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Research Paper

Animal studies supporting the inhibition of mast cell activation by *Eriobotrya japonica* seed extract

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Abstract

Objectives The potent antioxidant activity of *Eriobotrya japonica* seed extract (ESE) and its usefulness in the prevention and treatment of various disorders has been reported previously. Its antioxidant activity associated with β-sitosterol and polyphenols contained in the extract was also validated. In this study, anti-allergic activity of *Eriobotrya japonica* seed extract was investigated.

Methods The inhibition of histamine release-mediated type 1 allergy by *Eriobotrya japonica* seed extract was used as an index.

Key findings The administration of this extract inhibited histamine release from rat mast cells, suggesting its usefulness in allergic disease treatment. In an experiment using a guinea-pig allergic rhinitis model, this extract reduced the frequency of sneezing and nose-scratching.

Conclusions These results suggest that *Eriobotrya japonica* seed extract may contribute to the relief of allergic disease-related symptoms.

Keywords anti-allergic activity; *Eriobotrya japonica* seed extract; histamine release

Introduction

Eriobotrya japonica has long been used as a pharmaceutical plant. In particular, leaves of *E. japonica* have been employed as an ingredient of traditional Chinese herbal preparations to treat dermal disorders or relieve inflammation, pain, cough and sputum.[1] Leaves of *E. japonica* have recently been shown to contain polyphenols, and a blood glucose-lowering action,[2] anti-inflammatory action[3,4] and anti-cancer action[5] have been reported. Its seeds have been reported to contain nitrile aromatic compounds such as amygdalin. They have also been employed instead of Annin, a traditional Chinese herbal preparation. *E. japonica* seeds contain unsaturated fatty acids such as linoleic and linolenic acids, vegetable sterols such as β-sitosterol and polyphenols such as caffeic and chlorogenic acids other than aromatic compounds.[6] A 70% ethanol-extracted *E. japonica* seed extract (ESE) has been found useful for preventing and treating various disorders such as hepatopathy and nephropathy.[7,8] ESE prepared in this study may exhibit potent antioxidant actions, contributing to the prevention and treatment of oxidative stress-related disorders. As the involvement of oxidative stress in serious allergic disorders has been suggested,[9] the usefulness of this ESE in allergic disease treatment was investigated using the inhibition of histamine release-mediated type 1 allergy as an index.

Materials and Methods

Samples

A cell-counting kit, Compounds 48/80, histamine and o-phthalaldehyde were purchased from Wako Pure Chemical Industry Co., Ltd. (Japan). Evans blue was purchased from Nakalai Tesque Co., Ltd. (Japan). Anti-dinitrophenyl (DNP) antibody, dinitrophenyl-bovine serum albumin (DNP-BSA) and cedar pollen were purchased from Cosmo Bio Co., Ltd. (Japan).

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Preparation of *Eriobotrya japonica* seed extract

Eriobotrya japonica seeds were collected from Mogi loquats cultivated in Wakayama and Kochi Prefectures, and dried in the sun. One kilogram of seeds was crushed (1000 rev/min) using a crusher with a cooler, immersed in 2 l of 70% ethanol and continuously agitated for one week to extract their components. After filtration, the extract was concentrated using an evaporator and then diluted with purified water to prepare a volume of 200 ml. The extract was stored in a refrigerator until administration.

Investigation of active ingredients using high-performance liquid chromatography (HPLC)

The active components in ESE were analysed by HPLC (Figure 1). The data represent the polyphenol components and contents.

Animals

Seven-week-old Wistar rats and three-week-old Hartley guinea-pigs were purchased from Japan SLC. The animals were acclimated in a special facility under the following conditions: room temperature, 19–25°C; humidity, 30–70%; and lighting cycle, 12 h. Food and water were freely available. Experimental protocols used in this study were approved by the Experimental Animal Research Committee at Kochi University.

Preparation of rat peritoneal mast cell suspension

The suspension was prepared as described by Inagaki *et al.*[10] Under anaesthesia with diethyl ether, rats were exsanguinated, and 20 ml of Tyrode solution containing 10 mM HEPES was infused into the abdominal cavity. The abdomen was massaged for 90 s, and then laparotomy was performed to collect intraperitoneal exudate. The abdominal cavity was washed with 20 ml of the above solution, and centrifuged at 4°C and 55*g* for 8 min. Similarly, cells were washed in the above solution three times, and mixed with the same solution so that the final concentration of mast cells was 10^6 cells/ml. Intracellular granule staining with 0.05% toluidine blue N was performed to identify mast cells.

