



Long-term oral administration of *Gynostemma pentaphyllum* extract attenuates airway inflammation and Th2 cell activities in ovalbumin-sensitized mice

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ABSTRACT

Our previous report demonstrated that the oral administration of short-term high dose *Gynostemma pentaphyllum* extract (5 g/kg per day for 7 days) decreased allergic reactions in ovalbumin (OVA)-sensitized mice. The aim of this study was to determine whether long-term oral administration of *G. pentaphyllum* attenuated airway inflammation in OVA-sensitized mice.

Mice were sensitized and challenged with normal saline or OVA. OVA-sensitized mice were fed with 1.75 g/kg (low dose, GPL) or 5 g/kg (high dose, GPH) *G. pentaphyllum* extract, five days a week for 4 weeks. The airway hyperresponsiveness (AHR) and eosinophilia in bronchoalveolar lavage fluid (BALF) were examined. The cytokine levels or antibodies in BALF, serum and spleen cell culture supernatants were also determined. Both high and low dose extracts reduced AHR, serum OVA-IgE, and Th2-associated cytokine levels in spleen cell supernatants and BALF in OVA-sensitized mice. These results show that long-term orally administered *G. pentaphyllum* extract reduced allergic reactions in OVA-sensitized mice.

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1. Introduction

Asthma is an important public health problem in the world (Barrios et al., 2006). The symptoms of severe asthma include coughing, wheezing and breathlessness caused by contraction of the smooth muscles in the airways and by excessive mucus; these symptoms can result in suffocation (Barrios et al., 2006). Studies indicate that there is increased eosinophil infiltration and airway hyperresponsiveness (AHR) in the airways of asthma patients compared to unaffected individuals (Kay et al., 2004; Rothenberg and Hogan, 2006; Scichilone et al., 2005). Activated eosinophils release inflammatory substances that induce severe asthma symptoms (Rothenberg and Hogan, 2006), and Th2 cells secrete IL-4, IL-5, IL-9 and IL-13 to contribute to the pathogenesis of asthma, including increased AHR and eosinophil infiltration (Georas et al., 2005; Kips, 2001). Therefore, inhibiting Th2 cell activity might be effective in suppressing asthma symptoms.

Gynostemma pentaphyllum Makino (Cucurbitaceae) is a perennial creeping vine that grows mainly in the countryside of Southern China, Taiwan, Japan and Korea (Kuwahark et al., 1989). Traditional Chinese doctors use *G. pentaphyllum* to treat hypertension, hyper-

lipidaemia and lung inflammation. *G. pentaphyllum* is also commonly used as an herbal food or tea in China and Taiwan (Razmovski-Naumovski et al., 2005). Recent studies found that *G. pentaphyllum* has additional pharmacological effects that are useful for treating hyperlipidaemia, hepatitis, gastric ulcers and cancer as well as for regulating blood sugar (Cour et al., 1995; Lin et al., 2000; Norberg et al., 2004; Razmovski-Naumovski et al., 2005). We found previously that *G. pentaphyllum* extracts administered intraperitoneally or orally to BALB/c mice enhanced Th1-associated cytokine secretion by spleen cells (Huang et al., 2007a,b). With the consideration that asthma is a Th2 cell-mediated inflammatory disease, we fed OVA-sensitized mice with high doses (5 g/kg) of *G. pentaphyllum* for seven consecutive days. The results indicated that *G. pentaphyllum* suppressed AHR, eosinophil infiltration of the lungs, OVA-IgE in serum and Th2-associated cytokine production by spleen cells (Huang et al., 2008). Short-term administration of *G. pentaphyllum* decreased asthmatic symptoms in OVA-sensitized mice.

In this study, OVA-sensitized mice were treated with either a high dose *G. pentaphyllum* (GPH group, fed 5 g/kg) or a low dose *G. pentaphyllum* group (GPL, fed 1.75 g/kg) for a relatively longer period of time. Mice in each group were fed with *G. pentaphyllum* once a day, five days a week, for 4 weeks (a total of 20 doses) during the study. We found that long-term oral administration of both high and low dose *G. pentaphyllum* suppressed asthma symptoms in OVA-sensitized mice.

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2. Materials and methods

2.1. Animals

Six- to eight-week-old female BALB/cByJNarl mice were purchased from the National Laboratory Animal Center (Taipei, Taiwan). All mice were housed for one week before starting the experiments at the Animal Center of Chang Gung University. The mice were cared and handled according to the guidelines of the Animal Care Committee of Chang Gung University and the NIH Guidelines for the Care and Use of Laboratory Animals.

2.2. Preparation of *G. pentaphyllum* extract

The *G. pentaphyllum* extract was prepared as described previously (Huang et al., 2008). Dried whole *G. pentaphyllum* was boiled in water (50 g/L) for 50 min. The extract was centrifuged and the supernatant was lyophilized. The ratio of extract powder to original whole *G. pentaphyllum* was 36.2% (w/w). Then, the lyophilized extract powder was dissolved in phosphate-buffered saline (PBS), sterilized using 0.22- μ m filters, frozen and stored at -80°C until use. The mice were fed orally with a gastric feeding tube and the administered dose for each mouse was calculated and presented based on the weight of dried *G. pentaphyllum* herb.

2.3. Mouse sensitization, challenge and administration of *G. pentaphyllum*

Mice were sensitized and challenged as described previously (Huang et al., 2008). Briefly, mice were immunized by intraperitoneal injection of 50 μ g OVA/200 μ l in normal saline (Grade V; Sigma, St. Louis, MO, USA) with aluminum hydroxide on days 1–3 and on day 14. Mice were challenged with inhaled 2% OVA for 30 min using a nebulizer (DeVilbiss Pulmo-Aide 5650D, USA) on days 14, 17, 20, 23 and 27. The particle size ranged from 0.5 to 5 μ m and the nebulization rate was 0.15–0.35 ml/min.

