Study on the Mechanism of Gastrodia Rhizoma "FEATURE Identification based Quality Assessmen" based on Neuroprotection

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Abstract

Background: The theory of "Feature Identification based Quality Assessmen" is the essence of traditional Chinese medicine experience identification, and the theory is to identify the quality of Chinese Materia Medica by its properties. The mechanism of evaluating quality through "Feature " has not been clarified.

Methods: We used artificial intelligence sensory technology electronic nose, electronic tongue and other instruments to quantitatively determine the “feature” include "Shape, Color, Qi, taste" of Gastrodia Rhizoma. The relationship between fingerprint of chemical constituents and protective effects on OGD/R injury of SH-SY5Y cells of 30 batches of medicinal materials was analyzed, and the Pharmacodynamic Components Group (six compounds) of *Gastrodia* Rhizoma were determined. In vitro and in vivo pharmacodynamic experiments confirmed that the Pharmacodynamic Components Group had good protective effects on OGD/R injury of SH-SY5Y cells, spinal injury of zebrafish and cerebral ischemia of rats, and could be widely distributed in rats.

Results: The Pharmacodynamic Components Group could represent more than 90% of the whole pharmacodynamic effect of *Gastrodia* Rhizoma, which showed that the Pharmacodynamic Components Group was basically equivalent to the crude drug. Through the correlation analysis of “feature” and Pharmacodynamic Components Group, we revealed that the mechanism of “Feature Identification based Quality Assessmen” of Gastrodia Rhizoma was related to the six components of the Pharmacodynamic Components Group.

Conclusions: The mechanism of "Feature Identification based Quality Assessmen" of *Gastrodia* Rhizoma based on "Feature" is that "Shape", "Color", "Qi" and "Taste" of *Gastrodia* Rhizoma have significant correlation with the content of Pharmacodynamic Components Group. This study provides a new way of thinking for the interpretation of the scientific connotation of " Feature Identification based Quality Assessmen" and the quality evaluation of traditional Chinese medicine

1 Background

"Feature Identification based Quality Assessmen"(FIQA) [1] is the traditional experience identification theory of Traditional Chinese Medicine (TCM) varieties. It is to judge the quality of TCM according to its "Feature", such as "Shape", "Color", "Qi" and "Taste", and to clarify the essence of its quality. The "FIQA" of TCM has been formed through thousands of years of practice, which has been confirmed by many years of practice. It is a recognized standard for evaluating the quality of TCM in the field of TCM at present and a classic for identifying the quality of TCM. However, there are few reports on the internal mechanism of "FIQA", so "FIQA" has not been recorded as the standard of quality identification of TCM by legal institutions. The "Feature" in the theory of "FIQA" of TCM usually refers to the external macroscopic character. However, the team's previous research found that the micro shape microscopic characteristics of TCM are related to the quality of TCM [2–3]. For this reason, shape includes macroscopic and microscopic characteristics. In order to reveal the mechanism of FIQA of traditional Chinese medicine, it is necessary to clarify the correlation between the feature of traditional Chinese medicine and its internal effective components, However, current research mode of searching for effective components does not reflect the overall characteristics of multi-component synergistic effect of TCM. The quantification of feature is sensory evaluation, so it can’t objectively and accurately evaluate the quality of traditional Chinese medicine.

In order to reveal the close relationship between "Feature" and quality of TCM, and to explain the essence of "FIQA", *Gastrodia* Rhizoma (GR), a Orchidaceae plant, was selected as the research object in this experiment. GR is the dry tuber of the orchid plant *Gastrodia elata* BL., modern pharmacological studies have shown that GR has analgesic, sedative, anticonvulsant, anti-inflammatory, anti-aging, memory improving and immune enhancing effects [6–8]. Among them, neuroprotective effect is the main effect of GR [9–10]. The traditional standard of GR is "big, yellow and white, solid and heavy, with parrot mouth shape, bright cross-section and strong smell" [11], especially the "horse urine taste strong" of GR has become the generally accepted judgment standard in the current circulation field to evaluate the quality of GR. Based

on the above, this experiment is based on the neuroprotective effect, through the artificial sensory intelligence technology, to quantify the "Shape", "Color", "Qi", "Taste" of GR. Secondly, HPLC and LC-MS were used to establish the fingerprint of GR and to identify and quantify the common components. Finally, pharmacodynamic components group (PCG) of GR which can represent the overall efficacy of medicinal materials were screened through the Spectrum-Effect relationship, and verified by the in vitro and in vivo effects and absorption distribution. Through the correlation analysis of "Feature-Quantity-Effect", the mechanism of "FIQA" of GR was explained. This study provides new ideas for the interpretation of the scientific connotation of the theory of "FIQA" and the standardization of characters, as well as new thinking modes and new technical routes for the quality evaluation of TCM. At the same time, it provides the basis for the determination of quality markers of traditional Chinese medicine

2 Methods

2.1 Instruments, Experimental drugs and Animals.

2.1.1 Instruments.

OLYMPUS DP-72 microscope digital camera(Olympus Co., Ltd, JAPAN); Leica RM2126RT microtome(Shanghai Meisheng automation equipment Co., Ltd, Shanghai, CHINA); SC-10 colorimeter(Shenzhen sanenchi Technology Co., Ltd, Shenzhen, CHINA); Heracles II ultra fast gas chromatography electronic nose(Alpha MOS, FRANCE); Astree electronic tongue(Alpha MOS, FRANCE); Agilent 1100 high performance liquid chromatograph(Agilent Technology Co., Ltd, Beijing, CHINA); Waters 2690 analytical high performance liquid chromatograph(Milford, MA, USA); Three stage four stage mass spectrometer(Thermo Finnigan, USA); ECOSIL 120-5-C_{18}(4.6mm × 250 mm, 5 μm)chromatographic column (Guangzhou green herbal Biotechnology Co., Ltd, Guangzhou, CHINA); SANYO MCO175 Carbon dioxide incubator(Sanyo company, JAPAN); Type MR-96A Microplate Reader (Beijing hengaode Technology Co., Ltd, Beijing, CHINA); XB-K-25 blood cell counting board(Shanghai Qiujing biochemical reagent Instrument Co., Ltd, Shanghai, CHINA).

2.1.2 Experimental drugs.

30 batches of GR (Table 1) was collected. They were identified as the dried tuber of *Gastrodia* Rhizoma Bl. by Professor Zhai Yanjun from the teaching and Research Office of Liaoning University of traditional Chinese medicine.

Table 1
30 batches of GR from different sources.

No.	Region	Lot number
1	Guizhou Bijie	20160401
2	Guizhou Dafang	20160419
3	GUizhou Dejiang	20160401
4	Guizhou Dafang	20160420
5	Guizhou Dejiang	20160612
6	Yunnan Shaotong	20160401
7	Yunnan Shaotong	20160419
8	Yunnan Wenshan	20160420
9	Yunnan Lijiang	20160612
10	Sichuann Aba	20160401
11	Sichuan Qingzhou	20160419
12	Sichuan Liangshan	20160420
13	Sichuan Guangyuan	20160612
14	Shanxi Hanzhong	20160401
15	Shanxi Qinling	20160612
16	Shanxi Ankang	20160420
17	Shanxi Shangluo	20160612
18	Anhui Yuexi	20160325
19	Anhui Huoshan	20160419
20	Anhui Jinzhai	20160420
21	Anhui Anqing	20160612
22	Hubei Huanggang	20160401
23	Hubei Yichang	20160420
24	Hubei Xiangyang	20160612
25	Henan Nanyang	20160419
26	Henan Nanyang	20160612
27	Dabeishan	20160612
28	Jilin Changbaishan	20160419
29	Gansu Longnan	20160420
30	Hunan Huaihua	20160420

Adenosine, and protocatechuic acid (Protocatechuic) were obtained from the China Institute of Food and Drug (test batch number 110879–200202, 110809–201205, Purity ≥ 98%). Gastrodin, 4-Hydroxybenzyl alcohol, vanillin, parishin C, parishin

B, and parishin A were bought from Bioko Beijing Century Biotechnology Co., Ltd. (batch numbers: 110807, 111970, 150906, 150718, 150329, 150108, purity ≥ 98%). p-Hydroxybenzaldehyde (Hydroxybenzaldehyde) was purchased from the Beijing Mexican Altar Quality Technology Co., Ltd. (batch number 20151220, purity ≥ 98%). Eugenol was obtained from the Heng Yuan Kai-day Beijing Institute of Chemical Technology. (batch number 15052013, Purity ≥ 98%). Penicillin/Treptomycin double resistance solution was purchased from Shanghai sur Biotechnology Co., Ltd (batch number 15140-122). Gastrodia Tuder Halimasch Tablets were purchased from Fujian Sanming Tiantai Pharmaceutical Co., Ltd (batch number 20160501). Ginkgo biloba dropping pills were purchased from Zhejiang Wanbang Pharmaceutical Co., Ltd (batch number A01J151066). Acridine orange dye purchased from sigma Aldrich (Shanghai) Trading Co., Ltd (batch number 94-38-2). Human myeloneuroblastoma cell line (SH-SY5Y) was provided by Shanghai cell bank of Chinese Academy of Sciences (Shanghai, CHINA).

2.1.3 Animals.

24 hpf wild AB strain Zebrafish embryo was provided by Hangzhou Huante Biotechnology Co., Ltd (Batch number htsw20170622, htsw20170703, htsw20170731, htsw20170904). Sprague-Dawley (SD) rats (sex in half, 10–12 weeks old, weighing 200–220 g) were obtained from the Liaoning Changsheng Biotechnology co., Ltd. (Benxi, China). Animal welfare and experimental procedures were strictly in accordance with EU Directive 2010/63/EU for animal experiments and the Guidelines of the Committee on the Care and Use of Laboratory Animals of China (Liaoning University of Traditional Chinese Medicine, license: SYXK()2015-0009). Efforts were made to minimize the pain of animals and reduce the number of experimental animals. Reporting of this work complies with ARRIVE (animal research: reporting of in vivo experiments) guidelines. Before administration of drugs, the rats were fasted for 24 h with free access of water.

2.2 Sample Preparation

The GR(No.5) was extracted according to the literature method [13]. The extract was volatilized and added with the culture solution to prepare a solution with first the concentration (0.4, 0.08, 0.008, 0.004, 0.002 and 0.001 g/ml), second concentration (high 0.25 mg/ml, medium 0.1 mg/ml and low 0.05 mg/ml), third concentration (1 g/ml) according to the calculation of the original medicinal materials for standby.

By using the method of preparing liquid phase, the PCG selected by "spectrum effect" analysis were collected and prepared into the PCG solution and with the concentration of 0.4, 0.08, 0.008, 0.004, 0.002 and 0.001 g/ml respectively.

The other components were collected after the PCG were removed by the method of preparing liquid phase. After volatilization, the culture medium was prepared into a solution with the concentration same as above.

2.3 Quantitative analysis of "Feature" of GR.

2.3.1 Quantitative analysis of "Shape ".

Macro "shape". Each batch of GR was randomly taken 6 pieces, and the longest, widest and thickest parts were measured with Vernier Caliper, and weighed on electronic balance. The results of each batch of GR were recorded and averaged.

Microcosmic "shape". The GR power passing No. 5 sieve was selected and accurately weighed 200.0 mg in 6 parts. Chloral hydrate was added to the GR powder of each group, which was grinded and transferred to 10 ml volumetric flask for many times. 7 ml of glycerol was added to each group of samples, and the volume was fixed to the scale with chloral hydrate. After fully shaking the sample solution before each sampling, 0.08 ml of the solution was accurately absorbed, and 50 pieces were made in parallel, and the sclerenchyma cells were counted under the microscope.

Calculate the Microscopic Characteristic Index (MCI) according to the following formula.

MCI=(X × V)/ (V'×W) [12]

MCI (Quantity/mg)

X Number of microscopic characteristics of medicinal materials under each cover glass

V Total volume of quantitative drug suspension (mL)

V Volume of drug suspension under cover glass(mL)

W Weight of medicinal materials(mg,)(mg, Calculated by dry product)

2.3.2 Quantitative analysis of "Color", “Qi”, “Taste”.

The GR powder passing No. 3 sieve was sprea in a measuring dish for measurement, and the measured chromaticity values L*, a*, b* were recorded respectively. Accurately weighed 1.5 g GR powder passing No. 5 sieve was placed in a 20 ml electronic nose special headspace bottle. After automatic injection, each sample was tested 3 times, and each sample was tested 3 times in parallel. After treatment as required, GR sample was directly placed in a special beaker (25 ml) for electronic tongue measurement. Each sample was tested three times, and the response values of seven sensors were obtained on the asree electronic tongue according to the test procedure.

