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Original Article

Study on the mechanism of Huangqi Guizhi Wuwu Decoction in treating diabetes peripheral neuropathy based on network pharmacology and molecular docking

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Abstract

This study aimed to explore the effective substances and mechanism network of Huangqi Guizhi Wuwu Decoction in treating diabetes peripheral neuropathy. Based on the TCM systemic pharmacological analysis platform (TCMP) and UniProt database, the database of active Huqarqu Decoction was constructed, and the related targets of diabetic peripheral neuropathy were collected through the OMIM, CTD, DisGeNET, TTD and GeneCards databases. The intersection targets were obtained to construct the network diagram of Huangqi dis Guizhi Wuwu Decoction-Active Through the String database, the interaction between target proteins was analyzed, and molecular docking between active components and potential targets was carried out. Combined with the DAVID v6.8 database, GO function analysis and KEGG pathway analysis were performed on the targets. Guizhi Wuwu Decoction mainly acts on core targets such as IL6, MAPK3, VE GFA, JUN and ESR1 through quercetin, kaempferol and naringin and regulates the TNF signaling pathway, estrogen signaling pathway and MAPK signaling pathway, thus achieving the effect of treating diabetes peripheral neuropathy. Huangqi Guizhi Wu has multiple targets and regulates multiple signaling pathways in neuropathy, which lays a foundation for future pharmacological research.

Keywords: Huangqi Guizhi Wuwu Decoction; Diabetic peripheral neuropathy; Network pharmacology; Molecular docking

1. Introduction

Diabetic perpheral neropathy (DPN) is more common in the lower extremities, and the main clinical manifestations are numbness, pain, burning, electric shock sensation, and sensory and movement disorders in the distal extremities. It is one of the common complications of diabetes and belongs to the TCM category [1-3]. For the categories of “blood impediment” and “arthralgia”, at present, clinical Chinese and Western medicines mostly use insulin and calcium antagonists to relieve symptoms and pain in patients by lowering blood glucose and anticoagulation and improving metabolism. Injection Danshen injection, oral administration of traditional Chinese medicine for promoting blood circulation, foot bath fumigation and washing, and acupuncture and moxibustion.

Huangqi Guizhi Wuwu Decoction is compiled from “Synopsis of the Golden Chamber”, and it is composed of five Chinese medicines, i.e., astragalus, peony, jujube, ginger, and cinnamon sticks, and has the effects of harmonizing and replenishing qi and unblocking yang, which has been reported in recent years [4,5]. The treatment effect of DPN is significant [6]. Some scholars in China used meta-analysis to include 22 RCTs involving a total of 1,450 patients and confirmed that Huangqi Guizhi Wuwu Decoction was safe and effective in the treatment of DPN. The pathogenesis of DPN is still unclear. In addition, Huangqi Guizhi Wuwu Decoction has many and complicated components, and the pharmacological substances and pharmacological mechanisms of action remain to be studied further.

Network pharmacology is an emerging discipline that applies bioinformatics technology to analyze the collaborative relationships in complex biological networks among drugs, diseases, and targets. In recent years, it has been widely used in the field of traditional Chinese medicine research [7-9]. This research group used network pharmacology methods and molecular docking to construct and analyze the molecular network among Huangqi Guizhi Wuwu Decoction, active components, targets, diseases, signaling pathways, etc. The pharmacological substances and mechanism of action for the treatment of osteoporosis provide ideas and references for in-depth study of the pharmacological mechanism of Huangqi Guizhi Wuwu Decoction.

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1. Material and Methods

2.1. Ingredient acquisition and active ingredient screening

The five flavors of Astragalus and Guizhi Wuwu Decoction (astragalus, peony, jujube, ginger and osmanthus), using oral availability (OB≥30%) and drug-likeness (DL≥0.18) as the limiting conditions [10], were screened, and the screening ingredients were used as active ingredients of Huangqi Guizhi Wuwu Decoction.

2.2. Acquisition of targets for active ingredients

The targets of TCM ingredients obtained from the screening were collected in the TCMSP database, and the gene names of the targets were searched using the UniProt database (https://www.uniprot.org/).

2.3. Screening of diabetic retinopathy (DPN)-related targets

Using the OMIM database (http://www.omim.org/), the CTD database (http://ctdbase.org/), the DisGeNET database (http://www.disgenet.org/), the TTD database (http://database.idrb.cqu.edu.cn/TTD/), and the Genecards database (http://www.genecards.org/) with the search keyword "diabetic peripheral neuropathy" to obtain DPN-related targets. For the targets obtained from the CTD database, targets without direct evidence were excluded. Targets obtained from the Genecards database with a screening score lower than 20 were excluded, and the targets obtained from the above databases were merged as disease-related targets.

