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Review article

Review on the Potential Therapeutic Roles of *Nigella sativa* in the Treatment of Patients with Cancer: Involvement of Apoptosis

- Black cumin and cancer -

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Key Words

anti-proliferative, antioxidant, apoptosis, *Nigella sativa*, cancer, programmed cell death

Abstract

Nigella sativa (*N. sativa*, family Ranunculaceae) is a medicinal plant that has been widely used for centuries throughout the world as a natural remedy. A wide range of chemical compounds found in *N. sativa* expresses its vast therapeutic effects. Thymoquinone (TQ) is the main component (up to 50%) in the essential oil of *N. sativa*. Also, pinene (up to 15%), p-cymene (40%), thymohydroquinone (THQ), thymol (THY), and dithymoquinone (DTQ) are other pharmacologically active compounds of its oil. Other terpenoid compounds, such as carvacrol, carvone, 4-terpineol, limonenes, and citronellol, are also found in small quantities in its oil. The main pharmacological characteristics of this plant are immune system stimulatory, anti- inflammatory, hypotensive, hepatoprotective, antioxidant, anti-cancer, hypoglycemic, anti- tussive, milk production, uricosuric, choleretic, anti-fertility, and spasmolytic properties. In this regard, we have searched the scientific databases PubMed, Web of Science, and Google Scholar with keywords of *N. sativa*, anti-cancer, apoptotic effect, antitumor, antioxidant, and malignancy over the period from 2000 to 2017. The effectiveness of *N. sativa* against cancer in the blood system, kidneys, lungs, prostate, liver, and breast and on many malignant cell lines has been shown in many studies, but the molecular mechanisms behind that anti-cancer role are still not clearly understood. From among the many effects of *N. sativa*, including its anti-proliferative effect, cell cycle arrest, apoptosis induction, ROS generation, anti-metastasis/anti-angiogenesis effects, Akt pathway control, modulation of multiple molecular targets, including p53, p73, STAT-3, PTEN, and PPAR-γ, and activation of caspases, the main suggestive anti-cancer mechanisms of *N. sativa* are its free radical scavenger activity and the preservation of various anti-oxidant enzyme activities, such as glutathione peroxidase, catalase, and glutathione-S- transferase. In this review, we highlight the molecular mechanisms of apoptosis and the anti-cancer effects of *N. sativa*, with a focus on its molecular targets in apoptosis pathways.

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1. Introduction

Cancer is one of the most debilitating and traumatic diseases of modern life, for which no curative approach is presently available. Cancer is also the second leading cause of death [1, 2]. Due to the narrow therapeutic window of chemical drugs [3], particularly can-

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Table 1 Classification of caspases

Caspases	Type (s)	Role (s)
Initiators	2, 8, 9, 10	Cleave inactive pro-forms of effector caspases, thereby activating them
Effectors (executioners)	3, 6, 7	Cleave and inactivate proteins that protect living cells from apoptosis, such as the DNA repairing protein, PARP, ICAD/DFF45, and Bcl-2 proteins
Inflammatory	1, 4, 5	Have a role in the immune system
Others	11	Regulates apoptosis and cytokine maturation during septic shock
	12	Mediates endoplasmic-specific apoptosis and cytotoxicity by amyloid-β
	13	Is a bovine gene and is activated by caspase 8
	14	Is highly expressed in embryonic tissues, but not in adult tissues

PARP, poly ADP-ribose polymerase; Bcl2, B-cell lymphoma 2.

cer treatment agents, and the development of resistance against these drugs, a need exists to discover novel natural therapies for the treatment of chronic diseases [4-7], especially cancer [8]. Even though the recent therapies used to treat patients with various types of cancer have not been completely effective, adjuvant therapies, including the use of medicinal plants, may have some effect in achieving cancer treatment goals [9]. Cell survival and proliferation are due to many factors such as apoptosis, and each factor that disturbs the balance between the cell cycle and apoptosis can lead to cell malignancy [10].

Nigella sativa (*N. sativa*) has been used for medicinal purposes for centuries in traditional medicine, and its anti-cancer and anti-proliferative effects have been demonstrated in Unani, Ayurveda and Chinese medicine [11]. The aim of this review is to highlight the role of apoptosis in the progression of cancer and to evaluate the efficacy of *N. sativa* against malignancy development in both *in vitro* and *in vivo* models. In this regard, we have searched the scientific databases PubMed, Web of Science, and Google Scholar with keywords of *N. sativa*, anti-cancer, apoptotic effect, antitumor, antioxidant, and malignancy over the period from 2000 to 2017, and we have summarized the current scientific information available on the anticancer activities of *N. sativa* and its mechanisms of action.

2. Apoptosis

Nowadays, a great deal of interest has been focused on comprehending the inner workings of a particular style of cell death that occurs in different cells of the human body. Apoptosis is the process of programmed cell death (PCD), it usually affects scattered individual cells rather than all the cells in a particular area, and once it is initiated, it occurs quickly. Therefore, apoptosis is a gene-regulated phenomenon that causes cell changes such as alteration of the cell's morphology, blebbing, nuclear fragmentation, cell shrinkage, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay [12, 13]. It plays a prominent role in many neurodegenerative and autoimmune diseases and disorders, as well as cancer and AIDS [14-16]. Apoptosis is also induced by different injurious stimuli such as hypoxia, radiation, reactive oxygen species, heat, and cytotoxic anticancer drugs [17].

2.1. Morphological and Biochemical Features of Apoptosis

The definition of apoptosis was first given by Elmore and Kerr *et al*. [15, 18]. The beginning of apoptosis is characterized by shrinkage of the cell and the nucleus, as well as condensation of nuclear chromatin, membrane blebbing, and oligonucleosomal DNA fragmentation [14, 19]. During the early process of apoptosis, cell shrinkage and pyknosis (which is the most distinctive feature of apoptosis and results because of chromatin condensation) occur; thus, the cells are smaller, cytoplasm is dense, and organelles are tightly packed [19, 20]. Apoptotic bodies, sometimes called 'apobodies', are small sealed membrane vesicles that are produced from cells undergoing cell death by apoptosis. The formation of apoptotic bodies is a mechanism that preventing the leakage of potentially toxic or immunogenic cellular contents of dying cells, inflammation or autoimmune reactions, and tissue destruction [21, 22]. These bodies are subsequently phagocytized by macrophages, neoplastic cells, and parenchymal cells and are degraded within phagolysosomes. Because the apoptotic cells do not release their cellular components into the surrounding interstitial tissue, no inflammatory reactions related with apoptosis occur [20, 23].

