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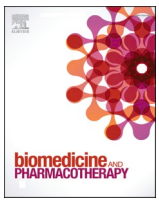

Review

Anethole in cancer therapy: Mechanisms, synergistic potential, and clinical challenges

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ABSTRACT

Cancer remains a major global health challenge, prompting the search for effective and less toxic treatments. Anethole, a bioactive compound found in essential oils of anise and fennel, commonly used as a food preservative, has recently garnered attention for its potential anti-cancer properties. This comprehensive review aims to systematically assess the anti-cancer effects of anethole, elucidating its mechanisms of action, pharmacokinetics, bioavailability, and synergistic potential with conventional cancer therapies. A detailed literature search was conducted across databases including PubMed, Embase, Scopus, Science Direct, Web of Science, and Google Scholar. Criteria for inclusion were experimental studies in peer-reviewed journals focusing on the anti-cancer properties of anethole. Extracted data included study design, intervention specifics, measured outcomes, and mechanistic insights. Anethole demonstrates multiple anti-cancer mechanisms, such as inducing apoptosis, causing cell cycle arrest, exhibiting anti-proliferative and anti-angiogenic effects, and modulating critical signaling pathways including NF-κB, PI3K/Akt/mTOR, and caspases. It enhances the efficacy of chemotherapeutic agents like cisplatin and doxorubicin while reducing their toxicity. *In vitro* and *in vivo* studies have shown its effectiveness against various cancers, including breast, prostate, lung, and colorectal cancers. Anethole shows significant potential as an anti-cancer agent, with its multi-faceted mechanisms of action and ability to synergize with existing chemotherapy. Further clinical research is essential to fully understand its therapeutic potential and application in oncology.

1. Introduction

Cancer is a widespread term used to describe a group of diseases with cells that deviate from their normal growth boundaries, present modified organic functions and may affect adjacent organs and body parts (metastasis) [1]. Cancer results from the interaction between individuals' genetic factors and ambiental factors from different categories: physical, such as UV and ionizing radiation; chemical

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constituents of tobacco smoke, alcohol, asbestos, aflatoxins and arsenic, among others; or biological, such as virus, bacteria or parasites infections [1,2]. Its incidence has been increasing worldwide, with an estimated upsurge from 32 % to 96 % between 2020 and 2040 [2]. Cancer is the second most prevalent cause of deathglobally, after cardiovascular disease, reaching about 10 million deaths in 2020 [1–3]. The burden of cancer impacts individuals, populations, and healthcare systems around the globe. This impact tends to continue growing if incidence and mortality continue to increase, compromising the public health system in several countries) [1,2]. It is estimated that about 40 % of cancers could be prevented by avoiding exposition to risk factors [1,4] and the mortality by cancer could be minimized by early detection and treatment [1,2,5].

Cancer treatment is highly specific to the individual and type of cancer, necessitating an accurate diagnosis for the selection of appropriate treatment modalities. The main treatment options include surgery, radiotherapy, chemotherapy, hormonal therapy, and targeted biological therapies [1,4]. These treatments, whether used alone or in combination, can be aggressive and often lead to undesirable adverse effects. Such adverse effects can be mitigated by integrating complementary therapies, such as herbal components, into the treatment regimen [1,6]. Research by Faal Maleki et al. has demonstrated that conventional treatments, such as surgery, radiotherapy, and chemotherapy, can negatively impact not only cancerous cells but also healthy cells in the body, leading to significant side effects [7]. This aggressiveness of traditional cancer treatments necessitates the exploration of supplementary approaches to reduce these adverse effects [8]. Additionally, Zimmermann-Klevid et al. have provided evidence supporting the efficacy of traditional medicine in cancer treatment, further emphasizing the potential of complementary therapies in oncology [9]. Herbal or phytopharmaceutical compounds have shown inhibitory effects on the rapid division of cancer cells,mitigatingthe adverse effects of chemotherapy and radiotherapy [6,10]. One of the prominent bioactive compounds is Anethole, known for its sweet licorice-like taste and aromatic flavor. It is found naturally in anise, fennel, star anise, and certain herbs like basil and tarragon. Anethole potentialbeneficial impacts on human health includeantioxidant, antifungal, antidiabetic, anti-inflammatory, immunomodulatory, neuroprotective, gastroprotective, anticarcinogenic, chemopreventive, and antithrombotic properties [11,12]. In the past, anise seed assisted in childbirth and stimulated [13] and is continuously recommended for diverse diseases in folk medicines. Trans-anethole in aniseed is used for traditional treatments such as cough relief, respirator congestions, migraines, gastrointestinal distress skin infections, and is used as a tranquilizer and aphrodisiac. Studies [13] have shown that there is the effectiveness of aniseed towards diabetics, dysmenorrhoea, and menopause hot flashes and elucidating its antioxidant, anti-inflammatory and antimicrobial properties. Its potential anti-cancer properties are seen in inhibiting cell growth and inducing apoptosis and cytotoxicity [14]. This review presents a comprehensive outline of insights into anetholes' anti-cancer and pharmacological properties found in the literature.

2. Review methodology

A comprehensive search of the literature was conducted to gather relevant studies on the anti-cancer properties of anethole. The following databases were utilized: PubMed/MedLine, Embase, Scopus, Science Direct, Web of Science, and Google Scholar. The inclusion criteria: studies published in English, experimental studies focusing on the anti-cancer properties of anethole, studies investigating the pharmacokinetics, bioavailability, and anti-cancer effects of anethole, peer-reviewed journal articles, theses, and books, and studies providing sufficient data for analysis.

The exclusion criteria: studies published in languages other than English, reviews, meta-analyses, conference abstracts, and editorials, studies not directly related to the anti-cancer properties of anethole, non-peer-reviewed articles, letters to the editor, and opinion pieces, and studies lacking sufficient data for comprehensive analysis. The search strategy included the use of Medical Subject Headings (MeSH) and keywords related to anethole and its anti-cancer properties. Boolean operators (AND, OR, NOT) were used to refine the search. Keywords and MeSH terms included "Anethole," "Cancer," "Tumor," "Neoplasm," "Carcinoma," "Treatment," "Chemotherapy," "Radiotherapy," "Biological therapy," and "Hormonal therapy." For example, a Boolean search was constructed as follows: ("Anethole" AND "Cancer") OR ("Anethole" AND "Tumor") OR ("Anethole" AND "Neoplasm") AND ("Treatment" OR "Chemotherapy" OR "Radiotherapy" OR "Biological therapy" OR "Hormonal therapy"). The scientific names and taxonomy of plant species containing anethole were validated using the World Flora Online (WFO) database. The chemical structures of anethole and related compounds were verified using PubChem. Data from selected studies were extracted and summarized in tables and figures. The extracted data included study characteristics (authors, year of publication, study design, and objectives), intervention details (concentrations of anethole used, duration of exposure, and combination with other treatments), outcomes measured (anti-cancer effects, including cell viability, apoptosis, and tumor growth inhibition), and mechanisms of action (molecular pathways involved in the anti-cancer effects of anethole).

3. Anethole: general characterization

3.1. Natural sources and traditional uses

Anethole is predominantly found in the essential oils of anise (Pimpinella anisum), fennel (Foeniculum vulgare), star anise (Illicium verum), basil (Ocimumbasilicum), tarragon (Artemisia dracunculus), and in lower concentrations in cilantro (Coriandrum sativum) and lemon balm (Melissa officinalis) [15]. *F.vulgare* (*Foeniculum vulgare*) is a perennial herb of the carrot family (Apiaceae) and has been proclaimed to contain percentages of fats, proteins, carbohydrates, fiber and moisture. Several minerals as: calcium, potassium, sodium, iron, and phosphorus are present while also vitamins such as thiamine, riboflavin, and ascorbic acid [16]. Anethole known as (1-methoxy-4-(1-propen-1-yl) benzene) can be found in essential oils and is aromatically distinctive in anise, star anise and sweet anise. Anethole is also present in low concentrations in basil, cilantro and lemon balm. Anethole has cis- and trans- isomers. The two isomers are known to have different properties. Essential oils protect host plants from pathogens and deflect herbivorous animals from ingesting the plants.Anethole is an essence that is used to add flavors to desserts, confectionaries, and certain alcoholic drinks such as arak, aguardiente, jagermeister, sambuca, anisado and raki [12, 17]. Anethole is also used as a concealing agent in various fragrances including airfreshners, toilet soaps, toothpastes, and mouthwashes.

3.2. Phytochemistry

Anethole is a derivative of alkoxyproppenybenzene and a flavoring component of essential oils obtained from (*Pimpinella anisum*), fennel (*Foeniculum vulgare*), star anise (*Illicium verum*), basil (*Ocimum basilicum*),tarragon (*Artemisia dracunculus*), and in lower concentrations in cilantro (*Coriandrum sativum*) and lemon balm (*Melissa officinalis*) [11, 18]. Anethole is an amber liquid, soluble in water slightly but highly soluble in methanol and ethanol. When water is added to certain anise-flavored liqueurs, it causes them to turn opaque, becoming whitish in colour due to the formation of a micro-emulsion [19]. The versatility of anethole owes to the fact that it is a naturally sourced compound from essential oil and it is a derivative of alkoxy propenylbenzene that can occur naturally in either trans- or cis- forms [11,20–22]. For example, the essential oil from anethole-rich *Clausenaheptaphylla* leaf was shown to have a high anethole content, which makes the plant a cheap source to extract anethole when exploitation is considered commercially [11, 20–22]. It is known that trans-anethole constitutes roughly 90 % of the

isomer form of anethole [23]. Traditional uses of plants that contain anethole include treating neurological disorders, and inflammatory conditions of the skin and respiratory tracts. Trans-anethole is conserved as a food grade and it masks unpleasant odors. It can be widely applied as a concealing agent in soaps, cosmetics, toothpaste and mouthwashes [24]. Anethole is also a food preservative, feed additive, industrial product of artificial flavors and as pesticide [25]. The benzene ring of the organic compound anethole (Fig. 1) has an ether group and an unsaturated hydrocarbon side chain. The para position of the ether group has a methoxy benzene with a prop-1-en-1-yl group. It belongs to the phenylpropanoid compounds and is synthesized from phenol.

Anethole is a plant metabolite characterized by a phenol group, where a benzene ring is attached to a hydroxyl group formed by replacing a hydrogen atom on the carbon with a hydroxyl group.The hydroxyl group in phenol makes the compounds ortho-para positions electron-rich. This is due to the lone pair in oxygen [26]. The O-H bond in phenols is more acidic than in aliphatic alcohols due to resonance stabilization of the phenoxide ion (Fig. 1). Therefore, the proton can be replaced with other groups in the phenol. Anethole exists as cis- and trans-isomers with a double bond outside the ring, with trans-anethole being more commonly used due to its abundance. Other primary constituents of *F. vulgare* are trans-anethole, fenchone, estragole, par-anisaldehyde, and alpha-phellandrene, Díaz-Maroto et al. [27] reported that the concentration of these compounds varied according to the origin of the fennel. Depending on the extraction methods, the chemodiversity of the essential oils of *F. vulgare* is shown. These volatile compounds vary in plants, as shown by Díaz-Maroto et al. [27]. The content and composition of essential oil varies at different stages of maturation of *F. vulgare*. As the fruit matures, the content of the essential oil declines. Telci et al. [28] showed that trans-anethole content varied between 18.6 % and 87.9 %. The major constituents of oleoresin (trans-anethole, phenylpropenes, and estragol) derived from the aerial parts of *F. vulgare* vary, showing maximum growth in flowers and developing mericarps.

