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Effect and mechanism of Yiqing decoction on hyperuricemia rats

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Abstract

This study aimed to experimentally compare the uric acid-lowering effect and renal protection of Yiqing Fang in a rat model of hyperuricemia. Additionally, we used network pharmacology to predict the potential active components, targets, and pathways of Yiqing Fang. Male SD rats were randomly divided into control, model, Yiqing Fang, allopurinol, and probenecid groups. Serum creatinine (Scr), blood urea nitrogen (BUN), serum uric acid (UA), alanine transaminase (ALT), complete blood count, and urinary NAG enzyme levels were measured. Standard pathology and electron microscopy samples were prepared from the left kidney to observe renal pathological changes, renal fibrosis, and collagen III expression levels. In addition, we employed network pharmacology to investigate the molecular mechanisms and pathways of Yiqing Fang. The Yiqing Fang group showed significantly lower levels of Scr, BUN, UA, ALT, urinary NAG enzyme, complete blood count, and liver function tests compared to the model group ($P < 0.05$). Furthermore, both the Yiqing Fang and allopurinol groups exhibited significant reductions in renal pathological changes compared to the model group, along with decreased expression of collagen III. Network pharmacology analysis identified a total of 27 specific sites related to hyperuricemia. The main active components were predicted to include quercetin, berberine, beta-sitosterol, epimedin C, and dioscin. The primary target sites were predicted to include TNF, IL-6, IL-17, IL-1B, and VEGFA. Yiqing Fang may exert its effects through regulation of drug response, urate metabolism, purine compound absorption, inflammation response, lipopolysaccharide response, cytokine activity, and antioxidant activity. These effects may be mediated through signaling pathways such as IL-17, HIF-1, and AGE-RAGE. Yiqing Fang offers potential as a treatment for hyperuricemia due to its multiple active components, targeting of various sites, and engagement of multiple pathways.

Keywords: Yiqing decoction, Hyperuricemia, Network pharmacology, Mechanism of action.

1. Introduction

Hyperuricemia (HUA) is a chronic metabolic disorder that occurs mainly involving a complex regulatory network of purine metabolism. In addition to causing obvious symptoms such as elevated blood uric acid levels and arthritis, the entero-renal pathway occupies a major role in regulating 99% of uric acid excretion and plays a crucial role in maintaining the balance of purine metabolism in the body. Long-term maintenance of a high uric acid state may lead to glomerulosclerosis, interstitial fibrosis and renal vascular sclerosis, accompanied by localized interstitial urate crystal deposition [1], which induces a variety of inflammatory responses, leads to endothelial damage, and stimulates vascular smooth muscle cell proliferation [2]. In addition, higher than normal physiological concentrations of uric acid not only disrupt the oxidation-reduction homeostasis system in the body but also contribute to the development of oxidative stress injury [3]. The activity of uric acid also activates the angiotensin system, promotes the generation of reactive oxygen species as well as the release of pro-inflammatory factors, increases lipid deposition and cell-cell-endothelial attachment [4], and ultimately leads to a series of cardiovascular system disorders including coronary artery disease and atherosclerosis.

According to the theory of Chinese medicine, hyperuricemia can be categorized as "dampness" and "turbid stagnation", and is usually treated by strengthening the spleen and resolving dampness, clearing heat and removing dampness, and draining turbidity and eliminating blood stasis [5]. In this study, Yiqing Fang (Patent No. 1350857), as a compound Chinese medicine composed of eight Chinese herbs, including Radix Rehmanniae Praeparata, Poria cocos, Radix Angelicae Sinensis, Radix Paeoniae Alba, Hederocallis sinensis, Zedoary, Rhizoma Atractylodes Macrocephalae, and Cyperus Rotundus, demonstrated remarkable efficacy in targeting the clinical manifestations of HUA such as Spleen Deficiency with Dampness, Phlegm and Turbidity Obstruction, Dampness-

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Heat Embeddedness, and Stasis Blood Stasis, which are commonly found in HUA. This experiment is planned to investigate the mechanism of action of Yiqing Fang by establishing a rat model of hyperuricemia and combining it with an innovative network pharmacology approach. In addition to exploring the molecular-level mode of action of traditional Chinese medicine treatments, we are also committed to comprehensively analyzing the effects of Yiqing Fang on multiple signaling pathways, in order to more fully understand the molecular level mechanisms of Chinese medicine treatments for hyperuricemia. This in-depth study aims to provide a new theoretical basis for the modernization of traditional Chinese medicine treatments and provide useful guidance for exploring new therapies in the future.

