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Pharmacodynamics, metabolomics and pathological studies on mechanisms of traditional benefits of *Angelica sinensis* in blood circulation

Xiujuan Yang[a,#], Yunxiang Hai[b,#], Zhijun Yang[b], Pengqian Wang[a], Jiameng Li[a], Danli Hao[a], Miao Zhang[a], Yi Deng[b] & Feng Sui[a,*]

[a]Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, China

[b]Gansu University of Traditional Chinese Medicine, Lanzhou, 730000, China

*E-mail: fsui@icmm.ac.cn

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Angelica sinensis is a rich source of medically important active molecules that need in-depth understanding on its action mechanisms. Therefore, through pharmacodynamics, metabolomics, and network pharmacology, the traditional benefits of *A. sinensis* in blood circulation was studied using 24 randomly selected Sprague-Dawley (SD) rats. Measurement of the blood rheological parameters for whole blood viscosity (WBV) and plasma viscosity (PV), and inspection of the heart and lung tissues pathological changes were undertaken using molecular and bioinformatic techniques. Multivariate statistical analysis and establishment of the model of the relationship between metabolite expression and sample categories to test the prediction of sample categories were performed. Screening was undertaken to find the potential metabolites for *A. sinensis* to treat blood stasis syndrome and find related metabolic pathways. Active ingredients of *A. sinensis* and targets and building of an "effect component-target" network was undertaken, *A. sinensis* was confirmed to improve blood stasis syndrome in rats and improve heart and lung pathology to varying degrees. Compared with the blood stasis model group, *A. sinensis* significantly reduced WBV and PV in hemorheology ($p<0.05$, $p<0.01$) and regulated blood stasis-induced changes in 22 metabolites including alpha-D-glucose, L-isoleucine, creatine and acetylcarnitine, which are involved in the metabolism of linoleic acid, linolenic acid, phenylalanine, ascorbic acid and uronic acid. Using the network pharmacology to build a "component-target-pathway" network of *A. sinensis,* 62 active ingredients, 169 active proteins and 18 metabolic pathways were obtained, among which linoleic acid metabolism, ascorbic acid and uronic acid metabolism were consistent with the metabolic pathways obtained by metabolomics.

Keywords: Activating blood, *Angelica sinensis*, Metabolomics and pathological studies, Pharmacodynamics, Traditional benefits

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Traditional Chinese Medicine (TCM) has a broad range of medicine practices that focus on sharing knowledge on common traditional medicinal concepts and their applications. In China, the knowledge has come from a tradition of more than 2,000 years, covering various forms of herbal medicine, acupuncture, massage, exercise and dietary therapy. Majorly, various forms of herbs have been developed from indigenous plants[1,2]. Angelica, root of *Angelica sinensis* (Oliv) Diels, originally published in "Shen Nong's Materia Medica", is one of the authentic indigenous herbs with numerous traditional medicines in Gansu. Traditionally, *A. sinensis* has been used in Chinese, Korean and Japanese medicine for thousands of years. Currently, it is one of the more popular traditional medicinal herbs used in China to treat painful menstruation, to aid recovery after childbirth and fatigue or low vitality, hormonal balance, digestive support and liver detoxification[1]. This product has the functions of nourishing blood and promoting blood circulation, mainly under blood deficiency syndrome such as the blood stasis syndrome, etc.[1,2].

Blood stasis syndrome is a common type of clinical symptom in TCM. Blood stasis refers to the symptoms of poor blood flow in the human body, obstructing the blood vessels and stagnating blood outside the veins[3], which is mainly manifested as pain, lumps, bleeding, purple tongue, astringent pulse[4,5]. Blood circulation disorders are typical manifestations of blood stasis, which can lead to the occurrence of various diseases[6,7]. Therefore, the symptoms of blood stasis syndrome are complex and

*Corresponding author
#Equal first authors

wide. The symptoms are similar to those of cardiovascular and cerebrovascular disease, connective tissue disease, and hyperlipidemia[8]. Metabolomics, as an emerging discipline, mainly performs dynamic panoramic detection of changes in the body's metabolites after receiving external stimuli, which can simultaneously reflect changes in upstream genes and downstream proteins[9]. As a current global hotspot research topic, metabolomics is not only used for disease typing and diagnosis but also in TCM syndromes to provide an appropriate understanding on traditional application of natural products and herbal medicine in human health improvement. For example, modern high-performance instruments are used to correlate with TCM syndromes of blood deficiency and blood stasis. Different metabolites in different syndromes have improved the scientific diagnostic and evaluation indicators. Metabolic pathways are used to analyze the material basis and pathological mechanisms of onset of disease and to provide new ideas for the diagnosis and search for new treatment approaches. It provides a feasible method for the research of syndrome standardization[10].

Network pharmacology focuses on the "drug-target-path" multi-angle, multi-level network and uses professional visual analysis software and algorithms to carry out computer-aided screening and prediction of database information. From the level of network prediction, the drug targets are known. The effect of points and pathways, and effective verification through experiments, reveal the interaction between drugs, genes and human diseases. The research of TCM can then use the network pharmacology research platform and related technologies to associate complex traditional Chinese medicines and prescriptions with modern diseases, explore the mechanism of complex diseases through multiple links and multiple targets, and clarify the substances that traditional Chinese medicines use in curing diseases. The foundation and mechanisms provide a research means for promoting the development of traditional application of the *A. sinensis* in Chinese medicine[9,10].

A. sinensis is an abundant source of chemical diversity from which we can discover active molecules. Thus, more studies on the pharmacological mechanisms of the predominant active compounds of *A. sinensis* are needed. In addition, given that *A. sinensis* is one of the most popular traditional herbal medicines, its main therapeutic aspects warrant further investigation. This study therefore focused on providing knowledge on mechanism of *A. sinensis* traditional use in blood activation by applying multiple perspectives, including pharmacodynamics, metabolomics, and network pharmacology and provides reference for Angelica's traditional pharmacological mechanism and clinical rational application.

