

## The effects of methanolic and chloroformic extracts of *Allium fistulosum* L. on cell-mediated immune response in mice

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### Abstract

Garlic and onions as dietary constituents and medicines have been used for many disorders which dates back to the early civilization. It has been shown that different species of *Allium* have immunomodulating effects. In this study, we sought to determine if methanolic and chloroformic extracts of *Allium fistulosum* L. (Leek) have any effect on cell-mediated immune response in mice. Methanolic and chloroformic extracts of *A. fistulosum* bulbs were prepared by maceration. To study the effects of *A. fistulosum* on acquired immunity, groups of Balb/c mice (n = 8-12) were used. Sheep red blood cell (SRBC) was injected (s.c.,  $1 \times 10^8$  cells/ml, 0.02 ml) and 5 days later, different extracts (1, 10, 100 and 1000 mg/kg), betamethasone (4 mg/kg) or normal saline were given i.p. After 1 h SRBC was injected to footpad (s.c.,  $1 \times 10^8$  cells/ml, 0.02 ml) and footpad swelling was measured up to 72 h. To see the effects of *A. fistulosum* on intrinsic immunity the same procedure was used, but animals only received one injection of SRBC 1 h after i.p. injection of test compounds. Our findings showed that SRBC induced an increase in paw swelling with maximum response at 7 h. Betamethasone inhibited paw thickness in both models. In both intrinsic and acquired immunity models, chloroformic and methanolic extracts of *A. fistulosum* bulbs significantly reduced paw thickness at the doses of 100 and 1000 mg/kg, but no clear dose-response was observed.

**Keywords:** *Allium fistulosum*; Leek; acquired immunity; intrinsic immunity; methanolic extract; chloroformic extract

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### INTRODUCTION

A variety of herbs have been used traditionally to prevent and treat diseases. Recent findings are clarifying important roles of immune functions in disease progression. Immune dysfunction may result in infectious diseases and cancers and hyper-immune reactions may cause autoimmune diseases, including allergy and rheumatoid arthritis. Thus, the development of an immune modifier that stimulates necessary functions and suppresses unnecessary functions is truly

desired (1). A variety of plants derived materials such as polysaccharides, lectins, peptides, flavonoids and natural sulfur compounds have been reported to modulate the immune system (2,3).

The scientific community has now become interested in the pharmacologic properties of different species of *Allium* and their chemical constituents. The genus *Allium* comprises more than 700 species and they are found throughout North America, Europe, North Africa and Asia. More than 139 *Allium* species have been reported in Iran from which 30 species are

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endemic (4,5). Onion (*Allium cepa*), garlic (*Allium sativum*), leek (*Allium fistulosum*), chives (*Allium schoenoprasum*), Welsh onion (*Allium fistulosum*) and shallot (*Allium ascalonicum*) are most widely used as food or supplements (6).

The use of garlic and onion as dietary constituents and medicines for many disorders dates back to early civilization. It has been shown that these plants and their constituents have immunomodulating effects (3,7). *A. fistulosum* is one of the most important flavoring vegetables in Chinese dishes and has been used as a Chinese traditional medicine to treat a variety of diseases, including common cold, arthritis, and headache (8).

Various researches have indicated that some *Allium* species can modulate immune responses (7,9-11) but, to the best of our knowledge, there is no previous report on the immunomodulating effects of *A. fistulosum*. In this study, we sought to determine whether *A. fistulosum* has any effect on cellular immune system in mice.

## MATERIALS AND METHODS

### *Plant material*

The bulbs of *A. fistulosum* were purchased from a local market of Isfahan province (center of Iran). The plant specimen was identified by Department of Medicinal Plant, Faculty of Agriculture, Isfahan University of Technology, Isfahan, Iran.

### *Preparation of extracts*

Air-dried and powdered bulbs of the plant (100 g) were percolated with 400 ml of methanol:water (75:25) or chloroform for 48 h. After filtration, the extracts were evaporated until dryness in a vacuum evaporator (12,13).

