Inhibitory Effects of *Selaginella tamariscina* on Immediate Allergic Reactions

Yue Dai  
*Department of Pharmacology, China Pharmaceutical University, China*

Paul Pui-Hay But, Lee-Man Chu and Yiu-Pong Chan  
*Department of Biology and Institute of Chinese Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong, China*

Abstract: The anti-allergic effects of a 70% ethanol extract from *Selaginella tamariscina* herb (EST) were evaluated in this study. EST given at the doses of 500 and 1000 mg/kg can inhibit mouse systemic anaphylactic shock induced by compound 48/80 in a dose-dependent manner. It can also dose-dependently block rat homologous passive cutaneous anaphylaxis and skin reactions caused by exogenous histamine and serotonin with a significant difference observed at the dose of 1000 mg/kg. In addition, EST can reduce histamine release from rat peritoneal mast cells triggered by compound 48/80 and an antigen *in vitro*. When incubated with rat mast cells, the extract (200 μg/ml) can significantly elevate the intracellular cAMP levels. The finding suggests that EST inhibits mast cell-dependent, immediate allergic reactions. Its effects appear to be mediated by reducing the release of vasoactive amines such as histamine from mast cells *via* stabilizing the cell membrane and weakening the inflammatory action of these amines. Based on these results, *Selaginella tamariscina* and one of its active components flavonoids may be useful as potential remedies for allergic rhinitis and other allergy-related diseases.

*Keywords:* *Selaginella tamariscina*; Anaphylactic Shock; Mast Cells; Passive Cutaneous Anaphylaxis; Histamine Release; cAMP.

Introduction

It has been suggested that allergic rhinitis, the most common atopic disease, is induced by immunoglobulin E (IgE)-mediated immediate allergic reactions to some specific allergens (e.g. pollens, molds, animal dander, and dust mites). The immune responses involve the
release of several inflammatory mediators from mast cells, as well as the activation and recruitment of cells to the nasal mucosa (Skoner, 2001). In Hong Kong, more than 30% of children are affected by allergic rhinitis. In order to identify some potential remedies for the disease, we screened several medicinal herbs and herbal products that are often used by some patients in Hong Kong to relieve the symptoms of allergic rhinitis. We found that Selaginella tamariscina was one of the active herbs.

In traditional Chinese medicine, the herb of S. tamariscina is often used to treat chronic trachitis, thrombocytopenic purpura and several forms of cancers (Jiangsu New Medical College, 1979; Zheng et al., 1998). Pharmacological studies have demonstrated that S. tamariscina possesses anti-bacterial, anti-hypertensive, anti-hyperglycemic, analgetic (Zheng et al., 1998; Miao et al., 1996), and anti-tumoral (Lee et al., 1999) activities. The major constituents in S. tamariscina herb are flavonoids (e.g. amentoflavone, hinokiflavone, sotetsuflavone and apogenin) (Cheong et al., 1998; Zheng et al., 1998) and saccharides (e.g. trehalose, D-glucose, D-fructose and D-rhamnose) (Shimada et al., 1984). In the present study, we described the inhibitory effects of the ethanol extract from S. tamariscina on experimental immediate allergic reactions.

**Materials and Methods**

**Preparation of Plant Extract**

Samples of S. tamariscina were purchased from a commercial herb market in Hong Kong. The identity was confirmed as the total herb of S. tamariscina (Beauv.) by anatomical observation, thin-layer chromatography (TLC) analysis and direct comparison with the authentic specimens, which are stored in the Herbarium in the Department of Biology, The Chinese University of Hong Kong. A voucher specimen (But 0104) is deposited in the Museum of the Institute of Chinese Medicine at The Chinese University of Hong Kong.

Two hundred grams of S. tamariscina herb were ground and refluxed with 70% ethanol (1.5 liter) for 1 hour. The refluxing process was repeated 3 times. Once filtered, the clear supernatants were pooled and concentrated under reduced pressure at 45°C by a vacuum rotary evaporator. Then, the concentrated extract solution was lyophilized until dry (14.5 g). The dried extract (EST) was freshly dissolved in distilled water or phosphate buffered saline (PBS) (NaCl 154 mM, KCl 2.7 mM, CaCl₂ 0.9 mM, Na₂HPO₄ 4 mM, and KH₂PO₄ 2.7 mM) before use.

**Chemicals and Reagents**

Compound 48/80, ovalbumin (chicken egg, Grade V), L-α-phosphatidyl-L-serine and 5-hydroxytryptamine hydrochloride (serotonin) were purchased from Sigma (St. Louis, MO). Inactive bacterial suspension of Bordetella pertussis, o-phthalaldehyde (OPT), prednisolone and histamine dihydrochloride were purchased from Wako (Osaka, Japan). Disodium cromoglycate (DSCG) was obtained from Biomol (PA, USA). 

