

Haussknechtia Elymatica: A Plant with Immunomodulatory Effects

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ABSTRACT

Background: Plant extracts have been widely investigated for possible immunomodulatory properties. **Objective:** To study the immunomodulatory functions of the methanol extract of *Haussknechtia elymatica* (Apioidae), an herb native to south-western Iran. **Methods:** Delayed type hypersensitivity (DTH) skin test and measurement of antibody titer after immunization with Sheep-RBC was performed. [³H]-thymidine incorporation assay on the human lymphocytes stimulated with PHA and determination of IL-2 production using ELISA method was carried out. **Results:** Treatment of mice with increasing concentrations of the extract decreased the footpad thickness indicating a dose-related inhibitory effect of *H. elymatica* on delayed hypersensitivity. The mean antibody titers for all concentrations of the extract at primary and secondary responses were significantly less than the control. Addition of the extract to the culture of human peripheral blood lymphocytes in the presence of mitogen decreased cell proliferation dose-dependently. A dose related decrease in production of IL-2 in extract-treated cells was also observed. **Conclusion:** The decline of antibody titer and DTH response indicates that *H. elymatica*, by acting on the lymphocyte proliferation and IL-2 secretion, inhibits both humoral and cell-mediated immune responses.

Keywords: Immunomodulation, *Haussknechtia elymatica*

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INTRODUCTION

Various immunomodulating compounds work to reduce immune function such as corticosteroids or the non-steroidal anti-inflammatory drugs (1-4). Drugs with more immunosuppressive properties such as cyclosporine are used to treat more severe autoimmune diseases or to suppress graft rejection (5-6). However, a major concern in recommending these drugs for therapeutic purposes is their adverse effects (7-10). Hence, the discovery and identification of safer new components, without severe side effects, has become an important goal of research in the biomedical sciences. There is an ever-increasing interest in research on different plant species to find out their therapeutic applications. In various studies, the immunomodulatory effects of medicinal plants have been investigated (11-15). *Satureja khuzistanica* Jamzad, *Lactuca sativa*, *Artemisia annua*, *Lavandula angustifolia* and *Glaucium grandiflorum* are among the plants that their anti-inflammatory and immunosuppressive effects have been reported from Iran (16-20). In the present study, the immunosuppressive effects of *Hausknechtia elymatica*, another native plant is reported. *H. elymatica* belongs to Apioideae family (21), represented by one species in the flora of Iran which grows in the Zagros Mountains in the southwest of Iran.

MATERIALS AND METHODS

Preparation of the Extract. Aerial parts of the plant were collected from Dena Mountain bordering Fars Province in June. The plant was identified by Dr. A. Jafari (Department of Botany, Center for Research in Natural Resource and Animal Husbandry, Yasuj University, Yasuj, Iran) and a voucher specimen (herbarium No. 5759) was deposited there. A Methanol extract was obtained by maceration of the plant in methanol for 48 h. The extract was filtered and concentrated and yielded 8.0% (w/w). Appropriate concentrations of the extract in RPMI 1640 and/or normal saline were prepared.

Delayed Type Hypersensitivity (DTH) Response. Balb/c mice (Pasteur institute, Tehran, Iran) were divided into 4 groups of 5 mice each. One, fifty or hundred mg extract/kg body weight were injected intraperitoneally to the 1st, 2nd or 3rd group, respectively, on days -2, -1, 0, 1 and 2. The 4th group received normal saline as the control. The animals were immunized with SRBC on day 0. The mice were then challenged by injection of SRBC in right hind foot pad at day 7. The thickness of the foot pad in each mouse after 24 h was taken as a measure of DTH.

Humoral Antibody Response. Five groups of 5 Balb/C mice were immunized with 5×10^9 SRBC on days 0 and +7 (IP). Three groups were injected with 1-100 mg/kg of the extract for five days (IP). The mice in the fourth group were injected with levamisol (2 mg/kg, IP) during the same five days. The fifth group was injected only with normal saline. Blood samples were obtained from each mouse on day +7 for primary response and on day +14 for secondary response. Antibody titers were determined by hemagglutination assay and the mean Log₂ of the titers were determined.

