Effect of regulating qi and invigorate blood circulation method on hemorrheology in rats with acute blood stasis syndrome and its network pharmacology research

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Abstract. To observe the effect of regulating qi and invigorate blood circulation method on hemorrheology in rats with acute blood stasis syndrome from the perspective of modern pharmacology, and use network pharmacology to explain its mechanism. Forty male Wistar rats were randomly divided into 6 groups. Except the blank control group, the other groups were injected with adrenaline hydrochloride and ice bath to establish the acute blood stasis syndrome model. Each group were administered Xuefu Zhuyu Decoction, Jinlingzi Powder, Taohong Siwu Decoction, and Yanhusuo Decoction, and the blank control group and model control group were given the same volume of saline, respectively. The blood rheology indexes such as red blood cell aggregation index of rats were measured. The TCMSP and Batman database were used to search for chemical components and targets of Jinlingzi Powder, and protein interaction analysis, GO enrichment analysis and KEGG analysis were performed on the common targets. The experimental results show that, compared with the model group, the blood rheology index levels of the Yanhusuo group and Taohong Siwu Decoction group are reduced, while the blood rheology index levels of the Xuefu Zhuyu Decoction group and the Jinlingzi San group are significantly reduced. The network pharmacology predicts that Jinlingzi Powder has 44 active ingredients and 34 drug-disease shared targets, and KEGG analyzes 61 signaling pathways. Conclusion: IN the case of blood stasis syndrome, the effect of regulating qi with invigorate blood circulation is better than that of regulating qi alone. Network pharmacologic prediction showed that TNF signaling pathway was strongly correlated with hemorrheology, and its action pathway was closely related to key targets IL6, TNF, CASP3.

1. Introduction
Regulating Qi and invigorate Blood Circulation is one of the common treatments in the clinical practice of traditional Chinese medicine. The combination of activating blood circulation medicine and regulating qi medicine can regulate Qi mechanism, make Qi flow and promote blood circulation, and treat qi stagnation and blood stasis syndrome [1]. The theory stems from the theory of Qi and Blood in TCM. The medical doctors of the past generations used to treat blood stasis syndrome with this method. The
clinical is often used to treat the pains of the flank and abdominal ribs, when the time is on and off, irregular menstruation, and postpartum lochia does not work.

There are many reports on the clinical research of the method of regulating qi and invigorate blood circulation, but few on its pharmacological experiment. Our research group based on the correlation between regulating qi and promoting blood circulation, this research group, taking Xuefu Zhuyu Decoction and Jinlingzi powder as carriers, discusses the influence of Liqi Huoxue Method on Hemorheology of acute blood stasis model rats from the perspective of modern pharmacology, and clarifies its mechanism by network pharmacology, and puts forward the scientificity and necessity of regulating qi and promoting blood circulation, so as to guide clinical application. Network pharmacology is based on the characteristics of traditional Chinese medicine in treating diseases with multiple targets and multiple links. Its advantage is to break the defect of a single drug corresponding to a single disease. By searching relevant databases, combining data analysis, computer simulation and other methods to build a multi-level network, and finally realizes a method to predict the drug target as a whole and the mechanism of action [2-3].

2. Materials and Methods

2.1. Animal
Forty Wistar male rats were purchased from Chongqing Tengxin Biotechnology Co., Ltd., with a body weight of (220±10) g and a mouse age of (60±5) d. They were fed adaptively for one week. The temperature of the laboratory is kept at about 25°C, and the rats are fed with basic feed, which is provided by Chongqing Jinxin Co., Ltd. (which contains 16% protein, 8% fat, and 50% carbohydrate).

