

RESEARCH ARTICLE

Effect of leaves of *Eriobotrya japonica* on anaphylactic allergic reaction and production of tumor necrosis factor- α

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Abstract

Leaves of *Eriobotrya japonica* Lindl. (Rosaceae) (LEJL) have been used as traditional medicines for inflammatory diseases and chronic bronchitis. However, its effect on mast cell-mediated anaphylactic reaction is not known. The anaphylactic allergic reaction is involved in many allergic diseases such as asthma and allergic rhinitis. In this report, we investigate the effect of LEJL on the anaphylactic allergic reaction and studied its possible mechanisms of action. LEJL inhibited compound 48/80-induced systemic anaphylactic reactions and serum histamine release in mice. LEJL dose-dependently decreased the IgE-mediated passive cutaneous anaphylaxis and histamine release from mast cells. Furthermore, LEJL decreased the production of tumor necrosis factor- α in phorbol 12-myristate 13-acetate and A23187-stimulated human mast cells. These findings provide evidence that LEJL could be a candidate as an anti-allergic agent.

Keywords: *Eriobotrya japonica*; anaphylactic reaction; mast cells; histamine; tumor necrosis factor- α

Introduction

Leaves of *Eriobotrya japonica* Lindl. (Rosaceae) (LEJL) have been used as traditional medicines for lung and stomach diseases, and have been found to be effective in chronic bronchitis, inflammation, and tumor.⁽¹⁾ Various triterpenes, flavonoids, tannins, and megastigmane glycosides have been found in the leaves of *E. japonica*, and some of these compounds such as oleanolic acid, 2- α -hydroxyursolic acid, maslinic acid, and 7-O- β -D-glucopyranoside are reported to have anti-inflammatory, anti-oxidant, anti-mutagenic, or anti-tumor activities.^(2–5)

Mast cells are important in the development of many physiologic changes during allergic and anaphylactic responses.^(6, 7) Anaphylactic reaction is a life threatening syndrome induced by the sudden systemic release

of inflammatory mediators from mast cells. Among the inflammatory substances released from mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity.^(8, 9) Activated mast cells also produce a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several proinflammatory and chemotactic cytokines.^(10, 11) Therefore, modulation of production of these cytokines and histamine from mast cells can provide a useful therapeutic strategy for allergic disease.

Mast cell activation by IgE induces the process of degranulation that results in the fusion of the cytoplasmic membranes with the plasma membrane. This is accompanied by both the fast external release of granule-associated stored mediators as well as by the generation and release of newly generated mediators.^(12, 13)

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Mast cell degranulation can be elicited by a number of positively charged substances, collectively known as the basic secretagogues of mast cells.^(14, 15) The synthetic compound 48/80 is known to be one of the most potent secretagogues. An appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis.^(16, 17)

In this study, we studied the effect of LEJL on the compound 48/80-induced systemic reaction, IgE-mediated local allergic reaction, and histamine release from mast cells. In addition, the effect of LEJL on phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187-induced production of TNF- α in a human mast cell line (HMC-1) cells was also investigated.

Materials and methods

Reagents and cell culture

Compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HSA), o-phthalaldehyde, PMA, calcium ionophore A23187, and metrizamide were purchased from Sigma (St. Louis, MO, USA). The human mast cell line (HMC-1) was grown in Iscove's media (Life Technologies, Grand Island, NY, USA) supplemented with 10% FBS and 2 mM glutamine at 37°C in 5% CO₂. The passage ranging 4–8 of HMC-1 cells was used throughout the study.

Preparation of LEJL

The plants of *Eriobotrya japonica* was purchased from the oriental drug store, Bohwa Dang (Jeonbuk, South Korea). A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The leaves of *E. japonica* were grinded (1000 rpm, 30 sec) at room temperature using Micro Hammer-Cutter Mill (Culatti Co., Zurich, Switzerland). The particle size was 0.5–2 mm after grinding. The sample (50 g) was extracted with purified water (500 ml) at 70°C for 5 h in water bath. The yield of dried extract from starting crude materials was about 12.5%. The extract was filtered and lyophilized. The dried extract was dissolved in PBS before use.

