

A combination of clinical study and Network Pharmacology approach to explore the efficacy and mechanism of Buxue Yimu Pills on gynecological anemia

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Research

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Abstract

Background

Iron supplement is the first-line treatment for gynecological anemia (GA). However, its performance is limited by common gastrointestinal reactions and some inadequate responses. Traditional Chinese medicine (TCM) has a long history in the treatment of gynecological conditions but has been restricted by limited high-quality research, unknown bioactive ingredients, and mechanisms.

Objectives

These studies aim to compare the clinical efficacy of Buxue Yimu Pills (BYP), ferrous sulfate, and the addition of BYP to ferrous sulfate on GA through oral administration, and to investigate the mechanisms of BYP using network pharmacology approach.

Design:

Prospective, open-label, comparative, randomized, multicenter clinical trial.

Setting:

Gynecology departments in three public hospitals.

Patients:

170 patients with hemoglobin of 70–110 g/L were recruited and randomized into three groups: BYP group, oral iron group, and combined BYP & oral iron group. After a four-week treatment, the complete blood count (CBC) along with the markers for iron metabolism were determined for 138 patients. Furthermore, network pharmacology was performed to identify the active ingredients and potential mechanisms of BYP.

Results

While iron-treated groups showed elevated hemoglobin in accompany with significant changes in iron metabolism biomarkers, the BYP group exhibited hemoglobin improvement without apparent changes in iron metabolism markers. The network pharmacology identified 27 bioactive compounds and 145 targets of BYP on GA. A number of biological processes and pathways were identified, including regulation of inflammation, regulation of steroid hormone, angiogenesis and hemostasis, response to decreased oxygen levels, effects on myeloma cell, and response to metal ions.

Conclusions

BYP contributes to the practical improvement of gynecological anemia potentially through multi-target mechanisms independent from increasing hemopoietic raw material-iron.

Background

Anemia, one of the major public health issues worldwide, affects about one-quarter of the world's population, especially among preschool children and young women¹. There are many common gynecologic diseases associated with anemia², such as uterine fibroids, adenomyosis, endometrial polyps, ovulation disorders, ectopic pregnancy, and abortion, affecting especially young women³. Besides, cancer-related anemia and therapy-induced anemia are common in patients with gynecological malignancies^{4–6}. Oral or intravenous iron supplements is the major therapeutic approach, while its common gastrointestinal side effects, including nausea, constipation, and diarrhea, can lower patient compliance. Over 40% of reproductive age women were reported to fail to adhere to medication⁷. Moreover, patients with gynecological anemia (GA) have been observed to be associated with inflammatory states⁸, causing limited iron absorption and utilization^{9,10}. Therefore, the exploration of alternative treatment options is encouraged. Traditional Chinese medicine (TCM) has been utilized to treat gynecological diseases and anemia for a long time, showing high safety, tolerability, and compliance even among the patients with malignancy, chronic kidney disease as well as the elderly¹¹. Due to the lack of high-quality randomized controlled trial (RCT) evidence and intensive study, the efficacy and therapeutic mechanisms of TCMs have yet to be well understood or appreciated.

Buxue Yimu Pills (BYP), a TCM with primary efficacy of Qi-reinforcing and nourishing blood, consists of *Angelicae Sinensis Radix (Danggui)*, *Hedysarum Multijugum Maxim (Huangqi)*, *Colla Corii Asini (Ejiao)*, *Leonuri Herba (Yimucao)*, and *Citrus Reticulata (Chenpi)* according to Pharmacopoeia of the People's Republic of China. Previous studies have shown that *Yimucao* promoted uterine contractions, thus reducing vaginal

bleeding; *Ejiao*, *Danggui*, *Huangqi*, and *Chenpi* could benefit Qi, activate blood circulation and promote hematopoiesis¹²; *Huangqi* and *Danggui* exerted anti-inflammatory effects^{13,14}. Therefore, we hypothesized that BYP could treat GA through a variety of mechanisms.

To comprehensively assess its clinical effect and herbal pharmacological mechanisms, we performed a randomized clinical trial at three centers, followed by a network pharmacology research.

Materials And Methods

Clinical study

Experimental design and setting.

To compare the effects of Buxue Yimu Pills (BYP), ferrous sulfate, and a combination of both treatments on GA, a prospective, randomized controlled trial was conducted between 2017 and 2019 at Gynecology departments of three public hospitals, including Peking Union Medical College Hospital (PUMCH), West China Hospital of Sichuan University, and Hangzhou Maternity and Child Health Care Hospital.

This study was approved by the Ethics Committee of PUMCH (No. ZS-1254) at January 2017, and registered at the United States National Institutes of Health (registration number NCT03232554, www.clinicaltrials.gov).

