Journal of Ayurveda and Integrative Medicine 7 (2016) 173–180

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Review Article

Recent advances on the anti-cancer properties of *Nigella sativa*, a widely used food additive

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ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form
18 July 2016
Accepted 27 July 2016
Available online 17 September 2016

Keywords:
Nigella sativa
Anti-cancer
Apoptosis
Cytotoxicity
NK cytotoxic activity

ABSTRACT

The use of naturally-occurring agents to regulate tumorigenesis is on the rise. Several herbal extracts, pure plant-derived active constituents, and food additives have been reported to possess potent anti-cancer properties and cancer-ameliorating effects. The wide-range anti-cancer effects of *Nigella sativa*, also known as black seed or black cumin, have been extensively studied using different *in vitro* and *in vivo* models. Here, we provide a comprehensive, analytical review of the reported anti-cancer properties of *N. sativa* seed extracts. This review focuses on analyzing experimental findings related to the ability of *N. sativa* to exert anti-proliferative, pro-apoptotic, anti-oxidant, cytotoxic, anti-mutagenic, anti-metastatic, and NK cytotoxic activity enhancing effects against various primary cancer cells and cancer cell lines. Moreover, we underline the molecular mechanisms of action and the signal transduction pathways implicated in the suppression of tumorigenesis by *N. sativa*. The major signaling pathway utilized by *N. sativa* to manifest its anti-cancer activity is the iNOS signaling pathway. This review underscores the recent developments that highlight an effective therapeutic potential of *N. sativa* to suppress tumor development, reduce tumor incidence, and ameliorate carcinogenesis. In sum, experimental findings reported in the last two decades strongly suggest that *N. sativa* fractions could serve, alone or in combination with known chemotherapeutic drugs, as effective agents to control tumor initiation, growth, and metastasis, and hence, treatment of a wide range of cancers.

1. Introduction

Many herbs have been shown to possess therapeutic potential towards several medical conditions, and hence, they are of a substantial medicinal value. For centuries, people around the globe have been using numerous medicinal herbs to alleviate the signs and symptoms of various disorders [1]. Herbal medicine, also known as botanical medicine, phytomedicine, phytotherapy, herbology, and herbalism, is a form of therapy that uses plants or plant extracts to prevent or treat different diseases and to boost the overall health status [1]. Herbal medicine is one of the oldest, if not the oldest, and probably remains to be of growing popularity. It is really intriguing that despite the great advancement in the fields of conventional medicine and drug discovery, the use of herbal formulations is still extremely widespread throughout the world, indicative of peoples' perception of the safety and therapeutic efficacy of such medicinal herbs. Although herbal medicine is more prevalent in Asia, Africa, and to a lesser extent in Europe, the use of medicinal herbs has witnessed a significant, gradual increase in North America [1,2]. It is most likely the gentle, nourishing, efficacious, synergistic, cost-effective, and safe properties of medicinal herbs that make them an attractive option for many people as therapeutic agents [1,2]. In fact, the discovery of the vast majority, if not all, conventional drugs is based on the chemical, physiological, and therapeutic actions of the bioactive constituents of many medicinal herbs. The recent advancement in pharma and medicine, manifested by the development of biotechnologies, and mass production of highly specific, chemically-synthesized drugs, has certainly revolutionized the therapeutic approach to health care and disease management worldwide. However, herbal medicine continues to be a primary ideology in many populations today and a very common practice in different parts of the world.

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Peer review under responsibility of Transdisciplinary University, Bangalore.

http://dx.doi.org/10.1016/j.jaim.2016.07.004

Herbs and spices are known to be major taste enhancers in most cuisines, primarily used as a source of flavor and aroma. Besides their use as food additives, a wide range of herbs and spices have been used to prevent or treat medical conditions including cancer. Over the past few decades, research investigating dietary factors and their effects on various medical conditions has been constantly growing. A large number of studies focused on these naturally-occurring products and reported a plethora of anti-cancer properties manifested through various molecular mechanisms. For instance, an ethanolic extract of *Piper nigrum* (black pepper) has been shown to induce DNA damage and reduce cell viability in MCF-7 human cancer cells [3]. Treatment with the ethanolic extract of *P. nigrum* inhibited cell proliferation by 57% and elevated ROS levels by 65%. Moreover, the same extract increased Bax and p53 levels, both of which are key proteins in regulating the cell cycle arrest. Another study used flow cytometric analysis to describe the anti-cancer effects exerted by *Fagonia cretica*, a tea herb and a food additive [4]. Indeed, the *F. cretica* extract caused a dose-dependent arrest of the cell cycle at G0/G1 phase and enhanced the rate of apoptosis in MCF-7 and MDA-MB-231 human cancer cells. Another example of a widely used active food constituent is sesamin, a major lignin in sesame seeds. Siao and colleagues showed that sasamin plays a strong preventive role against cancer by modulating apoptotic signaling pathways and restricting angiogenesis [5]. Hence, various herbs and food additives are becoming widely used for the treatment and/or the prevention of acute and chronic conditions ranging from mild allergies to more serious diseases including cancer. Yet, despite the intensive research efforts devoted to the identification of herbs with therapeutic properties, the exact molecular pathways and cellular mechanisms by which these herbs induce their therapeutic effects are not fully understood.