### *Animals*

Six- to eight-weeks old Balb/c male mice were purchased from Pasteure Institute (Tehran, Iran). Treatment of the

animals was in accordance with institutional guidelines. They were maintained in a temperature- and light-controlled environment with free access to standard rodent chow and water.

### *Sheep red blood cell-induced paw thickness*

To study the effects of *A. fistulosum* on acquired immunity, ten groups [receiving methanolic and chloroformic extracts (10, 100 and 1000 mg/kg), betamethasone (4 mg/kg) or normal saline], each composed of 8-12 Balb/c mice were used. Sheep blood was obtained from Shahrekord slaughterhouse and sheep red blood cell (SRBC) was prepared by centrifugation followed by washing it 3 times with normal saline. SRBC was injected subcutaneously (s.c.) on the shaved back with  $1 \times 10^8$  cells/ml, (0.02 ml) on day 0. The mice were challenged on day 5 by injecting  $10^8$  SRBC (20  $\mu$ l, s.c.) into the right hind footpad. Footpad thickness was measured with an engineer's caliper up to 72 h after antigen challenge, and the degree of footpad swelling was calculated as: percent increase = (footpad thickness after antigen challenge - footpad thickness before antigen challenge/footpad thickness before antigen challenge)  $\times$  100.

To block the effector phase of the SRBC-induced paw thickness methanolic and chloroformic extracts (10-1000 mg/kg), betamethasone (4 mg/kg) or normal saline were given i.p. 1 h before antigen challenge on day 5 (volume of injection = 100  $\mu$ l, i.p.). To see the effects of *A. fistulosum* on intrinsic immunity, animals received one injection of SRBC on day 0 into the footpad (s.c.) 1 h after i.p. injection of test compounds (14,15).

### *Statistical analysis*

SIGMASTAT™ (Jandel Software, San Raphael, CA) was used to perform statistical tests. The data are presented as means  $\pm$  S.E.M. The significance differences between various experimental

groups was determined by analysis of variance, followed by posthoc pairwise test using Dunns method. Significance was assumed at 5% level.

## RESULTS

Using percolation method for extraction, the extract yield of semi-solid masses after evaporation and solvent removal of methanolic and chloroformic extracts of *A. fistulosum* were 13% and 10%, respectively.

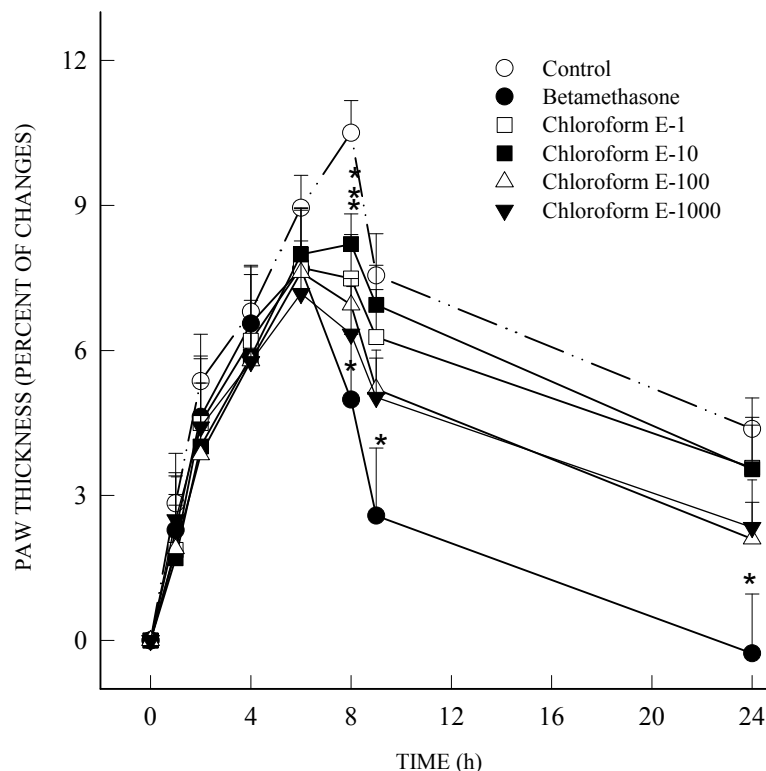
### *Effect of methanolic and chloroformic extracts of A. fistulosum on intrinsic immunity*

