



Physiological Effects of *Teucrium polium* Extract Ameliorates Ovalbumin-Induced Allergy in Rabbits

Aleem H.A. Nassar, Nasser J.H. Al-Mukhtar, Safaa H. Alturaihy

Department of Physiology and Medical Physics-College of Medicine, University of Babylon/Iraq.

Abstract

Objective: This present study aims to evaluation the effectiveness of *T.polium* extract and compares with oral corticosteroid drug (prednisolone) for reducing signs and symptoms of ovalbumin-induced allergy in rabbits. Also, to detect and confirm the use of *T.polium* as anti-allergic agent and to weather extent may replace as an herbal therapy instead of oral corticosteroids. **Methods :** Ovalbumin was used to induce allergy and sensitization challenge was confirmed by the presence of the clinical symptoms of sneezing, wheezing and shortness of breath, chest tightness, changes in the levels of eosinophils, neutrophils, Immunoglobulin E, Interleukin-4. Eight days after allergy induction, *Teucrium polium* in a dose of (4, 8 and 12 mg/kg) was administrated orally for eight days. Blood samples were collected at day 15, 30 of the experiment and complete blood count, IgE, IL-4 and levels were determined. **Results:** showed that ovalbumin could induce sensitivity and significantly increased eosinophils, Immunoglobulin E and Interleukin-4 levels ($p \leq 0.05$). Oral treatment with *Teucrium polium* showed a significant decrease in the number of eosinophils ($p \leq 0.05$) and serum levels of Immunoglobulin E and Interleukin-4 ($p \leq 0.05$). The effects of this plant extract were comparable to those observed in the prednisolone-treated group. **Conclusion:** *Teucrium polium* extract have significant results in preventing allergic complications and diminish allergic symptoms especially in asthmatic condition, Also, produced a significant decrease in total WBCs Neutrophils, Eosinophils, IgE and IL 4 on contrast with oral corticosteroid causes dimargination of neutrophils and a significant increase in total white blood cells.

Keywords: *Teucrium polium*; Asthma; Ovalbumin; Interleukin 4 and immunoglobulin E.

Introduction

Plants are the primary source of medicines, food, and shelter for humans. Besides curing ailments, various plant parts like roots, stems, leaves, flowers, fruit and seeds can act as a food resource for human [1]. These are an indispensable constituent of human diet providing the body with vitamins, minerals salts, and certain hormone precursors, also to protein and energy [2]. Plant-derived natural products can be used in the treatment of diseases; thus, can act as a base for development of natural blueprint of new drugs [3].

There is a high need for a constant search for new resources to alleviate hunger in developing countries. Predictions of the future need based on the current rates of increasing population emphasize the seriousness of this problem [4]. *Teucrium polium* Labiate (Ja'adah in Arabic) is a widely grows in Iraq-Also door/Diyala; the plant has medicinal properties [5]. It is a very polymorphous perennial plant 10-35 cm high.

The leaves are white, tomentose on both sides, with downwards rolled rounded-toothed margins. Flowers have a white or yellow corolla, in a globular inflorescence. The calyx is bell-shaped with five sub-equal flat, triangular or acuminate triangular teeth. The fruits are light brown to dark brown notelets with a latticed surface. The plant gives off a pleasant aromatic smell; Flowering takes place from April until June [6]. The development of drugs from plants continues, with pharmaceutical companies engaged in the pharmacological screening of herbs [7].

Teucrium polium L. is a dwarf shrub plant which grows wild in Mediterranean countries. This species is one of the most popular species used in the treatment of digestive and respiratory illnesses, abscesses, gout, and conjunctivitis, in the stimulation of fat and cellulite decomposition, and possess anti-inflammatory, antioxidative, antimicrobial, antidiabetic and anthelmintic effects [5]. Also, *T. polium* L. Has been traditionally used for

over 2000 years in traditional medicine outstanding to its diuretic, diaphoretic, tonic, antipyretic, antispasmodic and Cholagogic properties [8]. The tea preparation of the aerial parts of the plant is used for the treatment of abdominal colic, headache, and diabetes and as an astringent [8].

