Journal of Ethnopharmacology 136 (2011) 422–427

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Anti-inflammatory effects of ethanolic extract from *Lagerstroemia indica* on airway inflammation in mice

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ARTICLE INFO

Article history:
Received 4 March 2010
Received in revised form 13 May 2010
Accepted 25 May 2010
Available online 18 June 2010

Keywords:
Lagerstroemia indica Linn
Asthma
Anti-inflammatory effect

ABSTRACT

Aim of the study: In the present study, we investigated whether the *Lagerstroemia indica* Linn (LI) extract has an anti-inflammatory effect on lung inflammation in ovalbumin-induced asthmatic mice.
Materials and methods: The LI extract was obtained from dried and powdered whole plants of LI using 80% ethanol. ELISA was performed to evaluate cytokine concentration. BALB/c mice were used as a mouse model of asthma after asthmatic induction by ovalbumin sensitization and inhalation. We examined the effects of the LI extract on leukocyte infiltration and mucus secretion using cell count and histological stain.
Results: The amount of cytokines, such as interleukin (IL)-2, IL-4, IL-5, IL-13, and TNF-α, was increased in Jurkat cells using the extract from house dust mites. Increased cytokine concentrations were inhibited by the LI extract. The LI extract suppressed the increased expression of IL-6 after treatment with mite extract of EoL-1 cells and THP-1 cells. In an in vivo experiment using asthmatic mice, the LI extract significantly inhibited leukocytosis and eosinophilia in bronchoalveolar lavage (BAL) fluid and lung tissue samples. The LI extract inhibited the increase in mucus secretion by goblet cells, blocked the production of reactive oxygen species in BAL fluid cells, and blocked the protein expression of IL-5 in BAL fluid. The concentration of ovalbumin-specific IgE in BAL fluid was weakly inhibited by the LI extract.
Conclusions: These results suggest that the LI extract may be used as a valuable agent for treating allergic diseases such as asthma due to its anti-inflammatory property.

1. Introduction

Lagerstroemia indica Linn (LI) belongs to the *Lythraceae* family and is distributed in Korea, China, and Australlia. LI is known as an herb for treating asthma, hemostasis and detoxification in traditional oriental medicine. Although it has been reported that LI has anti-coagulation activity and contains biphenylquinolizidine alkaloids, however, the exact function of LI for the treatment of a disease is understood poorly (Chistokhodova et al., 2002; Kim et al., 2009).

Asthma is a chronic inflammatory disease of the lung. It begins in young children and persists in adulthood, resulting in high mortality and decreased quality of life (Braman, 2006). Allergic inflammation shows an increase in eosinophil infiltration into the airways, mucus production, bronchoconstriction, and reduction of respiratory function. Allergic inflammation is caused by eosinophils which destroy lung tissues by producing several toxic granule proteins. T helper (Th) 2 cytokines, such as IL-4 and IL-5, induce elevation of IgE in serum and BAL fluid; increased IgE then aggravates the clinical features of the disease (Gould and Sutton, 2008).

We performed the present study to determine the anti-inflammatory effect of LI on asthma. First, we examined the inhibitory effect of LI on cytokine production in Jurkat cells, EoL-1 cells, and THP-1 cells. To test the effect of LI in vivo, we investigated the inhibition of inflammatory response, production of ROS, mucus production, and IgE elevation after the treatment with LI of ovalbumin-induced asthmatic mice.

2. Materials and methods

2.1. Preparation of the LI extract

Whole plants of LI were collected and standard extract have been deposited at the Herbarium of the Department of Herbal

Abbreviations: LI, *Lagerstroemia indica* Linn; i.p., intraperitoneal; BAL, bronchoalveolar lavage; Th, T helper; IgE, immunoglobulin E; ELISA, enzyme-linked immunosorbent assay; ROS, reactive oxygen species.

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doi:10.1016/j.jep.2010.05.066

Pharmaceutical Development, Korea Institute of Oriental Medicine, Daejeon, Korea and Division of Life Science, Daejeon University (TUT), Korea. The dried and powdered whole plants of LI (36 g) were extracted with 80% ethanol (3× 0.5 l) for 2 days at room temperature. The combined liquid extracts (4.84 g) were concentrated under reduced pressure.

2.2. Cell culture

The human T cell line Jurkat cells and human monocytic cell line THP-1 cells were obtained from the American Type Culture Collection (Manassas, VA). The human eosinophilic leukemia cell line, EoL-1 cells were obtained from the Riken Cell Bank (Tsukuba, Japan). They were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml).

2.3. MTT assay

MTT assay was performed to determine cell viability using the cell proliferation kit (Roche, Penzberg, Germany). Both THP-1 cells and EoL-1 cells in 100 μl of the culture medium were plated into a 96-well culture plate. The LI extract was added to each individual well as a final concentration of 10 μg/ml. The plate was then incubated for 24 h at 37 °C in a CO_2 incubator. 10 μl of MTT solution was added in each well. After incubation of the plate at 37 °C for 4 h, 100 μl of solubilization solution was added to each well. After 24 h incubation, the absorbance was measured using an ELISA reader (Bio-Tek Instruments, Winooski, VT) at 550 nm.

