Network pharmacology-based strategy to investigate pharmacological mechanism of Liuwei Dihuang Pill against postmenopausal osteoporosis

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Abstract
Postmenopausal osteoporosis (PMOP) has become 1 of most prevalent bone disorders with aging population. Liuwei Dihuang (LWDH) Pill, a classical kidney-tonifying prescription, is extensively used to treat PMOP in China. The aim of this study is to explore the pharmacological mechanisms of LWDH Pill against PMOP via network pharmacological strategy. The active ingredients of LWDH Pill were screened out from the Traditional Chinese Medicine System Pharmacology, Encyclopedia of Traditional Chinese Medicine and Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine Databases, and their related target genes were fished in the UniProt database. Simultaneously, the GeneCards and DisGeNET databases were used to identify the target genes of PMOP. Through establishing a protein-protein interaction network, the overlapping genes between LWDH Pill and PMOP were identified to analyze their interactions and the hub target genes. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed to predict the underlying biological processes (BP) and signaling pathways, respectively. A total of 64 active ingredients and 653 related target genes were identified in LWDH Pill, and 292 target genes were closely associated with PMOP. After matching the target genes between LWDH Pill and PMOP, 84 overlapping targets were obtained and considered as therapeutically relevant. Through construction of a protein-protein interaction network, we identified 20 hub target genes including IL6, INS, tumor necrosis factor, AKT1, vascular endothelial growth factor A, IGF1, TP53, IL1B, MMP9, JUN, LEP, CTRNBN1, EGF, PTGS2, PPARC, CXCL8, IL10, CCL2, FOS and ESR1. Gene Ontology enrichment analysis suggested that LWDH Pill exerted anti-PMOP effects via regulating multiple BP including cell proliferation and apoptosis, oxidative stress, inflammation and angiogenesis. Kyoto Encyclopedia of Genes and Genomes enrichment analysis revealed several pathways, such as PI3K-AKT pathway, mitogen-activated protein kinase pathway, hypoxia-inducible factors-1 pathway, tumor necrosis factor pathway, interleukin-17 (IL-17) pathway and FoxO pathway that might be involved in modulating the above BP. Through network pharmacological approach, we investigated the potential therapeutic mechanism of LWDH Pill against postmenopausal osteoporosis in a systemic perspective. These identified multi-targets and multi-pathways provide promising directions for further revealing more exact mechanisms.

Abbreviations: BATMAN-TCM = Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine, BP = biological processes, DL = drug-likeness, EGF = epidermal growth factor, ETCM = Encyclopedia of Traditional Chinese Medicine, HIF-1 = hypoxia-inducible factors-1, IL-1 = interleukin-1, IL-17 = interleukin-17, IL-6 = interleukin-6, KEGG = Kyoto encyclopedia of genes and genomes, LWDH = Liuwei Dihuang, MAPK = mitogen-activated protein kinase, MDP = Mu Dan Pi, GO = gene ontology, OB = Oral bioavailability, OVX = ovarietomized, PMOP = postmenopausal osteoporosis, PPI = protein-protein interaction, SDH = Shu Di Huang, SY = Shan Yao, SZY = Shan Zhu Yu, TCM = Traditional Chinese medicine, TCMSP = Traditional Chinese Medicine System Pharmacology, TFN = tumor necrosis factor, VEGFA = vascular endothelial growth factor A, ZK = Ze Xie.

Keywords: Liuwei Dihuang pill, network pharmacology, pharmacological mechanism, postmenopausal osteoporosis, traditional Chinese medicine

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Postmenopausal osteoporosis (PMOP), 1 of most prevalent bone disorders, is characterized by low bone mineral density, microstructure deterioration and higher risk of fragility fracture.[11] It is estimated that 15% of postmenopausal women more than 50 years old are suffering in PMOP worldwide, which causes a huge medical and economic burden.[2] Currently, there are a variety of anti-osteoporosis drugs, such as estrogen receptor modulators, parathyroid hormone, bisphosphonates and active vitamin D, are applied to improve bone mineral density and alleviate symptoms.[13,14] However, most of them have varying side effects that limit the clinical application and efficacy.[14] Therefore, developing a safe and alternative treatment is largely needed.

