Nigella sativa Supplementation Improves Asthma Control and Biomarkers: A Randomised, Double-blind, Placebo-Controlled Trial

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Abstract

Poor compliance with conventional asthma medications remains a major problem in achieving asthma control. *Nigella sativa* oil (NSO) is used traditionally for many inflammatory conditions such as asthma. We aimed to investigate the benefits of NSO supplementation on clinical and inflammatory parameters of asthma. NSO capsules 500mg twice daily for 4 weeks was used as a supplementary treatment in a randomised, double-blind, placebo-controlled trial (RDBPCT) in asthmatics (clinicaltrials.gov: NCT02407262). The primary outcome was Asthma Control Test (ACT) score. The secondary outcomes were pulmonary function test, blood eosinophils, and total serum IgE. Between Jun 1 and Dec 30, 2015, 80 asthmatics were enrolled, with 40 patients in each treatment and placebo groups. After 4 weeks, 10 patients had withdrawn from each group. Compared to placebo, NSO group showed a significant improvement in mean ACT score 21.1 (SD=2.6) vs 19.6 (SD=3.7) (p=0.044) and a significant reduction in blood eosinophils by -50 (-155 – -1) vs 15 (-60 – 87) cells/ uL (p=0.013). NSO improved forced expiratory volume in 1 second as percentage of predicted value by 4 (-1.25 – 8.75) vs 1 (-2 – 5) but non-significant (p=0.170). This RDBPCT demonstrated that NSO supplementation improves asthma control with a trend in pulmonary function improvement. This was associated with a remakable normalisation of blood eosinophlia. Future studies should follow asthmatics for longer periods in a multicentre trial.

Keywords: Asthma, allergy, eosinophils, black seed, nigella sativa, clinical trial

Abbreviations

ACT: Asthma Control Test

BMI: Body Mass Index

CAM: Complementary and alternative treatments

COX-2: Cyclooxygenase 2

FEV1: Forced expiratory volume in 1 second

PEF: Peak expiratory flow

FEF 25-75%: Forced expiratory flow between 25-75%

FVC: Forced vital capacity

GAPP: The Global Asthma Physician and Patient

GMP: Good manufacturing practice

IgE: Immunoglobulin E

IQR: Interquartile range

ITT: Intention-to-treat

KAUH: King Abdulaziz University Hospital

LTB4: Leukotriene B4

LTC4: Leukotriene C4

NSO: *Nigella sativa* L. oil

NS: *Nigella sativa* L.

PGD2: Prostaglandin D2

RDBPCT: Randomised double-blind placebo-controlled trial

Saudi FDA: Saudi Food and Drug Authority

SCTR: Saudi Clinical Trials Registry

SD: Standard deviation

Th2: T helper cells type 2

TQ: Thymoquinone

Introduction

Asthma control is considered to be suboptimal regardless of the availability of conventional treatments (Demoly et al., 2012; Price, Fletcher & van der Molen, 2014). Data from the Recognise Asthma and Link to Symptoms and Experience (REALISE) survey in 2014 for 8000 European patients revealed that the level of asthma control remains low (Price, Fletcher & van der Molen, 2014).

A key concern is poor adherence to asthma medications (Horne et al., 2007; Haughney et al., 2008). Common medication-related reasons for non-adherence include difficulties with inhaler techniques, the complex course of therapy, adverse events, and cost of medications (Bateman et al., 2008; Dima et al., 2015).

The Global Asthma Physician and Patient (GAPP) Survey reported that 39% of asthma patients exchanged or stopped their asthma medication due to adverse events (GAPP, 2005). 76% of patients and 81% of physicians consider that new treatment options are required (GAPP, 2005). The introduction of novel treatment strategies (such as "add-on" treatments) is a key step for better asthma control (Lommatzsch & Stoll, 2016).

Up to 79% of adult patients with asthma used different complementary and alternative treatments including herbal medicines, but the evidence for such treatments' effectiveness in asthma is very limited (Slader et al., 2006). A review of plant-based medicines used in asthma management found that the evidence is still unconvincing and there is a need for a proper scientific research to support the use of plant-based medicines in asthma (Clarke, Lundy & McGarvey, 2015). Therefore, phytotherapy remains a commonly used but poorly investigated element of asthma treatment.

