

Quercetin Inhalation Inhibits the Asthmatic Responses by Exposure to Aerosolized-Ovalbumin in Conscious Guinea-pigs

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Effects of quercetin inhalation on immediate (IAR), late-phase (LAR) and late late-phase (LLAR) asthmatic responses by exposure to aerosolized-ovalbumin (AOA) (2w/v% in saline, inhalation for 3 min) were studied in conscious guinea-pigs sensitized with AOA. We measured specific airway resistance (sRaw), and recruitment of leukocytes, histamine and protein contents and phospholipase A₂ (PLA₂) activity in bronchoalveolar lavage fluid (BALF). Effects of quercetin (10 mg/mL, inhalation for 2 min) compared with cromolyn sodium, salbutamol, and dexamethasone inhalations, respectively. Quercetin inhalation decreased sRaw by $57.15 \pm 3.82\%$ in IAR, $57.72 \pm 7.28\%$ in LAR, and $55.20 \pm 5.69\%$ in LLAR compared with AOA-inhaled control. Salbutamol inhalation (5 mg/mL) significantly inhibited sRaw in IAR, but inhalations of cromolyn sodium (10 mg/mL) and dexamethasone (5 mg/mL) significantly inhibited sRaw in IAR, LAR and LLAR, respectively. Inhibitory activity of quercetin inhalation on sRaw was similar to effect of its oral administration (10 mg/kg) in asthmatic responses. Quercetin (10 mg/mL, inhalation for 2 min) significantly decreased histamine and protein contents, PLA₂ activity, and recruitments of leukocytes in BALF and also improved slightly infiltration of eosinophils and neutrophils in histopathological survey. Its anti-asthmatic activity was similar to cromolyn sodium and dexamethasone.

Key words: Quercetin inhalation, Anti-asthmatic action, Specific airway resistance, Recruitment of eosinophil, Bronchoalveolar lavage fluid, Guinea pigs

Abbreviations: AOA, Aerosolized ovalbumin; BALF, Bronchoalveolar lavage fluid; BSA, Bovine serum albumin; BSS, Balanced salt solution; DMSO, Dimethylsulfoxide; EOS, Eosinophils; H & E, Hematoxylin and eosin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IAR, Immediate-phase asthmatic response; IgE, Immunoglobulin E; LAR, Late-phase asthmatic response; LLAR, Late late-phase asthmatic response; OA, Ovalbumin; PLA₂, Phospholipase A₂; PMN, Polymorphonuclear leukocytes; 10-Pyrene PC, Pyrene-labeled phospholipid; sRaw, Specific airway resistance

INTRODUCTION

Flavonoids widely distributed in the plant kingdom are a large class of polyphenolic compounds which have anti-inflammatory (Pettinari *et al.*, 2006; Wu *et al.*, 2004; Li *et al.*, 2001; Skaltasa *et al.*, 2000; Ramesh *et al.*, 1998; Shibata *et al.*, 1991; Villar *et al.*, 1984; Fewtrell *et al.*, 1977), anti-allergic (Nishino *et al.*, 1984), anti-oxidant and spasmolytic (Omisore *et al.*, 2005; Morales *et al.*, 1994) activities. Dichromone flavonoid derivative, sodium chromo-

glycate, which is a similar chemical structure to flavonoids, inhibits the release of chemical mediators for food cold water exercise-induced anaphylaxis (Benhamou *et al.*, 2007), and has been used as anti-asthmatic agent, especially the prophylactic treatment of both allergic and exercise-induced asthma via the first dry-powder inhaler (Crompton G, 2006). Our previous study showed that the administration of quercetin and rutin at an oral dose of 30 mg/kg inhibits immediate-asthmatic response (IAR) and late-phase asthmatic response (LAR) by exposure to aerosolized ovalbumin (AOA) on conscious guinea pigs sensitized with ovalbumin (OA) injection (Jung *et al.*, 2007), but effects of its inhalation on the asthmatic responses are still unclear.

We studied effects of quercetin inhalation on asthmatic

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responses by exposure to AOA in the conscious guinea-pigs sensitized with AOA. For evaluation of effect of quercetin (10 mg/mL, inhalation for 2 min), we measured specific airway resistance (sRaw) in the double-chambered plethysmograph, and recruitment of leukocytes, histamine and protein contents, and phospholipase A₂ (PLA₂) activity in bronchoalveolar lavage fluid (BALF), and its activity was compared with reference drugs, cromolyn sodium, salbutamol and dexamethasone inhalations in IAR, LAR and late late-phase asthmatic response (LLAR), respectively.

MATERIALS AND METHODS

Materials and reagents

Quercetin, ovalbumin (type V), piprinhydrinate, dexamethasone, salbutamol, disodium cromoglycate, Wright's stain and bovine serum albumin (fraction V), OPT (O-phthalaldehyde) and 10-pyrene PC [1-palmitoyl-2-(10-pyrenyldecanoyl)-sn-glycero-3-phosphorylcholine] were purchased from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). Zoletil 50 was purchased from Virbac Lab. (06516 Carros, France). BCA protein assay kit was purchased from Pierce Co. (Rockford, IL, U.S.A.). Other reagents used reagent grade. HEPES-BSS/BSA (pH 7.4) was made by NaCl 132 mM, KCl 6 mM, HEPES 20 mM, KH₂PO₄ 1.2 mM, CaCl₂·H₂O 1.1 mM, MgSO₄ 1.0 mM, dextrose 5.5 mM, and bovine serum albumin (BSA) 1%. Test drugs (quercetin, ovalbumin, salbutamol, cromolyn sodium, and dexamethasone) were dissolved in saline with 10% DMSO. HBSS/BSA (pH 7.4) : NaCl 132 mM, KCl 6 mM, HEPES 20 mM, KH₂PO₄ 1.2 mM, CaCl₂·H₂O 1.1 mM, MgSO₄ 1.0 mM, dextrose 5.5 mM, EDTA 10 mM and BSA 1%.

