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Possible Anti-asthmatic Effect of Iraqi *Ammi majus* Seeds Extract Induced by Ovalbumin in Mice

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A B S T R A C T

Asthma is a chronic and complex condition of the respiratory tract, that occurs in both children and adults. Currently available medications have side effects; therefore, new therapies with fewer side effects are needed. Thus, present study was designed to evaluate the potential anti-asthmatic effects of alcoholic extract of *Ammi majus* on ovalbumin-induced asthma in mice. Forty-eight female Albino mice were randomly assigned to one of six groups with 8 animals each. Group I received distilled water as a negative control, Group II received ovalbumin as a positive control, Groups III and IV received *Ammi majus* orally at 64 and 128 mg/kg/day, respectively, with sensitization, and Groups V and VI received *Ammi majus* orally at 64 and 128 mg/kg/day, respectively, without sensitization. Bronchoalveolar lavage fluid (BALF) was obtained 24 h after the 1st challenge to measure the number of inflammatory cell counts. In addition, lung tissue was removed for histopathological examination. Oral administration of alcoholic extract of *Ammi majus* at both doses (64 and 128 mg/kg) significantly inhibited ovalbumin-induced increases in total and differential cell counts of eosinophile, neutrophile, monocyte, and lymphocyte) in BALF, in addition, improve histopathologic events of asthma in lung tissue of ovalbumin-induced asthma in mice. These results demonstrated that *Ammi majus* alcoholic extract has a potent anti-asthmatic activity that improved ovalbumin-induced asthma.

Keywords: asthma, Anti-asthmatic activity, *Ammi majus* alcoholic extract, ovalbumin

INTRODUCTION

Asthma is chronic respiratory condition of lung and more than 300 million people worldwide and 24 million in the United States are affected with asthma (1). approximately about 180,000 deaths are caused by asthma every year; in developed countries, this illness is higher as it is considered one of the primary causes of morbidity and mortality (2,3). Asthma is characterized by inflammation, hyperresponsiveness, obstruction of airway and remodeling states that refer to the alterations in structure of airways include: basement membrane thickening, increased smooth muscle mass, subepithelial fibrosis, angiogenesis, goblet cell and submucosal gland enlargement, and epithelial mucosa metaplasia, resulting in symptoms like cough, pain, dyspnea and chest tightness (4,5).

Many inflammatory cells are involved in asthma including eosinophile, mast cell, lymphocyte, neutrophil, and macrophage, however T lymphocyte play key role in pathogenesis of asthma, Th2 responds to stimulus through releasing pro inflammatory mediator like interleukins 3, 4, 5, 9 ,13 and GM-CS. On other hand, IL-4 is important in

regulation of growth and differentiation of Th 2 and production of IgE that bind to FC epsilon receptor (FcεR) receptor on mast cell leading to activation of mast cell which results in release of rapidly acting mediator (prostaglandin, histamine, and leukotriene) all of which act together to cause contraction of airway smooth muscle (ASM), vascular leakage and increased mucous production. Also mast cell producing mediators that are linked to allergic response such as IL-4, IL-5, IL-6 and TNF-α which stimulate inflammatory cell recruitment (neutrophile, eosinophile and T lymphocyte into site of damage to cause delay airway reaction (6,7). IL-5 is responsible for activation, maturation, survival of eosinophile which is important marker linked with asthma (8) and IL-13 is important in mucous production, subepithelial fibrosis and airways hyperresponsiveness (9). The most common therapy for asthma management is medicinal drug, like short, long-acting beta agonists and inhaled corticosteroids. These drugs reduce asthma attack by relaxation of smooth muscle cell and decreasing inflammation, but long-term use can cause toxic effect hence finding alternatives is necessary therapy for management of asthma with less adverse consequences and high efficacy and herbal plants is promising approach (10).

Ammi majus is herbal medicinal plant belonging to family of Apiaceous which is spread in Egypt and distributed to Europe, Mediterranean and western Asia. It is also grown in Indian and other Arabic countries like Oman (11,12). In Iraq, *Ammi majus* is frequently found in gardens and fields. It was gathered in Baghdad, Kut, Hawija and other areas (13). It is used for skin disorder such as psoriasis, vitiligo, tinea versicolor (14) for digestive problem, diabetes, angina pectoris, as antispasmodic (14,15). The seeds contain main active constituent namely (coumarin and flavonoid) which have anti-inflammatory and antioxidant activity that may be beneficial in treatment of asthma (16,17), thus this study was designed to assess anti-asthmatic effects of two doses of alcoholic extract of *Ammi majus* in management of ovalbumin-induced asthma in mice.

