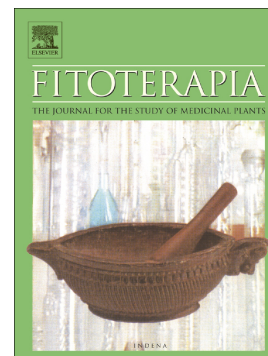


Accepted Manuscript

Ginsenoside F2 induces the release of mediators associated with Anaphylactoid reactions

Lu Wang, Fan Zhang, Zhengyu Cao, Ying Xiao, Suxia Li, Boyang Yu, Jin Qi



PII: S0367-326X(17)30743-8
DOI: doi: [10.1016/j.fitote.2017.07.010](https://doi.org/10.1016/j.fitote.2017.07.010)
Reference: FITOTE 3659

To appear in: *Fitoterapia*

Received date: 24 May 2017
Revised date: 16 July 2017
Accepted date: 19 July 2017

Please cite this article as: Lu Wang, Fan Zhang, Zhengyu Cao, Ying Xiao, Suxia Li, Boyang Yu, Jin Qi , Ginsenoside F2 induces the release of mediators associated with Anaphylactoid reactions, *Fitoterapia* (2016), doi: [10.1016/j.fitote.2017.07.010](https://doi.org/10.1016/j.fitote.2017.07.010)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Ginsenoside F2 Induces the Release of Mediators Associated with Anaphylactoid Reactions

Lu Wang,^{b,d} Fan Zhang,^b Zhengyu Cao,^b Ying Xiao,^c Suxia Li,^b Boyang Yu,^{a,b*} and Jin Qi^{a,b*}

^a State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China

^b Jiangsu Key Laboratory of TCM Evaluation and Translational Research, China Pharmaceutical University, Nanjing 211198, PR China

^c Department of Analytical Chemistry, China Pharmaceutical University, Nanjing 211198, PR China

^d Department of preparation, Nanjing General Hospital of Nanjing Military Region, PLA, Nanjing 210002, PR China

* Corresponding author

E-mail: boyangyu59@163.com (B. Yu).

qijin2006@163.com (J. Qi).

ABSTRACT

Recently, the allergenicity of ginsenosides, as main active components in ginseng, has attracted much attention. Ginsenoside Rb1 and Rd have been reported to induce anaphylactoid reaction. In this study, the allergenicity of a series of 20(S)-protopanaxadiol (PPD) type ginsenosides, including Rb1, Rd, F2, Compound K and 20(S)-PPD, was evaluated in rat basophilic leukemia 2H3 (RBL-2H3) cells. As a result, 20(S)-PPD had no effect on the mast cell degranulation, but other components showed anaphylactoid potential to different extent. The allergenicity was stronger and stronger according to the order “Rb1, Rd, F2, Compound K”. Then, F2 was further verified in RBL-2H3 cells, mouse peritoneal mast cells (MPMCs), Laboratory of Allergic Disease 2 (LAD2) human mast cells *in vitro* and mice *in vivo*. Results showed that F2 could induce a significant increase of histamine release and translocation of phosphatidylserine in RBL-2H3 cells. F2 also increased β -hexosaminidase release and the intracellular Ca^{2+} concentration of MPMC and LAD2 cells. In addition, histamine level in serum of mice was elevated dose-dependently. Our study revealed the potential structure-allergenicity relationship of 20(S)-PPD type ginsenosides and first verified the allergenicity of ginsenoside F2. This study could guide the establishment of quality standards for safe application of ginsenoside-containing preparations.

Key words : Anaphylactoid reaction; 20(S)-protopanaxadiol type ginsenoside; ginsenoside F2; mast cell degranulation.

1. Introduction

Anaphylactoid reactions (ARs), also known as pseudo-allergy, are caused by non-immunoglobulin E-based release of mediators from mast cells. The adverse effects occurred rapidly following the first ever administration of the drug with most affected patients becoming symptomatic in less than 30 min [1]. It is an acute life-threatening hypersensitivity reaction with severe clinical manifestations that resembles generalized anaphylaxis, e.g. urticaria, pruritus, angioedema, gastrointestinal symptoms or bronchospasm [2]. Many compounds have anaphylactoid potential, including basic secretagogues, fluoroquinolone antibiotics, radiocontrast media, non-steroidal anti-inflammatory drugs (NSAIDs) [3,4], polythoxylated pharmaceutical surfactants and vancomycin [5]. Recently, bioactive components in Traditional Chinese Medicine have also been reported to induce ARs, such as bacalin [6], chlorogenic acid, cryptochlorogenic acid [7], neoandrographolide [8] and ginsenosides [9].

Ginsenosides, as the main bioactive components in ginseng (*Panax ginseng* C.A. Meyer), exist in all kinds of preparations and are used in clinic. However, along with the wide application of these preparations, the toxicological effects of ginseng have recently attracted much attention [10]. In the previous studies, ginsenoside Rd was reported to induce ARs via activating mast cells [9]. Xuesaitong injection, a popular Traditional Chinese Medicine injection consisting of over 85% ginsenosides has also been reported to induce ARs [11,12]. Moreover, the allergenicity of ginsenoside Rb1 was confirmed in Xueshuantong injection [13]. Ginsenoside Rb1 and Rd are similar in structure and both

belong to the same deglycosylation pathway of protopanaxadiol (PPD) type saponins [14], which indicates that the allergenicity may be related to the structure to some extent. Therefore, it is necessary to clarify the potential structure-allergenicity relationship of ginsenosides, and find strong allergic components in ginseng for guiding the improvement of preparations and safe use in clinic.

