Research Article

Network Pharmacology-Based Prediction of Mechanism of Shenzhuo Formula for Application to DKD

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Background. Shenzhuo formula (SZF) is a traditional Chinese medicine (TCM) prescription which has significant therapeutic effects on diabetic kidney disease (DKD). However, its mechanism remains unknown. Therefore, this study aimed to explore the underlying anti-DKD mechanism of SZF. Methods. The active ingredients and targets of SZF were obtained by searching TCMSP, TCMID, SwissTargetPrediction, HIT, and literature. The DKD target was identified from TTD, DrugBank, and DisGeNet. The potential targets were obtained and PPI network were built after mapping SZF targets and DKD targets. The key targets were screened out by network topology and the “SZF-key targets-DKD” network was constructed by Cytoscape. GO analysis and KEGG pathway enrichment analysis were performed by using DAVID, and the results were visualized by Omicshare Tools. Results. We obtained 182 potential targets and 30 key targets. Furthermore, a “SZF-key targets-DKD” network topological analysis showed that active ingredients like M51, M21, M5, M71, and M28 and targets like EGFR, MMP9, MAPK8, PIK3CA, and STAT3 might play important roles in the process of SZF treating in DKD. GO analysis results showed that targets were mainly involved in positive regulation of transcription from RNA polymerase II promoter, inflammatory response, lipopolysaccharide-mediated signaling pathway, and other biological processes. KEGG showed that DKD-related pathways like TNF signaling pathway and PI3K-Akt signaling pathway were at the top of the list. Conclusion. This research reveals the potential pharmacological targets of SZF in the treatment of DKD through network pharmacology and lays a foundation for further studies.

1. Introduction

Diabetic kidney disease (DKD) is one of the most common chronic microvascular complications of diabetes. It may be caused and shaped by the interaction of many factors such as endoplasmic reticulum dysfunction, high sugar-mediated generation of terminal advanced glycation endproducts (AGE), increased activation of the renin angiotensin aldosterone system, increased generation of reactive oxygen species (ROS), and activation of extracellular matrix (ECM) and protein kinase C [1, 2]. It is reported that the incidence of DKD is about 40% in the diabetic population [3]. Furthermore, with the increasing incidence of diabetes, the incidence of DKD is increasing yearly [4]. Therefore, it is important to intensify studies of the pathogenesis of DKD and the search for effective intervention targets.

Shenzhuo formula (SZF) as a traditional Chinese medicine (TCM) prescription has certain advantages in the treatment of DKD [5]. It is created by Tong Xiaolin, an academician at the Chinese Academy of Sciences, and his team. This formula was based on the pathogenesis of qi deficiency blood stasis, and the classic prescription of Didang decoction. Years of clinical studies have shown that SZF can effectively increase the glomerular filtration rate,
reduce 24-hour urinary protein and kidney damage, and reverse kidney disease when used early [5, 6]. However, due to the diversity of TCM compounds and complexity of in vivo processes, the systematic mechanism research of SZF has been hindered.

Recently, network pharmacology has been developed rapidly with the use of multiomics, high-throughput screening, network visualization and analysis, or other techniques [7–9]. It can help to reveal the network structure of drug action [10] and provide possibilities for exploring the mechanism of action of TCM compounds. Therefore, this study aimed to shed light on the underlying mechanisms of SZF in DKD treatment using a network pharmacology approach.

2. Methods

2.1. Research Tools. The Chinese Traditional Medicine System Pharmacological Database Analysis Platform (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php) [11], Traditional Chinese Medicine Integrated Database (TCMID, http://www.megabionet.org/tcmid/) [12], SwissTargetPrediction (http://www.swisstargetprediction.ch/) [13], and HIT (http://lifecenter.biosino.org/hit/) [14] were used to access to SZF ingredients and targets. (2) The Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/group/cjttd/) [15], DrugBank (https://www.drugbank.ca/) [16], and DisGeNet (http://www.disgenet.org/) [17] were used to get the targets’ proteins of DKD. (3) The protein–protein interaction (PPI) network was obtained online using STRING (http://string-db.org) [18]. Compositional software Cytoscape 3.2.1 (http://www.cytoscape.org/) [19] was used to carry out network topology analysis and construct SZF-key targets-DKD network. The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.ncifcrf.gov) [20] was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The Omicshare Tools (https://www.omicshare.com/) were used for visual analysis of GO and KEGG results.

2.2. Collection of Major Chemical Constituents. We relied on TCMSP, TCMID database, and literatures mining to search for the chemical constituents of SZF (Hedysarum Multijugum Maxim, Radix Salviae, Hirudo, and Radix Rhei Et Rhizome).

2.3. Screening of Active Compounds. As we all know, TCM drugs enter human body and then take effect through absorption, distribution, metabolism, and excretion (ADME) processes. Among them, oral bioavailability (OB) and drug similarity (DL), the key parameters of ADME components, were used as the screening criteria for active ingredients in this study. In this section, we used TCMSP to collect active compounds and their ADME properties. And then the active compounds that meet “OB ≥ 30%, DL ≥ 0.18” were selected as potential active ingredients.

