REVIEW ARTICLE

Title: Progress of network pharmacology-based research on Chinese medicine compounds for hyperuricemia

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Abstract: Hyperuricemia represents a prevalent metabolic condition characterized by dysregulated purine metabolism, culminating in excessive uric acid production. This pathology may progress to more serious complications, including gout. The United Nations has recognized hyperuricemia as one of the twenty major chronic diseases in the 21st century, reflecting its global impact. In China, the incidence of this disease has been rising, yet long-term treatment strategies and drug development continue to face challenges. Traditional Chinese Medicine (TCM) offers an alternative approach to managing hyperuricemia, boasting characteristics such as holistic treatment principles, adaptability, and reduced side effects compared to conventional Western therapies. Despite these benefits, the precise mechanisms underlying the efficacy of certain TCM compound treatments remain enigmatic. In the current clinical landscape, network pharmacology has emerged as a powerful tool to dissect the intricate interactions between drugs, diseases, and molecular targets. It provides a systems-level perspective, elucidating the "multi-component, multi-target, and multi-pathway" synergistic relationships inherent in TCM compound treatments. This integrative approach holds promise for enhancing our understanding of TCM's therapeutic effects and could pave the way for more refined and personalized treatment strategies in the management of hyperuricemia. The current studies on the use of network pharmacology in the treatment of hyperuricemia by using Chinese medicine compounding, elucidated the active ingredients, targets, biological processes and pathway analysis of the single drugs in Chinese medicine compounding, and explored the prospect of network pharmacology research with a view to better developing the application of Chinese medicine compounding in clinical practice.

Keywords: Hyperuricemia; Network pharmacology; Herbal compounding; Active pharmaceutical ingredients; Drug targets; Pathway analysis

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1. Introduction

Hyperuricemia is a common disorder caused by abnormal purine metabolism leading to abnormal uric acid production [1]. The United Nations has identified hyperuricemia as one of the twenty preeminent chronic diseases of the 21st century, underscoring its significant public health implications. Contemporary epidemiological data reveal a concerning trend: despite enhancements in living standards, the prevalence of gout is escalating. This rise is attributable to dietary imbalances and the accelerated tempo of modern lifestyles [2]. Notably, the disease is manifesting younger ages with exacerbating the societal burden. Current therapeutic options for hyperuricemia has various risks and limitations. Allopurinol, while widely used, poses a risk of allergic reactions in susceptible individuals. Benzbromarone has been associated with an increased risk of fulminant hepatic necrosis in certain patient populations, necessitating careful monitoring. Febuxostat, although effective, is relatively costly

and has been linked to potential cardiovascular risks. These challenges highlight the imperative for developing more efficacious and safer treatment strategies to alleviate the burden of hyperuricemia and its progression to gout [3, 4]. In addition, gout is closely associated with the development of hypertension, hyperlipidemia, diabetes, and other diseases [5]. A recent trend in clinical treatment for metabolic syndrome involves the condition holistically. Therefore, the novel medicine development and treatment are necessary. Hyperuricemia is generally categorized as primary and secondary hyperuricemia [6]. Primary hyperuricemia is mainly due to unknown molecular defects or inborn impairment of purine metabolism. Secondary hyperuricemic uric acidemia shows production of blood uric acid or impaired excretion of uric acid caused by a variety of acute and chronic diseases, such as hematological disorders, malignant tumors, chronic intoxication, medications and high-purine diets. Chinese medicine treatment of hyperuricemia mainly focuses

on strengthening the spleen and kidneys, diuretic and clearing turbidity. Chinese medicine compound mainly exerts therapeutic effects through mechanisms such as inhibiting inflammatory reactions, protecting the kidneys, inhibiting xanthine oxidase or promoting uric acid excretion and elimination. The multi-target and multi-pathway action of Chinese medicine is consistent with the holistic and systemic nature of network pharmacology [7]. More and more researchers are exploring the mechanism of action of Chinese medicines through network pharmacology techniques. In this review, we will to explore the application of network pharmacology methods in the mechanism of Chinese medicine compounding for the treatment of hyperuricemia, which will provide a reference for an indepth and systematic study of Chinese medicine to prevent and treat hyperuricemia.

