# Anti-Inflammatory and Immune Regulatory Effects of *Salvia officinalis* Extract on OVA-induced Asthma in Mice

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**Abstract:** The study designed to evaluate the protective effect of *Salvia officinalis* (SO) water extract against OVA-induced asthma in mice. The OVA-induced asthmatic mice exhibited significant increase in total leukocytes count, IL-4 and IL-5 levels in bronchoalveolar lavage fluid (BALF) and eosinophils count in (BALF), in addition, to the serum total IgE level. Pathological changes in the lung were evaluated and compared to either treated OVA-challenged SO group or control- *Salvia Officinalis* oral administered group. *Salvia Officinalis* extract reduced significantly the number of inflammatory cells and cytokines in the BALF and the level of IgE as compared to OVA-challenged group. Moreover, *Salvia Officinalis* extract (SO) reduced the eosinophils infiltration in the lung tissue. These results suggest that *Salvia officinalis* extract via oral treatment may have anti-inflammatory and immune regulatory effects on bronchial allergic asthma.
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## 1. Introduction

The genus Salvia L. (Lamiaceae), with about 900 species, is one of the most widespread members of the family Lamiaceae. (Longaray Delamare *et al.,* 2007).*Salvia officinalis* (SO),a plant endemic to the Mediterranean region is the most popular herbal remedy in the Middle East to treat common health complications such as cold and abdominal pain (Gali-Muhtasib *et al.,* 2000). Salvia species are considered with curative properties (Kintzios, 2000). They possess antiseptic, antibacterial (Soković *et al.,* 2010), antinociceptive and anti-inflammatory (Baricevic *et al.,* 2001;Rodrigues *et al.,* 2012), antiviral (Geuenich *et al.,* 2008), Cytotoxic (Loizzo *et al.,* 2007), spasmolytic, anticonvulsant (Baricevic and Bartol, 2000) in addition, to the antimicrobial and antioxidant activities (Bozin *et al.,* 2007; Abu-Darwish *et al.,* 2012).

Allergic asthma is characterized by airway hyperesponsiveness to specific and/or nonspecific stimuli, chronic pulmonary eosinophilia, elevated serum IgE level, and excessive mucus production (Kuperman *et al.,* 1998) . The pathology associated with asthma is thought to be mediated by CD4+T lymphocytes producing the type 2 cytokines, Il-4 and IL-5, as both messenger RNA and protein levels of these cytokines are elevated in bronchial biopsies bronchoalveolar lavage (BAL) cells , and blood of allergic proteins as compared to normal individuals. Since these cytokines promote the accumulation and activation of eosinophils as well as IgE synthesis by B cells, this cytokine pattern has been thought to be important in the pathogenesis of asthma (Wills-Karp, 1999; Renauld, 2001; Elias *et al.,* 2003; Boyce *et al.,* 2012; Lommatzsch, 2012). Asthma is usually divided into allergic asthma and non-allergic asthma, and approximately two-thirds of asthma cases are allergic (Hamelmann, 2007 ; Bollag *et al.,* 2009). It is widely accepted that antigen-specific T helper cell type 2 (Th2) and their cytokines such as IL-5, IL-4, and IL-13 orchestrate the feature of asthma (Kay, 2001; Boyce *et al.,* 2012).

The study designed to evaluate the protective effects of *salvia officinalis*(SO)water extract on allergic asthma, so we used OVA-induced asthma mouse model which used as a reference method by other investigators (Secor *et al.,* 2008; Yim *et al.,* 2010).

## 2.Material and Methods

### Experimental animals

The study used Balb/c mice weighing 25-35g obtained from Theodor Bilharz Research Institute, AL-Giza Egypt. They were acclimatized to the environment for two weeks prior to experimental use and had access to water and food *ad libitum*. The Laboratory Animal Welfare including environment, housing and management are carried out with recommendations for the proper care and use of laboratory animals of American Association for Accreditation of Laboratory Animal Care (AAALAC) (1996).

