

Phytopharmaceutical and Anticancer Properties of Nigella Sativa Linn Seeds: A Comprehensive Review


Samajdar S.^{1*}

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^{1*} Saptarshi Samajdar, School of Pharmacy, The Neotia University, West Bengal, India.

Cancer is a growing health problem around the world and is second leading cause of death after heart disease. Although in the last century there has been effort to control the disease using various synthetic as well as herbal drugs but still not been controlled satisfactorily. Nigella sativa is one such herb which has very high potential in preventing cancer. There are various categories of phytochemicals that have been isolated from the plant and mainly terpene category of phytochemicals in the likes of Thymoquinone, carvone, germacrene, sabinene, alphahederin are prevalent. Some other phytochemicals that has been isolated are nigellone, nigellidone (alkaloids) cycloartenol, tirucallol, some fatty acids as well. Mainly two compounds have been concentrated upon for cancer, they are thymoquinone and alpha hederin N. sativa has been known to shown high efficacy in cancer and mainly works by promoting Caspase 8,9,3, suppressing Akt and NF-kB. Even some semisynthetic derivative like poloxin, kalapanaxosaponin I and also combination therapy has been found to be effective against cancer.

Keywords: Nigella Sativa, Thymoquinone, Anticancer, Phytochemicals

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Introduction

Medicinal plants have always been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Natural products have always been successful in history of medical science [1]. In the twentieth century, the use of secondary metabolites from plants and microorganisms has contributed in the improvement of human lives. They practically reduced pain and suffering, and revolutionized medication by enabling the transplantation of organs [2]. Although usage of medicinal plant is more prevalent in Asia, Africa and to some extent in Europe as well but in last one and half decade there has been a great boom in North America for the usage of medicinal plants [3].

Cancer is one of the biggest threats to modern human life, which is considered as the second cause of death right after myocardial infarction or heart attack. Millions die every year with cancer despite tremendous efforts to find methods of control and cure [4, 5].

In the previous century, modern medical science has achieved significant progress in the fight against cancer. But unfortunately, the pharmacological treatment and chemotherapeutics have shown limited potential in achieving a long-term solution for treatment of various types of cancer. So, to find out new areas in the research scientists are delving in the past by taking support of ethnomedicinal prospects comprising mostly of natural products. *Nigella sativa* has been used for medicinal functions for hundreds of years. It originated from South Eastern Asia and also employed in ancient Egypt, Greece and some parts of Mesopotamia.

In Islam, it's considered one among the best forms of healing medication out there. It's a flowering plant, of which seeds are generally employed as a spice in common practice [6]. In English, the seed is known as black cumin, while in Latin it is known as 'Panacea,' which means 'cure all,' and in Arabic it is known as 'Habbah Sawda,' or 'Habbat el Baraka,' which means 'Blessing Seeds.'. It's conjointly referred to as 'Kalo jeera' (in Bangladesh), 'Kalonji' (in India) and 'Hak Jung Chou' in (China). Each seeds and oil extracted from this plant square measure employed in healthful functions.

Kalonji (*Nigella sativa*) is a dicotyledonous seed of family ranunculaceae is an incredible herb usually grows within the Japanese, Europe, geographic area, and Western Asia [7]. The seeds is the main source of the active ingredient of this plant. It is also called black seed referred by Prophet Mohammed as a panacea that's a remedy for all ailments however cannot stop ageing or death. The utilization of black seeds has been mentioned in numerous religious and ethnic books. Even its mentions are found in Holy Bible [8]. From thousands of years, till now, many individuals from the Mediterranean region and much of Middle-East countries use the oil of *N. sativa* seeds daily as a natural protecting agent.

Pharmacognostical Characteristics

Taxonomic classification: Kingdom Plantae; Class Magnoliopsida; Order Ranunculales; Family Ranunculaceae; Genus *Nigella*; Species *N. sativa*

Common Names: English: fennel flower, nutmeg flower, Roman coriander, blackseed or black caraway, black sesame; India: Assamese - kaljeera or kolajeera, Bengali - kalo jeeray, Kannada - Krishna Jeerige, Tamil - karum jeerakam, Hindi/Urdu - kalaunji/mangrail; Russian: Chernushka; Hebrew: Ketzakh; [9,10]

Habitat: *N.sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia [11]

Characteristics of seed and flower: *N.sativa* is an annually flowering plant which grows from 20 cm to 90 cm in height, with finely divided leaf, the segments of the leaf are narrowly linear to threadlike [12]. The flowers are delicate and are usually white, yellow, pink, pale blue or purple in colour. Number of petals present varies from 5-10. The fruit is large green and capsulated containing 3-7 united follicles, each having numerous black seeds [13]. Some other species around the world include *N. damasena*, existing in Syria and have only ornamental purposes,with flowers having shades of blue. Some other species are *N. hispanica*, *N. orientalis*, *N.papilosa*, *N.integrifolia*, *N.arvensis*, *N.cilliaris*. Macroscopically, *N. sativa* seeds present are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5mm in height and 1-2 mm in breadth (Fig: 1b), black externally

