

Pharmacological and Immunological Effects of Individual Herbs in the Food Allergy Herbal Formula-2 (FAHF-2) on Peanut Allergy

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It was previously shown that a Chinese herbal formula, Food Allergy Herbal Formula 2 (FAHF-2) composed of nine herbs, blocked peanut-induced anaphylaxis in a murine model. The current study was designed to investigate the pharmacological actions of individual herbs comprising FAHF-2 on peanut-induced anaphylactic reactions in a murine model of peanut allergy and to determine if all nine herbs are necessary to prevent an anaphylactic reaction, or if a simplified formula containing fewer herbs would be equally effective. Some individual herbs reduced peanut-induced anaphylactic symptoms but no single herb offered full protection from anaphylactic symptoms equivalent to FAHF-2. The herbs had highly variable effects on histamine release, as well as peanut-specific serum IgE and IgG2a levels. The herbs also had variable effects on IL-4, IL-5 and IFN- γ levels. A simplified formula comprising the most efficacious tested individual herbs showed only partial efficacy and was not able to reproduce comparably the effects of FAHF-2, suggesting that component herbs of FAHF-2 may work synergistically to produce the curative therapeutic effects produced by the whole formula, which appears to be the best option for future clinical trials. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

Traditional Chinese medicine (TCM) has been used in Asia for centuries. A mainstay of TCM has been the use of herbal formulas usually consisting of multiple herbs that act in concert to alleviate symptoms recognized in the traditional Chinese system of medicine. Recently, TCM has attracted interest in Western countries as a source of alternative or complementary therapy for a variety of diseases. Growing scientific evidence suggests that Chinese herbal medicine has the potential to treat various diseases such as asthma and allergy (Li *et al.*, 2004; Li and Srivastava 2006b), cancer (Darzynkiewicz *et al.*, 2000; Melnick, 2006), liver diseases (Huang *et al.*, 2004; Lazar, 2004), Atherosclerosis and hypertension (Hou *et al.*, 2005), diabetes (Chan *et al.*, 2007; Zhao *et al.*, 2006), insomnia, anxiety (Brown and Gerbarg, 2001) and infectious diseases such as AIDS (Burack *et al.*, 1996; Wu, 2006) etc. Controlled clinical trials with TCM while few, can be found in Western medical databases, examples of which are studies with Tong-Xie-Ning for the treatment of irritable bowel syndrome (Wang *et al.*, 2006), demonstration of immunomodulatory properties of Yunzhi-Danshen

during radiotherapy of nasopharyngeal carcinoma (Bao *et al.*, 2006) and the use of Kaixin was shown to be efficacious for the treatment of ischemia in patients with diabetic heart disease (Xue *et al.*, 2006). However, for wider appreciation of TCM in Western medical practice, an evaluation of the claimed TCM benefits for disease therapy using rigorous evidence-based scientific methodology is needed. This is particularly important for diseases such as food allergy not historically reported in the TCM literature. In contrast to Asian countries where it is relatively rare, food allergy is a significant health problem in most Western countries including the United States. Peanut allergy (PNA) in particular is a major concern because it is rarely outgrown and is often life-threatening (Bock and Atkins, 1989). Self-reported PN allergy among children doubled between 1997 and 2002, and PN and tree nut allergies now affect more than 3 million Americans (Sicherer *et al.*, 2003). Early symptoms of food-hypersensitivity reactions include oral pruritus, colicky abdominal pain, nausea and vomiting, diarrhea, cutaneous flushing, urticaria and angioedema. Progressive respiratory symptoms, hypotension and dysrhythmias typically develop in fatal and near-fatal cases. PNA is an IgE mediated type I hypersensitivity, and Th2 cytokines are central to the pathogenesis of allergic disorders (Sampson, 1999). At the present time, there is no effective therapy for PN allergy. Other than immediate access to post-anaphylactic rescue medications, strict avoidance is the only way to manage this condition.

TCM formulas to specifically treat food allergy have not been described, however, there is a history of TCM

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use for the treatment of other atopic conditions such as asthma, rhinitis and atopic dermatitis (Li and Srivastava, 2006). Clinical trials utilizing an anti-asthma TCM formula, ASHMI (Wen *et al.*, 2005), are currently underway. It was noticed that an antiparasitic TCM preparation, Wu Mei Wan, is used to relieve symptoms also encountered in food allergy such as abdominal discomfort and vomiting after food ingestion (Bensky and Barolet, 1986). Wu Mei Wan is a well-known Chinese classical formula first documented by Zhang Zhong Jing in the classical Chinese medical text, the *Shang Han Lun*, compiled approximately 1500 years ago. This formula has been widely used in TCM practice. More recently Wu Mei Wan has been shown to be beneficial for the treatment of asthma and irritable bowel syndrome (Ruan, 1990; Yang, 1988) suggesting the possibility that this formula can potentially alter mucosal immune responses and thus may be a promising candidate for the treatment of food allergy. Randomized controlled trials with Wu Mei Wan appearing in Chinese medical databases have shown benefits in patients with various intestinal disorders and asthma (Chen, 2005; Huang *et al.*, 2004; Cui, 2004). With some modifications to Wu Mei Wan, we developed and investigated the efficacy of Food Allergy Herbal Formula-1 (FAHF-1) in a murine model of PN allergy. FAHF-1 blocked systemic anaphylactic symptoms after PN challenge of allergic mice and down-regulated pro-allergic Th2 immune responses (Li *et al.*, 2001). A refined version of this formula, FAHF-2, was found to be equally successful in blocking systemic anaphylactic symptoms after PN challenge of allergic mice (Srivastava *et al.*, 2005).