Materials and Methods

Material

Angelica has been identified as the root of *Angelica sinensis* (Oliv) Diels by Li Chengyi, professor of Department of Chinese Medicine Identification, Gansu University of Traditional Chinese Medicine. The materials used were compound Danshen Diwan (batch number: 16051Q, Tasly Pharmaceutical Group Co., Ltd., specifications: 27 mg per pill); adrenaline hydrochloride (batch number: 10160602; 1 $mg \cdot mL^{-1}$, Shanghai Hefeng Pharmaceutical Co., Ltd.); acetonitrile (Merck, 1499230-935); ammonium acetate (Sigma, 70221). Chloraldehyde hydrate (batch number 20160103, Tianjin Damao Chemical Reagent Factory); paraformaldehyde (batch number 20160222, Tianjin Damao Chemical Reagent Factory); fully automatic blood rheology detector (SA-6000, Beijing Sikexide Technology) Ltd). Agilent 1290 Infinity LC Ultra High-Pressure Liquid Chromatograph (Agilent); Triple TOF 5600+ Mass Spectrometer (AB SCIEX); Low Temperature High Speed Centrifuge (Eppendorf, 5430R); Waters Column (1.7 μm, 2.1 mm × 100 mm).

Angelica sample preparation

Precisely 200 g of *A. sinensis* was weighed, water was added 12 times its weight, cooked for 40 min, filtered, filtrate collected, residue extracted 2 times according to the above method, the 3 filtrates were combined, concentrated to 200 mL (containing 1 g of medicine) mL-1) and then kept in a refrigerator at 3-4°C for future analyses.

Laboratory animal and model preparation

The model animals were 24 specific pathogens-free (SPF) grade SD rats, half male and half male, weighing 180-220 g, which were sourced from the Animal Experiment Center of Gansu University of Traditional Chinese Medicine, Gansu, China, under animal license number: SCXK (Gan) 2015-0002. The experiment was commenced after one week of adaptive rearing. Acute blood stasis rat model was replicated according to the method by Li Weixia

et al.[10-12]. The normal control group was not stimulated. The model group was subcutaneously injected with epinephrine hydrochloride injection (0.8 mg kg^{-1}) 2 times on the 7th day. The interval was 4 h. 2 h after the first subcutaneous administration of epinephrine hydrochloride, the rats were placed in 0 to 1°C ice water for 4 min, removed, dried, and the body surface was again injected subcutaneously with 0.1% adrenaline hydrochloride (0.8 mg $\cdot kg^{-1}$) 2 h later. They were then fasted overnight to induce a rat model of acute blood stasis.

Grouping and administration

24 SD rats were reared for one week and randomly divided into normal control group, acute blood stasis model group and Angelica group of 8 rats each. The normal control group and the acute blood stasis model group were given 0.9% NaCl 20 mL$\cdot kg^{-1}$. According to the adult clinical dose of 0.81 g $kg^{-1} \cdot d^{-1}$, the equivalent dose was converted into the equivalent dose according to the body surface coefficient[8]. 73 mg$\cdot kg^{-1} \cdot d^{-1}$, in the administration group, angelica decoction 10.8 g$\cdot kg^{-1} \cdot d^{-1}$ was administered orally, once a day for 9 consecutive days. After the end of the administration on the 7th day, the normal control group was injected subcutaneously with normal saline, and the remaining groups were prepared with acute blood stasis model according to the method described earlier. On the 8th day after modeling, the rats were normally gavaged. Blood was collected from the abdominal aorta 30 min after the administration on the 9th day. During the administration, the changes in body weight and signs of the rats were recorded. The normal control group was marked as CG, the blood stasis model group was marked as MG and the Angelica group was marked as AG.

Biological sample collection

On the 9th day after modeling, 2 mL of blood was collected from the abdominal aorta and the blood was collected in an anticoagulated tube containing sodium heparin, centrifuged at 3000 rpm for 10 min at room temperature and the supernatant was collected and packed into a 1.5 mL centrifuge tube, each with 0.2 mL and refrigerated at -80°C for determination of metabolites in plasma. Rats were sacrificed after blood collection from the abdominal aorta, the heart and lungs were separated, and the surface of the tissue and the cavity were washed with ice-cold saline and then soaked and fixed with a 4% neutral paraformaldehyde solution. Hematoxylin and eosin (HE) stained pathological sections were made, and the tissue changes were observed under a microscope.

Chromatographic and mass spectrometric conditions

Samples were separated using Agilent 1290 Infinity LC Ultra Performance Liquid Chromatography System (UPLC) HILIC column; column temperature 25°C; flow rate 0.3 mL/min; injection volume 2 μL; mobile phase composition A: water + 25 mmol/L acetic acid Ammonium + 25 mM ammonia, B: acetonitrile; gradient elution procedure is as follows: 0-1 min, 95% B; 1-14 min, B linearly changes from 95% to 65%; 14-16 min, B linearly from 65% Change to 40%; 16-18 min, B remains at 40%; 18-18.1 min, B linearly changes from 40% to 95%, 18.1-23 min, B remains at 95%. The sample was placed at 4°C auto sampler during the entire analysis. In order to avoid the influence caused by the fluctuation of the signal detected by the instrument, the samples are continuously analyzed in a random order. QC samples are inserted into the sample queue to monitor and evaluate the stability of the system and the reliability of the experimental data.

