Research Article

Identification of the Allergenic Ingredients in Reduning Injection by Ultrafiltration and High-Performance Liquid Chromatography

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Reduning injection is a traditional Chinese medicine injection which has multiple functions such as clearing heat, dispelling wind, and detoxification. Although Reduning injection was widely utilized, reports of its allergenicity emerged one after another. However, there is little research on its allergenic substances. The aim of this study is to evaluate the sensitization of Reduning injection and explore the underlying cause of the anaphylactic reaction. The main ingredients in Reduning injection were analyzed before and after ultrafiltration. Ultrafiltrate Reduning injection, unfiltered Reduning injection, egg albumin, Tween-80, and nine effective components in Reduning injection were utilized to sensitize guinea pigs. The serum 5-hydroxytryptamine level was used to assess the sensitization effect of Reduning injection. We found a significant decrease in Tween-80 content comparing to other components in the injection after ultrafiltration. Unfiltered Reduning injection, Tween-80, chlorogenic acid, and cryptochlorogenin acid caused remarkable anaphylactoid reaction on guinea pigs while ultrafiltration Reduning resulted in a significantly lower degree of sensitization. Our results suggest that ultrafiltration could significantly reduce the sensitization of Reduning injection, which is likely due to the decrease of Tween-80. We also conjectured that the form of chlorogenic acid and cryptochlorogenin acid within the complex solution mixture may also affect the sensitizing effect.

1. Introduction

Reduning injection is a traditional Chinese medicine (TCM) injection refined from three Chinese herbal medicines, namely, honeysuckle, gardenia, and abrotani herba, and formulated for injection [1, 2]. This injection has multiple functions such as clearing heat, dispelling wind, and detoxification [3]. It is clinically used in the treatment of hyperpyrexia, slight aversion to cold, head and body pain, cough, yellow sputum, and other symptoms caused by respiratory tract infection (with external wind heat syndrome) [4, 5]. From the time Reduning injection was listed as a clinical treatment, it has been widely utilized with good clinical efficacy. However, there are also a few reports of severe anaphylaxis during the clinical application [6]. It is known that Reduning injection mainly contains Tween-80, chlorogenic acid, geniposide, caffeic acid, cryptochlorogenin acid, isochlorogenic acid (A, B, and C), neochlorogenic acid, and secoxyloganin [7–10]. Previous studies have shown that some components in TCM injections might be the cause of the allergic reaction and anaphylactoid reaction [11–13]. However, the specific compound responsible for the allergic reaction is still not clear.

Ultrafiltration is a separation technique in which a porous membrane is used as filtering medium and the molecules are separated according to their size [14]. After ultrafiltration, macromolecules such as pyrogen, microbes, proteins, pigments, resin, and tannin are excluded [15]. In this study, we used ultrafiltration to separate the compounds in Reduning injection to explore the cause of anaphylaxis.
Previous studies have identified some key cytokines as the mediator of antibody-mediated systemic anaphylaxis [16–18] and our studies had shown that 5-hydroxytryptamine (5-HT) can be detected rapidly by high-performance liquid chromatography (HPLC) and can be used as an index for anaphylaxis [19]. In our study, guinea pig plasma 5-HT level was measured to determine the sensitization of anaphylaxis [19]. In our study, guinea pig plasma 5-HT level was measured to determine the sensitization of anaphylaxis [19].

2. Materials and Methods

2.1. Animals. A total of 140 male guinea pigs were purchased from Nanjing Qinglongshan Animal Breeding Center. All animals were housed at 22 ± 5°C and 55% ± 5% relative humidity. All experiments were carried out according to the guidelines of the Animal Care Committee of Nanjing University of Chinese Medicine.

2.2. Reagents and Materials. Egg albumin and 5-HT were purchased from Sigma (St. Louis, USA). Chloral hydrate was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Heparin sodium injection was purchased from Jiangsu Wangbang Pharmaceutical Co., Ltd. (Jiangsu, China). Heptanesulfonate sodium was purchased from J&K Chemical Ltd. (Shanghai, China). Sodium dihydrogen phosphate was purchased from Shantou Xilong Chemical Industry Factory Co., Ltd. (Guangdong, China). Ethylene Diamine Tetraacetic Acid (EDTA) was purchased from Shanghai Fugi Industrial & Trading Co., Ltd. (Shanghai, China). Sodium chloride injection was purchased from Anhui Fenyguan Pharmaceutical Co., Ltd. (Anhui, China).