2.4 Quantitative analysis of chemical constituents of GR.

HPLC Gradient elution method was used to determine the common peaks of fingerprints from different sources of GR and the content of related effective components [13]. The adjusted mass spectrometry conditions were used. The injection volume was 10 μL. The structure of the common components of GR were demarcated according to the results.

2.5 Protective effect on OGD/R injury of SH-SY5Y.

The cells were divided into blank control group, normal control group, model group, treatment group and positive control group. According to the method of literature [13], the model of Oxygen Glucose Deprivation and Reoxygenation (OGD/R) was copied, and chemical components of twelve fingerprints of GR were separated. The protective effect of common components on the injury of SH-SY5Y OGD/R was determined.

The preparation methods of blank group, model group, positive control group and test group were as “2.2”.

According to the method of "2.4.1", the protective effect of PCG and other components on the injury of SH-SY5Y OGD/R were determined.

2.6 Protective effect on Zebrafish embryo OGD / R injury.

The embryos were incubated with water at 28 ℃. After Ao staining in vivo, the juveniles of Zebrafish were exposed to 0.64 mmol/L tricacaine methanesulfonic acid, and killed under anesthesia. The procedure of anesthesia was in accordance with the requirements of American Veterinary Association (AVMA). The model of Zebrafish embryo OGD/R injury was duplicated. Gastrodia Tuder Halimasch Tablets were ground and added with culture solution to prepare a solution of 0.005 g/ml. Ten 24 hpf Zebrafish were put into each hole of the 6-hole cell plate and randomly divided into normal control group, model group and treatment group. According to the literature operation [14–17], we used Image J processing software to process the photos taken, count the fluorescence absorption intensity of the injured spinal nerve cells of Zebrafish, and calculated the fluorescence intensity and neuroprotection rate. The medicinal material was GR 5.

2.7 Effect on infarct volume of midbrain in rats with local cerebral ischemia.

The model of persistent local cerebral ischemia induced by middle cerebral artery in infarcted rats was duplicated [18–25]. 90 SD rats (half male and half female) were randomly divided into normal control group, model group, positive drug group, No.5 GR extract group (high, medium and low dose), and PCG group (high, medium and low dose). After intraperitoneal

injection of Pentobarbital Sodium (dose 50 mg/kg) for 15 minutes, the cervical vertebrae of mice were dislocated when the respiratory rate was reduced and the touch reaction was basically unresponsive. After the brain was taken out, thick sections were prepared, stained with hematoxylin and eosin, and photos were taken. The infarct area was calculated by Image J image processing software. Infarct volume was calculated according to infarct area. Infarct volume = t (A1 + A2 + An) (t is slice thickness, A is infarct area).

2.8 Study on absorption and distribution in vivo.

SD rats were randomly divided into 10 groups (3 rats in each group) and given 4 ml of test solution by gavage. Blood samples (0.5 mL) were collected in heparinized tubes from the orbital vein, then the rats were killed at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 h after administration. The brain, heart, liver, spleen, lung and kidney were collected. After treatment, HPLC was injected to determine the content of effective components at each time point [26].

2.9 Statistical treatment.

The experimental results were analyzed by SPSS 19.0 software system, and the calculated data were expressed by mean ± standard deviation. If the variance is uniform, the data were designed with one-way ANOVA and LSD follow-up test. If the variance is not uniform, Welch was used for analysis, and Dunnett T3 was used for multiple comparison. $P < 0.05$ was the significant difference.

3 Results

3.1 Results of quantitative analysis of "Feature".

3.1.1 Determination results of "Shape".

The results of macroscopic shape show that (Table 2), there were some differences in the length, width and thickness of GR from different producing areas. The length was 86.1–116.0 mm, the width was 19.88–34.62 mm, and the thickness was 8.18–22.72 mm.

Table 2
Results of macroscopical "shape" determination of GR (n = 6).

No.	Length (mm)	Width (mm)	Thickness (mm)	Weight (g)
1	106.9 ± 10.8	25.70 ± 4.12	14.26 ± 2.24	27.50 ± 5.12
2	92.82 ± 8.41	24.56 ± 2.78	14.66 ± 1.96	37.81 ± 2.87
3	102.5 ± 10.3	22.62 ± 1.99	10.24 ± 0.74	24.21 ± 2.04
4	90.16 ± 7.01	25.16 ± 2.24	10.50 ± 0.87	26.10 ± 1.06
5	95.34 ± 8.04	30.70 ± 2.30	18.64 ± 1.44	29.63 ± 2.08
6	81.10 ± 7.01	27.74 ± 1.11	16.22 ± 0.86	26.72 ± 1.84
7	84.02 ± 8.07	23.54 ± 1.44	9.66 ± 0.87	24.54 ± 1.33
8	116.0 ± 9.98	34.62 ± 2.14	17.56 ± 1.08	44.29 ± 2.07
9	59.34 ± 4.44	19.88 ± 1.11	9.32 ± 6.87	10.66 ± 0.96
10	89.12 ± 7.74	30.62 ± 2.07	22.72 ± 1.66	37.45 ± 2.47
11	96.32 ± 8.87	21.72 ± 1.97	13.42 ± 0.55	21.50 ± 1.01
12	102.1 ± 8.79	32.02 ± 1.08	12.12 ± 0.96	34.28 ± 1.46
13	89.80 ± 7.86	25.36 ± 2.21	13.58 ± 1.18	26.75 ± 1.97
14	76.52 ± 6.64	22.20 ± 1.12	9.58 ± 1.05	19.84 ± 2.03
15	91.62 ± 7.89	30.54 ± 2.21	8.18 ± 0.78	25.99 ± 2.21
16	88.60 ± 7.99	26.20 ± 1.98	15.22 ± 1.07	25.21 ± 3.22
17	120.4 ± 10.5	21.38 ± 1.66	10.30 ± 0.87	27.66 ± 1.64
18	86.68 ± 6.98	35.18 ± 2.21	14.84 ± 1.05	42.63 ± 2.96
19	100.2 ± 10.4	23.20 ± 3.18	11.12 ± 0.96	25.96 ± 2.21
20	86.64 ± 6.88	26.20 ± 1.08	10.18 ± 0.55	23.24 ± 1.98
21	82.02 ± 7.77	30.58 ± 2.54	10.90 ± 0.97	20.50 ± 1.06
22	106.8 ± 9.47	28.12 ± 2.22	16.86 ± 1.47	47.14 ± 3.52
23	90.60 ± 7.71	25.46 ± 2.21	12.52 ± 1.44	25.25 ± 2.52
24	70.78 ± 5.28	20.16 ± 1.74	10.38 ± 0.87	14.96 ± 1.54
25	81.20 ± 7.88	20.26 ± 1.32	11.80 ± 0.97	20.08 ± 0.97
26	85.28 ± 7.39	25.76 ± 1.23	9.10 ± 0.87	21.60 ± 0.82
27	82.28 ± 8.49	25.22 ± 2.22	11.08 ± 0.87	22.74 ± 1.04
28	87.32 ± 9.04	35.40 ± 2.41	22.38 ± 1.07	53.84 ± 3.85
29	70.58 ± 6.65	25.92 ± 2.22	10.60 ± 0.87	21.94 ± 2.26
30	86.80 ± 7.71	27.84 ± 2.35	11.50 ± 0.99	28.52 ± 2.35

The results of microcosmic shape showed that (Table 3) there were significant differences in the microcosmic characteristic indexes of GR from different sources, among which the highest was No.7, reaching 74.66, and the lowest

was No.14, 13.23. The results might be related to the quality of GR.

Table 3
Results of MCI of sclerenchyma in GR (n = 3).

No.	Mean value of MCI(quantity /mg)	RSD (%)	No.	Mean value of MCI9(quantity /mg)	RSD (%)
1	22.82	0.22	16	43.77	0.18
2	40.58	0.19	17	33.22	0.17
3	24.66	0.32	18	32.56	0.08
4	36.45	0.1	19	46.36	0.15
5	25.29	0.23	20	50.42	0.11
6	27.75	0.28	21	53.29	0.11
7	36.87	0.27	22	27.49	0.27
8	33.84	0.17	23	23.87	0.3
9	30.23	0.55	24	37.59	0.41
10	45.19	0.17	25	30.46	0.2
11	18.92	0.44	26	74.66	0.07
12	26.01	0.45	27	56.64	0.46
13	22.74	0.3	28	20.15	0.42
14	13.23	0.54	29	26.85	0.69
15	31.28	0.19	30	33.21	0.56

3.1.2 Determination results of "Color".

The results (Table 4) of color analysis showed that the b* value of GR was greater than a * value, and the a * value color was close to the standard white, indicated that the color of GR was yellow and white, which was consistent with the description of "surface yellow white to yellow brown" in Chinese Pharmacopoeia (2015 Edition). It showed that it was feasible to determine the color of GR by using the Color Difference Instrument.

Table 4
− 1 Determination of the surface color of GR (n = 6).

NO.	ΔL*	Δa*	Δb*	ΔE*	NO.	ΔL*	Δa*	Δb*	ΔE*
1	-25.67	2.41	11.25	28.13	16	-28.40	2.93	12.10	31.00
2	-27.91	5.31	13.77	31.57	17	-28.69	4.53	13.28	31.94
3	-31.61	6.74	13.74	35.12	18	-27.60	3.37	12.02	30.29
4	-25.03	5.25	13.51	28.93	19	-28.98	4.49	13.18	32.15
5	-25.32	4.05	12.68	28.60	20	-27.27	4.02	12.55	30.29
6	-26.36	4.07	12.89	29.63	21	-23.74	4.83	14.43	28.20
7	-25.85	4.09	13.47	29.43	22	-25.14	2.95	12.27	28.13
8	-26.86	1.43	11.42	29.23	23	-29.38	5.13	13.50	32.74
9	-28.45	3.15	11.68	30.92	24	-24.90	2.79	11.69	27.65
10	-24.24	4.50	13.73	28.21	25	-30.19	3.85	12.28	32.82
11	-26.70	5.13	13.95	30.56	26	-31.34	3.43	11.51	33.56
12	-24.65	4.51	13.44	28.42	27	-28.44	3.48	13.06	31.49
13	-26.44	3.47	12.56	29.48	28	-32.15	2.61	11.27	34.17
14	-28.09	4.72	13.67	31.60	29	-26.50	3.10	11.42	29.02
15	-26.83	2.67	12.05	29.53	30	-23.80	4.49	13.24	27.61

Table 4
− 2 Determination of section color of GR (n = 6).

NO.	ΔL^*	Δa^*	Δb^*	ΔE^*	NO.	ΔL^*	Δa^*	Δb^*	ΔE^*
1	-28.70	6.41	12.37	31.90	16	-32.96	6.30	12.37	33.02
2	-28.89	6.84	13.57	32.64	17	-24.09	7.32	15.84	31.39
3	-28.57	7.49	13.95	32.66	18	-25.34	6.58	13.32	29.37
4	-29.50	8.46	14.67	34.02	19	-23.61	7.32	14.31	28.56
5	-31.23	6.78	12.78	34.42	20	-27.39	6.93	13.73	31.41
6	-31.79	5.71	12.76	34.73	21	-26.76	8.41	16.01	32.30
7	-29.33	7.77	15.27	33.97	22	-32.99	3.73	11.12	35.01
8	-29.12	6.96	13.46	32.82	23	-30.40	5.53	12.60	33.37
9	-33.07	5.62	12.24	35.71	24	-34.70	4.22	12.17	37.01
10	-33.15	5.90	12.91	36.06	25	-30.85	6.56	13.11	34.15
11	-28.76	3.94	14.72	32.55	26	-31.34	6.19	10.37	33.59
12	-31.20	6.76	14.27	34.99	27	-31.89	4.67	12.51	34.11
13	-27.92	6.18	14.73	32.17	28	-38.75	3.16	10.27	40.21
14	-25.40	7.35	15.25	30.52	29	-34.91	5.10	11.98	37.26
15	-28.47	6.82	14.90	32.85	30	-35.88	1.58	11.08	37.59

Table 4
− 3 Determination of powder color of GR (n = 6).