2. Materials and methods

2.1. Drugs and instruments

Oxonic acid potassium salt (OA) and uric acid (99%, UA) were purchased from Sigma Company, USA. Yiqing formula consisted of Radix Rehmanniae Praeparata, Poria cocos, Radix Angelicae Sinensis, Radix Paeoniae Alba, Rhizoma Hedera riae, Rhizoma Zedoariae, Rhizoma Atractylodis Macrocephalae, Rhizoma Cyperus rotundus, and the soup was provided by the General Hospital of People's Liberation Army. Anti-rat Col III polyantibody was purchased from Beijing Zhongshan Jinqiao Company (Beijing, China).

2.2. Animals and grouping

Fifty 2-month-old male SD rats weighing between (200±20) g were selected and purchased from the Animal Center of the Chinese People's Liberation Army. They were randomly divided into 5 groups, i.e., normal control group, model group, Yiqingfang group, Allopurinol group and Probenecid group, 10 rats in each group, and the rats were weighed once a week.

2.3. Modeling and drug administration method

In the model group, rats were gavaged with 3% potassium oxybate (1 ml / 100 g), in the allopurinol group, allopurinol 4 mg / 100 g was given orally along with 3% OA, in the probenecid group, probenecid 20 mg / 100 g was given orally along with 3% OA, and in the Yi-Qing-Fang group, 8 g/kg of Yi-Qing-Fang was given orally along with 3% OA, and the above four groups drank freely 0.1mmol/L aqueous solution of uric acid; the normal control group was weighed weekly, and each group had 10 rats each. All four groups were given 0.1 mmol/L uric acid solution daily; the normal control group was given saline gavage and free drinking water only. The dosage was adjusted weekly according to the body weight of the rats, and after 2 months of administration, the rats were killed to obtain samples.

2.4. Network pharmacology methods

2.4.1. Drug target collection

Through TCMSP (https: //tcmsp-e.com/) database, the main active ingredients and their pharmacokinetic parameters (absorption, distribution, metabolism and excretion, ADME) of each drug in YQF were searched separately, and the oral bioavailability (Oral bioavailability (OB) ≥30% and Drug-likeness (DL) ≥0.18 were set as the conditions for screening. The selected active ingredients were searched for the corresponding targets through TCMSP database, and the literature was consulted to supplement the targets of the ingredients, and all the targets were standardized through Uniport (https://www.uniprot.org), a universal protein database, to obtain the list of standard gene names of the active ingredients and their corresponding targets.

2.4.2. Collection of disease targets

Using "hyperuricemia" as the search term, the target names of the active ingredients and their corresponding targets were obtained from the human genome database (Genecards, https: //www. genecards. org/), the database of Mendelian Inheritance in Man (OMIM, https: //omim. org/), and the database of disease genes (Disgenetics, https: //omim. org/).) and the Disease Gene Database (DisGeNET, https://www.disgenet.org) were searched for disease gene information related to hyperuricemia, and a list of potential therapeutic target gene names for hyperuricemia was summarized.

2.4.3. Action target screening

The intersection of active ingredient targets and disease therapeutic targets was calculated by the Venny 2.1 analysis platform, which is the potential target of YQF for the treatment of hyperuricemia. The target list was uploaded to the protein interactions platform database STRING (https: //cn.string-db.org/), the species was selected as "Homo sapiensHomo sapiens", and the interaction score was set to ≥0.7 to obtain the Protein Interaction (PPI) network diagram.

2.4.4. Bio-enrichment analysis

The obtained genes were uploaded to Metascape (https: //metascape. org/gp/index. html#/main/step1) website for Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analysis.

2.4.5. Network construction

A drug-component-target-disease network was constructed using Cytoscape 3.7.1. In the network, "Node" represents drug, ingredient, target and disease. The Network Analyzer plug-in in Cytoscape 3.7.1 was used to analyze the topological features of the network and identify the core components of the network.

2.5. Observation indexes and measurement methods

2.5.1. Measurement of general condition

Observation of rats' spirit, urine volume, urine color, feces, joints and activities, body weight and so on.

2.5.2. Liver and kidney functions

Blood was collected from the abdominal aorta of rats, serum was separated (3000 r-min-1), and serum creatinine (Scr), urea nitrogen (BUN), blood uric acid (UA), and alanine aminotransferase (ALT) were measured by the Laboratory Department of the General Hospital of the People's Liberation Army (PLA) on an automatic biochemical analyzer.

2.5.3. Blood routine

Blood was taken from the abdominal aorta of rats, and the blood routine results were determined by the Laboratory Department of the General Hospital of the Chinese

People's Liberation Army on a blood routine analyzer.