Materials and Methods

Laboratory Animal

Thirty adult male rabbits (Breed: New Zealand white, (*Oryctolagus-cuniculus*) weighing: (2000-2500) grams and 12-16 months old were used in the present work. Animals were left four weeks for an adaptation before the experiments. Each four animals were housed in optimized steel fewer steel cages (1.50 m length, 0.25 m width, and 1.30 m high) and they were had been freely feeding on clean (green vegetables and chaw pellets) and water. Animals were kept under the same conditions of the temperature (22-25) C° and light regime of 12 hours' light, and 12 hours' darkness. Animals of the study were divided into six groups each group consists of 4 male rabbits used for the study design.

Extraction

The air-dried and powdered aerial parts of the *Teucrium polium* were extracted with ethanol 99.9 % (Avantor M). Using the Soxhlet apparatus 60 minutes for each cycle. The extract was evaporated by using a rotary evaporator; the powdered plant material was collected dried and stored at 5°C in an airtight container without light exposure. In an air tight container without light exposure.

Experimental Protocol

Induction of allergic airway inflammation was performed by intraperitoneal (i.p) injection of the immunization by Ovalbumin (OVA) 0.1 mg and 10 mg of aluminium hydroxide in 2 ml of phosphate buffer saline (PBS) in day 1 and challenge dose through second sensitization in day 14 of the immunization program [9].

Group 1

Control group consist of four rabbits injected (i.p) twice of the experiment with phosphate buffer slain (PBS) at the day 1 and 14 of the experiment.

Group 2

OVA group consist of four rabbits injected (i.p) twice of the experiment with OVA 0.1 mg

and 10 mg of aluminum hydroxide in 2 ml of PBS by immunization at day 1 of the experiment and challenge dose at day 14, and then all animals were euthanized on day 15 of the experiment.

Group 3

Prednisolone group consist of four rabbits injected (i.p) twice / experiment with OVA 0.1 mg and 10 mg of aluminum hydroxide in 2 ml of PBS by immunization at day 1 of the experiment and challenge dose at day 14, and treated begin from day 23 to 30 orally with 1mg/kg prednisolone, and then all animals were euthanized on day 31 of the experiment.

Treated Groups

Teucrium polium extract groups consist of four rabbits of each group injected (i.p) twice of the experiment with OVA 0.1 mg and 10 mg of aluminum hydroxide in 2 ml of PBS by immunization at day 1 of the experiment and challenge dose at day 14, and treated begin from day 23 to 30 orally with 4 mg/kg, 8mg/kg, 12mg/kg respectively and then all animals were euthanized on day 31 of the experiment. Blood samples were collected from all control and treated groups in the day 15 of the experiment for evaluation the effects of the OVA. Also, it obtained on day 30 after 1 hour from the last treatment dose.

Results and Discussion

Effect Received of Prednisolone on Neutrophils Estimation for Each of Control, Asthma Induce and Treated Rabbits

Distinct elevation of neutrophils in a significant manner ($P \leq 0.05$) in asthma induce rabbits (47 ± 1.414) % in compared with neutrophils of control (healthy) rabbits (32.75 ± 1.708) % as shown in Figure (1). Evidence show that neutrophils estimation was reduced. However, was remain significantly increase ($P \leq 0.05$) (43.5 ± 3.109) % in compared with control (healthy) rabbits.

Effects Received of *Teucrium polium* Extract Plant on neutrophils Estimation in Asthma Induce Rabbits

Various doses of *Teucrium polium* extraction as in figure (1) showed that significantly decrease ($P \leq 0.05$) in neutrophils measurement in asthma induce rabbits after receiving a dose of 4 mg/kg body weight of *Teucrium polium* extract (32.5 ± 291) % which

begin when asthma is induced and persist for 8 days after the immunization program of the experiment in compared with control (32.75±1.708) % (healthy) rabbits. The figure (1) show also, a significant decrease ($P \leq 0.05$) in neutrophils measurements through the

treatment with the second and third dose of (8 and 12 mg/kg body weight) of *Teucrium polium* extract (33±0.816) % and (30±4.082) % respectively in compared with control (healthy) rabbits.

