Integrated Multi-target Mechanisms for the Therapeutic Effects of Tripterygium Wilfordii Hook on Ankylosing Spondylitis Complicated by Inflammatory Bowel Disease

Hui Yang[1], Fenglian Ma[2], Jing Xie[3], Xi Chen[4], Yuanyuan Liu[5], Xu Chen[6], Jinhui Ji[7], Dayue Darrel Duan[8], Donggeng Guo[9]*

[1]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie,People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: yhui0925@163.com

[2]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: 5736111987@qq.com

[3]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: 279878409@qq.com

[4]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: chenxi1987_@163.com

[5]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: liuyuanyuan2949@163.com

[6]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: 1903816284@qq.com

[7]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: ji_jinghui@163.com

[8]Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: dduan@swmu.edu.cn

[9]Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Email: guodonggen@126.com

Abstract

Background: Ankylosing spondylitis is an inflammatory disease characterized by chronic inflammation that most frequently affects the spine and sacroiliac joints. Up to 10% of patients develop IBD. Tripterygium wilfordii Hook is a traditional Chinese medicinal herb whose crude extract exhibited strong anti-inflammatory and immunosuppressive effects. However, mechanisms underlying the therapy of AS complicated with IBD are not well clarified. Based on these ideas, this study intends to find out the molecular mechanism by which TWH has its anti-inflammatory effect on AS-IBD through network pharmacology, PPI analysis, and molecular docking techniques. **Methods**: Active components of TWH obtained from pharmacology databases, the AS-IBD-related targets were extracted from GeneCards and DisGeNET databases. Protein interaction analyses were carried out using the STRING platform, and a molecular docking study was performed to assess binding affinities of TWH compounds to relevant proteins. Gene Ontology and KEGG pathway enrichment analyses were also applied to examine relevant biological processes and signaling pathways. **Result**: Interestingly, nineteen bioactive compounds found in TWH have been reported to interact with core targets such as VEGFA, ESR1, CASP3, and MAPK8. The core pathways found include the signaling pathways of TNF and IL-17. Through molecular docking, strong binding affinities were confirmed mainly with VEGFA and ESR1. **Conclusion:** Therefore, the evidence presented here supports the therapeutic efficacy of TWH for AS-IBD through regulation of inflammatory and immune factors and thus warrants further exploration in the future.

Keywords: Ankylosing Spondylitis, Tripterygium Wilfordii Hook, AS-IBD, Network Pharmacology.

INTRODUCTION

Ankylosing spondylitis is a chronic inflammatory disease that mainly hits the axial spine with progressive stiffness and immobility of the joints in the thoracic and spine. Eventually, the fusion of the joints leads to severe impairment in mobility, with advanced cases resulting

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Address for Correspondence: Doctor, Department of Rheumatology and Immunology, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001. Doctor, Key Laboratory of Autoimmune Diseases and Precision Medicie, People's Hospital of Ningxia Hui Autonomous Region (The Third Clinical Medicine College, Ningxia Medical University), Yinchuan, China, 750001
Email: guodonggen@126.com

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in marked disability.[1] AS is classically associated with inflammatory changes in the sacroiliac joints and the peripheral joints but a high proportion of patients also have extra-articular manifestations, of which IBD is the most prominent.[2] Clinically, overt IBD occurs in only about 5% to 10% of patients with AS, but ileocolonoscopy can detect subclinical gut inflammation in up to 49% of AS patients. Histopathological studies of intestinal biopsies also demonstrate microscopic damage in 50-60% of AS patients, thus underscoring the close relationship between these diseases.[3,4] In contrast, IBD patients often have a range of extraintestinal manifestations, and the musculoskeletal manifestations, such as AS, are the most common. Approximately 3% of patients with IBD develop AS, and this would suggest that the pathogenesis between the diseases is not fundamentally different.[5]

The pathophysiological relation between AS and IBD suggests that the inflammatory mechanisms are similar in both diseases, involving immune dysregulation with a propensity to overproduce cytokines leading to tissue damage.[6] The conventional therapies for AS and IBD entail immunosuppressants and biologic agents, though effective have several drawbacks such as cost implications and the potential for infections.[7] This makes the requirement for an alternative and cost-effective therapy urgent, especially for patients suffering from AS-IBD who are considered one of the harder groups in the disease.