2.4. Prediction of Targets. SwissTargetPrediction and HIT databases were used to collect the drug targets. In addition, TTD, DrugBank and DisGeNet databases were used to search for DKD targets by entering the key words of “diabetic kidney disease” and “diabetic nephropathy.” Further, we matched SZF targets with DKD targets to obtain common targets.

2.5. Network Construction and Analysis. PPI network of common targets was obtained using STRING. Furthermore, the PPI network topology analysis was carried out using Cytoscape 3.2.1 software and then key targets were obtained. To further explore the interactions between the active ingredients and their related targets at a system level, a “SZF-key targets-DKD” network was constructed by Cytoscape 3.2.1.

2.6. GO and KEGG Analysis. GO analysis is widely used for gene function classification and mainly includes the molecular function (MF), biological processes (BP), and cellular components (CC) [21]. In this step, we used the DAVID tool for GO and KEGG pathway analysis. Then, we used Omicshare Tools for visual display.

3. Results

3.1. Screening of Candidate Components in SZF. Through TCMSP and TCMID database, a total of 87 active compounds of Hedysarum Multijugum Maxim, 210 of Radix Salviae, 35 of Hirudo, and 92 of Radix Rhei Et Rhizome were obtained. Then by ADME (OB ≥ 30%, DL ≥ 0.18) screening, a total of 101 active compounds were selected, including 20 active compounds of Hedysarum Multijugum Maxim, 65 of Radix Salviae, and 16 of Radix Rhei Et Rhizome (in this section, because Hirudo could not be found in TCMSP database, its ADME parameters could not be obtained and did not participate in screening). In addition, through literature mining, another 4 active compounds were collected, including 2 active compounds of Hedysarum Multijugum Maxim [22, 23], 1 of Radix Salviae [24], and 1 of Radix Rhei Et Rhizome [25].

3.2. Target Prediction. After matching SZF targets with DKD genes, a total of 182 common targets of SZF were obtained. We only show 50 of them in Table 1. And full information of 182 common targets is displayed in Table 2.

3.3. Construction and Analysis of Network Maps. The PPI network of the 182 common targets was obtained using STRING (Figure 1). Then, we used Cytoscape 3.2.1 to obtain 30 key targets by network topology analysis with inclusion criteria of “degree ≥ 2 times of the median, closeness centrality ≥ median, betweenness centrality ≥ median” (Table 3). Next, we constructed a “SZF-key targets-DKD” network by Cytoscape 3.2.1 (Figure 2).

3.4. GO and KEGG Analysis. The DAVID was used to carry out GO analysis. And the GO terms were visualized by the Omicshare Tools (Figure 3). The GO analysis results showed that targets were mainly involved in positive regulation of
| Serial number | Target                                      | Common name       | Uniprot ID |
|---------------|---------------------------------------------|-------------------|------------|
| 1             | Aldose reductase                            | AKR1B1            | P15121     |
| 2             | Acyl coenzyme A:cholesterol acyltransferase | CES1              | P23141     |
| 3             | Signal transducer and activator of transcription 3 | STAT3            | P40763     |
| 4             | Protein-tyrosine phosphatase 1C             | PTPN6             | P29350     |
| 5             | Vascular endothelial growth factor receptor 2 | KGFR              | P35968     |
| 6             | Epidermal growth factor receptor erbB1      | PIK3CA            | P42336     |
| 7             | c-Jun N-terminal kinase 1                   | MAPK8             | P45983     |
| 8             | LXR-alpha                                   | NR1H3             | Q13133     |
| 9             | Estrogen receptor alpha                     | ERα               | P03372     |
| 10            | Testis-specific androgen-binding protein    | SHBG              | P04278     |
| 11            | Cytochrome P450 2C19                        | CYP2C19           | P33261     |
| 12            | Protein-tyrosine phosphatase 1B             | PTPN1             | P18031     |
| 13            | Butyrylcholinesterase                      | BCHE              | P06276     |
| 14            | Vitamin D receptor                          | VDR              | P11473     |
| 15            | Glucose-6-phosphate 1-dehydrogenase         | G6PD              | P11413     |
| 16            | Peroxisome proliferator-activated receptor alpha | PPARA           | Q07869     |
| 17            | Peroxisome proliferator-activated receptor delta | PPARD         | Q03181     |
| 18            | Peroxisome proliferator-activated receptor gamma | PPARG         | P37231     |
| 19            | UDP-glucuronosyltransferase 2B              | UGT1B2            | P16662     |
| 20            | 11-Beta-hydroxysteroid dehydrogenase 2      | HSD11B2           | P80365     |
| 21            | NADPH oxidase 4                             | NOX4             | Q06101     |
| 22            | Tyrosine-protein kinase SYK                 | SYK              | P43405     |
| 23            | Glycogen synthase kinase-3 beta            | GSK3B             | P49841     |
| 24            | Matrix metalloproteinase 9                 | MMP9              | P14780     |
| 25            | Matrix metalloproteinase 2                 | MMP2              | P08253     |
| 26            | Matrix metalloproteinase 12                | MMP12             | P39980     |
| 27            | ATP-binding cassette sub-family G member 2 | ABCG2             | Q9UNQ0     |
| 28            | Arachidonate 12-lipoxygenase                | ALOX12            | P18054     |
| 29            | Cyclooxygenase-2                           | PTGS2             | P35354     |
| 30            | Insulin-like growth factor I receptor       | IGF1R             | P08069     |
| 31            | Myeloperoxidase                            | MPO               | P05164     |
| 32            | Matrix metalloproteinase 3                 | MMP3              | P08254     |
| 33            | Serine/threonine-protein kinase AKT         | AKT1             | P31749     |
| 34            | Beta-secretase 1                           | BACE1             | P56817     |
| 35            | Tyrosine-protein kinase receptor UFO        | AXL               | P30530     |
| 36            | NUAK family SNF1-like kinase 1             | NUAK1             | O60285     |
| 37            | Aldehyde reductase                         | AKR1A1           | P14550     |
| 38            | Plasminogen                                | PLG               | P00747     |
| 39            | PI3-kinase p110-delta subunit               | PIK3CD            | O00329     |
| 40            | PI3-kinase p110-gamma subunit               | PIK3CG            | P48736     |
| 41            | Hematopoietic prostaglandin D synthase      | HPGDS             | O60760     |
| 42            | Serine-protein kinase ATM                   | ATM               | Q33135     |
| 43            | Cytochrome P450 24A1                       | CYP24A1           | Q07973     |
| 44            | Mineralocorticoid receptor                  | NR3C2             | P08235     |
| 45            | Cannabinoid receptor 1                      | CNR1              | P21554     |
| 46            | Hepatocyte nuclear factor 4-alpha           | HNF4A             | P41235     |
| 47            | C-C chemokine receptor type 1               | CCR1              | P32246     |
| 48            | Histone-lysine N-methyltransferase EZH2     | EZH2              | Q13910     |

