The Mechanism of Jinqi Jiangtang Tablet in Treatment of Type 2 Diabetes Mellitus Based on Network Pharmacology

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Research

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Abstract

Background: Type 2 Diabetes Mellitus(T2DM) is an endocrine disease that caused mainly by insulin resistance (IR) and β cell dysfunction. The incidence of T2DM is quite high in the worldwide. To explore the molecular mechanism of Jinqi Jiangtang Tablet(JJT) in treating of T2DM based on Network Pharmacology.

Methods: The active compounds, targets of three Traditional Chinese medicines in JJT were obtained by the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database and Uniprot database; The targets of T2DM were screened through the Drugbank database; The compound-target network was constructed via the Cytoscape 3.7.2 software and used the built-in Network analyzer to analyze and select the key active compounds; The overlapping targets of drug and disease targets were gained by the Venny online tool and the targets were built by STRING website to select the key genes; Gene Ontology(GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were performed on the potential targets using DAVID6.8 online tool to study the mechanism of overlapping targets. Via Systems Dock platform to validate the interaction between compound and targets

Results: Twenty-five active compounds of JJT were screened, 101 drug targets, 142 disease targets and twenty-one overlapping targets. GO enrichment analysis showed that the biological processes (BP) mainly included the blood circulation ,etc. Cell composition(CC) mainly affected the integral component of plasma membrane, etc. Molecular functions(MF) mainly involved alpha-adrenergic receptor activity, etc. KEGG pathway analysis showed that there were twelve pathways related to T2DM, among which PPAR signaling pathway was related to T2DM mostly. RXRA is one of key targets of JJT and berberine performed well.

Conclusions: This study revealed the mechanism of JJT in treatment of T2DM preliminarily and supplied a further foundation for studying its mechanism.

1. Background

Diabetes mellitus(DM) is a chronic, life-long [1]_metabolic disease characterized by hyperglycemia_[2]. Nowadays, DM is one of the world's epidemic diseases and second only to the malignant tumors and cardiovascular diseases, which has become the third largest disease endangering to human health_[3]. DM is often divided into type I, type II and other types[2],and among them T2DM accounts for 85–95% of the total number of DM [3]. T2DM is an endocrine disease that caused mainly by insulin resistance (IR) and β cell dysfunction. The incidence of T2DM is quite high in the worldwide. In 2017, the World Diabetes Federation has reported that there were approximately 451 million diabetic patients around the global and it is expected to soar to 693 million in 2045[4]. It is important particularly to note that the clinical incidence of T2DM will clearly lead to the emergence of many complications, such as Diabetic Dephropathy_(DN) or Diabetic foot disease, etc., and these complications will cause more serious harm to

patients lately [5]. Therefore, it is imminent to find safe and effective drugs and methods for the treatment of patients with T2DM. However, there is still no curative medicine. The main treatment in clinical practice adopts synthetic drugs [6]. Synthetic drugs treat the T2DM with oral hypoglycemic drugs, insulin and supplementary diet control plus exercise therapy routinely. Although it has certain effects [3], it is easy to cause other adverse reactions, such as ketoacidosis, obesity, hypoglycemia, cardiovascular disease and so on [7–8]. Given many side effects caused by synthetic drugs treatment, so we consider that T2DM can be treated from the perspective of traditional Chinese medicine. Chinese medicine classifies DM as a "thirst quencher" category. The traditional Chinese medicine focuses on holistic treatment and it has multiple active compounds. It can interfere with the development of disease through the single-target addition, multiple-target synergy and toxic dispersion effect of multiple ways. Therefore, it becomes an option worth considering in the research and development of anti-diabetic drugs [9].

