Nigella sativa (Black Seed) Active Constituents and Therapeutic Applications

INTRODUCTION

Nigella sativa (N. sativa) is a plant native to, and cultivated in, North Africa, Turkey, and the Middle East has been used throughout the world medicinally and as a food seasoning for centuries. It is widely used in various traditional systems of medicine such as Ayurveda, Unani, and Siddha. The seeds of *N. sativa* are employed as a therapeutic tool for many different ailments and conditions. *N. sativa* has been shown to have antihypertensive, antimicrobial, immunomodulatory, diuretic, digestive, analgesic, antimicrobial, hypoglycemic, antioxidant, anti-inflammatory, anticancer, and antihistaminic properties, among others. Extensive preclinical, animal, and clinical research has been conducted on *N. sativa*'s properties. Due to its multitude of potential applications, *N. sativa* is among the top-ranked, evidence-based herbal medicines. Research has also revealed that most of the therapeutic properties of this plant are attributed to its essential oil constituent, thymoquinone. This paper endeavors to provide a detailed report on the characteristics, historical and modern uses, chemical composition, benefits of standardization, pharmacological activities, safety, toxicology, and recommended dosages of *Nigella sativa*.

DESCRIPTION, CULTIVATION, AND TAXONOMY

Nigella sativa, commonly known as Black Seed is an annual plant native to Northern Africa, Syria, Iraq, Iran, and Turkey[1-5] and is also cultivated commercially in various regions of Africa, the Middle East, India, and Paksitan.[6-8] *Nigella sativa* is the most well-known species of the Nigella genus and grows up to 35 inches tall with narrow, threadlike leaves. Its flowering season is brief but produces white, pale blue, or purple flowers that yield capsules containing many black seeds.[4] *Nigella sativa* is also known as kalonji (from the Arabic Unani system of medicine),[9] black cumin, and black caraway. However, the latter two names may be misleading as they refer to other common spice plants (*Cuminum cyminum* and *Carum carvi*, respectively) that are from completely different genera, each with its own unique properties.[10]

HISTORICAL USES

Sources report *Nigella sativa* seeds have been used medicinally in the Middle East and Southeast Asia for over 2,000 years.[5,11] Black cumin seeds are mentioned in the Bible as well as in the Koran. People think of *N. sativa* as a holy plant created by God in order to relieve difficult medical conditions and in Arabic it used to be called "the blessing seed." Greek pharmaco-botanist, Dioscorides (40-90 CE) described the use of black seeds from a plant now thought to be *N. sativa* as a remedy for breathing difficulties, inflammatory conditions, skin ailments, and parasites.[12] Nigella seeds were also historically thought to be useful for the afterlife journey and were found in the tomb of Egyptian Pharaohs.

Where Ayurvedic Medicine is accepted and utilized, Nigella seed is commonly found in preparations used as remedies for abdominal bloating, gas, diarrhea, and intestinal worm infestations.[13] In the Unani system of medicine practiced in India, Pakistan, Bangladesh, Malaysia, and Sri Lanka, dried Nigella seed (kalonji) is used for gastrointestinal conditions, asthma, headache and migraine, joint and low-back pain, nerve paralysis (hemiplegia, Bell's palsy), jaundice, vitiligo, and other skin conditions.[9] In the United States, the Food and Drug Administration (FDA) granted *Nigella sativa* Generally Recognized as Safe (GRAS) status for use as a spice, seasoning, or flavoring,[10] *Nigella sativa* cold press oil has been used in the US and European markets for the last 30 years.

CURRENT USES AND RESEARCH

Preclinical, animal, and clinical research has demonstrated *N. sativa*'s beneficial effects on inflammation, microbial infections, respiratory conditions and allergies, immune function, blood pressure and cardiovascular function, blood sugar modulation, liver function, wound healing, some aspects of cancer, among others.[14] There are approximately over 30 clinical trials evaluating the efficacy of Nigella seed preparations for a variety of conditions in humans including arthritis, cardiovascular conditions, diabetes,

metabolic syndrome and obesity, respiratory conditions, and skin or cosmetic applications. Other research has investigated its use in humans for gastrointestinal conditions, seizures, mood and cognitive function, Hepatitis C, male infertility, cyclical breast pain, thyroid conditions, and liver function. Also noteworthy is the important and ongoing preclinical research investigating the effects of *N. sativa*'s thymoquinone constituent in various human carcinoma cell lines and animal cancer models, which will be discussed below.

RAW MATERIALS AND STANDARDIZATION

RAW MATERIALS

Nigella sativa is typically available commercially as either seeds, seed powder, or seed oil. Both forms have their own unique benefits. Nigella seeds have a nutty or peppery taste and are used in food preparation as a spice or flavor component in many different dishes and breads.[15] The seed's skin and pulp contain unique healing compounds of their own. The compound nigellone is found in particularly high concentrations in the skin of the Nigella seed and exhibits powerful antihistaminic properties.[16] The whole Nigella seed has been proven to be beneficial for a variety of health conditions. Research on the effects of Nigella seeds consistently demonstrates a dose-dependent effect.[17] In other words, the more consumed, the greater the benefit. Unfortunately, when whole Nigella seeds are eaten and chewed, it is difficult to ingest enough of the seeds to obtain a therapeutic amount of its important constituents.

Nigella seed oil is the lipid component derived from either cold press or solvent extraction of the *Nigella sativa* plant's seeds. Nigella seed oil (NSO) is liquid at room temperature. The constituents in Nigella seed oil are much more highly concentrated compared to a similar volume of whole Nigella seed. Scientific analysis has found that the Nigella seed oil contains high concentrations of many different healing components; therefore, using a smaller quantity of Nigella seed oil enables one to get the concentrated lipid contents that would be found in a much larger volume of Nigella seeds.[18]

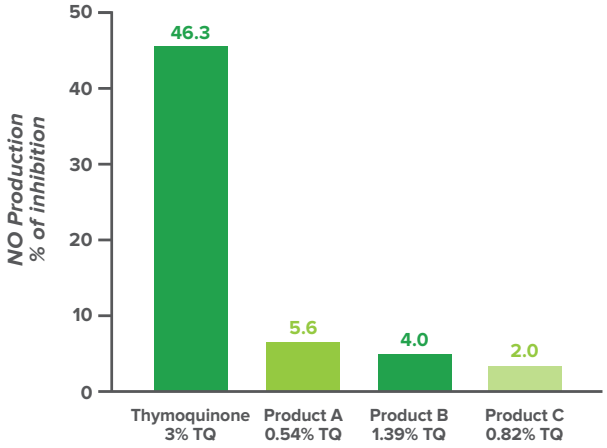
Cold-press extracted Nigella seed oil is the preferred form because it does not involve the use of solvents or heat.[19,20] Supercritical fluid extraction is another technique used to extract plant oils and may offer some favorable features such as isolation of specific desired constituents.[21] Generally speaking, when extracting oil with a regular TQ content of 0.3 - 0.7 percent, and trying to generate a higher-TQ content extract, selective extraction processes are required, which usually change the oil composition ratios to something other than that found in nature.[22]

Using heat during extraction causes some of the more sensitive active compounds to oxidize , diminishing the quality of the oil.[20] Ideally the pressing should be done slowly, as a fast pressing can generate excessive heat , which may discolor the oil and give it a bitter taste. Nigella seed oil extractions using solvents can create selective extraction and composition but will not maintain the whole oil composition as found in nature. Also, extraction chemicals such as benzene are undesirable as these solvents can contaminate the end-product and change the yield of various constituents.[21,22] The slow, cold-press extraction method of producing NSO has a long historical use prior to DSHEA and Novel Food legislation.

