The effect of allergenicity of *Artemisia aucheri* flowering taps in guinea pigs

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**ABSTRACT:** Allergic rhinitis or hay fever, is the most frequently occurring airway disease of our time. Therefore, the aim of this study was to evaluate the allergenicity effect of *Artemisia aucheri* extract. In this research, flowers of *Artemisia aucheri* were collected from Isfahan around, Iran in September 2012. Flowers were extracted using phosphate-buffered saline, PH 7.4. Three concentration of extracts (5%, 10%, 15%) were prepared. The experiments were intraperitoneally injection of the extracts 3 times, once every 10 days. The flowering taps allergenicity were detected using subcutaneous, skin test, serological and clinical tests. Tests were done on Hartley male guinea pigs. In treated and control groups the appearance of wheal, their diameter, serum IgE, eosinophilia, neutrophilia were compared. Means of triplicate measurements and standard errors were determined for each sample. Dates were analyzed using ANOVA test in the p <0.05. During the skin prick test, the maximum allergenic sensitivity was observed for %15 extracts, with an average wheal diameter of about 2.5 cm. The numbers of eosinophils and neutrophils were increased in the treated animals with extracts comparing control groups. The serum of treated animals with phosphate-buffered saline extracts contained more IgE than serum of control animals. Our results confirm that *Artemisia aucheri* is another allergenic plant. The confirmation of these aspects would facilitate the preparation of an effective extract, improving the diagnosis of the allergy to the *Artemisia aucheri*.

**Key words:** Allergicity, *Artemisia aucheri*, Eosinophil, IgE.

**INTRODUCTION**

The allergy, is the series of events which occurs when an antigen, causes an immune response, leading to symptoms and disease. An antigen that induces the allergic response is called an allergen. The allergic response is appeared on exposure to a harmless allergen is the result of a complex interaction of various immune cells and immunoglobulins (Ig) (Rezanejad and Majd, 2011). The allergy is mainly due to respiration of allergenic proteins from pollen, house dust or animal scurf. IgE is produced during an allergic response. It occurs when allergens bound with IgE receptors in the mucosa of sensitive humans. This leads to the release of inflammatory mediators from the effector cells (mast cells, basophils, dendritic cells, eosinophils) that cause the symptoms of allergic (Diethart et al., 2007).

Plants produce the microscopic grains called pollen. Pollen grains are male gametophytes which carry the male cells. Their major role is in sexual reproductive cycles in the plant world (Majd and Ghanati,1995). Pollen grains are of important allergic factors and 80-90 % of allergens have plant origin. Also, the allergic diseases are a important problem for the community (Amjad and Majd, 2009).

The genus *Artemisia* is the largest genus of the Asteraceae family, with more than 500 species. It is widely distributed mainly across the Northern Hemisphere. 34 species of the genus *Artemisia* are found in Iran (Pellicer et al., 2011; Abad et al., 2012). *Artemisia aucheri* is an aromatic plant from Asteraceae family, an native plant that distributed in Iran. In traditional medicine, this plant was used as astrigent, antiseptic, antiparasitic, antipoisoning, appetizers, stimulants and reduces the rheumatic pains. There are some researchs on chemical composition of essential oil from aerial parts of *A. aucheri*, 1.8-cineole (22.8%), chrysanthenone (18.16%), α-pinene (8.33%) and mesithylene (7.4%) were the major components. Also, the *A. aucheri* extract reduces of TG, TC, LDL cholesterol levels and increased HDL-cholesterol level. Also, the essential oil of *A. aucheri* have been antimicrobial and anti-
malarial activity (Mahboubi and Ghazian Bidgolz., 2009). According to 80-90% allergens have plant origin, the aim of this research was to study the allergenicity effect of *Artemisia aucheri* flowering taps extract.

**MATERIAL AND METHODS**

Collection of plants: The flowers of *Artemisia aucheri* were collected in west of Isfahan province (Kashan), Iran, in September 2012. The voucher specimen was deposited at the herbarium of the Research-Institute of Isfahan Forests and Rangelands.

