

Effect of Dexamethasone and *Nigella sativa* on Peripheral Blood Eosinophil Count, IgG₁ and IgG_{2a}, Cytokine Profiles and Lung Inflammation in Murine Model of Allergic Asthma

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It has been reported that *Nigella sativa* oil possesses anti-inflammatory and bronchodilator activities. Besides, non – toxic and wide margin of safety for therapeutic doses compared with Dexamethasone. This work aims to study the effect of *Nigella sativa* and Dexamethasone on different immune and inflammatory parameters in a mouse model of allergic asthma. Mice sensitized intraperitoneally and challenged intratracheally with conalbumin were treated with *Nigella sativa* 24 hours after the first intratracheal challenge. Dexamethasone treated and naïve mice served as controls. The effect of *Nigella sativa* and Dexamethasone treatment on peripheral blood eosinophil count, IgG₁ and IgG_{2a}, cytokine profiles and lung inflammation were evaluated. *Nigella sativa* was significantly reduced peripheral blood eosinophil count, IgG₁ and IgG_{2a} levels, cytokine profiles and inflammatory cells in lung tissue. These effects were equivalent to the effects of Dexamethasone except unchanged IFN- γ level. *Nigella sativa* exhibits anti-airway inflammation and immunoregulatory effect which may be useful for treatment of allergic asthma

Asthma is a major public health problem worldwide, and its morbidity and mortality have increased over the last two decades (Beasley et al., 2000).

Corticosteroids are the most potent nonspecific anti-inflammatory agents, and they are known to produce substantial improvement in objective lung functions of asthmatic subjects. However, many undesirable side effects are known for systemic corticosteroids, such as adrenal suppression, decreased bone metabolism and decreased growth in children, in whom asthma is increasing in frequency (Helms, 2000). Thus, there is a need for new or alternative approaches to the control of this disease. The seeds of *Nigella sativa* (Ranunculaceae), commonly known as black seed or black cumin, are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases and conditions that include asthma (Ferdous et al.,

1992; Salomi et al, 1992; Medenica et al., 2000; Gilani et al, 2001 and Kalus et al., 2003). The crude extract of *Nigella sativa* seeds exhibits spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade and this activity is concentrated in the organic fraction. Its usefulness for diarrhea and asthma in traditional medicine appears to be based on a sound mechanistic background (Gilani et al., 2001).

This work aims to study the effect *Nigella sativa* and Dexamethasone on different immune and inflammatory parameter on a well characterized mouse model of allergic asthma.

Material and Methods

Laboratory animals

Thirty one of approximately 6 weeks old male (CD1) albino mice, weighing 18-20 g were purchased from Experimental Research Center of Theodor Bilharz

Institute, Cairo, Egypt. They were maintained in animal facilities of Biotechnology Research Laboratory, Gastroenterology Surgery Center, Mansoura University and given food and water *ad libitum*.

Animal groups

Animals were divided into 4 groups. Group I (n=7) neither sensitized nor treated and served as controls. Group II (n=13) were sensitized and challenged with conalbumin (Sigma Chemical Co., USA). Group III (n=8) were treated with Dexamethasone. Group IV (n=12) were treated with *Nigella sativa* fixed oil.

Antigen sensitization, challenge and treatment

Mice were sensitized by two, one week apart, intraperitoneal injections of 200 µg of conalbumin and 2 mg of alum in 0.3 ml of PBS. Seven days after the second injection, mice were anesthetized and challenged intratracheally with 100 µg of conalbumin in 0.05 ml of PBS followed by two more intratracheal challenges on days 20 and 30. Intratracheal challenge was performed according to Keane-Myers et al. (1998). Specifically, mice were placed on a board in a supine position. The animal's tongue extended with lined forceps and 50 µl of a 0.2% solution of conalbumin is placed on the back of tongue. After 24 h of the first antigen challenge, group III mice received 0.5 mg/kg of Dexamethasone intraperitoneally. Group IV received *Nigella sativa* fixed oil at a dose of 5 ml/kg (Mahmoud et al., 2002) intragastrically by means of a 25-gauge stainless steel blunt feeding needle. Mice of groups III and IV were treated once per day for 17 consecutive days. After 24 h of the last dose of treatment, mice were sacrificed and the blood was collected, blood films were made directly then sera were separated and stored at -20°C. Lungs were removed and fixed in neutral buffered formaldehyde. Then, sectioned for histopathological studies.

Peripheral blood eosinophils

Peripheral blood eosinophils were examined in Leishmans stain film by light microscopy.

Measurement of serum IgG₁ and IgG_{2a}

Sera were examined for anti-conalbumin IgG₁ and IgG_{2a} in mice by enzyme linked immunosorbent assay (ELISA). Briefly, a polystyrene plate was coated with 50 µg/ml of conalbumin, incubated over night, then overlaid with diluted sera from control and treatment animals. After further incubation and washing, alkaline phosphatase labeled anti-mouse IgG₁ and IgG_{2a} (Sigma) were added. P-nitrophenyl phosphate in substrate buffer was added and the optical densities of the developed colors were read at 405 nm.