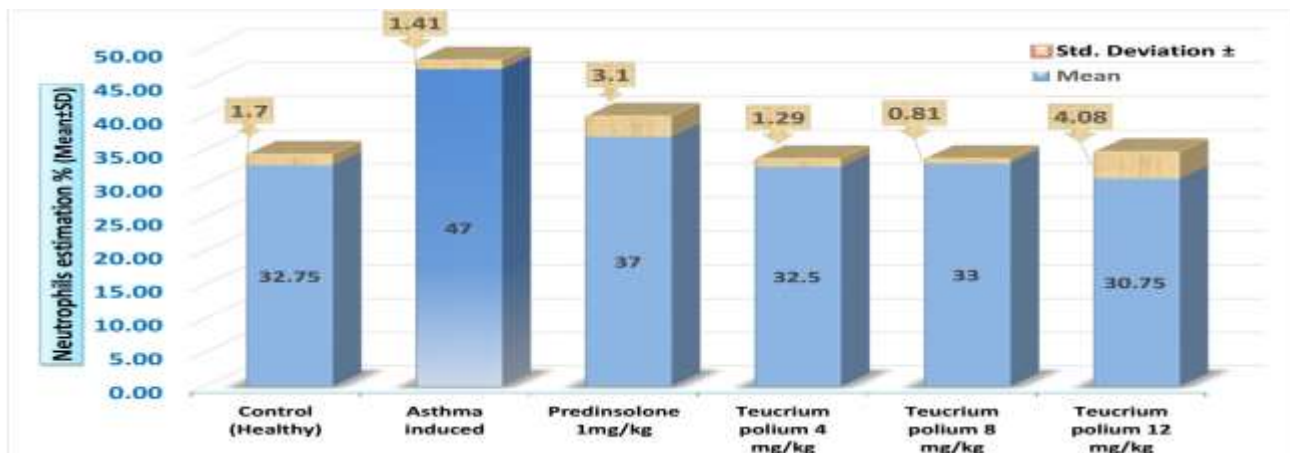


Figure 1: A comparison effect of prednisolone and *Teucrium polium* on the neutrophils estimation (Mean ± SD) in asthma induce and control rabbits

Effect Received of Prednisolone on Eosinophils Estimation for Each of control, Asthma Induce, and Treated Rabbits

The result in the table (2) is mostly associated with increase significant ($P \leq 0.05$) of eosinophils measurement in asthma induce rabbits (8.50 ± 0.577) % in compare with eosinophils measurement of control (healthy) rabbits (2 ± 0.816) % as it was shown in the figure (2). Also, that figure (2) illustrate a significant decrease ($P \leq 0.05$) in eosinophils measurement (1.75±0.500) % in the treated group that received 1mg/kg body weight of Prednisolone persist for eight days after the immunization program.

Various doses of *Teucrium polium* extraction as in figure (2) showed that significantly decrease ($P \leq 0.05$) in eosinophils measurement in asthma induce rabbits after receiving a dose of 4 mg/kg body weight of *Teucrium polium* extract (2.50±0.577) % which begin after present of asthma and persist for 8 days after the immunization program of the experiment in compared with control (2±0.816) 0% (healthy) rabbits. Meantime, figure (2) show also, significantly decrease ($P \leq 0.05$) in eosinophils measurements through the treatment with the second and third dose of (8 and 12 mg/kg body weight) of *Teucrium polium* extract (2±0.34) % and (1.5±0.577) % respectively in compared with control (2±0.816) % healthy rabbits.

Effects Received of *Teucrium polium* Plant Extract on Eosinophils Estimation for Each of Control, Asthma Induce and Treated Rabbits

