

Nigella sativa (black seed) oil does not affect the T-helper 1 and T-helper 2 type cytokine production from splenic mononuclear cells in allergen sensitized mice

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Received 5 July 2004; received in revised form 23 February 2005; accepted 4 March 2005

Available online 28 April 2005

Abstract

Nigella sativa Linn. (Ranunculaceae) is known to have beneficial effects on a wide range of diseases including asthma. However, the mechanism of action in asthma and other allergic diseases is not entirely clear. The present study was planned to evaluate the effects of *Nigella sativa* on cytokine production of splenic mononuclear cells in ova-sensitized mice. Nineteen two-month-old BALB/c mice were given 0.3 mL of *Nigella sativa* oil by oro-esophageal cannula once a day for a month. The control group consisting of 10 mice took 0.3 mL of 0.9% saline solution by the same route for the same period. In the third week of the study, all mice were sensitized by means of intraperitoneal injections of 20 µg of ovalbumin (OVA-Grade VI, Sigma). Ova injections were repeated three times with 7-day intervals. After another week, all mice were sacrificed by means of cervical dislocation. Then the splenic mononuclear cells (MNCs) of mice were cultured with OVA or Concanavalin A (Con-A). From the culture supernatants, IL-4, IL-10 and IFN-γ were assessed by means of ELISA. The cytokine production of splenic MNCs of mice that were given *Nigella sativa* for 30 days was not significantly different than those who took saline solution instead. In conclusion, *Nigella sativa* oil seems not to have an immunomodulatory effect on Th1 and Th2 cell responsiveness to allergen stimulation. © 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Nigella sativa*; Allergy; Asthma; Immunomodulation

1. Introduction

According to numerous epidemiological studies from different parts of the world, the prevalence of the atopic diseases has increased over the past 30–40 years (von Hertzen and Haahtela, 2004). Atopic asthma is the most common chronic disease of childhood and childhood mortality due

to bronchial asthma has doubled in the past decade (Burr et al., 1999).

A huge number of studies have focused on the aetiology of asthma. Many different hypotheses have been postulated including atopy, environmental allergen exposure, hygiene hypotheses, etc. Asthma has been associated with an exaggerated T-helper 2 (Th2) over Th1 responses to allergic and non-allergic stimuli. In the current concept of the hygiene hypothesis, many factors such as increased use of antibiotics, decline in family size, reduced exposure

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to endotoxins and intestinal parasites are accepted to cause to reduced stimulation of regulatory T cells and consequently lead to asthma (von Hertzen and Haahtela, 2004).

As parallel to the aetiological studies, prevention and treatment of allergic and atopic diseases have been investigated (Gore and Custovic, 2004). In addition to avoidance measures and pharmacological interventions, some types of naturopathic treatments have been attempted. Various derivatives from specific medicinal plants were identified as the anti-asthma components and some mechanisms of action were explored. The results show positive effects of these herbs on bronchodilation, pulmonary function tests, and antagonism of asthma mediators such as histamine and platelet activating factor, corticosteroid levels, and clearance of mucus (Bielory and Lupoli, 1999). One of these plants, *Nigella sativa* Linn. (Ranunculaceae) commonly known as “black seed”, has been traditionally used in The Middle East region as a natural remedy for asthma as well as some other diseases (Nadkarni, 1976; Sayed, 1980; Lautenbacher, 1997).

Nigella sativa seeds were demonstrated to contain 36–38% fixed oils, proteins, alkaloids, saponin and 0.4–2.5% essential oil (Ali and Blunden, 2003). The fixed oil is composed mainly of unsaturated fatty acids (Houghton et al., 1995). The major part of essential oil is thymoquinone of which various effects have been shown in vivo or in vitro by numerous studies. *Nigella sativa* oil was demonstrated to modify leukotriene synthesis and inhibit histamine release (Chakravarty, 1993). Some immuno-potentiating properties of *Nigella sativa* were also shown. However, the real mechanism of action on allergic diseases is still unclear. The purpose of this study was to evaluate the effects of *Nigella sativa* on cytokine production of splenic mononuclear cells in ova-sensitized mice.

2. Methods

Nineteen two-month-old BALB/c mice were given 0.3 mL of *Nigella sativa* oil by oro-eosophageal cannula once a day for a month. *Nigella sativa* oil was obtained by crude extraction of *Nigella sativa* seeds without using any solvent. Before the initiation of the study, different amounts of oil were given to a separate test group and the maximum dose, which was tolerated without adverse effect, was determined as 0.3 mL. Control group consisting of 10 mice took 0.3 mL 0.9% saline solution by the same route for the same period.

