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Original Article

Anaphylaxis effect and substance basis of honeysuckle extract

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ABSTRACT

Objective: To explore the anaphylaxis effect and anaphylaxis substances of honeysuckle.
Methods: Rat peritoneal mast cells (PMC) were separated and purified, the cells were incubated with compound 48/80 (0.02 g/L), physiological saline and honeysuckle extract (120 g/L) at 37 °C for 0, 15, 30, 45 and 60 min. Degranulation were observed by optical microscope and transmission electron microscope. Annexin V positive cell rate was detected by flow cytometry to reflect the degranulation rate of PMC. SD rats were supplied with honeysuckle extract by intravenous injection at a dose of 2.25 g/L. After administration, different parameters were analyzed, including the symptoms, histamine (HIS) and tryptase (MCT) levels, which were determined to explore the effect of anaphylaxis. Regression analysis was used to calculate the relationships between the peaks and the pharmacological effects to explore potentially anaphylactoid components.
Results: The percentage of Annxin V positive cells and the degranulation ratio were markedly elevated in PMC treated with honeysuckle extract for more than 15 min ($P < 0.05$). HIS and MCT level were significantly elevated after injection of honeysuckle extract for more than 15 min. Morphology of PMC and systemic symptoms were also changed compared with the controlled group ($P < 0.05$). Regression analysis was used to calculate the relationship between peaks and pharmacological effects, and to determine peaks 7, 10 and 13 as possible anaphylactoid ingredients.
Conclusion: This study established a prospective method to clarify the anaphylactoid components of honeysuckle extract, which would provide guidance for screening anaphylactoid components in traditional Chinese medicine injections containing honeysuckle in the prescription.

1. Introduction

Honeysuckle is the dry bud or the first blooming flower of *Lonicera japonica* Thunb. It is one of the most famous traditional Chinese medicinal herbs, which have been widely utilized in China for thousands of years. It has the function of relieving fever and usually appears in a number of antipyretic preparations of traditional Chinese medicine (TCM) (Guan et al., 2020). According to the analysis of TCM injections for the respiratory system that have been on the market, 20 compound preparations have been counted, honeysuckle has the highest frequency of medicinal flavors, accounting for 60%. chlorogenic acid compounds are the main active ingredients of honeysuckle, including chlorogenic acid and isochlorogenic acid (Huang, 2008), In the 1960 s, the Freedman group in Canada and the Layton group in the United States conducted research on the sensitization of chlorogenic acid, and reached diametrically opposite conclusions about whether it is an allergen (Freedman et al., 1961, 1962, 1964a, 1964b; Layton et al., 1963a, 1965b, 1966c, 1968d, 1966e; Lowell, 1966; Layton, 1966). Through the comprehensive analysis of the above two groups' literatures, there is no definite conclusion as to whether chlorogenic acid is an allergen. However, in the past decade, some other studies had found that it has anaphylaxis effect refers to non allergic sensitization and adverse effects occur at the first exposure to the drug (Xiao et al., 2013; Qin et al., 2010). They believed that chlorogenic acid might be the cause of the anaphylactoid reaction of some TCM injections which contained honeysuckle such as Shuanghuanglian Iinjection (SHLI) and Yinzhihuang Injection (Ye et al., 2010; Chen et al., 2014; Zeng et al., 2014; Ji et al., 2009; Huang et al., 2010). SHLI is a typical representative of preparations containing honeysuckle, Unfortunately, some serious anaphylaxis have occurred with the treatment of SHLI in recent years, numerous cases of anaphylaxis occurred in viral and bacterial infections treated with SHLI (Cui et al., 2018; Yang, 2016). However, whether chlorogenic acid is the only anaphylactoid substance in honeysuckle is still uncertain. Therefore, it is necessary to study the ana-

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phylactoid effect and anaphylactoid components of honeysuckle with chlorogenic acid as the main component.