The biochemical modifications of apoptotic cells are very extensive and include protein cleavage, phagocytic recognition, protein cross-linking, and DNA fragmentation [24]. Caspases (cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases) are a family of cysteine proteases that play important roles as catalysts for the central hydrolytic reactions of apoptosis, necrosis, and inflammation [25-27]. At least 12 of these enzymes are known. Caspases are extensively expressed in an inactive

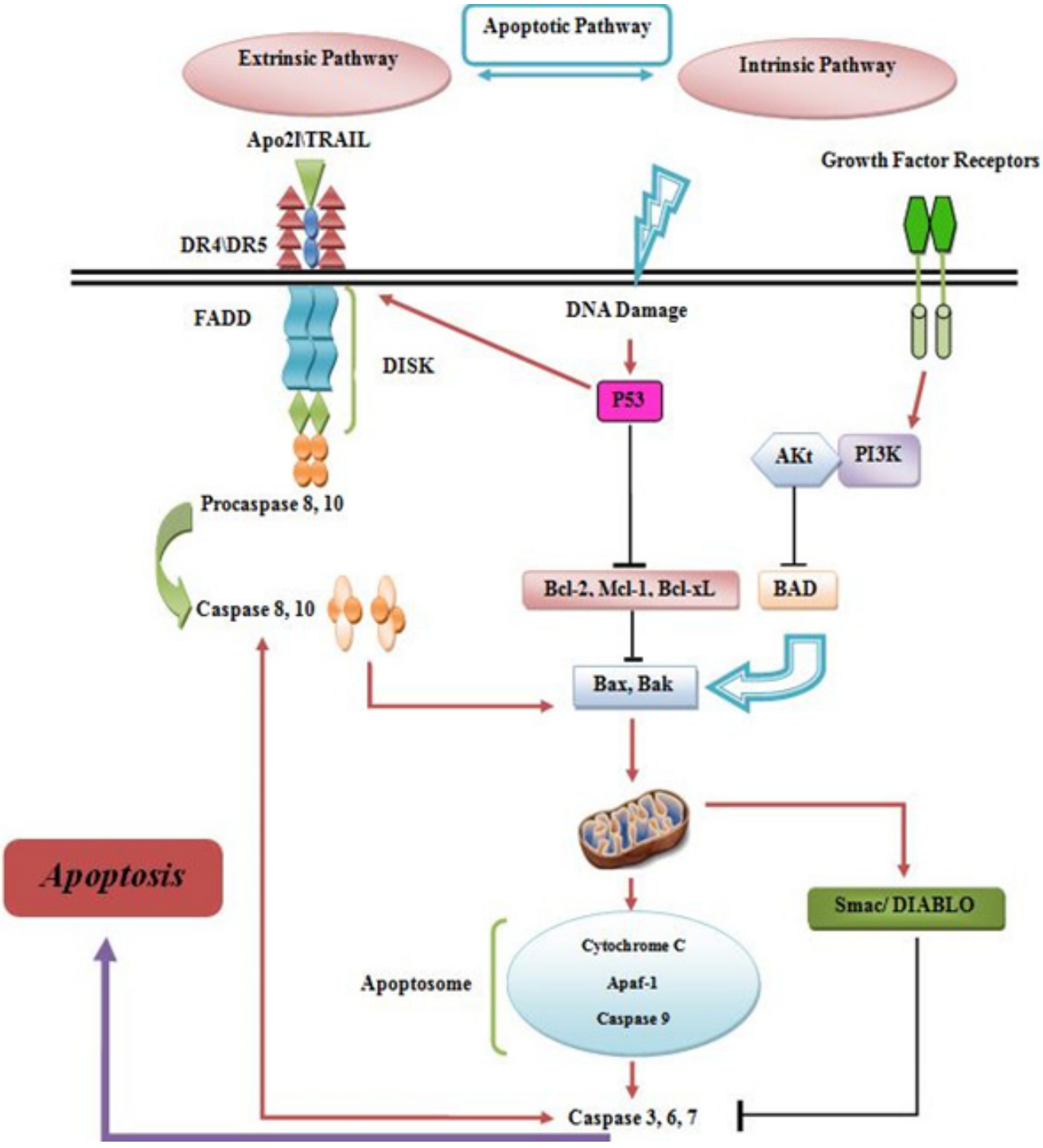

Figure 1 Apoptotic pathways.

proenzyme form in most cells and can often activate other procaspases, allowing initiation of a protease cascade [28, 29]. Table 1 shows the classification of caspases. Mitochondrial (intrinsic) and death receptor (extrinsic) pathways are two major apoptotic pathways that have been identified [30]. These pathways are in contact and share a general final phase of apoptosis that dismantles substrates critical for cell maintenance [31]. Fig. 1 shows the apoptotic pathways and important mediators in the apoptosis process.

