In vitro and *in vivo* effects of *Nigella sativa* in normal and cancerous cell

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Abstract

A growing body of evidence has shown over the past ten years that using medicinal plants may enhance the supportive management of a number of ailments. Among these plants, *Nigella sativa* (*N. sativa*, family Ranunculaceae) has garnered a lot of attention. Numerous studies on *N. sativa*'s extracts, seeds, and active ingredient thymoquinone have produced evidence that the plant may have some therapeutic promise for treating a variety of ailments, including cancer.

Through Pubmed, the terms "*N. sativa* and cancer," "*N. sativa*," "*N. sativa* and metastasis," and "*N. sativa* and cytotoxicity of natural killer cells" were used to apply the selection criteria for references.

With a focus on breast cancer, its anti-metastatic properties, and how *N. sativa* modifies the cytotoxicity of Natural Killer cells that are essential for tumor surveillance, the goal of this study was to summarize data discussing the effects of *N. sativa* in cancer generally.

In conclusion, the evidence points to the potential use of *N. sativa* as a cancer immune system stimulant, as well as for its anti-cancer and antimetastatic effects.

Keywords: *Nigella sativa*, alcoholic extracts, metaphase chromosomes, MCF-7 and HdFn cell line

Introduction

N. sativa, a member of the Ranunculaceae botanical family, is a common plant in Eastern Europe, the Middle East, and Western Asia. It is a tiny shrub with rosaceous white and violet blooms and tapering green leaves. The ripe fruit of this plant produces tiny black seeds known in English as black seed, black cumin, or black caraway in Arabic as "Al-Habba Al-Sauda" and "Al-Habba Al-Barakah."

Numerous ailments, such as fever, the common cold, headache, asthma, rheumatic diseases, microbial infections, and to expel worms from the intestines as well as "Sartan" are treated with the help of *N. sativa* seeds in folk medicine in the Middle East and some Asian nations (Unani, Ayurveda, Chinese, and Arabic Medicines) (cancer). Additionally, it is used to bread and pickles as a flavoring agent. It is reported that the prophet Muhammad advised using black seeds since all illnesses except "As-Sam," which results in death, may be cured by them.

Numerous researchers were inspired to separate *N. sativa*'s active compounds, including thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide, nigellicine, nigellidine, and alpha-hederin because of the plant's varied usage in traditional medicine. In order to investigate its pharmacological properties, such as immunostimulation, anti-inflammatory, hypoglycemic, antihypertensive, antiasthmatic, antimicrobial, antiparasitic, antioxidant, as well as anticancer properties, numerous in vitro and in vivo studies have been carried out on laboratory animals and humans. Thymoquinone, the most extensively researched active component, and *N. sativa*, its oil, have both been found to be quite safe, especially when administered orally, according to acute and chronic toxicity studies on laboratory animals. Only a small number of authors have previously examined the therapeutic benefits of *N. sativa*, describing some of the anticancer effects.

More research has since been done on *N. sativa*'s anticancer properties and its active ingredients, according to a literature review. Recent reviews of thymoquinone's molecular and clinical potential in treating cancer omitted research on the anticancer properties of *N. sativa* seed, oil, different extracts, and other active substances such alpha-hederin [1].

One of the most crippling and traumatizing illnesses of the contemporary era, cancer currently lacks a curative treatment. The second most common cause of mortality is cancer [2]. There is a need to find novel natural medicines for the treatment of chronic diseases [4-7], particularly cancer [8], due to the limited therapeutic window of chemical drugs [3], particularly cancer treatment agents, and the development of resistance against these drugs. Adjuvant therapy, such as the use of medicinal plants, may have some effect in reaching cancer treatment objectives even though modern medications used to treat patients with various types of cancer have not been entirely effective [9].

Apoptosis is just one of many elements that contribute to cell survival and proliferation; any factor that upsets the harmony between the cell cycle and apoptosis might result in cancerous cell growth [10]. *Nigella sativa* (*N. sativa*) has been used medicinally for centuries in traditional medicine, and studies in Unani, Ayurveda, and Chinese medicine have shown that it possesses anti-cancer and anti-proliferative properties [11]. The purpose of this review is to analyze the effectiveness of *N. sativa* against the development of malignancy in both in vitro and in vivo models, as well as to emphasize the significance of

apoptosis in the advancement of cancer.

In order to do this, we searched the scientific databases PubMed, Web of Science, and Google Scholar between the years 2000 and 2017 using the keywords *N. sativa*, anti-cancer, apoptotic effect, antitumor, antioxidant, and malignancy. We then compiled the most recent scientific data on *N. sativa*'s anticancer activities and its mechanisms of action.