Animals and sensitization

Pathogen-free male Dunkin-Hartley guinea pigs (250–300 g) were purchased from HanLim Lab. Animal Co. (Hwa Sung-Gun, Kyung Ki-Do, Korea), and were housed over one week in a normal environmentally controlled-animal room (temperature 25°C, humidity 55%, aeration 10–18/h, illumination 300–500 Lux) with the usual laboratory animal diet food (Superfeed Co., Won Ju City, Gang Won-Do, Korea) and water *ad libitum*. Animals were actively sensitized by exposure of AOA (1 w/v % in saline) for 3 min placed in the double-chambered plethysmograph (HSE type 855, Hugo Sachs Elektronik, Germany). This procedure was repeated 7 days later. Success of sensitization was confirmed after on interval of at least 14 day by the induction, under general anaesthesia of anaphylactic bronchoconstriction in two randomly chosen animals from the group upon exposure to AOA in the absence of any pretreatment. Twenty-one day after first sensitization, 1% OA (0.1 mL per site) was injected intradermally and skin

thickness was measured 1 h later. A doubling of skin thickness indicated the success of sensitization. Animals which did not respond to intradermal OA were not used (Sanjar *et al.*, 1990). All animal use procedures were approved by the Institutional Animal Care and Use Committee of Institute for Molecular-Based New Drug Development in Chung-Ang University.

Drug treatment and antigen challenge

After measuring the baseline airway function before the drug treatment, the animal was challenged with inhalation of 1% OA or saline for 5 min in the double-chambered plethysmograph (HSE type 855). Each sensitized animals were fasted overnight and treated with aerosolized drug solutions in the vehicle (saline with 10% DMSO) of quercetin (10 mg/mL), cromolyn sodium (10 mg/mL), salbutamol (5 mg/mL), dexamethasone (5 mg/mL) and vehicle were inhaled for 2 min at 1 h before and 6 h after last AOA challenge, additionally 12 and 20 h later, respectively. Thirty minutes before AOA challenge, the animals were pretreated with antihistamine, piprinhydrinate (5 mg/kg) to prevent sudden death by the systemic hypersensitivity. Twenty-five minutes later, each animal was placed in the double-chambered plethysmograph and permitted to acclimatize for 5 min. The animal was challenged with the aerosolized-OA (1.0 mg/mL in saline) or vehicle aerosol (saline containing 10% DMSO) by the jet nebulizer for 5 min (Matsumoto *et al.*, 1994). Quercetin (10 mg/mL in saline containing 10% DMSO) inhaled with jet nebulizer for 2 min, and its activity was compared with the inhaler of cromolyn sodium, dexamethasone and salbutamol.

Measurement of airway function

Airway function in conscious guinea-pigs was measured with double-chambered plethysmograph (Matsumoto *et al.*, 1994; Sanjar *et al.*, 1990). A conscious animal was positioned with its neck extending through the partition of a head and body chamber. At IAR and LAR, tidal volume, tidal airflow, respiratory rate and specific airway resistance (sRaw) were measured respectively by volumetric differential pressure transducer (PT5, Grass Co. U.S.A.), non-invasive respiratory analyzer and ink-writing oscilloscope with polygraph (7E, Grass Co. U.S.A.). The sRaw expressed mmHg×sec was analyzed by Pennock Program (Pennock *et al.*, 1979) following formula : $\tan\theta = \theta \times R \times C$ (θ , phase displacement; ω , $2\pi \times$ respiration rate; $R \times C$, time constant of the respiratory system; $C = V/P$, thoracic gas volume (V)/(P_{atm} - 47 mmHg).

The sRaw is therefore obtained as :

$$R \times V = (P_{\text{atm}} - 47) \times \tan\theta / (2\pi \times f) [\text{mmHg} \times \text{s}]$$

$$R \times V = (P_{\text{atm}} - 47) \times 13.6 \times \tan\theta / \omega [\text{mmHg} \times \text{s}]$$

The phase displacement is measured at the end of

inspiration on the steep change-over to the expiration phase. The evaluation unit can be linked to a computer for continuous data capture. The sRaw was expressed as a percentage of the baseline value before drug treatment. The increase of sRaw was expressed as a percentage of the baseline value before drug treatment and compared with control (AOA) :

$$\% \text{ increase in sRaw} = \{(\text{sRaw after challenge} / \text{sRaw before challenge}) - 1\} \times 100$$

Bronchoalveolar lavage and cell counting

At twenty-four hours after antigen challenge, the animals were terminally anaesthetized with Zoletil 50 (20 mg/kg i.p.) after measurement of lung function parameters. The trachea was exposed and cannulated with a polyethylene tube connected to a syringe, and the lungs were lavaged flushing with four 5-mL aliquote of Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS) containing EDTA (10 mM) and HEPES (10 mM), and collected all of BALF. BALF was centrifuged ($200\times g$ for 10 min at 4°C) to remove the supernatant, the cells resuspended in 1 mL of HBSS, and total leukocytes in BALF counted manually with hemocytometer. Differential cell counts were undertaken on cytocentrifuged preparations (Cytospin II; Shandon Southern Instruments, Pittsburgh, PA) stained with Wright's stain. A minimum of 300 cells were counted and classified as eosinophils, neutrophils, lymphocytes and macrophages based on normal morphologic criteria.

Histamine assay

Using the fluorometric method, histamine in BALF was measured (Shore *et al.*, 1959). Briefly, BALF (1 mL) are transferred to a test tube and added H_2O (1 mL). Four min after adding 0.4 mL of 1N-NaOH followed by 0.1 mL of OPT reagent, 0.2 mL of 3N-HCl is added. The mixture is thoroughly mixed after each addition. The solution is then transferred to a 96-well plate and the fluorescence measured at excitation 350 nm, emission 650 nm with fluorospectrophotometer (FL600 Microplate Fluorescence Reader, Bio-Tek, U.S.A.).

