Screening of Anti-inflammatory Active Ingredients from Gancao Qinlian Extract and Study of Its Efficacy

Maixun ZHU[1,2], Yang ZHANG[1], Yue XU[1], Hongmei TANG[1,2*], Tao WU[3], Yanda ZHANG[3]

1. National Center of Technology Innovation for Pigs, Chongqing 402460, China; 2. Chongqing Academy of Animal Sciences, Chongqing 402460, China; 3. Fujian Bedi Pharmaceutical Co., Ltd., Ningde, Fujian, 402460, China

Abstract [**Objectives**] To establish the chromatographic fingerprint of Gancao Qinlian Extracts (GQE) and reveal the possible material basis of the anti-inflammatory effect of GQE by the correlation analysis between the fingerprint chromatographic peaks of different components of GQE and its anti-inflammatory activity. [**Methods**] Ultra-performance liquid chromatography (UPLC) was used to detect the different ingredients of GQE to establish its chromatographic fingerprint and analyze the differences among the three medicine components; LPS stimulated RAW264.7 cells to construct an inflammatory cell model. The NO secretion of cells was detected by the Griess method. ELISA was used to detect the changes in TNF-α and IL-10 contents. RT-qPCR tested the mRNA expression levels of TNF-α and IL-10. Grey relational analysis was carried out by combining fingerprint chromatographic peak data and anti-inflammatory activity data. [**Results**] The GQE fingerprint was established, 34 fingerprint characteristic peaks were calibrated, and 33 related chromatographic peaks were screened out. The corresponding chromatographic peaks in the three components were obtained, and the content of the components was calculated; the anti-inflammatory results showed that the content of NO, TNF-α, and the expression of TNF-α mRNA in the high and medium-dose groups of GQE were significantly lower than those in the blank group ($P<0.01$). The NO content and TNF-α mRNA expression in the high-dose group of GQE I was considerably lower than those in the blank group ($P<0.01$). The secretion of NO, TNF-α, and the expression of TNF-α mRNA in the high, medium, and low dose groups of GQE II were significantly lower than those in the blank group ($P<0.01$); the results of grey relational analysis showed that the correlation degree of the three components was GQE II > GQE > GQE I, and the characteristic fingerprint peaks 12, 15, 22, 23, 28, 31, 33 may be closely related to the anti-inflammatory effect. [**Conclusions**] The best component of the anti-inflammatory effect in GQE is water-soluble component, and its main components are flavonoids and alkaloids. These components can alleviate cellular inflammatory damage by inhibiting the excessive secretion of NO and reducing the expression of TNF-α mRNA.

Key words Gancao Qinlian Extracts, Anti-inflammatory activity, Fingerprint, Grey relational analysis

1 Introduction

Gancao Qinlian Extract (GQE) is prepared based on the simplified prescription of Gancao Xiexin Decoction in *Treatise on Typhoid Fever and Miscellaneous Diseases*. It can be used to treat acute and chronic gastroenteritis and colonic ulcer, and has functions of resisting inflammation, regulating gastrointestinal peristalsis, repairing intestinal mucosal damage, *etc.*[1-2]. GQE prescription contains *Glycyrrhiza uralensis*, *Scutellaria baicalensis*, *Coptis chinensis*, *Zingiber officinale*, *Pinellia ternata* and other medicines, which mainly contain flavonoids, glycosides, alkaloids, phenolic acids and other ingredients[3-4]. It has been reported that compound Chinese medicines containing licorice, *S. baicalensis*, *C. chinensis* and other medicinal materials can affect the expression level of inflammatory cell-related factors and reduce the inflammatory symptoms of ulcerative colitis[5-6]. Previous studies have found that GQE has obvious anti-inflammatory effects, but the specific anti-inflammatory substance basis is not clear[7]. Quality control has always been the focus and difficulty in the research of traditional Chinese medicine and compound prescriptions. The establishment of fingerprint of traditional Chinese medicine provides a new idea for this[8-9]. However, fingerprint is specific and holistic, it is difficult to reflect the specific types of active ingredients, and the correlation with drug effect is not clear. Through the mathematical multivariate statistical method, the correlation research between fingerprint and drug effect data can directly correlate the "spectrum" of fingerprint and the "effect" of activity research, effectively solving the problem of precise control of active ingredients in traditional Chinese medicine compounds[10-12]. Based on this, this study will construct GQE traditional Chinese medicine fingerprint and screen characteristic fingerprint chromatographic peaks, at the same time compare the difference of the number and content of drug components in different extracts of GQE, obtain the anti-inflammatory effect results through *in vitro* inflammatory cell test, and use grey relational degree to analyze the relationship between "spectrum" and "effect", screen related chromatographic peaks, so as to clarify the best anti-inflammatory effect of GQE drug components and their anti-inflammatory activity mode.

