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# Inhibitory Effects of *Actinidia chinensis* and *Zizyphus jujube* on Histamine Release from Rat Peritoneal Mast Cells

Su Ok Yang[1] and Geun Eog Ji[1,2†]

[1]*Department of Food and Nutrition, Research Institute of Human Ecology, Seoul National University, Seoul 151-741, Korea*
[2]*Research Institute, Bifido Inc, Gangwon 250-804, Korea*

## Abstract

**Methanol extracts (80%, 10 μg/mL) of *Actinidia chinensis* (AC) and *Zizyphus jujube* (ZJ) inhibited histamine release from rat peritoneal mast cells (RPMCs) induced by compound 48/80. Evaluation of AC and ZJ solvent fractions (chloroform, ethylacetate, butanol and water) revealed that the butanol fraction of AC at 5 μg/mL and water fraction of ZJ at 1 μg/mL exhibited the highest anti-allergic effects. Combination of the butanol fraction of AC and water fraction of ZJ when combined showed higher inhibition of histamine release than either alone. The levels of cAMP in RPMCs treated with AC and ZJ were significantly increased compared to the compound 48/80 treated control. Our findings suggest that the extracts from AC and ZJ may alleviate immediate hypersensitivity reactions through the increase of cAMP in the mast cells.**

**Key words:** *Actinidia chinensis*, *Zizyphus jujube*, histamine, cAMP

## INTRODUCTION

Allergic diseases such as allergic rhinitis, asthma and atopic eczema are increasing in prevalence, which add considerably to the burden of health care costs. In Sweden, for example, the number of children with allergic rhinitis, asthma or eczema roughly doubled over a 12-year period, and in the United States the annual cost of treating asthma is about $6 billion (1). Moreover, allergic diseases are becoming an increasingly serious problem in the Korean with an incidence of 12~20%.

Bronchodilators, antihistamines, antispasmodic drugs and steroids are used as treatment drugs of allergy (2). Also, antiallergic drugs such as epinastine (3,4), disodium cromoglycate (DSCG), tranilast, ketotifen, azelastine, oxatomide and terfenadine have been used to treat allergic diseases. They inhibit the degranulation of mast cells and block the receptors for various chemical mediators including histamine H1 receptors (5). For example, epinastine showed a potent histamine H1-blocking effect and inhibited not only compound 48/80-induced histamine release from rat peritoneal mast cells (RPMCs) but also $Ca^{2+}$ release from the intracellular Ca store of RPMCs exposed to both compound 48/80 and substance P. Although these remedies provide treatments against the allergy symptoms, they do not cure the allergy nor remove the causative agents of allergies. Consequently, the development and search for the treatment of allergic diseases using traditional medicines and natural food materials is becoming an interesting field in research and product development. Our previous studies revealed that the extracts of *Actinidia chinensis* (AC) and *Zizyphus jujube* (ZJ) significantly reduce histamine release compared to other natural food materials (6). In this study, we investigated the active fractions of the AC and ZJ for their effect on compound 48/80-induced histamine release from RPMC and assessed the effect of the active fractions on the intracellular cAMP level in RPMC. The results of this screening may provide useful information for further discovery of functionally active compounds for reducing the occurrence of allergy.

## MATERIALS AND METHODS

### Animals

Seven-week-old male Wistar rats (200~250 g in weight) purchased from the Jungang Experimental Animal Center (Seoul, Korea) were kept in plastic cages, and allowed free access to water and food. The temperature and humidity were controlled at 22~24°C and 45~65%, respectively. They were maintained on a 12:12 hr light:dark cycle in an animal environmentally-controlled chamber.

### Materials

Percoll was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Isotonic Percoll solutions were prepared by dissolving 9 parts of Percoll with 1 part of

†Corresponding author. E-mail: geji@bifido.com
Phone: +82-2-880-8749, Fax: +82-2-880-6282

10-fold concentrated Hanks' solution (HS). Colour-markered beads obtained from Pharmacia Fine Chemicals covering the buoyant density range 1.016~1.178 g/mL were used for calibration of Percoll gradients and for checking equilibrium conditions. Compound 48/80, HS and bovine serum albumin (BSA) were from Sigma Chemical Co. (St. Louis, MI, USA). Histamine ELISA kit was purchased from Immunotech (Marseille Cedex 9, France), and cAMP kit was purchased from R&D Systems (Minneapolis, MN, USA).