The animals were randomly divided into ten groups, each composed of eight mice. The first eight groups received a single dose of methanolic or chloroformic extract of *A. fistulosum* (1-1000 mg/kg)

while the ninth and tenth group (negative and positive controls) received normal saline or betamethasone (4 mg/kg), respectively. After 1 h SRBC was injected into footpad and footpad tickness was measured up to 24 h. SRBC injection significantly increased paw thickness with maximum response at 6-8 h ( $P<0.05$ , Fig. 1 and 2). Administration of betamethasone (4 mg/kg) significantly inhibited paw thickness (Fig. 1 and 2). Chloroformic and methanolic extracts of *A. fistulosum* bulbs at the doses of 100 and 1000 mg/kg significantly reduced paw thickness ( $P<0.05$ , Fig. 1 and 2). However, there was not a clear dose-dependent response.

### *Effect of methanolic and chloroformic extracts of A. fistulosum on acquired immunity*

Different groups of mice (similar to intrinsic immunity model) were used. They

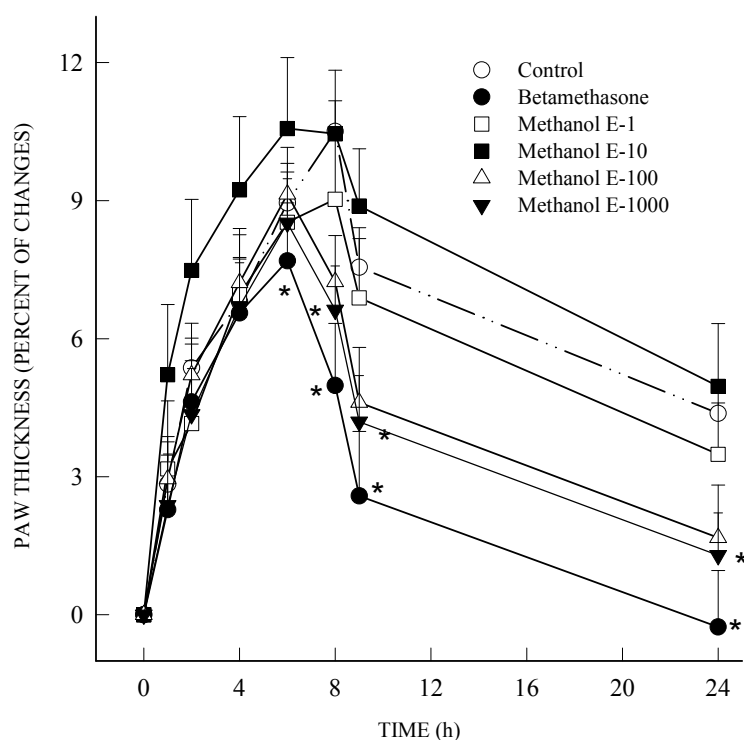


**Fig. 1.** Effect of chloroformic extracts of *A. fistulosum* on the intrinsic immunity. Animals received 20  $\mu$ l SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (1, 10, 100 and 1000 mg/kg, i.p.) one h before antigen challenge. Paw thickness was measured up to 48 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  S.E.M. Groups of 8-12 mice per condition were used. \* $P<0.05$  compared with control group.

were challenged with SRBC on day 0 and on day 5 one h after receiving i.p. injection of *A. fistulosum* extract, normal saline or betamethasone, paw thickness was measured up to 72 h. SRBC injection significantly increased paw thickness with maximum response at 3-5 h ( $P<0.05$ ) (Fig. 3 and 4). Percent of changes in paw thickness in control group was significantly higher than that of intrinsic immunity ( $P<0.05$ ). Administration of betamethasone (4 mg/kg) significantly decreased paw thickness (Fig. 3 and 4). Our findings showed that both methanolic and chloroformic extract of *A. fistulosum* bulbs at the doses of 10, 100 and 1000 mg/kg caused a significant decrease in paw thickness ( $P<0.05$ ).

