1 **Repository**

2 **Methods**

3 ***E1. FAHF-2 formula and product***

4 Dried aqueous extract of FAHF-2, produced in a good processing practice (GPP) certified
5 facility (Xiyuan Chinese Medicine Research Pharmaceutical Manufacturer, Beijing, China), was
6 obtained from Beijing Shen Hua Shi Di Medical Technology, Beijing China, and stored at room
7 temperature. The quality of the raw herbs was established and described in details in previous
8 publications. [1;2] In brief, based on organoleptic and microscopic examination, the raw herbal
9 materials used in FAHF-2 were identified as the fruits of *Prunus mume*, the skin of the fruits of
10 *Zanthoxylum schinifolium*, roots of *Angelica sinensis* , rhizome of *Zingiber officinalis*, twigs of
11 *Cinnamomum cassia*, bark of *Phellodendron chinense*, rhizome of *Coptis chinensis*, roots of
12 *Panax ginseng*, the fruiting body of *Ganoderma lucidum*. Product quality was monitored by
13 HPLC fingerprinting according to the FDA's Guidance for Industry Botanical Drug Products [3]
14 and as described in a previous publication.[2] The results of safety testing, including heavy metals,
15 pesticide residue and microbes all met the standards for botanical products.[4-6] . FAHF-2 and PN
16 preparations were tested for endotoxin using the Pyrogent Plus assay kit (Lonza, MA). They
17 were both undetectable with low limit of detection of <0.03 Endotoxin Unit/ml (EU/ml).[2]

18

19 ***E2. Extraction and Isolation of Fractions and Compounds from FAHF-2***

20 Powered FAHF-2 extract (5 g) was dissolved into distilled water (125 ml). The solution was
21 sonicated for 10 minutes followed by vortexing .This procedure was repeated for three times.
22 The solution was transferred to a seperatory funnel, and extracted with equal volume of butanol
23 (125 ml, Fisher Scientific, Pittsburgh, PA, USA). After the separation, the organic layer was

24 removed and saved. The aqueous layer was re-extracted with butanol for three more times. All
25 seperated butanol extracts were combined and washed with distilled water at ratio 3:1. The
26 butanol extrats were then evaporated using Rotorvaper R-210 (Buchi Corporation, New Castle,
27 DE, USA) under reduced pressure. The dried extract butanol FAHF-2 () was collected and stored
28 at 4˚C.
29 Sub-fractions of B-FAHF-2 were collected using Preparative-HPLC (pre-HPLC) system coupled
30 with UV detector (Waters, Milford, MA). Briefly, methanol dissolved B-FAHF-2 was loaded
31 onto prep-HPLC with Xbridge™ C18 reverse-phase column 150mm×19mm×5μm (particle size)
32 from Waters. The mobile phase used were 0.1% formic acid (mobile phase A) and acetonitrile
33 (mobile phase B). The separation gradient started at 97% of A to 75% of A for 10min and to
34 55% of A in another 20 min at a flow rate of 20mL/min. Four sub-fractions were collected based
35 on their polarities.
36 Liquid chromatography mass spectrometry (LC-MS) system and commercially available
37 standards were used to analyze the constituents in sub-fraction 2 of B-FAHF-2. Berberine and
38 palmatine were purchased from Sigma-Aldrich (St Louis, MO). Jatrorrihizine was purchased
39 from National Institute for the control of Pharmaceutical and Biological product of China
40 (Beijing, P. R. China). Exact mass of each constituent was measured by high-resolution
41 micromass LCT premier time-of- flight mass spectrometer (Waters Corporation, Milford, MA,
42 USA) coupled to Waters Alliance 2695 HPLC system. The mass spectra range was set to be
43 mass-to-charge ration (m/z) 50 to 1000. The mass spectrometer was set in positive mode with
44 source conditions as follow: capillary voltage of 3200v, cone voltage of 25v, desolvation gas
45 flow of 500L/h, cone gas flow of 40L/h, desolvation temperature of 350 ˚C , source temperature

46 of 110 ˚C. All spectra were obtained with an internal calibrant (lock mass with Leucine
47 Enkephalin as reference).

48 ***E3. Assessment of systemic anaphylactic symptoms***

49 Anaphylactic symptoms were evaluated approximately 30 minutes after the challenge dose
50 utilizing a previously described scoring system[7]: 0 - no signs; 1 - scratching and rubbing around
51 the snout and head; 2 - puffiness around the eyes and snout, redness around snout, diarrhea, pilar
52 erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3 - wheezing,
53 labored respiration, cyanosis around the mouth and the tail; 4 - no activity after prodding, or
54 tremor and convulsions; 5 - death. Rectal temperatures were measured approximately 30 minutes
55 following challenge using a thermal probe (Harvard Apparatus, Newark, NJ).