Jinqi Jiangtang Table (JJT) is a safe and effective Chinese patent medicine for the treatment of DM, developed by the Institute of Materia Medica, Chinese Academy of Medical Sciences based on ancient prescriptions combined with modern pharmacological research [10]. JJT is derived from Qianjin huanglian Pills of Qianjinfang, which is composed of *Coptidis Rhizoma*, *Hedysarum Multijugum Maxim*, and *Lonicerae Japonicae Flos*. It has the functions of clearing heat and nourishing qi, promoting hydration and quenching thirst [1], which is also a Chinese patent medicine used in the clinical treatment of T2DM [10]. At present, it is reported that the main constituents of traditional Chinese medicine or its extracted compounds or parts in JJT perform good anti-diabetic activity [12]. The pharmacological experiments have also proven that [11] a variety of chemical compounds in JJT can go through multiple links, for example, improving glycemic lipids metabolism and insulin resistance, antioxidation, immune regulation and the like, which plays a vital role in anti-diabetic at different pharmacological levels such as the whole, cells, molecules, etc.. Currently, it has reported that many researches on the pharmacological activity of JJT in the treatment of DM, but the molecular mechanism of JJT in treating T2DM based on network pharmacology has never been reported and it remains unclear, which is not conducive to the development and utilization of JJT, a classic prescription. British pharmacologist Hopkins [13] proposed the concept of "Network pharmacology" in 2007 and defined it as a branch of pharmacology, which used the network method to analyze the "multiple-component, multiple-target, multiple-path" synergy between drugs and diseases and targets relations. The research strategy of network pharmacology covers multiple targets. Combining drug-target networks with biological system networks can provide new approaches and strategies for new drug development [14]. The research philosophy of network pharmacology is to build a bioinformatic network by integrating the relevant information of multiple databases, analyzing the network topology further and selecting specific signal nodes to achieve multiple-target prediction of drug action [13]. In order to promote the development and utilization of JJT in the treatment of T2DM, this study explore multiple databases to mine and screen the active chemical compounds and drug targets of JJT comprehensively, thereby predicting the molecular mechanism and providing new ideas and new approaches for the study of JJT in the treatment of T2DM and applying it to the clinic. The flowchart of this study was shown in Fig. 1.

2. Materials And Methods

2.1 Screening the active compounds and potential targets of JJT

Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (<http://ibts.hkbu.edu.hk/LSP/tcmsp.php>) (TCMSP) is a unique Chinese herbal medicine system pharmacology platform, which can capture the relationship between drugs, targets and diseases, and research the drug target network and disease network on the whole level. [15–17].

In this study, TCMSP was used to screened the active and potential compounds. "Herb name" was selected in the TCMSP database and regarded the *Coptidis Rhizoma*, *Hedysarum Multijugum Maxim*, and *Lonicerae Japonicae Flos* as keywords to search all chemical constituents of three kinds of traditional Chinese medicine in JJT. Oral bioavailability (OB), drug-like (DL) and small intestinal epithelial cell permeability (Caco-2) were the core parameters for drug screening. Oral bioavailability (OB) $\geq 30\%$, and drug-like (DL) ≥ 0.18 [18] and small intestinal epithelial cell permeability (Caco-2) ≥ 0.4 [19] were the criteria for screening compounds with higher activity. Finally, the target proteins corresponding to the compounds were obtained by the TCMSP platform. At the same time, the protein database Uniprot Database(<http://www.Uniprot.org/>) was used and the search conditions were "Reviewed" and "Homo Sapiens" to obtain the information of compounds and related target genes.

2.2 Identification the related targets of T2DM

In order to explore the effects of drugs on diseases and clarify the mechanism of drug target receptor binding to disease targets, with "type 2 diabetes mellitus" as the key search term and the related target genes of T2DM were searched in the DrugBank database (<http://www.drugbank.ca/>). Finally, all target genes related to T2DM were obtained from it.

2.3 Construction the Component-Target network

Cytoscape(<https://cytoscape.org/>) is a software for graphically displaying networks that can analyze and edit networks[20]. The information of the compounds obtained from the TCSMP database and the Uniprot database(<https://www.uniprot.org/>) were employed to construct the corresponded relationship between the compounds and targets using Excel.xls format and the Excel file was imported into Cytoscape 3.7.2 software to complete the relevant network construction. Cytoscape 3.7.2 was used to construct the corresponding network according to the relation between the chemical compounds of JJT and the target. Finally, the key compounds of JJT acting on T2DM were screened based on the connection between the compounds and the target.

