

Immunomodulatory effects of Glycyrrhiza uralensis polysaccharide in glycinin-induced allergic mouse model

Jie Chen, Xinli Gu, Jianguo Chen, Yan Luo, Mengyuan Wang, Hongyang Yang, Xiao Guo & Xiaoqing Zhu

To cite this article: Jie Chen, Xinli Gu, Jianguo Chen, Yan Luo, Mengyuan Wang, Hongyang Yang, Xiao Guo & Xiaoqing Zhu (2017) Immunomodulatory effects of Glycyrrhiza uralensis polysaccharide in glycinin-induced allergic mouse model, Food and Agricultural Immunology, 28:2, 179-188, DOI: [10.1080/09540105.2016.1251393](https://doi.org/10.1080/09540105.2016.1251393)

To link to this article: <https://doi.org/10.1080/09540105.2016.1251393>



Published online: 18 Nov 2016.



Submit your article to this journal [↗](#)



Article views: 79



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 1 View citing articles [↗](#)



Immunomodulatory effects of *Glycyrrhiza uralensis* polysaccharide in glycinin-induced allergic mouse model

Jie Chen^a, Xinli Gu^a, Jianguo Chen^b, Yan Luo^a, Mengyuan Wang^a, Hongyang Yang^a, Xiao Guo^a and Xiaoqing Zhu^a

^aDepartment of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China; ^bUrumqi County Health and Epidemic Prevention Station, Urumqi, Xinjiang, People's Republic of China

ABSTRACT

This study aimed to investigate the therapeutic effects of *Glycyrrhiza uralensis* polysaccharide (GUP) on glycinin-induced allergic reactions in mouse model. Female BALB/c mice were pretreated for seven consecutive days with various concentrations of GUP aqueous solution or blank control. The mice were then orally administered with glycinin (10 mg/ml) three times and observed a week later. The survival rate and other indicators of the mice were measured, while IL-4, IL-13, plasma histamine and the IgE and IgG content in the serum were detected by enzyme-linked immunosorbent assay. The percentage of CD4+CD25+FoxP3+T cells was detected by flow cytometry. The results showed that IL-4, IL-13 and plasma histamine levels significantly decreased in the blood of mice treated with low GUP concentrations (20 mg/ml), while the specific glycinin-IgE, glycinin-IgG inhibition ratio and CD4+CD25+FoxP3+ T cells/CD4+ T cells ratio increased. However, the lowest, middle, high and the highest concentrations of GUP did not have significant effects on the immune reactions of glycinin-induced allergic mice. In conclusion, our study suggests that GUP is a promising agent to inhibit glycinin-induced allergy.

ARTICLE HISTORY

Received 18 May 2016
Accepted 16 October 2016

KEYWORDS

Crude *Glycyrrhiza uralensis* polysaccharide; glycinin; immunotherapy; allergy

Introduction

Food allergy is an adverse reaction mediated by the immune system (Pesek & Jones, 2014). Food allergies are often accompanied by a series of symptoms that can affect the gastrointestinal tract, skin and respiratory systems, as well as systemic reaction which could be fatal (Pesek & Jones, 2014). In animal husbandry, the feed protein antigen commonly causes allergic reactions in young animals, seriously hindering their growth and development, greatly reducing their survival, and even causing a narrow escape from death in small animals. Soybean is one of the most important legumes. Soybean protein and other protein sources are widely used in the diet of animals (Medic, Atkinson, & Hurburgh, 2014). However, soybean is one of the eight food allergens that can cause serious and even fatal allergic reactions in piglets, calves and other young animals (Cucu et al., 2012). Soybean protein can stimulate the body to produce an immune

response, and trigger a specific antigen-antibody reaction (type I allergic reaction) and T lymphocyte-mediated delayed type hypersensitivity reaction (type IV allergic reaction) (L'Hocine & Boye, 2007). Glycinin and β -conglycinin have the strongest immunogenicity of soy protein, accounting for 65–80% of soybean seed protein content, and glycinin is not susceptible to enzymatic hydrolysis (Cucu et al., 2013; Ma et al., 2010).