2.5.4. Renal histopathology

The rats were killed by decapitation at 24 h. The left kidney was immediately isolated and fixed, and the specimens were prepared for routine pathology and electron microscopy respectively [4], and observed under ordinary light microscope and transmission electron microscope.

2.5.5. Determination of urinary NAG enzyme

This enzyme is a sensitive index to react to renal tubular injury. Bladder puncture urine was used for immediate delivery and enzyme assay.

2.5.6. Indicator of renal fibrosis

After paraffin section of renal tissue, Col III, an indicator reflecting renal interstitial fibrosis, was detected by immunohistochemical ABC method.

2.5.7. Statistical analysis

Excel 2019 was used for data entry and Statistic Package for Social Science (SPSS) 25.0 for statistical analysis (IBM, Armonk, NY, USA). Measurement data that satisfied normal distribution were described using($\bar{X}$±SD), and comparisons between groups were made using t-test; continuous variables that were not normally distributed were described using median and quartiles, and comparisons between groups were made using Wilcoxon rank-sum test. Count data were described by frequency and percentage, and intergroup comparisons were made using the χ^2 test, with P<0.05 suggesting that the differences were statistically significant, and P<0.01 suggesting that the differences were highly significant.

3. Results

3.1. General condition

After 1 month, some rats in the model group and probenecid group showed red and swollen joints, lameness, dark yellow urine and hematuria, and 3 and 2 rats died respectively. In the Yiqingfang group, there was no death, no joint symptoms, and no hematuria, urine color was clearer than that of the model group and the Western medicine group, stools were slightly thinner, and the rats' mental state and activity were better than that of the model group, but there was no significant difference in food intake and body weight. It indicates that Yiqing formula can reduce the risk of arthritis (Figures 1 and 2).

3.2. Changes of blood uric acid

At 2 months of the experiment, compared with the normal control group (Table 1), the blood uric acid value of the model group was significantly higher, and the blood uric acid value of the rats in the Yiqingfang group, Allopurinol group, and Probenecid group was significantly lower, and the differences were all statistically significant (P<0.01). In addition, Scr and BUN in the model group were significantly higher than those in the normal control group (P<0.01), and blood creatinine and urea nitrogen in the Yiqingfang group and allopurinol group were significantly lower, with statistically significant differences between the Yiqingfang group and the model group (P<0.05), which indicated that Yiqingfang was able to improve renal dysfunction caused by hyperuricemia.

3.3. Changes of urinary NAGase

After modeling and treatment for 2 months, the urinary NAG enzyme of the model group was significantly higher than that of the normal control group (P<0.01), while the urinary NAG enzyme of the rats in both the Yiqing formula and allopurinol group was significantly lower than that of the model group (P<0.05), indicating that the Yiqing for-

Fig. 1. Probenecid group (rats with erythema and lameness of left lower limb joints).

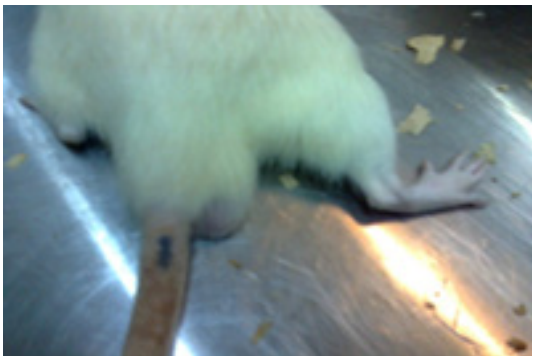

Fig. 2. Model group (rat right lower limb joints swollen and lame).

Table 1. Comparison of blood uric acid, blood creatinine, and urea nitrogen results after 2 months of intervention in each group of rats ($\bar{X}$±SD).

	Normal control group	Model group	Yiqingfang group	Allopurinol group	Probenecid group
BUA (mmol/l)	59.4±10.6	485.9±114.4**	168.6±38.2$^{\Delta\Delta}$	135.6±31.4$^{\Delta\Delta}$	199.6±60.1$^{\Delta}$
BUN (mmol/l)	5.0±1.2	18.6±5.8**	9.6±2.9*$^{\Delta}$	12.2±3.3*$^{\Delta}$	15.6±4.5**
Cr (umol/l)	30.6±3.9	121.5±23.6**	80.2±23.1*$^{\Delta}$	79.6±22.7*$^{\Delta}$	100.6±28.5*

Note: Compared with the normal group **P<0.01 *P<0.05; Compared with the model group ΔΔP<0.01 ΔP<0.05.

Table 2. Comparison of urinary NAG enzyme values after 2 months of intervention in each group of rats($\bar{X}$±SD).