MATERIALS AND METHODS

All procedures used in this study were reviewed and approved by The Scientific Committee of the College of Pharmacy and Toxicology, University of Baghdad in compliance with the ethical principles of animal welfare.

Ammi majus Seed Extract Preparation

The *Ammi majus* seeds were purchased from a local herbal market in Baghdad province, Iraq and authenticated by the botanist at the Iraqi National Herbarium, Directorate for Seed Testing and Certification, Ministry of Agriculture, Abu-Ghraib, Baghdad, Iraq. Ovalbumin powder was purchased from Sigma Aldrich, Germany.

The extraction was prepared by Soxhlet apparatus with 1000 mL of N-hexane until disappearing of yellowish colour. The remaining oil free residue was left at room temperature for 24 h, representing the defatted *Ammi majus* seeds and was extracted by alcohol via reflux method with 500 mL 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 evaporators at 40 °C until obtaining an ethanol free extract containing the active ingredient of *Ammi majus* seeds.

Animals

Forty-eight healthy Albino female mice, aged 6-8 weeks and weighing 25-30 g, were obtained from animal house of the College of Pharmacy, University of Baghdad, Baghdad, Iraq. Mice were housed under standard conditions of controlled temperatures, humidity, and photoperiods. Throughout the duration of the experiment, animals were fed *ad libitum* commercial pellets and tap water.

Study Design

The doses of *Ammi majus* alcoholic seeds extract (64 and 128 mg/kg) according to the previous studies (19) were considered. A total of six groups of mice were randomly assigned (8 mice of each) as follows. In Group I, eight mice were orally administrated distilled water (4 mL/kg) for 14 days and sacrificed at day 15 serviced as negative control group; Group II, eight mice were administrated ovalbumin (intraperitoneal injection on day 0 and inhalation for 14 and sacrificed at day 15 days as positive control group (ovalbumin group).**;** Group III, eight mice were orally administrated (64 mg/kg) *Ammi majus* extract with sensitization for 14 days and sacrificed at day 28 as treated group.**;** Group IV, eight mice were orally administrated (128 mg/kg) of *Ammi majus* extract with sensitization for 14 days and sacrificed at day 28 as treated group; Group V, eight mice were orally administrated 64 mg/kg of *Ammi majus* extract without sensitization for 14 days and sacrificed at day 15; Group VI, eight mice were orally administrated (128 mg/kg) of *Ammi majus* extract without sensitization for 14 days and sacrificed at day 15.

Sensitization Method

Mice were sensitized by intraperitoneal (IP) injection 1 mL of 10% ovalbumin on day 0, then exposed to 1% ovalbumin aerosol for 14 successive days, 30 min/day. Aerosolization was performed for 30 min by placing the mice in a chamber connected to the ultrasonic nebulizer (20,21).

Collection of Bronchoalveolar Lavage fluid (BALF)

Mice were sacrificed under deep anesthesia by cervical dislocation. The animal was placed on an operating table; the trachea was gently exposed with scissors and tweezers

after removed excess blood and gland. A suture was wrapped around the trachea, and a needle was used for its puncture. A 20 G venous catheter was inserted and sutured inside the trachea, and by using 1 mL syringe, 1 mL of normal saline was injected into the trachea and aspirated the solution gently within 60 sec and massaging of thorax. This process was repeated 3 times and the fluid was collected in a plain tube on ice, followed by the collection of BALF for white blood cells (WBC) count and differentiation (22).

White Blood Cells Count and Differentiation

BALF was collected and centrifuged for 7 min at 400 × g and 4 °C to separate entire cells. The deposited cells were isolated on ice after the supernatant had been removed. WBC counts were measured on the same day and manually distinguished under a light microscope by a specialist using a nebular hemacytometer (22).