\(^{125}\)I-cAMP
radioimmunoassay kit (Fang et al., 1986) was purchased from the Shanghai University of Traditional Chinese Medicine. The purity of all chemical reagents was of analytical grade.

Animals

Male Sprague-Dawley rats (weighing 180–220 g) and male ICR mice (18–20 g) were obtained from the animal centers of The Chinese University of Hong Kong and the China Pharmaceutical University. They were allowed to acclimate in an air-conditioned room at 23 ± 2°C with a 12-hour light cycle from 8:00 am to 8:00 pm and access to food and water ad libitum. The animals used in the studies were in compliance with the current ethical regulations on animal studies at the China Pharmaceutical University.

Systemic Anaphylactic Shock Induced by Compound 48/80 in Mice

The experiment was carried out according to the method described previously by Shin et al. (1999). Briefly, each mouse was given an intraperitoneal injection of 10 mg/kg of compound 48/80. Then the mice were monitored for mortality for 1 hour after the induction of systemic anaphylactic shock. Either EST or vehicle was orally administered 1 hour before the injection of compound 48/80 as the treatments.

Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats

Rat anti-ovalbumin serum containing IgE was prepared as described earlier (Dai et al., 2004). In brief, rats were immunized with 0.5 ml of solution containing 1 mg of ovalbumin, 10 mg of aluminum hydroxide gel (s.c.), and 1 ml of inactive bacterial suspension of Bordetella pertussis (2 × 10^{10} cells/ml, i.p.) on Day 0. Seven days later (Day 7), immunization was repeated. Fourteen days later (Day 14), rats were anesthetized with the mixture of ketamine and xylazine, and the blood was withdrawn from their carotid arteries. The rat anti-ovalbumin serum was separated by centrifugation, and the anti-ovalbumin IgE antibody in the serum was determined by PCA in rats. The titer (1:32) was expressed as the maximal dilution causing a skin lesion greater than 5 mm in diameter in rats.

The anti-ovalbumin serum diluted four-fold with saline (50 μl) was intradermally injected into two sites on the shaved dorsal skin of rats. After 48 hours, rats were challenged with 0.5 ml of saline containing 2 mg of ovalbumin and 5 mg of Evans blue via the tail vein. After 30 minutes, rats were sacrificed. The skin samples around the two injecting sites were removed and incubated with 1 N KOH for 24 hours. The blue dye that leaked into the skin was extracted with a mixture of acetone and phosphoric acid, and was determined colorimetrically (Katayama et al., 1978). For treatments, either EST or vehicle was orally administered 1 hour before the challenge of the antigen (ovalbumin). Unlike EST, DSCG or vehicle was injected intravenously just before the challenge.
Histamine Release Induced by Compound 48/80 or Ovalbumin from Rat Peritoneal Mast Cells

The experiments were conducted according to the methods described by Kubo et al. (1994) and Matsuda et al. (2001). Basically, intact or sensitized rats, which were immunized 24 hours before the assays by intraperitoneal injections of 1 ml of rat anti-ovalbumin serum prepared in the previous rat PCA, were used in compound 48/80 or ovalbumin assays. Mixed peritoneal cells were collected by a peritoneal lavage and were purified by centrifugation through a Ficoll density gradient in order to separate the mast cells. The purified mast cells were then washed and resuspended in PBS containing 5.6 mM glucose and 0.1% bovine serum albumin. The mast cells in the preparations presented about 92% of total cells by the toluidine blue-staining method. The viabilities were confirmed around 90% before and after experiments by the trypan blue exclusion method.

The purified rat peritoneal mast cells (2 × 10^6 cells/ml) were pre-incubated at 37°C for 10 minutes. Then vehicle, EST and DSCG in PBS were added into the cells 5 minutes before they were activated by compound 48/80 (0.5 μg/ml) or ovalbumin (1 mg/ml) plus L-α-phosphatidyl-L-serine (100 μg/ml). The mast cells continued to be kept in an incubator for 10 minutes until the reactions were stopped by chilling the cells in ice water. The cell pellets and supernatant were then separated by centrifugation. 0.05% Triton-100 was added to the cell pellets to liberate the residual histamine before 0.036% OPT methanol solution was mixed with the supernatant or the cell pellets. Histamine contents in the supernatant (Supernatant) and the cell pellets (Cell pellet) were determined spectrofluorometrically (Em 360 nm, Ex 450 nm). For the estimation of the spontaneous histamine release (Spontaneous), the assay was performed exactly as the above steps without treatments (EST or DSCG) or activators (compound 48/80 or ovalbumin) in the mast cells. The percentage of histamine release was calculated by the following equation.