Lymphocyte Proliferation Assay. Peripheral blood lymphocytes (PBL) from healthy individuals were separated and seeded in culture plate wells in triplicate. 10 µg/ml of phytohemagglutinin, PHA (Biotest, Germany) and 1 to 200 µg/ml of the extract were added to respective wells. After 3 days, 0.5 µCi/well of [³H]-Thymidine (Amersham, Germany) was added and the cells were harvested. Using a beta-counter (Pharmacia,

Sweden), counts were read and the stimulation indices (SI) were calculated. Cells treated with PHA and solvent without the extract were considered as negative controls. In the same experiment, culture supernatants were collected after 3 days and IL-2 production was examined by enzyme immunoassay (ELISA, Roche, Germany).

Statistical Analysis. Data were presented as mean \pm SD and the differences between groups were assessed using SPSS software and the Mann-Whitney test. P values less than 0.05 were considered as significant.

RESULTS

The results obtained for DTH reaction in the control and treated groups are shown in Table 1. The diameter of induration of mouse foot pad, showed a significant suppressive change with extract treatment. The overall statistical analysis indicated a suppressive effect at $p < 0.05$. The mean thickness of footpad at 24 h after immunization was 1.3 ± 0.8 mm for treated mice compared to 2.5 ± 0.5 mm for the control showing a significant decrease in delayed hypersensitivity due to exposure of mice to various concentrations of the extract ($p = 0.005$). The effect was dose dependent, as treating of mice with increasing concentrations of the extract decreased the footpad thickness.

Table 1. Effects of *Haussknechtia elymatica* extract on DTH and antibody response in mice. Values are expressed as mean \pm SD. P values were all below 0.05. NT: Non-treated. Positive control; Levamisol (2 mg/kg)

| Dose (mg/kg, IP) | DTH response (mm) | Primary antibody response | Secondary antibody response |
|--------------------|-------------------|---------------------------|-----------------------------|
| NT | 2.5 ± 0.5 | 8.3 ± 0.5 | 9.0 ± 0.4 |
| 1 | 2.2 ± 0.1 | 7.4 ± 0.6 | 7.6 ± 0.5 |
| 50 | 1.2 ± 0.3 | 4.2 ± 0.5 | 5.0 ± 0.7 |
| 100 | 0.6 ± 0.2 | 3.4 ± 0.6 | 3.8 ± 0.4 |
| All concentrations | 1.3 ± 0.8 | | |
| Positive control | - | 10.2 ± 0.5 | 11.2 ± 0.4 |

The mean antibody titer Log₂ for 1 mg/kg of the extract was 7.4 ± 0.6 compared to 8.3 ± 0.5 in non-treated mice at primary response ($p = 0.008$). As the concentration of the extract increased to 50 and 100 μ g/ml, the antibody titer was decreased as well (Table 1). Similar results were obtained for the secondary response. The mean antibody titer Log₂ for 1 mg/kg of the extract was 7.6 ± 0.5 compared to 9 ± 0.4 in non-treated mice at secondary response. The antibody titer was decreased at higher concentrations of the extract, showing a dose-related inhibition of humoral antibody response in extract treated mice.

In order to assess the effect of the extract on lymphocyte proliferation, cells were stimulated with mitogen and then treated with the extract. Results obtained showed an inhibitory effect of *H. elymatica* on human lymphocyte proliferation at concentrations more than 10 μ g/ml ($p < 0.05$, Figure 1). This inhibitory effect was dose dependent that is with an increase in the amount of the extract, the lymphocyte proliferation index decreased (SI range 0.55 - 0.04). The viability of cells determined by trypan blue dye test showed a significant decrease at 200 μ g/ml of the extract.