Nigella sativa is an annual flowering plant that is grown almost all over the world but is native to South and Southwest Asia and commonly found in Northern Africa, the Middle East, and Southern Europe [6,7]. *N. sativa* is also known as nigella, blackseed, black cumin, black caraway, Roman coriander, fennel flower, nutmeg flower, "kalonji" (in India), "Kalo jeera" (in Bangladesh), "Hak Jung Chou" (in China), and "habbat al-barakah" (in the Middle East). *N. sativa* belongs to the botanical family *Ranunculaceae* [8,9]. The mature plant grows to 20–90 cm of height with finely divided leaves, and white, pale blue, or pale purple delicate flowers containing 5–10 petals [10]. The follicles within the fruits contain many small (2.0–3.5mm × 1.0–2.0 mm) angular, black seeds with whitish interior [10]. Aside from its use as a food flavoring additive, *N. sativa* seeds oil and extracts have been used since ancient times to treat several diseases and medical conditions. *N. sativa* plant extracts have been commonly used in various traditional systems of medicine like Ayurveda, Siddha, Unani, Arabic, Islamic, etc. Several *N. sativa* crude extracts have been popularly used in traditional medicine as appetite stimulants, bronchodilators, liver tonics, and analgesics as well as to treat various conditions like diabetes, asthma, hypertension, cardiovascular disease, liver and kidney diseases, digestive problems, diarrhea, skin disorders, microbial infections, cancer, etc. [7–19] Such uses of *N. sativa* extracts in traditional medicine have been validated by well-designed experiments showing that such extracts possess cardio-protective, anti-microbial, anti-histaminic, anti-diabetic, antihypertensive, anti-hyperlipidemic, anti-diarrheal, hepato-protective, renal protective, gastro-protective, spasmolytic, immunomodulatory, anti-inflammatory, anti-oxidant, and anti-cancer properties [7–19]. Hence, traditional medicine uses that are validated by experimental evidence strongly suggest that *N. sativa* extracts can be of potent therapeutic efficacy in the prevention and treatment of various infectious and non-infectious diseases.

In this review, the *in vitro* and *in vivo* anti-cancer properties of *N. sativa* extracts are discussed. Special emphasis is given to the molecular and cellular mechanisms that mediate the anti-proliferative, pro-apoptotic, and anti-oxidant effects of *N. sativa*. Recent advances in the establishment of an effective therapeutic potential of *N. sativa* extracts, leading to suppressed tumor initiation and progression, are also underscored.

2. Anti-proliferative and pro-apoptotic effects of *N. sativa*

The potent anti-cancer potential of *N. sativa* is well established through *in vitro* and *in vivo* studies using different cell lines and animal models. Driven by traditional medical practices in Sri Lanka, a decoction (hot-water extract) comprised of *N. sativa* (seeds), *Hemidesmus indicus* (roots), and *Smilax glabra* (rhizome), a polyherbal mixture used to treat different types of cancer, has been shown to ameliorate diethylnitrosamine-induced hepatocarcinogenesis in male Wistar rats at a dose of 4–6 g/kg/day after 10 weeks of oral feeding [20]. The researchers of the aforementioned study indicated that the potential anti-cancer effects of the extracts of the individual plants in the decoction were not examined because only the decoction is traditionally used in cancer chemotherapy [20]. Subsequent studies suggest that flow cytometric analysis conducted using Annexin V and propidium iodide staining demonstrated that HepG2 cells were in the late stage of apoptosis and/or necrosis 24 h post treatment with the polyherbal mixture [21]. Consistently, oral administration (6 g/kg/day) of the polyherbal mixture of *N. sativa*, *H. indicus*, and *S. glabra* led to a long-term protection against diethylnitrosamine-induced hepatocellular adenoma in Wistar rats [22,23]. In fact, a great deal of literature underscores many *in vitro* and *in vivo* effects of pure *N. sativa* extracts. In an early *in vivo* study, topical application of *N. sativa* extract (100 mg/kg) inhibited the two-stage initiation/promotion of skin carcinogenesis and delayed the onset of skin papilloma in mice challenged with 7,12-dimethylbenzanthracene/croton oil [24]. The same study revealed that intraperitoneal administration of *N. sativa* extract significantly reduced methylcholanthrene (MCA)-induced soft tissue sarcomas in albino mice by about 70% following 30 days of subcutaneous administration of MCA [25]. Aqueous and ethanolic extracts of *N. sativa* seeds, both separately and in combination, were shown to exert potent anti-proliferative effects on MCF-7 human breast cancer cells in presence and absence of H_2O_2, which seems to play a synergistic role [26]. In another study, Salim and Fukushima examined the effects of *N. sativa* oil on the development of colon tumors in a murine model of 1,2-dimethylhydrazine (DMH)-induced colon cancer [27]. Fourteen weeks post DMH challenge, Fischer 344 rats that were treated with *N. sativa* oil at the initiation and post-initiation stages of colon carcinogenesis displayed significantly reduced DMH-induced aberrant crypt foci (ACF), which are putative pre-neoplastic lesions for colon cancer [27]. Immunohistochemical analysis revealed that *N. sativa* oil exerted potent anti-proliferative activity in the colonic ACF in rats that were treated with *N. sativa* oil at both the initiation and post-initiation stages of DMH challenge [27]. Similarly, using 7,12-di-methylbenz(a)anthracene (DMBA)-induced mammary carcinoma model, female Sprague–Dawley rats that were injected with DMBA were subsequently orally treated with *N. sativa* oil (4 g/kg/day) starting 2 weeks before or at the time of DMBA injection, and the experiment lasted for 3 months [28]. The frequency of mammary papillary, comedo, and cribriform carcinoma was reduced in rats treated with *N. sativa* oil at the time of DMBA injection, and this frequency was more potently recused in rats that were pre-treated with *N. sativa* oil for 2 weeks before DMBA challenge [28]. The reduced frequency of mammary carcinoma was associated with reduced serum levels of tumorigenicity