Patients

Adult females aged 18–50 years with hemoglobin of 70–110 g/L associated with gynecological disorders were enrolled following the informed consent. Exclusion criteria were: (1) patients with uncontrolled gastrointestinal inflammation or bleeding or urological bleeding; (2) patients presented with other diseases leading to iron deficiency; (3) pregnant women; (4) patients complicated with mental abnormalities or other severe diseases; (5) patients with a history of malignancy, chemotherapy or radiotherapy; (6) patients treated with iron supplements, blood transfusion or participating in any other clinical trial within the past month. Eligible participants were randomized into three groups: BYP group received Buxue Yimu Pills (24 g/day); oral iron group received ferrous sulfate tablets (0.9 g/day), and BYP & oral iron group received both drugs above simultaneously. All drugs were provided by ZhuZhou QianJin Pharmaceutical Co., Ltd. The total treatment periods were four weeks.

Assessments

Assessments were scheduled pre-and post-treatment. A basic physical examination including vital signs, weight, height, body mass index (BMI), blood pressure was performed. Venous blood samples were collected to detect complete blood count, including hemoglobin (Hb), mean cellular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells (RBC), hematocrit (HCT), reticulocyte (RET) count. Iron indexes of serum iron (SI), serum ferritin (SF), and total iron-binding capacity (TIBC) were analyzed. Moreover, the safety assessment, including liver function, renal function, and a blood coagulation index, were also evaluated (data not shown).

Statistical analysis

Statistical analysis was carried out using SPSS version 20.0 (IBM). Data from normally distributed parameters are presented as means plus or minus standard deviation (SD); paired t-tests were used to compare the intragroup difference pre- and post-treatment, and one-way analysis of variance (ANOVA) was applied for intergroup comparisons. Variables not normally distributed are expressed as median plus 25–75 interquartile range (IQR); intragroup and intergroup comparisons were evaluated with Wilcoxon matched-pairs signed-rank tests and Kruskal-Wallis tests. A *p*-value of less than 0.05 (*p* < 0.05) was considered significant in all statistical analyses.

Network pharmacology analysis

Bioactive compounds and compound targets of Buxue Yimu Pills

Composite compounds were searched manually with the major ingredients of BYP "*Angelicae Sinensis Radix*", "*Hedysarum Multijugum Maxim*", "*Colla Corii Asini*", "*Leonuri Herba*" and "*Citrus Reticulata*", in Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (TcmSP, <http://isp.nwu.edu.cn/tcmsp.php>)³, and Database of Traditional Chinese Medicine and chemical composition from Shanghai Institute of Organic Chemistry (http://www.organchem.csdb.cn/scdb/main/tcm_introduce.asp). Next, a combination of oral bioavailability $\geq 30\%$ along with drug-likeness ≥ 0.18 was applied to explore the bioactive compounds. Potential treatment targets of those bioactive compounds were subsequently obtained from TcmSP; and converted to target genes with official symbols using Uniprot Knowledgebase (<http://www.uniprot.org/>).

Target genes of GA

GA-associated targets were retrieved from the Online Mendelian Inheritance in Man (OMIM) database (<https://www.omim.org/>) and GeneCard Database (<https://www.genecards.org/>), using "gynecological anemia" as the keyword. Data were extracted and summarized without duplication.

Network construction

Potential therapeutic targets are the intersections between the compound targets of BYP and GA-associated targets, which were generated with the VennDiagram R package. After importing the main ingredients, bioactive compounds, and potential therapeutic targets, the compound-target-disease network was built and visualized by Cytoscape software (version 3.6.1, <http://cytoscape.org/>).

Functional analysis

Based on the obtained potential therapeutic targets, enrichment analysis was performed using the online tool Metascape (<https://metascape.org>)¹⁵, including Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

A protein-protein interaction (PPI) network was constructed via STRING (Version 11.0, <https://string-db.org/>) with a minimum required interaction score of 0.400. We subsequently used the Molecular Complex Detection tool (MCODE) plugin to identify significant clusters in this PPI network.

Results

Baseline characteristics

Out of 170 enrolled patients with GA, 138 patients (81.2%) completed the four-week treatment and were included in the data analysis: BYP group (n=58), oral iron group (n=37), and the combination group (n=43). The baseline characteristics of all three groups showed no statistical difference (Table 1).

Laboratory parameters

Table 2 shows the values of laboratory parameters before and after treatment. After four weeks of treatment, patients in three groups exhibited a significant increase in Hb, RBC, and HCT ($p < 0.01$), but no significant differences in RET. The changes of Hb (g/L) are listed in the following: BYP group (16.44 ± 14.12), oral iron group (30.84 ± 15.46), and the combination group (37.82 ± 16.9). Pairwise comparison show there are statistically significant differences between BYP group and oral iron group ($p < 0.01$) or BYP group and the combination group ($p < 0.01$), while differences between the two iron-containing groups were not remarkable ($p = 0.062$) (Figure 1).