2.4. Construction of an active pharmaceutical ingredient-target-disease network

The intersection of the targets obtained in 1.2 and 1.3 above was taken as the potential targets of Huangqi Guizhi Wuwu Decoction for the treatment of DPN, and the Huangqi Guizhi Wuwu Decoction-active ingredient-target-disease network was constructed using Cytoscape 3.7.2 software. In the network, nodes represent the active ingredients of the drug, targets, and diseases; edges represent the interaction relationship between the drug and the targets. The "NetworkAnalyzer" software was used to analyze the topology of the network, and the degree, BC and closeness centrality (CC) of the active drug ingredient nodes were calculated to screen key drugs. Effective molecule.

2.5. Target–protein interaction analysis

Potential targets were imported into the String database (https://string-db.org/) to analyze target–protein interactions. The target protein interaction (PPI) network was constructed in Cytoscape software, "NetworkAnalyzer" software was used to perform topological analysis on the network, and the topological parameters degree, BC, and CC of the nodes were calculated to screen core targets.

2.6. Molecular docking to verify the interactions between the active drug ingredient and potential target targets

The mol2 structure files of the top ten key pharmacological molecules were downloaded by Degree value from the TCMSP database and stored in the PDB database (http://www.rcsb.org/), and the pdb structure files of the top ten core target proteins in Degree value were downloaded. PyMol software was used to perform dehydration and hydrogenation on the receptor and ligand molecules, and AutoDockTools software was used to download the structure files of the pharmacological molecules and core target proteins. The images were converted to the pdbqt format, and the grid box coordinates of the ligands of the target proteins were set. Finally, Autodock_vina software was used for molecular docking simulation, the minimum binding energy between the active pharmaceutical ingredient and the target protein was calculated, and the molecular docking diagram was drawn using PyMol software.

2.7. Functional enrichment analysis of Gene Ontology (GO) and pathway enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG)

The potential targets were uploaded to the Davidv6.8 database (https://david-d.ncifcrf.gov/), with the species set to Homo species. The GO functional analysis and the KEGG pathway analysis were performed on the targets, with the threshold $P<0.05$. The active pharmaceutical ingredient-target-signaling pathway network was constructed by Cytoscape software.

3. Results

3.1. Retrieval and screening of drug ingredients

This study used oral availability (OB ≥ 30%) and drug-likeness (DL ≥ 0.18) as screening criteria and obtained 60 eligible active pharmaceutical ingredients from the TCMSP database, of which 55 had target predictions. There were 25 species of Astragalus, 15 species of Guizhi, 3 species of jujube, 7 species of ginger, and 5 species of peony, as shown in Table 1.

3.2. Prediction of potential targets of Huangqi Guizhi Wuwu Decoction in the treatment of DPN

Using the TCMSP database, a total of 142 targets for 55 active pharmaceutical ingredients were collected. A total of 103, 70, 1008, 39 and 97 disease-related targets were obtained from the OMIM database, CTD database, DisGeNET database, TTD database and Genecards database, respectively. After removing repetitive targets, a total of 1026 disease targets were obtained. The intersection analysis of the 142 targets of active pharmaceutical ingredients and 1026 disease targets was performed, and 46 intersection targets were obtained, which were used as potential targets for HGJWD in the treatment of DPN.

3.3. Construction of an active pharmaceutical ingredient-target-disease network

The active drug ingredient-target-disease network of Huangqi Guizhi Wuwu Decoction for the treatment of DPN had a total of 112 nodes and 528 edges. Diamond nodes represent diseases, square nodes represent drugs, hexagonal nodes represent active drug ingredients, and circular nodes represent the diameter and color depth of potential target and active ingredient nodes, which are proportional to the degree value. The results of network topology analysis showed that the median degree of the active ingredient node was 9, the median BC was 0.00297845, and the median CC was 0.41231156. There were a total of 24 active ingredients that met the above parameters at the same time; Table 2 lists the key pharmacodynamic molecules of DPN. The top ten active ingredients by degree were quercetin (MOL000098), kaemp-

Table 1. Information on compounds in Huangqi Guizhi Wuwu Decoction with good oral availability and drug-likeness.