At present, research on the causes of anaphylaxis caused by TCM injection is in an exploratory stage. Some scholars believe that it is necessary to conduct tests on the complete TCM injection formulation (Liu, 2010; Tian, 2007; Xu, et al., 2008; Yi, He & Wang, 2005; Zhang, Tian & Qiao, 2009), whereas others believe that screening time can be shortened and the screening process can be simplified by studying the anaphylactoid effect of extracted fractions. In the latter case, individual components of the TCM injection are isolated and structurally identified, and then can be tested in a model of anaphylaxis. This method has played a positive role in elucidating the anaphylactoid components of TCM injection in some cases (Hu, Xu & Ma, 2008; Yu, Hu, & Cui, 2008). Some researchers believe that the anaphylactoid effect of excipients in TCM injection should not be ignored since TCM injection containing Tween-80 has been shown to cause concentration-dependent anaphylactoid reactions in experimental animals (Liu, Pu, & Lei, 2008; Yan, 2009). Existing methods are not suitable for analyzing the multicomposition and multitarget character of the anaphylactoid reaction caused by TCM injection. Therefore, a new, combinative and powerful method is required for extensive characterization of this adverse reaction. In terms of biological activity, the pharmacology or pharmacological activity describes the beneficial or adverse impacts of TCM on living matter. Some have many chemical constituents, which can exert multichannel, multilevel and multitarget characteristics. In 2002, Li et al. proposed the concept of the fingerprint spectrum-effect relationship (Li, Yan, & Li, 2002). This is a scientific method that studies the correlations between fingerprint and pharmacological effect, which can be used to clarify the pharmacodynamic basis for the effect.

In order to explore the anaphylactoid components of honeysuckle, honeysuckle was first prepared into extracts according to the method of the 2020 version of "Chinese Pharmacopoeia". Rat PMC degranulation test is a detection method for anaphylactoid components in preliminary research. Results indicated that honeysuckle extract could cause degranulation of PMC and increase levels of HIS and MCT in the body to trigger anaphylactoid reactions. Recent years, studies have reported that honeysuckle extract could cause behavioral anaphylaxis reactions in laboratory animals (Feng, et al., 2008; Li, Zhou, & Li, 2016) and systemic anaphylaxis in human (Spiegel, 2004). Therefore, the research results are consistent with the literatures.

In this study, anaphylaxis effect of honeysuckle extract was studied systematically *in vivo* and *in vitro,* and spectrum-effect relationships were used to explore potentially anaphylactoid components. Peak assignment of the anaphylaxis component was investigated in the fingerprint of SHLI to prove whether the honeysuckle extract anaphylaxis components can be reduced or eliminated during the preparation process.

2. Materials and methods

2.1. Animals and materials

SD rats [half male and half female, (180 ± 20) g, certificate number: SCXK (Hei) 2008004] were provided by the Safety Evaluation Center of Heilongjiang University of Chinese Medicine and maintained under SPF conditions for at least 5 d before the experiments, Human care was given according to the 3R principle used in laboratory animals. The institute's open grain-based diet and fluoride - free tap water was fed, animal experiments were performed in accordance with the Experimental Animal Administrative Committee of Heilongjiang University of Chinese Medicine.

Honeysuckle was prepared in Pingyi County, Shandong Province, China and provided by the Tongrentang Medicine Co., (Harbin, China). Honeysuckle was extracted according to the Chinese Pharmacopoeia (2020 Edition). The herb was decocted twice for 1 h each time. The combined filtrates were concentrated to a relative density of 1.20–1.25 (70–80 °C) and cooled to 40 °C. Alcohol was added with stirring to bring the amount to 75%. After standing for 12 h, the mixture was filtered and the filtrate was collected. Ethanol was recovered completely. After three volumes of water were added, the mixture was allowed to stand for 12 h and then filtered. The filtrate was concentrated to a relative density of 1.10–1.15 (70–80 °C) and then cooled to 40 °C. Alcohol was added to bring the amount to 85%. The mixture was allowed to stand for 12 h and then filtered. The filtrate rotated and evaporated until there was no ethanol, and then collected. Before use, the content of chlorogenic acid was 12.959 mg/ml, as determined by HPLC.

