Research Article
A Network Pharmacology Approach to Investigate the Mechanism of Erjing Prescription in Type 2 Diabetes

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Erjing prescription (EJP) was an ancient formula that was recorded in the General Medical Collection of Royal Benevolence of the Song Dynasty. It has been frequently used to treat type 2 diabetes mellitus (T2DM) in the long history of China. The formula consists of *Lycium barbarum* L. and *Polygonatum sibiricum* F. Delaroche with a ratio of 1:1. This study aimed to identify the potential effects and mechanisms of EJP treatment T2DM. The target proteins and possible pathways of EJP in T2DM treatment were investigated by the approach of network pharmacology and real-time PCR (RT-PCR). 99 diabetes-related proteins were regulated by 56 bioactive constituents in EJP in 26 signal pathways by Cytoscape determination. According to GO analysis, 606 genes entries have been enriched. The PPI network suggested that AKT1, EGF, EGFR, MAPK1, and GSK3β proteins were core genes. Among the 26 signal pathways, the PI3K-AKT signal pathway was tested by the RT-PCR. The expression level of PI3K p85, AKT1, GSK3β, and Myc mRNA of this pathway was regulated by EJP. The study based on network pharmacology and RT-PCR analysis revealed that the blood sugar level was regulated by EJP via regulating the PI3K-AKT signal pathway. Plenty of new treatment methods for T2DM using EJP were provided by network pharmacology analysis.

1. Introduction

Type 2 diabetes (T2DM, Chinese name: Xiaoke) is a common chronic metabolic disease with a high prevalence. In 2019, over 463 million people suffered from diabetes. It is estimated that there will be 578 and 700 million diabetic patients in 2030 and 2045, respectively [1, 2], which would bring up a huge economic burden on society. In general, many cases may be undiagnosed in a long predetection period [3]. A lot of complications, such as heart failure, cardiovascular complications, and diabetic nephropathy, are always accompanied with the occurrence of diabetes [4–7]. Low immunity is a common feature for almost all forms of diabetes [8]. Therefore, the researcher devotes to exploring effective drugs to prevent and treat T2DM. Nowadays, traditional Chinese medicine (TCM) has been increasingly popular for the T2DM treatment [9]. For example, Liuwei Dihuang pills and Huanglian decoction have been widely researched for treating T2DM [10–12]. Alizarin, isolated from TCM (*Rubia cordifolia*), reduced blood sugar levels and alleviated insulin resistance through the PI3K/AKT pathway [13].

EJP (also known as Erjingwan) is a formula consisting of *Lycium barbarum* L. and *Polygonatum sibiricum* F. Delaroche, which is recorded in General Medical Collection of Royal Benevolence, early traced back to the Song Dynasty. Erjingwan, a classic formula of nourishing, showed antiaging effects in the skin through activation of Nrf2 and inhibition of NF-κB [14]. These two medicines possessed a certain hypoglycemic effect and mainly contained...
2.2. Ingredients Database Construction. All potential compounds of EJP were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database analysis platform database (http://lsp.nwu.edu.cn/tcmsp.php) [23]. In the TCMSP database, 500 Chinese herbal medicines and 30,069 constituents were registered from the Chinese Pharmacopoeia (2010 edition). A total of 56 compounds were gained from EJP, including 45 in L. barbarum and 12 in P. sibiricum. The details of each class of compounds are summarized in Table 1.

First, effective constituents that contributed to its efficacy were selected by absorption, distribution, metabolism, and excretion parameters (ADME), meanwhile those are ineffective or even toxic were removed [24]. Second, higher oral absorption, bioavailability, and biological properties were essential for candidate constituents. Therefore, these compounds needed to satisfy the 30% in oral bioavailability (OB) and 0.18 in drug-likeness (DL).

The related proteins of active components in EJP were acquired from TCMSP databases. Genes related to proteins were retrieved from the UniProt Knowledgebase (UniProtKB) (http://www.uniprot.org), which was a protein database containing 54,247,468 sequence entries [25].