Traditional Chinese medicine (TCM), mainly based on natural herbs, has attracted extensive attention due to its satisfactory curative effects and relative safety.[5] Liuwei Dihuang (LWDH) Pill, a classical prescription of tonifying kidney, was initially recorded in Song Dynasty (960-1279 AD). It consists of 6 herbs and have been used to treat PMOP for a long time in China.[6,7] Recent studies demonstrated LWDH Pill could attenuate bone loss in ovariectomized (OVX) animal models,[8,9] confirming its anti-PMOP effects. The theory of “kidney governing bones” can well account for its anti-PMOP effects from TCM perspective. Bone loss in PMOP is due to kidney deficiency and LWDH Pill can tonify kidney to strengthen bone.[6,10] Nevertheless, the exact pharmacological mechanism of LWDH Pill against PMOP is still unknown.

For the multi-components and multi-targets of TCM prescription, conventional animal or cellular research strategies can not meet the requirements to study massive gene targets simultaneously.[11] Network pharmacology is an emerging discipline that integrates pharmacology, system biology, bioinformatics, and computer science.[11-13] According to the study pattern of “Medicine – Target – Gene – Disease,” network pharmacology provides an integrative and systematic viewpoint to investigate the pharmacological mechanism of LWDH Pill.[13,14]

Here, through network pharmacology, we comprehensively reviewed the potential therapeutic targets of LWDH Pill in treating PMOP. These targets were further performed with bioinformatic analyses to determine the key therapeutic target genes, biological processes (BP) and signaling pathways. The protocol of our experimental procedures were shown in Figure 1.

2. Materials and methods

Ethical approval was waived or not necessary, all procedures performed in studies do not involve human participants or animals.

2.1. Identification of active ingredients

LWDH Pill is composed of 6 herbs including Shu Di Huang (SDH), Shan Zhu Yu (SZY), Shan Yao (SY), Mu Dan Pi (MDP), Fu Ling and Ze Xie (ZX) (Table 1). The herb names were inputted into the Traditional Chinese Medicine System Pharmacology (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php), Encyclopedia of Traditional Chinese Medicine (ETCM, http://www.tcmsp.cn/ETCM/) and a Bioinformatics Analysis Tool for Molecular mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/) to obtain the chemical ingredients. Oral bioavailability (OB) is a pharmacokinetic parameter that reflects the efficiency of drug delivery to the systemic circulation. Drug-likeness (DL) is used to assess drug properties including solubility and chemical stability. Oral bioavailability (OB > 30%) and drug-like (DL ≥ 0.18) were set as the screening criteria,[15] and a total of 64 active ingredients were identified in LWDH Pill.

2.2. Target prediction of LWDH pill

Then, we predicted the targets related to the active ingredients from TCMSP database through using the computer targeting technology developed by the TCMSP. The information of targets was further inputted into UniProt database (https://www.uniprot.org/) to obtain their gene symbols with the filter of “swiss-prot reviewed” and “Homo sapiens.” The target genes related to the active ingredients from BATMAN-TCM database and ETCPM database were directly obtained from respective databases.

2.3. Collection of PMOP-related genes

The “postmenopausal osteoporosis” was used as the keywords to retrieve disease-related genes in the GeneCards Database (https://www.genecards.org/) and DisGeNET Database (https://www.disgenet.org/). After filtered by score > 0.1 in DisGeNET and score > 10 in GeneCards,[14] a total of 292 PMOP-related genes were finally obtained.

2.4. Venn diagram

A Venn diagram was constructed on an online website (https://bioinfgp.cnb.csic.es/tools/venny/index.html) to identify the overlapping genes between PMOP and LWDH Pill.[11]

2.5. Protein-protein interaction (PPI)

The overlapping genes were imported into the STRING 11.0 (https://string-db.org/) for PPI analysis with the parameters of “confidence score > 0.4” and “Homo sapiens.” The node-node data were exported from STRING and their interconnection network were visualized and analyzed by using Cytoscape 3.7.1. Degree Centrality, Betweenness Centrality and Closeness Centrality, 3 topological properties of the network, were analyzed using plug-in “Network Analysis.”[11]

2.6. Gene ontology (GO) enrichment analysis

The overlapping genes were imported into the Annotation, Visualization and Integrated Discovery (https://david.ncifcrf.gov/home.jsp, version 6.8) for GO enrichment analysis.[16] Three sub-items including molecular function, cellular component and biological process (BP) were all analyzed. After filtered by FDR < 0.01, the information of top 40 BP terms were listed in a bubble diagram based on the ascending order of log P-value.[11]

2.7. KEGG enrichment analysis

The ID numbers of overlapping genes were imported into Kyoto Encyclopedia of Genes and Genomes (https://www.kegg.jp/) for pathway enrichment analysis. The top 32 signaling pathways were listed in a bar diagram according to the descending order of gene number enriched in each pathway.