Mice were divided into four groups: (1) sensitized and challenged with normal saline (N group, $n = 9$); (2) sensitized and challenged with OVA (OVA group, $n = 9$); (3) sensitized and challenged with OVA fed with 1.75 g/kg *G. pentaphyllum* (GPL group, $n = 9$); or (4) sensitized and challenged with OVA fed with 5 g/kg *G. pentaphyllum* (GPH group, $n = 8$). The mice in each group were fed once a day, five consecutive days per week for 4 weeks with saline (N and OVA groups) or *G. pentaphyllum* extracts (GPL and GPH groups) in addition to their regular diet.

2.4. Measurement of AHR

On day 28, 24 h after the last OVA challenge, AHR was assayed in a whole-body plethysmograph (Buxco Electronics, Troy, NY, USA) as described previously (Huang et al., 2008). Briefly, mice inhaled methacholine (6.25–50 mg/ml). The AHR was recorded and expressed as enhanced pause (Penh) that was calculated with the software provided by Buxco Electronics. Penh values were defined as $(\text{Te} - \text{Tr})/\text{Tr} \times \text{PIF}/\text{PEF}$ (Te, expiratory time; Tr, relaxation time; PIF, peak inspiratory flow; PEF, peak expiratory flow) (Park et al., 2007).

2.5. Collection of bronchoalveolar lavage fluid (BALF)

The mice were sacrificed with cervical spine dislocated method on day 29. BALF was collected from each mouse by lavage of the lungs (3×1 ml of normal saline). The total number of cells in the BALF was determined, and the percentage of eosinophils was measured by cytospin centrifugation and Liu staining. The BALF supernatant was subsequently collected, frozen, and stored at -80°C until use in the ELISA.

2.6. Serum collection and splenocyte culture

Blood was collected from the orbital vascular plexus and centrifuged at 6000 rpm for 5 min. The serum was collected and stored at -80°C until use. Spleen cells were prepared as previously described (Liou et al., 2004). Spleen cells (5×10^6 cells/ml) were cultured in a medium containing RPMI 1640 (Invitrogen-Gibco™, Paisley, Scotland) supplemented with 10% foetal bovine serum (FBS, Biological Industries, Haemek, Israel), 100 U/ml penicillin/streptomycin, and 2 mM L-glutamine. Cells (5×10^6 cells/ml) were cultured without or with 100 μ g/ml OVA for 5 days and the supernatants were collected for ELISA assays.

2.7. Determination of OVA-specific antibodies and cytokines

Serum OVA-specific IgG1, IgG2a and IgE were measured by ELISA as described previously (Wu et al., 2006). The units of OVA-specific IgG1 were determined by comparing the OD readings to a standard curve constructed using pooled serum. OVA-IgG2a levels (serum diluted 1000-fold) and OVA-IgE levels (serum diluted 5-fold) were measured directly using OD_{490nm} readings.

Cytokine levels in the BALF samples and splenocyte culture supernatant were determined using ELISA kits specific for TNF- α , IL-4, IL-6, IL-13 and IFN- γ (R&D Systems, Minneapolis, MN, USA) and IL-5 (BD Biosciences, San Diego, CA, USA).

2.8. Lung histology

The mice were sacrificed and their lungs were fixed in 10% formalin. The fixed tissue was embedded in paraffin, sliced into 6- μ m sections and stained with H&E (Mayer's haematoxylin & eosin) as described previously (Wu et al., 2006).

2.9. Statistical analysis

The data were evaluated using one-way analysis of variance (ANOVA) with a post hoc Dunnett's test. Values are presented as means $\pm$ standard error (SE). Statistical analysis was performed using SPSS12 software (SPSS Inc., Chicago, IL, USA). Probability values (p) less than 0.05 were considered to be significant.

3. Results

3.1. Long-term oral administration of *G. pentaphyllum* extract suppressed OVA-induced AHR

To determine the effect of *G. pentaphyllum* in OVA-induced airway inflammation, the mice were fed with *G. pentaphyllum* once a day, 5 days a week, for 4 weeks. On day 28, AHR was measured in all mice with the inhalation of methacholine (Fig. 1). No significant changes in AHR were observed in the OVA group compared to the GPL or GPH groups after inhalation of 0–25 mg/ml methacholine. However, the highest dose of methacholine (50 mg/ml) induced the highest AHR (presented as Penh values) in the OVA group (5.04 ± 0.39) compared to the N (2.56 ± 0.33), GPL (2.02 ± 0.20) and GPH (2.13 ± 0.25) groups ($p < 0.01$ for all groups). This indicated that both high and low doses of *G. pentaphyllum* extract significantly inhibited AHR. Notably, the Penh values from the GPL group and the GPH group were not significantly different at the 50 mg/ml methacholine concentration ($p = 0.41$). This suggests that the high and low doses of *G. pentaphyllum* extract have a similar effect on AHR.

