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A review on therapeutic potential of *Nigella sativa*: A miracle herb

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PEER REVIEW

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Comments

The current review on Nigella provides the detailed scientific information of this medicinal plant. Due to its miraculous medicinal properties *N. sativa* has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders. Extensive studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored, including antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastroprotective and antioxidant properties.
(Details on Page 349)

ABSTRACT

Nigella sativa (N. sativa) (Family Ranunculaceae) is a widely used medicinal plant throughout the world. It is very popular in various traditional systems of medicine like Unani and Tibb, Ayurveda and Siddha. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The seeds of *N. sativa* have been widely used in the treatment of different diseases and ailments. In Islamic literature, it is considered as one of the greatest forms of healing medicine. It has been recommended for using on regular basis in Tibb-e-Nabwi (Prophetic Medicine). It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders. Extensive studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, antioxidant properties, etc. Due to its miraculous power of healing, *N. sativa* has got the place among the top ranked evidence based herbal medicines. This is also revealed that most of the therapeutic properties of this plant are due to the presence of thymoquinone which is major bioactive component of the essential oil. The present review is an effort to provide a detailed survey of the literature on scientific researches of pharmacognostical characteristics, chemical composition and pharmacological activities of the seeds of this plant.

KEYWORDS

Nigella sativa, Miracle herb, Ranunculaceae, Habat-ul-Sauda, Thymoquinone, Tibb-e-Nabwi, Black seeds, Anti-diabetic, Antioxidant

1. Introduction

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Moreover, medicinal plants are also used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Many researchers are focusing on medicinal plants since only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies.

Among various medicinal plants, *Nigella sativa* (*N. sativa*) (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many

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researches revealed its wide spectrum of pharmacological potential. *N. sativa* is commonly known as black seed. *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[1].

The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. And it is an important drug in the Indian traditional system of medicine like Unani and Ayurveda[2,9]. Among Muslims, it is considered as one of the greatest forms of healing medicine available due to it was mentioned that black seed is the remedy for all diseases except death in one of the Prophetic hadith. It is also recommended for use on regular basis in Tibb-e-Nabwi (Prophetic Medicine)[3].

N. sativa has been extensively studied for its biological activities and therapeutic potential and shown to possess wide spectrum of activities *viz.* as diuretic, antihypertensive, antidiabetic, anticancer and immunomodulatory, analgesic, antimicrobial, anthelmintics, analgesics and anti-inflammatory, spasmolytic, bronchodilator, gastroprotective, hepatoprotective, renal protective and antioxidant properties. The seeds of *N. sativa* are widely used in the treatment of various diseases like bronchitis, asthma, diarrhea, rheumatism and skin disorders. It is also used as liver tonic, digestive, anti-diarrheal, appetite stimulant, emmenagogue, to increase milk production in nursing mothers to fight parasitic infections, and to support immune system[4-9]. Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. Black seeds are also used in food like flavoring additive in the breads and pickles because it has very low level of toxicity[10].

2. Pharmacognostical characteristics

2.1. Morphology of the plant

N. sativa is an annual flowering plant which grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are delicate, and usually colored white, yellow, pink, pale blue or pale purple, with 5-10 petals. The fruit is a large and inflated capsule composed of 3-7 united follicles, each containing numerous seeds[9,11].

Figure 1. *N. sative* (whole plant, flower and seeds) adopted from internet.

2.2. Characteristics of the seeds and powder

Macroscopically, seeds are small dicotyledonous, trigonus, angular, regulose-tubercular, 2-3.5mm×1-2 mm, black externally and white inside, odor slightly aromatic and taste bitter. Microscopically, 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[1,11].

3. Chemical composition of black seeds

Many active compounds have been isolated, identified and reported so far in different varieties of black seeds. The most important active compounds are thymoquinone (30%-48%), thymohydroquinone, dithymoquinone, p-cymene (7%-15%), carvacrol (6%-12%), 4-terpineol (2%-7%), t-anethol (1%-4%), sesquiterpene longifolene (1%-8%) α-pinene and thymol *etc*. Black seeds also contain some other compounds in trace amounts. Seeds contain two different types of alkaloids; *i.e.* isoquinoline alkaloids *e.g*. nigellicimine and nigellicimine-N-oxide, and pyrazol alkaloids or indazole ring bearing alkaloids which include nigellidine and nigellicine. Moreover, *N. sativa* seeds also contain alpha-hederin, a water soluble pentacyclic triterpene and saponin, a potential anticancer agent[12,13].

Some other compounds *e.g*. carvone, limonene, citronellol 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*. The seeds contain carotene which is converted by the liver to vitamin A. Root and shoot are reported to contain vanillic acid[12,14].

The seeds reported to contain a fatty oil rich in unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less. α-sitosterol 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)[15-17].

Examples of various other reported chemical components includes nigellone,avenasterol-5-ene, avenasterol-7-ene, campesterol, cholesterol, citrostadienol, cycloeucalenol, ,gramisterol, lophenol, obtusifoliol, stigmastanol, stigmasterol-7-ene, β-amyrin, butyrospermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabino-pyranosyl]-28-O-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-gluco-pyranosyl] 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, β-unsaturated hydroxy ketone, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, protein, reducing sugar, glycosidal saponin, 3-O-[β-D-xylopyranosyl-(1→2)-α-L-rhamno-pyranosyl-(1→2)-β-D-glucopyranosyl]-11-methoxy-16, 23-

dihydroxy-28-methy-lolean-12-enoate,stigma-5, 22-dien-3-β-D-gluco-pyranoside, cycloart-23-methyl-7, 20, 22-triene-3β, 25-diol, nigellidine-4-O-sulfite, N. mines A3, A4, A5, C, N. mines A1, A2, B1, and B2[18-22].

4. Traditional uses of folk remedies

N. sativa has been traditionally used for the treatment of a variety of 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[2,9].

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 their oil have a long history of folklore usage in Indian and Arabian civilization as food and medicine[11,23]. The seeds have been traditionally used in Southeast Asian and the Middle East countries for the treatment of several diseases and ailments including asthma, bronchitis, rheumatism and related inflammatory diseases. Its many uses have earned *Nigella* the Arabic approbation 'Habbatul barakah', meaning the seed of blessing. A tincture prepared from the seeds is useful in indigestion, loss of appetite, diarrhoea, dropsy, amenorrhoea and dysmenorrhoea and in the treatment of worms and skin eruptions. Externally the oil is used as an antiseptic and local anesthetic. Roasted black seeds are given internally to stop the vomiting[2,11,23,24].

5. Scientific researches and pharmacological potentials

The extensive researches using modern scientific techniques were carried out by various researchers on *N. sativa* since it is believed to be a miraculous herb that can cure multiple ailments and disorders. A number of pharmacological actions of *N. sativa* have been investigated in the past few decades.

5.1. Antibacterial activity

The antibacterial effect of ground black seeds was studied in a modified paper disc diffusion method. A clear inhibition of the growth of *Staphylococcus aureus* was observed by concentration of 300 mg/mL with distilled water as control, this inhibition was confirmed by using the positive control Azithromycin. The inhibition obtained was higher with *N. sativa* ground seeds from Hadramout than with *N. sativa* ground seeds from Ethiopia. The positive inhibition may be attributed to the two important active ingredients of *N. sativa*, TQ and melanin[25]. Different crude extracts of *N. sativa* were tested for antimicrobial effectiveness against different bacterial isolates which comprised of 16 gram negative and 6 gram positive representatives. These isolates showed multiple resistances against antibiotics, specially the gram negative ones. Crude extracts of *N. sativa* showed a promising effect against some of the test organisms. The most effective extracts were the crude alkaloid and water extracts. Gram negative isolates were affected more than the gram positive ones[26]. Antibacterial activity of *N. sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus* was investigated in 2008 by Hannan *et al*. All tested strains of methicillin resistant *Staphylococcus aureus* were sensitive to ethanolic extract of *N. sativa* at a concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/mL[27]. Antibacterial activity of *N. sativa* against and triple therapy in eradication of Helicobacter Pylori in patients with non-ulcer dyspepsia was carried out. It was showed that *N. sativa* seeds possess clinically useful anti *H. pylori* activity, comparable to triple therapy[28]. The antibacterial activity of TQ and its biofilm inhibition potencies were investigated on 11 human pathogenic bacteria. TQ exhibited a significant bactericidal activity against various human pathogenic bacteria especially Gram positive cocci (*Staphylococcus aureus* ATCC 25923 and Staphylococcus epidermidis CIP 106510). TQ prevented cell adhesion to glassslides surface[29].