2.8. Network construction

We constructed networks as follows: Herb-Ingredient network. Targets-Pathways network. All visualized graphs of above networks were established by Cytoscape 3.8.0 software (https://cytoscape.org/index.html).

3. Results

3.1. Active ingredients of LWDH pill

After screening herb names in the TCMSP databases with the thresholds of OB ≥ 30% and DL ≥ 0.18, a total of 64 active ingredients were identified in LWDH Pill, including 20...
ingredients in SZY, 16 in SY, 14 in MDP, 12 in FL, 8 in ZX and 2 in SDH. Then, we constructed a Herb-Ingredient network to visually present the relationship between the herbs and the active ingredients. As shown in Figure 2, beta-sitosterol (DL = 0.75, OB = 36.91) showed the highest frequency, and was contained in SZY, SY, ZX, MDP and SDH; stigmasterol (DL = 0.76, OB = 43.83) listed at second place was found in SZY, SY and SDH.

3.2. Target prediction and analysis

Then, we fished a total of 653 target genes on the 64 related ingredients using the Uniprot database, BATMAN-TCM database and ETCM database. Simultaneously, we integrated the PMOP related genes from GeneCards and DisGeNet databases, and 292 target genes were obtained. After the establishment of Venn diagram, 84 overlapping genes were identified between LWDH Pill and PMOP (Fig. 3).

3.3. PPI network and hub therapeutic targets

Next, these 84 overlapping genes were imported into the String database to build a PPI network, 84 nodes and 1458 edges were obtained. We plotted the PPI network according to the strength of evidence and confidence level (Fig. 4A and 4B), respectively. “Degree Centrality” and “Closeness Centrality” represent the
number of edge and the mean distance between target node and others, respectively.\textsuperscript{[17,18]} “Betweenness Centrality” is used to measure code centrality based on the shortest paths.\textsuperscript{[19]} These 3 parameters were used to select the pivot nodes. After the first screening round of Degree $\geq$ 20, Betweenness $\geq$ 0.003 and Closeness $\geq$ 0.57, 42 nodes and 710 edges were obtained. Through the second screening round of Degree $\geq$ 40, Betweenness $\geq$ 0.007 and closeness $\geq$ 0.702, only 20 nodes and 178 edges were left (Fig. 4C). Therefore, these remained genes, IL6, INS, tumor necrosis factor (TNF), AKT1, vascular endothelial growth factor A (VEGFA), IGF1, TP53, IL1B, MMP9, JUN, LEP, CTNNB1, EGF, PTGS2, PPARG, CXCL8, IL10, CCL2, FOS and ESR1 were considered as the hub therapeutic targets of LWDH Pill against PMOP. Their specific information were listed in Table 2.

3.4. GO biological process enrichment analysis

GO enrichment analysis was performed on these 84 overlapping genes by using the Annotation, Visualization and Integrated Discovery database. Based on the filter of FDR < 0.01, a total of 240 GO items were obtained, including 196 BP terms, 13 cellular component terms and 31 molecular function terms (Fig. 5A). As BP played a dominant role, we further build a bubble diagram for the top 40 BP terms (Fig. 5B). The diagram showed that these 40 BP terms were mainly summarized into 5 categories, including inflammation, oxidative stress, angiogenesis, cell proliferation and cell apoptosis. The category of cell proliferation included positive regulation of gene expression (GO:0010628), positive regulation of transcription DNA-templated (GO:0045893), negative regulation of gene expression (GO:0010629), positive regulation of transcription from RNA polymerase II promoter (GO:0045944), negative regulation of cell proliferation (GO:0008285) and positive regulation of cell proliferation (GO:0008284). There are 5 BP terms in the category of inflammation, including response to lipopolysaccharide (GO:0032496), inflammatory response (GO:0006954), lipopolysaccharide-mediated signaling pathway (GO:0031663), response to glucocorticoid (GO:0051384) and immune response (GO:0006955). Regarding to oxidative stress, there are response to hypoxia (GO:0001666), positive regulation of nitric oxide biosynthetic process (GO:0045429), positive regulation of nitric-oxide synthase activity (GO:0051000) and

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**Table 1**

The compositions of LWDH Pill.

