EFFECTS OF VITAMIN E ON COLORECTAL CANCER: A SYSTEMATIC REVIEW OF THE RECENT IN VITRO AND IN VIVO EXPERIMENTAL STUDIES

Shams Hashim NH[1], Md Yusoff NA[1], Mohamad S[1], Mohd Shahpudin SN[1], Silvaragi TGB[1] and Saifuddin SN[1].
[1]Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia

Correspondence:
Siti Nazmin Saifuddin,
Department of Toxicology,
Advanced Medical and Dental Institute,
Universiti Sains Malaysia,
13200 Kepala Batas, Penang, Malaysia
Email: sitinazmin@usm.my

Abstract
Colorectal cancer (CRC) is the second leading cause of cancer death worldwide. Although with advanced medical insight and care, patients with late-stage CRC have a poor prognosis due to a lack of efficient therapy. Numerous scientific studies on the effects of vitamin E on cancer have been undertaken over the last few decades, with mixed findings. Thus, the goal of this review was to compile and summarize the data on vitamin E in *vivo* and *in vitro* experimental trials. PubMed, Scopus, and Science Direct databases were used for automated search of the literature spanning the period from 2010 to February 2021 using the following keywords: vitamin E or tocopherol or tocotrienol; and colorectal cancer or colorectal carcinogenesis or colorectal tumorigenesis. The findings obtained from the ten selected papers on vitamin E, in both *in vitro* and *in vivo* models, exhibited the positive effects of vitamin E on colorectal carcinogenesis, thus, necessitating further research on the notable isomers and metabolites of this compound.

Keywords**:** Vitamin E, Tocopherol, Tocotrienol, Colorectal Cancer

Introduction

Colorectal cancer (CRC) is listed as the second most common cause of cancer death, accounting for 9.4% of all cancer deaths globally (1). The increased number of CRC cases among younger generations especially at the age of 50 and below implies a significant risk to public health. In developing nations, the rise in CRC incidences has been associated with heightened exposure to modifiable risk factors resulting from a shift in lifestyle towards westernization such as obesity, sedentary, poor diets, alcohol drinking, and smoking (2). The current outlook for CRC therapy is more favourable compared to before, which is attributable to the advances in understanding the pathophysiology of CRC and increased treatment options (3). However, survival for colorectal patients remains poor as currently there is no any effective treatment options for advanced-stage colorectal cancer.

Vitamin E plays an important role in nutrition-based illness improvement and prevention (4). Vitamin E can be divided into tocopherol and tocotrienol homologs based on their chemical structure. α, β, γ, and δ are the four homologs of each tocopherol and tocotrienol (5). Same mechanism is used for the absorption and metabolism of tocopherols and tocotrienols (6). Absorption occurs in tandem with dietary fat, and lymphatics transport it to peripheral tissues such as skin, adipose tissue, bone marrow and muscle. Alpha-tocopherol is the least metabolized because it is protected by the liver's alpha-tocopherol transfer protein (α-TTP). Vitamin E isoforms besides alpha-tocopherol are catabolized by CYP450-mediated x-hydroxylation or side chain oxidation, yielding 3'-carboxychromanol or 2'-carboxyethyl-6-hydroxychromans as the end metabolite. These eight lipophilic antioxidants of vitamin E are said to be the highly prominent lipid-soluble antioxidant in human plasma (7). Nuts and edible vegetable oils are known to be the major sources of vitamin E, with corn, soybean, and peanut oils being the most common ones (8, 9).

Until the previous decade, most studies focused on alpha-tocopherol, with only a few studies examining the role of other tocopherol isoforms and tocotrienols (10). Although numerous scientific investigations on the

effects of Vitamin E in *in vitro* and *in vivo* studies have been documented, a comprehensive evidence-based review of its modulation on colorectal cancer is inconclusive. Thus, this systematic literature review is conducted to summarize the most recent available *in vitro* and *in vivo* data on the effects and mechanisms of action of vitamin E on colorectal cancer.

Materials and Methods

This study was conducted according to the Reporting Standards for Systematic Evidence Syntheses (ROSES) protocol (11). PubMed, Scopus, and Science Direct databases were used for the automated search of the literature spanning the period from 2010 to February 2021. The following keywords were used to retrieve all the included studies; vitamin E or tocopherol or tocotrienol; and colorectal cancer or colorectal carcinogenesis or colorectal tumorigenesis. In addition, a manual search of the reference list of the shortlisted articles for additional data or forward citations was conducted. The title and abstract of each article obtained were assessed, and any duplicated articles were excluded from the list. Irrelevant articles were also omitted. The remaining articles were then evaluated further to ensure that they met the review's inclusion criteria. Articles in languages other than English, epidemiological studies, clinical studies, vitamin E used in combination with other treatments, conference proceedings, abstracts in symposiums and congresses, review articles, commentaries and letters were excluded. The Toxicological data Reliability Assessment Tool, also known as ToxRTool (12), was used for the appraisal of the selected articles.

Results

Characteristics of the Selected Studies

The literature search yielded 762 results (from the year 2010 to February 2021). The numbers of articles extracted from the respective databases are as follows: ScienceDirect (n=516), PubMed (n=70), and Scopus (n=176). After removing the 80 duplicate articles, 682 articles remained. The remaining studies were screened individually based on their titles and abstracts, where only eight studies were found to fulfill all the inclusion criteria. Two other studies were included after performing manual curation of the references of the selected papers. A total of ten articles were selected for final review. Out of these selected studies, four of them comprised both *in vitro* and *in vivo* experimental studies whereas three articles exclusively studied on *in vivo* and *in vitro,* respectively (Figure 1).

