Study on the Mechanism of Buyang Huanwu Decoction for the treatment of Ischemic Stroke Based on Network Pharmacology

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Research

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Abstract

Background: Buyang Huanwu Decoction (BYHWD) is one of the representative prescriptions for tonifying qi and promoting blood circulation. This formula has been widely used in Chinese clinical practice for treatment and prevention of ischemic cerebral vascular diseases. However, the mechanism and active compounds of BYHWD used in clinical practice for ischemic stroke are not well understood. The purpose of this study was to understand the potential active components of BYHWD and further explore its mechanism of improving ischemic stroke.

Methods: This study was based on network pharmacology and bioinformatics analysis. The compounds of BYHWD were obtained from public databases. Oral bioavailability as well as drug-likeness were screened by using absorption, distribution, metabolism, and excretion (ADME) criteria. Then, components of BYHWD, candidate targets of each component and known therapeutic targets of ischemic cerebral were collected. A network of compound-target genes and compound-ischemic cerebral was established by means of network pharmacology data sources. The enrichment of key targets and pathways was analyzed by using string database and DAVID database. In addition, we verified three key targets predicted by western blot analysis (IL6, VEGFA and HIF1A).

Results: Network pharmacology analysis results of BYHWD identified 7 herbs, 42 compounds and 79 target genes associated to cerebral ischemia. The 10 key compounds were baicalein, beta-carotene, Baicalin, kaempferol, luteolin, quercetin, hydroxysafflor yellow A, isorhamnetin, Bifendate, formononetin,Calycosin, AstragalosideIV, Stigmasterol, sitosterol, Z-ligustilide, Dihydrocapsaicin. Core genes in this network were IL6, TNF, VEGFA, HIF1A, MAPK1, MAPK3, JUN, STAT3, IL1B and IL10. And pathways TNF, IL-17, Apoptosis, PI3K-Akt, Toll-like receptor, MAPK, NF-kappa B and HIF-1 signaling pathway, etc. related to ischemic stroke were identified. In vitro experiments, The results showed that compared with the control group (no treatment), BYHWD could significantly inhibit the expression of IL6 and increased the expression of HIF1A and VEGFA.

Conclusions: Network pharmacology analysis can reveal close interactions between multi-components and multi-targets, and enhance our understanding of the potential effects of BYHWD in cerebral ischemia.

1. Background

Ischemic stroke is an acute cerebrovascular disease, also known as cerebral ischemia. It has the characteristics of high disability and high mortality[1, 2]. According to statistics compiled by the American heart association (AHA) in collaboration with the national institutes of health and other government agencies, stroke has become the third most common cause of death worldwide after cancer and heart disease in recent years, placing a huge psychological and financial burden on many families[3].

Buyang Huanwu Decoction (BYHWD) is one of the representative prescriptions for tonifying qi and promoting blood circulation in the "Correction on Errors in Medical Classic" by Wang Qingren, a famous doctor in the Qing Dynasty. It is composed of: Radix Astragali, the dried roots of *Astragalus membranaceus* (Fisch.) Bge; Angelicae sinensis Radix, the dried roots of *Angelica sinensis(Oliv.)*Diels; Paeoniae Radix Rubra, the dried roots of *Paeonia lactiflora* Pall; Chuangxiong Rhizoma, the dried rhizomes of *Ligusticum chuanxiong* Hort; Persicae Semen, the dried seeds of *Prunus persica*（L.）Batsch; Carthami Flos, the dried flowers of *Carthamus tinctorius* L; Pheretima, the dried bodies of *Pheretima aspergillum*（E.Perrier）, in the ratio of 120 : 6 : 4.5 : 3 : 3 : 3 : 3. According to the traditional Chinese medicine literature, BYHWD was used to promote blood circulation, and activate energy (qi) flow through energy meridians. This formula has been widely used in Chinese clinical practice for treatment and prevention of ischemic cardio-cerebral vascular diseases[4]. Many researches show that BYHWD has notable curative effectiveness in ischemic stroke and other vascular diseases[5]. However, the exact mechanism of BYHWD in improving ischemic stroke and its active components remain unclear. As such, there is a great interest in identifying the active compounds and molecular targets in BYHWD acting on ischemic stroke. The present study aimed to identify the effective constituents of BYHWD and to further explore its action mechanisms in the amelioration of ischaemic stroke.

Traditional Chinese medicine compound has the characteristics of “multi-component, multi-channel, multi-target”. It is a product of traditional Chinese medicine under the guidance of holistic view and dialectical theory. It has unique advantages for thetreatment of complex diseases, but because of the complex composition of traditional Chinese medicine compound to make its modernization process slow. Network pharmacology, a novel method which combined the system network analysis and the pharmacology, could clarify the synergistic effects and underlying mechanisms among the networks of compound-compound, compound-target and target-disease in the molecular level, which let us know the interactions among the compounds, genes, proteins and diseases. Due to its integrity and systematicness, which are consistent with the overall view of TCM and the principle of dialectical theory, it has been widely applied in TCM research[6]. Therefore, this study applied network pharmacology method to study and discuss the mechanism of BYHWD in the treatment of ischemic stroke, providing reference and theoretical basis for its experimental research and clinical application.

In this study, information on compounds in herbal medicines of BYHWD was acquired coming from public databases, and oral bioavailability (OB) and drug-likeness (DL) were evaluated utilizing ADME standards. Then, components of BYHWD, candidate targets of each component and known therapeutic targets of ischemic stroke were collected. By utilizing these data, compounds of BYHWD-compound targets and compounds – ischemic stroke target networks were established via network pharmacology databases. Key targets and pathway enrichment were analyzed by STRING database and DAVID database. In addition, we verified three of the key targets predicted in the network by western blot analysis. The workflow of this study on BYHWD against ischemic stroke based on network pharmacology was drawn (Fig. 1).

2. Materials And Methods

2.1. Chemical databases collection of BYHWD

The compound of buyang huanwu decoction was collected from two phytochemical databases: Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, http://ibts.hkbu.edu.hk/LSP/tcmsp.php) and TCM Database@Taiwan (http://tcm.cmu.edu.tw/). Bidimensional chemical structures were also gotten from NCBI PubChem (http://pubchem.ncbi.nlm.nih.gov/).

2.2. Pharmacokinetic ADME evaluation

OB represents the ability of a compound to circulate in the body after oral administration. OB can show whether the active compounds in a formula can be delivered through out the body and produce a physicochemical effect. DL is an indicator for determining the similarity or likeness of a compound, and its physicochemical properties, with conventional drugs. DL can help determine if a certain compound has a therapeutic effect[7]. All compounds were selected using the in silico integrative ADME model administered by the TCMSP Database. Chemicals without ADME information were removed from the final list. The ADME system used in this study includes predict OB and DL, and compounds were retained only if OB ≥ 30 and DL ≥ 0.18 to satisfy criteria suggested by the TCMSP database[8].