Phospholipase A_2 assay

Using pyrene-labeled phospholipids (10-pyrene PC), PLA_2 activity was measured in the presence of serum albumin with fluorospectrophotometer at excitation 345 nm, emission 398 nm. Spectrofluorometric analysis of pyrene phospholipids and fatty acids were carried out in reaction solution (Radvanyi *et al.*, 1989). The 10-pyrene PC was dried under nitrogen and suspended in ethanol at a concentration of 0.2 mM. The reaction solution was prepared by adding sequentially 1 mL of buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA; 10

μL of substrate (2 μM , final concentration); 10 μL of 10% bovine serum albumin solution (0.1%, final concentration) and 6 μL of 1 M CaCl_2 (6 mM, final concentration). The fluorescence of the reaction medium (blank) was recorded and the reaction was initiated by the addition of BALF. The specific activity in nanomoles per minute and per milligram protein (nmol/min/mg) was obtained by dividing the activity by the amount of protein in milligram. To calculate the specific activity, protein contents were analyzed with BCA (bicinchoninic acid) protein assay kit (Pierce Co., U.S.A.).

Histological staining

The lung tissues were fixed by inflating the lunge with phosphate-buffered formalin saline via tracheal cannula to a pressure of 15 cmH_2O and were then removed and prepared for staining with hematoxylin and eosin (H & E) before mounting canada balsam.

Data analysis

All the values were represented as mean $\pm$ SEM of six animals. Results of airway function measurement are expressed as changes (%) from baseline. The significance of differences of parameters between groups of drug-treated animals and their respective control (OA) groups were calculated by Student's *t*-test. Differences were considered significant if $P < 0.05$. All statistics were performed with a Sigma plot.

RESULTS

Increase of sRaw by AOA inhalation in AOA-sensitized guinea-pigs

Time-related changes of sRaw in AOA-inhaled guinea-pigs showed IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge (Fig. 1). AOA-challenged guinea-pigs had a significantly higher increase in sRaw than saline-challenged guinea-pigs in IAR (4.21 ± 0.53 to 13.92 ± 1.40 mmHg $\times$ sec), LAR (4.55 ± 0.58 to 11.80 ± 2.07 mmHg $\times$ sec), and LLAR (4.14 ± 0.56 to 11.10 ± 1.93 mmHg $\times$ sec), respectively ($P < 0.01$). AOA inhalation increased sRaw by 330.64% in IAR, 259.34% in LAR, and 268.12% in LLAR compared with saline inhalation (Fig. 1).

Recruitment of leukocytes into the lung by AOA inhalation in AOA-sensitized guinea-pigs

AOA-challenged guinea-pigs had significantly ($P < 0.05$) more total leukocytes in BALF compared with saline-challenged animals in IAR (7.34×10^5 to 12.17×10^5 cells), LAR (6.18×10^5 to 28.37×10^5 cells), and LLAR (7.11×10^5 and 27.69×10^5 cells), respectively ($p < 0.05$). The AOA-challenge caused leukocytosis, with neutrophils in BALF by 5.8 times in LAR (1.43×10^5 to 8.23×10^5 cells), and by

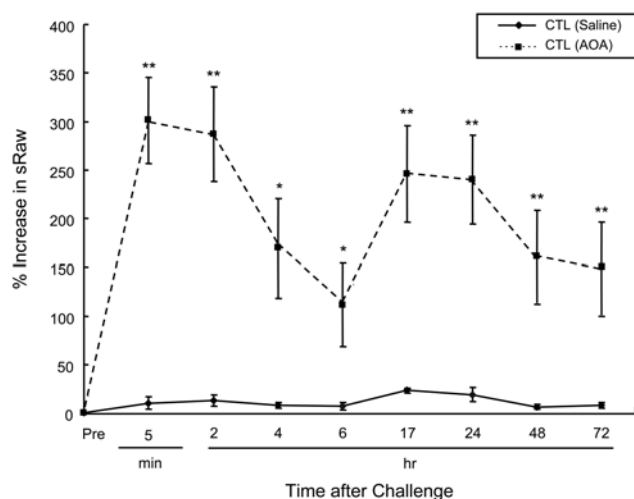


Fig. 1. Time-related changes of sRaw in control groups. Animals were actively sensitized by AOA inhalation (1 w/v % ovalbumin in saline) for 3 min on Day 1 and 7, and challenged with AOA inhalation on Day 21. sRaw was determined after AOA challenge. Time-related changes of sRaw shows IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge. % Increase in sRaw = (sRaw after challenge/sRaw before challenge-1) $\times$ 100. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from control (saline), *; $P < 0.05$ and **; $P < 0.01$. CTL: saline- or AOA-inhaled control.

3.4 times in LLAR (1.64×10^5 to 5.54×10^5 cells), and eosinophils in BALF by 8.9 time in LAR (0.89×10^5 to 7.94×10^5 cells), and by 13.9 times in LLAR (0.92×10^5 to 12.74×10^5 cells), respectively (Fig. 2).

AOA inhalation increase histamine, protein, and PLA₂ in BALF by AOA inhalation in AOA-sensitized guinea-pigs

Histamine contents in BALF of saline- and AOA-challenged guinea-pigs were 0.22 ± 0.03 and 1.79 ± 0.21 ng/mL in IAR, 0.25 ± 0.04 and 1.80 ± 0.28 ng/mL in LAR, and 0.21 ± 0.04 and 1.15 ± 0.31 ng/mL in LLAR, respectively, or an increase of 7.1 times in IAR, 6.2 times in LAR, and 4.5 times in LLAR by AOA challenge, respectively (Fig. 5).

Protein levels in BALF, a measure of exudates in saline- and AOA-inhaled animals were 643.10 ± 85.40 and 971.82 ± 106.11 μ g/mL in IAR, 625.83 ± 76.31 and 1713.20 ± 213.92 μ g/mL in LAR, and 633.96 ± 68.03 and 1282.22 ± 161.73 μ g/mL in LLAR, respectively, or an increase of $51.1 \pm 16.5\%$ in IAR, $173.8 \pm 34.2\%$ in LAR, and $103.3 \pm 25.0\%$ in LLAR by AOA challenge, respectively (Fig. 5).

