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Anticancer Effects of Nigella sativa Seeds in Cell Line

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Abstract

Nigella sativa L. (black cumin) has been recognized for its medicinal properties for centuries, and recent studies have highlighted its bioactive components for their anticancer potential. Key constituents such as thymoquinone, nigellone, and alpha-hederin have been shown to exert significant anticancer effects in animal cell lines. These effects include the modulation of apoptosis, cell cycle arrest, and the inhibition of metastasis, making these components promising candidates for cancer therapy. Preclinical studies indicate that *Nigella sativa* targets multiple cancer hallmarks, including evading apoptosis, promoting proliferative signaling, and modulating inflammation and oxidative stress. However, several challenges limit its clinical translation. The primary limitations include poor bioavailability of thymoquinone, variability in the concentration of bioactive components due to cultivation and extraction methods, and the lack of large-scale clinical trials. To address these issues, strategies such as nanoparticle encapsulation and liposomal formulations are being explored to enhance bioavailability. Standardizing extraction methods and further investigating the molecular mechanisms of *Nigella sativa*'s anticancer effects are also critical steps toward clinical application. Additionally, its potential to act synergistically with conventional chemotherapy offers opportunities for integrative cancer therapies. In conclusion, while *Nigella sativa* holds significant promise as a complementary therapeutic agent in cancer treatment, overcoming these challenges is crucial for its effective clinical use.

Keywords: Anticancer, Apoptosis, Bioavailability, Chemotherapy, Thymoquinone, *Nigella sativa*.

Introduction

Cancer continues to be one of the most critical health challenges worldwide, with millions of new cases and deaths reported annually. In 2018 alone, the disease caused approximately 9.6 million deaths globally, making it the second leading cause of mortality, as stated by the World Health Organization (WHO) (1). While conventional treatments such as chemotherapy, radiotherapy, and surgery have significantly improved, they often present considerable side effects and limitations. This has sparked growing interest in alternative and complementary therapies derived from natural products, which aim to minimize side effects while addressing multiple pathways involved in cancer progression (2). Nigella sativa L., also known as black cumin or black seed, has been used in traditional medicine for over 2,000 years, particularly in the Middle East and Southeast Asia. It is valued for its wide range of pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, and anticancer effects (3). The seeds are rich in bioactive components such as thymoquinone (TQ), nigellidine, alpha-hederin, and dithymoquinone, with thymoquinone emerging as the most potent and well-researched compound, especially for its anticancer properties (4). The anticancer potential of Nigella sativa has been demonstrated in various cancer types, including breast, colon, liver, and lung cancers, through both in vitro and in vivo studies (5). These findings indicate that its bioactive components can promote apoptosis, inhibit cell proliferation, modulate oxidative stress, and suppress inflammation—key processes in cancer development and progression (6). Thymoquinone, in particular, has been shown to target critical molecular pathways in carcinogenesis, such as the PI3K/Akt, NF-κB, and MAPK signaling pathways, making it a promising candidate for cancer treatment (7). However, translating the preclinical success of Nigella sativa into clinical practice presents challenges, including issues related to the bioavailability of its active components like thymoquinone, the lack of standardized formulations, and the need for robust clinical trials to confirm its efficacy and safety in humans (8). Overcoming these challenges is crucial to fully

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harness the therapeutic potential of Nigella sativa as an anticancer agent. Cancer continues to be a leading global health challenge, with an estimated 19.3 million new cases and nearly 10 million cancer-related deaths recorded in 2020 (9). Despite significant advancements in conventional cancer therapies, including chemotherapy, radiotherapy, and targeted treatments, these approaches are often accompanied by severe side effects, resistance, and limited long-term efficacy. This underscores the need for complementary therapeutic strategies that are effective, safe, and capable of targeting multiple cancer pathways simultaneously. The challenges include the poor bioavailability of thymoquinone, variability in the concentration of active components due to differences in cultivation and extraction processes, and a lack of well-designed clinical trials to validate its efficacy and safety in humans. Additionally, while several molecular pathways affected by *Nigella sativa* have been identified, a comprehensive understanding of its mechanisms of action is still lacking. This study aims to address these gaps by providing a detailed review of the anticancer properties of *Nigella sativa* components in animal cell lines, emphasizing their molecular mechanisms, therapeutic potential, and limitations. By highlighting the challenges in clinical translation and proposing future research directions, this study seeks to bridge the gap between preclinical findings and clinical applications, paving the way for *Nigella sativa* as a complementary treatment in oncology.

Cancer Statistics: A Growing Global Burden of Cancer

Cancer remains one of the leading causes of death worldwide, with 19.3 million new cases and nearly 10 million deaths reported in 2020, according to GLOBOCAN 2020. Projections indicate that the global cancer burden will increase by 47% by 2040, driven largely by population growth and aging demographics (10). The rising prevalence underscores the urgency to develop innovative and effective treatment strategies to manage this growing health crisis. Conventional cancer therapies such as chemotherapy, radiotherapy, and surgery have improved survival rates, but they remain limited by substantial drawbacks, including severe side effects and the risk of disease recurrence.

Side Effects of Conventional Therapies

Despite advancements, conventional cancer therapies are fraught with limitations that impact their efficacy and patients' quality of life. Chemotherapy, for instance, is non-specific, targeting both cancerous and healthy cells, leading to systemic toxicity. Common side effects include myelosuppression, gastrointestinal issues, fatigue, and cardiotoxicity, which often necessitate dose reduction or discontinuation of treatment (11). Radiotherapy, while precise, can cause collateral damage to healthy tissues, resulting in side effects like radiation-induced fibrosis, dermatitis, and chronic fatigue (12). Furthermore, resistance to chemotherapy and radiotherapy is a growing concern, as it contributes to disease progression and limits the success of these treatments.

