Research Article

Identification of the Active Compound of Liu Wei Di Huang Wan for Treatment of Gestational Diabetes Mellitus via Network Pharmacology and Molecular Docking

Yunqi Xiong, Qiutong Li, Xiuhui Chen, Ting Zhu, Qitian Lu, and Guojing Jiang

1Department of Obstetrics and Gynaecology, Shuguang Hospital Affiliated to Shanghai Traditional Chinese Medical University, Shanghai 200120, China
2Department of Obstetrics and Gynaecology, Nanjing Drum Tower Hospital Affiliated to Nanjing University Medical School, Nanjing 210008, China

Correspondence should be addressed to Guojing Jiang; jgj3137@shutcm.edu.cn

Received 22 November 2021; Accepted 5 May 2022; Published 28 May 2022

Copyright © 2022 Yunqi Xiong et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Liu Wei Di Huang Wan (LWDHW) is a well-known Chinese herbal compound, which has been prescribed for the treatment of gestational diabetes mellitus (GDM). We sought to clarify the potential therapeutic effects of LWDHW against GDM. Differentially expressed genes (DEGs) in GDM were firstly identified from the Gene Expression Omnibus (GEO) database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to reveal the biological functions of the DEGs. Subsequently, the LWDHW-compound–target network was constructed based on public databases to identify the relationship between the active components in LWDHW and the corresponding targets. Furthermore, gene functional analysis and protein–protein interaction (PPI) network construction were applied to investigate the function of potential targets and to evaluate hub genes. Finally, molecular docking was used to verify the binding activities between active ingredients and hub targets. Thirteen active components and 39 corresponding therapeutic target genes were obtained via network pharmacology analysis. The enrichment analysis demonstrated that the anti-GDM effect of LWDHW included oxidoreductase activity, involvement in renal system process, and regulation of blood pressure, which may be achieved through regulation of serotonergic synapses, vascular smooth muscle contraction, and neuroactive ligand–receptor interaction pathways. Additionally, molecular docking revealed that the main active component, Mu Dan Pi, exhibited the best affinity for proteins encoded by hub genes. This study applied network pharmacology analysis and molecular docking to display the multicomponent and multitarget characteristics of LWDHW in the treatment of GDM. Our findings provide novel insights into the pathogenesis of GDM and the therapeutic mechanisms of LWDHW against GDM.

1. Introduction

GDM is defined as diabetes diagnosed during pregnancy, with an approximately 2–10% morbidity in pregnant women [1, 2]. With the increasing frequency of obesity and type 2 diabetes, GDM is becoming more common. According to the results of a meta-analysis, the total incidence of GDM is 14.8% in China mainland. Older women had approximately twice the rate of GDM compared to younger women [3]. It was also proved that the incidence of GDM rose with advanced maternal age in another prospective cohort study in west China [4]. After the “two-child policy” put into effect in 2015, the prevalence of GDM has continued to increase during the past years and is likely to reach another peak in the future [5]. GDM is not only associated with adverse pregnancy outcomes, such as macrosomia and stillbirth [6], but it also increases the risk of metabolic syndrome in pregnant women and their offspring [7, 8]. Even though the multidisciplinary treatment of GDM includes modifications of lifestyle and pharmacological treatment, the most appropriate therapy for pregnancies complicated by GDM remains controversial, due to the unresolved safety issues.
of antidiabetic agents and uncertain treatment options [9, 10]. Therefore, the identification of effective molecular targets and elucidation of potential mechanisms related to GDM are of great importance for the improvement of the maternal and neonatal outcomes.

Traditional Chinese medicine (TCM) has been used for centuries to prevent and treat disease in China. The active components of several types of TCMs show great potential as effective therapy for GDM through different mechanisms, including the inhibitory effect on inflammation, enhancement of β cell function, and regulation of hepatic glucoseogenesis [11, 12].

Liu Wei Di Huang Wan (LWDHW) is a popular Chinese herbal formula and is commonly prescribed for the treatment of diabetes [13], hypertension [14], menopause [15], and systemic lupus erythematosus [16]. It is a formulation composed of six ingredients: Shu Di Huang (Radix Rehmanniae Preparata), Shan Zhu Yu (Fructus Corni), Mu Dan Pi (Cortex Moutan), Shan Yao (Rhizoma Dioscoreae), Fu Ling (Poria), and Ze Xie (Rhizoma Alismatis). However, the underlying mechanism of LWDHW in the treatment of GDM has not been fully clarified.

Network pharmacology, a methodology developed in recent years, can reveal the complex relational network of medicine, genes, and targets of diseases [17] and is able to predict and elucidate the potential mechanism of multiple components, targets, and pathways of TCMs, through the construction of the various complex networks and the analysis of multilevel connections [18]. Network pharmacology could be of great help in the development of cost-effective drug development [19] and in the identification of synergistic TCMs [20]. This would provide a novel approach to elucidating the molecular mechanisms by which LWDHW has therapeutic effects on GDM.

