

Mechanism of Banxia-Huanglian in the Treatment of Diabetes from the Perspective of Network Pharmacology Based on "Xin Kai Ku Jiang" Theory

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Abstract: Our purpose is to explore the mechanism of Banxia-Huanglian in the treatment of diabetes from the perspective of network pharmacology based on the theory of "Xin Kai Ku Jiang". Our method was to use Traditional Chinese Medicine Systems Pharmacology database (TCMSP) and Swiss Target Prediction database to search the chemically active components and their respective targets of Banxia and Huanglian, and to use GeneCards database, DisGeNET database, OMIM database and Drugbank database to collect the diabetes-related targets. The intersection targets of the Banxia-Huanglian and diabetes were input into the online database of STRING, thus the protein-protein interaction (PPI) network was obtained. Then GO and KEGG enrichment analysis was carried out by Metascape and the "Banxia-Huanglian active components - targets-pathways" network diagram was constructed by Cytoscape3.6.1 software. Finally, Autodock Tolls software was used for molecular docking. The results of this study were as follows: A total of 22 active components, 175 potential targets, 1812 targets of Diabetes, and 99 targets of intersection targets was obtained. The network analysis results showed that the key chemical components of Banxia-Huanglian in the treatment of diabetes mainly involve quercetin, baicalein and beta-sitosterol, and the key targets included MAPK1, AKT1 and PTGS2. We concluded that Banxia-Huanglian has an inhibiting inflammatory response, regulating glycolipid metabolism and reducing insulin resistance effect mainly by hypoglycemic, thereby playing a role in the treatment of diabetes.

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia. In traditional Chinese medicine, diabetes belongs to the category of Xiaoke disease, and its basic pathogenesis is Yin deficiency and dry heat, whose essence is deficiency syndrome and its surface is excess syndrome. However, with the change of modern living environment and the improvement of quality of life, diabetes is mainly manifested as damp heat, phlegm and blood stasis, and symptoms such as dry mouth and bitter mouth, dark red tongue, yellow and greasy tongue coating

are more common. Therefore, the TCM treatment of diabetes has also changed from "reinforcing" to "promoting" [1]. Xin-kai-Ku-jiang method was first developed by Zhang Zhongjing, a medical sage, and is commonly used in the treatment of many classic prescriptions, such as Banxia-Xiexin decoction, Xiaoxinxiong decoction, Huanglian-Wendan decoction, etc. The main purpose of this method is to combine two different flavors of Spicy-mild and bitter-cold drugs to adjust the air machine, dissipate dampness-heat, and dissipate phlegm, which is an important treatment for diabetes and its complications [2-3]. In this study, we will explore the mechanism of Banxia-Huanglian in the treatment of diabetes by means of network pharmacology, and verify the results by molecular docking technology, so as to lay the foundation for further research.

1. Materials and Methods

1.1 Screening of active ingredients and related targets of Banxia-Huanglian

Through TCMSP (<http://tcmospw.com/tcmosp.php>) to retrieval Banxia and Huanglian' active ingredient, with oral use of degrees (OB) $\geq 30\%$ and drug like (DL) ≥ 0.18 . The selected active ingredients are matched to their corresponding protein targets on TCMSP. Finally, all targets were merged and duplicated, and to standardize the protein target information with Uniprot database (<https://www.uniprot.org>).

1.2 Screening of diabetes-related targets

Using "diabetes mellitus" as the key word to mine disease targets in GeneCards (<https://www.genecards.org/>), DisGeNet (<https://www.disgenet.org/>), OMIM (<https://omim.org/>) and Drugbank (<http://www.drugbank.ca/>) database. The obtained targets are merged and eliminated to obtain potential targets of diabetes.

1.3 Network construction of drug pair-active ingredient-target

Venny2.1.0 was used to construct the Venn diagram of drug-disease targets, and obtain the intersection genes of "Banxia-Huanglian" and "diabetes", and to integrate the mapping relationship of drug-active component-intersection genes.

