



Black seed oil ameliorates allergic airway inflammation by inhibiting T-cell proliferation in rats

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ABSTRACT

The black seeds, from the *Ranunculaceae* family, have been traditionally used by various cultures as a natural remedy for several ailments. In this study, we examined the effect of black seed oil as an immunomodulator in a rat model of allergic airway inflammation. Rats sensitized to ovalbumin and challenged intranasally with ovalbumin to induce an allergic inflammatory response were compared to ovalbumin-sensitized, intranasally ovalbumin-exposed rats pretreated with intraperitoneally administered black seed oil and to control rats. The levels of IgE, IgG1 and ova-specific T-cell proliferation in spleen were measured by ELISA. The pro-inflammatory cytokine IL-4, IL-5, IL-6 and TGF- β 1 mRNA expression levels were measured by reverse transcription polymerase chain reaction.

The intraperitoneal administration of black seed oil inhibited the Th2 type immune response in rats by preventing inflammatory cell infiltration and pathological lesions in the lungs. It significantly decreased the nitric oxide production in BALF, total serum IgE, IgG1 and OVA-specific IgG1 along with IL-4, IL-5, IL-6 and TGF- β 1 mRNA expression. Black seed oil treatment resulted in decreased T-cell response evident by lesser delayed type hypersensitivity and lower T-cell proliferation in spleen. In conclusion, black seed oil exhibited a significant reduction in all the markers of allergic inflammation mainly by inhibiting the delayed type hypersensitivity and T-cell proliferation. The data suggests that inhibition of T-cell response may be responsible for immunomodulatory effect of black seed oil in the rat model of allergic airway inflammation.

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1. Introduction

Asthma prevalence is increasing around the world and glucocorticosteroids, the most important anti-inflammatory treatment for asthma, are rather non-specific in their actions and their use also raises concerns over side effects and compliance issues. Moreover, a significant number of asthmatic patients respond poorly or not at all to high-dose inhaled or systemic steroid treatment. Furthermore, many undesirable effects are known for systemic glucocorticosteroids, such as adrenal suppression, decreased bone metabolism and decreased growth in children, in whom asthma is increasing in

frequency. Therefore, many efforts are being made to develop novel, more specific and safer therapies for asthma [1,2].

Reviews published in recent years suggest that some of the folklore medicines have significant effect in reducing the severity of respiratory disease symptoms. Further, alternative medicines particularly plant extracts have gained acceptance by patients and physicians alike [3–5]. However, very few detailed scientific studies have been conducted to further the understanding of the anti-allergic mechanisms associated with these products. In spite of the lack of information, a substantial interest has been shown to alternative and supplementary medicines. Currently, closer to 2000 herbal products are available for the treatment of various ailments and the list is steadily growing [3,4]. For instance, the black seeds of *Nigella sativa* have been used in traditional medicine for the treatment of variety of diseases including asthma [6–8].

N. sativa, belonging to the botanical family *Ranunculaceae*, is a grassy plant with green to blue flowers and small black seeds. Black seeds have been employed for thousands of years as a spice and food preservative [9]. It was referred, by the Prophet Muhammad (PBUH) of Islam, as “Having healing powers for every illness except death itself” [10,11]. Extracts of the *N. sativa* seeds have been

Abbreviations: BSO, black seed oil; PBS, phosphate buffered saline; *N. sativa*, *Nigella sativa*; OD, optical density; ELISA, enzyme linked immunosorbent assay; NO, nitric oxide; IL, interleukin; DTH, delayed type hypersensitivity; PAS, periodic acid Schiff; Ig, immunoglobulin; Th2 cells, T-helper type 2 cells.

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used for many years for therapeutic purposes [12]. Many studies have been carried out in recent years on the pharmacological effects of *N. sativa* seeds that have uncovered their anti-inflammatory and immunological effects [13,14]. Oral administration of *N. sativa* oil is reported to decrease the disease scores in patients with allergic rhinitis and bronchial asthma [15]. It has been shown to possess 67 constituents, many of which are capable of inducing beneficial pharmacological effects in humans [16]. Now, black seeds (*N. sativa*) have attracted the attention of researchers due to its reported effectiveness in inflammatory and other disorders.

Here, we report our findings on the immunomodulatory effect of black seed oil in a rat model of allergic airway inflammation. Our results indicate that black seed oil downregulated Th2-driven immune responses by inhibiting the phenomenon of T-cell proliferation in spleen.

2. Materials and methods

2.1. Laboratory animals

Thirty male and female E3 rats (originated from the section of medical inflammation research, Lund University, Sweden), approximately 10 weeks of age were weighed and maintained at animal house with free access to OVA-free food and water. The animals were divided into 3 groups. In Group I ($n=10$), the rats were sham sensitized and exposed to phosphate buffered saline (PBS), served as Control. In Group II ($n=10$), the rats were sensitized and challenged with ovalbumin (Sigma Chemical Co., USA), named as OVA-Ch group. In Group III ($n=10$), the rats were sensitized and challenged with ovalbumin and treated with black seed oil, called as OVA-Ch + BSO group.