Many studies have demonstrated that essential oils exhibit pharmacological activities. Anethole is reported to be an active estrogenic agent and its polymers, dianethole and photoanethole are also estrogenic. Tognolini et al. [29] reported that anethole was a safe anti-thrombotic agent. Zeller and Rychlik [30] showed that estragole was found to be a potential carcinogen. When the essential oils of *Pimpinella anisum* and *F.vulgare* from mature fruits by hydro distillation, were analysed by gas chromatography-mass spectroscopy (GC-MS). The results showed that aniseed oil was characterized by large amounts of trans-anethole, which was 96.80 % and in fennel, it was 83.43 %.

Estragol, which is an isomer of trans-anethole, was found in both plants (in fennel at 1.36 % and in aniseed at 0.19 %). A study by Acimovic et al. [31] on sweet fennel and aniseed growing in northern parts of Serbia showed that trans-anethole was present in high amounts. Estragole was also present in aniseed and sweet fennel, but in small amounts. Punt et al. [32] and Martin et al. [33] studied the metabolism of anethole dithiolethione (ADT, 5- (p-methoxyphenyl)-3H-1,2-dithiole-3-thione) of microsomes from rats and human liver. The results revealed the formation of S-oxide. Estragole (1-ally-4-methylbenzene), derived from anethole, is an isomer which is a volatile phenylpropanoid. Since estragole is derived from anethole, their activities are similar. They are both involved in immunological processes and inflammation, which inhibits the effects of TNF-α. Estragole has anticancer, antioxidant, antiplatelet, anesthetic and antimicrobial activities and is also used in perfumes and beverages [34,35]. Trans-Anethole oxide {2-(4-methoxyphenyl)- 3 methyl –oxirane} and trans-asarone oxide {1-propenyl- 2,4,5- (trimethoxybenzene)} are derived from trans-asarone and trans-anethole by diethyldioxirane. As derivatives, they can be used as natural fragrances and flavouring agents [36]. Studies by Kim et al. [36] also showed that activities resulting in mutagenicity and carcinogenicity are linked to this volatile phenylpropanoid.

3.3. Semi-synthetic derivatives of anethole

Semi-synthetic derivatives of anethole are chemically modified compounds derived from the naturally occurring phenylpropene anethole, aimed at enhancing its pharmacological properties and therapeutic efficacy, particularly in the context of cancer treatment (Fig. 2). Research by Lam et al. in 2002 showed that anethole ditholethione reduced lung cancer progressionin smokers while eugenol and isoeugenol the two other tested substances can inhibit lipid peroxidation and oxidative levels [37]. Lubet et al. reported that anethole trithione significantly inhibited mammary cancer multiplicity in DMBA-induced rat mammary cancer models and azoxymethane - induced colon carcinogenesis [38]. Asarone is a carcinogenic propenylbenzene, which can also be metabolized to epoxide *in vivo* as it has similar properties to anethole [39]. Trans-anethole oxide and trans-asarone oxide have been synthesized (Fig. 2) by oxidation with dimethyldioxiran [40,41]. Every structure contains a pair of distinct chiral atoms, with variations possibly stemming from the employment of diverse oxidizing agents during synthesis and the potential formation of distinct adducts in reactions with various nucleophiles *in vivo*. However, the utilization of dimethyldioxirane in oxide synthesis did not yield structures presenting mixtures of slightly different isomers, as indicated by the absence of evidence in the proton NMR spectra of each compound. Marshall and Caldwell documented that the cytotoxic effects of trans-anethole in rat hepatocytes are due to its metabolic conversion into an electrophilic epoxide [41]. Their study further demonstrated that inhibiting cytosolic epoxide hydrolases with 4-fluorochalcone oxide significantly increased the cytotoxicity of anethole in cultured hepatocytes. Additionally, anethole-derived epoxides independently exhibited substantial

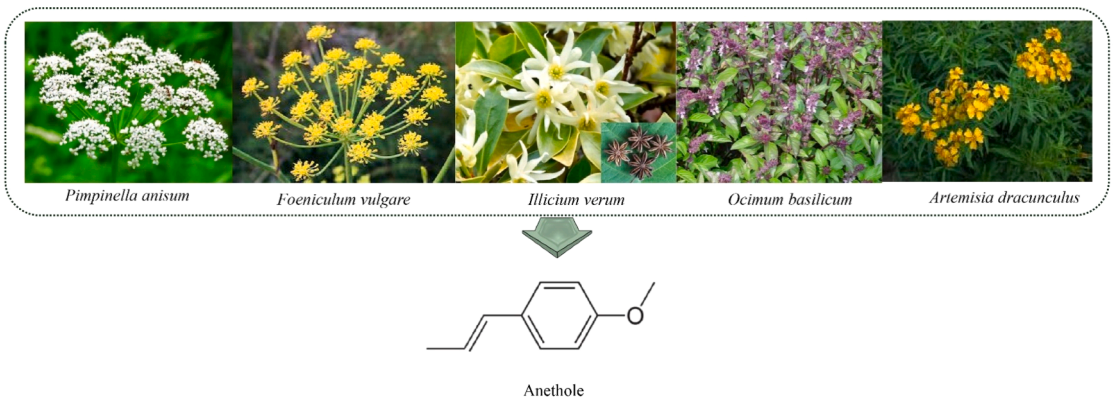

Fig. 1. Natural sources and chemical structure of Anethole.

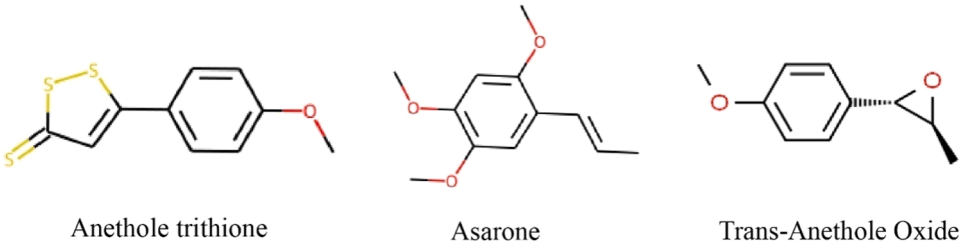

Fig. 2. Semi-synthetic derivatives of anethole.

cytotoxic effects [41]. Previous reports by Sangster et al. and Solheim and Schelineindicated that one of the primary metabolic pathways of anethole involves the formation of epoxide. It is suggested that anethole oxide might contribute to the mutagenic and weak carcinogenic properties associated with anethole [58,59]. Similarly, the oxidation of trans-asarone to its epoxide form could confer mutagenic and carcinogenic characteristics.

These semi-synthetic derivatives exhibit a range of biological activities, including enhanced antioxidant, anti-inflammatory, and gastroprotective effects. However, some derivatives have shown carcinogenic potential, indicating the need for careful evaluation of their safety profiles before considering therapeutic applications.

4. Bioavailability and pharmacokinetics data of anethole and its derivatives

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a thorough review of the metabolism of trans-anethole. The primary metabolic pathways identified include O-demethylation, ω-oxidation of the side chain, and side-chain epoxidation. Following these processes, the metabolites undergo further oxidation and hydration, which are then followed by conjugation with sulfate, glucuronic acid, glycine, and glutathione. Given that O-methylation serves as a deactivation pathway, the toxicity of trans-anethole is predominantly influenced by the varying amounts of metabolites produced through ω-oxidation and side-chain epoxidation. Bounds et al. [42] showed that in isolated rat hepatocytes trans-anethole was metabolized within 6 hours and 4-methoxybenzoic acid (33 %), 4-methoxycinnamic acid (7 %) and 4-methoxycinnamyl alcohol metabolites were identified [43]. The European Food Safety Authority (EFSA) [44] study showed that the excretion of metabolites of trans-anethole was rapid. After oral administration, anethole becomes absorbed in the gastrointestinal tract rapidly and undergoes biotransformation in the liver and it is excreted within 24 hours from the body as several identified metabolites in the urine and biliary excretion [17,45]. Anethole undergoes metabolism through three primary pathways: O-demethylation, ω-hydroxylation followed by side chain oxidation, and epoxidation of the 1,2-double bond [41,46]. Marshall and Caldwell attributed the cytotoxicity of trans-anethole in rat hepatocytes to its conversion into an electrophilic epoxide [41,46]. Similarly, Newberene et al., demonstrated that the biotransformation of anethole involves O-demethylation, N-oxidation, and epoxidation [17, 45]. In studies involving human volunteers administered with carbon-labeled trans-anethole, the metabolism primarily involved oxidation and methylation. Anethole 1, 2-epoxide metabolite was a result of metabolism of a minor portion of the administered dose [17,45]

