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Original Article

Immunomodulatory Activity of Aqueous Extract of *Heracleum persicum* Desf. in Mice

Fariba Sharififar[a*], Shirin Pournourmohammadi[b], Moslem Arabnejad[a], Ramin Rastegarianzadeh[a], Omid Ranjbaran[a] and Amin Purhemmaty[c]

[a]*Kerman University of Medical Sciences, Pharmaceutics Research Center, Department of Pharmacognosy, School of Pharmacy, Kerman, Iran.* [b]*Kerman University of Medical Sciences, Department of Toxicology-Pharmacology, School of Pharmacy, Kerman, Iran.* [c]*Bahonar University, Department of Botany, Kerman, Iran.*

Abstract

Studies have demonstrated that plant extracts possess various biological characteristics, including immunomodulatory activity. *Heracleum persicum* Desf. (Apiaceae), a medicinal plant native to Iran, was studied for its immunomodulatory activity**.** Immunomodulatory activity of different doses of an aqueous extract of *H. persicum*, was evaluated in female Swiss albino mice. Mice were treated with three doses (50, 100 and 200 mg/kg body weight) for 5 days. Body weight, relative organ weight, delayed type hypersensitivity (DTH) response and haemagglutination titre (HT) were studied in various groups of animals. No significant body weight gain differences were recorded in various groups of animals. The results obtained show a significant increase ($P < 0.05$) in relative organ weight of spleen and liver, at doses of 50 and 100 mg/kg. No elevation in the levels of liver function test (LFT) enzymes and kidney relative weight was observed with the plant doses examined. The *H. persicum* extract elicited a significant increase ($P < 0.05$) in the DTH response at doses of 100 and 200 mg/kg. In the HT test, the plant extract showed a stimulatory effect at all doses, however these changes were significant at doses of 50 and 100 mg/kg. No mortality occurred with the tested doses. Overall, *H. persicum* showed a stimulatory effect on both humoral and cellular immune functions in mice.

Keywords: *Heracleum persicum*; Immunomodulation; Delayed type hypersensitivity; Haemagglutination titre; Toxicity.

Introduction

The immune system is involved in the etiology, as well as pathophysiologic mechanisms of many diseases. Modulation of the immune responses to alleviate various diseases has been of interest for many years (1). Medicinal plants are a rich source of substances which are claimed to induce paraimmunity,which is the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions (2). Because of the concerns about the side effects of conventional drugs, 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 (3). 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

* Corresponding author:
E-mail:fsharififar@Kmu.ac.ir

anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, and immunomodulatory effects (4-8). Some of the plants with established immunomodulatory activity are *Viscum album*, *Panax ginseng*, *Asparagus racemosus*, *Azadirachta indica*, *Tinospora cordifolia*, *Polygala senega* and *Ocimum santum* (9-11). The present study was therefore undertaken to explore the immunomodulatory activity of an aqueous extract of the fruits of Heracleum persicum Boiss. (Apiaceae), *Heracleum persicum* Boiss (Apiaceae), known as "Golpar", is a large shrub which grows in Iran. Fruits of the plant are used as a condiment and as a constituent of the daily diet of general population in Iran. Its fruits are consumed widely as anti-flatulence and anti-microbial (12). *H. persicum* contains volatile oils, flavonoids and furanucoumarins (13, 14). Anti-convulsant and cytotoxicity of this plant has been reported (15, 16). However, there is no scientific data on the *in-vivo* immunomodulatory activity of the fruits of this plant. The objective of the present investigation was to study the immunomodulatory activity of the various doses of an aqueous extract of *H. persicum* in animal models.

Experimental

Plant extract

The plant was gathered from Lalehzar, Kerman in July 2005. The plant was identified and authenticated by Dr. Mirtajaldini, Bahonar University, Kerman, Iran. A voucher specimen of the plant materials (KP1185) was deposited at the Herbarium of Pharmacognosy Department, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. An aqueous extract of the fruits of *H. persicum* was prepared, using the maceration method and then freeze dried. The extracts with a suitable adsorbent were stored in a refrigerator until (4°C) use.