On the third week of the study, all mice were sensitized by means of intraperitoneal injections of 20 µg of OVA (Grade VI, Sigma). Ova injections were repeated three times with 7-day intervals. One week after from the last injection, all mice were sacrificed by means of cervical dislocation. This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986); 86/609/EEC).

2.1. In vitro production of cytokines

For in vitro cytokine production, splenic mononuclear cells (MNCs) isolated by means of density gradient centrifugation (Lymphoprep Axis-SHIELD PoC AS, OSLO) were suspended in culture medium (RPMI 1640 and 10% FCS). The cells were cultured in 96-well round bottom plates at 37 °C in a 5% CO₂ atmosphere with culture medium, Con-cavalin A (Con-A, 2.5 µg/mL, Sigma) or OVA (50 µg/mL, Sigma). At optimal time points assessed in preliminary experiments after 48 h for IFN-γ and after 96 h for IL-4 and IL-10 supernatants were harvested. The IFN-γ, IL-4 and IL-10 concentrations were determined by ELISA (Cytoscreen Mouse kits for IFN-γ, IL-4 and IL-10, Biosources International, Camarillo, CA, USA).

2.2. Proliferation assay with ³H thymidine

Splenic MNCs (5 × 10⁵/200 mL) were cultured for 96 h under the same conditions as described above. Proliferative responses were determined by means of ³H thymidine incorporation (0.5 µCi/200 mL; Amersham Buchler, Braunschweig, Germany) over the last 18 h of culture.

2.3. Statistical analysis

Results from study and control groups were compared with the Mann–Whitney *U*-test. Significance was set at a *p* value of 0.05.

3. Results

The cytokine levels produced by MNCs cultured with Con-A and OVA were found to be in a wide range. The cytokine production of splenic MNCs of mice that were given

Table 1
Cytokine production by splenic MNCs in vitro

Groups	Con-A IFN-γ (pg/mL)	OVA	
		IL-4 (pg/mL)	IL-10 (pg/mL)
Saline (<i>N</i> = 10)	292.7 ± 195.9	25.1 ± 28.1	390.1 ± 399.1
<i>Nigella sativa</i> (<i>N</i> = 19)	390.7 ± 166.6	7.8 ± 8.0	324.6 ± 255.4
Significance <i>p</i>	0.25 (NS)	0.15	0.96

Table 2
The results of proliferative responses of MNCs

Groups	n	Mean	S.D.	Minimum	Maximum
Saline					
OVA	10	10013.50	6743.74	1278.00	23532.00
Con-A		17173.30	8425.61	1178.00	26298.00
<i>Nigella sativa</i>					
OVA	19	12240.26	8457.90	224.00	26203.00
Con-A		17420.00	12083.33	1833.00	45572.00

³H thymidine incorporation.

Nigella sativa for 30 days was not significantly different than those who got saline solution instead (Table 1).

Table 2 shows the proliferative responses in control and test groups induced by ova and Con-A. We compared the proliferative responses between ova and Con-A with Wilcoxon test in the control group and we did not observe any significant differences ($z = -1.85$, $p = 0.06$). In the test group, the count of cells in Con-A was slightly but significantly higher than the ova group suggesting increased proliferative response to Con-A ($z = -2.19$, $p = 0.03$). Pairs of groups were compared with the Mann–Whitney *U*-test. We did not find any differences in ova stimulation between control and test group ($z = -0.73$, $p = 0.46$). Similarly, there was not any difference in Con-A stimulation between two groups ($z = -0.46$, $p = 0.65$).

4. Discussion

Our findings demonstrated that *Nigella sativa* oil (natural extract of *Nigella sativa* seeds) does not provide any effect on the Th1 and Th2 type cytokine profile as well as the proliferative responses of splenic mononuclear cells of ova-sensitized mice.

From a review of literature, it is likely to have a role for some herbal medicines in the treatment of asthma and allergic rhinitis. Usage of herbal medicines has increased in recent years. Many of these medicinal plants have provided relief of symptoms equal to allopathic medicines used (Bielory and Lupoli, 1999). But the amount of research on these products is limited. Although *Nigella sativa* is an important medical herb since very old times as a natural remedy for a wide range of diseases including allergy, its mechanism of action is still unclear (Ali and Blunden, 2003). There are not sufficient clinical data about its efficacy and the ways of action.