Histopathological Examination of Lung Tissue

The lung was removed from mice and washed with normal saline solution; tissue fixation was then done by putting the lung tissue in 10% formalin. The lung was followed by dehydration with successively stronger ethanol each for 1 min. The lung then was cleaned with Xylene to eliminate alcohol and to provide the lung with some degree of Transparency, then the tissue was saturated with paraffin wax, heated, and blocked by pouring in embedded models. Blocks were cut by microtome into 5 µm, thick sections, washed in a water bath, and left in the oven for dewaxing, then stained with hematoxylin and eosin, then examined by using a light microscope by a pathologist (23).

Statistical Analysis

Data were expressed as the mean ± 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 three or more than groups. Differences were considered statistically significant when $P \leq 0.05$.

RESULTS

As shown in Table1, the total WBC count in BALF for mice of group II (ovalbumin group) appeared highly significant elevation ($P<0.05$) in comparison to mice of group I (the negative control group). While mice that were treated with *Ammi majus* alcoholic extract (group III and group IV) at two doses (64 and 128 mg/kg, respectively) appeared highly significant reduction ($P<0.05$) in the total WBC count in BALF compared to mice of group II (ovalbumin group). Moreover, non-significant difference ($P>.0.05$) of the total WBC count in BALF for mice of group V, group VI without sensitization compared with group I (the negative control group).

There was significant elevation ($P<0.05$) in inflammatory cell count (lymphocyte, eosinophile, monocyte and neutrophil) in BALF for group II (ovalbumin group) compared to the negative control group (Table 1). Furthermore, mice that were treated with alcoholic extract of *Ammi majus* (group III and group IV) exhibited a significant decline ($P<0.05$) in differential cell count compered to sensitized group. Finally, treatment with alcoholic extract of *Ammi majus* (group III and group VI) without sensitization showed non-significant difference in differential cell count as compared with the negative control group.

Table 1. Effect of administration of alcoholic extract of *Ammi majus* on total and differential white blood cell count (cell/cm^3) in bronchoalveolar lavage fluid (BALF) for ovalbumin-induced asthma in mice

Groups	Parameter				
	White blood cells	Eosinophil	Neutrophil	Lymphocyte	Macrophage
Control	571.3±27.8	6.50±2.34	453.7±29.2	92.2±7.78	19.2±1.89
Ovalbumin	1401±175*	55.2±6.80*	1106±172.4*	171±9.98*	69.5±6.15*
OVA+A.M 64 mg/kg	815.5±27.9#	14.5±5.43#	671.3±30.1#	112±6.80#	29.8±10.0#
OVA+A.M 128 mg/kg	787.0±35.7#	10.6±4.00#	631.5±32.6#	121±6.99#	24.5±4.27#
A.M 64 mg/kg	608.7±79.7	8.16±4.49	486.0±85.1	95.0±7.00	20.0±3.10
A.M 128 mg/kg	629.8±42.2	8.50±2.16	504.1±48.2	94.2±9.26	23.0±3.68

Values are means±SD, n = 8 per treatment group. *Significantly different compared with the control group (P≤0.05). #Significantly different compared with ovalbumin group (P≤0.05). OVA=Ovalbumin, A.M= *Ammi majus*

Lung section of control group showed normal looking appearance of lung tissue (Figure 1a), ova challenged lung section showed heavy inflammatory cell infiltration into bronchial area and increased thickness of bronchiole and excessive mucous in bronchial cavity (Figure 1b). Treatment with extract of *Ammi majus* at two doses (64 and 128 mg/kg) showed mild inflammatory cell infiltration in interstitial tissue with nearly normal looking appearance of lung tissue (Figure 1c-e).

