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Mechanism of volatile oil from Chuanxiong (Chuanxiong Rhizoma) - Suhexiang (Styrax) - Bingpian (Borneolum) in treating angina pectoris based on network pharmacology and its protective effects on myocardial damage in rats

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ABSTRACT

Objective To explore the pharmacodynamic material basis and mechanism of action of volatile oil from Chuanxiong (Chuanxiong Rhizoma) - Suhexiang (Styrax) - Bingpian (Borneolum) (hereinafter referred to as C-S-B volatile oil) in treating angina pectoris based on network pharmacology and to detect its protective effects against rat myocardial damage.

Methods Gas chromatography-mass spectrometry (GC-MS) was used to determine the constituents of volatile oils from Chuanxiong (Chuanxiong Rhizoma), Suhexiang (Styrax), and Bingpian (Borneolum), and the targets of the three main constituents were found predicted and screened using the PharmMapper server, and GeneCards and CooLGeN databases. The STRING database and Cytoscape software were used to draw the protein-protein interaction (PPI) network, RStudio software was used to analyze Gene Ontology (GO) and Kyoto Encyclopedia of Genome and Genome (KEGG) pathways, and Cytoscape software was used to construct the component-target-pathway-disease network. The rat model of myocardial injury was established by intraperitoneal injection of a large dose of isoprenaline hydrochloride. After continuous intervention with C-S-B volatile oil for 14 d, the ejection fraction (EF) and short axis shortening rate (FS) of the left ventricle were detected. The indices of myocardial damage were detected after hematoxylin-eosin (HE) staining.

Results Fifteen volatile oil components from the C-S-B formula were identified. There are 470 targets of these volatile oil

components and 401 angina-related genes. There are 28 core targets, including CHRM4, ADRA1A, FGFR1, CHRM2, CYP2A6, CHRM5, CHRM1, CHRM3, HDAC2, and MPO, etc.. The results of the KEGG analysis indicated that the C-S-B formula probably interferes with the following pathways: neuroactive ligand-receptor interactions, calcium signaling, cytochrome P450 metabolism of heteropoietin, among others. The results of animal experiments showed that the C-S-B formula essential oil could significantly improve the following myocardial indices in rats with myocardial injury: EF, FS, left ventricular end-systolic diameter (LVIDs), left ventricular end-diastolic diameter (LVIDd), and stroke volume (SV), and all the differences were statistically significant ($P < 0.01$).

Conclusion The mechanism of action of volatile oil components in the C-S-B formula in treating angina pectoris was analyzed using multi-component, multi-target and multi-pathway systems, which has laid a foundation for further revealing its mechanism of action. Animal experiments have shown that the volatile oil of the C-S-B formula can improve EF, FS, and other indices of myocardial damage in a rat model, thus relieving myocardial damage caused by heart hyperactivity, improving cardiac function, and protecting against myocardial damage.

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1 Introduction

Cardiovascular and cerebrovascular diseases generally refer to ischemic or hemorrhagic diseases of the heart, brain, and systemic tissues caused by hyperlipidemia, changes in blood viscosity, atherosclerosis, and hypertension [1]. Modernization is accelerating, and cardiovascular and instances of cerebrovascular diseases are no longer limited to the elderly. The number of middle-aged and young people suffering from cardiovascular and cerebrovascular diseases is increasing [2]. Cardiovascular and cerebrovascular diseases have become a major threat to human health in today's society. There is an urgent need to prevent and treat these diseases. This can be achieved using aromatic drugs, whose efficacy in cardiovascular and cerebrovascular diseases cannot be ignored [2].

Chuanxiong (Chuanxiong Rhizoma) is warm, fragrant and dry. It promotes blood and Qi circulation, dispels wind, and relieves pain. It can improve atherosclerosis, myocardial injury, and cardiac function and has several other effects [3]. Suhexiang (Styrax) has the functions of inducing resuscitation, avoiding filth, warming and relieving pain, which can improve cardiac function, reduce the myocardial infarction area, slow down myocardial cell necrosis, and reduce myocardial enzyme activity, thus effectively improving cardiovascular diseases [4]. Bingpian (Borneolum) is refreshing, aromatic, and induces resuscitation and has other effects. Modern study has found that borneolum promotes bidirectional regulation of the central nervous system, protects from cerebral and cardiac ischemia, influences NO levels, regulates Ca^{2+} concentration, influences neurotransmitter content and has several other effects; it also has a therapeutic effect on myocardial damage and on NO synthase (NOS) functional defects [5]. ChuanXiong (Chuanxiong Rhizoma), Suhexiang (Styrax), and Bingpian (Borneolum) in ready-for-use traditional Chinese medicine (TCM) formulations, such as chest aerosol, the Guanxin Suhe Pill and the Suxiao Jiuxin Pill, which are currently commercially available, have shown therapeutic effects on coronary heart disease and angina pectoris. The purpose of this study was to prove that the essential oil of the C-S-B formula has a therapeutic effect on angina pectoris through network pharmacology and animal experiments, and to explore the mechanism of the essential oil components of the C-S-B formula in treating angina pectoris to provide a scientific basis for new drug development and clinical research.

2 Materials and methods

2.1 The source of TCM

The TCM formulas used to treat coronary heart disease, and the listed ready-for-use TCMs for angina pectoris were screened for aromatic drugs by frequency statistics and association rules [6] (IBM SPSS Modeler 14.1 Premium software, Apriori algorithm). Among these drugs, Chuanxiong (Chuanxiong Rhizoma), Suhexiang (Styrax), and Bingpian (Borneolum) have definite effects on promoting blood circulation, relieving pain, inducing resuscitation and awakening the mind, and have the benefits of wide availability and low price, so they were chosen as objects of this study.