In the present study, firstly, the allergenicity of a series of 20(S)-PPD type ginsenosides, including Rb1, Rd, F2, Compound K and 20(S)-PPD, was evaluated in rat basophilic leukemia 2H3 (RBL-2H3) cells. Results suggested that 20(S)-PPD had no effect on β -hexosaminidase release from RBL-2H3 cells, but other ginsenosides showed anaphylactoid potential to different extent. The allergenicity induced by ginsenosides was stronger and stronger according to the order “Rb1, Rd, F2, Compound K”. ARs usually occurred in a short-time, so most potential allergens were original ingredients rather than metabolites, such as Compound K [15,16]. Therefore, the allergenicity of ginsenoside F2 was further verified in mouse peritoneal mast cells (MPMCs), Laboratory of Allergic Disease 2 (LAD2) human mast cells *in vitro* and mice *in vivo*.

2. Materials and methods

2.1. Chemicals and reagents

Modified Eagle's Medium (MEM), StemPro-34 SFM Medium and trypsin were purchased from Gibco (Grand Island, NY, USA). Recombinant human stem cell factor was purchased from Peprotech (Rocky Hill, NJ, USA). Fetal bovine serum (FBS) was obtained from ScienCell (Carlsbad, CA, USA). Penicillin and streptomycin were

purchased from Beyotime Biotechnology (Shanghai, China). Compound 48/80 (C48/80), L-glutamine, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), *p*-nitrophenyl-N-acetyl- β -D-glucosaminide and poly-D-lysine hydrobromide (PDL) were purchased from Sigma Aldrich (Saint Louis, MO, USA). Triton X-100 was obtained from Amresco (Solon, OH, USA). Ginsenoside Rb1, Rd, F2, Compound K, 20(S)-PPD were purchased from Must Bio-technology (Chengdu, China). Pluronic acid F-127 was obtained from Invitrogen (Carlsbad, CA, USA). A Calcium 5 assay kit was obtained from Molecular Devices (Sunnyvale, CA, USA). An Annexin V-FITC fluorescence staining kit was purchased from KeyGEN BioTECH (Nanjing, China). ELISA kits for histamine were purchased from Biocalvin (Suzhou, China).

2.2. *Animals*

ICR male mice (18–22 g) were obtained from Yangzhou University (Yangzhou, China). Animals were housed with a 12-h light–dark cycle at 22 °C and relative humidity of $55 \pm 5\%$, and had free access to food and water. All experiments involving animals were carried out according to guidelines set by Institutional Animal Care and Use Committee of China Pharmaceutical University (Jiangsu, China).

2.3. *Cell lines and cell culture*

RBL-2H3 cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in MEM supplemented with 15% FBS, 100 U/mL penicillin, 100 μ g/mL streptomycin and 0.11 g/L sodium pyruvate in a

humidified atmosphere of 5% CO₂ at 37 °C.

LAD2 cells were provided by Dr. Kirshenbaum at National Institute of Allergy and Infectious Diseases (National Institutes of Health, America). Cells were cultured in StemPro-34 SFM medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and recombinant human stem cell factor (100 ng/mL). Cell suspensions were seeded at 1.0×10^5 cells/mL and maintained in a humidified atmosphere of 5% CO₂ at 37 °C. Cell culture medium was hemi-depleted every week with fresh medium.

2.4. Isolation of MPMCs

MPMCs were isolated by using a method adapted from Jensen et al [17]. Adult male mice were sacrificed. A total of 10 mL ice-cold phosphate-buffered saline (PBS) was used to make two sequential peritoneal lavages, which were combined and centrifuged at 800 *g* for 10 min at 4 °C. The supernatant was decanted to leave a small MC pellet ($\approx$ 2 ml), and 2 mL of 30% Percoll and 80% Percoll added successively to form an interface (final volume ratio: 1:1:1). After centrifugation at 600 *g* for 15 min at 4 °C, cells at the junction interface were collected and washed with PBS twice (200 *g*, 10 min, 4 °C). MPMCs were resuspended in DMEM with 10% FBS. Purity and viability were determined by toluidine blue staining and trypan blue exclusion.

2.5. β -Hexosaminidase release assay

β -Hexosaminidase release from mast cells was examined as described previously

with some modifications [18]. RBL-2H3 cells (1×10^4 cells/well) or MPMCs ($3-4 \times 10^4$ cells/well) in a 96-well plate were cultured for 24 h, washed with MEM/DMEM without FBS, respectively, and stimulated with different concentrations of sample solutions or the positive control C48/80 (30 $\mu\text{g/mL}$) for 30 min at 37 °C in an atmosphere of 5% CO_2 . Cells in negative control were incubated with MEM/DMEM without FBS.