This traditional Chinese medicinal herb, Tripterygium wilfordii Hook (TWH), has been used to treat several inflammatory and autoimmune diseases for more than a century. TWH is known to have anti-inflammatory, immunosuppressive, and anti-rheumatic properties. Hence, it has therapeutic potential in the treatment of conditions such as rheumatoid arthritis, AS, and IBD.[8] TWH is the most commonly used treatment in China for relief of joint pain syndrome, fever, local inflammation, and various other symptoms of autoimmune diseases. Researchers have demonstrated the ability of TWH to downregulate inflammatory mediators, inhibit fibrosis, and decrease collagen-induced arthritis.[9] A six-week study on AS patients demonstrated that treatment with TWH resulted in a significantly decreased BASDAI, and other disease markers, and the remission rates were significantly higher in AS patients treated with TWH as compared with controls.[10]

Though TWH is widely used, the mechanisms in its therapeutic activity on AS-IBD remain unclear. Many active compounds in TWH have been shown to participate in anti-inflammatory pathways, such as triptolide. Triptolide has been found to inhibit the IL-6/STAT3 signal pathway and reduce the content of IL-17, and suppress chemokine, which takes part in recruiting leukocytes and other immune cells, making this compound a potential AS-IBD therapeutic agent.[11] Nonetheless, the multi-target and multi-component characteristic of TWH raises questions that require further research to provide detailed mechanisms underlying its effects on AS-IBD.[12]

Recent breakthroughs in the developing science of network pharmacology have enlightened this multi-component and multi-target aspect of traditional Chinese medicine. Network pharmacology is an integration of bioinformatics with computational tools to predict a drug's interactions with targets and involved signaling pathways that operate in disease processes.[13] Thus, this approach is well aligned with the holistic aspect of traditional Chinese medicine in such diseases as AS-IBD where multiple targets may be required for treatment.[14] Aiming at these considerations, this research will scrutinize the mechanism of TWH therapy in AS-IBD with the help of network pharmacology, molecular docking, and pathway enrichment. Extending its treatment application in AS-IBD patients with essential information about bioactive compounds, target proteins, and signaling pathways related to TWH is within the scope of this research.

RESEARCH METHODOLOGY AND MATERIALS

Identification and Processing of Active Components and Targets of TWH

Active components of TWH were secured from the "Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP)."[15] OB is the percentage and rate of oral absorption of a drug after oral administration, while DL generally describes the drug with special functional groups or physical properties. After filtering those ingredients whose OB > 30% and DL >0.18, only high bioactivity ingredients were considered. The list of active ingredients was saved as "ingredients. txt." The TWH treatment targets were derived from the TCMSP bioinformatics database. This utilised Perl to parse out and identify TWH-related targets by full names and then convert TWH's gene name to appropriate standardised gene names using Uniprot database and exported to "uniprot.tsv". Finally, by using Perl analysis, active ingredient targets of TWH were identified, referred to as TWH-Tars.[16,17]

Screening Disease Targets

The gene data sets for ankylosing spondylitis (AS), AS with ulcerative colitis (UC), and AS with Crohn's disease were downloaded from GeneCards and DisGeNET databases using "ankylosing spondylitis" as the search term.[18,19] The duplicate entries were removed, and targets for AS-IBD were consolidated from the AS, AS-UC, and AS-Crohn's disease targets. The TWH-Tars overlap was then identified with the help of the Venny 2.1.0 tool, and the Venny algorithm would illustrate the possible targets of TWH, which can be used for the treatment of AS-IBD. The intersection results are taken as TWH-ASIBD-Tars and have been visualized with Wayne diagrams.

Construction of TWH-Active ingredient-ASIBD-Targets Network

A network diagram linking TWH, its active ingredients, and ASIBD targets was designed using CytoScape 3.8.2.[20] All the TWH-ASIBD-Tars were imported in the CytoScape and calculated by the internal tools in terms of effective components and network topology parameters, degree of connection, betweenness, and closeness. Results based on the network topology metrics indicated a core target and some key active components which mediate the effects of the drug's therapeutic action.

Identification of Potential Hub Targets and Protein-Protein Interactions Using STRING Network Platform

Via the STRING network platform, the selected targets were entered with the goal of finding probable hub targets for TWH and their interactions to initiate a "protein-protein interaction (PPI)" network.[21] The protein designation was established as Homo sapiens with a level of medium confidence at 0.400. Utilizing MCC method with the "Cytohubba" plug-in, the top 30 target proteins were ranked according to their network string interaction scores and then provided for further examination.