Rat PMC were isolated by density gradient centrifugation, cells were collected by lavage with Tryrode's buffer. The pooled cells were washed with Tryrode's buffer and resuspended. Percoll isotonic fluid (4 mL) were mixed with 1 mL cell suspension and covered by 1 mL Tyrode's buffer slowly. After centrifugation with 3000 r/min at 4 ℃, the supernatant was discarded. The precipitation was washed twice and resuspended in Tyrode's buffer. Then, cells were counted under the microscope and the cell concentration was adjusted to 10^5 cells/mL. Trypan blue dyeing, vitality is greater than 95%, and toluidine blue staining, purity greater than 95% is considered to meet the requirements. The viability and purity of the mast cell suspension were 95.8% and 95.2%.

$$\text{Living cell rate}\ (\%) = \text{Total number of living cells}/(\text{Total number of living cells} + \text{Total number of dead cells}) \times 100\%$$

Percoll cell separating liquid, heparin sodium and compound 48/80 were supplied by Sigma Co., Ltd. (USA). Rat HIS ELISA kit and MCT ELISA kits were purchased from R&D Co., Ltd. (USA). Napco 5410 Carbon Dioxide Incubator (NAPCO Co., Ltd., USA), CX41-32RFL Inverted Biological Microscope (Olympus company Co., Ltd.), FACS Calibur Flow Cytometry (BD Co., Ltd., USA), ACQUITY UPLC (Waters Co., Ltd., USA), multifunctional microplate (Molecular Devices Co., USA), EBH C_{18} columns (1.7 μm 4.6 × 250 mm, Waters Co., Ltd., USA) were the main instruments used in this study.

2.2. Cell viability of PMC

PMC were suspended in fresh medium and seeded into 96-well plates at (1×10^5) cells per well. Honeysuckle extract was added and diluted into final concentrations of 120, 180 and 240 g/L after 2 h incubation. The cells were then incubated for another 4 h. Subsequently, 100 μL DMEM were supplied with 20% MTT, and incubated for another 4 h. Cell viability was determined by multifunctional microplate reader at 490 nm.

$$\text{Inhibition rate}\ (\%) = (OD_{490\ nm}\ \text{of control group} - OD_{490\ nm}\ \text{of test group})/OD_{490\ nm}\ \text{of control group} \times 100\%$$

2.3. Morphological observation of PMC

The standard incubation was conducted in 100 μL of Tyrode's buffer containing PMC. The cells were stimulated by indicated amounts of compound 48/80 (0.02 g/L), physiological saline and honeysuckle extract (120 g/L) at 37 °C for 0, 15, 30, 45 and

60 min. Degranulation of PMC were observed under light microscope and electron microscope.

2.4. Degranulation rate of PMC

Cell treatment was the same as 2.3, after treatment, cells were harvested by centrifugation, and washed with cold PBS twice. Cells were resuspended with 500 μL binding buffer, stained with 10 μL Annexin V-FITC labeling reagent for 10 min in the dark and analyzed by a flow cytometer. The percentages of Annexin V-FITC positive cells were calculated using Cell Quest software.

2.5. Systemic anaphylaxis behavior score

The rats were administrated by compound 48/80, honeysuckle extract (2.25 mL/kg) and physiological saline respectively through intravenous injection. Symptoms of systemic anaphylaxis were observed 0, 15, 30, 45 and 60 min after administration. Symptoms were assessed as follows (Church and Levischaffer, 1997): 0 = no symptoms; 1 = scratching and rubbing around the nose; 2 = puffiness around the mouth and eyes; 3 = difficulty breathing, mouth and tail bun; 4 = convulsion; and 5 = death.

2.6. Plasma HIS and trypsin levels detected by ELISA

After injection, blood was collected 0, 15, 30, 45 and 60 min and serum samples were prepared and stored at − 80 °C. HIS, MCT levels were determined with ELISA kit under steps in instructions.

2.7. Fingerprint and HIS levels of 10 batches of honeysuckle extract serum

UPLC analysis of serum samples from rats treated with honeysuckle extract was performed through a Waters Acquity Solvent Delivery System (Waters Corporation, Milford, MA, USA), comprising a binary solvent delivery pump, an auto sampler manager, a column compartment and a photodiode array detector, connected to Waters Empower 2 software. Chromatographic separations were performed through a Dikma UPLC Endeavorsil C_{18} column (50 × 2.1 mm, 1.8 μm), operating at 30 °C with gradient elution. Mobile phase A was acetonitrile and mobile phase B was 0.1% aqueous formic acid solution. The linear gradient was as follow: 0–1 min, 5% A; 1–3 min, 5%–10% A; 3–8 min, 10%–39% A; and 8–14 min, 39%–65% A.