According to the iron indexes, it is expected that there were significant increases in SI and SF accompanied by a substantial decrease in TIBC in the oral iron group ($p < 0.01$), but no differences were detected in BYP group ($p > 0.05$). However, unexpectedly, the combination group, whose Hb was improved most significantly after treatment, showed noticeable increases in SI and SF but no apparent change in TIBC.

Compound-target-disease network

Thirty-two molecules, the bioactive compounds, with oral bioavailability $\geq 30\%$ and drug-likeness ≥ 0.18 were identified through network pharmacology analysis (Table 3), which belongs to the ingredients of *Huangqi* (20), *Yimucao* (8), *Chenpi* (5), and *Danggui* (2), respectively. Kaempferol (MOL000422, a unique given ID from TcmSP), quercetin (MOL000098), and isorhamnetin (MOL000354) are the three components common in *Huangqi* and *Yimucao*. ZINC14758732 (MOL000438), Jaranol (MOL000239), isoflavanone (MOL000398), isomucronulatol-7,2'-di-O-glucoside (MOL000439), and 5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside (MOL000374) in *Huangqi* were not included in this network. 1657 potential treatment targets of BYP with official symbols and 270 target genes of GA were identified, among which a number of 145 genes intersected (Figure 2(a)). The compound-target-disease network was constructed with 818 nodes and 1436 connecting edges and shown in Figure 2(b). After analyzing the network with the NetworkAnalyzer plugin in Cytoscape, quercetin (MOL000098, mol17), which is shared by *Huangqi* and *Yimucao*, was identified as the most important compound with the overwhelmingly highest degree of 107. Evidence from previous studies has shown that quercetin can improve inflammation and chemotherapy-related anemia through reducing oxidative stress, ameliorating the iron status and protecting red blood cells¹⁶⁻¹⁸.

Functional analysis

Figure 3 shows the results of functional enrichment analysis: KEGG pathway, Gene Ontology Biological Process (GOBP), Gene Ontology Cellular Component (GOCC), and Gene Ontology Molecular Function (GOMF), which were mostly associated with cancer, inflammation, steroid hormone regulation, oxidative stress, metabolic syndrome, nervous system diseases, and infectious diseases. Many of these terms are too general for annotation, so we took a deeper insight into their child function clusters and further summarized the biological processes into several underlying functional modules: regulation of inflammation, regulation of steroid hormone, angiogenesis and hemostasis, response to decreased oxygen levels, effects on myeloma cell and response to metal ion (Supplementary Table1). The biological processes with the highest enrichment score in each module were selected to build a compound-target-underlying biological process network (Figure 4), with 139 nodes, 483 edges.

Protein-protein interaction (PPI) network of intersecting targets was constructed with STRING, including 145 nodes and 3196 edges (Figure 5 (a)). According to the selection criteria of MCODE scores ≥ 5 , degree cutoff = 2, node score cutoff = 0.2, K-core = 2, and max depth = 100, a total of 6 significant clusters were identified (Figure 5(b), Supplementary Table 2).

Discussion

Consistent with previous studies, after four-week treatment, patients taking ferrous sulfate or ferrous sulfate &BYP showed an elevation of Hb in accompany with significant changes in iron metabolism markers, typically with elevated SI, Fer, and decreased TIBC (oral iron group). One unexpected finding was that we did not find a significant decline of TIBC in the combination group, even though it did show a most significant increase in Hb. BYP group also exhibited hemoglobin improvement without obvious changes in iron metabolism markers. This result may be explained by the fact that patients with GA are commonly found to have several closely related features: abnormal uterine bleeding (AUB), iron deficiency anemia (IDA), chronic inflammatory state¹⁹. On the one hand, an inflammatory state can upregulate the level of hepcidin, a liver-specific peptide, increasing the degradation of ferroportin (FPN) at enterocytes, hepatocytes, and macrophages, leading to iron overload in nonhematopoietic cells but the iron deficiency in erythroid precursors²⁰. In addition, chronic inflammation can cause unbalanced blood production of hematopoietic stem cells (HSCs), impaired HSC self-renewal as well as impaired erythroid production²¹⁻²³. The previous study has found a negative correlation between Hb concentration and inflammatory markers, especially interleukin 6 (IL-6)⁹. As mentioned before, Buxue Yimu Pills can reinforce qi and nourish the blood, whose main components have previously been observed to have different efficacies of promoting hematopoiesis and anti-inflammatory effects¹²⁻¹⁴. After treatment, with decreased inflammation and increased hematopoiesis, iron in nonhematopoietic cells can be transported to erythroid precursors more efficiently; subsequently, levels of circulating iron decrease. As a consequence, we did observe a slight upward trend of TIBC in the BYP group and an offsetting decline of TIBC in the combination group, while Hb in both groups elevated.