LAD2 cells were resuspended at 2.5×10^5 cells/mL in HEPES buffer (10 mM HEPES, 2.7 mM KCl, 137 mM NaCl, 1.8 mM $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.38 mM $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 5.6 mM glucose, 1.3 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, pH 7.4). Cell suspension (150 μL) was added to each well of 96 wells, and then stimulated with different concentrations of sample solutions or the positive control C48/80 (1.5 $\mu\text{g/mL}$) for 30 min at 37 °C in an atmosphere of 5% CO_2 [19]. Cells in negative control were incubated with HEPES buffer.

β -Hexosaminidase released into supernatants and cell lysates was quantified by hydrolysis of *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide in 0.1 M sodium citrate buffer (pH 4.5) for 60 min at 37 °C. Absorbance of each well was measured at 405 nm. Percentage of release of β -hexosaminidase was calculated as a percentage of total content, using the following formula:

$$\beta\text{-hexosaminidase}(\%) = \frac{\text{OD}(\text{supernatant})}{\text{OD}(\text{supernatant}) + \text{OD}(\text{lysate})} \times 100$$

2.6. MTT assay

After MPMCs or LAD2 cells were treated with different concentrations of test compounds in terms of method 2.5, the supernatant was discarded and cells were incubated with DMEM/ StemPro-34 SFM medium containing 0.5 mg/mL MTT at 37 °C

and 5% CO₂. After 3 h, the medium was removed and 150 µL dimethyl sulfoxide was added to each well. Absorbance was measured at 570 nm and 650 nm.

2.7. Histamine release assay

RBL-2H3 cells (5×10^4 cells /well) in a 24-well plate were cultured for 24 h, and washed with MEM without FBS. Cells were incubated with different concentrations of sample solutions for 30 min at 37 °C in an atmosphere 5% CO₂. Then, the supernatants were collected and centrifuged at 845 *g* for 10 min. Histamine content in the supernatant was determined using ELISA histamine kits according to the manufacturer's instructions.

Mice were randomly divided into five groups (*n*=8), including vehicle control group, positive control C48/80 group (2.5 mg/kg), F2 groups (10 mg/kg, 20 mg/kg and 40 mg/kg). Animals were challenged via intravenous injection with all test compounds dissolved in saline containing 20% propylene glycol. Blood was collected after 10min, and serum was obtained through centrifugation (825 *g*, 10 min, room temperature). Histamine content in the serum was determined using ELISA histamine kits according to the manufacturer's instructions.

2.8. Fluorescence microscopy

According to the instructions provided with the Annexin V-FITC detection kit. RBL-2H3 cells were treated with different sample solutions for 30 min and washed twice with cold PBS. Then binding buffer containing Annexin V-FITC (5 µL) was

added. After 5 min in the dark, cells were examined and photographed at 630× magnification on an laser scanning confocal microscope (Zeiss LSM700, Jena, Germany).

2.9. Measurement of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$)

$[Ca^{2+}]_i$ was measured using the fluorescence indicator calcium 5/AM. MPMCs or LAD2 cells were plated onto a 96-well plate, which has been coated with 50 µg/mL PDL and balanced at 37 °C in an atmosphere of 5% CO₂. After 6 h, cells were loaded with calcium-5 with 0.02% Pluronic F-127 for 45 min at 37 °C in an atmosphere of 5% CO₂, and washed five times with K25-Locke's buffer (10 mM HEPES, 4 mM NaHCO₃, 2.3 mM CaCl₂, 1 mM MgCl₂, 134 mM NaCl/ 25 mM KCl and 5 mM glucose , pH 7.4 at 37°C) [20] , to leave a final volume of 150 µL in each well. Then, the plate was transferred to the plate chamber of FLIPR (Molecular Devices, Sunnyvale, CA, USA). Cells were excited at 470–495 nm, and emission detected at 515–575 nm. Fluorescence readings were taken every 1 s for 120 s to establish the baseline. Then, 50 µl of different concentrations of sample solutions (4 ×) were added to different wells from the compound plate at 26 µL/s, yielding a final volume of 200 µL/ well. Fluorescence readings were taken for 20 min.

2.10. Statistical analysis

All the experimental data was presented as mean ± SEM. Statistical significance was assessed by ANOVA test. One-way analysis of variance followed by Dunnett's test was

used for multiple comparisons. $p < 0.05$ was considered significant. Each experiment was repeated at least thrice.

3. Results

3.1. Different ginsenosides in the same deglycosylation pathway “Rb1-Rd-F2-Compound K-20(S)-PPD” induced the β -hexosaminidase release of RBL-2H3 cells to different extent

β -Hexosaminidase is an enzyme contained in the cytoplasmic granules of mast cells, and the level of this enzyme released into the supernatant provides an indication of the degree of degranulation when mast cell is activated. As shown in Fig. 1, β -hexosaminidase release induced by ginsenoside Rb1 (Fig. 1A), Rd (Fig. 1B), F2 (Fig. 1C) and Compound K (Fig. 1D) was different, whose minimum effective concentration was 0.9 mM, 0.13 mM, 0.14 mM, 0.04 mM, respectively. While 20(S)-PPD (Fig. 1E) had no effect on β -hexosaminidase release of RBL-2H3 cells. These findings suggested that 20(S)-PPD couldn't induce mast cell degranulation, while 20(S)-PPD type ginsenosides possessed anaphylactoid potential to different extent. In addition, comparing the structures of these compounds (Fig. 1F), we could also find that the minimum effective concentration was lower and lower along with the decrease of the number of glucoses at C-3, which indicated that glucoses at this position had an effect on allergenicity of ginsenosides. In this section, the allergenicity of ginsenoside F2 and Compound K was confirmed for the first time, and the structure-allergenicity relationship of ginsenosides was first investigated.