The rats were administrated by 10 batches of honeysuckle extract (2.25 mL/kg calculated with chlorogenic acid), and plasma HIS levels were assayed using a commercial ELISA kit.

2.8. Spectrum-effect relationship evaluated by correlation analyses

The peak areas in the fingerprints analyzed by chromatography condition were respectively marked as X_1, X_2, X_3 . . . X_n, which were associated with the pharmacological results. Finally, components of correlated relationships to the pharmacological results were calculated by SPSS statistics software.

2.9. Peak assignments of anaphylactoid components in SHLI fingerprint

In this study, with investigating the peak assignment of the anaphylactoid component in the fingerprint of SHLI, it is further clarified whether the anaphylactoid component of honeysuckle will be reduced or eliminated during the preparation of preparations containing honeysuckle. First, analyze ten batches of SHLI according to the method under "2.7". Then fingerprints of honeysuckle and SHLI injection were compared.

2.10. Statistical analysis

All data were expressed as means ± SD and analyzed with SPSS 19.0 (SPSS, Chicago, USA). The X^2 test was used to analyze the positive rate of symptoms of systemic anaphylaxis. One way ANOVA and student's t test were used to analyze differences of plasma HIS, MCT and CH_{50} levels between the groups. $P < 0.05$ was considered as statistical significance. Canonical correlation analysis was used to study the spectrum–effect relationships between peak areas in the UPLC fingerprints and plasma HIS level to achieve a better dose–effect relationship.

3. Results

3.1. Inhibitory rate of rat PMC

As showed in Fig. 1, compared with the cell control group, honeysuckle extract in the dose of 120 g/L did not cause any cytotoxicity incubation. When the concentration was greater than 150 g/L, the cell Inhibitory rate was significantly increased. Honeysuckle extract 150 g/L group cell inhibitory rate was 33.09%, honeysuckle extract 240 g/L group cell inhibitory rate was 81.35%. Therefore, choose honeysuckle extract incubated concentration as 120 g/L and incubated time as 2 h for subsequent experiments.

3.2. Effect of honeysuckle extract on degranulation of PMC

Honeysuckle extract can cause a severe degranulation reaction in rat PMC. Normal rat PMC under the light microscope can be stained with neutral red to red, with clear edges and obvious nuclei. The particles in the cells are densely clustered, showing dark red after degranulation (Fig. 2A). The normal rat PMC under the electron microscope was bulky, with smooth membrane, clear cell outline, and containing more particulate matter cytoplasm. After being stimulated, degranulation occured, and the cell membrane presented irregularities, with the contents flowing out of the cell body (Fig. 2B). The percentage of AnnexinV-FITC in each group was detected by flow cytometry. Compared with treatment for 0 min, after treatment with 120 g/L of honeysuckle extract for 15 to 60 min, Annexin V positive population was markedly accumulated in a time-dependent manner. As shown in Table 1, the

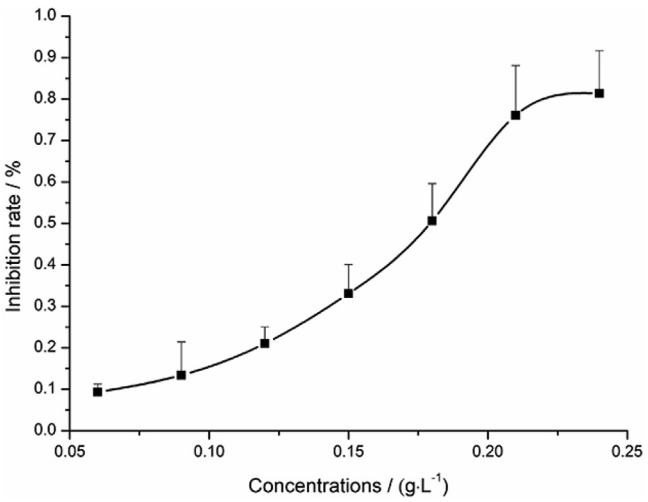

Fig. 1. Inhibition rate of rat PMC (means ± SD, n = 6). PMC were incubated with honeysuckle extract for 2 h. The cell inhibition was determined by MTT assay. Inhibition rate (%) = ($OD_{490\ nm}$ of control group − $OD_{490\ nm}$ of test group) /$OD_{490\ nm}$ of control group × 100%.