2.4 Construction the network of "active compounds-disease targets"

The overlapping targets of drug targets and disease targets were obtained by VENNY online tool (<http://bioinfogp.cnb.csic.es/tools/venny/>) and presented them with Venn diagram. According to the correspondence between the chemical compounds of JJT and the disease target of T2DM, using the Cytoscape 3.7.2 software to construct the network of active compounds-disease targets and analyze by topological analysis.

2.5 Building the PPI Network

In order to study the mechanism of JJT in treating T2DM deeper, the overlapping targets were imported into the STRING (<https://string-db.org/>) database for protein-protein interaction (PPI) network analysis. The screening condition was "Multiple proteins" and the research species was limited to "humans" ("Homo sapiens") and the next step was to analyze the PPI network.

2.6 Analysis the Gene ontology (GO) function and Kyoto gene and genome encyclopedia (KEGG) pathway enrichment

DAVID (<https://david.ncifcrf.gov/>) is an online database of gene and pathway function annotations. The database can be used to find out the gene enrichment pathway and the genes and proteins contained in the enrichment pathway [21]. The DAVID 6.8 database used in this research. In order to illustrate the role of target proteins in gene function and pathway in the PPI network of JJT, and the overlapping targets were imported into DAVID 6.8 database, and "Select identifier" was "Official gene symbol", "List type" was "Gene list", and species was "Homo Sapiens". The target protein was analyzed by GO classification enrichment analysis and KEGG pathway enrichment analysis. GO function analysis was mainly used to describe the function of gene targets, including biological process (BP), cellular component (CC), and molecular function (MF). KEGG enrichment analysis could obtain the signal pathways enriched by the overlapping targets of JJT and T2DM. Both GO and KEGG analysis were statistically significant at $P < 0.05$.

2.7 Molecular docking verification

The online platform system docking platform Systems Dock (<http://systemsdock.unit.oist.jp/iddp/home/index>) is used for further molecular docking verification of the selected core component targets. Systems Dock uses the score to evaluate the interaction between active compounds and targets [22]. The docking score is the negative logarithm of the experimental dissociation / inhibition constant value (pK_d / pK_i). The value is usually between 0 and 10 (weak to strong). When the docking score is > 4.25 , it is considered to have a certain binding activity; When the docking score is > 5 , the binding activity is considered to be good; when the docking score is > 7 , it is considered to have a strong binding property [23]. We can prove the performance of target and active compounds binding again.

3. Results

3.1 Screening the active compounds in JJT

A total of 371 chemical compounds of three traditional Chinese medicines in JJT were retrieved using TCMSP database, including forty-eight *Coptidis Rhizoma*, eight-seven *Hedysarum Multijugum Maxim*, and 236 *Lonicerae Japonicae Flos*. The result was that there were thirty-one compounds in the JJT totally. Six compounds was found to have no corresponding target after removing duplicated targets one by one. Therefore, there were twenty-five effective compounds JJT, including eight compounds in *Coptidis Rhizoma*, ten compounds in *Hedysarum Multijugum Maxim* and seven compounds in *Lonicerae Japonicae Flos*. The Detail information of the twenty-five active compounds were shown in Table 1.

3.2 Acquisition the target gene of T2DM

A total of 303 target genes related T2DM were retrieved from the Drugbank database. After removing duplicated targets (160) and invalid targets (1), 142 target targets related T2DM were finally obtained. The detail information of the 142 target targets related T2DM were shown in supplementary 1.

3.3 Construction the traditional Chinese medicine chemical compounds-Target (C-T network) network

Twenty-five active compounds of JJT from three flavors of traditional Chinese medicine and the 101 targets obtained by removing seventy-five duplicated targets of 176 targets, including thirty-six targets in *Coptidis Rhizoma*, sixty-two targets in *Hedysarum Multijugum Maxim* and seventy-eight targets in *Lonicerae Japonicae Flos* were imported into Cytoscape 3.7.2 software to build the "Compound-Target" network (Fig. 2). The result showed that there were 126 nodes in the network (eight nodes in *Coptidis Rhizoma*, ten nodes in *Hedysarum Multijugum Maxim*, seven nodes in *Lonicerae Japonicae Flos* and 101 target gene nodes) and 385 edges. Among them, the "node" was used to represent the chemical compounds of medicines and potential targets in JJT and the "edge" was used to indicate the relationship between Chinese medicine compounds and targets. Different shapes were used to present the network relationship between drug compounds and targets clearly and intuitively. The inner triangle represented the compounds, the outer ellipse represented the targets and the edge represented the interaction relationship. Among them, the green triangle was the compound in *Coptidis Rhizoma*, the yellow triangle was the compound in *Hedysarum Multijugum Maxim* and the red triangle was the compound in *Lonicerae Japonicae Flos* and the purple circle was the targets.