2.2. Drug
Yanhusuo group: Corydalis Rhizoma (9g); Jinlingzi Powder group: Melia Fructus, (9g) and Corydalis Rhizoma (9g), these two groups were from Jin Liu Wangsu, Suwenpathogenesis qi Yi Survival Collection, Jinlingzi Powder. Taohong Siwu Decoction group: Persicae Semen (12g), Carthami Flos (9g), Angelicas Sinensis Radix (9g), Rehmanniae Radix (9g), Chuanxiong Rhizoma (5g), Paeoniae Radix Rubra (6g), Xuefu Zhuyu Decoction group: Persicae Semen (12g), Carthami Flos (9g), Angelicas Sinensis Radix (9g), Rehmanniae Radix (9g), Chuanxiong Rhizoma (5g), Paeoniae Radix Rubra (6g), Achyranthis Bidentatae Radix (9g), Platycodonis Radix (5g), Bupleuri Radix (3g), Aurantii Fructus (6g), Glycyrrhizae Radix et Rhizama (6g), these two groups were from Xuefu Zhuyu Decoction in Yilin Gaicuo by Wang Qingren. All the drugs were purchased in Zhongshan Road, Zhilin Pharmacy, and Guiyang City.

2.3. Reagent
Epinephrine hydrochloride injection, produced by Fuzhou Haiwang Fuyao Pharmaceutical Co., Ltd., a nd 1mL: 1mg/piece (batch number: 101113) was purchased and placed in Guizhou Provincial People's Hospital.

2.4. Instrument
SA-5600 automatic hemorheology tester, produced by Beijing Secide Technology Development Co., Ltd. (Model: SA-5600), Department of Laboratory, First Affiliated Hospital of Guiyang University of Traditional Chinese Medicine.

2.5. Animal grouping and administration method
Forty rats were randomly divided into blank control group, model control group, Taohong Siwu Decoction group (0.58 g/mL), Xuefu Zhuyu Decoction group (0.92 g/mL), Yanhusuo group (0.10 g/mL), Jinlingzi powder group (0.20 g/mL). Rats in each group were administration the corresponding drug solution on concentration, blank group and model group were given the same volume of normal saline with the volume of 2 mL/kg, each group was given once in the morning and evening.
2.6. Animal modeling and index detection

Rats were given the Chinese medicine decoction by intragastric administration. On the 6th day, all rats except the blank control group were injected subcutaneously with adrenaline hydrochloride (Adrenaline, AD) injection 1.0 mg/kg, which was repeated once every 4 hours, and the interval was 2 hours. After modeling, the rats in each group were fasted for 12 hours without water, and the blood was collected from the abdominal aorta with a single-use intravenous blood collection needle the next morning, and sent to the First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine for hemorhology. Carcass viscosity, whole blood viscosity and plasma viscosity. Experimental data shows that the blank control group and the model group have significant differences in the five indexes of red blood cell aggregation index, carcassone viscosity, whole blood high-cut, low-cut viscosity, and plasma viscosity \( P<0.01 \), and each index of the model group is significantly increased, indicating successful modeling.

| Group       | n   | Red blood cell aggregation index | Carcassone viscosity | Whole blood high-cut viscosity (200/s) | Whole blood low-cut viscosity (1/s) | Plasma viscosity |
|-------------|-----|---------------------------------|----------------------|---------------------------------------|-----------------------------------|-----------------|
| Control group | 6   | 8.14±0.11                       | 4.39±0.07            | 6.78±0.09                             | 55.82±0.50                       | 1.56±0.07       |
| Model group  | 6   | 8.60±0.12                       | 4.89±0.06            | 7.30±0.07                             | 57.76±0.37                       | 1.82±0.09       |

Compared with the blank control group, the model group \( ^* P<0.01 \).

2.7. Statistical analysis

The experimental data was statistically analysed using SPSS17.0 software, and the data was expressed as mean ± standard deviation. One-way analysis of variance (One-Way ANOVA) was used to analyse the significance of the differences between the experimental data, and the multiple comparisons between the means of the experimental data of each group (Multiple Comparisons). The results showed that \( P<0.05 \) was significant difference and \( P<0.01 \) was extremely significant difference.

2.8. Acquisition of active components in Jinlingzi powder

TCMSP [4] and Batman database were used to screen the chemical components of *Melia toosendan* Si *eb. et Zucc. And Corydalis yanhusuo* W. T. Wang in Jinlingzi powder. The screening criteria of TCMSP database were oral bioavailability (OB) ≥ 30% and drug-like property (DL) ≥0.18. Searched the key words "Antithrombotic agents" and "hemorheology" in the GeneCard database to download the targets related to hemorheology, and integrate them to delete the duplicate targets. Use Veen's diagram (http://jvenn.toulouse.inra.fr/app/example.html) to intersect the Jinlingzi scattered target and the hemorheology target to obtain a common target. These common targets are there are 44 related to the chemical composition of Jinlingzi powder.