**Sample preparation**

AC and ZJ purchased from Kyung-Dong Market (Seoul, Korea) were dried and powdered. Fifty grams of each dried and powdered sample was mixed with 500 mL of 80% methanol (MeOH) and active compound were extracted from the solution after leaving it at room temperature for 24 hr and filtered using a Whatman 2 filter (Whatman Int. Ltd., Maidstone, UK). The above procedure was repeated twice and the filtrates were combined. Then, the 80% MeOH extracts were concentrated under a vacuum rotary evaporator (38~50°C) (Lab. Companion, Jeiotech, Korea) in order to remove MeOH and lyophilized in a freeze dryer (TFD5505, Ilshin Europe B.V, Korea). For the bioassay, the extracted samples were dissolved in dimethyl sulfoxide (DMSO) and further diluted with HEPES buffer.

**Solvent fraction of 80% MeOH extract**

Three hundred g each of AC and ZJ plant samples were extracted twice successively with 3 L 80% MeOH at room temperature for 24 hr. The 80% MeOH extracts were filtered, evaporated at 40~50°C under vacuum and lyophilized. The aqueous layer of the 80% MeOH extract was fractionated with chloroform ($CHCl_3$) followed by ethyl acetate and finally with butanol (5:3:2) (Fig. 1). The respective fractions and a residue fraction were evaporated at 50°C under reduced pressure, and then the aqueous fractions were lyophilized. The fractionated samples were dissolved in DMSO and further diluted with HEPES buffer. Then, the respective extracts were filtered through a 0.22 μm filter. All the above fractions were screened for anti-allergy activity *in vitro*.

**Isolation and purification of rat peritoneal mast cells**

Male Wistar rats weighing 200~250 g were anesthetized by ether. Then, twenty mL of HEPES buffer (137 mM NaCl, 5.6 mM glucose, 10 mM HEPES [N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic aci], 2.7 mM KCl, 0.4 mM $NaH_2PO_4$ and 1 mM $CaCl_2$, pH 7.4) supplemented with 50 IU/mL of heparin was injected into the abdominal cavity. The abdominal region was gently massaged for 90 sec, the peritoneal cavity was carefully opened, and

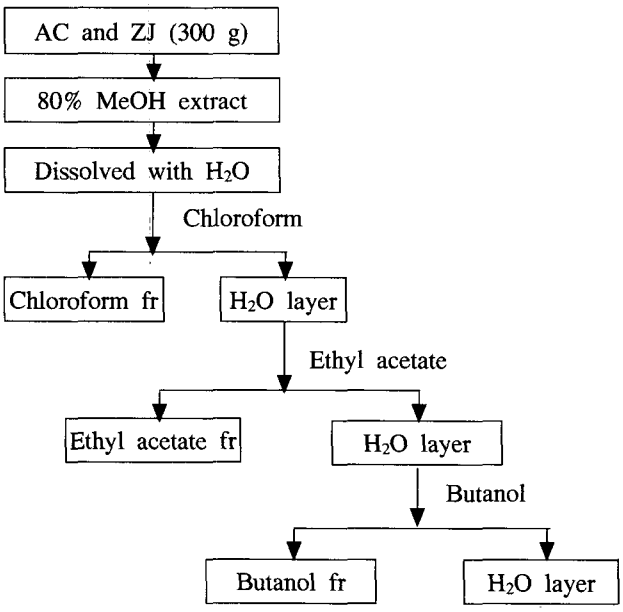


**Fig. 1.** Procedure for the fractionation of AC and ZJ with various solvents.

then peritoneal fluid was collected by pipette. Rat peritoneal mast cells were isolated and purified over Percoll density gradient as previously described (7). The mixed peritoneal cells were resuspended with 5 mL of 0.17 M ammonium chloride ($NH_4Cl$), which destroyed red cells with minimal effects on other cells. The cells were incubated at 4°C for 5 min with occasional shaking. The cells were centrifuged (400×*g*, 5 min, 4°C) and the lysis buffer was removed. The cells were resuspended in HEPES buffer, and the pellets were washed twice by centrifugation (400×*g*, 5 min, 4°C). To the pellets, 1 mL of HEPES buffer supplemented with 1 mg/mL of BSA was added. The cell suspension was mixed with 4 mL of 90% Percoll and 1 mL of BSA supplemented with HEPES buffer and then carefully layered over the Percoll-cell mixture. Purification was then performed by centrifugation (1900×*g*, 25 min, 4°C) which allowed cell separation and gradient formation simultaneously. The cell fraction (pellet) was then washed twice in HEPES buffer by centrifugation and finally resuspended to the desired cell density in HEPES buffer prewarmed at 37°C.

**Histamine release assay**

Mast cells pooled from three rats were resuspended to a cell density of $10^5$ cells/mL and were equilibrated at 37°C for 10 min with HEPES buffer. One hundred μL of the equilibrated cells was seeded into 96-well plates ($1\times10^3$ cells/well) and further incubated for 10 min at 37°C for stabilization. The peritoneal mast cells were incubated for 10 min with various concentration of samples (0~1000 μg/mL) and then challenged for 10 min with compound 48/80 (1 μg/mL). The supernatant

and cell pellet were separated by centrifugation at 900× *g* for 5 min at 4°C. The histamine content of the supernatant was assayed using a Histamine ELISA kit (Immunotech, France).