STANDARDIZED EXTRACTS

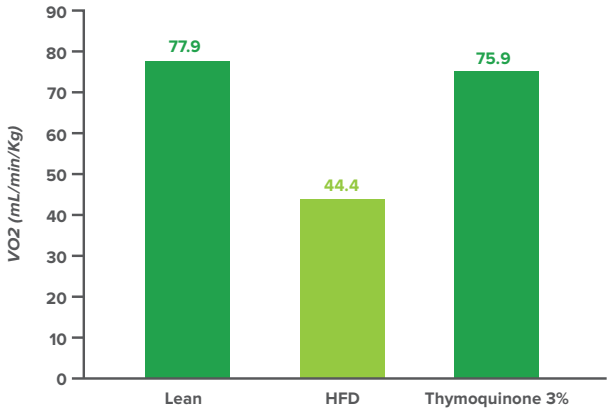
Two years ago, **TriNutra**™ began a *Nigella sativa* breeding program to yield high-concentration, Non-GMO thymoquinone varietals that thrive in the Israeli climate and yield 2-3 times the thymoquinone content of other varieties. TriNutra's™ Nigella seed oil, is extracted from these varietals via slow, cold-press extraction and is standardized to 3-percent thymoquinone, a higher concentration of this important therapeutic constituent. Cold-pressed NSO standardized to 3-percent thymoquinone offers superior performance in terms of its anti-inflammatory potential as compared to other Nigella seed oil preparations that are standardized to lower TQ concentrations or not standardized at all.

ThymoQuinone Concentration vs. Inflammation Inhibition (NO Production)



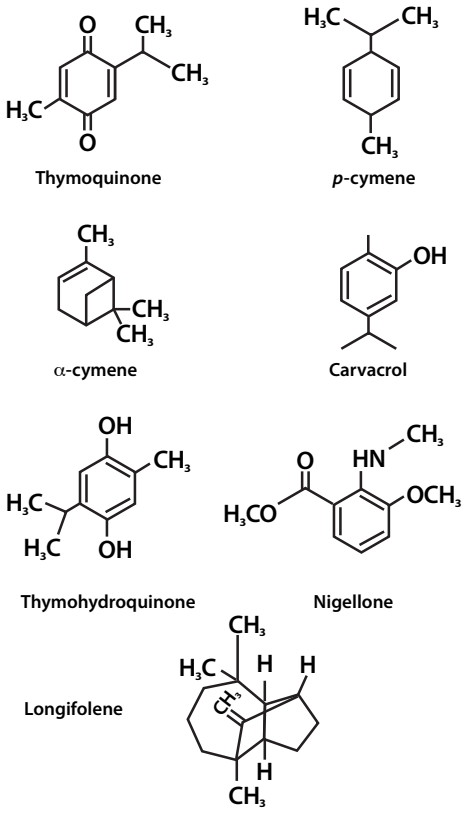
A recent study using a preclinical model of metabolic syndrome demonstrated Thymoquinone 3% administration to mice resulted in higher oxygen (VO2) consumption as compared to mice on a high-fat diet. Increased oxygen consumption is a marker of greater mitochondrial function and biogenesis, indicating a decrease in adipogenesis. Levels of oxygen consumption in the group of metabolic syndrome mice given Thymoquinone 3% was nearly equal to that of lean, healthy control mice. Significant improvements in blood pressure and blood sugar values were also observed in the Thymoquinone 3%-treated mice compared to controls.[2]

Nigella sativa (Thymoquinone 3% Effect on Oxygen Consumption in a Mouse Model of Metabolic Syndrome



ACTIVE CONSTITUENTS

Active Constituents of _Nigella sativa_ Essential Oil



Extensive research has been conducted to identity the composition of *Nigella sativa*. Primary constituents include: fixed oil, proteins, alkaloid, saponin and essential oil.[24] The fixed oil (32-40 %) contains unsaturated fatty acids which include: arachidonic, linoleic, linolenic, oleic, and palmitic acids, aliphatic hydrocarbons, and tocopherols, as well as beta-sitosterol, cycloeucalenol, cycloartenol, sterol esters and sterol glucosides.[25-27] The volatile/essential oil (0.4-0.45 %) is comprised mainly of monoterpenes, including thymoquinone (TQ) – the constituent responsible for the bulk of *N. sativa*'s therapeutic effects, thymohydroquinone (THQ), *p*-cymine, α and β-pinene, longifolene, and carvacrol. Nigellone is also a component of the essential oil and is thought to be polymer of TQ, while nigellin is thought to be an alkaloid.[25,26] Most of the pharmacological effects attributed to *N. sativa* are due to the thymoquinone constituent. TQ possesses anti-inflammatory,[27] anti-cancer,[28] antioxidant,[22] cardioprotective,[30] hypoglycemic,[31] antihistaminic,[32] antibacterial,[33] antifungal activity,[35] anticonvulsant activity,[21] in addition to numerous other properties.

PHARMACOKINETICS AND BIOAVAILABILITY

Nigella seed oil and thymoquinone are both very lipophilic and heat labile. As such, studying bioavailability is somewhat challenging, so consequently pharmacokinetic studies in humans are limited. Currently available clinical research has been aimed at improving absorption and bioavailability through liposomal and nanoparticle formulations of thymoquinone.[34,36] Despite the lack of pharmacokinetic research, it is apparent that NSO is absorbed from an oral dose and has some degree of bioavailability, as demonstrated by its beneficial effects on blood sugar, blood lipids, and markers of inflammation.

MECHANISMS OF ACTION

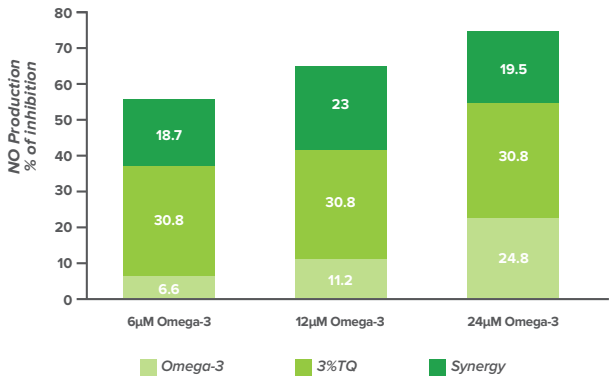
The oil and the seed constituents of *N. sativa*, but primarily thymoquinone (TQ), exhibit potent anti-inflammatory effects in several inflammation-based models. Via suppression of the inflammatory mediators, prostaglandins, and leukotrienes, *N. sativa* extracts have demonstrated inflammation-modulating properties in experimental encephalomyelitis, colitis, peritonitis, edema, and arthritis. Research has shown that 0.45 mg Nigella seed oil (3% TQ) has been shown in an *in vitro* model to inhibit NO production by LPS-stimulated macrophages to a degree equivalent to 1 mg Curcumin C3, and also has been shown to potentiate the anti-inflammatory capacity of omega-3 oils.[23]

Raw oil and the TQ constituent have been shown to augment T-cell and natural killer cell-mediated immune responses, demonstrating thymoquinone's beneficial immunomodulatory and respiratory-stimulating properties.[37-39] In addition, both the oil and its active ingredients expressed antimicrobial activity toward a variety of microbes[33] and inhibitory properties toward certain types of cancer.[28,40]

N. sativa's and TQ's cardiovascular benefits have been demonstrated in both preclinical studies and clinical trials. There are a number of mechanisms behind its beneficial cardiovascular effects. TQ

appears to modulate inflammation and oxidative stress, improve vascular contractile responsiveness, modulate lipid profiles, modulate blood pressure, and inhibit prothrombotic events.[41-44] Research has also demonstrated *N. sativa* constituents inhibit gluconeogenesis, regulate liver enzyme activity associated with glucose metabolism, preserve and improve proliferation of pancreatic beta cells, and prevent oxidative stress in both clinical trials and experimental models of hyperglycemia and diabetes.[45-48] Other mechanisms of action attributed to *N. sativa* constituents are neuroprotective activity via opioid receptor stimulation and reduction of neuronal degeneration by TQ,[49] hepatoprotective and nephroprotective activity via the antioxidant effects of thymoquinone,[50,51] and wound-healing activity via enhanced fibroblast proliferation and promotion of beta-fibroblast growth factor.[52] Numerous preclinical and clinical trials demonstrate these beneficial effects and underscore *Nigella sativa* constituents' multitude of potential therapeutic applications. Many studies confirm the pharmacological efficacy of *Nigella sativa* seed constituents, however, as a complex botanical containing more than 100 compounds, many of which have not yet been identified or studied, there is still much work to be done to reveal this plant's full potential.[53]

Synergy of Omega-3 and 3% ThymoQuin™ (Patent-Pending Composition)
Omega-3 Composition: EPA 46%, DHA 38%



Thymoquinone 3% has been demonstrated to potentiate the anti-inflammatory activity of omega-3 oils when the two are combined – an effect not observed with other NSO products on the market.