2 Materials and methods

2.1 Materials

2.1.1 Test drugs. Appropriate amounts of licorice, *S. baicalensis*, *C. chinensis*, *R. zingiberis* and *P. ternata* were weighed in 4∶3∶1∶3∶3 ratio. They were extracted by semi-bionic extraction method, water decoction method and solvent extraction method, respectively. Every 1 mL of extract was equivalent to 1 g of the original drug, and the extracts GQE, GQE I, GQE II were obtained; 16 batches of GQE, 3 batches of GQE I and GQE II were

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* Corresponding author. E-mail: tanghongmei2005@126.com

prepared, respectively.

2.1.2 Cell lines. Mouse mononuclear macrophage cell line RAW264.7 was purchased from the Kunming Cell Bank of the Type Culture Collection Committee of the Chinese Academy of Sciences, No. KCB200603YJ.

Reference substances: Coptisine hydrochloride (batch No.: 112026-201802, purity ≥ 94.0%), berberine hydrochloride (batch No.: 110713-201814, purity ≥ 86.7%), wogonoside (batch No.:112002-201702, purity≥98.5%), baicalein (batch No.:111595-201808, purity≥97.9%), ammonium glycyrrhetate (batch No.:110731-202021, purity≥96.2%), liquiritin (batch No.:111610-201908, purity≥95.0%), wogonin (batch No.: 110715-201821, purity ≥ 95.4%), gingerone (batch No.: 111807-201802, purity≥99.90%) were all purchased from China National Institute for Food and Drug Control; glycyrrhizin (batch No.: PS010083, purity ≥ 98.0%), 6-gingerol (batch No.: PS012566, purity≥99.90%) were purchased from Chengdu Push Biotechnology Co., Ltd.

2.2 Methodology

2.2.1 Detection of pharmaceutical ingredients in traditional Chinese medicines. GQE, GQE I, GQE II were dissolved in 50% methanol solution by ultrasonic treatment, the solution was made up to a constant volume of 0.1 g/mL, and filtration was used to obtain the test solution. Preparation of mixed reference substance: liquiritin, glycyrrhizin, ammonium glycyrrhetate, baicalin, wogonoside, baicalein, wogonin, berberine hydrochloride, coptisine hydrochloride, gingerone, 6-gingerol were precisely weighed, respectively, and 50% methanol solution was used to fix the volume to the concentration of 1 mg/mL. The above reference substance solutions were precisely weighed, respectively, and 50% methanol solution was used to prepare the solution at the concentration of 0.5 mg/mL. Chromatographic conditions refer to[13], the ultraviolet detector was selected to detect the wavelength of 277 nm, the flow rate was 0.3 min/mL, the column temperature was 15 ℃, and the sample injection volume was 3 μL. The repeatability and stability of the method were also investigated.

2.2.2 Construction of fingerprint of traditional Chinese medicine and analysis of its components. 16 batches of GQE chromatographic data were imported into the similarity evaluation system software of traditional Chinese medicine chromatographic fingerprint (2012 version), the median method was used for multi-point correction, the time window width was 0.5, the chromatographic peaks were screened according to the matching number≥2 for automatic matching and the characteristic fingerprint chromatographic peak was selected, to generate a common pattern of chromatographic fingerprint as a control fingerprint, and the similarity was calculated at the same time. The pharmaceutical components of GQE, GQE I and GQE II were analyzed by using the chromatogram of mixed control solution, and the content of some characteristic fingerprint chromatogram peaks was calculated by external standard method, and the differences of components were analyzed.

2.2.3 Determination of cytotoxicity of different extracts of traditional Chinese medicine on cells. RAW264.7 culture was performed through 96-well plates, and different traditional Chinese medicine extracts were diluted into 6 concentrations (1×10^{-1}, 1×10^{-2}, 1×10^{-3}, 1×10^{-4}, 1×10^{-5}, 1×10^{-6} g/mL), with 200 μL added per well and 8 wells repeated for each concentration. Cells without drugs were set as a blank group, and MTT method was used to determine the absorbance value of cells and calculate the relative survival rate of cells[14].