2. Materials and methods

Within the contemporary clinical application of Traditional Chinese Medicine (TCM), the elucidation of therapeutic efficacy is predominantly contingent upon two pivotal aspects: the pharmacodynamic material basis and the underlying mechanism of action. TCM adopts a "multi-component, multi-target, multi-pathway" approach, which has demonstrated commendable clinical outcomes. Nevertheless, the intricate interplay between TCM and disease states necessitates innovative conceptual frameworks and methodologies for comprehensive elucidation. Network Pharmacology (NP) emerges as a transformation paradigm, affording a novel perspective to investigate the complex interaction networks inherent in TCM. By mapping out these networks, NP facilitates the revelation and visualization of the multifaceted mechanisms underlying TCM's therapeutic interventions in multifactorial diseases [8]. The development and application of NP has facilitated the study of the safety, efficacy and mechanism of action of Chinese medicines, which has led to the increasing credibility and popularity of Chinese medicines. The current organ-centered medicine and the dogma of "one disease, one target, one drug" hinder the understanding of complex diseases and the development of effective drugs. And for chronic diseases represented by hyperuricemia, which may not only raise the risk of gout, but also the chance of diabetes as well as clinical diseases such as renal disease [9]. Therefore, in the future, more emphasis should be placed on the application of holistic thinking in TCM in the treatment of multifactorial diseases, and a shift from "phenotype and symptom" to "endophenotype and etiology" should be emphasized in the research and development of medicines and the elucidation of the mechanism of drug action. Over the past two decades, the integration of sophisticated intelligent technologies including metabolomics, proteomics, transcriptomics, single-cell genomics and artificial intelligence, which has propelled NP to new heights of sophistication and applicability. This evolution has underscored NP's substantial value and potential in drug discovery. Through rigorous investigation, NP not

only deciphers the intricate pathogenic mechanisms underpinning extant TCM formulas, furnishing a robust theoretical foundation for their clinical deployment, but also synergizes with multi-omics and interdisciplinary research avenues. This convergence extends the utility of NP into diverse clinical settings. The amalgamation of multi-omics data and interdisciplinary approaches has significantly enhanced our comprehension of drug mechanisms, offering fresh perspectives for the innovation of novel therapeutics and the assessment of drug toxicity. In synthesis, NP grounded in the tenets of systems biology, extrapolates drug action targets through interrogation of network databases. It constructs intricate drug-target-disease network correlations and establishes predictive models encapsulating the nuanced mechanisms of specific drug actions. This systems-level approach heralds a new era in precision medicine, where the complexity of biological systems is harnessed to inform more effective and targeted therapeutic interventions [8]. Analyzing the mechanism of drug action from the perspective of biological network equilibrium (Fig. 1). In this study, we searched the relevant literatures and summarized the composition, efficacy, and mechanism of action of Chinese herbal compound for the treatment of hyperuricemia (Table 1).
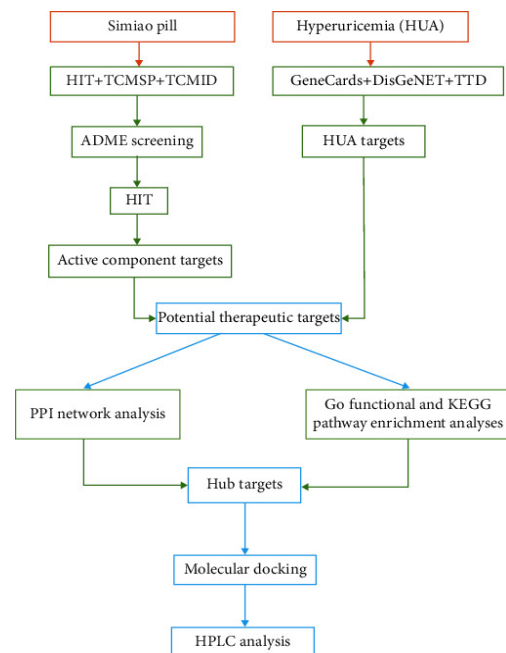

Fig. 1. Cyberpharmacology process (using the example of Si Miao Pill)[10]

3. NP in the treatment of hyperuricemia

3.1 Inhibition of inflammatory and antioxidant responses

3.1.1 Ermiao San

Yinhong Geng et al. utilized the screening of Ermiao San active compounds and their targets from the Traditional Chinese Medicine System Pharmacology

(TCMSP) database and analytical platform [10]. CTD, DisGeNET, DrugBank, GeneCards, OMIM, TTD and PharmGKB databases also be used to find disease targets related to hyperuricemia. Then the common potential targets of Ermiao San for hyperuricemia and gout were screened by passing the Venn diagram.