Traditionally, *Nigella sativa* L. (NS; Ranunculaceae), known as black seed, is used as a food supplement or herbal medicine for a range of inflammatory diseases such as asthma

(Lebling & Pepperdine, 2006). A survey of two hundred patients with asthma in Saudi Arabia found that 34.5% used unconventional therapies and NS was of the most common treatments recorded (Al Moamary, 2008).

In several preclinical and preliminary clinical studies, NS and its main active compound thymoquinone showed positive effects on clinical and biochemical markers of asthma inflammation. In mouse models of asthma, *Nigella sativa* oil (NSO) reduced airway hyperresponsiveness, total leukocytes, macrophages, eosinophils, and serum levels of total IgE (Balaha et al., 2012). NSO reduced peripheral blood eosinophil count in conalbumin sensitised mice (Abbas et al., 2005). NSO prevented inflammatory cell infiltration and pathological lesions in the lungs in ovalbumin sensitized rat (Shahzad et al., 2009). TQ was able to reduce eosinophilia in ovalbumin sensitised mice (El Gazzar et al., 2006a). In a randomised double-blind placebo-controlled trials, NS aqueous extract improved asthma symptoms and pulmonary function tests in 29 asthmatics (Boskabady et al., 2007). In a randomised double-blind controlled trial, NS aqueous extract improved pulmonary function tests of 15 asthmatics (Boskabady, Mohsenpoor & Takaloo, 2010). In non-randomised open-label trial, NS combined with bee honey improved lung forced vital capacity in 5 asthmatics. In a randomised single-blind controlled trial, NS powder enhanced asthma control in combination with immunotherapy in 31 Children asthmatics (Kardani et al., 2013). However, these clinical trials had some limitations. First, sample size was limited to a maximum of 29 asthmatics. Second, the investigated outcomes were limited in the measurement of only symptoms and pulmonary function except in one trial blood eosinophils and total serum IgE were investigated. However, they did not compare the results of these biomarkers with placebo. Third, limited information on the chemical composition and quality of the investigational NS preparation used in most trials.

Therefore, we aimed at conducting a clinical trial with a higher standard of trial design (RDBPCT) and sample size (more than 29 adult asthmatics), with inclusion of additional asthma biomarkers, and using chemically characterised preparation of NS.

Material and Methods

Trial design

The trial was prospective phase II randomised (1:1) double-blind placebo-controlled parallel-group clinical trial (RDBPCT).

Participants

The inclusion criteria for patients were: adult male/female (age 18-65 years), asthma diagnosis based on the Global Initiative for Asthma (GINA) guidelines (Global Intiative for Asthma, 2014), asthma symptoms not fully controlled based on ACT score from 5 to 24, no severe asthma exacerbation in the last four weeks, and able to obtain consent.

The exclusion criteria were: Serious co-morbid conditions, smoking history, pregnant women, taking any preparation containing NSO, known history of hypersensitivity to NSO, and taking medications that may interact with NSO such as: anticoagulant/antiplatelet, CNS depressants, and immunosuppressants.

Study settings

The trial was conducted at medical clinics of respiratory, allergy and family medicine at King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Ethical approval for the phase II RDBPCT was granted by both the ethical committees of University College London (ID: 6419/002) and King Abdulaziz University (ID: 95-15). Approval by the national regulatory framework of clinical trials in Saudi Arabia (Saudi

FDA) was obtained. The trial was registered with the clinicaltrials.gov database, identifier NCT02407262 and The Saudi Clinical Trials Registry (SCTR), identifier 15051902.

Interventions

In the treatment group, softgel capsules of cold pressed NSO (0.7% Thymoquinone, analysed by High Performance Liquid Chromatography (HPLC) at our labs at the UCL School of Pharmacy, London, UK) were used. The treatment product is produced according to pharmaceutical GMP standards by Marnys® (Cartagena, Spain; brand name: CUMINMAR; batch number: L885) and licensed as an herbal medicinal product in Saudi Arabia. The placebo group received similar capsules of virgin olive oil (produced in identical appearance by Marnys®, batch number: M398). Both groups received a dose of one capsules (500mg oil) twice daily for four weeks.