2.2.4 Anti-inflammatory test of extracts from different Chinese herbs *in vitro*. RAW264.7 cells with good growth were stimulated with 15 μg/mL LPS for 12 h to construct the inflammatory cell model. Inflammatory cells were divided into high, medium and low dose groups of GQE, GQE I and GQE II, with 8 wells for each group. After the model was constructed, the drugs in 1∶1.0, 1∶0.5, 1∶0.25 maximum non-toxic concentrations were added. The cells of the blank group and the model group were added with maintenance media containing 0.1% pancreatin and 2% serum as control. After treatment, the cells were cultured at 37 ℃ in a 5% CO_2 incubator for 24 h, and the cell supernatants of each group were collected. The content of NO, TNF-α and IL-10 was determined with reference to the instructions of the kit. The cells of each group were taken, the total RNA was extracted by Trizol method, then the mRNA was reversely transcribed into cDNA, and GAPDH was used as the internal reference gene. The forward primer was 5'-TG CAGT GGCAAAGTGGAGATT-3', and the reverse primer was 5'-GGCTT CCCGTTGATGACAAG-3'; the forward primer for TNF-α was 5'-CCAACGGCATGGA TCTC AAAG-3' and the reverse primer was 5'-AT AGCAAATCGGCTGACGGT-3'; the forward primer for IL-10 was 5'-AA GACCC AG ACATCA AGGCG-3' and the reverse primer was 5'-AATCGATGACAGCGC CGTAG-3'. Fluorescent quantitative PCR reaction was carried out using a 20 μL reaction system (cDNA 2.0 μL, SYBR Green PCR Mix 10.0 μL, forward and reverse primers 0.5 μL each, and RNase free dH_2O 7.0 μL). After vortex mixing, transient centrifugation was carried out. Reaction procedure: pre-denaturation at 95 ℃ for 5 min; denaturation at 94 ℃ for 30 sec, annealing for 40 sec, extension at 72 ℃ for 35 sec, a total of 42 cycles, extension at 72 ℃ for 10 min.

2.2.5 Grey relational analysis. Referring to[15], the fingerprint characteristic chromatographic peak area and anti-inflammatory activity related test data of different extracts were made to be dimensionless by means of mean method, the correlation degree between chromatographic fingerprint peaks and anti-inflammatory drug effect was calculated, and the GQE anti-inflammatory activity related components were screened.

The steps of the grey relational analysis method are as follows:

(i) selecting a reference sequence (parent sequence) Y_j, where $j=1, 2, 3, \cdots, m$; a comparative sequence (sub-sequence) X_i, where $i=1, 2, 3, \cdots, n$.

In this paper, the anti-inflammatory drug effect index was

used as the mother sequence (Y), then $j=1$ in Y_j; if the common peak area of the characteristic fingerprint was taken as the sub-sequence (X), then $i=1, 2, 3, \cdots, 34$ in X_i.

(ii) calculating the difference sequence, the maximum difference and the minimum difference. The absolute value of the difference between the reference sequence Y_j and the comparative sequence X_i on the kth index was the difference sequence value $\triangle 0_i(k) = |Y_j(k) - X_i(k)|$, $k=1, 2, 3, \cdots, 34$; $j=1$; $i=1, 2, 3, \cdots, 34$; the maximum difference $\triangle \max = \max Y_j(k) - X_i(k)$; the minimum difference $\triangle \min = \min |Y_j(k) - X_i(k)|$.

(iii) Calculating the correlation coefficient, see Formula (1).

(iv) Calculate the correlation degree r_i, see Formula (2). ri reflects the overall degree of correlation between the reference sequence Y_j and the comparative sequence X_i.

$$\xi_i(k) = [\Delta(\min) + \rho \times \Delta(\max)] / [\Delta 0i(k) + \rho \times \Delta(\max)] \quad (1)$$

$$r_i = \frac{1}{n}\sum_{k=1}^{n}\xi_i(k) \quad (2)$$

In Formula (1), k is the peak number $=1, 2, 3, \cdots, 34$; ρ is distinguishing coefficient, the value interval is $0-1$, usually 0.5 is selected; $\triangle 0_i(k)$ is the absolute difference between the drug effect index (reference sequence) and the characteristic peak area (comparative sequence) after averaging; $\Delta(\min)$ is the minimum difference, $\Delta(\max)$ is the maximum difference; in Formula (2), n is the number of data contained in the comparative sequence, $i=1, 2, 3, \cdots, 34$; $k=1$.

2.2.6 Data analysis. SPSS 20.0 software was used for one-way ANOVA and significance comparison, and Duncan method was used for multiple comparison; the results of fluorescent quantitative PCR were analyzed by $2^{-\triangle\triangle Ct}$ method; the expression intensity of the target gene was expressed by absolute copy number/absolute copy number of internal reference gene, and the expression amount of the gene in the blank group was set to $1\times$, and the expression level of the target gene was equivalent to that of the blank group.