While this formula appears to hold great potential as a therapy for food allergy, its complexity poses a considerable challenge for quality control and the elucidation of active pharmacological components. To address these issues, the contributions of each of the nine herbs that comprise FAHF-2's to the mixture's anti-allergic activity were evaluated. To our knowledge this study is the first to investigate these herbs for their effectiveness as anti-food allergy treatments. Using an established murine model of PN allergy, the ability of individual herbs comprising FAHF-2 to protect PN-allergic mice from anaphylaxis was tested following oral PN challenge and to inhibit Th2 immune responses. Also evaluated was a simplified formula comprising selected herbs, which appeared promising when tested individually.

MATERIALS AND METHODS

Mice and reagents. Five-week-old C3H/HeJ mice (Jackson Laboratory, Bar Harbor, ME) were maintained on PN-free chow under specific pathogen-free conditions. Standard Guidelines for the Care and Use of Animals were followed. Freshly ground, roasted, whole PN, termed PN was employed as the allergen. Crude PN extract, termed CPE was prepared as described previously. Cholera toxin was purchased from List Biological Laboratories, Inc (Campbell, CA). Concanavalin A (Con A) and albumin, dinitrophenyl human albumin (DNP-albumin) were purchased from Sigma (St Louis, MO). Antibodies for ELISAs were purchased from BD Biosciences (San Diego, CA).

Preparation of TCM herbs and formulas. All herbs used in this study were of Chinese origin, provided by Beijing Shen Hua Shi Di Medical Technology, Beijing, China. Consistent quality of each herbal medicine is the key to reproducing the efficacy of FAHF-2. All the herbs used in FAHF-2 were inspected for identity and quality by pharmacists trained and licensed in identification and processing of herbal medicines according to the Chinese Herbal Medicine Materia Medica and Pharmacopoeia of the People's Republic of China at the China-Japan Friendship Hospital, and The China Academy of Traditional Chinese Medicine (TCM) Sciences, Beijing, China. Only those herbs meeting the quality and safety standards in the Pharmacopoeia of P.R. of China (The State Pharmacopoeia Commission of The P.R. of China. Pharmacopoeia of The P.R. of China, 2000) were used. The general information of those individual herbs including geographic source of the plant material, time of harvest, processing and pesticide residue level is listed in Table 1. Based on organoleptic and microscopic examination, the raw herbal materials used in FAHF-2 were identified as the fruits of *Prunus mume* (Wu-Mei), the skin of the fruits of *Zanthoxylum schinifolium* (Chuan-Jiao), roots of *Angelica sinensis* (Dang-Gui), rhizome of *Zingiber officinalis* (Gan-Jiang), twigs of *Cinnamomum cassiae* (Gui-Zhi), bark of *Phellodendron chinense* (Huang-Bai), rhizome of *Coptis chinensis* (Huang-Lian), roots of *Panax ginseng* (Ren-Shen), and the fruiting body of *Ganoderma lucidum* (Ling-Zhi). Photographs of the herbs are shown in Fig. 1. Hot water extracts of individual herbs and formulas were prepared by boiling herbs in water twice and lyophilizing the resulting decoctions. For administration, the dried herb extracts were dissolved in tap water and dispensed by gavage in two daily feedings totaling 1 mL. The daily animal doses of individual herbs and formulas were calculated on the basis of dry yields from raw herb decoctions according to the prescribed dose for humans and normalized to the mouse body weight (Table 2). The dose of an individual herb given to each animal represents the amount of that herb present in each dose of the whole formula. Dried extract of FAHF-2 was provided by Beijing Shen Hua Shi Di Medical Technology, Beijing, China according to laboratory specifications was administered to mice at a dose equivalent to 228 mg of raw herb.

PN sensitization/challenge and herbal treatment. Mice were orally sensitized with PN (10 mg) and cholera toxin (20 µg), on three consecutive days at week 0, and once a week from weeks 1–5. Mice were boosted in weeks 6 and 8 with PN (50 mg) and Cholera toxin (20 µg). Treatment with herbs commenced at week 3 and was given as twice daily feedings for 7 weeks. Naïve mice were not sensitized or treated. All animals were challenged intragastrically with 200 mg of PN at week 14. The study includes data pooled from two separate experiments resulting in variable numbers of mice in each treatment group.