Outcomes

The primary outcome was the ACT score. The Global Initiative for Asthma guidelines recommends using one of the numerical asthma control tools such as ACT for scoring the level of asthma control and patients' progress (Global Intiative for Asthma, 2014). ACT is one of the best validated instrument to measure asthma control (Cloutier et al., 2012). ACT consists of a five questions in total scale of 5-25 with each question (Q1-Q5) scaled from 1 to 5. Full control is determined as total ACT score of 25 (Nathan et al., 2004). Questions of ACT are the following:

1. Q1=In the past 4 weeks, how much of the time did your asthma keep you from getting as much done at work, school or at home?
2. Q2=During the past 4 weeks, how often have you had shortness of breath?

3. Q3=During the past 4 weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?
4. Q4=During the past 4 weeks, how often have you used your rescue inhaler or nebulizer medication?
5. Q5=How would you rate your asthma control during the past 4 weeks?

The secondary outcomes were pulmonary function test, absolute peripheral blood eosinophils count, and total serum IgE.

Pulmonary function test was carried using an Easy on-PC spirometer (ndd Medical Technologies, Switzerland).

Randomisation and blinding

Both the investigator and the patients were blinded to treatment or placebo groups. An external medical scientist created a randomisation list with Sealed Envelope Ltd. online tool in random permuted blocks of sizes 2 and 4. These mixed block sizes were used to reduce the selection bias and achieve a better balance in the allocation of participants. The collaborating physicians enrolled participants from their clinics and the investigator allocated patients to their concealed study medications. The packaging label of study medications contained the randomisation code, study title, expiration date, date of packaging, and contact information.

Procedures and compliance check

Patients were recruited from the respiratory, allergy, and family medicine clinics at King Abdulaziz University Hospital in Jeddah, Saudi Arabia during the period of Jun 1 to Dec 30, 2015.

The study duration was four weeks for each patient. During the first visit and after signing the study consent form, patients' baseline demographics, symptoms, co-morbid conditions, medications were recorded. The relevant study outcomes were evaluated and the ACT was scored by the principal investigator by direct questioning of patients. Then, the study medication was dispensed to the patient. After four weeks, the patient returned for follow up and was assessed for the completion of outcomes. During the treatment period, the principal investigator contacted patients by phone after the first week to check compliance and any appearance of side effects.

Blood sampling and biomarkers measurement

Venous blood samples were collected twice (at the baseline and follow-up visits) for the assessment of absolute peripheral blood eosinophils count and total serum IgE. Four millilitres fresh blood was used for blood eosinophils counting. Three milliners blood was used for total serum IgE testing. The blood analysis was carried out using the Celldyn-3500 counter (Abbot) at KAUH's haematology laboratory. This flow cytometry counter provides differential white blood cell counts automatically and used to measure blood eosinophil count. Total serum IgE was measured with ImmunoCAP® total IgE (Phadia Laboratory Systems, Sweden) at the clinical immunology laboratory of KAUH.

Statistical analysis and sample size estimation

The study sample size (80) was calculated based on the expected improvement in primary outcome (ACT) from an average score of 17 to 20 (effect size = 0.638, power = 80%, the level of statistical significance=0.05 and two-sided independent t-test).

IBM SPSS Statistics 23 software was used for statistical data analysis. The normality of data was tested using Shapiro-Wilk test and Q-Q plot. Normally distributed data was represented as mean (SD) and independent t-test was used to compare the means of the outcomes between two groups. However, non-normal data was represented as median (IQR) and Mann–Whitney U testt was used. Intention-to-treat analysis was used for the primary outcome. Multiple regression-substitution was used for imputing primary outcome missing data, taking into account baseline values.

Results

Subjects

140 patients with asthma were assessed for eligibility. Of them, 80 patients were randomised into treatment and placebo groups. In each group ten patients did not complete the study. Reasons for drop-out were variable mainly non-medical such as loss of interest and inability to attend. Only four patients had medical reasons such as adverse events and asthma exacerbation. The recruitment flow chart and details of drop-out reasons are provided in figure 1. The baseline demographics of the recruited patients are presented in table 1. Patients were on routine asthma medications that were adjusted and optimised according to GINA guidelines for standard asthma management (Global Intiative for Asthma, 2014). These included: inhaled short-acting beta agonist (salbutamol), inhaled long acting beta agonist (salmetrol or formetrol), inhaled corticosteroids (budesonide or fluticasone), oral anti-leukotrienes (montelukast).