3.4 Construction the network of "Active compounds-disease target"

The 101 drug targets corresponding to the active compounds in JJT were matched with the 142 disease targets corresponding to T2DM and used VENNY online tool to obtain twenty-one overlapping targets (Fig. 3). Blue represented JJT targets; yellow represented T2DM targets; brown and yellow were common targets, that was overlapping targets. The relevant and detail information of the twenty-one overlapping targets of JJT-T2DM were shown in Table 2.

In order to obtain the molecular mechanism of JJT in treatment of T2DM, we constructed a network of the compounds and overlapping targets by Cytoscape 3.7.2 software. The relationship between the "active compounds-disease targets" interaction was shown in Figure 4. Among them, "node" was used to represent the chemical compounds of medicines and disease targets and the relationship between Chinese medicine compounds and targets was shown by "edge". As we could see from Figure 4 that there were a total of twenty-two drug compounds and twenty-one overlapping targets acting on the network. Among them, the green triangle was the *Coptidis Rhizoma*, the yellow triangle was the *Hedysarum Multijugum Maxim* and the red triangle was the *Lonicerae Japonicae Flos* and the purple circle was the overlapping targets.

3.5 Construction and analysis the protein-protein interaction(PPI) network

In order to study the mechanism of JJT in treating of T2DM further, the twenty-one overlapping targets of JJT-T2DM (Fig. 3) were imported into STRING (<https://string-db.org/>) database and selected "multiple proteins", entered the relevant target name, selected "homo sapiens" in "organism" to search and obtain protein interaction information and performed PPI network analysis (Fig. 5). The PPI network consisted of twenty-one nodes and forty-seven edges, which the nodes represented proteins and each edge represented the interaction between protein and protein. The thicker the line was, the greater the degree of association was and the average node degree value was 4.48. The results of PPI network analysis showed that the key proteins involve PPARG, RXRA, RXRB, CYP3A4, DPP4, etc.. That was, these targets protein could interact with multiple proteins to achieve the effect of treating T2DM.

3.6 Gene Ontology function enrichment analysis

In order to further explore the potential mechanism of JJT in treatment of T2DM, the DAVID 6.8 database was used to perform Gene Ontology(GO)function enrichment analysis on the overlapping targets. GO function analysis was mainly used to describe the function of gene targets, including biological processes (BP), molecular functions (MF) and cell composition(CC).The GO function enrichment analysis obtained 426 GO entries ($P < 0.05$),including 374 biological process (BP) entries, five CC composition-related entries and forty-seven molecular function (MF) entries. The information of GO entries were shown in Table 3 and Fig. 6._The P value was selected from small to large to identify the names of the top ten items of T2DM genes in each type of expression and the reliability of the conclusions was demonstrated using $-\log_{10} P\text{value}$ [24] as the standard. From the analysis of GO functional enrichment (Fig. 7), it could be seen that biological processes mainly included blood circulation, circulatory system process, phospholipase C-activating G-protein coupled receptor signaling pathway, regulation of blood vessel size and organic hydroxy compound transport, etc. Cell composition mainly affected integral component of plasma membrane, plasma membrane region, nonmotile primary cilium and primary cilium, etc. Molecular functions mainly involved alpha-adrenergic receptor activity, G-protein coupled amine receptor activity, molecular transducer activity, catecholamine binding, transmembrane signaling receptor activity, etc. GO enrichment function analysis showed that the disease target mainly occurred in the biological process (BP), such as the vascular process in circulatory system, blood circulation, etc.. The

active compounds of JJT will affect the cell composition (CC) such as the integral component of plasma membrane, intrinsic component of plasma membrane. In addition, it has molecular functions (MF) such as transmembrane receptor activity, receptor activity, adrenergic receptor activity, etc. GO function enrichment analysis suggested that JJT could play a role in treating T2DM by acting on multiple targets.