The bioavailability of anethole is limited due to several factors: it has poor water solubility, which affects its absorption when administered orally; it is slightly soluble in water but highly soluble in organic solvents like methanol and ethanol [19]. Anethole is rapidly metabolized in the gastrointestinal tract and liver upon ingestion, undergoing processes such as O-demethylation, ω-oxidation, and epoxidation [46]. These rapid metabolic transformations reduce the amount of active anethole available for therapeutic action. Anethole's stability in the gastrointestinal environment is compromised, further reducing its bioavailability [19]. As a result, only a small fraction of orally administered anethole reaches systemic circulation in its active form [19]. Data are important because any use of trans-anethole to improve health is affected by the quantity taken in and the nature of its bioactive components in target tissues. In human volunteers, trans-anethole was administered in doses of 1, 50 and 250 mg with an interval of three months [47]. These doses were the amounts of dietary intake of 50 mg to levels of 250 mg taken in several aniseed-containing drinks. Within 8 hours, metabolites such as 4-methoxy-hippuric acid and 4-methoxybenzenoic acid were detected in the urine. The given dose has no effect on the amount or route of elimination noticed, which is not the same with animals.Sangster et al. [48], showed similar results were obtained in human studies when 1 mg of trans-anethole was administered. In one volunteer, trans-anethole serum levels were determined at doses of 56.0, 93.4 and 168.1 mg at intervals of seven days [49]. In this experiment, levels of trans-anethole serum were at 15.5, 25.7 and 73 ng/ml, respectively. When breastfed young mothers were given 100 mg trans-anethole, it surfaced in breastmilk at 4.3 to 9.9 μg/l concentrations [50]. In humans, trans-anethole was metabolized by O-dimethylation; conjugated and unconjugated metabolites were excreted either as glycine or glucuronic acid [51]. Sutton D [83], observed that the metabolites found in human urine were only marginally different from those detected in rodent urine. The research demonstrated dose-dependent variations in urinary metabolites of trans-anethole in rodents, resembling the transformation into eugenol specifically in rats. The study highlighted that the formation of metabolites was contingent on the dosage administered, whereas eugenol exhibited fluctuations in its conjugates [51]. After the administration of trans-anethole orally in humans, some metabolites were oxidized [47,48] and they were eliminated via the lungs as carbon- dioxide. Efforts to enhance the bioavailability of anethole have focused on nanotechnology-based delivery systems, which significantly improve its solubility, stability, and absorption through techniques such as encapsulation in nanoparticles, nanoemulsions, and liposomes, demonstrating promising potential for advancing cancer treatment [52]. Watkins et al. [53] showed that natural products were bioavailable, targeted and controlled by using nanoparticles. A study aimed to prepare a characterized anethole that was loaded with body surface area (BSA) nanoparticles enriched with folic acid bonded chitosan (CS-FA). Investigations by Pandit et al. showed the formation of anethole-loaded BSA, nanoparticles, surface enriched with CS-FA [54]. Ant-BSA-CS-FA showed high toxicity of nanoparticles on breast and colon cancer cells in comparison to normal cells. Liposomes attracted attention as a result of their improved properties for amphiphilic characteristics, permeability and strong solubility. Polyethylene glycol (PEG) liposomal nanoparticles with trans-anethole were synthesized by the reverse phase evaporation technique, and it was the first study to include the encapsulation of trans-anethole in liposome nanoparticles [55].

5. Mechanism of antitumor action of anethole

5.1. Apoptosis induction

Apoptosis, a natural process inherent in the body, serves to eliminate abnormal or damaged cells, playing a pivotal role in maintaining

cellular homeostasis and removing cells that could potentially contribute to diseases such as cancer [56] (Fig. 3).

Anethole has been reported to facilitate apoptosis, a mechanism commonly referred to as programmed cell death within cancer cells, by triggering the activation of pro-apoptotic proteins Bax (BCL-2-associated X protein) and Bak (BCL-2-homologous antagonist killer), leading to their oligomerization and permeabilization of the mitochondrial membrane [54]. This permeabilization results in the release of Cyt C (cytochrome C), SMAC (second mitochondria-derived activator of caspase), and Omi (Omi/high-temperature requirement protein A2) from the mitochondria. These molecules then contribute to the formation of the apoptosome, which consists of procaspase-9 (inactive precursor of caspase-9), dATP (deoxyadenosine triphosphate), cytochrome c, and APAF-1 (apoptotic protease activating factor-1) [57]. The formation of the apoptosome subsequently leads to the activation of caspase-9, which then activates downstream caspases, resulting in the systematic breakdown of cellular proteins and structures and ultimately culminating in apoptosis. [58]. The specific mechanisms of apoptosis induction by anethole may vary based on the type of cancer cells and experimental conditions but exact mechanism of its induction is yet to be known and may vary depending on the specific type of cancer cells being targeted [59]. However, several proposed mechanisms have been investigated [58,60], they include the activation of caspases, mitochondrial dysfunction and modulation of apoptotic regulatory proteins, they are further described below.

5.2. Modulation of signaling pathways

Anethole can impact various signaling pathways involved in cell growth, survival, and proliferation. For example, it has been found to inhibit the Akt/mTOR pathway, which is frequently dysregulated in cancer and promotes cell growth [61] By inhibiting this pathway, anethole can impede cancer cell proliferation and promote apoptosis. This dual action on cell survival and apoptosis regulation makes anethole a potential therapeutic agent against cancer [61]

5.3. Activation of caspases

Caspases are a group of enzymes that are pivotal in initiating and executing apoptosis [62]. Anethole has been shown to activate caspases, particularly caspase-3, which is considered a key effector caspase in the apoptotic pathway; activation of caspase-3 leads to the cleavage of various proteins and DNA fragmentation, ultimately causing to cell death [60].

5.4. Mitochondrial dysfunction

The mitochondria, often referred to as the cell powerhouse, play a critical role in apoptosis [63]. Anethole was reported to induce mitochondrial dysfunction in cancer cells by disrupting the mitochondrial membrane potential, inhibiting anti-apoptotic proteins such as Bcl-2 and Bcl-xL, promoting the release of pro-apoptotic factors like cytochrome C, which in turn triggers a cascade of events leading to caspase activation and the initiation of apoptosis [64].

5.5. Modulation of apoptotic regulatory proteins

Anethole can affect the expression and activity of various proteins that take part in the regulation of apoptosis [65] For example, it was shown to downregulate the expression of survivin protein that promotes

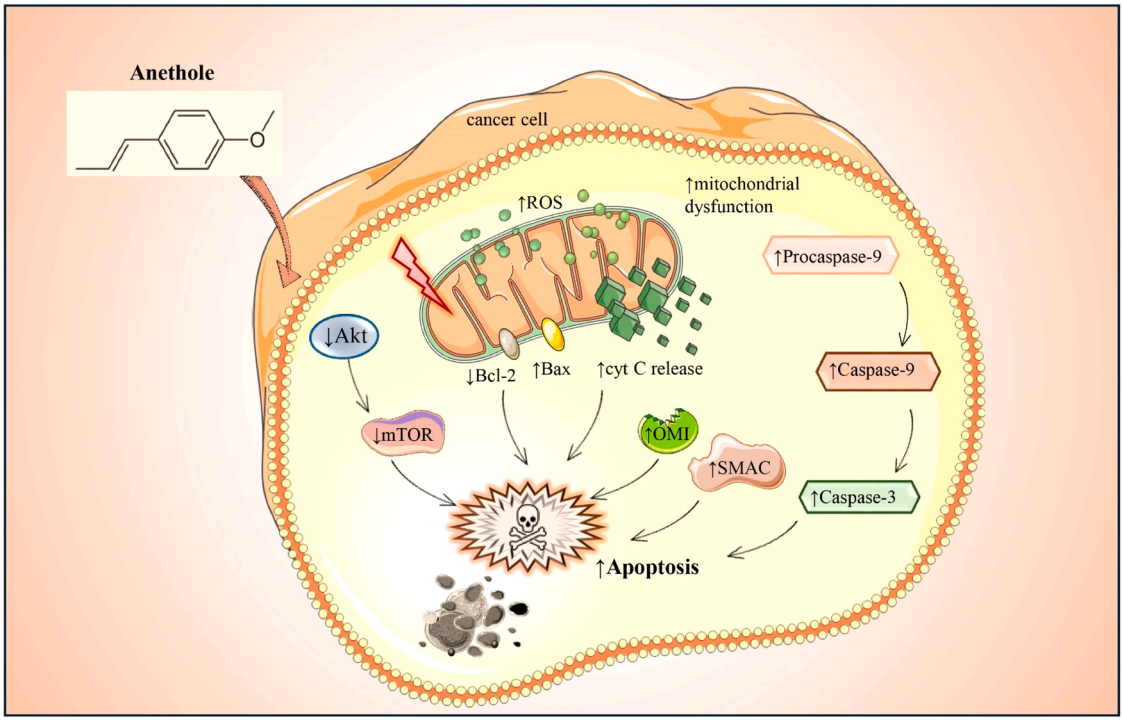

Fig. 3. Anethole-induced apoptotic pathways in cancer cells. This schematic illustrates the molecular mechanisms through which anethole induces apoptosis in cancer cells. Anethole enters the cancer cell and leads to mitochondrial dysfunction by increasing reactive oxygen species (ROS) and reducing Bcl-2 levels while increasing Bax levels. These changes promote the release of cytochrome c (cyt C) from the mitochondria into the cytosol. The released cytochrome c activates procaspase-9 to caspase-9, which in turn activates caspase-3, culminating in apoptosis. Additionally, anethole downregulates the mTOR/Akt pathway and promotes the activation of SMAC and OMI, which further enhance apoptotic signaling. The combined effects result in increased apoptosis, aiding in the suppression of cancer cell proliferation.Abbreviations and Symbols: Bax: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; Caspase-3: a critical executioner of apoptosis; Caspase-9: an initiator caspase in the apoptotic process; Cyt C: cytochrome c; mTOR/Akt: mammalian target of rapamycin/AKT signaling pathway; OMI: HtrA2 (a serine protease); Procaspase-9: the inactive precursor of caspase-9; ROS: reactive oxygen species; SMAC: second mitochondria-derived activator of caspases. ↑increase; ↓decrease.

cell survival and inhibits apoptosis [66]. By reducing the levels of survivin, anethole may promote apoptosis and inhibit the survival of cancer cells.

5.6. Anti-proliferation

5.6.1. Modulation of gene expression

Anethole can influence gene expression involved in cell growth, survival, and proliferation. It has been found to modulate the expression of various genes, including BCL-2 (B-cell lymphoma 2), Bax (Bcl-2-associated X protein), p53 (tumor protein p53), caspase-3, caspase-9, and cyclin D1. These changes lead to the inhibition of tumor cell proliferation, promotion of apoptosis, and reduction in cancer cell survival and metastasis [14].

5.6.2. Oxidative stress induction

While anethole also exhibits antioxidant activity, it has been reported that at higher concentrations, anethole can induce oxidative stress in cancer cells [14]. Excessive oxidative stress can damage the components of cells, thereby disrupting vital cellular processes and ultimately inhibiting cancer cell proliferation [67].

5.6.3. PI3K/Akt/mTOR pathway inhibition

Activation of the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway has been associated with cell proliferation, autophagy, apoptosis, angiogenesis, epithelial-to-mesenchymal transition (EMT), and chemoresistance in solid tumors. Growth factor (GF), epithelial-to-mesenchymal transition (EMT), and receptor tyrosine kinase (RTK) are all key components involved in these processes [68]. Studies by Choo et al. [69] showed that anethole derived from *F. vulgare* Mill, attenuated the metastasizes in human HT-1080 fibrosarcoma cells. Anethole led to an inhibition of the proliferation, attachment, adhesion, and invasion of HT-1080 cells, to the extracellular matrix (ECM) at nontoxic concentrations. Data by Choo et al. [69] also suggested that anethole possesses anti-metastatic activity and can be a very potent drug in cancer treatment. Using the MTT assay, the Anti-proliferation impact of anethole was evaluated. The IC50 value, representing the concentration of anethole required to reduce the viability of treated cell lines by 50 % compared to untreated cells, was determined to be 50 μM [70]. The groups that were treated with anethole and etoposide reduced the development of the cells. Compared to other groups, a decrease in cell development with less toxicity was seen in groups treated with anethole. Hence, anethole has Anti-proliferation activity towards cells. Anethole demonstrates Anti-proliferation effects specifically on skin cancer cells while exhibiting minimal toxicity towards normal human cells [71]. In the study conducted by Li and colleagues [124], the inhibition of skin cancer cells by anethole was evident as reductions in cell viability decreased from 100 % (control) to 10 %, with increase in treatment concentrations from 0 μM (control) to 100 μM. However, the viability of normal skin cells treated with the same graded doses (concentration) of anethole reduced from 100 % to 40 %. This confirms the selective inhibition of skin cancer cell growth by anethole. Observed apoptosis in skin cancer cells. Li et al., 2022 [71] suggested that more research needs to be done to find out if anethole has a potential in the treatment of skin cancer.

