

# The Role of *Zataria Multiflora* Essence (Iranian herb) on Innate Immunity of Animal Model

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## ABSTRACT

**Background:** Herbal medicines have been used since ancient times for treatment of a range of diseases and have represented stimulatory effects on the function of innate immunity. **Objective:** To evaluate the effects of *Zataria multiflora* (*Z. multiflora*) on the function of innate immunity including phagocytic activity and TNF- $\alpha$  secretion in animal model. **Methods:** Eight BALB/c mice were divided into two equal groups. In group A, *Z. multiflora* essence was injected intraperitoneally to the mice, in group B, distilled water was injected. Blood was obtained from 4 mice in each group, 4 and 7 days following injection. The amounts of phagocytosis (respiratory burst) and TNF- $\alpha$  secretion were assessed by chemiluminescence and ELISA method, respectively. **Results:** Significant increase in phagocytosis and TNF- $\alpha$  secretion was observed in group A compared with the control group at days 4 and 7. **Conclusion:** *Z. multiflora* essence can remarkably stimulate innate immunity function and it may be used to immunize individuals alone or in combination with other immunostimulatory agents.

**Keywords:** *Zataria Multiflora*, Phagocytosis, TNF- $\alpha$

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## INTRODUCTION

In the last 25 years frequency of life-threatening infections has increased dramatically among cancer patients, transplant recipients, AIDS patients, and those receiving broad-spectrum antibiotics, corticosteroids, and cytotoxic drugs (1). Numerous risk factors have been identified that can impair host defense, predisposing to serious diseases (1,2). Widespread efforts are made to identify immunomodulatory agents to combat infections as prophylactic or therapeutic regimens, or to enhance host immune mechanisms (2).

One class of immune modulators is known as herbal medicines. Herbal medicines have been used since ancient times for treatment of a range of diseases (3). Clinical studies have shown that various herbal products are effective in treating abnormalities of the immune system and balancing immune function in cases of chronic infection and toxicosis, preventing it from turning on the body and causing destruction (4-6). Herbal medicines have been studied by some investigators (6-9).

*Zataria multiflora* (Avishan-e-Shirazi in Persian and Sa'atar or Zaatar in the old Iranian medical books) is a thyme-like plant and a member of Labiata family that grows wild in central and southern Iran (10). In Iran, *Z. multiflora* is used in traditional folk remedies for its antiseptic, analgesic (pain relieving) and carminative (anti-flatulence and intestine soothing) properties (11,12). The essence is obtained by distilling or pressing the plant's leaves, roots, fruits, seeds, stems, or flowers. The essential oil contains the plant's essence, a complex chemical that provides its smell and other properties (12). In general, the essence was found to contain 26 types of different substances such as thymol (48.4 percent) and carvacrol (12.6 percent) which are antimicrobial and antifungal agents (13,14).

Knowing that human and animals are repeatedly exposed to different risk factors such as pathogenic agents and mycotoxins which impair immune function, immunomodulators are recommended to boost the immune system. The aim of this study was to evaluate the effects of Iranian herbal essence, *Z. multiflora*, on the function of innate immunity including phagocytic activity and TNF $\alpha$  production in animal model.

## MATERIALS AND METHODS

**Essence.** Standard *Z. multiflora* essence was obtained from Barij Essence Pharmaceutical Company (Kashan, Iran).

**Animals.** Eight 6-week-old female BALB/c mice were purchased from Razi Institute (Karaj, Iran). Animals were divided into two equal groups, were kept in cages, and fed under specific pathogen-free conditions.

**Administration Method.** In group A single dose of 100 mg/kg *Z. multiflora* essence was administered intraperitoneally (IP) and in group B or control group distilled water was used.

**Blood Sampling.** For evaluation of immune function, 4 mice in each group were anesthetized with chloroform at days 4 and 7, and blood was collected by cardiac puncture. Part of the blood was poured immediately into an Eppendorf centrifuge tube containing 5 units of heparin to determine respiratory burst by chemiluminescence method; the other part was poured into sterile tubes and placed at laboratory temperature for 20

min. Tubes containing non-heparinized blood were centrifuged at 2500 g for 20 min and serum was isolated to measure TNF- $\alpha$  level by ELISA method.