2.3. Predicting Targets of T2DM. The target proteins of T2DM were obtained from four sources: (1) 15,500 gene entries were included in OMIM database (http://www.omim.org/), which was focused on illustrating gene and genetic disorders [26]. (2) GeneCards (https://www.genecards.org/) is a comprehensive database that provides human genes of annotation and prediction. It could effectively establish the linkages of gene disease [27]. (3) DisGeNET (http://www.disgenet.org/) database, the current release contains more than 24,000 diseases and traits, 17,000 genes, and 117,000 genomic variants [28]. (4) Therapeutic target database (TTD) contained information from three aspects: (i) the microRNAs and transcription factors of target regulation, (ii) the proteins of target interaction, and (iii) targeting agents and targets, which can be easily retrieved and further enriched by the mechanisms of regulation or biochemical classes [29]. All targets linked to T2DM were only limited to Homo sapiens. The 1,260 genes totally were gained. The 99 same target proteins of compound and disease were selected as the main target of EJP in the treatment of T2DM (Table 2).

2.4. Network Construction

2.4.1. Compound-Target Network. The pharmacological mechanisms of actions were explored by the compound-target network, which was founded for the 57 compounds and relevant protein targets in T2DM utilizing Cytoscape 3.7.2. [30].

2.4.2. GO and KEGG Analyses. Gene ontology (GO) is to annotate genes and their expression products. It is mainly divided into 3 parts: cell component (CC), biological process (BP), and molecular function (MF) [31]. Kyoto Encyclopedia of Genes and Genomes (KEGG) can analyze the signal pathways of drug targets in order to search the disease signal pathways that have maximum correlation, which is significant for discovering the possible mechanism of EJP in the treatment of T2DM [32]. In this study, the enrichment analysis of GO and KEGG were performed through DAVID bioinformatics resources (https://david.ncifcrf.gov) [33] in order to explore the related CC, BP, MF, and pathways. It illustrated the connection of genes and target proteins in diabetes.
| MOL ID   | Molecule                                      | MW   | OB (%) | DL  | Medicine          |
|---------|-----------------------------------------------|------|--------|-----|-------------------|
| MOL001323 | Sitosterol α1                                 | 426.8| 43.28  | 0.78| L. barbarum       |
| MOL003578 | Cycloartenol                                  | 426.8| 38.69  | 0.78| L. barbarum       |
| MOL001494 | Mandenol                                      | 308.56| 42   | 0.19| L. barbarum       |
| MOL001495 | Ethyl linolenate                              | 306.54| 46.1  | 0.2 | L. barbarum       |
| MOL001979 | LAN                                           | 426.8| 42.12  | 0.75| L. barbarum       |
| MOL000449 | Stigmasterol                                  | 412.77| 43.83 | 0.76| L. barbarum       |
| MOL005406 | Atropine                                      | 289.41| 45.97 | 0.19| L. barbarum       |
| MOL005438 | Campesterol                                   | 400.76| 37.58 | 0.71| L. barbarum       |
| MOL006209 | Cyanin                                        | 411.66| 47.42 | 0.76| L. barbarum       |
| MOL007449 | 24-Methylidenelophenol                        | 426.8| 44.19  | 0.75| L. barbarum       |
| MOL008173 | Daucosterol_qt                                | 414.79| 38.87 | 0.72| L. barbarum       |
| MOL008400 | Glycitein                                     | 284.28| 50.48 | 0.24| L. barbarum       |
| MOL010234 | δ-Carotene                                    | 536.96| 31.8  | 0.55| L. barbarum       |
| MOL000953 | CLR                                           | 386.73| 37.87 | 0.68| L. barbarum       |
| MOL009604 | 14b-Pregnane                                   | 288.57| 34.78 | 0.34| L. barbarum       |