How to ease the symptoms of allergic reactions caused by soy protein has become a hot issue in recent years, especially in animal husbandry. *Glycyrrhiza uralensis*, a genuine regional drug in Xinjiang, not only has immunomodulatory activity, but also has anti-allergic effects (Shunsuke et al., 2014). *G. uralensis* polysaccharide (GUP) is the main active component of *G. uralensis* and may mediate the anti-allergic effects. To improve animal husbandry by avoiding fatal allergic reactions in young animals, we performed the present study to investigate the therapeutic effect of GUP on allergic symptoms caused by glycinin in mouse model.

Materials and methods

Reagents

One hundred and twenty female BALB/c mice aged 3 weeks were used. Crude GUP was extracted using the water-decoction-ethanol-precipitation method as previously described. The total polysaccharide was measured by Vitriol-anthracene ketone, using glucose without H₂O as a standard control, and the content (%) of GUP was found to be 83.3% (Cui et al., 2014; Liang, 2008; Zhang, Guo, Xue, Jian, & Lei, 2015). Glycinin was purchased from TransGen Biotech (Beijing, China). Enzyme-linked immunosorbent assay (ELISA) detection kits for IgE, IgG, IL-4, IL-13, histamine, and mouse anti-rat CD4 + FITC, Foxp3 PE, Foxp3 staining kit and CD25+PE-CY5 were all purchased from Sangon Biotech (Shanghai, China). All other chemicals and solvents of the analytical grade available were obtained from Wode Chemical Reagent (Shihezi, Xinjiang, China).

Establishment of mouse allergic model

One hundred and twenty healthy female BALB/c mice aged 3 weeks were selected and randomized into six groups, with 20 mice in each group. The mice were kept at 18°C with humidity of 50–70%. The mice were given free drinking water. They were then treated for seven consecutive days with various concentrations of GUP (1, 20, 50, 100, 200 mg/ml) aqueous solution or saline (blank control). During this pretreatment period, the mice were fed a controlled diet. Subsequently, 10 mice were randomly selected from each dose group and orally sensitized three times with 0.5 ml of 10 mg/ml glycinin and observed a week later. Then the remaining 10 mice in each dose group were orally administered with glycinin (1 mg/ml) three times a day, each time 0.5 ml, for a week.

Evaluation of allergic reaction

Allergy symptoms were observed for 30–40 min a week later after the mice were orally sensitized three times in each day with 10 mg/ml glycinin. The mice were categorized into the six following groups: (a) symptomless; (b) around the nose and head scratching;

(c) puffiness around the eyes and mouth, diarrhea, pillar installation, reduced activity and/or decreased with increase in respiration rate; (d) wheezing, forced breathing, cyanosis around the mouth and tail; (e) after prodding, no activity or tremors and convulsions and (f) death (Li, Schofield, Huang, Kleiner, & Sampson, 1999; Li et al., 2000). The incidence of allergic symptoms was calculated as follows: incidence of allergic symptoms (%) = The number of mice with allergic symptoms/Total number of mice in each group.

Separation of serum and plasma of animals

After seven days administration (1 mg/ml glycinin dose group), the eyeballs of the mice were removed under anesthesia to retrieve the blood. To get plasma, the blood was collected into 1.5 ml EDTA-coated Eppendorf tubes and gently mixed. The samples were centrifuged at 3000 rpm for 10 min at 4°C. The supernatants (plasma) were transferred into 0.5 ml Eppendorf tube for storage. To get serum, the blood was collected into regular 1.5 ml Eppendorf tubes and stored at room temperature for 1 h. The samples were centrifuged at 3000 rpm for 10 min at 4°C. The supernatants (serum) were collected and packed for analysis.

Detection of specific antibody levels in the serum

The glycinin-specific IgE and IgG levels in the serum of the mice were detected by ELISA following the manufacturer's protocols. The absorbance of the solution was measured at 450 nm. The inhibition ratio was calculated using the following equation: inhibition ratio (%) = (OD_{sample} - OD_{blank}) / OD_{blank} × 100%. OD_{sample} was the OD value of the well in which serum sample was added. OD_{blank} was the OD value of the well in which saline was added.