PLA₂ activity in BALF of saline-inhaled and AOA-inhaled guinea-pigs were 3.21 ± 0.62 and 6.92 ± 0.81 nmol/mg/min in IAR, 2.11 ± 0.10 and 8.72 ± 0.32 nmol/mg/min in LAR, and 3.14 ± 0.42 and 8.75 ± 0.85 nmol/mg/min in LLAR, respectively, or an increase of 2.16 times in IAR, 4.13 times in LAR, and 2.79 times in LLAR by AOA challenge, respectively (Fig. 5).

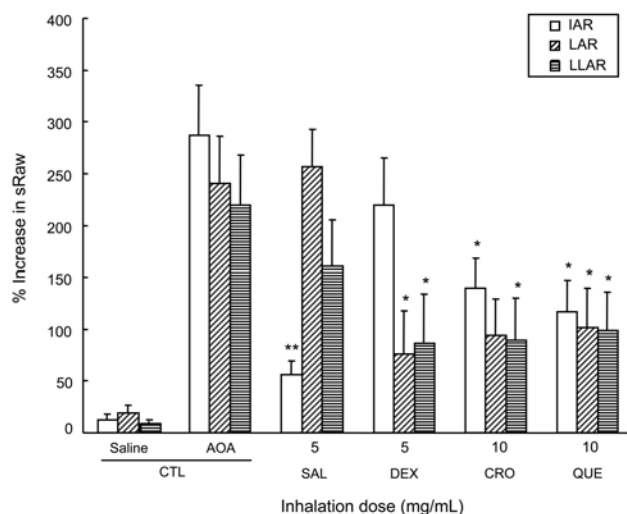


Fig. 2. Inhibitory activity of drug inhalation on sRaw in asthmatic responses. Animals were actively sensitized by AOA inhalation (1 w/v % ovalbumin in saline) for 3 min on Day 1 and 7, and challenged with AOA inhalation on Day 21. Animals were inhaled with aerosolized drug in saline for 2 min at 1 h before and 6 h after last AOA challenge, additionally 12 and 20 hr later, respectively. sRaw was determined after AOA challenge. Data shows IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge. % Increase in sRaw = (sRaw after challenge/sRaw before challenge-1) $\times$ 100. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from control (AOA), *; $P < 0.05$ and **; $P < 0.01$. CTL: saline- or AOA-inhaled control, SAL: salbutamol, DEX: dexamethasone, CRO: cromolyn sodium, QUE: quercetin.

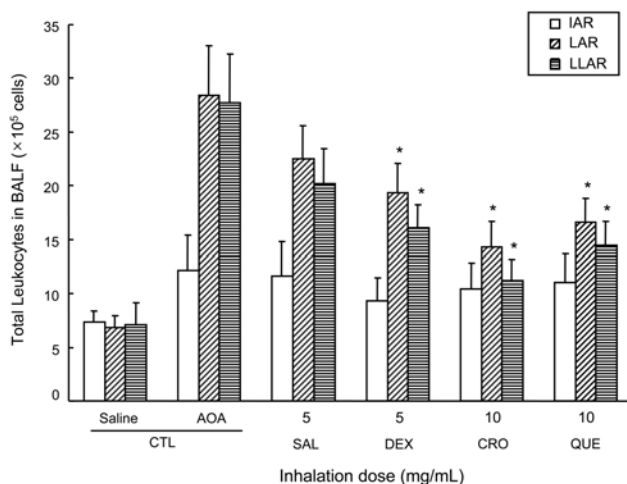


Fig. 3. The inhibitory activity of drug inhalation on recruitment of total leukocytes into lung. Animals were inhaled with aerosolized drug in saline for 2 min at 1 h before and 6 h after last AOA challenge, additionally 12 and 20 hr later, respectively. Data shows IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from control (AOA), *; $P < 0.05$. Abbreviations are the same as Fig. 2.

Histopathological changes by AOA inhalation

The lung of saline-challenged guinea-pigs showed normal architecture and did not seen any pathological changes