3.2. *G. pentaphyllum* reduced infiltration of eosinophils

The mice were sacrificed on day 29, BALF was collected from the lungs, and the total number of cells and the percentage of eosinophils in the BALF were determined (Fig. 2). The total number of cells was significantly increased in the OVA group. In particular,

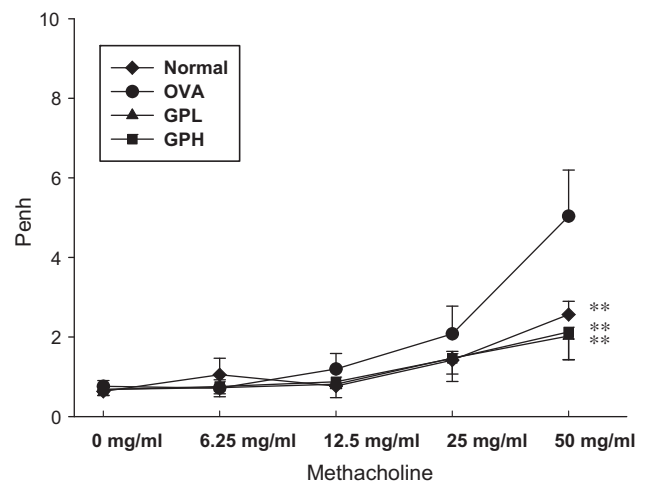


Fig. 1. Effect of long-term oral administration *G. pentaphyllum* extracts on airway hyperresponsiveness (AHR). AHR was measured 24 h after the final OVA challenge. The Penh values indicate AHR when mice inhaled the indicated doses of methacholine (6.25–50 mg/ml). Normal group (N; $\diamond$, $n = 9$), OVA-sensitive group (OVA; $\bullet$, $n = 9$), 1.75 g/kg *G. pentaphyllum* group (GPL; $\blacktriangle$, $n = 9$), 5 g/kg *G. pentaphyllum* group (GPH; $\blacksquare$, $n = 8$). Data are presented as means $\pm$ SE. $^{***}p < 0.01$ compared to the OVA group.

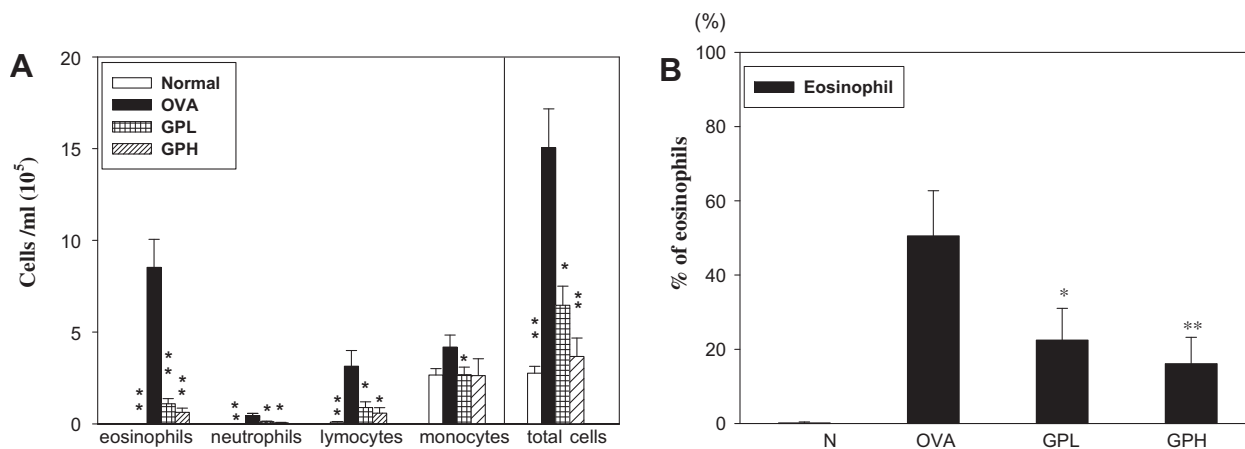


Fig. 2. *G. pentaphyllum* extracts reduced both total cell number (A) and the percentage of eosinophils (B) in bronchoalveolar lavage fluid (BALF) from OVA-sensitive mice. Data are presented as means $\pm$ SE. * $p < 0.05$ compared to the OVA group; ** $p < 0.01$ compared to the OVA group.

the percentage of eosinophils increased to $50.62 \pm 4.12\%$. Oral administration of *G. pentaphyllum* extract (GPL and GPH groups) significantly reduced the total number of cells. The percentage of eosinophils in BALF was also significantly inhibited with GPL ($22.54 \pm 3.10\%$, $p = 0.0003$) or GPH ($16.18 \pm 3.14\%$, $p = 0.001$). However, no significant difference was detected in the total cell number and the percentage of eosinophils in the GPL group compared to the GPH group ($p = 0.33$ and $p = 0.30$, respectively).

The lung tissue was removed, fixed with formalin, sectioned and stained. The distribution of eosinophils was analyzed in the sections (Fig. 3). The OVA group showed greater peribronchiolar and perivascular eosinophil infiltration compared to the N group.

In addition, low and high dose long-term oral administration of *G. pentaphyllum* extracts significantly suppressed eosinophil infiltration in the lungs.

3.3. *G. pentaphyllum* reduced Th2-associated and pro-inflammatory cytokine levels in BALF

To analyze the levels of Th2-associated cytokines and pro-inflammatory cytokine in BALF, levels of four cytokines were measured using ELISA (Fig. 4). The OVA group showed the highest levels of IL-5, IL-13, IL-6 and TNF- α . The GPL and GPH groups showed significantly decreased levels of IL-5 and TNF- α cytokines, but