2.9. Establishment of a common target PPI network

By searching the String database, 34 common targets are imported, the species is set to "human", and a PPI network of key targets is constructed. Import the resulting TSV file into Cytoscape 3.6.1 software to obtain the topological parameter Degree, Betweenness centrality, and Closeness centrality values of each target. Filter the above three topological parameter values to meet the targets that are greater than all medians, and list them in the form of a table. Go out and visualize the top ten targets in the comprehensive ranking.
2.10. Enrichment analysis of GO function and KEGG pathway

Use the DAVID (https://david.ncifcrf.gov/) database for the screened target targets for GO (Gene Ontology) enrichment analysis and KEGG pathway analysis, and use KEGG Mapper (https://www.genome.jp/kegg/mapper.html) Display the target target in the channel.

3. Results

3.1. Red blood cell aggregation index test results of each group

Compared with the erythrocyte aggregation index of the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi Powder group were significantly reduced \((P<<0.01)\); the Taohong Siwu Decoction group and the Yanhusuo group were significantly reduced \((P<<0.05)\), as shown in the table 2 shows. This indicated that Xuefu Zhuyu Decoction Quanfang and Jinlingzisan Quanfang were better than Taohong Siwu Decoction group and Yanhusuo group in improving the erythrocyte aggregation index of acute blood stasis rats.

Table 2. Measurement results of erythrocyte aggregation index of each group \(( \bar{x} \pm s)\).

| Group                           | n  | Erythrocyte aggregation index |
|---------------------------------|----|------------------------------|
| model group                     | 6  | 8.60±0.12                    |
| Xuefu Zhuyu Decoction Quanfang  | 7  | 8.29±0.07**                  |
| Taohong Siwu Decoction group    | 7  | 8.47±0.08*                   |
| Yanhusuo group                  | 7  | 8.49±0.08*                   |
| Jinlingzi powder group          | 7  | 8.32±0.05**                  |

Compared with the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi San group \(^{**}P<<0.01\). Compared with the model group, the Taohong Siwu Decoction group and the Yanhusuo group had \(^{*}P<<0.05\).

3.2. Carson viscosity test results of each group

Compared with the carcass viscosity of the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi Powder group were significantly reduced \((P<<0.01)\), and the Taohong Siwu Decoction group and the Yanhusuo group were significantly reduced \((P<<0.05)\), as shown in Table 3. This indicated that Xuefu Zhuyu Decoction Quanfang and Jinlingzisan Quanfang were better than Taohong Siwu Decoction group and Yanhusuo group in improving the carcass viscosity of acute blood stasis rats.

Table 3. Determination results of carcass viscosity of each group \(( \bar{x} \pm s)\).

| Group                           | n  | carcass viscosity |
|---------------------------------|----|------------------|
| model group                     | 6  | 4.89±0.06        |
| Xuefu Zhuyu Decoction Quanfang  | 7  | 4.51±0.14**      |
| Taohong Siwu Decoction group    | 7  | 4.80±0.05*       |
| Yanhusuo group                  | 7  | 4.80±0.04*       |
| Jinlingzi powder group          | 7  | 4.54±0.10**      |

Compared with the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi powder group \(^{**}P<<0.01\). Compared with the model group, the Taohong Siwu Decoction group and the Yanhusuo group had \(^{*}P<<0.05\).
3.3. Test result of whole blood viscosity (200/s) of each group

Compared with the model group’s whole blood high-cut viscosity (200/s), the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi Powder group were significantly reduced ($P<<0.01$); the Taohong Siwu Decoction group and the Yanhusuo group were significantly reduced ($P<<0.05$), as shown in Table 4. This indicated that Xuefu Zhuyu Decoction Quanfang and Jinlingzisan Quanfang were better than Taohong Siwu Decoction group and Yanhusuo group in improving the high blood viscosity (200/s) of a cute blood stasis rats.

