



ImmunoWin™  
Powered by ThymoQuin 3%  
Nigella Sativa Oil (Black Cumin Seed Oil)  
Full Spectrum, Standardized to 3%  
Thymoquinone

## *Nigella sativa* (Black Cumin Seed) Oil 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<sup>1-5</sup> and is also cultivated commercially in various regions of Africa, the Middle East, India, and Pakistan.<sup>6-8</sup> *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.<sup>4</sup> *Nigella sativa* is also known as kalonji (from the Arabic Unani system of medicine),<sup>9</sup> 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.<sup>10</sup>

### | HISTORICAL USES |

Sources report *Nigella sativa* seeds have been used medicinally in the Middle East and Southeast Asia for over 2,000 years.<sup>5,11</sup> 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.<sup>12</sup> *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.<sup>13</sup> 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.<sup>9</sup> 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,<sup>10</sup> *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.<sup>14</sup> 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.<sup>15</sup> 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.<sup>16</sup> 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.<sup>17</sup> 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.<sup>18</sup>

Cold-press extracted Nigella seed oil is the preferred form because it does not involve the use of solvents or heat.<sup>19,20</sup> Supercritical fluid extraction is another technique used to extract plant oils and may offer some favorable features such as isolation of specific desired constituents.<sup>21</sup> 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.<sup>22</sup>

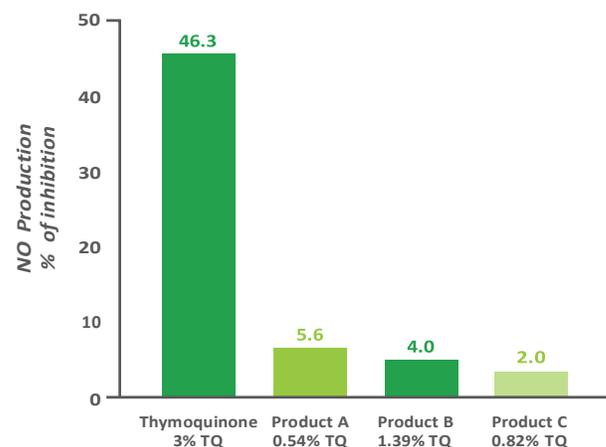
Using heat during extraction causes some of the more sensitive active compounds to oxidize, diminishing the quality of the oil.<sup>20</sup>

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.<sup>21,22</sup> The slow, cold-press extraction method of producing NSO has a long historical use prior to DSHEA and Novel Food legislation.

### STANDARDIZED EXTRACTS

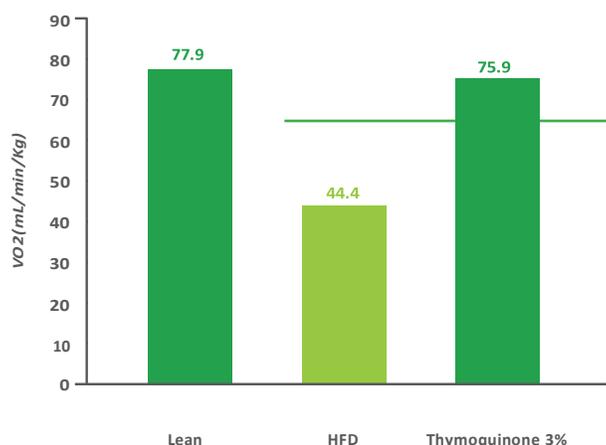
Two years ago, TriNutra™ began a *Nigella sativa* breeding program to yield high-concentration, Non-GMO thymoquinone varieties that thrive in the Israeli climate and yield 2-3 times the thymoquinone content of other varieties. TriNutra's™ Nigella seed oil, is extracted from these varieties via slow, 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)



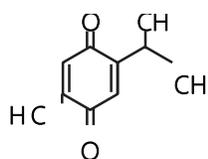
A recent study using a preclinical model of metabolic syndrome demonstrated Thymoquinone 3% administration to mice resulted in higher oxygen (VO<sub>2</sub>) consumption as compared to mice on a high-fat diet. Increased oxygen consumption is a marker of greater mitochondrial function and biogenesis, 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.<sup>2</sup>

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

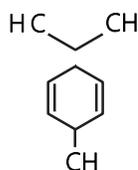


## | ACTIVE CONSTITUENTS |

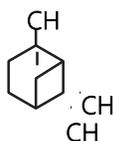
### Active Constituents of *Nigella sativa* Essential Oil



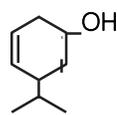
Thymoquinone



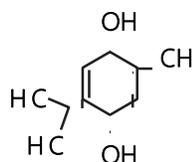
*p*-cymene



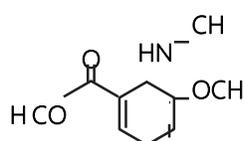
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Carvacrol

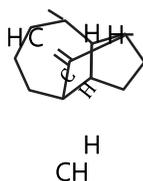


Thymohydroquinone



Nigellone

Longifolene



cycloartenol, sterol esters and sterol glucosides.<sup>25-27</sup> 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*-cymene,  $\alpha$  and  $\beta$ -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.<sup>25,26</sup> Most of the pharmacological effects attributed to *N. sativa* are due to the thymoquinone constituent. TQ possesses anti-inflammatory,<sup>27</sup> anti-cancer,<sup>28</sup> antioxidant,<sup>22</sup> cardioprotective,<sup>30</sup> hypoglycemic,<sup>31</sup> antihistaminic,<sup>32</sup> antibacterial,<sup>33</sup> antifungal activity,<sup>35</sup> anticonvulsant activity,<sup>21</sup> 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.<sup>34,36</sup> 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.<sup>23</sup>

