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# *Nigella sativa* Linn.– A comprehensive review

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*Nigella sativa* Linn. (Family-Ranunculaceae) is a widely used medicinal plant throughout India and popular in various Indigenous System of Medicine like Ayurveda, Siddha, Unani and Tibb. The seeds are used as astringent, bitter, stimulant, diuretic, emmenagogue and anthelmintic. They are also useful in jaundice, intermittent fever, dyspepsia, paralysis, piles and skin diseases. The present review is therefore, an effort to give a detailed survey of the literature on pharmacognosy, phytochemistry and pharmacological activities of the plant.

**Keywords:** *Nigella sativa*, Black cumin, Pharmacognosy, Phytochemistry, Pharmacology, Ranunculaceae, Medicinal Plant, Seeds.

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## Introduction

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human disease. India is considered as "Botanical Garden of the world" and more than 2200 species of medicinal and aromatic plants have been identified after studies. The revival of interest in natural drugs started in last decade mainly because of the wide spread belief that green medicine is healthier than synthetic products. Now-a-days, there is manifold increase in medicinal plant based industries due to the increase in the interest of use of medicinal plants throughout the world which are growing at a rate of 7-15% annually. Despite the major advances in the modern medicine, the development of new drugs from natural products is still considered important. This seems to be even more relevant for the developing countries, where the cost to develop a drug is prohibitive. Since 1980, the World Health Organization has been encouraging countries to identify and exploit traditional medicine and phytotherapy. The Indian Traditional systems of medicine namely Ayurveda and Siddha are primarily plant based system. The evaluation of new drugs especially phytochemically obtained materials has again opened a vast area for research and development. As per WHO, about 80% of the population in the world relays on the traditional medicine for the treatment of various diseases. Therefore, the evaluation of rich heritage of traditional medicine is essential. In this regard, one such plant is *Nigella sativa* Linn. which is a small elegant annual herb distributed and cultivated all over India[1]. In Islam, it is regarded as one of the greatest forms of healing medicine available. The Islamic prophet Muhammad once stated that the black seed can heal every disease except death. Avicenna, most famous for his volumes called The Canon of Medicine, refers to *Nigella* as the seed that stimulates the body's energy and helps recovery from fatigue and dispiritedness. It is also included in the list of natural drugs of 'Tibb-e-Nabavi', or "Medicine of the Prophet (Muhammad)", according to the tradition "hold onto the use of the black seeds for healing all diseases. In the Unani Tibb system of medicine, *N. sativa* is regarded as a valuable remedy for a number of diseases. In the Indian system of medicine, the seeds are used as astringent, bitter, stimulant, diuretic, emmenagogue, anthelmintic, jaundice, intermittent fever, dyspepsia, paralysis, piles and skin diseases, etc[2-4]. The aim of present review is to highlight the traditional uses, pharmacognostical, phytochemical and pharmacological investigation carried out on the plant so that more pharmacological studies could be conducted to investigate the unexploited potential.

*Nigella sativa* Linn. (Family-Ranunculaceae) commonly known as *Upakunchika*, *Ajaji, Kalvanjika*, *Kalika*, *Kunchika*, *Kalaunji* and Black cumin , is a small elegant herb, mostly found and cultivated in Punjab, Himachal Pradesh, Gangetic Plains, Bihar,

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Bengal, Assam, Maharashtra and also cultivated in Syria, Lebanon, Israel and Southern Europe. Annual herb about 45 cm in height. Leaves: 2.5-5.0 cm long, linear-lanceolate. Flower pale blue, 2.0-2.5 cm across, solitary on long peduncles; capsule 1.2 cm long; seeds flattened, oblong, angular, funnel shaped, small, 0.2 cm long and 0.1 cm wide, black in colour[8, 9] (Plate 1). Flowering and fruiting occur from January to April. It is generally cultivated on dry soil between November to April and seeds take about 10-15 days to germinate. It can also be propagated from the callus culture *in vitro* from leaf, stem and root explants from aseptically grown seedlings[1-12].

## Pharmacognostical studies on seeds

### Macroscopical characteristics

They are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5 × 1-2 mm, black externally and white inside; odor slightly aromatic and taste bitter[13].

### Microscopical and powder characteristics

Transverse section of seed shows single layered epidermis consisting of elliptical, thick walled cells, covered externally by a papillose cuticle and filled with dark brown contents. Epidermis is followed by 2-4 layers of thick walled tangentially elongated parenchymatous cells, followed by a reddish brown pigmented layer composed of thick walled, rectangular elongated cells. Inner to the pigment layer, is present a layer composed of thick walled rectangular elongated or nearly columnar, elongated cells. Endosperm consists of thin walled, rectangular or polygonal cells mostly filled with oil globules. The powder microscopy of seed powder shows brownish black, parencymatous cells and oil globules[14,15].


Plate 1: *Nigella sativa* seeds

### Physical constants

Foreign matter, 2% w/w; total ash, 6% w/w; acid insoluble ash, 0.2% w/w; alcohol soluble extractive, 20% w/w; water soluble extractive, 15% w/w; total fixed oil, 25-32% w/w; volatile oil, 0.42% w/w; organic matter, 3.91% w/w; loss on drying, 4% w/w[5, 6].

## Traditional uses

Traditionally the seeds and its oil are used in several diseases. The seeds are considered as bitter, pungent, aromatic, appetizer, stimulant, diuretic, emmenagogue, galactagogue, anthelmintic, acrid, thermogenic, carminative, anodyne, deodorant, digestive, constipating, sudorific, febrifuge, expectorant, purgative, abortifacient. They are used in ascites, cough, jaundice, hydrophobia, fever, paralysis, conjunctivitis, piles, skin diseases, anorexia, dyspepsia, flatulence, abdominal disorders, diarrohoea, dysentery, intrinsic hemorrhage and amenorrhea. Seed oil is a local anesthetic[4, 6, 9].

## Phytochemical studies

Very little phytochemical work has been carried out on this species. The seeds are reported to contain nigellone[16], nigellicine, nigellimine, nigellimine-N-oxide, avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol, 24-ethyl-lophenol, gramisterol, lophenol, 243-methyllophenol, obtusifoliol, sitosterol, stigmastanol, stigmasterol, stigmasterol-7-ene, beta-amyrin, butyrospermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]- 28-O-[α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl] hederagenin, volatile oil (0.5-1.6%), fatty oil (35.6-41.6%), oleic acid, esters of unsaturated fatty acids with C15 and higher terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol[6,19,20], nigellidine[21], carvone, d-limonene, cymene, α, β-unsaturated hydroxy ketone, steroids, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidal saponin, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16,23-dihydroxy-28-methylolean-12-enoate, stigma-5,22-dien-3-β-D-glucopyranoside, cycloart-23-methyl-7,20, 22-triene-

3β,25-diol, nigellidine-4-O-sulfite[22], nigellamines A3, A4, A5, C[23], nigellamines A1, A2, B1, and B2[24]. The structures of some major chemical compounds isolated from the plant are given in Plate 2.

**Seed oil**

The seed oil contains cholesterol, campesterol, stigmasterol, β-sitosterol, α-spinasterol, (+)-citronellol, (+)-limonene, p-cymene, citronellyl acetate, carvone[18], nigellone, arachidic, linolenic, linoleic, myristic, oleic, palmitic, palmitoleic and stearic acids. Fixed oil: linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%). Volatile oil: trans-anethole (38.3%), p-cymene (14.8%), limonene (4.3%), and carvone (4.0%)[25], 2-(2-methoxypropyl)-5-methyl-1, 4-benzenediol, thymol and carvacrol[26]. Root and shoot are reported to contain vanillic acid[27].

## Pharmacological studies

The popularity of the plant was highly enhanced by the ideological belief in the herb as a cure for multiple diseases. The detailed pharmacological activities of *N. sativa* are given below.