| Group                              | n  | whole blood viscosity(200/s) |
|------------------------------------|----|-----------------------------|
| model group                        | 6  | 7.30±0.07                   |
| Xuefu Zhuyu Decoction Quanfang group | 7  | 6.94±0.13**                  |
| Taohong Siwu Decoction group       | 7  | 7.15±0.13*                  |
| Yanhusuo group                     | 7  | 7.17±0.03*                  |
| Jinlingzi powder group             | 7  | 6.99±0.13**                  |

Compared with the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi powder group $^**P<<0.01$. Compared with the model group, the Taohong Siwu Decoction group and the Yanhusuo group had $^*P<<0.05$.

3.4. Test result of whole blood viscosity (1/s) of each group

Compared with the model group’s whole blood low-cut viscosity (1/s), the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi Powder group were significantly reduced ($P<<0.01$), and the Taohong Siwu Decoction group and the Yanhusuo group were significantly reduced ($P<<0.05$), as shown in Table 5. This indicated that Xuefu Zhuyu Decoction Quanfang and Jinlingzisan Quanfang were better than Taohong Siwu Decoction group and Yanhusuo group in improving the low blood viscosity (1/s) of acute blood stasis rats.

| Group                              | n  | whole blood viscosity(1/s) |
|------------------------------------|----|-----------------------------|
| model group                        | 6  | 57.76±0.37                  |
| Xuefu Zhuyu Decoction Quanfang group | 7  | 56.34±0.43**                |
| Taohong Siwu Decoction group       | 7  | 57.35±0.25*                 |
| Yanhusuo group                     | 7  | 57.38±0.09*                 |
| Jinlingzi powder group             | 7  | 56.59±0.12**                |

Compared with the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi powder group $^**P<<0.01$. Compared with the model group, the Taohong Siwu Decoction group and the Yanhusuo group had $^*P<<0.05$.

3.5. Test results of plasma viscosity of each group

Compared with the plasma viscosity of the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi Powder group were significantly reduced ($P<<0.01$), and the Taohong Siwu Decoction group and the Yanhusuo group were significantly reduced ($P<<0.05$), as shown in Table 6. This indicated that Xuefu Zhuyu Decoction Quanfang and Jinlingzisan Quanfang were better than Taohong Siwu Decoction and Yanhusuo in improving the plasma viscosity of acute blood stasis rats.
Table 6. Results of plasma viscosity in each group (\( \bar{x} \pm s \)).

| Group                                      | n  | Plasma viscosity |
|--------------------------------------------|----|------------------|
| model group                                | 6  | 1.82±0.09        |
| Xuefu Zhuyu Decoction Quanfang group       | 7  | 1.66±0.06**      |
| Taohong Siwu Decoction group               | 7  | 1.74±0.02*       |
| Yanhusuo group                             | 7  | 1.75±0.03*       |
| Jinlingzi powder group                     | 7  | 1.67±0.06**      |

Compared with the model group, the Xuefu Zhuyu Decoction Quanfang group and the Jinlingzi powder group **P<<0.01. Compared with the model group, the Taohong Siwu Decoction group and the Yanhusuo group had *P<<0.05.

From the statistics of the above data, it can be seen that for the improvement of various indexes of blood rheology in rats with acute blood stasis, the effect of combining blood circulation drugs with liqi drugs is the best. The experimental results show that the Jinlingzi San group and the Xuefu Zhuyu Decoction group have a good effect on improving the blood rheology indexes of acute blood stasis model rats (P<<0.01). Tang network pharmacology reports, and the research group mainly focuses on Jin Lingzi powder. Therefore, in order to conduct a deeper research on the follow-up Jinlingzi Powder, the author or conducted a network pharmacological prediction of Jinlingzi Powder, with a view to providing direction for the next experiment.