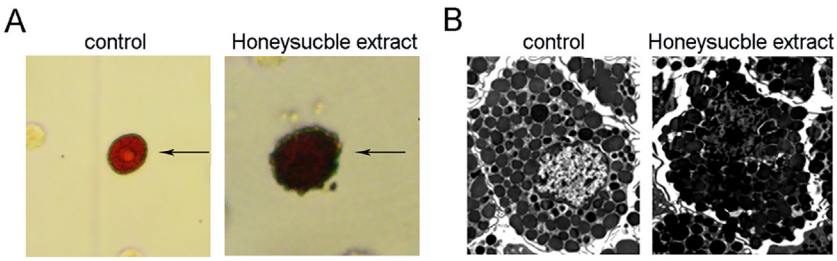

Fig. 2. Optical microscope (A, 100 ×) of PMC and electron microscope (B, 4200 ×) of PMC.

Table 1
Annexin V positive rate of PMC cell detected by FCM analysis (means ± SD, n = 6).

Groups	Annexin V positive rate/%
Control	6.83 ± 0.65
Compound 48/80	49.73 ± 5.11**
Honeysuckle extrat (0 min)	5.93 ± 0.65
Honeysuckle extrat (15 min)	18.74 ± 2.98**
Honeysuckle extrat (30 min)	21.98 ± 3.73**
Honeysuckle extrat (45 min)	30.05 ± 4.54**
Honeysuckle extrat (60 min)	37.57 ± 4.75**

** $P < 0.01$, compared with normal control group.

Table 2
Serum MCT, plasma HIS level of rat (means ± SD, n = 6).

Groups	Serum MCT level/ ($mg \cdot L^{-1}$)	Plasma HIS level/ ($mg \cdot L^{-1}$)
Control	0.263 ± 0.015	0.036 ± 0.0012
Compound 48/80	0.904 ± 0.0211**	0.863 ± 0.0162**
Honeysuckle extract (0 min)	0.288 ± 0.041	0.039 ± 0.0072
Honeysuckle extract (15 min)	0.566 ± 0.112*	0.103 ± 0.0019**
Honeysuckle extract (30 min)	1.166 ± 0.203**	0.143 ± 0.0022**
Honeysuckle extract (45 min)	1.198 ± 0.200**	0.160 ± 0.025**
Honeysuckle extract (60 min)	1.203 ± 0.303**	0.173 ± 0.032**

** $P < 0.01$, *$P < 0.05$, compared with control group.

percentage of degranulated cells was significantly increased in a time-dependent manner in PMC treated with 120 g/L of honeysuckle extract for 15 to 60 min.

3.3. Scores of systemic anaphylaxis behavior

Obvious systemic reactions in honeysuckle extract (2.25 mL/kg) injected rats were observed from 15 to 60 min after administration, a number of rats had puffiness around the mouth and eyes, while a number of rats had convulsions, while saline negative control group rats showed no reactions (Fig. 3).

3.4. HIS and trypsin levels

The mast cells were activated to degranulate and release anaphylaxis mediators into blood, such as HIS, MCT. As showed in Table 2, After injection with 2.25 mL/kg of honeysuckle extract for the same duration (15, 30, 45, 60 min), plasma HIS and serum MCT levels of the honeysuckle extract group were significantly higher than the control group, after injection with honeysuckle extract for 15 to 60 min, HIS and MCT release were significantly increased ($P < 0.05$). These results suggest that HIS and MCT are the major mediators of anaphylaxis, within a certain range, the concentration of HIS and MCT tends to increase with time.

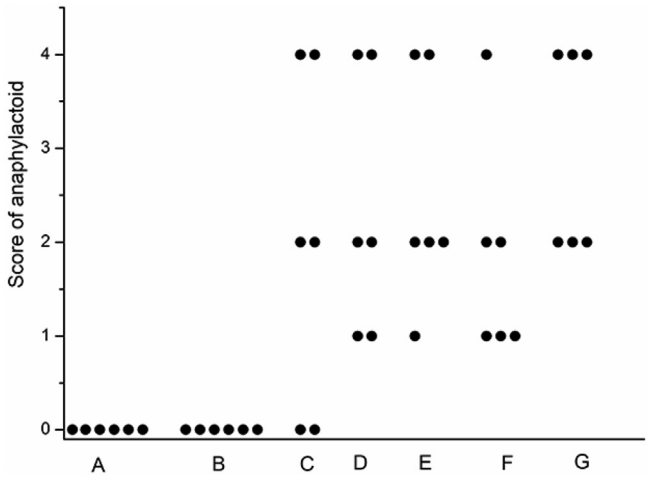

Fig. 3. System anaphylactoid symptom scores in rats. A, B, C, D, E, F and G: Control group, 0, 15, 30, 45 and 60 min after honeysuckle extract group (2.25 mL/kg) administration and Compound 48/80 group.