Mol ID	Molecule Name	OB (%)	DL	Herb
MOL000006	Luteolin	36.54	0.23	GSB, YYH
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-Dimethyl-17-[(2R,5S)-5-Propan-2-Yloctan-2-Yl]-2,3,4,7,8,9,11,12,14,15,16,17-Dodecahydro-1H-Cyclopenta[A]Phenanthren-3-Ol	36.34	0.76	JXT
MOL000296	Hederagenin	36.78	0.72	JXT
MOL000392	Formononetin	69.11	0.26	JXT
MOL000417	Calycosin	47.13	0.21	JXT
MOL000461	3,7-Dihydroxy-6-Methoxy-Dihydroflavonol	43.82	0.21	JXT
MOL000468	8-O-Methylreyusi	70.45	0.23	JXT
MOL000469	3-Hydroxystigmast-5-En-7-One	40.93	0.74	JXT
MOL000470	8-C-A-L-Arabinosylluteolin	35.67	0.62	JXT
MOL000471	Aloe-Emodin	83.89	0.25	JXT
MOL000483	(Z)-3-(4-Hydroxy-3-Methoxy-Phenyl)-N-[2-(4-Hydroxyphenyl) Ethyl]Acrylamide	121.31	0.22	JXT
MOL000490	Petunidin	30.24	0.32	JXT
MOL000492	(+)-Catechin	54.26	0.25	JXT, GSB
MOL000622	Magnograndiolide	63.25	0.18	YY
MOL001510	24-Epicampesterol	37.52	0.76	YY
MOL001645	Linoleyl Acetate	42.16	0.22	YY
MOL001771	Poriferast-5-En-3Beta-Ol	36.45	0.71	YY
MOL001792	DFv	32.72	0.15	YY
MOL003044	Chryseriol	35.98	0.22	YY
MOL003542	8-Isopentenyl-Kaempferol	38.15	0.35	YY
MOL004367	Olivier	62.15	0.43	YY
MOL004373	Anhydroicaritin	45.23	0.46	YY
MOL004380	C-Homoerythrinan, 1,6-Didehydro-3,15,16-Trimethoxy-, (3.Beta.)-	39.19	0.42	YY
MOL004382	Yinyanghuo A	56.52	0.73	YY
MOL004384	Yinyanghuo C	45.24	0.52	YY
MOL004386	Yinyanghuo E	51.19	0.51	YY
MOL004388	6-Hydroxy-11,12-Dimethoxy-2,2-Dimethyl-1,8-Dioxo-2,3,4,8-Tetrahydro-1H-Isochromeno[3,4-H]Isoquinolin-2-Ium	61.89	0.61	YY
MOL004391	8-(3-Methylbut-2-Enyl)-2-Phenyl-Chromone	48.35	0.22	YY
MOL004394	Anhydroicaritin-3-O-Alpha-L-Rhamnoside	41.25	0.64	YY
MOL004396	1,2-Bis(4-Hydroxy-3-Methoxyphenyl)Propan-1,3-Diol	52.28	0.23	YY
MOL004425	Icariin	41.48	0.62	YY
MOL004427	Icariside A7	31.77	0.82	YY
MOL001040	(2R)-5,7-Dihydroxy-2-(4-Hydroxyphenyl)Chroman-4-One	42.32	0.25	GSB
MOL001978	Aureusidin	53.65	0.24	GSB
MOL002914	Eriodyctiol (Flavanone)	41.43	0.26	GSB
MOL004328	Naringenin	59.56	0.22	GSB
MOL005190	Eriodictyol	71.11	0.22	GSB
MOL009061	22-Stigmasten-3-One	39.43	0.74	GSB
MOL009063	Cyclolaudenol Acetate	41.11	0.75	GSB
MOL009075	Cycloartenone	40.15	0.75	GSB
MOL009076	Cyclolaudenol	39.49	0.75	GSB
MOL000569	Digallate	61.82	0.23	GSB, LXC
MOL000422	Kaempferol	41.46	0.25	GSB, LXC, YYH
MOL003975	Icosa-11,14,17-Trienoic Acid Methyl Ester	44.11	0.26	LFZ
MOL010672	Icosa-8,11,14-Trienoic Acid Methyl Ester	44.19	0.22	LFZ
MOL000073	Ent-Epicatechin	48.45	0.21	XC
MOL000552	5,2'-Dihydroxy-6,7,8-Trimethoxyflavone	31.54	0.33	XC
MOL005320	Arachidonate	45.48	0.22	RCR
MOL005384	Suchilactone	57.47	0.55	RCR
MOL007563	Yangambin	57.35	0.82	RCR
MOL008871	Marckine	37.11	0.64	RCR
MOL000358	Beta-Sitosterol	36.24	0.72	RCR, JXT, GSB, LXC
MOL000098	Quercetin	46.37	0.22	RCR, LXC, YYH
MOL000449	Stigmasterol	43.45	0.71	SDH, JXT, GSB
MOL000359	Sitosterol	36.16	0.72	SDH, LXC, YYH, LFZ

Table 2. Key pharmacodynamic molecules and their topological parameters in the active pharmaceutical ingredient-target-disease network.