3.6. Active ingredient screening

Using TCMSP and Batman databases, screening to obtain 19 active compounds of Jinlingzi (L. chinensis), including Myristic Acid, TorachrysoneMethyl, and Tormentate, 21-O-Acetyl Toosendantriol and other compounds. Corydalis has 47 active compounds, namely Izoteolin, 13-methyldehydrocorydalmine, Corydine, CORYDALINE, Corydalmine, (1S, 8'R)-6, 7-dimethoxy-2-methylspiro [3, 4-dihydroisoquinoline-1, 7'- 6, 8-dihydrocyclopenta [g] [1, 3] benzodioxole]-8'-ol, isocorybulbine and other compounds.

3.7. Active target and disease screening

In the Genecards database, using "Antithrombotic agents" and "hemorheology" as keywords, a total of 456 targets related to hemorheology were retrieved, and 292 targets related to Jinlingzi. The screening results changed the blood rheology Match the learning target with the related targets of Jinlingzisan and draw a Venn diagram, as shown in Figure 1. There are 34 common targets, indicating that these 34 targets participate in the common system of the active ingredient of Jinlingzi Powder-hemorheology.

![Venn diagram of golden bells scattered intersection target.](image)
3.8. Construction of key target PPI network

Search the String database online, import 34 intersection targets, set the species "human", and take the confidence = 0.400, where number of nodes = 34, number of edges = 151, average node degree = 8.88, avg. local clustering coefficient = 0.745, Expected number of edges = 41, PPI enrichment p-value <<1.0e-16, the results are shown in Figure 2. The results are imported into Cytoscape 3.6.1 software in TSV format, and the three topological parameters are calculated by obtaining the topological parameters Degree (node connection degree), Betweenness centrality (node connection degree), and Closeness centrality (node tightness degree) of each target. The medians are 9, 0.00877577 and 0.56603774, respectively. Screening targets with Degree, Betweenness centrality, and Closeness centrality greater than the median, a total of 12 key targets were obtained, and 12 targets were displayed as data, as shown in Table 7. The relationship between the top ten targets in the comprehensive ranking is shown in the network diagram. As shown in Figure 2, the darker the red, the higher the ranking and the stronger the correlation.

Figure 2. PPI network diagram of key targets.
### Table 7. Related topological parameters of PPI directly acting on the target.

| Uniprot ID | Protein names                                | Name  | Degree | Betweenness Centrality | Closeness Centrality |
|------------|----------------------------------------------|-------|--------|-------------------------|-----------------------|
| P05231     | Interleukin-6, IL-6                          | IL6   | 25     | 0.2898                  | 0.8571                |
| P01375     | Tumor necrosis factor                        | TNF   | 21     | 0.0782                  | 0.7500                |
| P13500     | C-C motif chemokine 2                        | CCL2  | 20     | 0.0503                  | 0.7143                |
| P22301     | Interleukin-10, IL-10                        | IL10  | 17     | 0.0279                  | 0.6667                |
| P42574     | Caspase-3, CASP-3                            | CASP3 | 17     | 0.0760                  | 0.6818                |
| P05362     | Intercellular adhesion molecule 1, ICAM-1    | ICAM1 | 16     | 0.0201                  | 0.6522                |
| P19320     | Vascular cell adhesion protein 1             | VCAM1 | 14     | 0.0117                  | 0.6122                |
| P00747     | Plasminogen, EC 3.4.21.7                     | PLG   | 14     | 0.0667                  | 0.6122                |
| P37231     | Peroxisome proliferator-activated receptor gamma, PPAR-gamma | PPARG | 13   | 0.0103                  | 0.6000                |
| P19838     | Nuclear factor NF-kappa-B p105 subunit       | NFKB1 | 12     | 0.0645                  | 0.5882                |
| Q96EB6     | NAD-dependent protein deacetylase sirtuin-1  | SIRT1 | 11     | 0.0089                  | 0.5769                |
| P04150     | Glucocorticoid receptor, GR                  | NR3C1 | 11     | 0.0400                  | 0.6000                |