Electrospray ionization (ESI) positive and negative ion modes were used for detection. The samples were separated by UPLC and analyzed by mass spectrometry using Triple TOF 5600 mass spectrometers (AB SCIEX). ESI source conditions after HILIC chromatography were as follows: Ion Source Gas1 (Gas1): 60, Ion Source Gas2 (Gas2): 60, Curtain gas (CUR): 30, source temperature: 600°C, Ion Sapary Voltage Floating (ISVF) ± 5500 V (both positive and negative modes); TOF MS scan m/z range: 60-1000 Da, product ion scan m/z range: 25-1000 Da, TOF MS scan accumulation time 0.20 s / spectra, product ion scan accumulation time 0.05s/spectra; the secondary mass spectrum was obtained using information dependent acquisition (IDA) and high sensitivity mode; Declustering potential (DP): ± 60V (positive and negative modes), Collision Energy: 35±15 eV, IDA setting Exclude isotopes within 4 Da, Candidate ions to monitor per cycle.

Data processing

Effects on hemorheology in rats

After the last administration, 4 mL of blood was collected from the abdominal aorta, added to a centrifuge tube together with sodium heparin, and the whole blood viscosity (WBV) was measured using a fully automatic rheometer (1, 50, 100, 200 / s). shear rate), plasma viscosity (PV) and other hemorheological indicators[13].

Spectrum preprocessing and data analysis

Raw data was converted into mzML format by Proteo Wizard and then peak alignment, retention time correction, and peak area extraction were performed using XCMS program. Metabolite structure identification searched from a self-built standard library (secondary mass spectrometry data), using exact mass matching (<25 ppm) and second-order spectrum matching, for the data extracted by XCMS, deleting missing values within the group> 50% Ion peak[14,15]. Application software SIMCA-P 14.1 was used for pattern recognition. After the data was pre-processed by Pareto-scaling, multi-dimensional statistical analysis was performed, including unsupervised principal component analysis (PCA), supervised partial least squares discriminant analysis (PLS-DA) and positive Cross Partial Least Squares Discriminant Analysis (OPLS-DA). One-dimensional statistical analyses included multiple analysis of variance, T test and volcano map.

Result

Effects on Rat Whole Blood Viscosity (WBV) Plasma viscosity (PV), Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) on blood stasis rats

Compared with the control group, there was a statistically higher value of WBV (1, 50, 100, 200 / s) in both the model and Angelica groups than in the control group ($p<0.05$, $p<0.01$), indicating that the model of acute blood stasis was successfully executed (Fig. 1a). Additionally, compared with the control group, there was a statistically higher value of PV, PT and APTT in both the model and Angelica groups than in the control group ($p<0.05$, $p<0.01$), indicating that the model of acute blood stasis was also successfully executed (Fig. 1b).

Quality control

The total ion chromatograms of QC samples were spectrally overlapped (n=5), which showed that the response intensity and retention time of the chromatographic peaks basically overlapped, indicating that the variation caused by instrumental errors during the entire experiment was small. XCMS software was used to extract the ion peaks of the metabolites. The normal control group and the blood deficiency model group 2. The peaks obtained from Angelica group and QC samples were analyzed by PCA using Pareto-scaling. Taking the positive ion mode as an example, QC samples were tightly clustered in all samples, indicating that the repeatability of this test was good. The results are shown in Figure 2. In summary, the

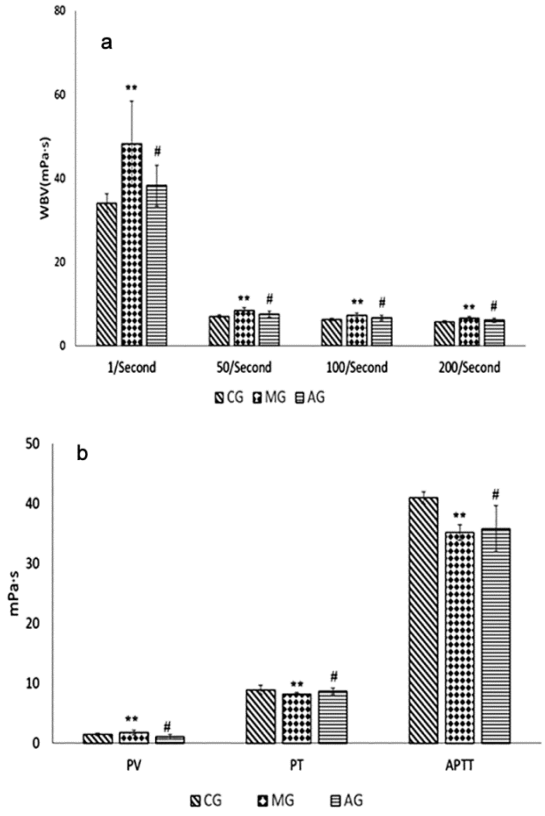

Fig. 1 — (a) Effects of treatment on WBV of different rates in blood stasis rats and (b) Effects of treatment on plasma viscosity (PV), prothrombin time (PT) and activated Partial Thromboplastin Time (APTT) on blood stasis rats. ($\bar{x} \pm s$, n=10). Compared with the normal control group, ** $p<0.01$; compared with the model group, # $p<0.05$; ## $p<0.01$

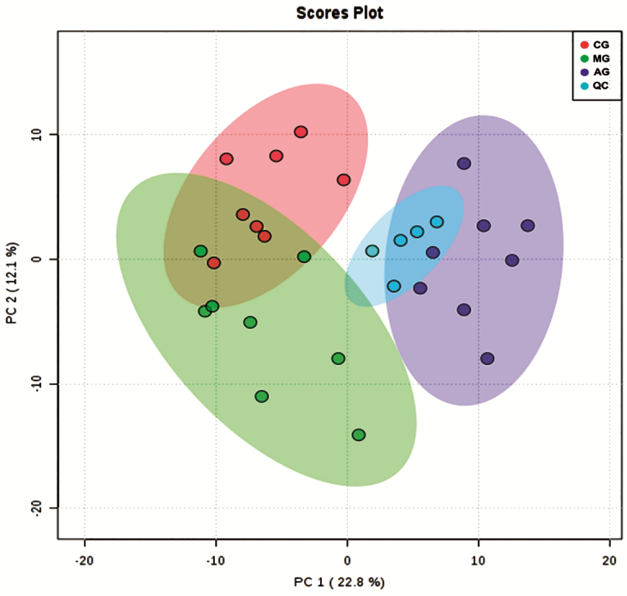

Fig. 2 — The PCA scores plot of sample in the positive ion mode (n=5)

stability of the instrumental analysis system in this test was good, and the test data was stable and reliable. The differences in the metabolic spectrum obtained in the test could therefore reflect the biological differences between the samples themselves.