And white on inside, odour slightly aromatic and tastes bitter. Microscopically, transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells. Inside the pigment layer, there is a layer composed of thick walled rectangular elongated or nearly columnar cells [14]. Endosperm consists of thin walled, rectangular or polygonal cells mostly filled with oil globules. The powder microscopy of seed powder shows brownish black, parenchymatous cells and oil globules.



(a)



(b)

Fig 1: Nigella Seed (a) crushed seed, and (b) full seed

Cultivation methods: The plant has seen to widely grow in different part of the globe and is a yearly herb cultivated in India as well as other south Asian nations and in gulfs. *N. sativa* is cultivated during winter season in the same way as wheat. The areas where maize, green gram or black grams are grown can be well used after harvesting these crops. Before sowing the seeds, 2 to 3 times ploughing soil is enough for good crops and weed control. Heavy soils need more ploughing than light soils. The seeds are sown 30 cm apart. The seeds should not be sown deep as because it delays germination [15]. About 12 to 15 kg seeds per hectare are sown. Three to five irrigation are required that is, pre-sowing, seeding stage, flowering stage, and fruit formation stage and seeds

Development stage. Crop matures during April and May. It should be harvested early in the morning. The crop is harvested when the fruit/capsule turn yellowish. The late harvesting may result in shattering the seeds. After harvesting and proper drying seeds can be threshed by trampling the crop with tractor or proper thresher. After threshing, the seeds should be properly stored in bags or containers [16].

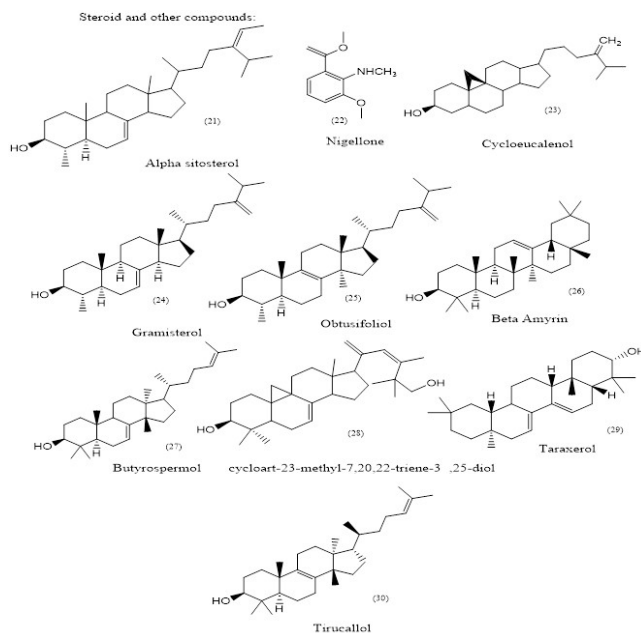
Chemical composition of black seeds: Many active compounds have been extracted isolated, identified and reported in different varieties of black seeds around the world.

Terpenoids: The most important active compounds in *N. sativa* are of terpenoids mainly thymoquinone (30%-48%)(1), thymohydroquinone (2), dithymoquinone (3), p-cymene (7%-15%)(4), carvacrol (6%-12%)(5), 4-terpineol (2%-7%)(6), t-anethol (1%-4%)(7), sesquiterpene longifolene (1%-8%)(8) α -pinene(9) etc. Black seeds also contain some other terpenoid compounds in trace amounts. These can be found in all polarities mainly in petroleum ether and chloroform extracts. Moreover, *N. sativa* seeds also contain alpha-hederin(13), a water soluble pentacyclic triterpene and saponin, a potential anticancer agent. Some other compounds e.g. carvone (10), limonene (11), citronellol (12) were also found in trace amounts. Most of the pharmacological properties of *N. sativa* are mainly attributed to quinine constituents, of which TQ is the most abundant. On storage, TQ yields dithymoquinone and higher oligocondensation products. The seeds of *N. sativa* contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fibre (8.4%) and total ash (4.8 %). The seeds are also containing good amount of various vitamins and minerals like Cu, P, Zn and Fe etc [17].