5.7. Inhibition of angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing ones, is a critical process in tumor growth and metastasis; by supplying oxygen and nutrients, angiogenesis supports the rapid proliferation of cancer cells [72].

Anethole has been shown to exhibit anti-angiogenic effects, which has been demonstrated to be a major step in cancer vascularization by inhibiting new blood vessels formation that are necessary for metastasis and tumor growth [73]. By targeting angiogenesis, anethole can restrict the blood supply to tumors, thus limiting their proliferation [74].

5.8. Mechanisms of angiogenesis inhibition by anethole

5.8.1. Suppression of VEGF signaling

Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis. Anethole has been shown to downregulate the expression of VEGF and its receptor, VEGFR-2, thereby inhibiting the VEGF signaling pathway. This suppression leads to reduced endothelial cell proliferation, migration, and tube formation, which are essential steps in angiogenesis [14].

5.8.2. Inhibition of matrix metalloproteinases (MMPs)

Matrix metalloproteinases are enzymes that degrade the extracellular matrix, facilitating endothelial cell migration during angiogenesis. Anethole has been reported to inhibit the activity and expression of MMP-2 and MMP-9, thereby preventing the degradation of the extracellular matrix and subsequent angiogenic processes [14].

5.8.3. Regulation of inflammatory cytokines

Inflammation plays a significant role in promoting angiogenesis. Anethole exhibits anti-inflammatory properties by reducing the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.; this reduction in inflammatory cytokines helps in diminishing the angiogenic signals within the tumor microenvironment [14].

5.8.4. Autophagy induction

One of the mechanisms through which anethole exerts its anticancer effects is by inducing autophagy, a cellular degradation process that plays a important role in maintaining cellular homeostasis [14]. Anethole can induce autophagy, it decreases the formation of reactive oxygen species (ROS) while the activity of intracellular glutathione (GSH) is increased [14]. In the potential therapy for oral cancer, when anethole is utilized, it inhibits the expression of oncogenes (cyclin D1) and up-regulated cyclin-dependent kinase inhibitor (p21WAF1), thereby leading to an increase in the expression of p53 gene, while inhibiting the epithelial-mesenchymal transition markers [6].

5.8.5. Modulation of signaling pathways

Anethole has been shown to influence several signaling pathways that regulate autophagy. It can modulate pathways such as NF-κB, MAPK, and Wnt, which are involved in cellular stress responses and autophagy regulation. By affecting these pathways, anethole promotes the formation of autophagosomes and enhances the autophagic flux [14].

5.8.6. Oxidative stress induction and autophagy

Anethole induces oxidative stress by increasing the production of reactive oxygen species (ROS) within cancer cells. This oxidative stress serves as a trigger for autophagy, leading to the degradation of damaged organelles and proteins. The induction of ROS and subsequent autophagy helps in reducing the proliferation of cancer cells and promoting apoptosis [14].

5.8.7. Activation of autophagic genes

Anethole has been observed to upregulate the expression of genes involved in the autophagy process, such as Beclin-1 and ATG proteins. This upregulation enhances the autophagic activity, facilitating the clearance of cellular debris and contributing to the cytotoxic effects on cancer cells [14].

5.9. Cell cycle arrest

The induction of cell cycle arrest by anethole is thought to be mediated by multiple mechanisms, including the modulation of various cell cycle regulatory proteins, such as cyclins, CDKs, and cyclin-

dependent kinase inhibitors (CKIs) [14]. Anethole's effects on these proteins can disrupt the finely tuned cell cycle regulation, leading to the arrest of the cell cycle and the inhibition of cancer cells proliferation [75]. Cell cycle arrest refers to the halting or slowing down of the normal progression of the cell cycle, which is the process by which cells grow and divide [76]. By interfering with the cell cycle, anethole can impede cancer cells' uncontrolled growth and proliferation; the specific effects of anethole on cell cycle arrest can vary depending on the type of cancer cells and experimental conditions [77]. Anethole can induce the arrest of cell cycle at different phases, such as G1, S, or G2/M phase [66]. By interfering with the normal progression of the cell cycle, anethole can halt the uncontrolled growth of cancer cells and prevent them from dividing [54].

5.9.1. G1 phase arrest

Anethole has been shown to induce cell cycle arrest at the first phase of the cell cycle - the G1 phase. During this phase, cells prepare for DNA replication. Anethole treatment can inhibit the activity of cyclin-dependent kinases (CDKs), which are enzymes involved in regulating the progression of the cell cycle [78]. By inhibiting CDK activity, anethole prevents the phosphorylation of the retinoblastoma protein (pRb) and other associated proteins, leading to the arrest of cells in the G1 phase.

5.9.2. S phase arrest

Anethole has also been reported to induce cell cycle arrest at the S phase, where DNA replication occurs [79]. By interfering with DNA synthesis, anethole treatment can trigger S phase arrest. The exact mechanisms by which anethole induces S phase arrest are not fully understood, but it may involve disruption of DNA replication machinery, inhibition of DNA polymerases, or alteration of key cell cycle regulatory proteins [80].

5.9.3. G2/M phase arrest

Anethole has been shown to induce cell cycle arrest at the G2/M phase transition, which is the checkpoint that ensures DNA integrity before cells enter mitosis [81]. Anethole treatment can lead to the accumulation of cells at the G2 phase by affecting the activity of CDKs and cyclins involved in G2/M progression. This arrest prevents cells from entering mitosis and undergoing cell division [66].(Fig. 4)

6. Pharmacological anti-cancer studies of Anethole

Multiple studies were performed in vitro and in vivo regarding Anethole anti-cancer activity, and different cancer types and their response to Anethole in diverse concentrations were explored (Table 1).

6.1. *In vitro studies*

Most studies exploring the interaction between Anethole and different types of cancer were performed as *in vitro* studies, breast cancer being the most studied one.

6.1.1. Breast Cancer

Triple-negative breast cancer (TNBC) is a heterogeneous subset of neoplasms that lack specific receptors: estrogen receptor (ER), progesterone receptor (PR), and HER2/neu [82]. Approximately 15 % of diagnosed breast cancers fall into this category globally [83]. Hudis and Gianni [84] noted that a percentage of drugs approved for treatment are derived from natural origins. Chen et al.analyzed MCF-7 and MDA-MB-231 breast cancer cells with Anethole in the concentration of 1×10^{-3} M [85]. In this concentration, the transcriptional activity of NF-κB was surpressed in both treated cell lines [85]. NF-κB is an upstream activator of p53, a protein known as a 'tumor suppressor.' In this sense, the stimulated transcription by the suppression of NF-κB is beneficial, given that p53 modulates cell apoptosis, reducing cancer cell survival. Other concentrations of Anethole were also tested, and in 1×10^{-3} M, more substantial results were found [85]. Other breast cancer cell lines and their response to Anethole were assessed by Shahbazian et al., in this case, MCF-7 and T47D [20]. In this case,

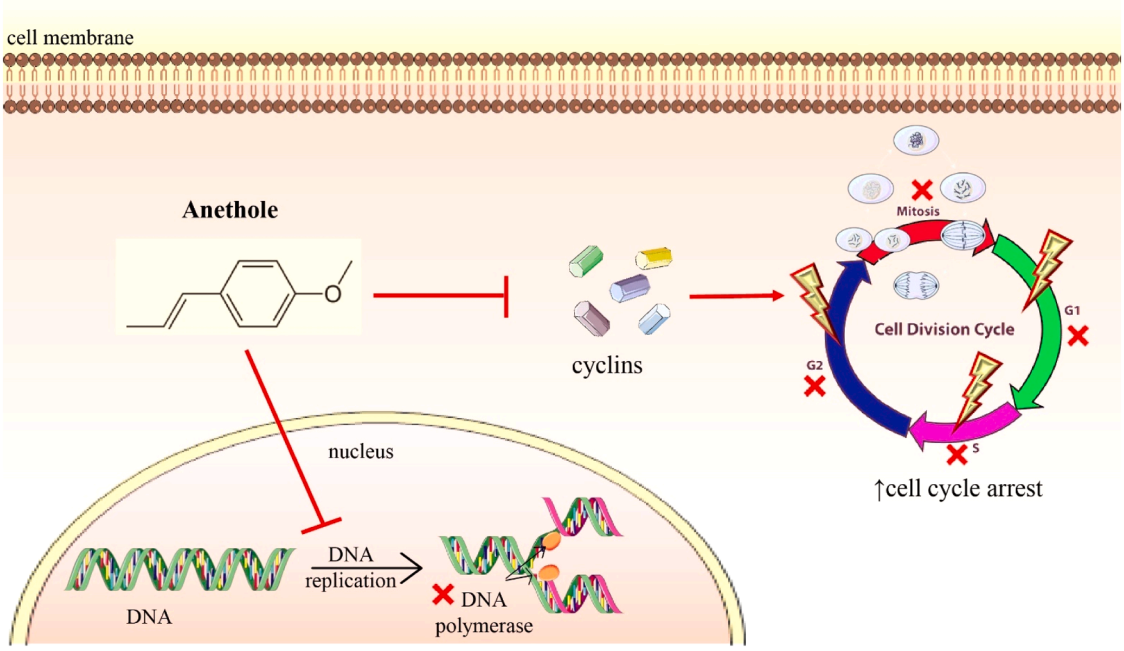

Fig. 4. Anethole-induced cell cycle arrest in cancer cells. This schematic illustrates how anethole induces cell cycle arrest in cancer cells. Anethole interferes with DNA replication by inhibiting DNA polymerase activity within the nucleus, thereby preventing DNA synthesis. Additionally, anethole inhibits the function of cyclins, essential proteins that regulate different phases of the cell cycle. This dual inhibition leads to a blockage of cell cycle progression at the G1, S, and G2 phases, ultimately preventing mitosis and leading to increased cell cycle arrest, thereby inhibiting cancer cell proliferation. Abbreviations and Symbols: DNA: Deoxyribonucleic Acid; X: Indicates inhibition or blockage; ↑: Increase.

Table 1
Pharmacological studies regarding the anethole anti-cancer activity.