**cAMP assay**

Mast cells were transferred to 96-well culture plates ($1\times10^5$ cells/100 μL/well) and preincubated for 10 min. Then, preincubated mast cells were reacted with each effective fraction for different time intervals, terminated by the addition of ice-cold acidified 0.1 N HCl. The supernatants and cell pellets were separated by centrifugation at 600× *g* for 5 min at 4°C. The supernatants were assayed immediately or stored at -80°C. cAMP content was assayed using an cAMP kit (R&D SYSTEMS, Germany).

**Statistical analysis**

Statistical significance was compared between each treated group and control by the Duncan's multiple range tests. Results with $p<0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION

**Dose-response of effective fractions of AC and ZJ on compound 48/80-mediated histamine release from RPMCs**

We investigated the inhibitory effect of AC and ZJ on compound 48/80-induced histamine release in RPMCs. To measure histamine release, RPMCs were preincubated with 80% MeOH extracts of AC and ZJ, 10 min prior to the addition of compound 48/80 (1 μg/mL). The inhibitory effect of AC and ZJ on histamine release was compared to the DSCG or compound 48/80 treated control. AC and ZJ significantly inhibited histamine release by compound 48/80 in RPMCs. We next assessed the effect of several solvent fractions (chloroform, ethylacetate, butanol and water) to determine which fractions have the active components responsible for the anti-allergic properties of AC and ZJ. The fractions with high histamine inhibitory activity were as follows: butanol fraction of AC and water fraction of ZJ (Table 1).

To assess the effect of the butanol fraction from AC and water fraction from ZJ on compound 48/80-induced histamine release, RPMCs were pre-treated with various concentrations (0.001～1000 μg/mL) for 10 min prior to compound 48/80 stimulation. As shown in Fig. 2, the histamine release was gradually decreased as the concentration of the butanol fraction of AC was increased from 0 to 5 μg/mL, but gradually increased as the concentration further increased up to 1000 μg/mL. At the concentration of 5 μg/mL, the inhibition rate reached up to 17.1%. On the other hand, the histamine release was gradually decreased as the concentration of the water fraction of ZJ was increased from 0 to 1 μg/mL, but gradually increased as the concentration further increased up to 1000 μg/mL. The degree of reduction of the histamine release was significant at the concentrations of 0.1～10 μg/mL. At the concentration of 1 μg/mL, the inhibition rate reached nearly 18% and was the most significant. However, at the 1000 μg/mL concentration of both AC and ZJ, histamine release level was similar to the level induced by compound 48/80.

**Table 1.** The effect of different fractions (10 μg/mL) from AC and ZJ on the histamine release by compound 48/80 (1 μg/mL)

| Treatment | Fractions | Inhibition (%) |
|---|---|---|
| None | – | 0 |
| DSCG (10 μg/mL) | – | 10.3 |
| AC | Methanol | 11.9 |
| | Chloroform | 8.0 |
| | Ethyl acetate | 8.6 |
| | Butanol | 16.4 |
| | Water | 5.5 |
| ZJ | Methanol | 13.2 |
| | Chloroform | 8.8 |
| | Ethyl acetate | 10.1 |
| | Butanol | 7.6 |
| | Water | 16.5 |

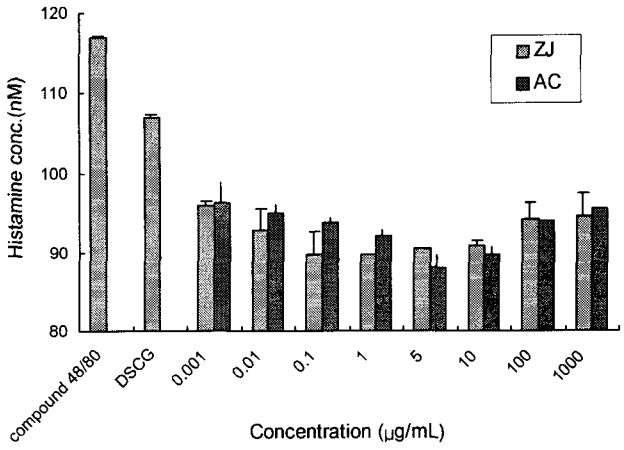


**Fig. 2.** The effect of butanol fraction of AC and water fraction of ZJ on the histamine release by compound 48/80. RPMCs ($1\times10^4$ cells/mL) were preincubated with butanol fraction of AC or water fraction of ZJ (0.001～1000 μg/mL) at 37°C for 10 min prior to the addition of compound 48/80 (1 μg/mL). Each data point represents the mean ± SD of three experiments.

