Name of the Ingredient: Nigella sativa

Nigella sativa, known as black seed or black cumin is very popular in various traditional systems of medicine. The seeds of N. sativa have been widely used in the treatment of different diseases and ailments[i] (asthma[ii], Hashimoto's thyroiditis[iii]). *N. sativa* has been studied extensively for its wide spectrum of activities; as antihypertensive[iv], antidiabetic[v], analgesic[vi], anti-inflammatory[vii], gastroprotective[viii], hypolipidemic[ix], and antioxidant[x]. It improves abnormal semen quality in infertile men without any adverse effects[xi]. It stabilizes mood, decreases anxiety and modulates cognition positively[xii]. It is beneficial for nasal mucosa symptoms due to aging[xiii]. It has been reported that, therapeutic properties of N. sativa may be due to Thymoquinone, the major active ingredient of Nigella sativa[xiv].

Thymoquinone (TQ) has been extensively studied in *in-vitro* experiments and animal studies for its various pharmacological activities. There are not many direct clinical studies that have been performed for Thymoquinone as a pure isolate or enriched in the plant extract for its pharmacological activities. As described above Thymoquinone possesses various pharmacological activities supported by *in-vitro* and animal studies. Thymoquinone is reported to possess potent anti-cancer properties in human cells[xv] (p53, NF-κB, PPARγ, STAT3, MAPK, and PI3K/AKT signalling pathways are among the most significant pathways through which thymoquinone mediates its anti-cancer activity)[xvi]. It has been reported that thymoquinone is useful against oxidative stress[xvii], inflammation[xviii] and infections. It might be a potential neuropharmacological agent. It features greater beneficial effects in toxin-induced neuroinflammation and neurotoxicity[xix]. It elicits antitussive, gastroprotective[xx], antihypertensive[xxi], antinociceptive[xxii], antihistaminic, antibacterial[xxiii], anthelmintic, immunomodulatory[xxiv], hepatoprotective[xxv], cardioprotective[xxvi], antidiabetic, ototoxicity protective[xxvii], anxiolytic[xxviii], arthritic[xxix], spermostatic[xxx] and nephroprotective[xxxi] effects [xxxii]. It improves learning[xxxiii] and memory[xxxiv]. It may improve fertility by means of increasing the healthy sperm number and preventing sperm anomalies[xxxv]. It decreases corneal neovascularization[xxxvi].

In clinical trials, with emphasis on thymoquinone was found to exhibit:

1. **Anti-epileptic effects in children with refractory seizures**[xxxvii].

In the reported clinical trial, "*thymoquinone was purchased from Sigma Chemical Co. The powder of thymoquinone was transformed to syrup by a pharmacologist experienced in industrial pharmacology. In this study, we chose the syrup form because of its easier preparation and use in children. The syrup was prepared in concentration of 25 mg/ml. The placebo was also prepared with the same specification (especially the color and taste of the solutions)*".

Summary: In a pilot, double-blinded crossover clinical trial on children, 22 patients were assigned in two groups and received either thymoquinone with dose of 1mg/kg or placebo for a period of four weeks and then during the two weeks of wash out period, they received only their pre-existing anti-epileptic drugs; then, after cross-overing, they received thymoquinone or placebo for a period of four weeks again. Results indicated that thymoquinone has anti-epileptic effects in children with refractory seizures.

2. **Beneficial effect as an adjunct to scaling and root planing in treating chronic periodontitis**[xxxviii].

In this trial, "*Formulation of 0.2% Thymoquinone gel was prepared using 99% pure extract of Thymoquinone (Extract from Sigma Aldrich Company – Product No. 274666) was obtained in crystal form which was converted to powdered form using a mortar and pestle. Then all the ingredients were accurately weighed and a total bulk of 500 gm of gel was prepared. Base of the gel was prepared by 14 gm of carbopol mixed with 485 ml of hot water heated at a temperature of 80–90° C and stirred for 30 min to facilitate hydration. 1 gm of Thymoquinone was added to the mixture to attain a 0.2% concentration of Thymoquinone in the gel prepared and was stirred until homogenization. The pH of the gel was adjusted to 7.0 with 1 N Sodium hydroxide. Then the mixture was cooled at the room temperature (35 °C) and 2 drops of the Strawberry flavour were added to the mixture. The drug was analyzed at 253 nm on UV–visible spectrophotometer for drug release estimation. The results showed that drug release from 0.2% Thymoquinone gel after 8 h was 95.01 ± 1.26*".