The Need for Complementary Therapies: The Role of *Nigella sativa*

Given the limitations of conventional therapies, there is increasing interest in complementary and integrative treatments to enhance cancer care. *Nigella sativa* (black cumin) has emerged as a promising natural remedy with significant anticancer potential. Its key bioactive component, thymoquinone (TQ), has demonstrated the ability to modulate apoptosis, inflammation, and oxidative stress, all of which are critical processes in cancer progression (13). Preclinical studies have shown that *Nigella sativa* targets multiple hallmarks of cancer, suggesting its potential as a complementary agent to mitigate the adverse effects of conventional therapies while enhancing their efficacy.

Potential Toxicity of *Nigella sativa* and Its Components

While *Nigella sativa* is widely regarded as safe and has been used traditionally for centuries in various medicinal systems, emerging studies suggest that certain bioactive components, particularly thymoquinone (TQ), may pose potential risks at high doses or in specific contexts. Understanding the therapeutic window and dosage limitations is crucial for ensuring its safe use in both preclinical and clinical applications.

Safety Profile at Therapeutic Doses

At low to moderate doses, *Nigella sativa* and its components have been shown to exhibit minimal

toxicity and high tolerability. Preclinical studies on animal models have reported that TQ, the primary bioactive compound, has a broad therapeutic window and is generally well-tolerated at doses ranging between 10–50 mg/kg body weight (14). These findings support its potential use in cancer therapy, where high efficacy and minimal side effects are desired.

Potential Toxicity at High Doses

At higher doses, however, *Nigella sativa* and its active compounds have demonstrated toxicity in specific organs, particularly the liver and kidneys. Studies have indicated that thymoquinone can induce hepatotoxicity and nephrotoxicity when administered at high concentrations over prolonged periods. Symptoms such as elevated liver enzymes, oxidative stress, and histopathological changes in the liver and kidneys have been observed in animal studies exposed to doses above 80 mg/kg body weight.

Context-Specific Risks

The potential for toxicity may also depend on individual patient factors, such as pre-existing liver or kidney conditions, concurrent medications, or hypersensitivity to specific components of *Nigella sativa*. For instance, patients undergoing chemotherapy may already experience hepatic or renal stress, making them more susceptible to additional toxicity from natural compounds like thymoquinone. Additionally, some studies have noted that *Nigella sativa* extracts could interact with certain drugs, such as anticoagulants, potentially leading to adverse effects (15).

Need for Dose Optimization and Safety Validation

To mitigate potential toxicity, it is essential to optimize the dose and duration of *Nigella sativa* treatments. Rigorous dose-response studies are needed to establish the maximum tolerated dose (MTD) and no-observed-adverse-effect levels (NOAEL) for human applications. Furthermore, clinical trials are critical to evaluate the long-term safety and potential interactions of *Nigella sativa* components with conventional cancer therapies. *Nigella sativa* shows immense therapeutic promise, careful consideration of dosage, patient-specific factors, and long-term safety is essential to minimize toxicity risks and maximize its benefits in clinical applications.

Mechanisms of Action of *Nigella sativa* Active Components

The anticancer potential of *Nigella sativa* L. seeds, especially through its active compound thymoquinone (TQ), has garnered considerable interest due to its ability to target multiple molecular pathways associated with tumor development and progression. Numerous in vitro and in vivo studies have highlighted the effectiveness of *Nigella sativa* components in influencing key processes such as apoptosis, cell cycle regulation, oxidative stress, and inflammation—all of which play crucial roles in cancer progression and metastasis (3, 4).

Induction of Apoptosis

One of the key mechanisms through which *Nigella sativa* exhibits its anticancer effects is by inducing apoptosis, or programmed cell death, in cancer cells. Apoptosis is a critical biological process that inhibits the proliferation of damaged or mutated cells, and its dysregulation is a hallmark of cancer (6). Thymoquinone (TQ), the primary bioactive compound in *Nigella sativa*, has been shown to induce apoptosis in a variety of cancer cell lines, including those of breast, lung, and colon cancers (7). TQ activates both intrinsic and extrinsic apoptotic pathways by modulating the expression of pro-apoptotic proteins such as Bax, caspases, and p53, while suppressing anti-apoptotic proteins like Bcl-2 (8). For instance, a study on human osteosarcoma cells demonstrated that TQ induced mitochondrial-mediated apoptosis by upregulating Bax expression and downregulating Bcl-2, resulting in the activation of caspase-9 and caspase-3 (3). Likewise, in breast cancer cell lines, TQ-induced apoptosis was accompanied by cell cycle arrest at the G2/M phase, suggesting that components of *Nigella sativa* can disrupt cancer cell proliferation and survival (5).