Reduning injection was purchased from Jiangsu Kangyuan Pharmaceutical Co., Ltd. (Jiangsu, China); the batch numbers are 120211, 120209, and 120703. Tween-80 was purchased from Sigma (St. Louis, USA), the batch number is 018K00941. Chlorogenic acid was purchased from China Institute of pharmaceutical and biological products (Beijing, China); the batch number is 0753-9909. Geniposide was purchased from National Institute for Food and Drug Control (Beijing, China); the batch number is 110885-200102. Neochlorogenic acid and cryptochlorogenic acid were purchased from Shanghai Yuanye Technology Co., Ltd. (Shanghai, China); the batch numbers are PA0819RA13 and ZF0226BA14. Isochlorogenic acid A, isochlorogenic acid B, isochlorogenic acid C, and secoxyloganin were prepared in saline, and their concentrations were 6.0 mg/mL, 3.7 mg/mL, 3.3 mg/mL, 7.0 mg/mL, 0.3 mg/mL, 0.3 mg/mL, 0.6 mg/mL, 0.5 mg/mL, and 1.0 mg/mL, respectively. The concentrations are equal to their respective concentrations in the Reduning injection.

After equilibration with two column volumes of Reduning injection, the ultrafiltration column with a molecular weight cut-off of 2,000 Da was used to filter the Reduning injection.

2.4. Detection of Major Components in Reduning Injection before and after Ultrafiltration

2.4.1. Simultaneous Detection of Chlorogenic Acid, Neochlorogenic Acid, Cryptochlorogenic Acid, Geniposide, Caffeic Acid, Isochlorogenic Acid A, Isochlorogenic Acid B, Isochlorogenic Acid C, and Secoxyloganin. Waters e2695 liquid chromatograph equipped with a Waters 2489 UV/visible light detector (Massachusetts, USA) was utilized for detection. The ODS-C18 (4.6 mm × 150 mm, 5 μm) column was used with a flow rate of 1.0 mL/min at a column temperature of 30°C. The mobile phases were methyl alcohol and 0.1% formic acid-water. The elution gradient program is shown in Table 1. The detection wavelength was 238 nm.

2.4.2. Detection of Tween-80. Waters e2695 liquid chromatograph equipped with an Alltech evaporative light scattering detection 2000 detector (Kentucky, USA) was used for detection. A TSK-GEL G2000 SWXL (7.8 mm × 300 mm, 5 μm) column was used for separation. The mobile phases were 5.0 mmol L−1 ammonium acetate: acetonitrile (90:10), pH = 4.0, and column temperature was 30°C. Flow rate was 0.6 mL·min−1 and the injection volume was 10 μL. As for the ELSD condition, the drift tube temperature was set at 110°C and nitrogen flow rate was set at 2.3 L·min−1.

2.5. Calculation of Componental Transmittance. The percentage of retention of each component following ultrafiltration was calculated according to

\[ R = \frac{C_f}{C_s} \times 100\% \]  

where \( R \) is the percentage of retention of the component, \( C_f \) is the concentration of components in filtrate (mg/mL), and \( C_s \) is the concentration of components in stock solution (mg/mL).

2.6. Animal Experiments. A total of 140 male guinea pigs with bodyweight of 300 ± 50 g were randomly divided into
Reduning the plasma was stored at −80°C.

Blood samples were centrifuged at 4,000 rpm for 5 min and the samples after the stimulation were collected at 30 min before (sample A) and after (sample B) the stimulation, and carotid artery and collected in a heparin anti-coagulation tube solutions, respectively. Blood (1 mL) was extracted from the jugular vein injection with 0.5 mL solution of each test used to anesthetize the guinea pigs. Animals were stimulated by jugular vein injection with a 0.5 mL solution of each test solutions, respectively. Blood (1 mL) was extracted from the carotid artery and collected in a heparin anticoagulation tube before (sample A) and after (sample B) the stimulation, and the samples after the stimulation were collected at 30 min. Blood samples were centrifuged at 4,000 rpm for 5 min and the plasma was stored at −80°C.

2.7. Detection of 5-HT. Plasma sample (100 μL) was thawed in the dark and mixed by vortex with an equal amount of methanol. The mixture was centrifuged at 3,000 rpm for 10 min. After freezing for 30 min, the solution was centrifuged at 1,000 rpm for 10 min and 20 μL supernatant was used for detection.