Here, we sought to clarify the mechanism by which LWDHW exerted an effect against GDM. We first identified genes that are differentially expressed in GDM from a Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/) [21]. Then, the active components of LWDHW and their corresponding target genes were predicted according to the Traditional Chinese Medicine Systems Pharmacology (TCMSP) and Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM) [22, 23]. The intersection of DEGs and drug-target genes was determined. We performed enrichment analysis and established a protein–protein interaction (PPI) network to identify the hub target genes and their corresponding pathways. A LWDHW-main active compound–GDM-target–signaling pathway network was constructed. Finally, the anti-GDM effect of LWDHW, as suggested by the network analysis, was further verified by molecular docking.

2. Materials and Methods

2.1. Identification of Targets of GDM. The GDM gene expression dataset was downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/) [21]. We systematically searched for microarray studies by using the terms “maternal diabetes” and “Homo sapiens.” The GSE51546 dataset (GPL 10558, Illumina Human HT-12 V4.0 expression bead-chip) was obtained, which included data from six women with GDM and six healthy pregnant controls. Then, we downloaded the platform annotation file and series matrix files. The data were normalized, and each corresponding gene probe was converted into a gene symbol by using the Bioconductor R package [24]. If there were multiple probes that could be mapped to the same gene symbol, the average was applied. Genes that were differentially expressed (DEGs) between the maternal diabetes tissues and normal control tissues in the microarray were identified by using the “limma” (linear models for microarray data) program in the R package [25]. In order to correct the P value and control the false discovery rate, the Benjamini–Hochberg method was applied for multiple comparisons. A \(|\log_{2}\text{fold change (FC)}| > 0.263\) and a \(P\) value < 0.05 were regarded as the cut-off criteria for DEGs. Heatmap and volcano plots of significant DEGs were generated using the R software.

2.2. Functional and Pathway Enrichment Analyses. Gene Ontology (GO) displays gene functions from three different aspects: molecular function (MF), cellular component (CC), and biological process (BP) [26]. Kyoto Encyclopedia of Genes and Genomes (KEGG) [27] is a practical database established in 1995 by Kyoto University Kanehisa Laboratory of Bioinformatics Center. GO and KEGG pathway enrichment analyses were performed for the DEGs using the clusterProfiler R package [28]. A \(P\) value < 0.05 was considered to be statistically significant. The results of GO and KEGG enrichment analyses were displayed as bubble charts generated by the ggplot2 R package.

2.3. Identification of the LWDHW Active Ingredients. The constituents of LWDHW were entered into the TCMSP database (https://tcmspw.com/tcmsp.php) [22] to obtain properties including the molecular name, molecular weight, lipid–water partition coefficient, number of donor atoms of H-bonds and acceptor atoms for H-bonds, oral bioavailability (OB), ability to cross the blood–brain barrier drug likeness (DL), and drug half-life (HF). In order to obtain the absorption, distribution, metabolism, and excretion (ADME) properties of compounds [29, 30], OB, DL, and HF were calculated to screen the potential active components. \(OB \geq 20\%\) and \(DL \geq 0.1\) were used as the criteria for choosing candidate compounds for further analysis.

2.4. Construction of PPI Network. The STRING database (http://string-db.org/) was used to establish a PPI network [31]. In the PPI analysis, a combined score > 0.15 was regarded as a significant interaction criterion. The PPI network was visualized with Cytoscape 3.8.0 (https://cytoscape.org/) [32]. Additionally, the novel Cytoscape plugin CytoHubba [33] was used to screen hub genes in the PPI network.

2.5. Active Compound of LWDHW and Corresponding Predicted Target Genes. The corresponding protein targets of each active ingredient in LWDHW were predicted using the BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm/)
database, which is the first online bioinformatics analysis tool specifically designed for research into the molecular mechanisms of TCMs [23]. Then, the LWDHW-compound and LWDHW-compound–target networks were constructed and visualized using Cytoscape software.

2.6. Functional Enrichment Analysis of LWDHW-Related Targets. The intersection of DEGs in GDM and the corresponding targets of the active compounds were obtained by using a Venn diagram. Then, to investigate the biological function of the potential targets, we performed GO and KEGG pathway enrichment analyses using the clusterProfiler R package and visualized the results by means of the ggplot2 R package, as before.