markers (total sialic acid (TSA) and lipid-bound sialic acid (LSA)), serum levels of endocrine derangement markers (prolactin, estradiol, and progesterone) and levels of apoptotic markers (serum tumor necrosis factor α (TNFα), tissue caspase-3 activity, and DNA fragmentation) [28]. Using the essential oil, an ethanolic extract, and a butanol extract of *N. sativa* and different cell lines (P815, IC01, Vero cells, and BSR cells), Ait Mbarek and colleagues demonstrated that the potency of the *in vitro* anti-cancer activity of *N. sativa* depends, at least partially, on the tumor cell type [29]. In the aforementioned study, the anti-cancer activity of *N. sativa* essential oil was also evaluated *in vivo*. Injection of 30–50 μl (28.5–47.5 mg/mouse) *N. sativa* essential oil into the tumor site of a DBA2/P815 (H2d) mouse model led to a significant suppression of solid tumor development (more than 10-fold decrease in tumor size) and resulted in a significantly delayed mortality of P815 mastocytoma tumor-bearing mice [29]. Recently, the administration of *N. sativa* ethanolic extract treatment was shown to improve the histopathological changes in the malignant liver tissue which were caused by diethylnitrosamine (DENA) treatment, without causing any direct cytotoxic effect [30]. In a similar study, the effects of a methanolic extract of *N. sativa* on the modulation of glyco-regulatory enzymes in an albino rat model of hepatocellular carcinoma were investigated [31]. Hepatocellular carcinoma was induced in albino rats by intraperitoneal injection of DENA and carbon tetrachloride (CCl_4), leading to a significant increase in the serum level of α-fetoprotein (AFP), the relative liver weight, and the activities of hexokinase, glyceraldehyde phosphate dehydrogenase, and G6P dehydrogenase in both the serum and liver homogenate of treated rats. Oral administration of a methanolic extract of *N. sativa* (1 g/kg/day) for 2 weeks prior to induction of hepatocellular carcinogenesis improved the histopathological changes associated with DENA and CCl_4 treatment, bringing the physiological and biochemical parameters indicated above back to normal levels [31]. Very recently, an *in vitro* study demonstrated that an aqueous extract of *N. sativa* (0.1–1.0% concentration) caused a significant decrease in cell proliferation and varying morphological changes including cell shrinkage and membrane damage in HepG2 cells, accompanied by DNA damage and cell death [32] (Fig. 1).