5.2. Antifungal activity

Methanolic extracts of *N. sativa* have the strongest antifungal effect followed by the chloroform extracts against different strains of *Candida albicans*. Aqueous extracts showed no antifungal activity. An intravenous inoculum of *Candida albicans* produced colonies of the organism in the liver, spleen and kidneys. Treatment of mice with the plant extract 24 h after the inoculation caused a considerable inhibitory effect on the growth of the organism in all organs studied. Khan *et al*. in 2003 reported that the aqueous extract of *N. sativa* seeds exhibits inhibitory effect against candidiasis in mice. 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 findings were also confirmed by Histopathological examination of the respective organs[30]. Antidermatophyte activity of ether extract of *N. sativa* and TQ was tested against eight species of dermatophytes: four species of *Trichophyton rubrum* and one each of *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis* using Agar diffusion method with serial dilutions of ether extract of *N. sativa*, TQ and griseofulvin. The MICs of the ether extract of *N. sativa* and TQ were between 10-40 and 0.125-0.250 mg/mL, respectively, while those of griseofulvin ranged from 0.00095 to 0.01550 mg/mL. These results denote the potentiality of *N. sativa* as a source for antidermatophyte drugs and support its use in folk medicine for the treatment of fungal skin infections[31]. The antiyeast activity of the black cumin seed quinines, dithymoquinone, thymohydroquinone, and TQ were evaluated *in vitro* with a broth microdilution method against six dairy spoilage yeast species. It was found that Antifungal effects of the quinones were compared with those of preservatives commonly used in milk products (calcium propionate, natamycin, and potassium sorbate) at two pH levels (4.0 and 5.5), while thymohydroquinone and TQ possessed significant antiyeast activity[32]. Two novel antifungal defensins named Ns-D1 and Ns-D2, were isolated from seeds of *N. sativa* and sequenced. The Ns-D1 and Ns-D2 defensins displayed strong divergent antifungal activity towards a number of phytopathogenic fungi[33].

5.3. Anti-schistosomiasis activity

The effect of NSO against the liver damage induced by *Schistosoma mansoni* (*S. mansoni*) infection in mice was studied by Mahmoud *et al*. When the NSO was given alone, it reduced the number of S. mansoni worms in the liver and decreased the total number of ova deposited in

both the liver and the intestine. When NSO was administered in combination with PZQ, the most prominent effect was a further lowering in the dead ova number over that produced by PZQ alone. Infection of mice with *S. mansoni* produced a pronounced elevation in the serum activity of ALT, GGT, with a slight increase in AP level, while reduce serum albumin level. Administration of NSO succeeded partially to correct the previous changes in ALT, GGT, AP activity, as well as the Alb content in serum. These results suggest that NSO may play a role against the alterations caused by *S. mansoni* infection[34]. Results of *in vitro* testing of *N. sativa* seeds against *Schistosoma mansoni*, *miracidia*, *cercariae*, and adult worms indicate its strong biocidal effects against all stages of the parasite and an inhibitory effect on egg-laying of adult female worms. *N. sativa* seeds also induced an oxidative stress against adult worms which indicated by a decrease in the activities of antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase, and glutathione reductase and enzymes of glucose metabolism, hexokinase and glucose-6-phosphate dehydrogenase. Disturbing of such enzymes of adult worms using *N. sativa* seeds could in turn render the parasite vulnerable to damage by the host and may play a role in the anti-schistosomal potency of the *N. sativa* seed[35]. The antioxidant and anti-schistosomal activities of the garlic extract (AGE) and NSO on normal and Schistosoma mansoni-infected mice was investigated. Result showed that, protection with 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. These results suggested that AGE and NSO may be promising agents to complement schistosomiasis specific treatment[36].

5.4. Antioxidant activity

The antioxidant and antiarthritic activity of TQ in Wistar rat by collagen induced arthritis was evaluated. TQ was administered at a dose of 5 mg/kg body weight once daily for 21 d. The effects of treatment in the rats were assessed by biochemical (articular elastase, myeloperoxdase (MPO), LPO, glutathione (GSH), catalase (CAT), SOD and NO), inflammatory mediators [IL-1β, IL-6, TNF-α, IL-10, IFN-γ and PGE(2)] and histological studies in joints. TQ was effective in bringing significant changes on all the parameters (articular elastase, MPO, LPO, GSH, CAT, SOD and NO) studied. Oral administration of TQ resulted in significantly reduced the levels of pro-inflammatory mediators [IL-1β, IL-6, TNF-α, IFN-γ and PGE (2)] and increased level of IL-10[37]. The antioxidant, anti-inflammatory, anticancer and antibacterial activities of the shoots, roots and seeds methanol extracts from *N. sativa* were studied. The three organs exhibited strong antioxidant activity using the oxygen radical absorbance capacity method and a cell-based assay[38]. TQ has been shown to suppress the Fe-NTA-induced oxidative stress, hyperproliferative response and renal carcinogenesis in Wistar rats[39]. It was suggested that dietary supplementation of black seeds powder inhibits the oxidative stress caused by oxidized corn oil in rats[40]. It was also reported that oral feeding of the diet containing black seed powder at 10% level antagonized the oxidative stress effects induced by hepato-carcinogens like dibutylamine and Sodium Nitrate ($NaNO_3$) in Swiss albino rats by normalizing GSH and NO levels[41]. The black seed oil and TQ by intraperitonial injection were found to shown protective effects on lipid peroxidation process during ischemia-reperfusion injury (IRI) in rat hippocampus[42]. Treating broiler chicks with black seed for 6 weeks prevented the liver from oxidative stress by increasing the activities of enzymes such as myeloperoxidase, glutathione-S-transferase, CAT, adenosine deaminase, myeloperoxidaseand bydecreasing hepatic lipid peroxidation[43]. The crude methanolic extract of black cumin seed cake was found to shown with significant antioxidant properties under *in vitro* systems[44].The modulatory effect of TQ on erythrocyte lipid peroxidation and antioxidant status during 1,2-dimethylhydrazine- (DMH-) induced colon carcinogenesis after initiation in male Wistar rats was investigated and The TQ pre-treatment restored the increased level of malondialdehyde and conjugated diene levels, and an augmentation of enzyme activities like CAT, glutathione peroxidase, and SOD activities due to exposure to DMH. TQ was a useful compound preventing DMH-induced erythrocyte damages[45].

5.5. Antidiabetic activity

The therapeutic potentials of α-lipoic acid (α-LA), L-carnitine, and *N. sativa* or combination of them in carbohydrate and lipid metabolism was evaluated in a Rat model of diabetes which was induced by single *i.p.* injection of streptozocin (STZ) 65 mg/kg. For evaluation of glucose metabolism, fasting blood glucose, insulin, insulin sensitivity, HOMA, C-peptide, and pyruvate dehydrogenase activity were determined. Either α-LA or *N. sativa* significantly reduced the elevated blood glucose level. The combination of 3 compounds significantly increased the level of insulin and C-peptide. Combination of α-LA, L-carnitine and *N. sativa* will contribute significantly in improvement of the carbohydrate metabolism in diabetic rats, thus increasing the rate of success in management of DM[46]. The effects of *N. sativa* aqueous extract and oil, as well as TQ, on serum insulin and glucose concentrations in streptozotocin diabetic rats were studied. Serum insulin and glucose concentrations, SOD levels, and pancreatic tissue malondialdehyde (MDA) were determined. Electron microscopy was used to identify any subcellular changes. Diabetes increased tissue MDA and serum glucose levels and decreased insulin and SOD levels. Treatment of rats with *N. sativa* extract and oil, as well as TQ, significantly decreased the diabetes-induced increases in tissue MDA and serum glucose and significantly increased serum insulin and tissue SOD. Ultrastructurally, TQ ameliorated most of the toxic effects of streptozotocine(STZ), including segregated nucleoli, heterochromatin aggregates (indicating DNA damage), and mitochondrial vacuolization and fragmentation. The aqueous extract of *N. sativa* also reversed these effects of STZ, but to a lesser extent. The *N. sativa* oil restored normal insulin levels, but failed to decrease serum glucose concentrations to normal. The biochemical and ultrastructural findings suggest that *N. sativa* extract and TQ have therapeutic and protect against STZ-diabetes by decreasing oxidative stress, thus preserving pancreatic β-cell integrity. The hypoglycemic effect observed could be due to amelioration of β-cell ultrastructure, thus leading to increased insulin levels. *N. sativa* and TQ may prove clinically useful in the treatment of diabetics and in the protection of β-cells against oxidative stress[47]. The protective effects of the volatile oil of *N. sativa* seeds on insulin immunoreactivity and ultrastructural changes of pancreatic β-cells in STZ-