Mol ID	Molecule Name	Degree	Betweenness, Centrality	Closeness Centrality
MOL000098	Quercetin	38	0.14569973	0.55223881
MOL000422	Kaempferol	24	0.05772909	0.48471616
MOL000006	Luteolin	21	0.03956325	0.46443515
MOL000358	Beta-Sitosterol	20	0.03522141	0.46443515
MOL004328	Naringenin	19	0.0428336	0.44939271
MOL000471	Aloe-Emodin	15	0.01980109	0.43873518
MOL000449	Stigmasterol	14	0.02315118	0.43529412
MOL000392	Formononetin	13	0.00806411	0.43190661
MOL001040	(2R)-5,7-Dihydroxy-2-(4-Hydroxyphenyl) Chroman-4-One	12	0.00551862	0.42528736
MOL000468	8-O-Methylreyusi	11	0.00462016	0.42528736
MOL000492	(+)-Catechin	11	0.00804329	0.42528736
MOL004373	Anhydroicaritin	11	0.00422206	0.42857143
MOL004391	8-(3-Methylbut-2-Enyl)-2-Phenyl-Chromone	11	0.0052663	0.42857143
MOL000417	Calycosin	10	0.00374107	0.42205323
MOL001792	DFv	10	0.00338867	0.42528736
MOL004396	1,2-Bis(4-Hydroxy-3-Methoxyphenyl) Propan-1,3-Diol	10	0.00338867	0.42528736
MOL005190	Eriodictyol	10	0.00360538	0.41886792
MOL000073	Ent-Epicatechin	9	0.00527765	0.42205323
MOL000296	Hederagenin	9	0.00476943	0.41886792
MOL000552	5,2'-Dihydroxy-6,7,8-Trimethoxyflavone	9	0.00527765	0.42205323
MOL004380	C-Homoerythrinan, 1,6-Didehydro-3,15,16-Trimethoxy-, (3.Beta.)-	9	0.00823189	0.42205323
MOL004384	Yinyanghuo C	9	0.00299495	0.42205323
MOL004386	Yinyanghuo E	9	0.00299495	0.42205323
MOL000359	Sitosterol	8	0.01983483	0.42205323

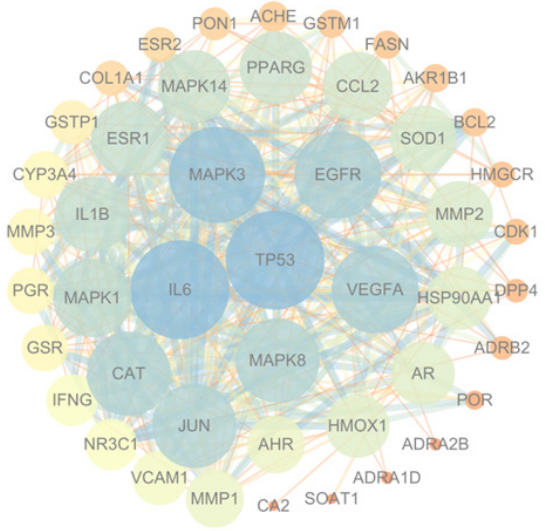

Fig. 1. PPI network of target proteins of Huangqi Guizhi Wuwu decoction in the treatment of DNP.

ferol (MOL000422), luteolin (MOL000006), β-sitosterol (MOL000358), naringenin (MOL004328), aloe-emodin (MOL000471), stigmasterol (MOL000449), formononetin (MOL000392), (2R)-5,7-dihydroxy-2-(4-hydroxyphenyl) chromium-4-one (MOL001040), and 8-O-methanol reduxin (MOL000468).