3 Results

3.1 Construction of GQE fingerprint and components of different extracts 34 characteristic fingerprint chromatographic peaks were obtained from the GQE control characteristic fingerprint chromatogram (Fig. 1). Referring to the mixed control sample chromatogram (Fig. 2), it was preliminarily determined that peak 12 was liquiritin, peak 15 was baicalin, peak 16 was gingerone, peak 22 was wogonoside, peak 23 was coptisine hydrochloride, peak 24 was glycyrrhizin, peak 27 was ammonium glycyrrhetate, peak 28 was berberine hydrochloride, peak 31 was baicalein, peak 32 was 6-gingerol, and peak 33 was wogonin (Fig. 3). In accordance with Table 1 and 2, the comprehensive analysis showed that the total number of components in GQE was 157, and there were 11 chromatographic peaks analyzed, accounting for 62.53% of the total components; there were 145 chromatographic peaks in GQE I, and there were 10 chromatographic peaks analyzed, accounting for 50.08% of the total components; there were 126 components in GQE II and 7 chromatographic peaks analyzed, accounting for 64.25% of the total components. The content of baicalin, wogonoside, coptisine hydrochloride, berberine hydrochloride, liquiritin was high in 3 extracts, and GQE II > GQE > GQE I.

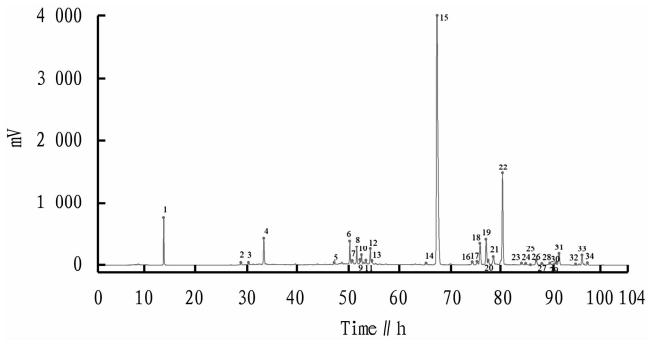
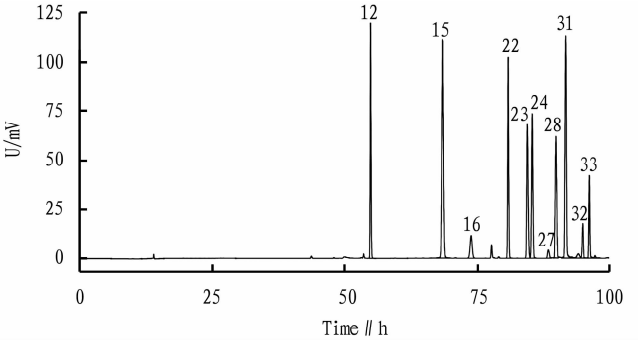


Fig. 1 Control characteristic fingerprint of GQE



NOTE 12. liquiritin; 15. baicalin; 16. gingerone; 22. wogonoside; 23. coptisine hydrochloride; 24. glycyrrhizin; 27. ammonium glycyrrhetate; 28. berberine hydrochloride; 31. baicalein; 32. 6-gingerol; 33. wogonin. The same below.

Fig. 2 Chromatogram of mixed control solution

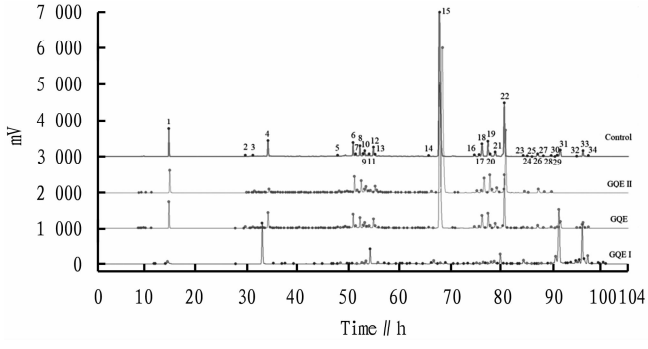


Fig. 3 Comparison of UPLC fingerprint of different extracts

Table 1 Difference analysis of extracts from different groups

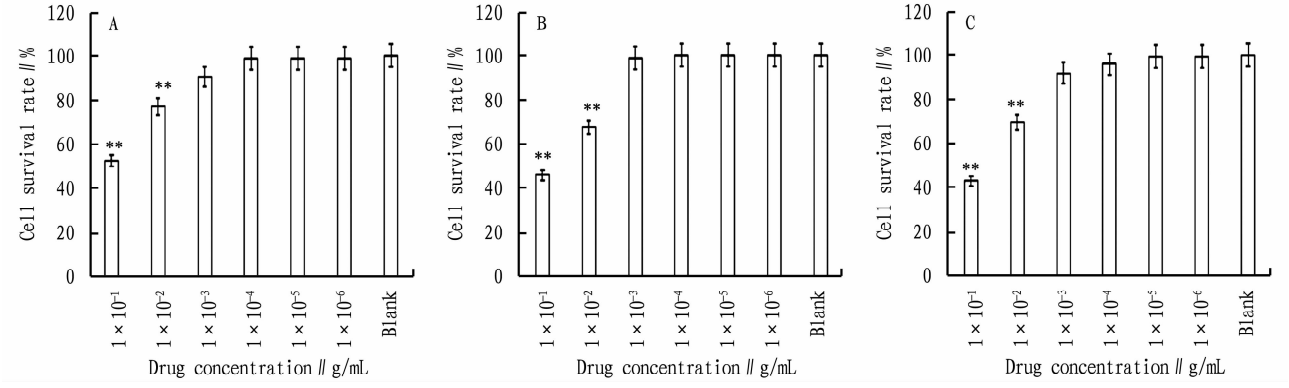
Chinese herbal extract	Total number of components	Number of components analyzed	Ratio of components analyzed to total peak area // %
GQE	157	11	62.53
GQE I	145	10	50.08
GQE II	126	7	64.25