Modulation of Oxidative Stress and Reactive Oxygen Species (ROS)

Cancer cells are known to experience elevated levels of oxidative stress due to increased production of reactive oxygen species (ROS), which contribute to cancer progression by causing DNA damage, activating oncogenes, and inhibiting tumor suppressor genes (6). The antioxidant properties of *Nigella sativa*, particularly those of its key compound thymoquinone (TQ), are well-documented. TQ has been shown to reduce oxidative stress by scavenging ROS and enhancing

the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (3). In hepatocellular carcinoma models, TQ significantly decreased ROS levels and inhibited oxidative DNA damage, leading to the suppression of tumor growth (7). Additionally, TQ has been shown to restore redox balance in cancer cells, thereby limiting their proliferation and metastatic potential. These findings highlight *Nigella sativa's* role as a powerful antioxidant with promising therapeutic potential in addressing cancer-associated oxidative stress (4)

Inhibition of Angiogenesis

Angiogenesis, the process by which new blood vessels form, plays a vital role in tumor growth and metastasis by providing oxygen and nutrients to rapidly dividing cancer cells (9). Targeting angiogenesis is considered a promising strategy for cancer treatment. *Nigella sativa* and its active compounds, particularly thymoquinone (TQ), have demonstrated anti-angiogenic properties in preclinical research. TQ suppresses angiogenesis by reducing the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), which are crucial regulators of angiogenesis in cancer (7). For example, a study on human glioblastoma cells revealed that TQ treatment significantly lowered VEGF levels, resulting in reduced angiogenesis and smaller tumor size in an animal model (4). *Nigella sativa* have significant potential as a complementary therapy, particularly for aggressive cancers characterized by high vascularization.

Anti-inflammatory Effects

Chronic inflammation is strongly associated with cancer development, as it promotes the proliferation, survival, and migration of malignant cells (10). *Nigella sativa* exhibits potent anti-inflammatory effects, primarily through the inhibition of nuclear factor-kappa B (NF-κB), a transcription factor that regulates the production of pro-inflammatory cytokines and enzymes involved in cancer progression (8). Thymoquinone (TQ), the key bioactive compound of *Nigella sativa*, has been shown to suppress NF-κB activation, leading to a decrease in pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (5).

In colorectal cancer models, TQ reduced inflammation-induced tumor formation by downregulating NF-κB and cyclooxygenase-2 (COX-2), both of which are key players in cancer-associated inflammation (3). These findings highlight *Nigella sativa's* therapeutic potential for preventing and treating inflammation-driven cancers.

Cell Cycle Arrest

Nigella sativa's bioactive components have been shown to inhibit cancer cell proliferation by inducing cell cycle arrest. Since uncontrolled cell division is a hallmark of tumor growth, regulating the cell cycle is a critical focus of cancer therapy. Thymoquinone has been reported to cause G1 or G2/M cell cycle arrest in various cancer cell lines by modulating cyclins and cyclin-dependent kinases (CDKs) (7). For example, in breast cancer cells, TQ treatment induced G2/M phase arrest, which was associated with the downregulation of CDK1 and cyclin B1 (4).

Synergistic Effects with Conventional Therapies

Research indicates that *Nigella sativa* components may boost the effectiveness of conventional cancer treatments when used in combination. Studies have shown that thymoquinone can enhance the efficacy of chemotherapeutic agents such as cisplatin and doxorubicin, allowing for reduced drug doses and fewer side effects (8). For instance, a study on ovarian cancer cells revealed that combining TQ with cisplatin significantly increased apoptosis and reduced tumor size compared to cisplatin alone (3). These synergistic effects suggest that *Nigella sativa* has potential as an adjuvant therapy in cancer treatment.

Bioactive Components of *Nigella sativa* L. Seeds

The seeds of *Nigella sativa*, also known as black cumin, have been extensively used in traditional medicine, especially in the Middle East and Southeast Asia. These seeds are rich in bioactive compounds, including essential oils, alkaloids, saponins, fatty acids, and phenolic compounds, with thymoquinone (TQ) being the most studied. These components exhibit a variety of pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer effects. Below is an overview of the major bioactive constituents of *Nigella sativa* and their potential roles in cancer prevention and treatment (11).

Thymoquinone (TQ)

Thymoquinone is the primary bioactive compound in the volatile oil of *Nigella sativa* seeds and is recognized as the key contributor to the plant's anticancer effects (4). Numerous in vitro and in vivo studies have demonstrated that TQ can induce apoptosis, inhibit cell proliferation, suppress angiogenesis, and mitigate oxidative stress. TQ's ability to target multiple molecular pathways, such as p53, NF-κB, and apoptosis-regulating proteins, makes it a promising candidate for anticancer drug development (5). One of TQ's significant advantages over traditional chemotherapy is its selective toxicity, as it targets cancer cells while sparing normal cells. This selective cytotoxicity has been observed in several cancer types, including breast, prostate, lung, and colon cancers (12).

Alkaloids

Nigella sativa seeds also contain alkaloids such as nigellidine, nigellimine, and nigellicine, which possess biological activities including anticancer effects. These alkaloids have been shown to inhibit cancer cell proliferation and induce apoptosis in vitro (13). Furthermore, these compounds may enhance the immune system's ability to combat cancer cells, making them potentially valuable in immunotherapy. Studies suggest that alkaloids work synergistically with other bioactive constituents, such as TQ, to enhance the therapeutic efficacy of *Nigella sativa* extracts (4).

Saponins

Saponins present in *Nigella sativa* seeds exhibit anticancer activity by promoting apoptosis, inhibiting tumor invasion, and modulating the immune system (14). One notable saponin, α-hederin, has been shown to suppress cancer cell proliferation and induce apoptosis in multiple cancer models. α-Hederin disrupts cancer cell membranes, increasing their permeability and leading to cell death. Additionally, it enhances the immune response against tumors, further supporting the potential role of *Nigella sativa* in cancer immunotherapy (15).

Essential Oils

The essential oils of *Nigella sativa* seeds, containing compounds such as p-cymene, thymohydroquinone, and carvacrol, contribute significantly to its anticancer potential. These oils inhibit cancer cell growth by regulating oxidative stress, inflammation, and apoptosis pathways (16). For example, thymohydroquinone induces apoptosis by increasing ROS production and activating caspases that regulate programmed cell death. Similarly, p-cymene inhibits cell proliferation and promotes apoptosis in various cancer cell lines (17), while carvacrol modulates signaling pathways involved in tumor growth and metastasis (18).

