Anti-allergic Effects and Related Active Constituents of Mung Bean (*Vignaradiatus* Linn) Sprouts

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Abstract The mung bean (*Vigna radiata*) is an important food crop with preventative effects against human diseases. The anti-allergic activities of mung bean sprouts of different lengths were evaluated by assaying *in vivo* antipruritic activity and *in vitro* hyaluronidase inhibitory effects. After 48 h of growth, sprouts were determined to have the best activity and extracted with petroleum (PeF), ethyl acetate (EaF), and n-butanol (nBF). The active EaF extracts were further assayed for *in vivo* effects on compound 48/80-induced mast cell degranulation and histamine release, as well as the anti-dinitrophenyl (DNP) IgE-induced passive cutaneous anaphylaxis (PCA) reaction. The main chemical constituents were further analyzed by UV spectrophotometry and high-performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS). EaF significantly protected against compound 48/80-induced mast cell degranulation and histamine release, and PCA. Flavonoids were determined to be the main contributors to the anti-allergic activity of the EaF extracts.

Keywords: mung bean sprouts, anti-allergic activity, mast cell, antihistamine, LC/MS/MS

Introduction

The mung bean (*Vigna radiata* Linn.) is a popular food worldwide with a long history of cultivation. Compared with soybean, mung bean has a high carbohydrate content, and starch is the main form of carbohydrate (1,2). Because of the high starch content, mung bean has typically been used to make the production of starchy noodles, muk, and mung bean sprouts (1). Modern pharmacological studies show that mung bean sprouts can help treat tumors, bacterial infections, and acne (3–5). The ethanol extracts of mung bean were also certificated to have anti-inflammatory effects through inhibition of proinflammatory cytokines including interleukin (IL)-1 β , IL-6, IL-12 β , tumor necrosis factor (TNF)- α , and inducible NO synthase (iNOS) in lipopolysaccharide (LPS)-induced activation of murine macrophages (J774). The proinflammatory cytokines usually are involved in series of cell immune response and local allergic reaction. The possible active components were suggested to be flavonoids vitexin, isovitexin, isouercitrin, kaempferol-3-O-rutinoside, and so on (1). The therapeutic functions of the mung bean are usually mediated by polyphenols, proteins, amino acids, carbohydrates, lipids, minerals, and other metabolites (3). A large proportion of active substances increased significantly during the sprouting process. In comparison with seeds, the sprouts contain more active substances and have more biological activity (6,7).

An allergic reaction involves the production of allergen-specific IgE, which binds to mast cells, and on subsequent cross-linking by allergen re-exposure, causes mast cell degranulation and release of signaling molecules such as histamine, eicosanoids, chemokines, and cytokines (8,9). Mast cells are major effector cells of allergic inflammation that are increasingly recognized for their roles in innate and adaptive immune responses. Mast cell degranulation can be induced by the compound 48/80. A mast cell degranulator has been used to study anaphylaxis mechanisms (10–12). In addition, anti-IgE antibodies that induce passive cutaneous anaphylaxis (PCA) are used as an experimental model for mast cell-dependent immediate-type allergic reactions (13). Considering that allergic response can be modulated by means of specific bioactive food components, inhibition of mast cell degranulation and PCA reaction is a reasonable strategy to modulate allergic response associated disease.

In the present study, the anti-allergic properties of mung bean sprouts (0–168 h) were evaluated by measuring *in vivo* antipruritic effects and *in vitro* hyaluronidase inhibitory activities. The extract of the 48-h sprouts was further extracted with petroleum (PeF), ethyl acetate (EaF), or n-butanol (nBF), and the EaF extract was determined to be the most active fraction. Experiments of compound 48/80-induced mast cell degranulation and histamine release, and anti-dinitrophenyl (DNP) IgE-induced passive cutaneous anaphylaxis (PCA) were conducted to evaluate the *in vivo* therapeutic effects of

EaF. Furthermore, the total flavonoid, polysaccharide, and protein contents of the EaF extract were determined by UV spectrophotometry. The main contributors to the anti-allergic activity of the EaF extract were quantitatively analyzed by LC/MS/MS. The results of this study show that mung bean sprouts are a promising anti-allergic food and a raw material that can be used in the development of medicines and cosmetics.