Table 2 Some of the proteins involved in the extrinsic pathway, along with their roles and abbreviations

Abbreviation (s)	Full name (s)	Role (s)
Apo2L, Apo3L	Apo2 ligand, Apo3 ligand	Acts as ligands for initiating apoptosis
DED	Death effector domain	Found in inactive procaspases and formed DISC
DR3, DR4, DR5	Death receptor 3, 4, 5	Interacts with ligands and initiates extrinsic pathway
Caspase 8	Cysteinyl aspartic acid-protease 8	Triggers execution phase of apoptosis
FasR	FAS receptor	Is an example of receptors in the extrinsic pathway
FasL	FAS ligand	Is an example of ligands in the extrinsic pathway
FADD	Fas-associated death domain	Is an adapter protein that is recruited to the DISK during signaling via death receptors
RIP	Receptor-interacting protein	Is a key effector in TNF signaling and is essential for ROS-induced cell death
TNF-α	Tumor necrosis factor alpha	Regulates immune cells functions and induces apoptotic cell death
TNFR1	Tumor necrosis factor receptor 1	Mediates actions of TNF-α
TRADD	TNF receptor-associated death domain	Acts as adaptor protein and mediates apoptosis signaling and NF-κB activation

2.2. Death Receptor (Extrinsic) Apoptotic Pathway

Death receptors (transmembrane receptors - mediated interactions) are members of the tumor necrosis factor (TNF) receptor gene super family and play the main role in extrinsic signaling pathways of apoptosis. Death signals from the cell's surface are dispatched to the intracellular space with these receptors, which have cysteine-rich extracellular domains and 80-amino-acid cytoplasmic domains. In this pathway, trimeric ligands, such as TNF-J, FasL, Apo3L, Apo2L, and Apo2L, bind to clustered receptors, after which cytoplasmic adapter proteins are recruited to exhibit corresponding death domains that bind with the receptors. The adaptor proteins Fas- associated protein with death domain (FADD) and tumor necrosis factor receptor type 1- associated DEATH domain protein (TRADD) are the consequences of the bindings of the FasL to the Fas receptor and of the TNF ligand to the TNF receptor, respectively [32, 33]. A combination of FADD and procaspase-8 in this pathway results in the formation of the death-inducing signaling complex (DISC) and tends to activate caspase-8, after which the execution phase of apoptosis is triggered [34]. Table 2 presents additional information about the major extrinsic pathway proteins, along with their full names and roles. Another important ligand for activating the extrinsic pathway is TNF-related apoptosis-inducing ligands (TRAIL), including TNF-α and FasL, the activations of which lead to apoptotic cell death interference with TRAIL- R1 (DR4) and TRAIL-R2 (DR5) in a wide range of cultured malignant cells [35].

2.3. Mitochondrial Apoptotic (Intrinsic) Pathway

The intrinsic pathway is independent of receptor involvement whereas intracellular signals that act directly on targets are mitochondrial-dependent events [36]. The intrinsic pathway initiates with positive or negative stimuli, and any stimulus that fails to cause suppression of death programs, including the absence of certain growth factors, cytokines and hormones, is categorized as a negative signal. Radiation, hypoxia, toxins, hyperthermia, and so forth that can trigger apoptosis are a subset of positive stimuli [14].

Changes in mitochondrial permeability transition (MPT) lead to the loss of mitochondrial transmembrane potential. Pro-apoptotic proteins, such as cytochrome C, Smac/DIABLO, and the serine protease (as the first group) and HtrA2/Omi AIF, endonuclease G, and caspase-activated DNase (CAD) (as the second group), are extricated from the intermembrane space of the mitochondria to the cytosol [37]. The first group activates the caspase-dependent mitochondrial pathway. In this collection, cytochrome C binds and activates Apaf-1, as well as procaspase-9, forming an "apoptosome" and Smac/DIABLO, and HtrA2/Omi promotes apoptosis by inhibiting IAP (inhibitors of apoptosis protein) activity [38]. Caspase-9 activation is the main consequence of releasing pro-apoptotic proteins [39]. The second group of pro-apoptotic proteins is released from the mitochondria during apoptosis. AIF and endonuclease G are caspase-independent proteins that translocate to the nucleus and cause DNA fragmentation. Nuclear condensation in this state, called "stage I" condensation, and caspase-dependent proteins that are cleaved by caspase-3

Table 3 Some of the proteins involved in the mitochondrial pathway, along with their roles and abbreviations

Abbreviation (s)	Full name (s)	Role (s)
AIF	Apoptosis inducing factor	Induces apoptosis in a caspase-independent death effector manner
Apaf-1	Apoptotic protease activating factor	Creates an apoptosome as a key mediator of the intrinsic pathway
Bcl-2, Bcl-10	B-cell lymphoma protein 2, 10	Acts as a pro- or anti-apoptotic protein and regulates the release of cytochrome C from the mitochondria
BAD	Bcl-2 antagonist of cell death	Acts as a pro-apoptotic protein
BAG	Bcl-2 associated athanogene	Enhances the anti-apoptotic effects of BCL2 and represents a link between growth factor receptors and anti-apoptotic mechanisms
BAK	Bcl-2 antagonist killer 1	Permeabilizes the mitochondrial outer membrane during the mitochondrial pathway
BAX	Bcl-2 associated X protein	Forms a heterodimer with BCL2 and functions as an apoptotic activator
Caspase-9	Cysteinyl aspartic acid- protease-9	Activates caspase-3, -6 and -7 and initiates a caspase cascade
IAP	Inhibitor of apoptosis proteins	Inhibits the activation of caspase 3,7,9
CAD	Caspase-activated DNAse	Degrades DNA during apoptosis, as well as its inhibitor ICAD
BID	BH3 interacting domain death agonist	Induces apoptosis via insertion of Bax into organelle membranes
BIK	Bcl-2 interacting killer	Induces apoptosis and acts as target for anti- apoptotic proteins
BIM	Bcl-2 interacting protein	Enhances the anti-apoptotic effects of Bcl-2
Blk	Bik-like killer protein	Is a pro-apoptotic member of the Bcl-2 family

produce advanced chromatin condensation, called "stage II" condensation [40, 41]. We have summarized the major intrinsic pathway proteins, along with their common abbreviations, and have stated their roles in Table 3.

3. *Nigella sativa*

N. sativa (family: Ranunculaceae) is a medicinal plant popularly called by different names, such as black seed, black cumin, and the seed of blessing (Habatul-barakah in Arabic) [42]. *N. sativa* is a 20- to 90-cm-tall bisexual plant that grows mainly in parts of Asia such as the Middle East and in southern Europe and northern Africa. Its blue solitary flowers are on long peduncles. When the fruit capsule forms, it consist of many white trigonal seeds that turn black in color when the fruit has matured and opened, exposing the black seeds to air [43, 44]. The seeds and oil of *N. sativa* are the main parts of the plants that have been used for medicinal purposes for thousands of years [45, 46].