Table 1. Composition, efficacy, application and mechanism of Chinese medicine compounding for the treatment of hyperuricemia

Medicine	Ingredient	Efficacy	Clinical application	Pathway
Ermiao San	Rhizoma Atractylodis (Cang Zhu) and Cortex Phellodendri (Huang Bo)	clearing heat and drying dampness	significantly reduces SUA, inhibits the synthesis of UA, dissolves urate, alleviates inflammatory reactions, and protects the kidney	nuclear factor-κB, interleukin-17 and tumor necrosis factor
Gardenia-Poria Soup	Gardenia jasminoides and Poria cocos	the inhibition of xanthine oxidase (XOD) activity	reduce the level of uric acid and alleviate liver and kidney injury caused by inflammatory response and oxidative stress	the TNF-α and IL-7 signaling pathway
Si Miao Pills	Rhizoma Atractylodis, Cortex Phellodendri, Radix Achyranthis Bidentatae, and Semen Coicis at the ratio	anti-HUA function,anti-inflammation	damp-removing agent with the function of eliminating heat and dispelling dampness	IL1B, IL6, IL10, TLR4, and TNF
Ten-flavored Frankincense Pills	Phyllanthus emblica L., Cassia obtusifolia L., Boswellia carterii Birdw., Tinospora sinensis (Lour.) Merr., Terminalia chebular Retz., Aucklandia lappa Decne., Terminalia billirica (Gaert.) Roxb., Abelmoschusmanihot (L.)Medic., Adhatoda vasica Nees. and Shilajit	inhibiting XOD activity and promoting renal excretion of uric acid	dispelling dampness and dispersing cold	the mitogen-activated protein kinase (MAPK) signaling pathway, nuclear factor kappaB (NF-κB) signaling pathway and NOD-like receptor signaling pathway
Plantago Ovata Soup	geniposidic acid, verbascoside, typhaneoside, isorhamnetin-3-o-neohesperidoside, β-ecdysterone and chlorogenic acid chemical composition standard (all purity >95%)	involvement in the regulation of inflammatory and apoptotic signaling pathways	treating HUA and alleviating kidney injury	ABCG2,NLRP3
Fresh ginseng paste	Radix ginseng (RG)-Ziziphus jujube (ZJ)	pharmacological activities of antibiosis, anti-tumor activities, antioxidation, anti-diabetes, and anti-cardiovascular diseases	liver and kidney protection	IL-17,TNF, IL-1β, and VEGFA
Qu-Zhuo-Tong-Bi decoction	Tufuling(Smilax glabra Roxb), Bixie (Dioscorea spongiosa J. Q. Xi, M. Mizuno et W. L. Zhao), Yumixu (Zea mays L), Yiyiren(Coix lacryma-jobi L. var. ma-yuen (Rom.Caill.) Stapf), Xixiancao (Siegesbeckia orientalis L),Jianghuang (Curcuma longa L), Sangjisheng (Taxillus chinensis (DC.) Danser), Yanhusuo (Corydalis yanhusuo (Y. H. Chou and Chun C. Hsu) W. T. Wang ex Z. Y. Su and C. Y. Wu) and Foshou (Citrus medica L. var. sarcodactylis Swingle)	gut microbiome recovery and intestinal immune homeostasis	hyperuricemia and gout	IL-1β, IL-6, TNF-α and IL-17 ,CD4 T cells via PI3K-AKT-mTOR pathway

| Cichorium intybus formulation | Cichorii radix (the root of Cichorium intybus, Juju), Gardeniae fructus (the ripe fruit of Gardenia jasminoides J.Ellis [Rubiaceae], Zhizi), Lilii bulbus (the fleshly scale leaf of Lilium brownii F.E.Br. ex Miellez [Liliaceae], Baihe), Mori folium (the folium of Morus alba L. [Moraceae], Sangye) and Puerariae lobatae radix (the root of Pueraria lobata (Willd.) Ohwi [Fabacea]) | Improving the biodiversity of the intestinal flora | hyperuricemia and gout | IL-6、TP53、TNF and JUN |

Protein-protein interaction networks were then constructed using Cytoscape software. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were then performed. Finally, some compounds and core targets were selected for molecular docking validation by Autodock Vina and Pymol software. A total of one or more active compounds, such as quercetin, hansenin and β-sitosterol, be identified.