Materials and Methods

Plant materials Mung beans were germinated using previously published methods (1). Germinating seeds were kept moist with sterile water and incubated in an incubator without light at 26°C.

Extraction Air-dried sprouts (0.5 kg) were extracted with 80% ethanol (v/v) and successively partitioned between water and organic solvents to obtain 4 fractions, petroleum (PeF), ethyl acetate (EaF), *n*-butanol (nBF), and water (WtF) fractions. The extracts (0–168 h) resolved in 80% ethanol (v/v) and diluted by water were tested for anti-allergic activity using *in vitro* hyaluronidase inhibition and *in vivo* anti-pruritus assays. The active EaF fraction resolved in 80% ethanol (v/v) and diluted by water was used for *in vivo* assays of the PCA reaction, mast cell stabilization, and histamine release, as well as for chemical analysis.

Chemicals and reagents Compound 48/80, anti-dinitrophenyl (DNP) IgE, DNP-human serum albumin (HAS), Evans blue, disodium chromoglycate (DSCG), histamine phosphate, hyaluronidase, sodium hyaluronate, *p*-dimethylaminobenzaldehyde, and trypan blue were purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI (Roswell Park Memorial Institute, Buffalo, NY, USA) 1640 medium was purchased from HyClone Laboratories, Inc. (Logan, UT, USA). Histamine enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). MS-grade methanol was purchased from Merck (Darmstadt, Germany). Ultra-pure water (18.2 MΩ) was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). The reference flavonoids isovitexin (1), kaempferol-3-O-rutinoside (2), and isoquercitrin (3) were purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China), and *p*-coumaric acid (4) was purchased from Beijing Banxia Tech. Co., Ltd. (Beijing, China). All of the reference compounds were determined to be greater than 98% pure by HPLC analysis. All other reagents were of analytical grade.

Animals Hartley guinea pigs (200±10 g), Sprague-Dawley rats (200–250 g), and BALB/c mice (20–25 g) were obtained from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). Mice were housed under standard conditions with a 12-/12-h light/dark cycle at a temperature of 22±1°C and 55±10% humidity, and were given standard laboratory feed (Beijing Jinmuyang Laboratory Animals,

Inc., Beijing, China) and water *ad libitum*. The animal certification number was SCXK (Jing) 2009-0017. Animal protocols were developed according to the institution's guidelines for the care and use of laboratory animals and were approved by the local animal care and use committee.

Hyaluronidase inhibitory activity Hyaluronidase inhibitory activity was determined by measuring the amount of β-*N*-acetylglucosamine formed from sodium hyaluronate with a spectrophotometer (14). Five hundred microliters of bovine hyaluronidase (7, 420 units/mL) dissolved in 0.1 M acetate buffer (pH 5.6) was mixed with 100 μL of 0.25 mM calcium chloride, and then incubated in a water bath at 37°C for 20 min. The mixture was treated with 500 μL of a designated concentration of a test sample, and incubated again in a water bath at 37°C for 20 min, after which 500 μL of sodium hyaluronate (0.5 mg/mL) dissolved in 0.1 M acetate buffer (pH 5.6) was added. After 30 min of incubation in a water bath at 37°C, 100 μL of 0.4 M sodium hydroxide was added to stop the reaction, and the mixture was placed in an ice water bath for 5 min. Next, 500 μL of acetylacetone (3.5 mL of acetylacetone dissolved in 50 mL of 1 M sodium carbonate solution) was added, and the mixture was incubated in boiling water for 30 min to produce a chromogenic reaction. After cooling to 25°C, 1.0 mL of *p*-dimethylaminobenzaldehyde solution (4 g of *p*-dimethylaminobenzaldehyde dissolved in 75 mL of absolute ethyl alcohol and 75 mL of 10 N hydrochloric acid) was added to the reaction mixture for 20 min at 25°C. The optical density of the reaction mixture was measured at 555 nm by using a microplate reader (SpectraMax 190; Molecular Devices LLC, Sunnyvale, CA, USA).