Assessment of hypersensitivity reactions. Anaphylactic symptoms were evaluated 30 min after challenge dose by two investigators using a scoring system described previously (Li *et al.*, 2000): 0, no signs; 1, scratching and rubbing around the snout and head; 2, puffiness around the eyes and mouth, pilar erection and reduced activity

Table 1. Description of individual herbs of FAHF-2 and doses used for mice. Mouse daily dose was calculated by weighing lyophilized extracts of human daily dose and normalizing for mouse body weight

Herbs	Harvest location	Harvest time	Processing	Pesticide residue level Cypermethrin (mg/kg)	Dichlorvosm (mg/kg)
Wu-Mei	Shehong, Si Chuan Province, Chian	May, June	Pick the mature fruit body, dried with low heat	ND*	ND
Chuan-Jiao	Bazhong, Si Chuan Province, China	Autumn	Pick the mature body, dried with low heat	ND	ND
Dang-Gui	Mingxian, Gan Su province, China	October, November	Pick up those that have been growing for more than 2 years, dried with low heat	ND	ND
Gan-Jiang	Bazhong, Si Chuan Province, China	Early winter	Dried with low heat or in the sun	ND	ND
Gui-Zhi	Yulin, Guang Xi province, China	March–June	Pick up the tender twig, cut into small pieces and dried	ND	ND
Hong-Shen	Ji'an, Ji Lin province, China	August, September	Pick up the fresh roots that have growing over 3 years, cut off branch roots, steam for 2–2.5 h, heat dry	ND	ND
Huang-Bai	Dandong, Liao Ning province, China	March–June	Pick the bark that have been growing over 10 years, dry in the sun	ND	ND
Huang-Lian	Hongchuan, Si Chuan province, China	July–September	Pick those have been growing over 5 years, heat dry	ND	ND
Ling-Zhi	Tongbai, He Nan province, China	July–September	Dried with low heat or at a well ventilated cool place in shade	ND	ND

ND, not detected.

**Figure 1.** Raw herbs used this study. The herbs are *Prunus mume* (Wu-Mei, WM), *Zanthoxylum schinifolium* (Chuan-Jiao, CJ), *Angelica sinensis* (Dang-Gui, DG), *Zingiber officinalis* (Gan-Jiang, GJ), *Cinnamomum cassiae* (Gui-Zhi, GZ), *Phellodendron chinense* (Huang-Bai, HB), *Coptis chinensis* (Huang-Lian, HL), *Panax ginseng* (Ren-Shen, RS) and *Ganoderma lucidum* (Ling-Zhi, LZ).

with an increased respiratory rate; 3, wheezing, labored respiration and cyanosis around the mouth; 4, no activity after prodding, or tremors, and convulsion; 5, death due to anaphylaxis

Measurement of plasma histamine levels and serum PN-specific IgE and IgG2a levels. Plasma was collected 30 min after challenge and histamine levels were determined by commercial ELISA (Beckman Coulter, MA),

Table 2. Information pertaining to the procurement of raw herbs

	TCM materia medica (pinyin)	Abbreviation	Latin name	Human daily raw herb dose (g)	Mouse daily raw herb dose (mg)	Mouse daily extract dose (mg)
1	Gan Jiang	GJ	<i>Zingiber officinalis</i>	6	15.5	1.60
2	Huang Lian	HL	<i>Coptis chinensis</i>	9	23.2	2.32
3	Gui Zhi	GZ	<i>Cinnamomum cassiae</i>	3	7.7	0.12
4	Chuan Jiao	CJ	<i>Zanthoxyl bungeani</i>	1.5	3.8	0.53
5	Dang Gui	DG	<i>Angelica sinensis</i>	9	23.2	4.63
6	Huang Bai	HB	<i>Phellodendron chinense</i>	6	15.5	2.04
7	Wu Mei	WM	<i>Prunus mume</i>	30	77.5	14.74
8	Ren Shen	RS	<i>Panax ginseng</i>	9	23.2	8.83
9	Ling Zhi	LZ	<i>Ganoderma lucidum</i>	15	38.7	0.96

as outlined by the manufacturer. For measurement of IgE and IgG2a venous blood samples were obtained from tail veins 1 day before treatment, 1 day before boosting and 1 day before challenge. Sera were collected and stored at -80°C until analysed. Levels of PN-specific IgE and IgG2a levels were determined by ELISA as described earlier with minor modifications (Bashir *et al.*, 2002; Lee *et al.*, 2001).

Cell culture and cytokine measurements. Pooled splenocytes from each treatment group were isolated after challenge and cultured in 24-well plates ($4 \times 10^6/\text{well}/\text{mL}$) in the presence of CPE (200 $\mu\text{g}/\text{mL}$), concanavalin A (ConA) (2.5 $\mu\text{g}/\text{mL}$) or medium alone (med) for 72 h at 37°C . After centrifugation at 1200 rpm for 7 min, the cell culture supernatants were harvested and stored at -80°C until use. Cytokine levels in the culture supernatants were determined by ELISA in triplicate according to the manufacturer's instructions (PharMingen, San Diego, Cal.)

Statistical analysis. Data were analysed using SigmaStat statistical software (SPSS, Chicago, IL). For normally distributed data ANOVA analysis with Bonferroni correction was used. For non-normal data, differences between the groups were analysed by Kruskal-Wallis one-way ANOVA on ranks or Mann-Whitney rank-sum test. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Ability of individual herbs treatment to protect against PN-induced anaphylaxis

Following sensitization and treatment, all mice were subjected to oral PN challenge at week 14 (5 weeks post therapy). Allergic symptoms were evaluated 30 min after challenge and scored using a scoring index described previously (Li *et al.*, 2000). As expected, almost all sham-treated mice displayed symptoms of anaphylaxis with a median score of 2 (Table 3). Consistent with our earlier observations, FAHF-2 treated mice were completely protected, with 0/8 mice presenting with symptoms of anaphylaxis. None of the constituent herbs when tested individually fully reproduced the effects of FAHF-2. *Phellodendron chinense* was found to have the most protective ability with only 1/4 mice showing anaphylactic reactions following challenge. Anaphylaxis rates in mice treated with any of the other herbs were above 50% with median scores in the range 2–3.