2.4. Enzyme-linked immunosorbent assay (ELISA)

After pre-treatment with LI or dexamethasone for 30 min, Jurkat cells were treated with the extract of house dust mite, *Dermatophagoides pteronissinus* supplied by Dr. Tai-Soon Yong (Yonsei University College of Medicine, Seoul, Korea). The concentrations of IL-2, IL-4, IL-5, and TNF-α in the supernatant were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using OptEIA Set (BD Biosciences, San Diego, CA) and the concentration of IL-13 was measured with a DuoSet ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions using an ELISA reader, BIO-TEK ELx808. Cytokine release of THP-1 cells and EoL-1 cells was measured using the method described by two reports (Lee et al., 2008, 2009). After pre-treatment with LI or dexamethasone for 30 min, the cells were treated with the extract of house dust mite, *Dermatophagoides pteronissinus*. The concentrations of IL-6, IL-8 and MCP-1 in the supernatant were measured by OptEIA Set (BD Biosciences). The cytokine concentrations were calculated using a linear-regression equation obtained from the standard absorbance values.

2.5. Asthma induction in BALB/c mice and drug administration

Six-week-old female BALB/c mice were purchased from Daehan Biolink Co. LTD (Seoul, Korea) and maintained in an air-conditioned room at room temperature. The mice were divided into six groups ($n = 10$), and airway inflammation was induced by ovalbumin (Grade III; Sigma–Aldrich, Korea) in five groups using the method described by Yuk et al. (2007). Each mouse was immunized by intraperitoneal (i.p.) injection of 20 μg of chicken ovalbumin (Grade III; Sigma–Aldrich, Korea) with 1 mg aluminum hydroxide (Sigma–Aldrich, Korea) on days 1 and 14. The mice were inhaled with 5% ovalbumin solution aerosolized using an ultrasonic nebulizer (ME-U12, Omrom, Tokyo, Japan) for 1 h per day from days 21 to 27 after the second sensitization. Four groups of asthma-induced mice were treated with oral injection of 50 mg/kg, 250 mg/kg or 500 mg/kg of LI extract, or with i.p. injection of 1 mg/kg of dexamethasone (Sigma–Aldrich, Korea) between days 14 and 27, respectively. LI extract and dexamethasone are dissolved in ethanol or DMSO, respectively and then was diluted to less than 1/100 with PBS. The normal group was sensitized and challenged with PBS without drug treatment.

All animal experiments used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Eulji University.

2.6. Collection of bronchoalveolar lavage (BAL) fluid and serum

BAL fluid was collected by lavaging the lung via the trachea with 1 ml of PBS. After five lavages, approximately 0.7 ml of BAL fluid was recovered. Blood was collected by heart puncture. BAL fluid and blood were centrifuged for 5 min at 4 °C. The supernatant was stored at −70 °C for the measurement of cytokines and IgE, respectively. The cells in the BAL fluid were resuspended in 100 μl of PBS for total cell and differential counts. After the measurement of total cell number using a Neubauer hemocytometer, the cells suspended in PBS were applied to a slide by cytospinning and stained with Wright stain solution. The percentage of each leukocyte was determined, and the absolute leukocyte count was calculated by multiplying each leukocyte percentage by the total cell count.

2.7. Measurement of intracellular ROS

The cells in the BAL fluid were washed and resuspended with PBS, and 3.3 μM of DCFDA was added to label the intracellular ROS. The cells were then incubated for 10 min at room temperature. Labeled cells were immediately observed using fluorescence-activated cell sorting (FACS) analysis (BD Biosciences).

2.8. Histological analysis

Lung tissues were fixed with Carnoy's solution overnight at 4 °C after isolation from the mice. The fixed tissues were embedded in paraffin and cut into 4-μm sections with a microtome (Leica, Nussloch, Germany). The sections were placed on slide glasses, deparaffinized, and stained with hematoxylin and eosin (Sigma-Korea) in order to examine the cells that had infiltrated into the peribronchial connective tissues or with periodic acid-Schiff stain (Sigma-Korea) to evaluate mucus production. A peribronchial cell count based on a five-point scoring system was performed as previously described (Duan et al., 2004). The scoring system was the following: 0 – no cells; 1 – a few cells; 2 – a ring of cells 1 cell layer deep; 3 – a ring of cells 2–4 cell layers deep; 4 – a ring of cells more than 4 cell layers deep. The extent of mucus production of goblet cells was evaluated using a five-point scoring system, as previously described (Kuperman et al., 2002). The scoring system was the following: 0 – no goblet cells; 1 – lower than 25%; 2 – from 25% to 50%; 3 – from 50% to 75%; 4 – higher than 75%. The scoring of the leukocytes and goblet cells was examined in three independent fields of lung section from each mouse.

2.9. Statistical analysis

The statistical significance of any difference was determined by one-way ANOVA followed by LSD and Turkey. The data are expressed as the means ± S.E.M. The SPSS statistical software package (Version 10.0, Chicago, IL) was used for the statistical analysis. A p value of less than 0.05 was considered statistically significant.