

Study on the Molecular Mechanism of Danggui Buxue Decoction in Intervention of Perimenopausal Syndrome Based on Network Pharmacology and Molecular Docking

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Abstract [Objectives] This study was conducted to explore the intervention mechanism of Danggui Buxue Decoction in perimenopausal syndrome based on network pharmacology and molecular docking. [Methods] The chemical components and targets of Danggui Buxue Decoction were acquired through the TCMSD database, and the main targets of perimenopausal syndrome were obtained through the GeneCards database. The component targets and disease targets were intersected, and combining with active components and Chinese herbs in the decoction, a traditional Chinese medicine-component-target network was constructed using Cytoscape 3.7.1 software. The STRING platform was employed for protein-protein interaction analysis. The DAVID analysis platform was used to conduct target GO and KEGG enrichment analysis, so as to predict the action mechanism Danggui Buxue Decoction. Finally, an active component-disease target-signal pathway network diagram was constructed. [Results] Twenty two components in Danggui Buxue Decoction related to perimenopausal syndrome and 120 corresponding targets were obtained, including active components such as 1,7-dihydroxy-3,9-dimercetol and kaempferol, and key targets such as TNF, ESRI and PPARG. The results of GO analysis and KEGG analysis indicated that Danggui Buxue Decoction might regulate the transcription of RNA polymerase II promoter, DNA templating, gene expression, signal transduction, hypoxia response and other biological processes by regulating multiple signal pathways such as chemical carcinogenesis-receptor activation, cancer pathways, lipid and atherosclerosis, tryptophan metabolism, malaria, steroid hormone biosynthesis and chemical carcinogenesis-DNA adduct. [Conclusions] Danggui Buxue Decoction intervenes in perimenopausal syndrome through multiple components, targets and pathways, providing a basis for elucidating the intervention mechanism of Danggui Buxue Decoction and expanding its clinical application.

Key words Danggui Buxue Decoction; Perimenopausal syndrome; Network pharmacology; Molecular docking

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Perimenopausal syndrome is a common symptom group in women before and after menopause. As women enter menopause, ovarian functions gradually decline, leading to a decrease in estrogen levels and dysfunction of the nervous, endocrine and immune systems, manifested as clinical symptoms such as menstrual disorders, hot flashes, irritability, palpitations, dizziness, headaches and insomnia^[1]. Due to the influences of modern social pressure, environmental pollution, eating habits and other factors, perimenopausal syndrome shows an incidence rate increasing year by year and a younger trend, which brings many problems to women's life and work. Research shows that about 75% of women will have some symptoms of menopausal syndrome before and after menopause^[2]. Among them, about 20% women have mild symptoms, about 25% women have moderate symptoms, and about 30% women have severe symptoms. In clinical practice, perimenopausal syndrome is often treated with Western medicine, such as hormone replacement and antidepressants, but there are many adverse reactions, such as increased vaginal discharge, breast distension,

edema, headache, pigmentation, and increased risk of cancer.

In recent years, Chinese medicine has received more and more attention in clinical research because it has definite clinical effect on various symptoms of perimenopausal syndrome and less adverse reactions and can minimize the adverse effects caused by hormone replacement therapy^[3]. Danggui Buxue Decoction is originated from *Theory of Differentiation and Confusion of Internal and External Injuries* written by Li Dongyuan, the originator of the school of invigorating the spleen and stomach, one of the four great medical schools in Jin and Yuan Dynasties. In the prescription, Radix Astragali is used to replenish the qi of spleen and lungs to nourish the source of blood production, as qi serves as the commander of blood. Meanwhile, coupling with Radix Angelicae Sinensis, which has the effects of nourishing blood and promoting blood circulation, the prescription mainly treats fatigue and internal injuries, as well as weak qi and blood deficiency. Modern pharmacological research has confirmed that Danggui Buxue Decoction has functions such as promoting hematopoiesis, regulating immunity, and protecting cardiovascular and cerebrovascular systems^[4]. It is commonly used in clinical practice to treat various types of hematological diseases such as anemia, cardiovascular and cerebrovascular system diseases, immune system diseases, various surgical and postoperative complications and tumors, and for adjuvant treatment for patients undergoing radiotherapy and chemotherapy^[5]. In recent years, scholars at home and abroad have gradually deepened their research on Danggui Buxue Decoction, but there is still a lack of intervention in perimenopausal

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syndrome. Therefore, in this study, research and exploration were conducted on the intervention of Danggui Buxue Decoction in perimenopausal syndrome, laying a foundation for better research on its intervention in perimenopausal syndrome in the future.