Summary: Twenty subjects were further divided into 2 groups. Group I comprised of study subjects (Thymoquinone 0.2% gel in addition to SRP (scaling and root planing)) and Group II comprised of control subjects (only SRP). Results showed 0.2% Thymoquinone gel was sensitive against *P. gingivalis*, *A. actinomycetemcomitans and P. intermedia*. Intracrevicular application of Thymoquinone could be a beneficial adjunct to SRP in treating chronic periodontitis.

In both studies reported, extracts or powder form N. sativa, were not used. Thymoquinone was purchased form 'Sigma Aldrich' as a pure compound and the formulation was prepared for use in clinical trial. In conclusion on the two above reported clinical trials Thymoquinone certainly has benefits on the children with refractory seizures and treating chronic periodontitis. Both studies have just had no more than 25 patients in trial. A large-scale clinical trial with multiple site using various gene pool shall be needed to strengthen the efficacy of Thymoquinone.

Phytochemistry of Thymoquinone in *N. sativa:*

Extracts from research article:

GC-MS Analysis[xxxix]: N. sativa oil GC-chromatogram has shown the presence of 9 major peaks (Figure 4A), and the components corresponding to the peaks have been determined: Beta-Pinene (9.1%), O-Cymene (8.7%), **Thymoquinone (7.8%)**, Palmitic acid (5.0%), Oleic acid (7.2%), Linoleic acid (9.5%), 9,12-Octadecadieneoic acid (Z-Z)-, methyl ester (7.1%), 1-Heptatriaco-tanol (6.9%), and Thymol (9.4%) (Table 2).
Moreover, **N. sativa capsule** GC-chromatogram has shown the presence of 5 major peaks (Figure 4B), and the components corresponding to the peaks have been determined: Beta-Pinene (10.0%), **Thymoquinone (8.6%),** Palmitic acid (5.6%), Oleic acid (8.1%), and Thymol (10.3%) (Table 2).
Furthermore, **N. sativa methanolic extract** GC-chromatogram has shown the presence of 2 major peaks (Figure 4C), and the components corresponding to the peaks have been determined: **Thymoquinone (3.7%)** and Thymol (6.4%) (Table 2).
Nevertheless, **N. sativa aqueous extract** GC-chromatogram has shown the presence of one major peak, **Thymoquinone (6.9%)** (Table 2). As shown in Table II, Thymoquinone is the common bioactive component of all N. sativa preparations, which is in agreement with the literature34and Beta-Pinene is the major constituent of N. sativa oil.

Furthermore, thymoquinone can be readily synthesized in gram quantities[xl]. Benzene extract of N. sativa has better solubility and percent composition of thymoquinone as compared to other solvents[xli].

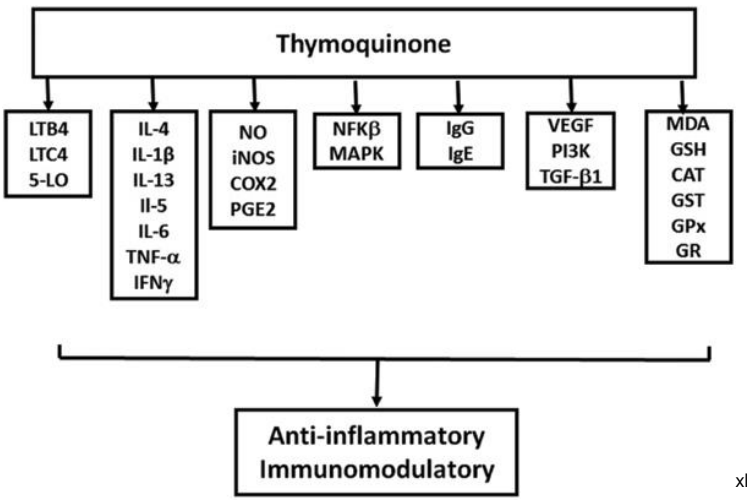
Toxicology in brief:

A variety of *in-vitro* and *in-vivo* studies has evaluated Thymoquinone toxicity profile. At higher doses, it is well tolerated by male and female rats. Thymoquinone at 10mg/kg body weight was found to have no observed adverse effect level. For long-term oral consumption, Thymoquinone at doses of 10mg/kg are safe in mice[xlii].

