

## Possible Anti-Asthmatic Effect of Iraqi Ammi Majus Seeds Extract Against Asthma Induced by Ovalbumin in Mice<sup>#</sup>

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### Abstract

Asthma is a chronic respiratory disorder of airways characterized by inflammation, hyperresponsiveness, inflammatory cell infiltration, mucous secretion, and remodelling. *Ammi majus* is a medicinal plant belong to the family of Apiaceous which has anti-inflammatory and antioxidant activities. This study was designed to investigate the anti-asthmatic activity of alcoholic extract of Ammi majus. Forty-eight previously untreated female mice were divided into six groups **Group I**: negative control group (distil water only) for 14 days, **Group II**: positive control group (ovalbumin group) for 14 days, **Group III**: Ammi majus (64 mg/kg/day) with sensitization for 14 days, **Group IV**: Ammi majus (128 mg/kg/day) with sensitization for 14 days, **Group V**: Ammi majus (64 mg/kg/day) without sensitization for 14 days and **Group VI**: Ammi majus (128mg/kg/day) without sensitization for 14 days. Mice were sacrificed by diethyl ether and blood samples were collected to prepare serum samples that were used in ELISA kits for measuring IL-4, IL-5, IL-33 and IgE. The level of all parameters (IL-4, IL-5, IL-33 and IgE) in mice of treated groups with alcoholic extract of Ammi majus were significantly reduced ( $p < 0.05$ ) in compared to ovalbumin group. In conclusion, our results demonstrated that alcoholic extract of Ammi majus has a potent anti-asthmatic activity that improves ovalbumin-induced asthma.

**Keywords:** Asthma, Anti-inflammatory activity, Ammi majus, Ovalbumin.

التأثير المحتمل المضاد للربو لمستخلص بذور الخلة الشيطانية العراقية ضد الربو المحفز بواسطة زلال البيض في الفئران<sup>#</sup>

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### الخلاصة

الربو هو اضطراب تنفسي مزمن يصيب الشعب الهوائية ويتميز بالالتهاب وفرط الاستجابة وتسلل الخلايا الالتهابية و تضيق في مجرى التنفسي وفرط الإفراز المخاطي وتغيرات تصميمية. الخلة الشيطانية هو نبات طبي ينتمي الى عائلة الخلة من جنس الخيميه التي لها أنشطة مضادة للالتهابات ومضادة للأكسدة.صممت هذه الدراسة للكشف عن الفعاليه المضاده للربو للمستخلص الكحولي لنبات الخلة الشيطانية تم اخذ ثمانية وأربعون فأراً من الإناث التي لم يتم علاجها سابقاً مقسمة إلى ست مجاميع المجموعة الأولى: اعطيت ماء نقي فقط لمدة 14 يوم، المجموعة الثانية: اعطيت ماده محسسه (مستخلص زلال الأبييض) لمدة 14 يوم، المجموعة الثالثة اعطيت الخلة الشيطانية (64 مجم / كجم / يوم) لمدة 14 يوم مع تحفيز الربو، المجموعة الرابعة اعطيت المستخلص الكحولي للخلة الشيطانية (128 مجم / كجم / يوم) لمدة 14 يوم مع تحفيز الربو، المجموعة الخامسة: اعطيت المستخلص الكحولي للخلة الشيطانية (64 مجم / كجم / يوم) لمدة 14 يوم بدون تحفيز الربو، المجموعة السادسة: اعطيت المستخلص الكحولي للخلة الشيطانية (128 مجم / كجم / يوم) لمدة 14 يوم بدون تحفيز الربو. في نهاية التجربه تم التضحية في حياة الفئران بواسطة ثنائي ايثيل ايترو جمع عينات الدم لاستخلاص السيروم لقياس عده متغيرات بجهاز الاليزا واطهرت النتائج نقصان معنوي للمتغيرات (IL4,IL5,IL33 and IgE) لفئران المجموعه المعالجه بالمستخلص الكحولي للخلة الشيطانية بالمقارنه مع المجموعه الثانيه، لذلك نستنتج بان المستخلص الكحولي للخلة الشيطانية يمتلك فعاليه مضاده للالتهاب قويه و مفيده في معالجه الربو الناتج من التحسس. الكلمات المفتاحية: الربو، نشاط مضاد للالتهابات، المستخلص الكحولي للخلة الشيطانية، زلال البيض.