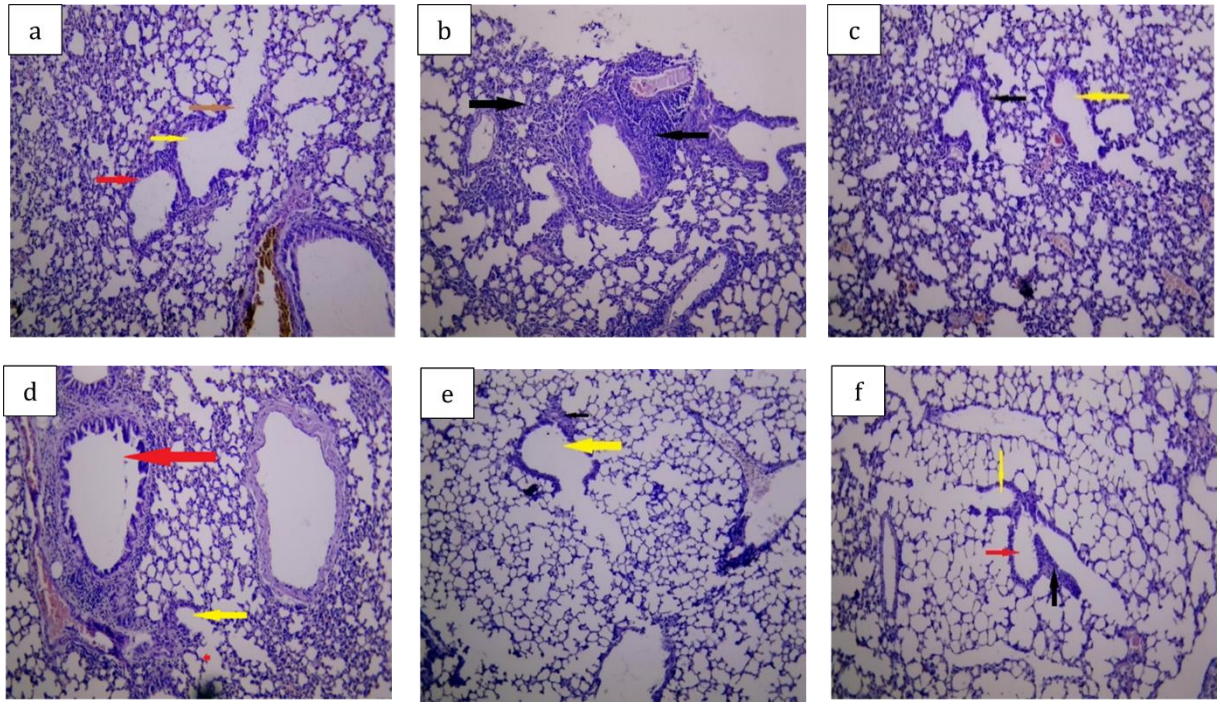

Figure 1. Effect of *Ammi majus* alcoholic extract on histopathological appearance of lung tissue for ovalbumin-induced asthma in mice (H&S, 40×). a, Group I served as a negative control and received distilled water; b, Group II received ovalbumin; c, Group III received *Ammi majus* at 64 mg/kg/day with sensitization; d, Group IV received *Ammi majus* at 128 mg/kg/day with sensitization; e, Group V received *Ammi majus* at 64 mg/kg/day without sensitization; f, Group VI received *Ammi majus* at 128 mg/kg/day without sensitization

DISCUSSION

In present study in order to support anti-asthmatic activity of herbal plants against allergic disease, we used ovalbumin as allergy trigger which acts through T lymphocyte that led to progression of inflammatory disease by expressing and producing cytokines that attract and activate inflammatory cells, resulting in additional cytokine production which is responsible for asthma development. Therefore, administration of ovalbumin to mice of group II resulted in inflammation of airways and characteristically associated with marked infiltration of neutrophils, eosinophil, lymphocyte, and monocyte into bronchial area. Other study showed asthmatic patients have a higher eosinophil count in their BALF than healthy individuals because eosinophils have IL-3, IL-4, IL-5, IL-13, IL-33, and GM-CSF receptors. When these mediators bind to their receptor on surface of eosinophil leading to eosinophil activation and production of proinflammatory mediators, like leukotriene C 4 and platelet activating factor, which increase the permeability of mucosal cells and act as powerful chemotactic factors that stimulate further eosinophil infiltration. Eosinophils also produce excessive amounts of proteins that are toxic to epithelial cell, so eosinophils are considered major biomarker associated to asthma (24). In present study treatment with *Ammi majus* alcoholic extract at 64 and 128 mg/kg significantly inhibited OVA induced increased in inflammatory cell count in BALF and reduced eosinophile infiltration. This suggests that *Ammi majus* extract has powerful anti-inflammatory activity. This effect is attributable to its richness in phytochemical ingredients (coumarin and flavonoid) which previously had proven to cause reduction in levels of pro-inflammatory cytokine and adhesions molecules via modulation of transcription factor NF-κB and protein kinase that have key role in control of gene expression of mediators that are responsible for activation of inflammatory cells and recruitment into site of inflammation such as IL-4, IL-5, IL-8, IL-13, IL-6, and TNF-α (25-27).