3.2. Ginsenoside F2 induced degranulation of RBL-2H3 cells

The allergenicity of ginsenoside F2 was verified in RBL-2H3 cells by determining the release of histamine and phosphatidylserine translocation of cells.

Histamine is also a classic mediator released concomitantly with β -hexosaminidase if mast cells are activated. As shown in Fig. 2A, F2 could also induce significant release of histamine ($p < 0.01$), which was consistent with that seen with β -hexosaminidase.

It has been reported that the membrane phospholipid phosphatidylserine translocates from the inner to outer leaflets of cytomembranes during mast cells degranulation [21]. Annexin V-FITC can bind to cells with exposed phosphatidylserine, causing the cells to appear green. As shown in Fig. 2B, normal cells were colorless, while the positive control C48/80 and ginsenoside F2 could cause the cells to appear green significantly, which suggested F2 could induce the phosphatidylserine translocation of RBL-2H3 cells.

3.3. Ginsenoside F2 induced degranulation of MPMCs

For further evaluation, degranulation induced by F2 was determined in MPMCs. As shown in Fig. 3A, F2 could induce the significant release of β -hexosaminidase in a concentration-dependent manner. In addition, cell viability was determined by the MTT assay simultaneously (Fig. 3B). F2 showed no cytotoxicity on MPMCs, which suggested that the release could be regarded as net degranulation.

Intracellular calcium ion ($[Ca^{2+}]_i$) played a crucial role for release of mediators. Change in $[Ca^{2+}]_i$ is an indication of the degree of mast cell degranulation [22,23]. So

we investigated the effect of F2 on Ca^{2+} mobilization in MPMCs. As shown in Fig. 3C and D, F2 could induce the response of Ca^{2+} , and the degree of which was dependent upon concentration of F2. Therefore, the mechanism of MPMCs activation induced by F2 might be related to an increase in $[\text{Ca}^{2+}]_i$.

3.4. Ginsenoside F2 induced degranulation of LAD2 cells

In order to confirm the degranulation observed in RBL-2H3 cells and MPMCs, we used human mast cell line LAD2 to further evaluate the anaphylactoid potential of F2. As shown in Fig. 4, consistent with MPMCs, F2 could dose-dependently induce the significant release of β -hexosaminidase (Fig. 4A), and the cell viability under different concentrations of F2 showed no significant difference (Fig. 4B). In addition, the calcium response of LAD2 cells increased when stimulated with F2 (Fig. 4C and D).

3.5. Ginsenoside F2 induced the increase of histamine level in mice serum

As shown in Fig. 5, after 10 min of intravenous injection, the histamine levels in serum of the F2 groups increased dose-dependently compared with that of the vehicle control group. Finally, our result showed that F2 could exhibit potential anaphylactoid activity *in vivo*.

4. Discussion

This is the first systematic evaluation on allergenicity of 20(S)-PPD type ginsenosides with different structures. We found that 20(S)-PPD had no effect on mediators release of RBL-2H3 cells, but ginsenosides from Rb1 to Compound K could induce the significant

release of β -hexosaminidase in RBL-2H3 cells, respectively with the minimum concentration of 0.9 mM, 0.13 mM, 0.14 mM and 0.04 mM. Our results indicated that the number and position of glucoses might influence allergenicity of PPD type ginsenosides. Meanwhile, the allergenicity of ginsenoside F2 was further verified in MPMCs, LAD2 cells and mice, which suggested that F2 had anaphylactoid potential and was also a strong allergenic ingredient in ginseng.

Previously, a similar study on allergenicity of Rd has been reported [9]. Their results suggested Rd was the direct inductor of RBL-2H3 cell degranulation, and cutaneous vascular permeability and plasma histamine level also significantly increased after Rd injection. Our study also confirmed the allergenicity of Rd. In addition, we found other potential allergens and clarified the structure-allergenicity relationship of 20(S)-PPD type ginsenosides in RBL-2H3 cells. The minimum sensitization concentration of ginsenosides from Rb1 to Compound K was lower and lower, which indicated that allergenicity of ginsenosides might be dependent on glucoses at C-20, and the number of glucoses at C-3 might have an effect on the allergenicity via influencing steric hindrance between components and mast cells. There was no glucose at C-20 in 20(S)-PPD so that it couldn't exhibit the anaphylactoid potential. However, whether the number of glucoses at C-3 was the only factor that influenced the allergenicity via steric-hindrance effect still remained unknown. Therefore, Ginsenosides 20(S)-Rg3 (Fig. 6A) and 20(S)-Rh2 (Fig. 6B) were also determined in RBL-2H3 cells (Results were not offered). We found that the minimum sensitization concentration of 20(S)-Rg3, as a

isomer of F2, was similar to F2, but 20(S)-Rh2 (one glucose less than 20(S)-Rg3 at C-3) had no effect on degranulation of RBL-2H3 cells, which indicated that the allergenicity of 20 (S)-PPD type ginsenosides might be related to other factors, such as physical and chemical properties of components. Therefore, more ginsenosides should be collected and analyzed in the future study.