3.7 KEGG enrichment pathway analysis

In order to illustrate the role of related targets of JJT in treatment of T2DM in the signal pathway, the study employed the DAVID 6.8 database to obtain the original data and the R language to perform KEGG pathway on twenty-one overlapping target genes analysis. KEGG pathway enrichment analysis found that twenty-one overlapping target genes were enriched to obtain fifteen signal pathways. Based on $P < 0.05$ and combined with relevant literature, twelve signal pathways related to T2DM were obtained. Among them, the top seven pathways with the most enrichment included Neuroactive ligand-receptor interaction, Calcium signaling pathway, cGMP-PKG signaling pathway, Thyroid cancer, Serotonergic synapse, Adrenergic signaling in cardiomyocytes and PPAR signal pathway, etc. The results of KEGG analysis suggested that JJT could play a vital role in treating T2DM by acting on multiple pathways. The relevant information of KEGG pathway were shown in Table 4 and Fig. 8. The enrichment results of the pathways were visually represented by a bubble chart. The color and size of the nodes were determined by the number of associated genes and the P value. The color changed from blue to red to reflect the P value from large to small and the node from small to large reflected the number of related genes from small to large, which showed that PPAR signaling pathway was the most significant pathway.

3.8 Molecular docking verification

For validation interaction between compound and targets, molecular docking is applied for testing the interaction. In this study, interaction between berberine and RXRA were reported by previous study. The mechanism of them has not been explained. RXRA is one of key targets of JJT we selected it to validate the mechanism. Molecular docking was performed to predict the binding modes between berberine and RXRA (Retinoid X Receptor alpha) in Schrödinger Software Suite (Schrödinger, LLC: New York, NY, 2015). The crystal structure of RXRA in complex with synthetic honokiol derivative 4 (PDB accession number: 5MKU) ((2017) ACS Chem Neurosci 8: 2065–2077) was derived from PDB database. The initial complex structure was prepared in the Protein Preparation Wizard module. The co-crystallized ligand in the complex structure was defined as the centroid of the binding sites and the protocol of Glide SP (standard precision) was applied for the molecular docking of berberine. The OPLS_2005 force field was set as the force field and the default parameters were applied throughout the process of molecular docking process. The result of molecular docking verification was shown in Fig. 9.

4. Discussion

Traditional Chinese medicine was guided by the idea of holistic view and dialectical treatment which was prescribed according to "Kun, Chen, Zuo, Shi". Therefore, the traditional Chinese medicine compound had

the characteristics of "multiple-target, multiple-direction, multiple-layer" and had unique advantages in terms of difficult disease to treat. However, the specific mechanism of JJT in treatment of T2DM has not been clarified so far. Therefore, this study was based on network pharmacology method, with the help of corresponding bioinformatic databases and software to construct networks and analyze the targets of pathway enrichment. The result was to scientifically explore the molecular mechanism of JJT in treatment for T2DM.