Effect of individual herb treatment on plasma histamine levels after challenge and PN-specific immunoglobulins

Histamine is a prominent mediator of anaphylaxis and its plasma levels shortly after challenge were markedly

Table 3. Anaphylactic reaction rates and median scores obtained 30 min after oral PN challenge

Herbal treatment	Anaphylactic reactions n/total	% of mice with a reaction	Anaphylactic score median (range)	<i>p</i> values vs sham
Sham	9/11	81	2 (0–4)	
FAHF-2	0/8	0	0 (0)	<0.001
HB	1/4	25	0 (0–2)	0.008
GJ	3/5	60	2 (0–2)	0.058
HL	3/5	60	2 (0–4)	0.058
CJ	5/8	63	2 (0–4)	0.140
LZ	3/4	75	2.5 (0–3)	0.590
WM	6/7	86	2 (0–4)	0.387
GZ	6/7	86	2 (0–3)	0.330
DG	5/5	100	3 (2–3)	0.640
RS	4/4	100	3 (2–3)	0.386
Naive	0/15	0	0 (0–0)	<0.001

elevated in sham-treated mice (Fig. 2A). Consistently, FAHF-2 treated mice, which were completely protected from anaphylaxis, showed significantly reduced plasma histamine levels compared with sham-treated controls. Only *Phellodendron chinense* treated mice showed a statistically significant reduction in plasma histamine compared with sham-treated controls ($p < 0.05$). The next lowest mean histamine levels occurred in mice treated with *Zingiber officinalis* and *Coptis chinensis*, but these values were not significantly different from those of the sham-treated mice.

The murine model of PN allergy used in this study mimics IgE-dependent food allergy, and a reduction in levels of PN-specific IgE is highly desirable (Li *et al.*, 2000). Of the herbs tested, *Ganoderma lucidum* treated-mice had the lowest average PN-specific IgE at the time of challenge, although the levels were not significantly different from sham ($p < 0.09$) (Fig. 2B). Treatment with whole formula, however, significantly reduced PN-specific

IgE as observed previously ($p < 0.001$). In contrast to IgE, IgG2a is a Th1-driven antibody that has been associated with beneficial effects of immunotherapy (Kowalski and Jutel, 1998). It was demonstrated previously that FAHF-2 treatment increases PN-specific IgG2a in our murine anaphylactic model. In this study, none of the individual herbs tested caused significant increases in PN-specific IgG2a, whereas animals treated with the whole formula showed a significant increase in PN-specific IgG2a ($p < 0.05$) (Fig. 2C).

Effect of individual herb treatment on PN-stimulated Th1 and Th2 cytokine production

Th2 cytokines such as IL-4, IL-5 and IL-13 play an important role in the establishment and maintenance of allergic responses. IL-4 and IL-13 are key cytokines for IgE synthesis and IL-5 has been implicated in allergic inflammation (Hamelmann and Gelfand, 2001; Izuhara *et al.*, 2002). In order to aid in selecting a few herbs to combine as a potentially simplified FAHF formula, some preliminary experiments were done on splenocyte production of cytokines upon PN challenge. The effects of individual herb treatments in mice on antigen-stimulated Th2 cytokine production in splenocytes were variable (Fig. 3A–C). Apart from *Cinnamomum cassiae* (which appeared to increase IL-4 production), *Prunus mume* and *Ganoderma lucidum*, all other herbs tested appeared to reduce antigen-stimulated IL-4 production by varying degrees. *Phellodendron chinense*, *Coptis chinensis*, *Zingiber officinalis* and *Angelica sinensis* treated mice showed 70–80% reduction in PN-stimulated IL-4 production compared with sham-treated animals and this decrease was comparable to the FAHF-2 treated group (Fig. 3A). Suppression of IL-13 production was less marked with no single herb matching the reduction in the FAHF-2 group. *Phellodendron chinense* appeared to be the most effective (Fig. 3B). None of the individual herbs suppressed PN-stimulated IL-5 production as efficiently as FAHF-2 which decreased IL-5 by 86%. Only *Phellodendron chinense* and *Ganoderma lucidum* treatments reduced IL-5 by more than 50% (Fig. 3C). Overall, splenocytes from *Phellodendron chinense*-treated mice produced the least IL-4, IL-13 and IL-5. The Th1 cytokine interferon (IFN)- γ has been shown to inhibit activity of Th2 cytokines and is often associated with positive outcomes of anti-allergy therapies (Lee *et al.*, 2001). It was demonstrated previously that treatment of PN-allergic mice with FAHF-2 increases antigen-specific IFN- γ from splenocytes and MLN cells (Qu *et al.*, 2007). None of the individual herb treatments tested increased the PN-stimulated IFN- γ to a level comparable to that of FAHF-2 (the largest increase among the individual herbs was in the *Ganoderma lucidum* group and that was less than half that produced by FAHF-2) and many appeared to decrease its production (Fig. 3).