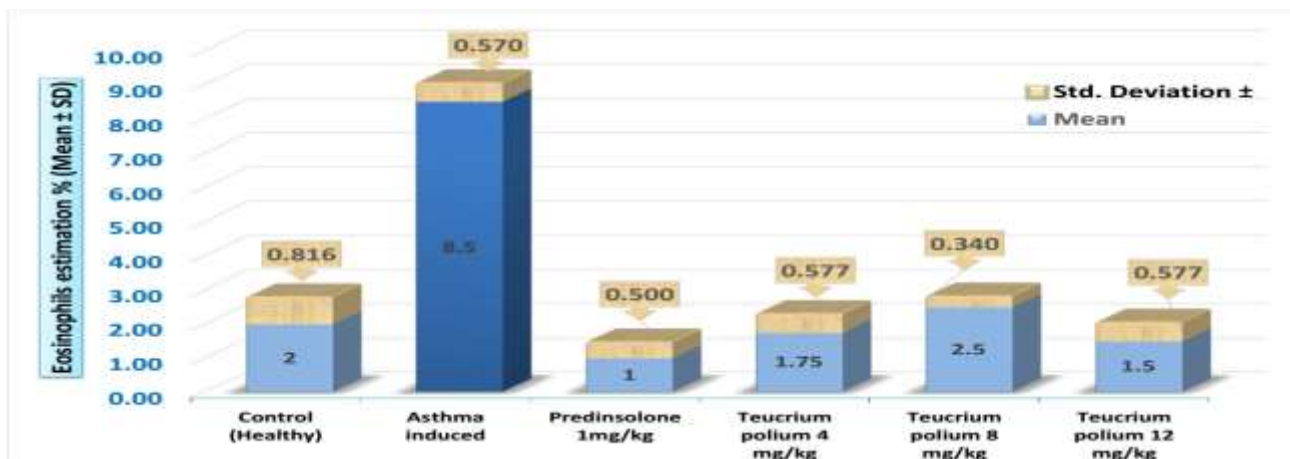


Figure 2: A comparison effect prednisolone and *Teucrium polium* on the Eosinophils estimation (Mean ± SD) in asthma induces, and control rabbits

Effect Received of prednisolone on Immunoglobulin E Level in Asthma Induce Rabbits

Result present in figure (3) show a significant increase ($P \leq 0.05$) in the levels of immunoglobulin E as a reflect of ovalbumin induce asthma in rabbits (374.50 ± 28.92) $\mu\text{g/ml}$ compared with the healthy (control) rabbits (47.90 ± 5.49) $\mu\text{g/ml}$. Meanwhile, the figure below shows significantly decrease ($P \leq 0.05$) in the levels of IgE (50.20 ± 3.17) $\mu\text{g/ml}$ on asthma rabbits.

Effects Received of *Teucrium polium* Plant Extract on Immunoglobulin E Level in Asthma Induce Rabbits

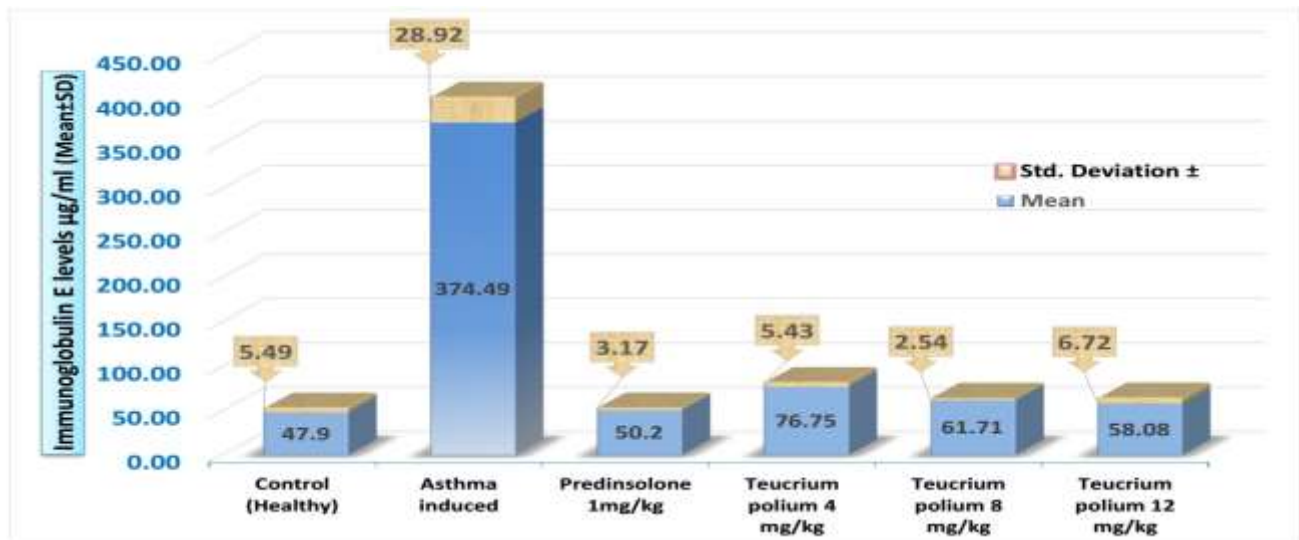


Figure 3: A comparison effect prednisolone and *Teucrium polium* on the levels of Immunoglobulin E levels (Mean \pm SD) in asthma induce and control rabbits