NO.	ΔL*	Δa*	Δb*	ΔE*	NO.	ΔL*	Δa*	Δb*	ΔE*
1	-4.40	13.23	15.48	20.84	16	38.60	37.86	7.11	38.60
2	3.47	24.81	9.84	26.96	17	4.76	28.64	9.07	30.42
3	2.37	28.94	9.68	30.61	18	0.87	18.30	12.98	22.45
4	-0.58	15.58	14.21	21.10	19	5.63	39.42	6.98	40.13
5	5.18	23.47	9.14	25.72	20	6.25	44.66	6.23	45.52
6	-2.94	22.50	16.54	28.08	21	1.44	27.15	11.66	29.58
7	-0.79	20.16	14.76	25.00	22	-5.32	14.29	16.71	22.62
8	-2.22	18.74	15.61	24.49	23	-2.72	17.83	16.58	24.50
9	-0.76	24.58	13.88	28.24	24	-2.80	17.36	15.70	23.57
10	-0.95	25.17	14.51	29.07	25	-3.05	15.33	15.88	22.28
11	-0.29	21.11	15.04	25.92	26	-3.54	16.13	16.74	23.52
12	-0.49	22.06	15.08	26.13	27	-5.02	19.12	17.84	26.63
13	-1.36	18.05	15.43	23.79	28	-8.93	13.16	14.43	21.47
14	-2.59	16.70	16.05	23.30	29	-2.83	13.52	14.92	20.33
15	-0.68	19.17	14.41	24.00	30	-4.40	13.56	15.75	21.24

3.1.3 Determination results of "Qi".

According to the principal components calculated by SPSS, the peak areas of 30 sources of Gastrodia elata were expressed as D1, D2……D30. 19 chromatographic peaks were reduced to 6 principal components. Using SPSS software, six principal components F1, F2……F6 were obtained. The factors of new principal components obtained by principal component load are new variables x1, X2……X30 (Table 5).

Table 5
New dimension reduction variable of "Qi" components of GR (n = 3).

	F1	F2	F3	F4	F5	F6
X1	10284	3236	30245	13405	4799	-661
X2	13154	5584	20822	10868	6197	-332
X3	12958	552	18173	16477	6244	6096
X4	17995	5542	30736	19094	-15	-4980
X5	10397	4150	14419	15540	4160	3635
X6	20995	1644	13667	11889	4754	6529
X7	12576	-1573	11460	15042	1919	20945
X8	9581	8031	48164	13178	7091	1353
X9	12277	1249	17043	15402	2426	3017
X10	7570	2901	11405	16843	1449	3139
X11	5510	958	7344	14688	3726	6277
X12	12309	511	7798	14836	3931	8058
X13	6084	2492	7900	15507	129	3168
X14	8830	1801	17621	17336	2568	3145
X15	11789	40	8257	14431	440	5138
X16	2077	2943	10225	14077	1997	360
X17	6599	1200	15836	16701	1125	2576
X18	6164	4489	19776	12618	2113	-1862
X19	1297	3903	20836	18246	3581	-2987
X20	6601	2066	12514	14710	3058	1553
X21	9200	1883	11403	14157	6560	2149
X22	5077	1822	9348	12191	-770	1038
X23	14135	1824	14127	11157	3830	2003
X24	9304	1482	11698	11634	5432	3797
X25	4289	963	18165	14552	7200	1785
X26	7732	1480	12557	13629	3648	1940
X27	2625	706	14662	18735	1974	418
X28	5442	15061	108246	9638	11802	-23807
X29	8249	361	12725	16417	2900	3424
X30	21967	2749	21288	13231	51	-1551

3.1.4 Determination results of "Taste".

The results showed that (Table 6) the response values of 7 sensors identified by electronic tongue were between 5.19 and 6.35. It could be seen that the "taste" of GR can be quantified by electronic tongue technology, the method was stable and feasible, and "Qi" could be used as the basis of "FIQA".

Table 6
Determination of "taste" of GR (n = 3).

	Sour		saline	freshness		Sweetness	bitterness
no	AHS_sourness	PKS	CTS_saltiness	-NMS_umami	CPS	ANS	SCS
1	7.2	4.8	5.5	5.8	6.1	6.0	1.7
2	8.9	4.5	4.2	7.8	4.3	4.5	1.6
3	8.7	4.5	4.0	7.5	4.5	4.6	1.7
4	10.0	4.6	2.5	8.2	3.6	3.7	2.3
5	7.6	4.5	3.5	7.0	5.0	4.5	2.1
6	8.1	4.5	4.2	7.6	4.5	4.5	4.8
7	6.8	4.6	4.9	6.9	5.0	4.9	5.8
8	6.7	4.5	4.9	7.3	4.7	4.4	5.8
9	7.3	4.7	5.1	7.2	4.8	4.7	5.5
10	8.2	4.5	3.4	7.7	4.3	4.1	4.7
11	7.2	4.6	4.0	7.2	4.9	4.8	5.5
12	7.9	4.5	3.7	7.5	4.5	4.2	5.0
13	7.3	4.7	4.5	7.1	4.9	4.8	5.5
14	8.7	4.5	3.3	8.1	3.8	3.8	4.4
15	7.6	4.7	4.0	7.1	4.9	4.9	5.3
16	7.0	4.6	4.8	7.1	4.6	4.5	5.6
17	9.1	4.5	3.1	8.5	3.4	3.2	4.2
18	8.3	4.5	3.3	7.9	4.0	3.9	4.7
19	5.4	6.2	5.7	5.0	7.2	7.1	6.5
20	4.2	6.1	6.5	4.5	7.7	7.6	7.5
21	3.6	6.2	7.6	4.1	8.0	7.8	7.9
22	3.5	6.0	8.2	4.0	8.1	8.0	8.0
23	3.9	6.0	8.1	4.1	8.1	8.0	7.7
24	3.8	6.1	7.8	4.4	7.8	7.7	7.8
25	3.3	6.1	7.7	4.0	8.1	8.3	8.2
26	3.0	6.2	8.5	3.9	8.3	8.5	8.4
27	3.9	6.2	8.1	4.2	7.9	8.0	7.7
28	4.0	6.1	8.0	4.1	8.0	8.0	7.7
29	4.8	6.1	7.3	4.2	7.9	7.7	7.1
30	4.7	6.1	7.2	4.1	7.9	7.9	7.2

3.2 Quantitative analysis results of chemical components.

In the fingerprint experiment, 12 common peaks were identified, and 30 batches of common peak areas of GR from different sources were obtained (Table 7). According to the results of liquid chromatography-mass spectrometry and related literature [27–28], 12 components were determined as: Citric acid, Methyl Citrate, Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Protocatechuic acid, p-Hydroxybenzaldehyde, Vanillin, Parishin B, Parishin C, Parishin A and 4,4 '-Dihydroxydibenzyl ether. The results of cell experiments of 12 common components showed that Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Protocatechuic acid, p-Hydroxybenzaldehyde, Vanillin, Parishin B, Parishin C and Parishin A had biological activities, so the contents of the above 9 components were determined [13]. And f1-f12 represented the above 12 components respectively

Table 7
− 1 Similarity results of common peak area of GR fingerprint (A: cosine of included angle, B: correlation coefficient).

No	1	2	3	4	5	6	7	8	9	10
f1	329	653.3	505.9	163.3	520.6	333.6	384.8	387.8	173.5	500.5
f2	556	572.6	378.8	397	1191	437.2	475.7	333.3	161.6	357.1
f3	2857	2256	1862	1590	3679	1661	2056	1486	1266	1438
f4	1054	933.3	882.5	1067	2922	826.5	1205	912.6	452.5	668.2
f5	661.6	439	259.8	381.6	872.8	1661	356.5	439.8	375.4	324.5
f6	188.2	192.6	177.7	173.4	181.4	155.9	246.7	184.5	90.2	115.2
f7	1121	1105	1002	910.6	137.5	1064	917.7	795.5	450.3	788.3
f8	214.4	144.1	168.5	205.5	291.6	86.2	150.6	175.5	138.2	89
f9	2120	1606	1451	1091	2252	1368	1833	1514	858.5	829.4
f10	442.6	319.6	269.8	246	594.5	350.3	436.1	297.3	203.2	247.9
f11	2814	2298	1896	1708	3799	2112	3046	2952	1420	1519
f12	303.8	187.8	162.7	88.1	257.1	75.2	41.7	163.4	201.6	117.2
A	0.9626	0.9501	0.9476	0.9507	0.9008	0.9578	0.9703	0.9779	0.9688	0.9372
B	0.9221	0.8947	0.8895	0.8967	0.8071	0.9111	0.9452	0.9609	0.9304	0.8665

Table 7
− 2 Similarity results of common peak area of GR fingerprint (A: cosine of included angle, B: correlation coefficient).

No	11	12	13	14	15	16	17	18	19	20
f1	511.3	407.3	330.8	388.8	615.2	371.4	460.4	217.1	604.2	273.1
f2	480.4	249.4	410.2	393.5	296.1	390.5	367.5	226	363.3	359.4
f3	2369	1380	1575	1178	2241	1834	1768	947.2	1924	1578
f4	1002	659.9	1102	1850	1063	174.6	1011	399.7	607.6	545.8
f5	518.9	260.7	433	313.1	666.3	447.9	544.4	220.5	485.4	333.6
f6	171.6	151.6	198.5	489.4	263.1	436.8	169.2	86.6	134	150.9
f7	1052	902.2	790.6	962.8	868.3	973.2	998	515.2	690.2	554
f8	196.6	108.9	195.6	363.6	233	284.5	247.7	121	201.8	125.4
f9	1576	981.1	1346	1685	1827	1574	1369	773.1	1478	1154
f10	96.3	178.7	314.4	328.1	411.3	308.9	325.8	213.8	324.5	215.9
f11	2965	1464	2662	2629	3176	2541	2127	1763	2680	1984
f12	229.7	129.6	201.2	187.4	215.1	108.8	125.8	90.1	264.6	178.7
A	0.9647	0.9356	0.9757	0.935	0.9698	0.9313	0.9692	0.9679	0.9716	0.9687
B	0.932	0.8645	0.9505	0.8626	0.9384	0.8651	0.934	0.9443	0.9415	0.9378

Table 7
− 3 Similarity results of common peak area of GR fingerprint (A: cosine of included angle, B: correlation coefficient).

No	21	22	23	24	25	26	27	28	29	30	Reference
f1	238.4	244	340.6	330.1	239.9	193.6	295.7	282.9	319.6	520.4	371.2
f2	177.3	369.5	340.1	329.9	141.5	264.5	112.1	306.8	338.7	295.5	369.1
f3	1069	2290	1266	1297	779.3	1230	1027	1308	1573	1675	1682
f4	644.3	754.2	532.7	943.3	362.9	368.6	548.8	773	761.6	951.2	866.1
f5	360	759.1	591.5	402.2	232.5	294.9	248.1	876.2	654.2	494	496.9
f6	170.1	180.6	125.8	224	79.4	71.5	103	114.4	107	178.5	177.1
f7	414.3	881.8	963.2	769.1	434.8	468	490.6	985.4	94.1	918.7	767.3
f8	146.5	225.5	168.7	201.9	93.9	110.3	105.7	279.5	234.4	163.2	182.4
f9	927.7	1521	893.4	1266	656	846.8	950.2	1637	1355	1179	1330
f10	168.2	232.4	289	263.9	176	176.9	164.4	449.2	239.7	318.4	286.8
f11	1862	2312	1267	2347	1309	938.7	1143	2339	1540	2128	2158
f12	118.9	287.3	90.4	128.4	80.8	110.6	71.4	263.9	83.6	117.5	156.1
A	0.9768	0.9684	0.9493	0.9776	0.9744	0.9432	0.9633	1.003	0.9343	0.9553	0.9742
B	0.9572	0.9328	0.8902	0.9538	0.9491	0.883	0.9235	1	0.8679	0.9067	0.945

3.3 Determination of PCG.

The partial correlation statistical method was used to analyze the spectral effect relationship with 12 components of GR as independent variables and the protective effect on OGD/R injury of SH-SY5Y as dependent variable The results of the protective effects of the common components on SH-SY5Y cells (Fig. 1) showed that 9 of the 12 common components had protective effects, which were Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Protocatechuic acid, p-Hydroxybenzaldehyde, Vanillin, Parishin B, Parishin C and Parishin A, respectively.