Measurement of plasma histamine level

Plasma histamine levels were analyzed by using a Histamine ELISA Kit (Immuno Sangon Inc, Shanghai, China) following the manufacturer's protocols.

Determination of IL-4 and IL-13 levels

IL-4 and IL-13 levels in the serum of the mice were measured by ELISA kit (Immuno Sangon Inc, Shanghai, China) following the manufacturer's protocols.

Flow cytometry analysis

The cells in the mesenteric lymph nodes of the mice were separated and adjusted to the concentration of 2×10^6 /ml. The 100 μ l of cell suspension was mixed with CD25+ isotype control, Foxp3 isotype control, CD4+CD25+, and CD4+CD25+Foxp3, respectively, and incubated at 4°C for 30 min in the dark. The cells were washed and suspended in 400 μ l staining buffer and subjected to flow cytometry analysis.

Statistical analysis

All the data were expressed as mean ± standard deviation. The differences between groups were analyzed by one-way ANOVA, followed by a homogeneity of variance test using SPSS 19.0 statistical software. *P* < .05 was considered statistically significant differences.

Results

The incidence of systemic allergic reactions

The degree and percentage of allergic reactions in mice in each group are summarized in Table 1. The allergic reaction induced by glycinin was successfully established in the mouse model. The mortality of the mice treated with GUP was significantly lower than that of the control group, and the health rate increased. Among them, the 20 mg/ml GUP group of mice had the highest health rate (42%) and the lowest mortality (4%) compared to the other groups. The 50 mg/ml GUP group had a health rate of 34% and a mortality rate of 16%. The concentration that was the least effective was 1 mg/ml GUP; which had a health rate of only 10% and a mortality rate of 24%.

Glycinin-specific IgE and glycinin-specific IgG

After mice were sensitized three times with 1 mg/ml glycinin by mouth over the course of a week, they were treated for seven consecutive days with various concentrations of GUP (1, 20, 50, 100, 200 mg/ml) or saline (blank control), the inhibition ratio of glycinin-specific IgG in serum of mice in each group increased, and the maximum inhibition rates of glycinin-specific IgE and IgG were 39.64 ± 0.4% and 31.00 ± 0.09% (Figure 1).

Histamine release rate

As shown in Figure 2, the release rate of histamine in mice treated with different concentrations of GUP was significantly lower than the control group, and GUP inhibited histamine release in a dose dependent manner (*P* < .05). The 200 mg/ml GUP pretreatment group had the lowest histamine release rate (14.77 ± 0.42%).

IL-4 and IL-13 contents

As shown in Figure 3, serum IL-4 and IL-13 contents showed a significant decrease in mice treated with different concentration of GUP compared to blank control group. At the GUP

Table 1. The incidence of allergic symptoms in each group.

Incidence of allergic symptoms (%)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>
Concentrations of GUP (mg/ml)						
0	0	8	12	18	12	50
1	10	18	12	14	22	24
20	42	22	16	8	8	4
50	34	14	26	4	6	16
100	24	22	12	8	12	22
200	22	16	16	18	2	26

Note: a–f: Six different types of allergic symptoms.

