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# The Effect of Shallot (*Allium Hirtifolium*) Extract on Profile of Th1/Th2 Cytokines in Balb/C Mouse Splenic Lymphocytes

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**A B S T R A C T**

Modulation of cytokine secretion by phytoconstituents offers a novel approach for treatment of diseases. A class of herbal medicines, known as immunomodulators, alters the activity of immune system through the dynamic regulation of cytokines secretion. Cytokines modulate functions of immune cells, including Th1 (T helper 1) or Th2 responses, responsible for cell-mediated and humoral immunity, respectively. Here, we evaluated the immunomodulatory effects of hydroalcoholic extract of shallot (*A. hirtifolium*) on immune response in BALB/c mouse. Extract of shallot bulbs were prepared and two doses of extract (80 and 400 mg/kg) were injected intraperitoneally within 10 days. Thereafter, splenic mononuclear cells (SMNC) were isolated and secreted IFN-γ (Interferon-gamma), Il-4 (Interleukin-4) as two prototypes of Th1/Th2 cytokines, and TNF-α (Tumor Necrosis Factor-alpha) levels were assayed using Enzyme-linked Immunosorbent assay (ELISA) kits. The results showed that shallot extract significantly reduced Th2 and Th1 responses by down-regulation of Il-4 and IFN-γ levels. The extract also reduced TNF-α, the major proinflammatory cytokine. These data indicated the suppressive potential of shallot extract on the signature cytokines of Th1/Th2 subsets and TNF-α.

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## Introduction

Cytokines, a large group of soluble extracellular proteins, are key regulators in immune responses. These proteins are critical to innate and adaptive immune responses, cell growth and differentiation and cell death. In addition, cytokines provide a link between organ systems, providing molecular cues for maintaining physiological stability [1]. The term immunomodulator has been used in the phytotherapy literature to describe botanical medicines to influence immunity [2]. In this regard, immunomodulators may be defined as the phytoconstituents that adjust the activities of the immune system via dynamic regulation of cytokines, hormones or neurotransmitters.

Medicinal plants display diverse pharmacologic activities and generally have advantages over synthetic drugs, including smoother action and a better tolerance. Shallot (*Allium hirtiffolium*) is a main ingredient of many eastern diets and is widely believed to be beneficial for health. *A. hirtifolium* has been known as "Moosir" in Iran and it is distributed in West, North-West and central parts of Iran, especially in the Zagros Mountains [3]. To date, there are few clinical reports about the pharmacologic properties of shallot. These include: *in vitro* analyses of the anti-oxidant / anti-inflammatory activities of shallot and *in vivo* analyses of the hypoglycemic effect of aqueous extracts of shallot (and garlic) in rats with fructose induced insulin resistance [4-6]. Shallot is rich in flavonoids such as quercetin, a potent antioxidant [7]. This compound was reported to be protective against oxidative stress induced by spontaneous hypertension [4].

Previously, we reported that hydroalcoholic extract of shallot could suppress angiogenesis *in vitro* and *in vivo* [8]. In addition, we reported the *in vivo* anti-inflammatory and anti-cancer activities of shallot extract [9]. Furthermore, we showed the effects of hydroalcoholic extract of shallot on induction of delayed type-hypersensitivity (DTH) responses and production of the key TH1 cytokine (IFNγ) [10]. Shallot contains considerable amounts of flavonoids that can exert significant immunomodulatory effects, in part, by modulating the production of Th1/Th2-derived cytokines IFN-γ and IL-4, respectively [11]. By these facts, here we attempted to evaluate the effect of hydroalcoholic extract of shallot (*A. hirtifolium*) on profile of Th1/Th2 cytokines in BALB/c SMNC.

## Materials and methods

### *Preparation of shallot bulb hydroalcoholic extract*

Fresh shallot bulbs were purchased from a local vegetable market at Kermanshah (West of Iran) and verified by Dr Masoumi (Agricultural College, Razi University, Iran). The bulbs were washed and cut into small slices, air dried and ground into a fine powder using a laboratory blender.

The hydroalcoholic extract was prepared by mixing one weight of shallot powder with 12 volumes (3×4 volumes) of 50% (v/v) ethanol by stirring at 4°C for 24 hr. The extract was filtered through Whatman paper No.1 and centrifuged at 10000 ×g for 20 min at 4 °C to remove any debris and allowed clear supernatant to evaporate to dryness under reduced pressure.

### *Animals*

Six to eight-weeks old BALB/c male mice were purchased from Pasteur Institute of Iran (Tehran, Iran). They were kept in standard conditions at 28°C with a 50% relative humidity and a 12-hr light / dark cycle. The mice were housed for one week to acclimate prior to any experiments. The Animal Care and Use Protocol Committee of Kermanshah University of Medical Sciences (Kermanshah, Iran) approved all experiments and protocols performed in this study on the animals.