Table 2 Content analysis of extracts from different groups

Peak number	Component name	Component content//mg/mL		
		GQE	GQE I	GQE II
12	Liquiritin	0.267 8	–	0.242 0
15	Baicalin	4.132 1	0.039 2	5.107 1
16	Gingerone	0.280 3	0.228 4	–
22	Wogonoside	1.485 6	0.032 7	2.153 1
23	Coptisine hydrochloride	0.253 5	0.085 4	0.291 6
24	Glycyrrhizin	0.018 4	0.058 0	0.015 5
27	Ammonium glycyrrhetate	0.181 7	0.137 4	0.180 7
28	Berberine hydrochloride	0.468 7	0.238 4	0.407 7
31	Baicalein	0.203 2	0.461 4	–
32	6-gingerol	0.085 9	0.508 3	–
33	Wogonin	0.392 8	0.601 1	–

3.2 Toxicity of different extracts on RAW264.7 cells As can be seen from Fig. 4, the cell survival rate was significantly lower than that of the blank group when the concentration of GQE, GQE I, GQE II was 1×10^{-1}, 1×10^{-2} g/mL ($P<0.01$). When the concentration of 3 extracts was lower than 1×10^{-3} g/mL, there was no significant difference in the cell survival rate ($P>0.05$), and the maximum safe concentration of 3 extracts was determined to be 1×10^{-3} g/mL.

3.3 Effect of different extracts on the content of inflammatory factors NO, TNF-α, IL-10 after LPS induction As can be seen from Table 3, compared with the blank group, the secretion of inflammatory factors NO, TNF-α, and IL-10 in the model group increased significantly ($P<0.01$). Compared with the model group, high and medium dose GQE groups and high, medium and low GQE II dose groups could significantly inhibit the release of NO and TNF-α ($P<0.01$); the low dose GQE group could significantly inhibit the secretion of NO ($P<0.05$) and TNF-α ($P<0.01$); the high dose GQE I group could significantly inhibit NO secretion ($P<0.01$), the medium and low dose GQE I groups significantly inhibited NO secretion ($P<0.05$), but there was no significant difference in the effect of three doses of GQE I on the secretion of TNF-α ($P>0.05$); there was no significant difference in the effect of GQE, GQE I and GQE II on the secretion of IL-10 ($P>0.05$).



NOTE A. GQE; B. GQEI; C. GQEII.

Fig. 4 Effect of different concentrations of GQE on cell viability

Table 3 Changes of NO, TNF-α and IL-10 levels in RAW264.7 cells treated with different extracts

Group	Drug concentration//mg/L	NO//μmol/L	TNF-α//pg/mL	IL-10//pg/mL
Blank	–	1.63±2.14	111.29±0.91	90.72±0.88
Model	–	45.53±3.42**	3757.22±2.08**	127.49±1.43**
GQE	50.0	13.62±3.89▲▲	1695.78±1.87▲▲	125.21±0.93
	25.0	25.81±2.93▲▲	2704.47±3.36▲▲	127.06±1.00
	12.5	37.81±3.70▲	2962.64±1.23▲▲	125.98±2.11
GQE I	50.0	28.05±1.34▲▲	3756.01±3.60	126.75±2.47
	25.0	36.99±2.95▲	3756.15±4.28	126.04±1.52
	12.5	37.40±1.48▲	3758.09±4.27	128.43±2.51
GQE II	50.0	9.55±2.24▲▲	1122.69±2.16▲▲	127.70±0.78
	25.0	22.97±2.72▲▲	1525.58±2.62▲▲	128.18±0.75
	12.5	31.71±2.56▲▲	2216.23±1.97▲▲	125.91±4.27

NOTE "**" means significant difference compared with the blank group ($P<0.05$), and "***" means extremely significant difference compared with the blank group ($P<0.01$); "▲" means significant difference compared with the model group ($P<0.05$), and "▲▲" means extremely significant difference compared with the model group ($P<0.01$). The same below.