### Introduction

Asthma is a chronic and complex inflammatory condition of lung affecting more than 300 million in worldwide and 24 million in the United States<sup>(1)</sup>. About 180,000 deaths caused by asthma every year, actually this condition has

become a major factor in morbidity and mortality in developed countries<sup>(2)</sup>. Prevalence of asthma is higher in high-income countries, but mortality of asthma is highest in low-and middle-income countries, While the prevalence and incidence of

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asthma are higher in children than adult, asthma-related mortality is lower and childhood asthma is more common in boys, while adult asthma is more common in women due to many factors that include: hormonal differences, environmental factors as well as biological sex differences that influence immune function, genetics and function of lung<sup>(3, 4)</sup>.

Asthma is characterized by inflammation, hyperresponsiveness, obstruction of the airways, leukocyte infiltration and remodelling state that refer to the structural alterations in airways among these are: basement membrane thickening, subepithelial fibrosis, goblet cell and submucosal gland enlargement, increased smooth muscle mass and epithelial mucosa metaplasia (mucous cells appearance in new areas of the airways with increased mucus production) so, resulting in symptoms like cough, pain, chest tightness and dyspnea<sup>(5) (6)</sup>.

In asthma, many immune cells are stimulated such as mast cells, eosinophils and activated T helper lymphocytes and macrophage which release mediators such as cytokine, chemokines, adhesions molecules, growth factors, lipid mediators, immunoglobulins, histamine and prostaglandin which lead to severe pathological change in airways<sup>(7)</sup>. However T helper 2 (TH2) plays a significant role in initiation and progression of asthma by its ability to release cytokine, Th2 cytokines control asthma inflammation and they can increase the production of more inflammatory mediators such as other cytokines and proteins through positive feedback processes such as IL- 4, IL- 5 & IL-13, these mediators plays a role in increasing the level of IgE, airways eosinophilia and mucous secretion<sup>(8, 9)</sup>.

The most popular kind of asthma management is drug therapy which reduces asthma episodes by causing the airway smooth muscle cell relaxation (bronchodilator) and reducing inflammation of airways (corticosteroid) but these have limitation and cause an adverse effect especially on long term use, hence there is a need to find alternative therapy for asthma with less adverse effects and high efficacy and using herbal therapy is promising approach.<sup>(10,11)</sup>

Ammi majus is medicinal plant from family of Apiaceous which began in Egypt and distributed to Europe, western Asia and Mediterranean. Also, it is cultivated in Indian and Arabic countries like southern part of Oman<sup>(12, 13)</sup>. In Iraq, Ammi majus is commonly found in fields and gardens. It was gathered in Baghdad Kut, Hawija, and other areas<sup>(14)</sup>. It is used for dermatological purpose such as psoriasis, tinea versicolor, vitiligo<sup>(15)</sup>, for digestive problems, diabetic, angina pectoris and as antispasmodic<sup>(15, 16)</sup>. Seeds contain important active constituents namely (coumarin and flavonoid). Flavonoid has potent anti-inflammatory activity through a variety

mechanisms including: inhibition of transcription factors such as NF- $\kappa$ B and regulatory enzymes such as (protein kinase), which play an important role in regulation of mediators involved in inflammation like IL4, IL5, IL-6, TNF $\alpha$  and IL1B<sup>(17, 18)</sup>. Flavonoid also has an effect on inflammatory cells of immune system by decreasing histamine and prostaglandin release from mast cell and inhibits formation of chemokines and cytokine in neutrophil, mast cell and other cells of immune system<sup>(19)</sup>. Coumarin can decrease inflammation and edema of the tissue and suppress prostaglandin formation<sup>(20)</sup>. This study was designed to evaluate anti-asthmatic effects of two doses of Ammi majus alcoholic extract in treatment of ovalbumin-induced asthma in mice.

