Journal of Ethnopharmacology 190 (2016) 372–386

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Review

Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone: A review

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ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form
26 June 2016
Accepted 26 June 2016
Available online 27 June 2016

Keywords:
Nigella sativa
Thymoquinone
Clinical effects
Antidiabetic
Antitumor
Hepatoprotective
Cardioprotective
Gastroprotective
Pulmonary protective
Neuroprotective

Chemical compounds studied in this article:
Thymoquinone (PubChem CID: 10281)
dithymoquinone or nigellone (PubChem CID: 398941)
thymol (PubChem CID: 6989)
carvacrol (PubChem CID: 10364)
p-cymene (PubChem CID: 7463)
4-terpineol (PubChem CID: 11230)
trans-anethol (PubChem CID: 637563)
alpha-pinene (PubChem CID: 6654)

ABSTRACT

Ethnopharmacological relevance: *Nigella sativa* (*N. sativa*) *L.* (Ranunculaceae), well known as black cumin, has been used as a herbal medicine that has a rich historical background. It has been traditionally and clinically used in the treatment of several diseases. Many reviews have investigated this valuable plant, but none of them focused on its clinical effects. Therefore, the aim of the present review is to provide a comprehensive report of clinical studies on *N. sativa* and some of its constituents.

Materials and methods: Studies on the clinical effects of *N. sativa* and its main constituent, thymoquinone, which were published between 1979 and 2015, were searched using various databases.

Results and discussion: During the last three decades, several in vivo and in vitro animal studies revealed the pharmacological properties of the plant, including its antioxidant, antibacterial, antiproliferative, proapoptotic, anti-inflammatory, and antiepileptic properties, and its effect on improvement in atherogenesis, endothelial dysfunction, glucose metabolism, lipid profile dysfunction, and prevention of hippocampus pyramidal cell loss. In clinical studies, antimicrobial, antioxidant, anti-inflammatory, antitumor, and antidiabetic properties as well as therapeutic effects on metabolic syndrome, and gastrointestinal, neuronal, cardiovascular, respiratory, urinary, and reproductive disorders were found in *N. sativa* and its constituents.

Conclusion: Extensive basic and clinical studies on *N. sativa* seed powder, oil, extracts (aqueous, ethanolic, and methanolic), and thymoquinone showed valuable therapeutic effects on different disorders with a wide range of safe doses. However, there were some confounding factors in the reviewed clinical trials, and a few of them presented data about the phytochemical composition of the plant. Therefore, a more standard clinical trial with *N. sativa* supplementation is needed for the plant to be used as an inexpensive potential biological adjuvant therapy.

Abbreviations: *N. sativa*, *Nigella sativa*; TQ, thymoquinone; NF-κB, nuclear factor-κB; STAT3, signal transducer and activator of transcription 3; MAPK, mitogen-activated protein kinases; PPAR-γ, peroxisome proliferator-activated receptor gamma; AKt, protein kinase B; LTC4, leukotriene C4; EAE, encephalomyelitis; NSPN, *N. sativa* and *Phyllanthus niruri*; DAS, disease activity score; ACR20, American College of Rheumatology 20%; EULAR, European League Against Rheumatism; RCT, randomized controlled trial; CD, cluster of differentiation; NK, natural killer; PBMC, peripheral blood mononuclear cells; MLC, mixed lymphocyte cultures; PWM, pokeweed mitogen; IL, interleukin; TNF-α, tumor necrosis factor alpha; IgE, Immunoglobulin E; HDL, high density lipoprotein; ACTH, adrenocorticotropic hormone; HECSI, Eczema Severity Index; DLQI, Dermatology Life Quality Index; MRSA, resistant *Staphylococcus aureus*; MIC, minimum inhibitory concentration; HCV, hepatitis C virus; MCF-7, human breast adenocarcinoma cell line; ACHN, human renal adenocarcinoma; GP-293 cell, normal renal epithelial; Bcl-2, B-cell lymphoma 2; BAL, bronchoalveolar lavage; LC, Lethal Concentration; ACHN, human renal adenocarcinoma; GP-293 cell, normal renal epithelial; SaOS-2, human osteosarcoma cell line; HUVEC, human umbilical vein endothelial cell; AMPK, adenosine monophosphate-activated protein kinase; LDL-C, low-density lipoprotein cholesterol; FBS, fasting blood glucose; 2hPG, 2-hour postprandial blood glucose; HbAlc, glycosylated hemoglobin; reactive ROS, oxygen species; TAC, total antioxidant capacity; SOD, superoxide dismutase; CAT, catalase; TBARS, thiobarbituric acid reactive substances; BMI, body mass index; GC–MS, gas-chromatography–mass spectrometry; TG, triglyceride; TC, total cholesterol; CYP, cytochrome P; DEX, dextromethorphan; DOR, dextrorphan; MM, methoxymorphinan; HPLC, High Performance Liquid Chromatography; MR, metabolic ratios; ALL, acute lymphoblastic leukemia; ALT, alanine transaminase; AST, aspartate transaminase; SBP, systolic blood pressure; DBP, diastolic blood pressure; PFT, pulmonary function test; FEV1, volume in one second; PEF, peak expiratory flow, MMEF, maximal mid expiratory flow;; MEF, maximal expiratory flow; sGaw, specific airway conductance; Th, T helper; ACT, asthma control test; foxp3, factor forkhead box P3; Treg, Regulatory T; PEFR, peak expiratory flow rate; PI, pulmonary index

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http://dx.doi.org/10.1016/j.jep.2016.06.061
0378-8741/

alpha-hederin (PubChem CID: 71464054)
kaempferol glucoside (PubChem CID: 12358425)

Contents

1. Introduction

1.1. Botanical, historical, and traditional characteristics

Nigella sativa (*N. sativa*) L. (Ranunculaceae) is an annual flowering plant, which is native to South and Southwest Asia and is cultivated and used in different parts of the world, such as the Mediterranean countries, southern Europe, and North Africa (Polat et al., 2011; Tembhurne et al., 2011). It is an annual grassy plant with green- to blue-colored flowers and black trigonal seeds. The seeds are the source of the active ingredients of the plants (El-Tahir and Bakeet, 2006). This plant is known all over the world by different common (folkloric) names, such as Habbat al-barakah in Arabic, Siah-Daneh in Persian, and black cumin or black seed in English. Based on historical records, this plant was known as far back as 1400 years ago and its seeds were extensively used for flavor (Zohary et al., 2012). In Unani traditional medicine, *N. sativa* is considered as a herbal medicine for a number of diseases, and in Islamic medicine, it has been named as a cure for all diseases except death and aging by a Hadith of the Holy Prophet Muhammad P.B.U.H (Tembhurne et al., 2011). The therapeutic use of *N. sativa* has been recommended not only in Islam but in the Bible and other religious sources as well (Chevallier, 1996).

N. sativa, as a herbal medicine with a rich historical background, has been traditionally used in the treatment of several diseases, including infertility, fever, cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, paralysis, hemiplegia, back pain, dysmenorrhea, obesity, diabetes, infection and inflammation, rheumatism, hypertension, and gastrointestinal disorders such as flatulence, dyspepsia, diarrhea, and dysentery (Ave-Sina, 1990; Durmuşkahya and Öztürk, 2013; Nasir et al., 2014). In addition, *N. sativa* oil has been used as an ointment for relief from abscesses, nasal ulcers, orchitis, eczema, and swollen joints. *N. sativa* in combination with honey has also been traditionally used to treat respiratory disorders such as asthma, bronchospasm, and chest congestion (Ave-Sina, 1990; Nasir et al., 2014).

1.2. Chemical composition of the seeds

The chemical composition of *N. sativa* seeds was reported for the first time in 1880 (Greenish, 1880), which were composed of oils, proteins, carbohydrates, fibers, ashes, moisturizers, etc. The oil component of *N. sativa* (36–38%) (Al-Jassir, 1992; Houghton et al., 1995) mostly consisted of linoleic (50–60%), oleic (20–23.4%), palmitic (12.5%), dihomolinoleic (10%), and eicosadienoic (3%) acids as well as arachidonic, stearic, and myristic acids; beta-sitosterol; cycloeucalenol; cycloartenol; sterol esters; and sterol glucosides (Al-Jassir, 1992; Ali and Blunden, 2003; Nickavar et al., 2003; Matthau and Ozcan, 2011) with some other minor lipid constituents such as methylnonadeca-15, 17-dienoate, pentyl hexadec-12-enoate, and pentyl pentadec-11-enoate (Nickavar et al., 2003). Their multipurpose preventive and relieving effects have been attributed to prominent constituents such as nigellicine, nigellidine, thymoquinone (TQ), dithymoquinone, thymol, and carvacrol (Ahmad et al., 2013). Many other active compounds have also been isolated and identified in different *N. sativa* varieties. The essential oil of the plant contains various pharmacologically active constituents, such as TQ (30–48%) (Fig. 1a), thymol (Fig. 1b), thymohydroquinone (Fig. 1c), dithymoquinone, p-cymene (7–15%), carvacrol (6–12%), sesquiterpene longifolene (1–8%), 4-terpineol (2–7%), *t*-anethol (1–4%), and α -pinene (Houghton et al., 1995; Ahmad et al., 2013). The seeds of the plant also contain many non-oily and non-caloric components in trace amounts, including pyrazole alkaloids (nigellidine and nigellicine, Fig. 1d), isoquinoline alkaloids (nigellicimine and nigellicimine-N-oxide, Fig. 1e), alpha-hederin (a water-soluble pentacyclic triterpene, Fig. 1f), saponin (a potential anticancer agent), vitamins (riboflavin, thiamin, niacin, pyridoxine, folic acid, and vitamin E), and minerals (potassium, sodium, calcium, phosphorus, magnesium, copper, and

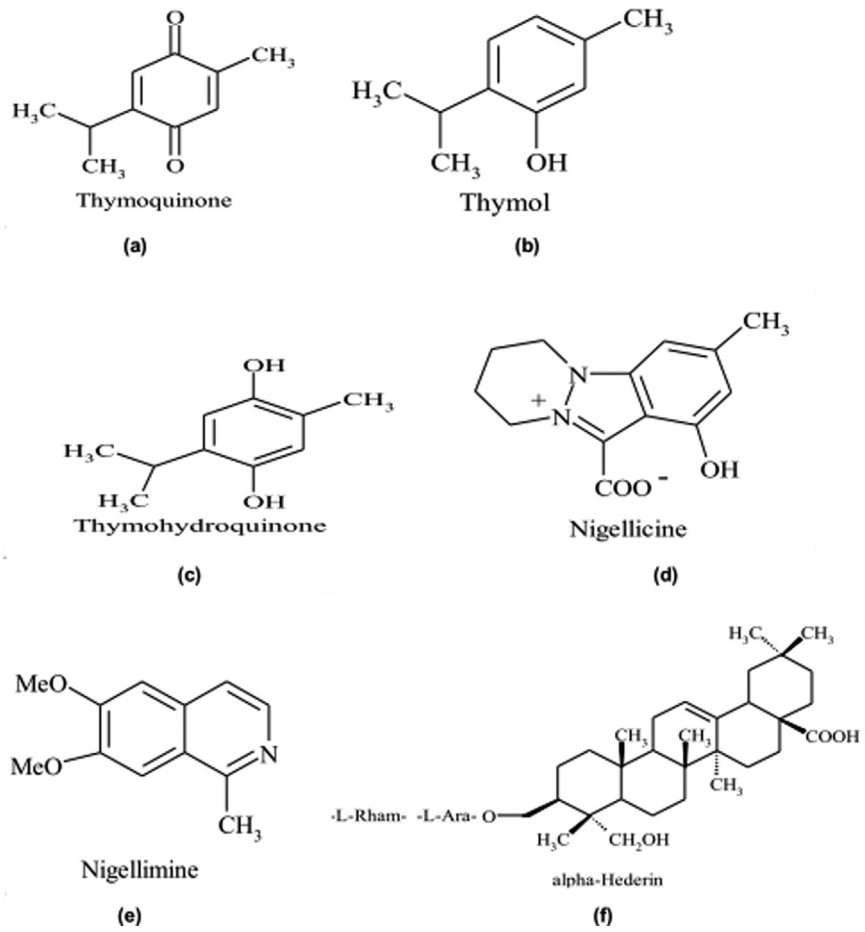

Fig. 1. Chemical structure of active ingredient of *N. sativa* essential oil.

iron) (Nergiz and Ötleş, 1993). However, the phytochemical composition of the *N. sativa* extract or oil has been determined and formulated in only a few clinical studies. In addition, free fatty acid or volatile oil contents of the plant were found in some of them (Boskabady et al., 2007, 2010; Dehkordi and Kamkhah, 2008; Fallah Huseini et al., 2013; Heshmati et al., 2015; Kolahdooz et al., 2014).

2. Methods

Databases such as PubMed, Science Direct, Scopus, and Google Scholar were searched for the terms of *N. sativa*, its different constituents, clinical effects, and different disorders between the years 1979 and 2015 to prepare this review. For validating the plant's scientific name, Plantlist.org and international plant name index databases were used.