1.4 Protein interaction (PPI) network construction

The intersection targets of Banxia-Huanglian and diabetes obtained by Venn diagram are uploaded to the String database (<https://string-db.org/>). In this database, we set the species to "Homo sapiens" and select minimum required interaction score > 0.9 . The output was TSV format file. The file was input into Cytoscape 3.6.1 software to obtain the key target proteins according to the degree value.

1.5 GO and KEGG analyses

The intersection targets were imported into the Metascape platform for GO enrichment Analysis and KEGG pathway analysis, respectively. The top 10 GO enrichment analysis results (biological process, molecular function, cell composition) and the top 20 KEGG enrichment analysis results were selected and imported into the wechat platform for visual analysis.

1.6 Construction of component-target-pathway network diagram

Mining the targets enriched in each of the top 20 pathways of KEGG enrichment analysis results, and matching the corresponding chemical components according to the results of 1.1. CytoScape3.6.1 was used to construct the "components-intersection target-pathway network diagram".

1.7 Molecular docking validation

According to the Degree value, the core targets and components are performed on docking. The compound structure are obtained from PubChem database (<https:// PubChem. Ncbi. While NLM. Nih. Gov />) and the protein structures are obtained from the PDB database (<https:// www.rcsb.org/>). Autodock Tolls software was used to achieve molecular docking with binding energy ≤ -5.0 kcal/mol. Finally, Pymol software was used to visualize the results.

2. Results

2.1 Acquisition of active Ingredients and related targets of Banxia-Huanglian

After preliminary screening, 13 active ingredients of Banxia and 14 active ingredients of Huanglian were obtained. Through the database prediction and supplement, we obtained 175 targets, as shown in Table 1.

Table 1: Basic information of active ingredients of Banxia and Huanglian

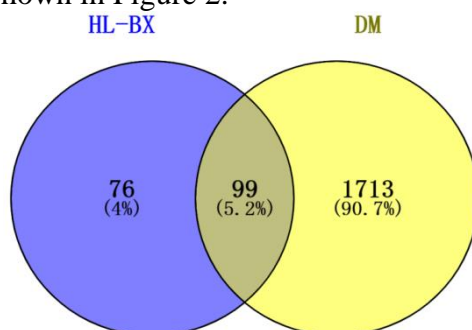
Mol ID	Active ingredient	OB (%)	DL	Drug
MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76	Banxia
MOL002670	Cavidine	35.64	0.81	Banxia
MOL002714	baicalein	33.52	0.21	Banxia
MOL000358	beta-sitosterol	36.91	0.75	Banxia
MOL000449	Stigmasterol	43.83	0.76	Banxia
MOL005030	gondoic acid	30.7	0.2	Banxia
MOL000519	coniferin	31.11	0.32	Banxia
MOL006936	10,13-eicosadienoic	39.99	0.2	Banxia
MOL006957	(3S,6S)-3-(benzyl)-6-(4-hydroxybenzyl)perazine-2,5-quinone	46.89	0.27	Banxia
MOL003578	Cycloartenol	38.69	0.78	Banxia
MOL006967	beta-D-Ribofuranoside, xanthine-9	44.72	0.21	Banxia
MOL001454	berberine	36.86	0.78	Huanglian
MOL002894	berberrubine	35.74	0.73	Huanglian
MOL002897	epiberberine	43.09	0.78	Huanglian
MOL002903	(R)-Canadine	55.37	0.77	Huanglian
MOL002904	Berlambine	36.68	0.82	Huanglian
MOL002907	Corchoroside A_gt	104.95	0.78	Huanglian
MOL000622	Magnograndiolide	63.71	0.19	Huanglian
MOL000785	palmatine	64.6	0.65	Huanglian
MOL000098	quercetin	46.43	0.28	Huanglian
MOL001458	coptisine	30.67	0.86	Huanglian
MOL002668	Worenine	45.83	0.87	Huanglian

2.2 Abtaining the disease targets of diabetes

Through searching in Genecards, DisGeNET, OMIM and Drugbank database, we have obtained 17,759, 2804, 225 and 57 disease targets respectively. Deleting the duplicate targets resulted in 1812 targets for diabetes.