Animals

The original stock of male ICR mice and male Sprague-Dawley rats were purchased from Dae-Han Biolink Co. Ltd. (Daejeon, South Korea). The animals were housed 5 per cage in a laminar air flow room maintained under a temperature of 22 $\pm$ 2°C and relative humidity of 55 $\pm$ 5% throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care

and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Compound 48/80-induced systemic reaction

Mice were given an intraperitoneal injection of 8 mg/kg, body weight (BW) of the mast cell degranulator, compound 48/80. LEJL was intraperitoneally administered 1 h before the injection of compound 48/80 ($n=10$ /group). Mortality was monitored for 1 h after induction of anaphylactic shock. After the mortality test, blood was obtained from the heart of each mouse to measure serum histamine content.

Passive cutaneous anaphylaxis (PCA)

An IgE-dependent cutaneous reaction was examined as previously described.⁽¹⁷⁾ PCA reaction was generated by sensitizing skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the mouse tail vein. The mice were injected intradermally with 0.5 μ g of anti-DNP IgE. After 48 h, each mouse was received an injection of 1 μ g of DNP-HSA containing 4% Evans blue (1:4) via the tail vein. Thirty minutes after the challenge, the mice were killed and the dorsal skin was removed for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with 1 ml of 1 M KOH and 9 ml of a mixture of acetone and phosphoric acid (5:13) based on the previous report.⁽¹⁸⁾ The intensity of absorbance was measured at 620 nm in a spectrophotometer (Ultrospec 2100, Amersham Biosciences, Piscataway, NJ, USA).

Preparation of rat peritoneal mast cells (RPMC)

RPMS were isolated as previously described.⁽¹⁹⁾ In brief, the peritoneal cells were collected and suspended in Tyrode buffer (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 0.1% bovine serum albumin), layered on 2 ml of metrizamide (22.5 w/v%), and centrifuged for 15 min at 400 g. The cells that remained at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode buffer. Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue exclusion.

Histamine assay

Histamine content from serum and RPMC were measured by the o-phthalaldehyde spectrofluorometric procedure.⁽²⁰⁾ The blood from the mice was centrifuged at 400 g for 10 min and the serum was withdrawn to measure histamine content. RPMC suspensions (2 $\times$ 10⁵ cells/ml)

were sensitized with anti-DNP IgE (10 µg/ml) for 16 h. The cells were preincubated with the LEJL (0.01 to 1 mg/ml) at 37°C for 10 min prior to the challenge with DNP-HSA (1 µg/ml). The cells were separated from the released histamine by centrifugation at 400 g for 5 min at 4°C.

Western blot analysis

HMC-1 cells (3×10^6 /6-well plate) were washed with PBS and resuspended in lysis buffer. Samples of protein were electrophoresed using 12% SDS-PAGE, as described elsewhere⁽²¹⁾ and then transferred to nitrocellulose membrane. The TNF- α was assayed using anti-TNF- α antibody (R&D Systems Inc. Minneapolis, MN, USA). Immunodetection was done using enhanced chemiluminescence detection kit (Amersham).

Statistical analysis

Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC, USA). Treatment effects were analyzed using one way analysis of variance followed by Duncan's Multiple Range test. A value of $p < 0.05$ was used to indicate significant differences.

Results

LEJL inhibits compound 48/80-induced systemic reaction

To determine the effect of LEJL on allergic reaction, an *in vivo* model of systemic reaction was used. Compound 48/80 (8 mg/kg BW) was used to induce a systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. As shown in Table 1, injection of compound 48/80 to mice induced

Table 1. Effect of LEJL on compound 48/80-induced systemic anaphylaxis.

LEJL treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (saline)	+	100
5	+	100
10	+	60
50	+	60
100	+	50
500	+	0
1000	+	0
1000	–	0

Groups of mice ($n=10$ /group) were intraperitoneally pretreated with 200 µl of saline or LEJL at various doses 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice $\times 100$ / total number of experimental mice.

fatal shock in 100% of animals. When LEJL was intraperitoneally administered at concentrations ranging from 5 to 1000 mg/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced. In addition, the mortality of mice administered with LEJL (1000 mg/kg) 5, 10, 15, and 20 min after compound 48/80 injection increased time-dependently (Table 2).