Organism: Homo sapiens. Only 50 potential targets’ information is shown here, and the whole is in Table 3.
Table 2: A total of 182 common targets.

| No. | Target                                                 | Common name          | Uniprot ID |
|-----|--------------------------------------------------------|----------------------|------------|
| 1   | Aldose reductase                                       | AKR1B1               | P15121     |
| 2   | Acyl coenzyme A:cholesterol acyltransferase           | CES1                 | P23141     |
| 3   | Signal transducer and activator of transcription 3    | STAT3                | P40763     |
| 4   | Protein-tyrosine phosphatase 1C                        | PTPN6                | P29350     |
| 5   | Vascular endothelial growth factor receptor 2         | KDR                  | P35968     |
| 6   | Epidermal growth factor receptor erbB1                | EGFR                 | P00533     |
| 7   | PI3-kinase p110-alpha subunit                          | PIK3CA               | P42336     |
| 8   | c-Jun N-terminal kinase 1                             | MAPK8                | P45983     |
| 9   | LXR-alpha                                             | NR1H3                | Q13133     |
| 10  | Estrogen receptor alpha                                | ESR1                 | P03372     |
| 11  | Testis-specific androgen-binding protein               | SHBG                 | P04278     |
| 12  | Cytochrome P450 2C19 13                               | CYP2C19              | P33261     |
| 13  | Protein-tyrosine phosphatase 1B                        | PTPNI                | P18031     |
| 14  | Butyrylcholinesterase                                 | BCHE                 | P06276     |
| 15  | Vitamin D receptor                                     | VDR                  | P11473     |
| 16  | Glucose-6-phosphate 1-dehydrogenase                   | G6PD                 | P11413     |
| 17  | Peroxisome proliferator-activated receptor alpha       | PPARA                | Q07869     |
| 18  | Peroxisome proliferator-activated receptor delta       | PPARD                | Q03181     |
| 19  | Peroxisome proliferator-activated receptor gamma       | PPARG                | P37231     |
| 20  | UDP-glucuronosyltransferase 2B7                       | UGT2B7               | P16662     |
| 21  | 11-beta-hydroxysteroid dehydrogenase 2                | HSD11B2              | P80365     |
| 22  | NADPH oxidase 4                                        | NOX4                 | Q9NPJH5    |
| 23  | Tyrosine-protein kinase SYK                            | SYK                  | P43405     |
| 24  | Glycogen synthase kinase-3 beta                       | GSKB                 | P49841     |
| 25  | Matrix metalloproteinase 9                            | MMP9                 | P14780     |
| 26  | Matrix metalloproteinase 2                             | MMP2                 | P08253     |
| 27  | Matrix metalloproteinase 12                           | MMP12                | P39980     |
| 28  | ATP-binding cassette sub-family G member 2             | ABCG2                | Q9UNQ0     |
| 29  | P-glycoprotein 1                                       | ABCB1                | P08183     |
| 30  | Arachidonate 12-lipoxygenase                          | ALOX12               | P18054     |
| 31  | Cyclooxygenase-2                                       | PTGS2                | P35354     |
| 32  | Insulin-like growth factor I receptor                 | IGF1R                | P08069     |
| 33  | Myeloperoxidase                                        | MPO                  | P05164     |
| 34  | Matrix metalloproteinase 3                            | MMP3                 | P08254     |
| 35  | Serine/threonine-protein kinase AKT                    | AKT1                 | P31749     |
| 36  | Beta-secretase 1                                       | BACE1                | P56817     |
| 37  | Tyrosine-protein kinase receptor UFO                  | AXL                  | P30530     |
| 38  | NUAK family SNF1-like kinase 1                        | NUAK1                | O60285     |
| 39  | Aldehyde reductase (by homology)                      | AKR1A1               | P14550     |
| 40  | Plasminogen                                           | PLG                  | P00747     |
| 41  | PI3-kinase p110-delta subunit                          | PIK3CD               | O00329     |
| 42  | PI3-kinase p110-gamma subunit                          | PIK3CG               | P48736     |
| 43  | Hematopoietic prostaglandin D synthase                | HPGDS                | O60760     |
| 44  | Serine-protein kinase ATM                              | ATM                  | Q13315     |
| 45  | Cytochrome P450 24A1                                   | CYP24A1              | Q07973     |
| 46  | Mineralocorticoid receptor                            | NR3C2                | P08235     |
| 47  | Cannabinoid receptor 1                                | CRC1                 | P21554     |
| 48  | Hepatocyte nuclear factor 4-alpha                     | HNF4A                | P41235     |
| 49  | C-C chemokine receptor type 1                         | CCR1                 | P32246     |
| 50  | Histone-lysine N-methyltransferase EZH2               | EZH2                 | Q13591     |
| 51  | MAP kinase p38 alpha                                   | MAPK4                | Q16539     |
| 52  | Bromodomain-containing protein 2                      | BRD2                 | P25440     |
| 53  | Aldehyde dehydrogenase                                | ALDH2                | P05091     |
| 54  | Fatty acid binding protein adipocyte                  | FABP4                | P15090     |
| 55  | Fatty acid-binding protein, liver                     | FABP1                | P07148     |
| 56  | Acyl-CoA desaturase                                    | SCD                  | O00767     |
| 57  | MAP kinase ERK1                                        | MAPK3                | P27361     |
| 58  | Short transient receptor potential channel 6          | TRPC6                | Q9Y210     |
| 59  | Mitogen-activated protein kinase kinase 5              | MAPK5                | Q96863     |
| 60  | Disintegrin and metalloproteinase domain-containing protein 17 | ADAM17 | P78536 |
Table 2: Continued.