induced diabetic rats was reported by Kanter *et al*. 2009. STZ was injected intraperitoneally at a single dose of 50 mg/kg to induce diabetes. Increased intensity of staining for insulin, and preservation of β-cell numbers were apparent in the *N. sativa*-treated diabetic rats. The protective effect of *N. sativa* on STZ-diabetic rats was evident by a moderate increase in the lowered secretory vesicles with granules and also slight destruction with loss of cristae within the mitochondria of β-cell when compared to control rats. It is evident that *N. sativa* treatment exerts a therapeutic protective effect in diabetes by decreasing morphological changes and preserving pancreatic β-cell integrity[48]. The antihyperglycemic potential of TQ on the activities of key enzymes of carbohydrate metabolism in streptozotocin (STZ)-nicotinamide (NA)-induced diabetic rats was evaluated. Oral administration of TQ at 20, 40, 80 mg/kg body weight for 45 d, dose dependently improved the glycemic status in STZ-NA induced diabetic rats. The levels of insulin, Hb increased with significant decrease in glucose and HbA (1C) levels. The altered activities of carbohydrate metabolic enzymes were restored to near normal. These results proved that TQ at 80 mg/kg body weight is associated with beneficial changes in hepatic enzyme activities and thereby exerts potential anti-hyperglycemic effects[49]. The *N. sativa* showed the synergistic effect with human parathyroid hormone in improving bone mass, connectivity, biomechanical behavior and strength in insulin-dependent diabetic rats and found to be more effective as compared to the treatment with *N. sativa* or human parathyroid hormone alone[50]. In a clinical study, the adjuvant effect of *N. sativa* oil on various clinical and biochemical parameters of the insulin resistance syndrome were investigated. *N. sativa* oil was found to be effective as an add-on therapy in patients of insulin resistance syndrome. *N. sativa* oil has a significant activity in diabetic and dyslipidemic patients[51]. *N. sativa* is of immense therapeutic benefit in diabetic individuals and those with glucose intolerance as it accentuates glucose-induced secretion of insulin besides having a negative impact on glucose absorption from the intestinal mucosa[52]. The effects of the TQ in STZ-induced diabetes in rats were investigated. The Effect of *N. sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus was investigated in 2010. *N. sativa* seeds were used as an adjuvant therapy in patients with diabetes mellitus type 2 added to their anti-diabetic medications. *N. sativa* at a dose of 2 gm/day caused significant reductions in fasting blood glucose, 2 h postprandially (2 hPG), and glycosylated hemoglobin (HbA1c) without significant change in body weight. The results indicate that a dose of 2 gm/day of black seed might be a beneficial adjuvant to oral hypoglycemic agents in type 2 diabetic patients[53]. The *in vivo* antidiabetic activity of *N. sativa* seed ethanol extract (NSE) was evaluated in diabetic Meriones shawi. Plasma lipid profile, insulin, leptin, and adiponectin levels were assessed. ACC phosphorylation and Glut4 protein content were determined in liver and skeletal muscle. NSE animals showed a progressive normalization of glycaemia. It was also demonstrate that *in vivo* treatment with NSE exerts an insulin-sensitizing action by enhancing ACC phosphorylation, a major component of the insulin-independent AMPK signaling pathway, and by enhancing muscle Glut4 content[54].

5.6. Anticancer activity

In vitro study of TQ to determine whether or not TQ can increase survival and sustain the expression of the homing receptor CD62L in antigen-specific T cells. The results showed that stimulation of OT-1 (transgenic CD+) T cells with OVA antigen resulted in activation, as shown by a decrease in the surface expression of CD62L which coincided with significant apoptosis measured three and five days after antigen stimulation. Addition of low concentrations of TQ during CD85+ T-cell activation resulted in enhanced survival of the activated T cells and sustained expression of CD62L. These effects coincided with enhancement in the capability of CD8+ T cells to produce the effector cytokine interferon-gamma (IFNgamma). This is concluded that TQ has a beneficial effect in conditioning T cells *in vitro* for adoptive T-cell therapy against cancer and infectious disease[55]. The cytotoxic effects of different *N. sativa* seed extracts as an adjuvant therapy to doxorubicin on human MCF-7 breast cancer cells was reported. The study showed *N. sativa* lipid extract is cytotoxic to MCF-7 cells with LC_{50} of 2.720 ± 0.232 mg/mL, while its aqueous extract cytotoxicity exhibited when the applied concentration is high as about 50 mg/mL[56].

The antitumor and anti-angiogenic effects of TQ on osteosarcoma *in vitro* and *in vivo* were investigated. Results showed that TQ induced a higher percentage of growth inhibition and apoptosis in the human osteosarcoma cell line SaOS-2 compared to that of control, and TQ significantly blocked human umbilical vein endothelial cell tube formation in a dose-dependent manner. It was found that TQ significantly downregulated NF-κB DNA-binding activity, XIAP, survivin and VEGF in SaOS-2 cells. Moreover, the expression of cleaved caspase-3 and Smac were upregulated in SaOS-2 cells after treatment with TQ. It was also found that TQ inhibits tumor angiogenesis and tumor growth through suppressing NF-κB and its regulated molecules. It was concluded that TQ effectively inhibits tumor growth and angiogenesis both *in vitro* and *in vivo*. Therefore, inhibition of NF-κB and downstream effector molecules is a possible underlying mechanism of the antitumor and anti-angiogenic activity of TQ in osteosarcoma[57].

The cytotoxicity of TQ in human cervical squamous carcinoma cells (SiHa) was investigated. TQ was cytotoxic towards SiHa cells with IC_{50} values of 10.67±0.12 and 9.33±0.19 μg/mL as determined by MTT assay and trypan blue dye exclusion test, respectively, after 72 h of incubation. TQ was found to be more cytotoxic towards SiHa cells compared to cisplatin. Interestingly, TQ was less cytotoxic towards the normal cells (3T3-L1 and Vero). Cell cycle analysis performed by flowcytometer showed a significant increase in the accumulation of TQ-treated cells at sub-G1 phase, indicating induction of apoptosis by the compound. TQ was more potent than cisplatin in elimination of SiHa cells via apoptosis with down-regulation of Bcl-2 protein[58].

The anticancer effects of TQ on breast cancer cells, and its potential effect on the PPAR-γ activation pathway was investigated and it was found that TQ exerted strong anti-proliferative effect in breast cancer cells and when TQ combined with doxorubicin and 5-fluorouracil, cytotoxicity was found to be increased. TQ was found to increase sub-G1 accumulation and annexin-V positive staining, indicating apoptotic induction. In addition, TQ activated caspases 8, 9 and 7 in a dose-dependent manner. Migration and invasive properties of MDA-MB-231 cells were also reduced in the presence of TQ. Interestingly, TQ was found to increase PPAR-γ activity and down-regulate the expression of the