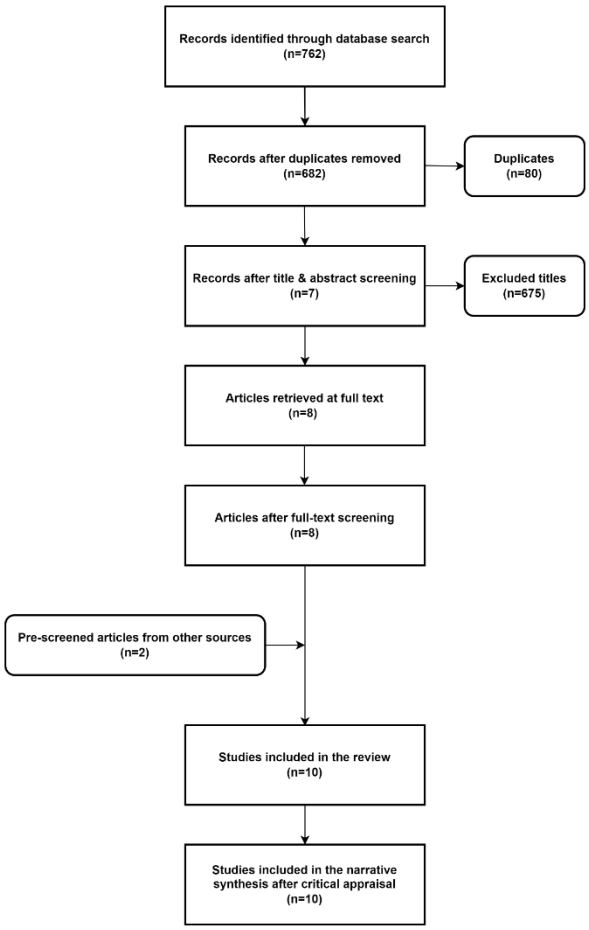

Figure 1: Article processing and selection process

Preclinical Evidence from In Vitro Studies

Of the ten studies included in this review, seven of which investigated the effects of vitamin E in *in vitro* model. The details of the experiment and the main findings of these studies are tabulated in Table 1.

A study by Zappe et al. (13) was the only study that used a mixture of Vitamin E that includes both tocopherols and tocotrienols groups from natural origin. Their research investigated on how vitamin E counteracted oxidative stress in the colorectal cancer cell line (Caco-2) at different glucose concentrations, which may need to be taken into account when studying relationships between antioxidative mechanisms and epigenetics. Antioxidants like vitamin E, supplemented in low concentrations, showed to be an effective way to neutralise hyperglycaemia-induced oxidative damage which has great therapeutic potential and may even be useful in the prevention of diseases like obesity and diabetes. It also has a cancer protective potential besides having an impact on epigenetic regulation that is involved in DNA repair. Furthermore, this study also demonstrated that

treatment of vitamin E on Caco-2 cells reduced hydrogen peroxide (H2O2) -induced lipid peroxidation as well as caused an upregulation of the DNA repair gene, MutL homolog 1 (MLH1) and DNA methyltransferase 1 (DNMT1) expressions. Loss of DNA methylation has been associated with genomic instability (Cheung et al., 2009). In this study, long interspersed nuclear elements 1, (LINE1) global methylation, was found to be increased by vitamin E treatment. All these results prove the advantageous effect of the low concentration of vitamin E in counteracting oxidative stress.

Jang et al. (14) observed the different impacts between delta-tocopherol, delta-tocotrienol, and gamma-tocopherols metabolites on apoptosis, pro-inflammatory and autophagy in human colorectal cancer cells, HCT-116 and HT-29. All treatments suppressed the HCT-116 and HT-29 cells viability in a dose- and time-dependent manner with the highest potential showed by delta-tocopherol metabolite followed by delta-tocotrienol metabolite. Both metabolites induced apoptosis and autophagy in HCT-116 cells with a stronger reaction observed in delta-tocopherol metabolite compared to delta-tocotrienol metabolite. On top of that, they are also superior compared to delta-tocopherol and gamma-tocopherol in terms of inhibiting COX-2 and 5-LOX enzyme activities. However, this effect on reducing inflammation was partially reversed by arachidonic acid. The presence of arachidonic acid reversed the effect of these metabolites on the modulation of COX-2 and 5-LOX enzymes, as well as other mechanisms independent of their suppression. This study found that those metabolites have anti-inflammatory and anticancer properties by inducing apoptosis and autophagy in colon cancer cells through sphingolipid modulation via targeting DEGS and potentially activating sphingomyelin hydrolysis, highlighting the impactful potential of new cancer preventive and therapeutic agents.

Gamma-tocotrienols, γ-T3 gained much attention in combating human CRC cells in a study by Prasad et al. (15) and Xu et al. (16). In the study by Prasad et al., treatment of γ-T3 affected the HCT-166 cell line by causing apoptosis dose-dependently as well as inhibiting HCT-166 colony-forming ability at the highest γ-T3 treatment concentration. Moreover, γ-T3 treatment obstructed the expression of proteins associated with cell proliferation, survival and invasion, as well as angiogenesis and metastasis. These findings are provided with support by Xu et al., a study that also demonstrated γ-T3's anticancer activities by reducing viability, preventing growth, and inducing apoptosis in HT-29 CRC cells. These effects of γ-T3 were modulated by β-catenin/T-cell factor signalling pathway.