We also used network pharmacology technology to investigate the mechanisms of Buxue Yimu Pills further. From the compound-target-disease network, 27 bioactive compounds in Buxue Yimu Pills were identified, among which quercetin, a common component of *Huangqi* and *Yimucao*, may play a critical role. One hundred forty-five potential therapeutic targets of these compounds were confirmed. Biological process and pathway enrichment analysis demonstrated that multiple mechanisms including regulation of inflammation, regulation of steroid hormone, angiogenesis and hemostasis, response to decreased oxygen levels, effects on myeloma cell as well as response to metal ions, may contribute to improving GA. Previous studies have demonstrated that reduction of inflammation and oxidative stress can alleviate the absorption and utilization of iron, increase the production of erythropoietin (EPO) and improve hematopoietic function^{24,25}. Metal ion transmembrane transport contributed to iron uptake and maintenance of iron homeostasis^{26,27}. In addition, ovulation disorders and coagulation dysfunction have been the main nonstructural etiologies of AUB^{28,29}. Therefore, the regulation of steroid hormone and hemostasis can have significant implications for this condition.

Since Buxue Yimu Pills may improve anemia through multiple targets, it could have potential applications in diseases needing to deal with oxidative stress damage, such as thalassemia and inflammatory anemia. But on the other hand, one crucial point that needs to be raised is that reduction of oxidative stress might reduce the therapeutic efficacy of anti-neoplastic drugs acting through mechanisms of reactive oxygen species (ROS)-induced injury³⁰, which was also indicated in KEGG enrichment (hsa01524, platinum drug resistance). Therefore, Buxue Yimu Pills may not be recommended to patients undergoing cancer treatment.

Conclusion

A combination of clinical study and network pharmacology approaches not only demonstrates that Buxue Yimu Pills contributes to the effective improvement of GA through multiple mechanisms but also provides us more comprehensive insights into integrated multidimensional relationships between drugs and diseases. Finally, some limitations need to be acknowledged. The sample size and the follow-up duration may have been insufficient to reveal apparent differences between cohorts, and inflammatory indicators were not monitored. Accordingly, based on the results of our study, continued efforts are needed to further verification through larger-scale, more prolonged follow-up cohort research with targeted designs, as well as molecular mechanism studies.

Abbreviations

ANOVA: one-way analysis of variance; AUB: abnormal uterine bleeding; BYP: Buxue Yimu Pills; CBC: complete blood count; EPO: erythropoietin; FPN: ferroportin; GA: gynecological anemia; GO: Gene Ontology; GOBP: Gene Ontology biological process; GOCC: Gene Ontology Cellular Component

GOMF Gene Ontology molecular function; Hb: hemoglobin; HCT: hematocrit; HSC: hematopoietic stem cell; IBM: International Business Machines Corporation; IDA: iron deficiency anemia; IL: interleukin; IQR: interquartile range; KEGG: Kyoto Encyclopedia of Genes and Genomes; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCODE: Molecular Complex Detection tool; MCV: mean cellular volume; OMIM: Online Mendelian Inheritance in Man; RBC: red blood cells; RET: reticulocyte; ROS: reactive oxygen species; SD: standard deviation; SF: serum ferritin; SI: Iron indexes of serum iron; TCM: Traditional Chinese medicine; TcmSP: Traditional Chinese Medicine System Pharmacology Database and Analysis Platform; TIBC: total iron-binding capacity

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Peking Union Medical College Hospital (No. ZS-1254) at January 2017, and registered at the United States National Institutes of Health (registration number NCT03232554, www.clinicaltrials.gov). A written informed consent was signed by all

participants.

Consent to publish

Not applicable.

Availability of data and materials

All relevant data and materials are within the paper and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

WYF implemented the research plan and was responsible for data curation, data mining, formal analysis, conceptualization and writing of the manuscript. DY, MRL, LD, LB, WX, DM participated in the enrollment of the patients and data collection. SAJ provided a critical contribution to the organization and cooperation of this multi-center task.

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Tables

Table 1. Comparison of patient characteristics and laboratory data at baseline.