3. Anti-oxidant and cytotoxic effects of *N. sativa*

Among the first reports pointing to the potential anti-cancer properties of *N. sativa*, Swamy and Tan demonstrated that an aqueous extract and an ethyl acetate chromatographic fraction of *N. sativa* seeds (50 μg/ml) caused significant cytotoxic effects against various types of cancer cell lines (HepG2, MOLT4, and LL/2), but not against normal, non-cancerous human umbilical cord endothelial cells [33]. Aside from their anti-proliferative effects, both aqueous as well as ethanolic extracts of *N. sativa* seeds were found to induce significant cytotoxic effects on MCF-7 cells in presence and absence of H_2O_2 [26]. However, the ethanolic extract of *N. sativa* exerted more potency against MCF-7 cells compared to the aqueous extract (LC_{50} values in presence of H_2O_2 were 377 μM and 725 μM, respectively). Also, the aforementioned anti-cancer polyherbal mixture, which is comprised of *N. sativa* (seeds), *H. indicus* (roots), and *S. glabra* (rhizome), was shown to exert cytotoxic effects in human hepatoma HepG2 cell line at 5–50 mg/ml concentration [21]. In fact, the three individual plant extracts exerted cytotoxic efficacy in the order *N. sativa* > *H. indicus* > *S. glabra* [21]. Such anti-cancer effects were confirmed by Samarakoon and colleagues who demonstrated that both the aqueous and ethanolic extracts of the polyherbal mixture of *N. sativa*, *H. indicus*, and *S. glabra* caused strong dose-dependent cytotoxicity in HepG2 cells [22]. However, most of these studies do not yield insightful results since *N. sativa* extracts were used in combination with *H. indicus* and *S. glabra* extracts, making it challenging to draw plausible conclusions regarding the anti-cancer activity of *N. sativa* itself. Nonetheless, several studies examined the effects of *N. sativa* and its extracts on various cell lines. An *in vitro* cytotoxic study showed that a crude methanolic extract of *N. sativa* caused about 50% cytotoxicity in Ehrlich ascites carcinoma (EAC), Dalton's lymphoma ascites (DLA), and Sarcoma-180 cells (S-180 cells) [24]. Another *in vivo* study demonstrated that 6-month oral administration of *N. sativa* seeds (0.2 g/rat/day) provided protective effects against methylnitrosourea-induced oxidative stress and colon carcinogenesis in Sprague Dawely rats due to reduced expression of malondialdehyde (MDA), a

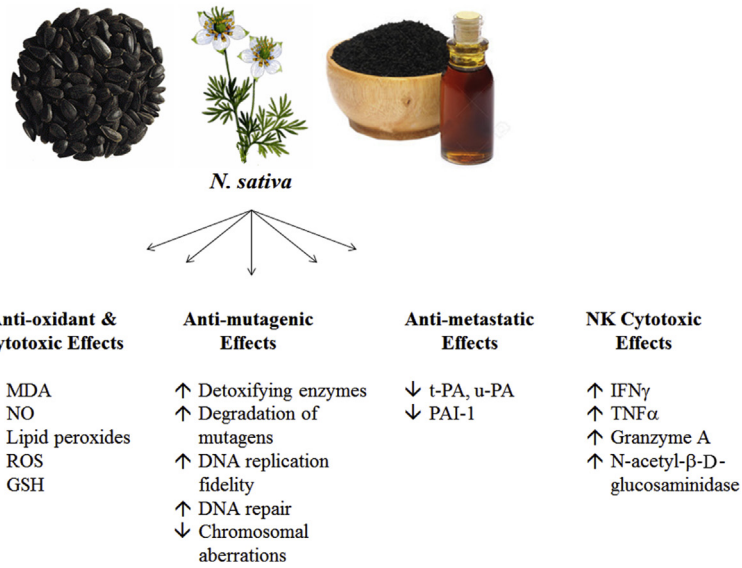

Fig. 1. A brief summary of the known molecular and cellular mechanisms underlying the anti-proliferative, pro-apoptotic, anti-oxidant, cytotoxic, anti-mutagenic, anti-metastatic, and NK-mediated cytotoxic effects of *N. sativa*. (TSA: total sialic acid, LSA: lipid-bound sialic acid, AFP: α-fetoprotein, TNFα: tumor necrosis factor α, IL-6: interleukin-6, MDA: malondialdehyde, NO: nitric oxide, ROS, reactive oxygen species, GSH: glutathione, t-PA: tissue-type plasminogen activator, u-PA: urokinase-type plasminogen activator, PAI-1: plasminogen activator inhibitor type 1, IFNγ: interferon γ).