2.2. Sensitization

Induction of allergic airway inflammation was performed by intraperitoneal (i.p.) sensitization and airway challenge through nasal inhalation. Groups II and III were sensitized on day 0 by intraperitoneal injection of 1 mg of OVA (Sigma–Aldrich, St. Louis, MO) in 50 mg Al (OH)₃ (adjuvant) (Pierce Biotechnology, Rockford, IL) in a volume of 1 ml PBS. Two weeks after the sensitization, the rats were subjected to intranasal challenge of OVA (1 mg/ml PBS) once daily for 7 days.

2.3. Treatment with black seed oil

We used 100% pure black seed oil which was obtained by cold pressing at very low temperatures from *N. sativa* seeds (Marhaba Laboratories, Pakistan). The Group III (OVA-Ch + BSO) rats received black seed oil at the dose of 4 ml/kg body weight intraperitoneally, once daily for 7 days preceding the first OVA challenge while control group was injected PBS. At the time of last challenge all the rats were injected with the OVA (1 mg/ml PBS) in the right ear to see inflammatory response and PBS in the left ear as control. Twenty-four hours after the last challenge, all the animals were weighed again and euthanized. The blood was collected; sera were separated and stored at -20°C . The lungs were removed and BAL was performed using ice cold PBS and stored at -20°C . Part of lung was fixed in neutral buffered formaldehyde and sectioned for histopathological studies. Remaining lung tissue was stored at -70°C for cytokine analysis. Spleens were processed for determining T-cell proliferation soon after the euthanization.

2.4. Inflammatory cells in Bronchoalveolar lavage fluid (BALF)

Lungs were lavaged by instillation and withdrawal of 2 ml ice cold PBS through trachea and BAL fluid was collected. Total cell

numbers were determined with crystal violet stain using a hemocytometer. For differential cell count, such as eosinophils, macrophages and lymphocytes, cytocentrifuged preparations were fixed, stained with Giemsa Wright stain and counted according to differentiated morphology.

2.5. Lung histology

Lungs removed from euthanized rats were insufflated with 10% buffered formalin, fixed in formalin solution and processed. Section were cut at 6 μm of thickness and stained with Hematoxylin and Eosin (HE) to observe inflammation, periodic acid Schiff (PAS) to detect mucus producing goblet cells and Masson stains to observe the presence of collagen fibers. Lung sections were evaluated microscopically for morphological changes including infiltration of eosinophils, lymphocytes and macrophages; goblet cell hyperplasia, alveolar thickening and presence of collagen fibers. The scoring of lung histology was performed in a blind fashion. We divided each pathological index into four grades. The grades were scored as 0, 1, 1.5 and 2 for each index according to the severity of inflammation. Then we added all the scores for each airway.

2.6. Determination of nitric oxide (NO) in BALF

NO concentration was measured in the BALF. Briefly, BALF mixed with 80 μl of 375 mM ZnSO₄ and 120 μl of 275 mM NaOH was centrifuged at 1300 rpm for 20 min. Supernatant was obtained and added with 400 mg of Cu (Copper) plated Cd (cadmium). Then 100 μl of 0.2 M glycine buffer was added. The whole mixture containing Cu plated Cd was put for shaking for 3 h. The obtained supernatant was added with the equal volume of Griess reagent (0.1% N (1-Naphthyl) ethyl-enediamine dihydrochloride mixed with equal volume of 1% sulfanilamide) to develop color. The absorbance was measured at 545 nm wavelength by micro plate reader (Thermo Electron Corporation, Finland).

2.7. Determination of OVA-specific antibodies

The serum level of OVA-specific IgG1 was measured by ELISA. Briefly, the 96-well round-bottom microtiter plates (Jet Biofil, Guangzhou) were coated for overnight at 4°C with 100 μl of 100 $\mu\text{g/ml}$ OVA in NaHCO₃ buffer (pH 9.6). The plates were washed three times with PBS–0.05% Tween 20, and then blocked by incubation with 200 μl 0.05% PBS–Tween and 2% BSA for 1 h at 37°C . After washing, different dilutions of serum samples and standard rat IgG1 (AbD Serotec, Munich) was added and the plates were incubated for 1 h at 37°C . OVA-specific IgG1 was detected with HRP-conjugated mouse anti-rat Kappa/Lambda chain (1 $\mu\text{g/ml}$) antibodies (AbD, Serotec) by incubation for 30 min at 37°C . Then, TMB and H₂O₂ were added as a substrate and incubated at 37°C for 20 min. The reaction was terminated with 2 N, H₂SO₄, and read at 450 nm by ELISA reader (Thermo Electron Corporation, Finland).

2.8. Total serum IgE and IgG1

Total serum IgE and IgG1 levels were determined in all rats after euthanization. The 96-well round-bottom microtiter plates were coated overnight at 4°C with mouse anti-rat IgE and IgG1 heavy chain (AbD Serotec, Munich). Different dilutions of serum samples were added. Total serum IgE and IgG1 were measured with HRP-conjugated mouse anti-rat Kappa/Lambda chain. All the steps were followed as above. The total IgE and IgG1 levels were expressed as $\mu\text{g/ml}$ after comparing the optical density (O.D. at 450 nm) values to rat IgE and IgG1 standards.