| MOL009612 | (24R)-4α-Methyl-24-ethylcholesta-7, 25-dien-3β-yl acetate | 482.87| 46.36 | 0.84| L. barbarum       |
| MOL009615 | 24-Methyleneoctoarctan-3β, 21-diol             | 456.83| 37.32 | 0.7  | L. barbarum       |
| MOL009617 | 24-Ethylecholesta-22-enol                     | 414.79| 37.09 | 0.75| L. barbarum       |
| MOL009618 | 24-Ethylecholesta-5, 22-dienol                | 412.77| 43.83 | 0.76| L. barbarum       |
| MOL009620 | 24-Methyl-31-norlanost-9 (11)-enol            | 428.82| 38    | 0.75| L. barbarum       |
| MOL009621 | 24-Methylenelanost-8-enol                    | 440.83| 42.37 | 0.77| L. barbarum       |
| MOL009622 | Fucosterol                                    | 412.77| 43.78 | 0.76| L. barbarum       |
| MOL009631 | 31-Norlanostadienol                           | 440.83| 38.68 | 0.81| L. barbarum       |
| MOL009633 | 31-Norlanostadienol-9 (11)-enol              | 414.79| 38.35 | 0.72| L. barbarum       |
| MOL009634 | 31-Norlanosteriolicheca-8-enol                | 412.77| 42.2  | 0.73| L. barbarum       |
| MOL009635 | 4, 24-Methyllophenol                         | 414.79| 37.83 | 0.75| L. barbarum       |
| MOL009639 | Lophenol                                      | 400.76| 38.13 | 0.71| L. barbarum       |
| MOL009640 | 4α, 14α, 24-Trihydroxymethylcholesta-8, 24-dienol | 426.8 | 38.91 | 0.76| L. barbarum       |
| MOL009641 | 4α, 24-Dimethylcholesta-7, 24-dienol          | 412.77| 42.65 | 0.75| L. barbarum       |
| MOL009642 | 4α-Methyl-24-ethylcholesta-7, 24-dienol       | 426.8 | 42.3  | 0.78| L. barbarum       |
| MOL009644 | 6-Fluoroindole-7-dehydrocholesterol           | 402.7 | 43.73 | 0.72| L. barbarum       |
| MOL009646 | 7-O-Methylbutenol-6-C-β-glucoside             | 318.3 | 40.77 | 0.3 | L. barbarum       |
| MOL009650 | Atropine                                      | 289.41| 42.16 | 0.19| L. barbarum       |
| MOL009651 | Cryptoxanthin monoepoxide                    | 568.96| 46.95 | 0.56| L. barbarum       |
| MOL009653 | Cycloeucalenol                                | 426.8 | 39.73 | 0.79| L. barbarum       |
| MOL009656 | (E, E)-1-Ethyl octadeca-3, 13-dienoate       | 308.56| 42    | 0.19| L. barbarum       |
| MOL009660 | Methyl (1R, 4αS, 7R, 7aS)-4α, 7-dihydroxy-7-methyl-1-[2S, 3R, 4S, 5S, 6R]-3, 4, 5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy-1, 5, 6, 7a-tetrahydrocyclopentanpyran-4-carboxylate | 406.43| 39.43 | 0.47| L. barbarum       |
| MOL009662 | Lantadene A                                   | 552.87| 38.68 | 0.57| L. barbarum       |
| MOL009664 | Physalin A                                    | 526.58| 91.71 | 0.27| L. barbarum       |
| MOL009665 | Physcion-8-O-β-D-gentiobioside                | 608.6 | 43.9  | 0.62| L. barbarum       |
| MOL009677 | Lanost-8-en-3β-ol                             | 428.82| 34.23 | 0.74| L. barbarum       |
| MOL009678 | Lanost-8-enol                                 | 428.82| 34.23 | 0.74| L. barbarum       |
| MOL009681 | Obtusifoliol                                  | 426.8 | 42.55 | 0.76| L. barbarum       |
| MOL000998 | Quercetin                                     | 302.25| 46.43 | 0.28| L. barbarum       |
| MOL001792 | DFV                                           | 256.27| 32.76 | 0.18| L. barbarum       |
| MOL002714 | Baicalein                                     | 270.25| 33.52 | 0.21| L. barbarum       |
| MOL002959 | 3′-Methoxydaidzein                            | 284.28| 48.57 | 0.24| L. barbarum       |
| MOL000358 | β-Sitosterol                                  | 414.79| 36.91 | 0.75| L. barbarum/P. sibiricum |
| MOL000359 | Sitosterol                                    | 414.79| 36.91 | 0.75| P. sibiricum      |
| MOL003889 | Methylprotopodioscin_gt                      | 446.74| 35.12 | 0.86| P. sibiricum      |
| MOL004941 | (2R)-7-Hydroxy-2-(4 hydroxyphenyl) chroman-4-one | 256.27| 71.12 | 0.18| P. sibiricum      |