biomarker of lipid peroxidation, and nitric oxide (NO) [34]. Zaoui and colleagues examined the possible biochemical and histopathological effects of *N. sativa* fixed oil in *Iops of a* mice and Wistar-Kyoto rats [35]. Acute toxicity of *N. sativa* fixed oil was assessed in mice that received a single oral or intraperitoneal dose, and the LD_{50} values were determined to be 28.8 ml/kg and 2.06 ml/kg, respectively [35]. The chronic toxicity of *N. sativa* fixed oil was assessed in rats receiving a daily oral dose of 2 ml/kg for a period of 12 weeks. It was demonstrated that chronic treatment with *N. sativa* fixed oil did not affect the level or catalytic activity of key hepatic enzymes including aspartate-aminotransferase, alanine-aminotransferase, and gamma-glutamyltransferase, nor it had any marked histopathological effects in the heart, liver, kidney, and pancreatic tissues [35]. The very low toxicity of the chronic treatment with *N. sativa* fixed oil was evidenced by biochemical stability and high LD_{50} values, suggesting that the indicated doses are sub-toxic and do not raise major safety concerns. Moreover, Islam and colleagues demonstrated that *N. sativa* oil exerts cytotoxic effects against a panel of four human cancer cell lines (SCL, SCL-6, SCL-37′6, and NUGC-4) and 3T6 fibroblast mouse cell line with LC50 values 155.02 ± 10.4, 185.77 ± 2.9, 120.40 ± 20.5, 384.53 ± 12.1, and 286.83 ± 23.3 μg/ml, respectively, with no significant cytotoxic effects on normal cells [36]. In another study, Ali assessed the ability of *N. sativa* oil to ameliorate the nephrotoxicity associated with gentamicin, an antibiotic, in rats [37]. Intramuscular injection of gentamicin was associated with proximal tubular damage, histopathological and biochemical signs of nephrotoxicity, elevated levels of creatinine and urea, as well as decreased level of glutathione (GSH) and total anti-oxidant status [37]. Such effects were abrogated by oral administration of *N. sativa* oil (1–2 ml/kg/day) for 10 days, without any detectable overall toxicity [37]. Similarly, oral *N. sativa* treatment (4 g/kg/day) of rats with DMBA-induced mammary carcinoma for a period of 3 months resulted in reduced tissue levels of oxidative stress markers (NO and lipid peroxides) [28]. Intragastric administration of *N. sativa* oil for 12 days in male albino rats potently reduced the hepatic and overall toxic effects associated with intraperitoneal administration of cyclophosphamide, an anti-cancer drug that causes a high degree of lipid peroxidation and reactive oxygen species (ROS) over-production [38]. Similarly, oral administration of *N. sativa* oil (90 mg/kg/day) in albino rats for 30 and 60 days significantly ameliorated, in a time-dependent manner, the toxic effects and pathological tissue damage in the spleen and thymus resulting from treatment with chloramphenicol, a potent antibiotic [39]. These findings suggest that *N. sativa* oil co-treatment could potentially reduce the toxicity-related side effects that accompany the bactericidal and anti-cancer chemotherapy. In a recent study, the hepatotoxic effects of *N. sativa* were evaluated in Spargue Dawley rats by measuring the catalytic activity of key liver enzymes (ALT and AST) and by histopathological assessment of liver tissue [40]. Rats were fed diet supplemented with 0.01–1 g/kg/day of *N. sativa* seeds powder for 28 days. It was demonstrated that *N. sativa* powder supplementation did not lead to a significant change in the catalytic activity of ALT and AST, histopathological abnormalities, inflammation, or necrosis in the liver tissue even at the highest dose of 1 g/kg/day [40]. This study showed that 0.01–1 g/kg/day doses of *N. sativa* seeds powder caused no marked toxic effects on liver function in rats and they are considered safe. Very recently, Hadi and colleagues performed a clinical trial to assess the anti-oxidant effects of *N. sativa* oil in patients with rheumatoid arthritis (RA) [41]. It was revealed that a daily dose of 1 g *N. sativa* oil for 8 weeks significantly reduced the serum levels of MDA and NO, suggesting that *N. sativa* can potentially be employed in the treatment of RA due to its ability to suppress RA-associated oxidative stress responses [41] (Fig. 1).

4. Anti-mutagenic effects of *N. sativa*

A few studies have examined the potential of *N. sativa* to exert anti-mutagenic activity against *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), a directly acting mutagen. Although an aqueous extract of *N. sativa* had no cytoprotective nor anti-mutagenic activity against MMNG in primary rat hepatocytes [42], an ethanolic extract of *N. sativa* exerted an inhibitory effect against MNNG mutagenicity due to significantly reduced chromosomal aberrations in primary rat hepatocytes [43]. The anti-mutagenic activity of the ethanolic extract of *N. sativa* was observed in MNNG-challenged primary rat hepatocytes that were pre-treated, co-treated, or post-treated with the extract, without inducing direct apoptosis [43]. Such anti-mutagenic effects against MNNG were attributed to possible induction of detoxifying enzymes that degrade MNNG, chemical interaction with or absorption of MNNG (or its electrophilic degradation products), enhanced fidelity of DNA replication, and/or improved DNA repair [43]. Such factors that prevent or reduce chromosomal aberrations [43]. Although some findings provide evidence of a potent anti-mutagenic activity of *N. sativa*, research is still in its early stages of establishing a direct link between the specific ingredients in *N. sativa* extracts and the anti-mutagenic activity of *N. sativa* (Fig. 1).

5. Anti-metastatic effects of *N. sativa*

Awad investigated the effect of *N. sativa* oil on HT1080 human fibrosarcoma cell lines with regard to their fibrinolytic potential, a hallmark of malignant tumors [44]. *N. sativa* oil (25–200 μg oil/ml) caused a significant dose-dependent down-regulation of key fibrinolytic products including tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), and plasminogen activator inhibitor type 1 (PAI-1), both in sub-confluent and confluent cell cultures [44]. This study highlights the ability of *N. sativa* to hinder local tumor invasion and metastasis. Ait Mbarek and colleagues reported similar findings, whereby injection of 30–50 μl (28.5–47.5 mg/mouse) *N. sativa* essential oil into the tumor site of a DBA2/P815 (H2d) mouse model resulted in inhibition of liver metastasis even after 30 days of treatment [29] (Fig. 1).

