, tumor 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)	*In vitro* method: Cells were treated with either capecitabine or different doses of γ-tocotrienol for 1, 3, and 5 days. *In vivo* method: Mice were 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 NFkB activation greater than capecitabine alone. Suppression of NFkB activation downregulated proteins involved in inflammation, tumour invasion, angiogenesis and metastasis, which led to reduction in tumor growth and size.
46	-	Human leukemia HL-60 cells	γ-tocotrienol from Sigma-Aldrich (USA)	*In vitro* method: 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

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
51	12- to 14-week-old CD2F1 male mice	Human primary hematopoietic CD34+ cells	δ-tocotrienol from Yasoo Health Inc. (Johnson City, Tennessee)	*In vitro* method: Cells were treated with 2 μM δ-tocotrienol for 24 hours *In vivo* method: 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.
52	-	Breast cell lines: MCF-7 triple negative MDA-MB-231 cell line and (NIH/3T3) cells	Tocotrienol-rich fraction (TRF) (Golden Hope Plantations, Selangor, Malaysia)	*In vitro* method: 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)). Oxazine derivatives of **δ**- and **γ**-tocotrienols derived from the pure compounds.	*In vitro* method: 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. *In vivo* method: 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 tumor size.
53	-	Adult T-cell leukemia (ATL) cell line: ED-40515 cells	α-,β-,γ- and δ-tocotrienol from Eizai Food & Chemical (Tokyo, Japan)	*In vitro* method: 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.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
28		Highly malignant +SA mouse mammary epithelial cells	γ-tocotrienol from First Tech International Ltd. (Hong Kong)	*In vitro* method: 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	*LSL-Kras^{G12D}; PDX-1-Cre* mice (pancreatic intraepithelial neoplasms (PanINs) genetically engineered mouse model)	-	δ-tocotrienol from Davos Life Science Ltd (Helios, Singapore).	*In vivo* method: 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 signaling pathway, AKT and NFκB activation, which in turn, increased the cell-cycle progression and pro-apoptotic markers, respectively.
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)	*In vitro* method: 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)	*In vitro* method: 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.	*In vitro* method: 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.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
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).	*In vitro* method: Cells were treated with 10 μM γ-tocotrienol for 4 hours or 10 μM capecitabine for 24 hours. *In vivo* method: 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 tumor 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)	*In vitro* method: 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. *In vivo* method: Study 1 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. Study 2 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.	γ- and δ-Tocotrienol inhibited NFκB activation, and subsequenty, downregulated Bcl-X_L, survivin, and XIAP (pro-survival factors in carcinogenesis)
42	-	Human non-small cell lung cancer cells (NSCLC) cell lines: A549 and H1299 cells	δ-tocotrienol from American River Nutrition, Inc (USA)	*In vitro* method: 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)	*In vitro* method: 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.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
56	BALB/c, C57BL/6, and Swiss albino strains mice	$CD4^+$ T cells and primary spleen lymphocyte cells	α- and γ-tocotrienol from Kyoto Prefectural University (Kyoto, Japan)	*In vitro* method: Cells were incubated with 10 to 50 μM α- and γ-tocotrienol for 4 or 12 hours. *In vivo* method: Lymphopenia induction Mice were treated with intraperitoneal injection of γ-tocotrienol (200 mg/kg) for 24 hours. Homeostatic proliferation study Mice were given purified CD4+ T cells and treated with 50 μM γ-tocotrienol. Graft-versus-host disease (GVHD) induction 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 NFkB and AP-1 activation. This wasfollowed by inhibition of expression of their gene products. However, with transient exposure to γ-tocotrienol, immunostimulatory effect involving NFkB 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.	*In vitro* method: 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).	*In vitro* method: Cells were treated with either 10 or 50 μmol/L γ-tocotrienol for 2, 4 and 6 days. *In vivo* method: 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.
31	-	Mouse +SA mammary epithelial cell lines	γ-tocotrienol from Carotech Bhd. (Malaysia).	*In vitro* method: 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.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
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)	*In vitro* method: 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 NFkB 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)	*In vitro* method: 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 downregulation 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)	*In vitro* method: 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)	*In vitro* method: 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 NFkB activation through suppressing EGF-R, which in turn induced apoptosis through PARP and caspase cleavage as well as inducing MKK/JNK pathways.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
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	*In vitro* method: 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 NFkB 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)	*In vitro* method: 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)	*In vitro* method: 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 tumor cell mitogenesis.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
58	-	RAW 264.7 macrophages and A20−/− and A20+/+ mouse embryonic fibroblasts	δ-, γ-tocotrienol from American River Nutrition DeltaGold	*In vitro* method: Study 1 Cells were treated with 5, 10 or 20 μM of δ- or γ-tocotrienol for 4, 8 or 16 hours Study 2 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	*In vivo* method: 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 signaling 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 signaling 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).	*In vitro* method: 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)	*In vivo* method: 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.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
59	Male BKS.Cg-*Dock7*$^{m+/+}$Lepr$^{db/J}$ (*db/db*) mice	Primary bone marrow-derived macrophages (BMDM) and iJ774 macrophages	γ-tocotrienol from Carotech (Edison, NJ, USA).	*In vitro* method: Cells were treated with 0 to 5 μM of γ-tocotrienol for 24 hours. *In vivo* method: 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 signaling pathway by reducing the A20 induction.
41	BALB/c mice	-	α-, δ- and γ-tocotrienol from Davos Life Science (Singapore)	*In vivo* method: 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.
65	-	Murine RAW 264.7 macrophages and primary bone marrow-derived macrophages (BMDM)	γ-tocotrienol from BASF (Germany)	*In vitro* method: 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)	*In vitro* method: Cells were treated with either vehicle or 5 mM γ-tocotrienol for 24 hours. *In vivo* method: Mice were treated with either vehicle or oral γ-tocotrienol (50 mg/kg) once daily for 4 weeks.	γ-tocotrienol reduced MAPK activation and IkBα 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).	*In vitro* method: 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 NFkB activation and C/EBPβ expression.