Table 1
Primer sequences and PCR conditions for amplification of cytokines.

Cytokine	Sense and antisense	Sequence	Amplified band (bp)	Annealing temp (°C)	Cycles
IL-4	Sense	5'-TGCTGTACCCCTGTTCTGC-3'	395	57.6	35
	Antisense	5'-ATTTCCTCGTAGGATGCTTT-3'			
IL-5	Sense	5'-TGCTTCTGTGCTTGAACGTTCTAAC-3'	298	62.3	37
	Antisense	5'-TCTCTTTTTGTCCGTCATGTATTC-3'			
IL-6	Sense	5'-CCTTCTGGGACTGATGTTGTG-3'	387	58.0	30
	Antisense	5'-TGACTCTGGCTTTGCTTTCTTG-3'			
TGF β 1	Sense	5'-AATGGTGGACCGCAACAAC-3'	331	55.7	33
	Antisense	5'-GTGAGCACTGAAGCGAAGC-3'			

2.9. RNA isolation and cDNA synthesis

Total RNA was isolated from whole lung tissue using TRIzol method. The integrity of the RNA was confirmed by denaturing agarose gel electrophoresis and RNA concentration was quantified by measuring optical density (CECIL Instrument, England). Total cellular RNA was examined for specific cytokine mRNAs by the reverse transcription (RT)-PCR method according to manufacturer's protocol (RevertAid™, Fermentas Life Sciences, International INC, Canada). Briefly, RNA (1 μ g) was reverse-transcribed into cDNA using Oligo (dT) and heating the mixture at 70 °C for 5 min. After that, 5 $\times$ reaction buffer and RiboLock™ Inhibitor added and incubated at 37 °C for 5 min. Then, Reverse Transcriptase was added and incubated at 42 °C for 60 min. Reaction was stopped by heating at 70 °C for 10 min and cDNA was stored at –20 °C until further use for PCR to measure the mRNA expression of different cytokines. The primer sequences for cytokines and PCR conditions can be found in Table 1.

2.10. Delayed type hypersensitivity (DTH)

Delayed type hypersensitivity (DTH) is a basic test to check the inflammatory response to the antigen *in vivo*. Twenty-four hours before the euthanization, the rats of all groups were injected with 20 μ l OVA (1 mg/ml PBS) in the right ear to see inflammatory response and 20 μ l PBS in the left ear as control. Soon after the euthanization of the rats, the right and left ears were separated and weighed. The difference of weight between both ears represented the magnitude of DTH.

2.11. OVA-specific T-cell proliferation

To check the T-cell proliferation, spleens isolated soon after killing the rats, were washed with Hank's balanced salt solution, 100 μ g/ml streptomycin and 100 U/ml penicillin. Then, spleens were grinded in 5 ml Hank's balanced salt solution. The digested tissue was filtered through a 45- μ m nylon membrane and equal volume of NH₄Cl solution was added for the lysis of RBC's. The resulting cell suspension was centrifuged at 1000 rpm for 10 min. Then, cells were coated in 96-well round-bottom plate (Jet Biofil, Guangzhou) by seeding 1×10^6 cells/well with 200 μ l of RPMI-1640 medium (RPMI-1640 containing glutamine, sodium pyruvate, penicillin–streptomycin and 10% heat inactivated fetal bovine serum). Cells proliferation was stimulated by treatment with OVA at different concentrations as 0, 1, 10 and 100 μ g/ml. Bromodeoxyuridine (BrdU, Sigma) was added into the wells. Cells were incubated at 37 °C and 5% CO₂. Then BrdU-DNA was measured by an ELISA described below [17].

After 72 h incubation, BrdU incorporation was terminated by centrifuging the cell culture plate at 2000 rpm for 20 min, aspirating the treatment medium and washing with 200 μ l/well of Hank's balanced salt solution (BSS). The cells were fixed by

replacing the BSS with 200 μ l/well of 70% ethanol for 20 min at room temperature. Next, the wells were washed with distilled water and DNA was denatured by incubation with 100 μ l/well of 2 M HCl for 10 min at 37 °C. Following aspiration wells were filled with borate buffer (0.1 M, pH 9) to neutralize the residual acid. After a wash with PBS, the cells were treated with 50 μ l of blocking buffer (PBS containing 0.1% Triton X-100 and 2% normal goat serum) for 15 min at 37 °C. Monoclonal rabbit anti-BrdU antibody (50 μ l/well; 1 μ g/ml) (BIOS, Beijing), diluted in blocking buffer was added for 60 min at 37 °C. Unbound antibody was then removed by three washes with PBS containing 0.1% Triton. Bound antibody was detected by Peroxidase-conjugated goat anti-mouse IgG (50 μ l/well; 2 μ g/ml) (BIOS, Beijing) diluted in blocking buffer and incubated for 30 min at 37 °C. After four washes with PBS/Triton and final rinse with PBS only, the wells received 100 μ l/well of 50 mM phosphate/citrate buffer at pH 5 containing substrate TMB and 3% H₂O₂. The reaction was terminated after 5–20 min by adding 40 μ l/well of 2 N Sulfuric acid. The absorbance was measured at 490 nm by ELISA reader (Thermo Electron Corporation, Finland).