| MOL008054 | Diosgenin                                     | 414.69| 80.88 | 0.81| P. sibiricum      |
| MOL006331 | 4′, 5-Dihydroxylavone                        | 254.25| 48.55 | 0.19| P. sibiricum      |
| MOL009760 | Sibiricoside A                                | 432.71| 35.26 | 0.86| P. sibiricum      |
| MOL009763 | (+)-Syringaresinol-O-β-D-glucoside            | 580.64| 43.35 | 0.77| P. sibiricum      |
| MOL009766 | Zhonghualiasone 1                             | 458.75| 34.72 | 0.78| P. sibiricum      |
| Symbol name                                      | Gene name | Symbol name                                      | Gene name | Symbol name                                      | Gene name |
|-------------------------------------------------|-----------|-------------------------------------------------|-----------|-------------------------------------------------|-----------|
| Prostaglandin G/H synthase 2                    | PTGS2     | Acetylcholinesterase                            | ACHE      | Mitogen-activated protein kinase 14              | MAPK1     |
| Prostaglandin G/H synthase 1                    | PTGS1     | Epidermal growth factor receptor                | EGFR      | Interstitial collagen                            | MMP1      |
| Mineralocorticoid receptor                      | NR3C2     | RAC-α serine/threonine-protein kinase           | AKT1      | Hypoxia-inducible factor 1-α                     | HIF1A     |
| β-2 Adrenergic receptor                         | ADRB2     | Vascular endothelial growth factor A            | VEGFA     | Signal transducer and activator of transcription 1-α/β | STAT1     |
| Urokinase-type plasminogen activator            | PLAU      | Apoptosis regulator Bcl-2                       | BCL2      | Receptor tyrosine-protein kinase erb-B2          | ERBB2     |
| Sodium channel protein type 5 subunit α         | SCN5A     | Bcl-2-like protein 1                            | BCL2L1    | Peroxisome proliferator-activated receptor gamma | PPARG     |
| 5-Hydroxytryptamine 2A receptor                 | HTR2A     | Proto-oncogene c-Fos                            | FOS       | Heme oxygenase 1                                 | HMOX1     |
| Sodium-dependent serotonin transporter           | SLC6A4    | Cyclin-dependent kinase inhibitor 1             | CDKN1A    | Cytochrome P450 3A                               | CYP3A4    |
| D (2) dopamine receptor                         | DRD2      | Apoptosis regulator BAX                         | BAX       | Cytochrome P450 1A                               | CYP1A2    |
| Estrogen receptor                                | ESR1      | 72 kDa type IV collagenase                      | MMP2      | Caveolin-1                                        | CAV1      |
| Androgen receptor                                | AR        | Interleukin-10                                  | IL10      | Muc proto-oncogene protein                       | MYC       |
| Peroxisome proliferator-activated receptor-γ    | KCNH2     | Proepidermal growth factor                      | EGF       | Tissue factor                                     | F3        |
| Estrogen receptor β                              | ESR2      | Retinoblastoma-associated protein               | RB1       | Gap junction α-1 protein                         | GJA1      |
| Mitogen-activated protein kinase 14             | MAPK14    | Tumor necrosis factor                           | TNF       | Intercellular adhesion molecule 1                | ICAM1     |
| Glycogen synthase kinase-3 α                    | GSK3β     | Transcription factor AP-1                       | JUN       | C-C motif chemokine 2                            | CCL2      |
| Cell division protein kinase 2                  | CDK2      | Caspase-3                                        | CASP3     | E-selectin                                       | SELE      |