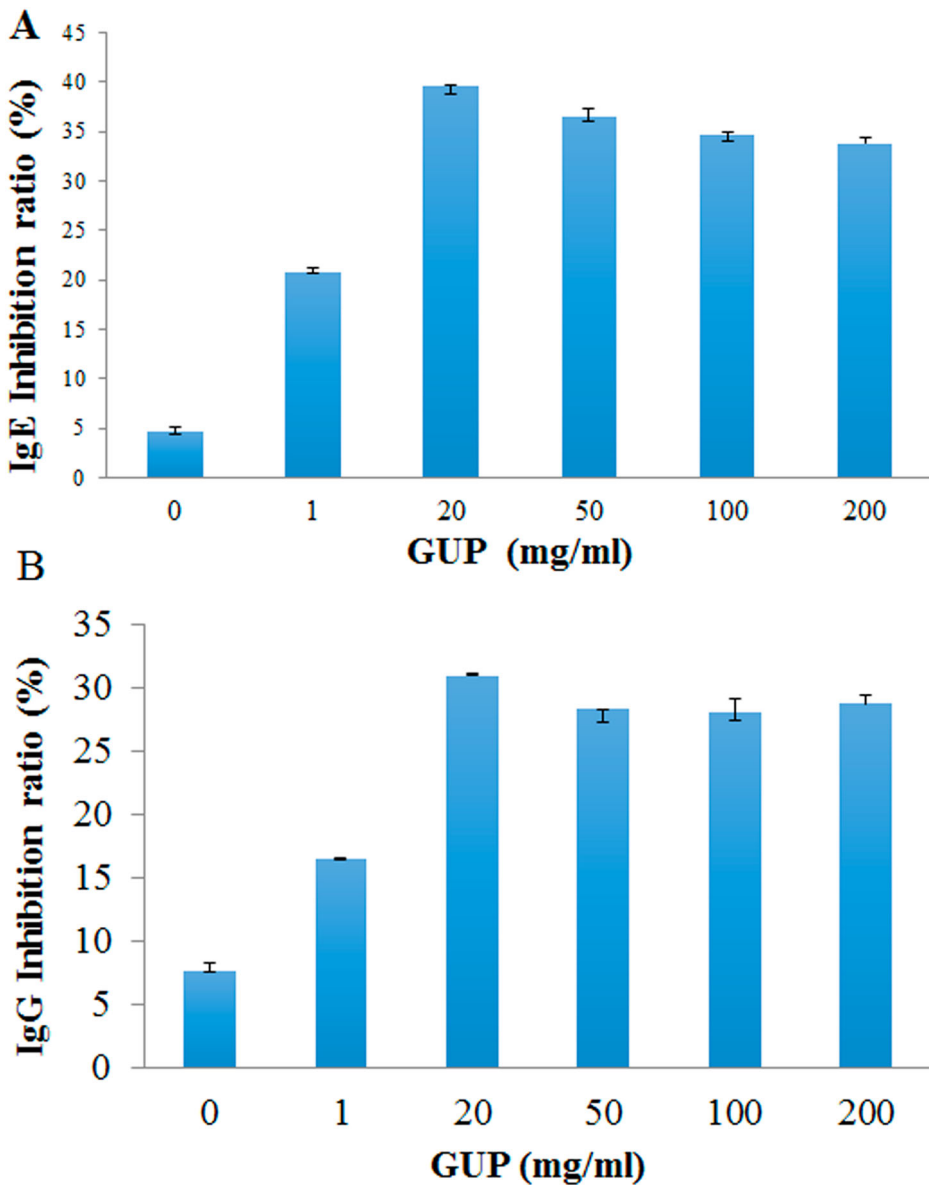


Figure 1. The inhibition ratio of glycinin-specific IgE (a) and glycinin-specific IgG (b) in serum of mice in each group.

concentration of 20 mg/ml, serum contents of IL-4 and IL-13 were the lowest at 89.57 ± 0.30 and 9.62 ± 0.16 pg/ml, respectively.

The proportion of CD4+CD25+ regulatory T cells in the mesenteric lymph nodes of mice

By flow cytometry, the proportion of CD4+CD25+FoxP3+T cells in the mesenteric lymph nodes of mice was higher in the mice pretreated with GUP than in blank control group