3. Biological activities and pharmacological properties (results)

Various studies have shown the role of this plant in the treatment of a wide spectrum of diseases, including asthma, diarrhea, headache, toothache, nasal congestion, and several types of cancer (Ali and Blunden, 2003; Salem, 2005). The seeds of *N. sativa* have proven to have antidiabetic, anticancer, anti-inflammatory, immunomodulatory, antioxidant, antimicrobial, analgesic, spasmolytic, bronchodilatory, and hepatoprotective properties, as well as therapeutic effects on renal, gastrointestinal, neurological, and cardiovascular disorders (Ahmad et al., 2013). Many basic and clinical studies have revealed that the extract of *N. sativa* seeds and its constituents could be used to suppress coughs (Boskabady et al., 2003; Mahfouz and El-Dakhakhny, 1960), dissolve kidney stones (Dollah et al., 2013a; Hadjzadeh et al., 2011), inhibit carcinogenic processes (Al-Sheddi et al., 2014; Randhawa and Alghamdi, 2011), reduce abdominal pain, cure diarrhea, and be gastro-protective (Gali-Muhtasib et al., 2006). In addition, the plant has been shown to have antimicrobial, anti-inflammatory, and antioxidant properties (Chakravarty, 1993; Landa et al., 2009; Rakhshandeh et al., 2011; Randhawa and Alghamdi, 2011; Salem, 2005). It was also shown that the essential oil of the plant has anthelmintic (Agarwal et al., 1979), antinematodal (Akhtar and Riffat, 1991), antischistosomal (Mahmoud et al., 1991), antimicrobial (Aboul-Ela et al., 1996; Hanafy and Hatem, 1991), and antiviral effects (Ahmad et al., 2013). Moreover, many studies have shown that *N. sativa* is an effective remedy in the treatment of allergic diseases (bronchial asthma and eczema) (Kalus et al., 2003) as well as neurological disorders (Ahmad et al., 2013; Akhtar et al., 2012).

The crude oil derived from the seeds of *N. sativa* exhibited a variety of pharmacological effects, such as diuretic and antihypertensive (El-Tahir et al., 1993; Zaoui et al., 2000), antioxytocic (Aqel and Shaheen, 1996), antinociceptive (Abdel-Fattah et al., 2000), respiratory stimulating (El-Tahir et al., 1999), hematological (Enomoto et al., 2001), hepatoprotective (Daba and Abdel-Rahman, 1998), hypoglycemic (Al-Hader et al., 1993), antihistaminic (Chakravarty, 1993; Mahfouz et al., 1965), and immunomodulatory (Swamy and Tan, 2000) effects. *N. sativa* oil has also been used as a therapeutic agent to treat headache, flatulence, blood hemostasis

abnormalities, rheumatism, and related inflammatory diseases (Boulos, 1983). In addition, *N. sativa* oil has been used as an ointment for relief from abscesses, nasal ulcers, orchitis, eczema, and swollen joints in traditional medicine (Ave-Sina, 1990).

According to the literature, most biological activities of *N. sativa* are mainly related to its essential oil components, mainly TQ (Woo et al., 2012). The beneficial effects of TQ on antioxidant enzymes, pro-inflammatory mediators/cytokines, nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK), peroxisome proliferator-activated receptor gamma (PPAR-γ), AKt, chemo-drug toxicity, metastasis, angiogenesis, proliferation, apoptosis, cell cycle arrest, tumor suppressors, and enhancement of chemo-drugs as well as its effect on the reactive oxygen species (ROS) system have been shown in recent years, which demonstrate the effectiveness of *N. sativa* in cancer and different inflammatory diseases (Woo et al., 2012).

The prophylactic effect of nigellone (a carbonyl polymer of TQ) has been demonstrated in asthma and bronchitis (Wienkotter et al., 2008). The antiasthmatic effect may be due to the inhibitory effect of nigellone on the release of histamine from the mast cells (Chakravarty, 1993), and its anti-inflammatory effects are due to inhibition of 5-lipoxygenase product synthesis in polymorphonuclear leukocytes (El-Dakhakhny et al., 2002).

In the present article, the clinical evidence regarding preventive and relieving effects of *N. sativa* on different diseases has been reviewed.

3.1. Anti-inflammatory effects

Inflammation is one of the main pathophysiological characteristics of many chronic and acute diseases. Infection and oxidative stress activate the expression of inflammatory genes, which result in promotion of the cascade of inflammatory mediators, including eicosanoids, oxidants, cytokines, and lytic enzymes. Therefore, introduction of a preventive and multipotential agent is promising in the treatment of inflammatory disorders.

According to several preclinical studies, *N. sativa* and TQ could suppress inflammatory mediators and oxidative stress (Salem, 2005). Plant oil (12.5–50 mg/ml), nigellone (6.25 and 50 μg/ml), or TQ (0.01 and 6.25 μg/ml) treatment inhibited the synthesis of 5-lipoxygenase products and 5-hydroxyeicosatetraenoic acid production in calcium- or ionophore-stimulated polymorphonuclear leukocytes in rats (El-Dakhakhny et al., 2002). In human blood cells, TQ (1, 3, 10, 100 μM/ml) inhibited eicosanoid generation through inhibition of both 5-lipoxygenase and LTC4 synthase pathways (Mansour and Tornhamre, 2004). The effective concentration of TQ needed for the inhibition of eicosanoid generation in human blood cells was 0.16–16.4 μg/ml, which is very close to the animal effective concentration range.

The anti-inflammatory effect of TQ in allergic encephalomyelitis (EAE) was demonstrated. TQ (1 mg/kg, injected into the tail vein) increased the glutathione level and reduced perivascular inflammation and EAE symptoms in rats (Mohamed et al., 2003). TQ (15 mg/kg, i.p. injection in mice) treatment showed 90% preventive and 50% curative effects in chronic relapsing multiple sclerosis (Mohamed et al., 2009).

In a comparative, parallel, randomized, double-blind, placebo-controlled clinical study, the effects of *N. sativa* and *Phyllanthus niruri* (NSPN) extract in 186 patients with acute tonsillopharyngitis were examined. The patients were orally administered NSPN capsules (360 mg *N. sativa* and 50 mg *Phyllanthus niruri*) t.i.d. for 7 days. On the first day of medication (14.4 mg/kg/day *N. sativa* and 2 mg/kg/day *Phyllanthus niruri*), swallowing, inflammation, and pain significantly decreased compared with the placebo group (Dirjomuljono et al., 2008). Despite applying valid methodology and evaluating the safety of therapy in this study, a more randomized clinical trial (RCT) is needed to meta-analyze such findings.

In a placebo-controlled study, the anti-inflammatory effect of *N. sativa* oil in patients with rheumatoid arthritis was examined. During supplementation, patients received placebo capsules (twice a day) for 1 month and treatment was followed by 1 month of *N. sativa* oil capsules (500 mg twice daily). After *N. sativa* supplementation (13.3 mg/kg/day), the white blood cell (WBC) count and the disease activity score (DAS-28) significantly decreased compared with the pretreatment results as well as those of the placebo group. In addition, the number of swollen joints and the duration of morning stiffness decreased and there was a marked improvement in the disease activity as shown by both ACR20 and European League Against Rheumatism (EULAR) response criteria after *N. sativa* treatment. According to those previous basic studies, TQ was mentioned as a candidate constituent for these therapeutic effects of the plant (Gheita and Kenawy, 2012). Acceptable sample size (40), taking into account inclusion and exclusion criteria, and placebo control evaluation validate the finding of this study. However, more RCT and *N. sativa* supplementation is needed for the plant to be used as an inexpensive potential adjuvant biological therapy in inflammatory disorders. Table 1 shows a summary of the anti-inflammatory effects of *N. sativa* and its constituents.

3.2. Immunomodulatory effects

It has been suggested that *N. sativa* and its constituents can improve immune response in humans (Salem, 2005). The effect of the plant seed therapy on cellular immunity was investigated in human volunteers. Subjects were treated with *N. sativa* of a dosage of 1 g (twice daily) for 4 weeks. In most of the subjects who received *N. sativa* (26.7 mg/kg/day), the $CD4^+/CD8^+$ T cell ratio and natural killer (NK) cell function were increased (El Kadi et al., 1990). The result of this study was presented in the 1st International Conference On Scientific Miracles of Quran and Sunnah, but there are no data about the methodology. In addition, the immunomodulatory effects of the whole and soluble fractions of *N. sativa* seeds (0.1–10 μg/ml) on human peripheral blood mononuclear cell (PBMC) responses to different mitogens were investigated. The effect of the whole plant and its purified proteins on mixed lymphocyte culture (MLC) was stimulatory as well as inhibitory (in different donors). However, in pokeweed mitogen (PWM)-stimulated lymphocytes, an inhibitory effect of *N. sativa* and all its four peaks at a concentration of 10 μg/ml was observed (Haq et al., 1999). The effect of *N. sativa* proteins on cytokine secretion was also evaluated. In non-stimulated PBMCs and allogeneic cells, the incubation of the whole plant increased IL-1β secretion, while the fractionated *N. sativa* was less effective as compared with whole plant proteins. IL-4 secretion was not significantly changed in non-activated, PWM-activated, or allogeneic cells. The whole *N. sativa* plant inhibited and stimulated the production of IL-8 in non-activated and PWM-activated PBMC, respectively. The effect of incubation of the whole *N. sativa* plant peaks (2 μg/ml) in PWM-activated cells was stimulatory on the induction of IL-8, but it had no effect in allogeneic PBMC. The whole plant and its fractionated proteins had stimulatory effects on the production of TNF-α in both non-stimulated and mitogen-stimulated cells (Haq et al., 1999). Moreover, the immunomodulatory effects of *N. sativa* extracts (0.1–5 mg/ml) on human PBMC (stimulated with phytohemagglutinin and concanavalin as mitogens) were investigated. In this study, two immunobiochemical pathways (tryptophan degradation and neopterin production) that are induced by pro-inflammatory cytokine interferon-γ have been evaluated. Incubation of *N. sativa* suppressed the production of neopterin and mitogen-enhanced degradation of tryptophan, which showed its inhibitory effect on

Table 1
Anti-inflammatory and immunomodulatory effect of *N. sativa* and its constituents.

Plant preparations	Study models	Effects	References
Anti-inflammatory			
N. sativa oil (500 mg twice/day)	Rheumatoid arthritis patients	↓Disease activity score, swollen joints, and the duration of morning stiffness	(Gheita and Kenawy, 2012)
Thymoquinone	Human blood cells	Inhibition of both 5-lipoxygenase and LTC4 synthase pathways	(Mansour and Tornhamre, 2004)
Immunomodulatory			
N. sativa (1 g b.i.d. for 4 weeks)	Human volunteers	↑$CD4^+$/$CD8^+$ T cell ratio and natural killer (NK) cell function	(El Kadi et al., 1990)
Whole *N. sativa* and its purified proteins	Human peripheral blood mononuclear cells (PBMC)	Stimulatory/suppressive effects on mixed lymphocyte cultures	(Haq et al., 1999)
Whole *N. sativa* and its purified proteins	Pokeweed mitogen (PWM) stimulated PBMC	Suppressive effects on lymphocyte, ↑IL-8, and TNFα	(Haq et al., 1999)
Whole *N. sativa*	non-stimulated PBMC	↑IL-1beta secretion and TNFα, ↓IL-8	(Haq et al., 1999)
N. sativa seed solution in RPMI	Human PBMC	Inhibitory effect on mitogen-stimulated T cells and macrophage	(Winkler et al., 2008)
N. sativa oil (40–80 mg/kg/day)	Patients with allergic rhinitis, bronchial asthma, and atopic eczema	↓IgE and eosinophil count, ↓in plasma T and ↑in HDL cholesterol, no change in lymphocyte subpopulations, endogenous cortisol levels, and ACTH release	(Kalus et al., 2003)
N. sativa ointments	Patients with hand eczema	Improvement in hand eczema and ↓ in dermatology life quality index I scores	(Yousefi et al., 2013)
N. sativa oil for 30 days	Patients with allergic rhinitis	↓Nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor	(Nikakhlagh et al., 2011)
N. sativa seed (2 g/day orally) for 30 days	Patients with allergic rhinitis	↑PMN functions, ↑ CD8 counts	(Isik et al., 2010)
N. sativa oil (twice/day) on lesions for 6 months	Patients with vitiligo lesions	↓ Size of patient's lesions	(Ghorbanibirgani et al., 2014)

mitogen-stimulated T cells and macrophage (Winkler et al., 2008). All these well-designed in vitro studies showed a potent potentiating effect of lipid-soluble components of the plant on T-cell-mediated immunity, while water-soluble components affected B-cell-mediated immunity. These effects could also change depending on the type of immune system stimulation.

The effect of *N. sativa* oil (40–80 mg/kg/day) as an adjuvant therapy in patients with allergic diseases (allergic rhinitis, bronchial asthma, atopic eczema in both adults and children) was evaluated. In four studies (two RCT and two open label), 152 patients were assessed (for 8 weeks) for subjective severity of target symptoms as well as biochemical parameters. *N. sativa* oil treatment decreased scores of subjective feeling, IgE, and eosinophil count. A mild decrease in plasma triglycerides and a discrete increase in high-density lipoprotein-cholesterol (HDL-C) occurred, but the lymphocyte subpopulations, adrenocorticotropic hormone (ACTH) release, and endogenous cortisol concentration did not change. In addition, no side effect was reported except in children receiving a high dose of 80 mg/kg. According to previous basic studies, it was suggested that TQ and nigellone may be responsible for the immunological effects of the plant (Kalus et al., 2003). Although the methodologies of these studies (randomized, placebo-controlled, double-blind trial) are acceptable, the type of randomization was not mentioned, and more studies are needed for evaluating the long-term effects of *N. sativa* adjuvant therapy.