2.12. Statistical analysis

Data was expressed as mean $\pm$ SD. Statistical analysis was performed using one-way analysis of variance followed by Tukey–Kramer test to determine the significant difference among groups and the value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. BSO arrested inflammatory cell infiltration in BALF

Total number of cells and differential number of eosinophils, macrophages and lymphocytes in the BALF of OVA-sensitized and challenged rats were markedly elevated as compared to control group. Notably, BSO treatment significantly decreased the total ($P < 0.001$) and differential numbers of eosinophils ($P < 0.01$), macrophages ($P < 0.05$) and lymphocytes ($P < 0.01$) in OVA-sensitized rats (Fig. 1). The data indicates that BSO plays a vital role during the inflammatory response by reducing the number of inflammatory cells in the airways.

3.2. Lung tissue lesions, eosinophilia and mucus production were abolished with BSO treatment

Lung tissue from Control, OVA-Ch, and OVA-Ch + BSO rats was examined for inflammation (Fig. 2). No pathological abnormalities were observed in the lungs of PBS-treated control group (Fig. 2 Panels A,D,G). While OVA-sensitized and challenged rats showed marked lung infiltration of eosinophil, numerous lymphocyte, macrophages and goblet cells containing mucus along with collagen fibers (Fig. 2 Panels B,E,H). OVA-challenged rats were also characterized by gross alteration in the structural integrity of the

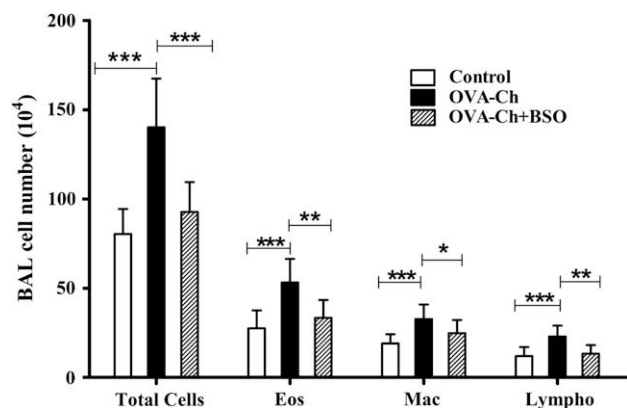


Fig. 1. Mean number of total cells, eosinophil (Eos), macrophage (Mac) and lymphocyte (Lympho) in BALF from three different groups. Data presented as mean $\pm$ SD for 10 rats each group. BSO significantly reduced total and differential number of eosinophil, macrophage and lymphocyte when compared with the increased number of these cells in OVA-challenged non-treated rats (OVA-Ch group). An asterisk (*) indicates $P < 0.05$, ** indicates $P < 0.01$ and *** indicating $P < 0.001$ comparing two groups.

airway walls and alveolar thickening. BSO treatment inhibited the lung inflammation by minimizing the inflammatory cell infiltration. Moreover, BSO administration resulted in less bronchial and alveolar epithelial hyperplasia with no goblet cells and collagen fiber (Fig. 2 Panels C,F,I). The total histological score of inflammation was significantly ($P < 0.01$) reduced by BSO treatment (Fig. 3).

3.3. BSO treatment resulted in significantly reduced NO production in BALF

Nitric oxide production is one of the markers of allergic lung inflammation. We found allergic inflammation resulted in the increase concentration of nitric oxide in the BALF of OVA-challenged rats which was significantly ($P < 0.01$) abated by black seed oil treatment (Fig. 4).

3.4. BSO decreased the elevated total IgE, IgG1 and OVA-specific IgG1 levels in the serum

We observed a boosted level of total serum IgE, IgG1 and OVA-specific IgG1 in the serum of rats exposed to OVA (OVA-Ch group) as compared to the control group rats. This rise was declined significantly ($P < 0.01$) in the rats challenged with OVA and treated with BSO (OVA-Ch + BSO group) (Fig. 5).

3.5. BSO diminished the inflated mRNA expression level of inflammatory cytokines

Total RNA extracted from whole lung cells was used in reverse transcription (RT)-PCR. The results showed that BSO significantly lowered the elevated levels of mRNA expression of IL-4 ($P < 0.01$), IL-5 ($P < 0.05$), IL-6 ($P < 0.01$) and TGF- β 1 ($P < 0.05$) in rats exposed to OVA, indicating that BSO affects Th2 cytokine response at gene expression level (Fig. 6A and B). There was no significant mRNA expression for IFN- γ in all three groups (data not shown).