| No. | Target                                      | Common name       | Uniprot ID |
|-----|---------------------------------------------|-------------------|------------|
| 61  | Hexokinase type IV                         | GCK               | P35557     |
| 62  | Intercellular adhesion molecule-1           | ICAM1             | P05362     |
| 63  | P-selectin                                  | SELP              | P16109     |
| 64  | Leukocyte adhesion molecule-1               | SELL              | P14151     |
| 65  | Matrix metalloproteinase 1                  | MMP1              | P03956     |
| 66  | Matrix metalloproteinase 8                  | MMP8              | P22894     |
| 67  | Endothelin-converting enzyme 1              | ECE1              | P42892     |
| 68  | Integrin beta-3                             | ITGB3             | P05106     |
| 69  | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta isoform | PIK3CB | P42338 |
| 70  | Sorbitol dehydrogenase                      | SORD              | Q00796     |
| 71  | MAP kinase ERK2                             | MAPK1             | P28482     |
| 72  | Vascular endothelial growth factor receptor 1 | FLT1          | P17948     |
| 73  | Matrix metalloproteinase 7                  | MMP7              | P09237     |
| 74  | Type-1 angiotensin II receptor              | AGTR1             | P30556     |
| 75  | Glucose transporter                         | SLC2A1            | P11166     |
| 76  | Nerve growth factor Trk-A                   | NTRK1             | P04629     |
| 77  | Tyrosine-protein kinase JAK1                | JAK1              | P23458     |
| 78  | Tyrosine-protein kinase JAK2                | JAK2              | O60674     |
| 79  | Sodium/glucose cotransporter 2              | SLCA2             | P31639     |
| 80  | Serine/threonine-protein kinase receptor R3 | ACVRL1            | P37023     |
| 81  | Epoxide hydratase                           | EPHX2             | P34913     |
| 82  | Cytochrome P450 11B2                        | CYP11B2           | P19099     |
| 83  | Endothelin receptor ET-A                    | EDNRA             | P25101     |
| 84  | Glutathione S-transferase Mu 1              | GSTM1             | P09488     |
| 85  | Interleukin-1 beta                          | IL1B              | P01584     |
| 86  | Insulin receptor                            | INSR              | P06213     |
| 87  | Protein tyrosine kinase 2 beta              | PTK2B             | Q14289     |
| 88  | Cyclooxygenase-1                            | PTGS1             | P23219     |
| 89  | Cytochrome P450 2C9                         | CYP2C9            | P11712     |
| 90  | Cytochrome P450 3A4                         | CYP3A4            | P08684     |
| 91  | Trypsin I                                   | PRSS1             | P07477     |
| 92  | C-C chemokine receptor type 5               | CCR5              | P51681     |
| 93  | Dopamine D2 receptor                        | DRD2              | P14416     |
| 94  | Cholesterol ester transfer protein          | CETP              | P11597     |
| 95  | Calcitonin gene-related peptide type 1 receptor | CALCRL         | Q16602     |
| 96  | Serotonin 2a (5-HT2a) receptor              | HTR2A             | P28223     |
| 97  | Disintegrin and metalloproteinase domain-containing protein 10 | ADAM10 | O14672 |
| 98  | TGF-beta receptor type I                    | TGBFR1            | P36897     |
| 99  | Nitric-oxide synthase, brain                | NOS1              | P29475     |
| 100 | Cathepsin (B and K)                         | CTSB              | P07858     |
| 101 | Bradykinin B1 receptor                      | BDKRB1            | P46663     |
| 102 | Potassium voltage-gated channel subfamily KQT member 1 | KCNQ1 | P51787 |
| 103 | Leukotriene A4 hydrolase                    | LTA4H             | P09960     |
| 104 | Apoptosis regulator Bcl-2                   | BCL2              | P10415     |
| 105 | Kininogen-1                                 | KNG1              | P01042     |
| 106 | Solute carrier family 22 member 2           | SLC22A2           | O15244     |
| 107 | Plasma retinol-binding protein              | RBP4              | P02753     |
| 108 | Histone deacetylase 4                      | HDAC4             | P56524     |