**Antitumor activity**

*In vitro* studies on pancreatic cancer cells revealed that preexposure of cells with thymoquinone (25 mumol/l) for 48 h followed by gemcitabine or oxaliplatin resulted in 60 to 80% growth inhibition compared with 15 to 25% when gemcitabine or oxaliplatin was used alone which suggest that the mechanism of thymoquinone could potentiate the killing of pancreatic cancer cells by down-regulation of nuclear factor-kappaB (NF-kappaB), Bcl-2 family, and NF-kappaB-dependent antiapoptotic genes[28]. 4-Acylhydrazones and 6-alkyl derivatives of thymoquinone (TQ) (the major constituent of seed oil extract) were tested for growth inhibition of human HL-60 leukemia, 518A2 melanoma, KB-V1/Vbl cervix and MCF-7/Topo breast carcinoma cells. The 6-hencosahexaenyl conjugate was most active in all resistant tumor cells, with $IC_{50}$ (72 h) values as low as 30 nM in MCF-7/Topo cells[29].

The combined dose of TQ and selenium produced decreased cell counts, increased cellular damage, decreased alkaline phosphatase levels and decreased glutathione levels on the proliferation of osteoblasts cells (MG 63) in tissue culture[30]. Oral administration of TQ (1, 2 and 4 mg/kg/day p.o.) for five days is effective in increasing the activities of quinone reductase and glutathione transferase in liver and makes TQ a promising prophylactic agent against chemical carcinogenesis and toxicity[31].

The essential oil ($IC_{50}$ = 0.6%, v/v) and ethyl acetate ($IC_{50}$ = 0.75%) extracts were more cytotoxic against the P815 cell line than the butanol extract ($IC_{50}$ = 2%). Similar results were obtained with the Vero cell line. Although all extracts had a comparable cytotoxic effect against the ICO1 cell line, with $IC_{50}$ values ranging from 0.2 to 0.26% (v/v); tests on the BSR cell line revealed a high cytotoxic effect of the ethyl acetate extract ($IC_{50}$ = 0.2%) compared to the essential oil ($IC_{50}$ = 1.2%). These data show that the cytotoxicity of each extract depends on the tumor cell type[32].

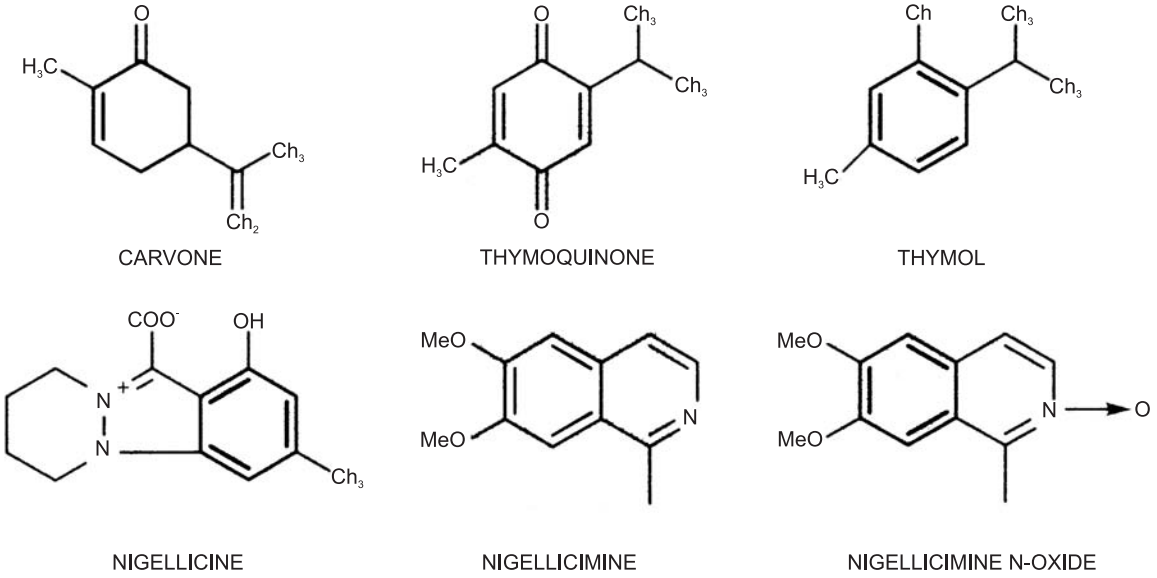


Plate 2: Chemical structures of some major compounds isolated from *Nigella sativa*

The long term (16 months) treatment with the decoction comprised of *N. sativa* seeds, *Hemidesmus indicus* R. Br. root bark and *Smilax glabra* Roxb. rhizome would be successful in inhibiting in rat livers, not only DEN-mediated expression of GST-P, but also the carcinogen mediated development of overt tumors or histopathological changes leading to tumor development[33, 34].

The separate effects of α-hederin and TQ on four human cancer cell lines [A549 (lung carcinoma), HEp-2 (larynx epidermoid carcinoma), HT-29 (colon adenocarcinoma) and MIA PaCa-2 (pancreas carcinoma)] were investigated and reported to possess cytotoxic effect against the tumor cell lines[35]. The cytotoxicity of the decoction of *N. sativa* seeds, *H. indicus* root and *S. glabra* rhizome and the individual plant extracts were tested on the human hepatoma HepG2 cell line. Results from MTT and SRB assays, and [$^{14}$C]-leucine and [$^{3}$H]-thymidine uptake demonstrated that the decoction had a strong dose-dependent cytotoxic activity[36].

Fractionation of the seed components yielded antioxidant compounds in both the water soluble and lipid soluble fractions. Black seed fractionated extracts and pure TQ showed markedly reduced levels of MDA in A549 cells in culture for 24, 48 and 72 hours[37].

The effect of TQ on ifosfamide (IFO)-induced Fanconi syndrome and its antitumor activity were investigated in rats and mice, respectively. TQ significantly prevented IFO-induced renal glutathione depletion and lipid peroxide accumulation. In mice bearing Ehrlich ascites carcinoma xenograft, TQ (10 mg/kg per day) administered in drinking water significantly enhanced the antitumor effect of IFO (50 mg/kg per day, i.p. on days 1-4 and 15-18). Furthermore, mice treated with IFO in combination with TQ showed less body weight loss and mortality rate compared to IFO single therapy suggesting that TQ may improve the therapeutic efficacy of IFO by decreasing IFO-induced nephrotoxicity and improving its antitumor activity[38].

The active principle of seeds containing certain fatty acids was studied for antitumor activities against Ehrlich ascites carcinoma, Dalton's lymphoma ascites and Sarcoma-180 cells. *In vitro* cytotoxic studies showed 50% cytotoxicity to Ehrlich ascites carcinoma, Dalton's lymphoma ascites and Sarcoma-180 cells at a concentration of 1.5 µg, 3 µg and 1.5 µg, respectively with little activity against lymphocytes[39].

The ethanol extract was found to inhibit cancer cells and endothelial cells progression *in vitro*[40, 41].The protective effect of seeds as nutraceuticals was studied on the oxidative stress and carcinogenesis induced by methylnitrosurea in rats. It provided 80% protection against methyl nitrosurea induced oxidative stress, inflammatory response and carcinogenesis[42]. Both TQ and dithymoquinone were found to be cytotoxic for several types of human cancer cells[43].

Topical application of seed extract inhibited skin carcinogenesis in mice and intraperitoneal administration delayed the onset of papilloma formation[44]. TQ inhibited benzopyrene induced carcinogenesis in mice due to its anti-inflammatory and antioxidant activity[45]. The aqueous and alcoholic extracts alone or in combination with $H_2O_2$ were found to be effective *in vitro* in inactivating MCF-7 breast cancer[46]. The fresh aqueous extract augmented natural killer cells (62.3%) in developing cytotoxicity against YAC *in vitro*[47,48]. Intraperitoneal administration of oil substantially decreased the cytomegalovirus load in liver and spleen. There was increase in interferon-γ, macrophages and CD4+ T cells and decrease both in number and function of NK cells[49]. α-Hederin produced significant tumor inhibition rates and the life span of treated rats increased by 153% as compared to dimethyl sulphoxide treated mice[50].