Effect Receiving of prednisolone on Interleukin-4 Levels in Each of Control (Healthy) and Asthma Induce Rabbits

The present result in the table (4) show that Interleukin-4 levels were increased significantly ($P \leq 0.05$) in asthma induce rabbits with ovalbumin injection (415.03 ± 44.88) pg/ml compared with healthy rabbits (42.173 ± 3.09) pg/ml . Whereas, the figure show a significant decrease ($P \leq 0.05$) in the levels of Interleukin-4 (54.92 ± 1.58) pg/ml when that asthma induce rabbits receiving prednisolone (treated) daily at a dose of 1 mg/kg of body weight.

Effects Received of *Teucrium polium* Plant Extract on the Interleukin-4 level in Each of Control (Healthy) and Asthma Induce Rabbits

Teucrium polium show a clear decrement in the levels of interleukin-4, but it does not

A valuable obtained data in the figure (3) showed asthma induce rabbits when treated with three different doses of *Teucrium polium* extract. Data showed a decrease in the level of Immunoglobulin E (76.753 ± 5.43) $\mu\text{g/ml}$ with the first extracted dose of *Teucrium polium* (4 mg/kg body weight). While, the other two doses of the extract of (8 and 12 mg/kg) were reveal significant decrease ($P \leq 0.05$) on the level of Immunoglobulin E (61.718 ± 2.54) $\mu\text{g/ml}$ and (58.083 ± 6.72) $\mu\text{g/ml}$ respectively in comparison with control levels of the immunoglobulin E (47.90 ± 5.49) $\mu\text{g/ml}$.

reach to significant value ($P \geq 0.05$) in inducing asthma rabbits (131.99 ± 9.41) pg/ml through the received a dose of 4 mg/kg body weight of *Teucrium polium* extract for eight days. While, after received treatment with a dose of 8 mg/kg of body weight of *Teucrium polium* extract for 8 days was shown a significant decrease ($P \leq 0.05$) in interleukin-4 levels (56.348 ± 5.73) pg/ml , also after treatment with a dose of 12 mg/kg body weight of *Teucrium polium* extract for 8 days a significant decrease ($P \leq 0.05$) was shown in interleukin-4 levels (38.051 ± 1.12) pg/ml which may give precise indication of the effectiveness of *Teucrium polium* extract in reduction of asthma. However, statistically the two values of 8 and 12 mg/kg body weight *Teucrium polium* extract compare with prednisolone were significant as shown in Figure (4).