According to statistics, ARs usually occurred in less than 30min [1], so ARs induced by preparations could mainly result from original ingredients but not metabolites. Although it has been confirmed that Compound K might possess the strongest anaphylactoid potential, it couldn't be involved in direct mast cell degranulation in injections due to the few content in preparations as a main metabolite. Therefore, the allergenicity of ginsenoside F2 was further verified in this study, but that of Compound K might be studied in the adverse drug reactions induced by oral preparations.

Nowadays, Ginseng has been widely used as a functional food or therapeutic supplement in the world, but the security of which attracted more and more attention in recent years. Except for ARs induced by ginsenosides, irreversible impairment of agonist-induced vascular contraction induced by ginsenoside Rg3 was also reported recently [24]. Ginsenoside F2 exists in many ginseng-containing preparations, and has been demonstrated great potential in terms of antioxidative [25], anti-cancer [26,27] and promoting hair growth effects [28,29]. Therefore, the toxicological effect of bioactive components should be paid more attention to ensure safe application in clinic.

5. Conclusion

Our study shows structure-allergenicity relationship of 20(S)-PPD type ginsenosides. Meanwhile, we first demonstrate the allergenicity of ginsenoside F2 by *in vitro* and *in vivo* experiments. This is the first systematic study on ARs induced by ginsenoside F2 and different ginsenosides in one deglycosylation pathway. Results reveal the potential rule of “structure-allergenicity”. Our study could guide establishment of quality standards for safe application of ginsenoside-containing preparations.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (81473317), Youth Foundation of Jiangsu Province (BK20140654), Qing Lan Project, 2011’ Program for Excellent Scientific and Technological Innovation Team of Jiangsu Higher Education and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

- [1] J.L. Weaver, M. Boyne, E. Pang, K. Chimalakonda, K.E. Howard, Nonclinical evaluation of the potential for mast cell activation by an erythropoietin analog, *Toxicol. Appl. Pharm.* 287 (2015) 246-252.
- [2] J. Ring, H. Behrendt, Anaphylaxis and anaphylactoid reactions, *Clin. Rev. Allergy Immunol.* 17 (1999) 387–399.
- [3] K. Farnam, C. Chang, S. Teuber, M.E. Gershwin, Nonallergic drug hypersensitivity

- reactions, *Int. Arch. Allergy Immunol.* 159 (2012) 327–345.
- [4] J. Szebeni, Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity, *Toxicol.* 216 (2005) 106-121.
- [5] Z. Weiszhar, J. Czúcza, C. Révész, L. Rosivalla, J. Szebeni, Z. Rozsnyay, Complement activation by polyethoxylated pharmaceutical surfactants: Cremophor EL, Tween-80 and Tween-20, *Eur. J. Pharm. Sci.* 45 (2012) 492-498.
- [6] S.L. Han, T. Zhang, J. Huang, R.H. Cui, L.C. He, New method of screening allergenic components from shuanghuanglian injection: with RBL-2H3/CMC model online HPLC/MS system, *J. Pharmaceut. Biomed. Anal.* 88 (2014) 602-608.
- [7] F. Wang, C.Y. Li, Y.G. Zheng, Y. Li, G.P. Peng, Study on the anaphylactoid of three phenolic acids in Honeysuckle, *J. Ethnopharmacol.* 170 (2015) 1-7.
- [8] X.G. Hu, Y. Wen, S.S. Liu, J.B. Luo, X.M. Tan, Z.H. Li, X.H. Lu, X.Y. Long, Evaluation of the anaphylactoid potential of *Andrographis paniculata* extracts using the popliteal lymph node assay and P815 cell degranulation in vitro, *J. Transl. Med.* 13 (2015) 2-9.
- [9] X.Y. Lu, X.P. Lian, J. Zheng, N. Ai, C. Ji, C. Hao, X.H. Fan, LC-ESI-TOF-MS-based metabolomic analysis of ginsenoside Rd-induced anaphylactoid reaction in mice, *RSC Adv.* 6 (2016) 19545-19554.
- [10] D.J. Paik, C.H. Lee, Review of cases of patient risk associated with ginseng abuse and misuse, *J. Gins. Res.* 39 (2015) 89-93.
- [11] L.L. Wang, Z. Li, X.P. Zhao, W. Liu, Y.F. Liu, J.H. Yang, X. Li, X.H. Fan, Y.Y.