The anti-tumor effect of TQ was investigated both *in vivo* and *in vitro* in male albino rats on fibrosarcoma induced by 20-methylcholanthrene. It was found to inhibit tumor incidence and tumor burden significantly[51]. TQ induced apoptosis and inhibited proliferation in pancreatic ductal adenocarcinoma (PDA) cells. TQ also increased p21 WAF1 expression, inhibited histone deacetylase (HDAC) activity, and induced histone hyperacetylation in comparison with that of trichostatin A. Anti-inflammatory activities of TQ in PDA cells, which are paralleled by inhibition of NF-kappa B are also reported. TQ acts as a novel inhibitor of proinflammatory pathways which combines anti-inflammatory and proapoptotic modes of action[52]. Methanol, n-hexane and chloroform extracts of the seeds effectively killed HeLa cells. The $IC_{50}$ values of methanol, n-hexane and chloroform extracts were 2. 28 µg/ml, 2.20 µg/ml and 0.41 ng/ml, respectively. All three extracts induced apoptosis in HeLa cells[53]. Polymer-based nanoparticle approach to improve upon TQ effectiveness and bioavailability for anti-inflammatory and anti-cancer activities were evaluated. TQ was encapsulated with 97.5% efficiency in biodegradable nanoparticulate formulation based on

poly (lactide-co-glycolide) (PLGA) and the stabilizer polyethylene glycol (PEG)-5000. Electrophoretic gel shift mobility assay showed that TQ nanoparticles (NP) were more active than TQ in inhibiting NF-kappaB activation and in suppressing the expression of cyclin D1, matrix metalloproteinase (MMP)-9, vascular endothelial growth factor (VEGF), those are markers of cell proliferation, metastasis and angiogenesis, respectively. TQ-NP was also more potent than TQ in suppressing proliferation of colon cancer, breast cancer, prostate cancer, and multiple myeloma cells. Esterase staining for plasma membrane integrity revealed that TQ-NP were more potent than TQ in sensitizing leukemic cells to TNF- and paclitaxel-induced apoptosis suggesting that TQ-NP enhances its anti-proliferative, anti-inflammatory and chemosensitizing effects[54].

The effect of Thymoquinone, on dendritic cells (DCs), key players in the regulation of innate and adaptive immunity by LPS was evaluated. LPS decreased and thymoquinone increased caspase 3 and caspase 8 activation and annexin V binding. Moreover, LPS-induced phosphorylation of prosurvival kinases Akt and ERK1/2 was abrogated by thymoquinone suggesting that it compromises the maturation, cytokine release and survival of DCs[55].

**Antidiabetic activity**

The effects of the crude aqueous extract of *N. sativa* seeds (0.1 pg/ml to 100 ng/ml) on intestinal glucose absorption *in vitro* using a short-circuit current technique and *in vivo* using an oral glucose tolerance test were investigated. It directly inhibits the electrogenic intestinal absorption of glucose *in vitro*. Together with the observed improvement of glucose tolerance and body weight in rats after chronic oral administration *in vivo*, these effects further validate the traditional use of these seeds against diabetes[56].

The highly active anti-retroviral therapy (HAART) regimen has considerably reduced the mortality rate in HIV-1 positive patients. However, long-term exposure to HAART is associated with a metabolic syndrome manifesting cardiovascular dysfunction, lipodystrophy and insulin resistance syndrome. Exposure to several different HIV protease inhibitors, nelfinavir (5-10 µM), saquinavir (5-10 µM) and atazanavir (8-20 µM) with *N. sativa* seeds extract decreases glucose stimulated insulin secretion from rat pancreatic beta-cells[57].

The protective effects of black seed oil (BSO) from *N. sativa* in Sprague-Dawley rats treated with a daily HAART regimen for 7 months which is associated with insulin resistance in HIV-1-positive patients was investigated. Chronic HAART may increase serum insulin levels by dysregulating both insulin production by beta cells and insulin action at the periphery. These deleterious effects may be prevented by dietary supplementation with BSO[58]. The effect of TQ on embryonic development in streptozotocin (STZ)-induced diabetic mice was evaluated. Mice receiving both STZ and TQ showed malformations and resorptions at 16.37 and 18.39%, respectively. MDA and GSH levels were significantly decreased and increased, respectively in the STZ and TQ group which suggest the use of TQ in pregnant diabetic females[59].

The possible beneficial effects of *N. sativa* (NS) and TQ on histopathological changes of sciatic nerves in streptozotocin-induced diabetic rats were evaluated. The treatment of both NS and TQ caused a sharp decrease in the elevated serum glucose and an increase in the lowered serum insulin concentrations in STZ-induced diabetic rats. NS and TQ treatment resulted in increased area of insulin immunoreactive beta-cells significantly. Histological evaluation of the tissues in diabetic animals treated with TQ and especially NS showed fewer morphologic alterations. Myelin breakdown decreased significantly after treatment with NS and TQ. The ultra structural features of axons also showed remarkable improvement suggesting the utility of NS and TQ as a potential treatment on peripheral neuropathy in STZ induced diabetic rats[60].

Combined treatment with NS and human parathyroid hormone (hPTH) is more effective than treatment with NS or hPTH alone in improving bone mass, connectivity, biomechanical behavior and strength in insulin-dependent diabetic rats was evaluated. NS treatment (alone or in combination with hPTH) significantly increased the area of insulin immunoreactive beta-cells in diabetic rats suggesting that NS might be useful in the treatment of diabetic osteopenia[61].

Oral administration of ethanol extract of the seeds (300 mg/kg body weight/day) to streptozotocin induced diabetic rats for 30 days significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products (TBARS and hydroperoxides) and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and glutathione peroxidase in liver and kidney[62, 63].

The insulin secretory effects of the defatted fraction which was divided into two sub fractions: the first containing acidic and neutral compounds and the second containing basic compounds were evaluated individually at different concentrations (0.01, 0.1, 1 and 5 mg/ml), *in vitro* in isolated rat pancreatic islets in the presence of 8.3 mmol/l glucose. The results show that addition of the defatted whole extract or of the basic sub fraction of the seed in the incubation medium significantly increased glucose-induced insulin release from the islets. However, a clear concentration-dependent increase in insulin release from isolated pancreatic islets was observed for the basic sub fraction. The result showed that the antidiabetic properties of these seeds may be, at least partly, mediated by stimulated insulin release, and that the basic sub fraction largely contributes to this stimulatory effect[64].

The effect of a 4-week intragastric gavage with a petroleum ether extract of *N. sativa* seeds on blood glucose, insulin and lipids in the normal rat was studied. Petroleum ether extract caused a 25% reduction in food intake that translated into a transient weight loss also lowered fasting plasma levels of insulin and triglycerides and higher HDL-cholesterol which suggest that the petroleum ether extract of seeds has a slight anorexic effect and that it contains the hypolipidemic activity[65].

The possible protective effects of NS for four week against beta-cell damage from streptozotocin (STZ)-induced diabetes in rats was studied. NS treatment has been shown to provide a protective effect by decreasing lipid peroxidation and serum NO and increasing antioxidant enzyme activity. Increased intensity of staining for insulin and preservation of beta-cell numbers were apparent in the NS-treated diabetic rats suggesting that NS treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity[66].

The mechanisms underlying the hypoglycemic effect of NS oil (400 mg/kg body weight; for 6 weeks) in streptozotocin induced diabetic hamsters, in terms of hepatic glucose production, and also the possible immunopotentiating effect on peritoneal macrophages were investigated. Treatment with this oil significantly increased the phagocytic activity and phagocytic index of peritoneal macrophages and lymphocyte count in peripheral blood compared with untreated diabetic hamsters suggesting that the hypoglycemic effect is due to, at least in part, a decrease in hepatic gluconeogenesis, and that the immunopotentiating effect of the oil is mediated through stimulation of macrophage phagocytic activity either directly or via activation of lymphocytes[67].

The possible insulinotropic properties of oil in streptozotocin plus nicotinamide-induced diabetes mellitus in hamsters were evaluated. Significant decrease in blood glucose level together with significant increase in serum insulin level were observed after treatment with NS oil for 4 weeks. The hypoglycemic effect was at least partly, from a stimulatory effect on beta cell function with consequent increase in serum insulin level and possesses insulinotropic properties in type 2-like model[68].