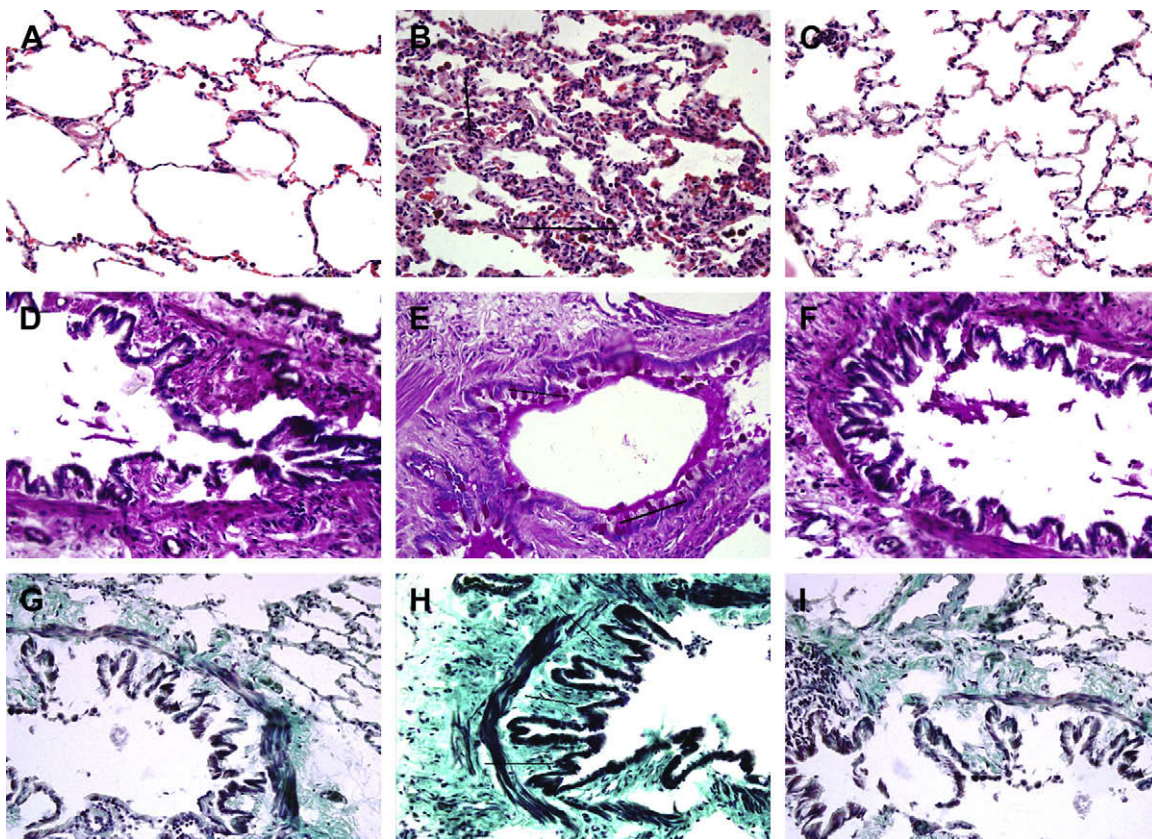


Fig. 2. Histopathology of lungs from different groups. A, D, G represent lung sections of normal control rats, showing no pathological changes (A: HE; D: PAS; G: Masson staining). B, E, H indicate lung sections of OVA-challenged rats (OVA-Ch). (B) Interstitial inflammatory infiltrates showing inflammatory cells (HE); (E) presence of goblet cells (PAS); (H) thickening of alveolar wall due to presence of collagen fibers (Masson). C, F, I show sections from OVA-challenged and BSO treated rats OVA-Ch + BSO. BSO treatment inhibited the infiltration inflammatory cells in the lungs (C), no goblet cell hyperplasia (F) and no collagen deposition (I) (C: HE; F: PAS and I: Masson staining). All pictures are taken at 40 $\times$.

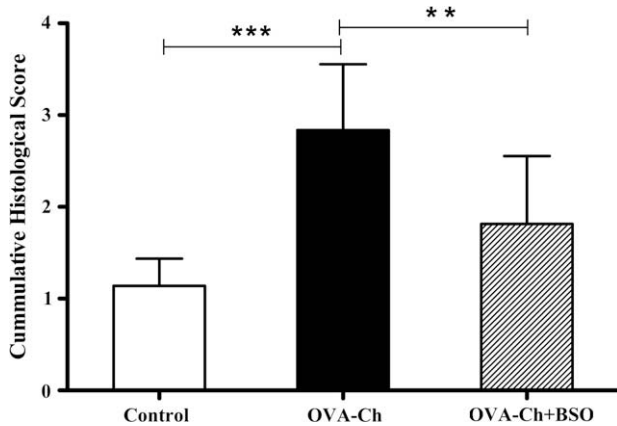


Fig. 3. BSO significantly reduced the histological score of inflammation in the OVA-Ch + BSO group. Data shown as mean $\pm$ SD for 10 rats each group. ** Indicates $P < 0.01$ comparing two groups.

3.6. BSO abrogated the OVA-specific T-cell response

The delayed type hypersensitivity is the response of T-cell to the antigen. We measured the DTH by weighing the ears of all rats. The high DTH was observed in the rats sensitized and challenged with OVA as compared to control group, which was significantly ($P < 0.05$) reduced by BSO treatment in OVA-Ch + BSO group (Fig. 7A).