Protection from PN-induced anaphylaxis by treatment with a simplified formula

Having observed that none of the individual herbs, when given at a dose equivalent to that in FAHF-2, successfully reproduced the effects of the whole formula, a

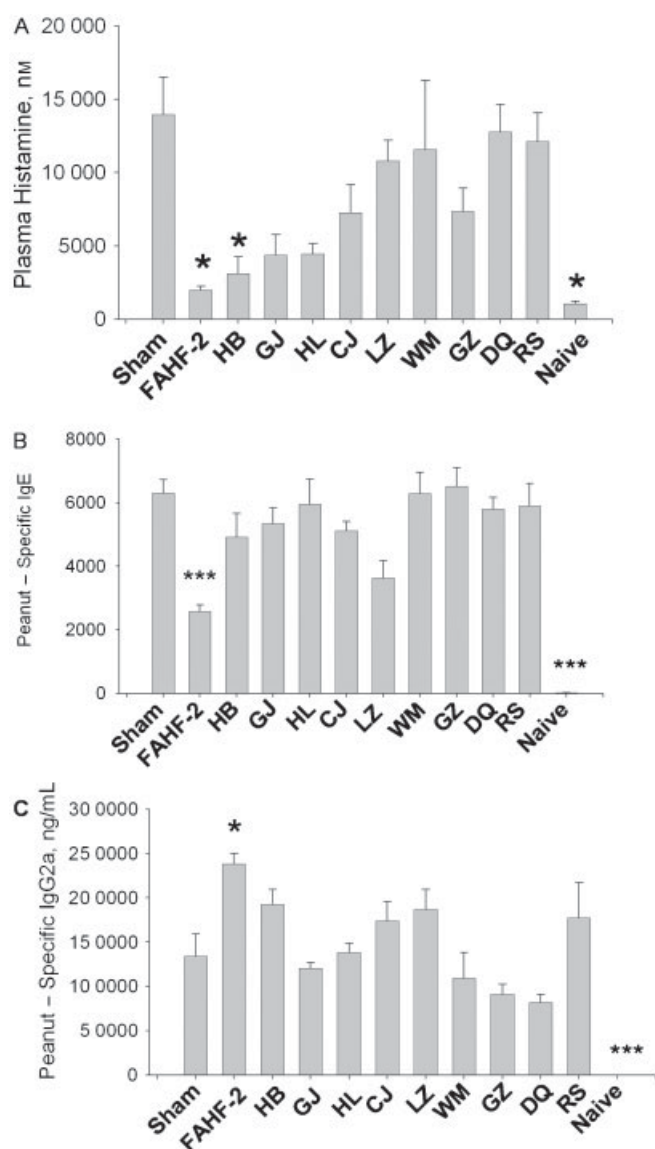


Figure 2. Plasma histamine levels (A). Blood was collected 30 min after oral PN challenge. Plasma histamine levels were measured by ELISA and data are shown as mean $\pm$ SEM. *, $p < 0.05$, vs Sham, n = as indicated in Table 2. PN-specific IgE (B) and IgG2a (C) in serum prior to the time of challenge measured by PN specific ELISA. Data represented as mean $\pm$ SEM. * $p < 0.05$; *** $p < 0.001$, vs Sham, n = as indicated in Table 2.