### Preparation of *Salvia officinalis L.* leaves aqueous extract.

Aqueous extracts were prepared as described previously by Nolkemper *et al.* (2006). Briefly, boiling water (100 ml) was added to dried leaves (10 g) and incubated for 15 min, subsequently filtered and cooled

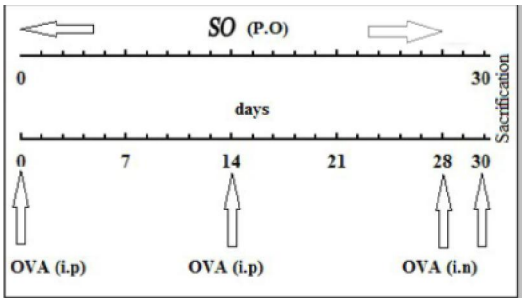
down. The resulting extracts were sterile filtered, aliquoted, and stored at -20°C. The extract was given orally (100 mg/ kg b.wt.).

**OVA-Induced Asthmatic Mouse Model**

According to the methods described by Henderson *et al.* (1996) and Erb *et al.* (1998) mice were injected intraperitoneally with 2µg OVA chicken egg albumin (Sigma Chemical Co., St. Louis,MO) in 100µl aluminum hydroxide (alum adjuvant) on day 0 (starting time) and boosted again intraperitoneally with 2µg OVA/alum at day 14. Fourteen days after the second intraperitoneal immunization mice were anesthetized by i.p. injection (0.2 ml) of a mixture of ketamine (0.44mg /ml) and xylazine (6.3mg/ml) then 100µg OVA in a 50 µl volume of phosphate buffered saline (PBS) was administered by intranasal challenge.

**Experimental design:**

Mice were divided into four groups, the 1st control mice and the 2nd control-*Salvia officinalis* (SO),while the 3rd and 4th groups were underwent OVA exposure as OVA-challenged, and OVA- challenged SO, respectively. The second and fourth groups received *Salvia officinalis* extract (100 mg/kg p.o.) from day 0 to the day of challenge. Sacrification of mice was done after 48hrs of intranasal OVA challenge (Figure 1).The blood collected and centrifuged for 10 min. and stored at -80°C.




**Figure(1):** Diagram of the experimental design

**Detection of Different Cell Types in Bronchoalveolar Lavage Fluid**

The mouse trachea was cannulated and lungs were washed 3 times with 1ml phosphate buffer saline (PBS). Bronchoalveolar lavage fluid (BALF) cells from a 0.05-ml aliquot of the pooled sample were counted using a hemocytometer to determine the total leukocytes count. The remaining fluid was immediately centrifuged at $4^0$Cfor 10 min at 200 g. The supernatant was collected for cytokines measurements, by ELISA. The cell pellets were re-suspended in normal saline containing 10% bovine serum albumin ( BSA). BALF cell smears were made on glass slides. The dried slides were stained for eosinophil's count with eosin and methylene blue according to Henderson *et al.* (1996).

**Detection of cytokines in BALF**

Cytokines, IL-4 and IL-5 were evaluated with an ELISA kit (California, USA) according to the manufacturer's protocol.

**Measurement of serum level IgE:**

Forty-eight hours post intranasal challenge, animals were sacrificed and Serum IgE was evaluating with an enzyme linked immune sorbent assay (ELISA) using Mouse Serum Anti-OVA IgE Antibody Assay Kit Catalog # 3010 Chondrex, Inc.

**Histological analysis**

Forty-eight hours after the intranasal challenge, the lungs were removed, washed with PBS and infused with 10% formalin in PBS for fixation. The lung tissue was sectioned and stained with hematoxylin and eosin (H& E)

**Statistical analysis**

Data were fed to the computer using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard error. Comparison between different groups was analyzed using F-test (ANOVA) and Post Hoc test (Scheffe) for pair wise comparison. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

**3. Results**

Data represented in table (1) showed that the number of total leukocytes and eosinophils cells as well as IL-4 and IL-5 levels in BALF and the serum total IgE were significantly increased in OVA-challenged group as compared to the control group values. The data of OVA-SO group showed significant decrease in the aforementioned parameters as compared to the corresponding values of OVA-challenged group.