## Material and Method

### Chemicals and kits

Ammi majus seeds purchased from market of Baghdad /AL-Rusafa. Ovalbumin powder was purchased from Sigma Aldrich, Germany. The cytokine (IL-4, IL-5 & IL-33) and IgE ELISA kits were brought from (elabscience. China).

### Extraction of plant

The seeds of Ammi majus were crushed and milled by electrical grinder, 100gm of powder seeds were defatted by Soxhlet apparatus with 1000 ml of n-hexane until disappearance of the yellowish color. The remaining oil free residue was left at room temperature for 24hrs, this residue represents the defatted Ammi majus seeds and was extracted by alcohol via reflux method with 2.5 liters of 80% ethanol at 40°C, then the mixture was allowed to cool and filtered by filter paper. The filtrate was evaporated by rotary vacuum evaporator at 40°C until obtained an ethanol free extract containing the active ingredients of Ammi majus seeds<sup>(21)</sup>.

### Preparation of extract for oral administration

The Stock solution of Ammi majus extract was prepared by dissolving 1.5 gm of extract in 100 ml of distilled water. The solution then was mixed to obtain a working solution. Using 1 ml syringe, for a dose of 64mg/kg (about 0.1\_0.128 ml of the solution according to animal weight) was given orally for each mouse for 14 successive days. For a dose of 128mg/kg (about 0.2\_0.256 ml of the solution according to animal weight) was given orally for each mice for 14 successive days<sup>(22)</sup>.

### Animal

Forty-eight (48) previously untreated albino female mice aged (6-8 week) and weighing (25-30 gm), were obtained from animal house of the College of Pharmacy / University of Baghdad. Mice were housed under standard condition of controlled temperatures, humidity and photoperiods. These animals were fed commercial pellets and tap water throughout the experiment period.

### Study design

Mice were randomly divided into six groups (8 mice per group). The doses of alcoholic

extract of Ammi majus seeds (64 and 128 mg/kg) according to the previous studies<sup>(22)</sup>.

**Group I:** Mice were administrated only distilled water orally (4 ml /kg) for 14 days as negative control group.

**Group II:** Mice were administrated ovalbumin intraperitoneal injection (IP) at day 0 and inhalation for 14 successive days as positive control group (ovalbumin group).

**Group III:** Mice were administrated (64 mg/kg) of Ammi majus extract orally for 14 days with use of ovalbumin for 14 days as treated group.

**Group IV:** Mice were administrated (128 mg/kg) of Ammi majus extract orally for 14 days with use of ovalbumin for 14 days as treated group.

**Group V:** Mice were administrated (64 mg/kg) of Ammi majus extract orally for 14 days.

**Group VI:** Mice were administrated (128 mg/kg) of Ammi majus extract orally for 14 days.

#### **Sensitization method**

Mice were sensitized by intraperitoneal (IP) injection of 1 ml of 10% OVA (10 g ovalbumin in 100 ml of phosphate buffer saline PBS ) on day 0 and exposed to 1% OVA aerosol (1 g of ovalbumin in 100 ml of PBS) for 14 successive days, 30 minutes/day.

Aerosolization was performed for 30 min by placing the mice in a chamber connected to an ultrasonic nebulizer<sup>(23, 24)</sup>. Doses of Ammi majus extract were given at 60 minutes after sensitization.

#### **Preparation of serum samples**

The blood sample was collected by puncturing the retro-orbital route with capillary tube, about 1-1.5 ml of blood was obtained and collected in the Eppendorf tube, it was let to completely coagulate for 20 to 30 min and then centrifuged in a cold centrifuge at 3000rpm for 20min at 4°C. Serum then was collected in Eppendorf tube and frozen at 20°C for analysis, then parameter (IL-4, IL-5, IL-33 and IgE) were measured by using Elisa kits.