2.2 Extraction of volatile oil from TCMs

The extraction of volatile oil from TCMs followed the determination for volatile oil extraction of the *Chinese Pharmacopeia* (2020 edition). The volatile oils of Chuanxiong (Chuanxiong Rhizoma) and Suhexiang (Styrax) were obtained by adding water, soaking for 2 h, slowly heating to boiling, boiling and refluxing, and cooling for 1 h.

2.3 Determination of volatile oil in TCMs

The volatile oil components in the mixture of Chuanxiong (Chuanxiong Rhizoma), Suhexiang (Styrax), and Bingpian (Borneolum) were determined by gas chromatography-mass spectrometry (GC-MS). Ligustilide [7] in the volatile oil of Chuanxiong (Chuanxiong Rhizoma), cinnamaldehyde [8] in the volatile oil of Suhexiang (Styrax), dextro-borneol [5] in Bingpian (Borneolum) are considered the main effective components against cardiovascular diseases; therefore, these three compounds were set as reference substances.

Volatile oil (100 μL) from the C-S-B formula was solubilized with anhydrous ether (10 mL), and then filtered before quantification. Endo-borneol (20 mg), cinnamaldehyde (20 mg), and ligustilide (20 mg) were solubilized in anhydrous ether (10 mL), filtered, and analyzed.

GC-MS conditions: The gas chromatographic column was an Agilent HP-5MS (30 m × 250 μm × 0.25 μm), the heating protocol was as follows: 50 °C (retained for 3 min), 70 °C to 180 °C (at 8 °C/min), up to 240 °C (at 5 °C/min); the equilibrium time was 0.3 min, the highest temperature was 320 °C, the temperature of the vaporization chamber was 250 °C, the carrier gas was high-purity He (99.999%), the sample volume was 1 μL, the pre-column pressure was 5.53 psi; the ion source of mass spectrometry was electron ionization (EI), and the ion source temperature was 230 °C; quadrupole temperature was 150 °C; Electromagnetic Volume (EMV) mode gain factor was 1.00; EMV was 1 000 V; and the mass scanning range was 50 – 650 amu [9-14].

2.4 Collection of TCM volatile oil component targets and angina pectoris targets

Simplified molecular input line entry specification (SMILES) numbers of each volatile oil component were retrieved from the PubMed database, and SMILES numbers were input into the SwissTargetPrediction database to obtain volatile oil component targets, which were extracted from the GeneCards, CoolGeN, and PharmMapper servers with the names of the volatile oil components. Next, the volatile oil targets of the C-S-B formula were compiled. Through Therapeutic Target Database (TTD), DisGENET, and Drugbank databases, angina-related targets were also obtained with "Angina" as the search term.

2.5 Screening of core targets

The target data of the volatile oil components of the C-S-B formula and those associated with angina pectoris were processed and imported into Cytoscape to build a network model of volatile oil components and disease targets, thus obtaining core targets.

2.6 Enrichment analysis

To better understand the mechanism of action of the C-S-B volatile oil components in the treatment of angina pectoris, RStudio software was used to carry out Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The species was limited to humans, and the threshold was set at $P < 0.05$.

2.7 Construction of the volatile oil component-target-pathway network model of TCM

Cytoscape software was used to construct a network model of volatile oil component core targets and main pathways.

2.8 Animal experiments

2.8.1 Animals Thirty-two healthy specific pathogen free (SPF) male Sprague-Dawley (SD) rats, weighing (180 ± 20) g, were purchased from Hunan Slack Jingda Experimental Animal Co., Ltd. [License No. SCXK (Xiang) 2019-0004]. The experimental unit License No. SYXK (Gan) 2017-0004. Each rat was fed with 12 g feed every day, and was housed with constant humidity and consistent light and environment; room temperature was set at 20 – 22 °C; rats were allowed

free access to drinking water, and were fasted for 12 h before the experiment.

2.8.2 Drugs and Instruments C-S-B volatile oil atomization solution: Chuanxiong (Chuanxiong Rhizoma) volatile oil∶Suhexiang (Styrax) volatile oil∶Bingpian (Borneolum) were mixed evenly (1∶1∶1), and the C-S-B volatile oil atomization solution was prepared in 1.364 g per kilogram rat weight, and placed in an atomization cup with 4 mL distilled water. Nitroglycerin spray was purchased from Shandong Jingwei Pharmaceutical Co., Ltd. (Batch No. 2008007); isoproterenol hydrochloride injection fluid was purchased from Shanghai Hefeng Pharmaceutical Co., Ltd. (Batch No. 41191203); the Ultra-high resolution ultrasound imaging system for small animals used was from VisualSocs Company of Canada; the paraffin embedding machine was from Leica Microsystems Shanghai Co., Ltd., and the slicer and automatic dyeing and sealing workstation were from Shanghai Leica Instrument Co., Ltd..

2.8.3 Model preparation Thirty-two rats were randomly divided into four groups: blank group, model group, nitroglycerin aerosol positive control group (nitroglycerin group) and C-S-B volatile oil atomization group (C-S-B group), with eight rats in each group. After the rats were weighed on the first day, each group except the blank group was injected intraperitoneally with isoproterenol hydrochloride at a dose of 20 mL/kg, while the blank control group was injected intraperitoneally with normal saline at the same volume. After injection, each group was exposed to treatment by fumigation for 30 min, the blank group was fumigated with blank atomization solution, the C-S-B group was fumigated with the volatile oil atomization solution of the C-S-B group, and the nitroglycerin group was given nitroglycerin spray in four squirts, with approximately 0.5 mg each. The dosages are listed in Table 1. On the 14th day of modeling, the heart was monitored by an echocardiogram (ECG) after administration. Ejection fraction (EF) and short axis shortening rate (FS) of the left ventricle were recorded at 1, 5, 15, and 30 min. After rat echocardiography, animals were sacrificed and the heart was immediately removed and divided into two halves, one half was soaked in formalin solution, and the other half was stored at −80 °C in a cryopreservation tube.