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
63	-	3T3-L1 adipocyte cells	γ-tocotrienol from Cayman Chemical (USA)	*In vitro* method: Cells were treated with 0.024 to 2.4μM 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)	*In vivo* method: Rabbits were treated with either placebo or oral tocotrienol (15 mg/kg) for 8 weeks.	Tocotrienol reduced NFκB expression, which leds to reduction in adhesion molecules and inflammatory markers.
68	-	Primary peritoneal macrophages.	Palm oil-derived TRF from Carotech Ltd. (Malaysia)	*In vitro* method: 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_2 and NO production, which may be due to inhibition of NFkB activation.
69	Wistar rat	-	Tocotrienol mixture from Golden-Hope	*In vivo* method: 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 signaling 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)	*In vivo* method: 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.
71	-	RAW 264.7 cells, primary peritoneal macrophages from C57BL/6, BALB/c, double knockout LMP7/MECL-1$^{-/-}$, and PPAR-α$^{-/-}$ knockout mice	Annatto δ-tocotrienol fraction from American River Nutrition (USA)	*In vitro* method: Cells were treated with either quercetin, riboflavin or δ-tocotrienol (5, 10, 20, or 40 μM) for 1 hour.	δ-tocotrienol inhibited NFkB translocation, which then suppressed production of inflammatory markers (TNF-α and NO)

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

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
72	White Leghorn female chicken	-	δ-tocotrienol from American River Nutrition, Inc. (USA)	*In vivo* method: 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. 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)	*In vitro* method: 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 NFkB 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.	*In vivo* method: 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 signaling pathway which reduced renal oxidative–nitrosative stress and inflammatory status.
75	Male Wistar rats	-	Tocotrienol mixture from Golden-Hope Bioganic (Malaysia)	*In vivo* method: 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 signaling 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)	*In vitro* method: Cells were treated either with TRF (0.5, 1.0, and 5.0 μg/mL) or LPS for 24 hours.	TRF suppressed NFkB 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 on the effect of tocotrienol on NFκB modulation in bone diseases models

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
22	-	Primary rheumatoid arthritis fibroblast-like synoviocytes and peripheral blood mononuclear cells.	Tocotrienol from Sigma Chemical Co. (USA)	*In vitro* method: 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)	*In vivo* method: 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)	*In vivo* method: 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)	*In vitro* method: 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 ostoeclastogenesis.
26	Sprague-Dawley rats	-	Tocotrienol mixture from Malaysian Palm Oil Board	*In vivo* method: 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 on the effect of tocotrienol on NFκB modulation in other disease models