3.4. Construction and analysis of the PPI network of target proteins

Treatment with Huangqi Guizhi Wuwu Decoction DNP The PPI network of the target proteins of ' is shown in Figure 1. The diameter of the node is proportional to the color depth and the degree value of the node, and the width of the edge is proportional to the color depth and the combined score of the edge. The network included 46 nodes and 419 edges. The median network topology parameter degree was 19, the median BC was 0.007877655, and the median CC was 0.62063523. There are a total of 19 targets that meet the above parameters at the same time and can be used as core targets. , including TP53, IL6, MAPK3, EGFR, VEGFA, MAPK8, JUN, CAT, MAPK1, IL1B, ESR1, MAPK14, PPARG, CCL2, SOD1, HSP90AA1, AR, HMOX1, and AHR, as shown in Table 3.

3.5. Molecular docking analysis of the interaction between the active drug ingredient and potential targets

Information on the top ten target receptor proteins by degree value obtained from the PDB database is shown in Table 4. The minimum binding energies between the core target and key pharmacological molecules obtained through molecular docking simulation are shown in Figure 2. It is generally considered that the absolute value of binding energy greater than 4.25 kcal·mol $^{-1}$ has certain bin-

Table 3. Core targets and their topological parameters in the PPI network of target proteins.

Gene Symbol	Target Name	Degree	Betweenness, Centrality	Closeness Centrality
TP53	Cellular tumor antigen p53	35	0.04277743	0.80357143
IL6	Interleukin-6	35	0.06145443	0.81818182
MAPK3	Mitogen-activated protein kinase 3	34	0.11626498	0.80357143
EGFR	Epidermal growth factor receptor	31	0.03026594	0.75
VEGFA	Vascular endothelial growth Factor A	31	0.02576015	0.75
MAPK8	Mitogen-activated protein kinase 8	30	0.01647123	0.73770492
JUN	Transcription factor AP-1	30	0.0261016	0.73770492
CAT	Catalase	30	0.04514023	0.73770492
MAPK1	Mitogen-activated protein kinase 1	28	0.01196028	0.71428571
IL1B	Interleukin-1 beta	27	0.01615379	0.69230769
ESR1	Estrogen receptor	27	0.0159526	0.703125
MAPK14	Mitogen-activated protein kinase 14	26	0.00795407	0.69230769
PPARG	Peroxisome proliferator activated receptor gamma	26	0.02718199	0.69230769
CCL2	CC motif chemokine 2	25	0.01136224	0.67164179
SOD1	Superoxide dismutase [Cu-Zn]	24	0.02183667	0.67164179
HSP90AA1	Heat shock protein HSP 90	23	0.00948957	0.66176471
AR	Androgen receptor	23	0.05355159	0.66176471
HMOX1	Heme oxygenase 1	23	0.0132568	0.65217391
AHR	Aryl hydrocarbon receptor	21	0.00826394	0.625

Table 4. Information on the top ten target receptor proteins by degree value.

Gene Name	Protein Name	PDB-ID	Ligand-ID
TP53	Cellular tumor antigen p53	6GGA	EY2
IL6	Interleukin-6	1ALU	TLA
MAPK3	Mitogen-activated protein kinase 3	3FHR	P40
EGFR	Epidermal growth factor receptor	4LRM	YUN
VEGFA	Vascular endothelial growth Factor A	5T89	NAG
MAPK8	Mitogen-activated protein kinase 8	4G1W	G1W
JUN	Transcription factor AP-1	5T01	5CM
CAT	Catalase	1DGH	HEM
MAPK1	Mitogen-activated protein kinase 1	3 W8Q	AGS
IL1B	Interleukin-1 beta	5R88	LWA

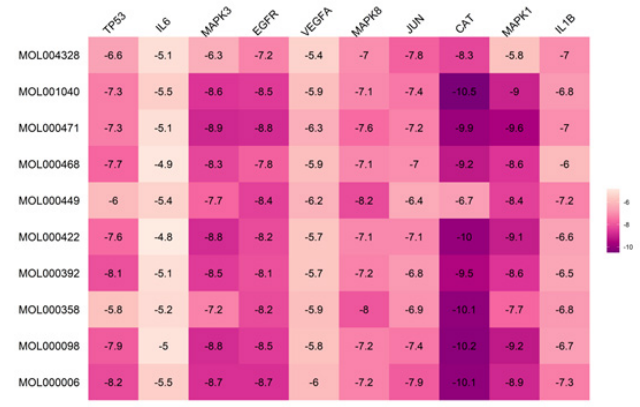

Fig. 2. Heatmap of the lowest binding energy between key pharmacodynamic molecules and core targets.