3.5. Effects of 10 batches of honeysuckle extract on serum chemical fingerprint data and HIS levels

For spectrum-effect relationship studies, Chinese herbal formulas are generally divided into different batches and samples are prepared through different extraction methods or different combinations, which refers to herbs, extracts or compositions in an orthogonal compatibility combination (Kalesnikoff & Galli, 2008; Hogan & Schwartz, 1997). In the present study, 10 batches of honeysuckle extract serum were prepared with chemical fingerprint data and HIS levels shown in Table 3. In a comparison of fingerprints of serum from rats treated with honeysuckle extract, 13 chromatographic peaks were characterized by relative retention time. In a comparison of 10 batches of fingerprints of honeysuckle extract (Fig. 4), 13 chromatographic peaks were characterized by relative retention time, peak 2 was chlorogenic acid.

3.6. Analysis of spectrum-effect relationships

The ENTER regression equations of Y (HIS Level) was established by analyzing the independent variables of relative peak area and the dependent variables of X (Table 3). Seven peaks were included in the equations, which were X_7, X_{10} and X_{13}, the equations of which were: Y = -0.503 X_1 – 0.267 X_5 + 1.025 X_7 – 0.359 X_9 + 0.536 X_{10} – 0.137 X_{11} – 0.627 X_{12} + 0.123 X_{13}

The residual statistics of Durbin-Watson reflected the independence between residuals in the range of (2 ± 1.2). The determination coefficient was 0.892, and the residuals statistics was 2.178. The results of variance analysis demonstrated statistical significance ($P < 0.05$).

Table 3
Chemical data of honeysuckle extract fingerprint (with chlorogenic acid as reference peak) and pharmacological data.

No.	Relative peak area of different batch samples										Average relative retention time/min
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	
1	0.175	0.184	0.179	0.189	0.193	0.177	0.169	0.172	0.177	0.168	0.979
2	1	1	1	1	1	1	1	1	1	1	1
3	0.416	0.455	0.477	0.437	0.424	0.437	0.444	0.401	0.43	0.40	1.047
4	2.448	2.477	2.494	2.436	2.077	1.981	1.849	2.328	2.361	2.543	1.051
5	0.750	0.721	0.802	0.767	0.736	0.751	0.784	0.728	0.629	0.721	1.174
6	0.947	0.881	0.898	0.989	0.789	0.949	0.909	0.826	0.804	0.800	1.349
7	0.263	0.327	0.267	0.294	0.331	0.203	0.363	0.212	0.325	0.298	1.519
8	0.292	0.248	0.381	0.337	0.299	0.366	0.233	0.319	0.275	0.301	1.560
9	0.253	0.315	0.278	0.271	0.302	0.282	0.308	0.289	0.276	0.307	1.650
10	0.317	0.321	0.298	0.365	0.331	0.378	0.299	0.289	0.308	0.342	1.791
11	0.223	0.268	0.295	0.241	0.209	0.287	0.299	0.212	0.209	0.211	1.821
12	0.261	0.250	0.229	0.287	0.221	0.278	0.293	0.254	0.271	0.238	1.857
13	0.336	0.401	0.342	0.359	0.329	0.311	0.311	0.309	0.341	0.321	1.883
HIS level	0.143	0.138	0.14	0.147	0.132	0.139	0.13	0.15	0.151	0.157	0.143