Alkaloids: Seeds contain two different types of alkaloids; i.e. isoquinoline alkaloids e.g. nigellicimine(14) and indazole alkaloids or indazole ring bearing alkaloids which include nigellidine (15) and nigellicine (16)[18][19]. These indazole alkaloids can only be seen in this plant. These are known to have antihyperglycemic activity proven to work against Type II diabetes mellitus in rat models at lower concentration. They are also known to have antimicrobial activities especially antifungal comparing with marketed fluconazole as well as anti-HIV drug.

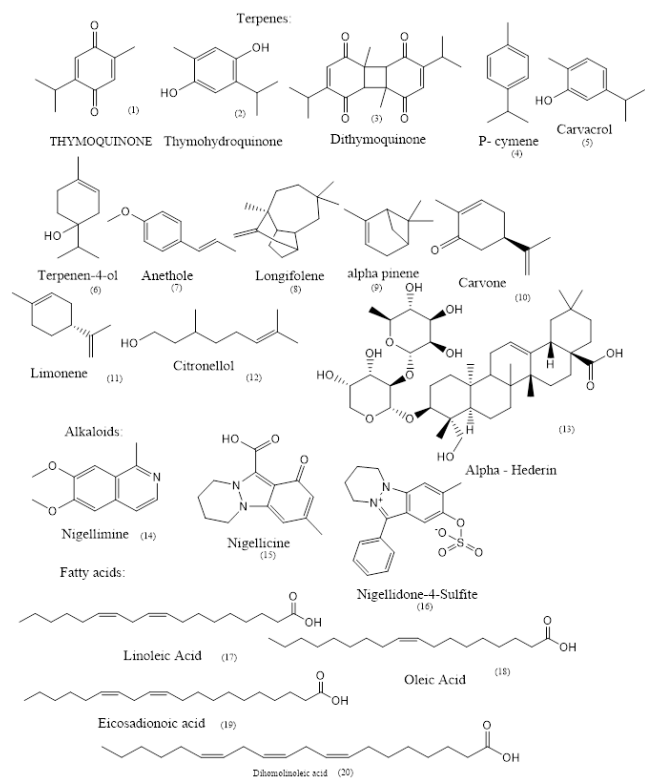
Fatty Acids: The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%) (17), oleic acid (20%) (18), eicosadienoic acid (3%) (19) and dihomolinoleic acid (10%)(20). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α -sitosterol (21) is a major sterol, which accounts for 44% and 54% of the total sterols in Tunisian and Iranian varieties of black seed oils respectively, followed by stigmasterol (6.57-20.92% of total sterols) [20]. They have shown to have effects as anti-inflammatory as well as enhance carcinogenic effects of different drugs

Steroidal and other compounds: Examples of various other reported chemical components includes nigellone (22), cycloeucalenol(23), gramisterol(24), obtusifoliol(25), stigmasterol-7-ene, β -amyrin(26), butyrospermol(27), cycloartenol(28), taraxerol(29), tirucalol (30), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol, β -unsaturated hydroxy ketone, hederagenin glycoside, bitter principle, tannin, resin, protein, reducing sugar and glycosidalsaponins. These are mostly found in methanolic and hydroalcoholic extracts and is known to have pulmonary protective activities and anti-asthmatic effects [21].



Ethnomedicinal Uses: N.sativa has been traditionally used for the treatment of various disorders, diseases and conditions pertaining to respiratory system, digestive tract, kidney and liver function, cardio vascular system and immune system support, as well as for general well-being. Avicenna refers to black seeds in the "The Canon of Medicine", as seeds stimulate the body's energy and helps recovery from fatigue and dispiritedness. Black seeds and its oil have a long usage as medicinal usage in Indian and Arabian civilization. The seeds have been traditionally used in Southeast Asian and the Middle East countries for the treatment of several ailments including asthma, bronchitis, rheumatism and related inflammatory diseases. Its many uses have earned Nigella the Arabic approbation 'Habbatul barakah', meaning the seed of blessings [22]. A tincture prepared from the seeds is useful in indigestion, loss of appetite, diarrhoea, dropsy and skin eruptions. Externally the oil is used as an antiseptic and local anesthetic. Roasted black seeds are given internally to stop the vomiting.

Anticancer effects of N. sativa seeds: The antitumor effects of N. sativa was first recognized by Ibn-Sina who generally used N. sativa for the treatment of tumors, particularly hard splenic mass. With regard to modern science, the anticancer activity of N. sativa was revealed, perhaps for the first time, when an enhancement of the natural killer (NK) cell activity, ranging from 200-300%, was observed in advanced cancer patients receiving multimodality immunotherapy program



In which *N. sativa* seed was one of the components. Later on, the antineoplastic effects of *N. sativa* seed and its extracts were investigated by a large number of researchers both in vivo using animal models and in vitro using cancer cell lines [23].