Wada et al. (17) and Husain et al. (18) methodically compared both vitamin E groups, tocopherols and tocotrienols, and their effects on CRC cells. All 8 isomers, at the same dose of 20μm, were used to treat mouse rectal polyploid carcinoma (CMT-93), human colon cancer cells (HT-29), murine colon adenocarcinoma cell line (Colon 26) and p53-deficient mouse embryonic fibroblasts (MEFs) (17). Gamma-tocotrienol, beta-tocotrienol and delta-tocotrienol inhibited the CMT-93 cell proliferation, with the most potent effect seen in delta-tocotrienol. Subsequent tests were focused on delta-tocotrienol, which was later found that at even a small dose of 10μM concentration, it had the capabilities of inhibiting cell proliferation in all CMT-93, HT-29, and Colon 26 cell lines. Meanwhile, at a concentration of 40μM, the treatment of delta-tocotrienol increased the number of early apoptotic cells. Treatment of delta-tocotrienol was further enhanced when CMT-93 was co-cultured with stimulated MEF thereby inhibiting proliferation by blocking the COX-2/PGE2 pathway, which involves carcinogenesis. A study conducted by Husain et al. (18) compared all 8 isomers of vitamin E and their respective impact on the proliferation of 4 types of CRC cells (HCT-116, HT-29, SW480, and SW620) with normal colonic mucosal cells (NCM460) as control. The findings showed that all isomers of tocotrienols, except alpha, inhibited HCT-116 cell proliferation at all concentrations. No significant effect was observed with any of the tocopherol isoforms, namely alpha-, beta-, gamma-, delta- tocopherol-treated cells. Delta-tocotrienol outstandingly inhibited all 4 types of CRC cells, dose-dependently while no cytotoxic effect was observed on the NCM460 proliferation. A subsequent test on delta-tocotrienol treatments found that it induced apoptosis in HCT-116, HT-29, and SW620 cells with no effect observed in normal cells. Delta-tocotrienol treatments were furthermore found to inhibit migration as well as invasion of SW620 and HCT-116 cells as well as suppressed the malignant transformation of HCT-116 and SW620 cells at a concentration of 50μmol/L.

As observed by Zhang et al., at concentrations greater than 5mol/L, delta-tocotrienol inhibited SW620 cell proliferation in a dose-dependent manner (19). This study also found that delta-tocotrienol also caused paraptosis-like cell death in SW620 cells, which was characterised by cytoplasmic vacuolization and swelling of mitochondria endoplasmic reticulum with intact nuclei. It was also noted that delta-tocotrienol down-regulated the expression of Wnt-signalling proteins, wnt-1 and β-catenin, dose-dependently as well as the downstream target proteins of the pathway, namely cyclin D1, c-jun and MMP-7.

Table 1: Summary of *in vitro* studies of vitamin E on colorectal cancer

References	Test model	Type of vitamin E	Dose/ concentration	Main findings
Prasad et al., 2016	Human colon cancer cells HCT 116, HT29 and Caco-2	γ - tocotrienol (γ-T3)	5-50μM	Treatment of γ-T3 • suppressed the growth of all 3 types of human CRC cell lines dose- and time-dependently. • induced apoptosis in HCT 116 dose-dependently. • inhibited the colony-forming ability of HCT 116 cells at the highest treatment concentration. • inhibited the expression of proteins involved in cell proliferation, survival, and invasion, as well as angiogenesis and metastasis. In capecitabine-treated HCT 116 cells • γ-T3 enhanced cell death and colony formation. • γ-T3 downregulated the survival, proliferation, and metastasis proteins' expressions.
Zappe et al., 2018	Human colorectal adenocarcinoma cell line Caco-2 under normal and high glucose cell culture conditions.	Vitamin E mixture (containing micellized d-α-tocopherol 20IU/ml, other tocopherols 15mg/ml and tocotrienols 2mg/ml from natural origin)	10 and 50μM	In the oxidative stress experiment • Vitamin E inhibited malondehyde (MDA) level in H_2O_2-induced oxidative stress of Caco-2 cells dose-independently with 10μM of vitamin E proved to be more efficient than 50μM. • No significant changes in ROS/superoxide levels in vitamin E-treated Caco-2 cells In MLH1 and DNMT1 gene regulation experiments • Vitamin E upregulated the expression of the MLH1 gene under both glycemic conditions in Caco-2 cells and H_2O_2-treated Caco-2 cells. • 10μM vitamin E increased DNMT1 expression under hyperglycemic conditions. • Combined incubation of vitamin E and H_2O_2 significantly increased DNMT1 expression under hyperglycemic conditions. In the LINE1 methylation experiment • LINE1 methylation decreased in vitamin E-treated Caco-2 cells in normoglycemic condition. • Vitamin E increased LINE1 methylation in H_2O_2-treated Caco-2 cells in hyperglycemic condition.
Husain et al., 2019	Human colon cancer cells (HCT 116, HT29, SW480 and SW620) and normal colonic	α-tocopherol (α-TP), β-tocopherol (β-TP), δ-tocopherol (δ-TP),	10-100μmol/L	Proliferation effect of α-TP, β-TP, δ-TP, γ-TP, α-T3, βT3, δ-T3, γ-T3 • βT3, δ-T3, γ-T3 inhibited HCT-116 cell proliferation at all concentrations whereas no significant effect was observed with α-T3, α-TP, β-TP, γ-TP and γ-TP-treated cells.