ding activity; greater than 5.0 kal·mol $^{-1}$ indicates good binding activity, and greater than 7.0 kal·mol $^{-1}$ indicates strong binding activity [11], where except for IL6, which had a minimum binding energy of kaempferol and 8-oxo-methylreduxin that was greater than -5 kal·mol $^{-1}$, the minimum binding energies of the other core targets and key pharmacological molecules were all less than -5 kal·mol $^{-1}$, indicating that the core target and key pharmacological molecules have good binding activity. We performed graphing analysis on some molecular docking results. The molecular docking diagram is shown in Figure 3. In the diagram, the key pharmacodynamic molecules and core target proteins can be formed through various intermolecular forces, such as hydrogen bonds, aromatic stacking interactions, and hydrophobic forces. more stable conformation.

3.6. Enrichment analysis of potential targets

Enrichment analysis of potential targets was performed using the DAVID database. During the functional enrichment analysis of GO, a total of 254 GO entries were identified, including 180 biological process entries, 32 cellular component entries, and 42 molecular function entries, including the regulation of sequence-specific DNA-binding transcription factor activity and apoptosis [5]. A total of

180 biological processes, including apoptosis, positive regulation of transcription, DNA templating and cell aging; 42 molecular functions, such as enzyme binding, steroid binding, steroid hormone receptor activity and identical protein aggregation; and 32 extracellular space, mitochondria, cytoplasm, and proteins related to cell composition, such as complexes, were included in the list. Figure 4 shows the GO function entries with the highest significance.

KEGG pathway enrichment analysis showed that a total of 36 metabolic pathways may be related to the molecular mechanism of Huangqi Guizhi Wuwu Decoction in the treatment of DNP. Figure 5 shows the top 20 metabolic pathways with significance. The results showed that 36 metabolic pathways were mainly enriched in the cell cycle, signal transduction, immune system, nervous system, and cancer pathways, including the tumor necrosis factor (TNF) signaling pathway and nucleotide-binding oligomerization domain (NOD)-like receptors. Somatic

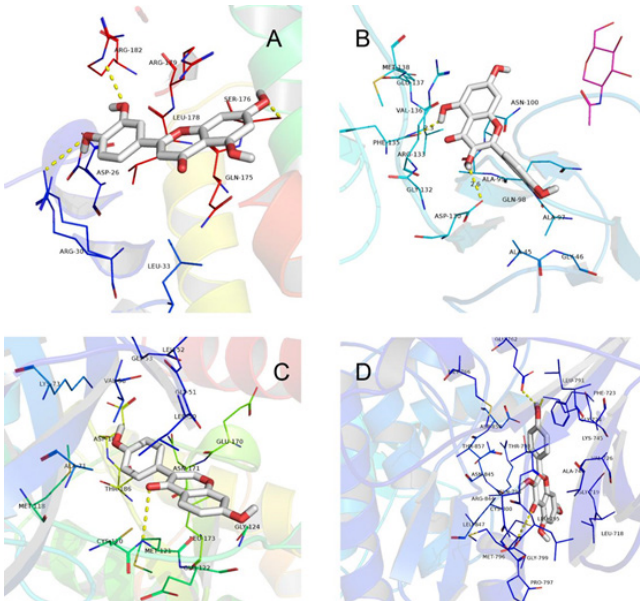

Fig. 3. Molecular docking schema of some key pharmacological molecules and core targets. (A: Docking model of luteolin and IL6; B: Docking model of quercetin and VEGFA; C: Docking model of formononetin and MAPK3; D: Docking model of kaempferol and EGFR).

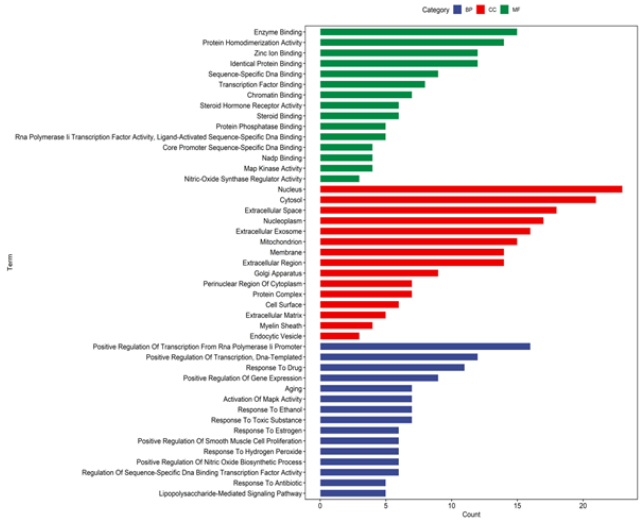

Fig. 4. Bar graph of GO enrichment analysis.