	Normal control group	Model group	Yiqingfang group	Allopurinol group	Probenecid group
Urinary NAG enzyme (mmol/l)	5.0±1.2	59.5±5.8**	18.7±3.9$^{\Delta}$	26.5±5.3$^{\Delta}$	46.1±6.5

Note: Compared with the normal group **P<0.01; Compared with the model group ΔP<0.05.

mula was able to improve the renal tubular injury (Table 2).

3.4. Blood routine and liver functions

After 2 months of intervention, the leukocyte count in the model group was significantly higher than that in the normal control group (P<0.05), whereas the leukocyte counts of rats in the Yi-Qing Fang group and the Allopurinol group were lower than those in the model group (P<0.05), and there was no significant difference when compared with that in the normal group; hemoglobin and platelet counts of the model group were lower than those of the normal group, but there was no statistically significant difference when compared with that in the other four groups (Table 3). In addition, ALT in the allopurinol group was higher than that in the normal control group (P<0.05), and the differences between the other four groups and the normal group were not statistically significant (Table 3).

3.5. Renal pathologic results

After 2 months of OA and uric acid gavage modeling, different degrees of proteinuria and erythrocytes can be seen in the tubular lumen of the kidney tissue of rats in all groups, and some of them have shedding of tubular cells, the lumen of the tubular lumen, especially the medullary part of the kidney and the collecting ducts have different degrees of dilatation, and urate crystals can be seen in some of the lumens, and the small arteries in the kidney can be seen to have thickened walls of the small blood vessels in some parts of the tubular lumina, glomerular collaterals are well opened, and a greater number of erythrocytes are seen in the globules, and a great number of inflammatory cells infiltration can be seen in the interstitium. Inflammatory cell infiltration was seen in the renal interstitium, and tubular atrophy and fibrosis were rarely seen. Among them, the lesions in the model group were particularly serious, and the renal pathological changes in the Yiqingfang group and Allopurinol group were significantly reduced compared with those in the model group, and the renal lesions in the Probenecid group were also reduced to a certain extent (Figure 3).

3.6. Expression of Col III in kidney tissue

Col III was less in the kidney tissue of normal rats, and the strongest expression of Col III was found in the kidney tissue of the model group at 2 months, while the expression of Col III in the Yiqingfang group, Allopurinol group and Probenecid group was significantly weaker than that in the model group. It indicated that Yiqingfang could reduce the production of extracellular matrix Col III in renal tissues and reduce the degree of fibrosis (Figure 4).

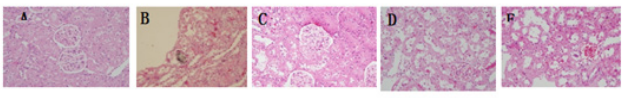

Fig. 3. Pathologic photographs of the kidneys of rats in each group at 2 months (HE×400); (A) Normal group; (B) Model group; (C) Yiqingfang group; (D) Allopurinol group; (E) Probenecid group.

3.7. Results related to potential targets

3.7.1. Prediction results of potential targets of Yiqing formula

A total of 67 compliant active ingredients were finally obtained from the 8 Chinese medicines (Radix Rehmanniae Praeparata, Poria cocos, Radix Angelicae Sinensis, Radix Paeoniae Alba, Rhizoma Atractylodis Macrocephalae, Rhizoma Atractylodis Macrocephalae, Rhizoma Cyperus rotundus) in Yicheng Formula. By searching TCMSP and STITCH databases, a total of 222 targets were predicted for the above 67 active ingredients. Using "Hyperuricemia" as the search term, we searched for therapeutic targets of related diseases through the databases Genecards and OMIM, respectively, and 194 targets were obtained from the database Genecards with the inclusion criterion of Relevance score ≥1. Venny2.1 online platform was used to draw the Wayne diagram of the intersection between drug targets and disease targets (Figure 5).

3.7.2. Construction and analysis of PPI network

Upload the above list of 27 targets to the protein interaction analysis platform STRING, set the species (Organization) as "Homo sapiens", the interaction score ≥ 0.4,

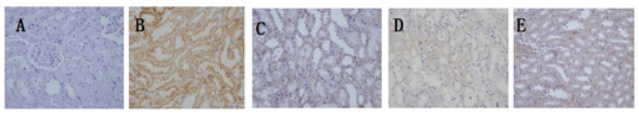

Fig. 4. Expression of renal Col III in rats of each group at 2 months (HE×400). (A) Normal group; (B) Model group; (C) Yiqingfang group; (D) Allopurinol group; (E) Probenecid group.