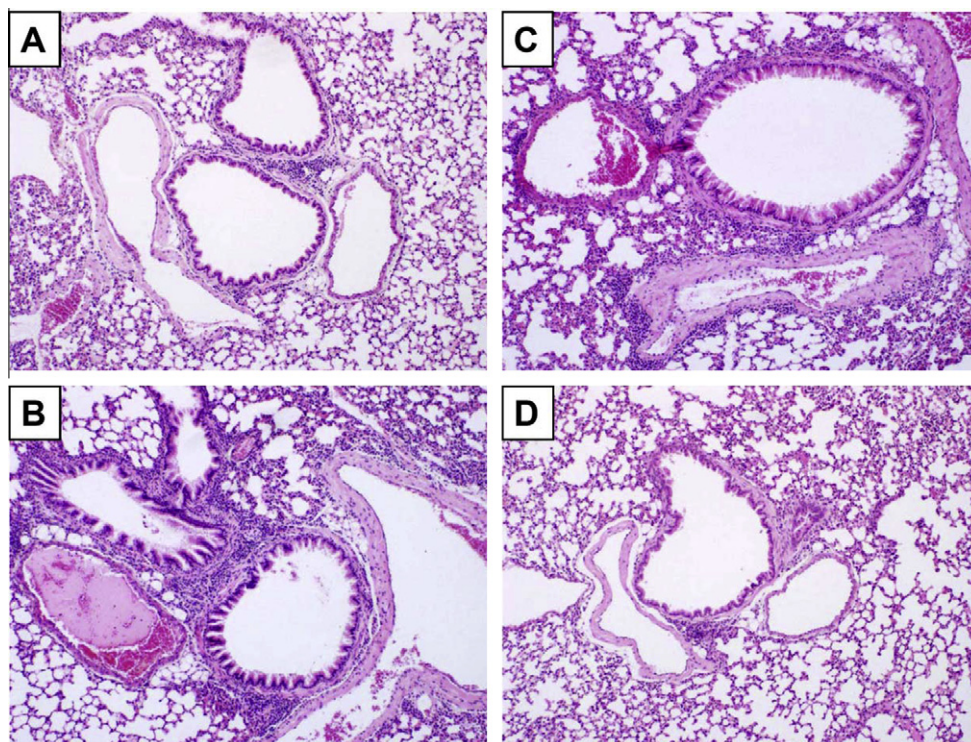


Fig. 3. Effect of *G. pentaphyllum* extracts on eosinophil infiltration in lung tissue. Lung sections were analyzed from mice in the Normal (N) group (A), OVA group (B), GPL group (C), and GPH group (D).

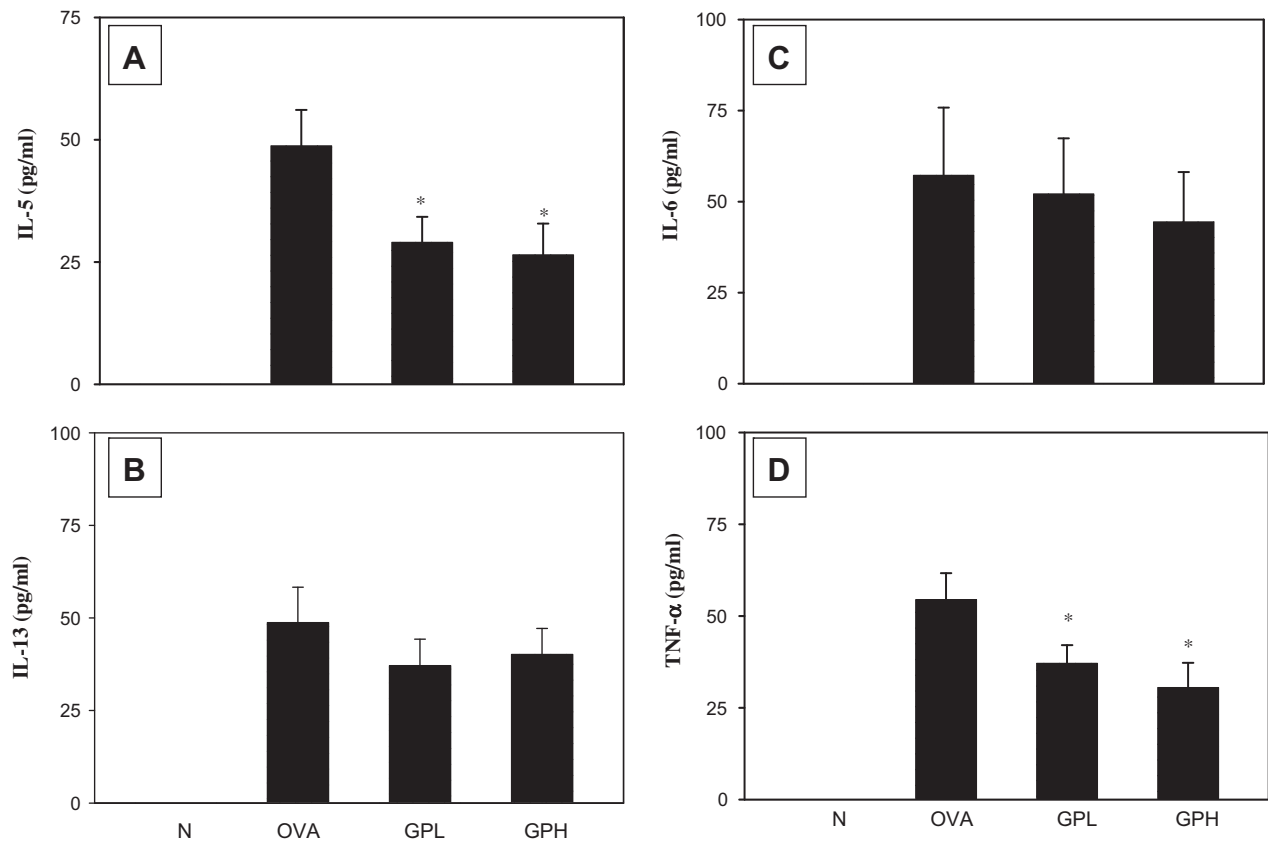


Fig. 4. *G. pentaphyllum* extract modulated the concentration of cytokines in bronchoalveolar lavage fluid (BALF). The concentrations of IL-5 (A), IL-13 (B), IL-6 (C) and TNF- α (D) were measured by ELISA. Data are presented as means $\pm$ SE. * $p < 0.05$ compared to the OVA group.

there was no significant suppression of IL-13 or IL-6. Therefore, not all Th2-associated cytokines or pro-inflammatory cytokines were suppressed when OVA-sensitized mice were given with high or low doses of *G. pentaphyllum*.