6. Effects of *N. sativa* on natural killer (NK) cytotoxic activity

Enhancement of NK cytotoxic activity has been proposed by several research groups to serve as a mechanism underlying the anti-cancer effects of *N. sativa* [45–50]. In an *in vivo* study performed on healthy volunteers, El-Kadi and colleagues showed that ingestion of *N. sativa* oil for 4 weeks enhanced the ratio of helper to suppressor T cells and significantly improved NK cytotoxic function [46]. In agreement, an *in vivo* study performed in mice revealed that 1-week oral administration of an aqueous extract of *N. sativa* caused a significant increase in the number of splenic NK cells and a significant enhancement of splenic NK cytotoxic activity against YAC-1 tumor cells [48]. These *in vivo* findings were supported by *in vitro* studies. Abuharfeil and colleagues demonstrated that a fresh aqueous extract of *N. sativa* (50 and 100 μg/ml) led to a significant increase in splenic NK cytotoxic activity against YAC-1 tumor cells (% cytotoxicity 43.7 ± 3.6 and 62.7 ± 5.6 at 200:1 E:T ratio, 45.7 ± 5.7 and 44.6 ± 6.2 at 100:1 E:T ratio, and 13.6 ± 2.7 and 18.3 ± 3.1 at 50:1 E:T ratio, respectively) [47]. Indeed, the fresh aqueous extract of *N. sativa* appeared to be more potent in inducing NK cytotoxic activity compared to the old dried aqueous extract or the ethanolic extract [47]. A similar study from our laboratory provided further *in vitro* experimental evidence indicating that an aqueous extract of *N. sativa* (50–100 μg/ml) significantly enhanced killing of YAC-1 tumor cells due to

augmented NK cytotoxic activity leading to 14% (3 folds) and 23% (4.5 folds) cytotoxicity 200:1 E:T ratio at 50 μg/ml and 100 μg/ml concentrations, respectively [50]. Importantly, enhanced killing of YAC-1 tumor cells is due to the ability of *N. sativa* extract to improve NK cytotoxic activity rather than inducing an immediate cytotoxic effect. This is evidenced by the findings that *N. sativa* extract had no significant, direct cytotoxic effect against YAC-1 tumor cells in absence of NK cells [50]. We have reported similar observations in which aqueous extracts (100 μg/ml) of black pepper (*P. nigrum*) and cardamom (*Elettaria cardamomum*) caused a significant increase (35% and 45% cytotoxicity, respectively) in the NK cytotoxic activity against YAC-1 tumor cells [51]. Therefore, it seems that boosting the cytotoxic potential of NK cells against tumor cells is at least one mechanism exploited by several plant extracts to exert their tumoricidal action. Interestingly, Abuharfeil and colleagues assessed NK cytotoxic activity in the presence of the aqueous extract of *N. sativa* using splenocytes obtained from BALB/c mice [47], whereas in our study the NK cytotoxic activity was assessed using splenocytes obtained from C57/BL6 mice [50]. Although the enhancement of NK cytotoxic activity caused by *N. sativa* does not seem to be strain-specific, more studies are required to confirm this argument using splenocytes from a wide range of mice strains and even different animal models. Along the same lines, an aqueous extract of *N. sativa* (10–500 μg/ml) was shown to significantly enhance the cytotoxic activity (26.6–67.7% cytotoxicity) of NK cells isolated from human blood against K562 tumor cells *in vitro* [49]. The improved cytotoxic potential of NK cells was primarily due to the ability of *N. sativa* extract to significantly enhance the production of interferon γ (IFNγ) and TNFα, immunostimulatory cytokines with potent tumoricidal activity, from NK cells [49]. Moreover, treatment of NK cells with *N. sativa* extract led to a significant increase in the release, and hence activity, of granzyme A and N-acetyl-β-D-glucosaminidase, key proteolytic enzymes involved in target cell killing [49]. These findings suggest that augmentation of NK cytotoxic activity against tumor cells serves as an effective immunomodulatory mechanism that may explain, at least partially, the reported *in vitro* and *in vivo* anti-cancer effects of *N. sativa*. An early *in vivo* study, however, demonstrated that intraperitoneal injection of *N. sativa* oil (100 μg/100 ml/mouse) for 7 days caused a significant decrease in the number of splenic NK cells in non-infected and CMV-infected BALB/c mice [52]. Interestingly, although *N. sativa* oil treatment had no effect on NK cytotoxic activity in non-infected mice, it caused a significant suppression of NK cytotoxic activity in CMV-infected mice [52]. The same study revealed that *in vitro* treatment of splenic NK cells isolated from non-infected mice with *N. sativa* oil (100 μg/ml) significantly decreased their cytotoxic activity against YAC-1 tumor cells [52]. These findings are inconsistent with those reported by El-Kadi and his colleagues [46] regarding the effects of *N. sativa* oil on NK cytotoxic effects against cancer cells. These inconsistent findings are most likely due to different experimental conditions including the dose of *N. sativa* extract or oil, cell type, species, incubation time, and method of detection. It is worth mentioning that *N. sativa* oil may exert toxic effects against NK cells, which could be another factor influencing the outcome of the reported experiments. Future studies, with a carefully-designed experimental approach that addresses the raised possible experimental variables, are required to shed more light on the potential modulatory effects of *N. sativa* oil on NK cytotoxic activity.