Study Type	Cancer Type	Model	Concentrations (IC50/ Doses)	Mechanisms/ Signaling Pathways	Results	Ref.
In Vitro	Breast Cancer	MCF−7 MDA-MB−231	1 × 10−3 M	Inhibition of NF-κB activation of p53	↓ Tumor cell survival, ↑ apoptosis	[85]
	Triple Negative Breast Cancer	MDA-MB−231	IC50 evaluated by MTT assay	Cytotoxicity and cell viability assays, comet assay	↑ Apoptotic potential, ↓ cancer cell proliferation	[85]
	Breast Cancer	MCF−7 T47D	Pegylated liposomal trans-anethole	Encapsulation and drug loading efficiencies measured	↑ Stability, controlled release of trans-anethole	[20]
	Oral Cancer	Ca9−22	0 to 0.3, 3, 10, and 30 μM	↓ NF-κB, ↓MAPKinases ↓Wnt, ↓caspases 3, 9 ↓PARP1, ↓ ROS ↑ glutathione activity ↓ oncogene expression	↑ Autophagy	[14]
	Ovarian Cancer	A2780 A2780cisR A2780ZD0473R	Combination studies with fixed IC50 ratios	↓ NF-κB	↑ cytotoxicity on cancer cells	[20]
	Prostate Cancer	PC−3	Flow cytometry, qPCR, Western blot analyses	Generation of ROS mitochondrial and lysosomal membrane permeabilization	↑ Apoptosis, ↓ cell proliferation, clonal growth, migration, ↓ NF-κB protein and gene transcription	[66]
	Skin Cancer	CRL−6475 HEMa-LP	Up to 100 μM	↓ miR−498/STAT4 axis	↓ Cell growth, ↓ colony formation in a concentration-dependent manner	[89]
	Gastric Cancer	AGS	12.5 or 25 μM of [Ru(η6-anethole)(en)l]PF6 (3)	↑caspases ↑ p53	↑ Apoptosis, ↓ cell viability	[86]
	Bone Tumor	MG−63	60.25 μM	Annexin V-FITC/PI assay flow cytometry ↓ mitochondrial membrane permeability ↑ ROS, ↑ p53 ↑ caspase−9/−3, ↓ Bcl-xL	↑ Apoptosis	[88]
	Colon Cancer	HCT 116 HT−29	25, 50, and 90 μg/ml (IC25, IC50, IC90)	MTT assay	↑ Cytotoxicity towards colorectal cancer cells ↓ cell migration ↓invasion ↓colony formation	[87]
In Vivo	Ehrlich Ascites Tumor (EAT)	Swiss albino mice	250, 500, and 1000 mg/ kg body weight	Cytotoxic effects in EAT cells ↓ cancer biomarkers ↑ NP-SH	↑ Survival duration, ↓ tumor volume and weight, similar histopathological alterations to cyclophosphamide, mitodepressive, non-clastogenic	[91]
	Sarcoma−180 Solid Tumor	Swiss albino mice	10, 20, and 40 mg kg−1	↓ Myelosuppression ↓hepatotoxicity ↓urotoxicity	↑ Tumoricidal activity ↓ tumoral growth	[92]
	DMBA-Induced Mammary Cancer	Rats with DMBA-induced mammary cancer	Anethole trithione (400 or 200 ppm)	↓ Tumor incidence ↓ tumor multiplicity	↓ Tumor multiplicity, limited ↑ in liver-to-body weight ratio	[38]
	Lung Cancer	NSCLC, A549 Xenograft model BALB/c nude mice	Orally, 25 - 50 mg/kg daily, 25 consecutive days	↓ PI3K-AKT ↓STAT3 ↓ Ki67 ↑ caspase 3	↓ proliferation ↑ apoptosis	[90]

Abbreviations: Akt - Protein Kinase B, CDK - Cyclin-Dependent Kinase, CXCR4 - C-X-C Chemokine Receptor Type 4, ER - Endoplasmic Reticulum, GSH - Glutathione, MAPKinase - Mitogen-Activated Protein Kinase, MDA - Malondialdehyde, MTT - Methylthiazolyldiphenyl-tetrazolium bromide, NF-κB - Nuclear Factor-kappa B, NP-SH - Non-Protein Sulfhydryls, PI3K - Phosphatidylinositol-3 Kinase, PTEN - Phosphatase and Tensin Homolog, ROS - Reactive Oxygen Species, STAT3 - Signal Transducer and Activator of Transcription 3. **Symbols**: ↓ - decrease, ↑ - increase.

Anethole was presented in pegylated nano liposomes (PEG). Nanoparticles with 247 nm and −28mV showed a 57 % release of trans-anethole in 48 h of administration, with a 78 % loading efficacy [20]. In those two breast cancer cell lines, the apoptotic capacity of Anethole was also found, suggesting that Anethole may be successfully utilized in humans in the form of PEG [20].

6.1.2. *Digestive cancers*

In the case where oral gingival epithelial cancer cells, CA9–22 were incubated with 0 to 30 μM concentrations of anethole for two weeks, inhibition of NF-κB transcription genes resulted in a dose-dependent way [14]. Expression of P53 gene and diminished proliferative signaling pathways were also observed in this treatment [14]. In a study conducted by Carrillo et al., AGS gastric cancer cell lines were treated with either 12.5 or 25 μM of [Ru(η6-anethole)(en)l]PF6 (3) for 48 h [86]. A multicaspase assay indicated that anethole induced apoptosis in cancer cells [86]. Also, in the same study, an upregulation of P53 was also found, a beneficial factor regarding cancer cell treatment, sought by many chemotherapic therapies [86]. Asif et al. performed an analysis of different essential oils. Trans-anethole was the major phenolic compound present in all essential oils[87]. Treatments with 25, 50 and 90 μg/ml of essential oils (IC25, IC50 and IC90) introduced in HCT 116 colorectal cancer cells in humans exhibited inhibition of cell migration, invasion, and colony formation effects in the treated cells that were directly proportional to the dose administered [87].

6.1.3. *Bone tumor*

MG-63osteosarcoma cells treated with 60.25 μM of trans-anethole. An inhibition of those osteosarcoma cells was found dose-dependent, given that anethole induced apoptosis and inhibited cell growth [88]. The same study indicated that cancer cells' mitochondrial membrane permeability was reduced with the implementation of trans-anethole, thus increasing the amounts of circulating ROS and impairing cancer cells' metabolism [88]. The expression of P53 and Caspase-9/-3proteins were upregulated while that of Bcl-xLwas downregulation in the same study [88].

6.1.4. Prostate cancer

Elkady et al. [66] showed that anethole repressed the rapid division, clonal growth and metastasis of PC-3 cells [66]. The pro-apoptotic capacities of anethole were also found to be associated with the down-regulation of NF-κB itself and its regulated transcriptional target genes [66].

6.1.5. Skin cancer

Both CRL-6475melanoma cell lines and HEMa-LP melanocytes of theepidermis of humans treated with 0 to 100 μManethole showed inhibited growthby targeting the miR-498/STAT4 axis [89]. However, anethole also inhibited the growth of healthy human cells, indicating possible collateral effects in skin cancer treatment [71]. In the case of CRL-6475 (melanoma cancer cell line), the suppression of cells' growth was responsive in a concentration dependent way [89].

6.1.6. Lung cancer

Regarding lung cancer cell lines, (NSCLC and A549), Anethole suppressed the multiplication of A549 cells [90]. There was an elevated rate of programmed cell death, a substantial presence of fragmented DNA, and the activation of caspase-3 [90]. Notably, essential proteins within the PI3K-AKT and STAT3 signaling pathways exhibited reduced levels in the anethole-treated group [90]. Furthermore, in the xenografted tumors of the anethole-treated group, growth was delayed, characterized by a reduction in Ki67 and an augmentation in cleaved caspase 3 expression [90].

6.2. In vivo studies

Compared to the numerous *in vitro* studies that have been conducted, there have been relatively few *in vivo* investigations. Three studies in animal models including Ehrlich ascites tumor (EAT), Sarcoma 180 solid tumor, and lung cancer—have been carried out. Regarding EAT, Swiss albino mice were treated with 250, 500 and 1000 mg/kg of solutions of Anethole [91]. Anethole was shown to have a significant cytotoxicity on EAT cells, while also reducing levels of nucleic acids and MDA, Malondialdehyde, a common biomarker for cancer [91]. Significant increase of glutathione (NP-SH), an important antioxidant in cancer treatment that reduces tumoral activity was also found in all treatments with anethole [91]. Other benefits from the treatment with anethole were also found, such as reduced tumor volume and weight [91]. Also, histopathological changes after anethole were similar to standard cytotoxic treatments, for example, cyclophosphamide.Anethole exhibited mitotic inhibition and non-clastogenic effects in the femoral cells of treated mice [91]. Concentrations of 10, 20 and 40 mg/kg of anethole in solid tumor of swiss albino mice (Sarcoma-180) werecombined with a traditional chemotherapic treatment of cyclophosphamide [92]. As a main result, treatments with anethole alone were able to decrease the growth speed of tumoral cells. However, another result was that anethole in combination with cyclophosphamide was significantly more efficient in suppressing tumoral growth, and promoting tumoricidal activity while also reducing common side effects of the traditional treatment, such as namelymyelosuppression, hepatoxicity and urotoxicity [92].