LEJL decreases compound 48/80-induced serum histamine release

The effect of LEJL on compound 48/80-induced serum histamine release was investigated. LEJL was given at concentrations ranging from 1 to 1000 mg/kg BW 1 h before ($n=10$ /group) compound 48/80 injection. After 30 min of compound 48/80 injection, blood was withdrawn from the mice and the histamine content was

Table 2. Time-dependent effect of LEJL on compound 48/80-induced systemic anaphylaxis.

LEJL treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Time (min)	Mortality (%)
None (saline)	+		100
1000	+	0	0
	+	5	0
	+	10	60
	+	15	60
	+	20	100

Mice ($n=10$ /group) were intraperitoneally pretreated with 200 µl of saline or LEJL. LEJL (1000 mg/kg) was given 5, 10, 15 and 20 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice $\times 100$ / total number of experimental mice.

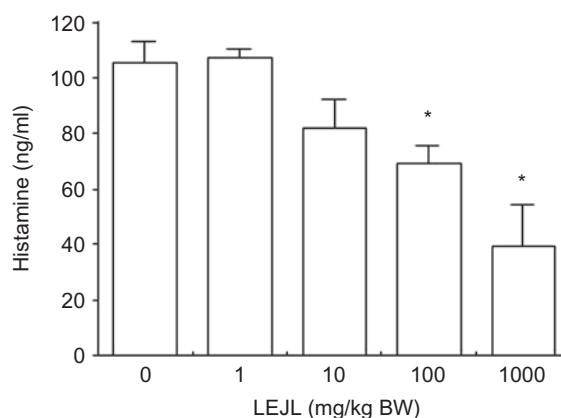


Figure 1. Effect of LEJL on compound 48/80-induced serum histamine release. Groups of mice ($n=10$ /group) were intraperitoneally pretreated with 200 µl saline or LEJL. LEJL was given at various doses 1 h before the compound 48/80 injection. The compound 48/80 (8 mg/kg, body weight) was intraperitoneally given to the group of mice. After the mortality test, blood was obtained from the heart of each mouse to measure serum histamine content. Each data represents the mean $\pm$ SEM of three independent experiments. * $p < 0.05$: significantly different from the saline value.

measured. LEJL dose-dependently decreased compound 48/80-induced serum histamine release (Figure 1).

LEJL inhibits IgE-mediated PCA reaction

Anti-DNP IgE was injected into the right dorsal skin sites. As a control, the left dorsal skin site of these mice was injected with saline alone. After 48 h, all animals were injected intravenously with DNP-HSA and Evans blue dye. LEJL was administered intraperitoneally 1 h prior to the challenge with antigen. The administration of LEJL (1–1000 mg/kg, BW) showed a dose-dependent inhibition in the PCA reaction (Figure 2).

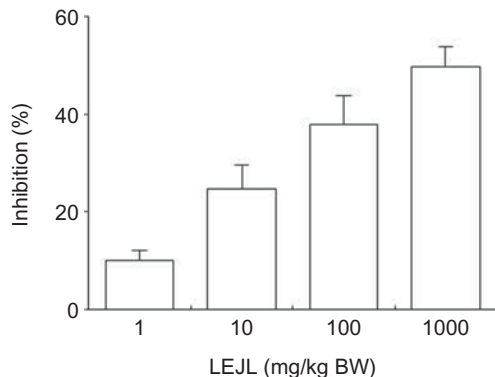


Figure 2. Effect of LEJL on the 48 h PCA. LEJL was intraperitoneally administered 1 h prior to the challenge with antigen. Each data represents the mean $\pm$ SEM of three independent experiments. * $p < 0.05$: significantly different from the saline value.

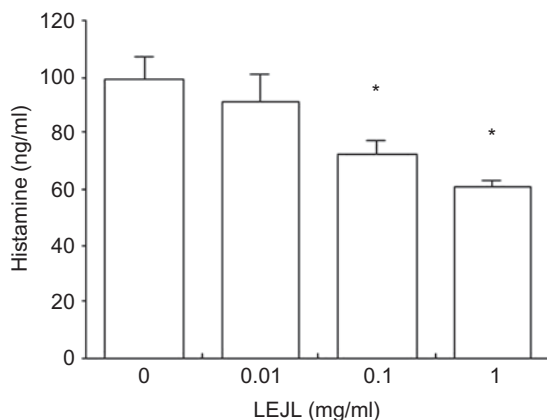


Figure 3. Effect of LEJL on IgE-mediated histamine release from RPMC. Cells (2×10^5 cells/ml) were preincubated with LEJL at 37°C for 10 min prior to incubation with DNP-HSA (anti-DNP IgE sensitized RPMC). Each data represents the mean $\pm$ SEM of three independent experiments. * $p < 0.05$: significantly different from the saline value.