30 batches of GR fingerprint peak area and the results (Table 8) of SH-SY5Y protection [13] were analyzed by Spectrum-Effect relationship. The results showed that D3, D4, D5, D9, D10 and D11 were the main components affecting the OGD/R injury of SH-SY5Y cells. It was preliminarily determined that there were 6 effective components related to the neuroprotection of GR (Fig. 2). The structural formula of six components were shown in Fig. 3.

Based on spectral screening and neuroprotective activities of common peaks, six components were identified as Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Parishin B, Parishin C and Parishin A.

Table 8
Results of SH-SY5Y protection of 30 batches of GR (n = 3, g/mL).

Source	Efficiency /%						
	0.4	0.08	0.008	0.004	0.002	0.001	Mean
1	101.63	106.22	99.48	95.05	92.03	94.25	98.11
2	100.31	91.26	88.31	65.65	83.89	91.22	86.77
3	97.59	93.52	96.02	95.72	95.95	91.54	95.06
4	102.06	91.77	90.29	91.53	90.34	105.63	95.27
5	48.56	72.42	57.76	103.94	103.35	23.06	68.18
6	92.44	71.81	93.84	92.77	93.01	92.88	89.46
7	69.05	131.56	93.54	94.00	93.90	90.90	95.49
8	91.07	87.08	95.26	91.86	92.59	93.38	91.87
9	95.59	84.16	72.19	90.13	90.53	97.62	88.37
10	98.09	132.31	91.57	90.07	84.88	91.93	98.14
11	75.85	54.01	102.11	108.71	104.65	107.68	92.17
12	98.27	93.01	91.62	92.04	89.99	92.58	92.92
13	205.09	87.27	80.10	82.73	83.41	86.73	104.22
14	49.98	73.07	76.42	79.07	80.87	81.75	73.53
15	96.50	70.45	76.98	83.69	92.56	85.95	84.35
16	71.27	80.17	76.27	79.43	86.39	89.28	80.47
17	66.72	82.28	91.79	84.72	93.42	87.64	84.43
18	90.17	55.60	99.39	64.90	179.74	120.81	101.77
19	72.03	63.24	220.95	204.95	69.86	84.79	119.30
20	60.94	52.37	69.80	71.50	89.84	137.75	80.37
21	40.95	59.17	86.37	84.19	91.49	42.99	67.53
22	79.44	71.86	99.62	100.83	107.03	108.74	94.59
23	81.89	38.04	88.96	77.64	91.21	93.56	78.55
24	85.38	94.67	99.74	119.60	106.09	108.23	94.59
25	105.94	80.29	104.22	108.10	98.05	104.71	100.22
26	101.06	97.11	91.79	94.15	90.71	92.84	94.61
27	110.16	77.12	109.76	104.81	106.88	105.02	102.29
28	90.23	86.22	88.44	85.81	86.32	87.75	87.46
29	88.79	86.76	89.97	88.57	91.79	90.14	89.34
30	100.64	97.85	86.66	93.46	93.39	87.99	93.33

Source	Efficiency /%						
	0.4	0.08	0.008	0.004	0.002	0.001	Mean
Mean	-	-	-	-	-	-	91.01

3.4 Validation of PCG.

3.4.1 Protective effect on OGD/R injury of SH-SY5Y.

The results showed that the selected PCG had good OGD/R injury protection effect, which could represent more than 95% of the overall efficacy of GE (Table 9). However, the efficacy of the remaining components of the PCG was only 46.4%, indicated that the protective effect of the PCG on OGD/R injury of SH-SY5Y cells could represent GE.

Table 9
− 1 Comparison of the neuroprotective effect of the PCG and GE in vitro(n = 3, g/mL).

source	Efficiency /%						
	0.4	0.08	0.008	0.004	0.002	0.001	mean
1	92.44	71.81	93.84	92.77	93.01	92.88	89.46
2	69.05	131.56	93.54	94.00	93.90	90.90	95.49
3	91.07	87.08	95.26	91.86	92.59	93.38	91.87
4	95.59	84.16	72.19	90.13	90.53	97.62	88.37
5	98.09	132.31	91.57	90.07	84.88	91.93	98.14
6	75.85	54.01	102.11	108.71	104.65	107.68	92.17
7	98.27	93.01	91.62	92.04	89.99	92.58	92.92
8	205.09	87.27	80.10	82.73	83.41	86.73	104.22
9	101.63	106.22	99.48	95.05	92.03	94.25	98.11
10	100.31	91.26	88.31	65.65	83.89	91.22	86.77
11	97.59	93.52	96.02	95.72	95.95	91.54	95.06
12	102.06	91.77	90.29	91.53	90.34	105.63	95.27
13	48.56	72.42	57.76	103.94	103.35	23.06	68.18
14	90.17	55.60	99.39	64.90	179.74	120.81	101.77
15	72.03	63.24	220.95	204.95	69.86	84.79	119.30
16	60.94	52.37	69.80	71.50	89.84	137.75	80.37
17	40.95	59.17	86.37	84.19	91.49	42.99	67.53
18	49.98	73.07	76.42	79.07	80.87	81.75	73.53
19	96.50	70.45	76.98	83.69	92.56	85.95	84.35
20	71.27	80.17	76.27	79.43	86.39	89.28	80.47
21	66.72	82.28	91.79	84.72	93.42	87.64	84.43
22	79.44	71.86	99.62	100.83	107.03	108.74	94.59
23	81.89	38.04	88.96	77.64	91.21	93.56	78.55
24	85.38	94.67	99.74	119.60	106.09	108.23	94.59
25	105.94	80.29	104.22	108.10	98.05	104.71	100.22
26	101.06	97.11	91.79	94.15	90.71	92.84	94.61
27	90.23	86.22	88.44	85.81	86.32	87.75	87.46
28	110.16	77.12	109.76	104.81	106.88	105.02	102.29
29	88.79	86.76	89.97	88.57	91.79	90.14	89.34
30	100.64	97.85	86.66	93.46	93.39	87.99	93.33

source	Efficiency /%						
	0.4	0.08	0.008	0.004	0.002	0.001	mean
mean	-	-	-	-	-	-	91.01

Table 9
− 2 Comparison of the neuroprotective effect of the remaining components and GE in vitro(n = 3, g/mL).

Source	Efficiency /%						
	0.4	0.08	0.008	0.004	0.002	0.001	Mean
1	100.73	46.96	21.09	12.17	17.09	10.40	34.74
2	18.89	12.61	10.06	31.77	42.11	30.94	24.39
3	36.79	33.19	28.86	19.58	21.64	93.92	39.00
4	59.95	35.35	27.65	48.80	69.17	382.25	103.86
5	28.52	27.90	17.69	55.42	79.14	83.46	48.69
6	37.13	38.83	62.99	59.34	40.08	38.60	46.16
7	27.15	25.13	46.66	95.95	114.36	253.27	93.75
8	25.71	20.68	42.40	6.22	7.54	17.72	20.05
9	7.24	8.40	3.10	27.72	14.88	33.11	15.74
10	87.80	7.84	18.37	12.79	8.89	1.73	22.90
11	2.31	-0.56	-1.91	1.04	78.24	18.07	16.20
12	57.81	13.67	75.02	9.56	16.78	14.93	31.30
13	71.90	9.69	20.61	27.27	51.47	54.49	39.24
14	18.80	22.93	32.10	28.04	20.77	36.39	26.50
15	45.35	28.23	98.08	1.34	-2.30	-3.64	27.85
16	23.10	12.58	7.06	21.78	25.77	90.61	30.15
17	38.52	38.41	54.65	74.33	24.74	50.41	46.84
18	43.83	31.62	73.68	71.10	205.84	159.84	97.65
19	85.12	41.76	111.39	124.60	66.97	49.02	79.81
20	40.48	31.37	51.56	121.85	47.15	75.67	61.35
21	30.02	25.76	30.82	69.43	28.94	20.98	34.32
22	1.26	0.67	20.85	9.87	16.52	18.45	11.27
23	44.94	33.89	44.43	55.85	18.15	105.51	50.46
24	39.32	8.11	12.85	18.96	103.53	21.00	33.96
25	18.55	30.23	32.54	-11.09	-0.85	-4.22	10.86
26	115.85	28.56	22.20	5.19	60.94	227.50	76.70
27	90.45	23.53	39.73	66.36	28.78	264.49	85.56
28	33.50	17.47	16.03	-7.79	22.97	29.20	18.56
29	11.99	13.14	20.65	-13.79	31.13	42.86	17.66

Source	Efficiency /%						
	0.4	0.08	0.008	0.004	0.002	0.001	Mean
30	49.36	19.35	37.41	40.92	32.14	525.66	117.47
Mean	-	-	-	-	-	-	45.43

3.3.3 Experimental results of protection of Zebrafish embryo against OGD/R injury.

The results were shown in Fig. 4 and Table 10. The results showed that the average fluorescence intensity of juveniles was 16.3 ± 1.8 in the normal group, 100.0 ± 2.5 in the model group, and 20.1–39.9 in the positive drug control group (0.05 g/ml). The effective rate of neuroprotection of the PCG was more than 92%, while the effective rate of the remaining components was less than 40%, which proved that the PCG could represent GR in the protection of Zebrafish spinal injury.

Table 10
Neuroprotective effects of different groups on zebrafish.

Groups	Dose(g/mL)	n	Relative fluorescence intensity	Neuroprotection rate	Relative protection rate
Blank	-	20	16.3 ± 1.8	-	-
Model	-	19	100.0 ± 2.5	-	-
Positive group	-	18	20.1 ± 1.7	94.5 ± 4.5	-
GR extract	0.40	20	32.9 ± 2.4	80.1 ± 3.7	-
	0.08	17	39.9 ± 4.1	71.7 ± 4.2	-
	0.008	19	36.8 ± 3.2	75.4 ± 2.8	-
	0.004	17	53.8 ± 1.8	55.1 ± 2.3	-
	0.002	17	57.4 ± 2.6	50.9 ± 3.0	-
PCG group	0.40	19	26.8 ± 3.3	87.4 ± 4.1	109.2
	0.08	18	29.9 ± 3.1	83.7 ± 2.9	116.7
	0.008	18	41.9 ± 2.7	69.4 ± 2.8	92.1
	0.004	18	43.3 ± 2.2	67.7 ± 3.8	122.8
	0.002	17	51.8 ± 4.6	57.6 ± 5.2	113.1
Remaining group	0.40	18	53.4 ± 4.2	55.7 ± 4.9	69.5
	0.08	19	69.7 ± 4.7	36.2 ± 5.3	50.5
	0.008	18	72.5 ± 3.6	32.8 ± 3.3	43.5
	0.004	17	90.9 ± 5.1	10.8 ± 4.2	19.7
	0.002	19	85.1 ± 3.6	17.7 ± 3.0	34.9

(Remarks: The relative protection rate is 100% of the ratio of the PCG and remainying ingredients to the protection rate of each concentration of GR extract.)

3.3.4 Protective effect on cerebral infarction in rats.

The results showed that (Fig. 5, Fig. 6), the PCG and GR extract had significant difference with the model group ($P < 0.05$). The infarct volume of high, middle and low dose group of GR extract and PCG were lower than that of model group, which indicated that GR extract and PCG could significantly reduce the infarct volume of hypoxic-ischemic brain tissue. The effective rate of each concentration group of PCG was similar to that of GR, indicating that the protective effect of PCG on cerebral infarction in rats could represent GR.

3.3.5 Absorption and tissue distribution in rats.

The results [26] shown that three components could be detected in plasma within 0.5–10 h after oral administration. The content of Adenosine and p-Hydroxybenzyl alcohol reached the peak value in the plasma for 2 h, and the content of Paliscin C reached the peak value in 3 h. Previous experiments showed that Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Paliscin B, Paliscin C and Paliscin A were the effective components of GE. However, only Adenosine, p-Hydroxybenzyl alcohol and Paliscin C were detected in vivo. The structure of Gastrodin contained the aglycone p-hydroxybenzyl alcohol and glucose. After entering the body, Gastrodin was degraded to aglycone p-Hydroxybenzyl alcohol and glucose, so Gastrodin was not detected. Paliscin was a kind of compound formed by Gastrodin, p-Hydroxybenzyl alcohol and their derivatives and citric acid at different carboxyl sites, which was easy to metabolize in vivo [27]. In vivo, because the substitution positions of two Gastrodin of Paliscin B were close to each other, combining with Paliscin A of three Gastrodin molecules, it was not easy to decompose the target protein due to the intramolecular crowding caused by Gastrodin [28], while Paliscin B and Paliscin A were superior to Gastrodin in metabolism [29]. The two Gastrodins in Paliscin C were more dispersed and can better combine with the target protein, thus ensuring the stability, so it could be detected in animal blood and tissues.