3.1. Chemical composition of *Nigella sativa*

A wide range of chemical compounds found in *N. sativa* express its vast therapeutic effects. Thymoquinone (TQ) is the main component (up to 50%) in the essential oil of *N. sativa*. Also, pinene (up to 15%), p-cymene (40%), thymohydroquinone (THQ), thymol (THY), and dithymoquinone (DTQ) are other pharmacologically active compounds of its oil. Other terpenoid compounds, such as carvacrol, carvone, 4-terpineol, limonenes, and citronellol, are also found in small quantities in its oil [47]. In addition to the volatile oil (0.5% - 2.5%), fixed oil (35.6% - 41.6%), proteins (22.7%), amino acids, mucilage, reduced sugars, tannins, organic acids, resins, glycosidal saponins, moisture, and Arabic acid are present. Two different types of alkaloids (isoquinoline alkaloids such as nigellicin and pyrazole[E1] [ترکد2] alkaloids) are found in the seeds [48]. Black cumin seeds contain unsaturated fatty acids [e.g., eicosadienoic[E3] [ترکد4] acid (3%), oleic acid (20%), dihomolinoleic acid (10%), and linoleic acid (55%)] and sat-

urated fatty acids (e.g., stearic acid (3%) and palmitic acid (14%)]. The seeds have also been found to contain crude fiber, vitamins, such as ascorbic acid, thiamine, niacin, pyridoxine, and folic acid, and minerals, such as Fe, Na, Cu, Zn, P, and Ca [49]. Moreover, free sterols, steryl glucosides, acylated steryl glucosides, and steryl esters have been isolated from the seed oil [50, 51]. β-carotene (pro-vitamin A) and tocopherol derivatives, as well as phytosterols, such as β-sitosterol, and in smaller amounts, Δ5-avenasterol, Δ7-avenasterol, campesterol, stigmasterol, and lanosterol, have been identified in black cumin seed oil [48]. The major phospholipid classes include phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol [52].

4. Traditional Uses and Pharmacological Properties of *N. sativa*

Historical and religious uses of *N. sativa* go back to antiquity. In ancient written sources, it is referred to as the melanthion (literally meaning little black seed) of Hippocrates and Dioscorides and as the gith of Pliny [53]. In the Bible, it is referred to as "the curative black cumin", and the prophet Mohammed described it as a plant with amazing healing powers [54, 55]. Treatments of fever, the common cold, asthma, rheumatic diseases, warts, headache, scorpion stings, and snake bites are examples of ancient applications of black cumin in folk medicine in the Middle and Far East. Ancient Egyptian and Greek physicians also used black cumin to treat nasal congestion, toothaches, and intestinal worms; they also used it as a diuretic and galactagogue. More recently, *N. sativa* has been used to treat infections, pain, obesity, hypertension, and gastrointestinal problems [56-62]. The seeds have also been used externally for many years to treat eczema, abscesses, nasal ulcers, seizures, orchitis, and rheumatism [62-66]. The stimulant, aromatic and carminative properties of *N. sativa*, as well as its beneficial effects in the treatment of patients with diarrhea, indigestion, loss of appetite, dysmenorrhea, and amenorrheaare the most important indications of this plant from the past to now [45, 47, 67].

Recent studies on the pharmacological properties of *N. sativa* have shown that the plant and its active constituent TQ have many beneficial effects, including anti-nociceptive, hypotensive, uricosuric, choleretic, anti-fertility, antihistaminic, immune stimulating, hypoglycemic, hepatoprotective, neuroprotective, spasmolytic, milk production, anti-tussive, and bronchodilator effects [45, 59, 68-80]. The anti-inflammatory, antioxidant, and apoptotic actions of *N. sativa* are the main mechanisms leading to its beneficial health effects [44, 45, 74, 81-83].

5. *N. sativa* and Apoptosis

Due to the indiscriminate and immoderate use of drugs and to their costs, side effects, and interactions, medicinal plants seem to have become appropriate alternatives for use in treating patients with diseases because of their availability and low costs; they also have fewer drug interactions. Currently, many medicinal plants have been found to possess remarkable beneficial properties with different mechanisms [3-5]. The mechanism of apoptosis is a novel therapeutic approach in the treatment of different diseases, especially cancer [84]. Recent scientific studies on plants used in ethnomedicine have led to the finding of many beneficial anticancer drugs, such as navelbine, vincristine, taxol, camptothecin, and so forth. [85, 86]. In the following subsections, we discuss the pharmacotherapy and the role of *N. sativa* as a valuable medicinal plant and the effects of its active compounds on various body systems.

5.1. *N. sativa* Effects on Apoptosis in *in vitro* Studies

As mentioned, *N. sativa* has been traditionally used as a tonic to prevent diseases and promote health [88]. *N. sativa* and its active compounds have also been shown to have possible anti-tumor activities. For many years, an extract of *N. sativa* seeds, *Smilax glabra* (rhizome) and *Hemidesmus indicus* (root), has been used for the treatment of cancer in Sri Lanka [88].

A recent study investigated the anti-cancer effect of *N. sativa* seeds, *Smilax glabra* (rhizome) and *Hemidesmus indicus* (root) extracts and reported that the greatest inhibitory effects on DNA synthesis were observed with *N. sativa* plant extract (88 ± 3.8%) even at low concentrations (5 mg/mL) [89]. Shafi *et al.* showed that methanolic, n-Hexane, and chloroform extracts of *N. sativa* were capable of killing human cervical carcinoma (HeLa) cells and that the IC50 values were 2.28 μg/mL, 2.20 μg/mL, and 0.41 ng/mL, respectively. In that study, the occurrence of apoptosis in HeLa cells was confirmed by using DNA fragmentation, western blot analyses, and terminal transferase mediated dUTP-digoxigenin-end labeling (TUNEL) assays. Based on their observations, the authors concluded that the expressions of pro- and anti-apoptotic genes were regulated by the extracts, indicating *N. sativa* to be a potential therapeutic medicinal plant for use in the treatment of patients with cervical cancer [90].