Apoptosis

Understanding the inner workings of a certain type of cell death that takes place in various cells of the human body is of significant interest right now. The process of programmed cell death (PCD), known as apoptosis, typically affects a few isolated cells rather than all the cells in a given location, and it proceeds swiftly once it is started. As a result, apoptosis is a gene-regulated process that results in morphological alterations, blebbing, nuclear fragmentation, cell shrinkage, chromatin condensation, chromosomal DNA fragmentation, and global mRNA degradation in cells [12, 13]. Along with cancer and AIDS, it is a significant factor in a number of autoimmune and neurological diseases and disorders [14-16]. Other harmful factors that cause apoptosis include heat, reactive oxygen species, radiation, hypoxia, and cytotoxic anticancer medicines [17].

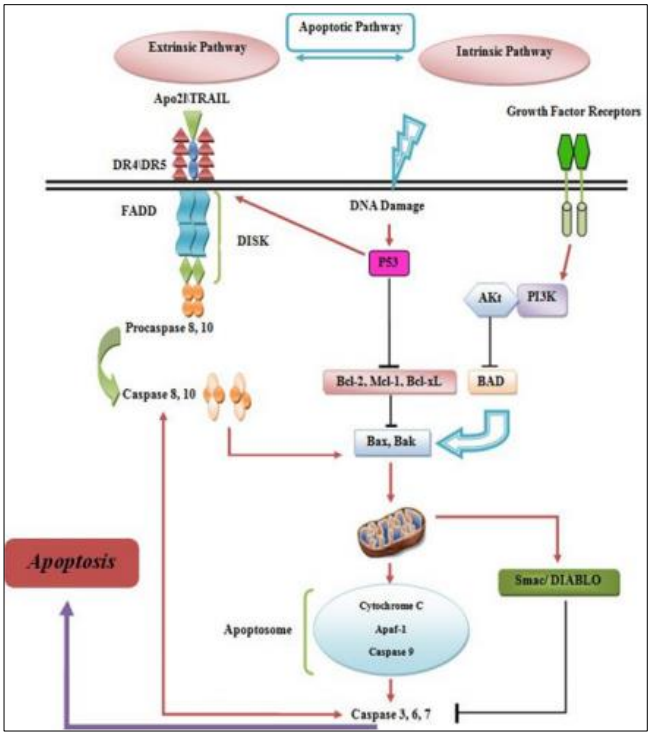


Fig 1: (Apoptotic pathways)

Nigella sativa

The medicinal plant *N. sativa* (family: Ranunculaceae) is also known as black seed, black cumin, and the seed of blessing (Habatul-barakah in Arabic) [18]. Bisexual *N. sativa* plants range in height from 20 to 90 cm and are primarily found in southern Europe, northern Africa, and regions of Asia, including the Middle East. Long peduncles support the blue flowers, which bloom alone. Many white trigonal seeds make up the fruit capsule when it first forms, however once the fruit has matured and opened, exposing the black seeds to air, they change color to black [19, 20]. The primary components of *N. sativa* plants that have been utilized for medical purposes for thousands of years are the seeds and oil [21, 22].

Materials and methods

Plant extracts

The dried *Nigella sativa* seeds were used to make the crude alcoholic extracts using a Soxhlet extractor. 100 g of the fine seed powder was taken after grinding and placed inside a thimble tube. 1000 ml of ethanol (ethyl alcohol) was added as the extraction solvent. The temperature of the mantle was fixed at the degree of heating (60-70 degrees Celsius). After nine to ten hours of extraction, a solution of the solute and solvent

Preparation of alcoholic extracts

After washing the *Nigella sativa* leaves in distilled water, 1% sodium hypochlorite solution, and sterile distilled water again, the alcoholic extract of *Nigella sativa* was created. 20 g of *Nigella sativa* were weighed and placed in a food processor. 100 ml of 95% ethanol was then added, mixed for 2–3 minutes, and left in the flask on a shaker for 24 hours to dissolve in ethanol. After that, the plant material was filtered through several layers of gauze to remove any remaining fibers and suspended plant matter, and then it was filtered once more through a Millipore microfiltration unit with a 0.45 mm When all the alcohol had evaporated, the mixture was then placed in an electric oven set to 40°C. The plants were washed in distilled water, 1% sodium hypochlorite solution, and then sterile distilled water one more to create the alcoholic extract of *Nigella sativa*.