Cytotoxicity test

Cytotoxicity was measured by lactate dehydrogenase (LDH) assay. LDH activity was measured by enzymatic test through the conversion to WST-8 formazan. The mast cell suspension was added to a serially diluted ESE to prepare a concentration of 10^3 cells/well. The solution was cultured in an incubator containing 5% CO_2 at 37°C for 48 h. Then, the absorbance of synthetic formazan pigment at 450 nm was measured using a cell-counting kit. The reaction was stopped by adding 10 μl of 0.1 M HCl.

Measurement of histamine release by rat peritoneal mast cells

Tyrode solution containing 10 mM HEPES was preincubated at 37°C for 5 min. The mast cell suspension was diluted using Tyrode solution to a final concentration of 10^5 cells/ml, and incubated at 37°C for 15 min. Then, as degranulation agents, Compounds 48/80 were added to the solution to prepare a final concentration of 10^{-6} M. The solution was incubated at 37°C for 20 min, and cooled on ice for 10 min to stop the reaction. After centrifuging at 4°C and 300*g* for 10 min, 0.5 ml supernatant was collected and mixed with

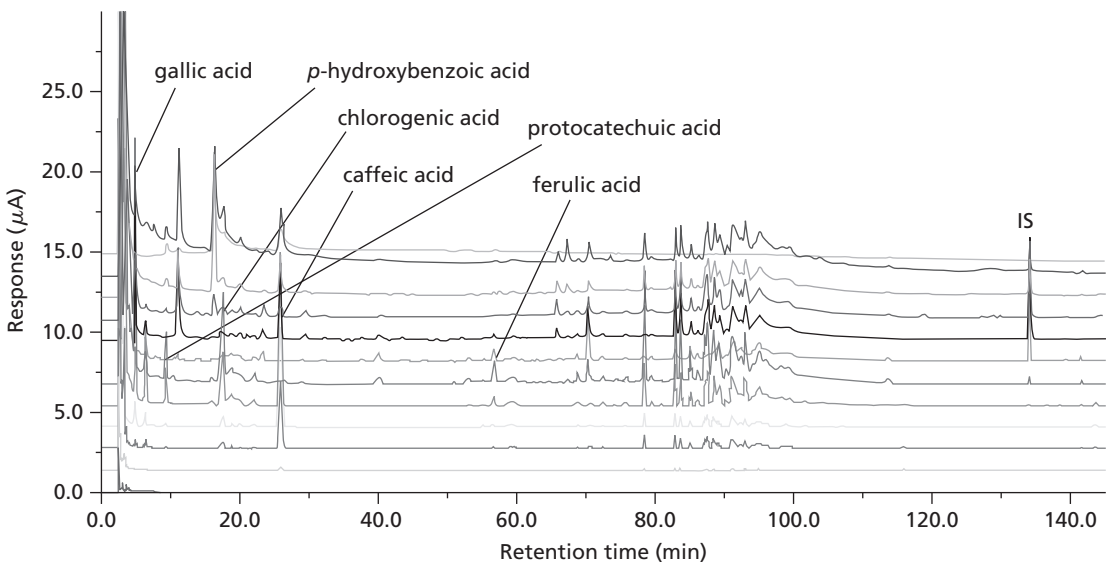

Figure 1 Representative HPLC chromatograms for polyphenols. Detector, CoulArray, Model 5600A (ESA Co., Ltd); applied potentials, –80 to 800 mV in + 80 mV increments; column, MCM (4.6 × 250 mm; 5 μm); flow rate, 1.0 ml/min; column temperature, 35°C; mobile phase A, 100 mM sodium phosphate, 5% methanol; mobile phase B, 100 mM sodium phosphate, 60% acetonitrile, 10% methanol. Gradient condition: linear increase of phase B from 0% to 6% from 0–45 min; linear increase of phase B from 6% to 14% from 45–60 min; linear increase of phase B from 14% to 30% from 60–90 min; linear increase of phase B from 30% to 40% from 90–120 min; linear increase of phase B from 40% to 77% from 120–140 min; isocratic at 85% phase B until 100 min. IS, internal standard.