#### **Statistical analysis**

Data were expressed as the mean  $\pm$  standard deviation (SD). Where, Unpaired Student t-test was used for testing the significant difference between the two groups. On other hand, one-way ANOVA analysis was used for testing the significant difference between the study groups. Differences were considered statistically significant when P-value less than 0.05.

## **Results**

### **1. Effect of Ammi majus alcoholic extract on pro inflammatory cytokine**

Table 1 and figure1-A, B, C show highly significant elevation ( $P < 0.05$ ) in serum IL-4, IL-5, and IL-33 levels for mice of ovalbumin group (group II) compared to negative control group (group I).

In contrast, mice that treated with Ammi majus extract (group III and group IV) exhibited significant decrease ( $p < 0.05$ ) in serum IL-4, IL-5 and IL-33 levels compared to ovalbumin group (group II).

However, non-sensitized Ammi majus treated groups (group V and group VI) did not reflect a significant difference ( $p > 0.05$ ) in serum IL-4, IL-5 and IL-33 levels compared to (group I).

Treated with Ammi majus extract (group III and group IV) exhibited significant difference ( $p < 0.05$ ) in serum IL-4 levels compared to (group I) and significant difference ( $p < 0.05$ ) in serum IL5 levels between (group III) and (group I). While non-significant difference ( $p > 0.05$ ) in serum IL-5 levels between (group IV) and (group I). Also, no difference ( $p > 0.05$ ) in serum IL33 levels of mice of groups that treated with Ammi majus extract (group III and group IV) compared to (group I).

There is a significant difference ( $P < 0.05$ ) in serum IL-4 levels for mice of (group V and group VI) compared to (group III and group IV).

However, there was no significant difference ( $P > 0.05$ ) in serum IL5 and IL33 levels for mice of (group V and group VI) compared to (group III and group IV).

While, highly significant elevation ( $P < 0.05$ ) in IL4, IL5 and IL33 levels in serum for mice of (group II) compared to (group V and group VI).

### **2. Effect of Ammi majus alcoholic extract on immunoglobulin E (IgE) level.**

Table1 and Figure1-D show that IgE levels (means  $\pm$  SD) in serum of mice in group II were significantly higher ( $p < 0.05$ ) than IgE levels in serum of mice in group I.

On other hand, the IgE levels in serum for mice of treated groups (III & VI) at doses (64&128mg /kg respectively) appeared highly-significant reduction ( $p < 0.05$ ) compared to group II.

Treated with Ammi majus extract (group III and group IV) exhibited a significant difference ( $p < 0.05$ ) in serum IgE levels compared to group I. However, there was significant difference ( $p < 0.05$ ) in serum IgE levels between (group V) with (group III and group IV) and a significant difference between (group VI) and (group IV) but there is no difference ( $P > 0.05$ ) between (group VI) and (group III).

Furthermore, IgE levels in serum for mice of group III (64 mg/kg Ammi majus extract) appeared non-significant different ( $P > 0.05$ ) from group IV (128 mg/kg Ammi majus extract). While, highly significant elevation ( $P < 0.05$ ) in IgE levels in serum for mice of group II compared to group V and group VI.

On other hand, IgE levels in serum for mice of (group V and group VI) without sensitization showing non-significant difference ( $p > 0.05$ ) compared to group I.