Topical application of *N. sativa* seed extract inhibited croton oil induced skin carcinogenesis in mice, delayed the onset of papilloma formation and reduced the number of papillomas per mouse. In the same study, intraperitoneal administration of 100 mg/kg of *N. sativa* extract restricted soft tissue sarcomas induced in albino mice by 20-methylcholanthrene to 33%. In another study, ethylacetate column chromatographic fraction (CC-5) of ethanolic extract of *N. sativa* was shown to possess cytotoxic effects against different classes of cancer cell lines, such as, P388, Hep G2, Molt4 and Lewis lung carcinoma cells. Moreover, *N. sativa* seed administered orally gave protection against methylnitrosourea induced oxidative stress and carcinogenesis in 80% and *N. sativa* seed with honey together in 100% of Sprague-Dawley rat [24].

Aqueous and ethanolic extracts of *N. sativa* seeds were shown to work well in MCF-7 breast cancer cells with low IC₅₀ value, while *N. sativa* oil given orally to rats, was shown to inhibit the induction and development of 1,2-dimethylhydrazine induced preneoplastic lesions for colon cancer, without any pathological changes in the liver, kidneys, spleen, etc. The volatile oil extracted from *N. sativa* also had cytotoxic effects against human cancer cell lines SCL, SCI-6, NUGC-4. Moreover, the protective potential of melatonin, retinoic acid and *N. sativa* seed was reported in terms of decreased levels of markers of tumorigenicity, endocrine damages and oxidative stress against 7,12-di-methylbenzene- α -anthracene that induced mammary carcinoma in rats.

Recently, publications mention that aqueous and ethanolic extracts of a similar plant like *N. sativa*, *H. indicus*, and *S. glabra* and their cytotoxic effects were shown on human hepatoma (HepG2) cell lines with the utilization of the 3-(4,5 dimethylthiazol-2yl)- 2, 5-biphenyl tetrazolium bromide (MTT) and Sulphorhodamine B (SRB) assays (El-Kadi and Kandil, 1986). Both tests showed that the two extract applied strong dose dependant cytotoxicity to HepG2 cells. The aqueous extract demonstrated a fundamentally higher cytotoxic

Potential than the ethanolic extract. Thymoquinone, a known cytotoxic compound isolated from *N. sativa* seeds was just seen in the ethanolic extract. In this way, extracts other than thymoquinone potentially intervened the cytotoxicity of the aqueous concentrate of this polyherbal preparation.

In a current report, methanolic, n-hexane and chloroform extract of *N. sativa* seeds are said to have viably killed HeLa cells, with an IC₅₀ estimations of 2.28 g/ml, 2.2 g/ml and 0.41 ng/ml, respectively. These extracts shown to cause apoptosis as affirmed by DNA breakdown in cells, Western blot and End terminal transferase-interceded dUTP-digoxigenin-end marking test.

The aqueous extracts of *N. sativa* mostly contain thymoquinone and other fatty acids which have been generally considered and proven to have anticancer action [25]. From all the references discussed earlier there has been no serious reports of any adverse effects from usage of *N. sativa*. The only reported ones are slowing down of blood clot for which it may be contraindicated during surgery. It may show symptoms of hypoglycemia in rare cases as well as uterine relaxation during parturition so it is contraindicated during pregnancy as well.

Active Components of *N. sativa*: Thymoquinone (1), dithymoquinone(3) and thymohydroquinone(2) are the main constituents isolated from the volatile oil of the *N. sativa* seed. Besides antimicrobial, anti-inflammatory and antioxidant activities, they have been reported to possess anticancer effects against a large number of cancer cell lines as well as in animal models. Another important active compound that has been shown to possess anticancer effects is alpha-hederin(13), a pentacyclic triterpene and a saponin, which is water soluble perhaps the major active component in the aqueous extract of *N. sativa*.

Thymoquinone and related compounds:

Once the anticancer effects of the *N. sativa* seed and its extracts were known, the scientists investigated its major active compounds, thymoquinone and dithymoquinone, etc. for their anticancer properties. Perhaps the first report of thymoquinone (1), which was isolated from the fatty acid component of *N. sativa*, for its cytotoxic activity was against Ehrlich's ascites carcinoma, Dalton's lymphoma ascites and sarcoma-180 cells [26].

Later, thymoquinone and dithymoquinone were reported to inhibit human tumor cell lines, which were resistant to doxorubicin and etoposide [27].

Thymoquinone (**1**) was investigated against benzopyrene (BP) induced fore-stomach tumor in female Swiss albino mice. Thymoquinone, administered for three weeks, was shown to reduce the incidence and multiplicity of BP-induced fore-stomach tumor.