genes for Bcl-2, Bcl-xL and survivin in breast cancer cells. More importantly, the increase in PPAR-γ activity was prevented in the presence of PPAR-γ specific inhibitor and PPAR-γ dominant negative plasmid, suggesting that TQ may act as a ligand of PPAR-γ. It was observed by using molecular docking analysis that TQ indeed formed interactions with 7 polar residues and 6 non-polar residues within the ligand-binding pocket of PPAR-γ that are reported to be critical for its activity. Thus, it was concluded that TQ may have potential implication in breast cancer prevention and treatment and anti-tumor effect of TQ may also be mediated through modulation of the PPAR-γ activation pathway[59]. It was also revealed in a study of the assessment of the chemo-preventive potential of crude oils in *N. sativa* on tumor formation using a well-established rat multi-organ carcinogenesis model featuring initial treatment with five different carcinogens that post-initiation administration of 1000 or 4000 mg/L *N. sativa* volatile oil in the diet of male Wister rats for 30 weeks significantly reduced malignant and benign colon tumor sizes, incidences and multiplicities. The treatment also significantly decreased the incidences and multiplicities of tumors in the lungs and in different parts of the alimentary canal, particularly the esophagus and fore stomach. It was shown that *N. sativa* administration exerts potent inhibitory effects on rat tumor development and on cellular proliferation in multiple organ sites like colon, lung, esophageal and fore stomach tumors in the post-initiation phase with no evidence of clinical side effects[60]. The potential immuno-modulatory effects of *N. sativa* are investigated in light of splenocyte proliferation, macrophage function, and NK anti-tumor activity using BLAB/c and C57/BL6 primary cells. NK cytotoxic activity against YAC-1 tumor cells was examined by JAM assay. The study demonstrated that the aqueous extract of *N. sativa* significantly enhances splenocyte proliferation in a dose-responsive manner. It was also evident that the aqueous extract of *N. sativa* significantly enhances NK cytotoxic activity against YAC-1 tumor cells[61]. The effect of TQ on pancreatic cancer cells and its effect on MUC4 expression were investigated. The MUC4-expressing pancreatic cancer cells FG/COLO357 and CD18/HPAF were incubated with TQ, and *in vitro* functional assays were also done. The results indicated that treatment with TQ down regulated MUC4 expression through the proteasomal pathway and induced apoptosis in pancreatic cancer cells by the activation of c-Jun NH(2)-terminal kinase and p38 mitogen-activated protein kinase pathways. The decrease in MUC4 expression correlated with an increase in apoptosis, decreased motility, and decreased migration of pancreatic cancer cells. Therefore, it was concluded that TQ has potential for the development of novel therapies against pancreatic cancer[62]. TQ alone was found to possess a weak anticancer constituent of black seeds oil. TQ Derivatives bearing terpene-terminated 6-alkyl residues were tested in cells of human HL-60 leukemia, 518A2 melanoma, multidrug-resistant KB-V1/Vbl cervix, and MCF-7/Topo breast carcinomas, as well as in non-malignant human foreskin fibroblasts. Derivatives with a short four-atom spacer between quinone and cyclic monoterpene moieties were more anti-proliferative than analogues with longer spacers. 6-(Menthoxybutyryl) TQ (3a) exhibited single-digit micromolar IC_{50} (72 h) values in all four cell lines. It was seven times more active than TQ (1) in 518A2 melanoma cells and four times in KB-V1/Vbl cervix carcinoma cells, while only half as toxic in the fibroblasts. Compound 3a was also not a substrate for the P-gp and BCRP drug transporters of the resistant cancer cells. The caryophyllyl and germacryl conjugates 3e and 3f specifically inhibited the growth of the resistant MCF-7 breast carcinoma cells. Conjugation of TQ with the triterpene betulinic acid via the OH group as in 3g led to a loss in activity, while conjugation via the carboxylic acid afforded compound 4 with nanomolar IC_{50} (72 h) activity against HL-60 cells. All anticancer-active derivatives of TQ (1) induced apoptosis associated with DNA laddering, a decrease in mitochondrial membrane potential and a slight increase in reactive oxygen species[63]. In another study, 4-Acylhydrazones and 6-alkyl derivatives of TQ were tested for growth inhibition of human HL-60 leukemia, 518A2 melanoma, KB-V1/Vbl cervix, and MCF-7/Topo breast carcinoma cells. It was revealed that unsaturated side chains conferred greater activities than equally long saturated chains. The number of C==C bonds was less decisive than chain length. The 6-hencosahexaenyl conjugate 3 e was most active in all resistant tumor cells, with IC_{50} (72 h) values as low as 30 nmol/L in MCF-7/Topo cells. The conjugates are likely to operate by mechanisms different from that of TQ. For instance, 3 e induced distinct caspase-independent apoptosis in HL-60 and 518A2 cells concomitant with a loss of mitochondrial membrane potential and a subsequent rise in the levels of reactive oxygen species[64]. The administration of *N. sativa* was found to reduce the carcinogenic effects of DMBA carcinogen in mammary carcinoma which indicated the protective role of *N. sativa* in mammary carcinoma[65]. TQ suppressed the migration and invasion of Panc-1 cells in a dose-dependent manner. It was also found that TQ significantly down-regulates NF-kappa B and MMP-9 in Panc-1 cells. In addition, metastatic model simulating human pancreatic cancer was established by orthotropic implantation of histologically intact pancreatic tumor tissue into the pancreatic wall of nude mice. And administration of TQ significantly reduced tumor metastasis compared to untreated control. Furthermore, the expression of NF-kappa B and MMP-9 in tumor tissues was also suppressed after treatment with TQ. TQ exerts anti-metastatic activity on pancreatic cancer both *in vitro* and *in vivo*, which may be related to down-regulation of NF-kappa B and its regulated molecules such as MMP-9 protein. Consequently, these results provide important insights into TQ as an anti-metastatic agent for the treatment of human pancreatic cancer[66]. The chemo-sensitizing effect of TQ and 5-fluorouracil (5-FU) on gastric cancer cells both *in vitro* and *in vivo* is reported by Lei *et al*. Pre-treatment with TQ significantly increased the apoptotic effects induced by 5-FU in gastric cancer cell lines *in vitro*. TQ also enhanced the 5-FU-induced killing of gastric cancer cells by mediating the down-regulation of the anti-apoptotic protein bcl-2, the up-regulation of the pro-apoptotic protein bax, and the activation of both caspase-3 and caspase-9. And further, the combined treatment of TQ with 5-FU represents a significantly more effective antitumor agent than either agent alone in a xeno-graft tumor mouse model. This study suggested that the TQ/5-FU combined treatment induces apoptosis by enhancing the activation of both caspase-3 and caspase-9 in gastric cancer cells[67].

5.7. Anti-inflammatory and analgesic activity

The aqueous extract of *N. sativa* was found to possess anti-inflammatory and analgesic but not antipyretic

activities in animal models while anti-inflammatory effect of the alcoholic extracts of *N. sativa* seeds and its callus on mix glial cells of rat with regard to their TQ content was investigated. The mix glial cells, inflamed by lipopolysaccharide, were subjected to anti-inflammatory studies in the presence of various amounts of TQ and the alcoholic extracts. Results confirmed that TQ content of the callus of leaf was 12 times higher than that measured in the seeds extract. Studies on the inflamed rat mix glial cells revealed significant reduction in the nitric oxide production in the presence of 0.2 to 1.6 mg/mL of callus extract and 1.25 to 20 μL/mL of the seed extracts[68]. Osteoporosis has been linked to oxidative stress and inflammation. The studies on the anti-osteoporotic effects of *N. sativa* and TQ were carried out. It was revealed that *N. sativa* and TQ were shown to inhibit inflammatory cytokines such as interleukin-1 and 6 and the transcription factor, nuclear factor κB. Both NS and TQ have shown potential as anti-osteoporotic agent[69]. Inflammation has been identified as a significant factor in the development of solid tumour malignancies. Studies show that TQ, induced apoptosis and inhibited proliferation in pancreatic ductal adenocarcinoma (PDA) cells. The anti-inflammatory potential of TQ in PDA cells was evaluated in comparison with that of a specific histone deacetylase (HDAC) inhibitor, trichostatin A. The effect of TQ on the expression of different pro-inflammatory cytokines and chemokines was analyzed by real-time polymerase chain reaction. TQ dose and time-dependently significantly reduced PDA cell synthesis of MCP-1, TNF-alpha, interleukin (IL)-1 β and Cox-2. At 24 h, Tq almost completely abolished the expression of these cytokines. TQ also increased p21 WAF1 expression, inhibited HDAC activity, and induced histone hyperacetylation. HDAC inhibitors have been shown to ameliorate inflammation-associated cancer. TQ as a novel inhibitor of proinflammatory pathways provides a promising strategy that combines anti-inflammatory and proapoptotic modes of action[70]. TQ exhibit a slight inhibitory effect on COX-1 expression and PGE2 production in a mouse model of allergic airway inflammation. This finding suggests that TQ has an anti-inflammatory effect during the allergic response in the lung through the inhibition of PGD_2 synthesis and Th2-driven immune response[71]. The antioxidant, anti-inflammatory, anticancer and antibacterial activities of the shoots, roots and seeds methanol extracts from *N. sativa* were studied. The seeds hexane fraction of the methanol extract showed significant anti-inflammatory activity, inhibiting nitric oxide release with an IC_{50} value of 6.20 μg/mL in lipopolysaccharide-stimulated RAW 264.7 macrophages[72]. A clinical trial study was conducted as prospective and double blind with descriptive analytic to investigate the anti-inflammatory effects of *N. sativa* in patients with allergic rhinitis symptoms. The sample included 66 patients (case and placebo) with allergic rhinitis exposed to *N. sativa* oil. Individual characteristics, including age and sex, and characteristics of the disease, including nasal congestion, runny nose, itchy nose, and sneezing attacks, were evaluated for a period of 30 d The results show that *N. sativa* could reduce the presence of the nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor during the first 2 weeks (day 15). The anti-allergic effects of *N. sativa* components could be attributed to allergic rhinitis. Moreover, *N. sativa* should be considered for treating allergic rhinitis when the effects of other anti-allergic drugs need to be avoided[73].

5.8. Immunomodulatory activity

The potential immunomodulatory effects of *N. sativa* were investigated in light of splenocyte proliferation, macrophage function, and NK anti-tumor activity using BLAB/c and C57/BL6 primary cells. Results demonstrated that the aqueous extract of *N. sativa* significantly enhances splenocyte proliferation in a dose-responsive manner. In addition, the aqueous extract of *N. sativa* favors the secretion of Th2, versus Th1, cytokines by splenocytes. The secretion of IL-6, TNF-α, and NO; key pro-inflammatory mediators, by primary macrophages is significantly suppressed by the aqueous extract of *N. sativa*, indicating that *N. sativa* exerts anti-inflammatory effects *in vitro*. Finally, experimental evidence indicates that the aqueous extract of *N. sativa* significantly enhances NK cytotoxic activity against YAC-1 tumor cells, suggesting that the documented anti-tumor effects of *N. sativa* may be, at least in part, attributed to its ability to serve as a stimulant of NK anti-tumor activity. It was anticipated that *N. sativa* ingredients may be employed as effective therapeutic agents in the regulation of diverse immune reactions implicated in various conditions and diseases such as cancer[74]. A group of medicinal plants including black seed were examined for their immunomodulatory effect in BALB/c mice. Treatment (intraperitoneal injection) with five doses of methanolic extract for Black seed was found to enhance the total white blood cells count [up to 1.2×10^4 cells/mm^3]. Bone marrow cellularity also increased significantly ($P<0.01$) after the administration of the Black seed extract. Spleen weight of the black seed treated groups was significantly increased ($P<0.01$). Two groups of mice were immunosuppressed with cyclophosphamide, the one which pretreated with the black seed extracts significantly ($P<0.01$) restored their resistance against lethal infection with the predominately granulocyte-dependant *Candida albicans*. These results confirmed the immunomodulatory activity of black seed, and may have therapeutical implications in prophylactic treatment of opportunistic infections and as supportive treatment in oncogenic cases[75]. The immunomodulating and cytotoxic properties of volatile oil of *N. sativa* seeds was investigated in a Long-Evans rat model designed to examine the effect of *N. sativa* seeds on selected immune components. Long-Evans rats were challenged with a specific antigen (typhoid TH) and treated with *N. sativa* seeds; Treatment with *N. sativa* oil induced about 2-fold decrease in the antibody production in response to typhoid vaccination as compared to the control rats but there was a significant decrease in splenocytes and neutrophils counts, but a rise in peripheral lymphocytes and monocytes in the these animals. These results indicated that the *N. sativa* seeds could be considered as a potential immunosuppressive cytotoxic agent[62]. Chronic administration of oxytetracycline (OXT) (incorporated at a level of 0.05 g/kg of feed for 50 d) to pigeons, significantly decreased total leukocyte and lymphocyte counts, increased heterophil: lymphocyte ratio and lysosomal enzyme activity, and decreased reticuloendothelial system function compared with controls. Coadministration of black seed at a level of 2.5% with OXT completely blocked the effects elicited by OXT and produced immunostimulant effects in pigeons. The addition of black seed to feed of pigeons could act as an immunoprotective agent when chronic administrations of antibiotics are considered[4]. The effect of TQ was tested on experimental autoimmune encephalomyelitis (EAE)