0.5 ml of 0.2 M HCl. The precipitate was mixed with 2 ml of 0.1 M HCl, and heated in a boiling water bath for 10 min. The solution was cooled on ice and then centrifuged at 4°C and 780*g* for 10 min. The supernatant at 1 ml was collected and each sample was used to quantify histamine. The histamine level was measured as described by Shore *et al.*[11] A 1-ml sample was mixed with 0.1 ml of 2 M NaOH and 0.05 ml of 1% o-phthalaldehyde MeOH solution. After standing at room temperature for 4 min, 0.1 ml of 2 M citric acid was added to stop the reaction. The fluorescence intensity was measured at an excitation wavelength of 356 nm and fluorescence wavelength of 440 nm. The inhibition rate was calculated in comparison with a control sample.

Vascular permeability related to antigen–antibody responses

Vascular permeability was evaluated based on passive cutaneous anaphylaxis (PCA) on the rat dorsal skin.[12] Anti-DNP antibody (titer: 1 : 2000 or more) at 100 μl was intracutaneously administered to the rat back. After 48 h, 1 ml of 50 mg/ml Evans blue solution containing 1 mg/ml of DNP-BSA as an antigen was intravenously administered. The ESE was orally administered at various doses (0.5, 1 and 2 ml) 1 or 2 h (dose, 2 ml) before antigen administration. As a control agent, physiological saline was administered. Rats were sacrificed 30 min after antigen administration and the dorsal skin was collected. It was placed in a test tube, mixed with 1 ml of 1 N NaOH, and incubated at 37°C for 24 h. Acetone–0.6 N phosphoric acid solution (13 : 5) was added, agitated and centrifuged at 1500*g* for 15 min. The absorbance of the supernatant at 620 nm was measured, and the inhibition rate was calculated in comparison with a control sample.

Measurement of nasal symptoms (sneezing, nose-scratching) caused by antigen administration

As described by Nabe *et al.*,[13] an allergic rhinitis model using cedar pollen in guinea-pig was prepared. For sensitization, a cedar pollen extract (3 μl) of 0.1 mg of $Al(OH)_3$ gel containing 0.1 μg/μl of cedar pollen-derived protein was administered into both nostrils twice a day for seven days. Then, guinea-pigs were made to inhale 1.8 mg of cedar pollen via a special hand-made inhalator once a week. The frequency of nasal symptoms (sneezing, nose-scratching) was measured for 10 min after inhalation. Cedar pollen inhalation was continued for 10 weeks. After the end of sensitization, the ESE was diluted at a ratio of 10, and given freely at a volume of 100 ml per day using a water supply bottle.

Results

Cytotoxicity test

LDH release by rat-derived mast cells varied among the doses of the ESE (Figure 2). In the 100-fold dilution group, some mast cells showed a higher LDH release in comparison with the ESE-free group, although there was no significant difference. Furthermore, there were no significant differences between the 200- to 500-fold dilution groups and ESE-free group.

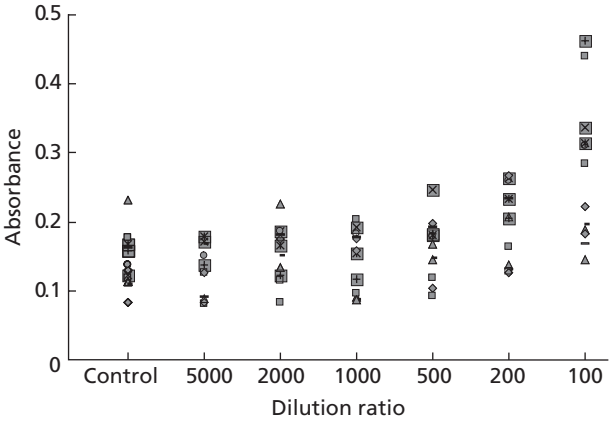

Figure 2 Cytotoxic effect of *Eriobotrya japonica* seed extract on rat peritoneal mast cells. We show absorbance of WST-8 formazan generated by enzyme reaction. Each point represents 10 experiments. Statistical analysis was performed using two-way analysis of variance followed by Dunnett's test.

Influence of the *Eriobotrya japonica* seed extract on histamine release by rat mast cells

The rate at which histamine release by rat mast cells was inhibited varied among the concentrations of this extract; it increased in a dose-dependent manner (Figure 3). In the 200-fold dilution group, in which there was no cytotoxicity, histamine release was inhibited in 75.8% of the mast cells.

Influence of this extract on vascular permeability on the rat dorsal skin

Vascular permeability on the rat dorsal skin depended on the administration time and dose of this extract. When administering this extract at different times before antigen administration, the tissue pigment levels 1 and 2 h before administration were significantly lower than those in the untreated group (Figure 4a). There was no marked difference between the levels 1 and 2 h before antigen administration.