\[ \text{Histamine release (\%)} = \frac{\text{Supernatant} - \text{Spontaneous}}{\text{Supernatant} + \text{Cell pellet}} \times 100\% \]

cAMP Levels in Rat Peritoneal Mast Cells

The cAMP level was measured according to the method previously reported (Kim et al., 1999). In brief, purified mast cells were resuspended in the prewarmed (37°C) PBS. An aliquot of cells (2 × 10^5 cells/50 μl) were added to the tubes containing 50 μl of prewarmed EST (200 μg/ml) and incubated for different periods. The reactions in the tubes were terminated by the addition of 0.9 ml of ice-cold acidified ethanol (86% ethanol: 1N HCl = 99:1). The cell samples were then snap-frozen in liquid nitrogen followed by a quick thawing. This freezing-thawing cycle was repeated 4 times to destroy the mast cell membranes. The debris was sedimented by centrifugation (400xg at 4°C for 5 minutes). Then 0.9 ml of the supernatants was transferred to fresh Eppendorf tubes and allowed to evaporate to dryness under reduced pressure. The dried samples were reconstituted in 1 ml of the assay buffer for cAMP quantification using a ^125I-cAMP radioimmunoassay (RIA) kit.
Skin Reactions Induced by Histamine and Serotonin in Rats

The method of histamine- and serotonin-induced cutaneous reactions was adapted from the previously described study (Kimata et al., 2000). Briefly, 50 μl of histamine (2 μg) or serotonin (0.02 μg) were intradermally injected into the sites on the shaved dorsal skin of rats. Then, 1 ml of saline containing 5 mg of Evans blue was immediately injected into the tail veins. EST and prednisolone were orally administered 1 hour before the challenge of histamine and serotonin. Animals were sacrificed 30 minutes after the induction of reactions, and the skin samples surrounding each reaction site were removed. The extravasated dye in the skin samples was extracted and measured by the method previously described in rat PCA.

Statistics Analysis

Data are expressed as means ± SD. Statistical analysis was done by comparing the treatment means with the control means using one-way analysis of variance (ANOVA) followed by Student’s t-test. The statistical significance was set as less than 0.05 (p < 0.05).

Results

Effects on Systemic Anaphylactic Shock Induced by Compound 48/80 in Mice

As shown in Table 1, compound 48/80 at the dose of 10 mg/kg (i.p.) can produce a 100% fatal systemic anaphylactic shock in mice. Pretreatments with EST (500 and 1000 mg/kg, p.o.) can dose-dependently reduce the mortality rate.

Effects on Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats

EST (500 and 1000 mg/kg), when orally administered to the rats 1 hour before the ovalbumin challenge, dose-dependently inhibited PCA by 28.1% and 43.9%, respectively.

Table 1. Effects of EST on Anaphylactic Shock Induced by Compound 48/80 in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Mortality Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>EST</td>
<td>200</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>10</td>
<td>40</td>
</tr>
</tbody>
</table>

Mice were orally given EST 1 hour prior to intraperitoneal injections of compound 48/80 (10 mg/kg). Mortality rate (%) within 1 hour after the injection of compound 48/80 was represented as number of dead mice × 100/number of total experimental mice.
A significant difference was observed at the higher dose of 1000 mg/kg. In addition, the reference drug DSCG (2 mg/kg), when intravenously given to the rats just before the ovalbumin challenge, potently inhibited PCA by 64.1% (Fig. 1).

Effects on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80 or Ovalbumin

The spontaneous releases of histamine from rat peritoneal mast cells were 9.5% and 8.3%, respectively in the compound 48/80 and the ovalbumin assays without any treatment and any activator. As shown in Fig. 2, compound 48/80 (0.5 μg/ml) can elicit 65.4% of histamine release from mast cells in the intact rats. Ovalbumin (1 mg/ml) can elicit 41.1% of histamine release from mast cells in the sensitized rats. EST clearly inhibited compound 48/80-induced histamine release at concentrations of 50, 200 and 500 μg/ml, and it also clearly reduced antigen-induced histamine release at concentrations of 50, 100 and 200 μg/ml. DSCG, a stabilizer of the mast cell membrane, markedly reduced histamine release induced by compound 48/80 and the antigen.