It has been demonstrated that Ermosan plays a therapeutic role in hyperuricemia and gout by modulating various biological processes, cellular compounds and molecular functions. The core targets of Ermiao San in the treatment of hyperuricemia and gout are, interleukin-2β, prostaglandin-endoperoxide synthase 17, and JUN. The key pathways were nuclear factor-κB, interleukin and tumor necrosis factor. The results of molecular docking analysis showed that the active compounds of Ermosan could bind well to the core protein receptor.

Combining the above research results, the researchers successfully constructed the network of Ermosan for the treatment of hyperuricemia. Through molecular docking, the researchers also verified that the active substance can bind well to the key receptor. This proves the mechanism of the treatment of hyperuricemia by Ermosan and provides a good theoretical basis for clinical application.

3.1.2 Gardenia-Poria Soup

This section provides details of the methodology used along with information on any previous efforts with corresponding references. Any details for further modifications and research should be included. Sufficient details should be provided to the reader about the original data source in order to enable the analysis, appropriateness and verification of the results reported in the study.

This study initiative initially targeted the two principal herbs, gardenia and poria, concentrating on their ability to inhibit the pivotal enzyme XOD. The investigators harnessed the power of molecular docking and network pharmacology techniques to unveil the uric acid-lowering mechanisms. Additionally, the incorporation of hyperuricemic animal models for drug screening added a layer of rigor to the experimental design. This approach not only showcased the multi-faceted effects characteristic of network pharmacology and TCM but also ensured the accuracy and validity of the findings through animal experimentation. The methodological innovations and the pharmacological insights derived from this study hold significant promise for future advancements in network pharmacology and the therapeutic management of hyperuricemia.

3.2 Suppression of the inflammatory response and diabetes comorbidities

Lei Zeng and colleagues conducted a systematic exploration of the active constituents and their corresponding targets in Si Miao Pill [10]. Employing the TCMSP, the Similarity Ensemble Approach (SEA), the Swiss ADME(Absorption, Distribution, Metabolism, Excretion) web tool, and the PharmMapper server, they predicted the pharmacologically active ingredients present in the formulation. Subsequently, to identify disease targets implicated in hyperuricemia, the researchers mined the GeneCards and Therapeutic Target Database (TTD). Through this extensive search, they pinpointed 28 bioactive compounds and 429 putative targets within Si Miao Pill, alongside 494 disease targets associated with hyperuricemia. Notably, 118 common targets were identified between the drug candidates and the disease condition.

To further elucidate the interactions among these targets, the authors utilized Cytoscape software version 3.6.1 to construct a protein-protein interaction network. This network was then subjected to GO enrichment analysis and KEGG pathway enrichment analysis on the STRING database platform. The KEGG pathway analysis illuminated the multifaceted therapeutic effects of Si Miao Pill on hyperuricemia, implicating several key signaling pathways such as TNF, apoptosis, and IL-17 signaling pathways.

In the final stages of their investigation, Zeng et al. focused on five pivotal targets: AKT1, IL-6, JUN, TNF, and CASP3. Using the SwissDock online tool, they conducted molecular docking studies to assess the binding affinities of these targets with selected compounds from Si Miao Pill, namely berberine, epiberberine, stigmasterol, and beta-sitosterol. The docking results indicated favorable binding interactions, suggesting that these targets might be instrumental in the therapeutic action of Si Miao Pill against hyperuricemia. Collectively, the study by Zeng et al. underscores the polypharmacological nature of Si Miao Pill, wherein the synergistic actions of multiple

components, targets, and pathways converge to deliver a therapeutic benefit in managing hyperuricemia [11]. In parallel, Qian Yue and colleagues approached the identification of Si Miao Pill targets through the use of the Herbal Ingredients' Targets (HIT), the TCMSP, and the Traditional Chinese Medicine Integrated Database (TCMID), thus providing a complementary perspective on the pharmacological landscape of this complex herbal formula [11]. HUA-related targets were retrieved from GeneCards, DisGeNET, and the Therapeutic Target Database (TTD). Protein-protein interaction (PPI) networks were constructed using the interacting gene/protein search retrieval search tool (STRING) database, ggraph, and igraph R package. GO and KEGG analyses were performed using ClusterProfiler.