2.3. Target genes related to the identified compounds

Collected all the genes related to the compound from the database, The official names of gene were taken from UniProt (http://www.uniprot.org/) by confining the species to'Homo sapiens', Subsequently, various ID forms of the targets were transformed into UniProt IDs.

2.4. Target genes collection of cerebral ischemia

Information on cerebral ischemia associated target genes was collected from the therapeutic targets database (TTD; http://bidd.Nus.edu.sg/group/cjttd/TTD_HOME.asp) and DisGeNETv6.0(http://www.disgenet.org/web/DisGeNET/menu/home),and simply "Homo sapiens"proteins connected to cerebral ischemia were selected.

2.5. Construction of networks and pathway analyses

The constructed herbal-chemical-protein networks were visualized using Cytoscape ver.3.6.1 (http://www.cytoscape.org/), Nodes in networks represent herbs and chemicals, and edges indicate interactions between herbs and chemicals, and between chemicals and target genes[9]. The functional pathways of BYHWD related to cerebral ischemia diseases were analyzed using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (http://www.genome.jp/kegg/pathway.html) and gene ontology (GO) enrichment evaluation based upon the database for annotation, Visualization and Integrated Discovery(DAVID) version 6.8 (https://david.ncifcrf.gov/). The value of $P < 0.05$ was screened from the enrichment analysis results.

2.6. Protein-Protein interaction (PPI) analyses

The screened drug component-disease common target was imported into the STRING (https://string-db.org/) database to build the protein-protein interaction (PPI) network model,and the protein category was set as "Homo sapiens", the minimum interaction threshold was set as the medium "highest confidence" (> 0.9), and the PPI network was obtained with the default setting of other parameters. In the network, thesize of the nodes represents the size of the degree.The higher the degree, the better the correlation between protein and therapeutic mechanism[10].

2.7. Pharmacological experimental verification

2.7.1. Materials

Buyang Huanwu Decoction is a granule made up of Astragali Radix, Angelicae sinensis Radix, Paeoniae Radix Rubra, Chuangxiong Rhizoma, Persicae Semen, Carthami Flos and Pheretima according to the ratio of 120 : 6 : 4.5 :3 : 3 : 3 : 3 (Manufacturer: Guangdong Yifang pharmaceutical co. LTD. Product batch:7070352). The rat Brain Microvascular Endothelial cells (BMECs) were purchased from the Cell Biologics Company (#C57-6023, Chicago, IL). Primary antibody against β-actin, VEGFA, HIF1A, IL6 and Secondary antibody were all purchased from Abcam technology (Cambridge, UK). All other reagents are analytical reagents and commercially available.

2.7.2. Cell culture and treatments

Cells were divided into three groups: control group, OGD group and OGD group treated with HH (100 μg/mL). Oxygen Glucose Deprivation (OGD) was established as follow: cells were rinsed once with glucose-free DMEM (Gibco, Rockville, MD) and transferred to an anaerobic chamber (Forma Scientific, Waltham, MA) containing a gas mixture composed of 7% CO2 and 93% N2 for 6 h at 37 °C. Then, the cells were returned to the normal culture condition. Control BMECs were cultured in complete DMEM under normal conditions. BYHWD groups were treated with 80 μg/mL BYHWD (dissolved in complete DMEM and filtered with a 0.22 μm membrane filter) for 12 h. The control group was treated without BYHWD.

2.7.3 Western blot assay

BMECs were lysed with cold RIPA buffer (Rockford, IL, USA) for 30 min. The whole cell lysates were fractionated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto PVDF membranes (Millipore, USA). The membranes were blocked with 5% bovine serum albumin (BSA) and incubated overnight with primary antibodies against VEGFA (1:1000,rabbit), HIF1A (1:1000,rabbit), IL6 (1:1000,rabbit) at 4℃. Subsequently, membranes were incubated with secondary antibody at a 1:10000 dilution at 37℃ for 1 h. The blots were visualized with ECL-Plus reagent (Santa Cruz, USA) and analyzed with Quantity One System image analysis software (Bio-Rad, USA).

2.7.4 Statistical analysis

The results were expressed as mean ± standard deviation (S.D.). Differences between multiple groups were analyzed by One-way analysis of variance (ANOVA) and differences between two groups were analyzed using the t-test. Significant difference was considered when $P < 0.05$.

3. Results

3.1. Compounds in herbal medicines

BYHWD consists of seven herbal medicines, namely Astragali Radix, Angelicae sinensis Radix, Paeoniae Radix Rubra, Chuangxiong Rhizoma, Persicae Semen, Carthami Flos and Pheretima. A total of 102 compounds were identified in BYHWD, among which 21 were Astragali Radix, 23 were Carthami Flos, 4 were Angelicae sinensis Radix, 29 were Paeoniae Radix Rubra, 9 were Chuangxiong Rhizoma, 23 were Persicae Semen and 5 were Pheretima. All identified compounds were subjected to ADME screening, among compounds below these requirements, six (Hydroxysafflor yellow A, AstragalosideIV, Ferulic acid, Ligustrazine, Z-ligustilide, Linoleic acid) were considered bioactive compounds. These compounds are the major components of BYHWD, and their effects on cerebral ischemia is have been investigated previously[11–16]. Remove duplicate components and remove components with ambiguous targets. The composition table of BYHWD was obtained, as shown in Table 1.

3.2. Combination of compound target genes and cerebral ischemia targetgenes

A total of 518 genes and compounds related to BYHWD were obtained from the database. A total of 274 genes related to cerebral ischemia were retrieved from the TTD and DisGeNETv6.0 database, and 79 overlapping genes were pinpointed through matching the mentioned 518 compounds genes with disease-associated genes (Fig. 2).

3.3. Potential target genes and network analysis

The constructed herbal-chemical-protein networks were visualized using Cytoscape, this network of 7 herbs 42 compounds and 79 target genes has 129 nodes and 490 edges (Fig. 3). Particularly, among the 16 compounds (baicalein, beta-carotene, Baicalin, kaempferol, luteolin, quercetin, hydroxysafflor yellow A, isorhamnetin, Bifendate, formononetin, Calycosin, AstragalosideIV, Stigmasterol, sitosterol, Z-ligustilide, Dihydrocapsaicin) were linked to more than five genes. Additionally, 33 genes (PTGS2, MMP9, NOS2, MAPK1, MAPK3, TNF, EGFR, APP, JUN, TP53, IL6, IL2, STAT3, NOS3, IL10, CYCS, VEGFA, HIFA, BCL2, CASP9, CAT, PARP1, CASP3, CCL2, MPO, NFE2L2, CSF2, RELA, ALB, NFKBIA, PPARG, NR3C2, ICAM1) were regulated by more than four compounds. The compound-target gene network showed intimate communications between several components and multiple targets, and facilitated a better understanding of the possible curative results of cerebral ischemia.