## DISCUSSION

Allicin, the most abundant compound in onions, is responsible for the pungent smell of onions and it has been shown to possess a variety of pharmacological effects (2,11,12). Allicin and garlic-related compounds, can affect certain aspects of the inflammatory response, probably independent of their known antioxidant activity (16,17). Several studies have indicated that garlic-related compounds modulate immune responses (9-11,18). Previously we reported that hydro-alcoholic extracts and polyphenolic fraction of *A. hirtifolium*, belongs to *Allium* genus, had immunosuppressive effects on mice (7). In this study, we demonstrated

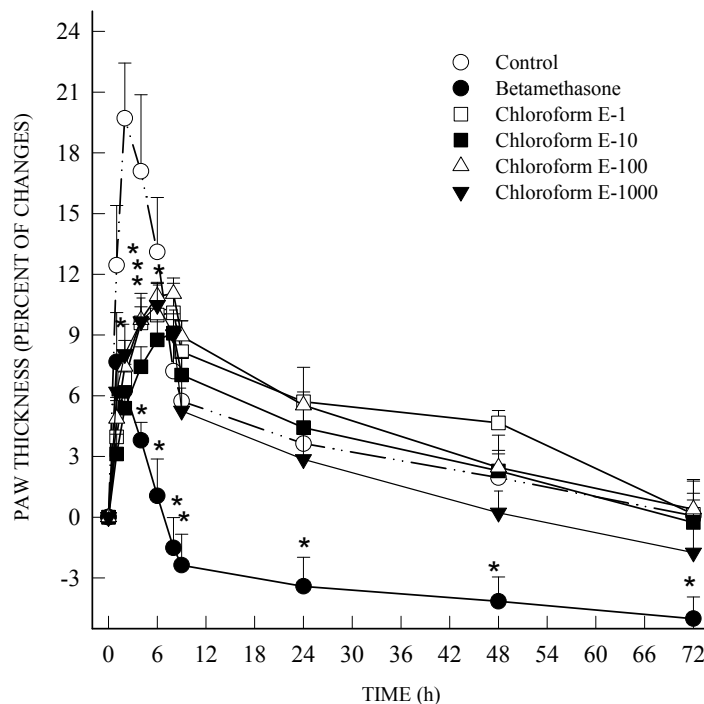


**Fig. 2.** Effect of methanolic extracts of *A. fistulosum* on the intrinsic immunity. Animals received 20  $\mu$ l SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (1, 10, 100 and 1000 mg/kg, i.p.) one h before antigen challenge. Paw thickness was measured up to 48 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  S.E.M. Groups of 8-12 mice per condition were used. \* $P<0.05$  compared with control group.

that *A. fistulosum*, another plant from *Allium* genus, attenuate cell-mediated immune response in mice.

Measuring delayed-typed hypersensitivity (DTH), we evaluated the immunomodulating effects of *A. fistulosum*. DTH is a well-defined *in vivo* model of cell-mediated response. DTH reaction can be quantified by measuring the amount of paw thickness after injection of antigen (14,15,19). Betamethasone (4 mg/kg), a well-known immunosuppressive drug, inhibited paw thickness in both intrinsic and acquired immunity models which indicates the accuracy of the method that was used in these experiments (Fig. 1-4). Methanolic and chloroformic extracts of *A. fistulosum* bulbs significantly reduced paw thickness in both models ( $P < 0.05$ ) (Fig. 1-4). Although there were differences among the inhibitory responses of

methanolic and chloroformic extracts of *A. fistulosum* on intrinsic and acquired immunity, there was not a clear dose-dependent response. The immunomodulating activities of *A. fistulosum* may depend on various chemical compounds, above all on sulphur-containing compounds like allicin and polyphenolic compounds like flavonoids. Sulphur-containing compounds may interfere with the function of the gamma-glutamyl cycle as well as inhibitors of some of the enzymes having SH-groups at the active site (2). Free radicals have been related to several age-related diseases, including cancer (20). Reduced glutathione is not only a co-factor for GST, but also peroxidase (GPX), an enzyme involved in natural protection by free radicals, in serves as a reductant for glutathione addition to superoxide dismutase and