When administering this extract at different doses 2 h before antigen administration, the tissue pigment level

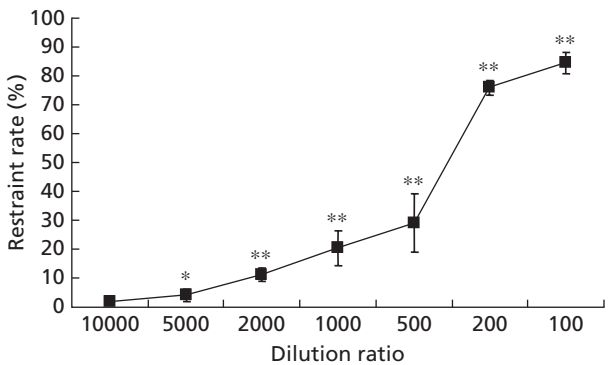

Figure 3 Effect of *Eriobotrya japonica* seed extract on histamine release by rat peritoneal mast cells. Each point represents the mean of six experiments. $*P < 0.05$, $**P < 0.01$, compared with control (*Eriobotrya japonica* seed extract untreated group). Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test.

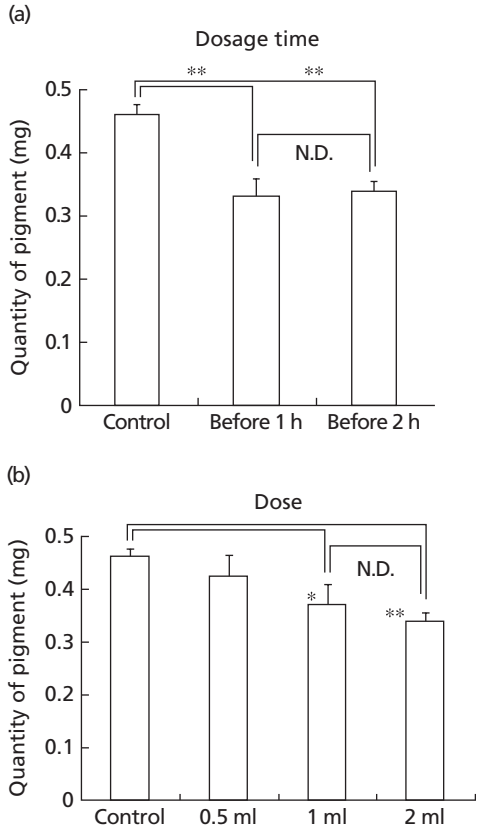

Figure 4 Effect of *Eriobotrya japonica* seed extract on blood vessel permeability of the rat back skin: vascular permeability related to antigen–antibody responses. We examined dosage time (a) and various doses (b) of *Eriobotrya japonica* seed extract. Each column represents the mean ± SEM of six experiments. $*P < 0.05$, $**P < 0.01$, compared with control. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test. N.D., no significant different.

decreased in a dose-dependent manner in comparison with the untreated group. There was a significant difference between the 1- and 2-ml groups (Figure 4b).

Influence of this extract on sneezing and nose-scratching

In our guinea-pig allergic rhinitis model, the frequency of sneezing and nose-scratching depended on the presence or absence of the administration of ESE. The frequency of sneezing decreased after four weeks of administration and frequency of nose-scratching reduced after five weeks. In the treatment group, the cumulative frequency after 10 weeks of treatment was half that of the untreated group (Figure 5). This persisted until week 25 (data not shown).

Discussion

Allergic disorders such as bronchial asthma, atopic dermatitis and allergic rhinitis are refractory, and recent environmental changes have increased the number of patients, raising a social issue. In particular, allergic rhinitis represents type 1 allergy and, currently, it is estimated to affect more than 20% of the population in Japan.[14] In the future, the number of patients will increase, raising an important national health problem.