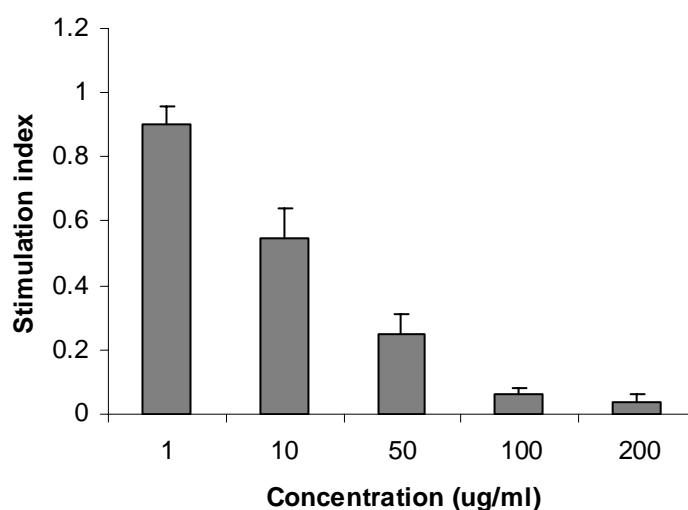


Figure 1. Effect of *Haussknechtia elymatica* on human lymphocyte proliferation in the presence of mitogen. Values represent the mean \pm SD of three experiments.

Production of IL-2 by the extract-treated cells indicated a dose dependant decrease ($p < 0.05$). Cultivated lymphocytes with mitogen and 100 $\mu\text{g/ml}$ of the extract showed nearly 60% decrease in IL-2 production as compared with that of non-treated cells (Table 2).

Table 2. Effect of different concentrations of *H. elymatica* on the secretion of IL-2 by mitogen-induced lymphocytes. Lymphocytes were exposed to suboptimal dose of PHA and different concentrations of the extract. Negative control; extract-untreated cells

| Extract Concentration ($\mu\text{g/ml}$) | IL- 2 Concentration (pg/ml) |
|--|--|
| 1 | 41 \pm 3.5 |
| 50 | 18 \pm 2.8 |
| 100 | 10 \pm 3 |
| Negative control | 32 \pm 2.9 |

DISCUSSION

In the present study the immunomodulatory effects of *H. elymatica* on immune response was investigated with both cell-mediated and antibody-mediated immune response. Results of our study showed that injection of the extract reduced the immune reaction to SRBC as a T cell dependent antigen. This result indicated that the extract could affect cellular components of the local immune reaction including lymphocytes and/or monocytes recruited at the site of interaction (22).

The extract also showed inhibitory effects on the humoral immune response. Injection of *H. elymatica* into mice caused a significant dose dependent decrease in anti-SRBC titer in both primary and secondary immune responses. The inhibition of humoral response to SRBC could be due to suppression of antigen processing and presentation, or might be influenced by relative amounts of different cytokines produced upon T cell and B cell stimulation (23).

The possible inhibitory effect of *H. elymatica* on the PHA-stimulated human peripheral blood lymphocytes was investigated. Studies have shown that PHA acts as a polyclonal activator of T cells. The interaction of T cells with PHA initiates a cascade of biochemical events including gene expression that induces the resting T cells to enter the cell cycle and start proliferation and differentiation (24). This process is then followed by production of IL-2. Our results show that, *H. elymatica* extract significantly inhibits proliferation of human lymphocytes in the presence of suboptimal concentration of PHA. A significant decrease in the production of IL-2 in cultures exposed to the extract was an indication of the inhibitory action of *H. elymatica* on both lymphocyte activation and cytokine production.

It is concluded that *H. elymatica* has the capacity to inhibit delayed hypersensitivity and antibody production in both primary and secondary immune response in mice and also inhibits the proliferation of human lymphocytes which are the predominant cell types modifying the immune system.

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