Waters 510 liquid chromatograph was used with a Waters 2465 electrochemical detector (Massachusetts, USA) and EC 2000 chromatographic work station (Dalian, China). Hedera ODS-2 chromatographic column (150 mm × 4.6 mm, 5 μm, Jiangsu Hanbang Science & Technology Co., Ltd.) was utilized at a column temperature of 25°C. Mobile phase was 25 mmol/L sodium dihydrogen phosphate (containing 0.5 mmol/L EDTA and 3 mmol/L sodium heptanesulfonate, pH 4.6) mixed with acetonitrile at a volume ratio of 85:15. The flow velocity was 0.8 mL/min. ISAAC (in situ silver/silver chloride) was used as the reference electrode. The detection potential was 0.6 V and the injection volume was 20 μL. The amount variation rate of 5-HT was calculated according to the following equation:

\[
\text{Sample (\%)} = \left(\frac{\text{value B} - \text{value A}}{\text{value A}}\right) \times 100\%, \quad (2)
\]

where value B is the amount of 5-HT in sample B and value A is the amount of 5-HT in sample A.

2.8. Statistical Analysis. Data were reported as mean ± SD for each group. All statistical analyses were performed using PRISM version 5.0 (GraphPad). Differences with P value of less than 0.5 were considered statistically significant.

3. Results

3.1. Calibration Curves of the Investigated Compounds. The mixture of standard solutions, Tween-80, and 5-HT reference solution were diluted to different concentrations. We assigned the compound concentration as the horizontal axis (X) and the peak area as the vertical axis (Y) and performed a linear regression to obtain the regression equations of each component. The results are shown in Table 2. The results showed that for all the standard solutions, there is a good linear relationship between the compound concentration and the area under the peak within a wide range.

The method precision was evaluated by intraday and interday variability. The intraday variability was performed by injection of the same sample six times in the same day. The interday variability was evaluated on two successive days using the same sample. The RSD values are summarized in Table 3. From the results, RSDs for intraday and interday precisions did not exceed 3%. To confirm the repeatability of the method, six independently prepared solutions from the same sample were analyzed. The stabilities of the sample solutions were analyzed at 0, 2, 4, 8, 12, and 24 h at room temperature. It was found that the sample solutions were stable within 24 h. Precision, repeatability, and stability of nine compounds (n = 6), the RSD values, are summarized in Table 3.

| Serial number | Component name   | Regression equation | Correlation coefficient (r) | Linear range (mg/mL) |
|---------------|------------------|---------------------|-----------------------------|----------------------|
| 1             | Neochlorogenic acid | \( Y = 13.232X - 6.236 \) | 0.9989                      | 0.05–5.05            |
| 2             | Chlorogenic acid  | \( Y = 10.758X + 20.156 \) | 0.9998                      | 0.04–10.00           |
| 3             | Cryptochlorogenic acid | \( Y = 10.002X + 5.3598 \) | 0.9991                      | 0.05–6.12            |
| 4             | Caffeic acid     | \( Y = 20.339X + 10.856 \) | 0.9995                      | 0.01–4.22            |
| 5             | Geniposide       | \( Y = 5.673X + 14.982 \) | 0.9993                      | 0.05–10.06           |
| 6             | Secoxyloganin    | \( Y = 12.912X - 5.32 \)  | 0.9992                      | 0.04–5.22            |
| 7             | Isochlorogenic acid A | \( Y = 14.008X + 20.703 \) | 0.9987                      | 0.01–3.00            |
| 8             | Isochlorogenic acid B | \( Y = 16.612X + 16.356 \) | 0.9995                      | 0.01–3.10            |
| 9             | Isochlorogenic acid C | \( Y = 16.103X + 35.311 \) | 0.9993                      | 0.01–4.00            |
| 10            | Tween-80         | \( Y = 1.5269X + 7.5201 \) | 0.9988                      | 0.50–4.23            |
| 11            | 5-HT             | \( Y = 0.5468X - 3.5868 \)  | 0.9989                      | 0.03 × 10⁻³–3.00 × 10⁻³ |