Reference	*In vivo* model	*In vitro* model	Source of tocotrienol	Groupings, treatment dose and duration	Outcomes (in relation to NFkB)
78	-	Human umbilical vein endothelial cells	Tocotrienol-tocopherol mixed fraction (TTMF) from Golden Hope Jomalina Sdn. Bhd (Malaysia) α-,β-,γ- and δ-tocotrienol from Davos Life Science (Singapore).	*In vitro* method: Cells were treated either with TTMF or tocotrienol isomers at concentration ranging from 0.3 μM to 10 μM for 16 hours.	Tocotrienol isomers, especially δ-tocotrienol, reduced monocytes adhesion by reducing NFκB(p50) and adhesion molecules expression.
80	Male *Sprague Dawley* rats	-	Annatto tocotrienol from American River Nutrition, Inc. (USA)	*In vivo* method: 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	α-,β-,γ- and δ-tocotrienol from Davos Life Science (Singapore)	*In vitro* method: Cells were treated with the tocotrienol isomers at concentration ranging from 0.3 μM to 10 μM for 16 hours.	Tocotrienol isomers, especially δ- followed by γ-tocotrienol, inhibited expression of adhesion molecules and inflammation through downregulation of NFκB(p50) expression.
79	-	Human umbilical vein endothelial cells (HUVEC)	α-tocotrienol from Malaysian Palm Oil Board (Malaysia)	*In vitro* method: Cells were treated with 5 to 50 μM of α-tocotrienol for 20 hours.	α-tocotrienol reduced NFkB activation (contains promoter binding sites for adhesion molecules) which led to downregulation of adhesion molecules expression.

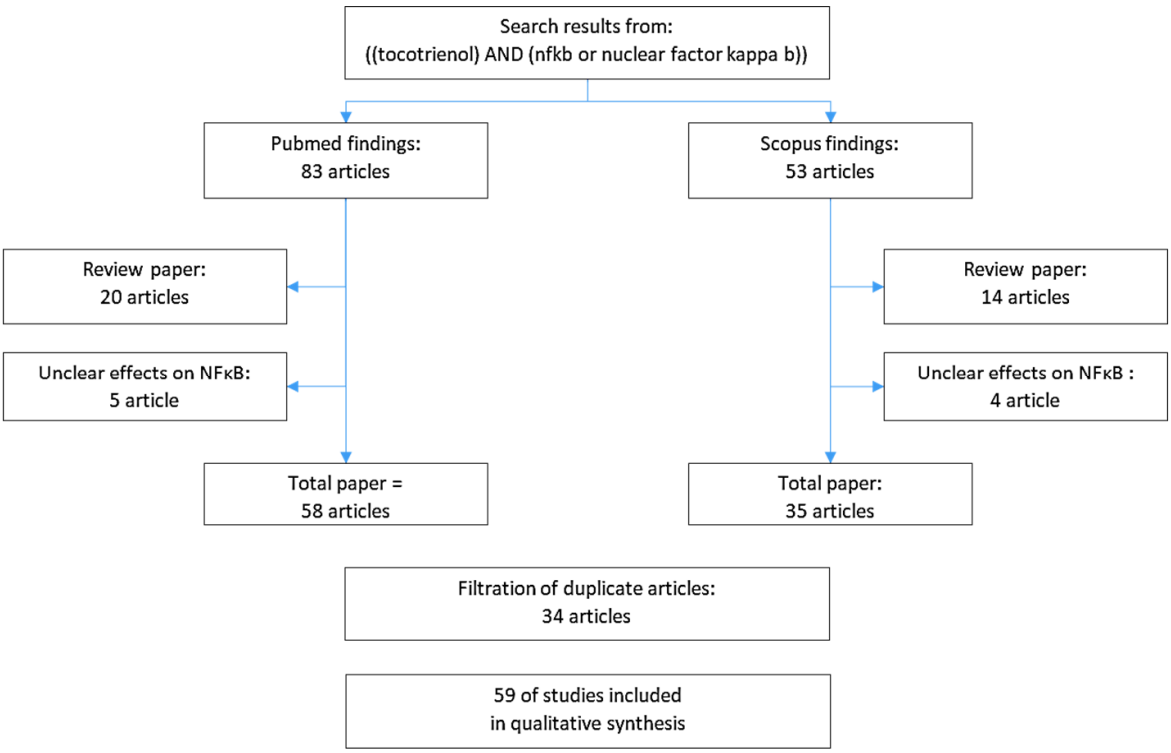

Figure 1. Flow chart showing the article selection process and the number of articles retrieved for this study.