In *ex vivo* experiments, we determined the T-cell proliferation in spleens from three groups at different OVA concentrations. *BrdU* uptake in the spleen cells of OVA-challenged rats was significantly higher as compared to control rats, in response to specific OVA stimulation. The effect was in a dose-dependent manner. While in the OVA-Ch + BSO group, a significant ($P < 0.01$) reduction in the T-cell proliferation was observed, indicating that BSO inhibited the T-cell proliferation which was stimulated by the OVA (Fig. 7B).

4. Discussion

Different animal models have been developed in order to investigate the therapeutic effect of this herb on allergic asthma. In this study, we examined the immunomodulatory effect of black seed oil in the rat model of allergic airway inflammation. Our model showed all the salient features of allergic airway inflammation. The results demonstrate that BSO attenuates OVA-induced airway inflammation by inhibiting T-cell proliferation. This inhibitory

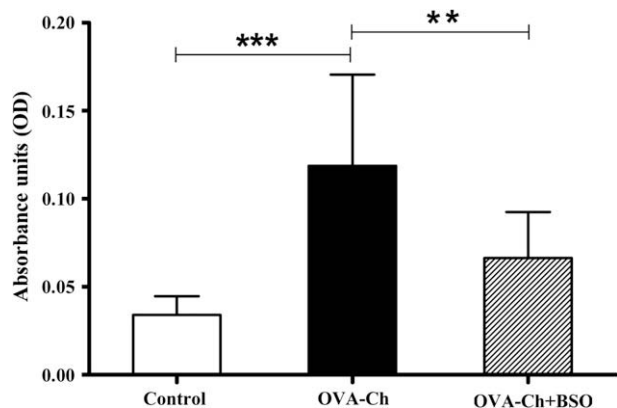


Fig. 4. Determination of nitric oxide concentration in BALF. Increased level of NO was found in the BALF of OVA-challenged non-treated rats (OVA-Ch group) which was significantly reduced in OVA-challenged and BSO treated rats (OVA-Ch + BSO group). Results represented as absorbance units measured by micro plate reader. Data given as mean $\pm$ SD for 10 rats each group. ** Indicates $P < 0.01$ comparing two groups.