Cytokine assays

Gamma interferon (IFN-γ), interleukin-2 (IL-2), interleukine-12 (IL-12) and interleukine-10 (IL-10) were measured as markers for Th1 and Th2 type patterns of cytokine response. A polystyrene flat bottom microtiter plate was coated with serum of treated and non treated mice overnight. After blocking, rat antimouse IFN-γ mAb, goat anti-mouse IL-2, IL-12 and IL-10 mAbs (Sigma) were added at 37 °C for 1h. After washing, goat anti-rat IgG alkaline phosphatase conjugate (for IFN-γ assay) and rabbit anti-goat IgG alkaline phosphatase conjugate (for IL-2, IL-12, and IL-10 assays) were added at room temperature for 2h. One mg/ml of p-nitrophenyl phosphate was used as a substrate, and the absorbance was read at 405 nm using microplate autoreader.

Histopathological examination

Paraffin blocks of lung tissues were prepared and sliced to 5-6 µm thick sections. The slides were stained with haematoxylin & eosin and were examined for the presence of peribronchial inflammation. The peribronchial inflammation was staged according to the severity of inflammation from 1+ to 3+ (1+; inflammation around the bronchiole occurs only in a focal place, 2+; inflammation surrounding the bronchiole is less than five cell lines and 3+; inflammation surrounding the bronchiole is more than five lines of cells) (Basdemir et al., 2001).

Statistical analysis

We used SPSS version 10.0.1, 1999 (SPSS Inc., Chicago, IL, USA). Our data showed nonparametric distribution by the Kolmogorov-Smirnov test. For comparison of continuous variables we used Mann-Whitney-U test, and for comparison of categorical variables we used Chi-Square test. Significance was considered when p-value is < 0.05 and all p-values reported are of the double sided type.

Results

Determination of peripheral blood eosinophil

As shown in table (1), the peripheral blood eosinophils were significantly increased in conalbumin sensitized mice than non sensitized mice (P<0.05).

Dexamethasonamithazon or *Nigella sativa* treatment result in significant decrease in peripheral blood eosinophil compared with sensitized non treated group (P<0.05).

IgG₁ and IgG_{2a} level

Our result showed that conalbumin sensitization and challenge resulted in significant elevation of IgG₁ and IgG_{2a} levels than in the control group ($P < 0.001$), (Table 1).

As shown in Table 1, IgG₁ and IgG_{2a} were significantly decreased after treatment with Dexamethasone ($P < 0.01$) and *Nigella sativa* ($P < 0.001$ and $P < 0.05$, respectively, compared with sensitized non treated group).

Table 1. Peripheral blood eosinophil percentage and serum levels of IgG₁ and IgG_{2a} in sensitized and treated mice groups.

Groups	Eosinophil	IgG ₁	IgG _{2a}
	Mean $\pm$ SD (%)	Mean $\pm$ SD (OD)	Mean $\pm$ SD (OD)
Control n = 7	0.67 $\pm$ 0.82	0.12 $\pm$ 0.025	0.02 $\pm$ 0.011
¹ Con.A n = 13	4.30 $\pm$ 1.70 $p < 0.05^*$	2.23 $\pm$ 0.07 $p < 0.001^*$	0.89 $\pm$ 0.35 $p < 0.001^*$
² Dex. n = 8	1.67 $\pm$ 2.0 $p < 0.05^{**}$	1.91 $\pm$ 0.29 $p < 0.01^{**}$	0.41 $\pm$ 0.25 $p < 0.01^{**}$
³ <i>N. sativa</i> n = 12	2.44 $\pm$ 1.59 $p < 0.05^{**}$	1.96 $\pm$ 0.21 $p < 0.001^{**}$	0.57 $\pm$ 0.23 $p < 0.05^{**}$

¹Conalbumin., ²Dexamethazone., ³*Nigella sativa*

* p value compared to control group., ** p value compared to Con-A group., P values < 0.05 are considered significant.

Cytokines assay

Conalbumin sensitized mice showed significant increase in the level of IFN- γ ($P < 0.05$), IL-2 ($P < 0.001$), IL12 ($P < 0.01$) and IL10 ($P < 0.01$) compared with non sensitized group. Twenty four hours after last dose of Dexamethasone treatment, serum levels of IL-2 were significantly decreased than sensitized

non treated group ($P < 0.05$). IL-12 and IL-10 were also decreased but not significantly while no change was observed in the level of IFN- γ . Treatment with *Nigella sativa* induce significant decrease in the level of IL-2 and IL-12 ($P < 0.001$ and < 0.05), respectively, and no change was noticed in the levels of IL-10 and IFN- γ (Table 2).