2.7. Construction of the LWDHW–Main Active Compound–GDM-Target-Signaling Pathway Network. To identify the potential GDM-related targets for treatment, a Venn diagram was applied to define the intersection of LWDHW targets and GDM-related targets. The corresponding chemical compounds of the intersecting targets were considered possible therapeutic ingredients for the treatment of GDM. Then, the LWDHW–main active compound–GDM-target-signaling pathway network was constructed to identify the potential relationship between the active compounds of LWDHW and their corresponding targets, which was then visualized as described above.

2.8. Verification through Molecular Docking. To further elucidate the underlying mechanism of LWDHW in the treatment of GDM, molecular docking was applied to predict and verify the binding activity of the core active compounds of LWDHW to proteins encoded by the hub genes with the top three Matthews correlation coefficient (MCC) scores. These genes were considered potential GDM-related targets. First, the SDF format file of the active component was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and converted into mol2 format by PyMol. Then, the PDB format file of the proteins encoded by the hub genes were downloaded from PDB database. Finally, Autodock Vina software [34], which improves the average accuracy of combined model prediction and performs ligand–protein molecular docking, was used to analyze the binding affinities and predict virtual docking.

2.9. Statistical Analysis. All statistical analysis were performed with R (version 3.6.3; https://www.r-project.org/) and were presented as mean ± standard deviation. All statistical tests were two-tailed. Differences were considered significant at \( P < 0.05 \).

3. Results

3.1. Target Prediction of GDM. We identified 1408 genes with differential expression in GDM from GSE51546. As shown in Figure 1, there were 574 upregulated genes and
834 downregulated genes. These DEGs were considered the potential GDM therapeutic targets and were further analyzed.

3.2. Enrichment Analysis of DEGs in GDM. The top 20 enriched GO terms are displayed as bubble plots (Figures 2(a)–2(c)) and a bar plot (Figure 2(e)). In terms of BP, DEGs were mainly enriched for renal system development, extracellular structure organization, and kidney development. In terms of CC, DEGs were mainly enriched for the collagen-containing extracellular matrix, endoplasmic reticulum lumen, and cortical cytoskeleton. For the MF category, the enriched terms included extracellular matrix structural constituent, glycosaminoglycan binding, and growth factor binding. Furthermore, as shown in Figure 2(d), KEGG pathway enrichment analysis demonstrated the top 20 enriched pathways of the DEGs. These mainly involved the regulation of the actin cytoskeleton, RAP1 signaling pathway, and vascular smooth muscle contraction.

3.3. Identification of Active Compounds of LWDHW and Construction of Networks. From the TCMSP database, there

![Figure 2: Enrichment analysis of the differentially expressed genes of gestational diabetes mellitus (GDM). (a) Bubble plot of the top 20 enriched biological process (BP). (b) Cellular component (CC) and (c) molecular function (MF) terms revealed by Gene Ontology (GO) functional enrichment analysis of differentially expressed genes (DEGs) in GDM. (d) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. (e) Bar plot of the top 20 enriched GO terms. The size of the bubble indicates the gene count, while colors indicate the significance of enrichment.]
were six types of herbs and 508 related components of LWDHW in total. According to the ADME thresholds, 169 active ingredients were screened (Supplementary Table S1). The LWDHW-compound network was constructed using Cytoscape 3.8.0 (Figure 3(a)). The predicted active ingredients with potential targets were selected from the BATMAN database. Finally, 19 active ingredients (Table 1) and 536 related targets of the active ingredients were obtained (Supplementary Table S2). The LWDHW-compound–target network was established and is displayed in Figure 3(b).

3.4. Prediction and Enrichment Analyses of the Targets. For further identification of the potential targets to improve treatment of GDM, the intersection of the DEGs of GDM and the active components of the LWDHW corresponding targets is shown in Figure 4. Thirty-nine genes were selected, and enrichment analysis of these genes was performed. The top 20 enriched GO terms were displayed as the bubble plots (Figures 4(a)–4(c)) and bar plot (Figure 4(e)). In terms of BP, genes were mainly enriched in renal system processes, response to antibiotics, and regulation of blood pressure. For the CC category, the enriched terms were basolateral plasma membrane, presynapse, and plasma membrane receptor complex. For the MF category, the enriched terms included oxidoreductase activity, retinol dehydrogenase activity, and SMAD binding.

The bubble plot of the top 20 enriched pathways is shown in Figure 4(d). The top three enriched pathways of the targets were the serotonergic synapse pathway, vascular smooth muscle contraction pathway, and neuroactive ligand–receptor interaction pathway. The detailed information of the pathways and related critical genes are shown in Figure 5.