animal model that mimic human multiple sclerosis. Myelin oligodendrocyte glycoprotein subcutaneously was used to induce chronic relapsing EAE. TQ intraperiotoneally was found to be almost 90% preventive and 50% curative in chronic relapsing EAE due to its antioxidant effect[76]. *N. sativa* oil is a promising natural radioprotective agent against immunosuppressive and oxidative effects of ionizing radiation[6]. Daily oral administration of *N. sativa* oil to rats before whole body gamma irradiation resulted in significant reversal of reduction of hemolysin antibodies titers.Potential immunomodulation effect of the extract of *N. sativa* on ovalbumin sensitized guinea pigs was evaluated. The effect of the extract of *N. sativa* on lung pathology and blood interleukin-4 (IL-4) and interferon-γ (IFN-γ) of sensitized guinea pigs was examined. Treatment of sensitized animals with the extract of *N. sativa* led to a significant decrease in pathological changes of the lung, except for the oedema in the sensitized group treated with low concentration of the extract, but an increased IFN-γ. These results confirm a preventive effect of *N. sativa* extract on lung inflammation of sensitized guinea pigs[77]. To determine the possible alleviating effect of *N. sativa* and TQ on food allergy, ovalbumin (OVA) -sensitized BALB/c-mice were pre-treated either with a hexanic *N. sativa* seed extract TQ and subsequently challenged intra-gastrically with OVA. All 4 treatments significantly decreased clinical scores of OVA-induced diarrhea. *N. sativa* seed extract, TQ decreased intestinal mast cell numbers and plasma mouse mast cell protease-1. It was demonstrated that *N. sativa* seed extract significantly improves symptoms and immune parameters in murine OVA-induced allergic diarrhea; this effect is at least partially mediated by TQ[78].

5.9. Cardiovascular activity

The acute (at 4 and 18 h) effects of diesel exhaust particles (DEP) on cardiopulmonary parameters in mice and the protective effect of TQ were investigated. Mice were given, intratracheally, either saline (control) or DEP (30 μg per mouse). At 18 h (but not 4 h) after giving DEP, there was lung inflammation and loss of lung function. At both 4 and 18 h, DEP caused systemic inflammation characterized by leucocytosis, increased IL-6 concentrations and reduced systolic blood pressure. SOD activity was decreased only at 18 h. DEP reduced platelet numbers and aggravated *in vivo* thrombosis in pial arterioles. *In vitro*, addition of DEP (0.1-1 μg/mL) to untreated blood-induced platelet aggregation. Pretreatment of mice with TQ prevented DEP-induced decrease of systolic blood pressure and leucocytosis, increased IL-6 concentration and decreased plasma SOD activity. TQ also prevented the decrease in platelet numbers and the prothrombotic events but not platelet aggregation *in vitro*[79].

5.10. Gastro-protective activity

The mechanism of gastroprotective effect of TQ was assessed. Animals were injected with vehicle, TQ (10, 20 mg/kg), omeprazole (10, 20 mg/kg) or their combination (10 mg/kg). Thirty minutes later, pyloric ligation was carried out and followed consequently with ischemia for another 30 min, abided by reperfusion for 120 min. The ischemia/reperfusion insult increased the gastric acid secretion, acid output, and pepsin, as well as the gastric mucosal content/activity of lipid peroxide, proton pump and myeloperoxidase, along with ulcer index. However, content/activity of gastric mucin, reduced glutathione, total nitric oxide, and SOD were decreased. TQ, especially the high dose level, corrected the altered parameters in a comparable manner to that of the reference drug used, omeprazole. In addition, when the low doses were combined they add to each other to reach the effect of the high dose of either drug. Besides the antioxidant property, TQ has novel gastroprotective mechanisms via inhibiting proton pump, acid secretion and neutrophil infiltration, while enhancing mucin secretion, and nitric oxide production[80]. The anti-ulcer potential of *N. sativa* aqueous suspension on experimentally induced gastric ulcers and basal gastric secretion in rats was examined to rationalize its use by herbal and Unani medicine practitioners. Acute gastric ulceration was produced by various noxious chemicals (80% ethanol, 0.2 mol/L NaOH, 25% NaCl and indomethacin) in Wistar albino rats. Anti-secretory studies were undertaken in a separate group of rats. Gastric wall mucus contents and non-protein sulfhydryl concentration were estimated, and gastric tissue was examined histopathologically. An aqueous suspension of black seeds significantly prevented gastric ulcer formation induced by necrotizing agents. It also significantly ameliorated the ulcer severity and basal gastric acid secretion in pylorus-ligated Shay rats. Moreover, the suspension significantly replenished the ethanol-induced depleted gastric wall mucus content levels and gastric mucosal non-protein sulfhydryl concentration. The anti-ulcer effect was further confirmed histopathologically. The anti-ulcer effect of *N. sativa* is possibly prostaglandin-mediated and/or through its antioxidant and anti-secretory activities[81]. Both and its constituent, TQ was found to possess Gastro protective activity against gastric mucosal injury induced by ischaemia/reperfusion in rats. Ischaemia/reperfusion (I/R) induced gastric lesion is known to be linked with free radical formation. Male Wistar rats were subjected to I/R and were injected with either NO (2.5 and 5.0 mL/kg, *p.o.*) or TQ (5, 20, 50 and 100 mg/kg, *p.o.*). The results showed that I/R elevated the levels of lipid peroxide and lactate dehydrogenase, while decreased those of reduced GSH and SOD. These biochemical changes were accompanied by an increase in the formation of gastric lesions, which was reduced by either treatment. This indicates that both NSO and TQ possess gastroprotective effect against gastric lesions which may be related to the conservation of the gastric mucosal redox state[82]. *N. sativa* prevents alcohol induced increase in lipid peroxidation (*i.e.* thiobarbituric acid reactive substances) and reduced gastric GSH content, enzyme activities of gastric SOD, GSH-S-Transferase[83]. TQ was found to protect gastric mucosa against the ulcerating effect of alcohol and mitigated most of the biochemical adverse effects induced by alcohol in gastric mucosa, but the effect of TQ was found to be a lesser than black seed whole. Both *N. sativa* and TQ did not affect the CAT activity in gastric tissue[5]. The beneficial effects of NSO on rats with necrotizing enterocolitis (NEC) were studied in newborn Sprague-Dawley rats. NEC was induced by enteral formula feeding, exposure to hypoxia-hyperoxia and cold stress. Pups in the NEC+NSO group were administered NOS at a dose of 2 mL/kg daily by intraperitoneal (*i.p.*) route from the first day until the end of the study. Proximal colon and ileum were excised for histopathologic, apoptosis (TUNEL) and biochemical evaluation, including xanthine oxidase, SOD, GSH peroxidase (GSH-Px), MDA, and MPO activities.

Pups in the NEC+NOS group had better clinical sickness scores and weight gain compared to the NEC group (P<0.05). In the macroscopic assessment, histopathologic and apoptosis evaluation (TUNEL), severity of bowel damage was significantly lower in the NEC+NOS group compared to the NEC group (P<0.05). Tissue GSH-Px and SOD levels were significantly preserved in the NEC+NSO group (P<0.05), whereas, tissue MDA, MPO levels of the NEC+NSO group were significantly lower than those in the NEC group (P<0.05). It is concluded that NSO significantly reduced the severity of intestinal damage in NEC[84]. A study was designed to determine whether treatment with TQ prevents and ameliorates colonic inflammation in a mouse model of inflammatory bowel disease. C57BL/6 murine colitis was induced by the administration of dextran sodium sulfate (DSS) (3% W/V) in the drinking water supplied to the mice for 7 consecutive d. The mice with colitis were treated with 5, 10, or 25 mg/kg TQ orally, and changes in body weight and macroscopic and microscopic colitis scores were examined. In addition, biochemical analyses were conducted. The treatment of mice with TQ prevented and significantly reduced the appearance of diarrhea and body weight loss. These results were associated with amelioration of colitis-related damage, as measured by macroscopic and microscopic colitis scores. In addition, there was a significant reduction in colonic myeloperoxidase activity and malondialdehyde levels and an increase in glutathione levels. These results indicate that TQ administration can prevent and improve murine DSS-induced colitis. These findings suggest that TQ could serve as a potential therapeutic agent for the treatment of patients with inflammatory bowel disease[85].