Similarly, thymoquinone administered one week before, during and after 20-methylcholanthrene treatment, significantly inhibited the fibrosarcoma tumor incidence. Thymoquinone (**1**) was also reported to possess chemotherapeutic effects on SW-626 colon cancer cells and the effect was comparable to 5-fluorouracil [28].

Moreover, thymoquinone showed promising anti-cancer activity against hepatocellular carcinoma by the inhibition of HepG2 cells in a dose-dependent manner. Recently, thymoquinone was shown to inhibit the proliferation of a set of human cancer cell lines (Caco-2, HCT-116, LoVo, DLD-1 and HT-29), with no cytotoxicity to normal human intestinal cells (FHs74Int) [29].

Alpha-Hederin: Early reports suggest that the in vitro anticancer effect of alpha-hederin (13), which was isolated from *Hedera helix*, against mouse (B16) melanoma cells and normal mouse (3T3) fibroblasts, which revealed that alpha-hederin, at very low concentrations (<5 µg/ml) and within a very short time (eight hours) inhibited the proliferation of both of these cell lines in a serum free medium. However, its cytotoxicity decreased in the presence of serum, due perhaps to the binding of alpha-hederin (a saponin) with serum proteins.

Later, alpha-hederin (13) obtained from the ethanolic extract of *N. sativa* was also evaluated for its in vivo anticancer activity against tumors formed by the subcutaneous implantation of LL/2 (Lewis lung carcinoma) cells in BDF1 mice. Given intraperitoneally at doses of 5 and 10 mg/kg body weight for seven days to these mice with formed tumors, alpha-hederin produced significant dose-dependent TIR values of 48% and 65%, respectively, on day 8 and 50% and 71%, respectively, on day 15; compared to 81% on day 8 and 42% on day 15 in the cyclophosphamide treated control group; demonstrating its dose-related antitumour effect comparable to cyclophosphamide [30].

In another study, alpha-hederin and thymoquinone separately induced a dose and time dependent cytotoxic and necrotic effects on the human cancer cell lines: A549 (lung carcinoma), HEP-2 (larynx epidermoid carcinoma), HT-29 (colon carcinoma) and MIA PaCa-2 (pancreas carcinoma) [31].

The results of these investigations clearly indicate that the beneficial effects of *N. sativa* against cancer are, at least partially, due to alpha-hederin.

But, as of now only these two compounds have shown potential against cancer but as extract *N. sativa* are shown to have better potential but the reason is yet to be determined whether any other single compound is acting or a group of compounds are acting synergistically to provide anticancer effects.

Mechanisms of *Nigella Sativa* as Anticancer Agents

Cancer is the irregular cell growth caused by genetic changes. In this way, any agent which has anticancer property, either shield genetic material from alteration or kill the genetically changed cancer cells. The active ingredients (for the most part TQ) from *N. sativa* follow up on cancer cells to inhibit them by a few molecular pathways.

Inhibition of BCL2 gene and promotion of Caspase 8,9,3:

Scientists have reported the apoptotic mechanism behind the anti-proliferative activity of TQ got from *N. sativa* on myeloblastic leukemia HL-60 cells. They reported that black seed extracts prompts apoptosis, disturbs mitochondrial layer potential and triggers the enactment of caspases 8, 9 and 3 in HL-60 cells. The apoptosis incited by black seed was hindered by a general caspase inhibitor, z-VAD-FMK; a caspase-3-specific inhibitor, z-DEVD-FMK; and additionally, a caspase-8-specific inhibitor, z-IETD-FMK (Fig: 3). In addition, the caspase-8 inhibitor hindered the TQ-mediated activation of caspase-3, PARP cleavage and the release of cytochrome C from mitochondria into the cytoplasm. Black seeds extract treatment of HL-60 cell lines caused a marked increment in Bax/Bcl2 proportions because of up regulation of Bax and down regulation of Bcl2 proteins. Their outcomes demonstrated that black seed-activated apoptosis is related with the activated of caspases 8, 9 and 3, with caspase-8 going about as an upstream activator and enacted caspase-8 starts the release of cytochrome C amid Black seed activated apoptosis.