JJT (Z10920027) (Tianjin Zhongxin Pharmaceutical Group Co., Ltd. Long shun Rong Pharmaceutical Factory, batch number FD87103) has been used clinically for many years and a large number of clinical studies have shown that JJT could improve the insulin resistance of T2DM patients significantly[25]. The group of prescriptions was a traditional Chinese medicine compound preparation consisting of *Coptidis Rhizoma*, *Hedysarum Multijugum Maxim* and *Lonicerae Japonicae Flos*. It had the effect of clearing heat and nourishing qi and had good blood sugar and blood lipid lowering effects. It was clinically used to treat mild and moderate non-insulin dependent diabetes mellitus [26]. The *Coptidis Rhizoma* of prescription had coldness, bitterness, and homeostasis, liver, gallbladder, spleen, stomach, large intestine meridian. It had the effects of clearing away heat and dampness, purging fire and detoxifying. Modern pharmacological studies have found [27] that the main chemical component was berberine, which could significantly improve the β -cell apoptosis. Ni Yanxia observed that the hypoglycemic mechanism of *Coptidis Rhizoma* was not only anti-glycemic hormone, but also related to the promotion of β -cell regeneration and functional recovery through animal experiments. *Hedysarum Multijugum Maxim* was slightly warm and sweet in the nature, which belonged to the lung, spleen, kidney, and liver meridians. It was one of the representative medicines for qi-enhancing medicine and had the effects of qi-enhancing yang and strengthened the body [28]. In modern pharmacological research[29], *Hedysarum Multijugum Maxim* contained many effective chemical compounds itself and its pharmacological effects were widely used in the field of diabetes. In terms of material metabolism, it could significantly reduce the blood glucose, blood lipids and low-density lipoprotein of T2DM, and effectively improve high-density lipoprotein. Especially in terms of glucose metabolism, it also had a two-way regulation effect, which had a reducing effect on the increase of blood glucose caused by various reasons and could also counteract the decrease of blood glucose mainly caused by phenformin. *Lonicerae Japonicae Flos* had a long history of medicinal use and remarkable efficacy. It had been known as a good medicine for clearing heat and detoxifying since ancient times. It was mainly used to treat carbuncle, scabies, erysipelas, fever toxins fever, colds fever. In modern pharmacological research shown that it also had effect of antibacterial, antiviral, anti-inflammatory, choleric, liver-protective, hypoglycemic and other effects[30]. Clinical observations indicated it played a significant role in Qi and Yin deficiency and fire and prosperous for patients with mild to moderate T2DM patients .

Some literature [31] reported that JJT had obvious hypoglycemic, lipid-lowering, and anti-oxidant effects on T2DM Qi-yin deficiency and fire deficiency and could significantly reduce acetylcholinesterase, serum islet levels and excessive hemoglobin. Therefore, it was an ideal Chinese patent medicine for the treatment of T2DM. This study systematically analyzed the active compounds of JJT and their corresponding hypoglycemic targets. According to the compound-target network (Fig. 3) and active

compounds-disease targets_(Fig. 5), it was found that there were twenty-five active compounds in JJT. There were 114 targets involved, each compound could correspond to multiple targets, and one target also corresponded to multiple components, which also reflected the characteristics of traditional Chinese medicine with multiple components and multiple targets. The study have shown that the key compounds in JJT to treat T2DM may be berberine, baicalein, berberrubine, β -carotene, stigmasterol, etc. It has been reported that berberine had obvious antioxidant, anti-inflammatory, hypoglycemic and lipid-lowering effects [32]. It could inhibit the activity of acetylcholinesterase (AChE) and activate $\alpha 7$ nicotinic type in human liver cancer (HepG2) cells. Acetylcholine receptor ($\alpha 7$ nAChR) could improve insulin resistance and reverse inflammation [33]. It could also block mitochondrial dysfunction and AMP accumulation caused by histone deacetylase 3 (SIRT3) and block glucagon signaling and degradation PEPCCK1, inhibit gluconeogenesis, promote glycolysis, thereby achieving the effect of reducing blood sugar [34], It also could reduce the inflammatory response, improve insulin resistance by inhibiting the binding of Toll-like receptors to lipopolysaccharides [35] and inhibit oxidation Stress and inflammatory responses inhibit the occurrence and development of T2DM [36]. Baicalein could reduce blood glucose by eliminating free radicals and antioxidants [37], lower blood lipids, inhibit α -glucosidase activity [35], regulate inflammatory cytokines [36] and protect β -cell function [38]. With the discovery of the hypoglycemic effect of berberrubine and the deepening of its mechanism research, researchers have used chemical synthesis to insert aminomethyl at the twelve position of berberrubine base to form a derivative. It's effect may exceed berberine and insulin [39]. β -carotene had strong anti-oxidant and immune-regulating effects [40], which could effectively scavenge oxygen free radicals, reduce lipid peroxide damage and effectively inhibit unsaturated fatty acid free radical chain reactions on cell membranes, reduce insulin resistance, lower blood sugar [41]. Stigmasterol was anti-diabetic, WARD et al [42] found that stigmasterol could improve the increase of free cholesterol caused by glucolipid toxicity in insulinoma cells (INS-1), which led to defects in insulin secretion, increased total insulin, and promoted stimulus protein recombination and also had been shown that played a beneficial role in the treatment of T2DM. Wang et al. [43] found that in vitro experiments, treating of L6 cells with different concentrations of stigmasterol had a significant effect on promoting glucose uptake. In the in vivo test, after oral administration of stigmasterol, fasting blood glucose levels and blood lipid indexes (such as triacylglycerol and cholesterol) in KK-A^y mice were significantly reduced, which significantly reduced insulin resistance and oral glucose tolerance in KK-A^y mice. The mechanism may be related to increase translocation and expression of glucose transporter 4 (GLUT4), suggesting that stigmasterol has potential benefits for the treatment of type 2 diabetes. The results of this study are consistent with the above report, which shows that JJT have the characteristics of multiple components acting together in the treatment of T2DM.

