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Research Article

Inhibition of Pro-inflammatory Cytokines by Ethyl Acetate Extract of *Scrophularia striata*

Abbas Azadmehr[1], Ghorban Maliji[2*], Reza Hajiaghaee[3], Mojtaba Shahnazi[4] and Ahmad Afaghi[5]

[1]Immunology Department, Qazvin University of Medical Sciences, Qazvin, [2]Immunology Department, Babol University of Medical Sciences, Babol, [3]Department of Pharmacognosy & Pharmaceutics Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran, [4]Parasitology Department, [5]Social Medicine Department, Qazvin University of Medical Sciences, Qazvin, Iran.

Abstract

Purpose: To investigate the effect of ethyl acetate extract of *S. striata* on pro-inflammatory production by macrophages.
Methods: Mouse peritoneal macrophages were cultured in solvent either alone or with 2 µg/ml lipopolysaccaride (LPS) with/without different doses of ethyl acetate extract of *S. striata*. Production of pro-inflammatory cytokines including interleukin 1β (IL-1β), tumor necrosis factor - α (TNF-α) and prostaglandin E2 (PGE2) were examined using ELISA.
Results: Ethyl acetate fraction of *S. striata* in doses of 10, 50, 100 and 200 µg/ml significantly ($p < 0.05$) inhibited pro-inflammatory mediators (IL-1β, TNF-α, and PGE2) production by LPS stimulated peritoneal macrophages.
Conclusion: The anti-inflammatory effect of the extract on pro-inflammatory cytokines may ameliorate inflammatory diseases, possibly via an immunomodulatory mechanism.

Keywords: Pro-inflammatory, Cytokines, Ethyl acetate extract, *Scrophularia striata*

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**Corresponding author: Email: aazadmehr@qums.ac.ir, ghmaliji@yahoo.com; Tel: +98-1112229591-5; Fax: 98-1112229591*

INTRODUCTION

Inflammation is the body's first defense mechanism against injury and infective agents such as bacteria and viruses. The process of inflammation is necessary in healing of damaged tissues. In response to inflammation, a variety of innate immune cells such macrophages can be activated. Macrophages have critical role in controlling of immune inflammatory responses [1, 2]. Cytokine production is a primary step of reaction of macrophages to inflammatory stimuli [3]. During inflammation, the over-production of inflammatory cytokines such as TNF-α and IL-1β by activated macrophages is a crucial event for triggering progressive activation of immune cells [4].

Modulation of immune cell response, such as macrophage, using medicinal plant may lead to discovery of new drugs. One of the plants proposed to have immunomodulatory and anti-inflammatory effects is *Scrophularia striata* Boiss (Scrophulariaceae). Several species of this genus have been used since ancient times as folk remedies for ailments such as scrophulas, scabies, eczema, psoriasis, tumors, etc. We have previously demonstrated the inhibitory effect of *S. striata* extract on nitric oxide production in vitro and ex vivo model [5]. Many *Scrophularia* species have been investigated and several compounds from different classes of secondary metabolites including iridoids, phenyl propanoids, phenolic acids, flavonoids, quercetin, isorhamnetin 3-O-rutinoside and saponins by column chromatography have been isolated [6-8]. In the present study, we investigated the effect of ethyl acetate extract of *S. striata* on the inhibition of pro-inflammatory cytokines (IL-1β, TNF-α, and PGE2) by LPS-activated macrophages.

EXPERIMENTAL

Plant material and preparation of extract

The aerial parts of *S. striata* were collected from Ruin region of in Northeastern of Iran in May 2009 and air-dried at room temperature. It was authenticated by Dr F Attar, and a voucher specimen was preserved in the herbarium of the Faculty of Sciences at Tehran University, Tehran, Iran (no: 36501). The dried and powdered aerial parts of the plant (500 g) were sequentially subjected to extraction with solvents of increasing polarity, namely, petroleum ether, chloroform and ethyl acetate (6.4 g dry weight corresponding to 1.2 %). The ethyl acetate extract of *S. striata* which was dissolved in dimethylsulfoxide (DMSO) to give 0.1 v/v concentration (was not toxic on the macrophages) and used at appropriate concentrations (1, 10, 50, 100 and 200 µg/ml).

Mice and Isolation of peritoneal macrophages from mice

Mice

The Balb/c mice (6–8 weeks old) were purchased from the Pasteur Institute of Iran (Tehran, Iran). All the animal experiments were approved by and performed according to the guidelines of the Ethical Committee of Institute of Medicinal Plants (IMP), (ACECR). The animals were maintained under standard laboratory conditions of a temperature of 25 ± 2 ◦C and a photoperiod of 12 h and received standard mouse chow and water ad libitum.

Isolation of peritoneal macrophages from mice

The Balb/c mice were scarified to provide peritoneal macrophages for in vitro experiment. Peritoneal macrophages were harvested immediately by lavaging with ice-cold sterile phosphate buffer sulfate (PBS). Cells were washed twice and plated in RPMI 1640 (Sigma Chemical Co) medium containing 10 % fetal bovine serum (GIBCO), 100 U/ml penicillin 100 µg/ ml streptomycin (Sigma Chemical Co) and incubated for 2 h at 37 °C in 5% CO_2 humidified incubator. After 2 h, non-adherent cells were removed by gently washing with PBS and freshly

prepared medium was added. Cell viability was checked by trypan blue exclusion technique. An aliquot of the cell suspension was mixed with an equal volume of 0.4% w/v trypan blue in PBS and incubated for 10 minutes. The cells failing to exclude the dye were counted and expressed as a percentage of the total cells present.