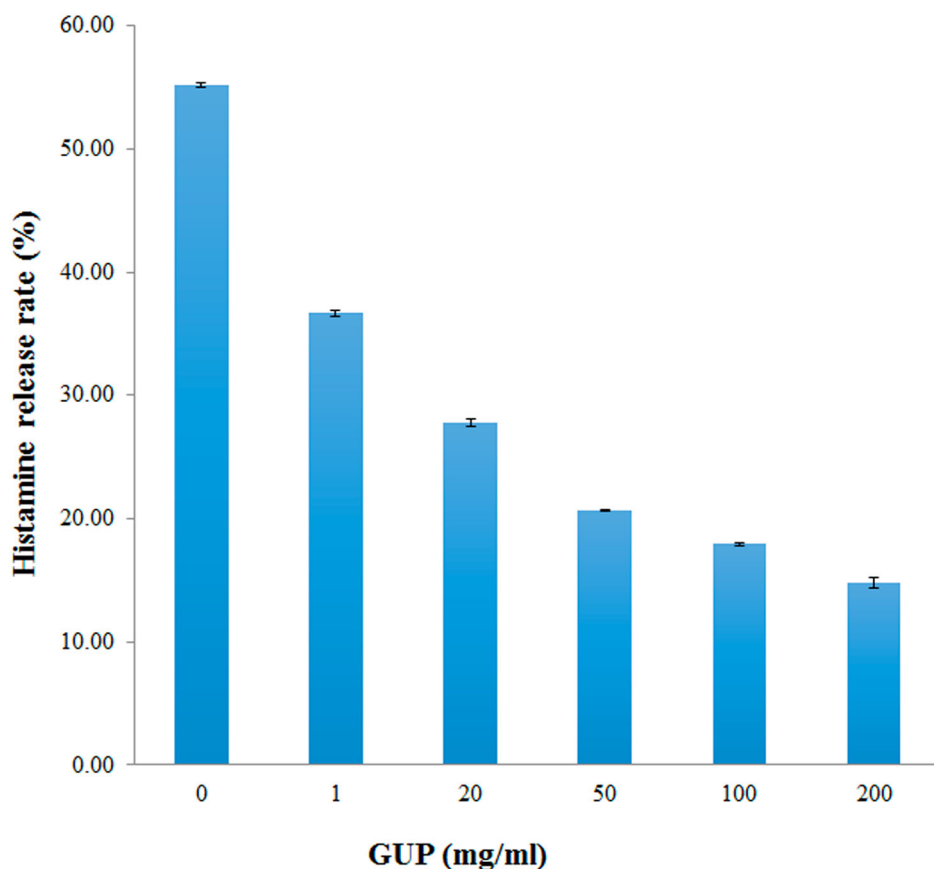


Figure 2. The release rate of histamine in mice treated with different concentrations of GUP.

(Figure 4). At the GUP concentration of 20 mg/ml, the percentage of CD4+CD25+FoxP3+T cells was the highest ($15.69 \pm 0.28\%$).

Discussion

In this study, a mouse model of oral administration of glycinin-induced allergic reactions was successfully established. The mice appeared to have different glycinin-specific allergy symptoms, including abnormalities of the hair, skin, respiratory tract, gastrointestinal tract, behavior, mental state, while the most serious reaction was death (Sicherer & Sampson, 1999). According to the different degrees of allergic symptoms, we determined the incidence of allergic reactions in mice. Interestingly, the incidence of various symptoms in mice did not decrease with increasing concentrations of GUP, which did not appear to be dose dependent. When the concentration of GUP was 20 mg/ml, the mortality of the mice was the lowest and the incidences of other allergic symptoms were relatively low. Interestingly, similar results were found in glycinin-specific IgE, glycinin-specific IgG inhibition ratio, IL-4 content, IL-13 content and percentage of CD4+CD25+FoxP3+T/CD4+T cells. The inhibition ratios of glycinin-specific IgE and glycinin-