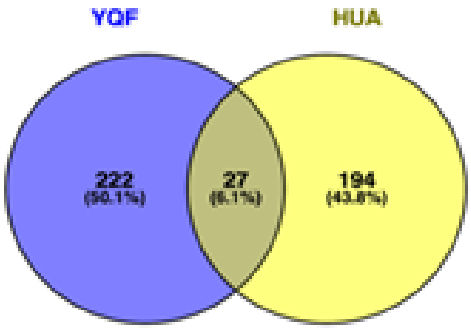

Fig. 5. Wayne diagram of common targets.

Table 3. Comparison of blood routine and ALT after 2 months of intervention in each group of rats (X±SD).

	Normal control group	Model group	Yiqingfang group	Allopurinol group	Probenecid group
WBC (*10⁹/L)	7.9±1.4	11.2±1.5*	8.7±1.3Δ	8.4±1.4Δ	10.5±1.4*
Hb (*g/L)	151.8±7.3	139.3±4.6	141.6±5.3	138.4±9.7	139.7±7.3
PLT (*10⁹/L)	655.7±40.9	830.7±40.2*	743.7±57.1Δ	748.0±70.1	788.5±58.8*
ALT (U/L)	159.2±15.0	189.4±16.8	172.2±24.6	216.7±28.2*	170.2±28.2

Note: Compared with the normal group *P<0.05; Compared with the model group ΔP<0.05.

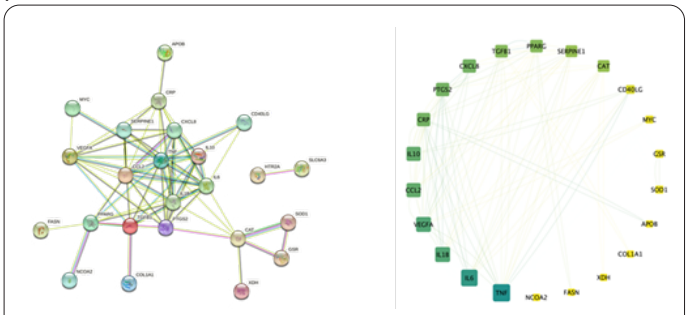

Fig. 6. Protein interaction network diagram.

the nodes with no interactions were hidden, and the rest of the parameters were selected as defaults, from the platform Download the tsv file about the analysis results and import it into Cytoscape3.9.1 software to draw the PPI network diagram (Figure 6). Analyzed using the Network Analysis plug-in in the Tools menu of Cytoscape 3.7.1 software, this network graph was obtained to consist of 22 nodes (Node) and 106 edges (Edge), with an average of 8.833 neighboring nodes present per node. The degree of the network refers to the number of edges associated with the node, i.e., the higher the degree indicates the more interactions at the point, which was used to initially screen the key targets, and the results suggested that tumor necrosis factor (TNF, degree:28), interleukin-6 (IL6, degree:26), and vascular endothelial growth factor (VEGFA, degree:22) might be the targets of Yiqingfang's therapeutic. targets may be the core targets of Yiqingfang for the treatment of HUA.

3.7.3. GO biofunctional annotation and KEGG pathway enrichment analysis

The above predicted list of 27 targets was uploaded to the metascape database for GO Biofunctional Annotation and KEGG Pathway Enrichment Analysis (Figure 7). According to the screening conditions, 20 entries of Biological Processes, 7 entries of Cellular Components, and 8 entries of Molecular Functions were obtained.BP involves the regulation of MAPK signaling pathway, inflammatory response, and the regulation of MAPK cascade. BP involves the regulation of MAPK signaling pathway (regulation of MAPK cascade), inflammatory response, response to lipopolysaccharide, etc.; CC involves neuronal cell body, peroxisome, RNA polymerase II transcription, and other functions. CC involves neuronal cell body, peroxisome, RNA polymerase II transcription regulator complex, etc.; MF involves cytokine activity, catecholamine binding, antioxidant activity, etc. MF involves cytokine activity, catecholamine binding, and antioxidant activity.

KEGG pathway enrichment analysis involved lipid and atherosclerosis pathway (AGE-RAGE), IL-17, NF-kB signaling pathway and so on. It was suggested that the regulation of inflammatory response and lipid metabolism might be the potential mechanism of Yiqingfang in the treatment of HUA (Figure 8).