- Cheng, A network study of Chinese medicine Xuesaitong injection to elucidate a complex mode of action with multicomponent, multitarget, and multipathway, *Evid-Based Compl. Alt. Med.* 2013 (2013) 652373.
- [12] Z. Xiang, T. Qiao, H. Xiao, T.G. Kang, D.Q. Dou, H.B. Li, H.X. Kuang, The anaphylactoid constituents in Xue-Sai-Tong injection, *Planta med.* 79 (2013) 1043-1050.
- [13] H.C. Li, Q.Y. Wu, Y.T. Fu, J.B. Luo, X.M. Tan, Study on screening method of allergenic ingredients of TCM injections, *Pharmacol. Clin. Chin. Mater. Med.* 30 (2014) 139-141.
- [14] H. Hong, C.H. Cui, J.K. Kim, F.X. Jin, S.C. Kim, W.T. Im, Enzymatic Biotransformation of Ginsenoside Rb1 and Gypenoside XVII into Ginsenosides Rd and F2 by Recombinant beta-glucosidase from *Flavobacterium johnsoniae*, *J. Gins. Res.* 36 (2012) 418-424.
- [15] X.P. Li, P. Wang, Y.X. Li, Progress in pharmacological actions of ginsenoside compound K, an active metabolite of protopanaxadiol type saponins, *Chin. J. Pharmacol. Toxicol.* 15 (2010) 97-101.
- [16] X.D. Yang, Y.Y. Yang, D.S. Ouyang, G.P. Yang, A review of biotransformation and pharmacology of ginsenoside compound K, *Fitoterapia* 100 (2015) 208-220.
- [17] B.M. Jensen, E.J. Swindle, S. Iwaki, A.M. Gifillan, Isolation, and Maintenance of Rodent Mast Cells and Mast Cell Lines, *Curr. Protoc. Immunol.* 3 (2006) 23.
- [18] J.M. Tang, J. Liu, W.B. Wu, Studies on degranulation of RBL-2H3 cells induced by

- Traditional Chinese Medicine Injections, *Chin. Med.* 3 (2012) 200-208.
- [19] B.D. McNeil, P. Pundir, S. Meeker, L. Han, B.J. Udem, M. Kulka, X.Z. Dong, Identification of a mast cell specific receptor crucial for pseudo-allergic drug reactions, *Nature* 519 (2015) 237-241.
- [20] Y. Guitierrez-Martin, F.J. Martin-Romero, F. Henao, C. Gutierrez-Merino, Alteration of cytosolic free calcium homeostasis by SIN-1: high sensitivity of L-type Ca^{2+} channels to extracellular oxidative/nitrosative stress in cerebellar granule cells, *J. Neurochem.* 92 (2005) 973-989.
- [21] S.D. Demo, E. Masuda, A.B. Rossi, B.T. Throdset, A.L. Gerard, E.H. Chan, R.J. Armstrong, B.P. Fox, J.B. Lorens, D.G. Payan, Quantitative measurement of mast cell degranulation using a novel flow cytometric Annexin-V binding assay, *Cytom.* 36 (1999) 340-348.
- [22] A.M. Gifillan, C. Tkaczyk, Integrated signalling pathways for mast-cell activation, *Nat. Rev. Immunol.* 6 (2006) 218-230.
- [23] Y. Gao, R. Hou, Q.L. Fei, L. Fang, Y.X. Han, R.L. Cai, C. Peng, Y. Qi, The three-herb formula shuang-huang-lian stabilizes mast cells through activation of mitochondrial calcium uniporter, *Sci Rep.* 7 (2017) 38736.
- [24] J.Y. Lee, K.M. Lim, S.Y. Kim, O.N. Bae, J.Y. Noh, S.M. Chung, K. Kim, Y.S. Shin, M.Y. Lee, J.H. Chung, Vascular smooth muscle dysfunction and remodeling induced by ginsenoside Rg3, a bioactive component of ginseng, *Toxicol. Sci.* 117 (2010) 505-514.

- [25] D. Liu, F.G. Pan, J.Y. Liu, Y. Wang, T. Zhang, E. Wang, J.B. Liu, Individual and combined antioxidant effects of ginsenoside F2 and cyanidin-3-O-glucoside in human embryonic kidney 293 cells, *RSC Adv.* 6 (2016) 81092-81100.
- [26] T.T. Mai, J. Moon, Y. Song, P.Q. Viet, P.V. Phuc, J.M. Lee, T.H. Yi, M. Cho, S.K. Cho, Ginsenoside F2 induces apoptosis accompanied by protective autophagy in breast cancer stem cells, *Cancer Lett.* 321 (2012) 144-153.
- [27] J.Y. Shin, J.M. Lee, H.S. Shin, S.Y. Park, J.E. Yang, S.K. Cho, T.H. Yi, Anti-Cancer Effect of Ginsenoside F2 against Glioblastoma Multiforme in Xenograft Model in SD Rats, *J. Gins. Res.* 36 (2012) 86-92.
- [28] H.S. Shin, S.Y. Park, E.S. Hwang, D.G. Lee, H.G. Song, G.T. Mavlonov, T.H. Yi, The inductive effect of ginsenoside F2 on hair growth by altering the WNT signal pathway in telogen mouse skin, *Eur. J. Pharm.* 730 (2014) 82-89.
- [29] H.S. Shin, S.Y. Park, E.S. Hwang, D.G. Lee, G.T. Mavlonov, T.H. Yi, Ginsenoside F2 Reduces Hair Loss by Controlling Apoptosis through the Sterol Regulatory Element-Binding Protein Cleavage Activating Protein and Transforming Growth Factor-beta Pathways in a Dihydrotestosterone-Induced Mouse Model, *Biol. Pharm. Bull.* 37 (2014) 755-763.

Figure captions

Fig. 1. Different ginsenosides in the same deglycosylation pathway “Rb1-Rd-F2-Compound K-20(S)-PPD” induced the β -hexosaminidase release of RBL-2H3 cells to different extent. (A) Ginsenoside Rb1. (B) Ginsenoside Rd. (C) Ginsenoside F2. (D) Ginsenoside Compound K. (E) 20(S)-PPD. (F) Change in structure of different ginsenosides. Data are presented as mean $\pm$ SEM of three independent experiments. * p < 0.05, *** p < 0.001, compared with control group.