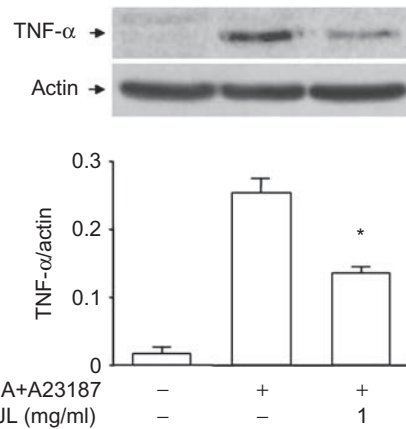


Figure 4. Inhibitory effect of LEJL on production of TNF- α in HMC-1 cells. HMC-1 cells were treated with LEJL for 30 min before being stimulated with PMA (20 nM) and A23187 (1 μ M). The production of TNF- α was analyzed by Western blot. Actin was used as a loading control. *Significantly different from the PMA and A23187 value at $p < 0.05$.

LEJL reduces IgE-mediated histamine release from RPMC

The inhibitory effect of LEJL on IgE-mediated histamine release from RPMC is shown in Figure 3. LEJL dose-dependently inhibited IgE-mediated histamine release at concentrations of 0.1–1 mg/ml.

LEJL inhibits production of TNF- α from HMC-1 cells

The effect of LEJL on the production of TNF- α was tested by Western blot. As shown in Figure 4, the production of TNF- α was increased by stimulation of PMA and A23187. Pretreatment of LEJL inhibited PMA and A23187-induced production of TNF- α . The concentration of LEJL used in the experiment did not interfere with the viability of cells as assessed by MTT assay (data not shown).

Discussion

Mast cell-induced anaphylactic allergic reaction is a life threatening syndrome induced by the sudden systemic release of inflammatory mediators, such as histamine, heparin, lipid-derived mediators and various cytokines from mast cells. Mast cells are located throughout the human body, and upon allergen exposure, they are stimulated via the IgE receptor.⁽⁶⁾

The results of this study demonstrated that LEJL has anti-allergic properties. Pretreatment with LEJL profoundly inhibited compound 48/80-induced systemic reaction and IgE-mediated local allergic reaction. LEJL inhibited IgE-mediated histamine release from RPMC. These results indicate that mast cell-mediated allergic reactions are inhibited by LEJL.

It is known that stimulation of mast cells with compound 48/80 or IgE initiates the activation of a signal transduction pathway which leads to histamine release. Several studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins.⁽²²⁾ The evidence indicates that the protein is Gi-like and that the activation is inhibited by benzalkonium chloride.⁽²³⁾ Tasaka et al.⁽²⁴⁾ reported that compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. LEJL might act on the lipid bilayer membrane thus preventing the prevention of the perturbation being induced by compound 48/80.

PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reactions. The mice administered with LEJL were protected from IgE-mediated PCA. This finding suggests that LEJL might be useful in the treatment of allergic skin reactions.

Mast cell-derived cytokines, especially TNF- α have a critical biological activity in the allergic reaction. It has been reported that mast cells are a principal source of TNF- α in human dermis, and degranulation of mast cells in the dermal endothelium is abrogated by the anti-TNF- α antibody.⁽²⁵⁾ This report may indicate that a decrease in TNF- α is a one of the key indicators of reduced allergic symptoms. The HMC-1 cell line is useful for studying cytokine activation pathways.⁽²⁶⁾ LEJL inhibited the production of TNF- α in PMA and A23187-stimulated HMC-1 cells. This result may suggest that one possible pathway of the anti-allergic effect of LEJL results from the reduction of TNF- α from mast cells. The effect of LEJL on TNF- α production by mast cells *in vivo* and the relative importance of mast cells as a source of TNF- α during inflammatory and immune responses are important areas for future studies.

Conclusion

In conclusion, the results obtained in the present study provide evidence that LEJL might contribute to the treatment of mast cell-mediated allergic diseases.

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