Antipruritic activity Hartley guinea pigs (200±10 g, half male and half female) were randomly divided into 16 groups of 10 animals each, which consisted of the negative control group (physiological saline) and the groups treated with the sprout extracts (0–168 h). The control treatment or the test samples (0.1 g cm⁻²) were applied to shaved dorsal skin sites (2 cm²) on the back right hind feet of the animals for 2 days. On the 3rd day, after the samples were applied to the shaved sites for 10 min, 0.05 mL of histamine phosphate (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.1%) was dripped onto the test site every 3 min. Scratching behavior induced by histamine phosphate was recorded and the itch threshold was determined based on the dosage of histamine phosphate required to produce itching (15).

Induction of the IgE-mediated passive cutaneous anaphylaxis (PCA) reaction Sprague-Dawley rats (200–250 g) were divided into 6 groups of 10 rats each, which consisted of the untreated control group, the model group, the positive control group (fluocinonide ointment, 50 mg cm⁻²), and the high-dose (50 mg cm⁻²), middle-dose (25 mg cm⁻²), and low-dose (12.5 mg cm⁻²) EaF groups. The IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of 0.5 μg of anti-DNP IgE into each of 3

dorsal skin sites, followed 48 h later by an injection of 100 µg of DNP-HSA containing 4% Evans blue into the tail vein. The DNP-HSA was diluted in phosphate-buffered saline (PBS). The sites were outlined with a water-insoluble red marker. One hour before the injection of anti-DNP IgE, fluocinonide ointment or the high, middle, or low dose of EaF were applied to the area of injection. The mice were sacrificed 30 min after the administration of DNP-HAS. The skin at the injection site was removed for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with a mixture of acetone and physiological saline (at a ratio of 1:1) (16). The absorbent intensity of the skin extraction was measured at 620 nm by using a spectrofluorometer (SpectraMax 190; Molecular Devices LLC), and the amount of dye in the dorsal skin was calculated using the Evans blue calibration line.

Mast cell stabilization activity (BALB/c) mice (20–25 g) were divided into 6 groups of 10 mice each, which consisted of the untreated control group, the model group, the positive control group (DSCG, 50 mg kg⁻¹, i.v.), and the high-dose (2.0 g kg⁻¹, p.o.), middle-dose (1.0 g kg⁻¹, p.o.), and low-dose (0.5 g kg⁻¹, p.o.) EaF groups. DSCG was administered intraperitoneally because of its poor oral absorption. After administration of the samples for 4 days, the mice were intraperitoneally administered 5.0 mL of cold phosphate-buffered saline. After gentle abdominal massage for 10 s, the peritoneal fluid containing mast cells was collected immediately in RPMI-1640 medium (pH 7.2–7.4). Macrophages in peritoneal fluid were adhered to the dish bottom for growth. Mast cell left in the suspension were transferred out and cultured in RPMI-1640 medium again. Toluidine blue indicator solution indicated that the purity of mast cell (>90%). Cell viability was assayed using the trypan blue dye test. The cells were washed 3 times with RPMI-1640 medium (3.0 mL) by centrifugation at low speed (4,500×g), the supernatants were discarded, and the mast cell pellets were resuspended in the RPMI-1640 medium. Mast cells from the treated and positive control groups were incubated with compound 48/80 (1.0 µg/mL) at 37°C for 10 min in a water bath. The cells were stained with toluidine blue (1%) and the numbers of degranulated and intact mast cells were counted in samples of 100 cells from each treatment group, which were observed under a high-power microscope (17). The percentage of intact mast cells was calculated by the following formula:

$$\text{Percentage of intact mast cells (\%)} = \frac{\text{total number of mast cells} - \text{total number of degranulated cells}}{\text{total number of mast cells}} \times 100$$

total number of degranulated cells/total number of mast cells×100

Assay of histamine release BALB/c mice (20–25 g) were divided into 6 groups of 10 mice each, which consisted of the untreated control group, the model group, the positive control group (DSCG, 50 mg kg⁻¹, i.v.), and the high-dose (2.0 g kg⁻¹, p.o.), middle-dose (1.0 g kg⁻¹, p.o.), and low-dose (0.5 g kg⁻¹, p.o.) EaF groups. The mice were given the test compounds every 24 h for 4 days. On the 5th day of administration, each group of mice was injected intraperitoneally with compound 48/80 (0.8 mg kg⁻¹), and after 10 min, retroorbital blood was collected and serum was prepared for histamine detection by ELISA.

Total flavonoid content The total flavonoid content of EaF was determined using a colorimetric method (18). Briefly, 0.4 mL of NaNO₂ (5%, v/v) was added to 1.0 mL of appropriately diluted EaF samples; to this, 0.4 mL of AlCl₃ (10%, v/v) was added after 6 min, and 4.0 mL of NaOH (4%, v/v) and ethanol (60%, v/v) were added to 10 mL of the resulting solution after 6 min. The absorbance was measured at 508 nm using a microplate reader (SpectraMax 190; Molecular Devices LLC) after 15 min. A standard curve was constructed using rutin. Total flavonoid content was expressed as mg of rutin equivalent per g of the EaF sample. The samples were analyzed in triplicate.

Total protein content The total protein content of EaF was measured according to previously described methods with minor modifications (19). First, 1.0 mL of the sample supernatant was mixed with 5.0 mL of Coomassie brilliant blue G-250 for 10 min. The sample was measured at 595 nm using a microplate reader (SpectraMax 190; Molecular Devices LLC). A standard curve was constructed using bovine serum albumin. Total protein content was expressed as mg of bovine serum albumin equivalent per g of the EaF sample. The samples were analyzed in triplicate.

Total polysaccharide content The total polysaccharide content of EaF was determined by the improved phenol-sulfuric acid method (20). Measurements were taken at 490 nm using a microplate reader (SpectraMax 190; Molecular Devices LLC). The total polysaccharide content of EaF was calculated as glucose/g from a calibration curve. The samples were analyzed in triplicate.

Table 1. Characterization of isovitexin, kaempferol-3-O-rutinoside, isoquercitrin, *p*-coumaric acid and the optimized MRM parameters for quantification by LC/QQQ/MS in EaF fraction

Compounds	Standards			MRM		
	RT (min)	M-H/M (m/z)	Lost ions	Quantification transition (m/z)	Frag (V)	CE (V)
Isovitexin	7.53	431	311;341	431311	148	18
Kaempferol-3-O-rutinoside	8.51	593	285;255	593285	180	40
Isoquercitrin	7.91	463	300;271	463300	170	30
<i>p</i> -Coumaric acid	7.40	163	119;93	163119	80	25

Quantification of the main constituents of EaF by LC/QQQ/MS

Quantification of the main constituents of EaF was performed using a QQQ 6410 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) coupled to a 1200 series HPLC system. First, 5.0 μ L of the sample was loaded onto an Eclipse Plus C₁₈ column (3.0 $\times$ 150 mm, 3.5 μ m, Agilent Technologies). The mobile phase consisted of (A) water: acetic acid (99.0:1.0, v/v) and (B) methanol with a gradient program of 15–45% (B) for 0–15 min and 100% (B) for 15–16 min. The flow rate was 0.4 mL/min. The column temperature was set at 35°C. The source conditions for electrospray ionization were as follows: gas temperature was 350°C with a drying gas flow rate of 10 L/min and a nebulizer pressure of 30 psig. The capillary voltage was 4.0 kV in the negative ion mode. The detection was performed by negative ion electrospray ionization in multiple reaction monitoring (MRM) mode. The transitions from molecular ion to dominant product ion were monitored (Table 1). Data was processed by Agilent Mass Hunter QQQ Quantitative Analysis. Isovitexin (1), kaempferol-3-O-rutinoside (2), isoquercitrin (3), and *p*-coumaric acid (4) were quantified using a calibration curve. The samples were analyzed in triplicate.