3.4. PPI network analysis of target genes

The drug component-disease common target was imported into the STRING database to construct the PPI network model of protein-protein interaction. With a interaction score of > 0.9 was selected, the network of PPI relationships has 79 nodes and 327 edges, the average node degree was 8.28, and PPI enrichment p-value was < 1.0e-16 (Fig. 4A). Cytoscape3.6.1 was used to analyze the degree and combined score of PPI network topological eigenvalues, the degree of node color and size reaction center, the edge thickness and colordepth reflect the combined score. The highest twenty target genes with a high degree of connectivity were selected as the hub genes for cerebral ischemia (Fig. 4B).

3.5. GO and KEGG enrichment analysis of potential target genes

To explore the molecular mechanism of BYHWD on cerebral ischemia, GO enrichment analysis and KEGG pathway enrichment of the 79 potential targets were executed. GO enrichment analysis results included biological process (BP), molecular function (MF) and cellular component (CC). Analyze in the DAVID database, take a value of $P < 0.05$, the results showed that the main biological processes have inflammatory response, negative regulation of apoptosis, angiogenesis, positive regulation of NF-kB transcription factor activity, apoptosis process, immuneresponse, and response to oxidative stress. The molecular function is mainly related to DNA binding, transcription factor activity, heparin binding, heme binding, cytokine activity and growth factor activity. Cell component analysis showed that nucleus and extracellular spacefor the higher proportion. An introduction of the GO analysis was discovered with the top 6 enriched conditions in the BP, CC and MF categories (Fig. 5) and the remarkable 20 biological processes in which they are involved were presented in Table 2. Subsequently, to examine the signaling pathways and functions of these target genes, we performed functional enrichment analysis using KEGG pathways. Obtained the signal pathway with statistical significance ($p < 0.05$), and target genes were found to interact mainly with the TNF signaling pathway, IL-17 signaling pathway, PI3K-Akt signaling pathway, Toll-like receptor signaling pathway, MAPK signaling pathway, NF-kB signaling pathway, and HIF-1 signaling pathway. Therefore, these signaling pathways appear to be closely associated with the potential effects of BYHWD against cerebral ischemia. Identified target genes were listed in Table 3 and take the top 20 signal pathways and draw KEGG bubble diagram (Fig. 6A). Additionally, main functional annotation clustersranked by the Biocarta functional annotation cluster tool are presented in Fig. 6B.

3.6. Experimental validation

To confirm the results of the network analysis and verify the key targets of BYHWD brain protection, we selected three key targets (IL6, VEGFA and HIF1A) for pharmacological validation (Fig. 7). Western blot results showed that compared with the control group (no treatment), BYHWD could significantly inhibit the expression of IL6 and increased the expression of HIF1A and VEGFA ($p < 0.05$).

4. Discussion

Ischemic stroke is one of the leading causes of death and disability worldwide. Studies have shown that the injury mechanism of ischemic stroke includes apoptosis, necrosis, inflammation, immune regulation, oxidative stress, etc[17]. However, no effective treatment has been found to prevent damage to the brain

in such cases except tissue plasminogen activator. This single compound or single target drug has limited effect on ischemic stroke. Therefore, a promising treatment approach for ischemic stroke is utilization of multiple-component agents with multiple targets[18].

Buyang huanwu decoction has the functions of supplementing qi, activating blood circulation and clearing collaterals. As a classic prescription for treating qi deficiency and blood stasis, it has been attached great importance by doctors of all generations. Traditional Chinese medicine cures disease through multi-approach, multi-target and integerconcept. It has unique advantages for thetreatment of complex diseases, but because of the complex composition of traditional Chinese medicine compound to make its modernization process slow. Furthermore, network pharmacology has provided a new way for exploring pharmacological mechanisms of TCMs. many scholars have been made an effort to apply network pharmacology to explore the complex of ingredients, targets, and mechanisms of herbal formulas[19].

In this study, network pharmacology was used to investigate the pharmacological mechanism of BYHWD related to cerebral ischemia, which improved the accuracy of targeted prediction to some extent. Network pharmacological analysis of BYHWD identified seven herbs, 42 compounds, and 79 target gene-regulated major pathways related to cerebral ischemia. In particular, the 16 compounds (baicalein, beta-carotene, Baicalin, kaempferol, luteolin, quercetin, hydroxysafflor yellow A, isorhamnetin, Bifendate, formononetin,Calycosin, AstragalosideIV, Stigmasterol, sitosterol, Z-ligustilide, Dihydrocapsaicin) were linked to more than five genes. A number of studies have reported that baicalein has potent neuroprote ctive properties under in vitro as well as in vivo systems[20]. Beta-carotene serve as an antioxidant, inhibiting free radical production. it may regulate cell growth anddeath[21]. Baicalin inhibited microglial cell activation and reduced inflammation, oxidative damage, and brain edema[22]. Additionally, Kaempferol has strong anti-inflammatory and antioxidant effects. Numerous scientific reports showed that it has beneficial role on different inflammatory-related diseases such as cardiovascular, and neurode generative diseases. Luteolin suppresses inflammation in the brain tissues and regulates different cell signaling pathways[23]. Quercetin has antioxidant stress and neuroprotective effects[24]. moreover, Hydroxysafflor yellow A protects brain microvascular endothelial cells (BMECs) against oxygen glucose deprivation/reoxygenation(OGD/R) induced injury by inhibiting autophagy via the Class IPI3K/Akt/mTOR signaling pathway[25]. Treatment of experimental stroke mice with isorhamnetin attenuated cerebral edema, improved blood-brain barrier function, and upregulated gene expression of tight junction proteins including occludin, ZO-1, and claudin-5[26]. Furthermore, calycosin protected the brain against ischemic injury through inhibiting calpain activation[27]. Dihydrocapsaicin treated cerebral I/R rats attenuate cerebral and BBB damage through inhibition of oxidative stress and inflammatory pathways[28]. In a word, these findings suggest that the main components of BYHWD are effective for treating cerebral ischemia.

Genes with high degrees of differential articulation were actually acquired depending on PPI system analysis, IL6, TNF, VEGFA, HIF1A, MAPK1, MAPK3, JUN, STAT3, IL1B and IL10 were recognized as center genes. Interleukin-6 (IL-6) is a multi-functional cytokine with a wide range of biological activities such as regulation of the immune system, generation of acute phase reactions[29]. Vascular endothelial growth factor (VEGF) is a pleiotropic growth factor that is crucially involved in neurovascular remodeling in the ischemic brain. VEGF promotes angiogenesis, protects ischemic neurons from injury, has potent anti-inflammatory actions, and promotes brain plasticity[30]. HIF1A regulates the expression of genes encoding molecules that participate in erythropoiesis, cell proliferation, and energy metabolism, and is closely associated with the regulation of neuronal survival in ischemia[31, 32]. HIF1A can up-regulate the gene expression of proteins related to the vascular system and promote the angiogenesis of VEGF and its receptors to increase blood flow and reduce ischemic injury[33]. TNF is a typical cytokine involved in the acute phase of systemic inflammation and is closely related to the severity of cerebral ischemia[34]. IL-10 is a potent anti-inflammatory mediator, and, if overexpressed, can suppress neuronal degeneration[35].