Hasan *et al.* reported the potential anti-cancer effects of *N. sativa* extract on human cervical cancer cells (SiHa) (88.3% inhibition, IC50 = 93.2 μL/mL) due to the expressions of caspase-3, -8 and -9 being increased several-fold [91]. Samarakoon *et al.* in their study showed that the extract could up-regulate the expression of the pro-apoptotic gene Bcl2-associated X protein, down- regulate the expression of the anti-apoptotic Bcl-2 gene, and enhance the activities of caspase-3 and caspase-9 in a time- and dose-dependent manner [92]. In another study, the impact of *N. sativa* on the growth of HeLa cells was investigated [93]. In that study, the apoptotic function of the ethanol extract was found to be associated with the release of mitochondrial cytochrome C, an increase in the Bax/Bcl-2 ratio, activations of caspases-3, -9 and -8, cleavage of PARP, increased expressions of p53 and p21, and decreased expressions of oncoproteins (c-Myc), human telomerase reverse

transcriptase (hTERT), cyclin D1, and cyclin-dependent kinase-4 (CDK-4). The results of that study were confirmed by Shahraki *et al*. in a separate study done on human renal adenocarcinomas and normal renal epithelial cells [94]. Shoieb *et al*. showed that TQ, the most abundant constituent present in *N. sativa*, was responsible for *in vitro* inhibition of growth and induction of apoptosis in cancer cell lines [95]. In that study, TQ (25 μM) induced apoptosis of COS31 (canine osteosarcoma) cells 6 h after treatment, decreased the number of COS31 cells in the S-phase, and increased the number of cells in the G1-phase, indicating cell- cycle arrest at G1. The results of that study suggest that TQ kills cancer cells through a process that involves apoptosis and cell-cycle arrest. Gali-Muhtasib *et al*. reported a similar result with a different mechanism for *N. sativa* [96]. They showed that TQ triggered human colorectal cancer cells via a p53-dependent mechanism (2.5- to 4.5-fold increase in mRNA expression of p53) and a significant inhibition of the anti-apoptotic Bcl-2 protein. Furthermore, using the TUNEL method and a flow cytometry analysis, they demonstrated that TQ inhibited the growth of colon cancer cells, which correlated with G1-phase arrest of the cell cycle, in a dose- and time- dependent manner. Thus, that study supports the potential use of the agent TQ for the treatment of patients with colon cancer.

Arafa *et al* reported that TQ greatly inhibited doxorubicin-resistant human breast cancer (MCF-7/DOX) cell proliferation through the following mechanisms: increases in the cellular levels of phosphatase and tensin homolog (PTEN) proteins and elevation of PTEN mRNA, resulting in a substantial decrease in the level of phosphorylated Akt (a known regulator of cell survival), increases in cellular levels of p53 and p21 proteins, an increase in the sub-G1 cell population, disruption of the mitochondrial membrane potential, activation of caspases, PARP cleavage, and an increase in the Bax/Bcl2 ratio via up-regulating Bax and down-regulating Bcl2 proteins. These results provide mechanistic insights for understanding the beneficial effects of TQ. Treating p53-null myeloblastic leukemia (HL-60) cells with TQ was found to trigger the activation of caspases -8, -9 and -3 and to cause a significant increase in the Bax/Bcl2 ratio due to up-regulation of Bax and down-regulation of Bcl2 proteins; these findings indicates that TQ may be a potential agent for the treatment of patients with cancer [97].

TQ was shown to exhibit an anti-proliferative effect, induce apoptosis, disrupt the mitochondrial membrane potential, and trigger the activations of caspases -8, -9 and -3 in HL-60 cells [98]. Gali-Muhtasib *et al* by using primary mouse keratinocytes, papilloma (SP-1) cells, and spindle (I7) carcinoma cells reported that non-cytotoxic concentrations of TQ could reduce the proliferation of neoplastic keratinocytes by 50% . The sensitivity of the cells to TQ treatment appeared to be stage-dependent, such that papilloma cells were twice as sensitive to the growth inhibitory effects of TQ as the spindle cancer cells. TQ treatment of SP-1 caused G0/G1 cell- cycle arrest, an increase in the expression of the cyclin-dependent kinase inhibitor p16, and a decrease in cyclin D1 protein expression. Also, TQ treatment of I7 induced G2/M cell-cycle arrest, increased the expression of the tumor suppressor protein p53, and decreased the expression of cyclin B1 protein. That study showed that the apoptotic effects of TQ were more pronounced in SP-1 than in I7 cells. Therefore, the findings support a potential role for TQ as a chemo- preventive agent [99].

In Rooney and Ryan's study, the effects of TQ on four human cancer cell lines [A549 (lung carcinoma), HT-29 (colon adenocarcinoma), HEp-2 (larynx epidermoid carcinoma) and MIA PaCa-2 (pancreas carcinoma)] were investigated. They reported that TQ induced a dose- and time-dependent apoptotic effect on the cell lines tested and that HEp-2 cells were the most sensitive to TQ [100]. In a similar study by them on HEp-2 cells treated with TQ, glutathione levels were significantly decreased in a dose-dependent manner and caspase 3-activation was mediated [101]. Sethi *et al* reported that treatment with TQ suppressed TNF-induced NF-κB activation in a dose- and time-dependent manner and inhibited NF-κB activation (correlated with the inhibition of the activatios of IκBA kinase, IκBA phosphorylation, IκBA degradation, p65 phosphorylation, p65 nuclear translocation, and NF-κB-dependent reporter gene expression) induced by various carcinogens and inflammatory stimuli. TQ also down-regulated the expressions of NF-κB–regulated anti-apoptotic (IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and survivin), proliferative (cyclin D1, cyclooxygenase-2, and c-Myc), and angiogenic (matrix metalloproteinase-9 and vascular endothelial growth factor) gene products. Overall, TQ may play the lead role in the apoptotic effects of TQ in the treatment of patients with cancer [102]. Ng *et al* in their study reported that TQ was more influential compared to cisplatin in eliminating SiHa. Treatment with TQ in the cells showed a significant elevation of p53 and a down- regulation of Bcl-2 (anti-apoptotic protein) in the treated cells without any changes in the expression of the Bax protein in a dose-dependent manner [103].