	BYP (n=58)	Iron (n=37)	BYP & Iron (n=43)	P value
Age (y)	40.68±7.43	40.62±7.03	39.17±7.37	0.579
BMI (kg/m ²)	23.26±3.22	21.94±2.63	23.05±3.52	0.076
Pulse(bpm)	81.96±9.27	82.33±10.75	79.59±8.69	0.385
SBP (mmHg)	116.15±13.64	116.88±14.27	116.66±16.05	0.969
DBP (mmHg)	71.79±9.23	73.18±9.62	72.68±8.67	0.906
Hb (g/L)	93.56±10.05	93.97±9.75	91.13±11.14	0.538
MCV (fl)	79.28±9.07	80.19±7.77	77.38±9.19	0.231
MCH (pg)	25.17±3.59	25.24±3.63	24.05±4.08	0.469
MCHC (g/L)	307.1±47.3	309.14±16.25	308.42±19.75	0.958
RBC (*109/L)	3.78±0.53	3.71±0.49	3.86±0.54	0.553
HCT (%)	29.85±2.78	30.49±2.58	29.36±2.85	0.28
RET	77.9(55.5,99.4)	70.1(35.3,97.9)	81.1(57.5,109.4)	0.091
SI (umol/L)	15.20(7.78,33.25)	20.00(10.35,26.50)	13.10(8.1,22.55)	0.129
Fer (ug/L)	9.7(4.1,22.6)	8.0(4.0,14.2)	9.9(4.0,33.5)	0.417
TIBC (g/L)	361.0(57.6,415.0)	344.5(58.9,406.5)	321.0(57.7,411.5)	0.898

Table2: The comparison of blood indices and iron metabolism markers between three groups before and after the 4-week treatment.

	BYP			Iron			BYP & Iron		
	Pre	4 weeks	P-value	Pre	4 weeks	P-value	Pre	4 weeks	P-value
Hb (g/L)	93.56±10.05	110±13.87*	<0.01*	93.97±9.75	124.81±13.87*	<0.01*	91.13±11.14	128.95±12.88*	<0.01*
MCV (fl)	79.28±9.07	78.5±8.73	0.322	80.19±7.77	84.97±7.42*	<0.01*	77.38±9.19	83.37±7.16*	<0.01*
MCH (pg)	25.17±3.59	24.78±3.33	0.23	25.24±3.63	27.53±3.00*	<0.01*	24.05±4.08	27.01±2.64*	<0.01*
MCHC (g/L)	307.1±47.3	314.32±11.48	0.347	309.14±16.25	324.59±13.5*	<0.01*	308.42±19.75	323.18±10.56*	<0.01*
RBC (*109/L)	3.78±0.53	4.46±0.42*	<0.01*	3.71±0.49	4.57±0.60*	<0.01*	3.86±0.54	4.8±0.47*	<0.01*
HCT (%)	29.85±2.78	34.72±3.45*	<0.01*	30.49±2.58	38.27±4.10*	<0.01	29.36±2.85	39.74±4.00*	<0.01
RET	77.9(55.5,99.4)	77.7(59.1,91.1)	0.826	70.1(35.3,97.9)	59.8(42.0,78.5)	0.788	81.1(57.5,109.4)	72.4(53.6,91.5)	0.517
SI (umol/L)	15.20(7.78,33.25)	19.80(8.78,32.1)	0.871	20.00(10.35,26.50)	38.80(12.23,59.30)	<0.01**	13.10(8.1,22.55)	26.10(13.90,59.90)	<0.01**
Fer (ug/L)	9.7(4.1,22.6)	8.0(5.0,18.5)	0.258	8.0(4.0,14.2)	26.5(14.5,31.8) *	<0.01**	9.9(4.0,33.5)	30.0(16.0,42.6) *	0.022**
TIBC (g/L)	361.0(57.6,415.0)	380.0(69.8,430.0)	0.12	344.5(58.9,406.5)	274.0(61.8,367.8)	<0.01**	321.0(57.7,411.5)	310.0(67.2,343.0)	0.081

Note: Significant difference with Paired T test (*) or Wilcoxon test (**)

Table3: Main ingredients of Buxue Yimu Pills and their bioactive compounds.