Table 1 Experimental group and dosage

Group	Abbreviation	Dose (g/kg rat weight)
Blank group	Blank	Blank atomized solution
Model group	Model	0
C-S-B group	C-S-B	1.364
Nitroglycerin group	Nitroglycerin	0.008

2.9 Index detection

2.9.1 Echocardiography indices After depilation, the rats were anesthetized with isoflurane, the limbs were fixed on the plate for inhalation and continuous anesthesia, the chest was smeared with coupling agent, and M-mode echocardiography was performed by connecting the rat with a small animal ultrasound instrument. The probe was placed in the middle of the sternum of the rats and shifted 1/3 to the left to obtain the long axis section of the left ventricle, and the images and data were collected for 1, 5, 15, and 30 min. Heart rate (HR), EF, FS, left ventricular end-systolic diameter (LVIDs), left ventricular end-diastolic diameter (LVIDd), and stroke volume (SV) were recorded to evaluate cardiac structure and left ventricular function.

2.9.2 Hematoxylin-eosin (HE) myocardial tissue staining index The heart fixed in formalin was cut into small pieces, put into an embedding box, washed in water under slow agitation for 12 – 16 h, and then sequentially dehydrated using 60% ethanol for 1.5 h, then 70%, 80%, 90%, and 95% for 1 h, and absolute ethanol twice for 0.5 h. Treatment continued with anhydrous ethanol-xylene (1∶1) for 15 min, anhydrous ethanol-xylene (1∶3) for 9 min, pure xylene for 5 min, and pure xylene for 2 min until the tissue became transparent. After treatment in a liquid paraffin water bath for 0.5 h, the sample was placed in a paraffin embedding machine for 2.5 h, and then embedded. The embedded wax block was then sliced every 5 μm, patched, and dried. HE staining was performed after dewaxing using an automatic staining and sealing workstation, and the sample was then sealed with neutral resin. After 24 h, the morphological changes and degree of myocardial cell injury were observed under an optical microscope.

2.9.3 Statistical analysis SPSS 21.0 statistical software was used to analyze and process the experimental data of each group, and the data were expressed as mean ± standard deviation (SD). The echocardiography indices were statistically analyzed by the general linear repetitive measure, and the serum biochemical indices were statistically analyzed using the comparative mean single factor. Differences were considered statistically significant at $P < 0.05$.

3 Results

3.1 C-S-B volatile oil composition collection

According to the GC-MS data, 15 volatile oil components from the C-S-B formula were isolated, as

shown in Table 2. Figure 1 shows the ion flow chromatogram of the volatile oil components in the C-S-B formula. The GC-MS ion flow diagram of the reference substances of endo-borneol, cinnamaldehyde, and trans-ligustilide is shown in Figure 2. The GC-MS results of endo-borneol, cinnamaldehyde and trans-ligustilide reference substance are shown in Table 3.

Table 2 GC-MS determination of volatile oil components of C-S-B formula

No.	Retention time (min)	Compound	Chemical Abstracts Service (CAS)
1	6.689	Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	143-22-6
2	6.795	3,6,9,12,15-Pentaoxanonadecan-1-ol	1786-94-3
3	7.631	Camphene	79-92-5
4	8.779	D-limonene	5989-27-5
5	10.032	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	586-62-9
6	10.367	1,6-Octadien-3-ol, 3,7-dimethyl-	78-70-6
7	11.237	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	464-48-2
8	11.361	Endo-borneol	507-70-0
9	11.448	6-Butyl-1,4-cycloheptadiene	22735-58-6
10	12.244	L-alpha-terpineol	10482-56-1
11	12.339	Cyclohexanol, 1-methyl-4-(1-methylethylidene)-	586-81-2
12	13.764	Cinnamaldehyde	104-55-2
13	17.372	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aβ)]-	17066-67-0
14	21.132	Ethanone, 2-(1-methylethoxy)-1,2-diphenyl-	6652-28-4
15	21.369	Trans-ligustilide	1000365-98-8

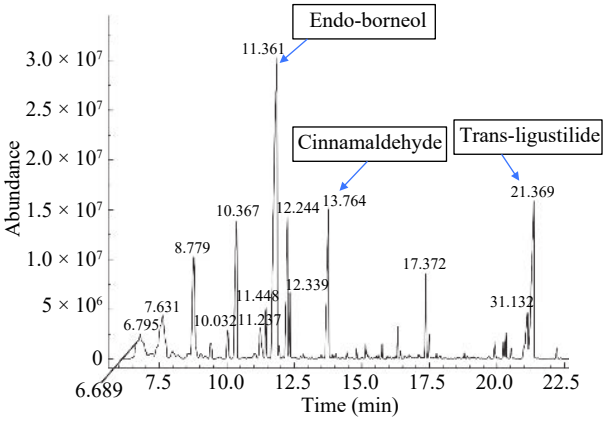

Figure 1 Ion chromatograms of volatile oil components of the C-S-B formula

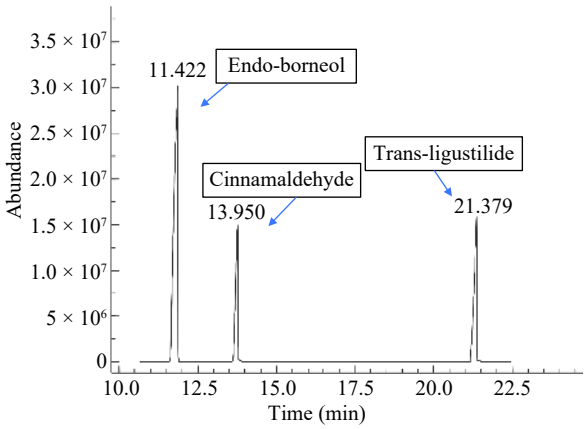

Figure 2 GC-MS total ion current diagram of the references for endo-borneol, cinnamaldehyde and trans-ligustilide