| Nitric oxide synthase, inducible                | NOS2      | Cellular tumor antigen p53                      | TP53      | Vascular cell adhesion protein 1                 | VCAM1     |
| Collagenase 3                                   | MMP13     | Ornithine decarboxylase                         | ODC1      | Interleukin-8                                    | CXCL8     |
| Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit, γ isoform | PIK3CG  | Xanthine dehydrogenase/oxidase                  | XDH       | Protein kinase C β type                          | PRKCB     |
| Dipeptidyl peptidase IV                         | DPP4      | Caspase-8                                        | CASP8     | Heat shock protein β-1                           | HSPB1     |
| Stromelysin-1                                   | MMP3      | RAF proto-oncogene serine/threonine-protein kinase | RAF1     | Transforming growth factor β-1                   | TGFBI     |
| Coagulation factor VII                          | F7        | Superoxide dismutase (Cu-Zn)                    | SOD1      | Malate-glucoamylase, intestinal                  | MGAM      |
| Nitric oxide synthase, endothelial              | NOS3      | Protein kinase C alpha type                      | PRKCA     | Interleukin-2                                    | IL2       |
| Tissue-type plasminogen activator               | PLAT      | Interferon gamma                                | IFNG      | Poly (ADP-ribose) polymerase 1                   | PARP1     |
| Thrombomodulin                                  | THBD      | Interleukin-1α                                  | IL1A      | Solute carrier family 2, facilitated glucose     | SLC2A4    |
| Plasminogen activator inhibitor 1               | SERPINE1  | Myeloperoxidase                                 | MPO       | transporter member 4                              | COL3A1    |
| Collagen α-1 (I) chain                          | COL1A1    | Nuclear factor erythroid 2-related factor 2     | NFE2L2    | Serine/threonine-protein kinase Chk2            | CHEK2     |
| C-reactive protein                              | CRP       | C-X-C motif chemokine 10                        | CXCL10    | Osteopontin                                      | SPP1      |
| Runt-related transcription factor 2             | RUNX2     | Cathepsin D                                     | CTSD      | Insulin-like growth factor-binding protein 3     | IGFBP3    |
| Insulin-like growth factor II                   | IGF2      | Serum paraoxonase/arylesterase 1                | PON1      | Cytosolic phospholipase A2                       | PLA2G4A   |
| CD40 ligand                                     | CD40LG    | Type 1 idiothyronine deiodinase                 | DIO1      | Canalicular multispecific organic anion transporter 1 | ABCC2    |
| Receptor tyrosine-protein kinase erbB-3         | ERBB3     | Catalase                                        | CAT       | Serine/threonine-protein kinase mTOR             | MTOR      |
| Insulin receptor                                | INSR      | Peroxisome proliferator-activated receptor-α    | PPARA     | Peroxisome proliferator-activated receptor δ     | PPARD     |
2.4.3. PPI Network. The effective components of drugs affected on the human body not only by directly acting on one target but also by indirectly acting on other targets. The prevention and control disease was regulated by multi-farious intricate signal pathways, which are interactions and transductions between upstream and downstream targets. The complex relationship between targets and proteins can be clearly displayed by constructing PPI. Target proteins related to diabetes were analyzed by online STRING 11.0 (https://string-db.org/cgi/input?sessionId=biiGmvCwYzjy&input_page_show_search=on) to construct the PPI network [34, 35].