| 109 | Dopamine D3 receptor                        | DRD3              | P35462     |
| 110 | C-C chemokine receptor type 2               | CCR2              | P41597     |
| 111 | Solute carrier family 22 member 12          | SLC22A12          | Q96537     |
| 112 | Glucagon-like peptide 1 receptor            | GLP1R             | P43220     |
| 113 | Dual specificity mitogen-activated protein kinase kinase 2 | MAP2K2 | P35507 |
| 114 | Death-associated protein kinase 2           | DAPK2             | Q9U1K4     |
| 115 | Bile acid receptor FXR                      | NRIH4             | Q96R1I     |
| 116 | Interleukin-6                               | IL6               | P05231     |
| 117 | Transcription factor AP-1                  | JUN               | P05412     |
| 118 | Vascular endothelial growth factor A         | VEGFA             | P15692     |
| 119 | Interleukin-10                              | IL10              | P22301     |
| 120 | Endothelin-1                                | EDN1              | P05385     |
| No. | Target | Common name | Uniprot ID |
|-----|--------|-------------|------------|
| 121 | Nitric oxide synthase, endothelial | NOS3 | P29474 |
| 122 | Urotensin II receptor | UTS2R | Q9UKP6 |
| 123 | 78kDa glucose-regulated protein | HSPA5 | P11021 |
| 124 | Galectin-3 | LGALS3 | P17931 |
| 125 | Macrophage migration inhibitory factor | MIF | P4174 |
| 126 | Serum paraoxonase/arylesterase 1 | PON1 | P27169 |
| 127 | Kallikrein 1 | KLK1 | P06870 |
| 128 | Rho-associated protein kinase 1 | ROCK1 | Q13464 |
| 129 | Sphingosine kinase 1 | SPPK1 | Q9NYA1 |
| 130 | Serine/threonine-protein kinase Sgk1 | SGK1 | O00141 |
| 131 | Low affinity sodium-glucose cotransporter | SLC5A4 | Q9NY91 |
| 132 | Neutrophil cytosol factor 1 | NCF1 | P14598 |
| 133 | Antileukoproteinase | SLPI | P03973 |
| 134 | Signal transducer and activator of transcription 1-alpha/beta | STAT1 | P42224 |
| 135 | Protein kinase C beta type | PRKCB | P05771 |
| 136 | Gap junction alpha-1 protein | GJA1 | P17302 |
| 137 | C-X-C motif chemokine 11 | CXCL11 | O14625 |
| 138 | Interleukin-8 | CXCL8 | P10145 |
| 139 | Superoxide dismutase [Cu-Zn] | SOD1 | P00441 |
| 140 | C-X-C motif chemokine 2 | CCL2 | P13500 |
| 141 | Hypoxia-inducible factor 1-alpha | HIF1A | Q16665 |
| 142 | Caveolin-1 | CAV1 | Q03135 |
| 143 | Interleukin-1 alpha | IL1A | P01583 |
| 144 | Nuclear factor erythroid 2-related factor 2 | NFE2L2 | Q16236 |
| 145 | C-X-C motif chemokine 10 | CXCL10 | P02778 |
| 146 | Plasminogen activator inhibitor 1 | SERPINE1 | P05121 |
| 147 | Osteopontin | SPPI | P10451 |
| 148 | Bone morphogenetic protein 2 | BMP2 | P12643 |
| 149 | Transforming growth factor beta-1 propeptid | TGFBI | P01137 |
| 150 | Cyclin-dependent kinase inhibitor 2A | CDKN2A | P42771 |
| 151 | Transcription factor E2F1 | E2F1 | Q01094 |
| 152 | Thrombomodulin | THBD | P07204 |
| 153 | Insulin-like growth factor II | IGF2 | P01344 |
| 154 | Catalase | CAT | P04040 |
| 155 | Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN | PTEN | P06034 |
| 156 | Pro-epidermal growth factor | EGFR | P01133 |
| 157 | ATP synthase subunit beta, mitochondria | ATP5F1B | P06576 |
| 158 | NAD-dependent protein deacetylase sirtuin-1 | SIRT1 | Q96EB6 |
| 159 | Angiotensin-converting enzyme | ACE | P12821 |
| 160 | Matrix metalloproteinase 10 | MMP10 | P09238 |
| 161 | Transketolase | TKT | P294501 |
| 162 | Dipeptidyl peptidase IV | DPP4 | P27487 |
| 163 | Nuclear factor NF-kappa-B p65 subunit | RELA | Q04206 |
| 164 | Nitric oxide synthase, inducible | NOS2 | P35228 |
| 165 | Protein kinase C alpha type | PRKCA | P17252 |
| 166 | Tumor necrosis factor | TNF | P01375 |
| 167 | Protein kinase C epsilon type | PRKCE | Q02156 |
| 168 | Renin | REN | P00797 |
| 169 | Axin1/beta-catenin | CTNNB1 | P35222 |
| 170 | Fibronectin | FN1 | P02751 |
| 171 | C-X-C chemokine receptor type 4 | CXCR4 | P01073 |
| 172 | Heparanase | HPSE | Q9Y251 |
| 173 | Glucagon | GCG | P01275 |
| 174 | Tumor necrosis factor receptor superfamily member 11B | TNFRSF11B | O00300 |
| 175 | Metalloproteinase inhibitor 1 | TIMP1 | P01033 |
| 176 | Metalloproteinase inhibitor 2 | TIMP2 | P16035 |
| 177 | Fibroblast growth factor 2 | FGF2 | P09038 |
| 178 | Lipoprotein lipase | LPL | P06858 |
| 179 | Coagulation factor V | F5 | P12259 |
Table 2: Continued.