Effects on cAMP Level in Rat Peritoneal Mast Cells

As shown in Fig. 3, when mast cells were incubated with EST (200 μg/ml), their intracellular cAMP levels increased significantly. Their cAMP levels peaked at 2 minutes after the addition of EST, and then they gradually returned to their basal level after 10 minutes.

Effects on the Cutaneous Reactions Induced by Histamine and Serotonin in Rats

Cutaneous reactions on rat skins can be elicited by intradermal injections of histamine (2 μg) and serotonin (0.02 μg), respectively. EST (500 and 1000 mg/kg, p.o.) prevented...
Figure 2. Effects of EST and DSCG on compound 48/80- or antigen-induced histamine release from rat peritoneal mast cells. Data are expressed as means ± SD from three experiments. *p < 0.05, #p < 0.01.

Figure 3. Effect of EST on the cAMP levels of rat peritoneal mast cells. Data are expressed as means ± SD from three experiments. *p < 0.05.

Figure 4. Effects of EST and prednisolone (Pred) on histamine- or serotonin-induced permeability in rats. Data are expressed as means ± SD (n = 7). *p < 0.05, #p < 0.01.
the cutaneous responses to the vasoactive amine (histamine) in a dose-dependent manner by 30.4% and 43.7%, and reduced the cutaneous reaction induced by serotonin by 15.2% and 25.9%, respectively. Significant differences were observed at the dose of 1000 mg/kg for EST. Prednisolone as a steroidal anti-inflammatory agent significantly inhibited the histamine- and serotonin-induced reactions at the dose of 20 mg/kg (Fig. 4).

Discussion

The present study demonstrated that the 70% ethanol extract from *S. tamariscina* (EST) inhibited compound 48/80-induced systemic anaphylaxis in mice and homologous passive cutaneous anaphylaxis (PCA) in rats. Compound 48/80, a classic secretagogue for mast cells, can induce a mast cell-dependent, non-specific anaphylactic reaction. The mechanism of the anaphylactic shock induced by compound 48/80 is due to the massive release of vasoactive amines such as histamine from mast cells and basophils (Kim *et al.*, 1999; Amir and English, 1991). Rat PCA, a typical animal model of Ig E-mediated immediate allergic reactions, is also mainly induced by vasoactive mediators such as histamine from mast cells. However, the release of the mediator in PCA differs from that caused by compound 48/80, which is mediated through the aggregation of specific IgE receptors (FcεRI) on the surface of mast cells by their corresponding antigens. In our *in vitro* studies, EST clearly reduced histamine release from rat peritoneal mast cells induced by compound 48/80 or an antigen. It has been reported that agents that induce and sustain elevations of the intracellular cAMP level can attenuate the stimulated release of mediators from mast cells and basophils (Weston and Peachell, 1998; Makino *et al.*, 1987). EST, when incubated with rat mast cells, dramatically increased the intracellular cAMP level in the rat peritoneal mast cells. Therefore, it was suggested that EST probably prevented the activation of mast cells and the release of mediators by elevating their intracellular cAMP levels. Based on the above findings, it can be reasonably concluded that EST inhibited IgE-dependent or -independent degranulation and exocytosis of mast cells, consequently inhibiting release and anaphylactic responses.

EST also inhibited skin reactions induced by histamine or serotonin in rats at doses of 500 and 1000 mg/kg, which suggested that EST directly reducing the inflammatory action of these mediators.

Several flavonoid compounds isolated from *S. tamariscina*, have been reported to inhibit immediate allergic reactions. Amentoflavone reduced the histamine release from rat peritoneal mast cells elicited by compound 48/80 or ionophore A23187 (Bronner and Landry, 1985). Apigenin reduced the antigen-IgE-mediated hexosaminidase release as well as tumor necrosis factor-alpha (TNF-α) and interleukin-4 (IL-4) production from RBL-2H3 cells (Cheong *et al.*, 1998; Matsuda *et al.*, 2002). These findings suggest that flavonoids might be active components responsible for the anti-allergic activities of *S. tamariscina*.

In conclusion, the ethanol extract of *S. tamariscina* has shown inhibitory effects on immediate allergic reactions, and its mechanism of action is probably that of reducing the release of mediators such as histamine from mast cells and weakening the inflammatory
S. TAMARISCINA ON IMMEDIATE ALLERGIC REACTIONS

actions of mediators. S. tamariscina and one of its active components flavonoids may be useful as potential remedies for allergic rhinitis and other allergy-related diseases.

Acknowledgments

Partial support of the study was received with gratitude from the Innovation Technology Fund (AF/281/97), Government of the Hong Kong Special Administrative Region.

References