Although some of the signaling molecules involved in mediating the immunostimulatory effects of *N. sativa* extracts in NK cells have been identified, the exact signaling pathways and molecular targets implicated in these pathways are largely unknown. Future *in vitro* and *in vivo* studies should focus on elucidating the targeted receptors and intracellular/extracellular factors involved in the signal transduction pathways that are modulated in NK cells by *N. sativa* extracts. Furthermore, we suggest that the stimulatory potential of *N. sativa* toward NK cytotoxic activity be further confirmed by *in vitro* and *in vivo* studies using a wide range of primary and transformed NK cells against numerous primary tumors and cancer cell lines (Fig. 1).

7. Signaling pathways underlying the anti-cancer effects of *N. sativa*

Several *in vitro* and *in vivo* studies were conducted in an attempt to elucidate the molecular and cellular mechanisms underlying the anti-cancer activity of *N. sativa*. The key mechanisms underlying the documented anti-cancer effects of *N. sativa* have been largely attributed to their ability to modulate the activity of key enzymes [31,43,44,49,53–58]. suppress inflammation [8,50,53,55,59–82], and induce apoptosis in tumor cells [21,41,54,55,72,83–101].

One mechanism that is implicated in tumorigenesis involves the inducible nitric oxide synthase (iNOS) pathway. NO, which is synthesized by iNOS or other nitric oxide synthase (NOS) isoforms during physiological reactions including inflammation, is an endogenous radical implicated in predisposition to tumor development. In a recent study, Fathy and Nikaido investigated the effect of an ethanolic extract of *N. sativa* on modulating the iNOS pathway in rats with DENA-induced hepatocarcinogenesis [30]. Oral administration of *N. sativa* ethanolic extract (250 mg/kg/day) for 5 days led to a significant reduction in the serum levels of AFP, NO, interleukin-6 (IL-6), and TNFα, factors whose production was significantly increased after treatment with DENA [30]. Very recently, Alhamzi and colleagues demonstrated that a methanolic extract of *N. sativa* seeds (50–100 μl/ml) induced apoptosis in MCF-7 cells in a time- and dose- dependent manner, as judged by TUNEL assay [97]. The methanolic extract of *N. sativa* led to a significant time- and dose-dependent increase in the expression of apoptotic factors including caspase-3, caspase-8, caspase-9, and p53 in MCF-7 cells, indicating that *N. sativa* manifests its anti-cancer activity by targeting the p53 and caspase signaling pathways [97].

A brief summary about the reported *in vitro* and *in vivo* anti-cancer activities of *N. sativa* is given in Table 1.

8. Anti-cancer effects of *N. sativa* phytoconstituents

Many of the anti-cancer activities of *N. sativa* have been attributed to its major active constituent, thymoquinone (TQ). TQ has been shown to exert anti-proliferative, pro-apoptotic, anti-oxidant, anti-oxidant, anti-mutagenic, anti-angiogenic, and anti-metastatic effects against cancer cells [6,12,14,16–19,38,53,55,63,64,66,70,71,77,82–100]. TQ seems to mediate its anti-cancer effects by targeting a number of cellular pathways involving p53, NF-κB, PPARγ, STAT3, MAPK, and PI3K/AKT transducing signals [67,69,72,83–100]. Besides TQ, other phytoconstituents of *N. sativa* have also been shown to contribute to the anti-cancer potential of *N. sativa* extracts. α-hederin is a pentacyclic triterpene saponin found in *N. sativa* seeds that exerts effective anti-cancer effects, both *in vitro* and *in vivo* [54,102–107]. Moreover, thymol, thymohydroquinone, dithymoquinone, nigellimine-N-oxide, nigellicine, nigellidine, and carvacrol are phytoconstituents of *N. sativa* that have been demonstrated to play anti-cancer and cytotoxic functions [13,108–117]. Yet, the exact molecular mechanisms underlying the anti-cancer effects of these phytoconstituents are not fully known, and future studies are needed to elucidate the detailed mechanisms of action that mediate the anti-cancer effects of *N. sativa* phytoconstituents.