GO and KEGG pathway analysis to better comprehend the interaction and action pathway of target genes. In results, GO analyses revealed that target genes were majorly associated with the biological processes of positive regulation of transcription from RNA polymerase II promoter, inflammatory response, transcription, DNA-templated, negative regulation of apoptotic process, positive regulation of transcription, DNA-templated, angiogenesis, response to hypoxia, response to hypoxia. The enriched molecular function ontologies were dominated through DNA binding transcription factor activity, sequence-specific DNA binding,sequence-specific DNA binding, identical protein binding, heparin binding and heme binding so on. KEGG pathway analysis were primarily pertaining to TNF, IL-17, Apoptosis, PI3K-Akt, Toll-like receptor, MAPK, NF-kappa B and HIF-1 signaling pathway. Our results suggest that these pathways might interact to exert their combined effects against cerebral ischemia, which could explain the apparent effects of BYHWD.

Cerebral ischemia results in decreased cerebral blood flow, decreased oxygen supply, induced activation of the HIF-1 signaling pathway, and upregulated HIF1A expression, which contributes to the recovery of blood circulation in the penumbra after cerebral ischemia and the transport of glucose, and mediates hypoxia adaptation after hypoxia, and plays a protective role in promoting cell survival and inhibiting apoptosis in brain tissues[36]. MAPK and PI3K/AKT signaling pathways are the main signaling pathways related to apoptosis after cerebral ischemia. Currently, it is believed that MAPK signaling plays a dual role in the process of cell apoptosis, while PI3K/Akt signaling is an important cell survival signaling pathway. Multiple neurotrophic factors inhibit apoptosis by activating the PI3K/Akt signaling pathway, thus playing a protective role in the brain[37]. The PI3K/Akt signaling pathway is involved in the regulation of various intracellular signaling pathways and plays a key role in promoting cell survival and proliferation, anti-apoptosis, regulating glucose metabolism and protein synthesis[38]. Toll-like receptors (TLRS), as inflammatory signal receptors, play an important role in the inflammatory cascade reaction triggered by cerebral ischemia and are closely related to the expression of various inflammatory mediators. Therefore, it is of great significance to intervene the TLRs signaling pathway in the initial stage of inflammatory response to effectively reduce the inflammatory injury in the acute stage of ischemic stroke[39].

In addition, we verified three of the key targets (IL6, VEGFA and HIF1A) that predicted in the network by using western blot analysis. Therefore, the pharmacological mechanism of BYHWD in the treatment of cerebral ischemia can be more clearly verified. The results showed that compared with the control group (no treatment), BYHWD could significantly inhibit the expression of IL6 and increased the expression of HIF1A and VEGFA.

5. Conclusion

In summary, network pharmacology analysis of BYHWD identified 7 herbs, 42 compounds and 79 target genes associated to cerebral ischemia. The 10 key compounds were baicalein, beta-carotene, Baicalin, kaempferol, luteolin, quercetin, hydroxysafflor yellow A, isorhamnetin, Bifendate, formononetin,Calycosin, AstragalosideIV, Stigmasterol, sitosterol, Z-ligustilide, Dihydrocapsaicin. Based on the results of pathwayenrichment, we determined that effects of BYHWD against cerebral ischemia may be due to that ingredients of BYHWD can simultaneously target multiple pathways like TNF, IL-17, Apoptosis, PI3K-Akt, Toll-like receptor, MAPK, NF-kappa B and HIF-1 signaling pathway and so on. Also, genes with high degrees of differential articulation were actually acquired depending on PPI system analysis, IL6, TNF, VEGFA, HIF1A, MAPK1, MAPK3, JUN, STAT3, IL1B and IL10 were recognized as center genes. Our results indicate that compound-target gene networks can reveal close interactions between multi-components and multi-targets, and enhance our understanding of the potential effects of BYHWD in cerebral ischemia.

Abbreviations

BYHWD, Buyang Huanwu Decoction; ADME, Absorption, Distribution, Metabolism, and Excretion; AHA, American heart association; TCM, Traditional Chinese medicine; OB, oral bioavailability; DL, drug-likeness; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; PPI, protein-protein interaction; BMECs, Brain Microvascular Endothelial cells; OGD, Oxygen Glucose Deprivation;BP, biological process; MF, molecular function; CC, cellular component; IL-6, Interleukin-6; VEGFA, Vascular endothelial growth factor-A; HIF1A, Hypoxia-inducible factor 1-alpha; TLRS, Toll-like receptors.

Declarations

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Author contributions:

WK, LL, CJ, QY designed the experiments; WK, LL conducted experiments and researched literature; WK, CJ, QY, LR collected and analyzed the data; WK wrote the manuscript; WK, CJ, YZ revised the manuscript. DJ, FZ, DY, MY conducted data interpretation; YZ, ZE conducted Funds Collection. All authors commented on the results and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Tables

Due to technical limitations, Table 1 is provided in the Supplementary Files.

Table 2

Biological processes of potential target genes based on GO enrichment analysis.