The effect of NS oil, nigellone and TQ were studied on insulin secretion of isolated rat pancreatic islets in the presence of 3, 5.6 or 11.1 mM glucose. It significantly lowered blood glucose concentrations in diabetic rats after 2, 4 and 6 weeks. The blood lowering effect was, however, not paralleled by a stimulation of insulin release in the presence of the oil, nigellone or TQ which suggest that the hypoglycemic effect of oil may be mediated by extra pancreatic actions rather than by stimulated insulin release[69].

The effect of NS oil and its active constituent thymoquinone (TQ) on oxidative stress in the heart and brain in an experimental model of diabetes mellitus using streptozotocin (STZ) were evaluated. The results suggested that NS and TQ correct STZ-diabetes-induced alterations in cardiac creatine kinase muscle and brain types (CK-MB) and brain monoamines due to their antioxidant properties[70]. The effect of TQ (20, 40 and 80 mg/kg b.w for 45 days) on the activities of key enzymes of carbohydrates metabolism in STZ-nicotinamide induced diabetic rats were evaluated. The results suggested that TQ at 80 mg/Kg b.w is beneficial in hepatic enzyme activities and exerts potential antihyperglycemic effect[71].

The seed ethanol extract induces an important insulin-like stimulation of glucose uptake in C2C12 skeletal muscle cells and 3T3-L1 adipocytes following an 18 h treatment. The extract increases activity of Akt, a key mediator of the effects of insulin, and activity of AMP-activated protein kinase (AMPK) suggesting its potent uncoupling activity. The extract behaves as an agonist of PPARgamma[72].

The possible protective effects of the volatile oil of *N. sativa* seeds on insulin immunoreactivity and ultra

structural changes of pancreatic beta-cells in STZ-induced diabetic rats were evaluated. NS treatment exerts a therapeutic protective effect in diabetes by decreasing morphological changes and preserving pancreatic beta-cell integrity thus suggesting it can be clinically useful for protecting beta-cells against oxidative stress[73].

Treatment with NS decreased the elevated glucose and MDA concentrations, increased the lowered GSH and ceruloplasmin concentrations and prevented lipid-peroxidation-induced liver damage in diabetic rabbits which suggest that it might be used in diabetic patients to prevent lipid peroxidation, increase anti-oxidant defense system activity and also prevent liver damage[74].

The plant mixture containing these seed revealed that blood glucose lowering effect was due to the inhibition of hepatic gluconeogenesis suggesting its use in non-insulin dependent diabetes mellitus[75]. An aqueous decoction of a plant mixture containing NS was found to lower blood glucose level after oral administration[76]. The intraperitoneal administration of volatile oil of seeds produced a significant hypoglycemic effect in normal and alloxan induced diabetic rabbit[77]. Similar results were obtained with seed extract after oral treatment for 2 months[74].

**Cardiovascular activity**

The effects of two months oral supplement of seeds of *N. sativa* to normal rats on cardiac haemodynamic *in vivo*, the ionotropic and chronotropic properties of the isolated hearts *in vitro*, and the cardiac responsiveness to progressive adrenergic stimulation by isoproterenol were investigated. The results showed the intrinsic cardiac contractile properties without evidence of an increased cardiac work load or energy consumption *in vivo* which makes these seeds an isotropic agent with hemodynamic profile[78-80].

In another study, the effects of aqueous and macerated extracts from *N. sativa* on heart rate and contractility of the isolated heart were examined. The results showed a potent inhibitory effect of both extracts on both heart rate and contractility of guinea pig heart that was comparable and even higher than that of diltazem which may be due to calcium channel inhibitory or an opening effect for the plant on potassium channels of the isolated heart[81,82]. The study was undertaken to evaluate the protective effect of TQ in rats after chronic inhibition of nitric oxide synthesis with N (omega)-nitro-l-arginine methyl esters (l-NAME). TQ inhibited the *in vitro* production of superoxide radical in enzymatic and non-enzymatic systems thus TQ is effective in protecting rats against l-NAME-induced hypertension and renal damage possibly via antioxidant activity[83].

The hypotensive effects of dichloromethane extract of seeds (0.6 ml/kg/day) in the spontaneously hypertensive rat were evaluated. The mean arterial pressure decreased, respectively by 22 and 18% in the *N. sativa* treated rat and nifédipine treated rat (0.5 mg/kg/day)[84]. The essential oil and unsaponifiable matter of oil exhibited a depressant effect on frog heart, produced a relaxant effect on isolated muscle of rat and also produced bradycardia[85]. The volatile oil and TQ produced cardio depressant effect which is mediated centrally via indirect and direct mechanism (both 5-hydroxytryptaminergic and muscarinic mechanism)[86]. The active ingredient Thymol has shown to lower blood pressure through blockade of calcium channels[87].

The effect of oral treatment of Wistar albino rats with different doses of powdered seeds (100, 200, 400 and 600 mg/kg/day) for four weeks on the levels of serum lipids were investigated. The result showed that it causes significant decrease in low density lipoprotein-cholesterol levels, triglyceride levels and increase in high density lipoprotein-cholesterol level[88]. Similar results were obtained when *N. sativa* in form of diet (30 mg/kg body weight) were fed to rats for 20 weeks, TQ for 5 days (i.p.) and oil (800 mg/kg orally) for 4 weeks[89,91].

The effect of fixed oil from the seeds on blood homeostatis, cholesterol and glucose level was investigated in rats. The serum cholesterol, triglycerides, glucose level, count of leukocyte and platelets decreased while hematocrit and hemoglobin levels increased significantly[92]. Similar results were obtained with both crushed seeds and total oil[93].

The extract of seeds was found to produce protection against cisplatin-induced fall in hemoglobin levels and leukocyte counts[94]. Methanol soluble portion of oil showed inhibitory effects on arachidonic acid induced platelet aggregation and blood coagulation which was purified to give 2-(2-methoxypropyl)-5-methyl-1, 4-benzenediol, thymol and carvacrol which showed very strong inhibitory activity when compared to the methanol soluble portion[26].

The antioxidant activities of the TQ-rich fraction (TQRF; 0.5-1.5 g/kg) extracted from *N. sativa* and its bioactive compound, thymoquinone (TQ; 20 -100 mg/kg b.w), in rats with induced hypercholesterolemia were investigated. TQRF and TQ effectively improved the plasma and liver antioxidant capacity and

enhanced the expression of liver antioxidant genes of hypercholesterolemia rats[95].

**Gastroprotective activity**

The effect of *N. sativa* aqueous suspension on experimentally induced gastric ulcers by various noxious chemicals (80% ethanol, 0.2 M NaOH, 25% NaCl and indomethacin) and basal gastric secretion in rats were evaluated. It significantly prevented gastric ulcer formation induced by necrotizing agents, also ameliorated the ulcer severity and basal gastric acid secretion in rats. The aqueous suspension significantly replenished the ethanol-induced depleted gastric wall mucus content levels and gastric mucosal non-protein sulfhydryl concentration which suggest that the anti-ulcer effect of NS is possibly prostaglandin-mediated and/or through its antioxidant and anti-secretory activities[96].

The effect of oil and TQ in an experimental model of ethanol induced ulcer in rats was evaluated. NS and TQ protected gastric mucosa against the injurious effect of absolute alcohol and promote ulcer healing. NS and TQ prevented alcohol-induced increase in thiobarbituric acid-reactive substances, tissue histamine level, myeloperoxidase level and decreased gastric glutathione content, enzymatic activities of gastric superoxide dismutase and glutathione-S-transferase[97,98] suggesting that both drugs, particularly NS could partly protect gastric mucosa from acute alcohol-induced mucosal injury and these gastroprotective effects could be due to their antiperoxidative, antioxidant and antihistaminic effects[98,99].

The antioxidant effects of *N. sativa* oil (N.O) and TQ on gastric mucosal redox state (Ischemia/reperfusion induced gastric lesion) and gastric lesions, 1 and 24 h after reperfusion was evaluated. N.O and TQ tended to normalize the level of LDH, GSH, LPX and SOD suggesting that both N.O and TQ possess gastroprotective effect against ischemia/reperfusion induced gastric lesions[100].

The aqueous extract of the seed decreased the volume of acid in gastric juice in acetyl salicylic acid treated rats exhibiting its antiulcer activity[101].The alcoholic extract was investigated for antiulcer activity by pyloric ligation and aspirin induced ulcer model in rats. The volume of gastric acid secretion, free acidity, total acidity and ulcer index were significantly reduced[102].