*Figure 3. The top ten targets with strong correlation.*
3.9. Enrichment analysis of GO biological functions
A total of 133 biological processes, 14 cellular processes, 26 molecular processes, and the first 5 biological processes were analyzed by GO enrichment analysis on 34 common targets of Jinlingzi powder composite target and hemorrhology-related targets. Enrichment includes inflammatory response, response to lipopolysaccharide, response to hypoxia, cellular response to lipopolysaccharide, response to amino acid. Quick Go Inflammatory response means the immediate defense of vertebrate tissues against infection or injury caused by chemical or physical substances, while response to lipopolysaccharide is any process of state or activity (movement, secretion, enzyme production, gene expression, etc.) induced by LPS stimulus. By screening \( P \leq 0.005 \), 35 biological processes were obtained, and the first 20 biological processes were presented in Table 5. It reflects that the mechanism of action between Jinlingzi powder and hemorrhology involves many biological processes in the body, and each biological process is interrelated.

![Figure 4. Jinlingzi power-hemorrhology biological process connection diagram.](image)

3.10. KEGG pathway analysis
34 potential targets were mapped into the database for KEGG pathway enrichment analysis, a total of 61 signal pathways were obtained. KEGG enrichment analysis \( P \leq 0.005 \), screened out 27 signal pathways mainly enriched by key targets. These pathways are closely related to the mechanism of action of Jinlingzi Powder and hemorrhology, including tumor necrosis factor signaling pathway, malaria, African trypanosomiasis, trypanosomiasis (American trypanosomiasis), amoebiasis, and Influenza and other pathways. The first 20 channels with significant differences are output in the form of a bubble chart, the results are shown in Figure 5.
Figure 5. KEGG enrichment analysis bubble chart.

Cytoscape 3.6.1 software was used to visually analyze 34 common targets, diseases, active ingredients and drugs, and an interactive network of Jinlingzi powder and hemorheology was constructed [5-6]. After screening out the corresponding interacting proteins and visualizing them with different colors and shapes, you can intuitively see the network relationship between the active chemical components and the target. There are 34 round green genes, representing the disease-drug shared genes, 44 in the inverted triangle, representing the active ingredients of Jinlingzi powder, red is the disease, the prism is the medicine composed of Jinlingzi powder, and purple is the golden bellflower. The results show that the greater the degree of connectivity, the greater the shape, as shown in Figure 6.
4. Discussion

According to Traditional Chinese medicine, blood stasis refers to stagnant or stagnant blood flow, forming "evil blood", "sepsemia" and "blood leaving the meridian", which is blocked in the meristem and zang-fu organs and causes a series of diseases, namely blood stasis syndrome, or blood stasis syndrome [7], which involves a wide range of diseases, including internal and external diseases, women, children, injuries and other diseases [8].

Aiming at the blood stasis syndrome, the principle of "relieving its qi and blood, allowing it to be adjusted, and bringing peace" [9] is emphasized during treatment, that is, treating blood and regulating qi. The relationship between qi and blood in Traditional Chinese medicine is often described as "Qi is the leader of blood" and "blood is the root of qi". "Qi is the leader of blood" includes qi can produce blood, qi can make blood and qi can drink blood. Among them, qi can make blood closely related to blood stasis, that is, the circulation of blood in the veins depends on the promotion of Qi, that is, "Qi can make blood, qi stagnation means blood stasis". "Theory of Blood Syndrome Vomiting Blood" [10]: "Qi knot leads to blood coagulation"; "Where there is blood stasis, not only blocked the airway, block vitality". The promotion of heart qi, the publicity and dissemination of lung qi, and the dredge of live qi are all closely related to the operation of blood. No matter which link is dysfunctional, it can lead to the obstruction of blood flow. If blood stasis blocks the arteries and veins, the blood running is not smooth, it will also affect the normal drainage of qi, which is manifested as qi stagnation. The two influence each other, and see the evidence of qi stagnation and blood stasis. Therefore, the treatment should promote blood circulation and regulate qi.