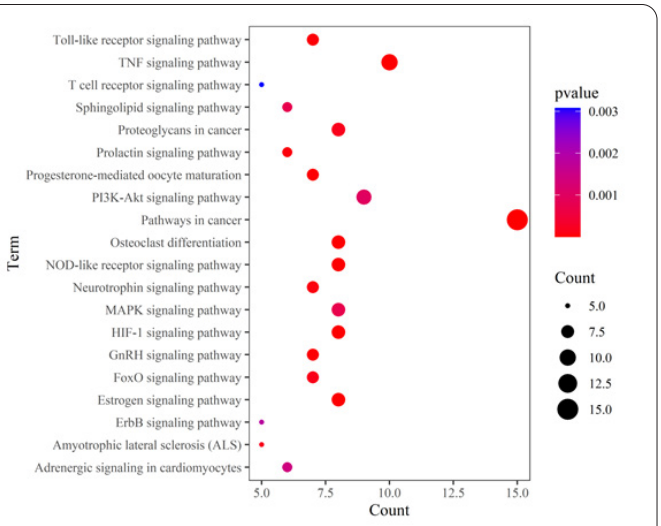

Fig. 5. Bubble plot of KEGG pathway analysis.

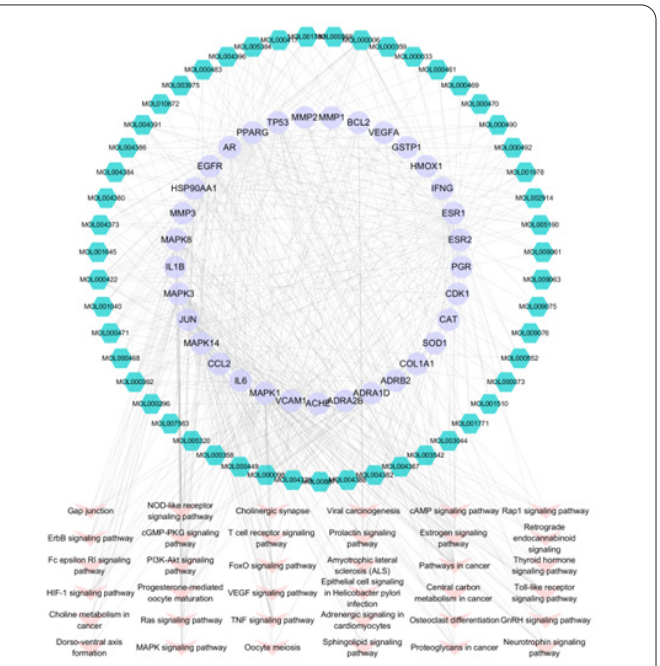

Fig. 6. Active pharmaceutical ingredient-target-signaling pathway network of Huangqi Guizhi Wuwu Decoction for the treatment of DNP.

signaling pathways, cancer pathways, estrogen signaling pathways, osteoclast differentiation pathways, mitogen-activated protein kinase (MAPK) signaling pathways, etc.

The active pharmaceutical ingredient-target-signaling pathway network was drawn in Cytoscape software (Figure 6). The network contained 120 nodes and 536 edges and involved 51 active drug ingredients, 33 targets, and 36 pathways. The network shows that the effect of Huangqi Guizhi Wuwu Decoction in the treatment of DNP has multiple components and multiple targets. point and multichannel characteristics.

4. Discussion

With the rapid economic development of the reform and opening up and the change in people's living standards and diet structure, diabetes has become a major health problem worldwide, and the incidence of DPN is also increasing each year [11-13]. In recent years, studies on the treatment of DPN with Chinese medicine have increased, showing great potential value and positive effects [14].

Huangqi Guizhi Wuwu Decoction is composed of five Chinese medicines, i.e., astragalus, peony, jujube, ginger and osmanthus twig, and the whole prescription has the effects of harmonizing ying and promoting numbness and replenishing qi and unblocking yang [15]. This group used the network pharmacology method to construct the active ingredient-target-disease network of Huangqi Guizhi Wuwu decoction for the treatment of DPN and analyzed the mechanism of action of Huangqi Guizhi Wuwu decoction in the treatment of DPN. Through the construction and analysis of the target proteins, the PPI network found that the targets of Huangqi Guizhi Wuwu decoction in the treatment of DPN cooperate with each other to regulate a complex protein interaction network. Molecular docking simulations showed that some active drug components of Huangqi Guizhi Wuwu Decoction had good binding activity to DPN-related targets. The above results suggest that Huangqi Guizhi Wuwu Decoction has multicomponent, multitarget and multi-pathway action modes.