| Sample | Linear range (mg/mL) | Correlation coefficient (r) | Regression equation | Component name   |
|--------|-----------------------|-----------------------------|---------------------|------------------|
| Sample A | 0.05–5.05 | 0.9989 | \( Y = 13.232X - 6.236 \) | Neochlorogenic acid |
| Sample B | 0.04–10.00 | 0.9998 | \( Y = 10.758X + 20.156 \) | Chlorogenic acid  |
| Sample A | 0.05–6.12 | 0.9991 | \( Y = 10.002X + 5.3598 \) | Cryptochlorogenic acid |
| Sample B | 0.01–4.22 | 0.9995 | \( Y = 20.339X + 10.856 \) | Caffeic acid     |
| Sample A | 0.05–10.06 | 0.9993 | \( Y = 5.673X + 14.982 \) | Geniposide       |
| Sample B | 0.04–5.22 | 0.9992 | \( Y = 12.912X - 5.32 \)  | Secoxyloganin    |
| Sample A | 0.01–3.00 | 0.9987 | \( Y = 14.008X + 20.703 \) | Isochlorogenic acid A |
| Sample B | 0.01–3.10 | 0.9995 | \( Y = 16.612X + 16.356 \) | Isochlorogenic acid B |
| Sample A | 0.01–4.00 | 0.9993 | \( Y = 16.103X + 35.311 \) | Isochlorogenic acid C |
| Sample B | 0.50–4.23 | 0.9988 | \( Y = 1.5269X + 7.5201 \) | Tween-80         |
| Sample A | 0.03 × 10⁻³–3.00 × 10⁻³ | 0.9989 | \( Y = 0.5468X - 3.5868 \) | 5-HT |

3.1. Calibration Curves of the Investigated Compounds. The mixture of standard solutions, Tween-80, and 5-HT reference solution were diluted to different concentrations. We assigned the compound concentration as the horizontal axis (X) and the peak area as the vertical axis (Y) and performed a linear regression to obtain the regression equations of each component. The results are shown in Table 2. The results showed that for all the standard solutions, there is a good linear relationship between the compound concentration and the area under the peak within a wide range.
Table 3: Precision, repeatability, and stability of nine compounds (n = 6).

| Serial number | Component name               | Precision (n = 6) | Repeatability (n = 6) | Stability (n = 6) |
|---------------|------------------------------|-------------------|-----------------------|-------------------|
|               |                              | Intraday RSD (%)  | Interday RSD (%)      | RSD (%) Intraday  |
| 1             | Neochlorogenic acid          | 1.32              | 1.46                  | 2.01              |
| 2             | Chlorogenic acid             | 1.78              | 2.74                  | 2.39              |
| 3             | Cryptochlorogenic acid       | 1.42              | 2.03                  | 2.72              |
| 4             | Caffeic acid                 | 1.09              | 1.62                  | 2.47              |
| 5             | Geniposide                   | 2.07              | 1.98                  | 1.59              |
| 6             | Secoxyloganin                | 2.01              | 2.41                  | 2.84              |
| 7             | Isochlorogenic acid B        | 1.37              | 1.93                  | 1.23              |
| 8             | Isochlorogenic acid A        | 1.96              | 2.34                  | 2.68              |
| 9             | Isochlorogenic acid C        | 1.79              | 2.63                  | 1.86              |
| 10            | Tween-80                     | 2.67              | 2.83                  | 2.40              |
| 11            | 5-HT                         | 1.39              | 1.57                  | 1.82              |

Table 4: Recovery of the compounds in Reduning injection.

| Serial number | Compounds       | Contained (mg/mL) | Added (mg/mL) | Found mean (mg/mL) | Recovery mean (%) | RSD (%) |
|---------------|-----------------|-------------------|---------------|--------------------|-------------------|---------|
| 1             | Neochlorogenic acid | 3.68              | 3.56          | 7.09               | 104.39            | 2.36    |
| 2             | Chlorogenic acid  | 5.83              | 5.93          | 11.98              | 96.42             | 1.87    |
| 3             | Cryptochlorogenic acid | 3.30              | 3.24          | 6.49               | 101.56            | 1.94    |
| 4             | Caffeic acid     | 0.34              | 0.34          | 0.67               | 103.03            | 2.50    |
| 5             | Geniposide       | 8.95              | 8.79          | 17.93              | 97.88             | 1.69    |
| 6             | Secoxyloganin    | 1.01              | 0.98          | 2.04               | 95.14             | 2.15    |
| 7             | Isochlorogenic acid B | 0.62              | 0.59          | 1.19               | 103.50            | 2.36    |
| 8             | Isochlorogenic acid A | 0.32              | 0.32          | 0.65               | 96.96             | 2.13    |
| 9             | Isochlorogenic acid C | 0.52              | 0.50          | 1.03               | 98.03             | 1.98    |
| 10            | Tween-80         | 2.20              | 2.00          | 4.25               | 97.56             | 2.69    |
| 11            | 5-HT             | 0.091 × 10⁻³      | 0.092 × 10⁻³  | 0.186 × 10⁻³       | 96.84             | 2.87    |