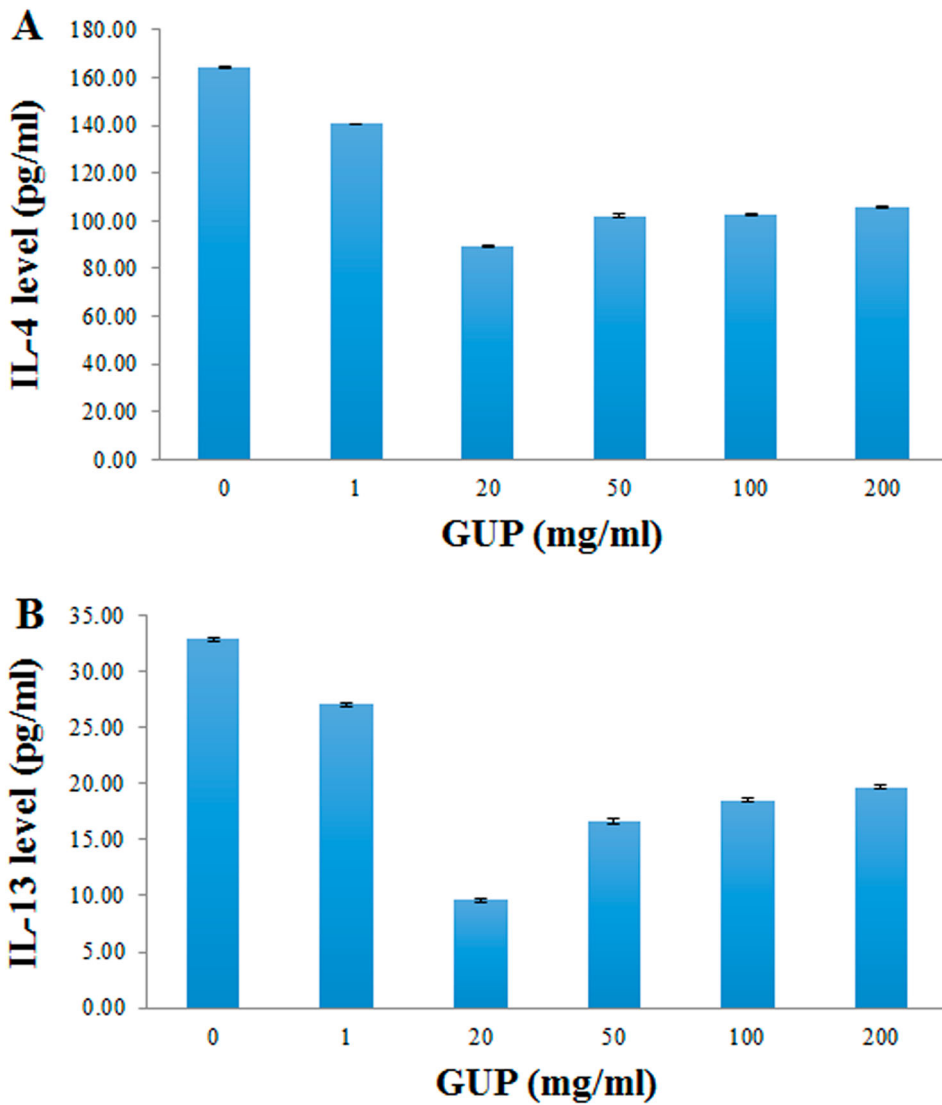


Figure 3. Serum content of IL-4 (a) and IL-13 (b) in mice treated with different concentration of GUP.

specific IgG were the highest when GUP was at a low concentration (20 mg/ml). In mice treated with low concentration of GUP (20 mg/ml), IL-4 and IL-13 levels in the blood were the lowest and the percentage of CD4+CD25+ regulatory T cells in the mesenteric lymph nodes of mice was the highest. However, there were no significant differences in these indexes among the lowest concentration (1 mg/ml), middle concentration (50 mg/ml), high concentration (100 mg/ml) and very high concentration (200 mg/ml) GUP groups. Nevertheless, the release rate of histamine was lower following an increase in the dose of GUP.

Our results showed that GUP is a kind of bidirectional immune modulator. Doses that are too low or too high do not give the best anti-allergic effects. By inhibiting glycinin antibody production and lowering histamine release levels, GUP could decrease IL-4 and IL-