3.7.4. Construction and analysis of the network of "Traditional Chinese Medicine - Ingredient - Target - Disease"

The Network Analyzer plug-in in Cytoscape 3.7.1 was used to analyze the network. The degree in the network refers to the number of edges associated with the node, i.e., the higher the degree is, the more interactions there are at that point, and the size of the color blocks in the graph

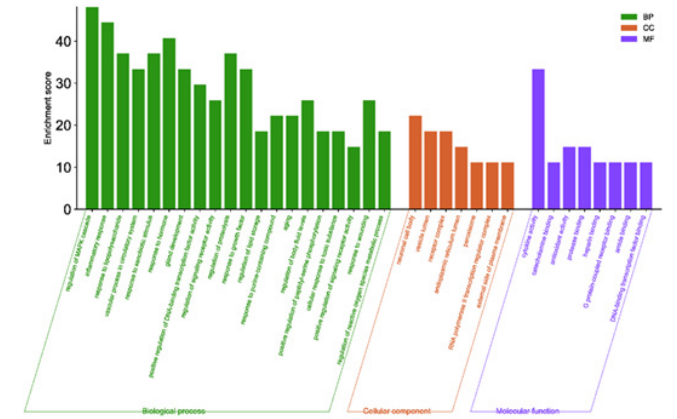

Fig. 7. GO enrichment pathway diagram.

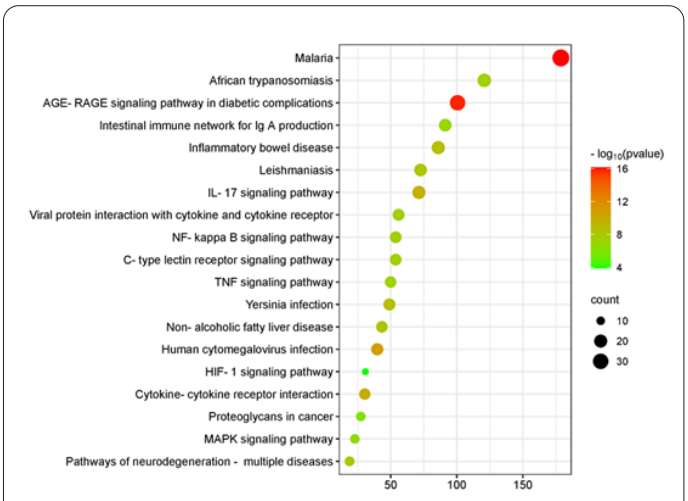

Fig. 8. KEGG enrichment analysis.

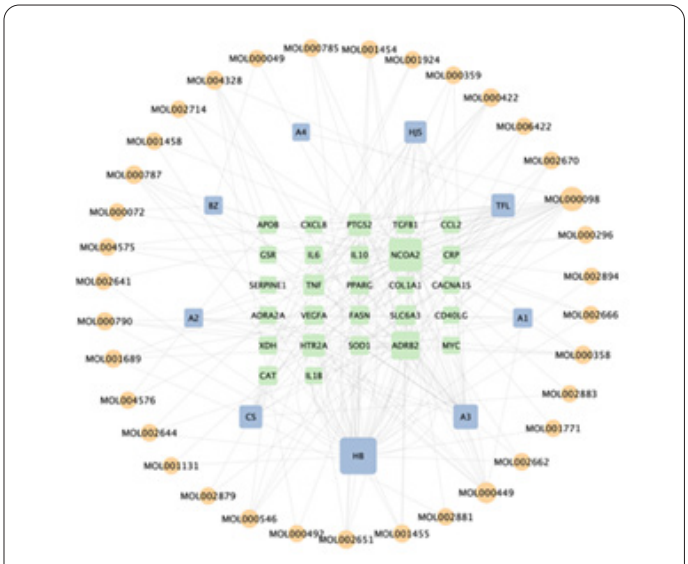

Fig. 9. Ingredient-drug-target map.

represents the degree value. From the perspective of active ingredients, the higher degree values of active ingredients such as astilbin, berberine, stigmasterol, quercetin, beta-sitosterol, and diosgenin suggest that these active components may be the core components of YQF in the treatment of HUA, and all of them are the main active components of Phellodendron Bark, suggesting that Phellodendron Bark plays a very important role in the treatment of HUA by YQF, see Table 4 and Figure 9.

Table 4. Drug composition.