The recovery was evaluated by adding standards into the sample. The mixture was extracted and analyzed by using the above method. Six replicates were performed for the determination. The RSD values are summarized in Table 4.

HPLC chromatogram of the standard solution mixture was shown in Figure 1, the chromatogram of Tween-80 reference solution was shown in Figure 2. The chromatogram of Reduning injection was shown in Figure 3.

3.2. Percentage of Retention of Major Components after Ultrafiltration. An ultrafiltration membrane was used to filter the Reduning injection. The permeation of molecular through the membranes was not entirely related to the monomolecular weight of composition, but to the molecular's existence formed (monomolecular, low polymolecular, and high polymolecular) in the solution. The concentrations and percentage of retention of the major components contained in the injection both before and after ultrafiltration are shown in Table 5. The percentage of retention of chlorogenic acid, geniposide, caffeic acid, neochlorogenic acid, and cryptochlorogenin acid is all greater than 90%, while the percentage of retention of secoxyloganin, isochlorogenic acid A, isochlorogenic acid B, and isochlorogenic acid C is all greater than 70%. The percentage of retention of Tween-80 is 20%.

3.3. Plasma 5-HT in Guinea Pigs. The chromatogram of 5-HT was shown in Figure 4. After the guinea pigs were sensitized to saline for 30 min by injection, the average increasing rate of plasma 5-HT was 4.46%. The average increasing
| Serial number | Name                     | Before ultrafiltration (mg/mL) | After ultrafiltration (mg/mL) | Percentage of retention (%) | RSD (%) |
|---------------|--------------------------|--------------------------------|-----------------------------|-----------------------------|---------|
| 1             | Neochlorogenic acid      | 3.71                           | 3.42                        | 92.18**                     | 1.79    |
| 2             | Chlorogenic acid         | 6.12                           | 5.46                        | 89.21**                     | 2.25    |
| 3             | Cryptochlorogenic acid   | 3.32                           | 3.00                        | 90.36**                     | 2.12    |
| 4             | Caffeic acid             | 0.31                           | 0.29                        | 93.54**                     | 2.10    |
| 5             | Geniposide               | 9.10                           | 8.90                        | 97.80**                     | 1.63    |
| 6             | Secoxyloganin            | 1.02                           | 0.68                        | 73.67**                     | 2.13    |
| 7             | Isochlorogenic acid B    | 0.60                           | 0.44                        | 73.33**                     | 1.98    |
| 8             | Isochlorogenic acid A    | 0.33                           | 0.25                        | 75.76**                     | 2.43    |
| 9             | Isochlorogenic acid C    | 0.51                           | 0.36                        | 70.58**                     | 1.59    |
| 10            | Tween-80                 | 2.00                           | 0.40                        | 20.00                       | 2.58    |

Values are expressed as mean ± SD; n = 3 in each group. **P < 0.01 versus Tween-80 group.

4. Discussion

Allergic reaction is the most common adverse reaction of herbal injection which could lead to life-threatening health problems. Commonly used methods such as murine passive
cutaneous anaphylaxis and measurement of the antibodies were expensive and time-consuming work. Previous studies have identified two types of antibody-mediated systemic anaphylaxis; one is mediated by IgE and the other mediated by IgG [20–23]. Mast cells are the major effector cells of IgE-mediated in allergic reaction and play a key role in allergic reaction diseases [24]; when anaphylactic reaction occurs, mast cells and basophils would degranulate and release β-hexosaminidase, histamine, serotonin, or other cytokines [25–28]. In this study, a simple and rapid method was discussed based on the measurement of serotonin with HPLC technique for predicting allergic reaction of all the analytes.

