International Journal of Medical Laboratory 2016;3(1):20-25.

Original Article

The Effect of Methanol Extract of *Echinops Lasiolepis* on TNF-α Production in LPS-activated J774 A.1 Mouse Macrophages

Fateme Sadat Dashti[1] M.Sc., Hossein Hadinedoushan[1,2*] Ph.D., Maryam Asadi[1] M.Sc.

[1]Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[2]Department of Immunology, Reproductive Immunology Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Article history
Received 2 Jan 2016
Accepted 25 Feb 2016
Available online 6 Mar 2016

Key words
Echinops lasiolepis
J774A.1 cell line
Tumor necrosis factor

A B S T R A C T

Background and Aims: Plants as medicines have always played a vital role in human life. Tumor necrosis factor alpha (TNF)-α is one of the macrophage-derived inflammatory cytokine with pleotropic effects in the inflammation process. Some studies have been demonstrated that some of the *Echinops* species have anti-inflammatory activity. In fact, *Echinops lasilepis* is introduced as one of the native plants of Yazd. Thus, the present study intended to assess the inflammatory activity of *Echinops lasiolepis* on TNF-α secretion in J774 A.1 mouse macrophages.

Materials and Methods: At first, methanol extract was prepared by maceration. 10^5 cells/ well were seeded in 96-well plate in triplicate and were treated with different concentrations of extract and 100 ng/ml Lipopolysaccharides. MTT cytotoxicity assay was used to determine the cell viability. Concentrations of extract with cell viability of more than 90% were used to evaluate the level of TNF-α in the macrophage culture using enzyme-linked immunosorbent assay.

Results: Viability of cells at different extract concentrations of 0.1, 1, 10, 50, 100 and 200 µg/ml were 91.68, 95.27, 94.2, 90.8, 85.38 and 71.38, respectively. Therefore, cells treated with 50 µg/ml and lower concentrates of extracts showed more than 90% of viability and their supernatants were used for TNF-α assay. The study results revealed that all concentrations of extract reduced the production of TNF-α.

Conclusions: Our findings showed that methanol extract of *Echinops lasiolepis* may have anti-inflammatory activity via reducing TNF-α production.

***Corresponding Author:** Department Of Immunology, Reproductive Immunology Research Center, Shahid Sadoughi University Of Medical Sciences Yazd, Iran. **Tel:** +98 3536285406, **Fax:** +98 3536238561.

Introduction

Plants as medicines have always played a vital role in the human life. Nowadays, the use of herbal medicine to treat diseases has been significantly increased [1]. Some of the medicinal plants with immunomodulatory effects have been reported to be used in the treatment of different autoimmune diseases such as rheumatoid arthritis [2]. The effectiveness of medicinal herbs on the inhibition of immune response could have useful applications in immune-mediated disorders [3]. Macrophage plays major roles in inflammatory and immunity responses. Activation of macrophage leads to production of cytokines, oxygen and nitrogen species [4]. Excessive activation of macrophages leads to extensive damage to tissues. Tumor necrosis factor (TNF)-α can be introduced as one of the macrophage-derived inflammatory cytokines. In its soluble form, TNF-α acts as a homotrimer with a subunit molecular mass of 17 kDa. This cytokine possesses such pleotropic effects as enhancing the immune response, activating lymphocytes and increasing neutrophil extravasion [5, 6].

Due to the diverse climate in Yazd, a great number of plants grow of which some species are already recorded as medicinal plants. *Echinops* genus belongs to Compositae family comprising over 120 species [7]. *Echinops lasiolepis* is distributed in the wilderness area around the Yazd known as "Shekartighal Ardestani". Several *Echinops* species have been used as a traditional herb to treat cough and lung irritation [8]. The effect of *Echinops lasiolepis* extract on proliferation of peripheral blood mononuclear cells and interferon gamma (INF-γ) secretion were investigated. The study results showed that most concentrations of *Echinops lasiolepis* extract produced inhibitory effect on peripheral blood mononuclear cells proliferation and INF-γ production [9]. Some *in vivo* studies have shown that some of the *Echinops* species have anti-inflammatory activity and reduce inflammation in rat or mouse [10, 11]. In the present study, for the first time, the anti-inflammatory activity of *Echinops lasiolepis* extract was evaluated in vitro. For this purpose, activity of methanol extract of *Echinops lasiolepis* on TNF-α secretion in J774 A.1 macrophages activated lipopolysaccharides (LPS) was checked.