Fatty Acids

Nigella sativa seeds are abundant in unsaturated fatty acids such as linoleic acid, oleic acid, and eicosadienoic acid, which not only promote overall health but also exhibit anticancer properties. These fatty acids modulate lipid metabolism, reduce inflammation, and enhance immune responses (19). For instance, linoleic acid has been shown to inhibit cancer cell proliferation by inducing lipid peroxidation and disrupting cell membrane integrity. Additionally, these fatty acids support cardiovascular health, which is often affected in cancer patients undergoing chemotherapy, making them an integral component of integrative cancer therapies (20).

Phenolic Compounds

Phenolic compounds, including flavonoids and tannins, are also present in *Nigella sativa* seeds and are known for their antioxidant and anticancer properties. These compounds neutralize free radicals, lower oxidative stress, and inhibit cancer initiation and progression (21). Flavonoids, for example, inhibit cancer cell survival enzymes like protein kinase C (PKC) and topoisomerase II, while simultaneously enhancing immune responses. These properties make flavonoids valuable in cancer immunotherapy (22). The diverse bioactive components of *Nigella sativa* seeds, such as thymoquinone, alkaloids, saponins, essential oils, fatty acids, and phenolic compounds, work through various mechanisms to prevent and treat cancer. These compounds collectively demonstrate significant potential for use in integrative and complementary cancer therapies.

Preclinical Studies in Animal Cell Lines: Anticancer Effects of *Nigella sativa*

Preclinical research has thoroughly examined the anticancer properties of *Nigella sativa* components across various cancer cell lines, revealing significant potential in both in vitro and

in vivo models. These studies have focused on key bioactive constituents, including thymoquinone (TQ), α-hederin, and nigellidine, evaluating their effects on different types of cancer. The anticancer mechanisms of *Nigella sativa* components involve multiple processes, such as inducing apoptosis, inhibiting cell proliferation, suppressing angiogenesis, reducing oxidative stress, and regulating key signaling pathways. Among these pathways, NF-κB, p53, and PI3K/Akt are particularly notable for their roles in cancer progression and treatment response (4). These findings underscore the therapeutic potential of *Nigella sativa* as a complementary or integrative approach in cancer management, warranting further exploration in clinical settings. Table 1 gives the overall view of the preclinical studies on the anticancer effects of *Nigella sativa* components on cell lines of various organs.

Table 1: Preclinical Studies on the Anticancer Effects of *Nigella sativa* Components

Cancer Type	Cell Line/Model	Mechanism of Action	Results	Ref
Breast Cancer	MDA-MB-231 (mouse)	Induced apoptosis, inhibited metastasis	Reduced tumor size and metastasis	(21)
Liver Cancer	HepG2 (rat)	Modulated oxidative stress and inflammatory pathways	Inhibited tumor growth	(3)
Colon Cancer	HCT-116 (mouse)	Induced apoptosis, inhibited cell proliferation	Reduced proliferation, induced caspase activation	(7)
Lung Cancer	A549 (rat)	Activated apoptotic pathways, reduced angiogenesis	Suppressed tumor growth	(8)

Preclinical Studies of Bioactive Compounds from *Nigella sativa*

Extensive preclinical studies have investigated the anticancer properties of various bioactive compounds from *Nigella sativa* in different cancer models. The few models are as

Thymoquinone (TQ) in Breast Cancer Cells

Thymoquinone has been shown to inhibit cell proliferation and promote apoptosis in breast cancer cell lines (MCF-7 and MDA-MB-231). This effect is mediated by the downregulation of the anti-apoptotic protein Bcl-2 and the upregulation of the pro-apoptotic protein Bax, leading to caspase activation and subsequent cell death (7).

***Nigella sativa* Oil in Colon Cancer Cells**

Nigella sativa oil demonstrated significant anti-proliferative effects in colon cancer cell lines (HT29 and HCT116). The oil induced G1 cell cycle arrest and triggered apoptosis through the activation of the p53 tumor suppressor pathway (23).

α-Hederin in Lung Cancer Cells

α-Hederin, a saponin derived from *Nigella sativa*, inhibited the proliferation of lung cancer cells (A549). The compound induced apoptosis by disrupting mitochondrial membrane potential and increasing reactive oxygen species (ROS) production, which contributed to cancer cell death (15).

Combination Therapy with Thymoquinone and Chemotherapeutic Agents

The combination of thymoquinone with conventional chemotherapeutic agents, such as doxorubicin and cisplatin, has demonstrated enhanced therapeutic efficacy. In hepatocellular carcinoma cell lines, the combination of TQ and doxorubicin increased cytotoxicity and reduced chemoresistance by modulating the NF-κB signaling pathway (24).

Nigellidine in Prostate Cancer Cells

Nigellidine, an alkaloid from *Nigella sativa*, showed anticancer effects in prostate cancer cells (PC-3). The compound inhibited cancer cell proliferation by inducing apoptosis and modulating the Akt/mTOR signaling pathway, resulting in significant tumor growth inhibition (8). These findings highlight the diverse anticancer mechanisms of *Nigella sativa* bioactive compounds, including apoptosis induction, cell cycle regulation, and enhancement of conventional therapies. Further clinical studies are needed to validate their therapeutic potential in cancer treatment. Table 2 gives the brief overview of the preclinical studies carried out for demonstrating anticancer effects of *Nigella sativa* components in selected animal cell lines.