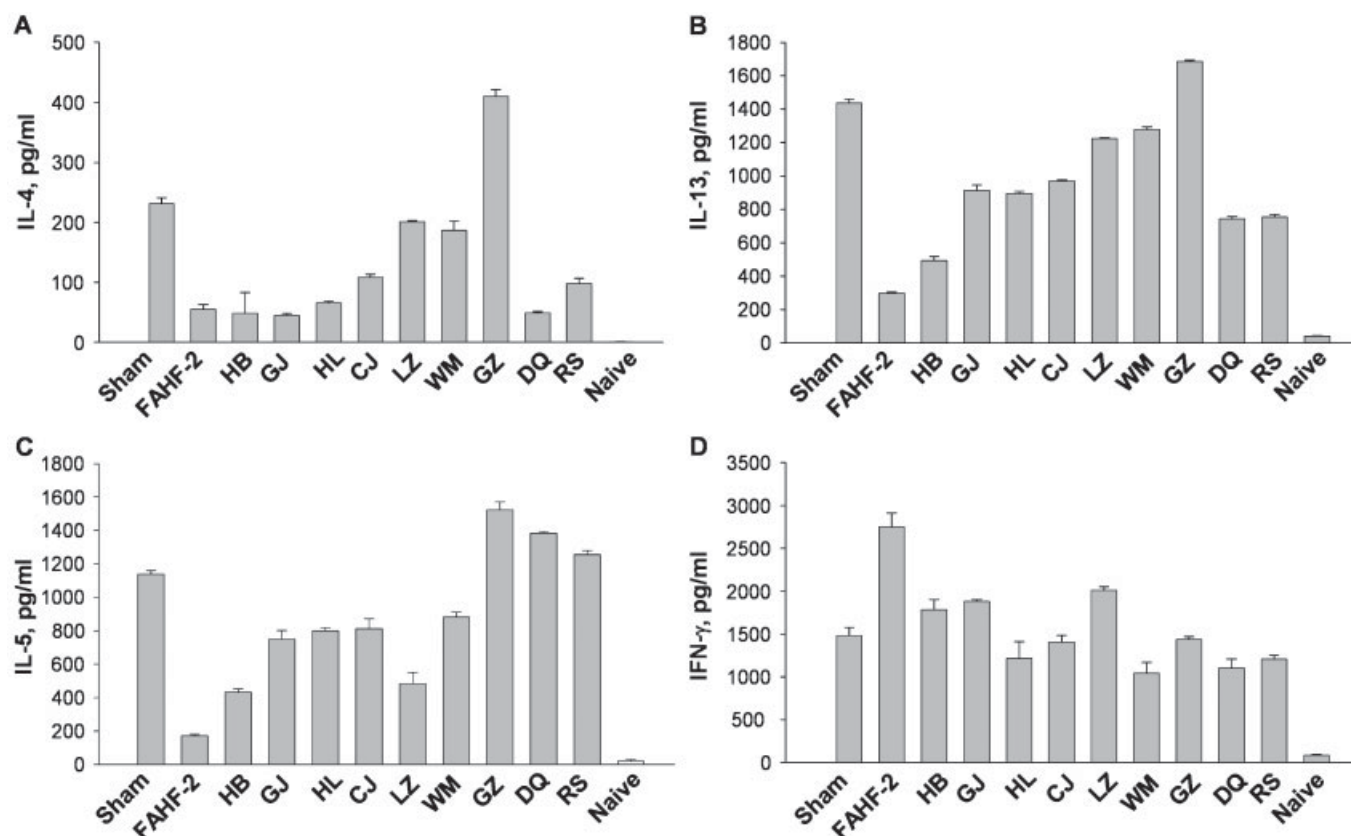


Figure 3. Cytokine levels in pooled splenocyte supernatants after 72 h stimulation with CPE. Cytokines were measured by commercial ELISA. Data are mean $\pm$ SEM of triplicate observations.

simplified FAHF formula (sFAHF) that included those herbs was tested, which appeared most effective in reducing anaphylaxis and/or Th2 cytokine production (Table 3, Fig. 2). If a simpler formula were shown to mimic the effects of FAHF-2, it would facilitate quality control and identification of the active therapeutic components. Among the individual herbs tested, *Phellodendron chinense* offered the most protection against anaphylaxis and had the best overall profile in reducing Th2 cytokine production in splenocytes. *Zingiber officinalis* and *Coptis chinensis* appeared equivalent in reducing anaphylaxis and in suppression of Th2 cytokines, but *Zingiber officinalis* stimulated increased production of IFN- γ , a Th1 cytokine with known anti-allergic properties. Another rationale for selecting *Zingiber officinalis*, but not *Coptis chinensis*, was that *Coptis chinensis*'s taste is extremely bitter. Thus, the sFAHF without *Coptis chinensis* would be more suitable for clinical use. Since, *Phellodendron*

chinense and *Zingiber officinalis* showed only modest reductions in IgE levels, which was considered important for an efficacious and lasting anti-allergy therapy, *Ganoderma lucidum* was included in our simplified formula because it appeared to be the most likely of the individual herbs to lower antigen-specific IgE. Thus the simplified FAHF-2 formula comprised *Phellodendron chinense*, *Ganoderma lucidum* and *Zingiber officinalis*. Using an experimental protocol identical to that used to test individual herbs, it was found that three of five mice treated with sFAHF were protected from anaphylaxis to PN challenge and the median symptom scores were significantly lower than the sham-treated group ($p < 0.05$) (Table 4). The symptoms were relatively mild with scores in the range 0–2. While somewhat effective, sFAHF did not replicate the results observed in FAHF-2 treated mice where all animals in the group (4/4) were protected ($p < 0.01$).

Table 4. Composition of simplified formula and symptom scores observed 30 min after oral PN challenge in treatment groups

Herbal treatment	Number of herbs in the formula	Herbs present in formula	Anaphylactic reactions n/total	Anaphylactic score median (range)	p values vs sham
Sham	NA	NA	7/8	2 (0–4)	
FAHF-2	9	GJ, HL, GZ, CJ, HB, RS, WM, DQ, LZ	0/4	0 (0)	0.009
sFAHF	3	HB, GJ, LZ	2/5	0 (0–2)	0.047
Naive	NA	NA	0/5	0 (0)	0.008