Multivariate statistical analysis

The results showed that the Angelica group and the blood stasis model group had a certain separation trend in the dimensions of the first principal component (PC1) and the second principal component (PC2). The model evaluation parameters R2Y and Q2 ≥ 0.5 obtained through 7 cycles of interactive verification indicated that the models between each group were stable and reliable (Table 1). After treatment, results for PCA, PLS-DA, OPLS-DA are shown in Figure 3.

Differential metabolite identification

Volcanic maps of Angelica group and blood stasis model group were established and T-tested. The points off-axis in the volcano map were VIP> 1 and $p<0.05$, $0.05 <p<0.1$. A total of 46 differential metabolites were screened in the normal control group and the blood stasis model group, and a total of 54 differential metabolites were screened in the Angelica and blood stasis model group. The metabolite changes were compared between the normal control group, the blood stasis model group, and the Angelica administration group. Venn analysis was performed on the differential metabolites identified in the serum of each group and 22 differential metabolites in the normal control group, model group and Angelica group were obtained. The multiples of change and the trend of change are shown in Table 2.

Analysis of potential metabolites and metabolic pathways

The 22 differential metabolites selected above were submitted to the Metaboanalyst 3.0 website, providing linoleic acid metabolism, linolenic acid metabolism, phenylalanine metabolism, ascorbic acid and uronic acid metabolism, fructose and mannose metabolism, arginine and proline 11 metabolic pathways of metabolism, starch and sucrose metabolism, glycolysis and gluconeogenesis, primary bile acid biosynthesis, galactose metabolism, amino sugar and nucleotide sugar metabolism. 4 metabolic pathways with impact value> 0.1 were selected *as A. sinensis* the most relevant major metabolic pathways for the treatment of blood stasis model are shown in Figure 4. Four different metabolites most relevant to this metabolic pathway were selected as potential metabolites for Angelica to treat blood stasis syndrome, including linoleic acid, alpha-linolenic acid and L-phenylalanine) and ascorbic acid.

***Angelica* active ingredient screening**

125 chemical constituents related to *Angelica* were found from TCMSP and other databases. The candidate compounds were screened with OB> 20% and DL> 0.03 as standard and combined with literature reports[16]. After screening, 62 Angelica

Table 1 — Evaluation parameters of PLS-DA and OPLS-DA in different medicinal parts of *Angelica sinensis* and blood stasis group

Group	Positive ion mode			Negative ion mode		
	A	R^2Y (cum)	Q^2 (cum)	A	R^2Y (cum)	Q^2 (cum)
PLS-DA	2	0.977	0.933	2	0.990	0.864
OPLS-DA	2	0.977	0.848	4	1	0.868

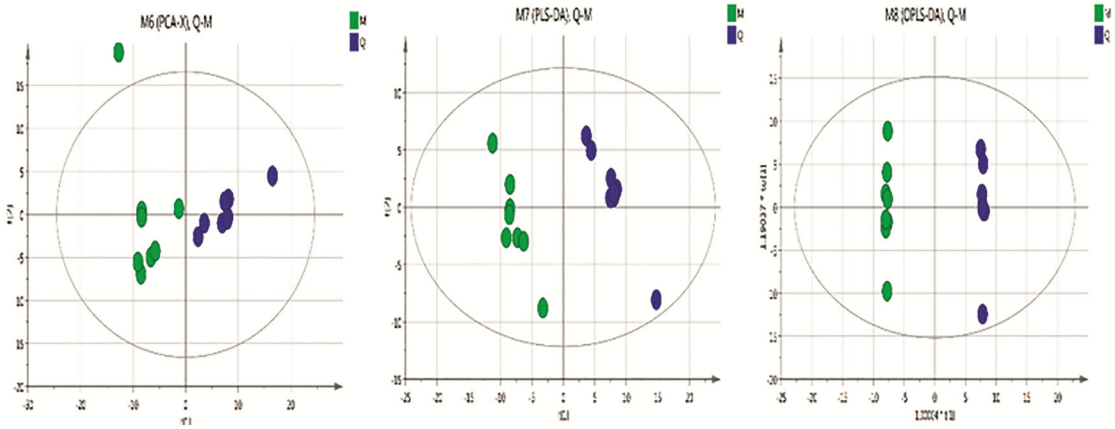

Fig. 3 — PCA, PLS-DA, OPLS-DA score plots of metabolites in *Angelica sinensis* and blood stasis group