ID	Biological Process	Genes	P-Value
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	HMGB1,TNF,RELA,PPARG,TP53,IGF1, NFKBIA,RB1,CD40, SIRT1, IL10,VDR, IL17A, APP,HIF1A,SP1,POU5F1,RIPK1, JUN, MAPK3	6.60E-12
GO:0006954	inflammatory response	PIK3CG, HMGB1, CCL2, PTGS2,RELA, TLR4, CD40, AGER, IL10, CXCL10, IL17A, JAK2, NFE2L2, CD14	7.35E-11
GO:0000122	negative regulation of transcription from RNA polymerase II promoter	EGR1,VDR,TNF,POU5F1,RELA,JUN, PPARG, TP53, NFKB1, RB1, SIRT1, DDIT3, EPO	3.56E-07
GO:0006351	transcription, DNA-templated	EGR1,VDR,HIF1A,CEBPB,JUN,MAPK3, PPARG, TP53, STAT3	5.98E-05
GO:0043066	negative regulation of apoptotic process	CASP3, ALB, BCL2, TP53, IGF1, CAT, SIRT1, STAT3	1.78E-04
GO:0045893	positive regulation of transcription, DNA-templated	CEBPB,JUN, PPARG,NFKB1, NFE2L2, STAT3, EPO	1.78E-04
GO:0031663	lipopolysaccharide-mediated signaling pathway	MAPK1, CCL2, TNF, MAPK3, NFKBIA, NOS3, TLR4	7.02E-10
GO:0001525	angiogenesis	PIK3CG,HIF1A,JUN,VEGFA,SERPINE, NOS3, SIRT1	2.14E-05
GO:0042127	regulation of cell proliferation	JUN,NFKBIA,JAK2,NOS2,CD40,PLAU, CXCL10	8.12E-05
GO:0006357	regulation of transcription from RNA polymerase II promoter	FOS,SP1,NFKB1, RB1, NFE2L2,STAT3, SOD2	0.001215038
GO:0001666	response to hypoxia	PLAT,VEGFA,NOS2,AGER, PLAU,EPO	1.96E-05
GO:0051092	positive regulation of NF-kappaB transcription factor activity	TNF, RELA, RIPK1, CAT, CD40, AGER	7.26E-05
GO:0006915	apoptotic process	APP, RIPK1, CYCS, TP53, RB1, EPO	2.98E-04
GO:0055114	oxidation-reduction process	GPX1, NOS3, CAT, NOS2, SOD1, SOD2	0.001431211
GO:0006955	immune response	CSF2, IL6, JUN, CD40, IL10, CXCL10	0.006954104
GO:0043491	protein kinase B signaling	CSF2, CCL2, TNF, IGF1, CD40	1.21E-05
GO:0071356	cellular response to lipopolysaccharide	JUN, SERPINE1, NOS2, IL10, CXCL10	1.82E-04
GO:0006979	response to oxidative stress	GPX1, APP, PTGS2, APOE, MPO	2.91E-04
GO:0045766	positive regulation of angiogenesis	HIF1A, VEGFA, SERPINE1, NOS3, SIRT1	6.75E-04
GO:0043524	negative regulation of neuron apoptotic process	CCL2, APOE, JUN, BCL2, SOD1	7.56E-04

Table 3

Functions of potential target genes based on KEGG pathway analysis.

ID	Pathway	Number of pathway gene
hsa05167	Kaposi sarcoma-associated herpesvirus infection	PTGS2/CALM1/PIK3CG/RELA/RB1/MAPK1/MAPK3/NFKBIA/CASP3/CSF2/CYCS/JUN/NFKB1/STAT3/TP53/VEGFA/IL6/CA
hsa04668	TNF signaling pathway	PTGS2/RELA/MMP9/MAPK1/MAPK3/NFKBIA/TNF/CASP3/CEBPB/CSF2/IL1B/JUN/NFKB1/IL6/ICAM1/CCL2/FOS/SELE/F
hsa05163	Human cytomegalovirus infection	PTGS2/CALM1/RELA/RB1/MAPK1/MAPK3/NFKBIA/TNF/CASP3/CYCS/IL1B/NFKB1/STAT3/TP53/VEGFA/IL6/CASP9/CCL
hsa04933	AGE-RAGE signaling pathway in diabetic complications	RELA/MAPK1/MAPK3/TNF/BCL2/CASP3/IL1B/JUN/NFKB1/STAT3/VEGFA/IL6/ICAM1/CCL2/SELE/AGER/EGR1/NOS3/AC
hsa04657	IL-17 signaling pathway	PTGS2/RELA/MMP9/MAPK1/MAPK3/NFKBIA/TNF/CASP3/CEBPB/CSF2/IL1B/JUN/NFKB1/IL6/CCL2/FOS/CXCL10/FOSB,
hsa05161	Hepatitis B	RELA/MMP9/RB1/MAPK1/MAPK3/NFKBIA/TNF/BCL2/CASP3/CYCS/JUN/NFKB1/STAT3/TP53/IL6/TLR4/CASP9/FOS/CRE
hsa05152	Tuberculosis	CALM1/NOS2/RELA/MAPK1/MAPK3/TNF/BCL2/CASP3/CEBPB/CYCS/IL1B/NFKB1/IL6/TLR4/CASP9/CD14/VDR/IL10/CR
hsa05145	Toxoplasmosis	NOS2/PIK3CG/RELA/MAPK1/MAPK3/NFKBIA/TNF/BCL2/CASP3/CYCS/NFKB1/STAT3/TLR4/CASP9/IL10/CD40/HSPA1A/
hsa04210	Apoptosis	RELA/MAPK1/MAPK3/NFKBIA/TNF/BCL2/CASP3/CYCS/JUN/NFKB1/TP53/CASP9/PARP1/BBC3/ATM/FOS/RIPK1/DDIT3
hsa05142	Chagas disease (American trypanosomiasis)	NOS2/RELA/MAPK1/MAPK3/NFKBIA/TNF/IL1B/JUN/NFKB1/IL6/TLR4/CCL2/FOS/IL10/ACE/SERPINE1/CCL3
hsa05418	Fluid shear stress and atherosclerosis	CALM1/RELA/MMP9/TNF/BCL2/IL1B/JUN/NFKB1/TP53/VEGFA/NFE2L2/ICAM1/CCL2/FOS/SELE/NOS3/PLAT
hsa05202	Transcriptional misregulation in cancer	PPARG/RELA/MMP9/CEBPB/CSF2/IGF1/MPO/NFKB1/PLAU/TP53/IL6/ATM/CD14/CD40/SP1/DDIT3/PLAT
hsa05169	Epstein-Barr virus infection	RELA/RB1/NFKBIA/TNF/BCL2/CASP3/CYCS/JUN/NFKB1/STAT3/TP53/IL6/CASP9/ICAM1/RIPK1/CD40/CXCL10
hsa05166	Human T-cell leukemia virus 1 infection	RELA/RB1/MAPK1/MAPK3/NFKBIA/TNF/CSF2/JUN/NFKB1/TP53/IL6/ICAM1/ATM/FOS/CD40/EGR1/CREB1
hsa04151	PI3K-Akt signaling pathway	PIK3CG/RELA/MAPK1/MAPK3/BCL2/IGF1/NFKB1/TP53/VEGFA/IL6/TLR4/CASP9/NOS3/CREB1/JAK2/EPO/CHRM1
hsa04620	Toll-like receptor signaling pathway	RELA/MAPK1/MAPK3/NFKBIA/TNF/IL1B/JUN/NFKB1/IL6/TLR4/CD14/FOS/RIPK1/CD40/CXCL10/CCL3
hsa05162	Measles	RELA/NFKBIA/BCL2/CASP3/CYCS/IL1B/JUN/NFKB1/STAT3/TP53/IL6/TLR4/CASP9/BBC3/FOS/HSPA1A
hsa05164	Influenza A	RELA/MAPK1/MAPK3/NFKBIA/TNF/CASP3/CYCS/IL1B/NFKB1/IL6/TLR4/CASP9/ICAM1/CCL2/CXCL10/JAK2
hsa05170	Human immunodeficiency virus 1 infection	CALM1/RELA/MAPK1/MAPK3/NFKBIA/TNF/BCL2/CASP3/CYCS/JUN/NFKB1/TLR4/CASP9/ATM/FOS/RIPK1
hsa04010	MAPK signaling pathway	RELA/MAPK1/MAPK3/TNF/CASP3/IGF1/IL1B/JUN/NFKB1/TP53/VEGFA/CD14/FOS/JUND/DDIT3/HSPA1A
hsa05133	Pertussis	CALM1/NOS2/RELA/MAPK1/MAPK3/TNF/CASP3/IL1B/JUN/NFKB1/IL6/TLR4/CD14/FOS/IL10
hsa04064	NF-kappa B signaling pathway	PTGS2/RELA/NFKBIA/TNF/BCL2/IL1B/NFKB1/PLAU/TLR4/PARP1/ICAM1/ATM/CD14/RIPK1/CD40
hsa04066	HIF-1 signaling pathway	NOS2/RELA/MAPK1/MAPK3/BCL2/IGF1/NFKB1/STAT3/VEGFA/IL6/TLR4/HIF1A/NOS3/SERPINE1/EPO