Statistical analysis All data are presented as mean $\pm$ standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test using the Statistical Package for Social Studies (SPSS) (IBM Corp., Armonk, NY, USA). A result of $p < 0.05$ was regarded as statistically significant.

Results and Discussion

Screening of mung bean sprouts by *in vitro* hyaluronidase inhibition and *in vivo* antipruritic activity Hyaluronidase, a mucopolysaccharide-splitting enzyme, has been implicated in allergic reactions, cancer cell migration, inflammation, and vascular system permeability (17). The extract of the 48-h sprouts had the best hyaluronidase inhibitory activity. The inhibition rates for 3 batches of 48-h sprouts were 80.47, 85.15, 74.26%, respectively (Fig. 1). *In vivo* pruritus induced by histamine phosphate was established

Fig. 1. The hyaluronidase inhibition rate of mung bean sprouts (0–168 h, each 12 h); (NC, negative control; ** $p < 0.01$, * $p < 0.05$, Duncan's multiple range test)

to evaluate the anti-allergic effects of the sprouts collected after 0–168 h of growth. The extracts of the sprouts increased the threshold for itch of the mice in comparison with the seeds. The highest threshold for itch (193.12 $\pm$ 15.80 μ g) was measured on 48-h sprouts (Fig. 2) ($p < 0.01$ vs. the control group). The results of the studies of *in vitro* hyaluronidase inhibition and *in vivo* antipruritic activity were correlated. Therefore, the active constituents were isolated from 48-h sprouts.

Screening of the active extracts *In vitro* hyaluronidase inhibition activity was used to further screen the active constituents isolated from 48-h sprouts. EaF showed better hyaluronidase inhibition activity than all of the other fractions. The inhibition rates of the fractions were ranked as follows: EaF (75.4%)>nBF (51.9%)>WtF (28.5%)>PeF (10.5%). Therefore, EaF was determined to be the most active fraction.

Effect of EaF on IgE-mediated PCA reaction The anti-allergic activity of EaF was evaluated by assaying the PCA reaction in mice. Local extravasation was induced by a local injection of anti-DNP IgE followed by an antigenic challenge (16). The results were visualized by the extravasation of dye. A normal application of EaF 1 h before injection of the antigen significantly suppressed the IgE-mediated PCA reaction in a dose-dependent manner; the inhibition rates were

Fig. 2. The effects of mung bean sprouts on the pruritus response (0–168 h, each 12 h); (NC, negative control; ** $p < 0.01$, * $p < 0.05$, Duncan's multiple range test), $n = 10$

Table 2. Influence of EaF fraction on PCA reaction¹⁾

Groups	Dosage	<i>n</i>	Dyes contents (μg/mL)	Inhibitory rate (%)
Blank group	-	10	3.01±0.31	-
Model group	-	10	124.93±7.67 ^{##}	-
Fluocinonide ointment	0.1 g/2 cm ²	10	102.53±8.54 ^{**}	17.93
High doses	0.1 g/2 cm ²	10	115.61±7.45 [*]	7.46
Middle doses	0.05 g/2 cm ²	10	118.32±5.64 [*]	5.29
Low doses	0.025 g/2 cm ²	10	123.94±6.70	0.79

¹⁾Data are expressed as mean±SD (*n*=3) using Microsoft Excel 2003. All one-way ANOVAs for difference were statistically significant, with ^{##}*p*<0.01 (compared with the blank group), ^{*}*p*<0.05 and ^{**}*p*<0.01 (compared with the model group).