2.4.4. Component-Target-Pathway Network. The relationships of component, target, and pathway were clarified utilizing Cytoscape 3.7.2.

2.5. Cell Culture. HepG2 cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin at 37°C, 5% CO₂ having 95% relative humidity atmosphere. The cells were seeded at a density of 1.0 × 10⁴ cells/well in a 96-well plate for 24 h, and then, the cells were treated with high concentrations of insulin (1 × 10⁻⁵) for 48 h. After the period, the cells were divided into four groups (control, model, DMBG, and HG group). The control and group were treated DMEM and DMBG, and the HG group was treated metformin (100 µg/mL) and EJP (100 µg/mL). After 24 h, the glucose content was measured using a spectrophotometric microtiter plate reader at 520 nm, and IR-HepG2 cells were prepared.

2.6. RT-PCR Analysis. The IR-HepG2 cells were extracted for RT-PCR analysis. First, the total RNA of IR-HepG2 was extracted with RNA rapid extraction solution, after treated as extracts of concentrations ranging from 100 to 500 ng/L. Second, the RNA was reverse-transcribed through a PCR instrument. Third, RT-PCR was performed using the PCR amplification instrument. The primer sequences were as follows: H-ACTIN-S: CCACCCAGCAATGAGATCAAGAT; H-ACTIN-A: CCAAGTCTTTAACCTGAGTCAAGC; H-PK3r1-S: GGAAGCAGAACCAGAAACAA; H-PK3r1-A: TCAGGCTCCACCTACAGA; H-AKT (1)-S: GCTCAAGCCAACCTTCAAG; H-AKT (1)-A: GTGTCACATTTGGTCAAGTGTT; H-GSK3β-S: GTTAGCAGAGACAGGACGGCA; H-GSK3β-A: GCATCTTTCTTGAGCCGA; H-MYC-S: CTGAGTACGACTCGGTGCA; H-MYC-A: CGGGTCGCAAGATGAAA; H-EF1-S: CCAGTTTTTAATTCCATCAG; H-EF1-A: GCTGTCATCTTGGTCAGGTGGT; H-GSK3β-S: GCTGTCATCTTGGTCAGGTGGT; H-GSK3β-A: GCATCTTTCTTGAGCCGA; H-MYC-S: CTGAGTACGACTCGGTGCA; H-MYC-A: CGGGTCGCAAGATGAAA CTCT.

3. Results

3.1. Compound-Target Network. Ninety-nine (99) kinds of candidate genes relevant to diabetes were excavated from OMIM, GeneCards, Dis-GeNET, and TTD databases. As shown in Figure 1, 130 nodes (56 compound nodes and 99 target nodes) and 288 edges were seen in the compound-target network. The yellow and blue nodes represented the compounds and targets, respectively. Furthermore, each edge represented the correlation of compound and target. In the network, larger degree represents a stronger interaction. Furthermore, the larger degree nodes may represent the key compound or target in the network. Quercetin, β-sitosterol, diosgenin, baicalein, and 3′-methoxydaidzein were found as the potential composition of treating diabetes in EJP. Quercetin was connected to 85 proteins, β-sitosterol was associated with 15 proteins, diosgenin was related to 12 proteins, baicalein was relevant in 16 proteins, and 3′-methoxydaidzein was connected to 10 proteins.

3.2. GO and KEGG Analyses. The DAVID 6.8 database (https://david.ncifcrf.gov) was utilized to elucidate the CC, BP, and MF annotations of the selected 99 proteins. There were 606 genes entries (FDR, Figure 2 shows the top 8 according to FDR <0.05), out of which 46 genes entries were relevant to CC including the extracellular region, plasma membrane, and cytosol. 477 items were related to BP, including the hypoxia and drug response, positive regulation of gene expression and transcription, DNA-templated, angiogenesis, inflammatory response, and positive regulation of cell proliferation. 83 items were related to MF, including protein binding, identical protein binding, and the homodimerization activity of protein. Therefore, the positive regulation of transcription with RNA polymerase II promoter, DNA-templated, and responision of hypoxia about the action of EJP were regulated through the binding of protein, identical protein, and enzyme, as well as plasma membrane, cytosol, and the homodimerization activity of protein. A target-pathway network was constructed from the data screened of DAVID for determining the relationship between T2DM proteins and related pathways. Furthermore, the key signal pathways (HIF-1, PI3K-AKT, and MAPK) were based on the KEGG analysis (FDR <0.05) and literature analysis (Figures 3 and 4).

3.3. PPI Network Analysis. The network of protein and protein was constructed for exploring the interaction of 99 antidiabetic protein. As shown in Figure 5, AKT1, EGF, EGFR, MAPK1, and GSK3β proteins were located at the core position. These five proteins mainly involved PI3K/AKT, MAPK, and VEGF signaling pathways. AKT1 regulated many processes including metabolism, proliferation, cell survival, growth, and angiogenesis. AKT1 was indirectly activated by insulin and other growth factors [36]. Inhibition of EGFR or HB-EGF intercepted the proliferative response to HB-EGF and glucose in rat islets [37]. MiR-133 may be an effective target for the treatment of diabetic nephropathy via the MAPK/ERK pathway [38]. The antidiabetic effect of SJE might be dependent on the AMPK pathway, which was indicated through the inhibition of gene expression in INS1 and GSK3β, and upregulating the hepatic phosphorylation of AMPKα in liver of mice [39].