Previous studies have reported that the chlorogenic acid in honeysuckle may cause anaphylaxis [29, 30]. In present study, we investigated sensitization to the major phenolic acid components in Reduning injection in guinea pigs. Our results indicate that chlorogenic acid causes sensitization, which is consistent with previous reports. At the same time, we found that cryptochlorogenin acid also could cause anaphylactoid reaction even stronger than chlorogenic acid. Other components, such as caffeic acid, neochlorogenic acid, isochlorogenic acid A, isochlorogenic acid B, isochlorogenic acid C, and secoxylloganin do not induce obvious sensitization. However, concentration of chlorogenic acid and cryptochlorogenin acid within the solution. The difference can likely be attributed to the form as an isolated compound and as a component in a mixed solution. The difference can likely be attributed to the form of chlorogenic acid and cryptochlorogenin acid within the complex solution mixture. In herbal medicine solution, the compounds exist in many forms such as ionic, molecular, and associated.

Tween-80 is a nonionic detergent and is mostly used in Chinese medicine injections to assist in solubilization [31, 32]. However, adverse reactions of Tween-80 have been observed with time. There are several Chinese medicine injections in clinical use that contain Tween-80, such as Yuxingcao injection and Xiangdan injection, both of which have been reported to have serious adverse reactions [13]. The percentage of retention of Tween-80 in ultrafiltered Reduning injection was low, resulting in decreased Tween-80 and decreased sensitization, which suggests that any observed sensitization to Reduning injection was likely due to the presence of Tween-80.

5. Conclusions

In this study, a simple HPLC technique was successfully used for predicting allergic reaction of all the analytes in Reduning injection before and after ultrafiltration and we suggested that Tween-80 might be the cause of anaphylaxis. Additionally, the form of chlorogenic acid and cryptochlorogenin acid within the complex solution mixture may also affect the sensitizing effect of them. Our findings might contribute to the safety evaluation of Reduning injection in clinical medication.

Disclosure

Cun-yu Li is the co-first author of this paper.

Conflict of Interests

The authors declare that there is no conflict of interests.

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References

[1] W. Xiao, Y. Ling, Y.-A. Bi, Z.-Z. Wang, and C.-F. Zhang, “GC/MS fingerprint of Reduning injection,” Chinese Journal of Natural Medicines, vol. 5, no. 2, pp. 127–129, 2007.
[2] Y. X. Wang, T. Liu, Z. Z. Wang, J. Zhang, and W. Xiao, “Compatible stability of reduning injection with solvents,” Zongguo Zhong Yao Za Zhi, vol. 35, no. 22, pp. 2990–2993, 2010.
[3] L. M. Sun, X. C. Chang, R. B. Zhao, Y. L. Cui, and L. J. Wang, “Reduning treatment of children with herpes angina efficacy,” China Medical Herald, vol. 8, article 149, 2011.
[4] G. Z. Feng, F. Zhou, and M. Huang, “Anti-respiratory syncytial virus (RSV, Long strain) effects of Reduning injection in vitro,” Acta Universitatis Medicinalis Nanjing, vol. 27, pp. 1009–1012, 2007.
[5] Y.-J. Li, Z.-Z. Wang, Y.-A. Bi et al., “The evaluation and implementation of direct analysis in real time quadrupole time-of-flight tandem mass spectrometry for characterization and quantification of geniposide in Re du Ning Injections,” Rapid Communications in Mass Spectrometry, vol. 26, no. 11, pp. 1577–1584, 2012.
[6] H. M. Xu, Y. Wang, and N. F. Liu, “Safety of an injection with a mixture of extracts from Herba Artemisiae annuae, Fructus Gardeniae and Flos Lonicerae,” Pharmacy World and Science, vol. 31, no. 4, pp. 458–463, 2009.
[7] J. C. Liang, Y. K. Huang, Z. Cai, and L. Jiang, “Determination of chlorogenic acid and geniposide in reduning injection by HPLC,” Chinese Journal of New Drugs, vol. 17, article 18, 2008.
[8] Y. Li, P. Wang, W. Xiao, L. Zhao, Z. Wang, and L. Yu, “Screening and analyzing the potential bioactive components from reduning injection, using macrophage cell extraction and ultra-high performance liquid chromatography coupled with mass spectrometry,” The American Journal of Chinese Medicine, vol. 41, no. 1, pp. 221–229, 2013.
[9] Y. A. Bi, Z. Z. Wang, A. H. Song, T. Liu, X. H. Fu, and W. Xiao, “The fingerprint research and multi-component qualitative analysis of reduning injection via HPLC,” World Science and Technology, vol. 2, pp. 11–13, 2010.
[10] W. L. Li, Z. W. Cheng, Y. F. Wang, and H. B. Qu, “Quality control of Lonicerae Japonicae Flos using near infrared spectroscopy and chemometrics,” Journal of Pharmaceutical and Biomedical Analysis, vol. 72, pp. 33–39, 2013.