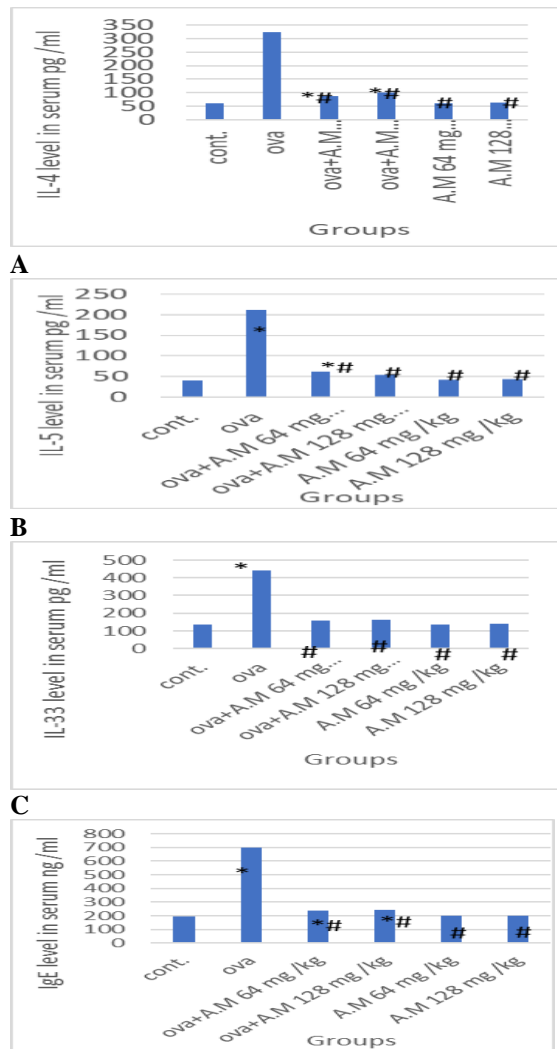
**Table 1: Effect of Ammi majus alcoholic extract on pro-inflammatory cytokine and antibody immunoglobulin in serum for ovalbumin induced asthma in mice.**

Serum cytokine	Group I	Group II	Group III	Group IV	Group V	Group VI
IL4 pg/ml	59.89±14.52	342.36±29.27*	88.44±13.04 <sup>#</sup>	101.5±13.47 <sup>#</sup>	62.84±12.39 <sup>#</sup>	68.21±9.36 <sup>#</sup>
IL5 pg/ml	39.66±12.2	212.10±20.85*	61.89±17.9 <sup>#</sup>	54.05±17.63 <sup>#</sup>	42.29±12 <sup>#</sup>	43.79±13.39 <sup>#</sup>
IL33 pg/ml	135.9±21.4	442.7±32.79*	157.4±24.03 <sup>#</sup>	161.3±21.18 <sup>#</sup>	136±18.77 <sup>#</sup>	139.6±15.6 <sup>#</sup>
IgE ng/ml	195.22±28.17	710.77±53.18*	235.51±26.8 <sup>#</sup>	243.56±28.04 <sup>#</sup>	206.63±21.3 <sup>#</sup>	210.38±18.36 <sup>#</sup>

Each value represents mean ± SD, n=8 in each group

\* Is significantly different compared with the control group (p<0.05).

# Is significantly different compared with ovalbumin group (p<0.05).



**D** Figure 1. Effect of Ammi majus alcoholic extract on pro-inflammatory cytokine and antibody immunoglobulin in serum for ovalbumin induced asthma in mice.

(Each value represents mean ± SD), n=8

\* Is significantly different compared with the control group (p<0.05).

# Is significantly different compared with ovalbumin group (p<0.05).

cont.=control, ova=ovalbumin, AM=Ammi majus extract.

## Discussion

Asthma is a chronic respiratory condition of airways, affecting a large number of populations, characterized by inflammation, mucous secretion, leukocyte infiltration and airways narrowing<sup>(25)</sup>.

Exposure to allergen known to causes airway inflammation and remodelling, these severe pathologic changes are highly predominated in ovalbumin induced asthma in mice. Ovalbumin was employed as allergen that causes immune reaction mediated by T lymphocyte which plays key role in asthma development through releasing pro inflammatory cytokines such as IL-4 and IL-5<sup>(26)</sup>. In this study, levels of IL-4, IL-5 and IL-33 were significantly increased after ovalbumin administration. In asthma, IL-33, IL-25, thymic stromal lymphopoietin (TSLP) and leukotriene cause activation of mast cell, TH2 and basophil leading to production of IL-4 which is essential in the regulation of growth and development of Th2 and inducing B cell isotype switching, resulting in IgE production that has a high affinity to bind to FcεRI receptor on mast cell leading to mast cell degranulation and releasing of rapidly acting mediators like (histamine, prostaglandin and leukotriene) all of which act together to contract airway smooth muscle cell and causing vascular leakage and mucous hypersecretion. Also, mast cells release mediators that are associated with allergic response such as IL-4, IL-5, IL-6 and TNFα which stimulate recruitment of inflammatory cells (neutrophile, eosinophile and T lymphocyte) to cause delay airway response<sup>(27) (28)</sup>.