Ingredients	English name	Latin	Mol	Molecule	OB (%)	DL	
(Chinese		name	ID	Name			
Pinyin name)							
Chenpi	Dried Tangerine peel	<i>Citrus</i>	MOL004328	naringenin	59.29	0.21	
			MOL005100	(Rac)-Hesperetin	47.74	0.27	
		<i>Reticulata</i>	MOL005815	Citromitin	86.9	0.51	
			MOL005828	nobiletin	61.67	0.52	
			MOL000359	sitosterol	36.91	0.75	
Danggui	Angelica	<i>Angelicae</i>	MOL000358	beta-sitosterol	36.91	0.75	
			<i>Sinensis Radix</i>	MOL000449	Stigmasterol	43.83	0.76
Huangqi	Astragalus	<i>Hedysarum</i>	MOL000392	formononetin	69.67	0.21	
			MOL000422	kaempferol	41.88	0.24	
		<i>Multijugum</i>	MOL000417	Calycosin	47.75	0.24	
			<i>Maxim.</i>	MOL000438#	ZINC14758732	67.67	0.26
				MOL000098	quercetin	46.43	0.28
		MOL000239#		Jaranol	50.83	0.29	
		MOL000398#		isoflavanone	109.99	0.3	
		MOL000378		7-O-methy-lisomucronulatol	74.69	0.3	
		MOL000354		isorhamnetin	49.6	0.31	
		MOL000380		Astrapterocarpan	64.26	0.42	
		MOL000371		3,9-di-O-methylnissolin	53.74	0.48	
		MOL000442		1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48	
		MOL000439#		isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62	
		MOL000387		Bifendate	31.1	0.67	
		MOL000374#		5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside	41.72	0.69	
		MOL000433		FA	68.96	0.71	
		MOL000296		hederagenin	36.91	0.75	
		MOL000211		Mairin	55.38	0.78	
		MOL000033		(24S)-24-Propylcholesta-5-ene-3beta-ol	36.23	0.78	
MOL000379	9,10-dimethoxypterocarpan-3-O-β-D-glucoside	36.74		0.92			
Yimucao	Motherwort	<i>Leonuri</i>	MOL001439	arachidonic acid	45.57	0.2	
			MOL000422	kaempferol	41.88	0.24	
		<i>Herba</i>	MOL000098	quercetin	46.43	0.28	
			MOL000354	isorhamnetin	49.6	0.31	
			MOL001422	iso-preleoheterin	66.29	0.33	
			MOL001421	preleoheterin	85.97	0.33	
			MOL001418	galeopsin	61.02	0.38	
			MOL001420	beta-Sitosterone	38	0.76	

Note: # indicates the component not involved in the following network.

Figures

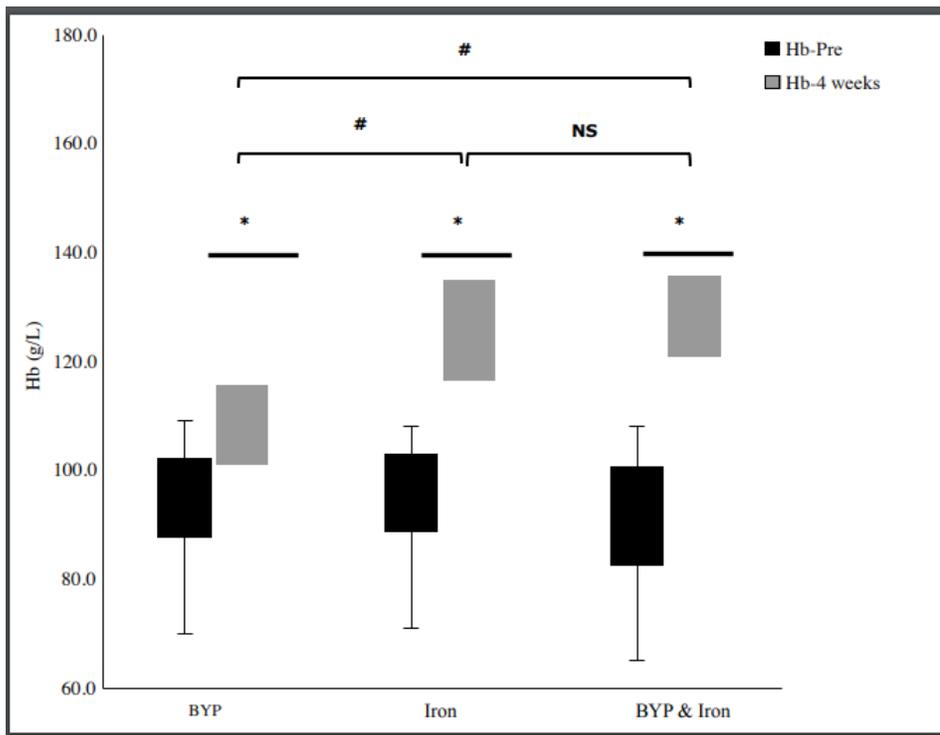


Figure 1

Changes in hemoglobin after 4-week treatment in three groups.