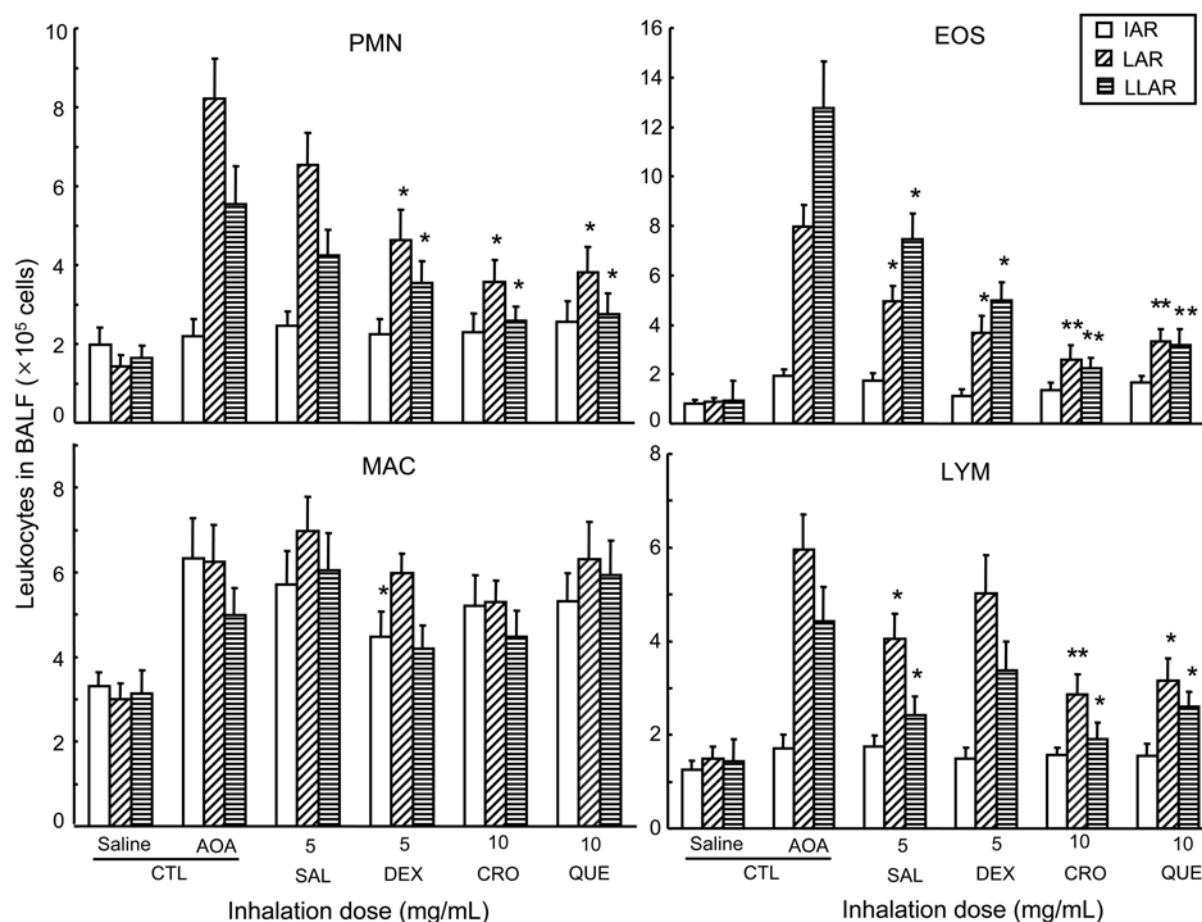


Fig. 4. Inhibitory activity of drug inhalation on recruitment of leukocytes into lung. Animals were inhaled with aerosolized drug in saline for 2 min at 1 h before and 6 h after last AOA challenge, additionally 12 and 20 h later, respectively. Data shows IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge. Each value represents the mean $\pm$ S.E.M ($n=6$). Significantly different from control (AOA), *; $P < 0.05$ and **; $P < 0.01$. PMN: Polymorphonuclear leukocytes, EOS: eosinophils, MAC: macrophages, LYM: lymphocytes. Other abbreviations are the same as Fig. 2.

(Fig. 7-A), but the lungs of AOA-challenged guinea pigs were seen marked infiltration of eosinophils and neutrophils in the alveolar sac and peribronchial connective tissue (Fig. 7-B).

Effect of quercetin inhalation on AOA-induced asthmatic responses

Decrease of sRaw

Quercetin (10 mg/mL, inhalation for 2 min) significantly decreased ($P < 0.05$) sRaw by $57.15 \pm 6.53\%$ (13.92 ± 1.40 to 7.47 ± 0.91 mmHg $\times$ sec) in IAR, $57.72 \pm 5.72\%$ (11.80 ± 2.07 to 6.75 ± 0.79 mmHg $\times$ sec) in LAR, and $55.20 \pm 7.36\%$ (11.10 ± 1.93 to 6.65 ± 0.81 mmHg $\times$ sec) in LLAR compared with AOA-inhaled control, respectively (Fig. 2). Salbutamol (5 mg/mL, inhalation for 2 min) significantly decreased sRaw in IAR by $51.90 \pm 5.82\%$ compared with AOA-inhaled control, but it has not activity in LAR and LLAR (Fig. 2). Dexamethasone (5 mg/mL, inhalation for 2 min) significantly decreased sRaw by 68.51

$\pm 6.36\%$ in LAR and $47.21 \pm 7.56\%$ in LLAR compared with AOA-inhaled control, but it has not effect in IAR, respectively (Fig. 2). Cromolyn sodium (10 mg/mL, inhalation for 2 min) significantly decreased sRaw by $51.20 \pm 6.75\%$ in IAR, $60.76 \pm 6.27\%$ in LAR, and $59.25 \pm 7.57\%$ in LLAR compared with AOA-inhaled control (Fig. 2).

Decreased recruitment of total leukocytes in BALF

Quercetin (10 mg/mL, inhalation for 2 min) significantly ($P < 0.05$) inhibited total leukocytes accumulation in BALF on IAR, LAR, and LLAR ($11.06 \pm 2.67 \times 10^5$, $16.60 \pm 2.19 \times 10^5$, and $14.47 \pm 2.28 \times 10^5$ cells, respectively) compared with AOA-inhaled control, respectively. Salbutamol (5 mg/mL, inhalation for 2 min) inhibited total leukocytes accumulation in BALF on IAR, LAR, and LLAR ($11.67 \pm 3.11 \times 10^5$, $22.48 \pm 3.09 \times 10^5$, and $20.17 \pm 3.29 \times 10^5$ cells, respectively). Dexamethasone (5 mg/mL, inhalation for 2 min) significantly inhibited total leukocytes accumulation in BALF on IAR, LAR, and LLAR ($9.31 \pm 2.13 \times 10^5$, $19.31 \pm 2.81 \times 10^5$, and $16.08 \pm 2.18 \times 10^5$ cells, respectively).

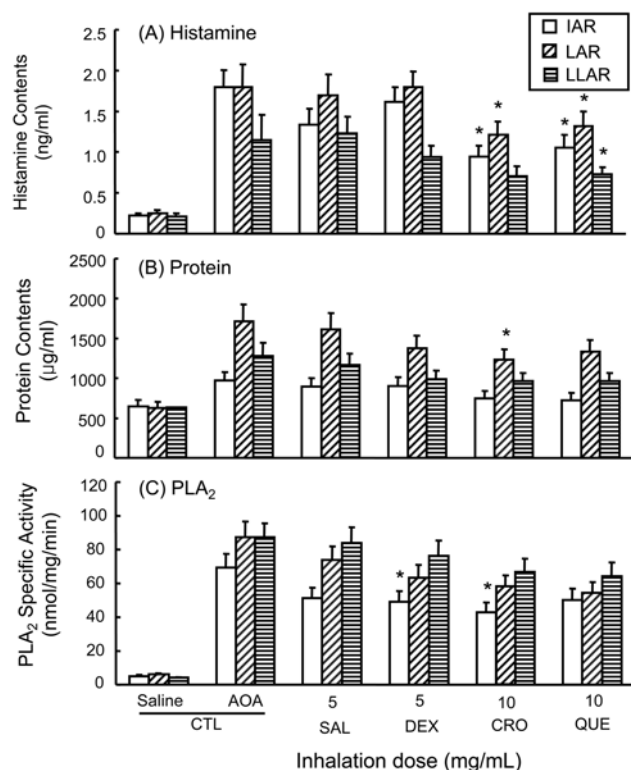


Fig. 5. Effect of drug inhalation on histamine and protein contents, and PLA₂ activity in BALF. Animals were inhaled with aerosolized drug in saline for 2 min at 1 h before and 6 hr after last AOA challenge, additionally 12 and 20 h later, respectively. Data shows IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from control (AOA), *; $P < 0.05$. Abbreviations are the same as Fig. 2.