	mucosal cells (NCM460) as control	γ-tocopherol (γ-TP), α-tocotrienol (α-T3), β-tocotrienol (β-T3), δ-tocotrienol (δ-T3), and γ-tocotrienol (γ-T3)		• δ-T3 inhibited the proliferation of SW620, HCT116, HT-29, and SW480 cells dose-dependently while no effect observed on the NCM460 cell proliferation. Treatment of δ-T3 • suppressed the malignant transformation of SW620 and HCT 116 cells at concentration of 50μmol/L. • induced apoptosis in HCT 116, HT-29, and SW620 cells with no effect observed in normal cells. • inhibits migration and invasion of SW620 and HCT 116 cells. • inhibits epithelial-to-mesenchymal transition in SW620 and HCT 116 cancer cells by increasing E-cadherin expression and decreasing the vimentin expression in both cells. • suppressed the expression of cancer angiogenesis, inflammation, metastasis, and β-catenin markers in SW620 and HCT 116 cells.
Jang et al., 2016	HCT 116 and HT-29 cells	Metabolites of δTP (δT-13'-COOH) and δT3 (δTE-13'-COOH), δT and γT	5μM, 10μM, 20μM of δTP and δT3 metabolites; 50μM of δTP and γTP	Effects of treatment on COX-2 and 5-LOX activities • Both δTP and γTP did not inhibit COX-2 or 5-LOX whereas both metabolites inhibited these enzymes. • Anticancer effect of both metabolites was partially reversed by arachidonic acid and their modulation of effect may result from the inhibition of COX-2 and 5-LOX as well as additional mechanisms independent of suppression of these enzymes. • All treatments suppressed the viability of HCT-116 and HT-29 cells time- and dose-dependently with the highest potential showed in δTP metabolite followed by δT3 metabolite and both tocopherols. Both metabolites • induced apoptosis and autophagy in HCT 116 cells with stronger reaction observed in δTP metabolite compared to δT3 metabolite. • modulated sphingolipids in a dose- and time-dependent manner. • suppressed DEGS1 enzyme activity without altering its protein expression.
Wada et al., 2017	Mouse rectal polyploid carcinoma (CMT93), human colon cancer cells (HT29), murine colon adenocarcinoma cell line (Colon	α-tocopherol (αTP), β-tocopherol (βTP), δ-tocopherol (δTP), γ-tocopherol (γTP), α-tocotrienol	20μM (all vitamin E isoforms); 5-40 μM of δT3 for subsequent test	In the cell viability assay, βT3, γT3 and δT3 inhibited the CMT93 cell proliferation with the most potent effect seen in δT3. Treatment with δT3 • inhibited cell proliferation in all CMT93, HT29, and Colon 26 cell lines at 10μM concentration. • increased the number of early apoptotic cells at a concentration of 40μM.

	26) and p53-deficient mouse embryonic fibroblasts (MEFs)	(αT3), β-tocotrienol (βT3), δ-tocotrienol (δT3), and γ-tocotrienol (δT3)		• anti-proliferative effects of δ-T3 at 5μM concentration on HT29 were reversed with application of caspase-3 and caspase-9 inhibitors. • suppressed the induction of NO and prostaglandin E2 in MEF cells. • inhibited CMT93 proliferation, which was enhanced when co-cultured with stimulated MEF, by blocking the COX-2/PGE2 pathway.
Xu et al., 2011	Human colon carcinoma HT-29 cell line	γ-tocotrienol (γT3)	0, 15, 30, 45 and 60 μmol/l	Treatment of γT3 • inhibited β-catenin/Tcf signalling in HT29. • down-regulated total β-catenin expression without affecting the level of β-catenin phosphorylation. • affected the intracellular localization of β-catenin. • decreased Wnt/β-catenin target gene expression, namely cyclin D1, c-myc and survivin • decreased the HT29 cell proliferation dose-dependently. • caused a dose-dependent increase in apoptotic cells and morphological changes in HT29 cells, such as chromatin condensation, deformation and nuclear fragmentation.
Zhang et al., 2011	Human colon cancer SW620 cells	δ-tocotrienol (δT3)	1-40 μmol/L of δT3	Treatment of δT3 • induced paraptosis-like cell death in SW620 cells (cytoplasmic vacuolization and swelling of mitochondria endoplasmic reticulum with intact nuclei). • inhibited SW620 cell proliferation in a dose-dependent manner at concentrations above 5μmol/L. • down-regulated the expression of Wnt signalling proteins, Wnt-1 and β-catenin, dose-dependently as well as the downstream target proteins of the pathway, cyclin D1, c-jun and MMP-7.

Preclinical Evidence from In Vivo Studies

Seven out of ten studies investigated the effects of vitamin E in *in vivo* model. The details of the experiment and the main findings of these studies are tabulated in Table 2. Before 2007, the study of vitamin E was overwhelmed by tocopherols in general, which mostly used alpha-tocopherol compared to tocotrienol and its isomers (20). A trend in the preference of vitamin E isomers for *in vivo* studies of CRC throughout the last decade could be observed. Up to 2015, the tocopherol group was still popular with the focus largely on gamma-tocopherols and a mixture of tocopherols isomers (21, 22). However, recently, studies focusing on tocotrienols isomers and a mixture of tocopherols and tocotrienols have started to gain interest.