56

57 ***E4. Histological assessment of mucosal and cutaneous mast cells degranulation***

58 For assessment of mucosal mast cell degranulation, sham treated and FAHF-2 treated mice were
59 sacrificed forty hours after the final challenge at wk 18. The proximal jejunum from each mouse
60 was snap frozen in OCT and 8μm transverse sections each were fixed in Mota's fixative
61 containing lead acetate and stained with acidic toluidine blue. Mast cells were counted in 400x.
62 Cutaneous mast cell degranulation during systemic anaphylaxis was assessed by examination of
63 skin samples collected after challenge as described previously.[7]

64

65 ***E5. Flow cytometry measurement of peripheral blood basophil numbers using FcεRI***
66 ***$^{+}$CD49b^{+} as marker to identify mouse basophils***

67 In addition to the previous method using the FcεRI $^{+}$CCR3^{-}Gr $^{-}$,[8-10] other methods such as

68 FcεRI $^{+}$CD49b^{+} have also been used recently as markers to identify mouse basophils. [11-13] To

69 further validate the study on peripheral blood basophils, and to determine prolonged inhibitory

70 effect of FAHF-2 on basophils, we conducted additional sets of experiments, in which mice were

71 sensitized with peanut and CT as previously described.[2;14] FAHF-2 treatment began at wk 16

72 (32 mg in 0.5 ml water , twice a day i.g) for 10 wks. Approximately 100µl blood samples were

73 collected from each mouse 23 wk post FAHF-2 therapy. PBLCs were processed and Fcγ

74 receptors were blocked with anti-mouse CD16/32 mAb (2.4G2). PE-conjugated anti-mouse CD3,

75 PE-conjugated anti-mouse B220, FITC-conjugated anti-mouse FcεRI, and APC-conjugated anti-

76 mouse CD49b were added to the cell suspension. CD49b^{+} FcεRI $^{+}$ CD3^{-}B220^{-} cells were

77 defined as mouse blood basophils.[13] Data were analyzed using Flowjo software (Tree Star, Inc.

78 Ashland, OR).

79

80 ***E6. Quantifying FcεRI subunit mRNA levels by real-time PCR (qPCR)***

81 Real-time reverse transcription quantitative PCR (qPCR) was performed using a SYBR green

82 protocol and an ABI7900 HT machine (Applied Biosystems, Foster City, CA, USA) as

83 previously described. [15] Briefly, 1x10^{6} MC/9 cells were cultured in the presence or absence of

84 FAHF-2 (20µg/ml) for 24 hr. Cells were then exposed to 2 µg/ml of mouse anti-DNP IgE for 5h.

85 Cells were harvested and RNA was extracted by using Trizol reagent (Invitrogen, Carlsbad, CA)

86 according to the manufacture’s instruction. The RNA was then deoxyribonuclease (DNase)

87 treated (Invitrogen). cDNA was prepared from these samples using oligo-dT primers (Invitrogen)

88 and Omniscript reverse transcript (QIAGEN, Valencia,CA).The cDNA was used to quantify the

89 mRNA levels of FcεRI α, β and γ subunits by using real-time PCR. The primers used were as
90 follows: FcεRIα (sense primer 5'-TGGGAACAATCACCTTCAAA-3 and anti-sense
91 5'CAGCCAATCTT GCGTTACAT-3'); FcεRIβ(sense 5'-
92 CATTAAAGGTCCAGACACTCCA-3' and anti-sense 5'-CGTCCCATATCAATTACCCA-3');
93 FcεRIγ (5'-CGCAGCTCTGCTATATCCTG-3' and antisense 5'-
94 ACAGCATCTGCTTTCTCACG-3'). The primers were designed based on National Center for
95 Biotechnology Information accession numbers NM01084 (α subunit), AB033617 (β-subunit)
96 and NM10185 (γ subunit).
97

98 ***E7. Western blot***

99 Determination of early FcεRI-mediated signal events was performed by Western blot analysis
100 using anti–Syk and anti-phospholated Syk antibodies (Cell Signaling, Danvers, MA) as
101 described previously.[16] Briefly, IgE-sensitized RBL-2H3 cells (7×10^6) were cultured with or
102 without B+P+J for 24 hours and stimulated with DNP (150μg/ml) for 5 minutes. The cells were
103 then lysed following manufacturer's instruction for Nuclear/Cytoplasmic Extract Kit (Active
104 Motif, Carlsbad, CA) and 50 μg of whole cell proteins was separated by SDS-PAGE and blotted
105 onto a nitrocellulose membrane. After overnight blocking with 5% fat-free milk in PBST, the
106 membranes were incubated with anti–Syk/phospho antibody for 2 hours. After washing with
107 phosphate buffered saline Tween-20 (PBST), the membranes were incubated with peroxidase-
108 conjugated anti–rabbit IgG. Immunoreactive bands were visualized by using the ECL Western
109 detection system (Amersham Pharmacia Biotech, Piscataway, NJ).