5.11. Hepato-protective activity

It is reported that *N. sativa* (0.2 mL/kg) intraperitoneally relieves the deleterious effects of ischemia reperfusion injury on liver. Biochemical parameters like the serum aspartate aminotransferase, alanine aminotransferase lactate dehydrogenase levels and total antioxidant capacity (TAC), CAT, total oxidative status (TOS), oxidative stress index (OSI) and MPO were determined in hepatic tissue in rats with hepatic ischemia. Results suggested that *N. sativa* treatment protects the rat liver against hepatic ischemia reperfusion injury[86]. *N. sativa* administration protects hepatic tissue from deleterious effects of toxic metals such as lead, and attenuates hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride[52]. Cadmium (Cd^{++}) causes alteration of the cellular homeostasis and oxidative damage. The protective role of TQ on the hepatotoxicity of Cd^{++} with special reference to its protection against perturbation of nonenzymatic and enzymatic antioxidants was investigated. The effect of TQ pretreatment was examined in post-nuclear supernatant prepared from liver of Swiss albino mice under *in vitro* conditions. $CdCl_2$ treatment (5 mmol/L) resulted in a significant increase in antioxidant enzymatic activities. It also caused a significant (P<0.001) increase in protein carbonyl and reduced glutathione content. Pretreatment with TQ (10 μmol/L) showed a significant protection as manifested by noticed attenuation of protein oxidation and rejuvenation of the depleted antioxidants of cellular fraction. These results strengthen the hypothesis that TQ exerts modulatory influence on the antioxidant defense system on being subjected to toxic insult[87].

5.12. Nephroprotective activity

The nephro-protective effect of vitamin C and *N. sativa* oil was observed against gentamicin (GM) associated nephrotoxicity in rabbits. Serum creatinine, blood urea nitrogen, and antioxidant activity were measured as indicators of nephrotoxicity for all the groups of rabbits. It was revealed that vitamin C and *N. sativa* oil both had nephroprotective effect as they lowered the values of serum creatinine, blood urea nitrogen, and antioxidant activity as compared to GM control group values. When these two antioxidants were given as combination, they proved to have synergistic nephroprotective effect[88]. Recenty, it was observed that there is an inherent lack in regulation of renal organic anion and cation transporters in cisplatin-induced nephrotoxicity. The effect of TQ on alterations in the renal expression of organic anion transporters and organic cation transporters, as well as multidrug resistance-associated proteins in rats treated with cisplatin was reported. Cisplatin-induced MDA and 8-isoprostane increase was found to be markedly reduced in rats treated with TQ. In cisplatin only treated rats, the induced renal injury increased protein levels of the efflux transporters MRP2 and MRP4 while expression of OAT1, OAT3, OCT1 and OCT2 was reduced. In combination TQ- and cisplatin-treated rats, expression of MRP2 and MRP4 proteins was decreased in the kidneys. Conversely, TQ treatment increased levels of OCT1, OCT2, OAT1 and OAT3 and decreased levels of 8-isoprostane and MDA levels in cisplatin-treated rats. This is concluded that TQ synergizes with its nephroprotective effect against cisplatin-induced acute kidney injury in rats[89]. The protective effects of *N. sativa* oil on methotrexate-induced nephrotoxicity were also studied in albino rats and this study revealed the protective effect of Black cumin in the methotrexate-induced nephrotoxcity[90]. The protective effects of *N. sativa* against ischemia-perfusion damage on kidney tissue were examined. TAC, CAT, TOS, OSI, and MPO in kidney tissue and blood were measured. Serum urea and creatinine levels were also determined. Kidney tissue histopathology was also evaluated. *N. sativa* was effective in reducing serum urea and creatinine levels as well as decreasing the tubular necrosis score. *N. sativa* treatment significantly reduced OSI and TOS levels and increased TAC levels in both kidney tissue and blood. Results revealed the protective effect of *N. sativa* against renal I/R injury in rat kidneys[91]. GM induced nephrotoxicity has been shown to involve the generation of oxygen free radicals. Nephrotoxicity was evaluated histopathologically and by measuring concentrations of urea, creatinine and total antioxidant status (TAS) in plasma and reduced GSH and TAS in kidney cortex. The effect of oral treatment of *N. sativa* oil (0.5, 1.0 or 2.0 mL/kg/day for 10 d) on GM (80 mg/kg/day given intramuscularly) induced nephrotoxicity in rats 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* increased TAS in plasma and reduced GSH concentrations in renal cortex and enhanced growth while it did not cause any over toxicity. The results suggest that *N. sativa* may be useful in ameliorating signs of GM nephrotoxicity in rats[92]. TQ supplementation prevents the development of GM-induced acute renal toxicity in rats. TQ was found to prevent the degenerative changes in kidney tissues against GM induced nephrotoxicity. TQ

supplementation resulted in a complete reversal of the GM-induced increase in serum creatinine, blood urea nitrogen, thibarbituric acid reactive substances, total nitrate/nitrite and decrease in GSH, glutathione peroxidase (GPx), CAT and ATP to control values suggesting that TQ prevents GM-induced degenerative changes in kidney tissues[93]. The protective effects of NSO in the 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. NSO protects kidney tissue against oxygen free radicals, preventing renaldysfunction and morphological abnormalities associated with chronic CsA administration[94]. Administration of *N. sativa* with GM intra-peritonealinjection resulted in significantly decreased creatinine, urea, MDA, NO generation and increased SOD and GSH-Px activities when compared with GM group suggesting nephro-protective activity. *N. sativa* acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of GM both in the biochemical and histopathological parameters[95]. *N. sativa* seeds had non-significant effects on biochemical parameters in Cisplatin-induced nephrotoxicity, although the histo-pathologic properties of the kidneys relatively recovered after *N. sativa* use[96].

5.13. Pulmonary-protective activity and anti-asthmatic effects

Wienkotter *et al*., reported the effect of nigellone and TQ on trachea (antispasmodic effect) and their influence on respiratory clearance. The effects on Ba^{++} carbachol- and leukotriene-induced trachea contractions and the transport of the fluorescence dye rhodamin B concerning ciliary action in the tracheal area were investigated using a micro dialysis technique. Nigellone and high concentrations of TQ had a concentration-dependent inhibitory effect on the trachea when being contracted by the depolarizing effect of Ba^{2+}. The trachea contractions induced by leukotriene-d (4) LT4 were inhibited by nigellone and by TQ. It was concluded that nigellone possesses an antispasmodic effect and an increase in mucociliary clearance but TQ do not have such effects. Therefore, it is suggested that nigellone but not TQ may be useful in treatment of different respiratory diseases[97]. The relaxant effects of four cumulative concentrations of n-hexane, dichloromethane, methanol and aqueous fractions of *N. sativa* (0.8, 1.2, 1.6 and 2.0 g%) in comparison with saline as negative control and four cumulative concentrations of theophylline (0.2, 0.4, 0.6 and 0.8 mmol/L) were examined by their relaxant effects on precontracted tracheal chains of guinea pig by 60 mmol/L KCl (group 1) and 10 microM methacholine (group 2). The results showed relaxant effect of most fractions from *N. sativa* on tracheal chains of guinea pigs which was more potent for methanol and dichloromethane fractions[98]. The protective effect of *N. sativa* on tracheal responsiveness (TR) and lung inflammation of sulfur mustard gas exposed guinea pigs was examined. Guinea pigs were exposed to diluent's solution (ethanol, control group), 100 mg/m^3 inhaled sulfur mustard (SME group), and SME treated with *N. sativa*, 0.08 g daily (SME+N), n=6 for each group. TR to methacholine, total white blood cell count of lung lavage, and differential white blood cell were done 14 d post exposure. The results showed a preventive effect of *N. sativa* on TR of sulfur mustard gas-exposed guinea pigs[99]. The possible beneficial effects of the seeds of *N. sativa* L. on experimental lung injury in male Wistar rats after pulmonary aspiration of different materials was investigated. Results showed that *N. sativa* treatment inhibits the inflammatory pulmonary responses, reducing significantly (P<0.05) peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudate, alveolar macrophages, interstitial fibrosis, granuloma and necrosis formation in different pulmonary aspiration models. Data 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 *N. sativa* therapy. It was concluded that *N. sativa* treatment might be beneficial in lung injury and have potential clinical use[100]. The beneficial effects of NSO on rats with hyperoxia-induced lung injury were evaluated since oxygen-induced lung injury is believed to lead to the development of broncho-pulmonary dysplasia in premature infants. NSO significantly reduced the severity of lung damage due to hyperoxia[101]. The prophylactic effect of boiled extract of *N. sativa* on asthmatic disease was examined. Twenty-nine asthmatic adults were randomly divided into control group (14 patients) and study group (15 patients), and they were studied for 3 months. In the study group 15 mL/kg of 0.1 g% boiled extract and in the control group a placebo solution was administrated daily throughout the study. Asthma symptom score, asthma severity, frequency of symptoms/week and wheezing were recorded in the beginning (first visit), 45 d after treatment (second visit), and at the end of the study (third visit). Pulmonary function tests (PFTs) were also measured, and the drug regimen of the patients was evaluated at three different visits. All asthma symptoms, frequency of asthma symptoms/week, chest wheezing, and PFT values in the study group significantly improved in the second and third visits compared with the first visit (P<0.05 to P<0.001). In addition, further improvement of chest wheezing and severity of disease on the third visit were observed compared with the second visit in this group (P<0.05 for both cases). In the third visit all symptoms in the study group were significantly different from those of the control group (P<0.01 to P<0.001). However, in the control group, there were only small improvements in some parameters in just the second visit. The usage of inhaler and oral β-agonists, oral corticosteroid, oral theophylline and even inhaler corticosteroid in the study group decreased at the end of the study while there were no obvious changes in usage of the drugs in control subjects. The results of phase I study generally suggested a prophylactic effect of *N. sativa* on asthma disease[102]. The anti-asthmatic (bronchodilatory) effect of the boiled extract of *N. sativa* in the airways of asthmatic patients was examined. The bronchodilatory effects of 50 and 100 mg/kg of boiled extract in comparison with 6 mg/kg theophylline were studied on 15 asthmatic patients. PFTs including forced expiratory volume in one second, peak expiratory flow, maximal mid expiratory flow (MMEF), maximal expiratory flow at 75%, 50% and 25% of the FVC [MEF(75), MEF(50), and MEF(25,) respectively] and specific airway conductance (sGaw) were measured before administration and repeated 30, 60, 90, 120, 150 and 180 min after administration of the oral extract and theophylline. The results showed that the extract caused significant increase in all measured PFTs, in most time intervals, (P<0.05 to P<0.001). However, the increase in forced expiratory volume in one second, MMEF and MEF (50) due to both doses of boiled extract and increase in MEF