Li et al. (22) used a mixture of tocopherols (γTmT) where nearly 60% was gamma-tocopherols together with delta-, alpha- and beta-tocopherols. These findings indicate that γTmT's antioxidant and anti-inflammatory properties in the colon are primarily attributed to tocopherols' direct role in trapping reactive nitrogen and oxygen species, rather than the antioxidant enzymes and anti-inflammatory proteins' activity regulated by Nrf2. The tocopherol metabolites' serum levels appear to be influenced by Nrf2 knockout signifying that tocopherol metabolism may be affected by Nrf2 knockout mice. This was reflected in Nrf2 mice having relatively higher serum levels of CMBHCs and CEH than wild-type mice. Seemingly, tocopherols poses the ability to directly trap RONS while also performing other anti-inflammatory functions. However, all these molecular basis needs further investigation.

Jiang et al. (21) compared the effects of gamma-tocopherols and a mix of tocopherol (gamma-, delta- and alpha-tocopherol) supplementation that was formulated on the basis that gamma-tocopherol and delta-tocopherol generally co-exist in various food sources, and both seems to be parallelly effective in anti-inflammatory activities. A different number of cycles of AOM/DSS was given to induce a different level of colitis among the different groups of mice. It was found that a gamma-tocopherols-supplemented diet and its protective benefits were only seen in moderate colon inflammation but reversed in severe conditions. Identifying biomarkers that address inflammation severity could be beneficial in predicting the possible consequences from a dietary intervention. Thus, they recommended that any future study should assess the severity of inflammation before starting dietary intervention to gain most of its benefit and predict the potential outcome.

Prasad et al. (15) discovered that gamma-tocotrienol can restrict the proliferation and growth of CRC cells via the suppression of proteins that regulate cell growth and down-regulation of several cell signalling molecules. Their findings also demonstrated its ability to inhibit NF-kB and related biomarkers involved in carcinogenesis' proliferation, angiogenesis, invasion and metastatic processes. Thus, gamma-tocotrienol has substantial potential to be useful for CRC patients.

Jang et al. (14) reported the high potential of metabolites delta-tocotrienol (δTE-13'-COOH) in suppressing the growth and multiplicity of colon tumours in mice. The amount given to the mice in their experiment corresponds to a daily intake of 200mg for a 70kg person and is considered a medium supplement dose. Their findings show that delta-tocotrienol metabolites poses anti-inflammatory and anticancer properties, which may contribute to *in vivo* anticancer effects and are also considered as promising novel cancer prevention agents.

A study by Wada et al. (17) using a diet rich in delta-tocotrienols found that this diet greatly reduced the development of colon cancer. Delta-tocotrienol had the great anti-proliferative effect of the four isoforms studied, and stimulated caspase-3 and caspase-9 to cause apoptosis in colon cancer cells. Delta-tocotrienol also had an indirect anti-proliferative effect on cancer stromal cells by decreasing the levels of the chemical cancer mediators COX-2 and nitrite oxide (NO). Greater research of delta-tocotrienol-rich diet's effect in cancer prevention is required.

Another study by Husain et al. (18) also investigated the effect of delta-tocotrienol on colorectal carcinogenesis where they found that delta-tocotrienol dramatically reduced colorectal polyps by 70% and colorectal cancer by almost 99%, and the cancer suppression effect was more potent than sulindac which only inhibited cancer at 50%. The findings suggest that delta-tocotrienol has a great potential for further elucidation of its effect as a chemopreventive agent in colorectal cancer.

The most recent study by Yang et al. (23) using delta-tocotrienol also supported these previous studies. They demonstrated that delta-tocotrienol and its metabolites, delta-13'-carboxychromanol were found to reduce cancer, lower pro-inflammatory cytokines, and modify the gut microbiota.

Table 2: Summary of *in vivo* studies of vitamin E on colorectal cancer

References	Test model & intervention	Dose & route of administration	Duration of study	Main findings
Husain et al., 2019	Azoxymethane-induced colorectal carcinogenesis model in Female Fisher rats (6 weeks old, 120–140 g). The animals (n=40) were randomized into 4 groups (10 rats/group) as follows: 1) untreated 2) vehicle (oral olive oil), 3) sulindac (20 mg/kg orally), 4) δT3 (200 mg/kg orally twice a day) The treatments were given orally twice daily.	δ-tocotrienol (δT3), 200 mg/kg, orally twice a day	20 weeks	δT3 shown to be significantly inhibited colorectal polyps by 70% and tumour formation by almost 99% compared with the vehicle treatment group, and the cancer inhibition effect of δT3was more potent than sulindac. In immunohistochemistry staining, no aberrant crypt foci (ACF) were observed in δT3-treated rat colons whereas all other groups showed positive staining for ACF.
Prasad et al., 2016	HCT116 cells tumour was implanted into the leg of 4-week-old male athymic mice. After 10 days, mice were randomised into four groups (5 mice/group), as follows: 1) corn oil vehicle (100 ml, daily) 2) γ-T3 alone (100 mg/kg, 5/week) 3) capecitabine alone (60 mg/kg, twice per week) 4) γ-T3 (100 mg/kg, 5 times per week) and capecitabine (60 mg/kg, twice per week) All treatments were administered orally for 2 weeks.	γ-tocotrienol (γT3) 100mg/kg	18 days	• γ-T3 inhibited the growth of human CRC • γ-T3 inhibited Ki-67 expression • γ-T3 inhibited the expression of cell survival proteins • γ-T3 suppressed cell proliferation proteins expression • γ-T3 inhibited metastatic proteins expression • γ-T3 downregulated inflammatory transcription factor NF-kB/p65
Jang et al., 2016	Azoxymethane (AOM)/dextran sodium sulfate (DSS)-induced colorectal carcinogenesis model of Male Balb/c mice (5-6 weeks old). AOM/DSS-induced mice were randomized into AIN-93G (control group) or δTE-13'-COOH-supplemented group (n=15-17).	δTE-13'-COOH (0.022% in diet) was prepared in the AIN93G-based diet. This dose is equivalent to 200 mg daily intake for a 70 kg person and represents a medium supplement dose.	43 days	δTE-13'-COOH significantly suppressed the growth and multiplicity of colon tumours in mice.