Animals

Study was conducted in Swiss albino female mice (20-25 g). The animals were bred and maintained under standard laboratory conditions (temperature 25 ± 2°C and lightperiod of 12 h). A commercial pellet diet and water were given ad libitum.

Treatment protocol

The plant extract was suspended in normal saline and administered i.p. for 5 days at doses of 50, 100 and 200 mg/kg body weight. The dose volume was 0.2 mL.The control animal group received the same volume of normal saline and left untreated respectively. Animals were divided into four groups (I–IV). Each group comprised of a minimum of five animals. Group I (control) received normal saline; group II, plant extract 50 mg/kg body weight; group III, plant extract 100 mg/kg; and group IV, plant extract 200 mg/kg. 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 within the experimental groups were challenged with 0.2 mL of 10% sheep red blood cells (SRBC), i.p., on the 10^{th} day of the initiation of experiment. The haemagglutinin titre was also studied in these animals.

Haemagglutinin titre assay

Haemagglutinin titre (HT) assay was performed based on the procedure stated by Bin-Hafeez et al. (17). On the fifth day after immunization, blood was collected from the heart of each mouse for serum preparation. Serial two fold dilution of serum was made in 50 µL of PBS (pH 7.2) in 96-well microtitre plates and mixed with 50 µL of 1% SRBC suspension in PBS. After mixing, plates were kept at room temperature for 2 h. The value of antibody titre was assigned to the highest serum dilution showing visible haemagglutination.

Delayed type hypersensitivity response

The delayed type hypersensitivity (DTH) response was determined using the method of Raisuddin et al. (18). On the day of termination of the treatment with plant extract, animals were immunized with 1×10^9 SRBC, subcutaneously. On the fifth day of immunization, all the animals were again challenged with 1×10^8 cells in the left hind footpad. The right footpad was injected with the same volume of normal saline, served

Table 1. Effect of the aqueous *H. persicum* extract on the relative organ weights of mice.

Group	Relative organ weight (mean ± SE) in grams		
	Spleen	Liver	Kidney
Plant extract (50 mg/kg)	0.5 ± 0.12	5.2 ± 0.4*	1.1 ± 0.1
Plant extract (100 mg/kg)	0.59 ± 0.1*	5.5 ± 0.2*	1.2 ± 0.1
Plant extract (200 mg/kg)	0.76 ± 0.14*	6. 0 ± 0.3*	1.3 ± 0.1*
Control	0.37 ± 0.07	4.6 ± 0.6	1.1 ± 0.2

Values are presented as mean ± SE of six mice.
*$P < 0.05$ considered to be significant difference, when compared with the control animals.

as the trauma control for non-specific swelling. Increase in footpad thickness was measured 24 h after the challenge, using a dial caliper.

Liver function and blood parameters

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

Determination of LD_{50}

An aqueous extract of plant fruits was administered by i.p. injection in increasing doses of 100, 200, 400 and 800 and 1600 mg/kg to six animals in each group. The number of dead animals within 48 h was recorded. LD_{50} was calculated using SPSS software the version 16 (19).

Statistical analysis

Statistical analysis was performed using the one-way ANOVA, followed by Tukey's post-test. The significance in difference was accepted at $P < 0.05$. The results are expressed as means ± SE (standard error).