Recently, Kalus et al. presented four studies indicating that the oil of *Nigella sativa* was effective on relieving symptoms of allergic diseases. But no laboratory evidence supporting the clinical effects was described in these studies. One study had shown that *Nigella sativa* oil inhibited the COX and 5-lipoxygenase pathways of arachidonic acid metabolism and decreased the synthesis of thromboxane and leukotrienes (Houghton et al., 1995). In another study, nigelone (an essential substance of *Nigella sativa* seeds) was demonstrated to inhibit the release of histamine from sensitized mast cells after prior antigen exposure (Chakravarty,

1993). Since leukotrienes are the potent mediators of asthma and histamine plays an important role in immediate hypersensitivity reactions, above findings might explain the efficacy of *Nigella sativa* on allergic diseases and asthma.

The immunopotentiating properties of *Nigella sativa* seeds on human T cells were shown firstly by El-Kad and Kandil in 1987. Eight years after, Haq et al. demonstrated that *Nigella sativa* seeds activated T lymphocytes to secrete the interleukin, IL-3 and increased IL-1 β production. However, Swamy and Tan (2000) reported that *Nigella sativa* extract has no immunomodulatory effect without mitogen activation.

Allergic diseases are accepted to occur with an imbalance in T-helper cells by means of a shift to Th2 subtype predominance and a decrease in Th1 subpopulation. Some strategies to prevent allergic diseases by immunomodulation, i.e. reducing Th2 proliferation and providing sufficient Th1 response have been proposed (Gore and Custovic, 2004). Allergen specific immunotherapy that specifically targets T cells over a long period of time has been used successfully in the treatment of some allergic diseases. Successful venom and aeroallergen immunotherapy was found to be associated with the induction of peripheral tolerance in T cells by generation of Tr cells that secrete the suppressive cytokines IL-10 and TGF- β , suggesting that generation of Tr1 cells might play a role in healthy immune response (Akdis et al., 1998; Jutel et al., 2003; Platt-Mills et al., 2001). Recently, immune responses in healthy and allergic individuals were shown to be characterized by a fine balance between allergen-specific T regulatory 1 and T-helper 2 cells (Akdis et al., 2004).

Several dietary supplements have been identified as being of possible benefit in the prevention and treatment of allergic disease (Baker and Ayres, 2000; Romieu et al., 2002; Mellis, 2002). Furthermore, appropriate bacteria are thought to act as natural Th1 “adjuvants” during the priming of T cells against newly encountered environmental antigens. *Mycobacterium vaccae*, lipopolysaccharide, *Lactobacillus* spp. and oral bacterial extracts are being examined for their proven or alleged atopy-preventing effect (Matricardi and Bonini, 2000). For example, adding probiotics (*Lactobacillus* GG) to the mother’s diet in the last month of pregnancy and during lactation as well as to the infant’s diet for the six months of life was shown to reduce the prevalence of atopic eczema at 2-year follow-up (Kalliomaki et al., 2003).

Our study aimed to evaluate whether *Nigella sativa*, which has been traditionally used for the treatment of allergic diseases and asthma, has an immunomodulatory effect on T cells in terms of Th1 and Th2 cytokine production. *Nigella sativa* supplement would be expected to cause an increase in IFN- γ (a Th1 type cytokine) and IL-10 (related to T regulatory cells) production and a decrease in IL-4 (Th2 type cytokine) from mononuclear cells of allergen-sensitized mice. Our results suggested that *Nigella sativa* oil does not affect the production of these three cytokines from mononuclear cells in allergen-stimulated mice. Naturally, this mouse model for immunologic study could not be regarded as an

undisputed example for human immune system. As mentioned above, the effects of *Nigella sativa* on human peripheral T cells were evaluated in vitro by Haq et al., (1995). The authors had demonstrated that *Nigella sativa* seeds activate T-lymphocytes to secrete IL-3 and to cause enhanced IL-1 β production. Four years later, the same authors fractionated *Nigella sativa* proteins by ion exchange chromatography and showed that some proteins have suppressive and others stimulatory properties in lymphocyte cultures (Haq et al., 1999). In that study, no effect on IL-4 secretion was seen while the production of TNF- α was found to be enhanced particularly in higher doses. In vivo evaluation of the effects of *Nigella sativa* on human peripheral T-lymphocytes in allergic and non-allergic individuals could be the subject of a new study and clarify the mechanism of action of this unique plant in ameliorating of allergic diseases.

In conclusion, *Nigella sativa* of which the efficacy on some chronic disorders including allergy and asthma has been accepted since ages seems not to have an immunomodulatory effect on Th1 and Th2 cell responsiveness against allergen stimulation. Certainly, the other probable ways of action of *Nigella sativa* on various diseases and asthma need to be elucidated.