The cytotoxicity and the anti-proliferative effects of TQ towards HeLa were investigated [104]. The authors of that study reported that TQ exhibited time-dependent cytotoxic and anti- proliferative activities towards the cells with IC50 values of 2.80 ± 0.10 mg/mL and 5.37 ± 0.12 mg/mL, respectively. The proposed mechanism for this activity was via the p53-dependent pathway. Harzallah *et al* reported that the apoptotic effects of TQ on Hep-2 cell lines (IC_{50} = 19.25 ± 1.60 μg/mL) were remarkably more potent than those of the essential oil of *N. sativa* (IC_{50} = 55.20 ± 2.10 μg/mL) [105].

TQ can affect cancer cell lines through different mechanisms. Kundu *et al* reported that TQ remarkably diminished the viability of human colon cancer cells (HCT116) in a concentration- and time-dependent manner. The proposed mechanisms of that study were up-regulation of Bax, inhibitions of Bcl-2 and Bcl-xL expressions, activations of caspase-9, -7, and -3, activation of PARP cleavage, diminished constitutive phosphorylation, nuclear localization and reporter gene activity of the signal transducer and the activator of transcription-3 (STAT-3), attenuations of the expressions of STAT-3 target gene products, such as survivin, c-Myc, and cyclin-D1, -D2, enhanced expressions of cell-cycle inhibitory proteins p27 and p21, and attenuations of the phosphorylation of upstream kinases, such as Janus-activated kinase-2 (JAK2), Src kinase, and

Table 4 Other studies that show the effect of N. sativa on cancer cell lines

Cancer cell line (s)	Roles of apoptosis	Reference
Human renal adenocarcinoma and normal renal epithelial	Bcl2 is under-expressed, P53 is over-expressed, and caspases 3, 8, and 9 are activated.	94
Human colon cancer cells (Caco-2, HCT-116, LoVo, DLD-1 and HT-29)	Apoptosis was induced via the generation of ROS. TQ increased the phosphorylation states of the MAPK, JNK and ERK.	117
Fibrosarcoma (HT1080)	NSO produced a concentration-dependent inhibition of t-PA, u-PA and PAI-1. Plasminogen activation system (modulation of the fibrinolytic potential of fibrosarcoma) is depleted.	118
Squamous cell carcinoma (SCC VII) and fibrosarcoma (FsaR)	RNA expression of p53 and the downstream p53 target gene inhibition of anti-apoptotic Bcdl-2 is increased several fold.	119
HL-60 cells	Apoptosis is induced by activating caspase-3 and 8.	120
PC3	Cell proliferation is inhibited by TQ, and the activations of AKT and extracellular signal-regulated kinase are suppressed. Vascular endothelial growth factor–induced extracellular signal- regulated kinase activation is inhibited. Acts as an angiogenesis inhibitor.	121
Human multiple myeloma cells	Both constitutive and IL-6-inducible STAT3 phosphorylation, which correlated with the inhibitions of c-Src and JAK2 activations, are inhibited. Signal transducer and activator of the transcription 3 activation pathway is suppressed.	122
Human lung cancer cell line	Cell viability is reduced and the cellular morphology of A-549 cells is altered in a concentration-dependent manner.	123
Osteosarcoma (SaOS-2)	TQ significantly blocked human umbilical vein endothelial cell tube formation in a dose-dependent manner. TQ significantly downregulated NF-κB DNA-binding activity, XIAP, survivin and VEGF. Expressions of cleaved caspase-3 and Smac were upregulated in SaOS-2 cells. NF-κB and its regulated molecules and anti-angiogenesis effects are suppressed.	124
Primary effusion lymphoma (PEL) cell lines	Constitutive activation of AKT via generation of ROS is downregulated and conformational changes in Bax protein, leading to the loss of mitochondrial membrane potential and the release of cytochrome c to the cytosol, are caused. Caspase-9, caspase-3, and polyadenosine 5′-diphosphate ribose polymerase cleavage are activated, leading to caspase-dependent apoptosis. TQ is a potent inducer of apoptosis in PEL cells via release of ROS.	125
Hepatic stellate cells	TQsignificantly attenuated the expression of CD14 and Toll-like receptor 4. TQ also significantly inhibited phosphatidylinositol 3-kinase and serine/threonine kinase-protein kinase B phosphorylation. Expressions of α-SMA and collagen-I were significantly decreased by TQ. TQ decreased XIAP and cellular FLIP expression, which are related with the regulation of apoptosis.	126

Mouse neuroblastoma (Neuro-2a) cells	Bax/Bcl-2 ratio is increased. Caspase-3 is activated, along with downregulation of XIAP.	127

AKT, protein kinase B; ERK, extracellular signal-regulated kinase; JNK, janus kinase; MAPK, mitogen- activated protein kinases; PAI-1, plasminogen activator inhibitor type 1; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis protein.

epidermal growth factor receptor (EGFR) tyrosine kinase. Therefore, TQ exhibited apoptotic effects mainly by blocking STAT-3 signaling via inhibition of Src and JAK2 [106]. Also, different mechanisms of TQ, such as activation of glycogen synthase kinase-3, an increase in the membrane localization of β-catenin, a reduction in the nuclear expression of c-myc [107], reductions in the expressions of tumor markers (PCNA, Ki67, cyclin D1, cyclin E, and Cdk4), an increase in the expression of p21, and down-regulation of the Bcl-2 protein [108], were also shown. Reductions in the expressions of XIAP, Bcl-2, Bcl-xL and survivin, an increase in the phosphorylation of p38 mitogen-activated protein kinase (MAP) [109], reductions in the expressions of AR, E2F1 and cyclin A [110], inhibition of MEK-ERK1/2 signaling and disruption of its pro-survival function and pERK1/2 loss [111], and a blocking of the PI3K/Akt signaling pathway [112] are the other reported anti-cancer mechanisms of TQ.