Table 2 — The trend changes of the difference metabolites in each group

metabolites	m/z	VIP	rt(s)	FC	M	Q
Alpha-D-Glucose	179.06	1.41	526.69	0.71	up	down
Lyso PC (18:1(9Z)	522.35	2.96	346.91	1.22	down	up
Lyso PC (14:0)	468.31	1.32	333.49	1.46	down	up
L-Isoleucine	132.1	4.11	505.73	0.66	up	down
Creatine	130.06	1.69	649.33	0.61	up	down
Acetylcarnitine	204.12	3.36	566.45	0.63	up	down
L-Carnitine	220.12	1.03	662.11	0.61	up	down
Indoxyl sulfate	212	10.52	45.74	2.57	down	up
Glycocholic acid	464.3	1.75	474.07	3.24	down	up
alpha-Linolenic acid	277.22	6.03	73.11	0.70	down	down
Lyso PC (16:0)	496.34	8.95	351.44	1.33	down	up
Linoleic acid	279.23	15.71	72.49	0.77	down	up
D-Mannose	179.06	2.11	558.81	1.18	down	up
3-Methylhistidine	170.09	1.92	718.8	0.67	up	down
1-Stearoyl-sn-glycerol3-phosphocholine	568.34	1.21	301.78	2.20	down	up
3-Methoxy-4-Hydroxyphenylglycol Sulfate	263.02	1.41	69.57	5.69	up	up
Cholic acid	426.32	1.13	349.14	0.50	up	down
Lyso PC (18:0)	524.37	2.69	345.22	1.44	down	up
1-Stearoyl-2-oleoyl-sn-glycerol 3-phosphocholine	810.6	5.2	247.56	1.53	down	up
L-Phenylalanine	164.07	2.24	472.31	0.77	up	down
L-Ascorbic acid	303.23	1.01	70.08	2.43	down	up
Acetylcholine	146.12	3.62	705.02	0.46	up	down

compounds were selected. The following compounds were analyzed, which were mainly lichen lactone C, lichen lactone D, lichen lactone E (senkyunolide-C, D, E), ligustilide (ligustilide) and α-pinene (alpha-pinene), camphoric acid, 3-butylidene-7-hydroxyphthalide, organic acids such as ferulic acid, beta-sitosterol, stigmasterol (stigmasterol), etc. The components of yangchuan lactone C, D, E, ligustilide and ferulic acid were considered as the main effective components in Angelica essential oil. Ferulic acid was an indicator ingredient of Angelica quality control in the 2020 Chinese pharmacopoeia. Therefore, the current screening conditions included most of the active ingredients in *A. sinensis* as reported in the literature[17,18].

Angelica component target prediction and network analysis

The candidate compounds in *A. sinensis* were searched through TCMSP, Drugbank, Swiss Target Prediction and other databases to find corresponding targets. The compound targets were entered into the protein Uniprot database and the names were corrected. The active ingredients and targets were imported into the Cytoscape network visualization software to construct the *A. sinensis* active ingredient-target targets (C-T) network. The results showed that in the component-target network, there were 223 nodes (62 TCM active ingredient nodes and 169 active target nodes) and 731 edges, of which the blue node represents the active ingredient of *A. sinensis* and the red node represents the drug target points, each side being the interaction relationship between the active ingredients of the TCM and the drug target. The results are shown in Figure 5.

Integration of metabolomics and network pharmacology results

Cytoscape software was used to establish the Angelica effect component-target-path network model, and 62 components, 169 targets and 18 metabolic pathways were obtained. Integration of the target predicted by the network pharmacology with the metabolites screened by the metabolomics was undertaken and the integrated metabolic pathway diagram of Angelica played the role of activating blood (Fig. 6). The results showed that: beta-selinene, aromadencrene and bicycloelemene regulate UGT2A1, UGT2A3, UGT2B10, UGT2B17, UGT2B4, UGT2B28, UGT2B15, UGT2B7, UGT2B11; ergapten, senkyunolide C, sedanolide and thymol regulate ALOX12B, ALOX12, ALOX15, ALOX15B, ALOX5 in the linoleic acid metabolism pathway.

Pathological changes in heart and lung tissue in rats

As illustrated in Figure 7a, in the normal rat (CG), the myocardial fibers appeared neatly arranged; myocardial and endometrial cells were continuously complete; myocardial cells were clear, uniform, with dense cell nucleus; and no edema, no significant expansion of the interstitial small blood vessel, no blood and no inflamed cells were seen. However,