CLINICAL RESEARCH AND INDICATIONS

INFLAMMATORY CONDITIONS

Because of the powerful inflammation modulating properties of TQ and other constituents, significant clinical research has been conducted on various inflammatory conditions ranging from rheumatoid and osteoarthritis, to pain associated with hormonal cycles in women, and obesity-associated inflammation.

Arthritis

Nigella sativa clinical research in osteo-and rheumatoid arthritis best illustrates its effect on inflammatory conditions, as evidenced by several clinical trials since 2012. A clinical trial in elderly subjects explored the benefits of *N. sativa* for symptoms of osteoarthritis of the knee. Forty elderly men and women (mean age 77 years) investigated the use of topical *N. sativa* oil or acetaminophen on knee osteoarthritis over a period of three weeks. Treatment or placebo was administered three times daily and pain was assessed. Results showed that both topical application of NSO and oral acetaminophen reduced pain in elderly subjects with knee osteoarthritis; but pain reduction was greater in those using *Nigella sativa* oil.[54]

Three clinical trials conducted between 2012 and 2016 evaluated *N. sativa*'s effects on rheumatoid arthritis (RA). In the earliest trial 40 women with RA were given placebo capsules twice daily for one month, then switched to 500 mg cold pressed *Nigella sativa* oil (NSO) twice daily for another month. Significant improvements were reported in participants' Disease Activity Scores (DAS-28), measuring joint swelling and tenderness, when compared to baseline and post-placebo ratings.[55] In a randomized, double-blind, placebo-controlled (RDBPC) trial, 42 subjects diagnosed with RA were randomized to receive either 500 mg NSO or placebo soft gels twice daily for eight weeks. Several markers of inflammation (serum TNF-α and the anti-inflammatory cytokine, interleukin -10 [IL-10]) and oxidative stress (serum malondialdehyde [MDA] and nitric oxide [NO]) were measured at baseline and at the end of the 8-week trial. A significant increase in IL-10, as well as significant decreases in MDA and NO were observed in the NSO group at the end of the trial, as compared to placebo, suggesting NSO may improve inflammation and oxidative stress in RA patients.[56] Another RDBPC, parallel group trial investigated NSO's immunomodulatory effect over a two-month period in 43 female patients with RA (age 20-50 years). Treatment with NSO significantly reduced serum high-sensitivity C-reactive protein (hs-CRP) level, a marker of inflammation, reduced overall DAS-28 score, and improved the number of swollen joints compared with baseline and placebo groups. NSO treatment also resulted in reduced CD8(+), and increased CD4(+)CD25(+) T cell percentage and the CD4(+)/CD8(+) ratio as compared to placebo and baseline indicating an improved immune response.[57]

Asthma

To date, four clinical trials have evaluated the efficacy of *N. sativa* in subjects with diagnosed asthma or asthma-like symptoms. Two of these studies were published in 2017. The first RDBPC trial investigated the effects of 500 mg cold pressed NSO (0.7% thymoquinone) or placebo given twice daily to 80 subjects with asthma for four weeks. Significant improvements in mean Asthma

Control Test scores were observed in those in the NSO group when compared to placebo. Blood eosinophils, a biomarker of inflammation in asthma were also significantly reduced in NSO subjects. Trends toward improved pulmonary function and peak expiratory flow were also observed in the treatment group.58 In a single-blind, randomized, placebo-controlled trial of *Nigella sativa* (NS) 50 subjects with asthma were divided into 3 groups: placebo (n=24), NS-1 (n=13, 1g NS daily), and NS-2 (n=13, 2g NS daily), along with maintenance inhaler therapy for three months The Asthma Control Test (ACT) score, peak expiratory flow (PEF) variability, fractional exhaled nitric oxide (FeNO) and other pulmonary function tests, IgE, serum cytokines, and exacerbation frequency were evaluated. Forced expiratory flow and volume increased significantly at mid- and endpoints in the NS-2 group compared to controls. In both treatment groups peak expiratory variability significantly improved and inflammation markers decreased significantly, compared to baseline and controls after 12 weeks. Both doses of NS significantly increased serum IFN-gamma and improved ACT scores compared to baseline. Significantly fewer patients had exacerbations in the NS-1 group.[59] Two earlier trials support these studies and further underscore the ability of *N. sativa* to be an effective modulator of many symptoms of asthma and asthma-like symtpoms.[60,61] Additional, long-term trials are warranted, perhaps designed to determine the plant constituents responsible for these effects.

Other Respiratory Conditions

In addition to asthma, *N. sativa*'s effects have been studied in other respiratory conditions. Three of these studies investigated its effect on allergic rhinitis and produced mixed results. Study durations were between 4-6 weeks and dosage varied significantly between studies, however, two of the three reported significant improvement in symptoms of allergic rhinitis.[62-64]

CHEMOTHERAPY-INDUCED FEBRILE NEUTROPENIA

Clinical research on *N. sativa*'s effects in cancer patients is scarce, but one trial explored its activity in preventing chemotherapy-induced febrile neutropenia (FN) in 80 children (aged 2-18 years) with brain tumors. In this study, 40 children were given five grams NS seeds daily (instructed to chew the seeds) for three months and showed a significant improvement in the incidence and course of febrile neutropenia as compared to 40 children in the control group who received no intervention. A 17.1-percent decrease in incidence and 2.5-day decrease in length of hospital stay, compared to the control group was reported. Children in the intervention group experienced significantly fewer FN episodes (2.2 percent) compared to the control group (19.3 percent). Those in the intervention group also experienced a shorter duration of hospital stay during FN episodes when compared to those in the control group who were hospitalized for longer periods (2.5 days vs 5 days). Additionally, children in the intervention group lost significantly less body weight (15.7 percent) compared to children in the control group (30.4 percent). Hematological indices were also assessed in both the intervention and control group. Children in the intervention group had a statistically significant decrease in incidence of neutropenia compared to the control group, but no statistically significant differences between the two groups regarding incidences of anemia, thrombocytopenia, and leucopenia.[65]

Preclinical research has also revealed the effects of crude oils of *N. sativa* on tumor formation in a rat multi-organ carcinogenesis model. Tumor-laden male rats were given 1000 or 4000 ppm *N. sativa* volatile oil (NSO) in the diet for 30 weeks. NSO significantly reduced malignant and benign colon tumor sizes, as well as incidences and multiplicities in the lungs, esophagus, and fore stomach. It was shown that *N. sativa* administration exerts potent inhibitory effects on rat tumor development and on cellular proliferation in multiple organ sites, with no evidence of side effects.[66] Numerous *in vitro* studies have demonstrated the cytotoxic effects of different *N. sativa* seed extracts. To date, studies have shown *N. sativa* extracts to be cytotoxic to MCF-7 breast cancer cells,[67] but the bulk of *in vitro* research has focused on the cytotoxic activity of the isolated thymoquinone constituent. In one study utilizing the human osteosarcoma cell line SaOS-2, TQ induced a higher percentage of growth inhibition and apoptosis than that of control, via inhibition of tumor angiogenesis and growth through suppressing NF-κB and associated molecules.[68] TQ cytotoxicity has also been demonstrated in human cervical squamous carcinoma cells,[69] T-cell lymphoma tumor cells,[70] pancreatic cancer cells,[71] human leukemia cells,[72] and MCF- breast carcinomas.[67]

MICROBIAL INFECTIONS

Preclinical and clinical research has demonstrated *N. sativa* seed powder and oil exhibits antibacterial, antifungal, antiviral, and antiparasitic activity, suggesting its potential as a useful tool in managing these infections and underscores its use against these organisms in traditional medicine systems.