Table 2: Chemical compositions of *N. sativa* preparations:oil, capsule, methanolic and aqueous extracts major peaks

N	Compound	RT	Oil	Capsule (%)	Methanolic extract (%)	Aqueous extract (K)
1	Beta-Pinene	3.1	9.1	10.0	_	_
2	O-Cymene	4.0	8.7	_	_	_
3	Thymoquinone	4.3	7.8	8.6	37	_
4	Palmitic acid	7.6	5.0	5.6	_	_
s	Oleic acid	10.2	7.2	8.1	_	_
6	Linoleic acid	12.1	9.5	_	_	_
7	9.12-Octadecadieneoic acid (Z-Z)-, methyl ester	16.1	7.1	_	_	_
8	1-Heptatriacotanol	17.2	6.9	_	_	_
9	Thymol	18.9	9.4	10.3	6.4	_

The chemical structure of thymoquinone (TQ) and a schematic diagram of the pharmacological activities of Nigella sativa and TQ [xliii]



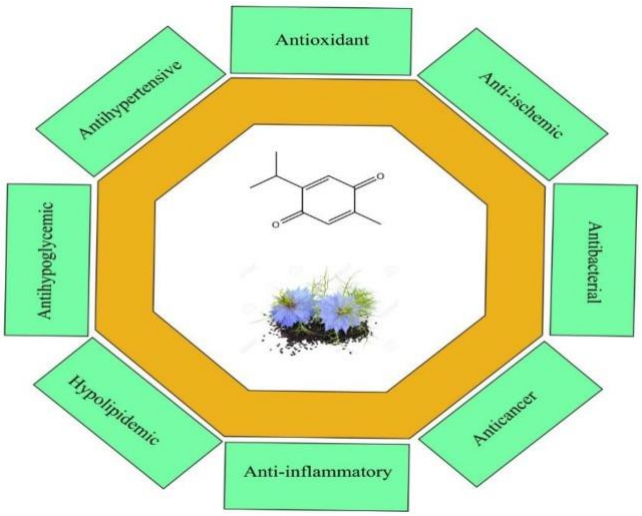
[xliv]

Clinical Trials on pharmacological activities of *Nigella sativa*:

- Individuals with knee pain were randomly selected and divided into experimental group (n = 30) and control group (n = 30). While the control group patients continued their routine prescription, For the experimental group, black cumin oil was applied by rubbing to their knees 3 times a week for 1 month. Results demonstrated that the pain-relieving properties of black cumin oil is effective on geriatric individuals living with knee pain[xlv].
- Nigella sativa oil capsules 500mg twice daily for 4 weeks were used as a supplementary treatment in a randomized, double-blind, placebo-controlled trial in asthmatics. Results demonstrated that NSO supplementation improved asthma control with a trend in pulmonary function improvement with associated normalization of blood eosinophilia[xlvi].
- Forty patients with Hashimoto's thyroiditis, aged between 22 and 50 years old, participated in the trial and were randomly allocated into two groups of intervention and control receiving powdered Nigella sativa or placebo daily for 8 weeks. Data showed a potent beneficial effect of powdered Nigella sativa in improving thyroid status and anthropometric variables in patients with Hashimoto's thyroiditis. Nigella sativa significantly reduced serum VEGF (Vascular Endothelial Growth Factor) concentrations in these patients[xlvii].
- In a double-blind placebo-controlled randomized clinical trial, 90 volunteers obese (body mass index = 30-34.9kg/m^2) women aged 25-50 years were randomly divided into two groups, an intervention group (n = 45) and a placebo group (n = 45). Each group received either: (1) a low-calorie diet with 3g/day of Nigella sativa oil or (2) a low-calorie diet with 3g/day placebo for 8 weeks. Results showed NS oil supplementation combined with a calorie-restricted diet may modulate systemic inflammatory biomarkers in obese women[xlviii].
- 114 type 2 diabetic patients on standard oral hypoglycaemic drugs were assigned into 2 groups. The control group (n = 57) received activated charcoal as placebo and NS group (n = 57) received 2g NS daily for one year in addition to their standard medications. Results indicated that long-term supplementation with Nigella sativa improved glucose homeostasis and enhanced antioxidant defence system in type 2 diabetic patients treated with oral hypoglycaemic drugs[xlix].
- In a randomised trial, hyperlipidaemic menopausal women were assigned to treatment (n=19) or placebo groups (n=18), and given N. sativa or placebo for two months. Results showed that N. sativa significantly improved lipid profiles of menopausal women (decreased total cholesterol, low density lipoprotein cholesterol and triglyceride, and increased high density lipoprotein cholesterol)[l].
- Infertile men with abnormal sperm morphology less than 30% or sperm counts below 20×10(6)/ml or type A and B motility less than 25% and 50% respectively were divided into N. sativa oil group (n=34) receiving 2.5ml N. sativa oil and placebo group (n=34) receiving 2.5ml liquid paraffin two times a day orally for 2 months. It was concluded that daily intake of 5ml N. sativa oil for two months improves abnormal semen quality in infertile men without any adverse effects[li].
- Forty-eight healthy adolescent human males aged between 14 to 17 years were randomly split into two groups: A (n=24) and B (n=24) receiving one capsule of 500mg placebo and 500mg NS respectively once daily for four weeks. The use of NS