| Chinese name (Abbreviation) | Botanical name | Common name | Parts | Proportion |
|----------------------------|----------------|-------------|-------|------------|
| Shu Di Huang (SDH)         | Rehmannia glutinosa (Gaertn.) | Medical Dogwood | Root | 32%        |
| Shan Zhu Yu (SZY)          | Cornus officinalis (Sieb.) | Tree Peony Bark | Fruit | 16%        |
| Mu Dan Pi (MDP)            | Paeonia suffruticosa (Andr.) | Common Yarn | Root | 12%        |
| Shao Yao (SY)              | Dioscoreae oppositifolia (Thunb.) | Tuckahoe | Root | 16%        |
| Fu Ling (FL)               | Poria cocos (Schw.) | Sclerotia | Tuber | 12%        |
| Ze Xie (ZX)                | Alisma orientale (Sam.) | Oriental Waterplantain Rhizome | Tuber | 12%        |

LWDH = Liuwei Dihuang.

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**Figure 2.** The network of Herb-Ingredient connection. The red square nodes represent herbs in LWDH Pill, and the blue circles represent active ingredients. The edges represent the direct relationship between them. LWDH = Liuwei Dihuang.
cellular response to hypoxia (GO:0071456). In the aspect of angiogenesis, there were angiogenesis (GO:0045766) and positive regulation of angiogenesis (GO:0045766) and positive regulation of endothelial cell proliferation (GO:0001938). The category of cell apoptosis included positive regulation of apoptotic process (GO:0043065) and negative regulation of apoptotic process (GO:0043066). These findings indicated that LWDH Pill exerted the anti-PMOP effects possibly through a multi-biological process synergetic way.

3.5. KEGG enrichment analysis

To further reveal the potential signaling pathways of LWDH Pill for PMOP treatment, we conducted KEGG enrichment analysis on the 84 overlapping genes. Based on the threshold of gene number ≥ 12, we screened out 32 signaling pathways (Fig. 6A), among which tumor necrosis factor (TNF) signaling pathway (hsa04668) and interleukin-17 (IL-17) signaling pathway (hsa04657) directly regulate inflammation; PI3K-Akt signaling pathway (hsa04151) and mitogen-activated protein kinase (MAPK) signaling pathway (hsa04068) plays an important role in oxidative stress; hypoxia-inducible factors-1 (HIF-1) signaling pathway (hsa04066) controls angiogenesis. Furthermore, a Targets-Pathways network was conducted to present the hub targets enriched in the above pathways (Fig. 6B).

4. Discussion

PMOP has become a public health disease worldwide with aging progression. The side effects caused by the long-term use of anti-osteoporosis drugs are calling for a more effective and safer treatment strategy. LWDH Pill has shown anti-osteoporosis effects both in animal model and PMOP patients. In this study, we comprehensively explored the potential mechanisms of LWDH Pill through network pharmacology approach.

Based on the Herb-Ingredient network, we identified 2 key ingredients of LWDH Pill: beta-sitosterol and stigmasterol. Through a literature search, beta-sitosterol is found to alleviate ovariectomy-induced osteoporosis through promoting osteoblast proliferation and osteogenic activity. Stigmasterol has an inhibition effect on osteoclastogenesis through anti-inflammation. These active ingredients are the material foundations for the anti-PMOP effects of LWDH Pill.

After collecting the overlapping genes between LWDH Pill and PMOP in the Venn diagram, we identified a total of 84 potential therapeutic targets. PPI topological screening with Degree Centrality, Closeness Centrality and Betweenness Centrality further revealed 20 hub targets that were regarded as key molecular targets of LWDH Pill for PMOP treatment. According to the findings in GO and KEGG enrichment analyses on the 84 overlapping genes, we speculated that LWDH Pill treating PMOP was possibly associated with the regulation of several BP (cell proliferation, cell apoptosis, inflammation, oxidative stress and angiogenesis) and signaling pathways (TNF signaling pathway, IL-17 signaling pathway, FoxO signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, and HIF-1 signaling pathway).

4.1. Cell proliferation and apoptosis

Bone homeostasis requires dynamic balance between bone formation and bone resorption. Osteoporosis occurs when osteoblast-mediated bone formation is slower than bone resorption. Thus, the abnormal proliferation and apoptosis of osteoblasts and osteoclasts directly result in osteoporosis. Epidermal growth factor (EGF), 1 of 20 hub overlapping
targets, can activate PI3K-AKT pathway to promote osteoblast proliferation for osteogenesis.\[31\] It is further reported that OVX-induced osteoporosis in rats can be treated through promoting PI3K-AKT pathway-mediated bone formation.\[32\] In contrast, MAPK signaling pathway contains 10 hub overlapping target genes (EGF, AKT1, FOS, IGF1, IL1B, INS, JUN, TNF, TP53 and VEGFA) and regulates osteoclastic bone resorption.\[33\] The down-regulation of MAPK signaling can attenuate postmenopausal bone loss by inhibiting osteoclast proliferation.\[34\] Therefore, it is reasonable to believe, cell proliferation regulated by PI3K-AKT pathway and MAPK pathway is a part mechanism of LWDH Pill against PMOP.