Fig. 2. Ginsenoside F2 induced degranulation of RBL-2H3 cells. (A) F2 induced the significant release of histamine of RBL-2H3 cells. Data are presented as mean $\pm$ SEM of three independent experiments. ** p < 0.01, compared with control group. ## p < 0.01, positive control C48/80 (30 μ g/mL) compared with control group. (B) Effect of ginsenoside F2 on phosphatidylserine translocation in RBL-2H3 cells.

Fig. 3. Ginsenoside F2 induced degranulation of MPMCs. (A) The effect of F2 on the β -hexosaminidase release of MPMCs. (B) The effect of F2 on the cytotoxicity of MPMCs. (C) Time-response relationships for F2 induced increase of $[Ca^{2+}]_i$ of MPMCs. (D) Quantification of concentration-response relationship for F2 induced increase of $[Ca^{2+}]_i$ of MPMCs. Data are presented as mean $\pm$ SEM. ** p < 0.01, *** p < 0.001, compared with control group. ### p < 0.001, positive control C48/80 (30 μ g/mL) compared with control group.

Fig. 4. Ginsenoside F2 induced degranulation of LAD2 cells. (A) The effect of F2 on the β -hexosaminidase release of LAD2 cells. (B) The effect of F2 on the cytotoxicity of

LAD2 cells. (C) Time-response relationships for F2 induced increase of $[Ca^{2+}]_i$ of LAD2 cells. (D) Quantification of concentration-response relationship for F2 induced increase of $[Ca^{2+}]_i$ of LAD2 cells. Data are presented as mean $\pm$ SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, compared with control group. $###p < 0.001$, positive control C48/80 (3 μ g/mL) compared with control group.

Fig. 5. Ginsenoside F2 induced the increase of histamine level in mice serum at 10 min post-injection. Data are presented as mean $\pm$ SEM of three independent experiments. $**p < 0.01$, $***p < 0.001$, compared with control group.

Fig. 6. Structures of ginsenoside 20(S)-Rg3 (A) and 20(S)-Rh2 (B).