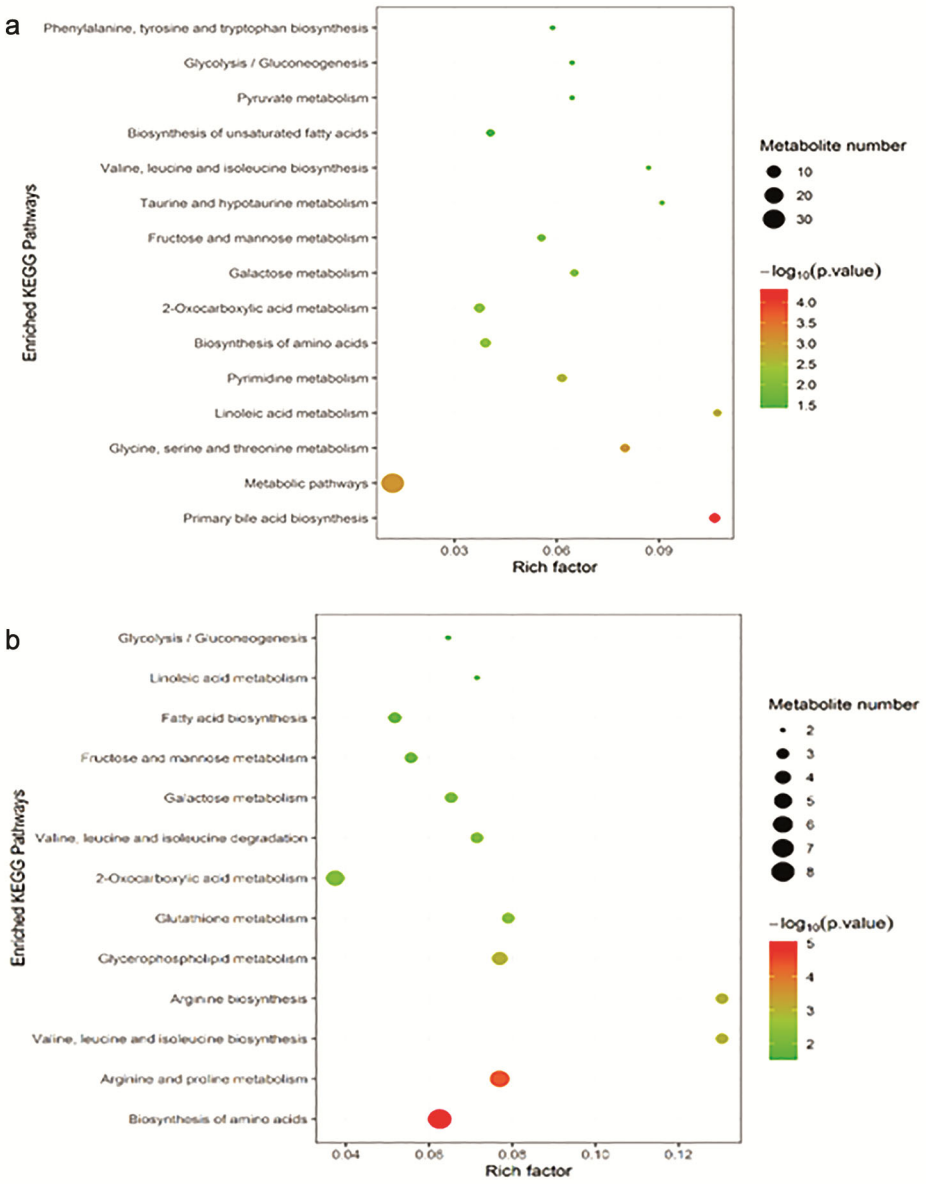

Fig. 4 — KEGG pathways of difference metabolites in *Angelica sinensis* and blood stasis group (a) MG vs CG and (b) AG vs MG

compared with the normal control group, the model rat (MG) heart muscle fiber had large-scale swelling, fracture, granular cell pulp clotting or empty bubble-like cell pulp dissolution, widened myocardial cells and round enlargement of cell nucleus. Moreover, compared with the model group, the *A. sinensis* group had visible mild disorder of cardiomyoblast arrangement, muscle fiber arrangement was closer than the model group, myocardial fiber was narrow, close to normal, around the visible mild edema, seepage and inflammatory cell immersion. All these indicated that the rat heart muscle injury was reduced under the Angelica group.

Additionally, compared with the normal control group, the model rats had severe lung bleeding, significant desalination necrosis and shedding of the analoids and inflamed cells. However, compared with the model group, the bleeding phenomenon improved to varying degrees with significant improved width of the velecule wall in each drug-given group (model and Angelica groups) Figure 7b.

Discussion

In order to reveal the mechanism of *A. sinensis* activating the blood, UPLC-Q-TOF / MS technology was used to screen the endogenous metabolites of

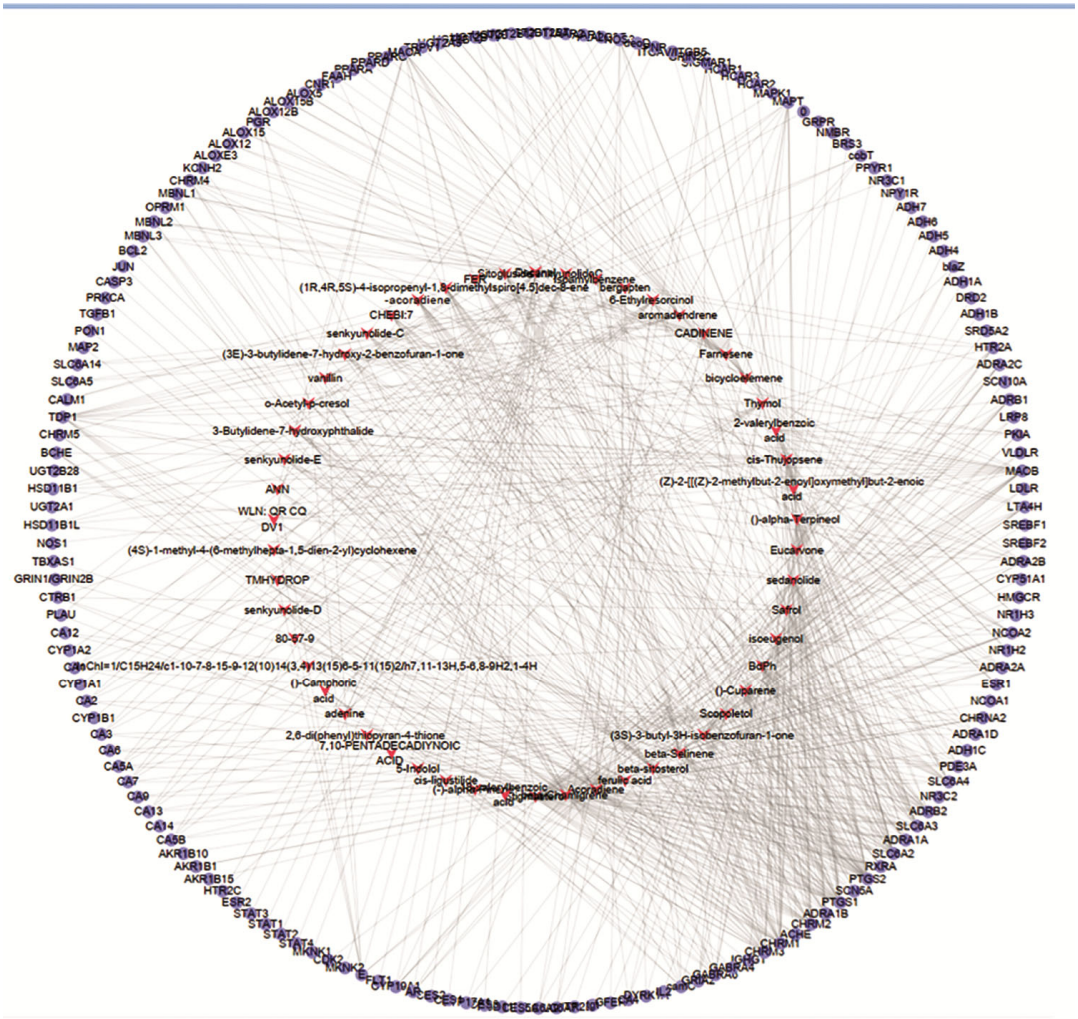

Fig. 5 — The network diagram of active components in *Angelica sinensis*-target (Blue: active ingredient, red: ingredient target)