Figures

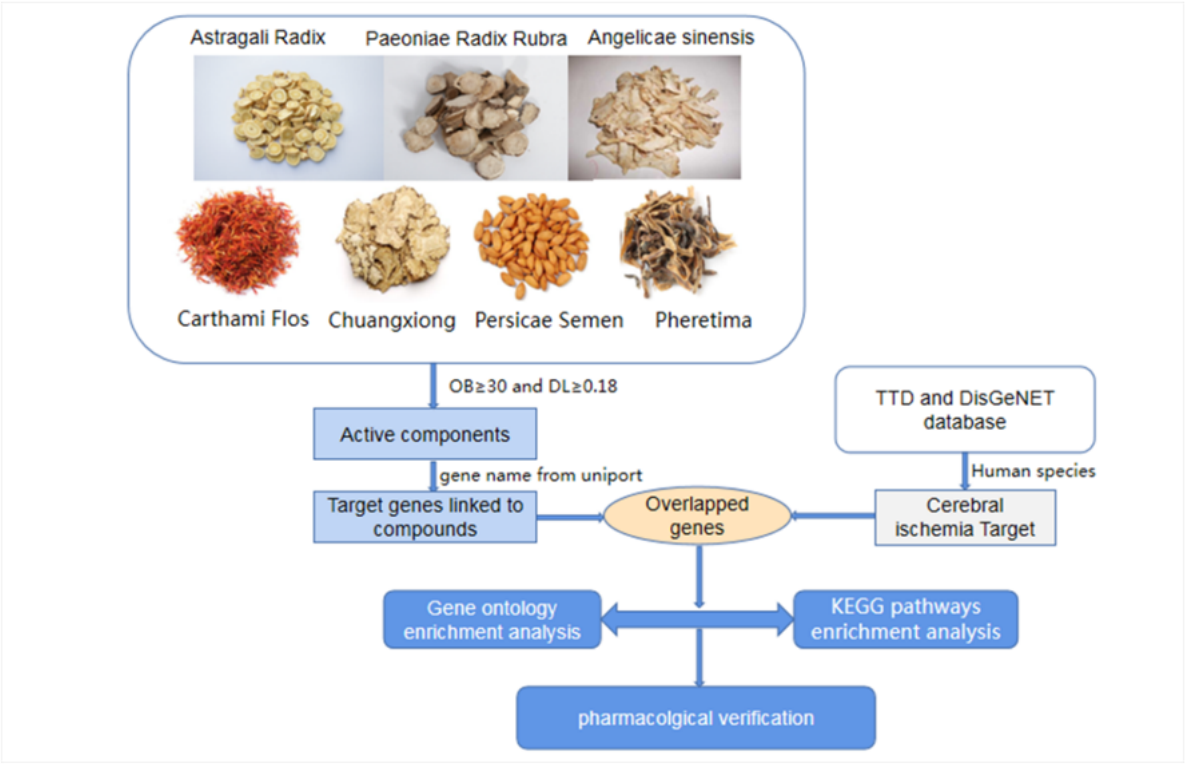

Figure 1

Workflow of network pharmacology analysis.

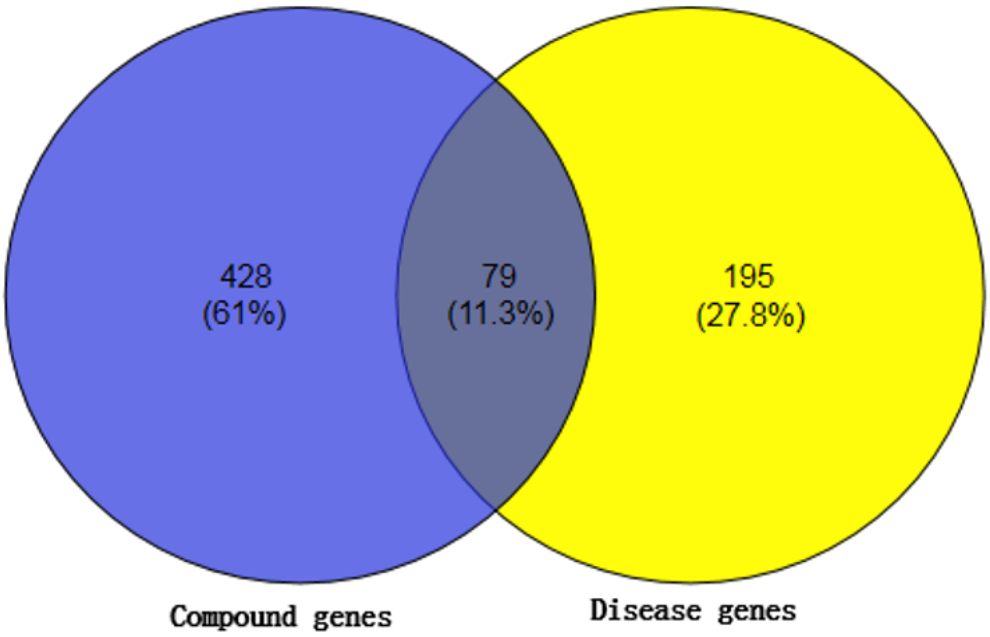

Figure 3

Venn diagram for compound genes and disease genes.