Table 3 Endo-borneol, cinnamaldehyde and trans-ligustilide reference substance GC-MS results

No.	Retention time (min)	Compound	Chemical Abstracts Service(CAS)
1	11.422	Endo-borneol	507-70-0
2	13.950	Cinnamaldehyde	104-55-2
3	21.379	Trans-ligustilide	Trans-ligustilide

3.2 Volatile oil targets, angina pectoris targets and core targets of the C-S-B formula

A total of 470 related volatile oil targets and 401 angina pectoris targets were screened and sorted using the PharmMapper server, GeneCards, and CooLGeN databases. The target volatile oil components of traditional Chinese medicine and angina pectoris

were introduced into Cytoscape to build a network model, as shown in Figure 3. The analysis shows that there are 28 intersection targets between the 15 volatile oil components and angina pectoris (Table 4).

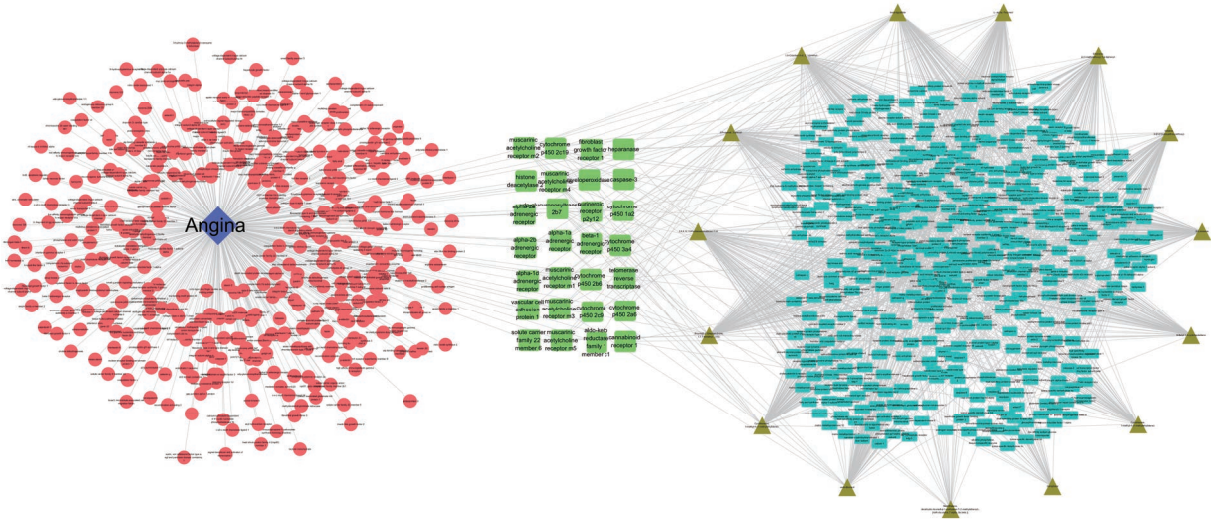

Figure 3 The volatile oil components of the C-S-B formula and the network model of angina pectoris targets
◆ stands for angina pectoris, ▲ stands for volatile oil components, ● stands for angina pectoris target, ▬ stands for volatile oil component target, ■ stands for volatile oil component and angina intersection target.

Table 4 Intersection targets of volatile oil components of the C-S-B formula and angina pectoris

Target	Symbol	Uniprot ID	Degree
Muscarinic acetylcholine receptor M4	CHRM4	P08173	12
Alpha-1a adrenergic receptor	ADRA1A	P35348	9
Fibroblast growth factor receptor 1	FGFR1	P11362	9
Muscarinic acetylcholine receptor M2	CHRM2	P08172	9
Cytochrome P450 2A6	CYP2A6	P11509	6
Muscarinic acetylcholine receptor M5	CHRM5	P08912	6
Muscarinic acetylcholine receptor M1	CHRM1	P11229	6
Muscarinic acetylcholine receptor M3	CHRM3	P20309	5
Histone deacetylase 2	HDAC2	Q92769	4
Myeloperoxidase	MPO	P05164	4
Aldo-keto reductase family 1 member C1	AKR1C1	Q04828	3
Caspase-3	CASP3	P42574	3
Heparanase	HPSE	Q9Y251	3
Cannabinoid receptor 1	CNR1	P21554	3
Cytochrome P450 2C19	CYP2C19	P33261	3
Telomerase reverse transcriptase	TERT	O14746	3
UDP-glucuronosyltransferase 2B7	UGT2B7	P16662	3
Solute carrier family 22 member 6	SLC22A6	Q4U2R8	2
Purinergic receptor P2Y12	P2RY12	Q9H244	2
Beta-1 adrenergic receptor	ADRB1	P08588	2
Cytochrome P450 1A2	CYP1A2	P05177	2
Cytochrome P450 2C9	CYP2C9	P11712	2
Cytochrome P450 3A4	CYP3A4	P08684	2
Alpha-2a adrenergic receptor	ADRA2A	P08913	2
Alpha-2b adrenergic receptor	ADRA2B	P18089	2
Vascular cell adhesion protein 1	VCAM1	P19320	2
Alpha-1d adrenergic receptor	ADRA1D	P25100	2
Cytochrome P450 2B6	CYP2B6	P20813	2