2.3 PPI Network Construction

The number of drug-disease intersection targets is 99, as shown in Figure 1. The 99 intersection genes were imported into the STRING database to obtain the PPI network graph. The results showed that there were 99 nodes and 227 edges in the PPI network graph, and the average node degree value was 4.59. MAPK1, AKT1, TP53, and TNF were selected as the top four genes with the largest Degree value according to degree. These genes play important roles through protein-protein interaction, as shown in Figure 2.



(HL-BX is the abbreviation for drug, DM is the abbreviation for diabetes mellitus)

Figure 1: Venn diagram of drug and diabetic targets

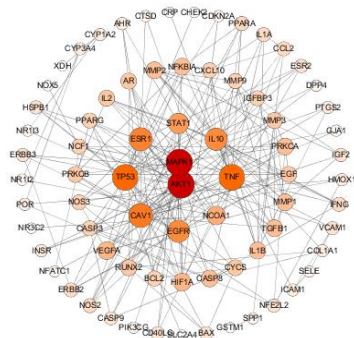


Figure 2: PPI network graph of drug-pair-disease targets

2.4 GO and KEGG analyses

There are 436 GO items in the result, of which 225 were biological process (BP), 132 were molecular function (MF), and 79 were cellular component (CC).

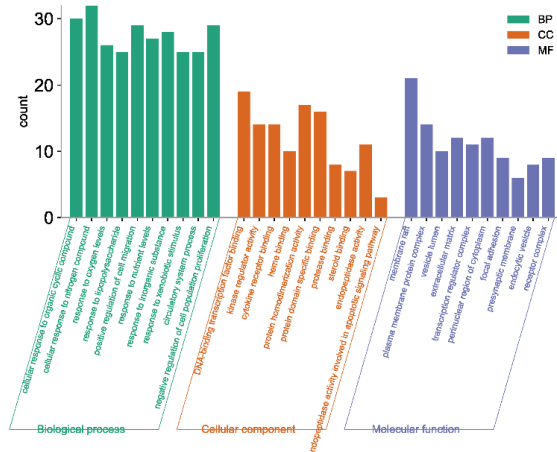


Figure 3: Three-in-one bar chart of BP, MF, and CC

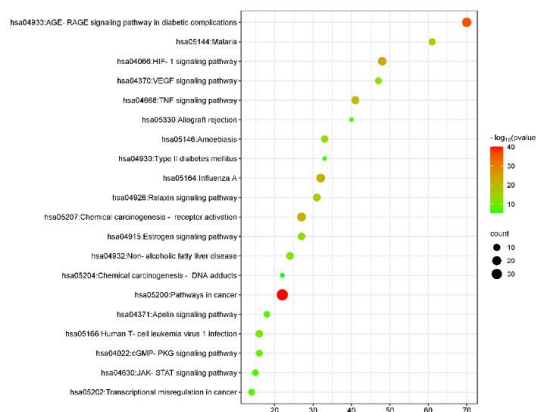
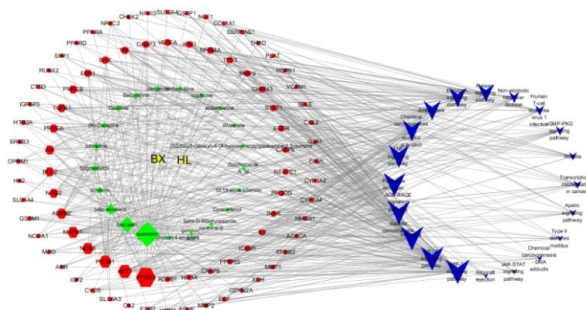


Figure 4: Bubble map of KEGG top 20 pathways

2.5 Construction of component-target-pathway network maps

According to the KEGG enrichment results, we obtained the component-target-key pathway network diagram, which with 125 nodes (20 pathways, 82 targets, 21 components, 2 medicinal materials) and 650 edges. As shown in Figure 5.