3.4. Component-Target Pathway Network. To further clarify the mechanism of action of EJP in the treatment of T2DM, the component-target-pathway network was established. As
shown in Figure 6, the network was composed of chemical components, protein targets, and pathways, including 156 nodes and 643 edges. 56 components interacted with 99 target proteins and were associated with 26 pathways. Only 66 proteins were related to 26 pathways, so these 66 proteins were potential key proteins of EJP for T2DM. This prediction provided a scientific evidence for further research into the mechanism of EJP in the T2DM treatment.

3.5. RT-PCR Analysis. Based on the prediction of network pharmacology, PI3K p85, AKT1, GSK3β, and Myc proteins in the PI3K-AKT signal pathway were selected and used to verify the mechanism of action of EJP in treating T2DM at the mRNA level by RT-PCR. As shown in Figure 7, compared to the control group, the expression level of the model group of PI3K p85 and AKT1 mRNA reduced while GSK3β and Myc mRNA increased. The expression level of DMBG and HG groups of PI3K p85 and AKT1 mRNA increased while GSK3β and Myc mRNA reduced compared with the model group. Thus, EJP controlled the blood glucose levels of T2DM mice via upregulating mRNA of PI3K p85 and AKT1 and downregulating mRNA of GSK3β and Myc in PI3K-AKT signal pathways.

4. Discussion

The prevalence rate of T2DM is gradually increasing due to the aging of the population and changes in lifestyle [40]. Long-term medication is inevitable for the treatment of such chronic metabolic disease. Due to TCM featured multitarget, multicomponent, and low toxicity became more and more popular in the treatment of diabetes. So, it is vital to elucidate the pharmacological mechanism of TCM formula. The
active ingredient and targets of TCM could be preferentially predicted by network pharmacology [41]. In this study, the bioactive component and the action mechanism of EJP for diabetes treatment was predicted via the network pharmacology method, and some proteins were verified through RT-PCR monitoring.

The compound-target network clarified that the treatment efficacy of EJP against T2DM was exactly related 56 compounds. Quercetin, β-sitosterol, diosgenin, baicalein, and 3′-methoxydaidzein were found as the potential composition of treating diabetes in EJP. The hyperglycemia was modulated through regulating the enzymes activities as for metabolism in glucose and improving the antioxidant status of pancreatic in rats of T2DM model from the results of Oyedemi’ studies [42]. Quercetin can be used for the treatment of T2DM by lowering the pancreatic iron deposition and PBC ferroptosis [43]. β-Sitosterol attenuates insulin resistance and high fat diet-induced detrimental changes via mediating IRS-1/AKT signaling for the management of T2DM. Diosgenin ameliorates cognitive deficits in T2DM, owing to its amelioration of astrogliosis, inflammation, and oxidative stress [45]. Baicalein (10^{-6} and 10^{-5} mol/L) regulated glucose uptake, glycolysis, and gluconeogenesis of hepatocytes to treat T2DM [46]. 3′-Methoxydaidzein has not been reported about the antidiabetic effect and may be as a potential T2DM drug. Therefore, T2DM treated by EJP was closely related to key target compound including quercetin, β-sitosterol, diosgenin, baicalein, and 3′-methoxydaidzein. The network pharmacology prediction of EJP for the T2DM treatment has been proved to be appropriate, and the screening of new compounds for the T2DM treatment provides ideas for the development of new drugs.

The component-target-pathway network depicted that the therapeutic effect of EJP on T2DM directly interacted with 99 genes. The results of KEGG pathway enrichment analysis of 66 proteins indicated that 26 pathways were exactly connected to the occurrence and progression of T2DM, suggesting that these pathways might be the molecular mechanism of EJP against T2DM. The relationships of some pathways with T2DM were succinctly discussed as follows. Hepatitis B: A study showed hepatitis B virus (HBV) coinfection was significantly related to blood glucose levels. The participants of 28% with HBV coinfection developed T2DM. It was an increasing evidence that infection of HBV is strongly associated with the development of T2DM [47]. Bladder and prostate cancer pathway: T2DM are becoming increasingly prevalent worldwide and is associated with the increased incidence of bladder cancer [48]. However, a personal history of T2DM is connected with a lower incidence of prostate cancer [49]. Apoptosis pathway: apoptosis plays important roles in the pathophysiology of T2DM. The prevention and revert of β-cell apoptosis by regulating the balance of Bcl family, and apoptotic genes against apoptosis might be a new path for prevention and therapeutic application on T2DM [50]. FoxO signaling pathway: forkhead
Figure 3: Target-pathway network constructed based on KEGG analysis from the DAVID database. Blue represents 26 pathways and green represents 66 protein targets.