Table 3. Influence of EaF fraction on mast cell degranulation and histamine release¹⁾

Groups	Dosage	<i>n</i>	The numbers of degranulated and intact mast cells	Percentage of intact mast cells (%)	Histamine contents (ng/mL)	Inhibitory rate (%)
Blank group	-	10	2.60±1.07	97.40±1.07	4.12±0.29	-
Model group	-	10	88.70±0.95	11.30±0.95 ^{##}	29.47±0.93 ^{##}	-
DSCG	50 mg/kg	10	30.00±2.87	70.00±2.87 ^{**}	7.59±0.30 ^{**}	74.24
High doses	2 g/kg	10	46.80±3.91	53.20±3.91 ^{**}	11.45±0.42 ^{**}	61.15
Middle doses	1 g/kg	10	65.70±3.89	34.30±3.89 ^{**}	17.58±0.55 ^{**}	40.33
Low doses	0.5 g/kg	10	76.00±2.58	24.00±2.58 ^{**}	22.27±0.88 ^{**}	24.41

¹⁾Data are expressed as mean±SD (*n*=3) using Microsoft Excel 2003. All one-way ANOVAs for difference were statistically significant, with ^{##}*p*<0.01 (compared with the blank group), ^{*}*p*<0.05 and ^{**}*p*<0.01 (compared with the model group).

Table 4. Contents of the main active chemicals in EaF fraction

sample	The contents of the chemicals			
	<i>p</i> -Coumaric acid (μg/g)	Isovitexin (mg/g)	Kaempferol-3- <i>O</i> -rutoside (μg/g)	Isoquercitrin (μg/g)
Batches (<i>n</i> =3)	207.3	46.1	194.8	0.932
	196.9	43.8	185.1	0.886
	213.5	47.4	200.1	0.960
mean±SD	205.9±8.0	45.8±1.8	193.3±7.6	0.926±0.04

7.46% for the high dose (50 mg cm⁻², *p*<0.01), 5.29% for the middle dose (25 mg cm⁻², *p*<0.05), and 0.79% for the low dose (12.5 mg cm⁻²) (Table 2).

Effect of EaF on mast cell degranulation Mast cells represent a major source of histamine, proteases, and other potent chemical mediators that are implicated in a wide variety of inflammatory and immunologic processes (21,22). After the induction of mast cell degranulation by compound 48/80, the model group showed significantly increased mast cell degranulation in comparison with the untreated control group. The cell integrity rate of the model group was 11.3±1.0%, whereas that of the untreated control group was 97.4±1.1% (*p*<0.01). The cell integrity rates of the groups treated with the high (2.0 g kg⁻¹, *p.o.*), middle (1.0 g kg⁻¹, *p.o.*), and low (0.5 g kg⁻¹, *p.o.*) doses of EaF were 53.2±3.9, 34.3±3.9, and 24.0±2.6%, respectively. EaF at different doses showed statistically significant mast cell stabilization activity (*p*<0.01) (Table 3).

Effect of EaF on compound 48/80-induced histamine release Histamine is an important molecular mediator released from

activated mast cells and the main mediator involved in early inflammatory reactions. EaF significantly reduced histamine release induced by compound 48/80 in a dose-dependent manner. The inhibitory rate at the high dose (2.0 g kg⁻¹, *p.o.*) of EaF was 61.2% (*p*<0.01) (Table 3).

Total flavonoid, protein, and polysaccharide contents The contents of total flavonoids, total polysaccharides, and total protein of EaF were 431.35, 91.47, and 32.64 mg g⁻¹, respectively. The flavonoids may be mainly attributed to the anti-allergic activity of EaF.

