Immunomodulatory activity of aqueous extract of *Achillea wilhelmsii* C. Koch in mice

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Immunomodulatory activity of aqueous extract of *Achillea wilhelmsii* (25, 50 and 100 mg/kg body weight for 5 days) was evaluated on body weight, relative organ weight, delayed type of hypersensitivity (DTH) response and haemagglutination titre (HT) in female Swiss albino mice. No significant body weight gain differences were recorded in various groups of animals. Significant increase in relative organ weight of spleen at 100 mg/kg was observed. No elevation in the levels of liver function test (LFT) enzymes and kidney relative weight was observed in tested doses of the plant. The extract of *A. wilhelmsii* elicited a significant increase in the DTH response at the dose of 100 mg/kg. In the HT test, plant extract showed stimulatory effect in all doses, however this changes were significant at 50 mg/kg. No mortality was occurred in tested doses. Overall, *A. wilhelmsii* showed a stimulatory effect on both humoral and cellular immune functions in mice.

Keywords: *Achillea wilhelmsii*, DTH, Haemagglutination titre, Immunity response, Toxicity

The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases. Modulation of the immune responses to alleviate the diseases has been of interest for many years. Medicinal plants are a rich source of substances which are claimed to induce paraimmunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades. Medicinal plants serve as therapeutic alternatives, safer choices, or in some cases, as the only effective treatment. A large number of these plants and their isolated constituents have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects. Some of the plants with established immunomodulatory activity are *Viscum album*, *Panax ginseng*, *Asparagus racemosus*, *Azadirachta indica*, *Tinospora cordifolia*, *Polygala senega*, and *Ocimum santum*. *Achillea wilhelmsii* C. Koch (Asteraceae), locally known as “Boomadaran” (Lavender cotton), is widely found in different parts of Iran. Top flowerings of the plant are used as anti-flatulence, spasmodytic, antinociceptive and diuretic. *A. wilhelmsii* contains volatile oils, flavonoids, terpenoids, alkaloids, saponins and sesquiterpen lactones. Antihypertensive and hypolipidemic effects of this plant have already been reported. However, there is no scientific data on the in vivo immunomodulatory activity of this plant. Therefore the present study has been undertaken to explore the immunomodulatory activity of various doses of aqueous extract of *A. wilhelmsii* top flowerings in animal models.

Materials and Methods

Plant extract — The plant was collected from Bidkhoon, Kerman during July 2006. The plant was identified and authenticated by Dr. Mirtajaldini, Bahonar University, Kerman, Iran. A voucher specimen of the plant materials (KP1155) was deposited at the Herbarium of Department of Pharmacognosy of School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. Aqueous extract of the top flowerings of *A. wilhelmsii* was obtained with maceration method and freeze dried. The extracts with suitable adsorbent were stored in the refrigerator until the time of experiments.
Animals — Swiss albino female mice (20 - 25 g) were bred and maintained under standard laboratory conditions (25° ± 2 °C; 12:12 h (photoperiod). Commercial pellet diet and water were given ad libitum. All experiments performed were in compliance with the Kerman University of Medical Sciences, Animal Experimentation Ethics Committee (AEESC) guidelines that follow Iran government regulations.

Dosage — The plant extract was suspended in normal saline and was administered i.p for 5 days at doses of 25, 50 and 100 mg/kg body weight. The dose volume was 0.2 ml. Control animals received the same volume of normal saline.

Body weight, lymphoid organ weight — Animals were divided into four groups (I–IV). Each group comprised of a minimum of six animals. Group I (control) received normal saline; groups II- IV received plant extract in 25, 50 and 100 mg/kg body weight dose respectively. The animals were humanized 24 h after the last dose. Body weight gain (percentage) and relative organ weight (organ weight/100 g of body weight) of kidney, liver and spleen were determined for each animal.

Assessment of humoral immune functions — Animals of all the groups treated in the above-described manner were challenged with 0.2 ml of 10% sheep red blood cells (SRBC), i.p on the 10th day of initiation of experiment and the haemagglutinin titre was studied as per Bin-Hafeez et al.17.

Haemagglutinin titre (HT) assay — Haemagglutinin titre assay was performed using the procedure of Bin-Hafeez et al.17. On the fifth day after immunization, animal was anesthesied and the blood was collected from cardiac of each mouse for serum preparation. Serum was diluted in 50 μl PBS (pH 7.2) with twofold serial dilution in 96-well microtitre plates and mixed with 50 μl of 1% SRBC suspension in PBS. Plates were kept at room temperature for 2 h. The value of antibody titre was considered the highest serum dilution showing visible aemagglutination.

Delayed type of hypersensitivity response — The DTH response was determined using the method of Raisuddin et al.18 with some modifications. On the day of termination of the treatment, animals were immunized with 1×10⁹ SRBC, subcutaneously. On the next day, the animals were again received 1×10⁹ cells in the left hind footpad by injection. The same volume of normal saline was injected to the right footpad as trauma control for nonspecific swelling. Increase in footpad thickness was measured 24 h after the challenge using dial caliper. Dexamethasone (0.2 mg/kg/day for 5 days) was used as positive group.

Liver function and blood parameters tests — Activities of serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and blood parameters (RBC, WBC and Hb) were estimated using the kits (Span Diagnostics, Surat, India). For this purpose, four groups of animals (one control + three treatments) as described above were used and treated for 5 days with respective doses of plant extract. Different blood parameters (RBC, WBC, Hb) were determined in four groups of animals (one control+ three treatments) as described above.

Statistical analysis — Statistical analysis was performed using one-way ANOVA, followed by Tukey’s test. The significance in difference was accepted at P <0.05. The values are expressed as means±S.E.