Cromolyn sodium (10 mg/mL, inhalation for 2 min) significantly inhibited total leukocytes accumulation in BALF on IAR, LAR and LLAR ($10.40 \pm 2.43 \times 10^5$, $14.28 \pm 2.37 \times 10^5$, and $11.18 \pm 1.97 \times 10^5$ cells, respectively) (Fig. 3).

Decreased recruitment of neutrophils, eosinophils and lymphocytes in BALF

Quercetin (10 mg/mL, inhalation for 2 min) significantly inhibited recruitment of neutrophils ($53.58 \pm 7.78\%$ in LAR and $50.36 \pm 9.57\%$ in LLAR, $P < 0.05$), eosinophils ($58.18 \pm 6.55\%$ in LAR and $75.04 \pm 5.18\%$ in LLAR, $P < 0.01$), and lymphocytes ($47.15 \pm 7.89\%$ in LAR and $41.31 \pm 7.22\%$ in LLAR, $P < 0.05$) in BALF compared with AOA-inhaled control, respectively (Fig. 4). Salbutamol (5 mg/mL, inhalation for 2 min) significantly inhibited recruitment of eosinophils ($37.66 \pm 7.93\%$ in LAR and $41.11 \pm 8.16\%$ in LLAR, $P < 0.05$) and lymphocytes ($32.05 \pm 9.06\%$ in LAR and $45.37 \pm 8.80\%$ in LLAR, $P < 0.05$) in BALF compared with AOA-inhaled control, respectively (Fig. 4). Dexamethasone (5 mg/mL, inhalation for 2 min) significantly inhibited recruitment of neutrophils ($43.74 \pm 9.36\%$ in LAR and 36.10

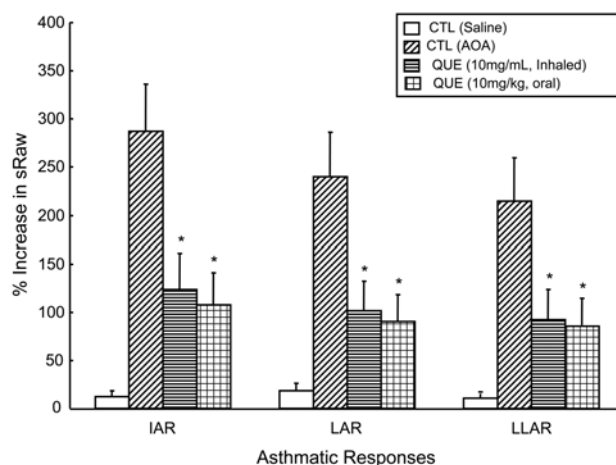


Fig. 6. Comparison of oral and inhaled quercetin on inhibition of sRaw. Animals were inhaled with aerosolized drug in saline for 2 min at 1 h before and 6 hr after last AOA challenge, additionally 12 and 20 h later, respectively. sRaw shows IAR at 5 min, LAR at 24 h and LLAR at 72 h after AOA challenge. Each value represents the mean $\pm$ S.E.M (n=6). Significantly different from control (AOA), *; $P < 0.05$. CTL: saline- or AOA-inhaled control, QUE: quercetin.

$\pm 10.29\%$ in LLAR, $P < 0.05$) and eosinophils ($37.66 \pm 8.69\%$ in LAR and $41.11 \pm 5.73\%$ in LLAR, $P < 0.05$) in BALF compared with AOA-inhaled control, respectively (Fig. 4). Cromolyn sodium (10 mg/mL, inhalation for 2 min) significantly inhibited recruitment of neutrophils ($56.62 \pm 2.24\%$ in LAR and $53.61 \pm 6.86\%$ in LLAR, $P < 0.05$), eosinophils ($67.63 \pm 7.30\%$ in LAR and $81.16 \pm 3.38\%$ in LLAR, $P < 0.01$), and lymphocytes ($35.23 \pm 7.38\%$ in LAR, $P < 0.05$ and $57.11 \pm 8.13\%$ in LLAR, $P < 0.01$) in BALF compared with AOA-inhaled control, respectively (Fig. 4).

Decrease of the chemical mediators in BALF

Quercetin (10 mg/mL, inhalation for 2 min) significantly inhibited histamine contents ($47.13 \pm 8.94\%$ in IAR, $31.61 \pm 10.00\%$ in LAR and $44.68 \pm 7.82\%$ in LLAR, $P < 0.05$) in BALF compared with AOA-inhaled control (Fig. 5). Cromolyn sodium (10 mg/mL, inhalation for 2 min) significantly decreased histamine contents ($53.50 \pm 7.26\%$ in IAR and $38.06 \pm 8.89\%$ in LAR, $P < 0.05$), and protein contents ($44.34 \pm 7.71\%$ in LAR, $P < 0.05$) and PLA₂ activity ($72.97 \pm 8.69\%$ in IAR, $P < 0.05$) in BALF compared with AOA-inhaled control (Fig. 5). Dexamethasone (5 mg/mL, inhalation for 2 min) significantly ($p < 0.05$) decrease PLA₂ activity in BALF ($54.05 \pm 8.69\%$ in IAR, $P < 0.05$) compared with AOA-inhaled control (Fig. 5).