The balance between osteoblast proliferation and apoptosis affects bone formation homeostasis, thereby osteoblast over apoptosis is a critical pathological mechanism of PMOP. It has been reported that inhibition of PI3K/AKT signaling pathway could promote osteoblast apoptosis in the OVX mice.\[35\] The Targets-Pathways network revealed 7 hub targets (EGF, AKT1, IGF1, IL6, INS, TP53 and VEGFA) enriched in PI3K/AKT signaling pathway, indicating that PI3K/AKT signaling inhibiting osteoblast apoptosis is involved in the therapeutic mechanism of LWDH Pill. IL-1A and IL1B were overlapping targets between LWDH Pill and PMOP, and were involved in MAPK signaling pathway. Previous study revealed that IL-1 could induce osteoblast apoptosis through activating MAPK pathways.\[36\] Thus, MAPK signaling pathway may be potential pharmacological mechanism when LWDH Pill treats PMOP.

### 4.2. Inflammation

Inflammation is 1 of most important pathological factors that contributes to the occurrence and development of PMOP. Most

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#### Table 2

| Uniprot ID | Gene Symbol | Description                  | Degree |
|------------|-------------|-------------------------------|--------|
| P05231     | IL6         | Interleukin-6                  | 72     |
| P01308     | INS         | Insulin                        | 72     |
| P01375     | TNF         | Tumor necrosis factor          | 70     |
| P31749     | AKT1        | RAC-alpha serine/threonine-protein kinase | 65     |
| P15692     | VEGFA       | Vascular endothelial growth factor A | 64     |
| P05019     | IGF1        | Insulin-like growth factor 1   | 60     |
| P04637     | TP53        | Cellular tumor antigen p53    | 60     |
| P01584     | IL1B        | Interleukin-1 beta             | 59     |
| P14780     | MMP9        | Matrix metalloproteinase-9     | 58     |
| P50512     | JUN         | Transcription factor AP-1      | 57     |
| P41159     | LEP         | Leptin                         | 57     |
| P35222     | CTNNB1      | Catenin beta-1                 | 57     |
| P01133     | EGFR        | Pro-epidermal growth factor    | 56     |
| P35354     | PTGS2       | Prostaglandin GH synthase 2    | 55     |
| P37231     | PPARG       | Peroxisome proliferator-activated receptor gamma | 56 |
| P10145     | CXCL8       | Interleukin-8                   | 53     |
| P22301     | IL10        | Interleukin-10                  | 52     |
| P13500     | CCL2        | C-C motif chemokine 2          | 51     |
| P01100     | FOS         | Protein c-Fos                   | 50     |
| P03372     | ESR1        | Estrogen receptor               | 50     |

Figure 5. GO enrichment analysis of the 84 overlapping genes. (A) Three types of GO items in DAVID database (Blue, GOTRIM_BP: biological process. Yellow, GOTRIM_CC: cellular component. Gray, GOTRIM_MF: molecular function). (B) The bubble diagram of the top 40 BP items. DAVID = the Annotation, Visualization and Integrated Discovery.
of pro-inflammatory cytokines can directly influence bone resorption though regulating osteoclast activity.\textsuperscript{[17]} TNF synthesized by T-lymphocytes can accelerate bone loss through 2 mechanisms which are promoting osteoclastogenesis and inhibiting apoptosis of osteoclasts.\textsuperscript{[15]} Interleukin-1 (IL-1), a multifunctional cytokine, is considered as “osteoclast activating factor.”\textsuperscript{[19]} It signals on osteoclast lineage cells to stimulate osteoclastogenesis and resorptive capacity in receptor activator of nuclear factors $\kappa B$ ligand-dependent way.\textsuperscript{[40]} IL-1 can also interact with TNF to protect osteoclast apoptosis. Interleukin-6 (IL-6) is highly increased after estrogen loss\textsuperscript{[41]} and closely related to low bone mineral density.\textsuperscript{[42]} IL-6 stimulates bone resorption by activating receptor activator of nuclear factors $\kappa B$ ligand/osteoprotegerin pathway.\textsuperscript{[43]} Based on hub Target-Pathway network, TNF, IL1B and IL-6 are enriched in TNF signaling pathway and IL-17 signaling pathway. Hence, the anti-PMOP effects of LWDH Pill may possibly be associated with the inhibition of inflammation-induced bone resorption.