3.5. PPI Network Visualization. The PPI network was constructed and visualized by using the Cytoscape software (version 3.8.0). As shown in Figures 6(a) and 6(b), the resulting network included 36 nodes and 110 edges. To identify hub target genes that may play key roles during the progression of GDM, CytoHubba was applied to rank the nodes in

| Molecule name       | MW  | OB  | DL  |
|---------------------|-----|-----|-----|
| Alisol B            | 472.78 | 34.47 | 0.82 |
| Alisol C            | 486.76 | 32.7  | 0.82 |
| Alisol B monoacetate| 514.82 | 35.58 | 0.81 |
| Rehmanniside A      | 524.53 | 25.95 | 0.87 |
| Pachymic acid       | 528.85 | 33.63 | 0.81 |
| Poricoic acid B     | 484.74 | 30.52 | 0.75 |
| Batatasin I         | 284.33 | 23.7  | 0.27 |
| Campesterol         | 400.76 | 37.58 | 0.71 |
| Mainin              | 456.78 | 55.38 | 0.78 |
| Quercetin           | 302.25 | 46.43 | 0.28 |
| Sitosterol          | 414.79 | 36.91 | 0.75 |
| Paeonidanin         | 492.57 | 24.64 | 0.78 |
| Kaempferol          | 286.25 | 41.88 | 0.24 |
| (+)-Catechin        | 290.29 | 54.83 | 0.24 |
| Tetrahydroalstonine| 352.47 | 32.42 | 0.81 |
| 20-Hexadecanoylingenol| 586.94 | 28.2  | 0.68 |
| 3,4-Dehydrolycopen-16-al| 548.92 | 46.64 | 0.49 |
| Aristolone          | 218.37 | 45.31 | 0.13 |
| Cornudentanone      | 378.56 | 39.66 | 0.33 |

Abbreviations: MW: molecular weight; OB: oral bioavailability; DL: drug likeness.
the PPI network according to the topological analysis method with MCC (Table 2). The top 10 hub genes and regulated genes are shown in Figure 6(c), while the top 20 hub genes and regulated genes are shown in Figure 6(d).

3.6. Construction of LWDHW-Main Active Compound-GDM-Target-Signaling Pathway Network. The LWDHW-main active compound-GDM-target-signaling pathway network was established for better elucidation of the therapeutic effect. As shown in Figure 7, the network contained five herbs: Mu Dan Pi, Shan Yao, Ze Xie, Shan Zhu Yu, and Fu Ling, 13 different ingredients, 39 target genes, and 31 corresponding pathways.

3.7. Verification by Molecular Docking. Molecular dynamics simulation offers novel insights into the stability and binding activities of protein–ligand complexes. Thus, to validate the potential GDM therapeutic targets, we performed molecular docking of LWDHW with the proteins encoded by the top three hub genes. Docking analysis predicted Vina scores...
successfully. Specific information is shown in Table 3. Furthermore, structure matching analysis was performed by using PyMoL software. Finally, we obtained four groups: AGT and aristolone, ADORA2A and mairin, ADORA2A and aristolone, and CNR1 and catechin, as shown in Figure 8. A Vina score $<-5$ was considered to be convincing. In particular, molecular docking between ADORA2A and mairin had the lowest Vina score: -8.6. ADORA2A was the target of Mu Dan Pi and Shan Zhu Yu. Mairin is an important ingredient of Mu Dan Pi. Overall, the results of molecular docking analysis indicated that the active components of LWDHW had good binding activities to the proteins encoded by the top three hub genes.

4. Discussion

In this study, we sought to clarify the potential therapeutic effects of LWDHW against GDM, by investigating the intersection of GDM DEGs and LWDHW targets, followed by virtual docking experiments for verification. Molecular docking analysis indicated that the active components of LWDHW bound well to the proteins encoded by the top three hub genes. Specifically, aristolone (an active compound of Shan Zhu Yu) targeted AGT and ADORA2A, mairin (a main ingredient of Mu Dan Pi) also targeted ADORA2A, while catechin targeted CNR1.

GDM is the commonest medical complication during pregnancy and imposes a marked economic and health burden with the increasing prevalence of obesity [5]. Since oral antidiabetic agents have side effects and safety issues, the management and treatment of GDM still remain challenging. Thus, a novel therapeutic strategy for GDM is urgently needed [35]. With a long history of use and effectiveness, TCM has increasingly attracted global attention for its unique insights into pathogenesis and multitarget treatment. Growing evidence has shown the potential function of LWDHW in the treatment of diabetes. A population-based case–control study of patients with type 2 diabetes has indicated that LWDHW can relieve diabetic nephropathy [36]. Another study in a mouse model showed its beneficial effects in diabetes-related renal failure [37]. The use of LWDHW and oral antidiabetic drugs have been reported to be associated with delayed use of insulin [38]. To elucidate the potential function and evaluate the beneficial effects of LWDHW on GDM further, the present study investigated the active ingredients and potential mechanisms of this TCM comprehensively using network pharmacology.
We identified 19 active compounds in LWDHW, 508 drug-related targets, and 1408 DEGs of GDM from public databases. Among these, there were 39 genes in common between the DEGs and the drug-corresponding targets, suggesting their potential role in anti-GDM action. In the PPI network of these 39 target genes, the proteins were not independent but were linked in a network. After evaluation of the compound and construction of the network, 13 active compounds, 39 targets, and 31 pathways were finally identified (Figure 7). These results suggested that LWDHW has multitarget biological function, with its multiple compounds, in the treatment of GDM.