Asthma Control Test

At baseline, the average levels of symptoms control, represented by the total ACT score, were similar between the treatment and placebo group. At the end of the study, the average total ACT score was significantly higher in the treatment group over the placebo group as shown in table 2. In addition to the total ACT score, the five individual parameters of ACT were also analysed. Significant improvement was found in the Q1 and Q2 of ACT. However, there was a positive improvement in the Q2, Q3 and Q4 of ACT but not statistically significant table 2.

Pulmonary function test

At the end of the study, there was a trend for improvement in the percentage of predicted FEV1 (Forced expiratory volume in 1 second) and PEF (Peak expiratory flow) but not

FEF25-75 (Forced expiratory flow between 25-75%) as shown in table 3. However, subgroup analysis of patients with low basal predicted FEV1% (<80%), significant improvement was found in the treatment subgroup (n=14) vs placebo subgroup (n=15) by 8% (3 – 13%) vs 1% (-2 – 5%) (p=0.018), respectively.

Blood outcomes

There was a marked significant reduction in the absolute peripheral blood eosinophil count in the treatment group compared to the placebo group by -50 (-155 – -1) vs 15 (-60 – 87) cells/ uL (p=0.013), as shown in figure 2. On the other hand, there was no significant change found on the total serum IgE level between both groups as shown in figure 3.

Safety and tolerability

Three patients (two from the treatment group and one from the placebo group) reported three adverse events including; stomach upset, headache, and insomnia. These adverse events were mild and self-limited (did not require any treatment or hospitalisation). The study treatment is considered to be generally tolerable as only two patients (one from the treatment group and one from the placebo group) discontinued the study due to adverse events.

Discussion

In this study NSO significantly improved asthma control over placebo and showed a trend toward pulmonary function improvement with an acceptable safety and tolerability profile among adult asthmatic patients. Therefore, this study helps to build up an evidence-base for using such supplements in the management of this disease. Unlike some other clinical trials, the chemical composition of the main active compound in NSO was determined by HPLC analysis and the product was GMP certified.

Our findings in asthma symptoms control, measured by ACT, were consistent with symptoms improvement in previous clinical studies of NS in asthmatic patients. NSO enhanced scores of subjective improvement in clinical symptoms of allergic conditions including asthma (Kalus et al., 2003). An aqueous extract of NS significantly improved the severity of asthma symptoms (Boskabady et al., 2007). The administration of powdered NS among asthmatic children with immunotherapy significantly increased ACT scores (Sugiono et al., 2013).

In this study, there was a trend toward pulmonary function improvement in FEV1 and PEF in the treatment group but did not reach statistical significance between the treatment and placebo groups as in previous studies. However, we found a significant improvement between both groups in pulmonary function only among subgroups of patients with below normal predicted FEV1% (<80%) at baseline. In a previous clinical study, an aqueous extract of NS significantly improved pulmonary function parameters between the treatment and placebo group only after 3 months treatment but all patients in this study had abnormal pulmonary function at baseline (Boskabady et al., 2007). Also, an aqueous extract of NS caused a significant improvement in short-term pulmonary function parameters in 30 min until 150min, but it was not compared to placebo and all patients

(15 asthmatic) had abnormal pulmonary function at baseline (Boskabady, Mohsenpoor & Takaloo, 2010). In non-RCT open-label clinical study, NS seeds combined with bee honey improved pulmonary function but this study was not placebo controlled and included only 5 asthmatics (Al Ameen et al., 2011).

Interestingly, this study discovered a remarkable reduction of peripheral blood eosinophil count. Eosinophil cell plays a major role in asthma inflammation and blood eosinophil count is considered to be a vital biomarker in asthma trials (Szefler et al., 2012). To our knowledge, this is the first RDBPCT study showed a significant reduction of blood eosinophilia by NSO among asthmatic patients.

The total IgE level in this study did not show any significant changes. This was consistent with findings of Kalus et al. (2003) who reported a non-significant IgE changes among NSO group, but this study including only 3 asthmatic patients was not statistically significant compared to placebo. However, in another clinical study, there was a significant reduction of serum total IgE in the group of NS, but this study was conducted in children, was not double-blinded placebo-controlled, and NS was combined with a probiotics and immunotherapy (Sugiono et al., 2013).