The results of tissue distribution showed that three components could be detected in each tissue after oral administration.

3.4 The result of the mechanism of FIQA.

The quantitative values of macroscopical "shape", microcosmic "shape", "color", "gas", "taste" and content of each component in the PCG of 30 batches of GR samples were input into SPSS statistical software respectively, and Pearson correlation analysis was used to reveal the mechanism of "FIQA" of GR.

3.4.1 The results of correlation between "Shape" and PCG.

The results of Table 11 showed that the six pharmacodynamic components were related to the macroscopic "shape". Gastrodin, Paliscin B, Paliscin C and Paliscin A were moderately related to the "Shape" of GR. Adenosine and p-hydroxybenzyl alcohol had low correlation with "Shape".

Table 11
Correlation between the “Shape” of GR and PCG

Component	Macro "Shape"		Microcosmic "Shape"	
	Pearson correlation	Significance (P)	Pearson correlation	Significance (P)
Adenosine	-0.096	0.056	0.031	0.066
Gastrodin	-0.344*	0.026	0.842**	0.000
p-Hydroxybenzyl alcohol	-0.093	0.058	-0.053	0.055
Paliscin B	-0.404*	0.027	0.620**	0.007
Paliscin C	0.419*	0.021	-0.161	0.058
Paliscin A	-0.345*	0.042	0.444*	0.020
(Remarks: *and** indicate that P < 0.05 and P < 0.01 respectively.)				
Gastrodin was highly correlated with the MCI of thick walled cells of GR, while Paliscin B was moderately correlated. According to the MCI, the contents of Gastrodin and Paliscin B can be predicted.				

3.4.2 The results of correlation between "Color" and PCG.

Table 12 showed that the brightness, yellow blue value and total color difference of GR appearance were moderately correlated with the content of Paliscin C. The brightness, red green degree and yellow blue degree of GR cross section were all moderately correlated with the content of Paliscin C. The brightness, red green value, total color difference of GR powder was correlated with the content of Gastrodin.

Table 12
Correlation between the "Color" of GR and PCG.

		1	2	3	4	5	6
surface	ΔL*	0.192	-0.069	0.022	-0.135	-0.386*	-0.017
	Δa*	0.174	0.047	-0.265	-0.175	-0.256	-0.211
	Δb*	0.071	0.027	-0.184	-0.23	-0.329*	-0.221
	ΔE*	-0.153	0.079	-0.083	0.08	0.323*	-0.044
section	ΔL*	0.032	0.075	0.051	-0.047	-0.430*	-0.141
	Δa*	0.162	0.192	0.056	0.029	-0.331*	-0.183
	Δb*	0.151	-0.079	0.095	-0.174	-0.358*	-0.213
	ΔE*	0.052	-0.133	-0.042	0.005	0.474*	0.101
powder	ΔL*	-0.113	0.322*	-0.159	-0.049	-0.189	-0.09
	Δa*	-0.076	0.467**	-0.055	0.157	-0.137	0.194
	Δb*	-0.134	-0.312*	0.12	-0.017	-0.015	-0.012
	ΔE*	-0.129	0.491**	-0.015	0.211	-0.093	0.268
(Remarks:1–6 were Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Paliscin B, Paliscin C, Paliscin A respectively, *and** indicate that P < 0.05 and P < 0.01 respectively.)							

3.4.3 The results of correlation between "Qi" and PCG.

The results were shown in Table 13. It could be seen that the main components 2, 3, 4, 5 and 6 have good correlation with Paliscin C. The main components 2, 3 were high positive correlation with Paliscin C, 6 was high negative correlation with Paliscin C, and 4, 5 were medium correlation with Paliscin C.

Table 13
Correlation between the "Qi" of GR and PCG.

	F1	F2	F3	F4	F5	F6
Adenosine	0.354	0.028	0.093	-0.131	0.197	-0.079
Gastrodin	-0.111	-0.269	-0.28	0.247	-0.144	0.100
p-Hydroxybenzyl alcohol	0	-0.017	0.02	0.070	-0.156	-0.077
Paliscin B	-0.022	-0.170	-0.106	0.116	-0.076	-0.084
Paliscin C	-0.136	0.747**	0.87**	-0.411*	0.551*	-0.736**
Paliscin A	-0.079	-0.107	-0.078	0.069	-0.119	-0.160

(Remarks: F1-F6 were the six principal components of GR flavor by principal component analysis respectively, *and indicate that P < 0.05 and P < 0.01 respectively.**)**

3.4.4 The results of correlation between "Taste" and PCG.

The results were shown in Table 14. It could be seen that Gastrodin, Paliscin B and Paliscin A had good correlation with all "taste" indexes.

Table 14
Correlation between the "Taste" of GR and PCG.

	Adenosine	Gastrodin	p-Hydroxybenzyl alcohol	Paliscin B	Paliscin C	Paliscin A
1	-0.106	-0.370*	-0.030	-0.412*	-0.257	-0.424*
2	0.183	0.415*	0.086	0.491**	0.283	0.518**
3	0.102	0.338*	0.035	0.416*	0.285	0.437*
4	-0.204	-0.360*	-0.063	-0.438*	-0.281	-0.442*
5	0.194	0.358*	0.069	0.440*	0.272	0.451*
6	0.209	0.363*	0.094	0.466**	0.272	0.464**
7	-0.081	0.303*	0.057	0.347*	0.239	0.404*

(Remarks: 1-F were AHS_sourness, PKS,CTS_saltiness, -NMS_umami, CPS, ANS, SCS respectively, *and indicate that P < 0.05 and P < 0.01 respectively.**)**

4 Discussion

4.1 Quantitative analysis of "Feature" of GR.

The four key elements of TCM characters are Shape, Color, Qi and Taste, especially the latter three are the direct expression of chemical components in the characters. Therefore, the research object of this study is "form, color, gas and taste". Among the microscopic characteristics of GR, including sclerenchyma, needle crystal, gelatinized polysaccharide, etc.,

sclerenchyma is the special characteristic of GR, which is the main difference between GR and its counterfeit. Therefore, sclerenchyma is selected as the microscopic "shape" of GR.

4.2 Quantitative analysis of chemical constituents of GR.

According to the analysis of the effective components of GR from different sources, there was a certain correlation among Gastrodin, p-Hydroxybenzyl alcohol and p-Hydroxybenzaldehyde. In terms of structure, p-Hydroxybenzyl alcohol is the aglycone of Gastrodin, and p-Hydroxybenzaldehyde is the oxidation product of p-hydroxybenzyl alcohol, which has a direct conversion relationship. From the analysis results, the content of p-Hydroxybenzyl alcohol and p-Hydroxybenzaldehyde in most of the GR plants with high content of Gastrodin was higher. This might be related to the different growth cycle of GR or the different degree of mutual transformation of effective components in different habitats.

4.3 Determination of PCG.

The neuroprotective effects of 12 common components showed that Gastrodin, Adenosine, p-Hydroxybenzyl alcohol, Paliscin B, Paliscin C and Paliscin A had better effects. According to the results of Spectrum-Effect relationship analysis, six components were selected as the PCG, which were Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Paliscin A, Paliscin B and Paliscin C.The PCG had good protective effect on OGD/R injury of SH-SY5Y cells. The effect of PCG can reach more than 90% of the whole effect of GR, the average value was 91.01%, which showed that the effective components group selected could replace the whole effect of GR. The activity of the remaining components was less than 30% of the whole effect of GR after removing the PCG, which indicated the rationality of the PCG. GR extract had a significant protective effect on the damage of Zebrafish OGD/R. The effect of the PCG was equivalent to that of the whole GR, but the effect of removing the remaining components of the PCG is relatively weak. The experimental results of infarct volume of brain tissue in the model of local cerebral ischemia showed that GR and PCG both had good protective effect. The pharmacodynamic effect of the PCG was equivalent to 98–105% of the whole effect of GR. The above experiments verified that the PCG was reasonable. Preliminary experiments showed that PCG such as Adenosine, p-Hydroxybenzyl alcohol and Paliscin C could be detected in vivo. All of the above had shown that the neuroprotective effect of PCG could represent the whole quality of GR.

4.4 Analysis of the mechanism of GR's "FIQA".

Macroscopically, Gastrodin and Paliscin A were negatively correlated with each other, and were moderately negatively correlated with Paliscin B, and moderately positively correlated with Paliscin C. The microcosmic "Shape" was highly correlated with Gastrodin in the PCG, while Paliscin B was highly correlated with Paliscin A. It could be seen that macroscopical "Shape" and microcosmic "Shape" can reflect the quality of medicinal materials. Most of the indexes of GR appearance color were moderately correlated with the content of Paliscin C. The color index of GR cross section was negatively correlated with the content of Paliscin C. The four indexes of powder color of GR have low correlation with Gastrodin. The "Qi" index of GR had a strong correlation with the group of effective components, mainly reflected in the good correlation between the main components 2, 3, 4, 5, 6 and the content of Paliscin C. The "Taste" of GR had a good correlation with the active ingredient group, which was embodied in the fact that the acid, salty, sweet and bitter taste of GR were mostly related to Gastrodin, Paliscin B and Paliscin A. Among them, acid, salty and sweet had a medium correlation with the three components, while bitter had a low correlation with the three components.

5 Conclusions

The six active components in the PCG are Adenosine, Gastrodin, p-Hydroxybenzyl alcohol, Paliscin A, Paliscin B and Paliscin C. Their effect is close to the whole effect of GR, which can represent the whole effect of GR and reflect the quality of GR. There is a significant correlation between the "Shape", "Color", "Qi" and "Taste" of GR and the PCG, which can fully demonstrate the mechanism of "FIQA".

Abbreviations

Feature Identification based Quality Assessmen FIQA

Traditional Chinese Medicine TCM

Gastrodia Rhizoma GR

Pharmacodynamic Components Group PCG

Microscopic Characteristic Index MCI

Oxygen Glucose Deprivation and Reoxygenation OGD/R

Declarations

Ethics approval and consent to participate

All animal experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Liaoning University of Traditional Chinese Medicine (license: SYXK (□)2013-0009). Euthanasia of mice conforms to the group standard of Chinese Society of Experimental Animals (T/CALAS 31-2017).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Kang TG designed the research, Wang Bing carried out the quantitative determination of *Gastrodia* Rhizoma, sun Yantao completed the pharmacological experiment of *Gastrodia* Rhizoma and its components; Zhang Hui analyzed the experimental data, and Pei Wenhan was responsible for the in vivo distribution experiment of *Gastrodia* Rhizoma.

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Not applicable.