LWDHW has displayed beneficial effects on alleviation of insulin resistance through regulating the PI3K/AKT signaling pathway, according to previous studies [39, 40]. In contrast, in our research, the GO and KEGG enrichment analyses suggested that the top three signaling pathways were the serotonergic synapse pathway, vascular smooth muscle contraction pathway, and neuroactive ligand–receptor interaction pathway, which are involved in the progression of GDM and could underlie the mechanism by which LWDHW exerts a therapeutic effect on GDM. It has been acknowledged that 5-hydroxytryptamine (5-HT) might play a significant role in the etiology of GDM [41], which could be a promising biomarker and potential risk factor of GDM [42]. A recent study explained that 5-HT uptake rates is decreased in GDM trophoblasts, resulting in defective insulin signaling and glycosylation [43]. Additionally, it

---

**Table 2: Top 10 hub genes according to the Matthews correlation coefficient (MCC).**

| Gene   | MCC score | Degree | Closeness | Betweenness |
|--------|-----------|--------|-----------|-------------|
| CNR1   | 416       | 15     | 24.5      | 192.09991   |
| ADORA2A| 404       | 12     | 22.83333  | 76.40726    |
| AGT    | 392       | 11     | 22.33333  | 53.54971    |
| PDE5A  | 386       | 9      | 20.83333  | 28.23189    |
| EDN1   | 296       | 15     | 24.33333  | 169.122     |
| HTR2B  | 242       | 7      | 19.16667  | 11.27673    |
| PTGS1  | 176       | 13     | 23.5      | 176.15779   |
| MAOB   | 132       | 9      | 20.25     | 44.90227    |
| BMP2   | 38        | 10     | 21.33333  | 81.15432    |
| ENPP1  | 34        | 7      | 20        | 33.68954    |
has been reported that arterial dilation in obese women with GDM is attenuated, which is likely due to both endothelial and smooth muscle dysfunction [44]. Thus, the regulation of 5-HT and vascular smooth muscle contraction may be promising therapeutic strategies for GDM. According to a recent network pharmacology analysis [45], LWDHW shows better effects on the function of neuroendocrine immunity, from a holistic point of view, than donepezil, memantine, and melatonin. Nevertheless, the correlation between the neuroactive ligand–receptor interaction pathway and GDM requires further investigation.

Additionally, molecular docking further verified the possible combinations of compounds and target hub genes (Figure 8). According to our analysis, mairin, which is the main component of Mu Dan Pi, was the most active compound with the highest binding affinity, for the target ADORA2A. Ample evidence has proven that Mu Dan Pi has a wide range of functions, such as antioxidant, antidiabetic, antitumor, and antioxidant effects [46]. In a study of type 2 diabetes mellitus, the active compounds from Mu Dan Pi were shown to activate the AMPK pathway and reverse metabolic abnormalities [47]. Consistently, we also found that mairin in Mu Dan Pi may be a promising component due to its affinity for GDM-related targets. ADORA2A encodes the adenosine receptor A2a. ADORA2A-mediated

**Table 3: List of the best models for molecular docking.**

| Herb name   | Active ingredient | Target gene  | Affinity |
|-------------|-------------------|--------------|----------|
| Mu Danpi    | Mairin            | ADORA2A      | -8.6     |
| Mu Danpi    | (+)-Catechin      | CNR1         | -5.9     |
| Shan Zhuyu  | Aristolone        | ADORA2A      | -7.6     |
| Shan Zhuyu  | Aristolone        | AGT          | -3.7     |
Figure 8: Molecular docking models of LWDHW binding to the proteins encoded by the top three hub target genes. (a) ADORA2A, (b) CNR1, (c) AGT, (d) catechin, (e) aristolone, (f) mairin, (g) ADORA2A and aristolone, (h) ADORA2A and mairin, (i) AGT and aristolone, (j) CNR1 and catechin, (k) ADORA2A and aristolone, (l) ADORA2A and mairin, (m) AGT and aristolone, and (n) CNR1 and catechin. Hydrogen bonded atoms in the receptor or atoms in close contact with atoms in the ligand are shown as spheres, and fragments of the secondary structure are shown as sequences of three or more residues interacting with the ligand in the receptor.
signaling results in the breakdown of the blood–brain barrier, which is induced by obesity. The mechanism implicates cerebrovascular dysfunction in diabetes [48]. A recent study has indicated that adenosine signaling via ADORA2A is increased in compensation of β cell proliferation, which could strengthen the expansion of β cells pharmacologically [49]. Taken together, the ADORA2A/mairin combination could be a promising therapeutic strategy for diabetes in future.