Eventually Yue Qian's study showed that the Shimuwan-HUA target network contains 80 targets. The key targets are mainly involved in the inflammatory response and their therapeutic effects. It was demonstrated that Si Miao Pill could alleviate hyperuricemia by inhibiting inflammation, mediating AGE-RAGE and HIF-1-related pathways, and targeting IL1B, IL6, IL10, TLR4 and TNF.

Synthesizing the above research results of the two, they constructed the therapeutic network of Si Miao Pill for hyperuricemia through not exactly the same database searching and analysis, respectively. Zeng discovered a large number of key targets of traditional Chinese medicines mainly by constructing protein interaction network and molecular docking, whereas Qian mainly focused on the analysis of the pathways. Together, they demonstrated the potential value of Si Miao Pill in the treatment of hyperuricemia. Moreover, their different focuses on the same prescription have potential significance for future network pharmacology research.

3.3 Inhibition of inflammatory response and minimization of renal injury
3.3.1 Ten-flavored frankincense pills
This section provides details of the methodology used along with information on any previous efforts with corresponding references. Any details for further modifications and research should be included. Sufficient details should be provided to the reader about the original data source in order to enable the analysis, appropriateness and verification of the results reported in the study.

Experimental finding indicated that the administration of SWR markedly alleviated the symptoms of hyperuricemia and renal impairment in mice. Through network pharmacological analysis, a total of 36 bioactive ingredients of SWR, including phenolic acids, terpenoids, alkaloids, and flavonoids, were identified and found to interact with 115 targets implicated in the treatment of hyperuricemia. These interactions are associated with various biological processes and multiple signaling pathways.

Pharmacological experiments demonstrated that SWR exerted its beneficial effects by modulating key signaling pathways in mice with hyperuricemic nephropathy, including the mitogen-activated protein kinase (MAPK), nuclear factor-κB (NF-κB), and NOD-like receptor signaling pathways. This research initiative commenced with animal studies on mice with hyperuricemia and renal insufficiency, confirming the significant improvement effects of the flavonoid components of SWR on the condition of these mice. This was followed by a novel and reliable approach using network pharmacology to analyze the active ingredients of the traditional Chinese medicine compound. The study conclusively demonstrated that SWR has a clear ameliorative effect on hyperuricemia and its associated nephropathy in mice. SWR not only exhibited an ameliorative effect on hyperuricemia but also provided superior renal protective effects, thereby presenting potential application value in clinical practice.

3.3.2 Plantago Ovata Soup
A study of carminatives soup (CQD), a compound formula consisting of four herbs that have been shown to have therapeutic effects on hyperuricemia but by an unknown mechanism [13]. Network pharmacology analysis revealed a total of 99 potential targets associated with Carbon Quantum Dots (CQD), indicating their involvement in the modulation of both inflammatory and apoptotic signaling pathways. These findings suggest that CQD may hold therapeutic potential for the treatment of hyperuricemia and the alleviation of renal injury. In vivo pharmacodynamic studies demonstrated that CQD treatment led to a significant decrease in serum uric acid (SUA), serum creatinine (SCr), and blood urea nitrogen (BUN) levels in comparison to the model group ($P < 0.05$). Additionally, there was a marked reduction in inflammatory factors following CQD administration ($P < 0.01$).

Real-time fluorescence quantitative PCR and Western blot analyses further elucidated the mechanism of action of CQD. The mRNA and protein levels of the ATP-binding cassette efflux transporter protein G2 (ABCG2) were significantly upregulated in the CQD-treated group compared to the model group ($P < 0.01$). Conversely, the mRNA expression of caspase 3 and NOD-like receptor family member 3 (NLRP3) was significantly downregulated ($P < 0.05$), while the protein expression of NLRP3 was also significantly reduced ($P < 0.01$).

Collectively, these data indicate that CQD promotes the excretion of Uric Acid (UA) by activating ABCG2 and reduces inflammation and apoptosis through inhibition of the NLRP3 inflammasome, thereby providing nephroprotective effects. This findings highlight the therapeutic potential of CQD in the management of hyperuricemia with associated renal injury.

3.4 Suppression of inflammatory response and improvement of renal metabolism

Analysis of past studies has shown that the kidney is one of the most important organs for uric acid metabolism. The kidney is not only involved in the excretion of urate, but also closely related to the metabolism of urate. Improvement of renal metabolism has become a hot spot of drug and clinical research.