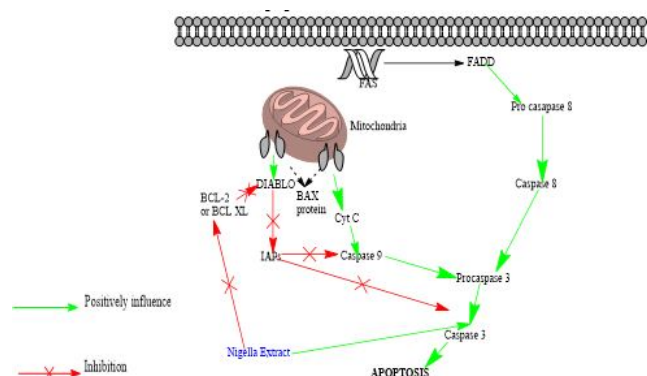


Fig 3: Mechanism of Nigella seed extract on CASPASE 8, 9, 3 and BCL-2 protein

TQ action was also found as pro-apoptotic against colon cancer cell line HCT116. It was showed that the apoptotic effects of TQ are modulated by Bcl-2 protein and are linked to and dependent on p53. It was demonstrated that the apoptotic impacts of black seed are modulated by Bcl-2 protein and are connected to and reliant on p53. TQ additionally down-regulates the declaration of NF-kappa B-directed antiapoptotic (IAP1, IAP2, XIAP Bcl-2, Bcl-xL, and survivin) gene product [32].

Inhibition of Akt: Scientists have discovered TQ prompting apoptosis by the activation of c-Jun NH(2)- terminal kinase and p38 mitogen-actuated protein kinase pathways in pancreatic cancer cell. TQ has likewise been accounted for to be dynamic in controlling Akt pathway. Xuan et al found that LPS (lipopolysaccharides: a bacterial segment)-prompted phosphorylation of prosurvival kinases Akt and ERK1/2 was annulled by TQ in dendritic cells [33].

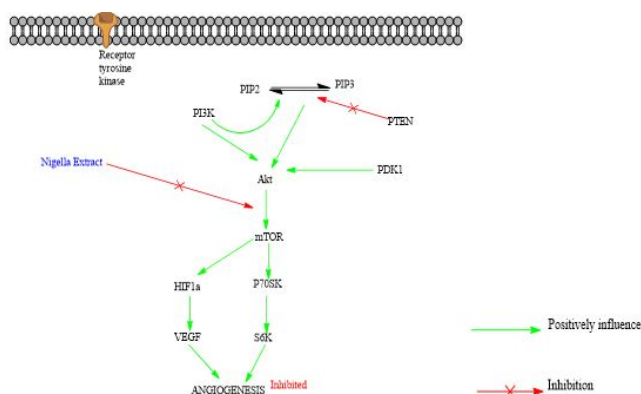


Fig 4: Effect of Nigella on Akt

Inhibition of NF-kB

NF-kappa B plays a key role in regulating the immune response, and irregularities of NF-kappa B has been found to be linked to cancer.

Sethi et al. discovered that black seed suppresses tumor necrosis factor-actuated NF-kappa B initiation in a dosage and time-dependent way and repressed NF-kappa B working incited by different cancer-causing agents and inflammatory stimuli. The supression of NF-kappa B pathway is connected with successive arrest to the pathway of I kappa B alpha kinase, I kappa B alpha phosphorylation, I-kappa-B-alpha degradation, p65 phosphorylation, p65 molecular translocation, and the NF-kappa B-dependent reporter gene expression. Additionally scientists revealed that a herbal melanin (HM) from N. sativa interferes cytokine generation and suggest it as a ligand for TLR4 (toll-like receptor 4). Their team researched the possibility that the HM-activated cytokine production is by means of NF-kappa B flagging pathway and found that HM initiated the degradation of I kappa B-alpha, a key to the activation of NF-kappa B (Fig: 5). In addition, expansion of I kappa B (IKK) specific inhibitors viably arrested the HM-induced production of IL-8 and IL-6 by TLR4-transfected HEK293 cells and THP-1 (Human acute monocytic leukemia) cells [33].

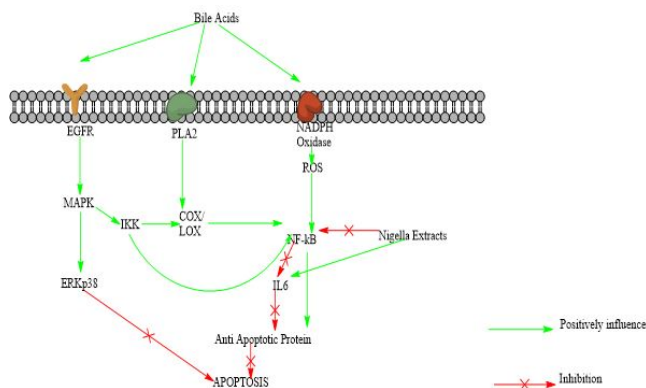


Fig 5: Mechanism of Nigella extracts on NF-kB and IL6 in facilitating Apoptosis

Maximization of antioxidant enzymes:

Many studies showed that N. sativa oil or the isolated TQ has antioxidant activity as well as maximises the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) etc. Antioxidant enzymes are found to be related to cancer, mostly their increased activities are beneficial against different types of cancer.