Table 2. Serum cytokine levels in sensitized and treated mice groups.

Groups	IFN- γ	IL-2	IL-12	IL-10
	Mean $\pm$ SD (OD)	Mean $\pm$ SD (OD)	Mean $\pm$ SD (OD)	Mean $\pm$ SD (OD)
Control n = 7	0.15 $\pm$ 0.1	0.23 $\pm$ 0.12	0.29 $\pm$ 0.08	0.29 $\pm$ 0.09
Con-A n = 13	0.33 $\pm$ 0.19 $p < 0.05^*$	0.56 $\pm$ 0.09 $p < 0.001^*$	0.5 $\pm$ 0.15 $p < 0.01^*$	0.56 $\pm$ 0.17 $p < 0.01^*$
Dex. n = 8	0.45 $\pm$ 0.14 $p > 0.05^{**}$	0.4 $\pm$ 0.11 $p < 0.05^{**}$	0.41 $\pm$ 0.09 $p > 0.05^{**}$	0.46 $\pm$ 0.14 $p > 0.05^{**}$
³ <i>N. sativa</i> n = 12	0.3 $\pm$ 0.19 $p > 0.05^{**}$	0.31 $\pm$ 0.1 $p < 0.001^{**}$	0.36 $\pm$ 0.11 $p < 0.05^{**}$	0.5 $\pm$ 0.13 $p > 0.05^{**}$

¹Conalbumin., ²Dexamethazone., ³*Nigella sativa*

* p value compared to control group., ** p value compared to Con-A group., P values < 0.05 are considered significant.

Histological finding

We observed a significant increase in the number of inflammatory cells invading the lungs in sensitized non-treated group when compared with control group, $P < 0.05$ (Table 3). We found a significant decrease in the

number of inflammatory cells when comparing Dexamethasone-treated group with sensitized non-treated group. Also, the group of mice treated with *Nigella sativa* showed a decrease in the number of inflammatory cells invading the lungs compared to the sensitized non-treated group.

Table 3. Histological examination of lung tissues in sensitized and treated mice groups.

Groups	No inflammation	Focal inflammation	All around inflammation (but less than 5 cell lines)
	n (%)	n (%)	n (%)
Control n = 6	3 (50)	3 (50)	0 (0)
Con-A n = 10	0 (0)	5 (50) $p < 0.05^*$	5 (50)
Dex. n = 6	3 (50)	3 (50) $p < 0.05^{**}$	0 (0)
³ <i>N. sativa</i> . n = 9	3 (33.3)	5 (55.6) $p > 0.05^{**}$	1 (11.1)

¹Conalbumin., ²Dexamethazone., ³*Nigella sativa*

* p value compared to control group., ** p value compared to Con-A group., P values < 0.05 are considered significant.

Discussion

The seeds of *Nigella sativa* have been used in traditional medicine for the treatment of variety of diseases including asthma (Medenica et al., 1977; Ferdous et al., 1992; Salomi et al., 1992; Gilani et al., 2001 and Kalus et al., 2003). Animal model of allergic asthma have been developed in order to investigate the therapeutic effect of this herb on allergic asthma.

In the present study, we have found a significant increase in serum IgG₁ and IgG_{2a} levels in the sensitized non-treated group when compared to the control group ($P < 0.05$). It well known that IgG₁ are antibodies produced under activation of Th2 (Ormstad et al., 2003) that play a central role in the pathogenesis of allergic asthma (Romagnani, 2000). Our results showed that the levels of IgG₁ were significantly higher than IgG_{2a}

($P < 0.05$) indicating the successful sensitization in our animal model. In one study, Steerenberg et al. (1999) demonstrated that pollen-specific IgG₁ were increased in serum of rats immunized with pollen grains. Another study by Li et al. (2000) demonstrated an increase in level of IgG₁ in a murine model of allergic asthma. Our study demonstrate that there was a significant decrease in serum IgG₁ and IgG_{2a} levels after treatment with Dexamethasone or *Nigella sativa* when compared with the sensitized non-treated group. From these results, we can suggest that *Nigella sativa* has an immunoregulatory effect similar to that of Dexamethasone and this effect may be favorable for the treatment of allergic asthma.

Peripheral blood eosinophilia was documented in bronchial asthma both in humans (Koller et al., 1995) and murine

models (Yu et al., 2002). The sensitized non-treated group of mice in this study showed a significant increase in peripheral blood eosinophilic percentage compared to control group indicating successful sensitization and development of an allergic model. The group of mice treated with Dexamethason during airway allergen challenge exhibited a significant reduction in percentage of circulating eosinophils compared to sensitized non-treated group ($P < 0.05$). The reduction of eosinophils in circulation and tissues, including airways and bone marrow, by systemically administrated corticosteroids has been reported previously in normal subjects (Butterfield et al., 1986), rats (Blenkinsopp and Blenkinsopp, 1967), mice (De Bie et al., 1996), and patients with asthma (Bentley et al., 1996). Also, the percentage of circulating eosinophils was significantly decreased in the *Nigella sativa*-treated group compared to sensitized non-treated group ($P < 0.05$) indicating an anti-inflammatory effect of *Nigella sativa*.