Table 2: Preclinical Studies on the Anticancer Effects of *Nigella sativa* Components in Animal Cell Lines

Study	Cell Line/Model	Bioactive Component	Mechanism of Action	Key Outcomes	References
Woo *et al.,* (2012)	Breast cancer (MCF-7, MDA-MB-231)	Thymoquinone (TQ)	Induced apoptosis via Bcl-2 downregulation and Bax upregulation	Inhibition of cell proliferation and increased caspase-mediated apoptosis	(7)
Gali-Muhtasib *et al.,* (2004)	Colon cancer (HT29, HCT116)	*Nigella sativa* oil	Promoted apoptosis through p53 activation	G1 cell cycle arrest, reduced cell proliferation, enhanced apoptosis	(25)
Mousavi *et al.,* (2015)	Lung cancer (A549)	α-Hederin	Increased ROS production, disrupted mitochondrial membrane potential	Induced apoptosis and reduced cell viability	(18)
Kundu *et al.,* (2014)	Hepatocellular carcinoma (HepG2)	Thymoquinone (TQ) + Doxorubicin	Inhibited NF-κB pathway, enhanced cytotoxicity	Synergistic anticancer effects with reduced chemoresistance in hepatocellular carcinoma models	(26)
Darakhshan *et al.,* (2015)	Prostate cancer (PC-3)	Nigellidine	Modulated Akt/mTOR signaling pathway, induced apoptosis	Inhibited cancer cell proliferation and induced apoptosis	(14)

Mechanistic Insights from Preclinical Studies

The anticancer effects of *Nigella sativa* are primarily linked to its ability to induce apoptosis and modulate critical signaling pathways in cancer cells. Thymoquinone (TQ), the most studied component, influences apoptotic regulators such as Bcl-2, Bax, and caspases, promoting programmed cell death. Additionally, TQ inhibits pro-inflammatory pathways like NF-κB, reduces oxidative stress, and enhances reactive oxygen species (ROS) production, all contributing to tumor suppression (12). *Nigella sativa* components also inhibit the PI3K/Akt and mTOR pathways, which are often overactivated in cancer cells to promote survival, proliferation, and resistance to therapy. These mechanisms highlight the potential of *Nigella sativa* as an effective adjuvant to conventional cancer treatments, with the ability to counteract drug resistance and enhance therapeutic efficacy (4). Although preclinical studies offer strong evidence of *Nigella sativa*'s anticancer potential, further research is essential to clarify the molecular mechanisms underlying these effects. Clinical trials are needed to validate preclinical findings in humans and to assess the safety and efficacy of *Nigella sativa* components as part of combination therapies in oncology. Such studies would help establish its role in integrative cancer treatment strategies.

Therapeutic Potential and Limitations of *Nigella sativa* in Cancer Treatment

Therapeutic Potential

Nigella sativa, commonly known as black cumin or black seed, has garnered attention for its therapeutic potential as an anticancer agent. Its bioactive components—including thymoquinone (TQ), α-hederin, nigellidine, and other phytochemicals—have demonstrated the ability to

target several cancer hallmarks, such as:Sustaining proliferative signaling, evading apoptosis, inducing oxidative stress and modulating inflammation. Numerous in vitro and in vivo studies support the anticancer effects of *Nigella sativa* in various cancers, including breast, lung, liver, colon, and prostate cancers (11, 19).Despite these promising findings, challenges remain, like poor bioavailability of *Nigella sativa* bioactive compounds, such as TQ, which may limit their therapeutic efficacy, A lack of standardized formulations and dosing protocols for clinical use and insufficient clinical trial data to confirm preclinical results and to determine the safety profile in human populations. Addressing these limitations through advancements in drug delivery systems, such as nanoparticles or liposomal formulations, and conducting comprehensive clinical trials are essential steps toward unlocking the full therapeutic potential of *Nigella sativa* in cancer treatment.

Induction of Apoptosis

Thymoquinone, the most studied bioactive compound derived from *Nigella sativa*, induces apoptosis in a variety of cancer cell lines by modulating both intrinsic and extrinsic apoptotic pathways. It enhances the expression of pro-apoptotic proteins like Bax and caspase-3 while suppressing anti-apoptotic proteins such as Bcl-2 (6). Additionally, thymoquinone reduces mitochondrial membrane potential, leading to the release of cytochrome c and subsequent caspase activation, which ultimately triggers programmed cell death (8).

Inhibition of Cancer Cell Proliferation

Nigella sativa and its constituents have demonstrated efficacy in suppressing cancer cell proliferation by targeting critical oncogenic pathways. Thymoquinone, in particular, has been found to downregulate the PI3K/Akt/mTOR pathway, which is essential for cancer cell growth, survival, and metabolism (7). Inhibiting this pathway induces G1 phase cell cycle arrest, thereby reducing the rate of cancer cell proliferation (5).

Anti-inflammatory and Antioxidant Effects

Chronic inflammation and oxidative stress are pivotal in cancer development and progression. *Nigella sativa* exhibits strong anti-inflammatory properties by inhibiting the NF-κB signaling pathway, a key regulator of pro-inflammatory cytokines and mediators involved in cancer progression (6). Moreover, thymoquinone has potent antioxidant effects, scavenging reactive oxygen species (ROS) and reducing oxidative stress. These properties prevent DNA damage and inhibit the initiation of cancer (11).