Weighed and placed in a food processor was Nigella sativa. After adding 100 ml of 95% ethanol, the liquid was processed for two to three minutes. After shaking the flask for 24 hours to help the ethanol dissolve, it was filtered. The remaining material was filtered once more using a Millipore microfiltration device with a 0.45 mm diameter to prevent bacteria from passing through the filter, after the remaining fibers and suspended plant bits were removed using numerous layers of gauze. To make the alcoholic extract, *Nigella sativa* was washed with distilled water, 1% sodium hypochlorite solution, and then sterile distilled water once more. Weighed and placed in a food processor was Nigella sativa. It was blended for a short while with 100 ml of 95% ethanol before being placed in a flask and agitated for 24 hours to dissolve in the ethanol. Following that, it was filtered. There were numerous layers of gauze employed to extract the leftover fibers and suspended plant parts. The residual fibers were then filtered once more using a 0.45 mm-diameter Millipore microfiltration machine. Alcohol extract sterilisation, stock preparation, dilution The mixture was pasteurized before the plant extract dilutions were made from the stock solution after adding 2 g of the powdered alcoholic and aqueous plant extracts to 5 ml of ethanol and distilled water, respectively, to form a stock solution with 400 mg/ml.

Laboratory animals

Swiss white mice, Mus musculus, both male and female, were utilized. They were procured from the animal house of the College of Veterinary Medicine/University of Mosul at ages ranging from (6-8) weeks and with weights between (25-30 g). The animals were kept in designated cages in rooms that are part of the animal house under environmental conditions that included a constant temperature of (25 2), continuous ventilation, and appropriate lighting, and they were continuously given access to water and a diet that was in line with a standard diet.

Determination of the lethal dose (LD 50)

The up and down approach provided by Mood and Dixon was used to calculate the LD50 of the alcohol extract of Nigella sativa [23].

Metaphase chromosomes analysis in bone marrow cells of albino mice.

Perform the test according to Brusick's method [24].

Cell culture

Breast cancer (MCF-7) and normal human newborn dermal fibroblast (HDFn) cells were the two cell lines used in this experiment. Using 10% fetal bovine serum (FBS), 1% antibiotic antimycotic, and Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, USA), the cells were kept alive in tissue culture flasks produced by Falcon in the USA (Invitrogen, USA). The incubation conditions for the cells were 37 °C, 5% CO2, and 95% relative humidity. Cell counts obtained using the trypan blue exclusion method were used to compute cell densities to a final level of 1 104 viable cells per ml. 1 103 cells were planted into each well of the 96-well microplates (Falcon, USA) that were used for the research. After the cells had been cultivated for 24 hours, the medication or plant extracts were added. The negative vehicle control was 0.2% DMSO in complete DMEM, and the negative controls were untreated cells. Following treatment, the cells were grown for 48 hours before being examined using the AlamarBlue® assay.

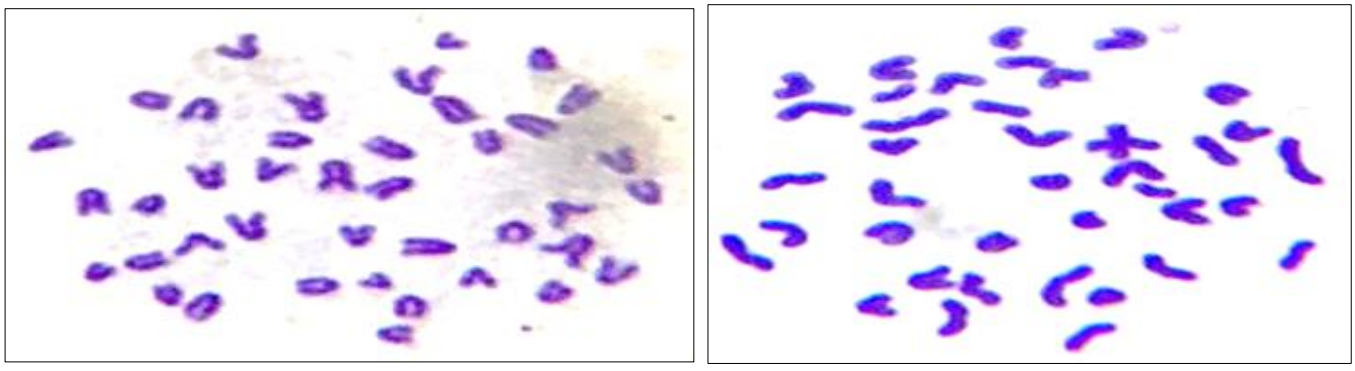
Statistical analysis

The Statistical Package of Social Science (SPSS) program was used to analyze the data statistically with a probability level ($P \leq 0.05$) ($P \leq 0.01$).