Ait Mbarek *et al*. evaluated the *in vitro* anti-cancer effect of *N. sativa* [85]. Their findings showed that the essential oil and the ethyl acetate extracts of *N. sativa* were more cytotoxic than the butanol extracts against the murine mastocytoma cell line (P815), a result similar to that for the kidney carcinoma cell line of monkeys (Vero). As a result, the data showed that the effect of each extract depended on the tumor cell type. Another study showed that an aqueous extract of *N. sativa* significantly enhanced natural killer cytotoxic activity against mouse lymphoma cells (YAC-1) [113]. The effects of TQ on the colon cancer (HT29), lymphoblastic leukemia (CEMSS), and promyelocytic leukemia (HL60) cell lines were investigated [115], and the IC_{50} values of TQ were found to be 8, 5 and 3 μg/mL, respectively, and those values were found to behave in a time-dependent manner. Although TQ could not arrest the cell-cycle phases of the cells, apoptosis was the main mode of HT29 and HL60 cell death induced by TQ [114].

Another agent isolated from *N. sativa*, α-hederin, has been reported to have a potent antitumor effect, as well. Swamy and Haut reported a dose- and time-dependent increase in the apoptosis of murine leukemia (P388) cells, with the mechanism being the release of cytochrome C from the mitochondria to cytosol, leading to caspase-3 activation [115]. Thus, the findings of that study provide a mechanism of α-hederin-induced cell death caused by changes in intracellular thiols and the redox status, leading to perturbations of mitochondrial functions.

The anti-inflammatory effects of TQ on pancreatic ductal adenocarcinoma cells (PDA) were investigated by Chehl *et al* [116]. The effects of TQ on the expressions of different pro- inflammatory cytokines and chemokines, which were analyzed by using the real-time polymerase chain reaction (PCR), showed significant reductions in PDA cell syntheses of MCP- 1, TNF-α, IL-1β and COX-2 in a dose-dependent manner. TQ also inhibited the TNF-α-mediated activation of NF-κB in PDA cells and reduced the transport of NF-κB from the cytosol to the nucleus. Thus, the use of TQ may be a promising strategy for inhibiting pro-inflammatory pathways [116].

Table 4 provides a summary of the results of studies that showed *N. sativa* could have an effect on cancer cell lines. The table lists the cell lines and the roles of *N. sativa* in the mechanism of apoptosis.

5.2. *N. sativa* Effects on Apoptotis in *in vivo* Studies

The published findings provide much information about the anti-tumor effects of *N. sativa*, particularly in *in vivo* studies. Ait Mbarek *et al* reported that in the DBA2/P815 ($H2^d$) mouse model, a meaningfully inhibition of solid tumor development was found when the essential oil of *N. sativa* was injected into the tumor site. When 30 μL (28.5 mg)/mouse and 50 μL (47.5 mg)/mouse of the essential oil were injected in the tumor every 48 h for six times, the tumor volumes of animals (2.5 ± 0.6 cm^3) were reduced 0.22 ± 0.1 and 0.16 ± 0.1 cm^3, respectively. In addition, the incidence of metastasis of the liver was suppressed by the administration of the essential oil into the tumor site, and the mouse's survival was increased [85].

Another study reported that *N. sativa* had a protective effect against mammary carcinomas induced by 7, 12-dimethylbenz (a) anthracene (DMBA) [128]. In that study, administration of *N. sativa* for 3 months was associated with decreased levels of markers of apoptotic activity (29.0 ± 1.7 *vs*. 20.9 ± 1.3 and $P < 0.05$ for the percentage of DNA fragmentation; 20.8 ± 1.1 vs. 13.4 ± 0.7 and $P < 0.01$ for caspase-3; and 9.4 ± 0.8 vs. 52.1 ± 3.3 and $P < 0.01$ for TNF-α). Therefore, *N. sativa* decreased the carcinogenic effects of DMBA, suggesting a protective role against cancer.

The chemo-preventive activity of *N. sativa* oil against rat colon carcinomas was evaluated in the model of 1, 2-dimethylhydrazine-induced aberrant crypt foci (ACF: clusters of abnormal tube-like glands in the lining of the colon and rectum in rats) [129]. The findings of that study showed that the oil of *N. sativa* had the ability to inhibit colon carcinogenesis with no evident adverse side effects and that the inhibition may be associated, in part, with the suppression of cell proliferation in the colonic mucosa. Lei *et al* reported that in the xenograft tumor mouse model for the treatment of gastric cancer, the combination of TQ/5-

FU induced apoptosis via activations of caspase-3 and -9 [130].

Modulation of inducible nitric oxide synthase (iNOS) pathway suppression of the inflammatory response mediated by TNF-α and IL-6 may be effective in the treatment of patients with a hepatocellular carcinoma by using *N. sativa* [131]. A combination of TQ and cisplatin in the treatment of lung cancer in a mouse xenograft model showed that TQ was able to inhibit cell proliferation (nearly 90%), reduce cell viability, induce apoptosis, and reduce tumor volume and tumor weight. As a result, TQ can regulate NF-κB expression and act with synergism activity with cisplatin. Therefore, TQ appears to be have active therapeutic potential for the treatment of patients with cancer [132].

The anti-proliferative and pro-apoptotic effects of TQ in the breast-tumor xenograft mouse model are mediated by p38 phosphorylation via ROS generation and potentiate the antitumor effect of doxorubicin [109]. Another study showed that the administration of TQ (10 mg/kg/i.p.) for 18 days inhibited lung cancer (LNM35) tumor growth by 39% ($P < 0.05$); this was associated with a remarkable increase in the expression of activated caspase-3 [133]. Hence, based on these experimental findings, we conclude that TQ has clinical potential as an anticancer agent.