3.4. *G. pentaphyllum* inhibited serum levels of OVA-specific IgG1 and IgE

To test the effect of *G. pentaphyllum* on serum levels of OVA-specific antibodies, blood was harvested, centrifuged and the serum used for ELISA analysis (Fig. 5). Serum from mice in the OVA group had the highest levels of specific IgG1 and IgE. OVA-specific IgG1 and IgE were significantly reduced in serum from mice in the GPL and GPH groups; and OVA-IgG2a levels (as Th1-associated IgG subtype) were significantly increased in the GPL and GPH groups. However, there was no significant difference in the antibody levels in the GPL and GPH groups.

3.5. *G. pentaphyllum* inhibited Th2-associated cytokines for OVA-activated spleen cells

Spleen cells (5×10^6 /ml) were cultured with 100 μ g/ml OVA for 5 days. The cells from mice in the OVA group produced more IL-4, IL-5 and IL-13 than did the cells from mice in the Normal group (Fig. 6). However, spleen cells from mice in the OVA group fed with *G. pentaphyllum* extract (i.e. cells from mice in the GPL and GPH groups) showed decreased IL-4, IL-5 and IL-13 levels and increased IFN- γ levels. The cytokine levels were not significantly different between the GPL and GPH groups.

4. Discussion

Bronchial asthma, a disease involving chronic inflammation of the airways, is a serious health problem in most developed countries (Rothenberg and Hogan, 2006). Asthma can cause breathlessness and even death by suffocation (Barrios et al., 2006; Busse and Lemanske, 2001). In Western medicine, steroids are often used to treat asthma (Barnes, 2006). Steroids acts as immunosuppressors that reduce inflammation, but they can also have many side effects (Barnes, 2006). Therefore, some investigators have tested alternative drugs or therapy models for treating asthma. Complementary treatments and alternative medicines that are used therapeutically include yoga in India, homeopathy in Europe, herbal medicine, acupuncture, and qigong in China (Galantino et al., 2008; Li et al., 2004; Mark, 2007; Schäfer, 2004).

Chinese herbal therapy is one of the most popular alternative medicines in China, Taiwan, Japan, Korea and many other countries (Li et al., 2004). In Western countries, researchers are interested in investigating the pharmacological effects of herbs for treating asthma and other diseases (Kimberly et al., 2007; Mark, 2007). Both complex formulas and pure compounds have been found to be effective for asthma, including Xiao-Qing-Long-Tang, Ding Chuan Tang, MSSM-002, *Platycodi Radix* and Glycyrrhizin (isolated from *Glycyrrhiza glabra*) (Kao et al., 2000; Li et al., 2000; Chan et al., 2006; Ram et al., 2006; Choi et al., 2009).

Although *G. pentaphyllum* has been used to treat hypertension, hyperlipidaemia and lung inflammation, the effective compounds have not yet been identified. Some studies reported that chemical composition, such as gypenoside or gynosaponin, was isolated from *G. pentaphyllum*. Gypenosides III and VIII have the protective effects