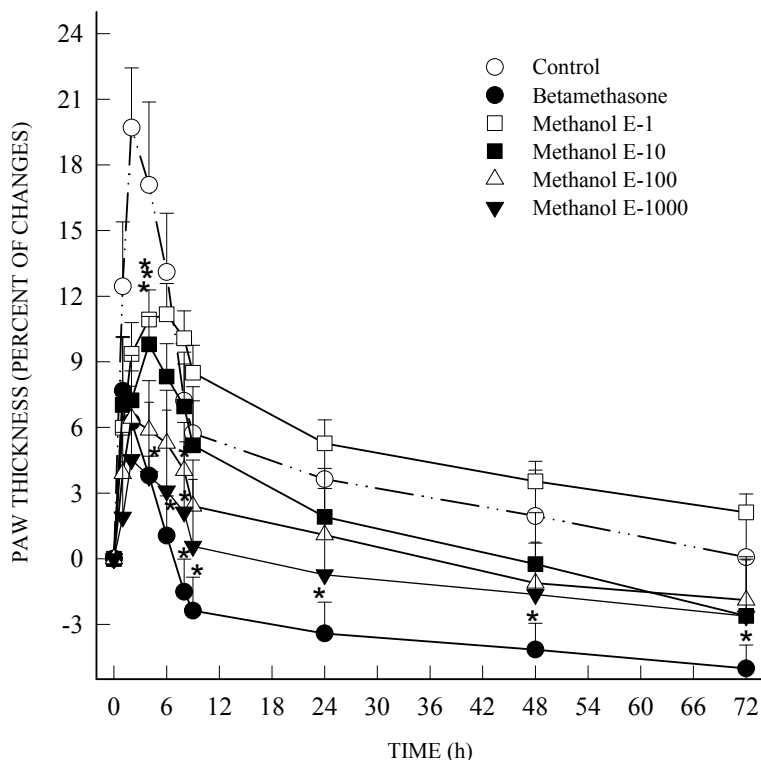


**Fig. 3.** Effect of chloroformic extracts of *A. fistulosum* on the acquired immunity. Animals received 20  $\mu$ l SRBC to the shaved back. After 5 days, a hypersensitivity response was elicited by injecting SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (1, 10, 100 and 1000 mg/kg, i.p.) one hour before antigen challenge. Paw thickness was measured up to 72 hours after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  S.E.M. Groups of 8-12 mice per condition were used.  $*P < 0.05$  compared with control group.

catalase. Garlic and onion oils stimulated the activity of GPX and inhibited the decreased ratio of reduced glutathione to oxidized one, produced by 12-*O*-tetradecanoylphorbol-13-acetate in epidermal cells (6,21). Further pharmacological and phytochemical studies are needed to identify the constituents of *A. fistulosum* and precisely evaluate their immunomodulatory activities and mechanisms.

### ACKNOWLEDGMENTS

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**Fig. 4.** Effect of methanolic extracts (PPE) of *A. fistulosum* on the acquired immunity. Animals received 20  $\mu$ l SRBC to the shaved back. After 5 days, a hypersensitivity response was elicited by injecting SRBC to the right paw. The left paw of each mouse received vehicle alone. Test compounds were given (1, 10, 100 and 1000 mg/kg, i.p.) one h before antigen challenge. Paw thickness was measured up to 72 h after antigen challenge. Results are shown as percent increase in paw thickness  $\pm$  S.E.M. Groups of 8-12 mice per condition were used. \* $P$ <0.05 compared with control group.

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