### *Experimental design and treatment*

To evaluate the effects of shallot extract on the profile of Th1/Th2 cytokines, the mice were weighed and grouped into tests and control. Then two doses of hydro-alcoholic extract (80 and 400 mg/kg) were injected intra-peritoneally in a 200 µl volume for ten consecutive days. The doses were selected based on the findings from the previous experiments [8, 12]. Control mice were injected with the same volume of physiological serum (85% w/v NaCl in DW)

### *Cell isolation and culture*

48 hr after the final injection, the mice were euthanized by cervical dislocation and their spleens were removed and mechanically disrupted in RPMI (pH 7.4) under sterile conditions.

The resulting suspension was passed through a 100-µm stainless steel mesh and red blood cells (RBC) were removed by incubation for 5 min in lysis buffer (150 mM $NH_4Cl$, 1 mM $KHCO_3$ and 0.1 mM $Na_2EDTA$) followed by centrifugation at 1000 ×g for 5 min at 4 °C.

The supernatant was discarded and the cell pellets were re-suspended in 1 ml RPMI 1640. After counting the cells, $5\times10^5$ live cells/ml containing 10% fetal bovine serum (FBS), 5 µg Concanavalin A (ConA)/ml, 100 U penicillin/ml and 100 µg streptomycin/ml (all reagents from Sigma, St. Louis, MO]) were cultured in wells of a 96-well plate. The plate was then incubated at 37 °C in a 5% CO2 incubator for 48 hr.

### *Cytokine assay*

To evaluate the effects of shallot extract on cytokine production by splenic SMNC, supernatants of treat and control wells were harvested for the determination of IFN-γ, IL-4 and TNF-α levels. All samples were analyzed using commercial ELISA kits (R&D Systems, Minneapolis, MN) according to manufacturer's protocols. The sensitivity of the IFN-γ, IL-4 and TNF-α kits were 2 pg/ml.

### *Statistical analysis*

All experiments were repeated at least three times in duplicates. All data were presented as mean ± SE. Statistical analyses were performed for each endpoint using a one-way analysis of variance (ANOVA). P-values <0.01 were considered statistically significant.

## Results

### *Effects of shallot extract on IL-4 production by SMNC or splenic lymphocytes*

We studied the effects of hydroalcoholic extract at doses 80 and 400 mg/kg on major Th2-derived cytokine, IL-4 production by splenic lymphocytes compared to control group. The results showed that level of IL-4 production decreased in the both test groups compared to control group; however, the decrease was significant at 400 mg/kg test group compared to control group (figure 1)

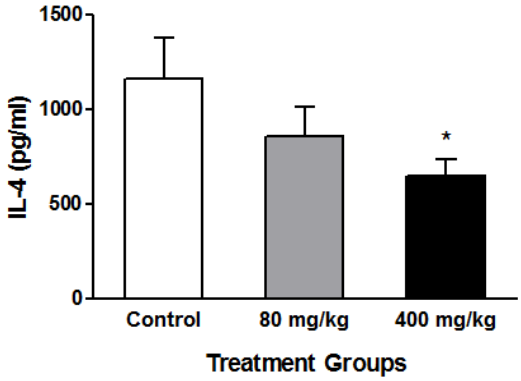


**Fig. 1.** Effect of hydroalcoholic extract of shallot on IL-4, production by splenic MNC. MNC were isolated from mice after following ten days of treatment. The data shown are the means (± SEM) of three independent experiments. N = 6/ group. *P < 0.01 vs control.

### *Effects of shallot extract on IFN-γ production by SMNC or splenic lymphocytes*

With respect to Th1-derived IFN-γ, treatments with shallot extract at 80 and 400 mg/kg decreased the level of IFN-γ in the test groups that received repeated injections of two dose of shallot extract compared to control, however the decrease was significant at 80 mg/kg treated group compared to control group (figure 2).

 

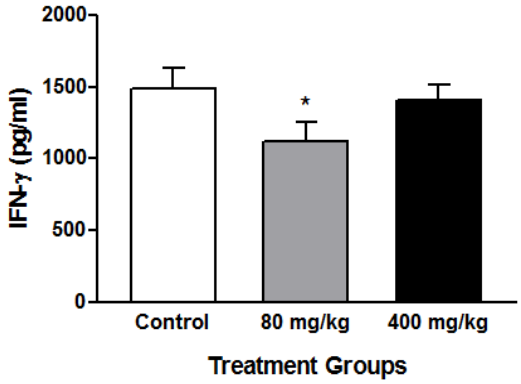


**Fig. 2.** Effect of hydroalcoholic extract of shallot on IFN-γ, production by splenic MNC. MNC were isolated from mice after following ten days of treatment. The data shown are the means (± SEM) of three independent experiments. N = 6/ group. *P < 0.01 vs control.