Pathogenic Bacteria

Several crude extracts of *N. sativa* were tested for antimicrobial efficacy against various bacterial isolates (16 gram-negative and six gram-positive representatives). Inhibitory effects on the growth of *Yersinia enterocolitica*, *Listeria monocytogenes*, *Corynebacterium pseudotuberculosis* and *renale*, *Brucella abortus*, *Pasteurella multocida*, *Mannheimia haemolytica*, *E. coli*, *Trueperella pyogenes*, and *S. aureus* are reported. Bacterial inhibition by *N. sativa* was more effective against gram-negative organisms than gram-positive.[73] The antibacterial activity of *N. sativa* against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) was investigated in 2008. All tested strains of MRSA were sensitive to

N. sativa at a concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/mL.[74] *N. sativa*'s thymoquinone constituent has also been shown to have significant bactericidal activity against gram positive *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* CIP 106510 strains.[75] A clinical trial investigated *N. sativa*'s inhibitory activity against *Helicobacter pylori* in 88 patients with non-ulcer dyspepsia. *N. sativa* demonstrated inhibition of *H. pylori* comparable to that of triple antibiotic therapy.[76]

Pathogenic Fungi

Certain extracts of *N. sativa* have strong inhibitory effects against various strains of *Candida albicans*. Mice inoculated with *Candida albicans* produced colonies of the organism in the liver, spleen and kidneys. Subsequent treatment with *N. sativa* extract 24 hours post-inoculation resulted in a significant inhibitory effect on the growth of the organism in all organs studied.[77]

Activity of *N. sativa* and TQ was tested *in vitro* against eight species of dermatophytes: four species of Trichophyton rubrum and one each of Trichophyton interdigitale, Trichophyton mentagrophytes, Epidermophyton floccosum and Microsporum canis . The inhibitory activity of *N. sativa* against these species supports the potential development of thymoquionone from *N. sativa* as a therapy and explains its successful use in folk medicine for the treatment of fungal skin infections.[78] Further research revealed two novel antifungal defensins (Ns-D1 and Ns-D2), isolated from seeds of *N. sativa* with strong antifungal activity against several pathogenic fungi.[79]

Viral Pathogens

A small clinical trial in Hepatitis C patients explored the potential benefits of *N. sativa* as well as efficacy, safety, and tolerability of supplementation as an alternative therapy in the management of HCV patients who are not candidates for IFN-α therapy. Thirty patients (16 male, 14 female), average age 47 years, were enrolled in the study. Fifteen patients had chronic liver disease, five had compensated cirrhosis, and 10 had decompensated cirrhosis. After a baseline evaluation, all subjects received 450 mg *N. sativa* oil three times daily for three months. The primary findings demonstrated that administration of *N. sativa* significantly decreased HCV viral load, increased total antioxidant activity, total protein and albumin levels, improved blood glucose levels, and improved lower-limb edema compared to baseline.[80]

Schistosomiasis

The effect of NSO against liver damage induced by infection with *Schistosoma mansoni* (*S. mansoni*), a water-borne blood fluke parasite, was studied in mice. Infection with *S. mansoni* resulted in a pronounced elevation in serum activity of liver enzymes, ALT and GGT, with a slight increase in AP level. Infection also reduced serum albumin levels. When NSO was given alone, it reduced *S. mansoni* fluke numbers in the liver and decreased the total number of ova deposited in both the liver and the intestine. When NSO was administered in combination with praziquantel (PZQ), a schistosoma drug therapy, the most prominent effect was a further lowering in the dead ova number over that produced by PZQ alone. Administration of NSO partially corrected schistosoma-induced changes in ALT, GGT, and AP activity, as well as serum albumin content, suggesting NSO may be effective at improving liver damage caused by S. mansoni infection.[81] *In vitro* research of *N. sativa* against other Schistosoma species support its strong effects against all stages of the parasite. *N. sativa* also induced oxidative stress in adult worms, which may render the parasite vulnerable to host damage and may be key to *N. sativa*'s anti-schistosomal potency.[82]

DIABETES

N. sativa is widely used in various traditional medicine systems as a remedy for diabetes.[83] Significant effects of *N. sativa* on blood sugar values have been confirmed and are likely attributed to the essential oil component, TQ.[84] Five clinical trials have investigated the benefits of *N. sativa* and its constituents on various aspects of hyperglycemia and diabetes, the most recent of which were published in 2017. An open-label, prospective, comparative, clinical trial evaluated efficacy and safety of *Nigella sativa* oil supplementation in patients with chronic kidney disease due to diabetic nephropathy. Patients were randomized into Group 1 (control) - conservative management of diabetic nephropathy, and Group 2 (intervention) who received oral *N. sativa* oil (2.5 mL once daily) in addition to conservative management for 12 weeks. Blood glucose, hematology, and kidney function were examined at baseline, 6, and 12 weeks of treatment. Decreases were observed in blood glucose, serum creatinine, blood urea, and 24-h total urinary protein levels. Glomerular filtration rate, 24-h total urinary volume, and hemoglobin levels increased in the treatment group compared to the control group, indicating improved kidney function.[85]

Oxidative stress plays an important role in pathogenesis of diabetes mellitus and its complications. A 2015 study explored the long-term (over one year) effects on blood glucose and redox status in 114 patients with type 2 diabetes taking oral hypoglycemic drugs. The control group (n = 57) received placebo and the NS group (n = 57) received 2g NS, daily, for one year in addition to their standard medications. Results in the NS group showed significant drops in fasting blood sugar from 195 to 172, and in HbA1c from 8.6 to 8.2, as compared to controls. Significant improvements were also observed in antioxidant status, insulin resistance, and β-cell activity in NS-treated patients when compared to controls and baseline values. Researchers concluded that long-term supplementation

with *Nigella sativa* improves glucose homeostasis and improves antioxidant status in type 2 diabetic patients treated with oral hypoglycemic drugs.[46] Other clinical trials of shorter duration using *N. sativa* preparations have demonstrated similar beneficial effect on blood sugar modulation.[47]

CARDIOVASCULAR CONDITIONS

A significant amount of clinical research has been conducted over the past decade to support *Nigella sativa*'s therapeutic benefits for cardiovascular disease, however, results generally fall into two outcome categories: management of lipid profiles and management of blood pressure, with some of this research having been conducted in diabetic populations.