Several mechanism of actions were suggested for NS or TQ, which may explain its effects in asthma. The aqueous extract of NS showed a competitive antagonistic activity on histamine H1 receptors (Boskabady & Sheiravi, 2002). TQ inhibited leukotrienes (LTC4 and LTB4) synthesis in human blood cells (Mansour & Tornhamre, 2004). TQ reduced leukotrienes (LTB4 and LTC4) levels by 5-lipoxygenase enzyme inhibition and decreased helper cell type 2 (Th2) cytokines level in a mouse model of allergic asthma (El Gazzar et al., 2006b). NSO was effective in decreasing histamine and leukotrienes

production and increasing PGE2 release in guinea pig model of allergic asthma (Saleh, ElDenshary & Mahran, 2012).

Limitations of our study include the following. The duration of the intervention is relatively short (four weeks only). The choice of outcomes was dependent on its applicability and availability at the study site. A relatively high drop-out rate which resulted from reasons not related to study design or medication, but rather due to unexpected reasons not considered initially including the geographical mobility of some of the study participants. In addition, the lack of incentives for participants (due to limited funding) along with the socioeconomic status of some the participants played a role in patient retention rate.

Conclusion

NSO supplementation appeared to be effective in enhancing the control of asthma symptoms with a trend in pulmonary function improvement. Remarkably, NSO showed a reduction of blood eosinophilia. These findings may provide an evidence for the potential benefits of NSO supplementation in the clinical management of asthma. To our knowledge, this is the first RDBPCT compared the effect of NSO on blood eosinophils, and serum total IgE in a relatively larger group of adult patients with asthma versus placebo. Future studies should follow patients for a longer period and use additional outcomes to validate the benefits of NSO in asthma.

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Conflict of Interest

The authors and collaborators disclose no conflicts of interest. The study medication as well as the placebo were produced by Marnys®, but the company had no input neither on the design of the study nor the interpretation of the results.

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Tables

Table 1. Baseline characteristics of the subjects.

Variable	Treatment (n=40)	Placebo (n=40)
Age	39 (13)	42 (15)
Men	15 (38%)	18 (45%)
Women	25 (62%)	22 (55%)
BMI	28 (5)	30 (8)
Predicted FEV1%	74(17)	72(16)
Total ACT	16.0 (3.9)	16.5 (3.6)
Blood eosinophil count, cells/uL	350 (187 – 711)	300 (135 – 415)
Total IgE, IU/mL	164 (112 – 468)	224 (57 – 682)

Values represented as mean (SD), n (%), or median (IQR).

Table 2. Asthma Control Test (ACT) scores.

Variable	Pre-treatment			Post-treatment		
	Treatment	Placebo	P	Treatment	Placebo	P
Total ACT	16.0 (3.9)	16.6 (3.6)	0.44	21.1 (2.6)	19.6 (3.7)	**0.04***
Q1 (daily functioning)	3.4 (0.9)	3.3 (1.1)	0.62	4.4 (0.6)	3.8 (0.9)	**0.01***
Q2 (SOB frequency)	3.1 (1.0)	3.4 (1.0)	0.22	4.2 (0.7)	3.9 (0.9)	0.28
Q3 (night symptoms)	3.3 (1.3)	3.6 (1.2)	0.27	4.4 (0.7)	4.0 (1.1)	0.14
Q4 (use of rescue medications)	3.8 (1.3)	3.7 (1.3)	0.77	4.5 (0.7)	4.2 (1.2)	0.17
Q5 (overall asthma control)	2.7 (0.7)	2.7 (0.9)	1.00	4.1 (0.6)	3.5 (0.9)	**0.01***

SOB, shortness of breath; Q1-Q5 are the individual questions of ACT. Values represented as mean (SD).

Table 3. Pulmonary function test scores.

	Change in placebo group (n=24)	Change in treatment group (n=25)	p
Predicted FEV1%	1 (-2 – 5) %	4 (-1.25 – 8.75) %	0.170
Predicted PEF%	2 (0 – 14.5) %	6.5 (0.25 – 22.75) %	0.279
Predicted FEF25-75%	3 (-6.5 – 16.5) %	2.5 (-7.75 – 18.75) %	0.992

Values represented as median (IQR). FEV1: Forced expiratory volume in 1 second. PEF: Peak expiratory flow. FEF 25-75%: Forced expiratory flow between 25-75%.