3.3 Biological function and pathway analysis of volatile oil component targets

A total of 50 results were obtained by GO biological process (BP) analysis. According to the *P* value, the first 15 enriched biological functions were selected, as shown in Figure 4. Among the biological functions involved in the pathological development of angina pectoris were: the catabolism of exogenous drugs such as that by adenylate cyclase, which inhibits the G protein-coupled acetylcholine receptor signaling pathway, phospholipase C activation of the G protein-coupled acetylcholine receptor signaling pathway, drug metabolism, steroid metabolism, and the G protein-coupled acetylcholine receptor signaling pathway.

KEGG pathway enrichment analysis showed 22 pathways. The first 10 pathways were selected according to *P* value as shown in Figure 5. These mainly involve neuroactive ligand-receptor interactions (11 targets), calcium ion signaling pathways (7 targets), cytochrome P450 metabolism of heterogenesis (7 targets), drug metabolism by cytochrome P450 (7 targets), retinal metabolism (6 targets), chemical carcinogenesis (6 targets) and cGMP-PKG signaling pathways (5 targets).

3.4 Volatile oil composition-target-pathway network model of the C-S-B formula

Using Cytoscape software, the network model of the volatile oil composition-target-pathway was generated as shown in Figure 6; it has 132 nodes and 449 edges. The analysis showed that the main volatile oil components were ligustilide, D-borneol, cinnamaldehyde, p-cymene, butenyl phthalide, and o-cymene. The main targets were CHRM2, UGT2B7, CYP2C19, CHRM4, CHRM3, and CYP3A4. The main pathways include neuroactive ligand-receptor interactions, calcium signaling pathways, and cytochrome P450 metabolism of heteropoietin.

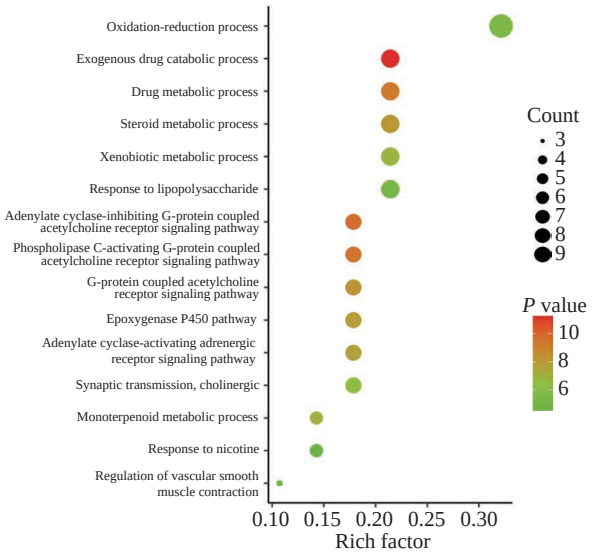

Figure 4 Enrichment analysis of GO BPs at the intersection of C-S-B volatile oil components and angina pectoris targets

3.5 Results of echocardiography by group

Compared with the blank group, EF and FS in the model group increased significantly ($P < 0.01$), while LVIDs, LVIDd, and SV decreased significantly ($P < 0.01$). In the C-S-B and nitroglycerin groups, EF and FS decreased significantly ($P < 0.01$), while LVIDd and LVIDs increased significantly ($P < 0.05$). There was no significant difference in SV in all groups, except in the C-S-B group ($P < 0.05$). The results are presented in Table 5 and Figure 7.

EF is the percentage of stroke volume to ventricular end-diastolic volume, and FS is an index of left ventricular systolic function. The EF is related to myocardial contractility. The stronger the myocardial contractility, the greater the stroke volume and EF. Compared with the model group, the EF and FS values of the C-S-B group were significantly reduced, which indicated that the C-S-B volatile oil may regulate excessive myocardial contractility and relieve heart hyperfunction. In the C-S-B group, LVIDd and LVIDs increased significantly, which enlarged the ventricular cavity, rendered the wall thinner and increased the ventricular contraction volume. The results of the echocardiography showed that the essential oil of the C-S-B formula significantly reduced EF and FS, and significantly increased LVIDd and LVIDs, thus alleviating myocardial injury caused by heart hyperfunction and improving cardiac performance.