The effect of NS on intestinal ischemia-reperfusion injury in rats was evaluated. Increase in total antioxidant capacity (TAC), catalase (CAT) and decrease in total oxidative status (TOS), oxidative stress index (OSI) and myeloperoxidase (MPO) in ileum tissue suggested that NS treatment protected the rat's intestinal tissue against intestinal ischemia-reperfusion injury[103].

**Pulmonary activity**

The relaxant effects of four cumulative concentrations of n-hexane, dichloromethane, methanol and aqueous fractions (0.8, 1.2, 1.6 and 2.0 g%) and four cumulative concentrations of theophylline (0.2, 0.4, 0.6 and 0.8 mM) were examined by their relaxant effects on precontracted tracheal chains of guinea pig by 60 mM KCl and 10 µm methacholine. The results showed potent relaxant effect of methanol and dichloromethane fractions on tracheal chains of guinea pigs[104].

The potential for *N. sativa* treatment to protect against lung injury after pulmonary aspiration of materials has been investigated. NS treatment inhibits the inflammatory pulmonary responses, reducing significantly peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudates, alveolar macrophages, interstitial fibrosis, and granuloma and necrosis formation in different pulmonary aspiration models. Further result indicated a significant reduction in the activity of inducible nitric oxide synthase and a rise in surfactant protein D in lung tissue of different pulmonary aspiration models after NS therapy which suggests that it might be beneficial in lung injury[105].

The effects on $Ba^{2+}$, carbachol and leukotriene-induced trachea contractions and the transport of the fluorescence dye rhodamin B concerning ciliary action in the tracheal area by nigellone and TQ were investigated. The trachea contractions induced by leukotriene were inhibited by nigellone and TQ. The rate of ciliary clearance was slightly modified by a high TQ concentration (153.0 vs. 505.0 sec/12 mm distance, respectively), and was highly increased by nigellone (217.5 vs. 505.0 sec/12 mm distance) which suggests an increase in mucociliary clearance for nigellone but not for TQ[106].

The effect of TQ on the *in vivo* production of prostaglandins PGs and lung inflammation in a mouse model of allergic airway inflammation were evaluated. Intraperitoneal injection of TQ for 5 days before the first OVA (ovalbumin) challenge attenuated airway inflammation as demonstrated by the significant decrease in $Th_2$ cytokines, lung

eosinophilia and goblet cell hyperplasia with concomitant to the inhibition of COX-2 protein expression and $PGD_2$ production. However, TQ had a slight inhibitory effect on COX-1 expression and PGE2 production suggesting that TQ has an anti-inflammatory effect during the allergic response in the lung through the inhibition of $PGD_2$ synthesis and $Th_2$-driven immune response[107].

Nigellone was found to inhibit effectively the histamine release from the mast cells suggesting its use in asthma[108]. The antianaphylactic effect of a polyherbal formulation containing *N. sativa* on mesenteric mast cells was studied. The antianaphylactic activity was possibly due to the membrane stabilizing potential, suppression of antibody production and inhibition of antigen induced histamine release[109].

The bronchodilatory effect of NS seeds has shown to be mediated possibly through calcium channel blockade[110]. In another study, the effect of the volatile oil and TQ were investigated and compared on the respiratory system of the urethane anaesthetized guinea pigs. Intravenous administration of volatile oil induced dose dependent increase in respiratory rate and intratracheal pressure whereas TQ induced significant increase in the intratracheal pressure without any effect in the respiratory rate suggesting that volatile oil induced respiratory effects were mediated via release of histamine with direct involvement of histaminergic mechanism and indirect activation of muscarinic cholinergic mechanism[111].

The prophylactic effect of TQ on tracheal responsiveness and white blood cell (WBC) count in lung lavage of sensitized guinea pigs was examined. The results suggested the preventive effect of TQ on tracheal responsiveness and inflammatory cells of lung lavage of sensitized guinea pigs[112].

TQ caused a concentration-dependent decrease in the tension of the pulmonary arterial rings precontracted by phenylephrine. The effects of TQ were not influenced by pretreatment of the rings with propranolol (a non-selective beta-blocker), atropine (a non-selective blocker for muscarinic receptors), theophylline (an adenosine receptor antagonist), indomethacin (a cyclooxygenase inhibitor), L-NAME (a NO synthase inhibitor), methylene blue (an inhibitor of soluble guanylyl cyclase) and nifedipine (a Ca (2+) channel blocker). TQ-induced relaxation of the precontracted pulmonary artery is probably mediated by activation of ATP-sensitive potassium channels and possibly by non-competitive blocking of serotonin, alpha1 and endothelin receptors[113].

**Nephroprotective activity**

The possible protective effect of NS against gentamicin (GM)-induced nephrotoxicity in rats was evaluated. Administration of NS with GS injection resulted in significantly decreased creatinine, urea, MDA, NO generation and increased SOD and GSH-Px activities when compared with gentamicin group suggesting neproprotective activity[114]. Similar experiment was conducted with TQ against gentamicin induced nephrotoxicity. TQ supplementation resulted in a complete reversal of the GM-induced increase in BUN, creatinine, TBARS, NOx and decrease in GSH, GPx, CAT and ATP to control values suggesting that it prevents GM-induced degenerative changes in kidney tissues[115].

The protective effects of *N. sativa* oil (NSO), in prevention of chronic cyclosporine A (CsA)-induced nephrotoxicity in rats were investigated. NSO significantly improved the functional and histological parameters and attenuated the oxidative stress induced by CsA[116].

Oral treatment of rats with NSO (0.5, 1.0 or 2.0 ml/kg/day for 10 days) on nephrotoxicity of GM (80 mg/kg/day given intramuscularly) were investigated. It produced a dose-dependent amelioration of the biochemical and histological indices of GM nephrotoxicity that was statistically significant at the two higher doses used. Treatments of rats with *N. sativa* did not cause any over toxicity and it increased total antioxidant status (TAS) in plasma and reduced glutathione (GSH) concentrations in renal cortex and enhanced growth[117].

The effect of TQ on the nephropathy and oxidative stress induced by doxorubicin (DOX) in rats was investigated. Treatment of rats with TQ (10 mg/kg per day) supplemented with the drinking water for 5 days before DOX and daily thereafter, significantly lowered serum urea, TG and TC. Similarly, TG, TC and lipid peroxides in the kidneys of TQ-treated rats were decreased significantly compared with DOX alone. Treatment with TQ significantly suppressed DOX-induced proteinuria, albuminuria and urinary excretion of NAG which suggests that TQ might be useful as protective agent for proteinuria and hyperlipidemia associated with nephrotic syndrome[118].

The seeds were found to reduce significantly the cisplatin-induced nephrotoxicity, blood urea nitrogen,

serum creatinine levels as well as cisplatin-induced serum total lipid increase[119]. Oral treatment with extract was found to be a potent chemopreventive agent causing the suppression of potassium bromate mediated renal oxidative stress; toxicity and tumor promotion response in rats[120]. In another study, the protective effects of NSO on methotrexate-induced toxicity were studied in albino rats[121].

**Hepatoprotective activity**

Oral administration of 1 ml/kg seed oil every day for one week prior to carbon tetrachloride ($CCL_4$) injection alleviated $CCl_4$-induced suppression of CYP2B, CYP3A2, CYP2C11 and CYP1A2 and also down regulated the $CCl_4$-induced iNOS mRNA and up-regulated IL-10 mRNA. These results suggest that this protective effect is partly due to the down regulation of NO production and up-regulation of the anti-inflammatory IL-10[122].

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) levels and total antioxidant capacity (TAC), catalase (CAT), total oxidative status (TOS), oxidative stress index (OSI) and myeloperoxidase (MPO) in hepatic tissue were determined. The results have shown that treatment with NS prevents hepatic ischemia-reperfusion injury to the liver[123].