Results (*in vivo)*

Metaphase chromosome analysis test

Figure (1) depicts a normal chromosomal karyotype and a chromosomal karyotype with structural abnormality in bone marrow cells in white mice of the negative control groups treated with Nigella sativa. The findings of the current study, which are summarized in Tables (1) and (2), supported the use of MTC as a positive control and *Nigella sativa* as a negative control. When compared to the negative and positive control group, all concentrations of *Nigella sativa* (25%, 50, 75, and 100% mg/kg-1) were able to cause structural and numerical chromosomal aberrations in the bone marrow cells of white mice, as well as their propensity to damage the DNA strand during cell division, One of the types of structural abnormalities is gaps, and because they are one of the types of genetic damage [28], it is important to record their appearance in order to estimate the susceptibility of chemical compounds to mutagenicity [27]. Additionally, because the repair process is performed incorrectly, many chromosomal aberrations are formed [25], and the high frequency of these aberrations may cause tumors [26]. As a result, the meta phase averages of structural chromosomal anomalies were determined both with and without the gaps [29].



(a) Normal chromosomal karyotype (b) A chromosomal karyotype containing Robertsonian transmission

Fig 2: (a,b) shows a normal chromosomal karyotype n=40 and a chromosomal karyotype with structural abnormality in bone marrow cells in white mice of the negative control group and the group treated with *Nigella sativa* (X100 Gimsa stain)

As for the concentrations of *Nigella sativa* (25%, 50, 75, and 100% mg/kg-1), the four extract concentrations did not significantly change the mean of chromosomal abnormalities in any of the groups when compared to the values of the negative and positive control group, indicating that the alcoholic extract of *Nigella sativa* reduces the numerical and structural abnormalities but does not stop them from occurring. The results listed in the table demonstrated this, where we find that the group that was given MTC reached an average numerical anomaly (1.51±4.40), while the four concentrations of the extract reached (1.50±0.40), (1.50±2.80), (1.50*±3.20) and (1.50±3.50), respectively, compared to the negative

control.

Table 1: Shows the mean values of differences for the numerical abnormalities of the chromosomes of the Meta phase in the bone marrow cells of white mice after treatment with different doses of alcoholic extract of *Nigella sativa*, negative control and MTC as positive control

No.	Numerical chromosomal anomalies (CAn) S.E ± MD	Treatment Dose/mg. kg-1. b.wt
1.	0.70±1.00	D.W
2.	1.25±0.20	N.S. 25%
3.	1.25±0.40	N.S. 50%
4.	1.25±0.50	N.S. 75%
5.	1.25±0.60	N.S. 100%
6.	**1.25±9.20	MTC Positive

* Significant at the level of significance ≤ 0.05 P Tukey LSD test
** Significant at significance level of 0.01 ≤ P Tukey LSD test

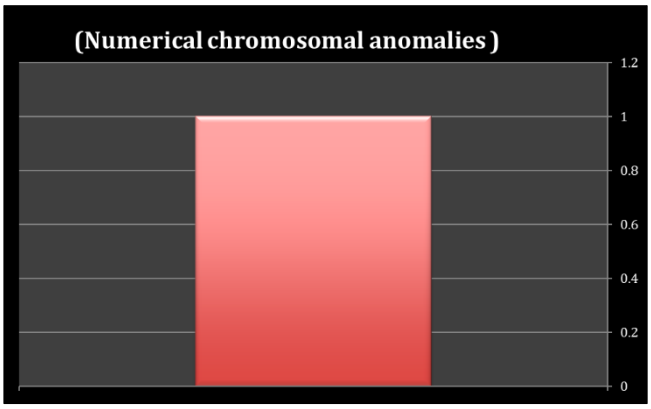


Fig 3: Shows the mean values of differences for the numerical abnormalities of the chromosomes of the Meta phase in the bone marrow cells of white mice after treatment with different doses of alcoholic extract of *Nigella sativa*, negative control and MTC as positive control.