Comparison of oral administration and aerosolized-inhalation of quercetin on decrease of sRaw

Both oral administration (10 mg/kg) and inhalation (10 mg/mL for 2 min) of quercetin significantly decreased sRaw by 62.56 ± 14.99 and $57.15 \pm 6.53\%$ in IAR, 62.40

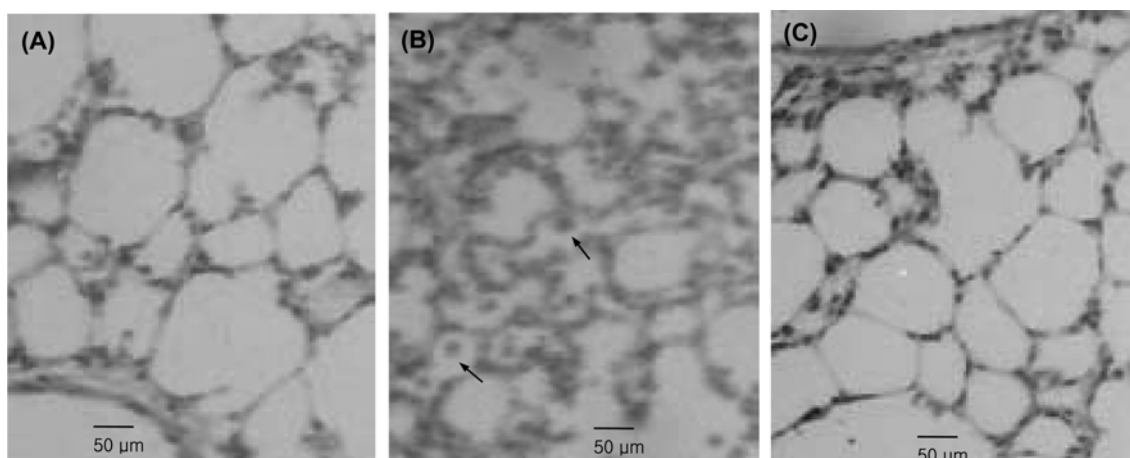


Fig. 7. Lung of guinea-pigs 24 h after saline challenge showed normal architecture and did not see any pathological changes (A), but lungs 24 h after AOA challenge were seen marked eosinophils and neutrophils infiltration in the alveolar sac and peribronchial connective tissue and cellular swelling (B). Quercetin (10 mg/mL, inhalation for 2 min) inhibited markedly infiltration of inflammatory cells, particularly recruitment of eosinophils and neutrophils in the alveolar sac and connective tissues (C) (H & E, $\times 200$). Scale bar, 50 μ m.

± 5.88 and $57.72 \pm 5.72\%$ in LAR, and 60.45 ± 5.68 and $55.20 \pm 7.36\%$ in LLAR compared with AOA-inhaled control, respectively (Fig. 6).

Improvement of histopathological changes

Quercetin (10 mg/mL, inhalation for 2 min) inhibited markedly infiltration of inflammatory cells, particularly recruitment of eosinophils and neutrophils in the alveolar sac, the peripheral vasculature, and the terminal bronchiole, as well as leukocytes in venules and arterioles (Fig. 7-C).

DISCUSSION

Asthmatic responses (IAR, LAR, and LLAR) with the elevation of sRaw and recruitments of leukocytes into lung by AOA exposure to lung in AOA-sensitized guinea pigs could be developed using other reports (Matsumoto *et al.*, 1994, Huston *et al.*, 1988). For preparing an asthmatic model in guinea-pigs by AOA challenge, guinea-pigs sensitized with AOA on Day 1 and 14, were same as guinea-pigs sensitized with a subcutaneous and intraperitoneal injection of ovalbumin for elevation of sRaw (Toward and Broadley, 2004; Su *et al.*, 2001). The inhibitory activity of quercetin inhalation (10 mg/mL for 2 min) on sRaw was same as its oral administration (10 mg/kg). And also quercetin and cromolyn sodium (10 mg/mL, inhalation for 2 min) had similar anti-asthmatic activity in IAR, LAR, and LLAR. Cromolyn sodium, dichromone flavonoid derivative which is chemically the disodium salt of 1,3-bis (2-carboxychromon-5-yloxy)-2-hydroxypropane, developed as mast cell stabilizer and anti-allergic agent to prevent the wheezing, shortness of breath, and troubled

breathing caused by asthma and exercise. Cromolyn sodium Inhaler is a metered dose aerosol unit for oral inhalation containing micronized cromolyn sodium. Each 14.2 g canister delivers at least 200 metered inhalations (100 doses) and each metered inhalation delivers approximately 0.8 mg cromolyn sodium through the mouthpiece to the patient. As comparison of inhalation dosages of cromolyn sodium and quercetin, these results indicated that quercetin inhalation can make less side effects than its oral administration for the treatment of asthma with flavonoids because the real inhaled dose on guinea-pig was about 0.14 mg for 2 min considering to jet nebulizer capacity (6.75 L/min, 1.5 bar), and tidal volume and respiratory rate of experimental animal. Now, quercetin can be developed as powder inhaler (Nora *et al.*, 2000) or liquid inhaler after pulverization or solubilization, respectively (Hirai *et al.*, 2007).

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REFERENCES

- Benhamou, A. H., Vanini, G., Lantin, J. P., and Eigenman, P. A., Antihistamine and sodium cromoglycate medication for food cold water exercise-induced anaphylaxis. *Allergy*, 62, 1471-1472 (2007).
- Crompton, G., A brief history of inhaled asthma therapy over the last fifty years. *Prim. Care Respir. J.*, 15, 326-331 (2006).
- Fewtrell, C. M. S. and Gomperts, B. D., Effect of flavone inhibitors of transport ATPases on histamine secretion from rat