3.4.1 Fresh ginseng paste

Literature extracting the active constituents of fresh ginseng paste from the TCMSP [14]. Subsequently, they curated disease targets associated with hyperuricemia from reputable databases such as GeneCards, OMIM, Pharmacogenomics Knowledgebase (PharmGKB), and TTD. Statistical analysis revealed an overlap of 25 targets between the herbal pair and hyperuricemia.

To delve into the underlying interactions, a PPI network was constructed using the STRING 11.0 database, followed by functional enrichment analysis through the David database. The PPI analysis pinpointed TNF, IL-1β, and VEGFA as pivotal genes in the network. Furthermore, KEGG pathway enrichment analysis indicated that the Toll-like receptor signaling pathway and IL-17 signaling pathway play predominant roles in the disease mechanism.

Concurrently, in vivo experiments demonstrated that fresh ginseng paste (FGP) ameliorated hyperuricemia in mice, evidenced by decreased serum uric acid (UA), blood urea nitrogen (BUN), xanthine oxidase (XO), and liver XO levels ($p < 0.05$, $p < 0.01$). High-performance liquid chromatography (HPLC) was employed to analyze the principal components of FGP. Molecular docking simulations substantiated the strong binding affinities of the major FGP components to the core targets implicated in hyperuricemia.

This segment of the research meticulously employed network pharmacology to dissect the signaling pathways and associated genes influenced by FGP. Subsequent in vivo experiments corroborated the therapeutic effects of FGP on hyperuricemic mice, affirming the methodological rigor and the potential clinical utility of fresh ginseng paste.

3.4.2 Jing Fang Granule

In the pursuit to elucidate the pharmacological mechanism of Jing Fang Granule (JFG) in combating hyperuricemia, Yu Yajie et al. [15]. employed a systematic approach integrating network pharmacology and in vivo experimental validation. Initially, the active constituents within JFG were identified using the TCMSP, while disease-related targets of hyperuricemia were aggregated from databases such as OMIM, GeneCards, and TTD. A comprehensive analysis of these targets yielded a set of common targets, which were further subjected to PPI network construction within the STRING data platform. Subsequent enrichment analyses, facilitated by the Metascape data platform, unveiled the biological processes and signaling pathways implicated in hyperuricemia, with a particular focus on the apoptosis signaling pathway, necrotic apoptosis, and hypoxia-inducible factor-1 (HIF-1) signaling pathway.

The in vivo component of this study leveraged a mouse model of hyperuricemia induced by otipyrimidine potassium [15]. Administration of JFG resulted in a marked reduction of serum uric acid, blood urea nitrogen, and serum creatinine levels, underscoring its efficacy in ameliorating renal dysfunction associated with hyperuricemia. Moreover, JFG modulated the expression of key regulatory proteins within the kidney, including Bcl-2-associated X protein (Bax), cleaved Caspase-3, and B-cell lymphoma-2 (Bcl-2), thereby providing insights into its anti-apoptotic effects. Complementing these findings, renal metabolomics analyses revealed that JF exerted a profound regulatory impact on various metabolic pathways, including those involving alanine, aspartate, and glutamate metabolism, as well as glycolysis and the tricarboxylic acid cycle.

This study represents a significant advancement over previous work, as it not only delineates the multifaceted target and active ingredient networks underpinning the therapeutic effects of JF but also introduces an innovative integration of in vivo experimentation with renal metabolomics. This approach pHUArovides a more rigorous and intuitive depiction of JF's synergistic multi-component, multi-target, and multi-pathway mechanisms of action in the treatment of.

3.5 Improvement of intestinal flora

Hyperuricemia is a metabolic disease caused by abnormalities in uric acid metabolism, and its causation as well as treatment is a complex physiological and biochemical process involving multiple organs of the body, including the liver, kidneys, and intestines [16]. In the past studies, the uric acid metabolic pathway involving the liver and kidney has been basically elucidated, but the mechanism of uric acid metabolism in the intestine has not been fully clarified. In a study by Qian Wang et al. found that intestinal flora can promote the metabolism and catabolism of purines as well as the excretion of uric acid, providing a reference for further pharmacological studies. Song and Li et al. also focused on the improvement of intestinal flora and studied the therapeutic mechanism of two pairs of traditional Chinese medicine compound prescriptions for hyperuricemia using network pharmacology, which is a novel and pioneering approach, and has inspirational significance and potential value for the future application of network pharmacology in traditional Chinese medicine compound prescriptions.