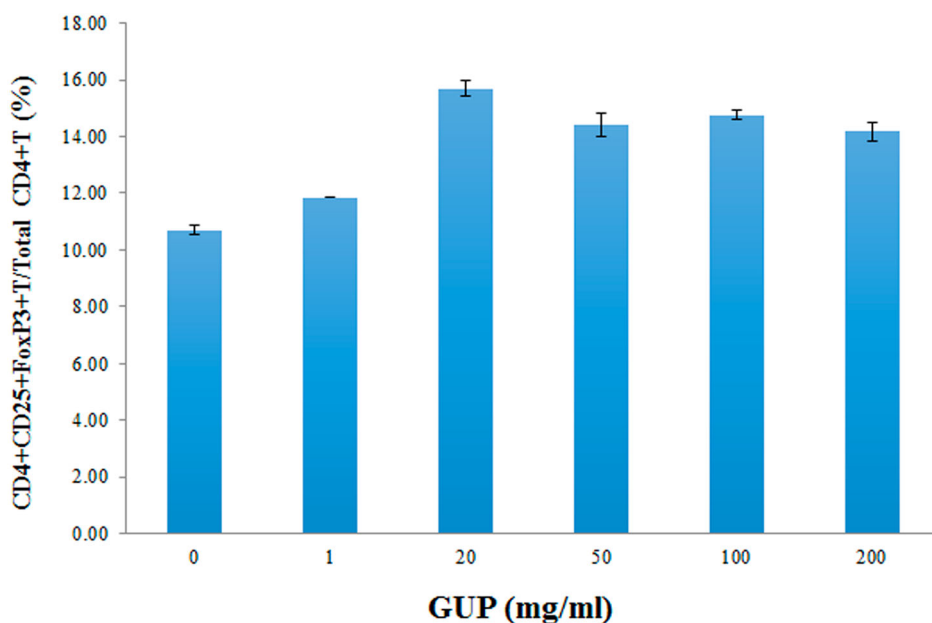


Figure 4. The percentage of CD4+CD25+FoxP3+T cells/total CD4+T cells in the mesenteric lymph nodes of mice treated with different concentrations of GUP.

IL-13 contents and elevate CD4+CD25+FoxP3+T/CD4+T cell ratio. IL-4 and IL-13 have been shown to play important role in the development and maintenance of allergic inflammation. Thus, our observation that GUP could decrease IL-4 and IL-13 contents indicated that GUP exerted anti-allergic effects.

Oral immunotherapy has been proposed as a novel treatment for food allergy (Khoriaty & Umetsu, 2013; Nelson, Lahr, Rule, Bock, & Leung, 1997; Sampson, 2013). In this study we investigated the effect of GUP on allergic symptoms caused by glycinin in mouse model. Our results showed that GUP could alleviate the symptoms and pathological indexes in glycinin-induced allergic mice. Therefore, GUP may be used via oral administration to inhibit glycinin-induced allergic response. Further studies are needed to validate the potential of GUP in allergy therapy.

Acknowledgements

We thank all staffs in the Institute of Animal Nutrition and Feed Science and Chinese Veterinary Medicine of Xinjiang Shihezi University for their assistances in the experiments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by Scientific Research Innovation Project of Graduate Education Innovation Fund from Xinjiang [grant number: XJGRI2014057].

Notes on contributors

Jie Chen (1986–), Doctor, Mainly engaged in animal nutrition and feed science research, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: zistef_001@sina.com.

Xinli Gu (1963–), Professor, Engaged in traditional Chinese veterinary medicine, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: xlg@shzu.edu.cn.

Jianguo Chen, Physician, Engaged in epidemiological studies, Urumqi County Health and Epidemic Prevention Station, Urumqi, Xinjiang, People's Republic of China. E-mail: 785193421@qq.com.

Yan Luo, Associate professor, Engaged in traditional Chinese veterinary medicine, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: 27752660@qq.com.

Mengyuan Wang, Master, Engaged in traditional Chinese veterinary medicine, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: 469592035@qq.com.

Hongyang Yang, Master, Engaged in traditional Chinese veterinary medicine, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: 1220523169@qq.com.

Xiao Guo, Master, Engaged in traditional Chinese veterinary medicine, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: 1498379318@qq.com.

Xiaoqing Zhu, Doctor, Engaged in veterinary and clinical sciences research, Department of Animal Science and Technology, Faculty of Animal Nutrition and Feed Science, Shihezi University, Shihezi, Xinjiang, People's Republic of China. E-mail: 86292432@qq.com.