(75) and MEF (25) due to its lower doses were significantly lower than those of theophylline (P<0.05 to P<0.001). The onset of brochodilatory effect of extract was similar to that of theophylline beginning 30 min, and the effect of extract decline after 150 min following administration similar to the effect of theophylline. The effect of both doses of the extract was also significantly less than that of salbutamol at 30 minutes post administration (P<0.001 for all cases). The results of the this study showed that *N. sativa* has a relatively potent antiasthmatic effect on asthmatic airways. However, the effects of boiled extract of this plant on most measured PFTs were less than those of theophylline at concentrations used[8].

5.14. Testicular-protective activity

The protective role of TQ on testicular toxicity of methotrexate on male C57BL/6 mice (6 weeks old, 20±2 g) was investigated. TQ treatment decreased TAC and prevented the increasein the myeloperoxidase activity. Light microscopy showed in mice that receiving methotrexate resulted in interstitial space dilatation, edema, severe disruption of the seminiferous epithelium and reduced diameter of the seminiferous tubules. Administration of TQ reversed histological changes of methotrexate significantly. It was suggested that TQ use may decrease the destructive effects of methotrexate on testicular tissue of patients using this agent[103].

5.15. Neuro-pharmacolgical activities

The aqueous and methanol extracts of defatted *N. sativa* L. seeds were shown to possess a potent central nervous system and analgesic activities, especially depressant action in the case of the methanolic extract[104]. An anxiolytic drug acts by increasing the 5-HT and decreasing the 5-HIAA (hydroxyindole acetic acid) levels in brain. A long term administration of *N. sativa* increases 5-HT levels in brain and improves learning and memory in rats[105]. Repeated administration of *N. sativa* decreases 5-HT turnover and produces anxiolytic effects in rats. The aqueous and methanol extracts of *N. sativa* L. seeds were evaluated for their effects on the central nervous system and NSO was used to study its effect on anxiety in rats. Open field and elevated plus maze models were selected for the evaluation of anxiolytic effect of drug. After four weeks of daily administration of drug, the rats exhibited an increase in open field activity. The drug also produced anti-anxiety effect in rats when tested in elevated plus maze. The oral administration of NSO increased brain levels of 5-HT (Serotonin), but the levels of brain 5-HIAA (hydroxyindole acetic acid) decreased significantly. Brain and plasma levels of tryptophan also increased significantly following oral repeated administration of NSO. Therefore, it may be suggested that NSO is a useful choice for the treatment of anxiety[106]. TQ produced antianxiety-like effects in mice through modulation of GABA and NO levels. The role of GABAergic and nitriergic modulation in the antianxiety effect of TQ was investigated in mice under unstressed and stressed conditions. TQ (10 and 20 mg/kg), methylene blue (1 mg/kg) and diazepam (2 mg/kg) were administered followed by behavioral testing using an elevated plus maze, the light/dark test and the social interaction test in both unstressed and stressed mice (mice subjected to 6 h immobilization). The effects of the above-mentioned drugs on plasma nitrite, a stable metabolite of nitric oxide and brain GABA content were also studied. TQ (10 and 20 mg/kg) produced significant antianxiety effects in unstressed mice without altering nitrite levels, but only the higher dose (20 mg/kg) of TQ increased the GABA content in unstressed mice. In stressed mice, TQ (20 mg/kg) showed anxiolytic effects, with a significant decrease in plasma nitrite and reversal of the decreased brain GABA content. Pre-treatment with methylene blue enhanced the antianxiety effect of TQ in both unstressed and stressed mice. Therefore, this is indicated an involvement of NO-cGMP and GABAergic pathways in the anxiolytic-like activity of TQ[107]. In 2011, Abdel-Zaher *et al.* reported that NSO can protect against tramadol-induced tolerance and dependence in mice. They found that repeated administration of NSO (4 mL/kg, *p.o.*) along with tramadol (50 mg/kg, *s.c.*) inhibited the development of tramadol tolerance and dependence as measured by the hot plate test and naloxone (5 mg/kg, *i.p.*)-precipitated withdrawal manifestations respectively. Concomitantly, nitric oxide overproduction and increase in brain malondialdehyde level induced by repeated administration of tramadol to mice or by administration of naloxone to tramadol-dependent mice were inhibited by co-administration of the black seed oil. Also, the decrease in brain intracellular reduced glutathione level and glutathione peroxidase activity induced by both treatments was inhibited by co-administration of the oil. The increase in brain glutamate level induced by both treatments was not inhibited by concurrent administration of the oil. The inhibitory effect of *N. sativa* oil on tramadol-induced tolerance and dependence was enhanced by concurrent intraperitonial (*i.p.*) administration of the NMDA receptor antagonist, dizocilpine (0.25mg/kg). Also, the inhibitory effect of the oil on naloxone-induced biochemical alterations in tramadol-dependent mice was enhanced by concurrent administration of dizocilpine. Similarly, concurrent i.p. administration of the NO synthase inhibitor, L-N (G)-nitroarginine methyl ester (10mg/kg) or the antioxidant, N-acetylcysteine (50mg/kg) enhanced these inhibitory effects of *N. sativa* oil. On the other hand, these effects were antagonized by concurrent i.p. administration of the NO precursor, L-arginine (300 mg/kg). These results provide evidence that *N. sativa* oil appears to have a therapeutic potential in tramadol tolerance and dependence through blockade of NO overproduction and oxidative stress induced by the drug[7]. Neuroprotective effects of Aqueous and hydroalcoholic extracts of *N. sativa* (400 mg/kg, orally) were evaluated for their neuroprotective effects on middle cerebral artery occluded (MCAO) rats. Locomotor activity and grip strength of animals were improved in both aqueous and hydroalcoholic extracts pretreated rats. Infarct volume was also reduced in both extracts pretreated rats as compared with MCAO rats. An elevation of thiobarbituric acid reactive substance and a reduction in glutathione and antioxidant enzymes, *viz.*, SOD and CAT levels were observed following MCAO. Pretreatment of *N. sativa* extracts showed the reduction in TBARS, elevation in glutathione, SOD and CAT levels as compared with MCAO rats. The neuroprotective effects of both the extracts of *N. sativa* in cerebral ischemia were observed. The neuroprotective effects could be due to its antioxidant, free radical scavenging, and anti-inflammatory properties[108].