The PPI network shown that there were twenty-one nodes in the PPI network (Fig. 6), which represented genes related to T2DM and forty-five edges represented interaction pairs between genes, indicating that T2DM was a complex genetic diseases. Combining the component-target network (Fig. 3) and active compounds-disease targets_(Fig. 5) and PPI network (Fig. 6) showed that RXRA, PPARG, RXRB, CYP1A2, CYP3A4, DPP4, etc. were the core targets for the treatment of T2DM. They were involved in the pathological process of T2DM and it was also the key targets of JJT in the treatment of T2DM.

Among them, RXRA gene was mainly expressed in liver, kidney, skin and other tissues and was the most abundant retinoic acid X receptor in the skin [44–45]. It was not only involved in a variety of physiological processes (cell development, apoptosis, and homeostasis) and also played an important role in cholesterol balance, intestinal cholesterol absorption, bile acid synthesis. The RXRA gene agonists had hypoglycemic, insulin-sensitizing and anti-obesity effects [46–47]. Peroxisome proliferator-activated receptor gamma (PPARG) bind to form heterodimers and helped regulate homeostasis in glucose and lipid metabolism [48]. Ying et al. [49] suggested that the potential functional polymorphisms of PPARG and RXRA genes may change the risk of T2DM by increasing the activity of the human ADIPO promoter. Some studies found that PPARG gene was located at 3p25, which belonged to the nuclear hormone receptor superfamily of transcription factors and played an important role in the differentiation of adipocytes and the expression of adipocyte-specific genes and the regulation of insulin sensitivity [50]. In the state of DM, the function and expression level of certain CYP450 (CYP3A4) enzyme subtypes changed, resulting in corresponding changes in the pharmacokinetics of the substrate drug, which affected the efficacy of the drug or led to the occurrence of adverse reactions [51–52]. CYP1A2 was one of the important members of the CYP450 family. It was mainly expressed in the liver, involved in the metabolism of a variety of endogenous substrates and drugs, activated and inactivated a variety of pre-carcinogens, which had important pharmacological and toxicological significance. Hu Nan et al. [53] conducted a preliminary exploration on the mechanism of liver CYP1A2 changed in diabetic state at the in vitro cell level and found that abnormal changes in fatty acid concentrations may be part of the reasons that affected the function and expression change of liver CYP1A2. The study of liver CYP1A2 change provided a basis to further explore the state of diabetes. DPP4 is an enzyme in the body, that is, enzymes. Its main function is to break down proteins in the body. One of the proteins broken down by DPP4 is called GLP-1. It is a hormone secreted by intestinal cells. GLP-1 can pass through DPP4 inhibitors that stimulate insulin, inhibit glucagon, inhibit gastric emptying, and regenerate islet cells to lower blood sugar; DPP4 inhibitors that cause DPP4 inactivation and do not break down GLP-1 have become one of the main directions for the treatment of diabetes [54].