3.4 Effect of different extracts on the relative mRNA expression of TNF-α and IL-10 As can be seen from Table 4, compared with the blank group, the mRNA expression levels of cytokines TNF-α and IL-10 in the model group significantly increased

($P<0.01$). Compared with the model group, high, medium and low dose GQE, GQE I, GQE II groups all inhibited mRNA expression of TNF-α significantly ($P<0.01$), and the inhibition effect was positively correlated with the dose, but there was no significant difference in the effect of GQE, GQE I, GQE II on mRNA expression of IL-10 ($P>0.05$).

3.5 Results of grey relational analysis As can be seen from Table 5, the correlation degree r value between GQE, GQE I, GQE II and anti-inflammatory drug effects was in the order of GQE II > GQE > GQE I. The correlation degree for correlation peaks 3, 4, 11, 12, 13, 15, 16, 17, 22, 23, 24, 27, 28, 29, 30, 31, 32, 33, 34 in GQE was greater than 0.80, the correlation degree for correlation peaks 7, 15, 18, 22, 29, 31, 34 in GQE I was greater than 0.75, and the correlation degree for correlation peaks 6, 7, 9, 10, 11, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24, 25, 29 in GQE II was greater than 0.90. According to the comprehensive analysis of the related chromatographic peaks of the three extracts, the peaks 12, 15, 22, 23, 28, 31, 33 were highly correlated with the anti-inflammatory efficacy. Based on the results of drug composition analysis, it can be considered that liquiritin, baicalin, wogonoside, coptisine hydrochloride, berberine hydrochloride, baicalein, wogonin and other components were the main substances to play the anti-inflammatory effect.

Table 4 Effect of different extracts on the mRNA expression of cytokines TNF-α and IL-10

Group	Drug concentration//mg/L	TNF-α	IL-10
Blank	–	1.03 ± 0.09	1.27 ± 0.24
Model	–	15.49 ± 0.25**	33.60 ± 0.80**
GQE	50.0	2.38 ± 0.26▲▲	32.47 ± 0.74
	25.0	5.38 ± 0.24▲▲	33.23 ± 0.62
	12.5	8.61 ± 0.25▲▲	33.23 ± 1.24
GQE I	50.0	4.38 ± 0.31▲▲	32.37 ± 0.71
	25.0	8.36 ± 0.31▲▲	32.32 ± 0.87
	12.5	12.44 ± 0.34▲▲	32.68 ± 0.81
GQE II	50.0	5.07 ± 0.09▲▲	32.89 ± 1.12
	25.0	9.57 ± 0.28▲▲	33.11 ± 0.66
	12.5	13.57 ± 0.34▲▲	32.74 ± 0.99