as a nutritional supplement was observed to stabilize mood, decrease anxiety and modulate cognition positively[lii].

- In this prospective, crossover randomized controlled trial, 42 geriatric patients with nasal dryness and related symptoms were randomized to receive either 2 weeks of isotonic sodium chloride solution (ISCS) followed by 2 weeks of N. sativa oil (NG oil) or the same treatment in the opposite order. There was a washout period of 3 weeks in between the treatment periods. It was seen that NSO is a better alternative to ISCS to treat nasal mucosa symptoms due to aging[liii].
- Thirty patients with hepatitis C virus (HCV) infection, who were not eligible for IFN (interferon)/ribavirin therapy received N. sativa for three successive months at a dose of (450mg three times daily). It was found to decrease viral load and improve oxidative stress[liv].
- In a double-blind, randomized study, 70 healthy volunteers aged 34 to 63 years with systolic BP from 110 to 140 mmHg and diastolic BP from 60 to 90 mmHg were randomly allocated to receive 2.5mL N. sativa oil or placebo two times a day for 8 weeks. N. sativa oil lowered systolic and diastolic BPs without any adverse effects[lv].
- 88 adult patients with dyspeptic symptoms and found positive for Helicobacter pylori infection were randomly assigned to four groups, receiving i) triple therapy (TT) comprising of clarithromycin, amoxicillin, omeprazole [n= 23], ii) 1g NS + 40mg omeprazole (OM) [n= 21], iii) 2g NS + OM [n= 21] or iv) 3 g NS + OM [n= 23]. Results showed that N. sativa seeds possess clinically useful anti-H. pylori activity, comparable to triple therapy[lvi].
- A randomized pre-test and post-test control group study including 80 children (2-18 years) with brain tumours undergoing chemotherapy were equally allocated into two groups. Intervention group received 5g of NS seeds daily throughout treatment while controls received nothing. NS seeds showed a decrease in incidence of FN (febrile neutropenia) in children with brain tumours[lvii].
- In this double-blind randomized controlled clinical trial, 90 obese women were randomly assigned to receive a low-calorie diet with 3g per day (1g before each meal) NS oil or placebo for 8 weeks. Results showed NS oil concurrent with a low-calorie diet can reduce cardiometabolic risk factors in obese women[lviii].



Dosage (references from clinical trial):

Thymoquinone 1mg/kg [lix]. Thymoquinone (99% pure extract) 0.2% gel[lx].

Conclusion:

Nigella sativa has been clinically proven for various indications as mentioned above in Ayurvedic medicine. Nigella sativa containing Thymoquinone has been reported for most of its therapeutic properties in Pre-clinical studies as mentioned above. There are no direct clinical evidences on Thymoquinone from N. sativa for its pharmacology activities in clinical trials. Based on two pilot studies performed, Thymoquinone (Isolated, pure form sourced from Sigma Aldrich) is indicated for its anti-epileptic effects in children with refractory seizures and chronic periodontitis; specific to 3 species of bacteria - *P. gingivalis*, *A. actinomycetemcomitans and P. intermedia.*

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