Methods

Acquisition of active components in Danggui Buxue Decoction and their targets

The chemical constituents of Danggui Buxue Decoction were obtained by searching the TCMSP database with the conditions defined as oral bioavailability (OB) $\geq 30\%$ and drug-likeness property (DL) ≥ 0.18 , and supplemented according to published literature reports. Names of target proteins corresponding to the active components were obtained in the TCMSP database. The target proteins were corrected for gene names through the Uniprot database.

Target screening for perimenopausal syndrome and PPI network construction

The key word "perimenopause" was searched in GeneCards database, and relevant targets of perimenopausal syndrome were obtained while deleting duplicate values. VENNY 2.1 was used to make a Venny diagram of component targets and disease targets, and common targets of the components and disease were obtained. The intersection targets were imported into the STRING database, and a protein-protein interaction network diagram was constructed, which was visualized and analyzed by Cytoscape 3.7.1 software.

Target function enrichment

The intersection targets were imported into the DAVID database for GO enrichment analysis and KEGG pathway enrichment analysis. The threshold was set to $P < 0.05$.

Construction of active component-disease target-signal pathway network

The active components, target genes and enrichment pathways were introduced into Cytoscape 3.9.1 software to construct a component-target-pathway network. And topological parameter analysis was conducted.

Molecular docking verification

The structure files of active components were downloaded from the Pubchem database, and the crystal structures of target proteins were downloaded from the RCSB PDB database. Molecular docking verification was conducted using AutoDock 1.5.6 software. The results were visualized using Pymol software.

Results

Active components in Danggui Buxue Decoction and their targets

In this study, a total of 22 active compounds were screened from Danggui Buxue Decoction. The TCMSP database predicted 526 component target proteins, and after correcting the gene names in the Uniprot database, 120 targets related to active components of Danggui Buxue Decoction were obtained.

Screening of targets related to perimenopausal syndrome

Targets of perimenopausal syndrome were screened from the

GeneCards database, while removing duplicate values. Ultimately, 177 targets related to perimenopausal syndrome were obtained.

Construction of protein-protein interaction network (PPI)

An intersection map of component targets and disease targets was created using the Venny diagram production platform, and 21 common targets were obtained for the active components of Danggui Buxue Decoction and perimenopausal syndrome, as shown in Fig. 1. The intersection targets were submitted to the STRING analysis platform, and the obtained data were imported into Cytoscape 3.9.1 software to create a protein-protein interaction network diagram. The PPI network diagram has 21 nodes and 102 edges, as shown in Fig. 2. According to the analysis results, TNF, ESR1, Degree, PPARG, TGF β 1, HMOX1, CYP3A4, SIRT1 and CYP1A1 had high degree values, and they might be key targets of Danggui Buxue Decoction in the treatment of perimenopausal syndrome.

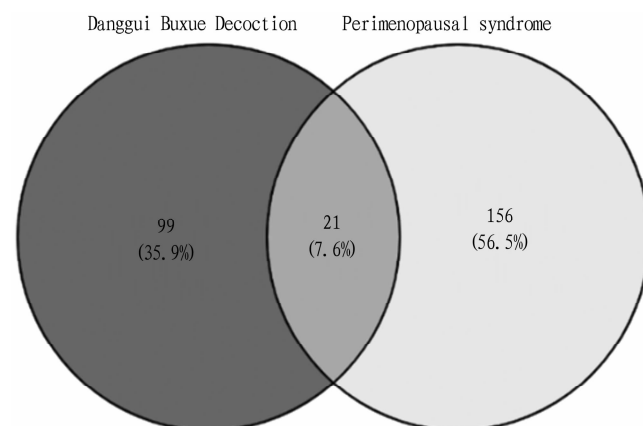


Fig. 1 Danggui Buxue Decoction active component-perimenopausal syndrome Venny diagram

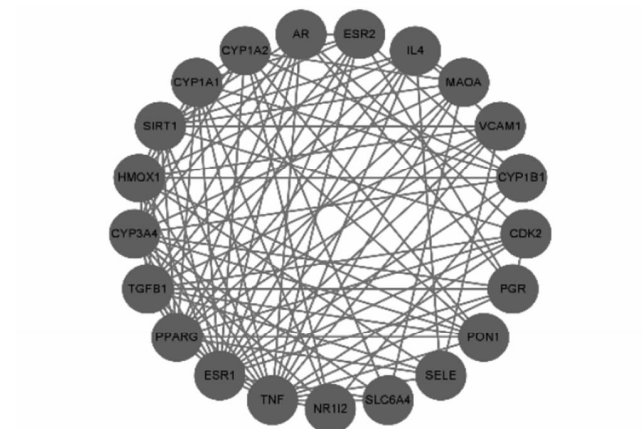


Fig. 2 Active component-disease common target protein-protein interaction network