Table 5 Results of grey relational analysis

Chromatographic peak number	GQE				GQE I				GQE II			
	NO	*TNF-α*	*TNF-α* mRNA	*r* average	*NO*	*TNF-α*	*TNF-α* mRNA	*r* average	*NO*	*TNF-α*	*TNF-α* mRNA	*r* average
1	0.684 0	0.778 1	0.930 2	0.797 5	0.651 0	0.688 4	0.776 8	0.705 4	0.853 3	0.886 1	0.852 9	0.864 1
2	0.635 5	0.583 9	0.634 2	0.617 9	0.000 0	0.000 0	0.000 0	0.000 0	0.495 8	0.509 9	0.494 7	0.500 1
3	0.890 2	0.806 2	0.789 6	0.828 7	0.000 0	0.000 0	0.000 0	0.000 0	0.715 0	0.733 6	0.708 3	0.718 9
4	0.871 0	0.877 0	0.815 9	0.854 6	0.000 0	0.000 0	0.000 0	0.000 0	0.907 0	0.944 5	0.899 5	0.917 0
5	0.716 0	0.696 1	0.696 1	0.702 7	0.784 9	0.769 3	0.515 1	0.689 8	0.868 0	0.931 0	0.880 9	0.893 3
6	0.723 1	0.699 9	0.566 6	0.663 2	0.000 0	0.000 0	0.000 0	0.000 0	0.898 4	0.939 3	0.896 4	0.911 3
7	0.604 9	0.565 8	0.612 7	0.594 5	0.758 2	0.864 6	0.636 8	0.753 2	0.876 5	0.942 9	0.891 3	0.903 6
8	0.623 4	0.669 7	0.561 2	0.618 1	0.696 7	0.778 0	0.695 0	0.723 3	0.883 7	0.924 8	0.877 4	0.895 3
9	0.446 5	0.451 8	0.474 9	0.457 7	0.652 7	0.716 9	0.768 9	0.712 8	0.922 8	0.954 5	0.905 5	0.927 6
10	0.592 9	0.695 3	0.777 0	0.688 4	0.731 9	0.826 0	0.654 1	0.737 3	0.903 9	0.962 2	0.913 7	0.926 6
11	0.920 4	0.845 1	0.716 0	0.827 1	0.815 4	0.808 8	0.619 2	0.747 8	0.874 1	0.949 1	0.896 1	0.906 4
12	0.829 7	0.796 8	0.807 2	0.811 3	0.000 0	0.000 0	0.000 0	0.000 0	0.919 2	0.910 3	0.868 6	0.899 4
13	0.808 2	0.808 1	0.846 6	0.821 0	0.470 7	0.511 0	0.444 9	0.475 6	0.862 3	0.897 8	0.968 5	0.909 5
14	0.811 7	0.762 7	0.756 9	0.777 1	0.692 7	0.824 0	0.665 6	0.727 5	0.845 6	0.922 3	0.945 2	0.904 4
15	0.816 7	0.938 8	0.830 7	0.862 1	0.821 1	0.991 2	0.589 5	0.800 6	0.912 1	0.969 7	0.919 1	0.933 6
16	0.938 7	0.791 6	0.693 1	0.807 8	0.697 5	0.838 4	0.632 8	0.722 9	0.000 0	0.000 0	0.000 0	0.000 0
17	0.842 0	0.915 6	0.827 0	0.861 5	0.532 4	0.622 1	0.611 0	0.588 5	0.910 1	0.971 7	0.923 1	0.935 0
18	0.652 8	0.623 2	0.716 7	0.664 3	0.772 6	0.872 9	0.632 6	0.759 4	0.900 6	0.961 6	0.913 6	0.925 3
19	0.844 8	0.820 9	0.671 4	0.779 0	0.000 0	0.000 0	0.000 0	0.000 0	0.903 6	0.959 1	0.911 3	0.924 7
20	0.594 4	0.585 3	0.503 8	0.561 2	0.651 7	0.767 2	0.702 2	0.707 0	0.907 8	0.963 5	0.914 2	0.928 5
21	0.691 6	0.601 6	0.552 3	0.615 1	0.576 7	0.647 2	0.800 4	0.674 8	0.905 0	0.952 6	0.906 0	0.921 2
22	0.877 8	0.859 9	0.811 1	0.849 6	0.903 1	0.898 5	0.570 9	0.790 8	0.925 2	0.955 9	0.927 8	0.936 3
23	0.847 7	0.848 8	0.842 3	0.846 3	0.701 9	0.844 0	0.632 5	0.726 1	0.842 9	0.942 6	0.941 9	0.909 1
24	0.840 1	0.802 6	0.803 3	0.815 3	0.675 0	0.802 3	0.664 4	0.713 9	0.872 3	0.946 6	0.894 0	0.904 3
25	0.616 7	0.714 5	0.812 9	0.714 7	0.627 5	0.731 0	0.748 5	0.702 3	0.751 6	0.806 4	0.769 5	0.775 8
26	0.795 3	0.758 6	0.735 3	0.763 1	0.546 6	0.621 5	0.854 4	0.674 2	0.000 0	0.000 0	0.000 0	0.000 0
27	0.873 6	0.779 3	0.762 5	0.805 1	0.705 3	0.798 6	0.679 6	0.727 8	0.848 5	0.886 8	0.844 9	0.860 0
28	0.893 3	0.830 7	0.804 5	0.842 8	0.731 4	0.890 5	0.607 5	0.743 1	0.892 4	0.935 3	0.888 9	0.905 6
29	0.767 3	0.810 7	0.904 3	0.827 4	0.815 3	0.956 4	0.602 9	0.791 5	0.940 3	0.942 0	0.931 1	0.937 8
30	0.820 7	0.816 6	0.844 0	0.827 1	0.000 0	0.000 0	0.000 0	0.000 0	0.000 0	0.000 0	0.000 0	0.000 0
31	0.898 3	0.845 5	0.794 4	0.846 1	0.808 9	0.903 3	0.609 9	0.774 0	0.000 0	0.000 0	0.000 0	0.000 0
32	0.924 3	0.801 4	0.682 1	0.802 6	0.810 7	0.771 4	0.633 6	0.738 6	0.000 0	0.000 0	0.000 0	0.000 0
33	0.874 3	0.861 2	0.814 4	0.850 0	0.673 5	0.798 0	0.677 3	0.716 3	0.000 0	0.000 0	0.000 0	0.000 0
34	0.837 2	0.860 2	0.849 5	0.849 0	0.786 3	0.845 3	0.621 7	0.751 1	0.000 0	0.000 0	0.000 0	0.000 0