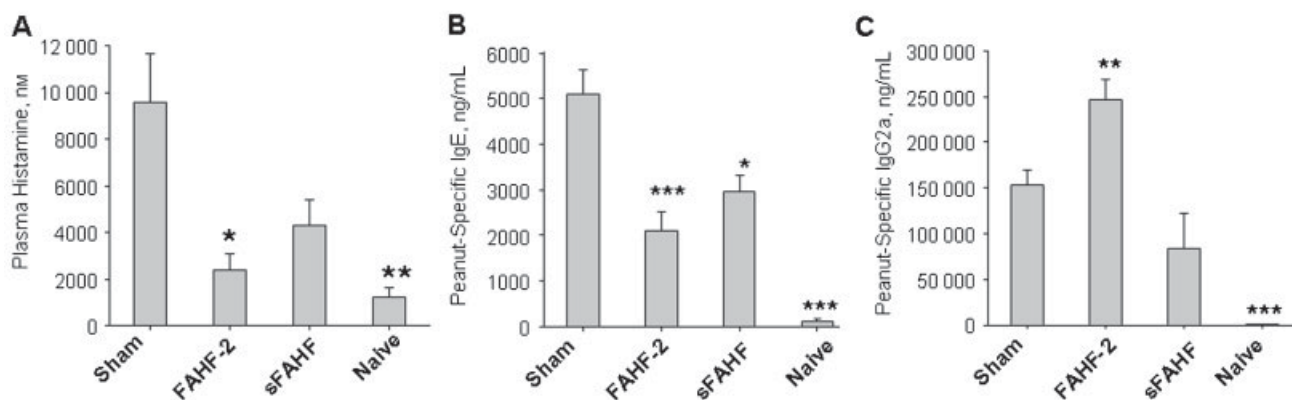


Figure 4. Plasma histamine levels 30 min after oral PN challenge (A). Histamine was measured by ELISA. * $p < 0.05$ vs Sham. $n =$ as indicated in Table 3. PN-specific IgE (B) and IgG2a (C) in serum prior to the time of challenge measured by PN specific ELISA. Data represented as mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs Sham. $n =$ as indicated in Table 3.

Plasma histamine levels after challenge and modulation of PN-specific immunoglobulins in mice treated with sFAHF

Plasma histamine levels in mice treated with sFAHF were not significantly reduced ($p = 0.071$) (Fig. 4A) compared with sham animals. In contrast FAHF-2 treated mice showed statistically significant decreases in plasma histamine ($p < 0.05$).

PN-specific IgE was significantly reduced in mice treated with sFAHF by about 40% of levels seen in sham-treated animals ($p < 0.05$), whereas IgE levels in FAHF-2 treated mice were decreased by almost 60%, consistent with our previous observations ($p < 0.001$) (Fig. 4B) (Srivastava *et al.*, 2005). Treatment of mice with sFAHF did not increase PN-specific IgG2a, whereas it was significantly increased in mice treated with FAHF-2 ($p < 0.05$) (Fig. 4C).

Modulation of Th1/Th2 cytokines by treatment with sFAHF

Mice treated with sFAHF showed decreased levels of PN-stimulated IL-4 and IL-13 but not IL-5 and reductions in IL-4 and IL-13 were not as great as those seen in animals treated with FAHF-2 (Fig. 5A–C). In contrast to treatment with FAHF-2, no elevation of PN-stimulated IFN- γ was observed in sFAHF treated animals (Fig. 5D).

DISCUSSION

Collectively the results suggest that the whole formula of FAHF-2 is more effective than any of its constituent herbs or a simplified formula comprising the best performing herbs. Among individual herbs the best results were seen in *Phellodendron chinense* treated mice where three of four mice were protected. No individual herb treatment reproduced the broad based immunological effects of FAHF-2 including suppression of IgE and Th2 cytokines and elevation of the Th1 cytokine IFN- γ and antibody IgG2a. Some individual herbs appeared to suppress PN-stimulated-Th2 cytokine production in

splenocytes, although the patterns of suppression differed among the herbs. For example, *Phellodendron chinense* treatment appeared to suppress all three Th2 cytokines tested, whereas *Angelica sinensis* and *Panax ginseng* treatment appeared to decrease IL-4 and perhaps IL-13, but not IL-5. *Phellodendron chinense*, although the most effective single herb overall, was insufficient as a stand alone treatment. In later experiments *Phellodendron chinense* at this dose was found to be non-protective following repeated challenges with PN in contrast to FAHF-2 which has been shown to protect from anaphylaxis to several challenges over 6 months (T. Zhang, unpublished observations; Srivastava *et al.*, 2006). Thus the actions of FAHF-2 seem to be attributable to multiple component herbs, with no single herb at a dose equivalent to that in FAHF-2 being able to reproduce the efficacy of the whole formula. These results are not entirely unexpected as the allergic condition is a result of activities and interactions of diverse cell types such as antigen presenting cells, B cells, T cells and effector cells such as mast cells and basophils. A single herb may possess the ability to influence one or more of these cell types. Our studies have highlighted the potential of the individual herbs studied to possess abilities as modulators of immunoglobulin and or cytokine synthesis and regulators of mast cell/basophil function. *Cinnamomum cassiae* was found to offer some protection from anaphylaxis but did not inhibit Th2 cytokines or IgE, suggesting that its target of action could be at the level of mast cell/basophil activity. Conversely *Ganoderma lucidum* while appearing to reduce IgE did not notably reduce IL-4 or IL-13 or protect from anaphylaxis indicating potential intervention in B cells rather than T helper cells or mast cell/basophils. Studies of *in vitro* cell culture systems of B cells, T cells and mast cells/basophils that will allow us to elucidate pharmacological actions of FAHF-2 in these cell types are planned. The findings regarding individual herb effects were not completely unexpected as TCM formulas often rely on the concerted action of multiple herb components for the principal therapeutic benefits and still others for the ability to synergize with the active components and blunt their possible adverse effects (Bensky and Gamble, 1986). While it would be interesting to test individual herbs at higher doses, the doses used in this study correspond to the prescribed