Construction of traditional Chinese medicine-component-target network

Potential active components and medicinal herbs corresponding to the intersection targets were screened out, and the components that do not act on these 21 targets were eliminated, and 19 active components that act on perimenopausal syndrome were obtained. A

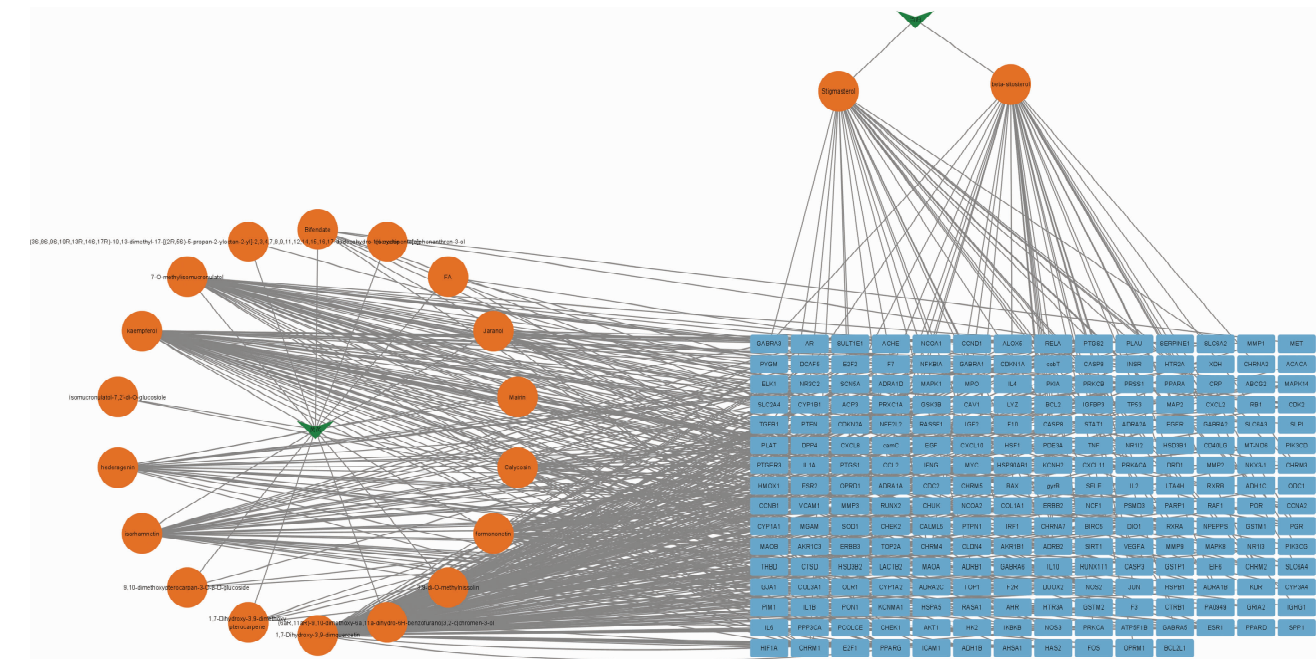


Fig. 3 "Traditional Chinese medicine-component-target" network diagram for Danggui Buxue Decoction

Table 1 Topological parameters of nodes in PPI network diagram

No.	Name of target	Degree value
1	TNF	18
2	ESR1	17
3	PPARG	15
4	TGFB1	13
5	HMOX1	12
6	CYP3A4	12
7	SIRT1	11
8	CYP1A1	10

traditional Chinese medicine-component-target network diagram was constructed and imported into Cytoscape 3.9.1 software for visualization, as shown in Fig. 3. In the diagram, blue rectangles represent targets, green V shapes represents names of Chinese herbs, and orange circles represent components in Chinese herbs.

Functional enrichment analysis

GO (gene ontology) functional enrichment analysis was conducted on intersection targets through the DAVID database. The results showed that a total of 200 GO items were obtained. In specific, the top BP results included negative regulation of RNA polymerase II promoter transcription, positive regulation of RNA polymerase II promoter transcription, positive regulation of transcription, DNA templating, positive regulation of gene expression, signal transduction, and response to hypoxia. The top CC results involved components of membranes, nucleoplasm, organelles bound to intracellular membrane, chromatin and extracellular space. And protein binding, enzyme binding, identical protein binding, sequence-specific DNA binding in the proximal region of the core promoter of RNA polymerase II and metal ion binding showed the most significant effects in MF analysis, as shown in Fig. 4.