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.<sup>37-39</sup> In addition, both the oil and its active ingredients expressed antimicrobial activity toward a variety of alkaloid, saponin and essential oil.<sup>24</sup> The fixed oil (32-40 %) contains unsaturated fatty acids which include: arachidonic, linoleic, linolenic, oleic,

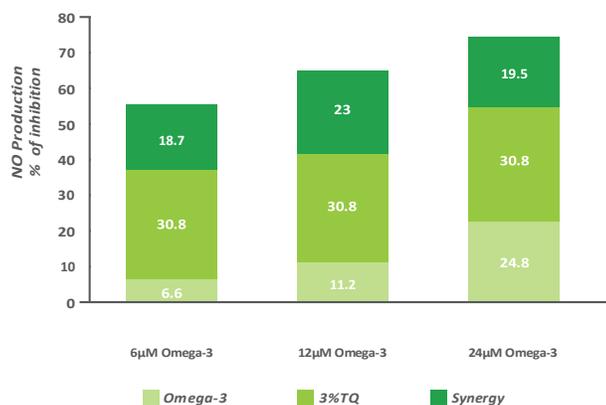
Extensive research has been conducted to identify the composition of *Nigella sativa*. Primary constituents include: fixed oil, proteins,

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and palmitic acids, aliphatic hydrocarbons, and tocopherols, as well as beta-sitosterol, cycloeucalenol, of microbes<sup>33</sup> and inhibitory properties toward certain types of cancer.<sup>28,40</sup>

*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.<sup>41-44</sup> 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.<sup>45-48</sup> Other mechanisms of action attributed to *N. sativa* constituents are neuroprotective activity via opioid receptor stimulation and reduction of neuronal degeneration by TQ,<sup>49</sup> hepatoprotective and nephroprotective activity via the antioxidant effects of thymoquinone,<sup>50,51</sup> and wound-healing activity via enhanced fibroblast proliferation and promotion of beta-fibroblast growth factor.<sup>52</sup> 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.<sup>53</sup>

**Synergy of Omega-3 and 3% Thymoquinone™  
(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.<sup>54</sup>

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.<sup>55</sup> 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- $\alpha$  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.<sup>56</sup> 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.<sup>57</sup>

### 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.<sup>58</sup> 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, 1gNS daily), and NS-2 (n=13, 2gNS 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.<sup>59</sup> 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 symptoms.<sup>60,61</sup> 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.<sup>62-64</sup>

### 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 88.6-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.<sup>65</sup>

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.<sup>66</sup> 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,<sup>67</sup> 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.<sup>68</sup> TQ cytotoxicity has also been demonstrated in human cervical squamous carcinoma cells,<sup>69</sup> T-cell lymphoma tumor cells,<sup>70</sup> pancreatic cancer cells,<sup>71</sup> human leukemia cells,<sup>72</sup> and MCF- breast carcinomas.<sup>67</sup>

### 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.<sup>73</sup> 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.<sup>74</sup> *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.<sup>75</sup> 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.<sup>76</sup>

### 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.<sup>77</sup>

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 thymoquinone from *N. sativa* as a therapy and explains its successful use in folk medicine for the treatment of fungal skin infections.<sup>78</sup> 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.<sup>79</sup>

### 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- $\alpha$  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.<sup>80</sup>

### 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.<sup>81</sup> *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.<sup>82</sup>

### DIABETES

*N. sativa* is widely used in various traditional medicine systems as a remedy for diabetes.<sup>83</sup> Significant effects of *N. sativa* on blood sugar values have been confirmed and are likely attributed to the essential oil component, TQ.<sup>84</sup> 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.<sup>85</sup>

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  $\beta$ -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.<sup>46</sup> Other clinical trials of shorter duration using *N. sativa* preparations have demonstrated similar beneficial effect on blood sugar modulation.<sup>47</sup>

### 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.<sup>86</sup>

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.<sup>87</sup>

#### 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.<sup>88</sup>

With clinical trials giving conflicting results on *N. sativa* powder's effect on blood pressure, Sahebkhari 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.<sup>89</sup>

#### Miscellaneous Cardiovascular Parameters

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

### 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 cardiometabolic risk factors.<sup>91</sup>

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.<sup>92</sup>

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.<sup>93</sup>

#### 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.<sup>94</sup> 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.<sup>95</sup>

#### Seizures

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

#### 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.<sup>98</sup>

#### 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.<sup>99</sup> Topical *N. sativa* treatment was as effective as common prescription medications and OTC creams for hand eczema in a four-week long trial.<sup>100</sup> In a RDBPC trial NSO applied topically for six months significantly decreased the size of vitiligo lesions compared to controls.<sup>101</sup>

#### | 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.<sup>102-104</sup> 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, higher 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.<sup>105,106</sup>

#### | 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.<sup>107</sup> *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.<sup>108</sup>

*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.<sup>31,47,87,89</sup>

## 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.

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