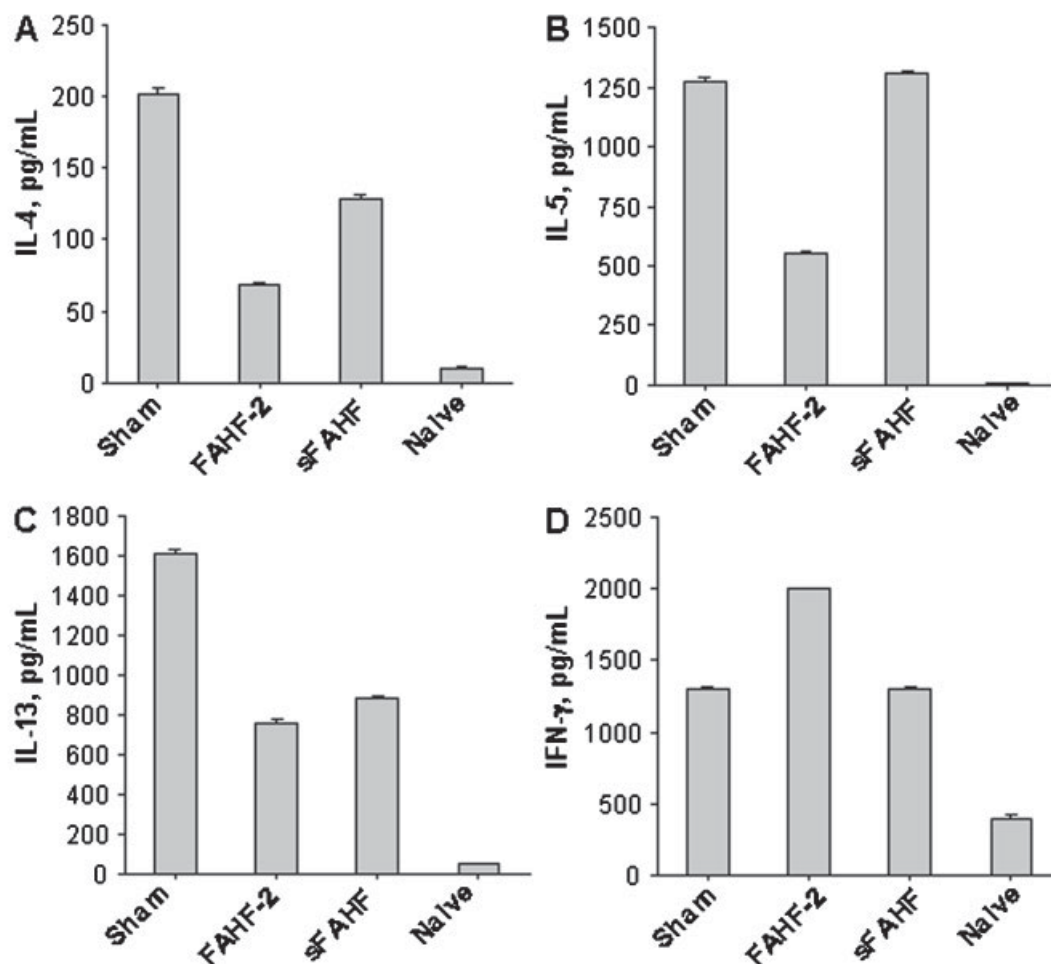


Figure 5. Cytokine levels in pooled splenocyte supernatants after 72 h stimulation with PN. Cytokines were measured by commercial ELISA kits. Data are mean $\pm$ SEM of triplicate observations.

dose for human use for each herb according to TCM practices, and thus generally recognized as safe.

Since no single herb was effective, the potential of using a simplified formula sFAHF which included *Phellodendron chinense*, *Zingiber officinalis* and *Ganoderma lucidum* was investigated. These three herbs collectively showed the most favorable responses in protection from anaphylaxis, suppression of IgE, and modulation of Th2/Th1 cytokines, presenting a potentially simpler alternative to FAHF-2. However, only partial success was achieved; sFAHF prevented anaphylactic symptoms in only three of five mice and plasma histamine levels were not significantly reduced following challenge. Although this formula did reduce PN-specific IgE and appeared to reduce some Th2 cytokines, it did not match the performance of FAHF-2 and had no effect on PN-specific IgG2a, which may potentially serve a beneficial role (Flicker and Valenta, 2003; Strait *et al.*, 2006).

Taken together the results suggest that all the herbs of FAHF-2 are likely required for the ability to provide

complete and lasting protection against anaphylaxis and alter Th2/Th1 immune responses in a manner beneficial for therapy of food allergy. Individual component herbs or a simplified formula were unable to reproduce all the effects of the whole formula. Efforts are currently underway to further analyse the chemical constituents of whole FAHF-2. Using only biologically active fractions of the whole formula may enable us to reduce the complexity and dose of FAHF-2 treatments for future use in the clinic. Should our animal study results be even partially reproducible in humans, FAHF-2 potentially represents a highly effective and long lasting therapy for food allergy and the potential for using Traditional Chinese herbal formulas for the therapy of diseases not originally characterized in TCM.

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