110 ***E8. IgE binding test by flow cytometry***

111 IgE binding test was performed as described previously.[17] RBL-2H3 Fc γ receptors were first

112 blocked with anti-FcγRII/III(2.4G2). 20μg/ml of berberine, palmatine or jatrorrhizine and then

113 1μg/ml mouse IgE antibody were incubated with RBL-2H3 ($1X10^6$/ml) at 4℃ for 1 hour. Cells

114 were washed twice with PBS, then FITC-anti-mouse IgE was added to detect binding of IgE on

115 RBL-2H3 cells (4℃, 30 minutes) and then analyzed. Cells were incubated without IgE in the

116 presence or absence of berberine, palmatine or jatrorrhizine ,and then stained with FITC-anti-

117 mouse IgE for use as negative controls.

118 **Results**

119 **E1. Reduction of basophil number and anaphylactic reactions**

120 In additional set of experiments, using the $CD49b^+Fc\varepsilon RI^+$ basophil criteria, numbers of basophil

121 in FAHF-2 treated mice remained significantly lower than sham treated mice 23 wks post

122 treatment (Figure E1A,B, $p<0.01$). Consistent with these findings, FAHF-2 treated mice were

123 protected following peanut challenge at this time point (Figure E1C,D,E, $p<0.05$ for all).

124

125

126

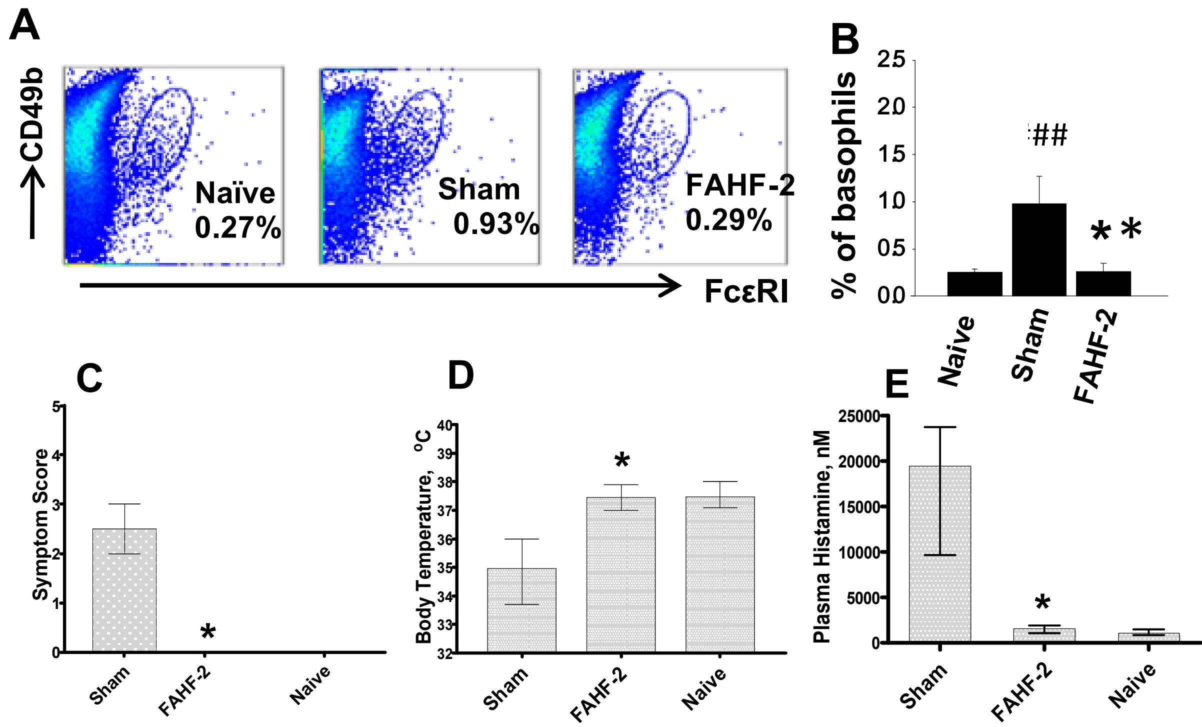
127

128

129

130 **E. Figure**

131 **Figure E1**



132

133
134

135 **E. Figure legend**
136
137 **E1: FAHF-2 induced prolonged inhibition of peripheral basophils and protection against**
138 **peanut anaphylaxis. A.** Dot plots are representative of individual mouse blood basophils from
139 each group 23 wks post therapy. **B.** Bar graph shows Mean ± SEM of 3 individual mouse from
140 each group. Data were analyzed by One-way ANOVA followed by Bonferroni test. **p<0.01,
141 Sham vs. FAHF-2; ## p<0.01, Sham vs. Naïve. **C-E.** Clinical reactions 30 mins following oral
142 peanut challenge. **C,** Anaphylactic symptom score**. D.** Rectal temperature**. E.** Plasma histamine
143 levels. Data were analyzed by Kruskal-Wallis One Way Analysis of Variance on Ranks followed
144 by Student-Newman-Keuls Method. Bars show median; error bars show range. n=4.*p<0.05,
145 Sham vs. FAHF-2.
146
147
148
149
150
151
152
153
154