Our study has yielded marked insights into the molecular mechanisms by which LWDHW exerts therapeutic effects in GDM. Nevertheless, the current study had some limitations. First, the one-sided results of single microarray analysis may lead to a high false positive rate; thus, it is necessary to improve the power of detection by integrating the results of analysis of multiple datasets. Second, although bioinformatics analysis is a powerful tool for the identification of potential targets in GDM and to improve mechanistic understanding, the therapeutic effect and potential mechanisms of active components still need to be validated in further experimental research. Third, to clarify whether LWDHW can be beneficial to maternal and neonatal outcomes, the safety of this drug should be evaluated.

5. Conclusions

In summary, our research elucidated the potential active ingredients and multiple target mechanisms of LWDHW in the treatment of GDM, which have not been published previously and which provide scientific evidence for clinical practice and application of LWDHW for further research. Our results clarified the potential therapeutic mechanism of the compound and verified this with molecular docking. The key targets, pathways, and ingredients identified here form a basis for further research and facilitate herbal medicine use for the treatment of GDM in future. However, due to the limitations of network pharmacology analysis, it needs to be established whether the active components themselves could be used in GDM treatment in future. Additionally, more clinical research and molecular experiments are necessary to validate our current findings.

Data Availability

The dataset (GSE1546) applied for this study can be found in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/). The data involved in this study are available from the corresponding author by request.

Conflicts of Interest

The authors declare they have no conflict of interest.

Authors’ Contributions

Yunqi Xiong and Qiutong Li contributed equally to this work.

Acknowledgments

This study was supported by the Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine through Si Ming Youth Fund (grant number: SGJ-202012) and Natural Science Foundation of Shanghai (No. 15401931400).

Supplementary Materials

Supplementary Table S1: active ingredients of LWDHW screened out from TCM SP. Supplementary Table S2: active ingredients and corresponding targets. (Supplementary Materials)

References

[1] D. R. McCance, “Gestational diabetes mellitus,” in The Evidence Base for Diabetes Care, pp. 243–284, Wiley, 2002.
[2] D. R. Coustan, “Gestational diabetes mellitus,” Clinical Chemistry, vol. 59, no. 9, pp. 1310–1321, 2013.
[3] C. Gao, X. Sun, L. Lu, F. Liu, and J. Yuan, “Prevalence of gestational diabetes mellitus in mainland China: a systematic review and meta-analysis,” Journal of Diabetes Investigation, vol. 10, no. 1, pp. 154–162, 2019.
[4] J. K. L. Mak, A. H. Lee, N. M. Pham et al., “Gestational diabetes incidence and delivery outcomes in Western China: a prospective cohort study,” Birth, vol. 46, no. 1, pp. 166–172, 2019.
[5] J. Juan and H. Yang, “Prevalence, prevention, and lifestyle intervention of gestational diabetes mellitus in China,” International Journal of Environmental Research and Public Health, vol. 17, no. 24, 2020.
[6] J. B. O’Sullivan, S. S. Gellis, B. O. Tenney, “The potential diabetic and her treatment in pregnancy,” Obstetrics and Gynecology, vol. 102, no. 1, p. 7, 2003.
[7] P. Damm, “Future risk of diabetes in woman and child after gestational diabetes mellitus,” International Journal of Gynaecology and Obstetrics, vol. 104, p. S25, 2009.
[8] T. A. Buchanan, A. H. Xiang, and K. A. Page, “Gestational diabetes mellitus: risks and management during and after pregnancy,” Nature Reviews. Endocrinology, vol. 8, no. 11, pp. 639–649, 2012.
[9] K. Vince, P. Perkovic, and R. Matijevic, “What is known and what remains unresolved regarding gestational diabetes mellitus (GDM),” Journal of Perinatal Medicine, vol. 48, no. 8, pp. 757–763, 2020.
[10] E. Kintiraki and D. G. Goulis, “Gestational diabetes mellitus: multi-disciplinary treatment approaches,” Metabolism, vol. 86, p. 91, 2018.
[11] R. Zhang, B. Xing, J. Zhao et al., “Astragaloside IV relieves gestational diabetes mellitus in genetic mice through reducing hepatic gluconeogenesis,” Canadian Journal of Physiology and Pharmacology, vol. 98, no. 7, pp. 466–472, 2020.
[12] Y. Li, B. Duan, Y. Li, S. Yu, and Y. Wang, “The isoflavonoid calycosin inhibits inflammation and enhances beta cell function in gestational diabetes mellitus by suppressing RNF38 expression,” Immunopharmacology and Immunotoxicology, vol. 42, no. 4, pp. 366–372, 2020.
[13] C. Y. Huang, Y. T. Tsai, J. N. Lai, and F. L. Hsu, “Prescription pattern of chinese herbal products for diabetes mellitus in taiwan: a population-based study,” Evidence-Based
Y. C. Ma, C. C. Lin, C. I. Li, J. H. Chiang, T. C. Li, and J. G. Lin,

Journal of Diabetes Research, vol. 12, Article ID 714805, 7 pages, 2012.