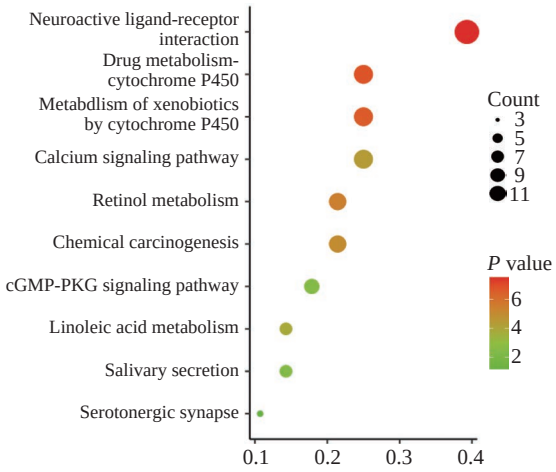

Figure 5 C-S-B volatile oil component target KEGG pathway enrichment analysis

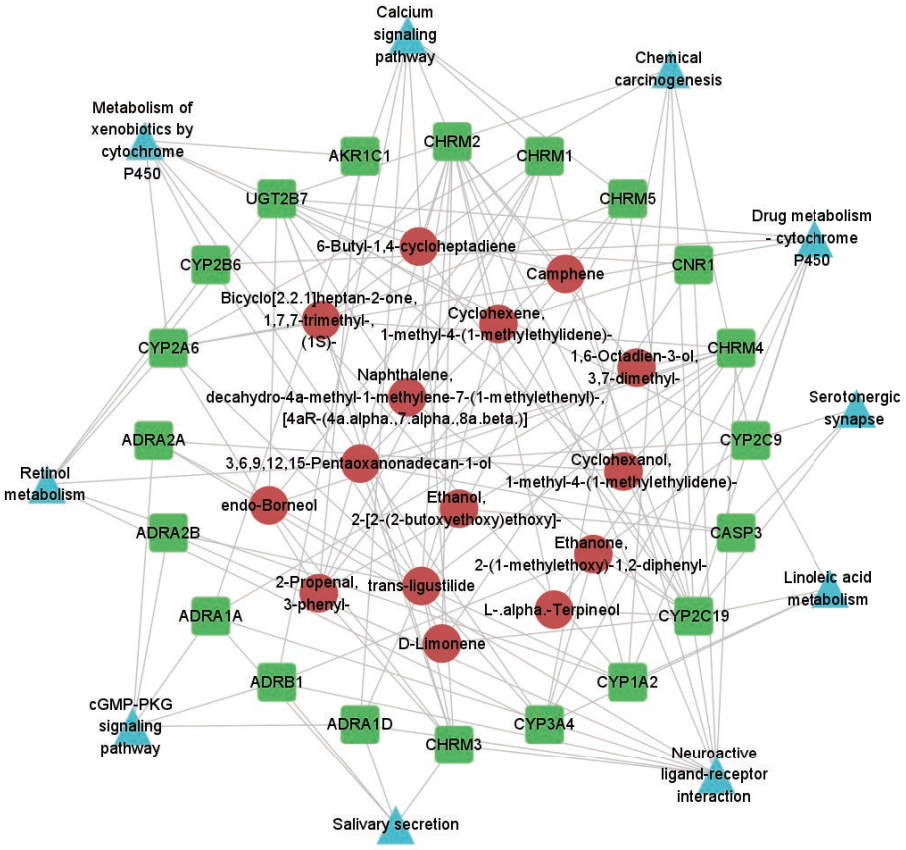

Figure 6 Volatile oil composition-target-pathway network model

▲ stands for pathway, ● stands for volatile oil components, ■ stands for intersection target

Table 5 Effects on echocardiography ($\bar{x} \pm s$, $n = 8$)

Group	EF (%)	FS (%)	LVIDs (cm)	LVIDd (cm)	SV (μL)
Blank group	75.14 ± 3.11	45.13 ± 2.82	3.70 ± 0.27	6.74 ± 0.37	173.15 ± 21.61
Model group	88.96 ± 6.72$^{\#\#}$	61.44 ± 8.91$^{\#\#}$	2.22 ± 0.78$^{\#\#}$	5.58 ± 0.85$^{\#\#}$	132.44 ± 36.12$^{\#\#}$
C-S-B group	81.10 ± 6.57**	51.83 ± 6.69**	2.93 ± 0.54*	6.13 ± 0.45*	156.15 ± 25.24*
Nitroglycerin group	81.84 ± 6.02**	52.03 ± 6.36**	3.04 ± 0.58*	6.21 ± 0.52*	146.24 ± 24.97

$^{\#\#}P < 0.01$, compared with the blank group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

3.6 Influence on the morphology of myocardial tissue

After HE staining, the histological changes in each group were observed under a microscope. In the blank group, myocardial fibers were compacted and arranged neatly, without inflammatory cell infiltration and no obvious pathological changes in the myocardial tissue. In the model group, myocardial fibers were disordered and broken, muscle fiber gaps were widened, the tissue was loose, there was edema and inflammatory cell infiltration. The myocardial fibers of the C-S-B and nitroglycerin groups were more compact, neatly arranged and had greater integrity. There was less myocardial tissue interstitium edema, narrower muscle fiber space, and a lower amount of inflammatory cell infiltration compared with the model group. There was obvious improvement, as shown in Figure 8.

4 Discussion

Angina pectoris is one of the most common types of coronary heart disease. In TCM, angina pectoris of coronary heart disease belongs to the scope of “sincere pain”, “chest obstruction” and “syncope heartache”. In the current study, 28 core targets, including CHRM4, ADRA1A, FGFR1, CHRM2, CYP2A6, and CHRM5, were identified. These targets are mainly related to neuroactive ligand-receptor interactions, calcium signaling pathways, and cytochrome P450 metabolism of heteropoietin. The results of animal experiments showed that the essential