Authors' information (optional)

References

1. Xie Zongwan. The theory of "Feature Identification based Quality Assessmen" in Traditional Experience Identification of Traditional Chinese Medicine varieties. Lishizhen Med Mater Med Res. 1994;5(3):19–21.
2. Liu Yunyun L, Li K, Tingguo, et al. Study on the Correlation between the Microscopic Characteristic Constant and Chemical Components of *Paeonia alba*. Chin Med Mat. 2010;33(4):559.
3. Chen, Conghui. Kang Tingguo. Study on the Correlation between the Microscopic Characteristic Constant and Chemical Components of *honeysuckle*. Chin Med Mat. 2011;34(9):1373.
4. Gong Jianting Z, Liying B, Bauer, et al. According to the theory of "Feature Identification based Quality Assessmen", the Traditional Chinese Medicine *Bitter Almond* leaves oil. Chin J Chin Mater Med. 2016;41(23):4375–81.
5. Wang Xiaoyu Z, Junning Wu, Ping, et al. Study on the Correlation between the Color of *Curcuma longa* and the Content of its main Chemical Components on the basis of "Feature Identification based Quality Assessmen". Chin Tradit Herbal Drugs. 2018;49(24):5929–37.
6. Zhao Min L, Li C, Xinhai, et al. A Study on the Analgesic Effect of Gastrodin Microinjection into the Nucleus Accumbens of rats on Neuropathic pain. Chongqing Medical. 2016;45(3):296–8.
7. Jun X, Ying ZJinjuan,X, et al. Effect of *Gastrodia elata* from Dejiang on Learning and Memory Ability of Scopolamine induced Memory Impairment Model mice. J Guiyang Med Coll. 2015;40(11):1194–6.
8. Wang H. Study on the Literature of *Gastrodia* in the Treatment of Headache and the Clinical Experiment of *Gastrodia Gegen Decoction* in the treatment of Solar Headache. Jinan: Shandong University of traditional Chinese Medicine; 2005.
9. Huang NK, Lin YL, Cheng JJ, et al. Gastridia elata prevents rat Pheocheomocytoma Cells from Serum-deprived Apoptosis the role of the MAPK family. Life Sci. 2004;75(13):1649.
10. Jinghong Hu, Yinchu S, Qingtao H, et al. The Protective Effect of Gastrodin on Endothelial cells in rats with Simulated Cerebral Ischemia in vitro. Chin J Tradit Chin Med Pharm. 2007;22(2):124–6.
11. Kang Tingguo. Identification of traditional Chinese Medicine. Beijing: China traditional Chinese Medicine Press; 2012. p. 211.
12. Yuan Dongmin. Study on the Correlation between the Microscopic Characteristic Constants and Chemical Components of five kinds of Traditional Chinese Medicines, such as *Phellodendron amurense* and the Microscopic Quantitative Analysis of Chinese Patent Medicine. Shenyang: Liaoning University of traditional Chinese Medicine; 2007.
13. Bing WANG, Yan-Tao SUN, Zhi-Dong PEI, Hui ZHANG, Ting-Guo KANG. Content Analysis the Compounds Found in Gastrodia elata and a Study of their Protective Effect on OGD/R Injury in Nerve Cells. Lat Am J Pharm. 2018;37(10):2092–100.
14. Widmer S, Moore FBG, Bagatto B. The effects of Chronic Developmental Hypoxia on Swimming Performance in Zebrafish. J Fish Biol. 2006;69(6):1885–91.
15. Han Liwen Y, Yanqiang H, Qiuxia, et al. Study on the Applicability of Zebrafish Model in the Activity Screening of Traditional Chinese Medicine. Chin Tradit Herbal Drugs. 2011;42(10):2037–41.

16. Wang Y, Binrui LShang,Y, et al. Application of Zebrafish model in Traditional Chinese Medicine. Chin J Integr Tradit West Med. 2012;10(11):1189–97.
17. Eimon PM, Ashkenazi A. The Zebrafish as a Model Organism for the study of Apoptosis. Apoptosis. 2010;15(3):331–49.
18. Xv Shuyun B, Rulian C. Xiu. Methodology of Pharmacological Experiment. Beijing: People's Health Press; 1985.
19. Wei W, Ximei Wu. Li Yuanjian. Methodology of Pharmacological Experiment. Beijing: People's Health Press; 2010.
20. Zhang Juntian Z. Qingzhu. Research techniques and methods of Neuropharmacology Chinese Medicine. Beijing: People's Health Press; 2005. pp. 10–3.
21. Zhang Juntian Z. Qingzhu. Research techniques and methods of Neuropharmacology Chinese Medicine. Beijing: People's Health Press; 2005. pp. 271–7.
22. Zhang Qingying C, Chuanhao S, Zhengren, et al. Anatomy of Internal Carotid Artery in rats and its Application in Cerebral Ischemia model. J Anatomy. 2002;25(6):581–3.
23. Szeplaki G, Szegedi R, Hirschberg K, et al. Strong complement activation after acute ischemic stroke is associated with unfavorable outcomes. Atherosclerosis. 2009;204(1):315–20.
24. Wu Ying C, Wenrong Wu, Chunze, et al. Establishment and improvement of focal cerebral ischemia model in rats with thread occlusion. Journal of Shantou University Medical College. 1999;12(1):23–5.
25. Yong L. Nong Weiwei. Experimental study on the model of focal cerebral ischemia-reperfusion in Wister rats. Journal of Chongqing Medical University. 2002;27(1):1–4.
26. Wang B, Yan-Tao S, Zhi-Dong P. Kang Ting-Guo and Zhang Hui. Pharmacokinetic and tissue distributions study of adenosine, 4-hydroxybenzyl alcohol and Parishin C from *Gastrodia elata* extract in rats. PakJPharmSci. 2018;31(5):2053–60.
27. Ha JH, Shin SM, Lee SK,et al. *In vitro* effects of hydroxybenzaldehydes from *Gastrodia elata* and their analogues on GABA ergic neurotransmission and a structure-activity correlation[J]. Planta Med. 2001;67(9):877–80.
28. Junko H Toshikazu, Shigeyoshi S. D,et al.Phenolic compounds from *Castrodia Rhizome* and relaxant effects of related compounds on isolated smooth muscle preparation[J]. Phytochemistry. 2002;59(5):513–9.
29. Lu Wei H, Yufang D. Xing. Study on the pathway of vanillin in Gastrodia elata into brain. Jiangsu tradit Chin Med. 2006;27(2):55–7.

Figures

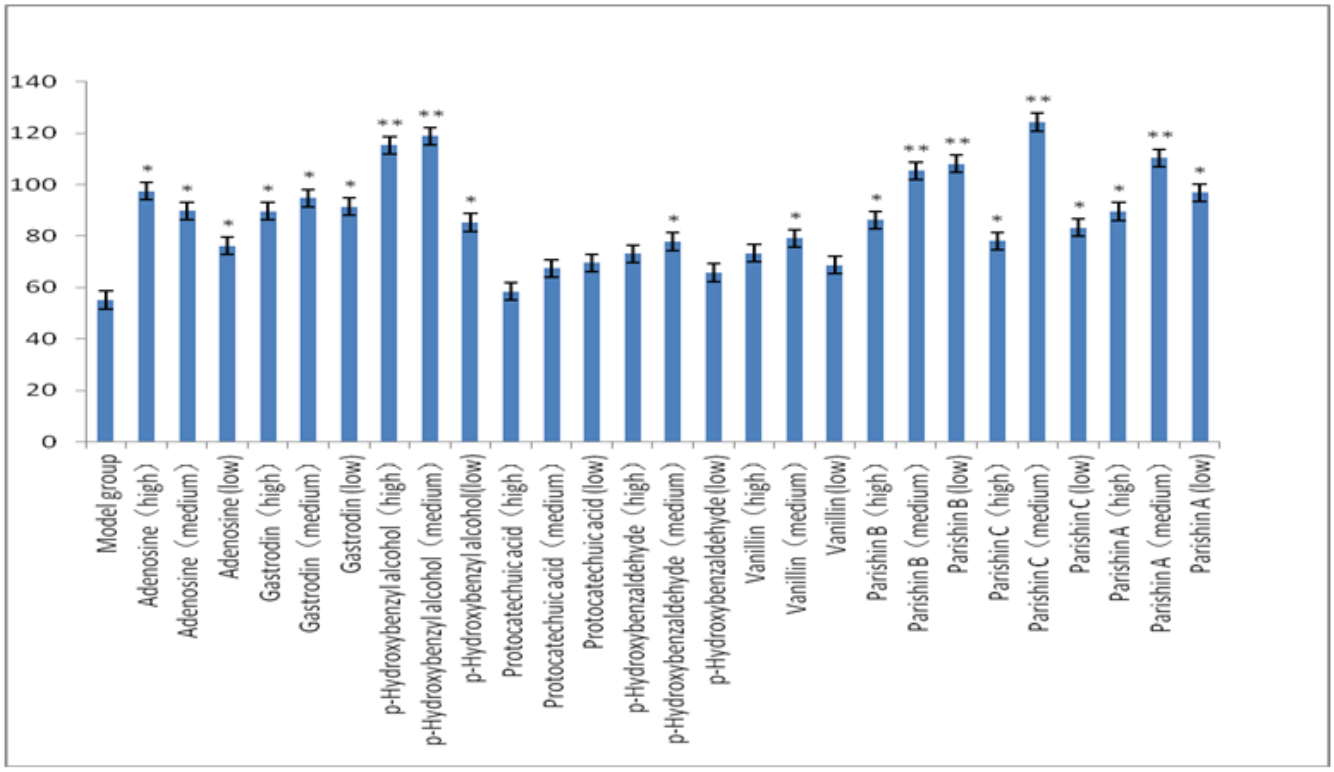
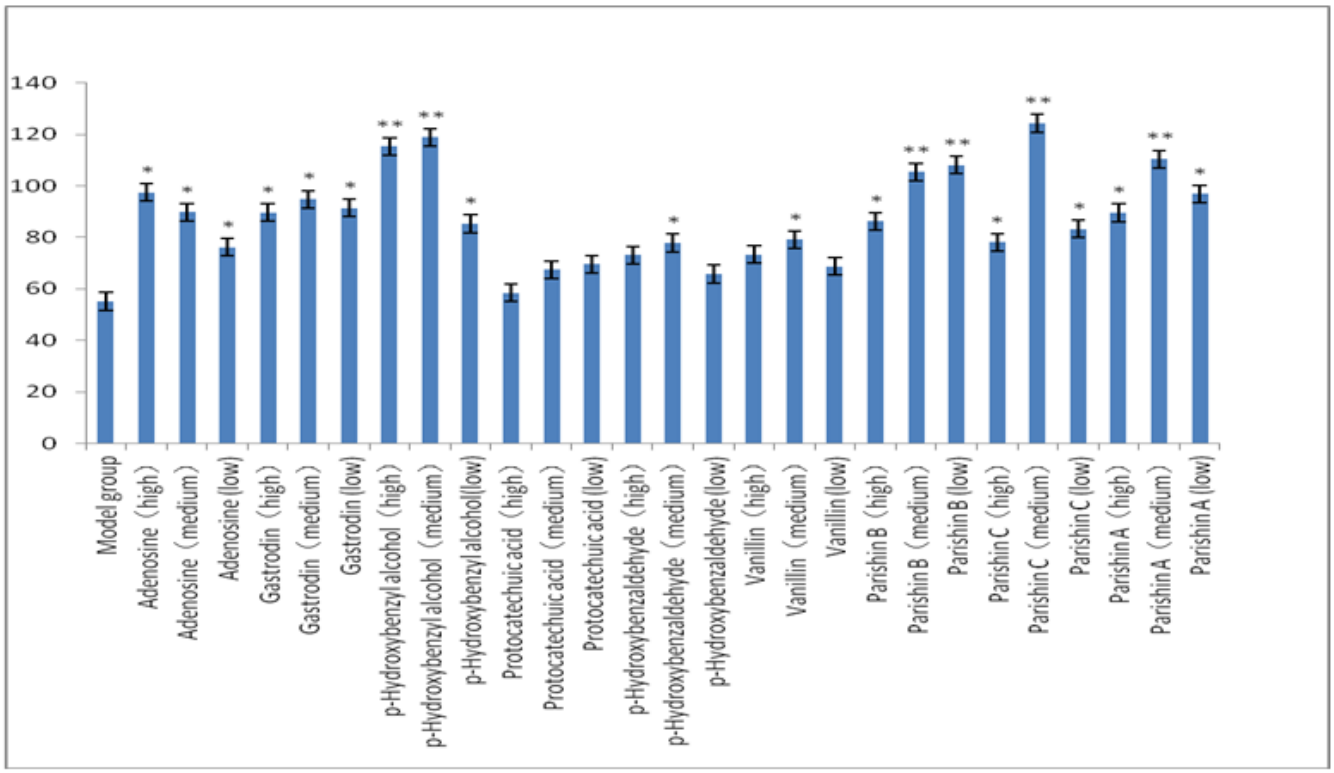


Figure 1

Protective effects of components in GR on OGD/R injury of SH-SY5Y.(*: P<0.05 was significant difference between treatment groups and the model group. **: P<0.01 was significant difference between treatment groups and the model group.)