3.5.1 Qu-Zhuo-Tong-Bi decoction（QZTBD）

Siyue Song and colleagues initially elucidated the therapeutic effects of the herbal compound QZTBD in a murine model of hyperuricemia and pulmonary injury, attributing its efficacy to the modulation of the intestinal microbiota and regulation of CD3 T cell differentiation via the PI4K-AKT-mTOR axis [17]. Although their findings underscored the potential of QZTBD in mitigating experimental hyperuricemia, the precise molecular underpinnings of this therapeutic action remained obscure. In pursuit of a deeper understanding of QZTBD's active

constituents and their targets implicated in gout, Siyue Song et al. harnessed the power of network pharmacology. This approach yielded the identification of 90 putatively active compounds within the QZTBD formulation and 262 corresponding gene targets. Leveraging extensive literature mining, they delineated 1,806 gout-associated target genes and pinpointed 240 overlapping targets between QZTBD and gout pathogenesis. These common targets were then subjected to rigorous bioinformatics analyses, including STRING interaction network construction and visualization via Cytoscape software. Application of the MCODE plugin facilitated the extraction of densely connected subnetworks, resulting in the recognition of 65 core targets. Notably, among these core targets were prominent molecules such as AKT1, VEGFA, and MMP9. Functional enrichment analyses, including Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway assessments, illuminated the involvement of these targets in diverse biological processes such as T cell activation, purine nucleotide metabolism, amino acid metabolism, ATP synthesis, and oxidative stress responses. Furthermore, KEGG pathway analysis highlighted the enrichment of key signaling pathways, including PI3K-Akt, IL-17, and TNF signaling cascades. The comprehensive compound-target network graphically depicted the multi-pronged interactions between QZTBD's active compounds and their pleiotropic effects on gout pathology, thereby substantiating the concept of synergistic therapeutic action. Collectively, these findings represent a significant advancement in unraveling the complex pathophysiological mechanisms of gout through a network pharmacology framework.

4. Summary, analysis, and outlook of studies on network pharmacology-based herbal compounding for hyperuricemia

4.1 Analysis of the content of the study (Table 2)

At present, most studies have explored the mechanism of action of compounding through modern pharmacological experimental animal models, but due to the complexity of the composition of traditional Chinese medicines have not been able to clarify the mechanism of action and the signaling pathway of intervention, and have not been able to analyze the active ingredients that play a major role in compounding. This is also one of the main reasons for the lack of international recognition of Chinese medicine compound preparations [19, 20]. The integrative multi-target approach inherent in network pharmacology aligns closely with the principles of Traditional Chinese Medicine (TCM) compounding. This systems-based methodology enables the rapid prediction of drug targets and mechanisms of action while simultaneously unveiling the primary bioactive components within TCM formulations. Notably, within each TCM compound designed for the treatment of hyperuricemia, a plethora of potent active ingredients have been identified. For instance,

compounds such as berberis red, berberine, stigmasterol, and sterol exhibit promising therapeutic potential. Furthermore, specific molecular targets, including AKT1, IL-6, JUN, TNF, and CASP3, are anticipated to play critical roles in the management of hyperuricemia.

Table 2 Summary and classification of research directions for various traditional Chinese medicine formulas

Research focus and direction	Name of Traditional Chinese Medicine Formula
Inhibition of inflammatory and antioxidant responses Suppression of the inflammatory response and diabetes comorbidities	Ermiao San Gardenia-Poria Soup Si Miao Pill
Inhibition of inflammatory response and minimization of renal injury	Ten-flavored frankincense pills Plantago Ovata Soup

In contrast to conventional medicines that typically focus on single disease targets or pathways, TCM compounds exert a broader regulatory impact on various tissues and organs, contributing to a holistic therapeutic effect on the entire physiological system. Clinically, this concept is exemplified by the diverse applications of certain TCM formulas. For example, Si Miao Pill is utilized in the treatment of anti-inflammatory responses and diabetes mellitus. Similarly, Ten-flavored Frankincense Pill and Psyllium Tang find application in mitigating renal injuries. Fresh Ginseng Paste and Thorny Prevention Granule are employed to enhance renal metabolism, while formulas like Turbidity Removal and Paralysis Soup hold promise in modulating the intestinal microbiota.