Jiang et al., 2013	AOM/DSS-induced colorectal carcinogenesis model in male BALB/c mice (5- 6 weeks). In AOM-DSS studies 1 and 2, mice were given tocopherols supplemented diet 7 days before AOM was injected. In AOM-DSS study 3, mice were given gamma tocopherols supplemented diet 3 days after AOM injected. The control group were fed the AIN-93G diet.	AIN-93G diet supplemented with γ tocopherol (γTP) or mixed tocopherol (mTP) at 0.1% diet, respectively. Mixed tocopherols were made by mixing 45% γTP, 45% δTP, and 10% α-tocopherol acetate.	AOM-DSS Study 1: 69 days. AOM-DSS Study 2: 147 days. AOM-DSS Study 3: 54 days	γTP or mTP did not exert any protective effects on tumorigenesis induced by AOM/DSS (3 cycles). Colon inflammation induced by three cycles of 2.5% DSS was not protected by γTP supplementation. γTP reduced tumour incidence in tumorigenesis induced by AOM/DSS (1.5%, 1 cycle) better than mTP. γTP supplementation inhibited tumorigenesis induced by AOM/DSS (1.5%, 2 cycles) even when the supplementation was started after AOM injection. γTP but not mTP reduced colon inflammation induced by one cycle of 1.5% DSS. Measurement of tocopherols and metabolites concentrations in the plasma and faeces showed that: • γTP and mTP supplementation showed an increase of γTP or both γTP and δTP in the plasma and faeces, respectively. • faecal excretion of γTP in mice fed a γTP-supplemented diet was 2-fold higher than that in those fed mT and greater excretion of γTP than δTP from the latter diet despite equal amounts of both types in this diet. • Supplementation of γTP or mTP led to marked increases in faecal excretion of all carboxychromanol metabolites with 13'-COOHs being the highest.
Wada et al., 2017	Male C57BL/6 mice (7 weeks old, n=109) were divided into five groups 1) 18 mice were treated with intraperitoneal (i.p.) saline injection, without DSS feeding (sham group) 2) 6 mice were treated with i.p. saline	Vitamin E content in the diets (per 100 g) was 50.2 mg tocopherol and 70.4 mg tocotrienol in the TRF group, and 23.6 mg tocopherol and 75.0 mg tocotrienol in the	70 days	δT3-enriched diet significantly inhibited colorectal cancer formation and COX-2 expression in AOM and DSS-induced colon cancer mouse models compared to TRF and control groups.

	injection, with DSS feeding (sham DSS group) 3) 30 mice were treated with 12.5 mg/kg AOM, by i.p. injection, with DSS feeding (control group) 4) 27 mice were treated with 12.5 mg/kg AOM, by i.p. injection, with DSS feeding, and fed 0.1% Mixed Tocotrienols 92® diet (TRF group) 5) 28 mice were treated with 12.5 mg/kg AOM, by i.p. injection, with DSS feeding, and fed 0.1% DeltaGold® (δT3 group)	δT3 group		
Li et al., 2012	Nrf2 knockout (Nrf2 -/-) female mice (C57BL/SV129) and wild-type (C57BL/6 J) female mice with matched age (5 weeks old). Nrf2 and wild-type mice were maintained on 0.03, 0.1, or 0.3% γ-TmT-enriched AIN93M or the AIN93M (control) diets. (n=5/group)	A mixture of tocopherols (γ-TmT) was used which contained 57% γTP, 24% δTP, 13% αTP, and 1.5% βTP.	24 days	Treatment of γ-TmT reduced the inflammation indexes, 8-oxo-dG levels, leukocyte infiltration and nitrotyrosine levels in the colon and serum levels of 8-isoprostane and PGE2 of both wild-type and Nrf knockout mice in a dose-dependent manner. Measurement of serum levels of tocopherols showed the γ-TmT treatment significantly increased the serum levels of AT, γTP and δTP in both Nrf2 (-/-) and wild-type mice, but the levels of the latter two were still much lower than those of AT. However, DSS treatment was also found to increase the γTP serum levels in both the wild-type and the Nrf2 mice that did not receive γ-TmT. γ-TmT supplementation increased the serum levels of the tocopherol's metabolites, carboxymethylbutyl hydroxychroman (CMBHC) dose-dependently with γ-CMBHC being the most prominent metabolite followed by δ-CMBHC and α-CMBHC. The levels of γ-, δ-, and α-carboxyethyl hydroxychroman (CEHC) were lower than the corresponding CMBHC metabolites, following the same rank order of concentrations as the latter.