Interleukin 5 is important factor in asthma pathogenesis produced by TH2 which is responsible for growth, survival, activation, and maturation of eosinophile which is hallmark associated with asthma. Also, IL-5 plays a role in releasing of superoxide from eosinophil. So, the increasing level of IL-5 may indicated for peak of eosinophils in airways<sup>(29)</sup>.

Recent studies have suggested that asthma is associated with differences in genes encoding IL-33 and IL-1RL1. IL-33 is cytokine from family of IL-1 released by endothelial and epithelial cell in response to cellular damage, in this way act as an alarm to stimulate immune system cell to release IL-4, IL-5 and IL-13 that led to severe pathological change in lung tissue such as: production of IgE, airway eosinophilia and mucous secretion. IL-33 activity is mediated by binding to the IL-33 receptor complex (IL-33R) and activation of MAPK and NF- $\kappa$ B signalling via the MyD88/IRAK/TRAF6 module resulting in formation and release of pro-inflammatory cytokines /chemokines<sup>(30)</sup>. Activation of NF- $\kappa$ B inducing transcription of numerous inflammatory genes that are expressed abnormality in asthma<sup>(31)</sup>. So, our study found that Ammi majus extract preventing ovalbumin induced airways inflammation in murine model of asthma, specifically by reducing the levels of IL-4, IL-5 and IL-33 in serum of treated groups compared to ovalbumin group. This result suggests that Ammi majus alcoholic extract-mediated anti-inflammatory effects against ovalbumin- induced asthma due to it is contents of phytochemical ingredients (coumarin and flavonoid) which previously demonstrated their ability in causing decline in the levels of pro-inflammatory cytokine and adhesions molecules by inhibition of transcription factor NF- $\kappa$ B and protein kinase which 5are responsible for the control of gene expression of inflammatory mediators such as IL-4, IL-5, IL-8, IL-13, IL-6 & TNF $\alpha$ <sup>(32, 33)</sup>. This is may explain the therapeutic effects of Ammi majus extract in asthma.

On other hand, IgE is important immune marker linked to asthma, Th2 cytokine IL-4 plays a major role in switching B cells to generate IgE<sup>(34)</sup>. In this study showed that IgE level in serum of ovalbumin group was highly elevated compered to negative control group, resulting from increased production of IL-4 from T lymphocyte upon ovalbumin exposure. In contrast, treatment with Ammi majus extract significantly decreased the level of IgE in serum compared to ovalbumin group, this effect due to reduced production of IL-4 by plant extract<sup>(35)</sup>. Results of our study was supported by other study that indicated flavonoid has inhibitory effect on allergic disease by decreasing levels of interleukins through the ability of flavonoid to block NF- $\kappa$ B signalling pathway<sup>(18)</sup>.

## Conclusion

In this study, pharmacological action of Ammi majus on inflammation of airway in asthma, has found a positive effect on management of inflammation associated with asthma, this is demonstrated by a significant decrease of proinflammatory cytokines (IL-4, IL-5& IL-33) and immune marker IgE antibody in serum. Further studies are required to evaluate the clinical effect of Ammi majus in asthmatic patient.

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## Ethics Statements

This study was approved by the scientific and ethical committees of the College of Pharmacy University of Baghdad.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Author contributions

Zainab T. Younis: contributed to data gathering, analysis, practical (follow the procedure) and written parts of the study. Shihab H. Mutlag gave final approval and agreement for all aspects of the study, supervision, revision, and rearrangement.

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