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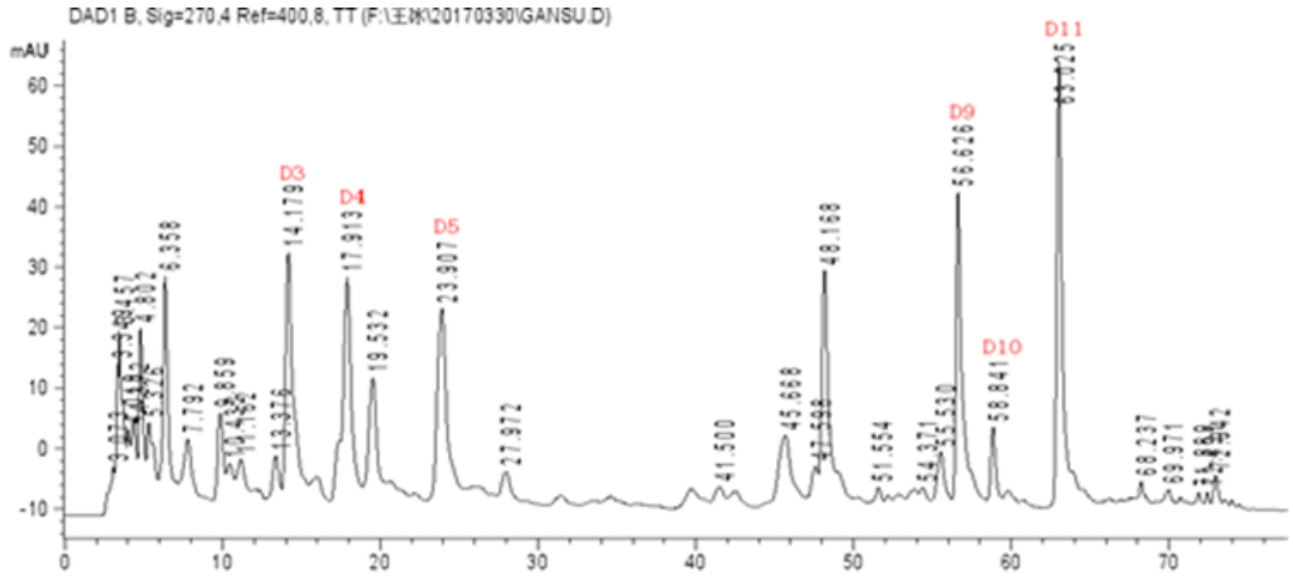
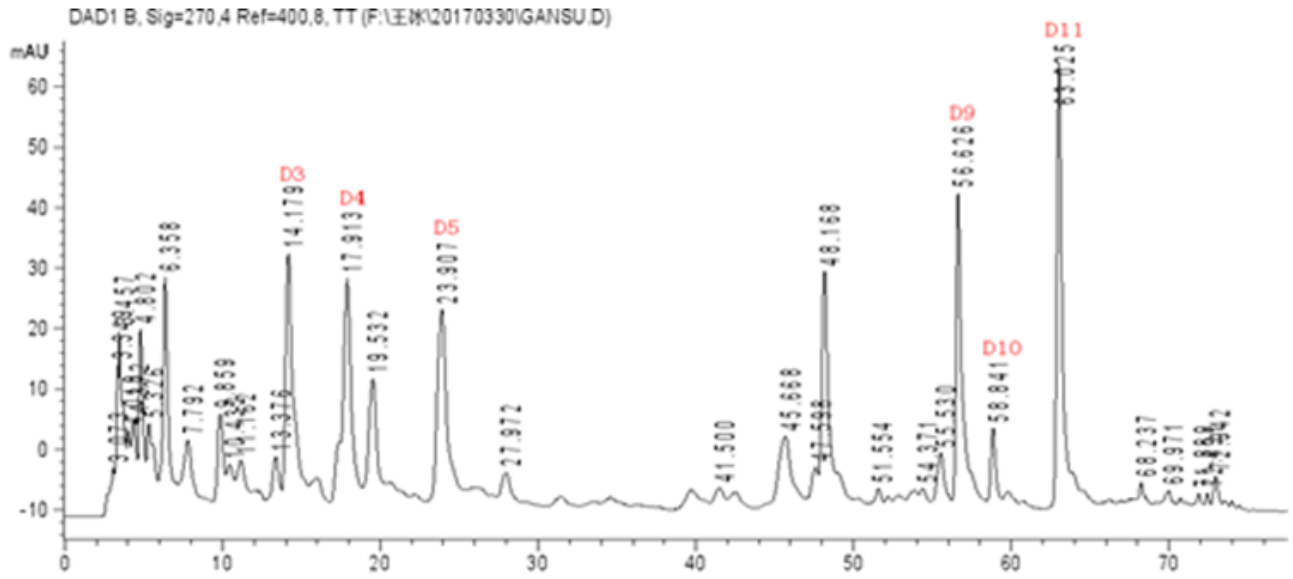


Figure 2

Schematic diagram of active components of Gastrodia Rhizoma.



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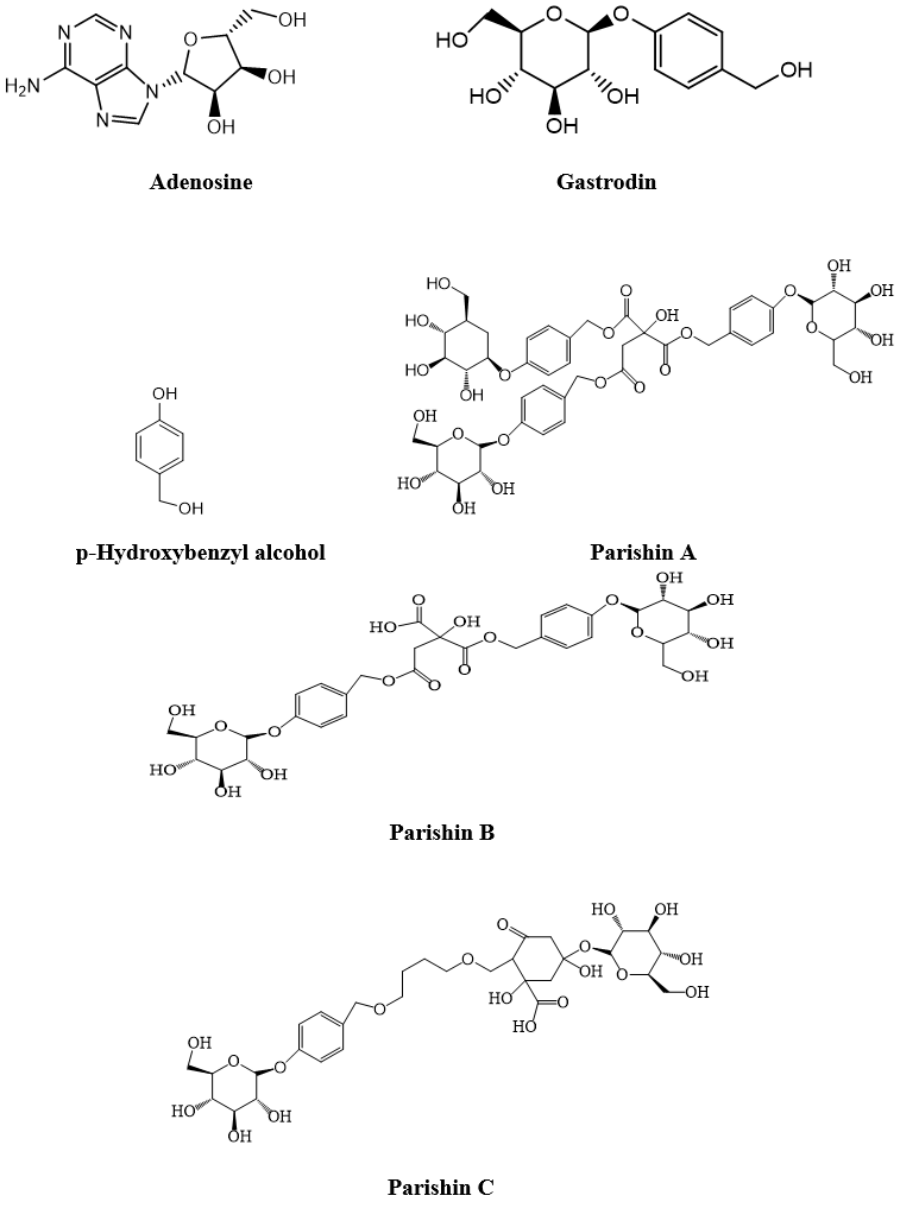
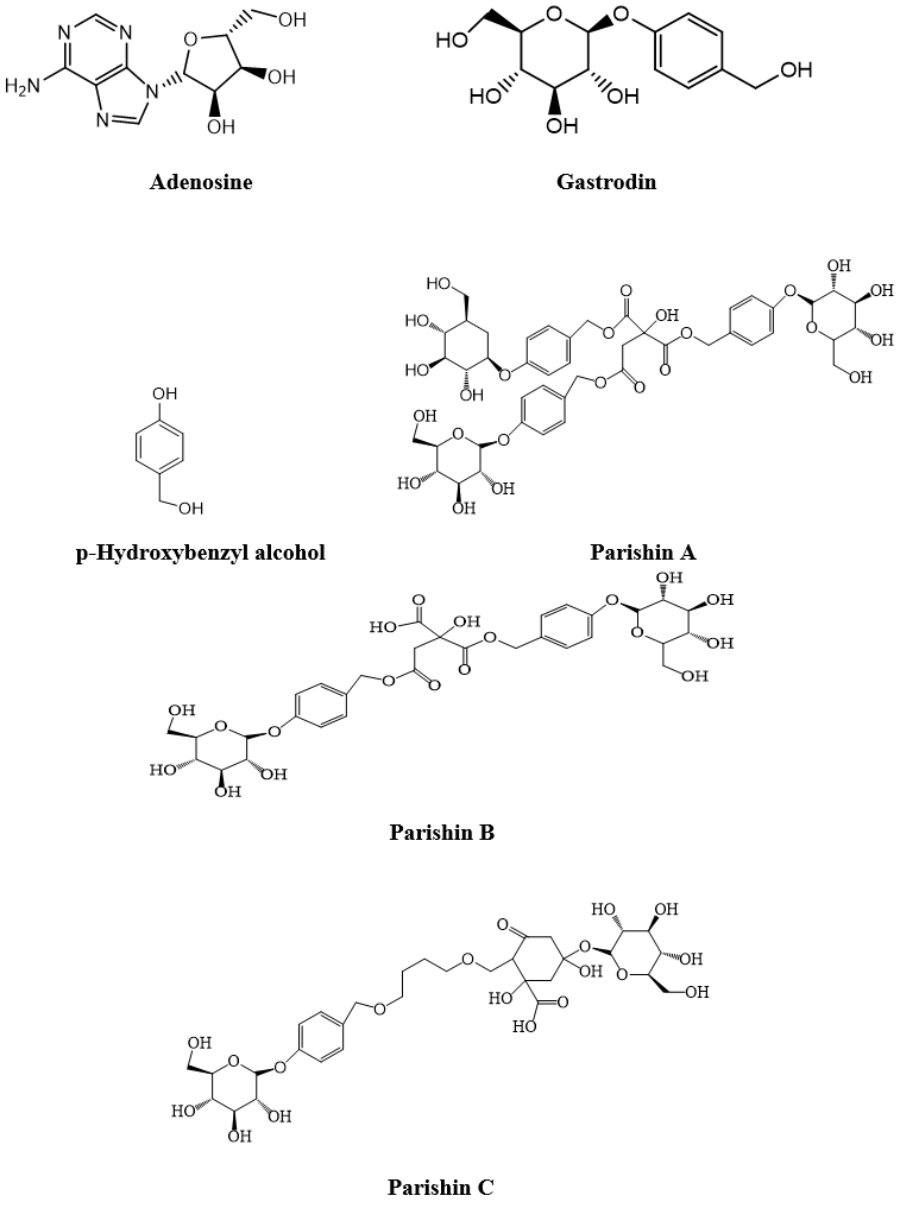


Figure 3

Structure of active ingredients.