Management of Dyslipidemia

In a recent single-blind, controlled trial, 57 type 2 diabetic patients were assigned to receive 2 g *N. sativa* daily for one year and 57 were assigned to receive an identical regimen of placebo, along with oral hypoglycemic agents. Lipid profiles were assessed at baseline and at 3-month intervals throughout the trial. The *N. sativa* group experienced significant declines in total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), ratio of total to high density lipoprotein cholesterol (TC/HDL-C), and LDL-C/HDL-C ratios, compared with the respective baseline and the control group data. HDL cholesterol levels were significantly elevated in the *N. sativa* group compared to controls. The *N. sativa* group also had a significant reduction in systolic and diastolic blood pressure, mean arterial pressure (MAP), and heart rate as compared with the control group. This study indicates that *N. sativa* supplementation improves cholesterol profiles, mean arterial pressure, and heart rate in type 2 diabetics taking oral hypoglycemic agents.[86]

A review and meta-analysis of other trials using *N. sativa* for lipid profile management in patients suffering from diabetes mellitus or metabolic syndrome revealed a total of 12 trials (6 human studies and 6 preclinical studies) that met search criteria. The majority of trials demonstrated improvements in serum lipid levels, including decreases in total lipids, triglycerides, and LDL levels. A greater effect of NS seed oil versus seed powder was observed on serum total cholesterol and LDL-C levels. Researchers concluded that *N. sativa* preparations may be a useful adjuvant to lipid lowering medications, however, further studies using standardized extracts and improved methodologies are warranted.[87]

Management of Hypertension

Nigella sativa seeds have a history of use in various systems of medicine as a remedy for hypertension. A RDBPC trial conducted in 2013 explored the effects of *N. sativa* oil on blood pressure (BP) in healthy volunteers. Seventy healthy volunteers (aged 34 to 63 years) with systolic BP (SBP) from 110-140 mmHg and diastolic BP (DBP) from 60-90 mmHg were randomly assigned to receive 2.5 mL *N. sativa* oil (standardization not noted) or placebo twice daily for eight weeks. The systolic and diastolic BPs, serum liver enzymes, creatinine, blood urea nitrogen, and body mass index were measured at baseline and endpoint. Subjects in the NSO group showed significant decreases in systolic and diastolic BPs compared with baseline and the placebo group at the endpoint. There were no significant changes in other parameters in either group at the endpoint and no adverse effects were reported. Oral daily administration of 5 mL *N. sativa* oil to healthy volunteers for eight weeks lowers systolic and diastolic BPs without any adverse effects.[88]

With clinical trials giving conflicting results on *N. sativa* powder's effect on blood pressure, Sahebkhar et al conducted a 2016 meta-analysis of available randomized clinical trials published before 30 August 2015, comparing *N. sativa* powder treatment with placebo or standard treatment. A total of 11 RCTs, including 860 hypertensive or normotensive individuals were eligible. Ten trials compared *N. sativa* versus placebo and one compared *N. sativa* versus standard treatment. Systolic and diastolic blood pressure (SBP and DBP) means decreased from 132.85 to 125.19 mmHg and from 82.63 to 77.74 mmHg after an average treatment duration of 8.3 weeks in *N. sativa* groups. This meta-analysis indicates short-term treatment with *N. sativa* powder can significantly reduce SBP and DBP levels. Additional studies are needed, however, to support existing evidence and to further explore the long-term BP-lowering effects.[89]

Miscellaneous Cardiovascular Parameters

Other preclinical studies have demonstrated beneficial effect on thoracic aorta contractile response,[90] irregular heart activity,[41] reduction in platelet numbers and the prothrombotic events,[42] decreases in serum cholesterol, triglycerides, hematocrit, hemoglobin, and cardiac tissue damage caused by ischemia-reperfusion.[43,44]

OBESITY AND OXIDATIVE STRESS

Obesity is often associated with an increased risk of cardiovascular and other diseases and is often characterized by long-term oxidative stress and inflammation. With the current epidemic of obesity (particularly in North America), research on therapies aimed at controlling body weight and metabolic profiles is much needed. In a DBRC trial, 90 obese women (aged 25–50 years old with body mass index (BMI) between 30-35) were randomly assigned to receive 3 g *Nigella sativa* oil (NSO) daily or placebo, along with a low-calorie diet for eight weeks. Anthropometric measurements and serum chemistry profiles were assessed at baseline and trial conclusion. At trial conclusion those in the NSO group experienced significant decreases in weight and

waist circumference measurements, compared with the placebo group. A significant decline in triglyceride and very low-density lipoprotein levels were also observed in the NSO group compared to the placebo group. NSO oil concurrent with a low-calorie diet in obese women may help with weight loss, decreased body measurements, and decreased in cardiometabolic risk factors.[91]

Researchers also examined the effects of NSO combined with a calorie-restricted diet on systemic inflammatory biomarkers in this same group of obese women. NSO significantly reduced levels of serum tumor necrosis factor-alpha by 40.8 percent in the treatment group, versus a 16.1 percent decrease in placebo subjects. High-sensitivity C-reactive protein was reduced by 54.5 percent in the treatment group versus 21.4 percent in the placebo group. These results suggest that the combination of a low-calorie diet and 3 grams NSO daily may modulate systemic inflammatory biomarkers in obese women.[92]

To explore the role of NSO on lipid peroxidation and oxidative stress status in obesity, a double-blind placebo-controlled randomized clinical trial of 50 volunteer obese women (body mass index = 30-35) aged 25-50 years old was conducted over an eight-week period. Participants were randomly divided into intervention (n = 25) and placebo (n = 25) groups. They received a low-calorie diet with 3 g/day NSO or low-calorie diet with placebo. NSO concurrent with a low-calorie diet decreased body weight in the NSO group compared to the placebo group (-4.80 ± 1.50 vs. -1.40 ± 1.90 kg; $p < 0.01$). Comparison of red blood cell superoxidase dismutase (SOD), an important antioxidant enzyme present in nearly all living cells, revealed significant increases in the NSO group compared to the placebo group at the end of the study (88.98 ± 87.46 vs. -3.30 ± 109.80 U/gHb; $p < 0.01$). No significant changes in lipid peroxidation, glutathione peroxidase, and total antioxidant capacity were observed, suggesting additional studies, possibly of longer duration, are warranted to confirm the beneficial effects of NSO on markers of oxidative stress in obesity.[93]

OTHER INDICATIONS

Nigella sativa has been studied in additional clinical trials and was found to have beneficial effects on various other health conditions via both oral and topical administration. These trials were somewhat limited in scope, but promising, indicating the need for additional research.

Neurological Conditions:

Nigella sativa's effect on various aspects of cognitive health was demonstrated in a RDBPC trial to stabilize mood, decrease anxiety, and improved cognition when given at 500 mg daily for 4 weeks to healthy males.[94] A second RDBPC trial in which 500 mg *Nigella sativa* was given twice daily, for 9 weeks demonstrated it was effective at enhancing memory, attention, and cognition in healthy adults.[95]

Seizures

Two different clinical trials revealed that thymoquinone or *N. sativa* for four weeks in children with refractory epilepsy reduced seizure frequency.[96,97]

Male Fertility

A RDBPC trial in 68 men with infertility and semen quality issues explored the benefits of NSO daily for 2 months. Sperm count, motility, morphology, and semen volume were improved significantly in the NSO-treated group compared with placebo.[98]

TOPICAL APPLICATIONS OF NSO

Topical application of NSO have also shown benefit in select conditions. In cyclic mastalgia, NSO was effective and superior to placebo at decreasing pain over a two-month period.[99] Topical *N. sativa* treatment was as effective as common prescription medications and OTC creams for hand eczema in a four-week long trial.[100] In a RDBPC trial NSO applied topically for six months significantly decreased the size of vitiligo lesions compared to controls.[101]

| SAFETY |

Nigella sativa has an excellent safety profile. When taken by mouth in food or in therapeutic amounts for short-term, *Nigella sativa* cold press oil has a history of safe consumption for the past 30 years. Studies in humans indicate a lack of significant adverse effects at oral dosages of 500 mg to 3 grams or 5mL daily. Human studies using more than 3 grams daily are not available, so safety at higher levels is unknown. *N. sativa* is also safe in children when given at lower doses.[102-104] Data in pregnant women is not available, but consuming *Nigella sativa* as a seed on food is thought to be safe.