Yang et al., 2021	AOM/DSS-induced colorectal carcinogenesis model of Male Balb/c mice (6-7 weeks old) were randomized into AIN-93G (control), delta-tocotrienol-supplemented (δTE) and 13'-carboxychromanol-supplemented (δTE-13) groups.	δTE/γTE (8/1) (0.035% diet, about 2.2 µm), daily. δTE-13 (0.045% diet, 2.3µm) daily. These doses are equivalent to a daily intake of 200 or 230mg for a 60kg adult.	2 months	δTE and δTE-13 inhibited AOM/DSS-induced colon tumorigenesis. δTE decreased IL1-β whereas δTE-13 inhibited GM-CSF and MCP-1, which are pro-inflammatory cytokines. In the experiment observing the effects of treatment on gut microbiota, • δTE and δTE-13 showed modulation of effect on gut microbes' composition but not their richness compared to the control. • δTE and δTE-13 enhanced potentially beneficial *Bacteroides* and *Lactococcus*, where the increment of the latter positively correlated with faecal concentrations of δTE-13 and its hydrogenated metabolite. • δTE-13 reversed AOM/DSS-induced depletion of *Roseburia* whereas δTE elevated *(Eubacterium) coprostanoligenes*.

Discussion

The focus of vitamin E investigation until the previous decade was on alpha-tocopherol and its anticancer characteristics (10). Large clinical trials with alpha-tocopherol, such as the SELECT study (24) and the ATBC trial (25), however, have failed to show meaningful results. The SELECT study, which ran from 2001 to 2004, in 427 states across the United States, Canada, and Puerto Rico, was an important interventional study. It was a randomised, double-blind, placebo-controlled trial that investigated the effects of selenium and alpha-tocopherol on cancer risk. The study found no evidence of a preventive benefit of alpha-tocopherol supplementation, but it did indicate a non-significant rise in the risk of prostate cancer (24). Meanwhile, the ATBC study looked at how alpha-tocopherol and beta-carotene affected the risk of lung cancer as well as other malignancies among male smokers. There was no association between alpha-tocopherol and lung cancer in this primary prevention trial with a statistically significant negative connection (25).

As a result of the dismal findings of alpha-tocopherols in SELECT & ATBC, isomers such as gamma-tocopherol, delta-tocopherol, and tocotrienol were explored. Gamma-tocopherol and delta-tocopherol, unlike alpha-tocopherol, can scavenge both reactive nitrogen and reactive oxygen species. On the other hand, tocotrienols' anti-cancer effects differ from those of other vitamin E isoforms due to their anti-angiogenic properties, regulation of the NF-kB pathway, and suppression of the HMG-CoA reductase enzyme (5). Pre-clinical studies on vitamin E of non-alpha tocopherol isoforms revealed promising anticancer benefits (10).

Earlier studies on vitamin E have been shown to have a variety of good and detrimental impacts on human health, raising questions about its usefulness and benefits (24, 25). Nevertheless, there were interventional studies that revealed the benefits of vitamin E as it possesses anti-inflammatory characteristics, anticancer properties, anti-diabetic, protective potential against eye disease, cardiovascular protective features and helps to delay the ageing process (26). The antioxidant, anti-inflammatory, anti-proliferative, anti-angiogenic, immunological modulatory, and HMG CoA reductase enzyme inhibitory pathways have all been linked to vitamin E's anticancer activities (5).

Although some studies indicated promising synergistic benefits between vitamin E and other medications in these cancer patients, adding vitamin E as a complementary treatment for patients suffering from various cancer types resulted in contentious consequences. This is not surprising given that the results of this research are based on various isoforms of vitamin E and/or mixing ratios, as well as varying doses, synthetic racemic or natural isomers, all of which result in varying biologically effective doses (9).

The results obtained in this systematic evaluation of available literature in the last ten years surmised that treatment of vitamin E in colorectal carcinogenesis both in *in vitro* and *in vivo* models produced good substantial data on positive outcomes for colorectal cancer. Most of the isomers studied showed a protective effect of vitamin E against colorectal cancer through modulations of multiple targets and pathways in different experimental models and under different experimental conditions. Collectively, in *in vitro* studies, vitamin E was found to be able to suppress the proliferation of cancer cell lines, induce apoptosis, inhibit colony formation, reduce lipid peroxidation, suppress malignant transformation, and restrain metastasis. Whereas, in *in vivo* studies, vitamin E was able to inhibit tumour formation and multiplicity, suppress colorectal polyps, and halt colon inflammation. Figure 2 depicted the possible mechanism of anticancer action of vitamin E and its isoforms.

The results obtained from this review also showed a good correlation between the *in vitro* and *in vivo* studies. Vitamin E isomers were able to inhibit the viability, proliferation, migration, invasion, metastasis, malignant transformation, and colony-forming ability of the colorectal cancer cell lines investigated in the *in vitro* models as well as other processes related to the carcinogenesis such as inflammation, angiogenesis and oxidative stress. In addition, induction of apoptosis, autophagy and other cell death mechanisms were also observed. These results correlate with the findings reported in the *in vivo* studies as the animals treated with vitamin E isomers also showed the inhibition of colorectal polyps, aberrant crypt foci and tumour formation. Inflammation and tumour multiplicity and incidences were also reduced. Investigations of protein expressions in both *in vitro* and *in vivo* models corroborate all these findings.