[11] L. Zeng, M. Wang, Y. Yuan et al., “Simultaneous multi-component quantitation of Chinese herbal injection Yin-zhi-huang in rat plasma by using a single-tube extraction procedure for mass spectrometry-based pharmacokinetic measurement,” Journal of Chromatography B, vol. 967, pp. 245–254, 2014.

[12] J.-X. Ye, W. Wei, L.-H. Quan, C.-Y. Liu, Q. Chang, and Y.-H. Liao, “An LC–MS/MS method for the simultaneous determination of chlorogenic acid, forsythiaside A and baicalin in rat plasma and its application to pharmacokinetic study of Shuang-huang-lian in rats,” Journal of Pharmaceutical and Biomedical Analysis, vol. 52, no. 4, pp. 625–630, 2010.

[13] K. Ji, J. Chen, M. Li et al., “Comments on serious anaphylaxis caused by nine Chinese herbal injections used to treat common colds and upper respiratory tract infections,” Regulatory Toxicology and Pharmacology, vol. 55, no. 2, pp. 134–138, 2009.

[14] X. L. Zhi, C. Y. Li, H. Y. Li, and G. P. Peng, “Depyrogenation mechanism of baicalin solution with activated carbon,” Asian Journal of Chemistry, vol. 25, no. 15, pp. 8821–8824, 2013.

[15] H. M. Li, C. Y. Li, Y. F. Zheng et al., “Effect of solution factors on endotoxin coagulation state,” Asian Journal of Chemistry, vol. 25, no. 12, pp. 6915–6919, 2013.

[16] E. D. Quakkelaar, M. F. Fransen, W. W. C. Van Maren et al., “IgG-mediated anaphylaxis to a Synthetic long peptide vaccine containing a B cell epitope can be avoided by slow-release formulation,” The Journal of Immunology, vol. 192, no. 12, pp. 5813–5820, 2014.

[17] V. Chirico, A. Lacquaniti, S. Vinci et al., “High-mobility group box 1 in allergic and non-allergic upper airway inflammation,” Journal of Biological Regulators & Homeostatic Agents, vol. 29, no. 2, supplement 1, pp. 55–57, 2015.

[18] S. H. Park, H.-J. Choi, S. Y. Lee, and J.-S. Han, “TLR4-mediated IRAK1 activation induces TNF-α expression via JNK-dependent NF-κB activation in human bronchial epithelial cells,” European Journal of Inflammation, vol. 13, no. 3, pp. 183–195, 2015.

[19] L. Yu, G. Peng, C. Li et al., “A rapid and low-cost approach to evaluate the allergenicity of herbal injection using HPLC analysis,” Fitoterapia, vol. 88, pp. 12–18, 2013.

[20] J. Y. Oh, W.-S. Choi, C. H. Lee, and H.-J. Park, “The ethyl acetate extract of Cordyceps militaris inhibits IgE-mediated allergic responses in mast cells and passive cutaneous anaphylaxis reaction in mice,” Journal of Ethnopharmacology, vol. 135, no. 2, pp. 422–429, 2011.

[21] I. Miyajima, D. Dombrowicz, T. R. Martin, J. V. Ravetch, J. P. Kinet, and S. J. Galli, “Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc γRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis,” Journal of Clinical Investigation, vol. 99, no. 5, pp. 901–914, 1997.

[22] R. T. Strait, S. C. Morris, M. Yang, X.-W. Qu, and F. D. Finkelman, “Pathways of anaphylaxis in the mouse,” Journal of Allergy and Clinical Immunology, vol. 109, no. 4, pp. 658–668, 2002.

[23] W. Jacoby, P. V. Cammarata, S. Findlay, and S. H. Pincus, “Anaphylaxis in mast cell-deficient mice,” Journal of Investigative Dermatology, vol. 83, no. 4, pp. 302–304, 1984.