Materials and Methods

Preparation of the extract

The aerial parts of *Echinops lasiolepis* was collected from different parts of Yazd, from April to May and then was confirmed by Research Center of Natural Resource, Yazd, Iran. Plant materials were shade dried and powdered. A methanol extract was obtained by maceration of the plant at room temperature for 72 hours [12]. The methanol extract was filtered and concentrated under reduced pressure. Dried extracts were dissolved in Dimethyl sulfoxide (DMSO), and Dulbecco's modified Eagle's medium (DMEM) (Gibco, BRL, USA). The culture medium was used to obtain 2000 µg/ml concentration. This solution was passed through 0.22 µm filters to be sterilized, and then diluted with the medium for preparing different

concentrations of plant extract (0.1, 1, 10, 50, 100 and 200 µg/ml).

Cell culture

The mouse macrophage cell line J774 A.1 was purchased from the National Cell Bank, Pasteur Institute of Iran (Tehran, Iran) and maintained by DMEM supplement with 10% of fetal bovine serum (FBS, Gibco, BRL), 100µg/ml streptomycin and 100 units/ml penicillin (all prepared from Sigma-Aldrich) incubated at 37°C in a humidified atmosphere containing 5% of CO2. The cells were grown to confluence in sterile culture flasks and counted by haemocytometer. The cells were seeded in triplicate at a density of 1×10^5 cells/ ml in 96-well flat-bottomed tissue culture plate for 2 hours at 37°C. Then the various concentrations of plant extracts (0.1, 1, 10, 50, 100 and 200 µg/ml) were added to the culture simultaneously with 100 ng/ml LPS (Sigma-Aldrich), and the cells were incubated at 37°C for 24 h.

MTT assay

Cell viability in J774 A.1 mouse macrophage culture was determined using Cell proliferation kit I (MTT), version 18 (Roche, Germany) according to the manufacturer's instruction. Briefly, 10 µl of MTT labeling reagent was added to each well and plates were incubated for 4 h at 37°C. 100 µl of solvent (DMSO) was then added to dissolve the formazan production (incubated for 16 h) and then optical density (OD) was measured on a microplate reader at 570 nm. Controls were extract-untreated cells stimulated with LPS and concentration of DMSO was equal to test wells in triplicate. Viability percentage was calculated using this formula: (OD of treated cells/ OD of corresponding control) × 100. Concentrations of extract with the cell viability of more than 90% were used to evaluate the effect of extract on TNF-α production by the activated macrophages.

TNF-α assay

The cells were incubated for 24 h with different concentrations of plant extract (0.1, 1, 10 and 50 µg/ml) and LPS (100 ng/ml) in triplicate and then supernatant was collected and stored at -70°C. Enzyme-linked immunosorbent assay (ELISA) procedure was performed according to the standard protocol of mouse TNF-α Platinum ELISA kit (eBioscience, Austria) to determine TNF-α concentration in supernatant. The sensitivity of the kit was 3.7 pg/ml. It should be mentioned that the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran approved this research.

Statistical analysis

Mann-Whitney U-test was used to assess the statistical significance of differences between mean secretions of TNF-α, which P value of <0.05 was considered significant.

Results

Effects of *Echinops lasiolepis* extracts on the viability of activated mouse macrophages were assessed by MTT colorimetric assay. During the 24-hour treatment of mouse macrophages, this extract greatly decreased the viability of cells at concentration of 100 µg/ml and 200 µg/ml. The effects of *Echinops lasiolepis* extract in different concentrations on the

viability of LPS-activated J774 A.1 mouse macrophage is shown in table 1.

Table1. The effects of *Echinops lasiolepis* extract on viability of LPS-activated J774 A.1 mouse macrophage after 24 hours

Concentration (µg/ml)	% Viability mean
0.1	91.68
1	95.27
10	94.2
50	90.8
100	85.38
200	71.38
control	93.67

The level of TNF-α in the extract-untreated control cells was 58.7 pg/ml. The mean concentration of TNF-α produced by extract-treated and LPS-activated J774 A.1 mouse macrophage is mentioned in Fig.1 compared to the untreated controls. The results revealed that extract of aerial part of *Echinops lasiolepis* at all concentrations decreased the level of TNF-α. Moreover, no significant differences were observed between the samples and the untreated controls at all concentrations (p>0.5).