The therapeutic effect of topical *N. sativa* (2% ointment) on the severity of hand eczema and the life quality of patients was compared with that of eucerin and betamethasone. Sixty patients received medication twice a day for 4 weeks in three therapeutic groups (*N. sativa*, betamethasone, and eucerin). Patients were evaluated at the beginning, on the 14th, and on the 28th day of the study by hand eczema severity index (HECSI) and dermatology life quality index (DLQI), respectively. *N. sativa* and betamethasone treatment caused a significantly more rapid improvement in hand eczema and also a significant decrease in DLQI scores as compared with eucerin. Topical administration of the plant did not result in any significant allergic and eczematous adverse effects. There was no significant difference in the mean DLQI and HECSI of *N. sativa* and betamethasone groups over time. TQ, dithymoquinone, and thymohydroquinone were mentioned in this study as effective constituents of the plant (Yousefi et al., 2013). This clinical trial (randomized, controlled, and double-blind) were well designed and the process of recruitment, random allocation, and analysis of participants ($n=68$) were mentioned. However, more studies with a larger sample size are needed for the determination of long-term therapeutic and side effects of topical *N. sativa* usage.

The therapeutic effect of *N. sativa* oil was compared with those of fish oil on vitiligo lesions, an autoimmune skin disorder. In this well-designed randomized, double-blind clinical trial, 52 patients participated and were divided into two equal groups, who applied oil twice a day on their lesions for 6 months. *N. sativa* and fish oils effectively reduced the size of lesions, but the plant oil was more effective than the fish oil and no significant side effects were reported (Ghorbanibirgani et al., 2014). Although valuable, these results could not be generalized because of some limitations, including conducting the study in one clinic, and uncontrollable factors that influenced skin lesions, such as patient nutrition.

The anti-inflammatory and immunomodulatory effects of *N. sativa* in patients with allergic rhinitis symptoms were investigated. Sixty-six patients (case and placebo) were treated with plant oil (6 mg/kg) for 30 days and individual characteristics, including age and sex, and characteristics of the disease, including nasal congestion, runny nose, itchy nose, and sneezing attacks, were assessed. The results indicated that *N. sativa* treatment decreased nasal mucosal inflammatory symptoms (congestion, itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor) during the first 2 weeks. The authors suggested an antiallergic effect for *N. sativa* and its components, which is in line with previous reports about the antihistamine properties of the plant. Therefore, the plant could be considered for treating allergic rhinitis (Nikakhlagh et al., 2011).

In another study, the effect of *N. sativa* adjuvant therapy was investigated in allergic rhinitis patients. An experimental group of 24 patients, randomly selected from 31 patients sensitive to house dust mites with allergic rhinitis, and a control group of 8 healthy volunteers were treated with allergen-specific immunotherapy at conventional doses for 30 days. The other 7 patients were given 0.1 ml saline solution subcutaneously once a week as placebo. At the end of immunotherapy, 12 individuals out of the 24 patients and the eight healthy volunteers received *N. sativa* seed

supplementation (26.7 mg/kg/day orally) for 30 days. The remaining patients continued only on immunotherapy during the same period. Before and after the treatment periods, the symptom scores, polymorphonuclear leukocyte (PMN) functions, lymphocyte subtypes, and other hematological factors were assessed. After immunotherapy, especially after the administration of *N. sativa* seed, there was a significant increase in the phagocytic and intracellular killing activities of PMNs of patients. Moreover, in patients receiving specific immunotherapy plus *N. sativa* seed supplementation, the CD8 counts significantly increased as compared with patients receiving only specific immunotherapy. *N. sativa* seed supplementation significantly increased PMN functions in healthy volunteers compared with the baseline. These data showed the therapeutic immunomodulatory and antiallergic effects for the plant as an adjuvant therapy (Isik et al., 2010). All these studies showed that both lipid- and water-soluble fractions of *N. sativa* are potent and safe immunomodulatory agents and could be recommended as prophylactic and therapeutic adjuvants in immune system diseases. Table 1 shows a summary of the immunomodulatory effects of *N. sativa* and its constituents.

3.3. Antimicrobial effects

The antibacterial effect of *N. sativa* essential oil (4 μl in pure or 1:200 dilution) as against various clinical isolates of bacteria resistant to a number of antibiotics was evaluated. The oil exhibited a potent dose-dependent antibacterial activity, which was more pronounced against Gram-positive than Gram-negative bacteria. Gram-positive bacteria such as *Staphylococcus aureus* (Oxford NCTC 6571, ATCC 25923), *S. epidermidis*, other coagulase-negative staphylococci, and *Streptococcus pyogenes* were sensitive, while *Enterococcus faecalis* and *Streptococcus agalactiae* were resistant to the oil. However, among the Gram-negative bacteria tested, only *Pseudomonas aeruginosa* (NCTC 10662, ATCC 27853) was sensitive to oil and *Acinetobacter baumannii*, *Vibrio cholerae*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *P. vulgaris*, and *Citrobacter freundii* were resistant (Salman et al., 2008). The antibacterial inhibitory effect of *N. sativa* against methicillin-resistant *Staphylococcus aureus* (MRSA), as one of the commonest pathogens encountered in clinical settings, was investigated. The plant ethanolic extract at a concentration of 4 mg/disc showed an antibacterial effect on all the tested strains of MRSA, and the extract had an minimum inhibitory concentration (MIC) range of 200–500 μg/ml, which showed the inhibitory effect of *N. sativa* on MRSA (Hannan et al., 2008). The traditional use of the plant as a natural remedy for treatment of skin infection was investigated. The antibacterial effect of *N. sativa* seed extract (33%) on pustules and staphylococcal skin infection in 40 neonates was compared with the standard medication mupirocin. The result showed no significant differences in recovery time between *N. sativa*- and mupirocin-treated groups, and the plant extract was as effective as mupirocin in the treatment of pustules in tissues in all patients (Rafati et al., 2014).

The bactericidal effect of TQ and its biofilm inhibitory activity on 11 human pathogenic bacteria were investigated. TQ (0–512 μg/ml) showed a significant antibacterial activity against most of the bacteria tested (MIC values ranged from 8 to 32 μg/ml), especially Gram-positive bacteria (*Staphylococcus epidermidis* CIP and 106510 *Staphylococcus aureus* ATCC 25923). The cellular oxidative activity was influenced by TQ, which prevented cell adhesion to the surface of the glass slide (Chaieb et al., 2011).

In a clinical study, the effects of ethanolic extracts of *N. sativa*, *Zingiber officinale* (*Z. officinale*), and their mixture in patients with hepatitis C virus (HCV) infection were evaluated. Patients were divided into five groups: I) healthy subjects; II) (HCV) as HCV control; III) HCV+ a capsule containing 500 mg of the plant extract administered twice daily; IV) HCV+ a capsule containing 500 mg *Z. officinale* extract administered twice daily; and V) HCV+ a capsule containing 500 mg of each extract administered twice daily. The results showed that ethanolic extracts of *N. sativa* (13.3 mg/kg/day) and *Z. officinale* had a potent effect in HCV patients, as it decreased the viral load and altered the liver function (Adel et al., 2013). In another study, administration of *N. sativa* oil (16.88 mg/kg/day for 3 months) in patients with HCV decreased the viral load and improved oxidative stress, clinical condition, and glycemic control. *N. sativa* administration was safe in all patients, and only one patient reported epigastric pain and hypoglycemia (Barakat et al., 2013).

All these studies demonstrated that *N. sativa* seed extract and oil are potent antimicrobial agents, which probably do not elicit resistance in microorganisms (Hannan et al., 2008). In addition, antimicrobial activity of TQ at low concentrations suggested the necessity of further in vivo studies. Therefore, isolation and the formulation of new antimicrobial components from this herb should be carried out in the future, and more clinical trials should be designated before marketing.

3.4. Antitumor effects

The antitumor activity of *N. sativa* seed extract and oil against a human lung cancer cell line (A-549 cells) showed that incubation of *N. sativa* seed extract (0.25, 0.5, and 1 mg/ml) and *N. sativa* seed oil (0.1, 0.25, 0.5, and 1 mg/ml) significantly reduces the viability and changes the cellular morphology of A-549 cells (lose their typical morphology and appear smaller in size) in a concentration-dependent manner (Al-Sheddi et al., 2014). The plant oil could also regulate the cell growth and differentiation in human monocyte and monocyte-derived macrophages. Incubation (24 h) of *N. sativa* oil (140 ng/ml) reduced macrophage growth and increased the suppressive effect of low-density oxidized lipoprotein on CD11b expression (Mat et al., 2011). Moreover, the lipid fraction of the *N. sativa* seed extract was cytotoxic to MCF-7 cells at low concentrations (LC50 of 2.72 ± 0.232 mg/ml), while cytotoxicity of the aqueous extract was clear at high concentrations (50 mg/ml) and its low concentrations had a hormetic rather than a cytotoxic effect (Mahmoud and Torchilin, 2013). It was reported that the plant's hydroalcoholic extract (50–2000 mg/ml) and its fractions reduced the cell viability of ACHN (human renal adenocarcinoma) in a dose- and time-dependent manner but had no significant cytotoxic effect on the GP-293 cell (normal renal epithelial) (Shahraki et al., 2015).

There are many reports about the antitumor activity of TQ (the main lipid constituents of *N. sativa*) in both in vitro and in vivo studies (Woo et al., 2012). The inhibitory effects of TQ (10–200 μM) on the growth of colon cancer cells were shown. The apoptotic effects of TQ could be mediated by Bcl-2 protein by increasing the mRNA expression of p53 (Gali-Muhtasib et al., 2004). In addition, the inhibitory and apoptotic effects of TQ (20, 40, and 80 μmol/l) on human osteosarcoma cell line (SaOS-2) and blocking the human umbilical vein endothelial cell (HUVEC) tube formation were shown to be dependent on dose. Different mechanisms, including inhibition of tumor growth and tumor angiogenesis (through suppressing NF-κB and its regulated molecules), were responsible for this effect (Peng et al., 2013). It was also shown that TQ reduced cell survival in a dose-dependent manner and this effect was more marked in p53-null MG63 cells (IC(50) = 17 μM) as compared with p53-mutant MNNG/HOS cell (IC(50) = 38 μM) (Roepke et al., 2007). Moreover, the cytotoxic effect of TQ (IC(50) = 10.67 ± 0.12 and 9.33 ± 0.19 μg/ml) in SiHa (cervical squamous carcinoma) cells was more pronounced as compared with cisplatin, but it was less cytotoxic towards the normal cells (3T3-L1 and Vero) (Ng et al., 2011).

Cytotoxic and apoptotic effects on different human cell lines,

Table 2
Antitumor effect of *N. sativa* and its constituents.

Plant preparations	Study models	Effects	References
N. sativa seed extract and oil	Human lung cancer cell line	↓Viability and change in the cellular morphology of cancer cells	(Al-Sheddi et al., 2014)
N. sativa oil	Human monocyte and macrophages	Regulatory effect in cell growth and differentiation in monocyte and monocyte-derived macrophage	(Mat et al., 2011)
Lipid fraction of *N. sativa* seed extracts	Human MCF-7 breast cancer cells	Cytotoxic to MCF-7 cells at low concentrations	(Mahmoud and Torchilin, 2013)
Aqueous extract of *N. sativa* seed	Human MCF-7 breast cancer cells	Cytotoxicity of aqueous extract at high concentration and hormetic effect at low concentrations	(Mahmoud and Torchilin, 2013)
Adjuvant therapy of oil nanoemulsion	Human MCF-7 breast cancer cells	↑ Antitumor activity of doxorubicin	(Mahmoud and Torchilin, 2013)
Hydroalcoholic extract of *N. sativa* and its n-hexane and ethyl acetate fractions	ACHN (human renal adenocarcinoma) and GP-293 (normal renal epithelial) cell lines	↓Cell viability of ACHN dependent on dose and time More pronounced morphological changes and apoptotic effect of total extract in ACHN cells compared with the GP-293 cells	(Shahraki et al., 2015)
Thymoquinone	HCT-116 human colon cancer cells	Apoptotic effects of TQ on HCT-116 (by ↑ Bcl-2 protein and mRNA expression of p53)	(Gali-Muhtasib et al., 2004)
Thymoquinone	Human osteosarcoma cell line (SaOS-2)	Apoptotic effect (↓tumor angiogenesis and tumor growth through suppressing NF-κB)	(Peng et al., 2013)
Thymoquinone	Human umbilical vein endothelial cell	Apoptotic effect (↓tumor angiogenesis and tumor growth through suppressing NF-κB)	(Peng et al., 2013)
Thymoquinone	Human osteosarcoma cell lines	p53-independent apoptosis in human osteosarcoma cells	(Roepke et al., 2007)
Thymoquinone	Human cervical squamous carcinoma cells	Cytotoxic effect (elevation of p53 and downregulation of the antiapoptotic Bcl-2 protein)	(Ng et al., 2011)

including lung cancer, breast cancer, renal adenocarcinoma, colon cancer, osteosarcoma cell, and cervical squamous carcinoma, were shown in the above mentioned in vitro studies. However, more clinical trials are needed to recommend *N. sativa* derivatives as potential anticancer products. Table 2 shows a summary of the antitumor effects of *N. sativa* and its constituents.