Quantitation of the main constituents of EaF Isovitexin, kaempferol-3-*O*-rutoside, isoquercitrin, and *p*-coumaric acid in EaF were quantified by LC/QQQ/MS (Table 4). As expected, flavonoids isovitexin, kaempferol-3-*O*-rutoside, and isoquercitrin were present in EaF at concentrations higher than that of the phenolic acid, *p*-coumaric acid. Isovitexin (45.8±1.8 mg g⁻¹) was the most abundant monomeric flavonoid in EaF.

In Traditional Chinese Medicine (TCM), mung beans were considered to be cool and sweet, and have been used for clearing the

heat associated with thirst, irritability, and fever in a variety cuisines and in folk remedies. These effects were thought to be correlated with the effects of mung beans on inflammatory responses and immune modulation (6). The extract of mung bean, which mainly comprises of polyphenols, gallic acid, vitexin, and isovitexin has been certificated to markedly reduce the activity of murine macrophages through the prevention of proinflammatory interleukin (IL)-1 β , IL-6, IL-12 β , tumor necrosis factor (TNF)- α , and inducible NO synthase (iNOS) (1) gene expression without cytotoxicity. The mung bean ethanol extracts had great potential to improve the clinical symptoms of inflammation-associated diseases, such as allergies (1).

Through the evaluation of the *in vitro* inhibition of hyaluronidase activity and *in vivo* antipruritic activity induced by histamine phosphate, it was found that germinated mung bean sprouts showed enhanced biological activities owing to the production of secondary metabolites by relevant biosynthetic enzymes during the initial stages of germination (23). The results showed that sprouts collected 48 h after germination had the best anti-allergic activity. Various solvents were used to isolate the active constituents of 48-h mung bean sprouts. The hyaluronidase inhibition test showed EaF to be the most active extract.

PCA reaction usually was induced by a rapid local release of inflammatory mediators such as histamine, serotonin, and various cytokines from mast cells (24). In the present study, PCA animal model was used to investigate the effect of mung bean sprouts on localized allergic reactions *in vivo*. Active constituents isolated from mung beans sprouts effectively suppressed the local allergic reaction in the PCA model in a dose-dependent manner. Mast cells are the major effector cells in type I hypersensitivity reaction through the release of numerous proinflammatory mediators. Histamine is one of the important bioactive mediators released by mast cells, which are the main mediators involved in early hypersensitivity reaction. Numerous reports have established that stimulation with compound 48/80 initiates the activation of signal transduction pathways that lead to histamine release (10,11). In literature, Inula japonica extract (IFE) was reported to attenuate the mast cell-mediated PCA reaction in IgE-sensitized mice in a similar animal model (25). In the present paper, EaF showed obvious mast cell stabilization and antihistamine activities against compound 48/80-induced allergic responses in mice. Type-I hyper-sensitivity is thought to involve mast cell degranulation, which is followed by the release of mediators such as histamine, leukotrienes, and prostaglandins from activated cells (26,27). Our results show that active constituents from mung bean sprouts relieve pruritus by increasing mast cell stability and inhibiting histamine release. In addition, our results suggest that mung bean sprout extracts can alleviate skin redness and swelling by inhibiting the PCA reaction.

Flavonoids, proteins, and polysaccharides have been isolated from the sprouts of mung beans (3). In order to elucidate the main bioactive constituents responsible for the anti-allergic activity of mung bean sprouts, total flavonoid, total protein, and total polysaccharide

contents were determined. The results indicated that the effects of EaF were primarily produced by flavonoids. Among the active constituents, isovitexin, kaempferol-3-O-rutinoside, isoquercitrin, and *p*-coumaric acid were detected in EaF and quantified. The results suggest that flavonoids isovitexin, kaempferol-3-O-rutinoside, and isoquercitrin are the main contributors to the anti-allergic activity of mung bean sprouts.

Germination is thought to improve the nutritional and medicinal qualities of mung beans. Use of mung beans guided by empirical evidence will be beneficial to the application of mung beans in health foods, medicines, and cosmetics.

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