| SIDE EFFECTS | TOXICITY |

In clinical trials, highe r doses of Nigella seed (>40 mg/kg body weight) have occasionally been associated with unspecified gastrointestinal complaints including constipation, burning sensation, vomiting, or mild nausea. [105,106]

| CONTRAINDICATIONS | INTERACTIONS |

N. sativa may affect the intestinal availability and pharmacological effect of certain drugs. *N. sativa* has been shown to enhance amoxicillin availability in both *in vivo* and *in vitro* studies.107 *In vitro* and preclinical research suggests that Nigella seed extract may inhibit platelet aggregation and clotting so care should be used when taking blood-thinning or platelet medications.[108]

N. sativa, especially its constituent thymoquinone, can have hypoglycemic and blood-pressure lowering effects, so caution should be used when taking it with diabetes and hypertension medications. Dose adjustments to diabetes and hypertension medications or botanical supplements might be necessary.[31,47,87,89]

| DOSAGE | SUGGESTED USE |

Dosages of *N. sativa* vary widely depending on the form ingested. (seed or oil) Most clinical trials giving oral doses to adults have utilized dosages between 500 mg to 3 grams or 5 mL NSO daily without significant adverse events. Something important to take into account is the standardization of the preparation. If taking NSO standardized to 3% thymoquinone (more concentrated than most commercially available material), smaller doses should be effective.

REFERENCES:

1. The Plant List (2013). Version 1.1 Available at: *http://www.theplantlist.org/tpl1.1/record/kew-2381679* Accessed June 15, 2018.
2. Corncanu CG, Corncanu M. Considerations on human evolution and on species origin centers. *Oltenia Journal for Studies in Natural Sciences* 2011;27:210-217.
3. Raunkiaer C. *The Life Forms of Plants and Statistical Plant Geography: Being the Collected Papers of C. Raunkiaer. Oxford*, UK; Oxford University Press; 1934.
4. Ansari Z, Sarish T. Traditional uses of *Nigella sativa* in Malegaon region of Nashik – a review. *Int J Pure App Biosci* 2013;1:19-23.
5. Heiss AG, Stika HP, De Zorzi N, Jursa M. Nigella in the mirror of time: a brief attempt to draw a genus' ethnohistorical portrait. *Offa-Zeitschrift Berichte und Mitteilungen zur Urgeschichte Frubgeschichte und Mittelalteraarchaologie* 2012;13:69/70:147-169.
6. Toma CC, Simu GM, Hanganu D, et al. Chemical composition of the Tunisian *Nigella sativa*. Note II. Profile on fatty oil. *Farmacia* 2013;61:93-108.
7. Kapital B, Feyissa T, Petros Y, Mohammed S. Molecular diversity study of black cumin (*Nigella sativa L.*) from Ethiopia as revealed by inter simple sequence repeat (ISSR) markers. African J Biotech 2015;14:1543-1551.
8. Paarakh PM. *Nigella sativa* Linn. – a comprehensive review. *Indian J Natural Prod Resources* 2010;1:409-429.
9. Unani Pharmacopoeia Committee. The Unani Pharmacopoeia India. Vol. 1, 1st ed. New Delhi, India, Deparatment of Ayurvedic Yoga and Naturopathy, Unani, Siddha, and Homeopathy. AYU Ministry of Health & Family Welfare, Govt. of India: 2007.
10. Food and Drug Adminstration (FDA)182.10 Spices and natural seasonings and flavorings. Code of Federal Regulations 21 (21 CFR). Washington, DC: US Government Printing Office; 2016:474-475.
11. Germer R. *Handbuch der altagyptischen Heilpflanzen*. Wiesbad, Germany: Otto Harrassowitz; 2008.
12. Beck LY. De Materia *medica by Pedanius Dioscorides*. Hildesheim, Germany: Olms-Weidman; 2005.
13. Ayurvedic Pharmacopoeia Committee. *The Ayurvedic Pharmacopoeia India*. Vol. 1, 1st ed. New Delhi, India: The Controller of Publications; 2001.
14. Shariq IM, Israil AM, Iqbal A, Brijesh P. Morpho-physiological characterization of seeds and seedlings of *Nigella sativa Linn*.: Study on Indian germplasm. *International Res J Biol Sci* 2015;4:38-42.
15. Amin B, Hosseinzadeh H. Black cumin (*Nigella sativa*) and its active constituent, thymoquinone: an overview on the analgesic and anti-inflammatory effects. *Planta Med* 2016;82:8-16.
16. El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. *J Ethnopharmacol* 2002 ;81:161-164.
17. Yoruk O, Tatar A, Keles ON, Cakir A. The value of *Nigella sativa* in the treatment of experimentally induced rhinosinusitis. *Acta Otorhinolaryngol Ital* 2017;37:32-37.
18. Mohammed NK, Abd Manap MY, Tan CP, et al. The effects of different extraction methods on antioxidant properties, chemical composition, and thermal behavior of black seed (*Nigella sativa l.*) Oil. E*vid Based Complement Alternat Med* 2016;2016:6273817.
19. Kiralan M. Volatile compounds of black cumin seeds (*Nigella sativa* L.) from microwave-heating and conventional roasting. *J Food Sci* 2012;77:C481-C484.
20. Kiralan M., Özkan G., Bayrak A., Ramadan M. F. Physicochemical properties and stability of black cumin (*Nigella sativa*) seed oil as affected by different extraction methods. *Industrial Crops and Products.* 2014;57:52–58.
21. Akanda MJH, Sarker MZI, Ferdosh S, Manap MYA, et al. Applications of supercritical fluid extraction (SFE) of palm oil and oil from natural sources. *Molecules.* 2012;17(2):1764–1794.
22. Solati Z, Baharin BS, Bagheri H. Antioxidant property, thymoquinone content and chemical characteristics of different extracts from *Nigella sativa L.* seeds. *J Am Oil Chem Soc* 2014;91:295–300.
23. Licari M. Universita Degli Studi di Cantania, Sicily. August 2018. Unpublished Research.
24. Gharby S, Harhar H, Guillaume D, et al. Chemical investigation of *Nigella sativa L.* seed oil produced in Morocco. *J Saudi Soc Agric Sci* 2015;14:172-177.
25. Tembhurne SV, Feroz S, Sakarkar DM. A review on therapeutic potential of *Nigella sativa* (kalonji) seeds. *J Med Plants Res* 2014;8:166–167.
26. Ahmad A, Husain A, Mujeeb M, et al. A review on therapeutic potential of *Nigella sativa:* A miracle herb. Asian Pac J Trop Biomed 2013;3:337–352.
27. Staphylakis PK, Gegiou D. The sterols of *Nigella sativa* seed oil. *Phytochemistry* 1986;25:761–763.
28. El Gazzar M, El Mezayen R, Marecki JC, et al. Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation. Int Immunopharmacol 2006;6:1135–1142.
29. Gali-Muhtasib H, Roessner A, Schneider-Stock R. Thymoquinone: a promising anti-cancer drug from natural sources [J]. *Int J Biochem Cell Biol* 2006;38:1249-1253.
30. Liu H, Liu H, Jiang Y, Li N. Protective effect of thymoquinone improves cardiovascular function, and attenuates oxidative stress, inflammation and apoptosis by mediating the PI3K/Akt pathway in diabetic rats. *Molecular Medicine Reports* 2016;13, 2836-2842.
31. Fararh KM, Shimizu Y, Shiina T, et al. Thymoquinone reduces hepatic glucose production in diabetic hamsters. *Res Vet Sci* 2005;79:219-223.
32. Duncker SC, Philippe D, Martin-Paschoud C, et al. *Nigella sativa* (black cumin) seed extract alleviates symptoms of allergic diarrhea in mice, involving opioid receptors. *PLoS One* 2012;7:e39841.
33. Toppozada HH, Mazloum HA, el-Dakhakhny M. The antibacterial properties of the *Nigella sativa L.* seeds. Active principle with some clinical applications. *J Egypt Med Assoc* 1965;48:Suppl:187-202.
34. Khan MA, Aljarbou AN, Khan A, Younus H. Liposomal thymoquinone effectively combats fluconazole-resistant *Candida albicans* in a murine model. *Int J Biol Macromol* 2015;76:203-208.37.
35. Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. *Phytomedicine* 2004;11:56-64.
36. Surekha R, Sumathi T. An efficient encapsulation of thymoquinone using solid lipid nanoparticle for brain targeted drug delivery: physicochemical characterization, pharmacokinetics and bio-distribution studies. *Int J Pharm Clin Res* 2016;8:1616-1624.
37. Shabsoug B, Khalil R, Abuharfeil N. Enhancement of natural killer cell activity in vitro against human tumor cells by some plants from Jordan. *Journal of Immunotoxicology* 2008;5:279-285.
38. Haq A, Lobo PI, Al-Tufail M, et al. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *Int J Immunopharmacol* 1999;21:283-295.
39. Isik AF, Kati I, Bayram I, Ozbek H. A new agent for treatment of acute respiratory distress syndrome: thymoquinone. An experimental study in a rat model. *Eur J Cardiothorac Surg* 2005;28:301-305.
40. Badary OA, Al-Shabanah OA, Nagi MN, et al. Inhibition of benzo(a)pyrene-induced forestomach carcinogenesis in mice by thymoquinone. *Eur J Cancer Prev* 1999;8:435-440.