Assessment of pro-inflammatory cytokines

Balb/c mice were used to provide peritoneal macrophages for *in vitro* experiment. Peritoneal macrophages were harvested immediately by lavaging with ice-cold sterile PBS. The cells were washed twice, adjusted to a density of 10^6 cells/ ml, and cultured at 37 ºC in 5% CO_2 for 24 h in RPMI either alone or with 2 µg/ml LPS with/ without increasing doses of the ethyl acetate extract. After 24 h, supernatants were collected by centrifugation at 2500 rpm for 20 minute and assayed for TNF-α and IL-1β using ELISA kits (R & D system, Abingdon, UK) according to the manufacturer's instructions.

Assessment of prostaglandin E2

Peritoneal macrophages were harvested immediately by lavaging with ice-cold sterile PBS. The cells, at a density of 10^6 cells/ml were cultured at 37 ºC in 5 % CO_2 for 24 h in RPMI either alone or with 2 µg/ml LPS with/without increasing doses of the ethyl acetate extract. The control cells were incubated with solvent alone. Supernatants were collected by centrifugation at 2500 *g* for 15 min, and the production of PGE2 was measured by ELISA kit (Cayman Chemicals).

Statistical analysis

The data are presented as mean ± standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA). All analyses were performed using SPSS 16 software. *P* values < 0.05 were considered as significant.

RESULTS

Ethyl acetate extract of *S. striata* inhibits LPS-stimulated TNF-α, IL-1β and PGE2 production by peritoneal macrophages. As shown in Fig 1, LPS activated macrophages increased levels of both TNF-α, IL-1β. Treatment of cells with ethyl acetate extract of *S. striata* induced a dose-dependent inhibition of the production of both pro-inflammatory cytokines by LPS activated macrophages. The extract significantly inhibited the production of both cytokines by LPS activated macrophages in the concentrations 1 0- 200 µg/ml. In addition, the ethyl acetate extract suppressed LPS-induced PGE_2 production in a dose dependent manner.

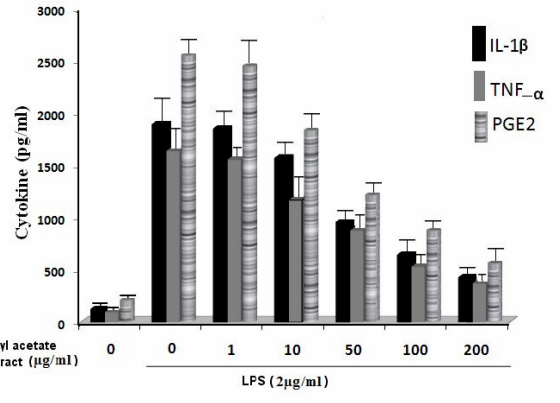

Fig 1: Effect of ethyl acetate fraction of *S.striata* on LPS- activated pro-inflammatory cytokine production.

DISCUSSION

Scrophularia striata is a traditional plant in Iran and has been used to treat various inflammatory diseases such as rheumatics and inflammatory disorders. Some species in this genus have shown anti-inflammatory activity [10]. We have previously demonstrated the inhibitory effect of *S. striata* extract on matrix metalloproteinases (MMPs) in Wehi-164 cell line [11]. Also, in our recent study, flavonoids, cinamic acid, phenylpropanoid, nepitrin, flavonoid glycoside, acteoside1 and phenylpropanoid glycoside

were identified from aerial parts of *S. striata* using 80 % methanol fraction [8]. In controlling of immune inflammatory responses, macrophages have critical roles [2]. Cytokine production is a primary step of response of macrophages to inflammatory stimuli [3]. On the other hand, activated macrophages are known to be an important source of other soluble factors such as PGE2 and nitric oxide [12]. During inflammation, over-production of inflammatory cytokines TNF-α and IL-1β by macrophages are crucial events for triggering progressive activation of immune cells [4]. In addition, we have previously demonstrated the inhibitory effect of *S. striata* extract on nitric oxide production by macrophages as an inflammatory mediator in vitro and ex vivo [5]. Therefore, in this study, we investigated the inhibitory effects of ethyl acetate extract of *S.striata* on the other pro-inflammatory cytokines and mediator including TNF-α, IL-1β and PGE2, respectively. Our results in this study showed that ethyl acetate extract of *S. striata* suppressed the production of pro-inflammatory cytokines and mediator including TNF-α, IL-1β and PGE2 by LPS activated macrophages in dose dependent manner.

CONCLUSION

This study shows that ethyl acetate extract of *S. striata* can be suppressed to produce of pro-inflammatory cytokines by macrophages. Moreover, the anti-inflammatory activity of some species in this genus such as *Scrophularia scorodonia* has shown showed [6, 7]. The inhibition of pro-inflammatory cytokines of ethyl acetate extract of *S. striata* may be ascribed to natural compounds such as flavonoids in this extract of plant that has potential anti-inflammatory effect for inhibition of pro-inflammatory mediators by LPS activated macrophages. Our findings suggest that the beneficial and anti-inflammatory activity of the ethyl acetate extract may be used in down-regulation of the inflammatory mediator's production by macrophages in the inflammatory diseases. Further investigations are necessary to identify the active compounds in the extract and the other anti-inflammatory related mechanisms.

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