4 Discussion

Fingerprint of traditional Chinese medicine is a quantifiable multi-component comprehensive analysis method. Its notable feature is that it has an overall comprehensiveness and can quickly screen out possible active ingredients of drugs. The mathematical multivariate statistical analysis can combine the "spectrum" of the fingerprint with the "effect" of the drug effect test, fully relate the relationship between the composition and effect of the traditional Chinese compound medicine, and can more accurately determine the material basis of its drug effect[16]. GQE fingerprint was established by UPLC, and the chromatograms of GQE, GQE I and GQE II extracts were compared. 34 fingerprint chromatographic peaks were screened out by comprehensive analysis of drug components. In accordance with the results of anti-inflammatory drug effect, the main effective components in GQE were screened by grey relational analysis. The results showed that although the number of total components of GQE II was the least, the correlation degree for fingerprint chromatographic peaks was the highest, 7 components were preliminarily analyzed by comparison with chromatogram, and the area of chromatographic peaks accounted for about 65% of the total peak area. At the same time, the content of 7 components was calculated. It was found that the content of baicalin, wogonoside, coptisine hydrochloride, berberine hydrochloride and liquiritin was relatively high. It can be considered that these components had the greatest correlation with anti-inflammatory drug effects, and the dosage and drug effects are positively correlated. In addition, baicalein and wogonin accounted for a large proportion of GQE and GQE I, and were positively correlated with anti-inflammatory drug effect. Therefore, it was speculated that baicalin, wogonoside, coptisine hydrochloride, berberine hydrochloride, liquiritin, baicalein and wogonin in the extract were the main anti-inflammatory substances of GQE, which can be used as the main ingredients for accurate evaluation of drug effect, and also provide a theoretical basis for the study of drug production technology. Through further analysis, it was found that these components were flavonoids and alkaloids from licorice, *S. baicalensis*, *C. chinensis*. At the same time, referring to the literature[17-18], it was found that the main anti-inflammatory components in Gancao Xiexin Decoction were also derived from licorice, *S. baicalensis*, *C. chinensis* and other medicinal materials, and the experimental results were consistent with the main components reported in the literature.

Anti-inflammation is a research focus of the immune response[19] and plays a central role in the regulation of health and disease of the body's tissues. The persistence of inflammation is the pathological basis of acute and chronic gastritis, inflammatory bowel disease, and upper and lower gastrointestinal diseases[20]. LPS is the main component of the cell wall of Gram-negative bacteria, which can induce cell activation, regulate the production of different inflammatory cytokines, and lead to inflammatory reactions. In the initial stage of inflammatory reaction, NO has anti-inflammatory effect and can reduce the occurrence of inflammatory reaction; when the inflammatory response intensifies, the NO is released in large quantities, leading to further deterioration of the inflammatory response[21]. In this experiment, the NO content of the cells in the model group increased sharply, and the cell activation reaction was violent. After receiving different components of drugs, the NO content decreased to varying degrees, suggesting that GQE, GQE I, GQE II had inhibitory effects on the secretion of NO. TNF-α can regulate the activity of NOS system-related enzymes, promote NO synthesis, and intensify cascade reactions at all levels of inflammation. It is one of the most important cytokines to promote inflammatory response, and can cause acute inflammatory diseases and cause tissue damage. IL-10 is an anti-inflammatory landmark cytokine in the inflammatory response, which can inhibit the inflammatory response and the immune response of Th1 cell mediators, and repair the damaged tissues of the body[22]. The balance of anti-inflammatory factors and pro-inflammatory factors in inflammation is broken, which is an important factor causing inflammatory response. Therefore, the use of drugs to reduce the production of pro-inflammatory factors or increase the production of anti-inflammatory factors has an inhibitory effect on the inflammatory response. The results of this experiment showed that the content of cytokines TNF-α and IL-10 in the model group significantly increased, the mRNA expression level of TNF-α and IL-10 sharply increased, the balance of anti-inflammatory and pro-inflammatory cytokines was destroyed, and a typical cellular inflammatory reaction was formed. High, medium dose GQE groups and high, medium and low dose GQE II groups could significantly reduce the secretion of pro-inflammatory factors TNF-α and NO, and inhibit the mRNA expression of TNF-α, while high dose GQE I group only reduced the secretion of NO and the mRNA expression of TNF-α, but had little effect on the secretion of TNF-α, so it was inferred that the effect on cell activation was relatively small. At the same time, GQE, GQE I, GQE II basically had no effect on the secretion and mRNA expression level of anti-inflammatory factor IL-10. It was inferred that GQE, GQE I, GQE II mainly reduced the production of pro-inflammatory factors in the process of inhibiting inflammatory reaction. Therefore, the correlation study of this test mainly analyzed the correlation degree between fingerprint chromatographic peaks and inflammatory factors NO, TNF-α.

To sum up, the anti-inflammatory activity of GQE, GQE I and GQE II was in the order of GQE II > GQE > GQE I. It was speculated that the anti-inflammatory active components of GQE II were flavonoids and alkaloids. The anti-inflammatory action mode of GQE II was mainly by inhibiting the large amount of NO secretion, reducing the secretion and mRNA expression of pro-inflammatory factor TNF-α, so as to achieve the effect of inhibiting inflammatory reaction. According to the production technology of the preparation, it was considered that the treatment effect of GQE extraction and decoction was good.

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