No.	Mol ID	Molecule Name	OB(%)	DL	Herb Name
1	MOL001454	berberine	62.4	0.22	Phellodendron chinense
2	MOL001458	coptisine	60.31	0.31	Phellodendron chinense
3	MOL002641	Phellavin	63.37	0.3	Phellodendron chinense
4	MOL002644	Phellopterin	39.51	0.76	Phellodendron chinense
5	MOL002651	Dehydrotanshinone II A	36.23	0.78	Phellodendron chinense
6	MOL002662	rutaecarpine	54.07	0.22	Phellodendron chinense
7	MOL002666	Chelerythrine	35.95	0.21	Phellodendron chinense
8	MOL000449	Stigmasterol	46.43	0.28	Poria cocos, Sparganium stoloniferum, Paeonia lactiflora, Rehmannia glutinosa (A1)
9	MOL002670	Cavidine	37.6	0.74	Phellodendron chinense
10	MOL000358	beta-sitosterol	30.68	0.23	Poria cocos, Sparganium stoloniferum, Paeonia lactiflora, Sparganium stoloniferum, Phellodendron chinense (A2)
11	MOL000785	palmatine	36.91	0.75	Phellodendron chinense
12	MOL000787	Fumarine	36.91	0.75	Phellodendron chinense
13	MOL000790	Isocorypalmine	36.91	0.75	Phellodendron chinense
14	MOL000098	quercetin	41.88	0.24	Poria cocos, Phellodendron chinense (A4)
15	MOL001131	phellamurin	43.83	0.76	Phellodendron chinense
16	MOL001455	(S)-Canadine	54.83	0.24	Phellodendron chinense
17	MOL001771	poriferast-5-en-3beta-ol	63.71	0.19	Phellodendron chinense
18	MOL002894	berberrubine	35.36	0.65	Phellodendron chinense
19	MOL006422	thalifendine	64.6	0.65	Phellodendron chinense
20	MOL000049	3β-acetoxyatractylone	59.26	0.83	Atractylodes macrocephala
21	MOL000072	8β-ethoxy atractylenolide III	35.77	0.59	Atractylodes macrocephala
22	MOL000359	sitosterol	34.47	0.82	Poria cocos, Paeonia lactiflora, Alisma orientale, Rehmannia glutinosa(A3)
23	MOL001689	acacetin	35.58	0.81	Sparganium stoloniferum
24	MOL002879	Diop	32.52	0.82	Sparganium stoloniferum
25	MOL002881	Diosmetin	32.43	0.77	Sparganium stoloniferum
26	MOL002883	Ethyl oleate (NF)	36.76	0.82	Sparganium stoloniferum
27	MOL000296	hederagenin	32.7	0.82	Sparganium stoloniferum
28	MOL000422	kaempferol	33.06	0.83	Sparganium stoloniferum
29	MOL001002	ellagic acid	35.58	0.81	Paeonia lactiflora
30	MOL001924	paeoniflorin	43.06	0.43	Paeonia lactiflora
31	MOL002714	baicalein	56.6	0.39	Paeonia lactiflora
32	MOL004355	Spinasterol	36.86	0.78	Paeonia lactiflora
33	MOL000492	(+)-catechin	53.83	0.77	Paeonia lactiflora
34	MOL004328	naringenin	30.67	0.86	Poria cocos
35	MOL004575	astilbin	33.55	0.42	Poria cocos
36	MOL004576	taxifolin	34.97	0.24	Poria cocos
37	MOL000546	diosgenin	36.91	0.75	Poria cocos

4. Discussion

The body's uric acid level depends on the balance of uric acid production and excretion, and impaired renal excretion is the main reason for the occurrence of hyperuricemia, and the reduction of renal uric acid excretion in the state of hyperuricemia is associated with the abnormal expression of urate transporters [6]. Molecular cloning and genome-wide association studies (GWAS) have identified relevant proteins responsible for urate transport, and it is believed that the kidneys mainly regulate the expression of urate transporter 1 (URAT1) and other proteins in order to achieve balance of uric acid reabsorption or secretion [7-10]. When renal excretion is impaired, long-term accumulation of uric acid causes renal inflammatory infiltration. In the renal inflammatory response, NF-κB and NLRP3 inflammatory vesicles are involved. NLRP3 inflammatory vesicles are activated in the high uric acid state, and then stimulate the secretion of IL-1 and IL-18 through the NF-κB pathway; at the same time, the activated NF-κB participates in the transcription of NLRP3 as a transcription factor, and the two factors interact with each other to produce a vicious circle accelerating the occurrence of renal damage. The results of the network pharmacology in this paper showed that Yiqingfang could treat HUA through NF-κB signaling pathway, and one of its main components, berberine, could inhibit NF-κB signaling pathway, improve renal inflammatory response, and enhance antioxidant capacity, so as to reduce the serum uric acid level of hyperuricemic mice, and the mechanism

of which is related to the down-regulation of the expression of URAT1 [11]. Another component of Yiqingfang, luoxinwuoside, can simultaneously inhibit NLRP3 inflammatory vesicles and NF-κB signaling pathway, inhibit the release of downstream inflammatory factors, with significant anti- inflammatory and analgesic and diuretic effects, so Yiqingfang can alleviate the symptoms of acute gouty arthritis in the late stage of HUA to a certain extent [12]. Other components of Yiqingfang, such as naringenin and diosgenin, can reduce the expression of HIF-1 and VEGFA, decrease lipid synthesis and inflammatory response, and enhance the level of autophagy [13,14].