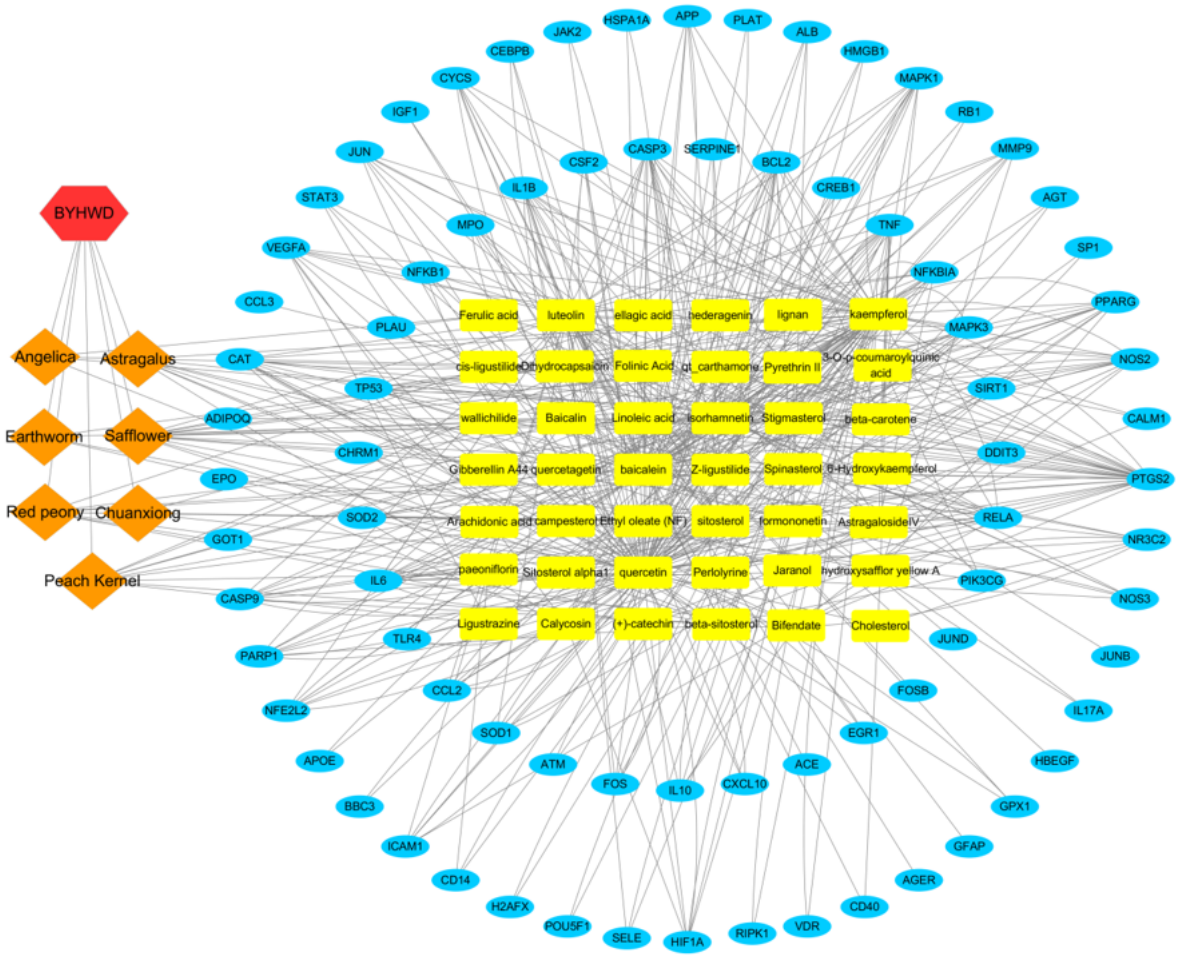

Figure 5

Network of BYHWD, seven herbal medicines and 42 compounds predicted to have 79 common potential protein targets. The blue ellipses represent the protein targets, orange diamonds delineate the herbal medicines, yellow ellipses represent the compounds and the red hexagon delineates the BYHWD.

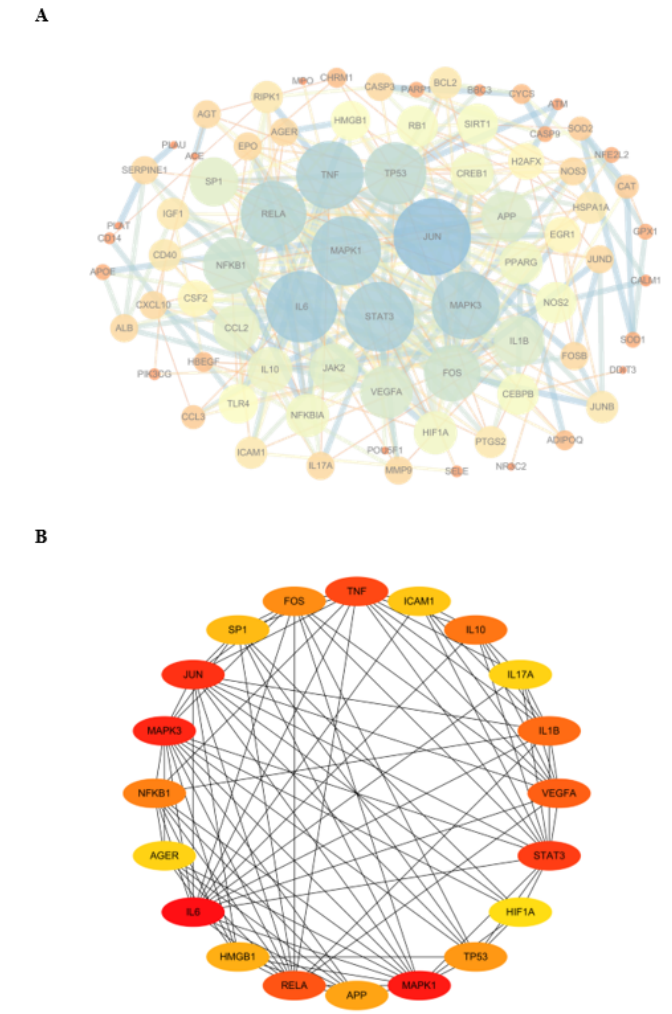

Figure 7

Protein–protein interaction (PPI) networks of active ingredients of BYHWD for the treatment of cerebral ischemia. (A) Each node represents the relevant gene, the edge means line thickness indicates the strength of data support. (B) Hub top 20 genes in PPI network, the darker the color, the higher the score.