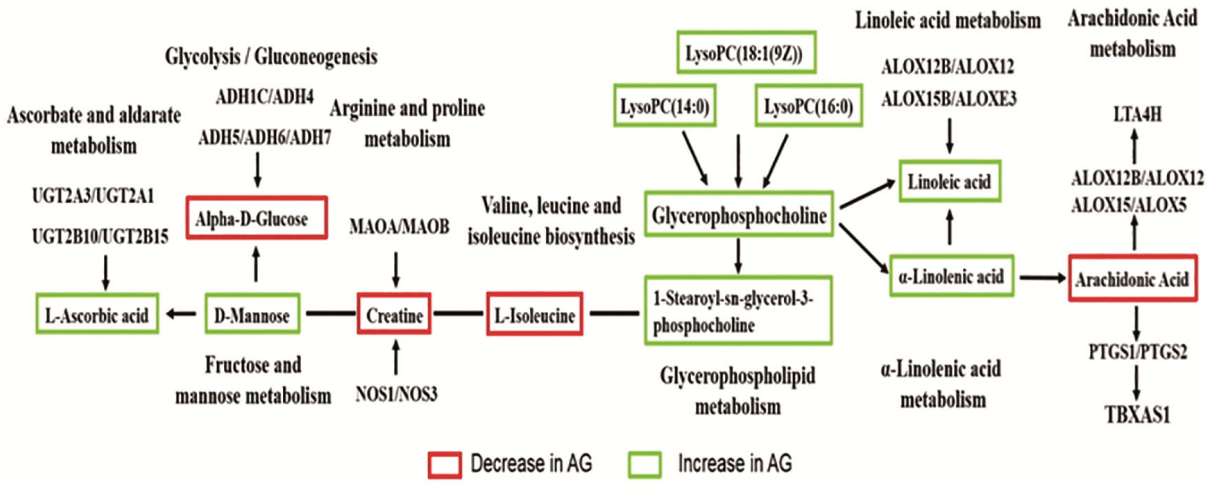

Fig. 6 — Metabolic pathway of activating blood in *Angelica sinensis* and *Angelica sinensis* tail

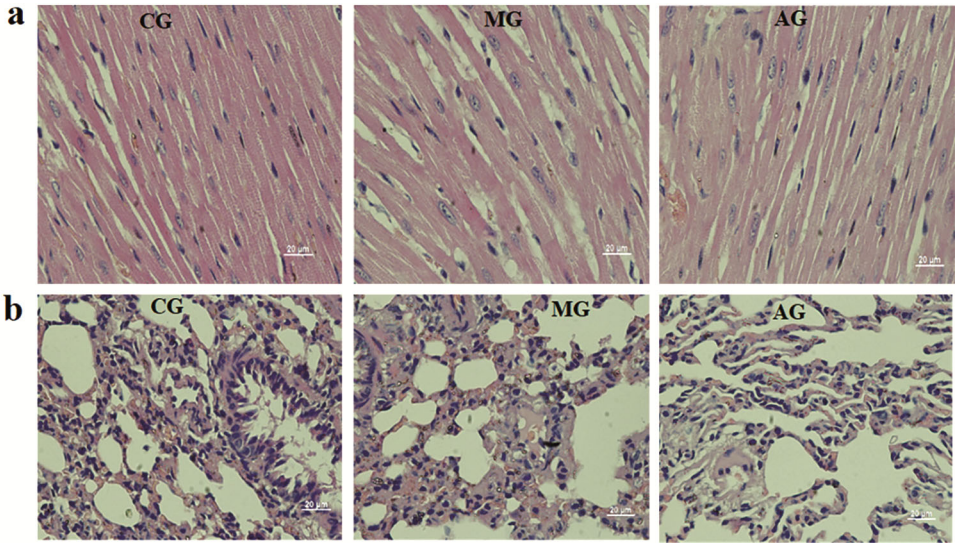

Fig. 7 — (a) Effects on cardiac muscle in blood stasis rats (×40) for CG, MG and AG and (b) Effects of on pulmonic tissue in blood stasis rats (×40) for CG, MG and AG

A. sinensis and acute blood stasis model group, to search for related metabolic pathways, and to clarify the mechanism of blood circulation of *A. sinensis*. In this study, UPLC-Q-TOF / MS technology was used to detect rat plasma samples between groups. Acute blood stasis model group and normal control group were screened for phenylalanine, D-glucose, L-tryptophan, ascorbic acid, 46 differential metabolites such as oleic acid, linolenic acid, arachidonic acid, etc. Then, 54 differential metabolites were screened out from Angelica group and blood stasis model group. According to Venn analysis, there were 22 potential metabolites in normal group, model group and Angelica group. Compared with the blood stasis model group, Angelica group reduced 8 metabolites including Alpha-D-glucose, L-isoleucine, creatine, acetylcarnitine, lysolecithin (18: 1 (9Z), sulfuric acid Fourteen metabolites such as indolene were significantly up-regulated, all of which turned to normal. The main metabolic pathways of Angelica for treating blood stasis syndrome included linoleic acid metabolism, linolenic acid metabolism, phenylalanine metabolism, ascorbic acid and uronic acid metabolism. Potential metabolites include linoleic acid, linolenic acid, phenylalanine and ascorbic acid[14-18].