Results

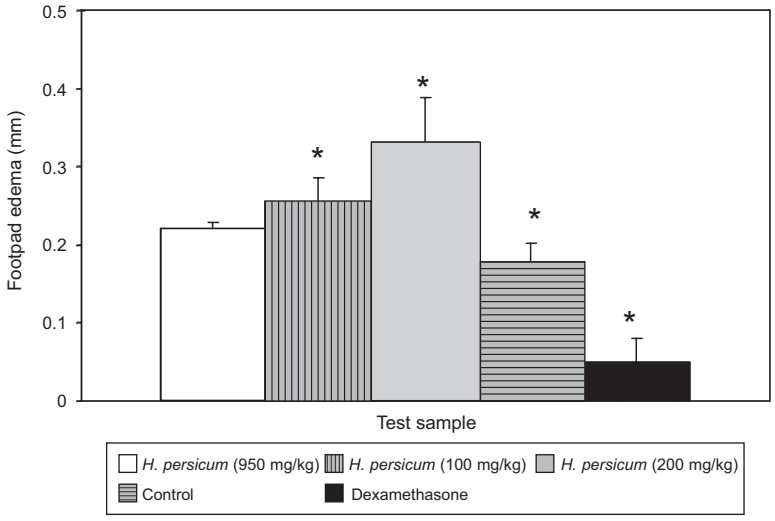

Figure 1. Effects of various doses of *H.persicum* extract on DTH response in mice, compared with dexamethasone and the control group.
*P < 0.05 considered to be significant difference, when compared with the control animals.

Table 2. Effect of different doses of aqueous *H. persicum* extract on HT titre, using SRBCs as an antigen in mice-5 days pretreatment.

Group	Treatment for 5 days	HT (Mean ± SE)
I	Plant extract (50 mg/kg)	5 ± 1*□
II	Plant extract (100 mg/kg)	7 ± 1*
III	Plant extract (200 mg/kg)	4 ± 1□
IV	Control	2 ± 1

Values are presented as mean ± SE of five mice.
* $P < 0.05$ considered to be significant difference, when compared with the control animals.
□ $P < 0.05$ considered to be significant difference, when compared with the other test animals.

Acute toxicity and LD_{50} determination

The LD_{50} of extract was recorded to be more than 1600 mg/kg. Hence,this plant was not lethal up to a dose of 1600 mg/kg.

effect of plant extract on body weight and lymphoid organ weight

None of the doses of *H. persicum* extract showed toxicity or mortality in the extract-treated animals. No significant body weight gain differences were recorded in various groups of animals. The plant did not alter the weight of kidney at the tested doses, never the less, increased the weight of liver with all doses. A significant increase was observed in the relative weight of spleen at doses of 100 and 200 mg/kg,compared with the control group ($P < 0.05$) (Table 1).

Effect of plant extract on humoral immunity parameters

In the haemmaglutination titre (Table 2), doses of 50, 100 and 200 mg/kg showed titre values of 1:4.8, 1:6.5 and 1:3.6, respectively, while the titre value of control was 1:2.4, thus showing a significant increase in the titre values with doses of 50 and 100 mg/kg in the treated groups ($P < 0.05$).

Effect of plant extract on cell-mediated immunity parameters

The plant extract at doses of 100 and 200 mg/kg elicited a significant ($P < 0.05$) increase in DTH response (Figure 1), compared to the control animals. In this study, dexamethasone decreased DTH response significantly, compared to the control group ($P < 0.05$).

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 *H. persicum* at any of the doses used in this study ($P < 0.05$) (Table 3). No significant difference in blood parameters was recorded in various test groups. The dose of 50 mg/kg increased the WBC count, compared with the control group ($P < 0.05$) (Table 3).

Discussion

H. persicum has primarily been described as an anti-microbial and anti flatulence herb in traditional medicine. This plant has been suggested for treatment of epilepsy (20) in folk medicine of Iran. Its anti-convulsant effect is also well established (16). In the present study, *H. persicum* showed an overall stimulatory effect on the immune function in mice. Stimulatory effects were observed on both humoral and cellular immunity. In the HT test, the plant showed an increased response with all the tested doses, but this increase was only significant dose of 100 mg/kg. This activity could be due to the presence of flavonoids or coumarins, which can augment the humoral response by stimulating the macrophages and B-lymphocytes involved in antibody synthesis (21). It appears that 100 mg/kg is the optimum humoral immunity dose in mice. An increase in dose could induce a down-regulation of immune function. The increase in spleen weight may be partly due to the stimulatory effect of the plant extract on this immune organ. Estimation of the LFT enzymes did not reflect any toxicity, despite the increase in liver weight. Results of the present study also revealed no significant difference in the blood parameters. In the DTH test, the DTH response, which directly correlates with cell-mediated immunity (CMI), was found to be the highest at the maximum dose of the extract of the plant extract (200 mg/kg) tested. The mechanism behind this elevated DTH during the CMI responses could be due to the sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblasts and secrete a variety of molecules including proinflammatory lymphokines, attracting more

Table 3. Effect of the aqueous extract of *H. persicum* on liver enzymes, WBC and blood parameters.