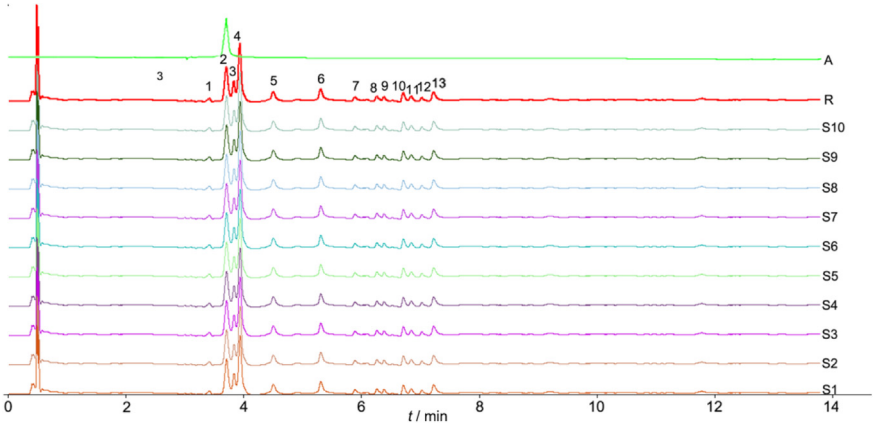

Fig. 4. Ten batches of honeysuckle extract fingerprints (A and R are the relative fingerprints of honeysuckle extract and chlorogenic acid solution, respectively).

3.7. Peak assignment of anaphylactoid components in SHLI fingerprint

The fingerprint of SHLI were obtained (Fig. 5), and the similarities were all greater than 0.9, indicating that the similarity is good and meets the requirements of fingerprints. A total of 17 common peaks were calibrated in the map, and the peaks were numbered in sequence. The relative retention time and relative peak area of common fingerprint peaks in each fingerprint were counted with chlorogenic acid (peak 5) as the reference peak (Table 4). The RSD were less than 3%, which indicating that the quality of ten batches of SHLI is stable. Fingerprints of honeysuckle and SHLI were compared, it was found that the relative area of peak 7 of the suspected anaphylactoid component in honeysuckle was significantly elevated in the fingerprint of SHLI.

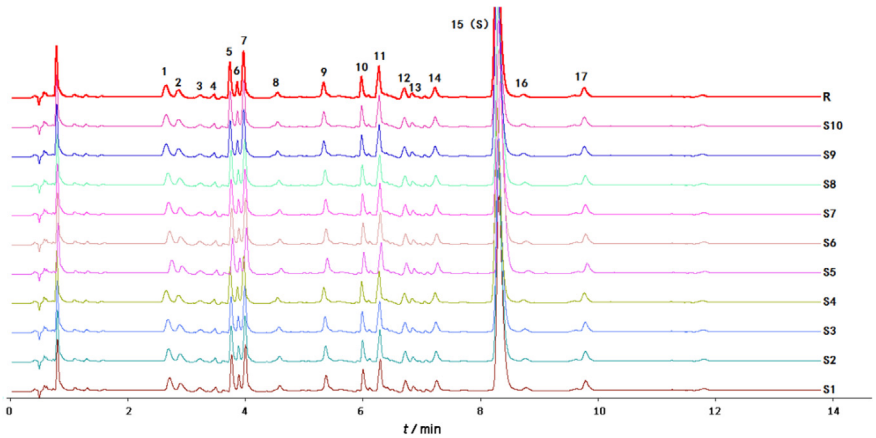

Fig. 5. Ten batches of SHLI fingerprints (R is the relative fingerprint of SHLI).

Table 4
Chemical data of SHLI fingerprint (with chlorogenic acid as reference peak).