Linoleic acid is involved in linoleic acid metabolism, and alpha-linolenic acid is involved in linolenic acid metabolism. Linoleic acid has the effects of lowering blood pressure, softening blood vessels, lowering blood lipids, and promoting microcirculation and can inhibit arterial thrombosis. At the same time, linoleic acid is also a precursor of linolenic acid and arachidonic acid[19]. In the blood stasis model group, the content of fatty acids such as lysolecithin, linoleic acid and linolenic acid decreased, which indicates that fatty acid metabolism is abnormal and fatty acid and phospholipid metabolism are closely related to energy metabolism, which indicates abnormal energy metabolism. In the process of causing the blood stasis model, adrenaline can enhance energy utilization and ice water stimulation can also increase productivity, resulting in large consumption of fatty acids, enhanced metabolism, and abnormal energy metabolism. Consistent with literature reports[20], linoleic acid lowers LDL cholesterol, slightly increases HDL cholesterol, and predicts cardiovascular disease risk[21]. Ascorbic acid is involved in the metabolism of ascorbic acid and uronic acid. Ascorbic acid (vitamin C) is related to immune function and the prevention and treatment of coronary heart disease. It can reduce total serum cholesterol and lower blood pressure. It is used for the repair of coronary smooth muscle and can interact with glutathione. It acts as an effective free radical scavenger to remove reactive oxygen species in plasma. If ascorbic acid is deficient, arterial vascular smooth muscle cannot be repaired in time and lipoproteins produced by the liver can be deposited on the damaged arterial wall, causing plaques on the blood vessel wall[22,23]. After the acute blood stasis model was created, ascorbic acid content decreased, suggesting that immune function was low, and coronary blood vessels and smooth muscles were

damaged. Phenylalanine and tryptophan are involved in amino acid metabolism. Phenylalanine is an essential amino acid in the human body. In normal organisms, tyrosine can be metabolized in the liver and other tissues to synthesize certain substances in the nervous system and adrenal medulla. For neurotransmitters, studies have found that immune, liver and kidney and other diseases can cause phenylalanine metabolism disorders. After causing blood stasis, the conversion of phenylalanine to tyrosine is blocked, causing excessively high concentrations of phenylalanine in the blood and blocking the phenylalanine metabolism pathway[24-26]. After administration, Angelica can restore disordered linoleic acid, linolenic acid, phenylalanine, and ascorbic acid to normal.

Network pharmacology mainly obtains real data by analyzing databases and laboratories results such as drugs, genes/proteins, diseases, etc., and integrates multidisciplinary technologies and content such as computational biology, pharmacology, systems biology and network analysis to build a multi-level network. Research studies have been more systematic, holistic, and structured to explore the association between drugs and disease[27,28]. TCM exerts its effects through the synergy of multiple ingredients and multiple targets. In order to further explain its mechanism of action, based on pharmacodynamics and metabolomics research, this study finds the active ingredients and effects of *A. sinensis* through platforms such as TCMSP, PUBCHEM and Swiss target prediction. For targets, Cytoscape software is used to build a "component-target" network. The selected targets are input to DAVID software for path enrichment and a "component-target-channel" network model is constructed. In this study, a “component-target” network of *A. sinensis* was constructed. The network predicted that *A. sinensis* had 62 active ingredients, 169 active proteins and 18 metabolic pathways. The predicted two pathways of linoleic acid metabolism, ascorbic acid and uronic acid metabolism were consistent with those obtained by *A. sinensis*. Arachidonic acid 12-lipoxygenase (ALOX12B, ALOX12, ALOX15B, ALOXE3) mainly regulates linoleic acid metabolism; UGT2A3, UGT2A1, UGT2B10, UGT2B15, UGT2B17, UGT2B28, UGT2B4, UGT2B7, etc., and participates in ascorbic acid and uronic acid metabolism. *A. sinensis* can regulate different signaling pathways and metabolic pathway groups, involving multiple links and each link is connected to targets, which reflects the synergy of multiple targets. The use of metabolomics verification network prediction results is just one of the methods. The signal pathways predicted by the network can be re-verified through various means such as analysis of cells, proteomics, and transcriptomics and provide a true understanding on the action of TCM in an holistic manner. This study has also revealed the multi-dimensional regulatory role of Angelica, confirming it as one of the Chinese herbs with multiple traditional medicine values.

Conclusion

From this study, it can be concluded that the *A. sinensis* improved whole blood viscosity and plasma viscosity of acute blood stasis model rats. The metabolomics results also showed that compared with the model group, *A. sinensis* can regulate the four metabolic pathways of the disorder caused by blood stasis to normal and play a role in promoting blood circulation. Potential metabolites were found to be linoleic acid, linolenic acid, phenylalanine and ascorbic acid. Through the network pharmacology to build Angelica "component-target-pathway" network, 62 active ingredients, 169 active proteins and 18 metabolic pathways of Angelica were obtained. Among them, linoleic acid metabolism, ascorbic acid and uronic acid metabolism were consistent with the two metabolic pathways of *A. sinensis* activating blood metabolomics. Therefore, the study has helped to confirm the medicinal actions of *A. sinensis,* hence making is one of the Chinese herbs with multiple traditional medicinal values. Moreover, further studies that incorporate detailed analytical method are recommended for each analyte quantified with partial validation data.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

All authors played equal roles in the formulation of the study, sampling analysis and manuscript production.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. These experiments were performed in accordance with the relevant animal ethics guidelines and regulations granted by Gansu University of Traditional Chinese Medicine (Number GUTM/approval 1232112/2020).

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