4.3. Oxidative stress

Oxidative stress is associated with PMOP, as excessive reactive oxygen species are able to exert oxidative damage to mitochondria and DNA in osteoblast and osteoclast.\textsuperscript{[44]} Currently, inhibition of oxidative stress is regarded as a therapeutic target for treating PMOP. Many studies have revealed the regulating effects of FoxO signaling pathway on oxidative stress. FoxO1 was found to promote osteoblast proliferation by resistance to oxidative stress.\textsuperscript{[45]} Loss function of FoxO signaling increased osteoclast-mediated bone resorption by releasing $H_2O_2$.\textsuperscript{[46]} There are 12 hub targets (EGF, AKT1, IGF1, FASLG, IL6, INS, MAPK1 and PTEN) were enriched, indicating that inhibition of oxidative stress regulated by FoxO signaling pathway is an important mechanism of LWDH Pill against PMOP.

4.4. Angiogenesis

Bone is a highly vascularized tissue with a wide network of capillaries and blood vessels. New bone formation needs a spatial and temporal involvement of vascularization to provide essential nutrients, oxygen and growth factors.\textsuperscript{[47]} More remarkably, angiogenesis directly promotes bone formation, which is named angiogenesis-osteogenesis coupling.\textsuperscript{[48,49]} VEGF, a major driver of angiogenesis, can activate a series of well-orchestral vascularization processes including endothelial cell proliferation, migration, sprouting vessel pruning and anastomosis.\textsuperscript{[50]} HIF-1$\alpha$ is a transcription factor that can bind to VEGF gene promoter and induce VEGF gene expression.\textsuperscript{[51]} KEGG enrichment analysis and Targets-Pathways network revealed that VEGFA and HIF-1 pathway were both identified in the anti-PMOP event of LWDH Pill, and VEGFA was contained in HIF-1 pathway. Thus, promotion of angiogenesis regulated by HIF-1 pathway may be a potential mechanism of LWDH Pill in treatment of PMOP.

4.5. Limitations

There are several limitations in the present study. First and foremost, network pharmacology is a discipline of calculation and prediction, which is bound to have some false positives in ingredients, BP and signaling pathways. Secondly, we screened 3 databases to obtain active ingredients and 2 databases for disease-related targets, while there still might leave out some of ingredients and targets. Finally, in vivo and in vitro experiments need to be performed further to verify these predicted BP and signaling pathways.

5. Conclusion

By utilizing network pharmacology, we explored the potential therapeutic targets of LWDH Pill, and investigated its underlying mechanisms against PMOP. As we discovered, it might be based on 3 key BP: regulation of cell proliferation and apoptosis, promotion of angiogenesis, inhibition of inflammation and oxidative stress. KEGG pathway analysis revealed the pathways involved in anti-PMOP event of LWDH Pill as follows: TNF signaling pathway, IL-17 signaling pathway, FoxO signaling pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, and HIF-1 signaling pathway. Therefore, it can be concluded that the pharmacological mechanism of LWDH Pill against PMOP is a direct or indirect synergy way of multi-targets and multi-pathways. Although it still needs further experimental validation to determine their exact pharmacological mechanism, our results provide promising directions for future research.
All our main data used to support the findings of this study have been deposited by the corresponding author.

Author contributions

Conceptualization: Qingchan Wang, Chenjie Xia, Danqing Fu. Data curation: Qingchan Wang, Chenjie Xia, Danqing Fu. Formal analysis: Ping Huang, Danqing Fu. Funding acquisition: Chenjie Xia, Danqing Fu. Methodology: Qingchan Wang, Ping Huang, Danqing Fu. Project administration: Qingchan Wang, Ping Huang, Danqing Fu. Software: Qingchan Wang, Ping Huang, Chenjie Xia. Supervision: Ping Huang. Validation: Qingchan Wang, Ping Huang, Chenjie Xia. Visualization: Qingchan Wang, Chenjie Xia, Danqing Fu. Writing – original draft: Chenjie Xia. Writing – review & editing: Qingchan Wang, Chenjie Xia, Danqing Fu.

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