W. Limopasmanee, S. Chansakaow, N. Rojanasthien, M. Manorot, C. Sangdee, and S. Teekachunhatean, “Effects of the Chinese herbal formulation (Liu Wei Di Huang Wan) on the pharmacokinetics of isoflavones in postmenopausal women,” BioMed Research International, vol. 2015, Article ID 902702, 8 pages, 2015.

Y. C. Ma, C. C. Lin, C. I. Li, J. H. Chiang, T. C. Li, and J. G. Lin, “Traditional Chinese medicine therapy improves the survival of systemic lupus erythematosus patients,” Seminars in Arthritis and Rheumatism, vol. 45, no. 5, pp. 596–603, 2016.

A. L. Hopkins, “Network pharmacology,” Nature Biotechnology, vol. 25, no. 10, pp. 1110-1111, 2007.

S. Li and B. Zhang, “Traditional Chinese medicine network pharmacology: theory, methodology and application,” Chinese Journal of Natural Medicines, vol. 11, no. 2, pp. 110–120, 2013.

C. Hao da and P. G. Xiao, “Network pharmacology: a Rosetta stone for traditional Chinese medicine,” Drug Development Research, vol. 75, no. 5, pp. 299–312, 2014.

H. Yuan, Q. Ma, H. Cui et al., “How can synergism of traditional medicines benefit from network pharmacology?,” Molecules, vol. 22, no. 7, p. 1135, 2017.

T. Barrett, S. E. Wilhite, P. Ledoux et al., “NCBI GEO: archive for functional genomics data sets–update,” Nucleic Acids Research, vol. 41, no. D1, pp. D991–D995, 2012.

J. Ru, P. Li, J. Wang et al., “TCMSP: a database of systems pharmacology for drug discovery from herbal medicines,” Journal of Cheminformatics, vol. 6, no. 1, 2014.

Z. Liu, F. Guo, Y. Wang et al., “BAMMAN-TCM: a bioinformatics analysis tool for molecular mechanism of traditional Chinese medicine,” Scientific Reports, vol. 6, no. 1, 2016.

A. Colaprico, T. C. Silva, C. Olsen et al., “TCGAAbiols: an R/Bioconductor package for integrative analysis of TCGA data,” Nucleic Acids Research, vol. 44, no. 8, article e71, 2016.

M. E. Ritchie, B. Hippon, D. Wu et al., “limma powers differential expression analyses for RNA-sequencing and microarray studies,” Nucleic Acids Research, vol. 43, no. 7, article e47, 2015.

M. Ashburner, C. A. Ball, J. A. Blake et al., “Gene Ontology: tool for the unification of biology,” Nature Genetics, vol. 25, no. 1, pp. 25–29, 2000.

M. Kanehisa and S. Goto, “KEGG: Kyoto Encyclopedia of Genes and Genomes,” Nucleic Acids Research, vol. 28, no. 1, pp. 27–30, 2000.

G. Yu, L. G. Wang, Y. Han, and Q. Y. He, “clusterProfiler: an R package for comparing biological themes among gene clusters,” OMICS, vol. 16, no. 5, pp. 284–287, 2012.

P. Ertl, B. Rohde, and P. Selzer, “Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties,” Journal of Medicinal Chemistry, vol. 43, no. 20, pp. 3714–3717, 2000.

F. Yamashita and M. Hashida, “In silico approaches for predicting ADME properties of drugs,” Drug Metabolism and Pharmacokinetics, vol. 19, no. 5, pp. 327–338, 2004.

D. Szklarczyk, A. Franceschini, S. Wyder et al., “STRING v10: protein-protein interaction networks, integrated over the tree of life,” Nucleic Acids Research, vol. 43, no. D1, pp. D447–D452, 2015.

P. Shannon, A. Markiel, O. Ozier et al., “Cytoscape: a software environment for integrated models of biomolecular interaction networks,” Genome Research, vol. 13, no. 11, pp. 2498–2504, 2003.