Objective indicators in the diagnosis of blood stasis syndrome are all related to blood circulation, that is, vessels, blood and blood flow and their interactions. In a broad sense, they are all closely related to homorheology, which has become one of the important and practical means for the diagnosis of blood stasis syndrome and the study of promoting blood circulation and removing blood stasis [11]. Wu Xiaomei et al. [12] study of blood stasis syndrome is associated with the changes in the hemodynamics and microcirculation, blood flow in vascular, cycling, as a fluid, when its rheological properties change, mainly in predominantly blood viscosity abnormal homorheology indexes, all kinds of promoting blood circulation to remove blood stasis of TCM compound also had an improved the effect of blood rheol
ological property. Whole blood high-cut, low-cut viscosity, all high, indicating that the whole blood viscosity increased. Whole blood viscosity refers to the viscosity of a liquid. The greater the viscosity, the slower the flow. Conversely, the faster the flow. Increased whole blood viscosity indicates increased hematocrit or plasma viscosity, increased aggregation of red blood cells, poor deformability or elasticity of red blood cells, and hardened and rough blood vessel walls, causing increased resistance to blood flow, slowing blood flow velocity, and finally leading to blood flow stagnation and formation "Blood stasis", a disorder of blood circulation and microcirculation in the whole body or part [13], directly affects the blood supply to the organs, resulting in disease. Experiments have shown that Jinlingzi Powder has a good therapeutic effect on hemorrheology, but the mechanism of Jinlingzi Powder's intervention in hemorrheology is still unclear. According to online pharmacological predictions, Jinlingzi powder is mainly composed of Jinlingzi (L. chinensis) and Yanhusuo. The chemical components of Jinlingzi are Mandenol, Ethyl linolenate, etc., and the chemical components of Yanhusuo are Isocorypalmine, bicuculline, quercetin. Among them, studies have reported that quercetin combined with adenosine diphosphate and thromboxane A inhibitor can effectively inhibit platelet extension [14]. These active components related to hemorrheology are mainly related to IL6, AKT1 CASP3 and other genes on the TNF signaling pathway. There are also reports of TNF signaling pathway related to hemorrheology and other diseases [15-16], as shown in Figure 7.

Figure 7. TNF signalling pathway target annotation diagram.

Pharmacodynamics experiments and network pharmacology prediction results show that after modeling, the five indexes of erythrocyte aggregation index, carcass viscosity, whole blood high-cut, low-cut viscosity, and plasma viscosity are significantly increased, indicating that the model of blood stasis model success. After medication, the blood indexes of rats in each group were significantly reduced except for the blank control group and the model group. The indexes of Xuefu Zhuyu Decoction group and Jinlingzi San group were significantly different from the model group (P<<0.01) The Taohong Siwu Decoction group and Yanhusuo group were different from the model group (P<<0.05). It shows that the
use of blood circulation drugs and qi-regulating drugs can reduce the index levels of whole blood viscosity in hemorheology, and the two types of drugs are effective when used alone. The combination of Huoxue medicine and Liqi medicine can reduce the level of whole blood viscosity and other indicators, that is, Xuefu Zhuyu Decoction and Jinlingzi San have a significant improvement in blood flow in acute blood stasis rats. The effect of metamorphic index is better than that of blood circulation medicine or Qi medicine alone. Network pharmacological predictions indicate that TNF signaling pathway is closely related to hemorheology. Tumor necrosis factor (TNF), as an important cytokine, can induce multiple signaling pathways in the cell, including apoptosis, Cell survival, inflammation and immunity. After being activated, TNF is assembled into homotrimers, resulting in trimerization of TNFR1 or TNFR2 [17]. Almost all cells express TNFR1, which is the main receptor of TNF (also known as TNF-alpha). In contrast, TNFR2 is expressed in limited cells, such as CD4 and CD8 T lymphocytes, endothelial cells, microglia, oligodendrocytes, neuronal subtypes, cardiomyocytes, thymocytes, and human mesenchyme stem cell. It is a receptor for TNF and LTA (also known as TNF-beta). When binding to a ligand, TNFR mediates the binding of some adapter proteins (such as TRADD or TRAF2), which in turn initiates the recruitment of signal transducers [18]. It can be deduced from this: ① the use of blood-activating drugs or qi-regulating drugs alone can reduce the level of blood rheology indicators of the blood stasis model, and can help the blood stasis dissipate and improve the blood flow. In particular, the application effect of the medicine for regulating qi proves that the theory of "qi leads to blood" in Traditional Chinese medicine is correct and reasonable. ②The effect of reducing the index level is more clear and significant after the compatibility of activating blood circulation and regulating qi, indicating that the use of activating blood circulation is more effective than the use of activating blood circulation or regulating qi alone. Therefore, in various types of blood stasis syndrome, especially in the high incidence of cardiovascular diseases, the results of the experimental research of our research should be able to better guide clinicians to use the combination of qi and activating blood therapy to treat clinical blood stasis. The card provides an important treatment method and also lays the foundation for the follow-up research of Jinlingzi powder.