### *Effects of shallot extract on TNF-α production by SMNC or splenic lymphocytes*

The results for TNF-α assay showed significant decrease in 80 and 400 mg/kg treated groups compared to control group. Between the two tests groups, TNF-α concentration was lower in 80 mg/kg than 400 mg/kg treated group (figure 3).

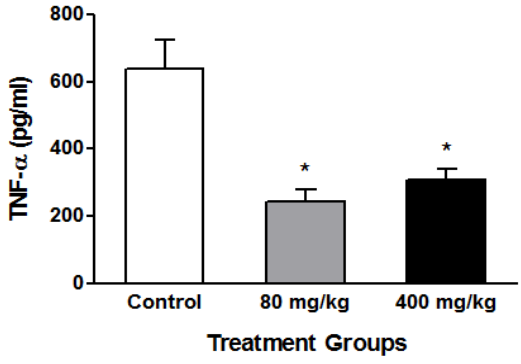


**Fig. 3.** Effect of hydroalcoholic extract of shallot on TNF-α, production by splenic MNC. MNC were isolated from mice after following ten days of treatment. The data shown are the means (± SEM) of three independent experiments. N = 6/ group. *P < 0.01 vs control.

## Discussion

Collectively, our results indicated that shallot extract could significantly decrease the production of Th1 and Th2 signature cytokines and pro-inflammatory cytokine TNF-α by mouse splenic lymphocytes after treatment of the animals for ten consecutive days. These data suggest that shallot extract may directly and/or indirectly modify splenic T cell function. According to the results, inhibitory effect of the extract on production of IL-4 and TNF-α by splenic lymphocytes was more pronounced at 80 mg/kg compared to IFN-γ that more inhibited at 400 mg/kg treated mice. In accordance to our results, Jafarian *et al,* showed that hydroalcoholic extracts of *A. hirtifolium* decreased acquired immune response in a dose dependent manner [13]. In a study on garlic, a different species of Allium genus, Zamani et al [14] reported that consumption of garlic may exert regulatory effects on rat splenic lymphocytes and promote a Th2 response. In contrast to our study, they showed that garlic consumption increase IL-4 secretion by splenic lymphocytes. Also, Hodge et al. [15] showed that garlic extract inhibits secretion of pro-inflammatory cytokines, IFN-γ and Il-2 from human blood mononuclear cells *in vitro.* They suggested garlic extract for resolving bowel disease inflammation. In previous studies, we showed that hydroalcoholic extract of shallot could exert considerable anti-inflammatory activity in mice and anti-growth effect against cancer cells including Jurkat, K562 and Wehi-164 [9]. These data collectively suggest the similar suppressive potential of shallot and garlic, the two different species of allium plants, against autoimmune and inflammatory disease. However, their responsible component/s have not been identified to date.

Shallot is a rich source of biologically active compounds such as polyphenolic compounds with low toxicity. Several biological activities have been described for polyphenolic compounds including modulatory effects on different populations of immune cells [16]. In addition, the results of several population-based studies have indicated that intake of Allium vegetables is inversely associated with the risk of cardiovascular, diabetes and infectious diseases as well as certain types of

 

cancer [17]. Shallot contains a number of chemical constituents such as flavones and polyphenolic derivatives with anti-cancer, anti-angiogenic and anti-inflammatory properties [12, 8]. We hypothesize that some components of *A. hirtifolium* like flavonoids exert immune-modulatory effects by modulating Th1 and Th2 derived cytokines.

As mentioned above, when the results at 80 and 400 mg/kg treated mice were compared, we saw that decrease in Th1 response in 80 mg/kg treated group is more than 400 mg/kg treated group. On the other hand, for Th2 response, the response is more inhibited in 400 mg/kg than 80 mg/kg treated group. These findings demonstrated that treating with shallot extract at different doses may lead to the differential activity of Th1/Th2 subpopulations. Regarding the present results and also dose-dependent behavior of the hydroalcoholic extract of shallot, a regulatory effect on Th1/Th2 responses can be suggested. For example, higher doses (400 mg/kg or more) of shallot extract polarize Th0 toward Th2 responses and this situation can be applied in treating allergic diseases. On the other hands, lower doses of shallot extract (80 mg/kg) suppress Th1 response and can be used in autoimmune and inflammatory diseases such as multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease. Therefore, the evaluation of molecular mechanisms of shallot-mediated immunomodulatory effects may be a promising area for the development of botanical pharmaceutical agents for the treatment of certain autoimmune and inflammatory diseases.

## Conclusion

Herein, we indicated that hydroalcoholic extract of shallot could cause a significant decrease in production of Il-4, IFN-γ and TNF-α by mouse splenic lymphocytes. These data suggest the suppressive potential of shallot extract at different doses on both Th1/ Th2 cytokines. Low toxicity and dose-dependent behavior of shallot extract, compared to synthetic compounds, *su*ggest potential uses of shallot in treatment of some inflammatory diseases.

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### Conflict of interest

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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