Administration of N. sativa oil or TQ can lower the toxicity of other anticancer drugs (for example, cyclophosphamide) by an up-regulation of antioxidant mechanisms, indicating a potential

Clinical application for these agents to minimize the toxic effects of treatment with anticancer drugs [34].

Adding to these cancer inhibiting properties, components of *N. sativa* have cancer protective roles. Ibrahim et al. reported that *N. sativa* oil administration has a protective effect against the CCl₄-mediated suppression of CYP (drug metabolizing cytochrome P450 enzymes). And genetic mutations and polymorphisms of CYP enzymes are associated with cancer.

Radiotherapy is one of the most common strategies for treating human cancers but this treatment is somehow risky for normal tissue. Cemek et al. researched that *N. sativa* and glutathione treatment significantly antagonize the effects of radiation.

Therefore, *N. sativa* may be a beneficial agent in protection against ionizing radiation-related tissue injury. Assayed investigated the radio-protective potential of *N. sativa* crude oil against hemopoietic adverse effects of gamma irradiation. He found that irradiation resulted in significant reduction in hemolysin antibodies titers and delayed type hypersensitivity reaction of irradiated rats, in addition to significant leukopenia and significant decrease in plasma total protein and globulin concentration and depletion of lymphoid follicles of spleen and thymus gland [35].

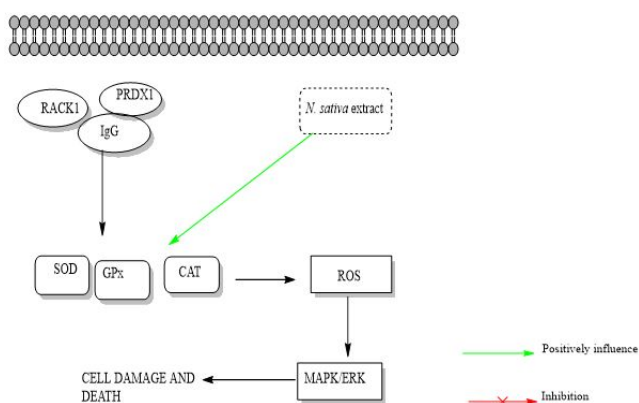


Fig 6: Effect of Nigella on Superoxide

But oral administration of *N. sativa* oil before irradiation considerably normalized all the above-mentioned criteria; and produced significant regeneration in spleen and thymus lymphoid follicles. Thus *N. sativa* oil is recognized as a promising natural radioprotective agent against immunosuppressive and oxidative effects of ionizing radiation.

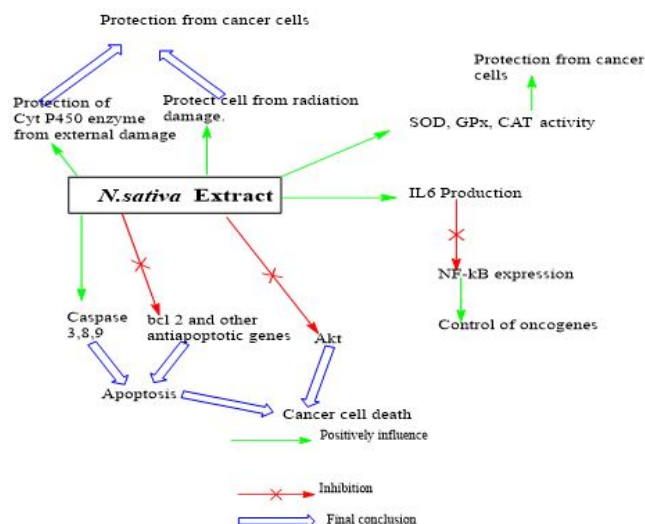


Fig7: Possible mechanisms of thymoquinone (TQ) action against Cancer.

Derivatives of Thymoquinone and Alpha-Hederin as Anticancer

Poloxin, a thymoquinone derivative, deactivates Plk1 and decreases its capacities in vitro, subsequently causing Plk1 mislocalization, chromosome disruption, mitotic arrest, and apoptosis in HeLa cells (Reindl et al., 2008). 4 acylhydrazones and 6-Alkyl derivatives of thymoquinone were likewise tried for hindrance in human HL-60 leukemia, 518A2 melanoma, KB-V1/Vb1 cervix and MCF-7 breast carcinoma cells. Of these, 6-hencosahexaenyl conjugate 3e was observed to be the most dynamic with an IC₅₀ values as low as 30 nmole/ml in MCF-7 cells [36].