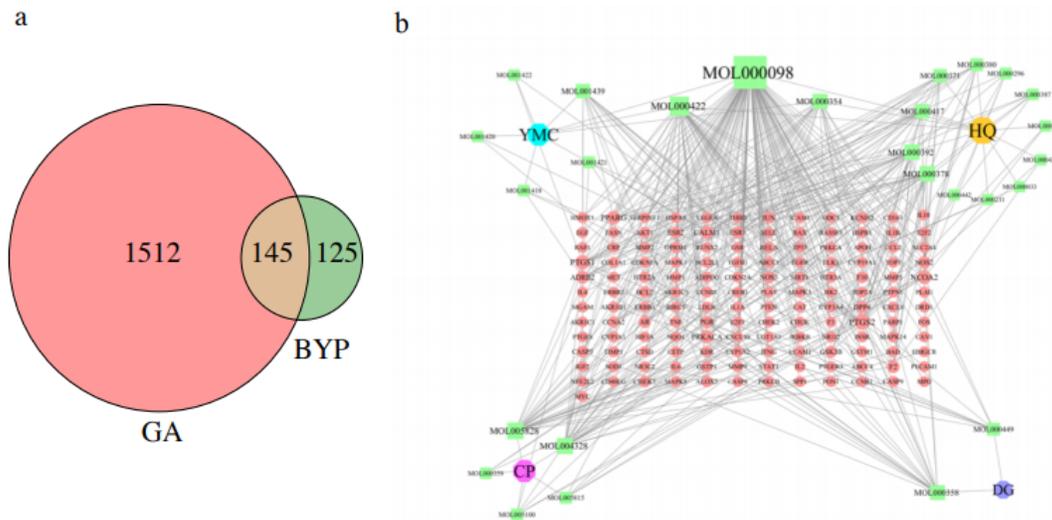


Figure 2

The pharmacology network generated for BYP. (a) Intersection of drug-target and disease-target. (b) Compound-target-disease network with 176 nodes, 409 edges. Red oblong nodes represent target genes; green square nodes represent bioactive compounds; polygon nodes of different colors represent main ingredients of Buxue Yimu Pills. The node size is proportional to degree. Notes: YMC, Yimucao; HQ, Huangqi; CP, Chenpi; DG, Danggui.

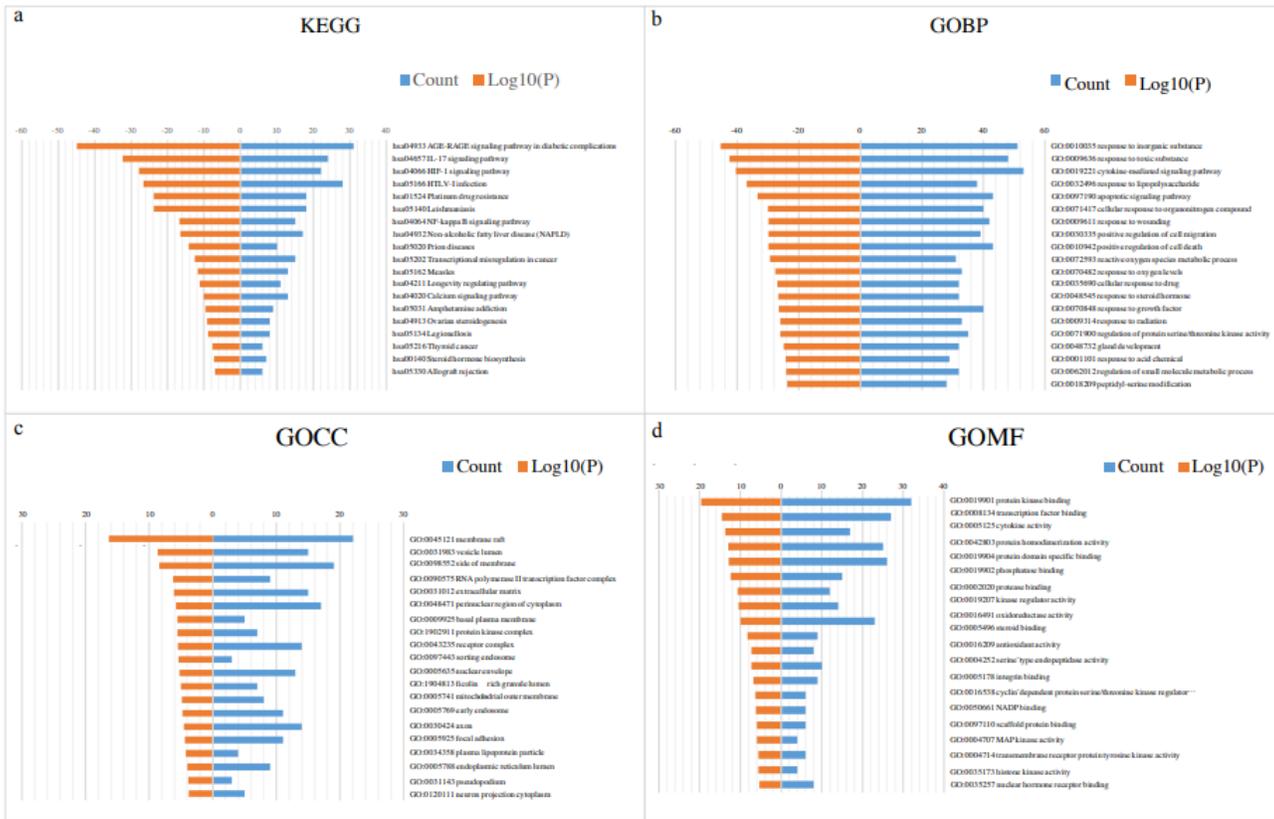


Figure 3

Results of functional enrichment analysis: KEGG pathway, GOBP, GOCC and GOMF.