| No. | Target                                                | Common name | Uniprot ID |
|-----|-------------------------------------------------------|-------------|------------|
| 180 | Cyclic AMP-responsive element-binding protein 1       | CREB1       | P16220     |
| 181 | Phosphatidylinositol 3,4,5-trisphosphate 5- phosphatase 2 | INPPL1      | O15357     |
| 182 | Tumor necrosis factor ligand superfamily member 6     | FASLG       | P48023     |

Figure 1: PPI network of the 182 common targets.

Table 3: Thirty key targets obtained by network topology analysis.

| Serial number | Node  | Degree | Closeness centrality | Betweenness centrality |
|---------------|-------|--------|----------------------|------------------------|
| 1             | PIK3CA| 40     | 0.49508197           | 0.09370214             |
| 2             | STAT3 | 40     | 0.5                  | 0.0863086              |
| 3             | AKT1  | 35     | 0.49025974           | 0.1311921              |
| 4             | KNG1  | 33     | 0.44023324           | 0.2618185              |
| 5             | VEGFA | 33     | 0.49185668           | 0.06953442             |
| 6             | JUN   | 32     | 0.48089172           | 0.17229449             |
| 7             | MAPK3 | 30     | 0.4617737            | 0.09420476             |
| 8             | MAPK1 | 30     | 0.4689441            | 0.09420476             |
| 9             | EGFR  | 27     | 0.4617737            | 0.09420476             |
| 10            | EDN1  | 27     | 0.46604938           | 0.09420476             |
| 11            | EGFR  | 26     | 0.44023324           | 0.09420476             |
| 12            | JAK1  | 26     | 0.44940476           | 0.09420476             |
| 13            | IL6   | 26     | 0.45209581           | 0.09420476             |
| 14            | CXCL8 | 25     | 0.43768116           | 0.09420476             |
| 15            | RELA  | 24     | 0.45757576           | 0.09420476             |
| 16            | FN1   | 23     | 0.4351585            | 0.09420476             |
| 17            | JAK2  | 23     | 0.44940476           | 0.09420476             |
| 18            | CTNNB1| 23     | 0.45841928           | 0.09420476             |
| 19            | TNF   | 22     | 0.44281525           | 0.09420476             |
| 20            | TGFBI | 21     | 0.44281525           | 0.09420476             |
| 21            | MMP9  | 20     | 0.40921409           | 0.09420476             |
| 22            | CCR4  | 19     | 0.41032609           | 0.09420476             |
| 23            | TIMP1 | 19     | 0.41712707           | 0.09420476             |
| 24            | MAPK14| 19     | 0.44411765           | 0.09420476             |
| 25            | BDKRB1| 18     | 0.3994709            | 0.09420476             |
| 26            | PIK3CB| 18     | 0.40921409           | 0.09420476             |
| 27            | MAPK8 | 18     | 0.42296919           | 0.09420476             |
| 28            | ITGB3 | 18     | 0.42296919           | 0.09420476             |
| 29            | CCR5  | 16     | 0.39841689           | 0.09420476             |
| 30            | PLG   | 16     | 0.40266667           | 0.09420476             |
Figure 2: “SZF-key targets-DKD” network. The nodes were visualized with degree. The larger and the redder the node, the higher the degree it was. M1-75 stand for the active ingredients whose full names are shown in Table 4.