7. Synergistic anticancer activity of anethole in combination with chemotherapeutic drugs

There are several traditional chemotherapy treatments, but their clinical outcomes are mixed given the varied side effects [93]. In this sense, the combination of natural compounds, such as anethole, are recommended, since they are known to both decrease side effects and to act in a synergistic way with conventional treatments [94]. In the study conducted by Semlali et al., a dose of 3 μM of anethole was combined with cisplatin, a conventional treatment for oral squamous cancers. In general, the combination of both compounds showed positive results, with reduced proliferation of cancer cells and an increased cytotoxic potential of cisplatin [95]. The combination of both compounds seem to be activating caspase, increasing the amount of reactive oxygen species in the cancer cells mitochondria, thus inducing their apoptosis [95]. The combined use of anethole and cisplatin led to the inhibition of important pathways involved in cancer signaling, including MAPKase (Mitogen-Activated Protein Kinase), beta-catenin, and NF-κB (Nuclear Factor-kappa B) pathways. These pathways play significant roles in regulating various cellular processes, including cell proliferation, survival, and inflammation, making them key targets for cancer treatment strategies. The inhibition of these pathways by the combination of anethole and cisplatin suggests potential synergy in impeding cancer progression and highlights the therapeutic promise of this combined approach [95]. Doxorubicin, an anthracycline chemotherapy drug, is commonly used in the treatment of breast cancer with attendant side effects [59]. In this sense, the combination of this drug with anethole induced oxidative stress disrupting cellular functions and resulting in a significant rise in the levels of reactive oxygen species (ROS). This increase can destabilize proteins within the endoplasmic reticulum, a vital cellular organelle involved in protein synthesis and folding. As a consequence, this disruption can lead to inhibited cell division and migration of cancer cells. The impact of elevated ROS levels on cellular processes is a field of active research in understanding cancer biology and developing targeted therapies[59]. Such synergistic action of both compounds was evident at different stages of tumor cell cycle[59]. Arumugam et al. [96] studied the cytotoxic effect of anethole when combined with doxorubicin on MDA-MB-231 cells by apoptosis, DNA damage, cell cycle, and cell proliferation. They also assessed the endoplasmic reticulum, mitochondrial membrane potential stress and the level of reactive oxygen species in cells with or without anethole and doxorubicin. Initial assessment of cytotoxicity of anethole on MDA-MB-231 cells showed the suppression of cell proliferation, but treatment with doxorubicin released arrest at different stages of the cell cycle. The combination of anethole and doxorubicin effectively induced the loss of mitochondrial membrane potential, leading to the production of reactive oxygen species. This, in turn, triggered the unfolded protein response by damaging the endoplasmic reticulum. When MDA-MB-231 cells were exposed to doxorubicin and anethole, a combined anticancer effect was observed in inducing cell death [59]. The combination of etoposide and anethole in the drug treatment of the group revealed more cell shrinkage in comparison to other groups. Staining using acridine orange/ethidium bromide showed that anethole compound induced apoptosis in MDA-MB-231 cancer cells. When the chemical structure of anethole and its anti-cancer effect is considered, the nuclear region was shown to have viable cells which were green, while the apoptotic cells had bright dots of chromatin and were orange in colour. The study demonstrated that when combined with etoposide, anethole triggers cell death in MDA-MB-231 cancer cells by causing membrane damage. These findings indicate that anethole influences cytotoxicity, cell growth, and apoptotic responses in triple-negative breast cancer cells [22]. Prostate cancer DU145 cells were under a sequenced treatment of 50 μM of lactacystin or with 200 μM of chloroquine with the addition of 100 μM of anethole for 24 h [97]. In both treatments, anethole reduced the RNA and protein levels of CXCR4, whereas most concentrated doses of anethole performed more successfully without inducing cytotoxicity [97]. The mechanism was based on the decreased phosphorylation of AKT and phosphatidylinosiltol-3kinase (PI3K), reducing the metastatic progression of DU145 prostate cancer cell lines [97]. In ovarian tumor models, Anethole was studied with curcumin (an isolated compound from turmeric) in cancer cell lines A2380 and two variants, $A2780^{cisR}$ and $A2780^{ZD0473R}$ [98]. In all studied concentrations, the product resulting from the combination of anethole and curcumin was successful in inhibiting the expression of NF-κB, in a similar way to what was reported in other studies utilizing anethole in different concentrations [98]. In combination, cisplatin and oxaliplatin in ovarian cancer treatmentcommonly encounter resistance requiring higher doses and, therefore, increasing side effects[98]In this sense, in the study

conducted by Meher et al., an important finding concerns not only the effectiveness of anethole to treat this type of cancer but also concerning the order of presentation of compounds in the treatment sequence [98]. Thus, more positive results were found when platinum-based drugs were administered 2 h after administration of the combination of anethole and curcumin [98]. Furthermore, when combined with cyclophosphamide, anethole could reduce markers of tumor growth, such as cell proliferation, cell growth and migration [92]. However, despite the combination of anethole with cyclophosphamide allowing a reduction in the concentration of traditional chemotherapy, doses from 40 mg/kg of anethole significantly increased the proportion of liver size in relation to the body[92].(Table 2)

8. Clinical studies

Most studies on the antitumor properties of anethole have been conducted invitro (in laboratory settings) or in animal models, and there is a need for further research to fully understand its effectiveness and safety in humans. In human clinical studies, in order to ascertain the efficacy of anethole as a natural plant, its mechanism of action alone or in a combination needsto be well investigated. It was recently shown that anethole can enhance the chemosensitivity of cancer cells when used with cisplatin drug by acting synergistically in the treatment of patients with oral squamous cell carcinoma [100]. Other studies show that anethole antioxidant effects can be attributed to its sequestering-ability on free radicals and decrease the concentrations of reactive oxygen species. Owing to this effect, anethole has the ability to block both carcinogenesis and inflammation. However, the mediation of these effects is not fully known [101,102]. The antioxidant activities present in star anise (*Illicium verum*) essential oil, can be attributed to trans-anise [103]. Antioxidant activity of anethole is similar to those found in other phenolic compounds, with conjugated double bonds. Henceforth, anethole can increase glutathione and glutathione-S-transferase at intracellular levels, thereby inhibiting the peroxidation of lipids with chemopreventive efficacy in cancer rat models [21,104]. In this regard, *trans*-Anethole might be able to decrease seizures and neurodegenerationcaused by free radicals; however, pharmacological studies will be needed to better inform the multiple mechanisms that are involved in neuroprotective and antiseizure activities of *trans*-Anethole in humans [105]. Anethole is a potential antitumor agent, since it can interfere with cancer cells, and have pro-apoptotic, anti-metastatic, and anti-inflammatory effects [106]. The authors showed that anethole and its synthetic analogs exhibit therapeutic activity in humans by suppressing invasive and non-invasive adenocarcinomas. Even though there are many documented studies on the medicinal properties of *Illicium verum* (star anise fruit), but few studies on the anticancer potential of ethanol extract of *I. verum* on different types of cancers in humans are documented. The utilisation of *I. verum* and its extract as a medical plant is cost effective with few side effects when used as a drug in human patients having Luminal-A breast cancer[107]. Therefore, as suggested by the authors in this recent research, it will be vital to conduct further clinical trials on human patients for the treatment option for Luminal-A type breast cancer represented by MCF-7 cell line.Another investigation in humans showed that anethole can be effective in prostate cancer therapy since it can suppress the growth of PC-3 derived cancer stem cells [66]. In some related studies on human prostate cancer, anethole was suggested to regulate the invasion and migration of metastatic prostate cancers through C-X-C chemokine receptor type 4 (CXCR4) suppression, and tumor suppressor phosphatase and tensin homolog (PTEN) augmentation which affectsthe phosphorylation of phosphatidylinositol-3kinase (PI3K)-AKT negatively [108].Similarly, anti-oral cancer proprieties are induced with anethole treatment to trigger apoptosis, autophagy and oxidative stress in the treatment of gum cancer [6].

9. Other pharmacological properties of Anethole

9.1. Antioxidant activity

Anethole has been recognized for its antioxidant activity and exhibits antioxidant properties through several mechanisms, such as:

Table 2
Synergistic anticancer activity of anethole in combination with chemotherapeutic drugs.

Combination	Cancer Type	Mechanism of Action	Results	Ref.
Anethole + Cisplatin	Oral Squamous Cancer	↑ Caspases ↑ ROS in mitochondria ↓ MAPK ↓ beta-catenin ↓ NF-κB	↓ Cancer cell proliferation ↑ Cytotoxic potential of cisplatin ↑ Apoptosis	[95]
Anethole + Doxorubicin	Breast Cancer	↑ Oxidative stress, disruption of cellular functions, ↑ ROS, destabilizing proteins in ER	↓ Cell division and migration, affected tumor cell cycle stages	[59]
Anethole + Etoposide	Triple-Negative Breast Cancer	↑ Mitochondrial membrane damage, ↑ ROS	↑ Apoptosis, ↑ Cell shrinkage, viable cells green, apoptotic cells orange, triggered cell death	[71]
Anethole + Curcumin + Platinum-based drugs	Ovarian Cancer	↓ NF-κB and its regulated transcriptional target genes effective sequence of administration	↑ Effectiveness of treatment, ↓ Side effects, optimal results with specific dministration order	[114]
Anethole + Cyclophosphamide	Sarcoma−180 Solid Tumor (Swiss albino mice)	↓ Tumor growth markers, modulation of cell proliferation, growth, and migration	↓ Concentration of traditional chemotherapy, ↑ Liver size with higher anethole doses	[121]
Anethole + Doxorubicin	Breast Cancer (MDA-MB−231 cells)	Apoptosis, DNA damage, cell cycle arrest, ↑ ROS, loss of mitochondrial membrane potential, unfolded protein response	↓ Cell proliferation, ↑ Cytotoxicity, induced cell death, membrane damage	[41]
Anethole + Lactacystin/ Chloroquine	Prostate Cancer (DU145 cells)	↓ RNA and protein levels of CXCR4, ↓ phosphorylation of AKT and PI3K	↓ Metastatic progression, no cytotoxicity at higher anethole doses	[39]
Anethole + Curcumin	Ovarian Cancer (A2780, A2780cisR, A2780ZD0473R)	↓ NF-κB and its regulated transcriptional target genes	optimal when platinum-based drugs are administered 2 h after anethole and curcumin	[99]
Anethole + Cyclophosphamide	Cancer Model (Sarcoma−180 solid tumors)	↓ Tumor growth markers, modulation of cell proliferation, growth, and migration	↓ Concentration of traditional chemotherapy, ↑ Liver size with higher anethole doses	[121]

Abbreviations and symbols:AKT - Protein Kinase B; ER - Endoplasmic Reticulum; MAPK - Mitogen-Activated Protein Kinase; NF-κB - Nuclear Factor-kappa B; PI3K - Phosphatidylinositol-3-Kinase; ROS - Reactive Oxygen Species; ↑ increase; ↓ decrease

- Free radical scavenging: Anethole has the ability to directly scavenge free radicals, particularly reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide and superoxide radicals. By donating an electron to these free radicals, anethole can stabilize them and prevent them from causing damage to cellular structures [109].
- Activation of antioxidant enzymes: Anethole stimulates the activity of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes play vital roles in the defense against oxidative stress by converting harmful free radicals into less reactive or non-toxic forms in the body.
- Lipid peroxidation inhibition: Anethole in fennel essential oil inhibits lipid peroxidation by preventing the formation of lipid radicals and interrupting the chain reaction of lipid oxidation [110]. This action preserves the integrity of cell membranes and prevents oxidative damage to cellular structures.
- Chelation of metal ions: Anethole is capable of binding or chelating to iron or copper metal ions that can participate in free radical generation through Fenton and Haber-Weiss reactions. By chelating these metal ions, anethole can prevent their involvement in generating free radicals and subsequent oxidative damage. Anethole-induced cell death occurs as a result of oxidative stress-dependent apoptosis via typical mitochondrial death cascades in fungi [111].

Fennel seed was evaluated by two lipid model systems: i) a modified thiobarbituric acid reactive species assay and ii) a spectrophotometric detection of hydroperoxydienes from linoleic acid in micellar systems and compare to reference antioxidants α-tocopherol and butylated hydroxytoluence. Ethanol and water extracts of fennel seeds showed peroxidation inhibition in linoleic acid. This was observed in a study by Oktay et al. [112]. Free radicals are produced continuously by metabolic processes during normal physiological function and reactive oxygen species (ROS) increase during stress conditions. Di Matteo and Esposito [113] showed that the presence of non-radical species such as singlet oxygen, ozone and peroxide. These highly reactive molecules are regulated by enzymatic and non-enzymatic antioxidant, which chelates free catalytic metals and prevent free radicals of oxygen to be formed. In organisms these activities lead to an imbalance between radical-generating and radical-scavenging effects and the development of oxidative stress. Cellular and extracellular components such as carbohydrates, lipids, proteins and nucleic acids are damaged, leading to human diseases such as neurodegenerative disorders, arteriosclerosis and cancer [113]. Trans-anethole is lipophilic and can be absorbed by the skin. Galicka et al. [114] determined a correlation between anethole and anti-aging. The nuclear factor kappa B (NF-κB), an oxidant-sensitive transcriptional factor and is responsible for matrix metalloproteinases-2 (MMP-2) expression is activated by oxidative stress. It is responsible for degrading collage in fibroblasts of the skin, leading to skin aging. Intrestingly, anethole can suppress lipid peroxidation and chelate with zinc ions from MMP-2 and block the activation of NF-κB.