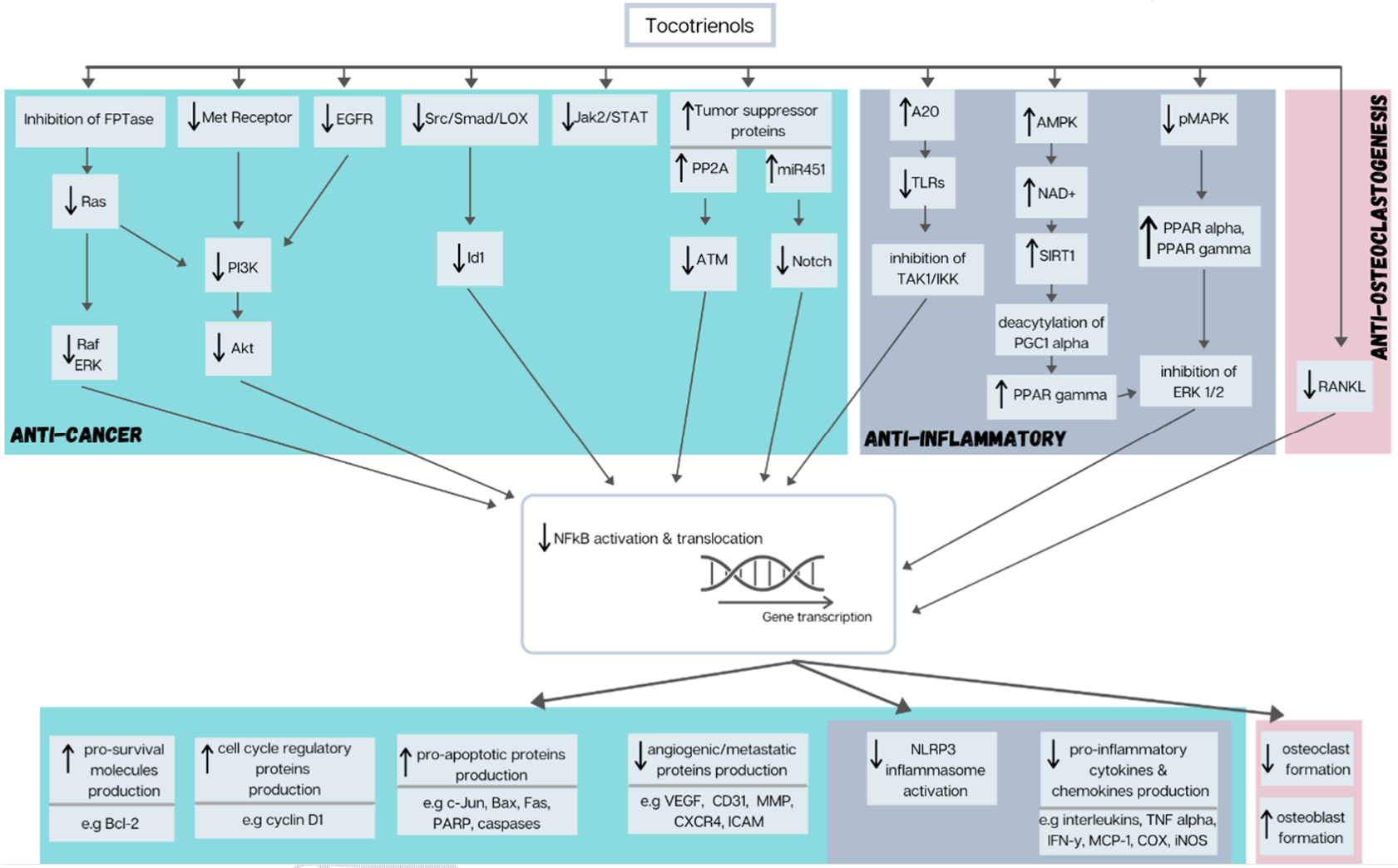

Figure 2. Modulation of NFκB signalling pathways by tocotrienol.