Table 4: The information of active ingredients.

| No. | Active ingredients                                                                 | Code name |
|-----|-------------------------------------------------------------------------------------|-----------|
| 1   | Isoimperatorin                                                                      | M1        |
| 2   | 1,2,5,6-Tetrahydrotanshinone                                                        | M2        |
| 3   | 5,6-Dihydroxy-7-isopropyl-1,1-dimethyl-2,3-dihydrophenanthrene-4-one                | M3        |
| 4   | (E)-3-[2-(3,4-Dihydroxyphenyl)-7-hydroxy-benzofuran-4-yl]acrylic                    | M4        |
| 5   | 2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxy-3-benzofuranboxaldehyde | M5        |
| 6   | Przewaquinone c                                                                     | M6        |
| 7   | Cryptotanshinone                                                                    | M7        |
| 8   | Dihydrotanshinlactone                                                               | M8        |
| 9   | Isotanshinone II                                                                    | M9        |
| 10  | Miltipolone                                                                         | M10       |
| 11  | Miltirone                                                                          | M11       |
| 12  | Danshenaldehyde                                                                    | M12       |
| 13  | Danshenol B                                                                        | M13       |
| 14  | Danshenol A                                                                        | M14       |
| 15  | Deoxyneocryptotanshinone                                                            | M15       |
| 16  | Dihydrotanshinone I                                                                 | M16       |
| 17  | Miltionone I                                                                       | M17       |
| 18  | Miltionone II                                                                       | M18       |
| 19  | Neocryptotanshinone ii                                                              | M19       |
| 20  | Neocryptotanshinone                                                                 | M20       |
| 21  | Luteolin                                                                           | M21       |
| 22  | Salvilenone I                                                                       | M22       |
| 23  | Salviolone                                                                         | M23       |
| 24  | Epidanshenspiroketalactone                                                          | M24       |
| 25  | Tanshinone iia                                                                      | M25       |
| 26  | α-Amyrin                                                                            | M26       |
| 27  | Dan-shexinkum d                                                                    | M27       |
| 28  | Sclareol                                                                            | M28       |
| 29  | Dehydrotanshinone II A                                                               | M29       |
| 30  | Baicalin                                                                            | M30       |
| 31  | 2-Isopropyl-8-methylphenanthrene-3,4-dione                                          | M31       |
| 32  | Formyltanshinone                                                                    | M32       |
transcription from RNA polymerase II promoter, inflammatory response, lipopolysaccharide-mediated signaling pathway, positive regulation of peptidyl-serine phosphorylation, and other biological processes. As the top 20 GO enrichment items listed, DKD is relevant to kinds of BP in body abnormalities, and SZF is likely to regulate these items and then play an anti-DKD role.

KEGG pathway enrichment analysis showed that a total of 104 pathways were obtained. The top 20 pathways are displayed in Figure 4, which include TNF signaling pathway, HIF-1 signaling pathway, Toll-like receptor signaling pathway, FoxO signaling pathway, NOD-like receptor signaling pathway, and so on.

**Table 4: Continued.**

| No. | Active ingredients | Code name |
|-----|-------------------|-----------|
| 33  | 3-Beta-Hydroxymethylnetanshiquinone | M33       |
| 34  | Methylnetanshinquinone | M34       |
| 35  | (2R)-3-(3,4-Dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid | M35       |
| 36  | (6S)-6-(Hydroxymethyl)-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g]benzofuran-10,11-dione | M36       |
| 37  | Tanshinone VI | M37       |
| 38  | Przewalskin b | M38       |
| 39  | 6-o-Syringyl-8-o-acetyl shanzhiside methyl ester | M39       |
| 40  | Prolithospermic acid | M40       |
| 41  | (Z)-3-[(E)-2-(3,4-Dihydroxyphenyl)vinyl]-3,4-dihydroxyphenyl]acrylic acid | M41       |
| 42  | Salvianolic acid g | M42       |
| 43  | Salvianolic acid j | M43       |
| 44  | Danshenspiroketallactone | M44       |
| 45  | 1-Methyl-8,9-dihydro-7H-naphtho[5,6-g]benzofuran-6,10,11-trione | M45       |
| 46  | 3,9-di-O-MethylnissolinM | M46       |
| 47  | (6αR,11αR)-9,10-Dimethoxy-6α,11α-dihydro-6H-benzofuran-3,2-c]chromen-3-ol | M47       |
| 48  | (3R)-3-(2-Hydroxy-3,4-dimethoxyphenyl)chroman-7-ol | M48       |
| 49  | Isorhamnetin | M49       |
| 50  | Kaempferol | M50       |
| 51  | Quercetin | M51       |
| 52  | Jaranol | M52       |
| 53  | Bifendate | M53       |
| 54  | Formononetin | M54       |
| 55  | Isoflavanone | M55       |
| 56  | Calycosin | M56       |
| 57  | Hederagenin | M57       |
| 58  | Sennoside E_qt | M58       |
| 59  | Toralactone | M59       |
| 60  | Palmidin A | M60       |
| 61  | Daucosterol_qt | M61       |
| 62  | Eupatin | M62       |
| 63  | Procyanidin B-5,3’-O-gallate | M63       |
| 64  | Rhein | M64       |
| 65  | Beta-sitosterol | M65       |
| 66  | Aloe-emodin | M66       |
| 67  | Lipase | M67       |
| 68  | Gardnerilin a | M68       |
| 69  | Hirudin | M69       |
| 70  | o-Desulfated heparin | M70       |
| 71  | Ursolic acid | M71       |
| 72  | Heparin | M72       |
| 73  | Geniosidic acid | M73       |
| 74  | Genipinic acid | M74       |
| 75  | Nadropratin | M75       |