(Circular nodes represent drugs, diamond nodes represent differential components, hexagonal nodes represent targets, and "V" shapes represent signaling pathways)

Figure 5: Drug-component-target-pathway network diagram

2.6 Results of molecular docking

As shown in Table 2. The visualization of the key targets and the compounds with the best binding activity is shown in Figure 6.

Table 2: Molecular docking results

target	PDB	Binding energy/kcal mol ⁻¹		
		Quercetin	Baicalein	beta-sitosterol
PTGS2	5f19	-5.01	-5.86	-5.14
AKT1	2uzs	-6.00	-5.38	-6.95
MAPK1	1PME	-5.04	-5.84	-5.75

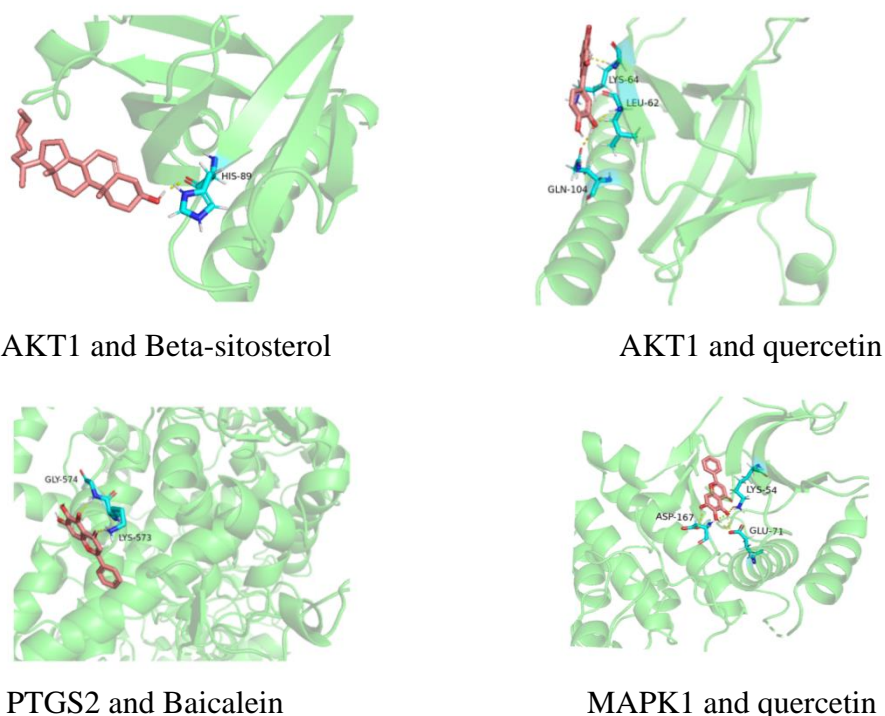


Figure 6: Visualization of optimal docking results

3. Discussion

The method of Xin Kai Ku Jiang has been used in the treatment of diabetes for a long time. Ye Tianshi pointed out in the *Medical Records of the Clinical Guidelines* that "a little bitterness can be used to clear down, and a little spicy can be used to promote publicity." Compatibility of spicy and bitter drugs makes Yin and Yang coordinate, the middle coke qi machine flows smoothly, and the body fluid is carried out, which can not only prevent the accumulation of dampness to produce phlegm, but also dissipate the already formed phlegm [4]. Banxia-Huanglian, as the representative drug pair of Xin-Kai-Ku-Jiang method, is widely used in clinical practice. The *MingYiBieLu* was the first to record that Huanglian had the function of "stopping wasting-thirst". In the *Treatise on Materia Medica*, it was mentioned that Banxia had the function of "eliminating phlegm and saliva, invigorating the spleen and appetizing the stomach". Therefore, the combination of Banxia and Huanglian, which can dispel phlegm but not warm and dry, dissipate heat but not damage Yin liquid.