A total of 31 signal pathways were collected from KEGG analysis results. The top 20 disease-related signal pathways were screened, mainly including chemical carcinogenesis-receptor

activation, cancer pathways, lipid and atherosclerosis, tryptophan metabolism, malaria, steroid hormone biosynthesis, chemical carcinogenesis-DNA adduct, as shown in Fig. 5.

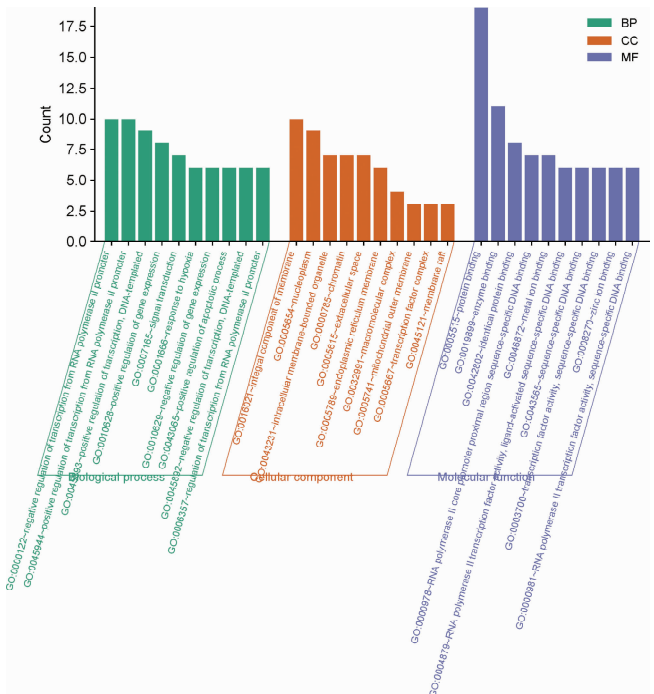
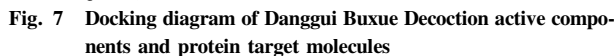


Fig. 4 GO enrichment analysis on the treatment targets of Danggui Buxue Decoction

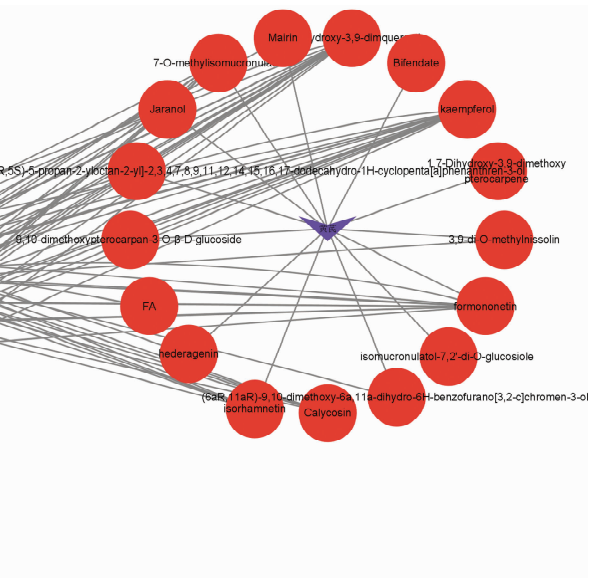
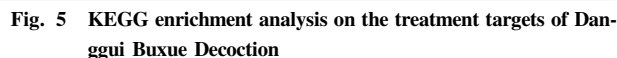
Construction of active component-disease target-signal pathway network diagram

The selected 19 active components, 21 target genes and 20 PD-related pathways were imported into Cytoscape 3.9.1 software to construct an "active component-disease target-signal pathway"

The core targets TNF, ESRI, PPARG, TGFBI, HMOXI and CYP3A4 with the top 6 degree values in the active component-disease common target PPI network diagram were selected for molecular docking with the active components with the top 5 degree values in the active component-disease target-signal pathway network diagram, as shown in Fig. 7 and Table 2. The results showed that the values of docking binding energy were all smaller than -5.0 kcal/mol.



indicating that the active components could bind well with the target protein receptors, forming relatively stable structures after binding.