According to the "renal overload" theory [15], the intestinal tract plays a key role in the homeostatic regulation of blood uric acid levels, and abnormalities in intestinal and renal function firstly lead to a sustained increase in uric acid levels, which in turn triggers a vicious cycle of tissue damage and uric acid accumulation in the body [16]. In recent years, it has been found that the antioxidant effect of uric acid has limitations, and when the concentration of uric acid in the body is too high, uric acid becomes a strong oxidizing agent to disrupt the oxidation-reduction homeostasis system in the body, which further promotes oxidative stress injury [3] and acts as an endogenous irritant to induce vascular endothelial injury [17]. A study found that high concentrations of fructose-induced hyperuricemia model rats increased the level of oxidative stress, intestinal and renal uric acid excretion decreased, intestinal and renal uric acid excretion impairment is related to oxidative stress-induced oxidative damage in intestinal and renal tissues [18], and oxidative stress plays a key role in inflammatory response as a "second messenger". In this paper, we found that one of the main components of Yiqing Fang is quercetin, which can significantly reduce the mRNA expression of colonic inflammatory factors IL-1, IL-17 and IL-6, and reduce the mRNA expression level of VEGF in mice [19], inhibit oxidative stress and inflammation, improve the damaged intestinal epithelial cells, and promote uric acid efflux from the intestinal tract [20]. Naringenin pretreatment in Yiqing formula can inhibit the overexpression of TNF-α and IL-8, improve the inflammatory damage of intestinal epithelial cells, and maintain the integrity of intestinal epithelial barrier. The results of network pharmacology showed that Yiqingfang acted on HUA through targets such as IL-6 and VEGF, as well as the IL-17 signaling pathway; therefore, Yiqingfang may promote uric acid excretion by improving oxidative damage in renal tissues and intestinal epithelial injury.

Recent studies have shown that a variety of diseases, including atherosclerosis, diabetes, etc., are independent risk factors for the development of HUA, and HUA patients are more likely to be complicated by metabolic syndrome, resulting in dyslipidemia, insulin resistance, etc. Insulin resistance is one of the factors causing HUA. Insulin resistance is one of the factors causing HUA, under insulin resistance, the intermediate products of glycolysis to 5-phosphate ribose and ribose phosphate transfer, resulting in an increase in the production of blood uric acid, but also increase the concentration of triglycerides [21], the latter cause more free fatty acid production, accelerate the decomposition of ATP, also caused by an increase in the production of blood uric acid [22]; insulin resistance can increase the synthesis of hepatic fat, resulting in purine metabolism disorders, and increase the production of fatty acids, which can lead to the development of HUA. Insulin resistance can also increase hepatic fat synthesis, leading to purine metabolism disorders and increasing blood uric acid levels [23]. Berberine in Yiqing formula reduces the damage of free radicals to the biofilm by increasing the antioxidant activity, activating the peroxisome proliferator-activated receptor γcoactivator 1 (PGC-1) signaling pathway, inhibiting lipid accumulation [24], inhibiting lipid peroxidation to enhance the antioxidant function of the body, thus increasing insulin sensitivity and improving insulin resistance [25] and decreasing uric acid level in the rat. Uric acid decreased.

5. Conclusion

Yiqingfang demonstrated the potential to reduce the risk of arthritis in rats, with the effect of lowering blood uric acid, BUN, urinary NAG enzyme, Scr and ALT levels, and improving renal injury and liver function. Pathological analysis and immunohistochemical results demonstrated that Yiqingfang significantly reduced the symptoms of proteinuria and urate precipitation, as well as inhibited inflammatory cell infiltration, in addition to having a decreasing effect on extracellular matrix Col III produced by renal tissues, which led to an improvement in renal fibrosis. The results of network pharmacology showed that the active ingredients of Yiqingfang, such as luoxinoside, berberine, quercetin, naringenin, stigmasterol, and diosgeninogen, successfully reduced inflammatory responses, decreased vascular endothelial adhesion, anti-lipid deposition, improvement of renal fibrosis, and inhibition of disease progression in HUA rats.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of Hubei University of Chinese Medicine Animal Center.

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

YX and JZ designed the study and performed the experiments, LS collected the data, JH analyzed the data, YX and JZ prepared the manuscript. All authors read and approved the final manuscript.

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