Table 2: Shows the mean values of the differences for the structural abnormalities with and without the gaps for the Meta phase chromosomes, and the percentages of the types of structural abnormalities in the bone marrow cells of the white mice

Treatment /mg. kg-1. b.wt	Aberration with gap (CAs Wg) S.E	Aberration without gap (CAs Wog) S.D	Gaps %	Breaks %	Fragments %	Centromere join%	Robertsonian translocation%	Tails meet%
D.W	1.51±4.40	1.51±3.40	22.72	27.27	31.81	0	9.09	9.09
N.S. 25%	1.50±0.40	1.22±0.00	35.29	17.64	38.23	0	8.82	0
N.S. 50%	1.50±2.80	1.22±2.20	46.15	28.2	25.64	0	0	0
N.S. 75%	1.50*±3.20	1.22*±2.80	27.08	25	45.83	0	2.08	0
N.S. 100%	1.50±3.50	1.22±3.00	34.37	21.87	29.68	0	12.5	1.56
MTC Positive	1.51±4.40	1.51±3.40	26.92	30.76	38.46	0	3.84	0

When compared to the negative and positive controls, the effects of treatment with Nigella sativa's alcoholic extract in its four concentrations did not significantly alter the mean values of the numerical anomalies. These acids exhibit protective properties against oxidative stress brought on by MTC as well as working to break free radicals and stop the start of a cycle of free radicals [30].

Results *in vitro*

The rising *Nigella sativa* concentrations and controls affect the cytotoxicity of breast cancer cells, as shown in the following set of tables and figures. The determined percentages of methanol and aqueous suppression of cell viability are shown in (Table 3) A linear decrease in optical density and an increase in cellular growth inhibition are observed with increasing doses of the two extracts. An ANOVA analysis revealed that there was a significant difference (*p* value 0.001) from the negative control at concentrations of 30, 60, 125, 250, and 500 g/ml for the two extracts, but no significant difference from the positive controls at any of the concentration levels.

Table 3: The cytotoxic effect of *Nigella sativa* in methanol extract on HdFn and MCF-7 cell line at 72H

Concentration µg mL-1	Mean viability (%) ± SD	
	HdFn	MCF-7
500	57.523±5.8	52.08±1.63
250	62.92±2.33	63.31±1.5
125	77.662±1.40	76.15±1.7
60	87.4±1.42	86.227±3.3
30	94.71±0.67	94.44±0.50

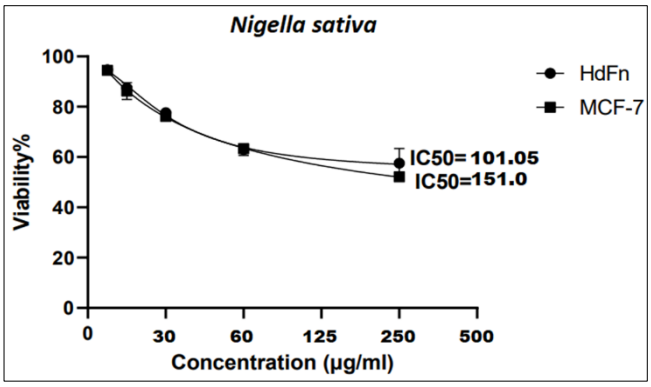


Fig 4: Determine of mtt on HdFn and MCF-7 cell line in methanol *Nigella sativa* extract

The log-linear regression dose-response curves for methanol given in Figures 4 were utilized to calculate the IC50 using the slope, linearity index (r2), and accompanying confidence intervals. The *Nigella sativa* extract dramatically decreased cell viability more than the other extracts in a dose-dependent manner. The IC50 values for HdFn and MCF-7 were 151.0 and 101.05 g/ml, respectively, for the methanol extracts. These values are lower than the predicted IC50 for mytomycine but higher than the IC50 for colchicine and 5-fluorouracil.

Treatment of HDFn with the identical concentrations of *Nigella sativa* extracts and controls used for MCF-7 cell line allowed researchers to gauge the effect on a typical human cell line. The cytotoxic effects of the extracts and the controls on the normal cells are shown in Table 3. Log-linear regression was used to establish the parameters for the dose-response curve and calculate the IC50. The findings demonstrated that pharmacological controls had far more lethal effects on normal cells than did plant extracts. The data show that HDFn normal cells are not cytotoxic to *Nigella sativa* extracts (IC50 > 100 g/ml).

Cancer is almost often treated with chemotherapy and/or radiation. However, these cytotoxic treatments carry a risk of serious immunological deficits, cardiomyopathies, and the emergence of cancers linked to the malignancy itself. The search for safer therapeutic alternatives is still a problem for science. Some extracts have been found to have anti-cancer properties in recent study on plant-based goods.