Perimenopausal syndrome belongs to the category of "pre- and post-menopausal symptoms" in traditional Chinese medicine, which mostly occurs in women around the age of 49 (7 × 7), and may be found earlier in those with a history of ovarian surgery, radiotherapy and chemotherapy. When women enter the perimenopausal syndrome, the viscera function is gradually weakened, and the source of qi and blood is lacking, leading to unbalanced yin and yang, "weak Tai-Chong vessel" and "menopause", resulting in menstrual disorder or even amenorrhea, or a series of pre- and post-menopausal symptoms such as metrorrhagia, palpitation, headache, insomnia, emotional anxiety or depression. Professor He pointed out that the hypofunction of zang-fu organs is the basic pathogenesis leading to perimenopausal syndrome, mainly involving the kidney, liver, spleen and heart. Before and after menopause, women often suffer from qi and blood deficiency, so Professor

Table 2 Molecular docking results between active components in Danggui Buxue Decoction and key targets

kcal/mol

Hub target	Docking affinity with hub target proteins					
	TNF	ESR1	PPARG	TGFB1	HMOX1	CYP3A4
1,7-Dihydroxy-3,9-dimquercetin	−5.75	5.89	6.39	4.46	5.62	7.77
Kaempferol	7.11	5.21	7.23	5.26	6.16	7.06
Formononetin	6.55	5.85	7.66	6.09	5.63	7.88
7-O-Methylisomucronulatol	5.23	5.49	7.32	4.98	5.32	7.11
Isorhamnetin	6.46	5.32	5.02	4.44	5.64	7.34

He's ointment is often used to nourish blood with four herbs in Siwu Decoction and replenish qi with four herbs in Sijunzi Decoction, and those with severe qi deficiency are replenished by wild ginseng, *Radix Pseudostellariae*, *Radix Astragali*, *etc.* , "to supplement the qi deficiency of the five internal organs"; and *Colla Corii Asini* and *Radix Angelicae Sinensis* are used to replenish blood and nourish blood for those with severe blood deficiency^[6]. Therefore, Danggui Buxue Decoction is an excellent choice to intervene in perimenopausal syndrome.

Danggui Buxue Decoction is a classic prescription for tonifying both qi and blood, and the clinical practice of traditional Chinese medicine shows that it has definite curative effect on the syndrome of qi weakness and blood deficiency and the syndrome of blood deficiency after menstruation and childbirth in women. Modern clinic has extended it to treat anemia, climacteric syndrome, cardiovascular and cerebrovascular diseases, adjuvant treatment of cancer patients and diseases caused by immune dysfunction^[4].

The results of network pharmacological analysis showed that components such as 1,7-dihydroxy-3,9-dimquercetin, kaempferol, formononetin, 7-O-methylisomucronulatol and isorhamnetin were potential active components for intervention in perimenopausal syndrome. The targets TNF, ESR1, Degree, PPARG, TGFB1, HMOX1, CYP3A4, SIRT1 and CYP1A1 were frequently connected in the PPI network, and considered important potential targets for Danggui Buxue Decoction to intervene in perimenopausal syndrome. TNF is a multifunctional pro-inflammatory signaling factor that can bind to specific receptors on the cell membrane, participate in the activation, function and differentiation of immune regulatory cells, and regulate malignant bacterial infections, immune system, cell apoptosis, and other functions^[7]. TNF can participate in the immune response by activating T cell immune activity, and has the ability to kill transformed cells and cells infected with certain viruses^[8]. TNF-α is a kind of inflammatory cytokine with various biological activity, mainly secreted by hypertrophy, endothelium and macrophages. TNF-α can transmit biological information to nuclei through specific receptors present on the cell membrane, leading to the production of biological activity such as promotion of cell proliferation and differentiation, cytotoxicity, apoptosis, immune regulation, anti-tumor, antiviral, and inflammatory mediation. The concentration of inflammatory factors such as TNF in the endometrium of patients with dysmenorrhea is relatively high, so it is closely related to the occurrence of dysmenorrhea^[9].

In summary, in this study, with active components of the classic formula Danggui Buxue Decoction as the research object, network pharmacology was applied to establish "traditional Chinese medicine-component-target" and "component-pathway-target" compound networks to identify potential active components, related therapeutic targets, and signal pathways of Danggui Buxue Decoction. The results revealed that Danggui Buxue Decoction can intervene in perimenopausal syndrome through multiple components, targets and pathways, laying a foundation for further research on the mechanism of Danggui Buxue Decoction's intervention in perimenopausal syndrome.

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