The effects of NS and *Urtica dioica* Linn. (UD) on lipid peroxidation, antioxidant enzyme systems and liver enzymes in $CCl_4$-treated rats were evaluated. NS or UD treatment (alone or combination) for 60 days decreased the elevated lipid peroxidation and liver enzyme levels and also increased the reduced antioxidant enzyme levels. NS and UD decrease the lipid per-oxidation and liver enzymes and increase the antioxidant defense system activity in the $CCl_4$-treated rats[124-127].

The effect of the aqueous suspension of NS (250 and 500 mg/kg) for five days on $CCL_4$-induced liver damage in rats was determined. Serum levels of AST and ALT were slightly decreased while LDH was significantly increased in animals treated with $CCL_4$ when compared to the control group. LDH was restored to normal but ALT and AST levels were increased in animals pretreated with NS[128].

Effects of TQ, p-cymene and α-pinene, on $CCl_4$-indued acute liver injury were investigated in mice. Pretreatment of mice with different doses of TQ 1 h before $CCl_4$ injection showed that the only dose of TQ that ameliorated hepatotoxicity of $CCl_4$ was 12.5 mg/kg i.p. as evidenced by the significant reduction of the elevated levels of serum enzymes as well as hepatic MDA content and significant increase of the hepatic nonprotein sulfhydryl(-SH) concentration. Treatment of mice with the other volatile oil constituents, p-cymene or α-pinene did not induce any changes in the serum ALT measured[129]. Similar results were obtained with oral administration of TQ in a single dose (100 mg/kg) which provided significant protection against the hepatotoxic effects of $CCl_4$[130].

TQ was tested in isolated rat hepatocytes as a hepatoprotective agent against tert-butyl hydroperoxide (TBHP) toxicity. Preincubation of hepatocytes with 1 mM of either TQ or silybin resulted in the protection of isolated hepatocytes against TBHP induced toxicity evidenced by decreased leakage of ALT and AST and by decreased trypan blue uptake in comparison to TBHP treated hepatocytes. Both TQ and silybin prevented TBHP induced depletion of GSH to the same extent[131].

TQ was applied to primary rat hepatocyte cultures and both cyto- and genotoxic effects were tested. TQ induced significant anti-proliferative effects at 20 µM and acute cytotoxicity at higher concentrations. It significantly increased the rates of necrotic cells at concentrations between 2.5 and 20 µM. Furthermore, it induced significant genotoxicity at concentrations 1.25 µM suggesting that TQ causes glutathione depletion and liver damage, but contradicts the reports indicating antioxidant and anti-clastogenic effects[132].

Administration of NS in the rats with biliary obstruction resulted in inhibition of necro-inflammation by decrease in gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and LDH activities suggesting a therapeutic effect on cholestatic liver injury in bile duct ligated rats[133].

**Antiinflammatory activity**

The antiinflammatory effects of TQ on arthritis in rat models were investigated. Signs of inflammation on the claw and radiological signs were searched for and TNF-α and IL-1β were measured. The results showed that TQ suppressed adjuvant-induced arthritis in rats[134,135].

The analgesic and anti-inflammatory effects of polyphenols from seed were studied in mice and rats using the acetic acid-induced writhing, formalin, light tail flick, carrageenan-induced paw edema and croton oil-induced ear edema tests. These results suggest that

NS polyphenol have potent analgesic and anti-inflammatory effects[136].

Black cumin seed essential oil (BCSEO) was found to produce a significant analgesic effect in acetic acid-induced writhing, formalin and light tail flick tests. Naloxone, an opioid antagonist, could not reverse the analgesic effect observed in the formalin test. Although oral administration of BCSEO at doses of 100, 200 and 400 µl/kg did not exert a significant anti-inflammatory effect in the carrageenan test, i.p. injection of the same doses significantly inhibited carrageenan-induced paw edema. BCSEO at doses of 10 and 20 µl/ear could also reduce croton oil-induced edema. It seems that mechanism other than opioid receptors is involved in the analgesic effect of BCSEO since naloxone could not reverse this effect. Both systemic and local administration of BCSEO showed anti-inflammatory activity[137].

The *in vitro* effect of aqueous extract of seeds on nitric oxide (NO) production by murine macrophages was studied. These results indicate that the aqueous extract of seeds exhibits an inhibitory effect on nitric oxide production by murine macrophages. This study validates the traditional use of the seeds for the treatment of rheumatism[138].

In another study, NSO, nigellone and TQ were studied to evaluate their effect on the formation of 5-lipoxygenase (5-LO) products from polymorphonuclear leukocytes (PMNL). $IC_{50}$ values of inhibition of 5-LO products for NSO and TQ were found to be 25 µg/ml and 0.26 µg/ml. $IC_{50}$ values of inhibition of 5-hydroxy-eicosa-tetra-enoic acid (5-HETE) production for NSO, nigellone and TQ were found to be 24 µg/ml, 11.9µg/ml and 0.36 µg/ml, respectively. The data may in part explain the effect of the oil, its derived TQ and nigellone in ameliorating inflammatory diseases[139].

The aqueous extract of NS was investigated for anti-inflammatory, analgesic and antipyretic activities in animal models. The extract has an anti-inflammatory effect demonstrated by its inhibitory effects on carrageenan induced paw edema. It also produced significant increase in the hot plate reaction time in mice indicating analgesic effect. However, NS crude suspension had no effect on yeast induced pyrexia[140]. The crude fixed oil of *N. sativa* and TQ both have been found to inhibit the eicosanoid generation and measure lipid peroxidation, though the inhibition of cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism, thus responsible for its anti-inflammatory activity[141].

**Immunomodulatory activity**

The volatile oil of *N. sativa* seeds (NSVO) was investigated for its immunomodulating and cytotoxic properties in rats. Antibody titre for the experimental animal was found to be 1280 as compared to the 2560 in the control rats. There was a significant decrease in splenocytes and neutrophils counts, but a rise in peripheral lymphocytes and monocytes in the experimental animals. $LC_{50}$ values for NSVO were 155.02, 185.77, 120.40, 384.53 and 286.83 µg/ml, respectively against the SCL, SCL-6, SCL-37'6, NUGC-4 cancer lines and 3T6 fibroblast line. Results indicate NSVO as a potential immunosuppressive cytotoxic agent[142].

*In-vitro* cytotoxic screening of extracts of seeds indicated cytotoxicity in the ethyl-acetate fraction (EAF) against different classes of cancer cell lines, P388, Molt4, Wehi 164, LL/2, Hep G2, SW620 and J82, as measured by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The ethyl-acetate column chromatographic fraction (CC-5) showed selectivity against Hep G2, Molt4 and LL/2. CC-5 was relatively non-toxic against human umbilical cord endothelial cells at 50 µg/ml. These data indicate that CC-5 possesses a potent cytotoxic effect as well as a potentiating effect on the cellular immune response[41].

The radioprotective potential of *Nigella* crude oil against hemopoietic adverse effects of gamma irradiation was evaluated. Oral administration of *N. sativa* oil before irradiation considerably normalized significant increase in malondialdehyde concentration with a significant decrease in plasma glutathione peroxidase, catalase and erythrocyte superoxide dismutase activities promising natural radioprotective agent against immunosuppressive and oxidative effects of ionizing radiation[143].

**Central nervous system activity**

The possible beneficial effects of NS in comparison to methylprednisolone on experimental spinal cord injury (SCI) in rats were investigated. Methylprednisolone and NS treatment decreased tissue MDA and PC levels and prevented inhibition of SOD, GSH-Px and CAT enzymes in the tissues. The morphology of neurons in methylprednisolone and NS-treated groups were well protected which suggest that NS might be beneficial in spinal cord tissue damage[144].

The effect of aqueous and methanol extracts of defatted seeds on the central nervous system (CNS)

and on analgesic activity were evaluated. The observations suggest that both extracts possesses a potent CNS depressant and analgesic activity[145].

Similar results were obtained with oil treatment also. It was found to potentiate pentobarbitone – induced sleeping time[132]. The aqueous and methanol extract of seeds produced an alteration in general behaviors, significant reduction of spontaneous motility, normal body temperature and analgesic activity against hot plate suggesting its CNS depressant activity[146].