Allergic rhinitis develops via the following process of capturing antigens in the mucosal layer: antigens are absorbed by the nasal mucosa, treated by antigen-presenting cells such as macrophages, and recognized by T cells, leading to the production of cytokines such as interleukin-4. These cytokines produce IgE antibody by acting on B cells. The IgE antibody binds to the mast cell membrane of the nasal mucosa, achieving sensitization. After a specific period, antigens invade the host again, and bind to IgE antibody, releasing chemical mediators such as histamine and cysteinyl leukotrienes (CysLTs). These mediators attack the tissue, causing allergic rhinitis.[15,16]

In patients with allergic rhinitis, antigen induction in a topical nasal area immediately causes sneezing, and enhances the secretion of nasal discharge, leading to nasal obstruction. In addition, repeated exposure to antigens results in chronic symptoms, the enhancement of nasal discharge secretion and nasal obstruction.[17–19]

Produced/released histamine may stimulate the trigeminal terminal, causing sneezing by exciting the motor nerves and simultaneously secreting nasal discharge by exciting the parasympathetic nerves.

The ESE inhibited histamine release by rat mast cells. Therefore, this extract contains a component that inhibits histamine release and may be useful for relieving the symptoms of various allergic disorders by suppressing immediate responses related to type 1 allergy. Antihistamine agents inhibit sneezing and the enhancement of nasal discharge secretion.[20,21]

Furthermore, this extract inhibited the antigen–antibody response-related enhancement of vascular permeability. It may have reduced vascular permeability induced by histamine released from mast cells via antigen–antibody responses, suggesting its usefulness in allergic disease treatment *in vivo*.

Considering the clinical application of this extract, ESE was administered in a guinea-pig allergic rhinitis model. It was useful for relieving nasal symptoms due to cedar pollen-related antigen stimulation. There are two reasons to choose this model. The first is that this model resembles allergic rhinitis in man. The second is that this model evaluates protective efficacy of ESE. However, it is difficult to explain such a decrease in the frequency of sneezing and nose-scratching only by the antihistaminic actions of this extract, because the tissue level of this extract given freely did not rapidly increase and because its high concentration was not maintained. The influence of this extract on the frequency of sneezing and nose-scratching appeared in weeks three to four of treatment. After 10 weeks of treatment, its influence became marked, suggesting the influence of other factors, such as the relief of hypersensitivity to antigens, on a decrease in the frequency of sneezing and nose-scratching. This issue is being immunologically reviewed.

Conclusions

These results suggest the usefulness of ESE in allergic disease treatment. However, it remains to be clarified which component exhibits these pharmacological actions. It has been reported that this extract contains various polyphenols. Some polyphenols have been reported to exhibit anti-allergic actions.[22–25] Currently, we hypothesize that the efficacy of this extract is associated with these polyphenols, not with a single component.

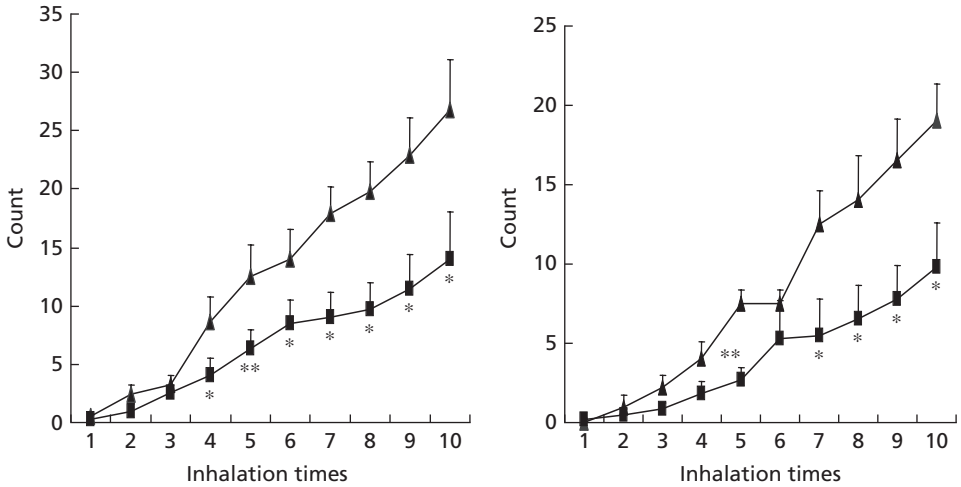

Figure 5 Effect of *Eriobotrya japonica* seed extract on nose symptoms (sneezing, nose-scratching) in guinea-pigs. We measured frequency of sneezing and nose-scratching caused by inhalation of the pollen from one to ten weeks and showed it by accumulation: ▲, control; ■, *Eriobotrya japonica* seed extract group. Each point represents the mean ± SEM of eight experiments. $*P < 0.05$, $**P < 0.01$, compared with control. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's test.

In the future, active ingredients contained in ESE should be investigated in addition to immunological examination.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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