Figure 4: KEGG enrichment analysis of predicted pathways.
box protein O1 (FOXO1) played important roles in β-cell growth and function. FOXO1 mRNA levels were increased in the islets of patients with T2DM [51]. TNF signaling pathway: there was a growing evidence that tumor necrosis factor-α (TNF-α) involved in insulin resistance, and it is associated with the development of T2DM [52]. Glioma pathway: it was inverse relations between diabetes and glioma risk [53]. Thyroid hormone signaling pathway: the downregulation of FT3 was significantly related to the prevalence of DR in T2DM with normal thyroid function [54, 55]. Neurotrophin signaling pathway: the changes in the serum neurotrophic factor levels were associated with metabolic syndrome components in T2DM [56, 57]. T cell signaling pathway: in a study, it showed that the presence of senescent T cells exerted a detrimental influence on immune function during T2DM [58]. VEGF signaling pathway: the redox environment influences vascular endothelial growth factor (VEGF) production in response to proinflammatory

**Figure 5:** The network of protein-protein interaction.
stimuli in T2DM [59]. Toll-like receptor signaling pathway: toll-like receptor, the central of innate immunity, was significantly involved in progression of T2DM [60]. MAPK and PI3K-AKT signaling pathways: MAPK and PI3K-AKT are essential for glucose homeostasis. The PI3K-AKT signaling pathway is the major effector of metabolic insulin action.

Figure 6: Component-target-pathway network was structured. From left to right, the components of EJP, target, pathways, and component represent the green, red, and blue, severally. The edges show the relationship of the component-target-pathway.

Figure 7: The RT-PCR analysis of the PI3K-AKT signal pathway. The expression level of PI3K p85, AKT1, GSK3β, and Myc mRNA. *P < 0.05, **P < 0.01.
5. Conclusion

99 targets and 58 signal pathways were screened via network pharmacology. Only 66 targets and 26 pathways were directly related to diabetes with literature analysis. In the compound-target network, quercetin, β-sitosterol, diosgenin, baicalein, and 3′-methoxydaidzein were core compounds. Furthermore, PI3K-AKT, MAPK, and VEGF signaling pathways might be significant. It implied that the hyperglycemia of T2DM model rats were alleviated via the PI3K-AKT pathway. In conclusion, EJP was proved to treat T2DM. It is helpful for researchers to further design pharmacodynamics experiments on these components in the next protocol. Meanwhile, it provided a reference to study the effective constituents of EJP and mechanism of treating T2DM. These specific targets were also worth researching deeply in future.

Abbreviations

EJP: Erjing prescription
T2DM: Type 2 diabetes mellitus
RT-PCR: Real-time PCR
CC: Cell component
BP: Biological process
MF: Molecular function
PPI: Protein-protein interactions
TCM: Traditional Chinese medicine
TCMSP: Traditional Chinese medicine systems pharmacology
OB: Oral bioavailability
DL: Drug-likeness
GO: Gene Ontology
TTD: Therapeutic target database
KEGG: Kyoto Encyclopedia of Genes and Genomes.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

All authors participated in this study. Liming Zhang designed this manuscript. Jiexin Wang and Haiqing Chu conducted the experiments, the literature survey, and drafted the manuscript. Hangying Li revised the manuscript. Wenqian Yang, Yu Zhao, and Tong Shen processed the data and checked the manuscript. John Cary checked the grammar of the manuscript and revised it. Jieixin Wang and Haiqing Chu contributed equally.

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Supplementary Materials

The graphical abstract is the overall flowchart of this study and may be found online in the supporting materials section at the end of the article. (Supplementary Materials)

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