In this study, quercetin, baicalein and β -sitosterol were preliminarily screened as the core

components of Banxia-Huanglian in the treatment of diabetes. Studies have shown that quercetin can regulate GLUT4 (glucose transporter 4) by activating AMPK signaling pathway, which improve the function of small intestinal endothelial cells, and increase the absorption of glucose by cells, thus maintaining the stability of glucose metabolism^[5-6]. Studies have found that baicalein can significantly reduce kidney damage in diabetic rats and alleviate renal oxidative stress, thereby improving the structural changes of renal tissue^[7]. Baicalein can also significantly inhibit monocyte adhesion, TGF- β 1 mRNA expression and vascular inflammation caused by hyperglycemia, thereby reducing or delaying diabetic vascular damage^[8]. β -sitosterol has a similar effect to metformin, which can reduce blood glucose by reducing intestinal glucose absorption, promoting glycogenolysis, and accelerating gluconeogenesis^[9]. Rahim-ifard^[10] found that β -sitosterol can enhance the function of islet β cells, promote insulin secretion and improve insulin activity, thereby enhancing the hypoglycemic effect.

In terms of targets, the core targets of Banxia-Huanglian in the treatment of diabetes are MAPK1, AKT1 and PTGS2, which are involved in the pathogenesis of diabetes and its complications by positive or negative regulation. MAPK1 (mitogen-activated protein kinase 1) is a key kinase regulating glucose and lipid metabolism in the body^[11], and also a key kinase in the MAPK signaling pathway, which is involved in the occurrence of diabetes and its various complications^[12]. AKT1 is a kind of protein kinase and a key protein in the AKT signaling pathway^[13], which can not only regulate glucose and lipid metabolism, but also activate downstream response molecules to play a role in anti-oxidative stress^[14]. Studies have found that the activation of AKT signaling pathway can reduce inflammatory response^[15]. PTGS2 is an inducible rate-limiting enzyme and a key enzyme in prostaglandin synthesis. Studies have confirmed that PTGS2 expression is enhanced in tissue damage and inflammation conditions. By inhibiting PTGS2 expression, diabetes and its complications can be improved by reducing inflammatory response^[16]. In addition, PTGS2 can negatively regulate insulin secretion and reduce insulin sensitivity^[17].

In addition, Banxia-Huanglian treatment of diabetes involves multiple complex signaling pathways. AGEs can cause diabetic kidney injury, diabetic cardiovascular disease, diabetic peripheral vascular disease and other complications. AGE-RAGE signaling pathway can reduce the expression of AGE, reduce the production of oxygen-causing free radicals and the release of pro-inflammatory factors^[18]. TNF signaling pathway is mainly involved in inflammatory response, cell apoptosis and the regulation of some hormone levels^[19]. Increased TNF in blood can lead to serine phosphorylation, destroy insulin signaling, and induce insulin resistance in adipocytes and surrounding tissues^[20]. HIF1 signaling pathway plays a significant role in glucose and lipid metabolism, which can inhibit the catabolism of fatty acids by inhibiting medium-chain and long-chain acyl-CoA dehydrogenase^[21].

In conclusion, Banxia-Huanglian mainly acts on the core targets of MAPK1, AKT1 and PTGS2 through quercetin, baicalein and β -sitosterol, and regulates the diabetic complications AGE-RAGE signaling pathway, TNF signaling pathway and HIF1 signaling pathway. It reflects the characteristics of multiple active components, multiple targets and multiple action pathways of traditional Chinese medicine in the treatment of diseases.

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