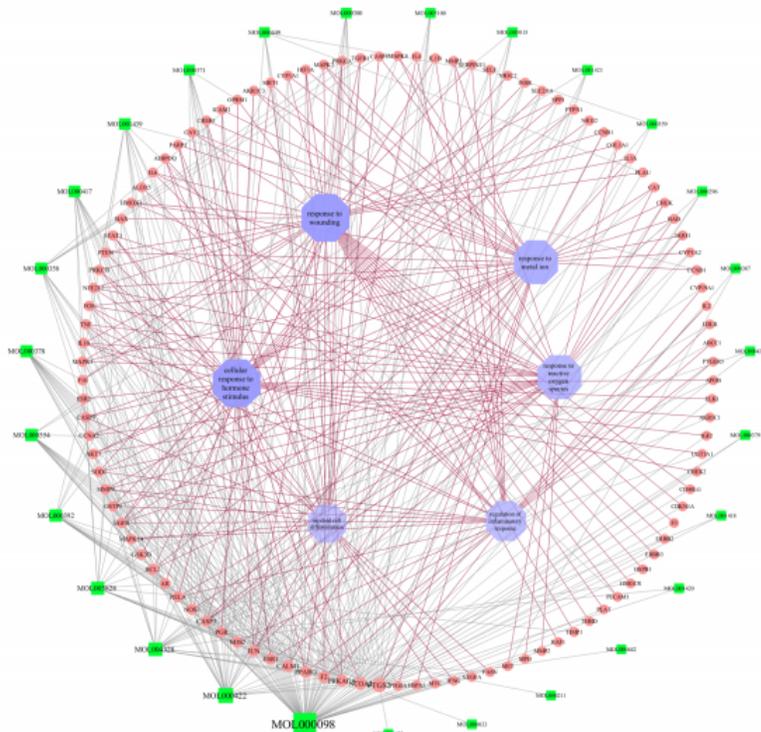


Figure 4

Compound-target-underlying biological process network with 139 nodes, 483 edges. Red oblong nodes represent target genes; green square nodes represent bioactive compounds; Blue polygon nodes represent main underlying biological processes. Edges between biological processes and targets

are shown in red; edges between bioactive compounds and targets are shown in grey.

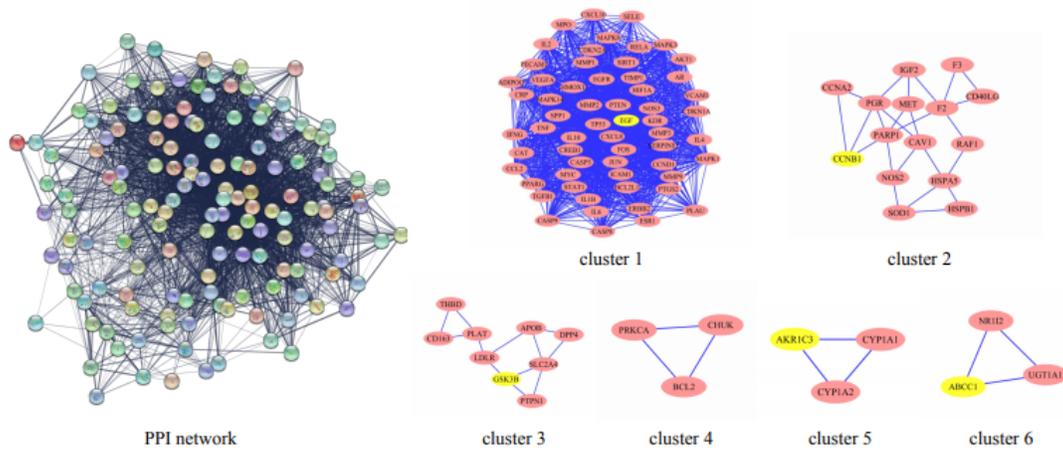


Figure 5

PPI network (145 nodes, 3196 edges) and 6 clusters were identified using MCODE. Seed target proteins are represented in yellow.

Supplementary Files

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- [Additionalfile1.pdf](#)
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