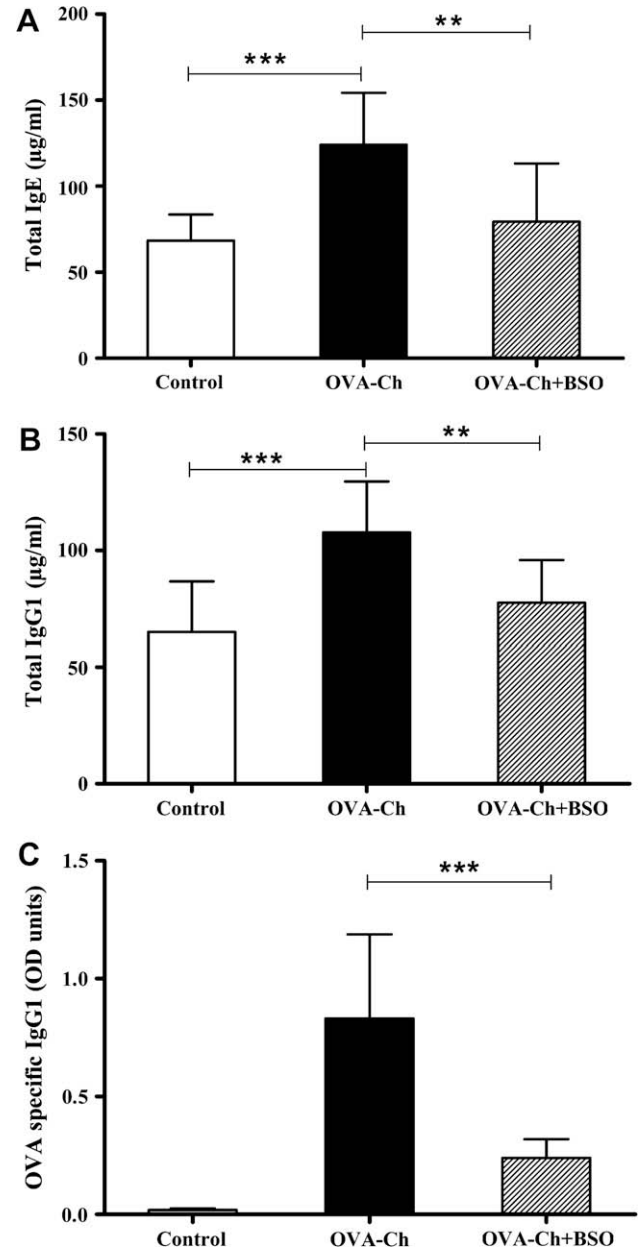


Fig. 5. Total serum IgE, IgG1 and OVA-specific IgG1. (A) Level of total serum IgE was measured in the three groups by ELISA. Data represents mean $\pm$ SD for 10 rats each group. Significant increased total Serum IgE in the OVA-challenged rats (OVA-Ch group) as compared to control group. BSO significantly ($P < 0.01$) reduced the raised level of total serum IgE in OVA-challenged and BSO treated rats (OVA-Ch + BSO group). (B) Total serum IgG1 measured in the three groups by ELISA. Data represented as mean $\pm$ SD for 10 rats each group. A significant increase in total Serum IgG1 in the OVA-challenged rats (OVA-Ch group) was observed as compared to control group. BSO significantly ($P < 0.01$) lowered the level of total serum IgG1 in OVA-challenged and BSO treated rats (OVA-Ch + BSO group). (C) OVA-specific IgG1 measured in the three groups by ELISA. Data represents mean $\pm$ SD for 10 rats each group. BSO significantly ($P < 0.001$) reduced the raised level of OVA-specific IgG1 in OVA-challenged and BSO treated rats (OVA-Ch + BSO group).

effect resulted in a significant decrease in delayed type hypersensitivity, Th2 cytokines expression, lung eosinophilia, goblet cell hyperplasia, nitric oxide production, Igs level in OVA-sensitized and challenged rats.

Actually, Th2 cells play an important role in the pathogenesis of allergic airway inflammation through the production of cytokines [18–23]. Our results indicate that administration of BSO significantly lowered the levels of IL-4, IL-5, IL-6 and TGF- β 1 in

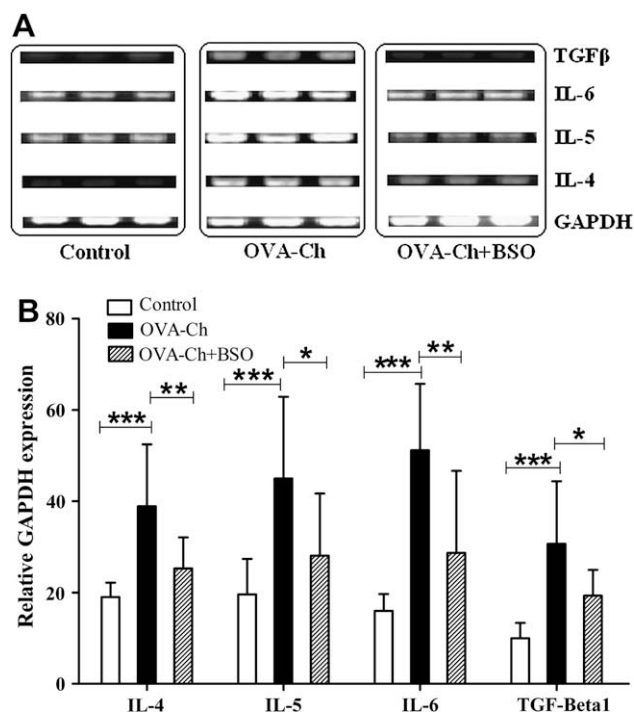


Fig. 6. Effect of BSO on cytokine mRNA expression. (A) Total RNA was extracted and used in RT-PCR to assess IL-4, IL-5, IL-6, and TGF-β1 gene expression. GAPDH was amplified as an internal control. Representative bands from gel electrophoresis of PCR products are shown. Results from three rats per each group are given. Data is given for Control, OVA-Ch and OVA-Ch + BSO group, respectively. (B) Cytokines mRNA expression in rat lungs expressed as a percent ratio to GAPDH mRNA determined by RT-PCR. Data is shown as mean ± SD for 10 rats each group. A significant increase in the mRNA expression for all cytokines was noted in OVA-sensitized rats (OVA-group) as compared to PBS-treated controls. BSO significantly reduced mRNA expression of IL-4 ($P < 0.01$), IL-5 ($P < 0.05$), IL-6 ($P < 0.01$) and TGF-β1 ($P < 0.05$).

OVA-challenged rats. Consequently, all the inflammatory parameters under the effect of Th2 cytokines including inflammatory cells infiltration such as eosinophils, lymphocytes, and macrophages [24]; goblet cell hyperplasia and the levels of IgE and IgG1 were diminished. This result strengthened the view that Th2 type inflammatory events are mainly induced by the cytokines profile. As IL-4 and IL-5 have previously shown to increase the levels of IgE and IgG1 and promote eosinophil formation, differentiation, survival and transmigration across endothelium [25–28]. On the other hand, IL-6 is important because it induces the expansion of Th2 effector cells, which play major roles in adaptive immune response [29]. While, TGF-β has been shown to enhance goblet cell proliferation and mucus hyper-secretion [30].

Nitric oxide is another important marker of lung inflammation and increased exhaled NO levels have been found in patients with asthma [31–33]. In fact, arginine acts as a substrate for arginase to produce NO and the expression of arginase is also strongly induced by Th2 cytokines, particularly IL-4 and IL-6 [34]. This NO has a key role in the eosinophilic infiltration and more production of cytokines [35,36]. As shown by our results, BSO treatment significantly reduced the production of NO in the OVA-sensitized and challenged rats. It has been previously reported that the presence of different alkaloids (nigellidine, nigellimine and nigellidine) in *N. sativa* seeds could influence the production of NO [37]. Therefore, alkaloids should also be considered as important constituents of *N. sativa*.