41. Meral I, Donmez N, Baydas B, et al. Effect of *Nigella sativa L.* on heart rate and some haematological values of alloxan-induced diabetic rabbits. *Scand J Lab Anim Sci* 2004;31:49-53.
42. Nemmar A, AlSalam S, Zia S, et al. Contrasting actions of diesel exhaust particles on the pulmonary and cardiovascular systems and the effects of thymoquinone. *Br J Pharmacol* 2011;164:1871-1882.
43. El Tahir KE, Ashour M, Al-Harbi MM. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: elucidation of the mechanism of action. *Gen Pharmacol* 1993;24:1123-1131.
44. Zaoui A, Cherrah Y, Lacaille-Dubois M, et al. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapie 2000;55: 379-382.
45. Pari L, Sankaranarayanan C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin– nicotinamide induced diabetic rats. *Life Sci* 2009;85:830-834.
46. Kaatabi H, Bamosa AO, Badar A, et al. *Nigella sativa* improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: placebo-controlled participant-blinded clinical trial. *PLoS One* 2015;10:e0113486.
47. Bamosa AO, Kaatabi H, Lebdaa FMet al. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. *Indian J Physiol Pharmacol* 2010;54:344-354.
48. Houcher Z, Boudiaf K, Benboubetra M, et al. Effects of methanolic extract and commercial oil of *Nigella sativa L.* on blood glucose and antioxidant capacity in alloxan-induced diabetic rats. *Pteridines* 2007; 18:8-18.
49. Kanter M. Protective effects of *Nigella sativa* on the neuronal injury in frontal cortex and brain stem after chronic toluene exposure. *Neurochem Res* 2008;33:2241-2249.
50. Omran OM. Effects of thymoquinone on STZ-induced diabetic nephropathy: an immunohistochemical study. *Ultrastruct Pathol* 2014;38:26-33.
51. Hamed M, El-Rigal N, Ali S. Effects of Black seed oil on resolution of hepato renal toxicity induced by bromobenzene in rats. *Eur Rev Med Pharmacol Sci* 2013;17:569-581.
52. Osama A. Abu-Zinadah. Using *Nigella sativa* oil to treat and heal chemical induced wound of rabbit skin. *JKAU Sci* 2009;21:335-346.
53. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa L.* seed. *Int Immunopharmacol* 2005;5:1749-1770.
54. Kooshki A, Forouzan R, Rakhshani MH, Mohammadi M. Effect of topical application of *Nigella sativa* oil and oral acetaminophen on pain in elderly with knee osteoarthritis: a crossover clinical trial. *Electron Physician* 2016;8:3193-3197.
55. Gheita TA, Kemway SA. Effectiveness of *Nigella sativa* oil in the management of rheumatoid arthritis patients: A placebo-controlled study. *Phytother Res* 2012:26:1248-1248.
56. Hadi V, Kheirouri A, Alizadeh M, et al. Effects of *Nigella sativa* oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis; a randomized, double-blind, placebo-controlled clinical trial. Avicenna J Phytomed 2016;6:34-43.
57. Kheirouri S, Hadi V, Alizadeh M. Immunomodulatory effect of *Nigella sativa* oil on T lymphocytes in patients with rheumatoid arthritis. *Immunol Invest* 2016;45:271-283.
58. Koshak A, Wei L, Koshak E, et al. *Nigella sativa* supplementation improves asthma control and biomarkets: a randomized, double-blind, placebo-controlled trial. *Phytother Res* 2017;3:403-409
59. Salem AM, Bamosa AO, Qutub HO, et al. Effect of *Nigella sativa* supplementation on lung function and inflammatory mediatorsin partly controlled asthma: a randomized controlled trial. *Ann Saudi Med* 2017; 37:64-71.
60. Boskabady MH, Javan H, Sajady M, Rakhshandeh H. The possible prophylactic effect of *Nigella sativa* seed extract in asthmatic patients. *Fundam Clin Pharmacol* 2007;21:559-566.
61. Boskabady MH, Farhadi J. 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* 2008;14:1137-1134.
62. Alsamarai AM SM, Alobaidi AHA. Evaluation of therapeutic efficacy of *Nigella sativa* (Black Seed) for treatment of allergic rhinitis. In Kowalski ML, ed Allergic Rhinitis. Rijeka, Croatia: *Intech;*2012:197-214.
63. Isik H, Cevikbas A, Gurer US, et al. Potential adjuvant effects of *Nigella sativa* seeds to improve specific immunotherapy in allergic rhinitis patients. *Med Princ Pract* 2010;19:206-211.
64. Nikakhlagh S, Rahim F, Aryani FH, et al. Herbal treatment of allergic rhinitis: the use of *Nigella sativa*. *Am J Otolaryngol* 2011;32:402-407.
65. Mousa HFM, Abd-El-Fatah NK, Darwish OA, et al. Effect of *Nigella sativa* seed administration on prevention of febrile neutropenia during chemotherapy among children with brain tumors. *Childs Nerv Syst* 2017;33:793-800.
66. Salim EI. Cancer chemopreventive potential of volatile oil from black cumin seeds, *Nigella sativa* L., in a rat multi-organ carcinogenesis bioassay. *Oncol Lett* 2010;1:913–924.
67. Mahmoud SS, Torchilin VP. 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* 2012;25:1392–1398.
68. Peng L, Liu A, Shen Y, et al. et al. Antitumor and anti-angiogenesis effects of thymoquinone on osteosarcoma through the NF-κB pathway. *Oncol Rep* 2013;29:571–578.
69. Ng WK, Yazan LS, Ismail M. Thymoquinone from *Nigella sativa* was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. *Toxicol In Vitro* 2011;25:1392–1398.
70. Majdalawieh AF, Hmaidan R, Carr RI. *Nigella sativa* modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. *J Ethnopharmacol* 2010;131:268–275.
71. Torres MP, Ponnusamy MP, Chakraborty S, Smith LM, Das S, Arafat HA, et al. et al. Effects of thymoquinone in the expression of mucin 4 in pancreatic cancer cells: implications for the development of novel cancer therapies. *Mol Cancer The*r 2010;9:1419–1431.
72. Effenberger K, Breyer S, Schobert R. Terpene conjugates of the *Nigella sativa* seed-oil constituent thymoquinone with enhanced efficacy in cancer cells. *Chem Biodivers* 2010;7:129–139.
73. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. *Acta Microbiol Pol* 2000; 49:63-74.
74. Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU. Anti bacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad 2008;20:72-74.
75. Chaieb K, Kouidhi B, Jrah H, et al. Antibacterial activity of Thymoquinone, an active principle of *Nigella sativa* and its potency to prevent bacterial biofilm formation. *BMC Complementary and Alternative Medicine* 2011;11:29.
76. Salem EM, Yar T, Bamosa AO, et al. Comparative study of *Nigella sativa* and triple therapy in eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia. *Saudi J Gastroenterol* 2010;16:207-214.
77. Bita A, Rosu AF, Calina D, et al. An alternative treatment for Candida infections with *Nigella sativa* extracts. Eur J Hosp Pharm 2012;19:162.
78. Aljabre SH, Randhawa MA, Akhtar N, et al. Antidermatophyte activity of ether extract of *Nigella sativa* and its active principle, thymoquinone. *J Ethnopharm*. 2005;101:116–119.
79. Rogozhin EA, Oshchepkova YI, Odintsova TI, et al. Novel antifungal defensins from *Nigella sativa L.* seeds. *Plant Physiol Biochem* 2011;49:131–137.
80. Barakat EM, El Wakeel LM, Hagag RS. Effects of *Nigella sativa* on outcome of Hepatitis C in Egypt. World *J Gastroenterol* 2013;19:2529-2536.
81. Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *J Ethnopharmacol* 2002;79:1–11.
82. Mohamed AM, Metwally NM, Mahmoud SS. *Nigella sativa* seeds against Schistosoma mansoni different stages. *Mem Inst Oswaldo Cruz* 2005;100:205–211.
83. Ali B, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* 2003;17: 299-305.
84. Al Yahya M. Phytochemical studies of the plants used in traditional medicine of Saudi Arabia. *Fitoterapia* 1986;57:179-182.