Upon analyzing multiple TCM compound formulations, it becomes evident that they commonly possess the ability to inhibit inflammatory reactions, oxidative stress, and related processes. Inflammation, in particular, may not only precipitate hyperuricemia but also manifests as a symptom in a myriad of metabolic, renal, and cardiovascular diseases. Collectively, these observations underscore the efficacy of TCM compounding in the treatment of various ailments, showcasing its unique strength in addressing multi-targeted and multi-pathway pathologies. This systems pharmacology perspective not only validates the traditional wisdom of TCM but also highlights the potential of network pharmacology in advancing our understanding and application of TCM compounding strategies.

4.2 Analysis of research methodology
4.2.1 Basic research methods (Fig. 1)

At present, most of the network pharmacology research ideas are to first find the Chinese medicine compound that

has clear efficacy on the disease. Chinese medicine database was used to find the active ingredients contained in the Chinese medicine compound first. The similarity search of the active ingredients with the target database was carried out, and finally get the important active ingredients and their action targets. The disease gene database and the known disease-related network were combined with the drug target database. The drug targets of the disease-related gene therapy disease targets and the disease network were obtained. The signaling pathways and their networks regulated by the TCM targets were derived from the above studies, and the effects of TCM on the disease networks were finally verified to draw conclusions.

In recent years, network pharmacology combined with other methods or techniques has provided new ideas to study the mechanism of action of Chinese medicine compounding. For example, UPLC/O-TOF-MSO [21] and other analytical techniques can be used to clarify the material basis of TCM compounding, machine learning algorithms, virtual screening and other technologies can further narrow the scope of database screening; molecular docking methods can be focused on the predictive metabolomics of individual compounds [22] and proteomics [23] The core of the predictions can be verified.

4.2.2 Innovative research methods

Network pharmacology in the study can also be fully integrated with other fields of disease or research methods, so as to further elucidate the material basis and mechanism of action of Chinese medicine compound for the treatment of hyperuricemia disease. Further proving the scientific and reliability of Chinese medicine compound prescription, such as in gardenia-poria soup, ten flavors of frankincense powder, fresh ginseng paste, Jing anti-granule, drain turbid and pass paralysis soup and so on many compound mechanism of action of the research were used respectively in different rat and mouse model for auxiliary animal experiments. The network of pharmacological research results are more persuasive, more rigorous and accurate. For example, the introduction of the intestinal flora in the study of the formula of Cichorium intybus and the Turbidifier and Paralysis Soup to explore the action target of the Chinese medicine compound from multiple angles not only focuses on the influence of the Chinese medicine on the intestinal flora during the absorption process, which is not only innovative, but also further elucidates the mechanism of action of the drug, which is more persuasive. In summary, network pharmacology can be linked to a variety of histological studies as well as experimental studies to assist in corroborating the correctness of the conclusions.

4.3 Deficiencies and Prospects for the Development of Cyber Pharmacology

Inconsistencies observed in target discovery and active ingredient analysis within the context of hyperuricemia by network pharmacology investigations may stem from the lagging updates in biological and annotation databases, alongside concerns regarding the precision and breadth of extant active ingredient inventories. Moreover, the outcomes of inter-platform data screening may be skewed. Additionally, the distribution of herbal compounds within bodily tissues subsequent to oral administration and entry into the bloodstream can be heterogeneous, leading to variable target affinities and compromising the integrity of screened components and targets. Consequently, there remains an imperative to augment the research applications of network pharmacology, integrating it with complementary disciplines such as pharmacokinetics, pharmacodynamics, the pharmacology of traditional Chinese medicine, and metabolomics. Such integration aims to enhance the efficacy and veracity of identifying action targets and discovering active ingredients.

In synthesis, network pharmacology has been extensively employed in the exploration of Chinese medicine compounding for hyperuricemia, underpinning the empirical foundation and scientific rigor of its therapeutic application. Prospectively, an increasing number of network pharmacology studies across various drugs will delve into an expanded array of drug pathways and mechanisms from diverse perspectives, adopting a multi-component, multi-target, and multi-pathway approach. This evolution promises to yield substantial added value and serve as a significant reference for drug development and clinical research endeavors.

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