Synergistic Effects with Chemotherapy

Several studies highlight the potential of *Nigella sativa* components, especially thymoquinone, to enhance the efficacy of conventional chemotherapy agents. In combination with drugs such as doxorubicin, cisplatin, and 5-fluorouracil, thymoquinone has been shown to increase cytotoxicity and promote apoptosis in cancer cells. Additionally, it helps overcome drug resistance and reduces the required doses of chemotherapeutic agents, thereby minimizing their toxic side effects (8, 22). These synergistic effects position *Nigella sativa* as a promising adjuvant therapy in cancer treatment.

Bioavailability and Pharmacokinetics

A major challenge with *Nigella sativa*, particularly thymoquinone, is its low bioavailability and poor solubility. After oral administration, only a small fraction of the compound reaches systemic circulation, limiting its therapeutic efficacy (7). Strategies like nanoparticle delivery systems, liposomal formulations, and other advanced drug delivery methods are being explored to improve TQ's bioavailability and clinical potential (23).

Lack of Clinical Trials

Despite promising preclinical results, the clinical application of *Nigella sativa* is hindered by a lack of well-designed clinical trials in humans. Most evidence comes from laboratory and animal studies, and robust clinical research is needed to confirm safety, efficacy, and optimal dosage in humans (8). Large-scale trials are essential for translating preclinical success into practical oncology therapies.

Variability in Active Component Concentration

The concentration of active compounds like thymoquinone in *Nigella sativa* seeds can vary significantly due to factors such as geographic origin, cultivation conditions, and extraction methods (27). This variability poses challenges in achieving consistent therapeutic outcomes. Standardized extraction and processing methods are necessary to ensure uniformity and reproducibility in therapeutic applications (11).

Potential Side Effects and Toxicity of *Nigella sativa* and Its Components

While *Nigella sativa* is widely recognized for its safety and has been used in traditional medicine for centuries, emerging evidence suggests that its bioactive components, particularly thymoquinone (TQ), may cause side effects or toxicity at high doses or during long-term use. Understanding these potential risks is essential for establishing safe therapeutic applications.

Safety at Low to Moderate Doses

At low to moderate doses, *Nigella sativa* and its components are generally considered safe. Preclinical studies have demonstrated high tolerability in animal models, with doses of TQ ranging between 10–50 mg/kg body weight showing minimal side effects (14). Human studies on *Nigella sativa* seed oil and powder for various conditions have reported few adverse effects, such as mild gastrointestinal discomfort and allergic reactions (15). This safety profile supports its use as a complementary therapeutic agent in short-term interventions.

Toxicity at High Doses

At higher doses, however, TQ and other components of *Nigella sativa* have been associated with organ-specific toxicity. Studies in animal models have shown that doses above 80 mg/kg of TQ can lead to hepatotoxicity and nephrotoxicity. Symptoms include elevated liver enzymes (e.g., ALT, AST), oxidative stress, and histopathological changes in liver and kidney tissues (28). These toxic effects are believed to result from excessive oxidative stress and disruption of cellular redox balance.

Long-Term Use and Chronic Toxicity

Long-term use of *Nigella sativa* or its bioactive components raises concerns about cumulative toxicity, especially in vulnerable populations such as cancer patients who may already have compromised liver or kidney function. While there is limited data on chronic toxicity, some studies suggest that prolonged high doses could exacerbate oxidative damage and impair organ function. Further research is needed to establish the long-term safety profile of *Nigella sativa* in both healthy individuals and cancer patients (29).

Context-Specific Risks

The potential for side effects may also vary depending on the context of use. For instance, *Nigella sativa* components may interact with conventional medications, such as anticoagulants, leading to increased bleeding risk. Additionally, cancer patients undergoing chemotherapy or radiotherapy may experience heightened susceptibility to the toxic effects of *Nigella sativa* due to pre-existing organ stress. This emphasizes the need for careful dose management and monitoring in clinical applications. (7)

Strategies to Minimize Toxicity

To minimize the risk of toxicity, several strategies should be considered like dose optimization in which the determining the maximum tolerated dose (MTD) and establishing no-observed-adverse-effect levels (NOAEL) through preclinical and clinical studies, Advanced Drug delivery system includes utilizing nanoparticles or liposomal formulations to improve the bioavailability of TQ and reduce systemic exposure, and patient specific guidelines includes the tailoring dosages based on patient-specific factors, such as age, comorbidities, and concurrent medications. Although *Nigella sativa* is generally considered safe at low to moderate doses, high concentrations of thymoquinone may cause toxicity, particularly in the liver and kidneys (23). Studies have reported hepatotoxicity at elevated doses, emphasizing the importance of determining optimal dosages that maximize efficacy while minimizing adverse effects (8). Careful dose management and monitoring will be critical for clinical applications.

Limited Understanding of Mechanisms

Although research has identified key molecular pathways targeted by *Nigella sativa*, a comprehensive understanding of its anticancer mechanisms remains incomplete. Additional studies are required to uncover the full spectrum of molecular interactions and identify biomarkers that can predict patient responses to *Nigella sativa*-based therapies (22). A deeper understanding could facilitate the development of personalized cancer treatments incorporating *Nigella sativa* in integrative strategies.*Nigella sativa* holds immense promise as a natural anticancer agent, thanks to its ability to induce apoptosis, inhibit proliferation, reduce inflammation, and synergize with chemotherapy. However, challenges such as bioavailability, variability in active component concentration, and limited clinical trials must be addressed to translate preclinical findings into effective clinical

applications. With advancements in drug delivery systems, rigorous clinical studies, and standardization protocols, *Nigella sativa* could become a vital component of integrative cancer therapies. Table 3 describes the therapeutic potential possessed by *Nigella sativa* in anticancer therapy.