Taking together, our results indicate that the inhibitory effects by BSO on airway inflammation are due to decrease in Th2 cytokine levels. To understand the reason by which BSO abrogated the levels of Th2 cytokines and subsequently attenuated all the inflammatory markers, we measured T-cell response by delayed type hypersensitivity

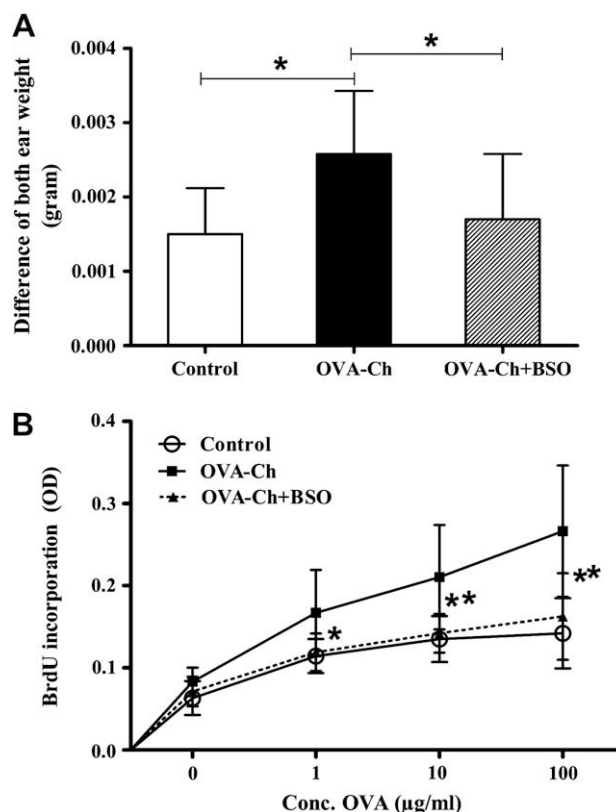


Fig. 7. (A) DTH (delayed type hypersensitivity). At the time of last challenge OVA was injected in the right ear to see the inflammatory response and PBS was injected in the left ear as a control. After sacrificing, the ears were separated and weighed. DTH is shown as mean ± SD for 10 rats each group in all the three groups represented by the difference of right and left ear weight. DTH found increased in the OVA-sensitized and challenged rats (OVA-Ch group) when compared with healthy rats in control group. BSO treatment significantly ($P < 0.05$) reduced the DTH in OVA-Ch + BSO group. (B) T-cell proliferation was determined after culturing the cells from spleen 1×10^6 cells/well with 200 μl of RPMI-1640 medium and stimulating with different OVA concentrations i.e. 0, 1, 10 and 100 μg/ml. BrdU was added in the wells to measure the proliferation by ELISA. Data given as mean ± SD for 10 rats each group. BSO significantly ($P < 0.05$ and $P < 0.01$) inhibited the OVA-specific T-cell proliferation at different OVA concentrations in OVA-challenged and BSO treated rats (OVA-Ch + BSO group) when compared to increased proliferation of T-cell in OVA-challenged rats (OVA-Ch group).

test *in vivo*. While, T-cell proliferation in spleen was determined by *ex vivo* experiment. The results demonstrate that BSO clearly abolished the OVA-specific T-cell response. The allergen inhalation has been reported to induce inflammatory cell proliferation [38,39] as seen that OVA could induce the splenocytic proliferation in a dose-dependent manner [40]. In contrast, *N. sativa* oil has been reported to have significant anti-proliferating activity [41]. In another study, BSO treatment suppressed the function of macrophages in spleen [42]. While in recent years, evidence has increased to suggest that activation and proliferation of antigen-specific Th2 cells play a central role in the pathogenesis of bronchial asthma [43]. In fact, T cells are differentiated into Th2 cells under the action of Th2 cytokines. If by any reason, the T cells fail to proliferate or differentiate, the whole inflammatory process may shut down as shown by our study in which BSO administration abrogated the OVA-specific T-cell response by inhibiting their proliferation. Our results clearly indicate that BSO inhibited the T-cell proliferation in rats challenged with OVA. This resulted in the depletion of Th2 cytokine levels which subsequently led to the attenuation of all the inflammatory markers including delayed type hypersensitivity, invasion of inflammatory cells in the lung, goblet cell hyperplasia, NO level, total serum IgE, IgG1 and OVA-specific IgG1 production. This phenomenon of inhibition of T-cell proliferation by BSO is an

important and novel finding by which BSO ameliorated the allergic airway inflammation.

5. Conclusion

In summary, the results presented in this report clearly indicate that the capacity of black seed oil to improve the allergic airway inflammation is by the inhibition of T-cell response and proliferation. However, the exact mechanism by which the inhibition of T-cell proliferation occurred is not clear and further studies are needed to understand it.

6. Conflict of interests

The authors declare that they have no competing interests.

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