**Table (1):** Effect of *Salvia officinalis* extract on BALF cells migration, cytokines production (IL-4, IL-5) and IgE level

| | Control (n=5) | control-SO (n=5) | OVA-challenged (n=5) | OVA-SO challenged (n=5) | *p* |
|---|---|---|---|---|---|
| **Total leukocyte ($10^6$/ml)** | 0.43 ± 0.05 | 0.46 ± 0.05 | $6.51^a$ ± 0.12 | $3.22^b$ ± 0.36 | <0.001* |
| **Eosinophil ($10^5$/ml)** | 0.25 ± 0.04 | 0.23 ± 0.01 | $5.27^a$ ± 0.08 | $2.32^b$ ± 0.08 | <0.001* |
| **IL_4 (pg/ml)** | 10.4 ± 0.64 | 10.54 ± 1.14 | $80.46^a$ ± 0.35 | $12.40^b$ ± 1.46 | <0.001* |
| **IL_5(pg/ml)** | 11.34 ± 0.58 | 11.28 ± 0.13 | $41.22^a$ ± 0.5 | $13.34^b$ ± 0.13 | <0.001* |
| **Total IgE (ng/ml)** | 14.52 ± 0.66 | 15.30 ± 1.55 | $70.26^a$ ± 0.11 | $13.56^b$ ± 1.79 | <0.001* |

*Salvia officinalis* extract was given orally from the first day of immunization to the day of intranasal challenge
Data was expressed in mean ± SE; *p*: *p* value for F test (ANOVA)
pair wise between any two groups was assessed using Post Hoc test (Scheffe);
a: Significant with control group; b: Significant with OVA- challenged group; *: Statistically significant at $p \leq 0.05$

**Histological analysis**

Lungs in the OVA-challenged group showed a large increase in eosinophils recruitment compared to the control group. The lungs of mice treated with *Salvia officinalis* (SO) extract showed significantly fewer eosinophils than in the OVA-challenged group (Figure 2).

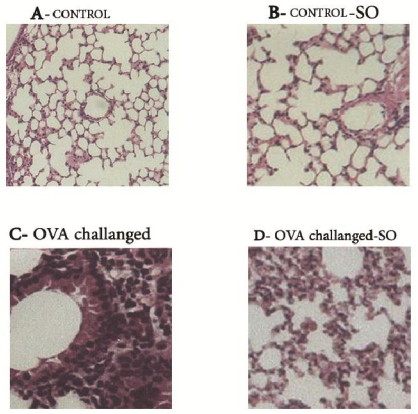



**Figure (2):** Histological analysis of mouse lung sections. Mouse lung sections were stained with hematoxylin and eosin. Lung tissue from the OVA challenged-SO mouse group (D) showed fewer eosinophils than the OVA challenged(C), scale bars = 100μm.

**4.Discussion**

Airway inflammation is the key factor in the pathogenesis of asthma and current strategies for the management focus on suppressing airway inflammation (Kumar, 2001; Walsh, 2006).

In oriental medicine, herb was widely used to treat bronchial asthma (Stockert *et al.,* 2007), theoretically by clearing the pathogenic factors and reinforcing the body's resistance. In the present study, *Salvia officinalis* (SO) extract was used for the herbal treatment. To investigate the effect of *Salvia officinalis* on allergic bronchial asthma, the OVA-induced asthmatic mouse model was used.

The cellular infiltrate that characterizes asthma and the ability to control leukocyte migration and eosinophils infiltration into the lungs is viewed as the key to regulating disease severity. (Lukacs, 2001) .

After infiltration at site of allergic inflammation, these inflammatory cells release mediators, e.g., (cytokines and chemokines). *Salvia officinalis* decreased the total leukocyte and eosinophils infiltration in BALF of OVA- induced asthmatic mice. From these results, *Salvia officinalis* may have therapeutic effect in allergic asthma via inhibiting leukocytes and eosinophils migration in the lungs.