5.16. Anticonvulsant activity

The antioxidant effects of curcumin, NSO and valproate

on the levels of malondialdehyde, nitric oxide, reduced glutathione and the activities of CAT, Na^+, K^+-ATPase and acetylcholinesterase in the hippocampus of pilocarpine-induced animal model of epilepsy was evaluated and left for 22 d to establish the chronic phase of epilepsy. The animals were then treated with curcumin, NSO or valproate for 21 d. Treatment with curcumin, NSO or valproate ameliorated most of the changes induced by pilocarpine and restored Na^+, K^+-ATPase activity in the hippocampus to control levels. Results indicated the anticonvulsant and potent antioxidant effects of curcumin and NSO in reducing oxidative stress, excitability and the induction of seizures in epileptic animals and improving some of the adverse effects of antiepileptic drugs[109]. The antiepileptic effects of aqueous extract, fixed oil, volatile oil of *N. sativa* seeds and its major constituents *i.e.* TQ, α-pinene and p-cymene against PTZ and maximal electroshock (MES)-induced convulsions were investigated. The potential of these constituents to induce minimal neurological deficit (MND) was also evaluated by using chimney test. All of the *N. sativa* seed constituents protected mice effectively against PTZ-induced convulsions except fixed oil. The antiepileptic activity of the volatile oil in this model maybe attributed mainly to its content of TQ and p-cymene and to a lesser extent, α-pinene. Volatile oil and its component p-cymene effectively suppressed convulsions induced by MES. All of the *N. sativa* seed constituents induced varying degrees of MND in the chimney test. MND induced by volatile oil may pertain to its contents of TQ (63%), p-cymene (23%) and α-pinene (<14%). Exploration on the role of receptors suggests that picrotoxin and bicuculline-sensitive GABA receptors, most probably GABAA receptors, mediate an increase in GABAergic response. In the part dealing with the interaction of valproate with TQ, it can be mentioned that TQ increased the potency of valproate in both PTZ and MES models[110]. The antiepileptic effect of curcumin and *N. sativa* oil in the pilocarpine model of epilepsy in comparison with valproate was evaluated by Noor *et al*. Epilepsy was induced by *i.p.* injection of pilocarpine, and the animals were left for 22 d to establish spontaneous recurrent seizures. They were then treated with curcumin, NSO or valproate for 21 d. Pilocarpine induced a significant increase in hippocampal aspartate and a significant decrease in glycine and taurine levels. In the cortex, a significant increase in aspartate, glutamate, GABA, glycine, and taurine levels was obtained after pilocarpine injection. Treatment of pilocarpinized rats with curcumin and valproate ameliorated most of the changes in amino acid concentrations and reduced the histopathological abnormalities induced by pilocarpine, while *N. sativa* oil failed to improve the pilocarpine-induced abnormalities[111].

5.17. Contraceptive and anti-fertility activity

Oral administration of Hexane extract of *N. sativa* seeds L. prevented pregnancy in Sprague-Dawley rats at a dose of 2 g/kg daily on day's 1-10 postcoitum. While column fractions and sub-fractions of Hexane extract of *N. sativa* seeds also showed significant anti-fertility activity. At contraceptive dose, the active hexane extract exhibited only mild uterotrophic activity comparable almost to 0.002 mg/kg dose of 17 varies; is directly proportional to-Ethinylestradiol, but was devoid of any estrogenicity in the immature rat bioassay[112]. The ethanolic extract of *N. sativa* seeds was found to possess an anti-fertility activity in male rats which might be due to inherent estrogenic activity of *N. sativa*[113].

5.18. Antioxytocic activity

The antioxytocic properties of *N. sativa* were reported in some preliminary studies. *N. sativa* seeds inhibit the uterine smooth muscle contraction induced by oxytocin stimulation. The volatile oil of *N. sativa* seeds inhibited the spontaneous movements of rat and guinea piguterine smooth muscle and also the contractions induced by oxytocin stimulation which suggest the anti-oxytocic potential of *N. sativa* seeds oil[114].

5.19. Toxicological studies

Many toxicological studies have been carried out on *N. sativa* seeds. It has been shown that no toxic effects were reported when *N. sativa* fixed oil was given to mice via the stomach in an acute study. In a chronic toxicity study rats treated daily with an oral dose for 3 months caused no changes in key hepatic enzyme levels particularly aspartate-aminotransferase, alanine-aminotranferase, and gammaglutamyl-transferase. Moreover, the histopathological results also showed to be normal for the tissues of heart, liver, kidneys and pancreas LD_{50} values of fixed oil of *N. sativa* obtained by single doses orally and intraperitoneally in mice, were reported to be 26.2-31.6 and 1.86-2.26, respectively. The low toxicity of *N. sativa* fixed oil, evidenced by high LD_{50} values, key hepatic enzyme stability and organ integrity, suggests a wide margin of safety for therapeutic doses of *N. sativa* fixed oil[115]. In another study, the LD_{50} of TQ was found to be 104.7 mg/kg (89.7-119.7) and 870.9 mg/kg (647.1-1 094.8) after intra-peritoneal injection and oral ingestion respectively. Whereas, LD_{50} in rats was found to be 57.5 mg/kg (45.6-69.4) and 794.3 mg/kg (469.8-1 118.8) after intra-peritoneal injection and oral ingestion respectively. The LD_{50} values presented here after intra-peritoneal injection and oral gavages are 10-15 times and 100-150 times greater than doses of TQ reported for its anti-inflammatory, anti-oxidant and anti-cancer effects. These observations revealed that TQ is a relatively safe compound, particularly when given orally to experimental animals[10,116].

5.20. Drugs-nigella interaction

There is a possibility that *N. sativa* may interact with co-administered drugs and affect their intestinal availability and pharmacological effect. *In vitro* studies have shown that *N. sativa* extracts inhibit cDNA-expressed human cytochrome P-450 3A4, 2C9, 3A5 and 3A7-mediated metabolism of marker substrates therefore may affect and/or inhibit the metabolism of a wide range of drugs (117). Further, the effect of *N. sativa* on bioavailability of amoxicillin was investigated in everted rat intestinal sacs. The *in vitro* studies both with methanol and hexane extracts of *Nigella* increased the permeation of amoxicillin significantly ($P<0.001$) as compared to control. Permeation was also found to be significantly higher for the hexane extract ($P<0.001$) in comparison to methanol extract at the same dose levels. *in vivo* experiments revealed that Cmax of amoxicillin in rat plasma when administered orally alone and in combination with hexane extract increased correspondingly from 4 138.251±156.930 to 5 995.045±196.280 ng/mL while as AUC $0\rightarrow t$ increased from 8 890.40±143.33 to 13 483.46±152.45 ng/mL/h. *Nigella* enhanced amoxicillin availability in both *in vivo* and *in vitro* studies[117].

6. Conclusion

The use of herbal drugs as complementary medicine is prevalent and gaining world wide popularity. Many drugs are derived directly from plants; while the others are chemically modified natural products. The original research articles published so far have confirmed the pharmacological potential of *N. sativa* seeds, its oil and extracts and some of its active principles, particularly TQ and alpha-hederin, possess remarkable *in vitro* and *in vivo* pharmacological activities against a large variety of diseases and found to be relatively safe.

7. Future perspectives

Further investigations are required to study the mechanism of actions of *N. sativa* seeds and its constituents by which they exert their therapeutic effects. Chemical modifications in the molecular structure of TQ, alpha-hederin and other constituents of *N. sativa* seeds could lead to more effective and safer drugs for the treatment of wide variety of diseases in the future. *N. sativa* seeds, its oil, constituents of *N. sativa* seeds like TQ, alpha-hederin or others could be used in suitable combinations with existing chemotherapeutic agents for an effective treatment of many infectious diseases and to overcome the resistance problem. Moreover, further researches should focus and explore the specific cellular and molecular targets of various constituents of *N. sativa*, particularly TQ. This review article is dedicated to all those researchers who are interested in focussing their research on this miracle herb and hope, this review article would help them in investigating and conducting further preclinical and clinical studies on the use of *N. sativa* for the treatment of variety of diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The seeds of *N. sativa* Linn are used in various traditional systems of medicines and folk medicine all over the world for the treatment and prevention of a variety of diseases. This article contains wide spectrum researches and authors well done to collect and compile current research data. The article is well written which includes current researches from all over the world. The scientific data in this article will help the researchers to get updated information about *N. sativa*.

Research frontiers

A detailed literature survey on *N. sativa* has been collected, nicely compiled and presented by the authors and Many researches indicated the mechanism of action, chemicals responsible for the medicinal uses of *N. sativa* TQ, some of its analogues and alpha hedrine are the major chemical constituents are responsible for the therapeutic potential actions of black seeds.

Related reports

Many researchers focus on *Nigella* due to its miraculous power of healing. There are tremendous researches on *N. sativa* are being carried out all over the world & continuously published. One can found many research articles in different peer reviewed journals. This review article is a good attempt to compilation researches in the recent past.

Innovations and breakthroughs

A review on therapeutic potential of *N. sativa*: A miracle herb included a wide range of therapeutic potential of this medicinal plant and well supported by scientific documents Data regarding the medicinal uses, chemical constituents and pharmacological actions. This article is well organized and presented in scientific manner. Most importantly, this article included recent advances on the topic.

Applications

It is highly recommended to publish this article since it explains various new discoveries on mechanism of action, therapeutic potential about this miracle herb and let the world community knows about the scientific facts of this medicinal plant.

Peer review

The current review on *Nigella* provide the detailed scientific information of this medicinal plant. Due to its miraculous power of healing *N. sativa*, It has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders. Extensive research studies on *N. sativa* have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which include antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastroprotective and antioxidant properties.

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