Table 3: Therapeutic Potential of *Nigella sativa* in Cancer Treatment

Therapeutic Potential	Description	References
Induction of Apoptosis	Promotes cancer cell apoptosis by modulating Bcl-2, Bax, and caspase proteins	(30, 4)
Inhibition of Cancer Cell Proliferation	Downregulates oncogenic pathways such as PI3K/Akt/mTOR	(4)
Anti-inflammatory and Antioxidant Effects	Reduces inflammation by suppressing NF-κB and scavenges free radicals	(12, 7)
Synergistic Effects with Chemotherapy	Enhances efficacy and reduces drug resistance in combination with chemotherapy	(21, 24)

Drug Delivery Systems and Standardized Formulations for *Nigella sativa*

Advanced Drug Delivery Systems

Nigella sativa have a poor bioavailability of bioactive components, particularly thymoquinone (TQ), has prompted extensive research into innovative drug delivery systems. Nanoparticle-based carriers, liposomal formulations, and self-nanoemulsifying drug delivery systems (SNEDDS) have shown promise in enhancing the solubility, stability, and systemic absorption of these compounds. For instance, researchers (19) developed a nano-based carrier for *Nigella sativa* essential oil, demonstrating significant improvements in stability and bioavailability. The use of nanoparticles has also been shown to facilitate targeted delivery, reducing systemic toxicity and enhancing therapeutic efficacy (20, 21). Additionally, SNEDDS systems have been successfully applied for other natural compounds, such as curcumin, to improve their bioavailability, suggesting potential applications for *Nigella sativa* components as well (22).

Standardized Formulations

The variability in *Nigella sativa*'s bioactive compound concentration due to differences in cultivation, extraction, and processing remains a major challenge for its clinical application. Standardization of extraction techniques and quality control protocols is essential to ensure consistency and reproducibility. There is a need of harmonized guidelines in cultivating and processing *Nigella sativa* to improve the reliability of its pharmaceutical formulations (20). Furthermore, novel positioning of *Nigella sativa* from "farm to pharma" underscores the importance of incorporating these standardized practices into pharmacopeia to facilitate its integration into modern medicine (23). Table 4 shows the limitations associated with the use of *Nigella sativa* in cancer treatment. The information is enough to work on the shortcomings and utilize its maximum benefits.

Table 4: Limitations of *Nigella sativa* in Cancer Treatment

Limitations	Description	References
Low Bioavailability and Poor Solubility	Limits systemic absorption and therapeutic efficacy	(7)
Lack of Clinical Trials	Few human studies available to validate preclinical findings	(8)
Variability in Active Component Concentration	Variability in thymoquinone content due to cultivation and extraction methods	(24)
Potential Side Effects and Toxicity	High doses of thymoquinone may cause hepatotoxicity	(23)
Limited Understanding of Mechanisms	More research needed to fully elucidate molecular mechanisms and biomarkers	(22)

Enhancing Bioavailability

Thymoquinone, the primary active component of *Nigella sativa*, suffers from poor bioavailability when administered orally, leading to low systemic absorption and reduced therapeutic efficacy. To address this limitation, researchers are developing innovative drug delivery systems, such as nanoparticle encapsulation, liposomal formulations, and other advanced delivery methods. These approaches aim to enhance thymoquinone's bioavailability, stability, and targeted delivery; ensuring higher concentrations reach cancerous tissues (7).

Conducting Rigorous Clinical Trials

Despite promising preclinical data, the lack of large-scale clinical trials evaluating the anticancer effects of *Nigella sativa* remains a significant barrier to its clinical application. Translating laboratory findings into clinical efficacy requires well-designed studies to assess safety, optimal dosage, and therapeutic potential in human populations (8). Such trials are essential to establish *Nigella sativa* as a viable component of integrative cancer therapy.

Standardization of Extraction Processes

Variability in the concentration of bioactive compounds in *Nigella sativa* seeds due to differences in geographic origin, cultivation practices, and extraction methods presents a major challenge to reproducibility. Developing standardized protocols for extraction and processing is crucial to ensuring consistent therapeutic effects and facilitating the development of reliable treatments based on *Nigella sativa* (24, 27).

Exploration of Molecular Mechanisms and Synergistic Effects

While preclinical research has identified some molecular pathways targeted by *Nigella sativa* components—such as NF-κB, PI3K/Akt, and apoptosis-related proteins like Bax and Bcl-2—more detailed studies are needed to fully understand these mechanisms. Furthermore, the potential synergistic effects of *Nigella sativa* with conventional cancer treatments, such as chemotherapy and radiotherapy, warrant further exploration. If these interactions are proven, *Nigella sativa* could serve as a valuable adjuvant therapy, improving the efficacy of existing treatments while minimizing their side effects.