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References
[1] Gao H. Discussion on the Treatment of Blood Stasis Syndrome in "A Brief Introduction to the Golden Chamber" [D]. Shandong University of Traditional Chinese Medicine, 2008.
[2] Chen R D. Study on the Mechanism of "Guben Tongluo Decoction" for Chronic Atrophic Gastritis Based on Internet Pharmacology [D]. China Academy of Chinese Medical Sciences, 2019.
[3] Hopkins A L. Network pharmacology [J]. Nat Biotechnol, 2007, 25 (10): 1110-1111.
[4] Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines [J]. J Cheminform, 2014, 6 (1): 13.
[5] Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application [J]. Chinese Journal of Natural Medicines, 2013, 11 (2): 110-120.
[6] Sun J, Wu Y, Xu H, et al. DTome: a web-based tool for drug-target interactome construction [J]. Bmc Bioinformatics, 2012, 13(9): 1-10.
[7] Wu C Y. On Development of Researches on Blood Stasis [J]. Journal of Nanjing University of Traditional Chinese Medicine (Natural Science), 2004, 20 (3): 133-136.
[8] Huo H, Su H C. Blood Circulation the Rapeutic Clinical Applications for Cardiovascular Disease [J]. China Journal of Chinese Medicine, 2014, 29 (5): 740-742.
[9] Wu K. Sun Guozhong. Huang Di Neijing Su Wen Wuzhu [M]. Beijing: Xueyuan Press, 2007, 02.
[10] Wang M M, Li L. Tang Rongchuan Medical Book [M]. Beijing: China Traditional Chinese Med
icine Press, 1999, 08.

[11] Liao L F, Chen K J. Biomechanopharmacology Annotation of Action of Promoting Blood and Removing Blood-stasis [J]. Chinese Journal of integrated traditional and western, 2006,26 (1 0):869-870.

[12] Wu X M, Zhang M. Research Overview of Blood Stasis Syndrome and Activating Blood Circulation to Remove Blood Stasis [J]. Journal of Chinese Medical, 2005,3 (3):92-94.

[13] Wang Z. On the relationship between hemorheology and the principle of "blood stasis" in traditional Chinese medicine [J]. Journal of Shanxi traditional Chinese medicine, 2003,23 (5):426-427.

[14] Navarro-Núñez L, Lozano , Martinez C, et al. Effect of quercetin on platelet spreading on collagen and fibrinogen and on multiple platelet kinases [J]. Fitoterapia, 2010, 81 (2) : 75-80.

[15] Su S H, Zhang L, Zhang J F. Total Glucosides of Paeony Has Effect on TNF-α/P38MAPK/NF-κ B/RBP4 Signaling Pathways in Rats with Atherosclerosis and Its Regulating Effect on IL-7, IL-27 and IL-33 in Serum [J]. Journal of New Chinese Medicine, 2019,51 (07):18-21.

[16] Tang Lirong, Xu Yu'e,Wei Ying,He Xu. Uric acid induces the expression of TNF-α via the ROS-MAPK-NF-κB signaling pathway in rat vascular smooth muscle cells [J]. Molecular medicine reports, 2017, 16(5):18-21.

[17] Hao Z Y. Correlation analysis of mRNA-miRNA-circRNA expression profile of hepatocellular carcinoma HepG2 induced by cytokine TNF-α [D]. Southeast University, 2018.

[18] Qi Z L. Study on the molecular mechanism of phosphorylated HSP27 against TNF-α-induced apoptosis in HeLa cells [D]. Nanjing Normal University, 2014.