**Chemiluminescence Method.** Chemiluminescence method was used to study the respiratory burst of stimulated neutrophils according to the light intensity. 0.5 ml of heparinized blood was diluted with dextran (Pharmacia, Uppsala, Sweden) in ratio of 2:1 (dextran: blood) and maintained at laboratory temperature for 30 min, allowing the erythrocytes to sediment. Supernatant containing neutrophils was poured into a fresh tube and 0.19 ml of Ficoll (Sigma Chemical Co., St. Louis, USA) was added to the supernatant, then re-suspended in 10 ml of phosphate buffer saline (PBS) with pH of 7.2. Suspension was centrifuged at 1800 g for 10 min. Subsequently, the supernatant was harvested, brought to a final volume of 1 ml with PBS, and neutrophils were counted. 0.5 ml of PBS, 0.2 ml of luminol (Sigma Chemical Co., Deisenhofen, Germany), 0.2 ml of phorbol 12-myristate 13-acetate (PMA) solution (Sigma Chemical Co., Deisenhofen, Germany), and 0.1 ml of neutrophil suspension were added to a special cuvet and its value was determined by the luminometer set (Biorbit 1251, Finland) (15).

**Determination of TNF- $\alpha$  in Serum.** Serum samples were collected 4 and 7 days after injection and murine TNF- $\alpha$  level was measured by ELISA method using a commercial kit (RF 51017, GIB-Co., Ludwigshafen, Germany).

**Statistical Analysis.** Results were expressed as mean  $\pm$  standard deviation (SD). Unpaired Student's t-test was performed using SPSS software (version 12). P-values less than 0.05 were considered significant.

## RESULTS

As depicted in table 1, the value of phagocytosis in group A in comparison with the control group was statistically significant (P=0.002 for day 4 and P=0.001 for day 7). Moreover this value in experimental group at day 7 was higher than day 4 (P=0.03).

**Table 1. Quantitative parameters of Phagocytosis in mice treated with essence**

Test	Phagocytosis (mv <sup>*</sup> )		
	Mean $\pm$ SD		P-value
Day	Group A	Group A	
Day 4	122.26 $\pm$ 29.47	73.75 $\pm$ 18.79	0.002
Day 7	170.62 $\pm$ 49.09	72.38 $\pm$ 17.74	0.001

\* mv: millivolt

The mean optical density (OD) for TNF- $\alpha$  at day 4 was 1.64 $\pm$ 0.61, in comparison with mean OD of 0.49 $\pm$ 0.28 for control group. When the serum level of TNF- $\alpha$  was measured 7 days after injection, the amount of TNF- $\alpha$  was higher than the control group (mean OD: 1.84 $\pm$ 0.63 versus 0.51 $\pm$ 0.29, P: 0.0005). The serum level of TNF- $\alpha$  at day 7 was slightly higher than day 4 but the difference was not statistically significant. (Table 2)

**Table 2. TNF- $\alpha$  amount in mice treated with essence**

Test	TNF- $\alpha$ (OD)		P-value
	Mean $\pm$ SD		
Day	Group A	Control	
Day 4	1.64 $\pm$ 0.61	0.49 $\pm$ 0.28	0.001
Day 7	1.84 $\pm$ 0.63	0.51 $\pm$ 0.29	0.0005

## DISCUSSION

In recent years different factors such as AIDS, cancers, chemotherapy, corticosteroid therapy, and other underlying diseases have been known that can compromise immune defenses, predisposing to serious and fatal infections (1). Regarding the above mentioned points, immunomodulators that enhance specific and non-specific components of immune system may represent a reasonable approach to protect human and animals exposed to pathogens (16,17). Immunity can be boosted by immune-enhancing herbs such as Garlic, Echinacea, Licorice, and Ginseng (18-20).

In this study the effects of Iranian herbal essence, *Z. multiflora*, on immune system function were investigated. No previous study has been carried out on immunostimulatory effects of *Z. multiflora* extracts, except our own study (7). Results of this study indicate that in animals injected (IP) with *Z. multiflora*, mean respiratory burst of group A at days 4 and 7 were 122.2 and 170.62 mv, indicating a 1.6-fold (P=0.002) and 2.3-fold increase (P=0.001) compared with the control group. The essence enhanced respiratory burst at day 7 compared with day 4 (1.4-fold, P=0.03). For the first time in Iran, Khosravi *et al.* showed that subcutaneous administration of Iranian herbal essences such as *Z. multiflora* and *Geranium* stimulates a significant cellular immunity in rabbits. Moreover, *Z. multiflora*, *Myrth*, and *Lemon peel* essence had considerable effect on phagocytosis, whereas *Geranium* had no significant effect (7).

In conclusion, some Iranian herbal essences such as *Z. multiflora* can be used to enhance the immune function in patients with immune disorders.

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