Salim in his study reported that post-initiation administration of 1,000 or 4,000 ppm *N. sativa* volatile oil in the diet of male rats for 30 weeks remarkably reduced the incidences of benign and malignant colon tumor and their sizes, especially those in the lungs, murine colon, esophagus, and forestomach [134]. This finding shows the potent inhibitory effects on rat cellular proliferation and tumor development in multiple organ sites. Kundu *et al*. reported that the pretreatment of hairless mouse skin with TQ attenuated 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of COX-2 and the phosphorylations of Akt, c-Jun-N-terminal kinase, and p38 MAPK [135]. It also diminished nuclear translocation and the DNA binding of NF-κB by blocking the phosphorylation and subsequent degradation of IκBA. Fig. 2 shows the roles of apoptosis in cancer treatment using *N. sativa*.

6. Conclusion

Due to the increased worldwide popularity, safety, and low cost of medicinal plants, their uses to treat patients

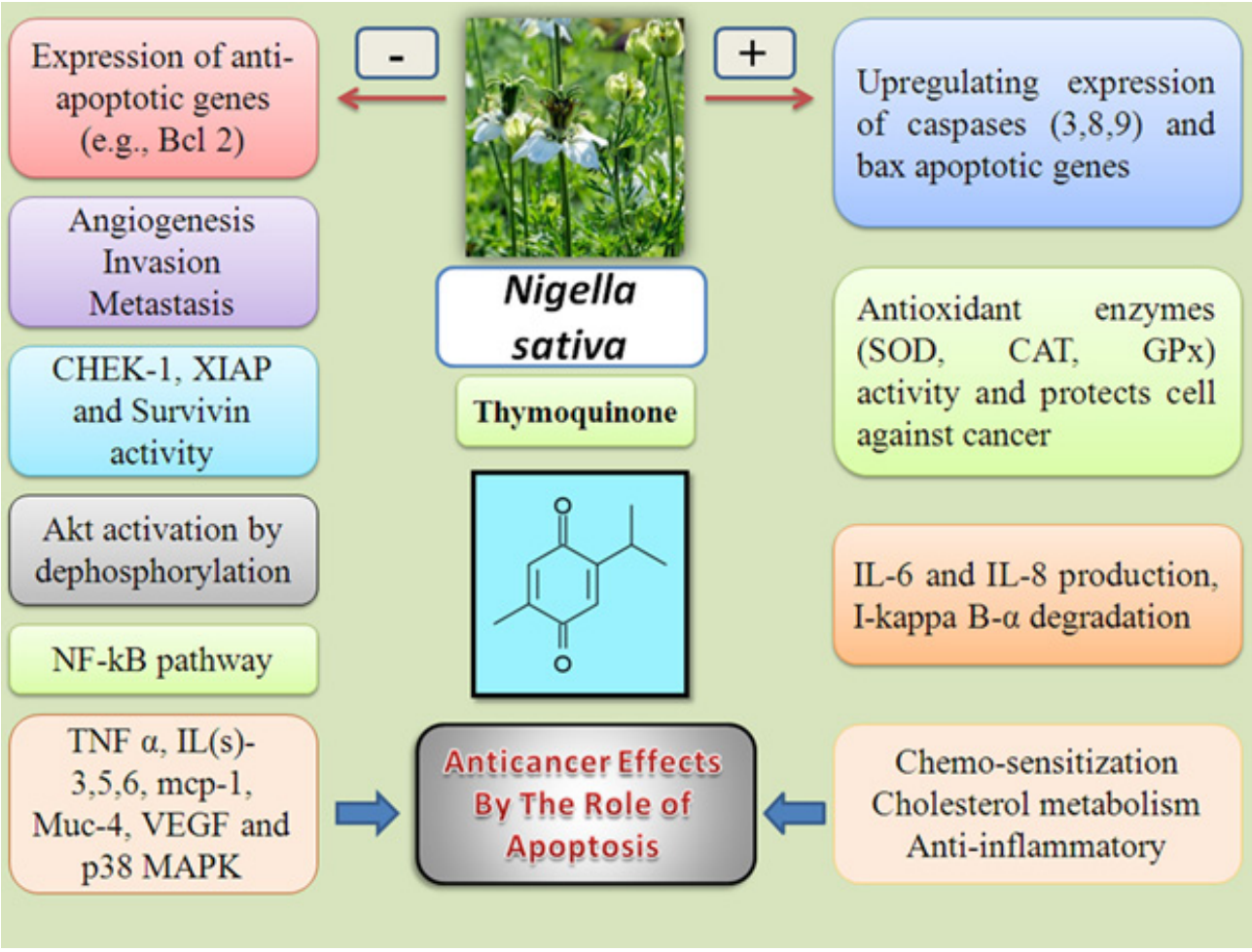

Figure 2 Role of apoptosis in the treatment of patients with cancer by using *N. sativa*.

with disorders ranging from simple aliments to more complex ones such as cancer are on the rise. The published findings show that *N. sativa*, especially TQ its predominant bioactive constituent, shows anticancer properties with apoptotic effects; for that reason, it can be used to treat patients with various diseases and disorders, especially cancer. Induction of apoptosis by TQ was shown through the up-regulation of p21 and p53, together with the inhibition of Bcl-2, the activations of caspases -8, -9 and -3, and increases in the Bax/Bcl-2 ratio. Up-regulation of tumor suppressors, along with a decrease in p-Akt, is another apoptotic effect of TQ. TQ was shown to suppress IAP1, IAP2, Bcl-2, Bcl-xL, XIAP, survivin, COX-2, cyclin D1, and VEGF as NF-κB-regulated gene products due to its inhibitory effects on TNF-α. Inhibition of both VEGF-dependent ERK and Akt activation are other anti-cancer mechanisms of *N. sativa*.

These results are important because they highlight the potential effects of *N. sativa* in the treatment of patients wi th cancer; thus encouraging researchers to conduct further studies in order to develop various and more effective formulations to treat an array of diseases, including cancer. Lately, *N. sativa* has become an important topic for research worldwide, but more studies need to be done to discover the different apoptotic mechanisms that further show the therapeutic efficiency of the plant against cancer.

Conflict of interest

The authors declare that they have no conflicts of interest.

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