3.5. Effects on metabolic disorders

Several animal and clinical studies showed antidiabetic and antihyperlipidemic activities of *N. sativa* and its effects on other metabolic disorders. Many possible mechanism(s) have been proposed for these effects. It was seen that the antioxidant property of the plant contributed to its effect of reducing insulin resistance and increasing insulin sensitivity by improving the intracellular pathways of insulin receptors and increasing their sensitivity to insulin (Le et al., 2004; Rchid et al., 2004). Various reports on the effect of *N. sativa* on body weight showed an association between weight loss and improvement in the lipid profile and glucose status (Haque et al., 2011; Heshmati et al., 2015; Najmi et al., 2008). *N. sativa* could act as an agonist of PPAR-γ gene and increase the PPAR-γ activity (Benhaddou-Andaloussi et al., 2010). It was proposed that the plant could decrease glucose absorption by inhibiting the sodium-glucose co-transporter; in addition, its polyphenol ingredients could have suppressive effects on glucose absorption (Meddah et al., 2009). The *N. sativa* seed ethanol extract could also inhibit gluconeogenesis by the liver and muscle activation of adenosine monophosphate-activated protein kinase (AMPK) (Benhaddou-Andaloussi et al., 2011). It was demonstrated that TQ (the main constituent of *N. sativa*) could reduce the expression of gluconeogenic enzymes (glucose-6-phosphatase and fructose 1, 6 bisphosphatase) and hepatic glucose production (Al-Rasheed et al., 2014; Alimohammadi et al., 2013). TQ could increase the uptake of low-density lipoprotein-cholesterol (LDL-C) by upregulation of hepatic receptors of LDL-C (Ibrahim et al., 2014a). Phytosterols such as beta-sitosterol (cholesterol-lowering effect), polyunsaturated fatty acids, polyphenol components (with antioxidant activity), TQ, thymol, nigellamine (lowering triglyceride levels in primary cultured mouse hepatocytes), lipase, and tannins were responsible for *N. sativa* metabolic effects (Sabzghabaee et al., 2012; Ibrahim et al., 2014a; Heshmati et al., 2015).

3.5.1. Antidiabetic effects

Therapeutic effect of *N. sativa* on carbohydrate and lipid metabolism disorders was indicated in previous studies. Several animal and clinical studies have shown the therapeutic effect of the plant on metabolic parameters in diabetes (Heshmati and Namazi, 2015). According to the evidence and traditional usage, in many clinical studies, hypoglycemic and hypolipidemic effects of *N. sativa* in patients suffering from diabetes and metabolic syndrome have been reported (Bamosa et al., 2010; Sabzghabaee et al., 2012). Adjuvant therapy of *N. sativa* seed (26.7 mg/kg/day for 12 weeks) in patients with type 2 diabetes resulted in reduction in fasting blood glucose (FBS), 2-h postprandial blood glucose (2hPG), glycosylated hemoglobin (HbAlc), and insulin resistance, but it did not cause any adverse effect on renal or hepatic functions of the diabetic patients (Bamosa et al., 2010).

In diabetes, chronic elevation of blood glucose results in the production of reactive oxygen species (ROS), which enhance cellular damage and contribute to the development and progression of diabetic complications. The effect of *N. sativa* treatment in this regard was examined. The effect of long-term supplementation of the plant (1 year) on glycemic control and oxidant/antioxidant status in patients with type 2 diabetes was determined. In diabetic patients, *N. sativa* (seeds powder) add-on therapy (26.7 mg/kg/day) significantly reduced FBG and HbA1c in all the samples taken in 1 year compared with the baseline that represented the glycemic control of the patients. The difference in C-peptide was not significant, but the insulin resistance was lower and β-cell activity was higher in *N. sativa*-treated patients than in the placebo group. Plant supplementation caused a significant increment in total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), and glutathione and a significant reduction in thiobarbituric acid-reactive substances (TBARS). There were no significant changes in the results of renal and liver functions between the two groups and the complete blood count remained normal. Therefore, long-term *N. sativa* supplementation enhanced glucose homeostasis and improved antioxidant balance in patients with type 2 diabetes receiving oral hypoglycemic drugs (Kaatabi et al., 2015).

The antihyperglycemic effect of plant oil adjuvant therapy in type 2 diabetes in an RCT was investigated. Patients received 2.5 ml of *N. sativa* oil (30 mg/kg/day) in addition to their antidiabetic drug, and FBS, 2hPG, HbA1C, lipid profile, body mass index (BMI), and liver and renal function tests were measured at the baseline and after 3 months. In *N. sativa*-treated patients, all the measured parameters were decreased compared with baseline and placebo groups and no side effect was detected. Although there was some limitations, such as lack of identification of plant constituents, the high content of linoleic and oleic acid in the oil and its lipase activity were implicated in the hypolipidemic effect of *N. sativa* according to previous basic studies (Hosseini et al., 2013). In another study, the effect of the plant oil (40 mg/kg/day) on serum levels of lipids and glucose metabolism in patients with type 2 diabetes was evaluated in a double-blind, randomized, controlled clinical trial. The fatty acid and TQ concentration of *N. sativa* oil was determined by gas chromatography–mass spectrometry (GC–MS) technique and presented in the result. Both intervention and placebo groups were advised to continue the antidiabetic medication. After 12 weeks, weight and BMI decreased in the *N. sativa*-treated group compared with those of the baseline group, without any significant differences between the two groups. No side effects were reported throughout the intervention except mild gastrointestinal problems. The amount of diet intake in both groups changed compared with that of the baseline group. FBS, HbA1c, triglyceride (TG), and LDL-C were significantly decreased in the plant-treated group compared with those in the placebo group, but there were no significant changes in the total cholesterol (TC), HDL-C, and insulin secretion between the two groups. The authors concluded that unsaturated fatty acids such as linoleic and oleic acids and polyphenol components improved glucose and lipid profile parameters (Heshmati et al., 2015). In their study, Memon et al. showed that the add-on therapy of type 2 diabetes mellitus patients with *N. sativa* seed (3.3 mg/kg) in combination with *Trigonella foenum-graecum* increased HDL-C, but TG and creatinine levels remained unchanged in comparison with the group that received a routine dose of glibenclamide (Memon et al., 2012). In all the studies mentioned, despite demonstrating valuable findings, there were confounding factors and limitations that made data interpretation difficult. Lack of designing of the placebo control group or mentioning the type of randomization, uncontrolled patient diet or psychological status and physical activity, self-testing blood glucose, small sample size, short duration of study, and lack of identification of active constituents of the plant are examples of those limitations. Table 3 shows a summary of the antidiabetic effects of *N. sativa* and its constituents.

3.5.2. Antihyperlipidemic and antimetabolic syndrome effects

The effect of *N. sativa* on the glycemic control in patients with metabolic syndrome and poor glycemic control (HbA1c > 7%) was shown. Dietary supplementation of the plant (seed powder or oil) could improve dyslipidemia, as it decreased the total lipid, TG, and LDL levels in diabetic patients, but an increase in the HDL level is under question (Qidwai and Ashfaq, 2014). *N. sativa* (6.67 mg/kg/day seed powder) treatment as an add-on therapy in metabolic syndrome patients ($n=80$) for 2 months significantly reduced the FBG, PPBG, HbA1c, and LDL-C levels, which showed the effectiveness of the plant for glycemic control in metabolic syndrome, and the presence of unsaturated fatty acids as well as TQ, thymol, lipase, and tannins could also be responsible for these effects (Najmi et al., 2012). In this study, there was no placebo-controlled group and the type of randomization was not mentioned, which could decrease data validation. In addition, *N. sativa* treatment (2 g/day seed powder) in hyperlipidemic patients decreased TC, LDL, and

Table 3
Antidiabetic, antihyperlipidemic, hepatoprotective, and other effects of *N. sativa* and its constituents on metabolic syndrome.

Plant preparations	Study models	Effects	References
N. sativa seed 2 mg/day for 12 weeks	Patients with type 2 diabetes Adjuvant therapy	↓ FBS, 2hPG, HbAlc, and insulin resistance	(Bamosa et al., 2010)
N. sativa seed 2 g/day for 1 year	Patients with type 2 diabetes Adjuvant therapy	↓FBG, HbA1c, and insulin resistance; ↑β-cell activity; ↑TAC, SOD, CAT, and glutathione, ↓TBARS	(Kaatabi et al., 2015)
N. sativa oil (2.5 ml) for 3 months	Patients with type 2 diabetes Adjuvant therapy	↓FBS, 2hPG, HbA1C, lipid profile, body mass index (BMI)	(Hosseini et al., 2013)
N. sativa oil (3 g/day) for 12 weeks	Patients with type 2 diabetes Adjuvant therapy	↓Weight and BMI (NS), FBS, ↓HbA1c, TG, and LDL-C; NS changes in TC, HDL-C, and insulin secretion	(Heshmati et al., 2015)
N. sativa seed (250 mg)+*Trigonella foenum-graecum*	Patients with type 2 diabetes Adjuvant therapy	↑HDL-C; NS changes in triglycerides and creatinine	(Memon et al., 2012)
N. sativa (500 mg/day seed powder)	Patients with metabolic syndrome	↓FBG, PPBG, HbA1c, and LDL-C	(Najmi et al., 2012)
N. sativa oil (2.5 ml twice daily) for 6 weeks	Patients with metabolic syndrome	↓FBG, TC, and LDL-C	(Haque et al., 2011)
N. sativa treatment (2 mg/day)	Hyperlipidemic patients	↓TC, LDL-C; NS changes in FBS and HDL-C	(Sabzghabaee et al., 2012)
N. sativa powder (1 g/day) for 2 months	Hypercholesterolemic patients	↓TC, LDL-C, HDL-C, and TG	(Bhatti et al., 2009)
N. sativa powder (2 g/day) for 8 weeks	Overweight females	↓TC, LDL-C, TG, and ↑ HDL-C	(Farzaneh et al., 2014)
N. sativa powder (1 g/day) for 2 months	Menopausal women	↓TC, LDL, TG and ↑HDL-C	(Ibrahim et al., 2014b)
N. sativa seed (1.6 g/day) for 12 weeks	Premenopausal women	↓BG and LDL; NS changes in TC, TG, and HDL-C	(Latiff et al., 2014)
N. sativa seed (500 mg)+ Allium sativum oil (250 mg)	Psoriasis-induced dyslipidemia	↓Non-HDL, TG, LDL and cholesterol, and ↑HDL	(Ahmad Alobaidi, 2014)
Ethanolic extract (10–100 mg/ml)	Human liver microsomes (in vitro)	↓Formation of CYP3A4 and CYP2D6 metabolites	(Al-Jenoobi et al., 2010)
Ethanolic extract (2.5 g twice daily) for 7 days	Healthy volunteers	↓Urinary metabolic ratios of DEX/DOR and DEX/3-MM	(Al-Jenoobi et al., 2010)
N. sativa oil (80 mg/kg/day)	Methotrexate therapy in ALL children	↓Total, direct, and indirect serum bilirubin; serum ALT, AST, and alkaline phosphatase levels; and prothrombin time	(Hagag et al., 2013)

FBS (fasting blood glucose), 2hPG (2-h postprandial blood glucose), HbAlc (glycosylated hemoglobin), TAC (total antioxidant capacity), SOD (superoxide dismutase), CAT (catalase), TBARS (thiobarbituric acid reactive substances), NS (Not Significant), DEX (dextromethorphan), DOR (dextrorphan), 3-MM (3-methoxymorphinan), ALT (alanine transaminase), and AST (aspartate transaminase).

TG levels, but had no effect on FBG and HDL levels (Sabzghabaee et al., 2012). In this randomized, placebo-controlled trial, limitations such as short-term duration of the study and self-reporting information about diet and exercise made interpretation of result difficult.

The effectiveness of *N. sativa* oil on various biochemical parameters of the metabolic syndrome was determined. The plant oil (60 mg/kg/day for 6 weeks) add-on therapy in metabolic syndrome patients improved FBG, total, and LDL-C values. These results suggest that *N. sativa* could be a therapeutic agent in patients with hyperlipidemia and hyperglycemia (Najmi et al., 2008). In this randomized, placebo-controlled trial, limitations such as short-term duration of the study and self-reporting information about diet and exercise made interpretation of result difficult. In another study, *N. sativa* oil (60 mg/kg/day) was used as an adjuvant therapy in patients with metabolic syndrome and the results were compared with the standard drugs. *N. sativa* add-on therapy improved FBS, 2hPG, fasting lipid profile, BMI, waist circumference, hip circumference, body weight, and waist hip ratio, and it was more effective than the standard medication. These results indicated the therapeutic effects of the plant on hyperglycemia and dyslipidemia. The antiobesity effect of the plant contributed to its lipase content (Haque et al., 2011). Although in this parallel group, interventional, randomized, open-labeled, active control, and comparative study was done, the active constituents of the oil were not determined. However, the plant's insulin-sensitizing action and its anti-inflammatory agent (TQ, thymol, various unsaturated fatty acids, lipase, and tannin) were the probable causes of the findings described.

The antihyperlipidemic effects of *N. sativa* were also shown in hypercholesterolemic patients. Subjects received the plant seed powder (13.3 mg/kg/day) before breakfast for 2 months. This supplementation reduced serum concentrations of TC, LDL-C, HDL-C, and TG significantly, which showed the therapeutic effect of the plant on the lipid profile (Bhatti et al., 2009). This study had a very small sample size ($n=10$), and no control group and other parameters of scientific methodology were designed, which limited the reliability of the findings.