- mast cells. *Nature*, 265, 635-636 (1997).
- Hirai, S., Kim, Y. I., Goto, T., Kang, M. S., Yoshimura, M., Obata, A., Yu, R., and Kawada, T., Inhibitory effect of naringenin chalcone on inflammatory changes in the interaction between adipocytes and macrophages. *Life Sci.*, 81, 1272-1279 (2007).
- Huston, P. A., Chuch, M. K., Clay, T. P., Miller, P., and Holgate, S. P., Early and late-phase bronchoconstriction after allergen challenge of nonanesthetized guinea pigs. I. The association of disordered airway physiology to leukocyte infiltration. *Am. Rev. Respir. Dis.*, 137, 548-557 (1988).
- Hutson, P. A., Holgate, S. T., and Church, M. K., The effect of cromolyn sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of nonanesthetized guinea pigs. *Am. Rev. Respir. Dis.*, 138, 1157-1163 (1988).
- Jiang, J. S., Shih, C. M., Wang, S. H., Chen, T. T., Lin, C. N., and Ko, W. C., Mechanisms of suppression of nitric oxide production by 3-O-methylquercetin in RAW 264.7 cells. *J. Ethnopharmacol.*, 103, 281-287 (2006).
- Jung, C. H., Lee, J. Y., Cho, C. H., and Kim, C. J., Anti-asthmatic action of quercetin and rutin in conscious guinea-pigs challenged with aerosolized ovalbumin. *Ach. Pharm. Res.*, 30, 1599-1607 (2007).
- Li, W. G., Zhang, X. Y., Wu, Y. J., and Tian, X., Anti-inflammatory effect and mechanism of proanthocyanidins from grape seeds. *Acta Pharmacol. Sin.*, 22, 1117-1120 (2001).
- Matsumoto, T., Ashida, Y., and Tsukuda, R., Pharmacological modulation of immediate and late airway response and leukocyte infiltration in the guinea pig. *J. Pharmacol. Exp. Ther.*, 269, 1236-1244 (1994).
- Morales, M. A., Tortoriello, J., Meckes, M., Paz, D., and Lozoya, X., Calcium-antagonist effect of quercetin and its relation with the spasmolytic properties of *Psidium guajava* L. *Arch. Med. Res.*, 25, 17-21 (1994).
- Nishino, H., Iwashima, A., Fujiki, H., and Suginuma, T., Inhibition by quercetin of the promoting effect of teleocidin on skin papilloma formation in mice initiated with 7,12-dimethylbenz [a]anthracene. *Jpn. J. Cancer Res.*, 75, 113-116 (1984).
- Chew Nora Y. K., Bagster Ddvid F., and Chan Hak-Kim, Effect of particle size, air flow and inhaler device on the aerosolisation of disodium cromoglycate powders, *Int J Pharmaceut.*, 206, 75-83 (2000).
- Omisore, N. O., Adewunmi, C. O., Iwalewa, E. O., Ngadjui, B. T., Adenowo, T. K., Abegaz, B. M., Ojewole, J. A., and Watchueng, J., Antitrichomonal and antioxidant activities of *Dorstenia barteri* and *Dorstenia convexa*. *Braz. J. Med. Biol. Res.*, 38, 1087-1094 (2005).
- Pennock, B. E., Pennock, C. P., Rogers, R. M., Cain, W. A., and Wells, J. H., A noninvasive technique for measurement of changes in specific airway resistance. *J. Appl. Physiol.*, 46, 399-406 (1979).
- Pettinari, A., Amici, M., Cuccioloni, M., Angeletti, M., Fioretti, E., and Eleuteri, A. M., Effect of polyphenolic compounds on the proteolytic activities of constitutive and immunoproteasomes. *Antioxid. Redox. Signal.*, 8, 121-129 (2006).
- Radvanyi, F., Jordan, L., Russo-Marie, F., and Bon, C., A sensitive and continuous fluorometric assay for phospholipase A₂ using pyrene-labeled phospholipids in the presence of serum albumin. *Anal. Biochem.*, 177, 103-109 (1989).
- Ramesh, M., Rao, Y. N., Rao, A. V., Prabhakar, M. C., Rao, C. S., Muralidhar, N., and Reddy, B. M., Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. *J. Ethnopharmacol.*, 62, 63-66 (1998).
- Sanjar, S., Aoki, S., Kristersson, Smith, D., and Moley, J., Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitized guinea-pigs: the effect of anti-asthma drugs. *Br. J. Pharmacol.*, 99, 679-686 (1990).
- Shibata, S., Inoue, H., Iwata, S., Ma, R. D., Yu, L. J., Ueyama, H., Takayasu, J., Hasegawa, T., Tokuda, H., and Nishino, A., Inhibitory effects of licochalcone A isolated from *Glycyrrhiza inflata* root on inflammatory ear edema and tumour promotion in mice. *Planta Med.*, 57, 221-224 (1991).
- Shore, P. A., Burkhalter, A., and Cohn, V. H. Jr., A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.*, 127, 182-186 (1959).
- Skaltsa, H., Bermejo, P., Lazari, D., Silvan, A. M., Skaltsounis, A. L., Sanz, A., and Abad, M. J., Inhibition of prostaglandin E₂ and leukotriene C₄ in mouse peritoneal macrophages and thromboxane B₂ production in human platelets by flavonoids from *Stachys chrysantha* and *Stachys candida*. *Biol. Pharm. Bull.*, 23, 47-53 (2000).
- Su, Y. C., Peng, H. J., Wang, S. R., Han, S. H., and Tsai, J. J., Effect of BCG on ovalbumin-induced bronchial hyperreactivity in a guinea pig asthma model. *J. Microbiol., Immunol. Infect.*, 34, 25-34 (2001).
- Toward, T. J. and Broadley, K. J., Early and late bronchoconstrictions, airway hyper-reactivity, leucocyte influx and lung histamine and nitric oxide after inhaled antigen: effects of dexamethasone and rolipram. *Clin. Exp. Allergy*, 34, 91-102 (2004).
- Villar, A., Gasco, M. A., and Alcaraz, M. J., Anti-inflammatory and anti-ulcer properties of hypolaetin-8-glucoside, a novel plant flavonoid. *J. Pharm. Pharmacol.*, 36, 820-823 (1984).
- Wu, M. J., Wang, L., Ding, H. Y., Weng, C. Y., and Yen, J. H., *Glossogyne tenuifolia* acts to inhibit inflammatory mediator production in a macrophage cell line by down-regulating LPS-induced NF-kappa B. *J. Biomed. Sci.*, 11, 186-199 (2004).