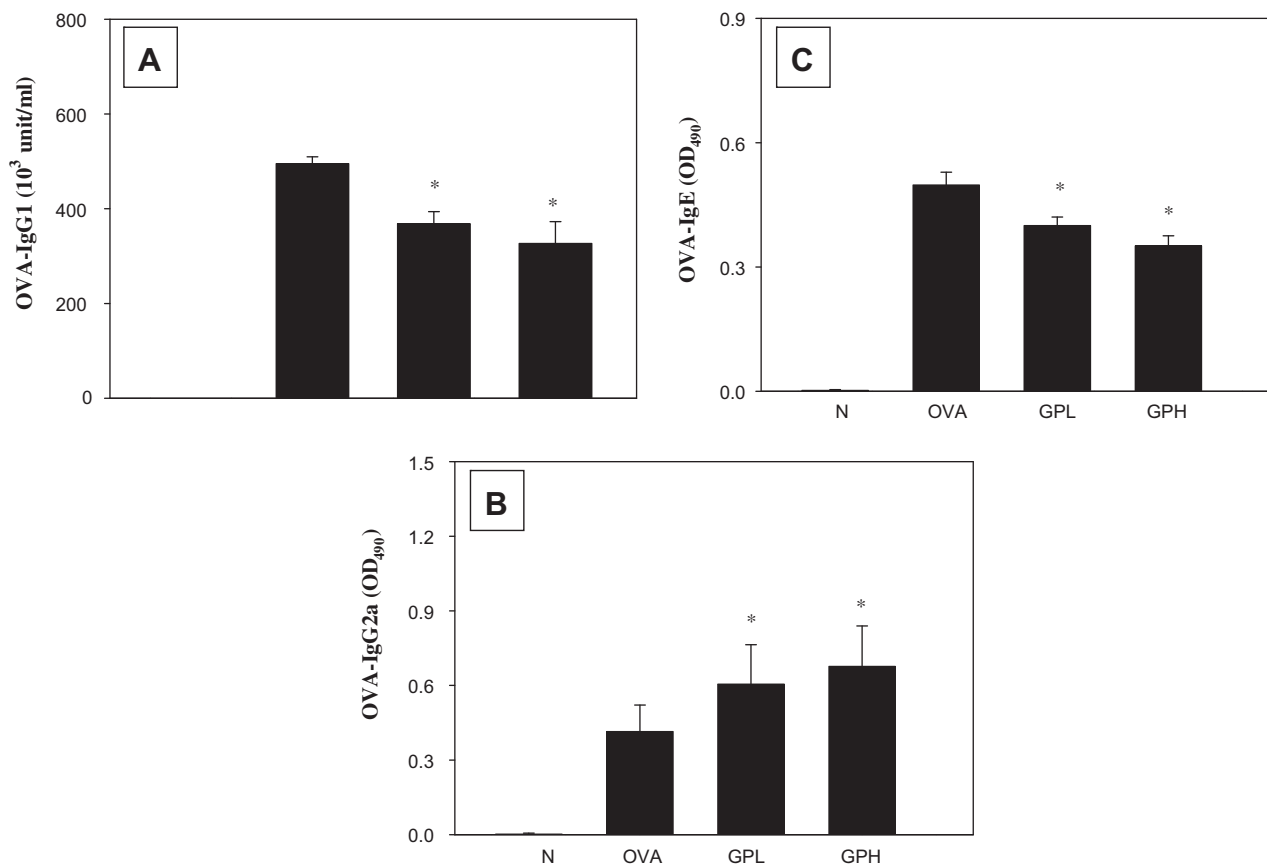


Fig. 5. Serum levels of OVA-specific IgG1 (A) and IgE (C) decreased in mice fed with *G. pentaphyllum* extract, while serum levels of IgG2a antibodies (B) increased. Serum components were analyzed using ELISA. The OVA-IgG1 levels were determined based on a standard curve constructed from pooled serum. OVA-IgE was diluted 5-fold and OVA-IgG2a was diluted 1000-fold; these are reported as OD_{490nm} readings. Data are presented as means $\pm$ SE. * $p < 0.05$ compared to the OVA group.

on cardiovascular system (Circosta et al., 2005). One gypenoside, named as phanoside, was found to induce insulin release in rats (Norberg et al., 2004). Thus, *G. pentaphyllum* might have a potential to improve hyperglycemia. Some gypenosides were described to induce apoptosis of human hepatoma or lung cancer cells (Wang et al., 2002; Lu et al., 2008). The effective compound(s) that attenuates asthmatic inflammatory responses requires further studies.

In this study, we aimed to determine whether long-term low or high oral doses of *G. pentaphyllum* suppressed AHR and other asthma symptoms in OVA-sensitized mice. In our previous study, mice fed with a high dose (5 g/kg) of *G. pentaphyllum* extract for seven consecutive days showed reduced AHR. However, OVA-sensitized mice fed with a low dose (1.25 g/kg) of *G. pentaphyllum* extract for seven consecutive days showed no reduction in AHR or eosinophil infiltration (data not shown). In the present study, we used two dosages of *G. pentaphyllum*, a high dose (5 g/kg; GPH group) and a low dose (1.75 g/kg GPL), that were fed to mice once a day, five times a week, for four consecutive weeks. Both dosages significantly reduced AHR, eosinophil infiltration, OVA-IgG1 and OVA-IgE levels in serum, Th2-associated cytokine levels from spleen cells, and IL-5 and TNF- α levels in BALF. However, there were no significant differences between the low and high doses in terms of inhibiting asthma indicators. Therefore, it seems that long-term oral administration of low doses of *G. pentaphyllum* extract attenuates airway inflammation and asthma symptoms.

Th2-associated cytokines enhance eosinophil activity and the pathological symptoms of asthma, including AHR, airway remodeling, smooth muscle contraction and overproduction of mucus. Therefore, analysis of Th2-associated cytokines is important in monitoring the effectiveness of asthma therapy (Kips, 2001). How-

ever, we could not detect IL-4 expression because the level of this cytokine was too low in the BALF samples (data not shown). Our data showed that IL-5 and IL-13 were suppressed significantly in the BALF of OVA-sensitized mice treated with *G. pentaphyllum*. IL-5 could induce eosinophils in the bone marrow to migrate toward the trachea and lungs, resulting in more eosinophils in BALF (and thus more inflammation in the lungs). As a result, it was important to demonstrate that *G. pentaphyllum* extract could inhibit IL-5 and decrease eosinophils in BALF. Our experiments confirmed that there was not much eosinophil infiltration in lung sections from OVA-sensitized mice treated with *G. pentaphyllum*. However *G. pentaphyllum* extract did not affect IL-13 production. We thought that *G. pentaphyllum* extract would inhibit inflammation of the airways via inhibition of the IL-5 expression pathway.

We also found that OVA-sensitized mice treated with *G. pentaphyllum* had decreased TNF- α level in BALF. However, the level of IL-6 was not decreased significantly. Among the cells that produce TNF- α and IL-6, including T cells, epithelial cells and macrophages. Macrophages are the major cells that secrete TNF- α and IL-6. In our OVA-sensitized model, the number of macrophages did not increase significantly in BALF. Therefore, it seems that *G. pentaphyllum* does not suppress all inflammation mechanisms in this experimental model.

Consumption of *G. pentaphyllum* suppressed production of OVA-IgG1 and OVA-IgE and promoted production of OVA-IgG2a. Th1 cell-associated cytokines induce IgG2a secretion from B cells, and cytokines produced by Th2 cells enhance IgG1 and IgE secretion from B cells. On the other hand, among the Th2-associated cytokines, only IL-4 and IL-13 were decreased significantly in spleen cells cultured with OVA from OVA-sensitized mice treated