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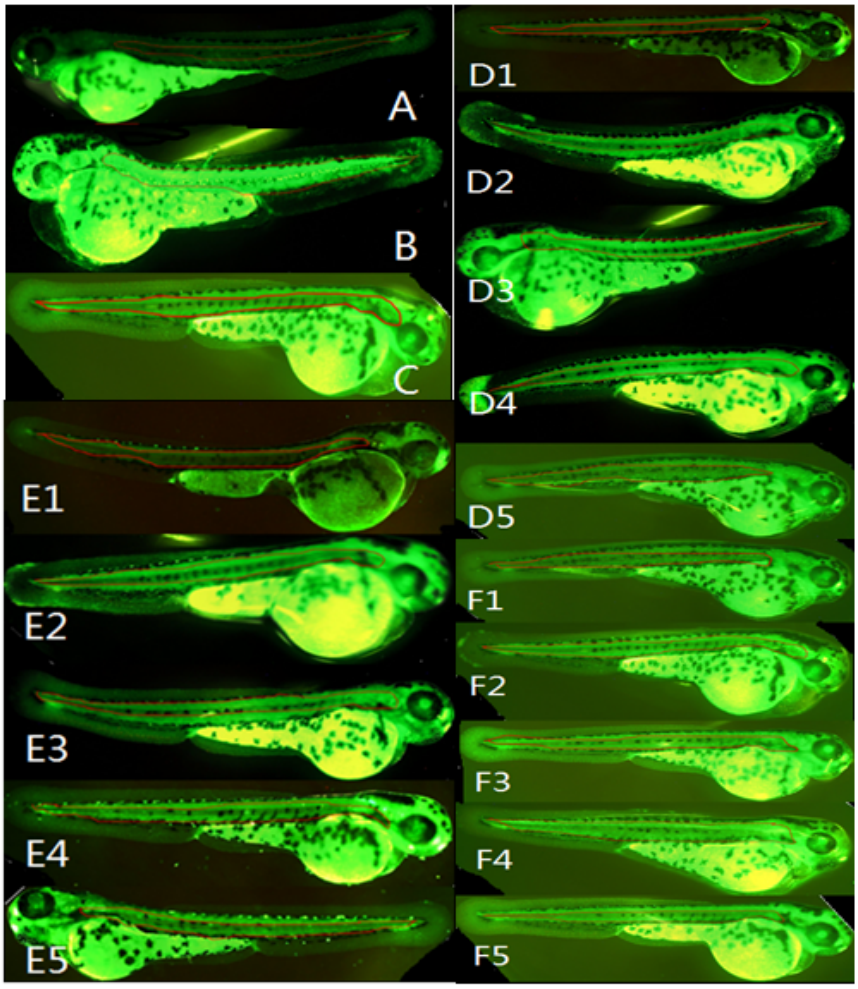
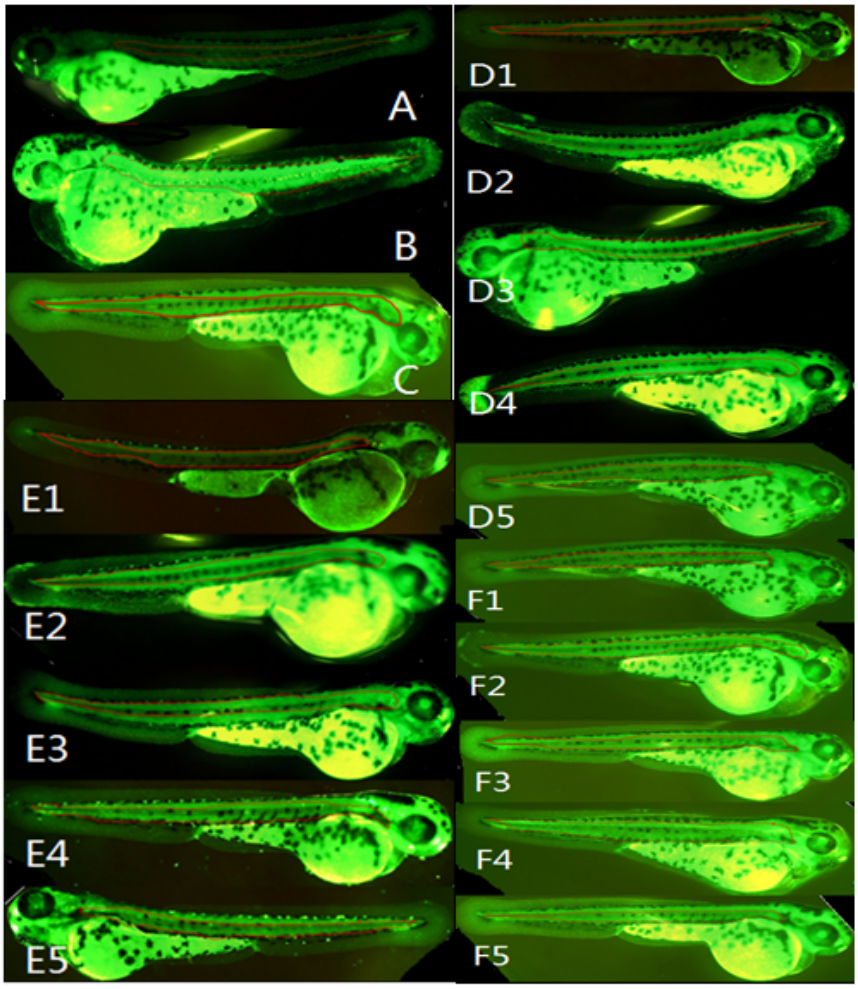


Figure 4

Neuroprotective effect of each group of drugs on Zebrafish. (A: Blank group, B: model group, C: positive drug, D: GR group, e: PCG group F: residual component group,1-5 were 0.4, 0.08, 0.008, 0.004, 0.002 g / ml respectively).



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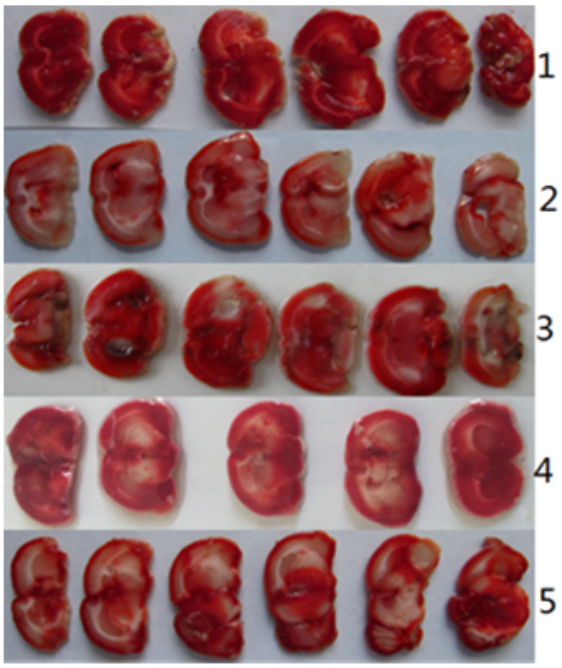
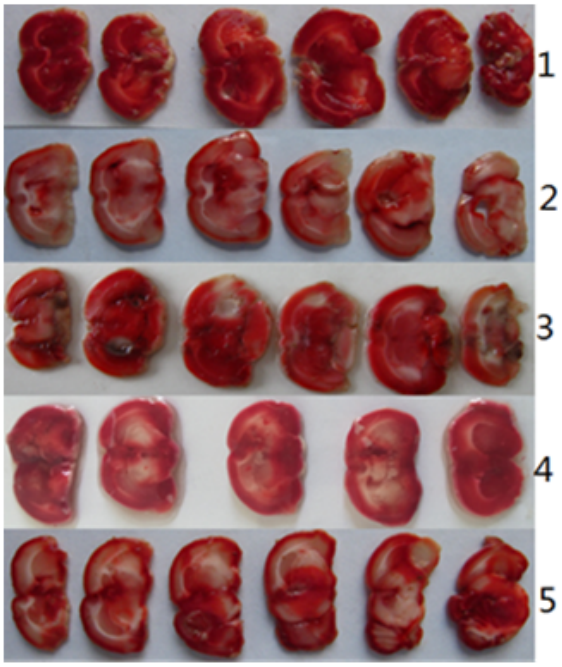


Figure 5

Brain slice of rats. (1-5 were blank group, model group, positive drug group, GR and PCG group respectively).



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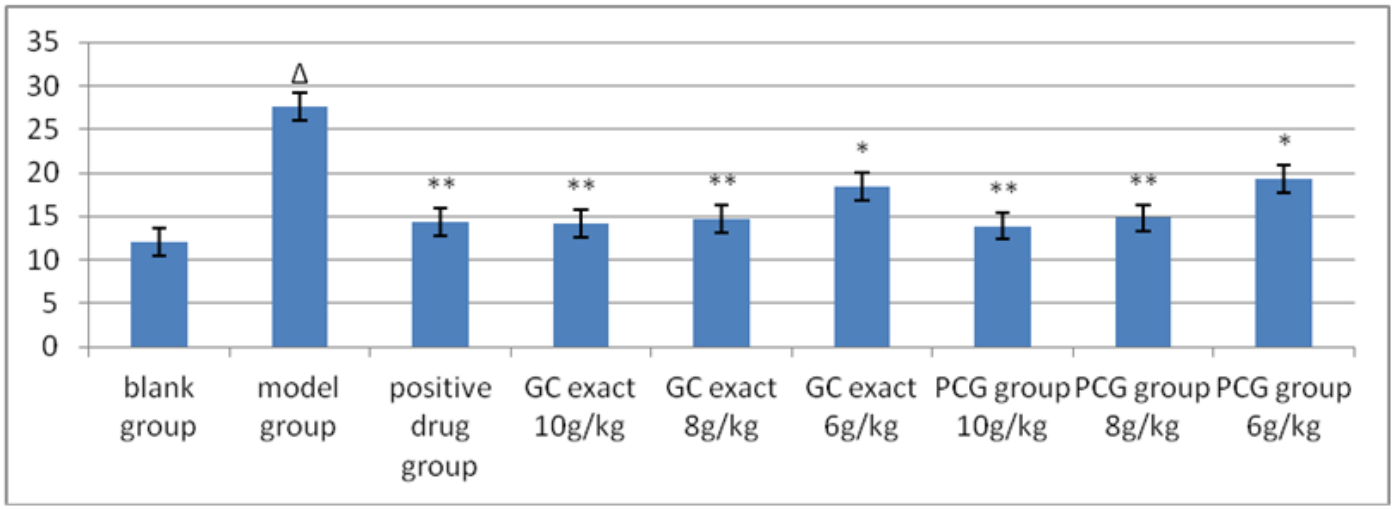
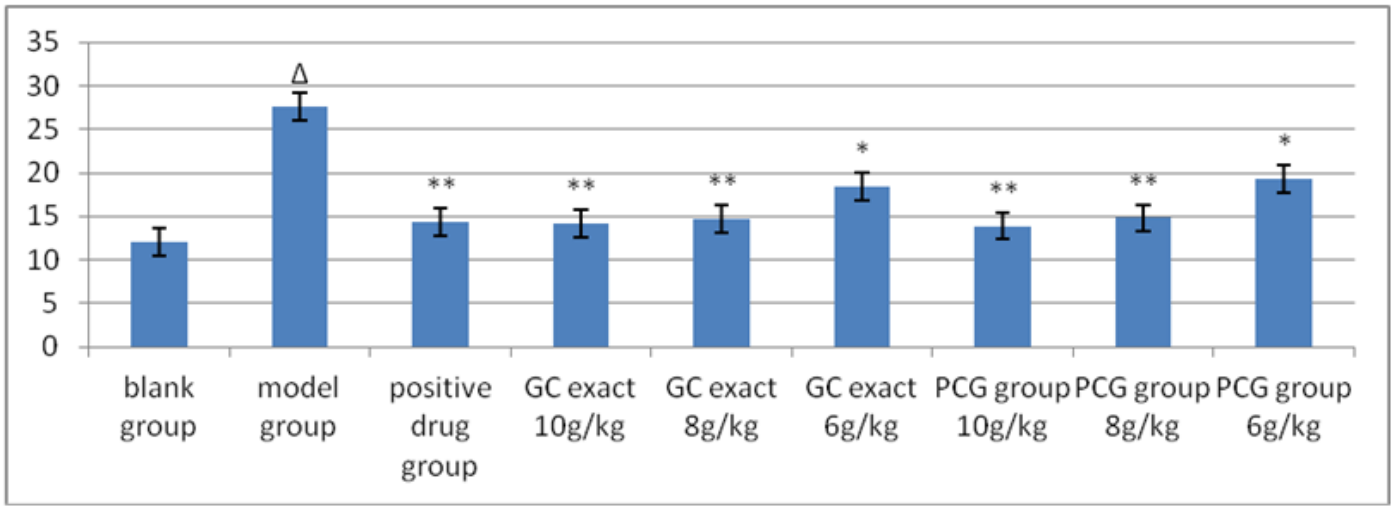


Figure 6

Protective effect of drugs in each group on cerebral infarction in rats. (Δ:P<0.05 was significant difference between model group and the blank group. *: P<0.05 was significant difference between treatment groups and the model group. **: P<0.01 was significant difference between treatment groups and the model group.)



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