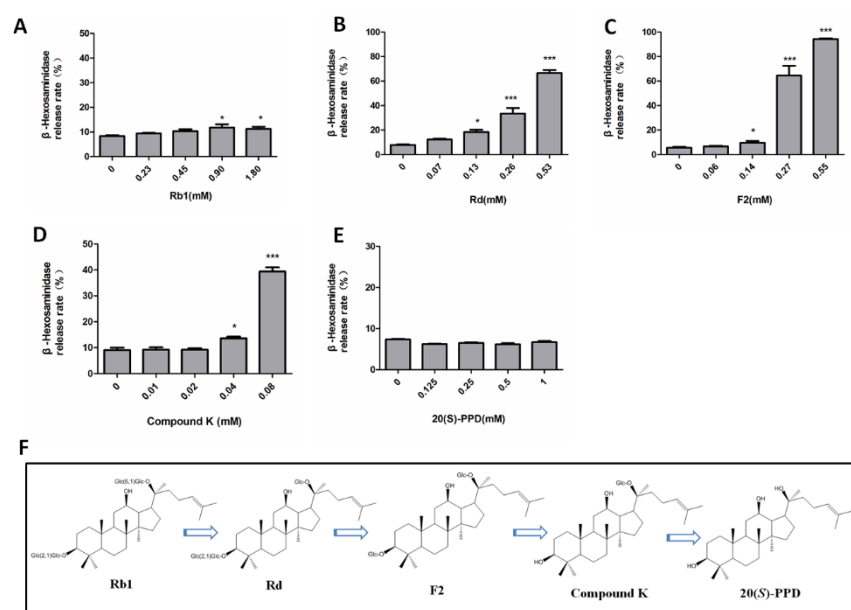


Figure 1

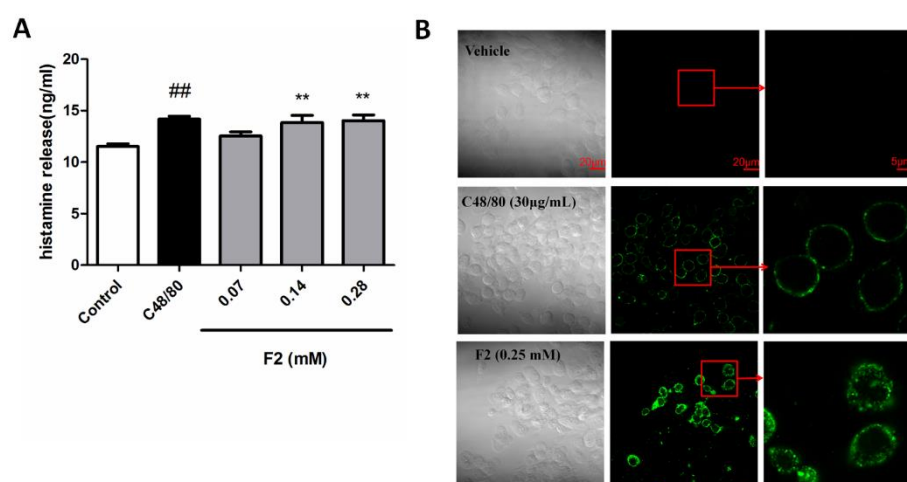


Figure 2

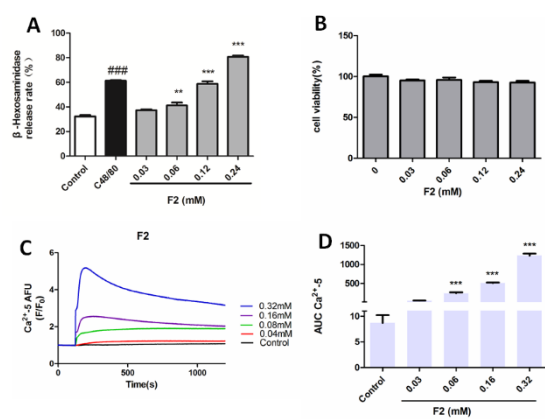


Figure 3

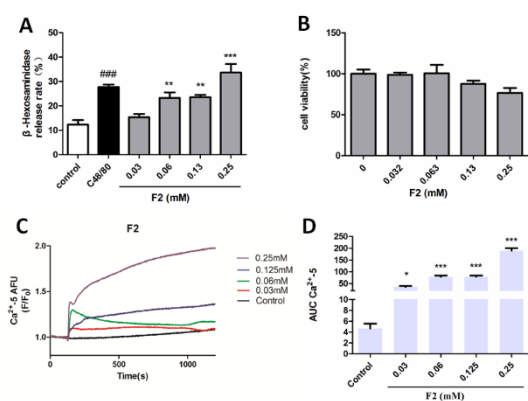


Figure 4

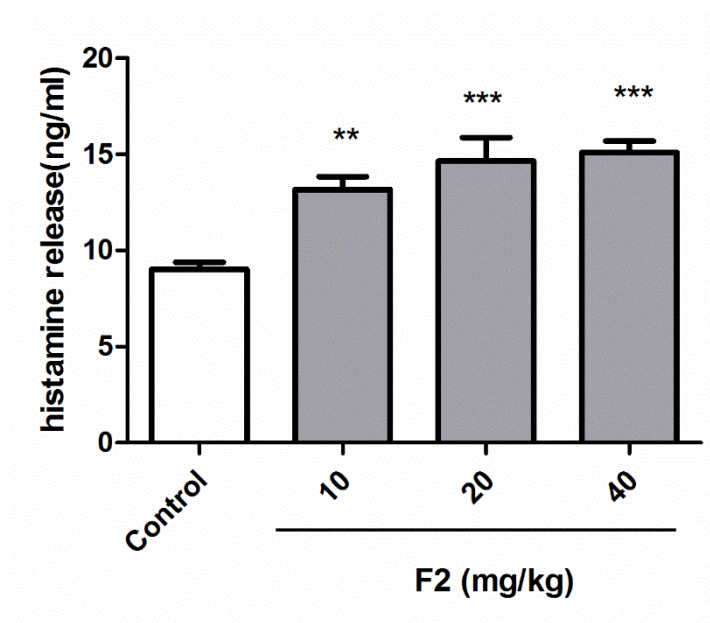


Figure 5

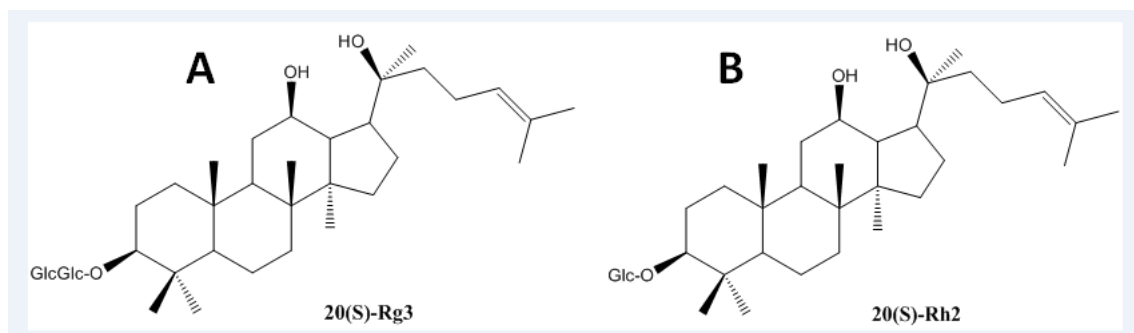
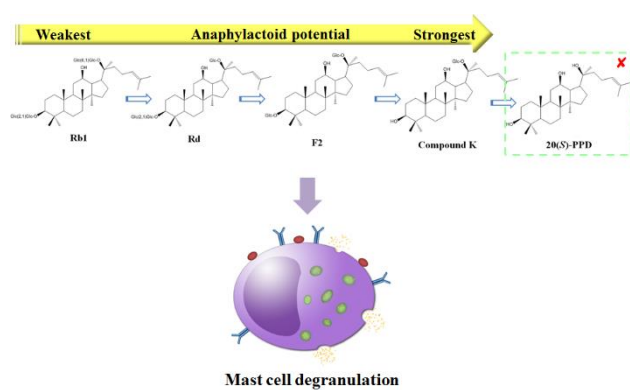


Figure 6



Graphical abstract