The protective effects of NS (15.62-250 µg/ml) and TQ (1.17-150 µm) on cell viability and reactive oxygen species (ROS) production in cultured PC12 cells were investigated under serum/glucose deprivation (SGD) conditions was evaluated. NS (250 µg/ml, $P<0.01$) and TQ (2.34, 4.68, 9.37 µm, $P<0.01$) pretreatment reversed the increased ROS production following ischemic insult suggesting that NS extract and TQ protects the PC12 cells against SGD-induced cytotoxicity via antioxidant mechanisms and its application for managing cerebral ischemic and neurodegenerative disorders[147].

**Anticonvulsant activity**

The anticonvulsant and antioxidant activities of NSO on pentylenetetrazol (PTZ) kindling seizures in mice were investigated. NSO showed anti-epileptogenic properties as it reduced the sensitivity of kindled mice to the convulsive and lethal effects of PTZ induced oxidative injury in the brain. The effect was comparable to that of valporate[148]. In PTZ-induced epileptic seizures, the i.c.v. injection of thymoquinone at doses of 200 and 400 µm prolonged the time until onset and reduced the duration of tonic-clonic seizures. The results indicate that thymoquinone may have anticonvulsant activity, probably through an opioid receptor-mediated increase in GABAergic tone[149].

Similar results were obtained when intraperitoneal injection of TQ with doses of 40 and 80 mg/kg were administered. Moreover, TQ (40 and 80 mg/kg) did not have any hypnosis effect in the pentobarbital-induced hypnosis, but impaired the motor coordination and reduced the locomotors activity suggesting that TQ may have anticonvulsant activity in the petit mal epilepsy[150].

**Antinociceptive activity**

The antinociceptive effects of NSO and TQ were examined in mice. The p.o. administration of NSO (50-400 mg/kg) dose-dependently suppressed the nociceptive response in the hot-plate test, tail-pinch test, and acetic acid-induced writhing test and in the early phase of the formalin test. The systemic administration (2.5-10 mg/kg, p.o. and 1-6 mg/kg, i.p.) and the i.c.v. injection (1-4 µg/mouse) of TQ attenuated the nociceptive response in not only the early phase but also the late phase of the formalin test which suggests that the oil and TQ produce antinociceptive effects through indirect activation of the supraspinal µ (1)-and kappa-opioid receptor subtypes[151].

**Anxiolytic activity**

The aqueous and methanol extracts of *N. sativa* seeds for four weeks were evaluated for their anxiolytic activity in rats by open field and elevated plus maze models. The rats exhibited an increase in open field activity and produced anti-anxiety effect in elevated plus maze. Oral administration of *N. sativa* oil increased brain levels of 5-HT and tryptophan but the levels of brain 5-HIAA decreased significantly suggesting its anxiolytic use[152].

**Antioxidant activity**

The effects of NSO on the antioxidant enzyme status and myocardium of cyclosporine-A-treated rats was evaluated. Pre-treatment with NSO reduced the subsequent cyclosporine A injury in rat heart, demonstrated by normalized cardiac histopathology, decrease in lipid peroxidation, improvement in antioxidant enzyme status and cellular protein oxidation suggesting antioxidant activity as mechanism[153].

The essential oil of NS was tested for a possible antioxidant activity by diphenylpicrylhydracyl assay. A rapid evaluation for antioxidants, using two TLC screening methods, showed that TQ and the components carvacrol, anethole and 4-terpineol demonstrated respectable radical scavenging property. They were also effective OH radical scavenging agents in the assay for non-enzymatic lipid peroxidation in liposomes and the deoxyribose degradation assay[154].

TQ was found to exhibit renal protective effect in rats through its antioxidant action[38,118] and also provide protection against hepatotoxicity induced by $CCl_4$ in mice[130], rats and rabbits[98]. The free radical scavenging effects of thymol, TQ and dithymoquinone were studied on reaction generating reactive oxygen species such as superoxide anion

radical, hydroxyl radical and singlet oxygen using chemiluminescence and spectrophotometric methods[155].

The hepatoprotective effects of oil and TQ were found to be via antioxidant mechanism. Similarly the protective effect of TQ against doxorubicin induced nephrotoxicity[118] and that against doxorubicin induced cardiotoxicity[156,157] were found to be due to antioxidant activity. The modulating effect of TQ on benzopyrene induced cancer in mice[45] and its antitumor effect on 20-methylcholanthrane induced fibrosarcoma tumor genesis were partly due to its antioxidant activity[51].

**Antioxytocic activity**

The effects of the volatile oil of seeds on the uterine smooth muscle of rats and guinea pigs were tested *in vitro* using isolated uterine horns. The volatile oil inhibited the spontaneous movements of rat and guinea pig uterine smooth muscle and also the contractions induced by oxytocin suggesting its anti-oxytocic potential[158].

**Post-coital contraceptive activity**

Hexane extract of the seeds of NS prevented pregnancy in Sprague-Dawley rats treated orally at 2 g/kg daily dose on day's 1-10 post-coitum. The active hexane extract exhibited only mild uterotrophic activity comparable to ethinylestradiol, but was devoid of any estrogenicity in the immature rat bioassay[159]. The ethanol extract of seeds showed antifertility effect in male rats that is probably due to inherent estrogenic activity[160].

**Abortifacient activity**

Hot water extract of NS as well as whole seeds in large oral doses causes abortion in human pregnant females[161,162].

**Anti-implantation activity**

Powder of seed (500 mg/kg) has shown to possess antiimplantation activity in pregnant rats[163]. The ethanolic extract showed inhibition of ovulation when administered at 200 mg/kg in female rabbits[164].

**Diuretic activity**

The diuretic effects of dichloromethane extract of seeds in rat were determined. An oral dose of extract (0.6 ml/kg/day) and furosemide (5 mg/kg/day) increased significantly the diuresis by 16 and 30%, respectively after 15 days of treatment; urinary excretion of Cl-, Na+, K+ and urea is also increased[84].

**Antiurolithatic activity**

TQ significantly decreased the number and size of calcium oxalate deposits in the renal tubules in ethylene glycol-induced kidney calculi in rats[165]. Treatment of rats with ethanol extract of NS reduced the number of calcium oxalate deposits in rats in ethylene glycol-induced kidney calculi in rats and also lowered the urine concentration of calcium oxalate suggesting the use as antiurolithatic agent[166].

**Antispasmolytic activity**

The aqueous extract of seed caused mild to moderate dose dependent relaxation effects, increased the sensitivity of the ileum to acetylcholine and interacted with serotonin in a dose dependent manner[167] and also showed spasmolytic activity mediated through calcium antagonist effect justifying the traditional use in diarrhoea[110]. The volatile oil and ethanol extract inhibited spontaneous movements of rabbit jejunum as well as agonist induced contractions suggesting the calcium channel blockade as spasmolytic mechanism[168].

**Opioid dependence treatment**

NS is effective in long-term treatment of opioid dependence (35 patients). It not merely cures the opioid dependence but also cures the infections and weakness from which majority of addict's suffer[169].

**Experimental autoimmune encephalomyelitis (EAE)**

TQ afforded protection (90%) in experimental autoimmune encephalomyelitis (EAE) in rats suggesting the role in treating the human chronic relapsing multiple sclerosis phase. Treatment of the rats with NS inhibited ROS production induced by EAE showing diminished levels of MDA of both brain and medulla spinalis tissues and decrease in brain NO level suggesting that it provides protection against oxidative stress induced by experimental autoimmune encephalomyelitis[170-172].

**Antibacterial activity**

The ethanol extract of seeds has inhibited the growth of Methicillin resistant *Staphylococcus aureus* at a concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/ml[173].

The seed essential oils obtained by hydro distillation (HD), dry steam distillation (SD), steam distillation of crude oils obtained by solvent extraction (SE-SD) and supercritical fluid extraction (SFE-SD) were tested for their antibacterial activities. The MICs values were 256 and 32 µg/ml for HD and SD, respectively) and MIC values for both SE-SD and SFE-SD were 4 µg/ml. All oil samples were significantly more active against Gram-positive than against Gram-negative bacteria[174].

Different crude extracts of NS were tested for antimicrobial activity against different bacterial isolates, viz. 16 Gram negative and 6 Gram positive bacterias. The most effective extracts were the crude alkaloid and water extracts. Gram negative isolates were affected more than the Gram positive ones[175]. Filter paper discs impregnated with the diethyl ether extract of seeds (25-400 µg extract/disc) caused concentration-dependent inhibition of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and a pathogenic yeast *Candida albicans*[176,177].