Asthma pathogenesis begins with changes in the structure of the airway such as thickening of the epithelial and subepithelial mucosa, goblet cell metaplasia, epithelial folding and sluffing, blood vessels dilation and increase mucus secretion into the airways (10). The results of our study showed that the ovalbumin sensitization led to heavy inflammatory cell infiltration in bronchial area, thickening of the epithelial layer, mucosa, and submucosa of the lung tissues and increased mucous production, indicating that ovalbumin successfully established the model for airway remodeling. On the other hand, *Ammi majus* treatment showed it is effective for alleviating asthma associated histopathological alternation and reducing airways inflammation by inhibition of transcription factor and reducing gene expression for chemokine and cytokine that associated with these pathological changes in asthma (28). These inhibitory effects of plant extract were supported by

previous studies that demonstrated that flavonoid (quercetin) have the capacity to decrease the most significant pathological changes in asthma diseases like neutrophil and eosinophil recruitment, epithelial cell activation, hyperresponsiveness of the airways and mucus production (29). Thus, these results suggest that alcoholic extract of *Ammi majus* has inhibitory action that protects against airway inflammation induced by ovalbumin.

The results of this study clearly demonstrated that treatment with two doses of *Ammi majus* seeds extract produce anti-asthmatic effects against ovalbumin-induced asthma. This is demonstrated by significant decrease in total and differential WBC count in BALF. Furthermore, it improved histopathologic events of asthma in lung tissue of mice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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التأثير المحتمل المضاد للربو لمستخلص بذور الخلة الشيطانية العراقية المحفز بواسطة زلال البيض في الفئران

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الخلاصة

الربو هو حالة مزمنة ومعقدة في الجهاز التنفسي ، ويحدث في كل من الأطفال والبالغين. الأدوية المتاحة حاليًا لها آثار جانبية، وبالتالي نحتاج للبحث عن علاج جديد مع آثار جانبية أقل، لذلك تم تصميم الدراسة الحالية لتقييم الآثار المضادة للربو للمستخلص الكحولي من الخلة الشيطانية المحفز بواسطة زلال البيض في الفئران. ثمانية وأربعون فأرًا من الإناث الصحية مقسمة إلى ست مجاميع المجموعة الأولى: اعطيت ماءً نقياً فقط لمده ١٤ يوماً، المجموعة الثانية: اعطيت مادة محسسة (مستخلص زلال الأبيض) لمده ١٤ يوماً، المجموعة الثالثه: اعطيت المستخلص الكحولي للخلة الشيطانية (٦٤ ملغم/كغم) لمده ١٤ يوماً مع التحسس، المجموعة الرابعة: اعطيت المستخلص الكحولي للخلة الشيطانية (١٢٨ ملغم/كغم) لمده ١٤ يوماً مع التحسس ، المجموعة الخامسة: اعطيت المستخلص الكحولي للخلة الشيطانية (٦٤ ملغم/كغم) لمدة ١٤ يوماً بدون تحسس، المجموعة السادسة: اعطيت المستخلص الكحولي للخلة الشيطانية (١٢٨ ملغم/كغم) لمده ١٤ يوماً بدون تحسس. تم التضحية بالفئران عن طريق خلع الرقبة وتم الحصول على سائل غسيل القصبات الهوائية لقياس عدد الخلايا الالتهابية بالإضافة إلى ذلك، تمت إزالة الرئة لفحص الأنسجة. واظهرت النتائج ان تناول المستخلص الكحولي من الخلة الشيطانية عن طريق الفم بجرعتين (٦٤ و ١٢٨ ملغم/كغم) أدى بشكل كبير إلى تثبيط الزيادات التي يسببها زلال البيض في عدد كريات الدم البيضاء بالإضافة إلى تحسين الأحداث النسيجية المرضية في الرئة، لذلك نستنتج ان المستخلص الكحولي للخلة الشيطانية له نشاط قوي مضاد للربو يحسن الربو الناتج من التحسس بواسطة زلال البيض في الفئران.

الكلمات المفتاحية: الربو، النشاط المضاد للربو، الخله الشيطانيه، زلال البيض