The main active components of Huangqi Guizhi Wuwu Decoction are quercetin, kaempferol, luteolin, naringin, and icariin. All have anti-inflammatory, antioxidant, immune regulation, and estrogen-like effects [16]. In the PPI network analysis results, the degree values of TP53, IL6, MAPK3, EGFR, VEGFA, JUN, CAT, MAPK1, IL1B, ESR1, etc., were high, indicating strong interactions with other proteins and being at the core position of the network. In docking analysis, it had good binding activity to the main active ingredient of the drug, indicating that there is a synergy between the active ingredient and the target protein to jointly complete the treatment of DPN. In-depth studies in recent years have found that inflammatory cytokines are directly involved in the occurrence and development of DPN. Inflammatory cytokines such as IL-1 and IL-6 are correlated with susceptibility to the development of DPN and have gene polymorphisms [17,18]. JUN is a rapid-response gene that acts as a transcription factor by forming a homodimer with its own JUN protein or the AP-1 dimer with the Fos protein and binds to the AP-1 locus in the regulatory region of target genes to regulate the expression of DPN-related genes. Vascular endothelial growth factor (VEGF) is a growth factor. Clinical tests have shown that VEGF levels in DPN patients are far lower than those in normal individuals.

The results of enrichment analysis showed that the biological functions involved in Huangqi Guizhi Wuwu Decoction included the regulation of the activity of sequence-specific DNA-binding transcription factors, the apoptosis process, the positive regulation of transcription, enzyme binding, steroid binding, and steroid hormone receptor activity. The signaling pathways involved in the potential targets included the TNF signaling pathway, NOD-like receptor signaling pathway, estrogen signaling pathway, osteoclast differentiation pathway, MAPK signaling pathway, etc.; pathways have an impact. Activation of the TNF signaling pathway can promote the expression of proinflammatory cytokines, chemokines, growth factors, and TNF-α itself, causing immune system dysfunction and inflammation and affecting the biochemical progression of DPN [19,20]. The MAPK signaling pathway is an important signal transduction pathway that regulates cell proliferation and differentiation, and the main pathways include extracellular signal-regulated kinase (ERK), p38, and c-Jun amino-terminal kinase (JNK). ERK can regulate osteoblast differentiation [21], inhibition of the hyperphosphorylation of p38MAPK can protect cells and inhibit their apoptosis [22], and JNK can affect late-stage cell differentiation by inducing the expression of activating transcription Factor 4 (ATF4) [23]. The MAPK signaling pathway is also interrelated with the phosphatidylinositol 3-kinase-protein kinase (PI3K-Akt) signaling pathway and plays a key regulatory role in cell proliferation, differentiation and apoptosis [24]. Studies have shown that ER can affect cell maturity and mineralization through the ERK and JNK MAPK signaling pathways [25], and estrogen receptor α can activate insulin-like growth Factor 1 receptor (IGF-1R) and downstream MAPK/ERK signaling cascade reactions and the PI3K/AKT pathway.

This study used the network pharmacology method to conduct GO biological function enrichment analysis and KEGG on the potential targets by constructing and analyzing the collaboration network among Chinese medicines, active drug ingredients, targets, diseases, protein molecules, and signaling pathways. Pathway analysis showed that Huangqi Guizhi Wuwu Decoction mainly acts on core targets such as IL6, MAPK3, VEGFA, JUN, and ESR1 through active components such as quercetin, kaempferol, and naringin to regulate the TNF signaling pathway, MAPK signaling pathway, estrogen signaling pathway and other pathways and can be used to treat DPN, and the molecular mechanism of Huangqi Guizhi Wuwu Decoction in the treatment of DPN is preliminarily elucidated. However, there are still few studies in this area, and therefore, a large number of clinical and experimental studies are still needed for exploration and validation. In the meantime, this study provides a reference for future basic experiments and drug development.

Conflict of Interests
The authors declared no conflict of interest.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed Consent
The authors declare not used any patients in this research.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
BA and GL designed the study and performed the experiments, BA and WZ collected the data, GL and WZ analyzed the data, BA and GL prepared the manuscript. All authors read and approved the final manuscript.

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