The antimicrobial activity of the volatile oil of seeds was studied. The antimicrobial principle has been isolated from volatile oil, identified as TQ and found to be active against gram-positive bacteria and yeasts[178]. The methanol extract of seed was found to exhibit anti-plaque action by inhibiting *Streptococcus mutants*, thus preventing dental caries[179]. Alcoholic extract showed antibacterial activity against *Micrococcus pyogenes* var. *aureus, Shigella dysenteriae, S. sonnei, S. boydii, Vibrio cholerae* and *E. coli*[180]. In another study, it was found to exhibit antibacterial activity against *Bacillus pumilus, B. subtilis, Streptococcus mutants, Staphylococcus aureus, S. lutea* and *P. aeruginosa*[181].

**Antifungal activity**

The antifungal activity of ether extract of seeds and TQ were tested against eight species of dermatophytes: four species of *Trichophyton rubrum* and one each of *Trichophyton interdigitale, Trichophyton mentagrophytes, Epidermophyton floccosum* and *Microsporum canis*. The MICs of the ether extract of NS and TQ were between 10 and 40 and 0.125 and 0.25 mg/ml, respectively, while those of griseofulvin ranged from 0.00095 to 0.0155 mg/ml[182].

The effect of an aqueous extract of seeds was studied on candidiasis in mice. An intravenous inoculum of *Candida albicans* produced colonies of the organism in the liver, spleen and kidneys. A 5-fold decrease in *Candida* in kidneys, 8-fold in liver and 11-fold in spleen was observed in the groups of animals post-treated with the plant extract. These results indicate that the aqueous extract of seeds exhibits inhibitory effect against candidiasis[183].

Antifungal activities of the seeds oil were tested against twenty fungi including pathogenic and industrial strains. All the oils were found to have significant activities against the fungi, but the volatile oil showed stronger and wider range of antifungal activities. MIC values of the volatile oil were also determined against three pathogenic fungi and lowest MIC was found against *Aspergillus fumigatus*[184].

**Anti-schistosomiasis agents**

The antioxidant and anti-schistosomal activities of the garlic extract (AGE) and *N. sativa* oil (NSO) on normal and *Schistosoma mansoni*-infected mice was investigated. AGE (125 mg/kg, i.p.) and NSO (0.2 mg/kg, i.p.) were administrated separately or in combination for successive 28 days, starting from the 1st day post infection (pi). AGE and NSO prevented most of the hematological and biochemical changes and markedly improved the antioxidant capacity of schistosomiasis mice compared to the infected-untreated ones. In addition, remarkable reduction in worms, tissue eggs and alteration in oogram pattern were recorded in all the treated groups[185, 186].

The schistosomicidal properties of seeds were tested *in vitro* against *Schistosoma mansoni miracidia*, *Schistosoma mansoni cercariae* and adult worms. Results indicate its strong biocidal effects against all stages of the parasite and also showed an inhibitory effect on egg-laying of adult female worms and also decrease in the activities of both antioxidant enzymes, superoxide dismutase, glutathione peroxidase and glutathione reductase and enzymes of glucose metabolism, hexokinase and glucose-6-phosphate dehydrogenase[187].

**Anthelmintic activity**

Larvicidal activity has been reported against *Culex pipiens* when ether extract is used at 151.7 ppm. Hydro alcoholic extract is highly effective against *Entamoeba histolytica* at a concentration of 125 µg/ml[188-189].

**Toxicology**

The drug is traditionally considered to be safe in the dosage mentioned. Acute toxicity of fixed oil was investigated in mice and rats. The $LD_{50}$ values by single doses orally and intraperitoneally administered in mice were 28.8 ml/kg b.w., p.o. and 2.061 ml/kg b.w. i.p., respectively. The $LD_{50}$ of the alcoholic extract was reported to be 561 mg/kg i.p. in albino mice. In a chronic toxicity study in rats, the fixed oil in oral doses of 2 ml/kg b.w administered daily for 12 weeks showed changes in hematological parameters. However, there was no change in histological and biochemical parameters in liver, heart, kidney and pancreas[96].

The $LD_{50}$ in mice after intraperitoneal injection was determined to be 104.7 mg/kg and after oral ingestion

was 870.9 mg/kg. Whereas, $LD_{50}$ in rats after intraperitoneal injection was determined to be 57.5 mg/kg and after oral ingestion was 794. 3 mg/kg[190].

In this study, acute and sub acute toxicity of the aqueous, methanol and chloroform extracts of the seeds have been investigated. To determine their $LD_{50}$, the aqueous, methanol and chloroform extracts were administered orally, in 4 different doses, 6, 9, 14 and 21 g/kg. Mortality rate and weight changes have also been measured in all groups for 3 and 7 days, respectively. Degenerative changes in hepatic cells have been observed only with aqueous extract of the seeds suggesting the hepatic toxicity with the extract[191].

The safety of *N. sativa* fixed oil (BCFO) and essential oil (BCEO) in Sprague Dawley rats at a dose of 4 and 0.3%, respectively were evaluated. The serological indices like liver and kidney functioning tests, serum protein profile, level of cardiac enzymes, electrolytes balance, indices of red and white blood cells remained in normal range indicating its safe use[192].

#### Clinical evaluation

In the clinical trial with female UTI patients, there was decrease in pus cells in the urine after the patients were treated with seed extract. Alcoholic extract of seeds depressed both the systolic and diastolic blood pressure (statistically highly significant) starting from 1 h till the end of 5 h after oral intake. Anticestodal effect of seeds studied in children indicated positive results without any adverse side effects[6,193].

A comparative, parallel, randomized, double-blind, placebo-controlled study of acute tonsillopharyngitis patients with a treatment period of 7 days was conducted to examine clinical effectiveness of *N. sativa* and *Phyllanthus niruri* extract (NSPN extract). At the end of treatment (Day 7), a significantly greater proportion of patients in the NSPN group than in the placebo group had their sore throat completely relieved[194].

A double-blinded crossover clinical trial conducted on children with refractory epilepsy, the aqueous extract of black seed was administered as an adjunct therapy. The mean frequency of seizures decreased significantly during treatment with extract. The prophylactic effect of NS (15 ml/kg of 0.1 g% boiled extract) for 3 months on asthmatic patients was examined. All asthma symptoms, frequency of asthma symptoms/week, chest wheezing and PFT values in the study group were significantly improved. Similar results were obtained when boiled extract of N.Sativa was administered at the dose of 50 and 100 mg/kg in 15 asthmatic patients[195,196].

The effect of seed supplement on symptom levels, polymorphonuclear leukocyte (PMN) functions, lymphocyte subsets and hematological parameters of allergic rhinitis during allergen specific immunotherapy for 30 days in 24 allergic rhinitis patients was evaluated. It was found to be a potential adjuvant therapy for allergic rhinitis[197].

A randomized double blind controlled trial on 123 patients for effectiveness; safety and tolerability of powdered seed in capsules on serum lipid levels, blood sugar, blood pressure and body weight in adults were evaluated. Favourable impact of powdered seeds in capsules was noted in almost all the variables[198].

## Conclusion

In recent years, ethnobotanical and traditional uses of natural compounds, especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use. They obviously deserve scrutiny on modern scientific lines such as phytochemical investigation, biological evaluation on experimental animal models, toxicity studies and investigation of molecular mechanism of actions of isolated phytoconstituents[199]. *N. sativa* is reported to possess antitumor, antidiabetic, cardiovascular, pulmonary, gastroprotective, antifertility,diuretic, CNS depressant, antispasmolytic, anti-inflammatory, antimicrobial, antioxidant, anticonvulsant, antinociceptive, antiurolithatic, anxiolytic, nephroprotective, hepatoprotective, immunomodulatory and anthelmintic activities but number of other pharmacological activites are yet to be explored. In future studies, the isolated principles from seeds needs to be evaluated in scientific manner using specific experimental animal models and clinical trials are to be done to understand the molecular mechanism of action, in search of lead molecule from natural resources.

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