Farzaneh et al. reported that *N. sativa* powder supplementation along with concurrent aerobic exercise decreased TC, LDL-C, and TG levels and increased HDL-C level in overweight females after 8 weeks (Farzaneh et al., 2014). Limitations such as small sample size ($n=20$) and lack of plant's active component determination decreased the validity of this well-defined randomized, double-blind, controlled trial study. *N. sativa* treatment (13.3 mg/kg/day capsulated plant powder) in menopausal women for 2 months ameliorated the lipid profile by decreasing TC, LDL, and TG levels, and the increase in the HDL level was more marked than that in the placebo group. The synergistic action of various constituents of *N. sativa*, including TQ, nigellamine, soluble fiber (mucilage), sterols, flavanoids, and high content of polyunsaturated fatty acids, contributed to this hypolipidemic effect (Ibrahim et al., 2014b). *N. sativa*-treated premenopausal women received 21.3 mg/kg/day encapsulated seed powder for 12 weeks, which led to improvement in blood glucose and low-density lipoprotein in the treatment group as compared with the placebo group, but TC, TG, and HDL concentration changes were not significant between two groups. The prevalence and severity of menopausal symptoms were reduced in the treated group (Latiff et al., 2014). Although the small sample size was a limitation of these studies, the similar study design helped in interpreting and approving the results.

A well-designed randomized, double-blind, placebo-controlled, two-arm parallel study with large sample size, which had a planned 4-week diet stabilization period including a 4-week baseline evaluation phase, followed by an 8-week treatment period, was conducted. In this study, the synergic effect of *N. sativa* seed (6.67 mg/kg) and *Allium sativum* oil (3.3 mg/kg) on the improvement of dyslipidemia in patients with psoriasis was determined. The combination of these herbal remedies as an add-on therapy with simvastatin caused a significant reduction in non-HDL, TG, LDL, and cholesterol and increased the HDL (more than simvastatin alone). Although the chemical composition of the plant could not be determined, the cholesterol-lowering effects of the plant contributed to the ability of beta-sitosterol to inhibit dietary cholesterol absorption. The recorded side effect was diarrhea in about 4% of patients, while biochemical markers did not show any significant changes following treatments (Ahmad Alobaidi, 2014).

Several mechanisms have been proposed for the antihyperlipidemic activity of *N. sativa*, including preventing the absorption of dietary cholesterol in the intestines by the anti-absorptive activity of beta-sitosterol or by increasing the flow of bile acids (Farzaneh et al., 2014). The other possible mechanisms are a slight anorexic effect and reducing the appetite property of the plant. In addition, the plant inhibits de novo cholesterol synthesis by downregulating 3-hydroxy-3-methylglutaryl-coenzyme A reductase genes in HepG2 cells and the decrease in LDL-C could be due to upregulation of LDL receptor gene (Tauseef Sultan et al., 2009).

All these studies demonstrated the positive effects of *N. sativa* on glycemic control and lipid profile, but the differences in the dose and type of plant extract, the amount of dietary intake, physical activity level, baseline biochemical profile, duration of study, type of disease, ethnicity, and genotype could have affected the results. Table 3 shows a summary of the antihyperlipidemic and antimetabolic syndrome effects of *N. sativa* and its constituents.

3.5.3. Hepatoprotective effects

The effects of *N. sativa* on the metabolic activities of CYP3A4 and CYP2D6 in human liver microsomes and in subjects using dextromethorphan as a probe drug were evaluated. In in vitro experiments, the formation of CYP2D6-mediated O-demethylation and CYP3A4-mediated N-demethylation of dextromethorphan (DEX) to dextrorphan (DOR) and 3-methoxymorphinan (3-MM) DEX in the absence or presence of plant extract (10–100 μg/ml) was measured by high-performance liquid chromatography (HPLC). In a clinical study, four healthy volunteers were treated with a single oral dose of DEX (30 mg) alone in the first phase and with *N. sativa* (66.7 mg/kg/day for 7 days) in the second phase, and at the end of the study, the urinary metabolic ratios (MRs) were evaluated. The plant extracts significantly suppressed the formation of both metabolites in microsomes in a concentration-dependent manner. The urinary MRs of DEX/DOR and DEX/3-MM increased after consumption of *N. sativa* by volunteers, which indicates that it has the potential to interact with substrates of CYP2D6 and CYP3A4 (Al-Jenoobi et al., 2010). Therefore, more caution should be exercised in the plant co-administering with conventional drugs metabolized by CYP2D6 and CYP3A4 enzymes. The plant constituents such as TQ, nigellone, and nigellamine were suggested to have been responsible for these inhibitory effects by the authors, which need further investigations.

The protective effect of *N. sativa* oil against methotrexate-induced hepatotoxicity in children newly diagnosed with acute lymphoblastic leukemia (ALL) was investigated. Twenty eight males and 12 females (9.17 ± 3.81 years) were divided into 20 patients of ALL under methotrexate therapy, delayed leukovorin rescue (10 mg/m^2 orally or IV every 6 h for five doses beginning 2 days after the start of methotrexate infusion), and three divided doses of *N. sativa* oil (80 mg/kg/day) for 1 week after each methotrexate dose (Group I) and 20 patients of ALL under methotrexate therapy, delayed leukovorin rescue (10 mg/m^2 orally or IV every

6 h for five doses beginning 2 days after the start of methotrexate infusion), and placebo for 1 week after each methotrexate dose (Group II). Serum bilirubin, transaminase (ALT), aspartate transaminase (AST), alkaline phosphate concentration, and prothrombin time were not significantly different between the intervention (I) and placebo (II) groups, but there was a significant difference between groups I and II as compared with controls. No significant difference in clinical manifestations (pallor, purpura, and fever followed by hepatomegaly, splenomegaly, and lymphadenopathy) was found on studying patients of groups I and II. The difference in the total protein, albumin, and globulin levels and the albumin/globulin ratio among the studied groups was not significant. After methotrexate and *N. sativa* oil therapy, the result showed a non-significant increase in the total serum bilirubin (direct and indirect), ALT, AST serum concentrations, alkaline phosphatase levels, and prothrombin time in group I, but there was a significant increase in group II after treatment with methotrexate and placebo with a significant difference between groups I and II after therapy. There were significant differences in the overall and disease-free survival between groups I and II. These results demonstrated the anti-hepatotoxic effect of *N. sativa* oil (Hagag et al., 2013). Table 3 shows a summary of the hepatoprotective effects of *N. sativa* and its constituents.

3.6. Gastrointestinal protective effects

The effect of *N. sativa* seed in comparison with triple therapy, including clarithromycin, amoxicillin, and omeprazole against *H. pylori* in patients with non-ulcer dyspepsia, was evaluated. Patients were randomly (not defined) divided into four groups: I) triple therapy, II) *N. sativa* (13.3 mg/kg/day)+40 mg omeprazole, III) *N. sativa* (26.7 mg/kg/day)+40 mg omeprazole, and IV) *N. sativa* (40 mg/kg/day)+40 mg omeprazole for 4 weeks. The results indicated that the plant at a dose of 26.7 mg/kg/day+40 mg omeprazole has a potential effect on *H. pylori* activity. The antibacterial activity of essential oil content comprising TQ, dihydrothymoquinone, and terpenes could be responsible for this effect of the plant. The authors reported that the side effects in the patients consuming *N. sativa* and antibiotics were similar with only a minor short duration of gastrointestinal irritation (Salem et al., 2010).

3.7. Effects on neurological disorders

The therapeutic effect of *N. sativa* on aging and memory impairment has been demonstrated in an animal study as it prevented pyramidal cell loss in hippocampus and improved consolidation of the recall capability of stored information and spatial memory (Azzubaidi et al., 2011). Therefore, clinical studies have been designated to evaluate the effects of the plant on memory, attention, and cognition. Forty healthy elderly volunteers were randomly divided into an intervention group that received 13.3 mg/kg/day *N. sativa* capsule for 9 weeks and a control group that received placebo. At the end of the study, neuropsychological tests, including logical memory test, digit span test, Rey–Osterrieth complex figure test, letter cancellation test, trail making test, and Stroop test as well as a biochemical safety assay test, were performed. There were significant differences in all neuropsychological tests between the treated and placebo groups, but biochemical markers of cardiac, liver, and kidney functions did not significantly change after 9-week intervention, which showed the safety of the administered dose. According to these results, *N. sativa* supplementation could prevent or slow down Alzheimer's disease complications (Bin Sayeed et al., 2014). Conducting clinical trials with a larger sample size would help the safety and efficacy of plant in cognitive disorders.

The antiseizure effect of the plant oil (40–80 mg/kg/day) add-on therapy was compared with antiepileptic drugs in children suffering from intractable epileptic. In this randomized, single-blind, controlled, crossover pilot study, thirty intractable epileptic children were randomly assigned to either group I or group II. Intervention included receiving placebo for 4 weeks, followed by a 2-week washout period, and subsequently *N. sativa* oil given for 4 weeks in one group and the reverse order in another. After 4-week treatment with the plant oil, there were no significant differences in seizure frequency, severity, or oxidative stress markers (TAC and MDA) in epileptic children compared with baseline and placebo therapy. Although the oxidative stress markers were higher in intractable epileptic children compared with healthy children, *N. sativa* oil supplementation could not improve it, but no side effects were reported during the study (Shawki et al., 2013). However, in another similarly planned study, administration of TQ (the main constituents of the plant) showed antiepileptic effects in children with intractable epilepsy. In this double-blind crossover clinical study, the effects of TQ (1 mg/kg/day) adjuvant therapy on the frequency of seizures were compared with those of the placebo. TQ adjuvant therapy for 4 weeks reduced the frequency of seizures in comparison with baseline and placebo group, which showed its anticonvulsant effects (Akhondian et al., 2011). Table 4 shows a summary of the clinical effects of *N. sativa* and its constituents on neurological disorders.

3.8. Effects on cardiovascular disorders

Several in vitro and in vivo animal studies have reported the therapeutic effect of *N. sativa* on diabetes, metabolic syndrome, lipid profile disturbance, atherogenesis, endothelial dysfunction, cardiac mass and contractility abnormality, platelet aggregation, heart rate, blood pressure disorder, and cardiotoxicity. Therefore, *N. sativa* as a safe multipotential plant with potent antioxidant and anti-inflammatory properties could be used as a preventive and therapeutic agent in cardiovascular disorders (Shabana et al., 2013). A few studies have evaluated the cardioprotective activity of the plant. Controversial findings have reported about the cardiovascular effects of the plant and its constituents in both human and animal studies. Dehkordi et al. showed that oral *N. sativa* seed extract supplementation in patients with mild hypertension for 2 months may have a blood pressure-lowering effect. After treatment with 2.7 and 5.3 mg/kg/day of the plant extract, both systolic blood pressure (SBP) and diastolic blood pressure (DBP) values significantly reduced compared with the baseline. In addition, *N. sativa* supplementation significantly reduced the total and LDL-C concentrations compared with the baseline data. No complication was reported throughout the study in both test and placebo groups. In this study, the plant's essential oil phytochemical composition was determined by HPLC, but was not presented in the results of the published paper (Dehkordi and Kamkhah, 2008).

The effectiveness, safety, and tolerability of *N. sativa* powdered seeds (in capsules) on serum lipid levels, blood sugar, blood pressure, and body weight of 123 adult patients were evaluated in a randomized, double-blind trial. Patients of the intervention group were administered *N. sativa* (26.7 mg/kg/day) and the control group received powdered calcium lactate as placebo. In this long-term evaluation (from February 2006 to January 2007), 39 patients in the intervention group and 34 in the control group completed the study. Although the authors found favorable effects of the plant supplementation on all the measured parameters, the results were not statistically significant because of the small sample size. The safety and tolerability of the *N. sativa* supplementation were demonstrated in this study and no adverse effect on liver and kidney functions was found (Qidwai et al., 2009).

The antihypertensive effect of the plant oil (60 mg/kg/day) was

Table 4
Effects of *N. sativa* and its constituents on neurological, cardiovascular, and respiratory disorders and their anti-infertility properties.