Preparation of extract: The flowers of *Artemisia aucheri* were air-dried under shade. Flowering taps were extracted using phosphate-buffered saline, PH 7.4. Three concentration of flowering taps extracts (5%, 10%, 15% w/v) were prepared. The resulting was mixedtured on a shaker for 24h and finally was centrifuged in 10000 g for 24h (Ghaemi et al., 2010).

Animals: The allergenicity experiments were done on male guinea pigs (Hartley strain, 4-6 week-old, 350-500g weight). They were obtained from the Iran Pastor Institute. The protocol in this study were carried out in accordance with the institutional scientific procedures for animals and was approved by the Institutional animal care and use committee. In the present study, a total of 36 guinea pigs were used. They were divided in to 6 groups of 6 animals each. The animals were maintained under 25±2°C temperature. Animals were sensitzed and treated by injection of 75µl flowering tap extract. Treatments were repeated three times, for 30 days, once every 10 days (Sharif-Shooshtari et al., 2007).

Skin test and clinical and serological tests: In this research the flowering taps allergenicity were detected using subcutaneous, serological and clinical tests. Each animal was tested with 75µl of the flowering tap total extract diluted with phosphate-buffered saline, pH 7.4. The negative control was buffered saline. Skin reactions were read 72h from the beginning of the test and quantified on the basis of wheal diameter (Chehregani et al., 2004) .

Blood samples were drawn directly from the animals heart (Aberg, 1989). After treatment with flowering tap extracts, blood was obtained directly from the heart. Sera from all samples were stored at -20°C until analyzed. In clinical tests, the numbers of eosinophils, neutrophils and IgE were assayed (Chehregani and Kouhkan, 2008; Irian et al., 2012).

Statistical analysis: Collected data were analyzed using one way ANOVA in SPSS. Duncan’s test was used. All data were reported as mean±SD. P-value < 0.05 indicates significant difference.

**RESULTS AND DISCUSSION**

Results of the skin test in guinea pigs are summarized in Table 1. Flowering taps extract had an allergenic effect. During the skin test, the maximum allergenic sensitivity was observed for 15% extract, with an average wheal diameter of about 2.5 cm. There are significant changes of allergenicity effects between treatment and control groups (P<0.05).

Results of blood smears from different concentrations are summarized in Table 2. The numbers of eosinophils, neutrophils were increased in the animals treated with flowering taps extracts (Fig 1,2). Data for determination of IgE in different concentrations are given in Table 2 and Fig. 3. All flowering tap extracts caused an increase in IgE levels in the blood of treated animals. Serum of animals treated with 15% extract contained more IgE than serum of control animals. The results indicate that the amount of IgE increased significantly in the animals treated with 15% extract. Statistical analysis indicates that differences between 15% and control are significant (P< 0.001).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wheat diameter (cm)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>5% extract</td>
<td>2.2 ± 0.78</td>
</tr>
<tr>
<td>10% extract</td>
<td>1.5± 0.23</td>
</tr>
<tr>
<td>15% extract</td>
<td>2.5 ± 0.89</td>
</tr>
</tbody>
</table>

Table 1. Results of skin test for different concentrations of *Artemisia aucheri* flowering taps extract: X±SD, (p<0.05)
Table 2. Results of serological studies, determination of numbers of eosinophils and neutrophils and evaluation of blood IgE in different experimental and control animals

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5% extract</th>
<th>10% extract</th>
<th>15% extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eosinophils</strong></td>
<td>0.001 ± 0.001</td>
<td>0.33 ± 0.51</td>
<td>0.001 ± 0.0001</td>
<td>1 ± 1.095</td>
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<tr>
<td>((\times 10^4) cells/ml blood)</td>
<td></td>
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<tr>
<td><strong>Neutrophils</strong></td>
<td>2.170 ± 0.408</td>
<td>7 ± 1.89</td>
<td>5 ± 1.09</td>
<td>5.50 ± 1.64</td>
</tr>
<tr>
<td>((\times 10^4) cells/ml blood)</td>
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<tr>
<td><strong>IgE</strong></td>
<td>0.256 ± 0.005</td>
<td>0.22 ± 0.13</td>
<td>0.1 ± 0.00001</td>
<td>0.39 ± 0.00001</td>
</tr>
<tr>
<td>(IU/ml)</td>
<td></td>
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Eosinophilia in the peripheral blood of different experimental groups and control group