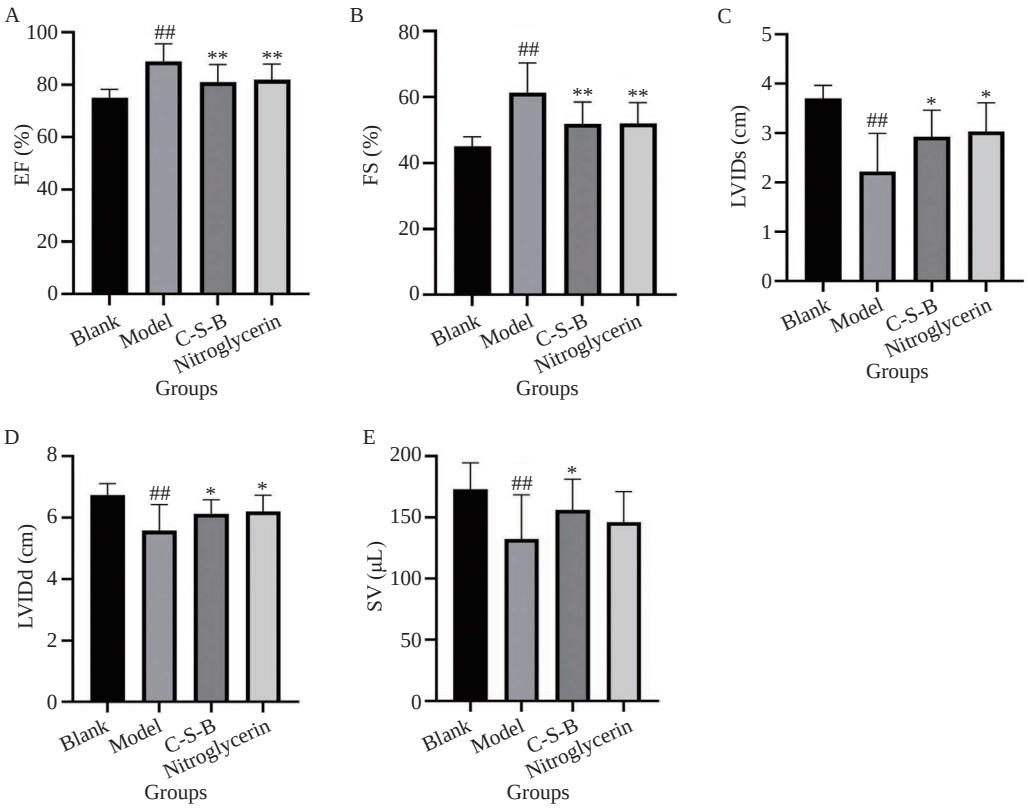

Figure 7 EF, FS, LVIDs, LVIDd, and SV by echocardiography in each group

A, EF results in each group. B, FS results in each group. C, LVIDs results in each group. D, LVIDd results in each group. E, SV results in each group. $^{\#\#}P < 0.01$, compared with the blank group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the model group.

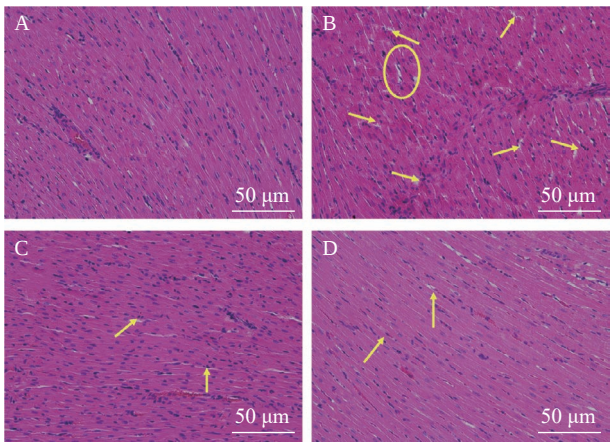

Figure 8 Effects of C-S-B volatile oil on myocardial tissue

A, blank group. B, model group. C, C-S-B group. D, nitroglycerin group.

oil of the C-S-B formula could significantly improve EF, FS, LVIDs, LVIDd and SV myocardial indices in rats with myocardial injury ($P < 0.01$). After component-target-pathway analysis of C-S-B formula volatile oil compounds associated with the treatment of angina pectoris, experiments confirmed that C-S-B formula volatile oil can improve myocardial damage in rat models according to EF, FS, and of other indicators, alleviating myocardial injury caused by hyperfunction, improving cardiac function, and protecting against myocardial damage.

The interaction network of key targets of volatile oil components showed that CHRM4, ADRA1A, CHRM2, CYP2A6, CHRM5, CHRM1, CHRM3, CYP2C9, and CYP2C19 play leading role in the treatment of angina pectoris. CHRM2, UGT2B7, CYP2C19, CHRM4, CHRM3, CYP3A4 and other targets are important according to the pathway enrichment analysis. Studies have shown that the CYP2C subfamily is mainly expressed in the vascular endothelium and catalyzes the production of EETs [15, 16]. EETs mainly have anti-inflammatory effects, reducing vascular endothelial cell adhesion, oxidative stress and apoptosis. The effect of CYP2C subfamily enzymes on EET concentration in coronary heart disease may lead to angina pectoris. The CYP2C9 gene product participates in histamine metabolism, and histamine released by myocardial mast cells under emergency conditions is widely involved in the development of a series of cardiovascular diseases [17]; CYP2C19 is an important homologous sequence of the CYP2C9 gene, whose product plays an important role in cardiovascular drug metabolism and promotes cardiovascular diseases. Dopamine inhibits the release of acetylcholine through the DA receptor (dopamine receptor), the CHRM4 receptor can bind to Gi, inhibit the

activity of AC, thereby counteracting the role of the D1 receptor signaling pathway. That is, M4 activation can hinder the inhibitory effect of dopamine on the release of acetylcholine, and acetylcholine itself can protect from atherosclerosis. Also, acetylcholine can activate CHRM5, promote the release of dopamine, and increase the simultaneous secretion of both dopamine and acetylcholine, thus treating angina pectoris to a certain extent [18]. ADRA1A plays an important role in the sympathetic nervous system by combining endogenous epinephrine, catecholamine, and norepinephrine [19]. Endogenous epinephrine can prevent the increase in pulmonary artery pressure (PAP) during acute hypoxia, and the lack of endogenous β-epinephrine leads to a decrease in ascending aorta blood flow in rats [20]. CHRM1 plays an important protective role in myocardial ischemia-reperfusion injury, and upregulates the expression level of p-Akt (Ser473), which can improve the survival of hypoxic-reoxygenated myocardial cells [21], thus playing a role in protecting these cells from apoptosis. The targets in the network interact and cooperate with each other to participate in the treatment of angina pectoris, which is of great significance in drug development and clinical research.