Plant preparations	Study models	Effects	References
Neurological effects			
N. sativa seed (500 mg twice/day for 9 weeks)	Healthy elderly volunteers	Improvement in all neuropsychological tests	(Bin Sayeed et al., 2014)
N. sativa oil for 4 weeks	Intractable epileptic children	No significant changes in seizure frequency, severity, or oxidative stress markers (TAC and MDA)	(Shawki et al., 2013)
Thymoquinone (1 mg/kg)	Intractable epileptic children	Antiepileptic effects	(Akhondian et al., 2011)
Cardiovascular effects			
N. sativa seed extract (100/200 mg twice a day) for 2 months	Patients with mild hypertension	↓SBP and DBP; ↓TC and LDL	(Dehkordi and Kamkhah, 2008)
N. sativa seed	Adult patients	No significant decrease in serum lipid levels, blood sugar, blood pressure, and body weight	(Qidwai et al., 2009)
N. sativa oil (2.5 ml two times/day) for 8 weeks	Healthy volunteers	↓SBP and DBP	(Fallah Huseini et al., 2013)
Respiratory effects			
N. sativa powder & immunotherapy	Children with mild asthma	No effect on the Th17 cell number Improvement in clinical symptoms	(Kardani et al., 2013)
N. sativa powder & immunotherapy	Children with mild asthma	No effect on $CD4^+CD25^+$ $foxp3^+$Treg and $CD4^+$ $IL\text{-}10^+$ Improvement of clinical symptoms	(Susanti et al., 2013)
Boiled aqueous extract	Asthmatic patients	Improvement in all asthmatic symptoms, asthma symptom/week, chest wheeze, and PFT values Reducing the usage of inhaler and oral β-agonists, oral corticosteroid, oral theophylline, and inhaler corticosteroid reduction	(Boskabady et al., 2007)
Boiled aqueous extract	Chemical war victims	Decreasing the use of inhaler and oral β-agonists and oral corticosteroid in the study group	(Boskabady and Farhadi, 2008)
Boiled aqueous extract	Asthmatic patients	Lesser effectiveness on FEV_1, PEF, MMEF, MEF_{75}, MEF_{50}, MEF_{25}, and sGaw than theophylline	(Boskabady et al., 2010)
N. sativa oil	Asthmatic patients	PI decrement PEFR improvement	(Ahmad et al., 2010)
Anti-infertility properties			
N. sativa oil (5 ml/12 h) for 2 months	Infertile men	Improves sperm count, motility, morphology and semen volume, pH, and round cells	(Kolahdooz et al., 2014)

PFT (pulmonary function test), FEV1 (volume in one second), PEF (peak expiratory flow), MMEF (maximal mid expiratory flow), MEF (maximal expiratory flow), sGaw (specific airway conductance), Th, (T helper), foxp3 (factor forkhead box P3), Treg (Regulatory T), PEFR (peak expiratory flow rate), PI (pulmonary index).

also investigated in healthy volunteers. In this double-blind, randomized study, 70 healthy volunteers with systolic blood pressure (BP) from 110 to 140 mm Hg and diastolic BP from 60 to 90 mm Hg were randomly divided into intervention (2.5 ml *N. sativa* oil) and placebo (2.5 ml mineral oil) groups, who received medication two times a day for 8 weeks. The total phenol (HPLC method) and fatty acid (GC–MS) concentrations in the plant oil were measured and presented. At the baseline and the end of the study, the systolic and diastolic BPs, BMI, and blood concentrations of AST, ALT, alkaline phosphatase, creatinine, and blood urea nitrogen were measured. The SBP and DBP significantly reduced compared with baseline and placebo groups, while the other parameters did not change significantly. TQ, polyphenols, flavonoids, and unsaturated fatty acid contents of *N. sativa* were responsible for these antihypertensive effects. The results also showed that a daily supplementation of the plant oil did not result in any side effects of hepatic and renal functions. (Fallah Huseini et al., 2013). Table 4 shows a summary of the clinical effects of *N. sativa* and its constituents on cardiovascular disorders.

3.9. Effects on respiratory disorders

The preventive or prophylactic effect of *N. sativa* has been studied in a number of clinical research. The prophylactic effect of the plant boiled extract was shown in asthmatic patients. In the study group and control group, 15 mg/kg/day of 0.1 g% boiled extract and a placebo solution, respectively, were administrated daily throughout the study. The constituents of the essential oil of *N. sativa* were assessed by the HPLC method, but these results were not presented in the published paper. It was concluded that all asthma symptoms, chest wheeze, and pulmonary function test (PFT) values in a 3-month treatment period improved. In addition, the need for inhaled and oral β-agonists, oral corticosteroid, oral theophylline, and even inhaled corticosteroid decreased in *N. sativa*-treated patients (Boskabady et al., 2007). Regarding the relieving effect of this plant, the bronchodilatory effect of the boiled extract of the plant (50 and 100 mg/kg/day) in comparison with theophylline (6 mg/kg/day) was studied in 15 asthmatic patients. The quality of boiled extract of *N. sativa* was characterized by HPLC and documented by a fingerprint in the "Results" section of the paper. PFTs, including forced expiratory volume in 1 s (FEV_1), peak expiratory flow (PEF), maximal midexpiratory 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 at 30, 60, 90, 120, 150, and 180 min after oral administration of the extract and theophylline. The results of this study showed that *N. sativa* had a bronchodilatory effect, but this effect on most measured PFTs was less than that of theophylline at the concentrations used (Boskabady et al., 2010). The prophylactic effect of daily administration of the plant boiled aqueous extract (187 mg/kg/day of 50 g%) in chemical war victims was also examined for 2 months. Forty patients were randomly divided into two equal control and study groups. The use of inhaler, oral β-agonists, and oral corticosteroid after treatment in the study group was reduced at the end of the study, while there were no obvious changes in the amount of drug usage in untreated patients. All respiratory symptoms, chest wheezing, and PFT values in the study group significantly improved during the study visits, and no adverse effect was reported by the patients during the study period

(Boskabady and Farhadi, 2008).

The effect of immunotherapy in combination with probiotics and/or *N. sativa* (15 mg/kg/day) did not reduce the number of peripheral blood Th17 but induced a significant difference in the asthma control test (ACT) score compared with before intervention. No side effect was reported at the end of the study (Kardani et al., 2013). In another study, the effect of a combination of immunotherapy of house dust mites and probiotics or *N. sativa* (15 mg/kg/day) on the induction of $CD4^+$ IL-10^+ and $CD4^+CD25^+$ $foxp3^+$ Treg or the control of asthma symptoms in mild asthmatic children was also evaluated. The asthma symptoms were significantly reduced, but no significant reduction in other factors was observed in the treated groups. The authors attributed these bronchodilatory and anti-inflammatory activities to TQ. Immunotherapy and administration of *N. sativa* and probiotic caused no adverse effects in the patients (Susanti et al., 2013).

The therapeutic role of *N. sativa* oil (0.09 mg/kg/day) in wheezing associated with lower respiratory tract illness was demonstrated by Ahmed et al. In this study, the peak expiratory flow rate (PEFR) and pulmonary index (PI) were increased in 84 patients on the 3rd, 7th, 10th, and 14th days. The PI significantly decreased in treated groups as compared with the control group on all days of treatment. There was also a significant improvement in the PEFR of the test group as compared with the control group. Taking into account the bronchodilatory as well as anti-inflammatory effects of TQ and its high content in the plant oil, it was found to be responsible for those effects (Ahmad et al., 2010). Table 4 shows a summary of the clinical effects of *N. sativa* and its constituents on lung diseases.

3.10. Effect on infertility

The traditional use of *N. sativa* for treatment of infertility was evaluated in a randomized, double-blind, placebo-controlled clinical trial. Sixty-eight infertile men with abnormal semen quality were chosen with the inclusion criteria, such as abnormal sperm morphology $< 30\%$ or sperm count below 20×10^6/ml or type A and B motilities $< 25\%$ and 50%, respectively. The patients were randomly divided into *N. sativa* oil ($n=34$) and placebo group ($n=34$), who received medication orally two times a day for 2 months. The sperm count, motility, morphology, semen volume, pH, and round cells as primary outcomes were determined at the baseline and the end of the study. The results indicated that the daily intake of 5 ml *N. sativa* oil (60 mg/kg/day) for 2 months significantly improved the sperm count, motility, morphology, semen volume, pH, and round cells compared with the placebo group, without any side effects. Fatty acid content of fixed oil and chemical composition of the volatile oil components of the plant oil were determined and presented in this study. The antioxidant activity of TQ, vitamin E, selenium, and unsaturated fatty acid contents of the *N. sativa* oil may be responsible for this effect of the plant (Kolahdooz et al., 2014). Table 4 shows a summary of the anti-infertility effects of *N. sativa* and its constituents.

3.11. Toxicological studies and safety of N. sativa

Several studies have evaluated the acute and chronic toxic effects of *N. sativa* seeds and its fixed oil as well as of TQ in rats and mice, as given in detail below, and no toxic effects were reported. For acute toxicity, LD_{50} values of the plant's fixed oil (oral and intraperitoneal single dose administration to mice) were reported to be 26 and 1.9 mg/kg, respectively. In addition, hepatic enzyme levels, including AST, ALT, and gamma-glutamyltransferase, as well as histopathological modifications (heart, liver, kidneys, and pancreas) were not observed in rats treated with *N. sativa* (oral dose of 2 ml/kg) after 12 weeks of treatment (Zaoui et al., 2002). Moreover, the liver enzyme level did not change after supplementation of *N. sativa* up to a dose of 1 g/kg for 28 days, and no toxic effects on the liver function could be seen (Dollah et al., 2013b). The LD_{50} of TQ was determined to be 870.9 and 104.7 mg/kg in mice and 794.3 and 57.5 mg/kg in rats in terms of oral and intraperitoneal administration, respectively (Al-Ali et al., 2008; Khader et al., 2009). These doses are much greater than therapeutic doses and represent the relative safety of *N. sativa* and TQ, especially following oral administration. In addition, no serious side effects were reported in clinical trials (Paarakh, 2010).

4. Conclusion

The preclinical and clinical effects of *N. sativa* and its main constituent, TQ, on various diseases were reviewed. The reviewed papers showed the following pharmacological and clinical effects of the plant and its constituents:

1) Anti-inflammatory effects of the plant, TQ, and nigellone on basic and clinical studies.
2) Immunoregulatory effects of *N. sativa* and its lipid (affecting T-cell immunity) and water-soluble (affecting B-cell immunity) fractions, with TQ and nigellone being implicated in the clinical immunoregulatory effects of the plant.
3) Antimicrobial effects of the whole plant and its constituent, TQ.
4) Antitumor effects of ethanolic and aqueous extract and the fractions as well as its constituent, TQ, in various cancer cell lines.
5) Effects on metabolic disorders, including antidiabetic, antihyperlipidemic, metabolic syndrome, and hepatoprotective effects.
6) Effect on gastrointestinal *H. pylori*, which could be due to TQ, dihydrothymoquinone, and terpenes.
7) Effects on neurological disorders such as effect on aging and memory impairment in both animals and humans for the plant and antiepileptic effect for TQ.
8) Cardiovascular effects, mainly effect on hypertension, which was suggested to be due to TQ, polyphenols, flavonoids, and unsaturated fatty acids of the plant.
9) Effects on respiratory disorders, including bronchodilatory effect on asthmatic patients, preventive effect on asthma, and prophylactic effect on respiratory disorders of chemical war victims, all respiratory effects being exerted by its constituent, TQ.
10) Effect on infertility.

All the above findings demonstrated in various studies support the traditional use of *N. sativa*. In addition, a wide range of standard preparations (seed powder: 2-mg/day–3 g/day, oil: 40–10 ml/day, and TQ: 1 mg/day) were used as oral supplementation in different clinical studies, and no side effects and toxicity were reported.

However, only in a few clinical studies the phytochemical composition of the *N. sativa* extract or oil was assessed and in some of them the total phenol, fatty acid, and volatile oil components of *N. sativa* oil were presented. In addition, the clinical effects of phytochemical compositions of the plants were not studied. According to the described clinical studies, *N. sativa* and its constituents have health-promoting properties by exhibiting therapeutic effects on different disorders. In all the mentioned studies, despite demonstrating valuable findings, there were confounding factors and limitations that made data interpretation difficult. Lack of designing placebo control group, baseline biochemical profile, identification of active constituents as well as phytochemical assessment and formulation of the applied agents,

mentioning the type of randomization, uncontrolled patient diet or psychological status and physical activity, self-testing, small sample size, short duration of study, type of disease, ethnicity, and genotype are examples of those limitations. In addition, there are few studies about the pharmacological effects of other constituents of *N. sativa* such as nigellone, nigellamine, and alpha (α)-hederin. Therefore, more precise clinical studies regarding the effect of *N. sativa* and its constituents on various diseases are needed to ensure their exact clinical efficacy as well as the mechanisms of each effect.

References

Abdel-Fattah, A.M., Matsumoto, K., Watanabe, H., 2000. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone in mice. Eur. J. Pharmacol. 400, 89–97.

Aboul-Ela, M.A., El-Shaer, N.S., Ghanem, N.B., 1996. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. Pharmazie 51, 993–994.

Adel, A.-M., Morsy, B.M., Mahmoud, A.M., AboSeif, M.A., Zanaty, M.I., 2013. Beneficial therapeutic effects of *Nigella sativa* and/or Zingiber officinale in HCV patients in Egypt. EXCLI J. 12, 943–955.

Agarwal, R., Kharya, M.D., Shrivastava, R., 1979. Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn. Indian J. Exp. Biol. 17, 1264–1265.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A., Anwar, F., 2013. A review on therapeutic potential of *Nigella sativa*: a miracle herb. Asian Pac. J. Trop. Biomed. 3, 337–352.

Ahmad Alobaidi, A.H., 2014. Effect of *Nigella sativa* and *Allium sativum* coadministered with simvastatin in dyslipidemia patients: a prospective, randomized, double-blind trial. Antiinflamm. Antiallergy Agents Med. Chem. 13, 68–74.

Ahmad, J., Khan, R.A., Malik, M.A., 2010. A study of *Nigella sativa* oil in the management of wheeze associated lower respiratory tract illness in children. Afr. J. Pharm. Pharmacol. 4, 436–439.