C. H. Chin, S. H. Chen, H. H. Wu, C. W. Ho, M. T. Ko, and C. Y. Lin, “cytoHubba: identifying hub objects and subnetworks from complex interactome,” BMC Systems Biology, vol. 8, no. S4, 2014.

O. Trott and A. J. Olson, “AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading,” Journal of Computational Chemistry, vol. 31, no. 2, pp. 455–461, 2010.

D. Simmons, “GDM and nutrition-answered and unanswered questions—there’s more work to do!,” Nutrients, vol. 11, no. 8, p. 1940, 2019.

P. C. Hsu, Y. T. Tsai, J. N. Lai, C. T. Wu, S. K. Lin, and C. Y. Huang, “Integrating traditional Chinese medicine healthcare into diabetes care by reducing the risk of developing kidney failure among type 2 diabetic patients: a population-based case control study,” Journal of Ethnopharmacology, vol. 156, p. 358, 2014.

J.-h. Huang, D. He, L. Chen et al., “A GC-MS-based metabolomics investigation of the protective effect of Liu-Wei-Di-Huang-Wan in type 2 diabetes mellitus mice,” International Journal Of Analytical Chemistry, vol. 2020, Article ID 1306439, 9 pages, 2020.

H.-H. Chen, C.-T. Wu, Y.-T. Tsai, C.-W. Ho, M.-C. Hsieh, and J.-N. Lai, “Liu Wei Di Huang Wan and the delay of insulin use in patients with type 2 diabetes in Taiwan: a nationwide study,” Evidence-Based Complementary and Alternative Medicine, vol. 2021, Article ID 1298487, 8 pages, 2021.

Z. Qiu, J. Dong, C. Xue et al., “Liwei Dihuang pills alleviate the polycystic ovary syndrome with improved insulin sensitivity through PI3K/Akt signaling pathway,” Journal of Ethnopharmacology, vol. 250, article 111965, 2020.

B. Dai, Q. Wu, C. Zeng et al., “The effect of Liwei Dihuang decoction on PI3K/Akt signaling pathway in liver of type 2 diabetes mellitus (T2DM) rats with insulin resistance,” Journal of Ethnopharmacology, vol. 192, p. 382, 2016.

M. Vlaiu, J. Lafond, and C. Vaillancourt, “Expression of placental serotonin transporter and 5-HT2A receptor in normal and gestational diabetes mellitus pregnancies,” Reproductive BioMedicine Online, vol. 19, no. 2, pp. 207–215, 2009.

M. Leitner, L. Fragner, S. Danner et al., “Combined metabolomic analysis of plasma and urine reveals AHBA, tryptophan and serotonin metabolism as potential risk factors in gestational diabetes mellitus (GDM),” Frontiers in Molecular Biosciences, vol. 4, 2017.

Y. Li, C. Hadden, P. Singh et al., “GDM-associated insulin deficiency hinders the dissociation of SERT from ERp44 and down-regulates placental 5-HT uptake,” Proceedings of the National Academy of Sciences of the United States of America, vol. 111, no. 52, pp. E5697–E5705, 2014.

S. Brewster, B. Zinman, R. Retnakaran, and J. S. Floras, “Cardiometabolic consequences of gestational dysglycemia,”
Journal of the American College of Cardiology, vol. 62, no. 8, pp. 677–684, 2013.

[45] J. Zeng, X. Zhang, J. Wang, X. Cheng, Y. Zhang, and W. Zhou, “Comparison of donepezil, memantine, melatonin, and Liuwei Dihuang decoction on behavioral and immune endocrine responses of aged senescence-accelerated mouse resistant 1 mice,” Frontiers in Pharmacology, vol. 11, 2020.

[46] Z. Wang, C. He, Y. Peng, F. Chen, and P. Xiao, “Origins, phytochemistry, pharmacology, analytical methods and safety of Cortex Moutan (Paeonia suffruticosa Andrew): a systematic review,” Molecules, vol. 22, no. 6, 2017.

[47] D. T. Ha, T. N. Trung, T. T. Hien et al., “Selected compounds derived from Moutan Cortex stimulated glucose uptake and glycogen synthesis via AMPK activation in human HepG2 cells,” Journal of Ethnopharmacology, vol. 131, no. 2, pp. 417–424, 2010.

[48] M. Yamamoto, D. H. Guo, C. M. Hernandez, and A. M. Stranahan, “Endothelial Adora2a activation promotes blood-brain barrier breakdown and cognitive impairment in mice with diet-induced insulin resistance,” The Journal of Neuroscience, vol. 39, no. 21, pp. 4179–4192, 2019.

[49] N. Schulz, K. C. Liu, J. Charbord et al., “Critical role for adenosine receptor A2a in β-cell proliferation,” Molecular Metabolism, vol. 5, no. 11, pp. 1138–1146, 2016.