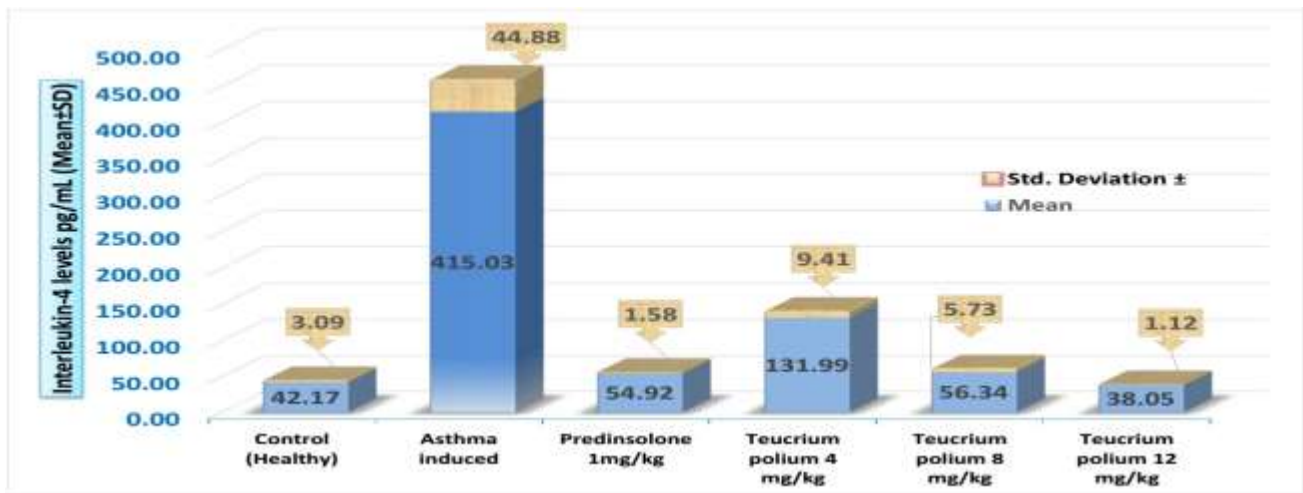


Figure 4: A comparison effect of prednisolone and *Teucrium polium* on the levels of Interleukin-4 (Mean \pm SD) in asthma induce and control rabbits

The aim of the present study was to investigate the effect of *Teucrium polium* as an agent to relieve the asthma-related hematological and immunological changes that occur in the rabbit airway following OVA-induced allergy. The effects of *Teucrium polium* on inflammatory cell response. Although many studies have used mice as a model, we used the rabbit as our model system to imitate human chronic lung disease because it is phylogenetically closer to humans and offers a better understanding of lung structure than smaller animal models [9]. This study agreed with (Kennedy *et al.*, 2000), about the glucocorticoid treatment leads to increase in neutrophils and decrease in eosinophils, lymphocyte, and basophils, that such results were in matched with present obtained issue, in addition to associated immunosuppression, also leaves patients susceptible to invasive diseases and fungal infections [10].

The outcomes data were showed a significant increase in neutrophils and eosinophils measurements and extend their role in the expression of asthma symptoms by the release of inflammatory mediators of cytokines, chemokines that reflect the use of OVA to play finally a major role in distinctive allergy and asthma signs [9].

However, it also showed a significant decrease in the eosinophil that estimated in treated groups and not has demargination effect on the marginal neutrophils; all this contribute to the inhibitory effect of flavonoids of *Teucrium polium* plant extract in the present study. Flavonoids that play the main role in inhibitions of β -hexosaminidase release from mast cells and to suppress cysteinyl leukotriene synthesis through

inhibition of phospholipase A (PLA2) and 5-lipoxygenase. As for the suppressive effect of flavonoids on cytokine expression. The intracellular calcium is the critical functional compartments in the degranulation of mast cells. Calcium movements across the membranes of mast cells represent a major target for effective anti-allergic drugs, as these are main events that link stimulation to secretion. ADP-ribosylates G-protein binding protein modifies the transduction pathways modulating intracellular calcium. Other results showed an attenuation of intracellular calcium in mast cells with flavonoids treatment is well consistent with other reports [11], [12], [13], [14]. According to these observations, we speculate that decreased intracellular calcium might be involved in the inhibitory effect of flavonoids on histamine release, and flavonoids might have membrane stabilizing activity through G-protein [15].

Immunoglobulin E (IgE)-mediated sensitization to domestic inhalant allergens, such as dust mites, cockroaches, and pets are the most important risk factor for asthma, particularly in children [14]. IgE-mediated immune responses consist of a sensitization phase and an effector phase, both of which have been shown to be affected by the anti-allergic properties possessed by flavonoids.

Fentress and Gomperts first identified the inhibition by flavones of transport ATPase on histamine secretion from mast cells [15], which was followed by the discovery of the inhibitory effect of quercetin on allergen-stimulated human basophils [16,17]. The inhibitory activity of flavonoids on IL-4 and CD40 ligand expression was mediated by their suppressive action on transcriptional

factors, such as activator protein 1 (AP-1) and the nuclear factor of activated T-cells (NFAT) [18, 19]. For the differentiation of B-cells into IgE producing cells, both the interaction of the CD40 ligand with CD40 and the effect of IL-4 or IL-13 on B cells are required [12], so that the inhibitory properties of flavonoids, such as luteolin,

apigenin, and fisetin, indicate that they are potentially natural IgE inhibitors. Apart from histamine release by degranulation, mast cells play differential roles in the allergic inflammation by introducing and orchestrating immune responses by the release of cytokines and chemokines through differential intracellular signaling pathways.

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