Akhondian, J., Kianifar, H., Raoofziaee, M., Moayedpour, A., Toosi, M.B., Khajedaluee, M., 2011. The effect of thymoquinone on intractable pediatric seizures (pilot study). Epilepsy Res. 93, 39–43.

Akhtar, M., Maikiyo, A.M., Khanam, R., Mujeeb, M., Aqil, M., Najmi, A.K., 2012. Ameliorating effects of two extracts of *Nigella sativa* in middle cerebral artery occluded rat. J. Pharm. Bioallied Sci. 4, 70–75.

Akhtar, M.S., Riffat, S., 1991. Field trial of Saussurea lappa roots against nematodes and *Nigella sativa* seeds against cestodes in children. J. Pak. Med. Assoc. 41, 185–187.

Al-Ali, A., Alkhawajah, A.A., Randhawa, M.A., Shaikh, N.A., 2008. Oral and intraperitoneal LD50 of thymoquinone, an active principle of *Nigella sativa*, in mice and rats. J. Ayub Med. Coll. Abbottabad 20, 25–27.

Al-Hader, A.A., Aqel, M.B., Hasan, Z.A., 1993. Hypoglycemic effects of the volatile oil of *Nigella sativa* seeds. Int. J. Pharmacogn. 31, 96–100.

Al-Jassir, M.S., 1992. Chemical composition and microflora of black cumin (*Nigella sativa* L.) seeds growing in Saudi Arabia. Food Chem. 45, 239–242.

Al-Jenoobi, F.I., Al-Thukair, A.A., Abbas, F.A., Ansari, M.J., Alkharfy, K.M., Al-Mohizea, A.M., Al-Suwayeh, S.A., Jamil, S., 2010. Effect of black seed on dextromethorphan O- and N-demethylation in human liver microsomes and healthy human subjects. Drug Metab. Lett. 4, 51–55.

Al-Rasheed, N., Bassiouni, Y., Faddah, L., Mohamad, A.M., 2014. Potential protective effects of *Nigella sativa* and Allium sativum against fructose-induced metabolic syndrome in rats. J. Oleo Sci. 63, 839–848.

Al-Sheddi, E.S., Farshori, N.N., Al-Oqail, M.M., Musarrat, J., Al-Khedhairy, A.A., Siddiqui, M.A., 2014. Cytotoxicity of *Nigella sativa* seed oil and extract against human lung cancer cell line. Asian Pac. J. Cancer Prev. 15, 983–987.

Ali, B.H., Blunden, G., 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. 17, 299–305.

Alimohammadi, S., Hobbenaghi, R., Javanbakht, J., Kheradmand, D., Mortezaee, R., Tavakoli, M., Khadivar, F., Akbari, H., 2013. Protective and antidiabetic effects of extract from *Nigella sativa* on blood glucose concentrations against streptozotocin (STZ)-induced diabetic in rats: an experimental study with histopathological evaluation. Diagn. Pathol. 8, 137.

Aqel, M., Shaheen, R., 1996. Effects of the volatile oil of *Nigella sativa* seeds on the uterine smooth muscle of rat and guinea pig. J. Ethnopharm. 52, 23–26.

Ave-Sina, 1990. Law in Medicine. Ministry of Guidance Publication, Teheran, p. 314.

Azzubaidi, M.S., Talib, N.A., Saxena, A.K., Ahmed, Q.U., 2011. Mnemonic effects of Fixed Oil of Black Cumin (*Nigella sativa*) seeds on aged rats with memory impairment. In: Proceedings of Malaysia-Australia Research Colloquium on Exercise, Nutrition, Health and Wellness, 19th – 21st september, Penang.

Bamosa, A.O., Kaatabi, H., Lebdaa, F.M., Elq, A.M., Al-Sultanb, A., 2010. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J. Physiol. Pharmacol. 54, 344–354.

Barakat, E.M.F., El Wakeel, L.M., Hagag, R.S., 2013. Effects of *Nigella sativa* on outcome of hepatitis C in Egypt. World J. Gastroenterol. 19, 2529–2536.

Benhaddou-Andaloussi, A., Martineau, L., Vuong, T., Meddah, B., Madiraju, P., Settaf, A., Haddad, P.S., 2011. The in vivo antidiabetic activity of *Nigella sativa* is mediated through activation of the AMPK pathway and increased muscle Glut4 content. Evid.-Based Complement. Altern. Med. 2011, 9.

Benhaddou-Andaloussi, A., Martineau, L.C., Vallerand, D., Haddad, Y., Afshar, A., Settaf, A., Haddad, P.S., 2010. Multiple molecular targets underlie the antidiabetic effect of *Nigella sativa* seed extract in skeletal muscle, adipocyte and liver cells. Diabetes Obes. Metab. 12, 148–157.

Bhatti, I.U., Ur Rehman, F., Khan, M.A., Marwat, S.K., 2009. Effect of prophetic medicine kalonji [*Nigella sativa* l.] on lipid profile of human beings. An invivo approach. World Appl. Sci. J. 6, 1053–1057.

Bin Sayeed, M.S., Shams, T., Fahim Hossain, S., Rahman, M.R., Mostofa, A., Fahim Kadir, M., Mahmood, S., Asaduzzaman, M., 2014. *Nigella sativa* L. seeds modulate mood, anxiety and cognition in healthy adolescent males. J. Ethnopharmacol. 152, 156–162.

Boskabady, M.H., Farhadi, J., 2008. The possible prophylactic effect of *Nigella sativa* seed aqueous extract on respiratory symptoms and pulmonary function tests on chemical war victims: a randomized, double-blind, placebo-controlled trial. J. Altern. Complement. Med. 14, 1137–1144.

Boskabady, M.H., Javan, H., Sajady, M., Rakhshandeh, H., 2007. The possible prophylactic effect of *Nigella sativa* seed extract in asthmatic patients. Fundam. Clin. Pharmacol. 21, 559–566.

Boskabady, M.H., Kiani, S., Jandaghi, P., 2003. Comparison of antitussive effect of *Nigella sativa* with codein in guinea pig. Iran. J. Med. Sci. 28, 111–115.

Boskabady, M.H., Mohsenpoor, N., Takaloo, L., 2010. Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. Phytomedicine 17, 707–713.

Boulos, L., 1983. Medicinal Plants of North Africa. Reference Publications, Algonac, pp. 103.

Chaieb, K., Kouidhi, B., Jrah, H., Mahdouani, K., Bakhrouf, A., 2011. Antibacterial activity of Thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. BMC Complement. Altern. Med. 11, 29.

Chakravarty, N., 1993. Inhibition of histamine release from mast cells by nigellone. Ann. Allergy 70, 237–242.

Chevallier, A., 1996. The Encyclopedia of Medicinal Plants. DK Publishing, New York, pp. 237.

Daba, M.H., Abdel-Rahman, M.S., 1998. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. Toxicol. Lett. 16, 23–29.

Dehkordi, F.R., Kamkhah, A.F., 2008. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundam. Clin. Pharmacol. 22, 447–452.

Dirjomuljono, M., Kristyono, I., Tjandrawinata, R.R., Nofiarny, D., 2008. Symptomatic treatment of acute tonsillo-pharyngitis patients with a combination of *Nigella sativa* and *Phyllanthus niruri* extract. Int. J. Clin. Pharmacol. Ther. 46, 295–306.

Dollah, M.A., Parhizkar, S., Izwan, M., 2013a. Effect of *Nigella sativa* on the kidney function in rats. Avicenna J. Phytomed. 3, 152–158.

Dollah, M.A., Parhizkar, S., Latiff, L.A., Bin Hassan, M.H., 2013b. Toxicity effect of *Nigella sativa* on the liver function of rats. Adv. Pharm. Bull. 3, 97–102.

Durmuşkahya, C., Öztürk, M., 2013. Ethnobotanical survey of medicinal plants used for the treatment of diabetes in Manisa, Turkey. Sains Malays. 42, 1431–1438.

El-Dakhakhny, M., Madi, N.J., Lembert, N., Ammon, H.P., 2002. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. J. Ethnopharmacol. 81, 161–164.

El-Tahir, K.E., Ashour, M.M., Al-Harbi, M.M., 1993. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: elucidation of the mechanism of action. Gen. Pharmacol. 24, 1123–1131.

El-Tahir, K.E.H., Ashour, M.M., Al-Harbi, M.M., 1999. The respiratory effect of the volatile oil of the black seed (*Nigella sativa*) in guinea-pig: elucidation of the mechanism(s) of action. Gen. Pharmacol. 24, 1115–1122.

El-Tahir, K.E., Bakeet, D.M., 2006. The black seed *Nigella sativa* Linnaeus - a mine for multi cures: a Plea for urgent clinical evaluation of its volatile oil. J. Taibah Univ. Med. Sci. 1, 1–19.

El Kadi, A., Kandil, O., Tabuni, A.M., 1990. *Nigella sativa* and cell mediated immunity. Arch. AIDS Res. 1, 232–234.

Enomoto, S., Asano, R., Iwahori, Y., Narui, T., Okadam, Y., Singab, A.N., Okuyama, T., 2001. Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. Biol. Pharm. Bull. 24, 307–310.

Fallah Huseini, H., Amini, M., Mohtashami, R., Ghamarchehre, M.E., Sadeqhi, Z., Kianbakht, S., Fallah Huseini, A., 2013. Blood pressure lowering effect of *Nigella sativa* L. seed oil in healthy volunteers: a randomized, double-blind, placebo-controlled clinical trial. Phytother. Res. 27, 1849–1853.

Farzaneh, E., Nia, F.R., Mehrtash, M., Mirmoeini, F.S., Jalilvand, M., 2014. The effects of 8-week *Nigella sativa* supplementation and aerobic training on lipid profile and VO2 max in sedentary overweight females. Int. J. Prev. Med. 5, 210–216.

Gali-Muhtasib, H., Diab-Assaf, M., Boltze, C., Al-Hmaira, J., Hartig, R., Roessner, A., Schneider-Stock, R., 2004. Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. Int. J. Oncol. 25, 857–866.

Gali-Muhtasib, H., El-Najjar, N., Schneider-Stock, R., 2006. The medicinal potential of black seed (*Nigella sativa*) and its components. In: Mahmud, T.H.K., Arjumand, A. (Eds.), Advances in Phytomedicine. Elsevier, pp. 133–153.

Gheita, T.A., Kenawy, S.A., 2012. Effectiveness of *Nigella sativa* oil in the management of rheumatoid arthritis patients: a placebo controlled study. Phytother. Res. 26, 1246–1248.

Ghorbanibirgani, A., Khalili, A., Rokhafrooz, D., 2014. Comparing *Nigella sativa* oil and fish oil in treatment of vitiligo. Iran. Red Crescent Med. J. 16, e4515.