**cAMP assay**

We measured the concentration of cAMP in the mast cells to assess the mechanism by which active fractions

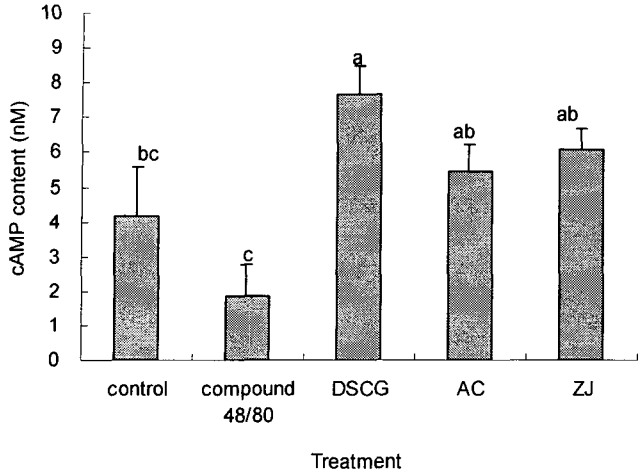


**Fig. 3.** The effect of AC and ZJ on cAMP levels from RPMCs. RPMCs ($1\times10^3$ cells/mL) were preincubated with butanol fraction of AC (5 μg/mL) and water fraction of ZJ (1 μg/mL) at 37°C for 10 min. Each data point represents the mean ± SD of three experiments. Means with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.

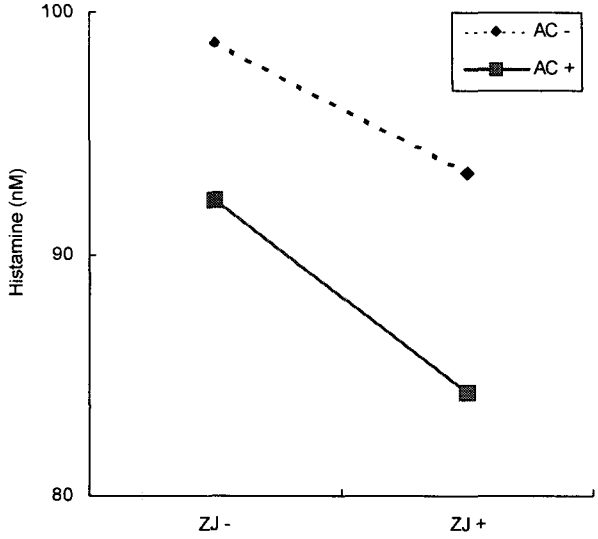


**Fig. 4.** The synergistic effect of AC and ZJ on histamine content. The analysis of the synergistic effect between AC and ZJ was significant at the level of $\alpha$ =0.05 by 2-way ANOVA.

inhibited histamine release from RPMCs (Fig. 3). When RPMCs were incubated with 5 μg/mL butanol fraction of AC or 1 μg/mL water fraction of ZJ, the cAMP content was high compared to that of control, and increased about 4~5 fold compared to that of compound 48/80 control. Both AC and ZJ significantly increased cAMP content.

It was suggested that stimulation of mast cells with compound 48/80 initiates the activation of a signal transduction pathway, which leads to histamine release, and activates G proteins (8). Tasaka et al. (9) reported that compound 48/80-induced increase in the permeability of the lipid bilayer membrane was an essential trigger for the release of the mediator from mast cells. The possible mechanism of this effect appears to be related to the activation of adenylate cyclase or inhibition of cAMP phosphodiesterase, and a subsequent increase in intracellular cAMP. The release of histamine is depressed by an increase in the intracellular cAMP. Since agents which inhibit a decrease in cAMP decrease histamine release, it is apparent that cAMP is an important part of the control mechanism of histamine secretion (10). In this context, the mode of the action of the effective natural food materials that inhibit histamine release might be related to the prevention of calcium release from the calcium stores of mast cells due to elevation of the intracellular cAMP level by the inhibition of the cAMP phosphodiesterase.

### Synergistic effect

The effects of the mixtures of the most active fractions on compound 48/80-induced histamine release from RPMCs are shown in Fig. 4. When RPMCs were incubated with the combination of butanol fraction of AC and water fraction of ZJ, the mixtures (AC+ZJ) inhibited histamine content (Fig. 4) and increased cAMP level (data not shown) compared to compound 48/80 treated group. While the synergistic increase in cAMP was not shown in the experimental condition at the level of $\alpha$ =0.05 by 2-way ANOVA, the inhibition of histamine release was synergistic.

Further studies are required to characterize the effective components of AC and ZJ and the mechanism of action to utilize them for the development of functional food with anti-allergy activity.

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