Group	Treatment	SGPT	SGOT	WBC	Hb (g/dL)	RBC (× 10^6/mm^3)
I	Plant extract (50 mg/kg)	56.63 ± 19.02	112.30 ± 37.04	6.60 ±1.32*	13.64 ± 0.57	9.42 ± 0.48-
II	Plant extract (100 mg/kg)	54.5 ± 27	129.41 ± 33.01	4.32 ± 2.13	13.34 ± 0.42	9.44 ± 0.72
III	Plant extract (200 mg/kg)	53.71 ± 25.01	120.81 ± 29.03	5.02 ± 1.90	13.30 ± 0.92	9.18 ± 0.51
IV	Control	72.84 ±16.87	106.64 ± 20.14	4.92 ± 1.63	15.04 ± 1.63	9.73 ± 1.32

*$P < 0.05$ considered to be significant difference, when compared with the control animals.

scavenger cells to the site of reaction (22). The infiltrating cells are probably immobilised to promote the defensive (inflammatory) reaction (23). An increase in DTH response indicates that the *H. persicum* extract has a stimulatory effect on lymphocytes and the accessory cell types required for the expression of the reaction (24). The main chemical constituents of *H. persicum* are furanocoumarins, flavonoids and some alkaloids (13, 14). It is suggested that some of these constituents might have immunostimulatory effects, which in turn lead to stimulatory effects on the immunocompetent cells. 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, gama-interferon and macrophages and are thereby useful in the treatment of several diseases caused by immune dysfunction (25). Some reports have suggested that these compounds affect the health as immuno-stimulating agents, i.e. directly enhancing the lymphocyte activation and/or secretion of multipotent cytokine IFN-γ. Some of these constituents also possess antioxidant properties and they may induce the immunostimulant effect, as several antioxidants have been reported to possess immunomodulatory properties (26-28). In another studies, the immunosuppressive effects of the flavonoids and coumarins have been reported (29, 30).

H. persicum has stimulated both humoral, as well as cellular arms of the immune system. *H. persicum* fruits are a rich source of furanocoumarins, which might act as an immunostimulatory agent (31). There are some studies which have confirmed the immunostimulatory effects of the two other species of *H. maximum and H. nepalense*. The extract of *H. maximum* Bartr. stimulate the production of IL-6. IL-6 production is a well-established and reliable marker of macrophage activation (31). Aqueous extracts of *H. maximum* showed an increased production of IL-6 in the immune assay and exhibited a steep dose-response curve (32). It has been shown that the methanol is extract of *H. nepalense*, at a dose of 1000 mg/kg, results in a four-fold increase in HT, compared to the untreated control groups. The DTH response of this plant was found to be the highest at a dose of 1000 mg/kg (33).

Findings of the present study establish that *H. persicum* also has appreciable immunostimulatory activity, but it is not possible at this juncture to single out the most effective immunostimulatory constituent of this plant. However, based on the published studies, furanocoumarins and flavonoids seem to be the most likely candidates eliciting immunostimulating effects. Administration of *H. persicum* in human is simple, since its fruits are used as common dietary constituents in the Iranian household. Its reported immunomodulatory effects warrant further investigation for its use in the cases of clinical immunosuppression. We have reported cytotoxicity of this plant using Brine shrimp lethality assay (15). However,based on the results obtained in this study,it seems that more detailed studies on the mechanisms of immunomodulation of this plant and its probable use in immunocompromised individuals are still to be investigated. Finally, we suggest that *H. persicum* has potential for newer therapeutic applications in the future.

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