No.	Relative peak area of different batch samples										Average relative retention time/min
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	
1	0.331	0.320	0.338	0.319	0.293	0.297	0.316	0.272	0.287	0.248	0.373
2	0.214	0.220	0.216	0.209	0.199	0.218	0.208	0.220	0.215	0.213	0.432
3	0.170	0.174	0.179	0.179	0.183	0.177	0.163	0.172	0.167	0.169	0.979
4	0.161	0.153	0.147	0.158	0.149	0.154	0.160	0.163	0.159	0.160	0.561
5	1	1	1	1	1	1	1	1	1	1	1
6	0.316	0.355	0.367	0.347	0.354	0.356	0.376	0.400	0.383	0.379	1.047
7*	2.399	2.387	2.454	2.506	2.132	2.081	2.049	2.238	2.354	2.467	1.051
8	0.764	0.721	0.785	0.749	0.760	0.749	0.766	0.739	0.699	0.701	1.174
9	0.881	0.947	0.898	0.989	0.750	0.929	0.909	0.826	0.784	0.772	1.349
10	0.978	0.941	0.967	0.894	0.934	0.923	0.963	0.902	0.925	0.998	1.519
11	1.299	1.248	1.383	1.337	1.309	1.326	1.232	1.314	1.275	1.303	1.560
12	0.346	0.390	0.338	0.365	0.371	0.358	0.305	0.299	0.301	0.348	1.791
13	0.293	0.287	0.290	0.224	0.239	0.279	0.269	0.221	0.218	0.216	1.821
14	0.359	0.399	0.352	0.353	0.332	0.322	0.331	0.329	0.340	0.323	1.883
15	20.113	19.346	21.355	20.153	21.033	20.932	20.789	21.099	21.103	21.126	2.185
16	0.758	0.732	0.780	0.799	0.706	0.756	0.776	0.783	0.701	0.709	2.146
17	0.343	0.388	0.346	0.364	0.370	0.356	0.355	0.303	0.304	0.309	2.421

4. Discussion

Mast cells are important mediators and effectors of anaphylactoid reactions. The mechanism of anaphylactoid reaction is complex, and it is generally agreed that the drug component directly acts on mast cells. The release of the active medium, such as HIS, MCT, β-hexosaminidase and a wide variety of other inflammatory cytokines causing a series of symptoms similar to allergies (Xu et al., 2014; Gao et al., 2018). HIS, one of the biogenic amines present in mast cells and basophils, is constituted from histidine by histidine decarboxylase. It is one of main biologically active substances resulting in anaphylaxis and anaphylactoid reactions. HIS has become a characteristic marker indicating activation and degranulation of mast cells and basophils (Hogan & Schwartz, 1997). MCT, a neutral serine protease, is the principal enzyme selectively located in mast cells. On mast cell activation, it is released in parallel with HIS and β-hexosaminidase (Hogan & Schwartz, 1997). Serum MCT levels, which are related to the degree of anaphylaxis and allergic reactions, have become a useful diagnostic tool indicating mast cell degranulation (Hogan & Schwartz, 1997). Thus, MCT, a characteristic constituent in mast cells, is shown to be a precise clinical marker of mast cell degranulation (Hogan & Schwartz, 1997). Annexin V is one of proteins involved in mast cell degranulation. To mast cell activation, exogenous Annexin V, specifically binds to secretory granules on the cell surface is in proportion to the extent of degranulation. Annexin V has been used as a direct quantitative indicator to monitor mast cell degranulation (Demo et al., 1999). Therefore, these characteristic markers were employed to determine the degree of degranulation of PMC in the present study.

In this study, anaphylactic symptoms were observed within 60 min after intravenous injection. In our present study, after treated with honeysuckle extract, the percentage of Annexin V positive cells in rat PMC was significantly increased after 15 min. Additionally, morphological changes of rat PMC treated with Honeysuckle extract were observed by optical microscope and electron microscope. Honeysuckle extract induced cell degranulation in rat PMC in a time-dependent manner. In this study, anaphylaxis effects and fingerprinting of honeysuckle extract were systematically evaluated to elucidate the basis for the anaphylaxis.

The assignment study of anaphylaxis components in the fingerprint of SHLI found that the relative area of peak 7 of the suspected anaphylaxis component in honeysuckle was significantly elevated in the fingerprint of SHLI. The results showed that the honeysuckle extract anaphylaxis components can be reduced or eliminated during the preparation process, but whether changes in process conditions or differences in the source of medicinal materials can cause the original anaphylaxis components to increase leading to the occurrence of anaphylaxis reactions, these issues still need to be explored.

5. Conclusion

In summary, multiple ingredients in honeysuckle instead of just one ingredient in honeysuckle can cause anaphylaxis reactions in rats. Because anaphylactoid reactions are dose- and concentration-dependent, and this study showed that honeysuckle is able to induce degranulation concentration dependently, caution should be exercised when using TCMs containing honeysuckle. It is recommended that drug labels should be followed strictly, doses should not be increased casually, and adverse events should be treated rapidly in order to avoid the occurrence of anaphylactoid allergy. This study provides new ideas and methods for the study of the safety evaluation methodology of TCM injections.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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