Our findings after GO enrichment analysis of volatile oil targets in the C-S-B formula show that enrichment of biological functions by key targets mainly involves metabolism, signal transduction and regulation of smooth muscle contraction. The enrichment analysis of KEGG pathways associated with volatile oil components of the C-S-B formula shows that key target pathways mainly involve nerve signal ligand-receptor interactions, calcium signaling pathways and cGMP-PKG signaling pathways. The results showed that the C-S-B formula volatile oil components can regulate a variety of biological functions, and the key targets are distributed in different angina pathways, which provides an important direction for experimental and clinical research.

According to the volatile oil component-target-pathway network model obtained, several important volatile oil components were selected, including ligustilide, dextrobornol, cinnamaldehyde, p-cymene and butenyl phthalide. A study have shown that ligustilide has antioxidant effects and can inhibit the expression of various inflammatory factors, thus preventing atherosclerosis, and can inhibit the contraction of the coronary artery ring induced by KCl, $CaCl_2$, and histamine [22]. It has been found that ligustilide can improve myocardial ischemia and prolong its anti-myocardial ischemia effect, which is the most effective way to relieve angina pectoris in coronary heart disease [22]. Cinnamaldehyde has anti-inflammatory, anti-platelet aggregation, anti-apoptotic effects, among others; it can also improve myocardial fibrosis by stimulating the expression of miR-1252 and regulating the TGF-β/SMAD signaling pathway [23].

To verify the results of network pharmacological analysis in this study, the effects of the C-S-B volatile oil on echocardiographic indices and myocardial pathological morphology were studied using a rat model of myocardial damage. The results showed that C-S-B oil can significantly reduce EF and FS values, significantly increase LVIDd and LVIDs, enlarge ventricular cavity, thin ventricular wall, and enlarge ventricular contraction volume, which could significantly improve cardiac function. The results of histopathological sections showed that the myocardial fibers in the C-S-B group were compact, arranged neatly, had greater integrity, with less edema in the myocardial interstitium, smaller muscle fiber gaps and that there was a small amount of inflammatory cell infiltration, which indicates a better outcome than the model group. The results showed that the essential oil of the C-S-B formula had a significant effect on the improvement and treatment of angina pectoris.

In summary, the volatile oil components of the C-S-B formula play an important role in the treatment of angina pectoris, providing a new direction for drug development. There are few reports on the treatment of angina pectoris by volatile oil components, and these need to be further studied experimentally.

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Competing interests

The authors declare no conflict of interest.

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基于网络药理学探究“川芎-苏合香-冰片”组方挥发油治疗心绞痛作用机制及其对心肌受损大鼠的保护作用

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【摘要】目的 基于网络药理学探讨“川芎-苏合香-冰片”组方挥发油(以下简称 C-S-B 组方挥发油)治疗心绞痛药效物质基础及其作用机制,并探讨 C-S-B 组方挥发油对心肌受损的保护作用。**方法** 通过 GC-MS 测定川芎、苏合香、冰片混合挥发油成分并进行筛选,利用 PharmMapper、GeneCards 和 CoolGeN 数据库预测和筛选三者活性成分的作用靶点。采用 STRING 数据库和 Cytoscape 软件绘制蛋白相互作用 (PPI) 网络图,借助 Rstudio 软件对靶点进行基因本体论 (GO) 分析和京都基因与基因组百科全书 (KEGG) 通路分析,采用 Cytoscape 软件构建成分-靶点-通路-疾病网络。通过大剂量腹腔注射盐酸异丙肾上腺素建立心肌受损大鼠模型,使用 C-S-B 组方挥发油连续干预 14 d 后,分别检测大鼠射血分数(EF)和左心室短轴缩短率(FS)等各项超声心动指标;并通过苏木精-伊红染色法 (HE) 染色对心肌组织指标检测。**结果** 共收集 C-S-B 组方挥发油成分 15 个;C-S-B 组方挥发油成分靶点 470 个;心绞痛相关基因 401 个;核心靶点有 CHRM4、ADRA1A、FGFR1、CHRM2、CYP2A6、CHRM5、CHRM1、CHRM3、HDAC2、MPO 等 28 个。KEGG 分析结果主要涉及神经活性配体 - 受体相互作用,钙离子信号通道,细胞色素 P450 对异生素的代谢等通路。动物实验结果表明 C-S-B 组方挥发油可明显改善心肌受损模型大鼠的 EF、FS、左心室收缩末内径 (LVIDs)、左心室舒张末期内径 (LVIDd)、每搏输出量 (SV) 等心肌指标,且差异均具有统计学意义($P < 0.01$)。**结论** 本研究从多成分-多靶点-多途径分析 C-S-B 组方挥发油成分治疗心绞痛的作用机制,为进一步深入揭示其作用机制奠定基础。动物实验表明,C-S-B 组方挥发油能够改善心肌受损模型大鼠的 EF、FS 等指标,从而缓解心功能过分亢进导致的心肌损伤,改善心脏功能,预防心肌损伤。

【关键词】川芎;苏合香;冰片;挥发油;心绞痛;气相色谱-质谱;网络药理学;心肌受损;大鼠模型