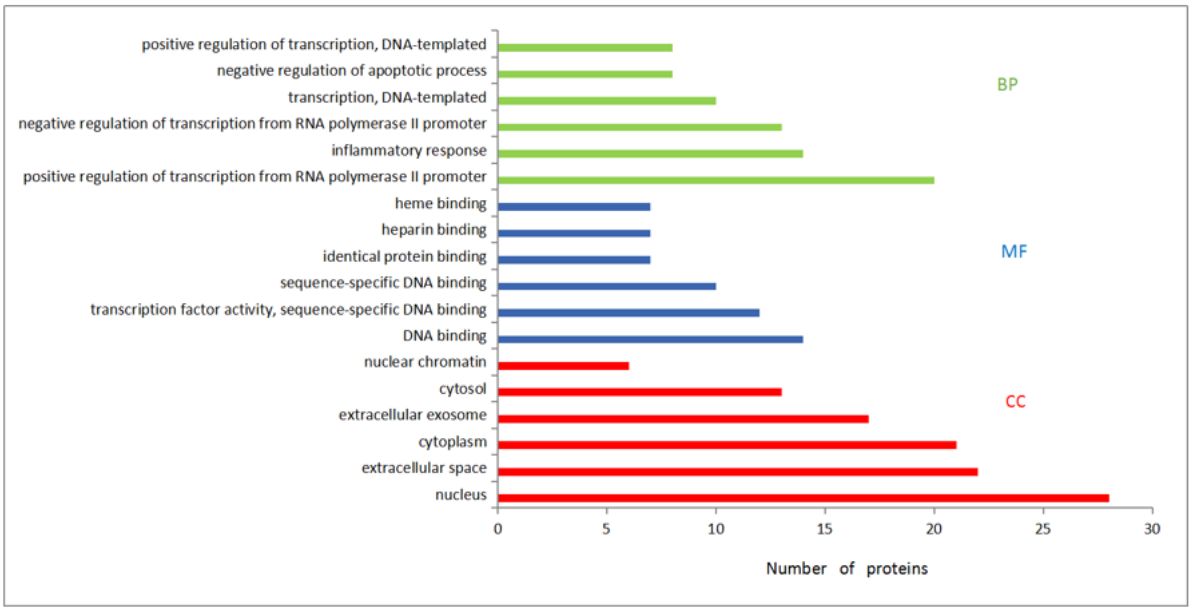

Figure 9

GO analysis of candidate targets. Database showed the six remarkably enriched items in the biological processes (BP), cell component (CC) and molecular function (MF).

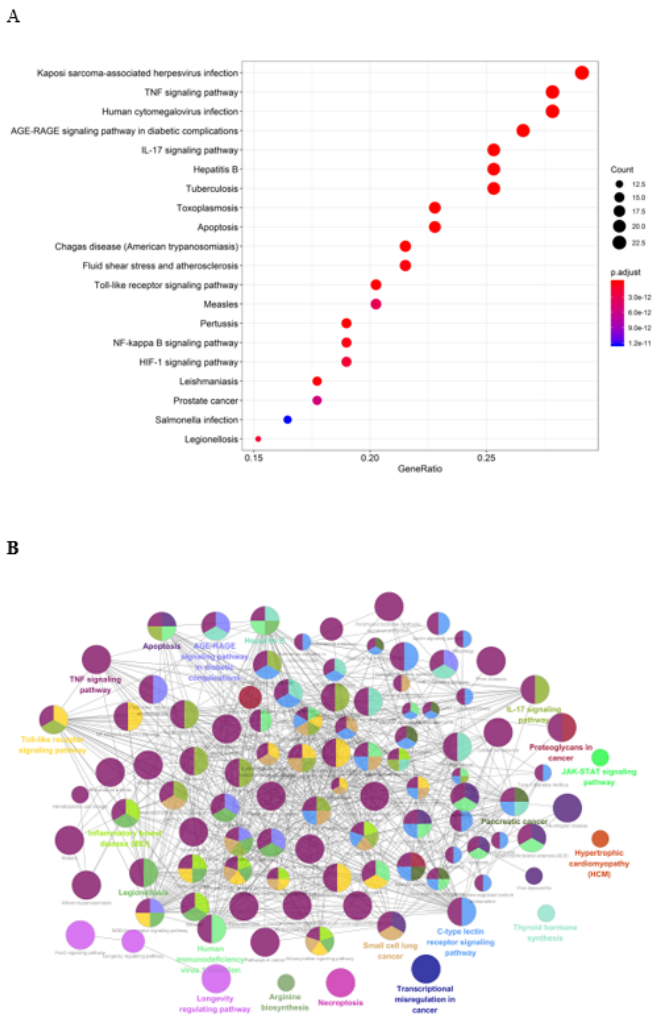

Figure 11

(A) KEGG pathways of target genes. (B) Main functional annotation clusters by Biocarta analysis.

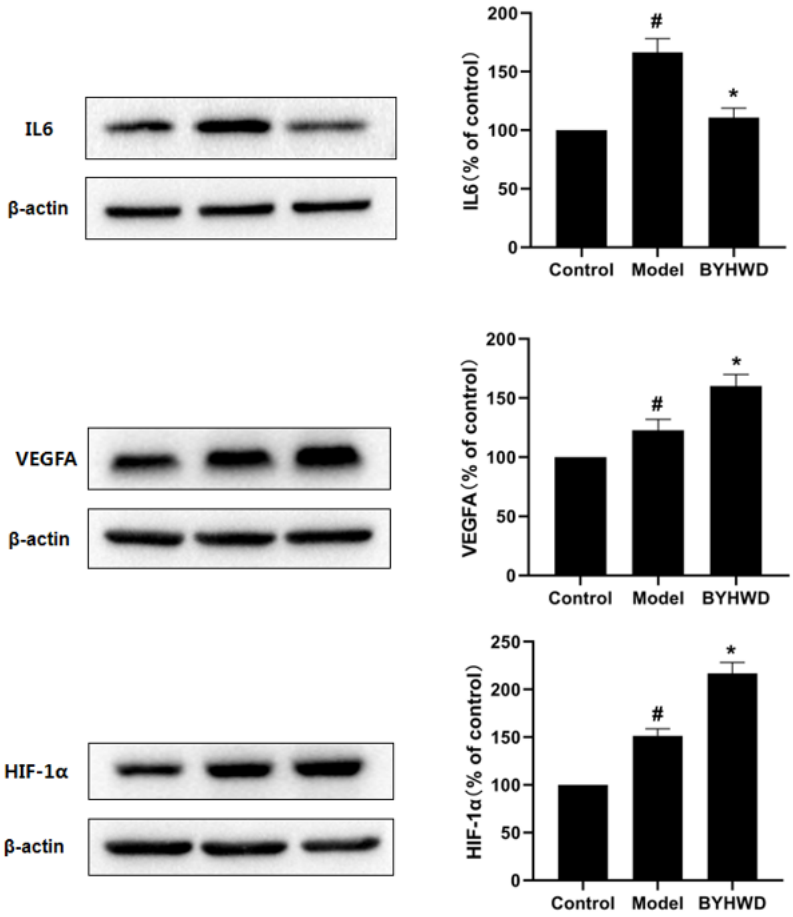

Figure 13

Effects of BYHWD on the levels of IL6, VEGFA and HIF-1α in BMECs. (#p<0.05compared with control group; *p<0.05compared with model group).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.docx
- Table.docx