155 E. References
156

157 (1) Kattan JD, Srivastava KD, Zou ZM, Goldfarb J, Sampson HA, Li XM.
158 Pharmacological and immunological effects of individual herbs in the Food Allergy Herbal
159 Formula-2 (FAHF-2) on peanut allergy. Phytother Res 2008; 22(5):651-9.

160 (2) Srivastava KD, Qu C, Zhang T, Goldfarb J, Sampson HA, Li XM. Food Allergy
161 Herbal Formula-2 silences peanut-induced anaphylaxis for a prolonged posttreatment period via
162 IFN-gamma-producing CD8+ T cells. J Allergy Clin Immunol 2009; 123(2):443-51.

163 (3) The US Food and Drug Administration (FDA), Center for Drug Evaluation and
164 Research. Guidance for Industry Botanical Drug Products. Revised ed. 2004.

165 (4) Raman P, Patino LC, Nair MG. Evaluation of metal and microbial contamination
166 in botanical supplements. J Agric Food Chem 2004; 52(26):7822-7.

167 (5) Dolan SP, Nortrup DA, Bolger PM, Capar SG. Analysis of dietary supplements
168 for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry. J
169 Agric Food Chem 2003; 51(5):1307-12.

170 (6) Caldas ED, Machado LL. Cadmium, mercury and lead in medicinal herbs in
171 Brazil. Food Chem Toxicol 2004; 42(4):599-603.

172 (7) Li XM, Serebrisky D, Lee SY, Huang CK, Bardina L, Schofield BH et al. A
173 murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic
174 human responses. J Allergy Clin Immunol 2000; 106(1 Pt 1):150-8.

175 (8) Luccioli S, Brody DT, Hasan S, Keane-Myers A, Prussin C, Metcalfe DD. IgE(+),
176 Kit(-), I-A/I-E(-) myeloid cells are the initial source of Il-4 after antigen challenge in a mouse
177 model of allergic pulmonary inflammation. J Allergy Clin Immunol 2002; 110(1):117-24.

178 (9) Dvorak AM. The mouse basophil, a rare and rarely recognized granulocyte. Blood
179 2000; 96(4):1616-7.

180 (10) Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajima I, Kinet JP et al. IgE
181 regulates mouse basophil Fc epsilon RI expression in vivo. J Immunol 1997; 158(6):2517-21.

182 (11) Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomin PR
183 et al. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-
184 dependent immunity. Nat Immunol 2009; 10(7):697-705.

185 (12) Zaidi AK, MacGlashan DW. Regulation of Fc epsilon RI expression during
186 murine basophil maturation: the interplay between IgE, cell division, and Fc epsilon RI synthetic
187 rate. J Immunol 2010; 184(3):1463-74.

188 (13) Torrero MN, Larson D, Hubner MP, Mitre E. CD200R surface expression as a
189 marker of murine basophil activation. Clin Exp Allergy 2009; 39(3):361-9.

190 (14) Srivastava KD, Kattan JD, Zou ZM, Li JH, Zhang L, Wallenstein S et al. The
191 Chinese herbal medicine formula FAHF-2 completely blocks anaphylactic reactions in a murine
192 model of peanut allergy. J Allergy Clin Immunol 2005; 115(1):171-8.

193 (15) Busse PJ, Zhang TF, Srivastava K, Schofield B, Li XM. Effect of ageing on
194 pulmonary inflammation, airway hyperresponsiveness and T and B cell responses in antigen-
195 sensitized and -challenged mice. Clin Exp Allergy 2007; 37(9):1392-403.

196 (16) Han EH, Park JH, Kim JY, Jeong HG. Houttuynia cordata water extract
197 suppresses anaphylactic reaction and IgE-mediated allergic response by inhibiting multiple steps
198 of FcepsilonRI signaling in mast cells. Food Chem Toxicol 2009; 47(7):1659-66.

199 (17) Ishikawa Y, Tokura T, Nakano N, Hara M, Niyonsaba F, Ushio H et al. Inhibitory
200 effect of honeybee-collected pollen on mast cell degranulation in vivo and in vitro. J Med Food
201 2008; 11(1):14-20.
202
203
204