As of now, 27 analogues of thymoquinone were synthesized by change at carbonyl and benzenoid sites and tried for their biological activity against different cancer cell lines. Many of these compounds were observed to be potent than thymoquinone as far as inhibition of cell growth, induction of apoptosis and regulation of transcription factor, NF-kB is concerned. The novel analogs additionally bettered the activity of gemcitabine and oxaliplatin induced apoptosis in gemcitabine resistant pancreatic carcinoma cells, MiaPaCa-2 [37]. Correspondingly, in another current study, in which antiproliferative impact of 19 analogs of thymoquinone were screened by MTT assay against six human cell lines; HCT116, MCF7, MDA231, T74d and MRCS and the lead compound, ON01910, obstructed the cancer cells at G2/M stage, improved caspase-3 activity and caused the development of multipolar mitotic spindle which is reliable with the impacts of Plk1-PBD inhibition.

Additionally, computer-based drug design helped in designing of the compound OC2-23 which was ten times more potent than the lead compound, ON01910. One of the substitutes of alpha-hederin, kalopanaxsaponin-I, was found to have anticancer action. The tests of alpha-hederin and kalopanaxsaponin-1 for their cytotoxic effects against cancer cell lines, got from 30 people of European origin (Center d'Etue du Polymorphisme Huma) and 30 tribes of African descent (Yoruban), uncovered that they were moderately more successful in the Yoruban populace [38].

Combination of Thymoquinone and Alpha-Hederin with Cytotoxic Drugs

In the endeavor to build effective and low adverse effects drugs especially with the development of resistance, a vast majority of the anticancer drugs are recommended in combinations. Other than the anticancer properties, thymoquinone and alpha-hederin are both known to have different properties like antioxidant agent and cytoprotective activities. It is normal that their combination with currently marketed anticancer drugs would not just expand their useful impacts and lessen the odds of resistance yet in addition effectively don't have effects on the normal body cells. Thymoquinone was found to improve the antitumor activity of ifosfamide in rats bearing Ehrlich ascites carcinoma and lessen the ifosfamide prompted Fanconi syndrome in rats [39]. It was likewise appeared to increase the antitumor action of gemcitabine and oxaliplatin against pancreatic cancer. The experiment uncovered that pre-exposure of cells to thymoquinone took after by gemcitabine or oxaliplatin brought about 60% to 80% growth inhibition contrasted with 15% to 25% when gemcitabine or oxaliplatin were utilized alone. In addition, thymoquinone could potentiate the killing of pancreatic cells by down regulation of NF-Kappa-B, Bcl-2 family and NF-Kappa-B controlled antiapoptotic activities (X-linked inhibitor of apoptosis, surviving and cyclooxygenase-2) [39,40].

In a current report, doxorubicin, thymoquinone and equimolar mix were tried for cytotoxicity on human cells of HL-60 leukemia, 518A2 melanoma, HT-29 colon, KB-V1 cervix and MCF-7 breast carcinoma and on their multi drug resistant variations and non-malignant human fibroblasts (HF). Thymoquinone enhanced the anticancer properties

Of doxorubicin, especially against HL-60 leukemia cells and multi drug resistant MCF-7 cells. Even investigation on *Azadirachta indica* and *Nigella sativa* yield good result on HCT116, MDA-MB- 231 cell lines [41].

Current study has showed that there is very little or no toxicity shown in invitro studies on different parts of rat and has been termed safe. Different evidence of high LD50 values, key hepatic enzyme stability, and organ integrity, suggests a wide margin of safety for therapeutic doses of *Nigella sativa* fixed oil, but the changes in hemoglobin metabolism and the fall in leukocyte and platelets count must be taken into consideration.

Conclusion

Hence, it can be said that *N. sativa* of family ranunculaceae, which is mentioned as panacea in Holy Bible and Holy Quran that is, it can cure anything except ageing and death, has proven to be highly potent against cancer. The two of the reported isolated compounds shown to have worked against cancer i.e, Thymoquinone and Alpha hederin. By its four prominent mechanism of working against cancer i.e, by activating caspase 3,8,9 and inactivating Bcl2 and other antiapoptotic genes, suppression of Akt to prevent mTOR formation, increase IL6 formation to arrest production of NF-kB and lastly promotion of enzymes like SOD, GPx to increase superoxides thereby prohibiting cell growth. It is also known to protect cytochrome P450 from external damage as well has protect from radiatiation. Even some of the synthetic analogues like poloxin, kalapanaxogenin I and other analogues have very good anticancer potential. As adjuvant to doxorubicin it is shown to have much better therapeutic action in cases of doxorubicin resistant breast cancer. However, there are no defined action of compounds other than the above mentioned Thymoquinone and Alpha hederin but extracts show better action than these compounds so extensive research needed to find out the reason behind this phenomenon. So, *Nigella sativa* is one of the finest herbs which has high potential against cancer at par with synthetic analogues available.

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