Late phase pulmonary inflammation in allergic asthma is modulated by Th cells that have been classified into Th1 and Th2 types in accordance with their cytokine patterns. The Th1 cells are characterized by increase secretion of IL-2 and IFN- γ , whereas the Th2 subset secrete IL-4, IL-5, IL-6, IL-9 and IL-10 (Mosmann et al., 1986). Our results demonstrate that IFN- γ , IL-2, IL-12 and IL-10 were significantly elevated after conalbumin sensitization and challenge.

Although several studies reported that allergic asthma is associated with elevated Th2 pattern of cytokine production (Robinson et al., 1992., Walker et al., 1992; Del Pret et al., 1993), our results demonstrate that cytokines of both Th1 (IFN- γ , IL-2, IL-12) and Th2 (IL-10) were activated, in addition to the elevation of both IgG₁ and IgG_{2a}. This discrepancy may be accounted by several reasons, one of these is the strain of used mice. Ovalbumin elicited Th1 cytokines in C57BL/6 mice, while the

same dose of ovalbumin elicited Th2 cytokines in BALB/c mice (Morakata et al., 2000). Other reason is related to the dose of sensitizing antigen, Sakai et al. (1999) reported that the dose of antigen is assumed to be one of the important factors in the polarized development of helper T cell subsets, i.e. Th1 or Th2 cells. Environmental factor as lypopolysaccharides which present in dust can induce a strong antibody response of both IgG₁ and IgG_{2a} when introduced with allergen in mice (Ormstad et al., 2003). Other study by O'Donnell and Openshaw. (1998) found that infection with respiratory viruses powerfully augments the cellular and humoral immune responses to aeroantigen.

Our results showed that treatment with *Nigella sativa* has induced significant decrease in the levels of IL-2 and IL-12 ($P < 0.05$). The levels of IL-10 and IFN- γ were also decreased, but not significantly. Dexamethasone treatment resulted in a significant decrease in the serum level of IL-2 than in sensitized non-treated mice ($P < 0.05$). Serum levels of IL-12 and IL-10 were also decreased, although not significantly. No change was found in the level of IFN- γ . These results were supported by the findings of Li et al. (2000) who found that Dexamethasone, one of the most potent cortico-steroids, can exhibit immunomodulatory function for treatment of allergic asthma.

Accordingly, manipulation of cytokine levels can modify the intensity of the inflammatory response. These findings suggest that *Nigella sativa* may offer immunoregulatory effect which can control allergic asthma. Haq et al., (1999) reported that besides the immunomodulatory effect of *Nigella sativa*, its purified proteins were found in mixed lymphocyte cultures to cause changes in the levels of IL-1 β , IL-8 and TNF- α .

In our study we observed a significant increase in the number of inflammatory cells invading the lungs in sensitized non-treated

group when compared with control group ($P < 0.05$). These observations are consistent with some previous studies that revealed eosinophils, lymphocytes, neutrophils and monocytes invading of the lung tissues after sensitization in murine models of atopic asthma. (Blyth et al., 1996; Fujitani and Trifilieff, 2003; Kumar et al., 2003).

A significant decrease in the number of inflammatory cells, when comparing Dexamethasone treated group with sensitized non-treated group, were shown in the present work. This observation is consistent with other previous findings that corticosteroids (different preparations) inhibited inflammatory cellular infiltration of the lungs in allergic models of bronchial asthma. (Trifilieff et al., 2000; Shen et al., 2002; Kumar et al., 2003; Konduri et al., 2003).

The group of mice treated with *Nigella sativa* showed a decrease in the number of inflammatory cells invading the lungs compared to the sensitized non-treated group indicating the anti-inflammatory activity of *Nigella sativa* as was previously mentioned by Ali and Blunden, (2003) who found that much of the biological activity of *Nigella sativa* seeds has been shown to be due to thymoquinone, the major component of the essential oil, and it would appear that the beneficial effects of the use of the seeds and thymoquinone might be related to their cytoprotective and antioxidant actions, and to their effect on some mediators of inflammation.

In conclusion, *Nigella sativa* can induce a significant reduction of peripheral blood eosinophil, inflammatory cells invading lung tissue and down regulation of IgG isotypes and cytokines response in murine model of allergic asthma. So, *Nigella sativa* may be used as an effective treatment for allergic asthma and that experimental studies may be useful in selecting candidate antiasthma herbal formulas for future clinical evaluation.

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