9.2. Anti-inflammatory effect

Inflammation is noted to be a complex biological response that has a role to play in the defense against harmful stimuli. However, chronic inflammation contributes to the development of various diseases [115]. Studies have demonstrated that anethole has the capacity to impede the production and release of diverse inflammatory mediators, including prostaglandins, cytokines, and leukotrienes [116]. These mediators play important roles in initiating and amplifying an inflammatory response. Anethole inhibits enzymatic activities that are involved in inflammatory mediator synthesis. For example, anethole inhibits the activity of cyclooxygenase (COX) enzymes, responsible for prostaglandin production from arachidonic acid.; by inhibiting COX enzymes, anethole can reduce pro-inflammatory prostaglandins [70]. Anethole exhibits antioxidant properties, and oxidative stress is closely linked to inflammation, as it can activate inflammatory pathways and promote the release of inflammatory mediators [117]. Anethole can help mitigate inflammation by neutralizing free radicals and reducing oxidative stress [99] and can disrupt the nuclear factor-kappa B (NF-κB) pathway, a pivotal regulator of inflammatory responses. It functions by inhibiting the activation of NF-κB, thereby diminishing the expression of cytokines and genes associated with inflammation[118]. Anethole can also suppress the activation of immune cells, such as monocytes and macrophages, which are important in immune response to inflammation; by reducing the activation of these immune cells, anethole can help attenuate inflammation [119]. Anethole's anti-inflammatory properties have been showcased in preclinical studies and laboratory experiments [120]. However, it's important to note that translating these findings to clinical applications in humans requires further research and clinical trials. Moreover, the effectiveness of anethole as an anti-inflammatory agent may depend on factors such as dosage, bioavailability, and the specific inflammatory condition being targeted.

9.3. Sedative effect

According to a study [121], mice administered with essential oil exhibited an increase in pentobarbital-induced sleeping time. Ritter et al. in 2014 revealed similar results with trans-anethole. There is however no conclusive evidence to support a similar observation in human studies [116].

9.4. Spasmolytic and secretolytic effect

In a study, Cambar and Avaidro [122] demonstrated that alcoholic extracts and essential oils derived from aniseed had a notable relaxing effect on both tracheal and ileal muscles. When the essential oil, dissolved in 12 % ethanol, was administered intra-gastrically to anesthetized guinea pigs at a dosage of 50 mg/kg body weight, it led to an elevation in respiratory tract fluids within the initial two hours. Comparable outcomes were observed when anesthetized rats received oral doses of the essential oil at 0.0015 ml/kg, increasing the respiratory fluid by 28 %. Boyd and Sheppard, in 1968 [123] administered an emulsion of a few drops of essential oil intra-gastrically to cats and this resulted in hypersection of mucus in the airways with stimulation of ciliary mucus removal. Li and co-workers [124] revealed from their studies an increase in mucociliary transport velocity after 200 μl of aniseed infusion was applied to isolated ciliated frog esophagus epithelium.

9.5. Gastroprotective effect

Birdane et al. [125] studied the antioxidant and anti-ulcerogenic effects of *F. vulgare* extracted with water on ethanol-induced gastric lesions in rats. *F. vulgare* doses at 50, 150, and 300 mg/kg were administered and compared with 200 mg/kg of famotidine. It was found that both inhibited ethanol-induced gastric mucosal injury. *F. vulgare*'s effect of protection was statistically measurable in the 300 mg/kg group and was more than in the famotidine group. It can be concluded from these results that, gastro protective and antioxidant effects were shown by *F. vulgare.*

9.6. Anti-aggregation effects on platelet activity

Tognolini et al. [29], studied the anti-aggregation activities of essential oils with phenols on platelets of guinea pigs. By using platelet aggregation and clot retraction assays, the essential oil presented inhibitory concentration values (IC50). The coagulation protagonist, adenine di-phosphate, arachidonic acid and U46619 were added to the platelet aggregation assay. Apiaceace displayed the highest activity against arachidonic acid, which acts as a precursor of thromboxane A2 -

an intrinsic coagulation factor. It suppresses blood coagulation induced by adenosine di-phosphate. The pathway leading to thrombin formation involves the interaction of platelets and leukocytes with the primary layer of collagen, which triggers thrombin generation. Anethole affects U46619, leading to the formation of blood clots [29,126].

9.7. Anti-diabetic activity

The essential oil of *F. vulgare* shows hypoglycaemic activities in diabetic rats induced with streptozotocin. El-Soud et al. [127] revealed that *F. vulgare* essential oil ingested by diabetic rats reduced hyperglycaemia.The polymers derived from anethole, such as, photoanethole, fenchone, dianethole estragole, and P-anisaldehyde, have been identified as biologically active compounds exhibiting strong estrogenic and antithrombotic properties.

9.8. Immunoprotective effects

In their study on mice, Wiirzler et al. [93] investigated the impact of test compounds on cell and humoral-mediated immune responses. Their findings revealed that trans-anethole and estragole exhibited protective effects against cyclophosphamide-induced immune suppression. Additionally, Wiirzler et al. [93] highlighted the utility of phenylpropenes in developing novel plant-based products with immunomodulatory properties.

9.9. Anticonvulsant and neuroprotective effects

Karimzadeh et al. [128]demonstrated that anise oil containing trans-anethole prolonged the seizure latency and reduced the amplitude and duration of epileptiform discharge induced by pentylenetetrazol. Abdul-Ghani et al. [129] and Pourgholami et al.[130] reported the anticonvulsant properties of trans-anethole and its protective effects against hypoxic conditions, attributed to its anti-excitotoxic activity. Bhadra et al. [131] revealed that trans-anethole inhibits acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes

10. Toxicity and safety

The application of anise for human use largely depends on the clinical trials conducted to prove its efficacy while ensuring it safety with limited or no side effects. Essential oils, being a mixture of compounds in aromatic plants such as anethole, are made up of hydrophobic fragranced volatile oily liquid concentrated with mixtures of compounds that were produced as secondary metabolites [25]. Anethole in essential oil is utilized for various treatments in alternative medicine, as more people are interested in the efficacy of natural sources for treatments. Anethole is natural and less toxic when compared to synthetic variants of essential oils. This makes a strong case for its use as a natural product by virtue of the current trend to greener alternatives with less toxicity.

Anethole was reported to be non-clastogenic and will not disturb normal DNA-related processes in an *in-vitro* chromosome aberration assay [132]. Also, other in-vivo micronucleus studies, observed negative results for its non-clastogenicity as trans-Anethole (CAS # 4180–23-8) was mainly metabolized via O-demethylation and epoxidation of its side chain, followed by the formation of diols in rodents and humans [43]. However, there are safety concerns of some trans-anethole metabolites. For example, when trans-anethole is exposed to high temperature or light, it is converted to cis-anethole (Z-anethole), which is highly toxic [133]. The epoxide of anethole was shown to exhibit cytotoxicity, hepatotoxicity and genotoxicity in some studies, but a comprehensive review of the available data by the Expert Committee on Food Additives in 2000 concluded that trans-anethole was not likely to be genotoxic *in-vivo* [44]. Trans-anethole oxide, was shown to be more toxic to animals than trans-anethole. The application of trans-anethole oxide to CD-1 mouse produced skin sores [134]while bacterical cells showed more resistance to cytotoxicity of tran-anethole oxide than hepatocytes. Kim et al. (1999) have shown that trans-anethole oxide is mutagenic to bacterial cells and induces hepatomas [134]. There were no genotoxic effects in rat hepatocytes with trans-anethole epoxide as shown in a study by Marshall and Cadwell [46]. However, Kim et al. [134] found that the Salmonella typhimurium strains had positive Ames tests with point and frameshift mutations. Carcinogenicity is negligible in humans when treated with low doses of trans-anethole. A study have shown anticarcinogenic and genoprotective effects of trans-anethole [135]. One of the main metabolic fates of anethole is the formation of epoxide, according to Sangster et al. and Solheim and Scheline, anethole oxide may be responsible for the mutagencity and weak carcinogenicity to anethole [136]. The mutagenicity of anethole has been questionable. This warrants studies at microbial levels, such as those performed in *Salmonella*, anethole was negative without metabolic activation. Metabolic activities leading to mutagenicity varies in different biological systems, there was mutagenicity with an activation of metabolism in some studies by Sekizawa and Shibamoto, To et al. and Abel G. but not in others as shown by Gorelick in 1995 [135,137–139]. In microbial and animal tests, Gorelick and Heck demonstrated that anethole exhibited favorable outcomes when metabolic activation was involved, particularly in the mouse lymphoma assay [135,140]. However, other mutagenic assays, such as the *Escherichia coli* point mutatio and *Saccharomyces cerevisiae* reversion assays yielded negative results [141]. These conflicting outcomes were attributed to the instability of anethole oxide.