Results

Effect of the plant extract on body weight and lymphoid organ weight — None of the studied doses of A. wilhelmsii extract showed toxicity or mortality in the extract-treated animals. No significant difference in the body weight gain were recorded in various groups of animals. The extract did not alter the relative weight of kidney and liver in tested dose, however a significant increase was observed in the relative weight of spleen in group IV (P<0.05) (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Relative organ weight (g)</th>
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<tbody>
<tr>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td>A. wilhelmsii extract (25 mg/kg)</td>
<td>0.5 ± 0.09</td>
</tr>
<tr>
<td>A. wilhelmsii extract (50 mg/kg)</td>
<td>0.51± 0.08</td>
</tr>
<tr>
<td>A. wilhelmsii extract (100 mg/kg)</td>
<td>0.65± 0.16*</td>
</tr>
<tr>
<td>Control</td>
<td>0.37 ± 0.07</td>
</tr>
</tbody>
</table>

* P<0.05 when compared with control

Table 1 — Effect of different doses of aqueous extract of A. wilhelmsii on the relative organ weight (g) of mice. [Values are mean ± SE from 6 mice in each group].
Effect of plant extract on humoral immunity parameters — In haemmaglutination test, doses of 25, 50 and 100 mg/kg showed titre values of 1:4.0, 1:5.1 and 1:4.0, respectively, while the titre value of control was 1:2.0. The increase in the titre values was significant ($P<0.05$) in 50 mg/kg.

Effect of plant extract on cell-mediated immunity parameters (DTH) — Plant extract at dose of 100 mg/kg elicited a significant increase in DTH response in comparison to control animals ($P<0.05$) (Fig.1). In this experiment, dexamethasone has decreased DTH response significantly in comparison to control ($P<0.05$).

Effect of plant extract on liver enzymes and blood parameters — There was no significant elevation in the levels of SGOT and SGPT as a result of treatment with *A. wilhelmsii* at any of the doses used in this study ($P<0.05$) (Table 2). No significant differences in blood parameters and WBC were recorded between test groups, however a significant decrease in RBC and Hb were observed ($P<0.05$) (Table 2).

Discussion

Boomadarn has primarily been described as an anti-spasmodic and anti flatulence herb in traditional medicine. This plant has been suggested for treatment of hypertension. In the present study, Boomadaran showed an overall stimulatory effect on the immune functions in mice. Stimulatory effects were observed on both humoral and cellular immunity. In HT test, the plant showed an increase response in all doses, but this increase was significant only in dose 50mg/kg. This activity could be due to the presence of flavonoids or saponins which augment the humoral response, by stimulating the macrophages and B-lymphocytes subsets involved in antibody synthesis. It appears that 50 mg/kg is the optimum dose in mice in humoral immunity. An increase in dose might have induced down-regulation of immune functions. Estimation of the LFT enzymes did not reflect any toxicity which was concomitant with any significant increase in relative weight of liver. All doses of the plant decreased the SGPT, however this difference was significant in 100 mg/kg dose which may be due to the hepatoprotective effect of the plant. Results of the present study also revealed no significant difference in the blood parameters. In DTH test, the DTH response, which directly correlates with cell-mediated immunity (CMI), was found to be the highest at the maximum dose (100 mg/kg) tested in the extract. The mechanism behind this elevated DTH during the CMI responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lymphokines, attracting more scavenger cells to the site of reaction. An increase in DTH response indicates a stimulatory effect of the plant which has occurred on the lymphocytes and accessory cell types required for the

![Fig. 1— The effects of various doses of aqueous extract of *A. wilhelmsii* on delayed type hypersensitivity (DTH) response in mice immunized with sheep red blood cells in comparison to dexamethasone and control [values are mean ± SE of footpad thickness from 6 mice in each group. * $P<0.05$ when compared with the control animals]](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>WBC ($10^3$/mm$^3$)</th>
<th>Hb (g/dL)</th>
<th>RBC ($10^6$/mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><em>A. wilhelmsii</em> extract (25 mg/kg)</td>
<td>50.2 ± 17.4</td>
<td>141.7 ± 39</td>
<td>4.8 ± 1.0</td>
<td>11.8 ± 1.4*</td>
<td>7.8 ± 0.6*</td>
</tr>
<tr>
<td>II</td>
<td><em>A. wilhelmsii</em> extract (50 mg/kg)</td>
<td>44.6 ± 25.6</td>
<td>115.0 ± 29.2</td>
<td>3.3 ± 1.1</td>
<td>13.8 ± 0.7</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>III</td>
<td><em>A. wilhelmsii</em> extract (100 mg/kg)</td>
<td>42.9 ± 25*</td>
<td>107.8 ± 25.5</td>
<td>4.6 ± 2.4</td>
<td>13.7 ± 1.3</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>IV</td>
<td>Control</td>
<td>72.8 ± 17</td>
<td>106.6 ± 20</td>
<td>4.9 ± 0.6</td>
<td>15.0 ± 1.6</td>
<td>9.7 ± 1.3</td>
</tr>
</tbody>
</table>

* $P<0.05$ when compared with control animals (significantly different)
expression of this reaction. The main chemical constituents of *A. wilhelmsii* are flavonoids, terpenoids, saponins and some alkaloids. Recent reports indicate that several types of flavonols stimulate human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T cells, cytokines, interleukin 2, g-interferon and macrophages and are thereby useful in the treatment of several diseases caused by immune dysfunction. The immunosuppressive effects of the flavonoids and saponins have been reported. Boomadran has stimulated both humoral as well as cellular arms of immune system. This plant also is rich source of terpenoids which may act as immunostimulatory. Another species, *A. taleagonica* has shown a suppression effect on humoral immunity system of animal. Findings of the present study showed an overall stimulatory effect of *A. wilhelmsii* flowers extract on both humoral and cellular immunity in mice.

Acknowledgement

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References