![Eosinophilia](image1)

*Figure 1. Eosinophilia (\(\times 10^4\) cells/ml blood) in the peripheral blood of guinea pigs treated with different extracts and phosphate-buffered saline. 5% extract caused an increase in eosinophils characteristic of allergic, but 15% extract was more effective (P<0.05).*

Neutrophilia in the peripheral blood of different experimental groups and control group

![Neutrophilia](image2)

*Figure 2. Neutrophilia (\(\times 10^4\) cells/ml blood) in the peripheral blood of guinea pigs treated with different extracts and phosphate-buffered saline. 10% and 15% extracts caused an increase in eosinophils characteristic of allergic, but 5% extract was more effective (P<0.001).*
Increasing of total blood IgE in animals treated with different extracts and control group.

![Graph showing the increase of IgE in blood of animals treated with different extracts.](image)

Figure 3. Amount of IgE (IU/ml) in blood of animals treated with different concentrations extract and phosphate-buffered saline. Result showed that both extracts induce an increase in blood IgE as allergy inducers, but 15% extract with more effective (P<0.001).

According to the results in this study, the numbers of eosinophils, neutrophils and amount of IgE were increased in the treated animals with phosphate-buffered saline extracts, so that, Jaggi and Gangal with study on pollen extracts of *Artemisia scoparia* were concluded that amount of IgE in all patients was increased (Jaggi and Gangal, 1987). In the other research, with study on eleven species of *Artemisia* were observed that all species tested had significant levels of group 1 allergens which was associated with the ability bind to IgE (Plunkett and Jimeno, 2006). Pastorello et al. (2002) also were concluded in IgE immunoblotting the 10 patients without seasonal respiratory symptoms reacted only to a 9-kd allergen of Mugwort (*Artemisia vulgaris*) (Pastorello, 2002). The other researcher were observed that Mugwort pollen contain a number of cross-reactive allergens, among the major allergen Art v 1. In addition Mugwort pollen extract induced histamine release and reduction of nasal air flow in a patient with IgE reactivity against the Mugwort major allergen Art v 1 (Hirschwehr et al., 1998). Whereas Kim et al. (2005) with the research on *Artemisia iwayomogi* were observed that, this plant decreased IgE reaction and dose dependently attenuated histamine release by compound 48/80 or IgE. Compound 48/80 (8 mg/kg BW) was used for induction of a systemic fatal allergic reaction (Kim et al., 2005). Other researchers were studied the *Artemisia annua* leaves and stems extracts in 52 subjects sensitive to *Artemisia* pollen, 92.3% gave positive responses in skin prick tests, 100% gave positive responses in intradermal tests, 66.7% gave positive responses in intranasal challenge and 59.3% gave positive responses in bronchial provocation tests also the rate of IgE was increased (Leng and Tai, 1987). Stach et al. (2007) with study on *A.vulgaris, A. campestris* and *A.absinthium* were concluded 12% of patients had a positive skin prick tests reaction to *Artemisia* species. Their symptoms were rhinitis and conjunctivitis (15%), atopic dermatitis (15%), chronic urticaria (14.3%), bronchial asthma (2.4%), facial and disseminated dermatitis (1.3%). Also the IgE concentrations were detected in the sera of 10.1% of patients (Stach et al., 2007).

According to the results of the present study, the flowering taps of *Artemisia aucheri* have allergenicity effects and it is a delayed type hypersensitivity. Therefore we attribute the sensitivity of phosphate-buffered saline 15% extracts to presence of allergenicity proteins.

**ACKNOWLEDGEMENT**

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**REFERENCES**