Greenish, H.G., 1880. Contribution to the chemistry of *Nigella sativa*. Pharmac. J. Trans. 10, 909–911.
Hadjzadeh, M.A., Rad, A.K., Rajaei, Z., Tehranipour, M., Monavar, N., 2011. The preventive effect of N-butanol fraction of *Nigella sativa* on ethylene glycol-induced kidney calculi in rats. Pharmacogn. Mag. 7, 338–343.
Hagag, A.A., Elaal, A.M.A., Elsheik, A., Elzamarany, E.A., 2013. Protective effect of *Nigella sativa* oil against methotrexate induced hepatotoxicity in children with acute lymphoblastic leukemia. J. Leuk. 1, 123.
Hanafy, M.S., Hatem, M.E., 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J. Ethnopharmacol. 34, 275–278.
Hannan, A., Saleem, S., Chaudhary, S., Barkaat, M., Arshad, M.U., 2008. Anti bacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant Staphylococcus aureus. J. Ayub Med. Coll. Abbottabad 20, 72–74.
Haq, A., Lobo, P.I., Al-Tufail, M., Rama, N.R., Al-Sedairy, S.T., 1999. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. Int. J. Immunopharmacol. 21, 283–295.
Haque, S.F., Nasiruddin, M., Najmi, A., 2011. Indigenous herbal product *Nigella sativa* proved effective as an anti-obesity therapy in metabolic syndrome. Int. J. Med. Res. 1, 133–176.
Heshmati, J., Namazi, N., 2015. Effects of black seed (*Nigella sativa*) on metabolic parameters in diabetes mellitus: a systematic review. Complement. Ther. Med. 23, 275–282.
Heshmati, J., Namazi, N., Memarzadeh, M.-R., Taghizadeh, M., Kolahdooz, F., 2015. *Nigella sativa* oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. Food Res. Int. 70, 87–93.
Hosseini, M.S., Mirkarimi, S.A., Amini, M., Mohtashami, R., Kianbakht, S., Fallah Huseini, H., 2013. Effects of *Nigella sativa* L. seed oil in type II diabetic patients: a randomized, double-Blind, placebo - controlled clinical trial. J. Med. Plants 12, 93–99.
Houghton, P.J., Zarka, R., de las Heras, B., Hoult, J.R., 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med. 61, 33–36.
Ibrahim, R.M., Hamdan, N.S., Ismail, M., Saini, S.M., Abd Rashid, S.N., Abd Latiff, L., Mahmud, R., 2014a. Protective effects of *Nigella sativa* on metabolic syndrome in menopausal women. Adv. Pharm. Bull. 4, 29–33.
Ibrahim, R.M., Hamdan, N.S., Mahmud, R., Imam, M.U., Saini, S.M., Rashid, S.N., Abd Ghafar, S.A., Latiff, L.A., Ismail, M., 2014b. A randomised controlled trial on hypolipidemic effects of *Nigella sativa* seeds powder in menopausal women. J. Transl. Med. 12, 82.
Isik, H., Cevikbas, A., Gurer, U.S., Kiran, B., Uresin, Y., Rayaman, P., Rayaman, E., Gurbuz, B., Buyukozturk, S., 2010. Potential adjuvant effects of *Nigella sativa* seeds to improve specific immunotherapy in allergic rhinitis patients. Med. Princ. Pract. 19, 206–211.
Kaatabi, H., Bamosa, A.O., Badar, A., Al-Elq, A., Abou-Hozaifa, B., Lebda, F., Al-Khadra, A., Al-Almaie, S., 2015. *Nigella sativa* improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: placebo controlled participant blinded clinical trial. PLoS One 10, e0113486.
Kalus, U., Pruss, A., Bystron, J., Jurecka, M., Smekalova, A., Lichius, J.J., Kiesewetter, H., 2003. Effect of *Nigella sativa* (black seed) on subjective feeling in patients with allergic diseases. Phytother. Res. 17, 1209–1214.
Kardani, A.K., Fitri, L.E., Barlianto, W., Olivianto, E., Kusuma, H.M.S.C., 2013. The effect of house dust mite immunotherapy, probiotic and *Nigella sativa* in the number of Th17 cell and asthma control test score. IOSR-JDMS 6, 37–47.
Khader, M., Bresgen, N., Eckl, P.M., 2009. In vitro toxicological properties of thymoquinone. Food Chem. Toxicol. 47, 129–133.
Kolahdooz, M., Nasri, S., Modarres, S.Z., Kianbakht, S., Huseini, H.F., 2014. Effects of *Nigella sativa* L. seed oil on abnormal semen quality in infertile men: a randomized, double-blind, placebo-controlled clinical trial. Phytomedicine 21, 901–905.
Landa, P., Marsik, P., Havlik, J., Kloucek, P., Vanek, T., Kokoska, L., 2009. Evaluation of antimicrobial and anti-inflammatory activities of seed extracts from six Nigella species. J. Med. Food 12, 408–415.
Latiff, L.A., Parhizkar, S., Dollah, M.A., Hassan, S.T., 2014. Alternative supplement for enhancement of reproductive health and metabolic profile among perimenopausal women: a novel role of *Nigella sativa*. Iran. J. Basic Med. Sci. 17, 980–985.
Le, P.M., Benhaddou-Andaloussi, A., Elimadi, A., Settaf, A., Cherrah, Y., Haddad, P.S., 2004. The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. J. Ethnopharmacol. 94, 251–259.
Mahfouz, M., Abdel-Maguid, R., El-Dakhakhny, M., 1965. The effect of "nigellone therapy" on the histaminopexic power of the blood sera in asthmatic patients. Arznei. Forsch. Drug Res. 15, 1230–1234.
Mahfouz, M., El-Dakhakhny, M., 1960. The isolation of a crystalline active principle from *Nigella sativa* seeds. J. Pharm. Sci. UAR 1, 9–19.
Mahmoud, M.R., El-Abhar, H.S., Saleh, S., 1991. The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. J. Ethnopharmacol. 79, 1–11.
Mahmoud, S.S., Torchilin, V.P., 2013. Hormetic/cytotoxic effects of *Nigella sativa* seed alcoholic and aqueous extracts on MCF-7 breast cancer cells alone or in combination with doxorubicin. Cell Biochem. Biophys. 66, 451–460.
Mansour, M., Tornhamre, S., 2004. Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. J. Enzym. Inhib. Med. Chem. 19, 431–436.
Mat, M.C., Mohamed, A.S., Hamid, S.S., 2011. Primary human monocyte differentiation regulated by *Nigella sativa* pressed oil. Lipids Health Dis. 10, 216.
Matthau, S.B., Ozcan, M.M., 2011. Fatty acids, tocopherol, and sterol contents of some Nigella species seed oil. Czech J. Food Sci. 29, 145–150.
Meddah, B., Ducroc, R., El Abbes Faouzi, M., Eto, B., Mahraoui, L., Benhaddou-Andaloussi, A., Martineau, L.C., Cherrah, Y., Haddad, P.S., 2009. *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J. Ethnopharmacol. 121, 419–424.
Memon, A.R., Shah, S.S., Memon, A.R., Naqvi, S.H.R., 2012. Effect of combination of *Nigella sativa* and Trigonella foenum-graecum with glibenclamide on serum triglycerides, HDL, and creatinine levels in type 2 diabetes mellitus patients. Pak. J. Pharmacol. 29, 1–6.
Mohamed, A., Shoker, A., Bendjelloul, F., Mare, A., Alzrigh, M., Benghuzzi, H., Desin, T., 2003. Improvement of experimental allergic encephalomyelitis (EAE) by thymoquinone; an oxidative stress inhibitor. Biomed. Sci. Instrum. 39, 440–445.
Mohamed, A., Waris, H.M., Ramadan, H., Quereshi, M., Kalra, J., 2009. Amelioration of chronic relapsing experimental autoimmune encephalomyelitis (cr-eae) using thymoquinone - biomed 2009. Biomed. Sci. Instrum. 45, 274–279.
Najmi, A., Nasiruddin, M., Khan, R.A., Haque, S.F., 2008. Effect of *Nigella sativa* oil on various clinical and biochemical parameters of insulin resistance syndrome. Int. J. Diabetes Dev. Ctries 28, 11–14.
Najmi, A., Nasiruddin, M., Khan, R.A., Haque, S.F., 2012. Therapeutic effect of *Nigella sativa* in patients of poor glycemic control. Asian J. Pharm. Clin. 5, 224–228.
Nasir, A., Siddiqui, M., Mohsin, M., 2014. Therapeutic uses of shoneez (*Nigella sativa* Linn.) mentioned in unani system of medicine – a review. Int. J. Pharm. Phytopharm. Res. 4, 47–49.
Nergiz, C., Ötleş, S., 1993. Chemical composition of *Nigella sativa* L. seeds. Food Chem. 48, 259–261.
Ng, W.K., Yazan, L.S., Ismail, M., 2011. Thymoquinone from *Nigella sativa* was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. Toxicol. Vitr. 25, 1392–1398.
Nickavar, B., Mojab, F., Javidnia, K., Amoli, M.A., 2003. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. Z. Nat. C. 58, 629–631.
Nikakhlagh, S., Rahim, F., Aryani, F.H.N., Syahpoush, A., Brougerdnya, M.G., Saki, N., 2011. Herbal treatment of allergic rhinitis: the use of *Nigella sativa*. Am. J. Otolaryngol. 32, 402–407.
Paarakh, P.M., 2010. *Nigella sativa* Linn. A comprehensive review. Indian J. Nat. Prod. Resour. 1, 409–429.
Peng, L., Liu, A., Shen, Y., Xu, H.Z., Yang, S.Z., Ying, X.Z., Liao, W., Liu, H.X., Lin, Z.Q., Chen, Q.Y., Cheng, S.W., Shen, W.D., 2013. Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-kappaB pathway. Oncol. Rep. 29, 571–578.
Polat, R., Satil, F., Cakilcioglu, U., 2011. Medicinal plants and their use properties of sold in herbal market in Bingoel(Turkey) district. Biol. Divers. Conserv. 4, 25–35.
Qidwai, W., Hamza, H.B., Qureshi, R., Gilani, A., 2009. Effectiveness, safety, and tolerability of powdered *Nigella sativa* (kalonji) seed in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults: results of a randomized, double-blind controlled trial. J. Altern. Complement. Med. 15, 639–644.
Qidwai, W., Ashfaq, T., 2014. Effect of dietary supplementation of black seed (*N. Sativa* L.) on lipid profile of patients suffering from diabetes. Antiinflamm. Antiallergy Agents Med. Chem. 13, 3–8.
Rafati, S., Niakan, M., Naseri, M., 2014. Anti-microbial effect of *Nigella sativa* seed extract against staphylococcal skin Infection. Med. J. Islam. Repub. Iran. 28, 42.
Rakhshandeh, H., Vahdati-Mashhadian, N., khajekaramadini, M., 2011. In vitro and in vivo study of the antibacterial effects of *Nigella sativa* methanol extract in dairy cow mastitis. Avicenna J. Phytomed. 1, 29–35.
Randhawa, M.A., Alghamdi, M.S., 2011. Anticancer activity of *Nigella sativa* (black seed) – a review. Am. J. Chin. Med. 39, 1075–1091.
Rchid, H., Chevassus, H., Nmila, R., Guiral, C., Petit, P., Chokairi, M., Sauvaire, Y., 2004. *Nigella sativa* seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. Fundam. Clin. Pharmacol. 18, 525–529.
Roepke, M., Diestel, A., Bajbouj, K., Walluscheck, D., Schonfeld, P., Roessner, A., Schneider-Stock, R., Gali-Muhtasib, H., 2007. Lack of p53 augments thymoquinone-induced apoptosis and caspase activation in human osteosarcoma cells. Cancer Biol. Ther. 6, 160–169.
Sabzghabaee, A.M., Dianatkhah, M., Sarrafzadegan, N., Asgary, S., Ghannadi, A., 2012. Clinical evaluation of *Nigella sativa* seeds for the treatment of hyperlipidemia: a randomized, placebo controlled clinical trial. Med. Arch. 66, 198–200.
Salem, E.M., Yar, T., Bamosa, A.O., Al-Quorain, A., Yasawy, M.I., Alsulaiman, R.M., Randhawa, M.A., 2010. Comparative study of *Nigella sativa* and triple therapy in eradication of Helicobacter Pylori in patients with non-ulcer dyspepsia. Saudi J. Gastroenterol. 16, 207–214.
Salem, M.L., 2005. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int. Immunopharmacol. 5, 1749–1770.
Salman, M.T., Khan, R.A., Shukla, I., 2008. Antimicrobial activity of *Nigella sativa* Linn. Seed oil against multi-drug resistant bacteria from clinical isolates. Nat. Prod. Radiance 7, 10–14.
Shabana, A., El-Menyar, A., Asim, M., Al-Azzeh, H., Al Thani, H., 2013. Cardiovascular benefits of black cumin (*Nigella sativa*). Cardiovasc. Toxicol. 13, 9–21.
Shahraki, S., Khajavirad, A., Shafei, M.N., Mahmoudi, M., Tabasi, N.S., 2015. Effect of total hydroalcholic extract of *Nigella sativa* and its n-hexane and ethyl acetate fractions on ACHN and GP-293 cell lines. J. Tradit. Complement. Med., 1–8.
Shawki, M., El Wakeel, L., Shatla, R., El-Saeed, G., Ibrahim, S., Badary, O., 2013. The clinical outcome of adjuvant therapy with black seed oil on intractable paediatric seizures: a pilot study. Epileptic Disord. 15, 295–301.
Susanti, N., Barlianto, W., Kalim, H., Kusuma, H.M.S.C., 2013. Asthma clinical improvement and reduction in the number of CD4+CD25+foxp3+Treg and CD4+IL-10+cells after administration of immunotherapy house dust mite and

adjuvant probiotics and/or *Nigella sativa* powder in mild asthmatic children. IOSR-JDMS 7, 50–59.
Swamy, S.M., Tan, B.K., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. J. Ethnopharmacol. 70, 1–7.
Tauseef Sultan, M., Butt, M.S., Anjum, F.M., 2009. Safety assessment of black cumin fixed and essential oil in normal Sprague Dawley rats: Serological and hematological indices. Food Chem. Toxicol. 47, 2768–2775.
Tembhurne, S.V., Feroz, S., More, B.H., Sakarkar, D.M., 2011. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. J. Med. Plants Res. 8, 167–177.
Wienkotter, N., Hopner, D., Schutte, U., Bauer, K., Begrow, F., El-Dakhakhny, M., Verspohl, E.J., 2008. The effect of nigellone and thymoquinone on inhibiting trachea contraction and mucociliary clearance. Planta Med. 74, 105–108.
Winkler, C., Schroecksnadel, K., Ledochowski, M., Schennach, H., Houcher, B., Fuchs, D., 2008. In vitro effects of *Nigella sativa* seeds extracts on stimulated peripheral. Blood Mononucl. Cells Pteridines 19, 101–106.
Woo, C.C., Kumar, A.P., Sethi, G., Tan, K.H., 2012. Thymoquinone: potential cure for inflammatory disorders and cancer. Biochem. Pharmacol. 83, 443–451.
Yousefi, M., Barikbin, B., Kamalinejad, M., Abolhasani, E., Ebadi, A., Younespour, S., Manouchehrian, M., Hejazi, S., 2013. Comparison of therapeutic effect of topical Nigella with Betamethasone and Eucerin in hand eczema. J. Eur. Acad. Dermatol. Venereol. 27, 1498–1504.
Zaoui, A., Cherrah, Y., Lacaille-Dubois, M.A., Settaf, A., Amarouch, H., Hassar, M., 2000. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapie 55, 379–382.
Zaoui, A., Cherrah, Y., Mahassini, N., Alaoui, K., Amarouch, H., Hassar, M., 2002. Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomedicine 9, 69–74.
Zohary, D., Hopf, M., Weiss, E., 2012. Domestication of Plants in the Old World: The Origin and Spread of Domesticated Plants in Southwest Asia, Europe, and the Mediterranean Basin. OUP, Oxford.