Among all the isomers investigated in both *in vitro* and *in vivo* studies, delta-tocotrienol and its metabolites garnered much interest from the researchers due to their superior effects on colon carcinogenesis compared to others.

Conclusion

Vitamin E isomers could be a promising alternative as chemopreventive agents in colorectal cancer though further investigation in the clinical setting is strongly advised. The reviewed literature has several limitations.

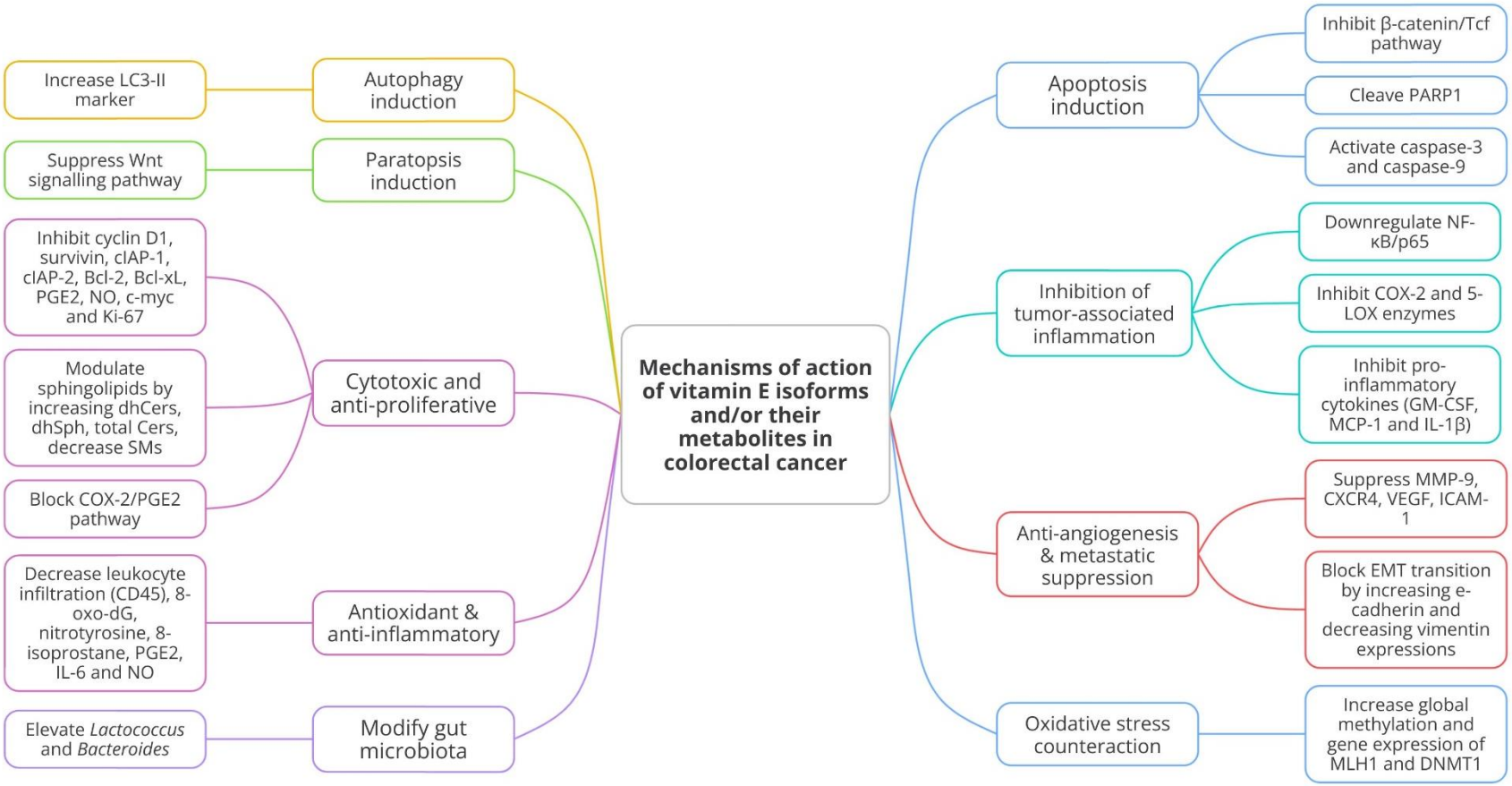

Figure 2: Mechanisms of anticancer action of vitamin E and its isoforms

Further evaluation of the mechanism of actions of vitamin E and its isomers/metabolites based on the severity of cancer needs to be conducted to gain deeper insight into their effects towards colorectal cancer. Some studies took a new path of looking into the effect of this compound on the gut microbes due to the synergistic effect observed on colorectal cancer and this field is worth to be investigated further. It is recommended to conduct more in-depth studies on each of the vitamin E isomers before commencing clinical trials in colorectal cancer patients.

However, there are a few limitations of this study. The major one was the limited availability of research articles being included as all non-available full-text articles were excluded. In addition, grey literature and non-English articles were also excluded from this study.

Conflict of Interest

All authors declare no conflicts of interest.

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