KEGG pathway analysis shown that a total of twelve signal pathways with $P < 0.05$ were screened out. Among them, the most significant pathways included Neuroactive ligand-receptor interaction, Calcium signaling pathway, cGMP-PKG signaling pathway, Serotonergic synapse, Adrenergic signaling in cardiomyocytes and PPAR signal pathway. The neuroactive ligand-receptor interaction signaling pathway is a collection of all receptor ligands on the plasma membrane that are related to intracellular and extracellular signaling pathways and is most closely related to neural function [55]. DM is a long-term progressive developmental disease that is mainly manifested by abnormal glucose and lipid metabolism. DM can cause coronary heart disease and diabetic cardiomyopathy. Diabetes can cause obvious myocardial cell apoptosis, which may cause heart damage caused by diabetes. It plays a very important role, and the accumulation of reactive oxygen species (ROS) and oxidative stress are important factors in the occurrence of cardiomyocyte apoptosis. Since the abnormal "calcium signal" of atrial muscle cells is a key link in the regulation of atrial fibrillation, and diabetes is related to oxidative stress, the cardiac calcium channels RYR and IP3 contained in the heart are more sensitive to oxidative stress, because RYR

and IP3 regulatory proteins contain A large amount of free thiol, which is the target of ROS and nitro substances, so diabetic hyperglycemia can produce oxidative stress and may affect intracellular calcium channels[56].The PPAR signaling pathway may play an important role in improving the fat level of T2DM patients by JJT. PPAR was a peroxisome proliferator-activated receptor, which mainly included three isoforms of α , β / δ , and γ . PPAR α was mainly expressed in brown adipocytes with fast fatty acid catabolism, followed by liver, kidney, heart, and skeletal muscle cells. PPAR α could regulate cholesterol and free fatty acids through multiple pathways [57]. PPAR β was widely expressed and it played a role in fatty acid catabolism, energy metabolism and reverse cholesterol transport. PPAR- γ regulated glucose metabolism mainly by increasing the sensitivity of peripheral tissues to insulin, thereby improving insulin resistance [58].

5. Conclusion

In summary, a network pharmacological method was used to explore the chemical compounds, targets and pathways of JJT for T2DM in this study. It can be seen from the results that the treatment of T2DM by JJT was effective through the synergy of multiple compounds, multiple targets, and multiple pathways. This study not only provided ideas and reference for further experimental research and clinical application and also provided a new direction for the mechanism of action of traditional Chinese medicine compounds. However, the limitation of this study was that network pharmacology was based on predictions made by big data and data collection. Whether it was comprehensive or not and the formulation of active ingredient screening criteria could not be completely accurate. In addition, the effect of drug dosage and decoction methods on treatment results had not been considered in this study and its hypoglycemic effect needed to be confirmed by further experimental research.

Abbreviations

Type 2 Diabetes Mellitus

T2DM

Diabetes Mellitus

DM

Diabetic Dephropathy

DN

Oral bioavailability

OB

Drug-like

DL

Caco-2 permeability

Caco-2

Biological Process

BP

Cellular Component

CC

Molecular Function

MF

Compounds-Target network

C-T network

Jinqi Jiangtang Tablet

JJT

Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

TCMSP

Gene Ontology

GO

Kyoto Encyclopedia of Genes and Genomes

KEGG

Protein-Protein Interaction

PPI

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Publicly available datasets were analyzed in this study. This data can be found here:

TCMSP: <http://tcmspw.com/tcmsp.php>

DrugBank: <https://www.drugbank.ca/>

Uniprot: <https://www.uniprot.org/>

STRING: <https://string-db.org/>

DAVID: <https://david.ncifcrf.gov/>

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Mingjun Yang and Zhitong Bing formulated the idea of the paper and supervised the research. Zhitong Bing and Juxiang Liu performed the research. Boni Song and Rui Li analyzed the data and wrote the manuscript. Jingyun Zhang and Lijuan Yang designed molecular docking by computer. Lingyan Yuan made the figures. Zhitong Bing and Mingjun Yang participated in revising the data and improving manuscript writing. And all authors read and approved the final version of the manuscript.

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Tables

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Figures

Figure 1

Flowchart for JJT in treating T2DM

Figure 3

The overlapping targets of the active compounds JJT and the targets of T2DM

Figure 4

Network of "Active compounds-disease target" interaction

Figure 5

The PPI network based on STRING database

Figure 6

The number of GO function analysis entries Note: Green represented the number of BP entries, red represented the number of MF entries, and blue represented the number of CC entries

Figure 7

The bar chart of GO function enrichment analysis of JJT

Figure 8

Annotated map of KEGG main pathways

Figure 9

Supplementary Files

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