Asthma is generally regarded as a T-cell mediated disease. Antigen cause the differentiation of naïve T-cell into helper cells, which then secrete cytokines such as IL-4and IL-5. IL-4, which is pivotal in the pathogenesis of allergic disorder acts on B cells to facilitate (Switch) IgE production (Haas *et al.,* 1999; Ramshaw *et al.,* 2001). Increased IgE production in response to common environmental antigens is the hallmark of atopic diseases such as bronchial asthma (Hamelmann *et al.,* 1999).

IL-4 also induces the rolling and adhesion of circulating eosinophils to endothelial cells.IL-4, which at least in murine system is essential in instructing B cells to switch to IgE production. Therefore, inhibitors of IL-4 signaling pathway have been suggested as therapeutic targets (Barnes, 2000; Romagnani, 2002).

The present work showed that *Salvia officinalis* (SO) extract significantly decreased IL-4 in BAL. the eosinophils migration observed here showed that (100mg/kg),*Salvia officinalis* extract (SO) caused the reduction of eosinophils infiltration in the BAL and this may be due to decrease in IL-4 by *Salvia officinalis* (SO) extract. Based on these results, it is inferred that *Salvia officinalis*(SO) extract may have an anti-allergic effect on allergic asthma by suppressing IL-4 secretion and, in consequence, inhibiting IgE secretion from lymphocyte B cells. Further, reduced IL-4 may reduce eosinophils infiltration into lungs by inhibition the adhesion of circulating eosinophils to endothelial cells.

IL-5 is a Th2-type cytokine that promotes the recruitment and activation of eosinophils, IL-5 stimulates the release of chemical mediators from eosinophils (Broide 2001). Airway eosinophils inflammation is one of the characteristics of allergic asthma. Therefore IL-5 has been implicated in the pathogenesis of allergic diseases (Rothenberg *et al.,* 1997; Hogen *et al.,* 2001), and many studies appeared that IL-5 is an important target for improved asthma therapy.(Costa *et al.,* 1997; Okudaira and Mori, 1998; VanWauwe *et al.,* 2000). Furthermore, activated Th2- type cells, which can produce IL-5 and several other cytokines, are activated in a greater proportion in asthmatic individuals, in this study, *Salvia officinalis* significantly reduced IL-5 in BAL and reduced eosinophils infiltration and activation in OVA-induced asthmatic mice.

The present data demonstrate that *Salvia officinalis* can inhibit the excess activity of T- helper cells cytokines which play an important role in the maintenance of bronchial asthma not only by **inhibiting IL-4** production from T cells to suppress IgE production from B cells but also by **reducing IL-5** to decrease eosinophils infiltration.

In addition, histological analysis of lung tissue showed that *Salvia officinalis* inhibited eosinophil infiltration in the lung. From these results, *Salvia officinalis* extract may have therapeutic activity against eosinophilic infilammation in allergic asthma via inhibiting cellular infiltration in the lung.

The control *Salvia officinalis* extract group did not show any considerable differences from the control group this result indicates that *Salvia officinalis* extract does not cause any changes in normal condition, so it does not interrupt the homeostasis of healthy subjects.

Moreover, the present study showed that water extract of *Salvia officinalis* had an anti-inflammatory effect on asthmatic mice induced by OVA.

The anti-inflammatory activity of plant has been attributed to their triterpene (Ghannadi *et al.,* 2005), or flavonoid contents (Palgan and Bartuzi, 2011). Biflavonoids, and triterpenoids produced significant anti-inflammatory activities (La Casa *et al.,* 2000; Calixto *et al.,* 2000 ; Sabu and Kuttan, 2002 ; Silva *et al.,* 2005)

Previous studies showed that Salvia species are rich in phenolic compounds, such as phenolic acids and flavonoids, in addition to terpenoids (LaCasa *et al.,* 2000).

Therefore, the presence of anti-inflammatory activity may be due to phenol and terpenoid contents in the plant.

In summary, We conclude that *Salvia officinalis* may be used to bronchial allergic asthma to reduce airways hyperreactivity by suppressing IL-4, IL-5 and IgE. The present data suggest that *Salvia officinalis*(SO)has a therapeutic effect on bronchial asthma by inhibiting T cells activation and reducing eosinophils infiltration in the airway.

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