More research is needed because of the *Nigella sativa* extract's potent anti-colorectal cytotoxic action. For HdFn and MCF-7, the IC50 for methanol was 151.0 g/ml and 101.05 g/ml, respectively. Given the pharmacological restrictions, these IC50 values for MCF-7 are acceptable. This cancer cell line has very little cytotoxic activity, as evidenced by the IC50 values from methanol extracts, which are noticeably higher than the pharmacological controls.

Furthermore, both *Nigella sativa* extracts did not significantly harm healthy HDFn cells, as was demonstrated. Further research should be done on the protective mechanisms and effectiveness of *Nigella sativa* extracts against these cancer cells. When compared to pharmacological controls, the IC50 levels for MCF-7 and HdFn generated by the *Nigella sativa* extract were significantly lower. Several cancers, including lung, breast, colon, and prostate cancers, are inhibited by *Nigella sativa* [31-33].

The anti-cancer properties of pomegranates have been attributed to a variety of mechanisms in these studies. In the LAPC4 xenograft model, *Nigella sativa* reduces prostate cancer cell lines' NF-kBand cell survival in a dose-dependent way in vitro [34].

The important androgen-synthesizing enzymes SRD5A1 (steroid 5alpha reductase type 1), AKR1C3 (aldo-ketoreductase family 1 member C3), and HSD3B2 (3beta-hydroxysteroid dehydrogenase type 2) were lowered by *Nigella sativa* polyphenols, ellagitannin-rich extract, and whole juice extract [35].

Due to its inhibition of CYP activity/expression, which is necessary for procarcinogen activation, *Nigella sativa* may have anti-carcinogenesis properties. Some pomegranate metabolites, including 3,8-dihydroxy-6H-dibenzo[b, d]pyran-6-one (urolithin A, UA), produced by ellagic anthracens (ETs), may also have anti-cancer potential. After receiving (50-150 g/mL) pomegranate fruit extract (PFE) for 72 hours, lung cancer was discovered to be strongly suppressed.

The effects of this treatment included the dose-dependent arrest of cells in the G0/G1 phase of the cell cycle, induction of WAF1/p21 and KIP1/p27, reduction of the protein expression of cyclins D1, D2, and E, and reduction of the expression of cyclin-dependent kinase (PE also altered Bax and Bcl-2 levels in the PC-3 cell line).

Angiogenesis can be stopped by Pg, according to a recent study. By assessing VEGF, IL-4, and migration inhibitory factor (MIF) in the conditioned media of immortalized normal human breast epithelial cells, estrogen-sensitive (MCF-7) or estrogen-resistant (MDA-MB-231) human breast cancer cells, and both.

Assessed Pg's capacity for anti-angiogenic activity. As VEGF was dramatically reduced in MCF-10A and MCF-7 while MIF was elevated in MDA-MB-231, human umbilical vein endothelial cells (HUVEC) demonstrated great potential for inhibitory effects of angiogenesis by *Nigella sativa* fractions. The strongest anti-mutagenic action is seen in the 15 mg/plate methanolic extract of *Nigella sativa* in TA 100 cells.

The use of medicinal plants to treat patients with illnesses ranging from minor ailments to more serious ones like cancer is on the rise as a result of their rising global popularity, safety, and low cost. According to the published research, *N. sativa*, particularly TQ, its main bioactive component, exhibits anticancer qualities with apoptotic effects. As a result, it can be utilized to treat patients with a wide range of illnesses and disorders, particularly cancer.

The up-regulation of p21 and p53, as well as the inhibition of Bcl-2, the activations of caspases -8, -9, and -3, and elevations in the Bax/Bcl-2 ratio, were used to demonstrate that TQ induces apoptosis. Another apoptotic impact of TQ is the up-regulation of tumor suppressors together with a reduction in p-Akt. TQ's effects on TNF- have been demonstrated to reduce IAP1, IAP2, Bcl-2, Bcl-xL, XIAP, survivin, COX-2, cyclin D1, and VEGF as NF-B-regulated gene products. Other anti-cancer

actions of *N. sativa* include inhibition of both VEGF-dependent ERK and Akt activation.

These findings are significant because they shed light on the potential benefits of *N. sativa* for the treatment of cancer patients. This motivates researchers to carry out additional research in order to create new and more potent formulations for the treatment of a variety of illnesses, including cancer. *N. sativa* has recently gained prominence as a research topic across the globe, but more research is required to understand the various apoptotic processes that further demonstrate the plant's effectiveness as a cancer treatment.

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