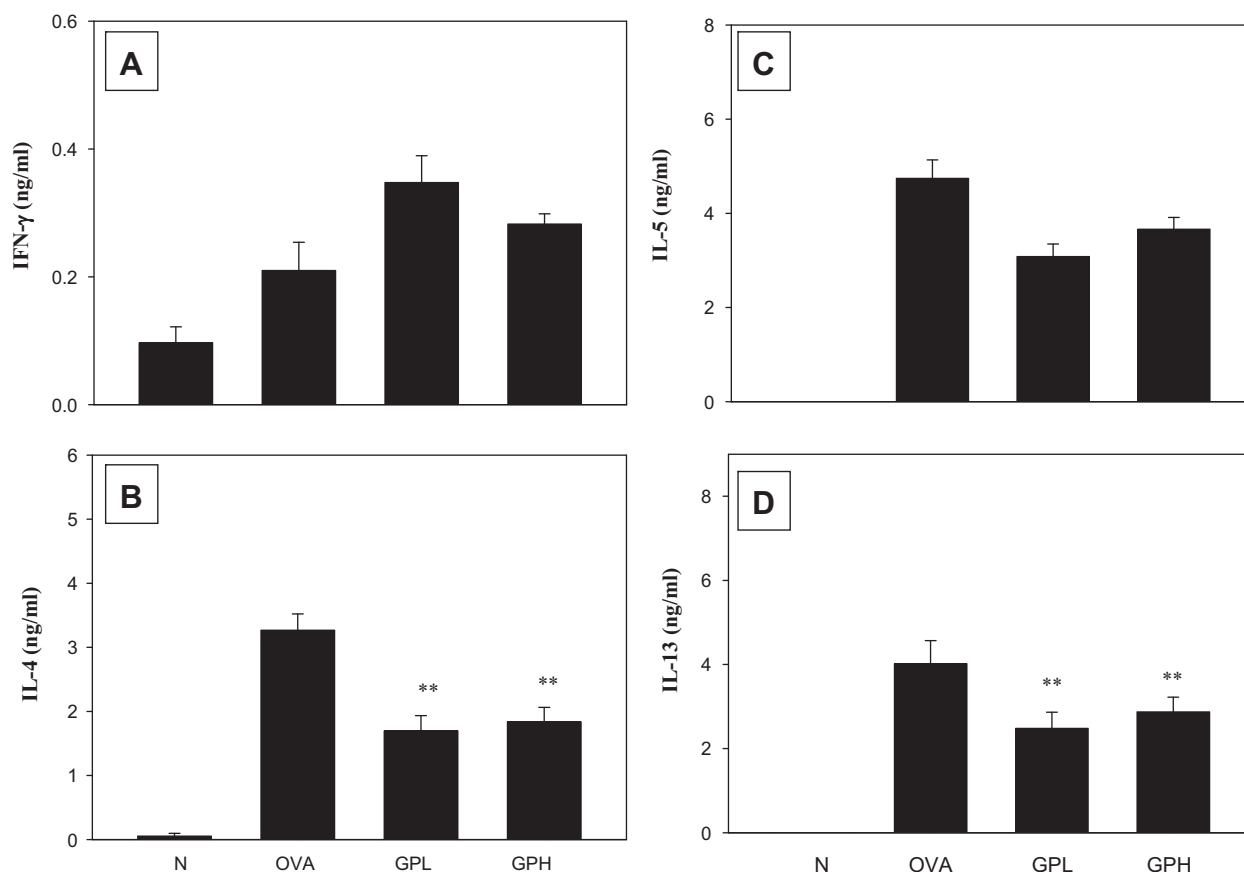


Fig. 6. *G. pentaphyllum* extract altered cytokine production in OVA-activated spleen cell cultures. IFN- γ (A), IL-4 (B), IL-5 (C) and IL-13 (D) levels were measured by ELISA. Data are presented as means $\pm$ SE. ** $p < 0.01$ compared to the OVA group.

with *G. pentaphyllum* extract (including cells from mice in the GPL and GPH groups). Some evidence indicates that IL-4 and IL-13 enhance IgE and IgG1 production by B cells. Therefore, IL-4 and IL-13 appeared to play major roles in inhibiting serum OVA-IgG1 and OVA-IgE levels in this experimental model.

Taken together, these results imply that long-term oral administration of low doses of *G. pentaphyllum* extracts decrease airway inflammation in OVA-sensitized mice. Currently, *G. pentaphyllum* is usually marketed as tea bags that are consumed in China and Taiwan to promote good health. Although further study is needed, such consumption may help prevent or attenuate symptoms of asthma.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References

- Barnes, P.J., 2006. Corticosteroids: the drugs to beat. *Eur. J. Pharmacol.* 533, 2–14.
- Barrios, R.J., Kheradmand, F., Batts, L., Corry, D.B., 2006. Asthma: pathology and pathophysiology. *Arch Pathol. Lab. Med.* 130, 447–451.
- Busse, W.W., Lemanske, R.F., 2001. Advances in immunology: asthma. *N. Engl. J. Med.* 344, 350–362.