Acknowledgement

This study was supported by The Istanbul University Research Fund.

References

- Ali, B.H., Blunden, G., 2003. Pharmacological and toxicological properties of *Nigella sativa*. *Phytotherapy Research* 17, 299–305.
- Akdis, C.A., Blesken, T., Akdis, M., Wüthrich, B., Blaser, K., 1998. Role of IL-10 in specific immunotherapy. *Journal of Clinical Investigation* 102, 98–106.
- Akdis, M., Verhagen, J., Taylor, A., Karamloo, F., Karagiannidis, C., Cramer, R., Thunberg, S., Den, z.G., Valenta, R., Fiebig, H., Kegel, C., Disch, R., Schmidt-Weber, C.B., Blaser, K., Akdis, C.A., 2004. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *Journal of Experimental Medicine* 199, 1567–1575.
- Baker, J.C., Ayres, J.G., 2000. Diet and asthma. *Respiratory Medicine* 94, 925–934.
- Bielory, L., Lupoli, K., 1999. Herbal interventions in asthma and allergy. *Journal of Asthma* 36, 1–65.
- Burr, M.L., Limb, E.S., Andrae, S., Barry, D.M., Nagel, F., 1999. Childhood asthma in four countries: a comparative survey. *International Journal of Epidemiology* 23, 341–347.
- Chakravarty, N., 1993. Inhibition of histamine release from mast cells by niggellone. *Annals of Allergy* 70, 237–242.
- El-Kadi, A., Kandil, O., 1987. The black seed (*Nigella sativa*) and immunity: its effect on human T cell subset. *Federation Proceedings* 46, 12–22.
- Gore, C., Custovic, A., 2004. Can we prevent allergy? *Allergy* 59, 151–161.
- Haq, A., Abdullatif, M., Lobo, P.I., Khabar, K.S.A., Sheth, K.V., Al-Sedairy, S.T., 1995. *Nigella sativa*: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. *Immunopharmacology* 30, 147–155.
- Haq, A., Lobo, P.I., Al-Tufail, M., Rama, N.R., Al-Sedairy, S.T., 1999. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *International Journal of Immunopharmacology* 21, 283–295.
- Houghton, P.J., Zarka, R., de las Heras, B., Houlst, J.R.S., 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Medica* 61, 33–36.
- Jutel, M., Akdis, M., Budak, F., Aebischer-Casaulta, C., Wryszcz, K., Blaser, K., Akdis, C.A., 2003. IL-10 and TGF- β cooperate in regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *European Journal of Immunology* 33, 1205–1214.
- Kalliomaki, M., Salminen, S., Poussa, T., Arvilommi, H., Isolauri, E., 2003. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 361, 1869–1871.
- Kalus, U., Pruss, A., Bystron, J., Jurecka, M., Smekalova, A., Lichius, J.J., Kiesewetter, H., 2003. *Phytotherapy Research* 17, 1209–1214.
- Lautenbacher, L.M., 1997. Schwarzkümmelöl. *Deutsche-Apotheker Zeitung* 137, 68–69.
- Matricardi, P.M., Bonini, S., 2000. Mimicking microbial ‘education’ of the immune system: a strategy to revert the epidemic trend of atopy and allergic asthma? *Respiratory Research* 1, 129–132.
- Mellis, C.M., 2002. Is asthma prevention possible with dietary manipulation? *The Medical Journal of Australia* 177, 78–80.
- Nadkarni, A.K., 1976. *Indian Materia Medica*. Popular Parkishan, Bombay India, p. 854.
- Platt-Mills, T., Vaughan, J., Squillace, S., Woodfolk, J., Sporik, R., 2001. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 357, 752–756.
- Romieu, I., Sienra-Monge, J.J., Ramirez-Aguilar, M., Tellez-Rojo, M.M., Moreno-Macias, H., Reyes-Ruiz, N.I., et al., 2002. Antioxidant supplementation and lung functions among children with asthma exposed to high levels of air pollutants. *American Journal of Respiratory and Critical Care Medicine* 166, 703–709.
- Sayed, M.D., 1980. Traditional medicine in health care. *Journal of Ethnopharmacology* 2, 19–22.
- Swamy, S.M.K., Tan, B.K.H., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *Journal of Ethnopharmacology* 70, 1–7.
- von Hertzen, L.C., Hahtela, T., 2004. Asthma and atopy—the price of affluence? *Allergy* 59, 124–137.