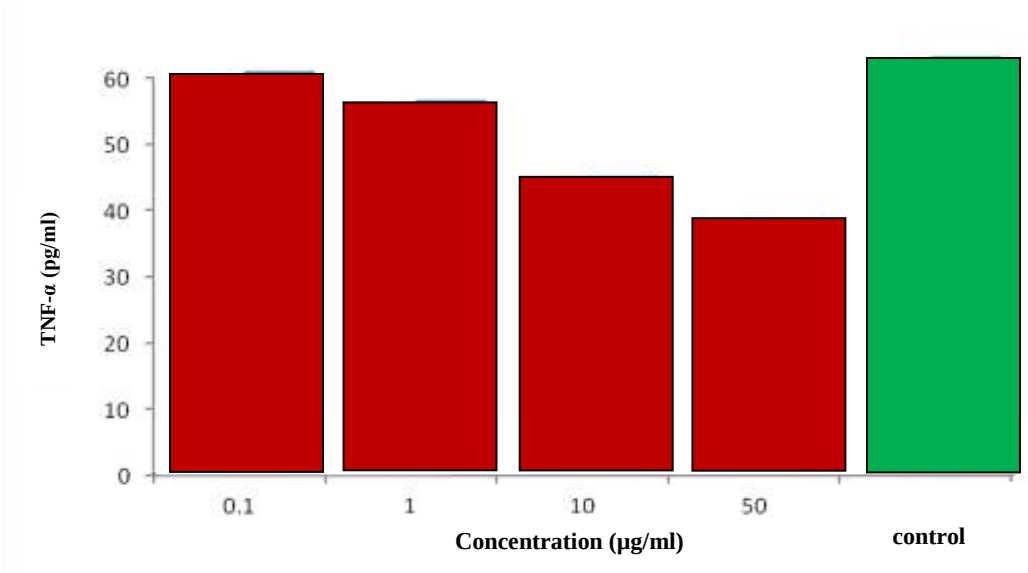

Fig. 1. comparison of TNF-α production in extract-treated and LPS-activated J774 A.1 mouse macrophages and untreated control

Discussion

In the present study, J774 A.1 mouse macrophages were used as an *in vitro* model to evaluate the anti-inflammatory effect of methanol extract of *Echinops lasiolepis*. TNF-α is represented as one of the most important cytokines in regard with the maintenance and

development of inflammation. TNF-α has an important role in some inflammatory diseases such as rheumatoid arthritis. It was demonstrated that usage of TNF-α antibodies reduces the inflammation in this disease [5]. Different species of *Echinops* genus have secondary plant products such as Flavonoids and Terpenoids specially Sesquiterpens [6, 13]. Terpenoids represent the largest and most diverse class of chemicals among the numerous compounds produced by plants. Terpenoids metabolites were utilized regarding a variety of basic functions in growth and development in plants. Traditionally, humans apply plant-based terpenoids in the food, pharmaceutical, and chemical industries [14]. The findings of different studies revealed that some terpenoids have inhibitory effects on production of proinflammatory cytokines [15]. It has been demonstrated that activation of NF-κB involves the inhibitory mechanism of Sesquiterpens [16]. NF-κB is a protein complex that regulates transcription of DNA, which is almost present in all cell types of animals. NF-κB has a key role in regulating immune responses. Moreover, malfunction of NF-κB was observed in cancer, autoimmune diseases and septic shock. As a matter of fact, NF-κB regulates transcription of inflammatory cytokines and other inflammatory factors [17]. Flavonoids have low molecular weight found almost in all plants, which is recognized as the pigment responsible for the colors. They are regarded as potent antioxidants that reduce the risk of cardiovascular diseases [18]. Flavonoids were held to suppress serum levels of TNF-α and IL-1β *in vivo* [19]. Researchers have stated that some of flavonoids have the ability to reduce the production of TNF-α and nitric oxide by NF-κB inhibition [20].

As it was mentioned earlier, secondary products such as flavonoid and terpenoids were isolated from different species of *Echinops*, *which* were likely to be present in *Echinops Lasiolepis* due to the inhibitory effect of *Echinops lasiolepis* extract on TNF-α production in LPS-activated J774 A.1 mouse macrophage. The mechanism of this effect is probably inhibition of NF-κB. Further studies are demanded on the composition of the extract and its mechanisms to reduce production of TNF-α.

Conclusion

The findings of the present study demonstrated that methanol extract of *Echinops lasiolepis* may have anti-inflammatory activity via reducing TNF-α production which is presumably due to secondary components such as flavonoids and terpenoids isolated from different species of *Echinops.*

Acknowledgements

This manuscript is a part of the M.Sc thesis of Ms. Fateme Sadat Dashti, that Shahid Sadoughi University of Medical Sciences provided the grant for this research project. Authors would like to thank the staff of Stem cell Research Center, Mrs. Fateme Sadeghiyan, for her technical assistance.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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