85. Ansari ZM, Nasiruddin M, Khan RA, Haque SF. Protective role of *Nigella sativa* in diabetic nephropathy: a randomized clinical trial. *Saudi J Kidney Dis Transpl* 2017;28:9-14.
86. Badar A, Kaatabi H, Bamosa A, et al. Effect of *Nigella sativa* supplementation over a one-year period on lipid levels, blood pressure and heart rate in type-2 diabetic patients receiving oral hypoglycemic agents: nonrandomized clinical trial. *Ann Saudi Med* 2017;37:56-63.
87. Sahebkar A, Beccuti G, Simental-Mendia LE, et al. *Nigella sativa* (black seed) effects on plasma lipid concentrations in humans: A systematic review and meta-analysis of randomized placebo-controlled trials. *Pharmacol Res* 2016;106:37-50.
88. Fallah Huseini H, Amini M, Mohtashami R, et al. Blood pressure lowering effect of *Nigella sativa L.* seed oil in healthy volunteers: a randomized, double-blind, placebo-controlled clinical trial. *Phytother Res* 2013;27:1849-1853.
89. Sahebkar A, Soranna D, Liu X, et al. A systematic review and meta-analysis of randomized controlled trials investigating the effects of supplementation with *Nigella sativa* (black seed) on blood pressure. *J Hypertens* 2016;34:2127-2135.
90. Roughani M, Vaez MM, Vaseei M. The effect of long-term oral administration of Nigella sativum on the contractile reactivity of thoracic aorta in diabetic rats. *Koomesh* 2006;7:153-157.
91. Mahdavi R, Namazi N, Alizadeh M, Farajinia S. Effects of *Nigella sativa* oil with allow-calorie diet on cardiometabolic risk factors in obese women: a randomized controlled trial. *Food Funct* 2015;6:2041-2048.
92. Mahdavi R, Namazi N, Alizadeh M, Farajnia S. *Nigella sativa* oil with a calorie-restricted diet can improve biomarkers of systemic inflammation in obese women: a randomized double-blind, placebo-controlled clinical trial. *J Clin Lipidol* 2016:10:1203-1211.
93. Namazi N, Mahdavi R, Alizadeh M, Frrajnia S. Oxidative stress responses to *Nigella sativa* oil concurrent with a low-calorie diet in obese women: a randomized, double-blind, controlled clinical trial. *Phytother Res* 2015;29:1722-1728.
94. Bin Sayeed MS, Shams T, Fahim Hossain S, et al. *Nigella sativa L.* seeds modulate mood, anxiety and cognition in healthy adolescent males. *J Ethnopharmacol* 2014;152:156-612.
95. Bin Sayeed MS, Asaduzzaman M, Morshed H, et al. The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. *J Ethnopharmacol* 2013 Jul 30;148(3):780-786.
96. Akhondian J, Kianifar H, Raoofziaee M, et al. The effect of thymoquinone on intractable pediatric seizures (pilot study). *Epilepsy Res* 2011;93:39-43.
97. Akhondian J, Parsa A, Rakhshande H. The effect of *Nigella sativa L.* (black cumin seed) on intractable pediatric seizures. *Med Sci Monit* 2007;13:CR555-9.
98. Kolahdooz M, Nasri S, Modarres SZ, et al. Effects of *Nigella sativa L.* seed oil on abnormal semen quality in infertile men: a randomized, double-blind, placebo-controlled clinical trial. *Phytomedicine* 2014;21:901-905.
99. Huseini HF, Kianbakht S, Mirshamsi MH, Zarch AB. Effectiveness of topical *Nigella sativa* seed oil in the treatment of cyclic mastalgia: a randomized, triple-blind, active, and placebo-controlled clinical trial. *Planta Med* 2016;82:285-288.
100. Yousefi M, Barikbin B, Kamalinejad M, et al. Comparison of therapeutic effect of topical Nigella with Betamethasone and Eucerin in hand eczema. *J Eur Acad Dermatol Venereol* 2013;2:1498-1504.
101. Ghorbanibirgani A, Khalili A, Rokhafrooz D. Comparing *Nigella sativa* oil and fish oil in treatment of vitiligo. *Iran Red Crescent Med* J 2014;16:e4515.
102. *http://naturaldatabase.therapeuticresearch.com/nd/search asp?cs=&s=ND$pt=100&id=901&ds=&name=Nigella+sativa+(BLACK+SEED)&searchid=63895884* Accessed June 14, 2018
103. Mohtashami R, Huseini HF, Heydari M, et al. Efficacy and safety of honey-based formulation of *Nigella sativa* seed oil in functional dyspepsia: A double blind randomized controlled clinical trial. *J Ethnopharmacol* 2015;175:147-52.
104. Shawki M, El Wakeel L, Shatla R, et al. The clinical outcome of adjuvant therapy with black seed oil on intractable paediatric seizures: a pilot study. *Epileptic Disord* 2013;15:295-301.
105. Kalus U, Pruss A, Bystron J, et al. Effect of *Nigella sativa* (black seed) on subjective feeling in patients with allergic diseases. *Phytother.Res* 2003;17:1209-1214.
106. Qidwai W, Hamza HB, Qureshi R, Gilani A. 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* 2009;15:639-644.
107. Ali B, Amin S, Ahmad J, et al. Bioavailability enhancement studies of amoxicillin with *N* 2012;135:555–559.
108. Enomoto S, Asano R, Iwahori Y, et al. Hematological studies on black cumin oil from the seeds of *Nigella sativa L. Biol.Pharm.Bull* 2001;24:307-310.