**4. Discussion**

Previous studies have suggested that SZF has a therapeutic effect on DKD [5,6]. However, the potential mechanisms of SZF treating in DKD have not been fully explained. In this study, we mainly applied network pharmacology to explore it. Firstly, a total of 140 potential active compounds and 182 common targets of SZF and DKD were obtained after screening of active compounds and mapping of targets. Then, we constructed two networks, including the PPI network of 182 common targets and SZF-key targets-DKD network, and then applying GO and KEGG enrichment analysis to explore the regulation mechanism of SZF in treating DKD.
Through the SZF-key targets-DKD network, we could know that most active ingredients were linked with no less than one target, which indicated the character of multi-target of TCM active ingredients. In the meanwhile, different active compounds from different herbs acted on the same targets, which demonstrated that SZF had a synergistic effect in treating DKD. In addition, there were 8 active ingredients whose degrees were greater than 2 times of average in SZF-key targets-DKD network topology analysis. Interestingly, 3 of them had been proven to have kidney protection effect by experiments. For example, quercetin liposomes had renal protective effects of reducing oxidative stress, attenuating AGE expression, and delaying the progression of DKD [26]. Luteolin attenuated DKD mainly via suppression of inflammatory response and oxidative response [27]. Ursolic acid alleviated renal damage in type 2 diabetic db/db mice by downregulating proteins in the angiotensin II type 1 receptor-associated protein/angiotensin II type 1 receptor signaling pathway to inhibit extracellular matrix accumulation, renal inflammation, fibrosis, and oxidative stress [28]. These results were coincident with our predictions, which suggested that active ingredients with higher degree might play an important role in the treatment of DKD. Meanwhile, we discovered five active ingredients (M5, M27, M28, M60, and M70) that were likely to have renal protection effect but had not been verified up to now.

Moreover, the results of the SZF-key targets-DKD topology analysis also showed that there were 5 targets whose degrees were greater than 2 times of the average. Particularly, 3 of these had been proven to be closely related with DKD. For instance, EGFR activation had a significant role in activating pathways that mediate podocyte injury and loss in diabetic nephropathy [29]. Downregulated expression of MMP-9 could promote the process of DKD [30]. STAT3 inhibition could hinder the development and progression of DKD in diabetic patients [31].
As shown in GO analysis, the potential targets of SZF acting on DKD were mainly associated with various biological processes, such as lipopolysaccharide-mediated signaling pathway, inflammatory response, positive regulation of cyclase activity, protein kinase B signaling, positive regulation of MAP kinase activity, and response to estradiol, which had a strongly direct correlation with the pathogenesis of DKD [32–38].

Similarly, KEGG pathway enrichment analysis showed that SZF took an anti-DKD effect by multiple pathways. Through further research, we found that some pathways had been already verified to exert anti-DKD potential by experiments, such as TNF signaling pathway [39], HIF-1 signaling pathway [40], Toll-like receptor signaling pathway [41], FoxO signaling pathway [42], focal adhesion [43], and NOD-like receptor signaling pathway [44]. These results were also consistent with what we predicted. In addition, SZF might have potential therapeutic effects on diseases such as cancer, hepatitis, influenza, leishmaniasis, pertussis, and tuberculosis according to the KEGG enrichment analysis. Just as it was reported that different diseases had common or similar pathological changes and could be treated with the same prescription [45], the above results suggested that SZF concentrated more on the systematicness of the body when treating DKD. In other words, SZF possibly regulated the body to reach the balance state, then reaching the aim of treatment.

5. Conclusion

In conclusion, this study based on the network pharmacology had preliminarily explained the anti-DKD mechanism of SZF from the perspective of multi-active ingredients, multi-targets,
and multi-pathway. In the future, we will further investigate its mechanism by molecular docking, using in vitro or in vivo studies.

**Data Availability**

The data used to support the results of this study can be obtained from the corresponding author upon reasonable request.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Authors’ Contributions**

Wang Xin-miao, Yang Hao-yu, and Zhang Li-li contributed equally to this work.

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