Trans-anethole induces the liver cytochrome p450 enzyme.Rompelberg [142] in 1993, studied the administration of trans-anethole and found that the activity of cytochrome enzymes was increased. The weight of the liver and expression of cytochrome p450 isoenzymes also increased when trans-anethole was administered. Trans-anethole at high doses induces monooxygenase enzymes, causing the proliferation of smooth endoplasm. Research by Newberne et al. [17], Hazelton [143], and Truhaut et al. [144] showed pathological changes when trans-anethole treated male mice displayed considerable hepatocellular hypertrophy characterized by enlarged livers, due to adaptive physiological alteration to the enzyme–inducing characteristics of trans-anethole. Rats treated with trans-anethole at concentrations higher than 200 mg/kg showed sinusoidal dilation and nodular hyperplasia [144]. When almost double the amount of trans-anethole was administered, hepatocellular carcinoma occurred in 6 out of 52 females. Studies by Hazelton et al. [143] showed elevated levels of alanine transaminase, gamma-glutamyltransferase, aspartate transaminase and alkaline phosphatase, when 120 mg/kg of anethole were administrated. Truhaut et al. [144] further showed that when 1 % of trans-anethole was administrated, it decreased body weight gain, lethargy and a reduction of adiposity in animals. According to Goggelmann and Achimmer, they reported that asarone is mutagenic for *Salmonella* bacteria [138]. Further work done by Hasheminejad and Caldwell showed that asarone was genotoxic to hepatocytes [134]. However, there are few studies on the metabolism of asarone and since it has a similar structure to anethole it is unclear if they will have the same metabolic fate.Kim et al. highlighted also the mutagenic effects of trans-asarone and trans-anethole oxide on bacterial cells, demonstrating their potential to induce skin papillomas or hepatomas [138]. A better understanding of anethole's impact on human reproduction safety necessitates delving into its mechanisms, particularly its effects on important factors governing human sperm function, such as intracellular calcium concentration ([Ca2+]i) and tyrosine phosphorylation. Research findings indicate that anethole may impede human sperm function by decreasing sperm [Ca2+] through a predominant calcium influx channel (CATSPER) and by dampening tyrosine phosphorylation *in vitro*. Therefore, caution is advised against excessive intake of anethole in substantial doses [145]. The reproductive toxicity was highlighted in a study conducted by Marinov and Valcheva-Kuzmanova [16] showed anti-implantation activity could be induced in adult female rats by administering

trans-anethole on days 1–10 of pregnancy. These results were compared to controls, which delivered normal offspring. When 50, 70 and 80 mg/kg of trans-anethole were administered, it inhibited implantation. The administration of trans-anethole during days 1–2 of pregnancy resulted in regular implantation and successful delivery. Inhibition of pregnancy occurred when trans-anethole was administered on days 3–5. When trans-anethole was given on days 6–10, the results showed that 3 out of 5 rats did not deliver at the right time. These results demonstrate that trans-anethole has antifertility activity. It was also observed that when male and female pups were treated with 700 mg/kg of trans-anethole, the second, third and fourth generations had low weight [121]. Similar findings were reported by Anges Research Laboratories when rats were treated with doses of 175 and 350 mg/kg. It is yet to be ascertained if these results can be extrapolated for humans. In their research, Nakagawa et al. [146] investigated the estrogenic activity in human breast cancer cells expressing estrogen receptors and prone to estrogen-induced proliferation. Their findings revealed that trans-anethole and its metabolite 4-methoxycinnamic acid exhibited minimal to no estrogenic activity. Tabanca et al. [147] also showed estrogenic activity in *Saccharomyces cerevisiae* cultures that expressed the human alpha receptor. A more comprehensive research on the reproductive toxicity of anethole in humans will be required in the future[90]. Acute liver injuries were observed when a dose of 695 mg/kg was given to mice for four days [148]. mInanition syndrome was caused when 60–120 mg/kg/day was administrated for 90 days due to the lack of food and water consumption after the 3rd week of treatment. When 30–60 mg/kg/day was administered for 90 days, decreased liver glycogen content and total organ weight correlated with decreased cellularity [148]. When mice were treated with doses of 120, 240, 360 and 500 mg/kg/day, death was observed, whereas when 1200 mg/kg/day were administered to rats, no deaths occurred [148]. The lethal dose (LD50) was 1326 mg/kg/ for fennel oil. Ostad and co-workers [149] reported observations of sedation, movement disorders, respiratory distress, unresponsiveness to external stimuli, limb weakness, tremors, and muscle twitching occurring within 24 hours post-administration. Importantly, no significant tissue damage was quantifiable.

Trans-anethole toxicity was found to be closely associated with the dose-dependent formation of trans-anethole epoxide. Epoxidation rates are higher in rats than in mice. The European Commission Panel on Additives and Products or Substances used in Animal Feed also confirmed the efficacy and safety of the so called 'chemical group 18' as food and feed [44]. The 'chemical group 18' includes trans-anethole, otherwise known as 1-methoxy-4-(prop-1(trans)-enyl) benzene, and three other additives i.e.,eugenyl acetate, 4-allyl-2,6-dimethoxyphenol and eugenol are found naturally in various herbs and plants, currently they are authorised for use as flavours in foods. *Illicium verum,* Star anise, often described as an eight-pointed seed, is widely available in global markets, it has a high content of anethole and essential oil with a flavour similar to anise seed [150]; the chemical compounds of *I. verum* include its phenylpropanoids essential oil, flavonoids, neolignans, sesquiterpenoids and monoterpenoids.For human safety, the acceptable maximum concentrations (isomer unspecified) in finished products for anethole were established for various products by the International Fragrance Association (IFRA) as a guide on safety [151]. Concerns have been raised regarding the potential side effects of anethole in food and beverages. Despite this, the Flavor and Extract Manufacturers Association (FEMA) recognizes trans-anethole, the primary component of *I. verum* essential oil, as generally safe (GRAS). However, the Food and Drug Administration (FDA) in the United States cautioned against consuming teas containing *I. verum* fruit due to reported side effects like vomiting, convulsions, hypertonia, hypothermia, nausea, and rapid eye movements, despite anethole's GRAS status. The FDA also highlighted the risk of contamination of such teas with toxic I. anisatum. Animal studies by Nakamura et al. demonstrated acute toxicity upon oral ingestion of veranisatin A, veranisatin B, and veranisatin C. The primary compound in the essential oil of *I. verum* fruit is the phenylpropanoid trans-anethole, comprising an average of 72–92 % [151]. The European Medicines Agency (EMA) confirmed the safety of *I. verum* fruit and its essential oil, recommending human daily intake averages of 3 g for the fruit and 0.3 g for the essential oil. They further suggested a 2.9 % concentration of *I. verum* fruit, along with other active ingredients, in veterinary preparations. The EMA also approved the use of *I. verum* fruit in human consumption, such as in spicing alcoholic beverages, sweets, or toothpaste, and as an expectorant [152].

11. Limitations and clinical gaps of anethole as adjuvant in oncological management

Despite the promising potential of anethole as an adjuvant in oncological management, several limitations and clinical gaps need to be addressed to fully harness its therapeutic benefits:

i. One of the primary limitations of anethole is its poor bioavailability. As previously discussed, anethole's limited water solubility, rapid metabolism, and instability in the gastrointestinal tract significantly reduce the amount of active compound available for therapeutic action. Although nanotechnology-based delivery systems show promise in enhancing bioavailability, further research is needed to optimize these technologies and ensure consistent and efficient delivery of anethole in clinical settings
ii. Most of the current evidence supporting the anticancer properties of anethole comes from *in vitro* and animal studies. There is a notable lack of comprehensive clinical trials evaluating the efficacy and safety of anethole in human subjects. Without robust clinical data, it is challenging to determine the optimal dosage, therapeutic window, and potential side effects of anethole when used as an adjuvant in cancer treatment.
iii. While anethole has demonstrated cytotoxic effects against cancer cells, its potential toxicity to normal cells remains a concern. Studies have shown that anethole can be metabolized into electrophilic epoxides, which may exert cytotoxic effects on non-cancerous tissues. Further research is needed to delineate the safety profile of anethole and develop strategies to mitigate any adverse effects.
iv. Anethole may interact with conventional chemotherapeutic agents, potentially altering their pharmacokinetics and efficacy. Understanding these interactions is important for integrating anethole into existing cancer treatment regimens. Detailed pharmacokinetic studies and clinical evaluations are necessary to identify and manage potential drug-drug interactions.
v. The therapeutic efficacy of anethole can be influenced by the variability in its concentration within natural sources. Standardization and quality control of anethole preparations are essential to ensure consistent therapeutic outcomes. This requires the development of stringent extraction and purification protocols, as well as regulatory guidelines for its use in clinical practice.
vi. While several mechanisms of anethole's anticancer activity have been proposed, including apoptosis induction, cell cycle arrest, and anti-inflammatory effects, a comprehensive understanding of its molecular targets and pathways is still lacking. Advanced research is needed to elucidate the detailed mechanisms of action and identify biomarkers for predicting patient response to anethole-based therapies.

12. Conclusions and future prospects

Anethole, a major component of essential oils from anise, fennel, and star anise, has demonstrated significant anti-cancer properties through various *in vitro* and *in vivo* studies. These studies have shown that anethole exerts its effects through multiple mechanisms, including the

induction of apoptosis, inhibition of cell proliferation, and modulation of key signaling pathways such as NF-κB, PI3K/Akt/mTOR, and MAPK. Furthermore, anethole possesses anti-inflammatory, antioxidant, and chemopreventive properties, underscoring its potential as an adjuvant in cancer therapy. Despite these promising findings, anethole's poor bioavailability and rapid metabolism pose significant challenges to its therapeutic application, necessitating further research to optimize its delivery and efficacy. Future research should prioritize conducting comprehensive clinical trials to evaluate the safety, efficacy, and optimal dosing of anethole in human subjects, particularly focusing on its use as an adjuvant in various cancer types such as breast, ovarian, prostate, and colon cancers. Additionally, the development and optimization of nanotechnology-based delivery systems, including nanoparticles, nanoemulsions, and liposomes, are important to enhancing the bioavailability, stability, and targeted delivery of anethole, which will improve its therapeutic index and reduce potential side effects. Detailed investigations into the molecular mechanisms underlying anethole's anti-cancer activity, including its effects on additional signaling pathways, gene expression, and the tumor microenvironment, are essential for a broader understanding of its therapeutic potential and to identify biomarkers for predicting patient response. Exploring the synergistic potential of anethole in combination with conventional chemotherapeutic agents and other natural compounds could enhance overall treatment efficacy while minimizing adverse effects. Establishing standardized extraction and purification protocols for anethole to ensure consistent quality and concentration in therapeutic formulations, along with developing regulatory guidelines, will facilitate its clinical application and ensure reliability. Moreover, investigating strategies to improve the bioavailability of anethole, such as prodrug development, formulation with bioenhancers, and structural modifications, will be key to maximizing its therapeutic potential. Conducting thorough toxicity and safety profiling of anethole and its derivatives is necessary to delineate their therapeutic window and identify potential adverse effects, with long-term studies required to assess the chronic toxicity and carcinogenicity of anethole, ensuring its safe use in cancer therapy. By addressing these future research directions, anethole can be effectively integrated into cancer treatment protocols, offering a complementary approach to conventional therapies and potentially improving patient outcomes.

Abbreviations

Akt	Protein Kinase B
BSA	Bovine Serum Albumin
CDK	Cyclin-Dependent Kinase
CXCR4	C-X-C Chemokine Receptor Type 4
DMBA	7,12-Dimethylbenz[*a*]anthracene
ER	Endoplasmic Reticulum
GSH	Glutathione
IC	Inhibitory Concentration
MAPKinase	Mitogen-Activated Protein Kinase
MDA	Malondialdehyde
miR	MicroRNA
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
NF-κB	Nuclear Factor-kappa B
NP-SH	Non-Protein Sulfhydryls
PARP1	Poly (ADP-Ribose) Polymerase 1
PI3K	Phosphatidylinositol−3 Kinase
PTEN	Phosphatase and Tensin Homolog
qPCR	Quantitative Polymerase Chain Reaction
ROS	Reactive Oxygen Species
STAT3	Signal Transducer and Activator of Transcription 3

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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