Table 1
A brief summary of the reported *in vitro* and *in vivo* anti-cancer activities of *N. sativa*.

Activity	*N. sativa*
Anti-proliferative and pro-apoptotic effects	• Stimulation of anti-proliferative effects on MCF-7 cells [26]. • Reduction in frequency of mammary papillary, comedo, and cribriform carcinoma in DMBA-induced carcinoma model [28]. • Reduction in serum levels of total sialic acid (TSA), lipid-bound sialic acid (LSA), prolactin, estradiol, progesterone, serum TNFα, tissue caspase-3 activity, and DNA fragmentation [28]. • Amelioration of diethylnitrosamine-induced hepatocarcinogenesis [20]. • Induction of late-stage apoptosis and/or necrosis as well as inhibition of both DNA synthesis and cell proliferation in HepG2 cells [21,32]. • Protection against diethylnitrosamine-induced hepatocellular adenoma [23]. • Reduction of serum AFP levels, relative liver weight, and activities of hexokinase, glyceraldehyde phosphate dehydrogenase, and G6P dehydrogenase [31]. • Inhibition of the two-stage initiation/promotion of skin carcinogenesis and delays the onset of skin papilloma [25]. • Reduction of methylcholanthrene (MCA)-induced soft tissue sarcomas [25]. • Reduction in formation of pre-neoplastic lesions for colon cancer [27]. • Delay in mortality of P815 mastocytoma bearing cells [29].
Anti-oxidant and cytotoxic effects	• Induction of cytotoxic effects against HepG2, MOLT4and LL/2 cells but no effects on normal cells [33]. • Induction of cytotoxic effects against MCF-7 cells [26]. • Induction of cytotoxic effects against EAC, DLA, S-180 cells [24]. • Induction of cytotoxic effects against SCL, SCL-6, SCL-37′6, NUGC-4 cells [36]. • Reduction in expression of MDA and NO [34,41]. • Reduction in lipid peroxides and NO levels [28]. • No effect on not affect the level or catalytic activity of aspartate-aminotransferase, alanine-aminotransferase, and gamma-glutamyltransferase [35]. • Amelioration of nephrotoxicity through reduction in creatinine and urea as well as elevation of GSH levels [37]. • Amelioration of anti-cancer drug-induced hepatic cytotoxicity [38]. • Amelioration of antibiotic-induced cytotoxicity in the thymus and spleen [39].
Anti-mutagenic effects	• Inhibition against MNNG mutagenicity [43]. • Enhancement of DNA replication and reduction in chromosomal aberrations [43].
Anti-metastatic effects	• Down regulation of t-PA, u-PA, and PAI-1 [44]. • Inhibition of liver metastasis [29].
Effects on NK cytotoxic activity	• Enhancement of helper to suppressor T cell ratio and improvement of NK cytotoxic activity [46]. • Improvement and increase in cell numbers [48]. • Enhanced killing of YAC-1 tumor cells [50]. • Increase in production of IFNγ and TNFα [49]. • Increase in production and activity of granzyme A and N-acetyl-β-D-glucosaminidase [49]. • Suppression of NK cytotoxic activity in CMV-infected mice [52].

9. Conclusions

N. sativa is among the most commonly used herb in the history of mankind. *N. sativa* is considered by many to be a "miracle" herb due to its effective therapeutic potential to alleviate signs and symptoms of many diseases including cancer. The anti-cancer properties of *N. sativa* have been mainly attributed to its ability to exert potent anti-proliferative, pro-apoptotic, anti-oxidant, anti-mutagenic, and anti-metastatic roles. The protective effects of *N. sativa* against tumor initiation and progression have also been attributed, at least in part, to their ability to suppress inflammation and exert immune-boosting effects. Enhancement of NK cytotoxic activity against cancer cells and regulation of signaling pathways, such as iNOS, p53, and caspases, mediate the potential of *N. sativa* to subdue tumorigenesis and cancer. *In vitro* and *in vivo* experimental findings suggest that *N. sativa* extracts can potentially be employed in the development of effective therapeutic agents that can be employed in the regulation of various stages of tumorigenesis and treatment of many types of cancer. Further studies are definitely needed to shed more light on the molecular and cellular mechanisms underlying the anti-cancer effects of *N. sativa*. Such research endeavors will hopefully elucidate the exact signaling pathways implicated in the suppressive role that *N. sativa* extracts play in tumorigenesis and cancer. Moreover, although the preclinical, experimental evidence suggesting potent anti-cancer effects of various *N. sativa* extracts is compelling, preventive and clinical studies that directly point to the anti-cancer potential of *N. sativa* extracts are still lacking. Future studies should focus on establishing a direct link between the reported anti-cancer effects of *N. sativa* extracts and cancer prevention/treatment in preclinical and clinical settings.

Sources of support

None.

Conflicts of interest

There are no conflicts of interest.

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