References

- Cucu, T., Devreese, B., Kerkaert, B., Rogge, M., Vercruysse, L., & Meulenaer, B. D. (2012). ELISA-based detection of soybean proteins: A comparative study using antibodies against modified and native proteins. *Food Analytical Methods*, 5, 1121–1130.
- Cucu, T., Platteau, C., Taverniers, I., Devreese, B., Loose, M. D., & Meulenaer, B. D. (2013). Effect of partial hydrolysis on the hazelnut and soybean protein detectability by ELISA. *Food Control*, 30, 497–503.
- Cui, G. T., Zhang, W. X., Wang, Q. J., Zhang, A., Mu, H. B., Bai, H. J., & Duan, J. Y. (2014). Extraction optimization, characterization and immunity activity of polysaccharides from *Fructus Jujubae*. *Carbohydrate Polymers*, 111, 245–255.
- Khoriaty, E., & Umetsu, D. T. (2013). Oral immunotherapy for food allergy: Towards a new horizon. *Allergy, Asthma and Immunology Research*, 5, 3–15.
- L'Hocine, L., & Boye, J. (2007). Allergenicity of soybean: New developments in identification of allergenic proteins, cross-reactivities and hypoallergenization technologies. *Critical Reviews in Food Science and Nutrition*, 47, 127–143.

- Li, X. M., Schofield, B. H., Huang, C. K., Kleiner, G. I., & Sampson, H. A. (1999). A murine model of IgE-mediated cow's milk hypersensitivity. *Journal of Allergy and Clinical Immunology*, 103, 206–214.
- Li, X. M., Serebrisky, D., Lee, S. Y., Huang, C. K., Bardina, L., Schofield, B. H., ... Sampson, H. A. (2000). A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *Journal of Allergy and Clinical Immunology*, 106, 150–158.
- Liang, R. J. (2008). Optimization of extraction process of *Glycyrrhiza glabra* polysaccharides by response surface methodology. *Carbohydrate Polymers*, 74, 858–861.
- Ma, X., Sun, P., He, P. L., Han, P. F., Wang, J. J., Qiao, S. Y., & Li, D. F. (2010). Development of monoclonal antibodies and a competitive ELISA detection method for glycinin, an allergen in soybean. *Food Chemistry*, 121, 546–551.
- Medic, J., Atkinson, C., & Hurburgh, C. R. (2014). Current knowledge in soybean composition. *Journal of the American Oil Chemists' Society*, 91, 363–384.
- Nelson, H. S., Lahr, J., Rule, R., Bock, A., & Leung, D. (1997). Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *Journal of Allergy and Clinical Immunology*, 99, 744–751.
- Pesek, R. D., & Jones, S. M. (2014). Immunomodulatory effect of active treatment options in food allergy. *Current Treatment Options in Allergy*, 1, 133–144.
- Sampson, H. A. (2013). Peanut oral immunotherapy: Is it ready for clinical practice? *The Journal of Allergy and Clinical Immunology: In Practice*, 1, 15–21.
- Shunsuke, F., Indree, T., Bayart, M., Battseren, M., Takuhiro, U., Osamu, M., & Yukihiro, S. (2014). Screening of *Glycyrrhiza uralensis* Fisch. ex DC. Containing high concentrations of glycyrrhizin by eastern blotting and enzyme-linked immunosorbent assay using anti-glycyrrhizin monoclonal antibody for selective breeding of licorice. *Journal of Natural Medicines*, 68, 717–722.
- Sicherer, S. H., & Sampson, H. A. (1999). Food hypersensitivity and atopic dermatitis: Pathophysiology, epidemiology, diagnosis, and management. *Journal of Allergy and Clinical Immunology*, 104, S114–S122.
- Zhang, Z., Guo, Y. L., Xue, J., Jian, H. C., & Lei, F. F. (2015). Extraction optimization and biological properties of a polysaccharide isolated from *Gleostereum incarnatum*. *Carbohydrate Polymers*, 117, 185–191.