- Chan, C.K., Kuo, M.L., Shen, J.J., See, L.C., Chang, H.H., Huang, J.L., 2006. Ding Chuan Tang, a Chinese herb decoction, reduced the severity of airway hyper-responsiveness in children with asthma: a double-blind and placebo-controlled clinical trial. *Pediatr. Allergy Immunol.* 17, 316–322.
- Choi, J.H., Hwang, Y.P., Lee, H.S., Jeong, H.G., 2009. Inhibitory effect of *Platycodi Radix* on ovalbumin-induced airway inflammation in a murine model of asthma. *Food Chem. Toxicol.* 47, 1272–1279.
- Circosta, C., De Pasquale, R., Occhiuto, F., 2005. Cardiovascular effects of the aqueous extract of *Gynostemma pentaphyllum* Makino. *Phytomedicine* 12, 638–643.
- Cour, B., Molgaard, P., Yi, Z., 1995. Traditional Chinese medicine in treatment of hyperlipidaemia. *J. Ethnopharmacol.* 46, 125–129.
- Galantino, M.L., Galbavy, R., Quinn, L., 2008. Therapeutic effects of yoga for children: a systematic review of the literature. *Pediatr. Phys. Ther.* 20, 66–80.
- Georas, S.N., Guo, J., Fanis, U.D., Casolaro, V., 2005. T-helper cell type-2 regulation in allergic disease. *Eur. Respir. J.* 26, 1119–1137.
- Huang, W.C., Kuo, M.L., Li, M.L., Yang, R.C., Liou, C.J., Shen, J.J., 2008. *Gynostemma pentaphyllum* decreases allergic reactions in a murine asthmatic model. *Am. J. Chin. Med.* 36, 579–592.
- Huang, W.C., Kuo, M.L., Li, M.L., Yang, Y.C., Liou, C.L., Shen, J.J., 2007a. The extract of *Gynostemma pentaphyllum* enhanced the production of antibodies and cytokines in mice. *Yakugaku Zasshi* 127, 889–896.
- Huang, W.C., Shen, J.J., Liou, C.L., Kuo, M.L., Chang, Y.P., Yang, Y.C., Li, M.L., 2007b. Enhancing Th1 cell activities in mice by short-term oral administration of *Gynostemma pentaphyllum* extracts. *BioFormosa* 42, 9–16.
- Kao, S.T., Wang, S.D., Wang, Y.J., Yu, C.K., Lei, H.Y., 2000. The effect of Chinese herbal medicine, xiao-qing-long-tang (XQLT), on allergen-induced bronchial inflammation in mite-sensitized mice. *Allergy* 55, 1127–1133.
- Kay, A.B., Phipps, S., Robinson, D.S., 2004. A role for eosinophils in airway remodelling in asthma. *Trends Immunol.* 25, 477–482.
- Kimberly, S.A., Yoos, H.L., McMullen, A., Kitzman, H., 2007. Complementary and alternative medicine use in children with asthma: prevalence and sociodemographic profile of users. *J. Asthma* 44, 169–175.
- Kips, J.C., 2001. Cytokines in asthma. *Eur. Respir. J.* 18, 24s–33s.
- Kuwahara, M., Kawanishi, F., Komiya, T., Oshio, H., 1989. Dammarane saponins of *Gynostemma pentaphyllum* Makino and isolation of malonylginsenosides-Rb1, -Rd, and malonylgypenoside V. *Chem. Pharm. Bull.* 37, 135–139.
- Li, X.M., Huang, C.K., Zhang, T.F., Teper, A.A., Srivastava, K., Schofield, B.H., Sampson, H.A., 2000. The Chinese herbal medicine formula MSSM-002 suppresses allergic

- airway hyperreactivity and modulates Th1/Th2 responses in a murine model of allergic asthma. *J. Allergy Clin. Immunol.* 106, 660–668.
- Li, X.M., Zhang, T.F., Sampson, H., Zou, Z.M., Beyer, K., Wen, M.C., Schofield, B., 2004. The potential use of Chinese herbal medicines in treating allergic asthma. *Ann. Allergy Asthma Immunol.* 93, S35–44.
- Lin, C.C., Huang, P.C., Lin, J.M., 2000. Antioxidant and hepatoprotective effects of *Anoectochilus formosanus* and *Gynostemma pentaphyllum*. *Am. J. Chin. Med.* 28, 87–96.
- Liou, C.J., Li, M.L., Tseng, J., 2004. Intraperitoneal injection of ginseng extract enhances both immunoglobulin and cytokine production in mice. *Am. J. Chin. Med.* 32, 75–88.
- Lu, H.F., Chen, Y.S., Yang, J.S., Chen, J.C., Lu, K.W., Chiu, T.H., Liu, K.C., Yeh, C.C., Chen, G.W., Lin, H.J., Chung, J.G., 2008. Gypenosides induced G0/G1 arrest via inhibition of cyclin E and induction of apoptosis via activation of caspases-3 and -9 in human lung cancer A-549 cells. *In Vivo* 22, 215–221.
- Mark, J.D., 2007. Integrative medicine and asthma. *Pediatr. Clin. N. Am.* 54, 1007–1023.
- Norberg, A., Hoa, N.K., Liepinsh, E., Van Phan, D., Thuan, N.D., Jornvall, H., 2004. A novel insulin-releasing substance, phanoside, from the plant *Gynostemma pentaphyllum*. *J. Biol. Chem.* 279, 41361–41367.
- Park, S.J., Shin, W.H., Seo, J.W., Kim, E.J., 2007. Anthocyanins inhibit airway inflammation and hyperresponsiveness in a murine asthma model. *Food Chem. Toxicol.* 45, 1459–1467.
- Ram, A., Mabalirajan, U., Das, M., Bhattacharya, I., Dinda, A.K., Gangal, S.V., Ghosh, B., 2006. Glycyrrhizin alleviates experimental allergic asthma in mice. *Int. Immunopharmacol.* 6, 1468–1477.
- Razmovski-Naumovski, V., Huang, T.S.W., Tran, V.H., Li, G.Q., Duke, C.C., Roufogalis, B.D., 2005. Chemistry and pharmacology of *Gynostemma pentaphyllum*. *Phytochem. Rev.* 4, 197–219.
- Rothenberg, M.E., Hogan, S.P., 2006. The eosinophil. *Annu. Rev. Immunol.* 24, 147–174.
- Schäfer, T., 2004. Epidemiology of complementary alternative medicine for asthma and allergy in Europe and Germany. *Ann. Allergy Asthma Immunol.* 93, S5–S10.
- Scichilone, N., Messina, M., Battaglia, S., Catalano, F., Bellia, V., 2005. Airway hyperresponsiveness in the elderly: prevalence and clinical implications. *Eur. Respir. J.* 25, 364–375.
- Wang, Q.F., Chen, J.C., Hsieh, S.J., Cheng, C.C., Hsu, S.L., 2002. Regulation of Bcl-2 family molecules and activation of caspase cascade involved in gypenosides-induced apoptosis in human hepatoma cells. *Cancer Lett.* 183, 169–178.
- Wu, C.J., Chen, L.C., Kuo, M.L., 2006. Attenuated *Salmonella typhimurium* reduces ovalbumin-induced airway inflammation and T-helper type 2 responses in mice. *Clin. Exp. Immunol.* 145, 116–122.