Translating Preclinical Findings to Clinical Settings: Challenges and Strategies

Despite the promising anticancer properties demonstrated by *Nigella sativa* and its bioactive components in preclinical studies, translating these findings into clinical applications faces several challenges. One major hurdle is the lack of large-scale, well-designed clinical trials to validate the safety, efficacy, and optimal dosages of *Nigella sativa* in humans. Most of the existing evidence is derived from in vitro experiments and animal models, which, while valuable, may not fully capture the complexities of human physiology and cancer heterogeneity (13). Another challenge is the poor bioavailability of thymoquinone (TQ), the most studied component of *Nigella sativa*. Low solubility and rapid metabolism limit its systemic absorption and therapeutic efficacy. Furthermore, variability in the concentration of bioactive components due to differences in cultivation conditions, extraction methods, and processing techniques complicates the standardization of formulations. This inconsistency poses difficulties in achieving reproducible results in clinical settings (22). To overcome these challenges, several strategies can be proposed. First, conducting rigorous clinical trials, including randomized controlled studies, is essential to assess the efficacy and safety of *Nigella sativa* in diverse patient populations. Second, advanced drug delivery systems, such as nanoparticles, liposomal formulations, and hydrogels, should be developed to improve the bioavailability and targeted delivery of thymoquinone. These systems can enhance therapeutic efficacy while minimizing off-target effects (7). Third, the development of standardized cultivation, extraction, and quality control protocols can ensure consistent concentrations of active components, which is critical for therapeutic reliability. Lastly, exploring *Nigella sativa*'s synergistic effects with conventional cancer therapies, such as chemotherapy and radiotherapy, could enhance its integrative potential and improve treatment outcomes.

Future Research Directions for *Nigella sativa* in Cancer Therapy

To fully harness the therapeutic potential of *Nigella sativa* and its bioactive components in

cancer treatment, a range of critical research areas needs to be addressed. These include the conduct of clinical trials, development of standardized formulations, and exploration of advanced drug delivery mechanisms. Below, these key future directions are elaborated.

Need for Clinical Trials

While numerous preclinical studies have demonstrated the efficacy of *Nigella sativa* in cancer therapy, the translation of these findings to human applications remains limited due to a lack of large-scale, well-designed clinical trials. The majority of available evidence stems from in vitro and animal model studies, which may not accurately reflect the complex physiological conditions in humans. Clinical trials are essential to:

- Validate the safety, efficacy, and optimal dosage of *Nigella sativa* components, particularly thymoquinone (TQ), in cancer patients.
- Investigate the potential interactions of *Nigella sativa* with conventional therapies, including chemotherapy and radiotherapy.
- Assess the long-term safety of *Nigella sativa* in diverse patient populations, particularly those with pre-existing conditions or undergoing multimodal cancer treatments (15).

Development of Standardized Formulations

The variability in the concentration of bioactive components in *Nigella sativa* due to differences in cultivation, harvesting, and extraction methods remains a significant challenge for its therapeutic application. To address this, future research must focus on:Establishing standardized cultivation and extraction protocols to ensure consistent concentrations of key bioactive compounds such as TQ, α-hederin, and nigellidine. Developing quality control measures for *Nigella sativa* products to ensure reproducibility and uniformity in clinical studies and therapeutic use (22).

Exploration of Novel Drug Delivery Mechanisms

The poor bioavailability of thymoquinone, primarily due to its low solubility and rapid metabolism, limits its therapeutic efficacy. Advances in drug delivery systems can play a pivotal role in overcoming this limitation. Future research should focus on:

Nanoparticle-based Delivery Systems: Encapsulating TQ in nanoparticles can enhance its solubility, protect it from rapid degradation, and improve its systemic bioavailability. This approach has already shown promise in preliminary studies (7).

Liposomal Formulations: Liposomal carriers can deliver TQ directly to tumor tissues, reducing systemic toxicity and enhancing its therapeutic index.

Hydrogel-based Delivery Systems: Hydrogels could provide sustained and localized release of TQ, improving its retention at the tumor site and minimizing off-target effects.

Combination Delivery Platforms: Developing dual delivery systems to co-administer TQ with chemotherapeutic agents or other natural compounds could enhance their synergistic effects (28).

Molecular Mechanisms and Biomarker Identification

Understanding the detailed molecular mechanisms through which *Nigella sativa* exerts its anticancer effects is critical for its successful application. Future research should to investigate the role of *Nigella sativa* components in modulating key cancer pathways such as PI3K/Akt/mTOR, NF-κB, and MAPK signaling and to identify predictive biomarkers that can help stratify patients who are most likely to benefit from *Nigella sativa*-based therapies (29). Addressing these future research directions—through clinical trials, standardization of formulations, advanced drug delivery systems, and in-depth molecular studies—will be crucial for realizing the full potential of *Nigella sativa* as an effective complementary therapy in cancer treatment. By overcoming current challenges, *Nigella sativa* could play a pivotal role in integrative oncology, improving both treatment efficacy and patient outcomes.

Conclusion

The bioactive components of *Nigella sativa* seeds, particularly thymoquinone, have shown significant anticancer potential in preclinical studies by inducing apoptosis, suppressing cell proliferation, and modulating key signaling pathways like NF-κB and PI3K/Akt. However, translating these findings into clinical practice requires overcoming critical challenges, including improving bioavailability, conducting rigorous clinical trials, and standardizing extraction methods. Addressing these obstacles through

continued research and innovation will be essential in advancing *Nigella sativa* as a complementary therapeutic agent in cancer treatment. With its potential to enhance treatment efficacy and reduce side effects, *Nigella sativa* could become an integral part of integrative oncology in the future.

Abbreviations

TQ: Thymoquinone, NF-Kb: Nuclear Factor Kappa B, PI3K: Phosphoinositide 3-Kinase, Akt: Protein Kinase B, mTOR: Mechanistic Target of Rapamycin, ROS: Reactive Oxygen Species, Bcl-2: B-cell Lymphoma 2 (anti-apoptotic protein), Bax: Bcl-2-associated X protein (pro-apoptotic protein).

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Author Contributions

Imran Khan conducted this study and contributed in study design, conceptualization, and final draft of the manuscript. Dr. J.D. Shaikh reviewed the study and suggested modifications.

Conflict of Interest

None of the Co-authors expressed Conflict of Interest.

Ethics Approval

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