Pathway Enrichment Analysis Using DAVID and Visualization

For enrichment analysis, DAVID Database imported targets.[22] The type of recognition framework designated was to be the official gene symbol, the list type identified as GeneList, and species was restricted to Homo sapiens. The analysis revealed GO and KEGG pathways to significantly contrast with each other. Distribution of the targets in these pathways was analyzed. In turn, enriched GO terms were broadly classified based on BP, CC, and MF. Further knowledge on the position of these targets in the context of metabolism, signal transduction, and other relevant processes was possible through KEGG pathway analysis.[23] Furthermore, R software version 4.2.0 for Windows was used to draw a bubble plot showing the pathways. The most significantly enriched pathways were obtained from the DAVID Database, which highlighted the enriched genes using red stars.

Ligand and Receptor Processing for Molecular Docking Analysis

The structure of TWH was retrieved from PubChem in the mol2 format for the ligand preparation of 3D structure. Once we hydrogenated the ligand molecule and set up the charges along with the roots and the rotatable bonds, AutodockTools 1.5.6 was enlisted, and we stored the ligands in pdbqt files.[24]

Receptor Processing: Structures of the target proteins' docking receptors were taken from the RCSB Protein Database. Preparation encompassed set-up in AutodockTools 1.5.6 by adding hydrogens, evaluating Gasteiger charges, merging nonpolar hydrogens, and finally saving receptors as pdbqt files.

Docking Parameter Setting: The boxesize and molecular docking coordinates for Vina are adjusted. The exhaustiveness parameter is 15. Alternatively, parameters are defined by their preset defaults.

Operations and Output: The semi-flexible docking procedure is carried out using Autodickvina 1.1.2.[25] A conformation with the best affinity will serve as the final docking result. The evaluation of ligand-receptor interaction utilised binding energy. When the binding energy is found to be lower than -4.25 kcal•mol-1, there is an inclination towards moderate binding activity; any binding energy that is less than -5.0 kcal•mol-1 is deemed good; and significantly below -7.0 kcal•mol-1 signifies strong binding activity.[26,27] Increased interaction reliability tends to correlate with higher conformational instability between chemical molecules and receptors, which implies better docking reliability.

RESULTS

Screening for Bioactive Compounds and Related Targets of TWH in the Treatment of ASIBD and the "TWH-Active Ingredient-ASIBD-Targets" Network and Core Targets

Table 1: Information of 19 Bioactive Compounds Screened in TWH.

No.	Mol ID	Bioactive Compounds	OB%	DL
1	MOL000422	kaempferol	41.88	0.24
2	MOL005828	nobiletin	61.67	0.52
3	MOL003187	triptolide	51.29	0.68
4	MOL000296	hederagenin	36.91	0.75
5	MOL000358	beta-sitosterol	36.91	0.75
6	MOL000449	Stigmasterol	43.83	0.76
7	MOL002058	40957-99-1	57.2	0.62
8	MOL003184	81827-74-9	45.42	0.53
9	MOL003192	Triptonide	67.66	0.7
10	MOL003217	Isoxanthohumol	56.81	0.39
11	MOL003225	Hypodiolide A	76.13	0.49
12	MOL003229	Triptinin B	34.73	0.32
13	MOL003231	Triptoditerpenic acid B	40.02	0.36
14	MOL003248	Triptonoterpene	48.57	0.28
15	MOL004443	Zhebeiresinol	58.72	0.19
16	MOL003185	(1R,4aR,10aS)-5-hydroxy-1-(hydroxymethyl)-7-isopropyl-8-methoxy-1,4a-dimethyl-4,9,10,10a-tetrahydro-3H-phenanthren-2-one	48.84	0.38
17	MOL003199	5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin	61.85	0.54
18	MOL003283	(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	66.51	0.39
19	MOL009386	3,3'-bis-(3,4-dihydro-4-hydroxy-6-methoxy)-2H-1-benzopyran	52.11	0.54

Figure 1.MOA of TWH for AS-IBD and the bioactive compounds and related targets of TWH and the comprehensive “TWH-Active ingredient-ASIBD-Targets” network and core targets. (A) The veen diagram of this paper is the following: Analysis of the target of TWH and the target of AS-IBD. 64 TWH targets and 1011 AS-IBD targets were identified. The cross-section of the 2 circles represents the target of TWH for AS-IBD. (B) By way of conclusion, the network connecting bioactive compounds and their targets within the therapeutic framework of TWH for patients with AS-IBD has been described. Red represents AD-IBD disease, green represents target protein, blue represents TWG, and yellow represents TWH active compounds. (C) Network of potential targets of TWH that could be used in the treatment of AS-IBD. Each node in the network represents proteins, standing for all proteins yielded from a singular protein-encoding location. Margins form protein-protein associations, meaning that the proteins are involved in a collective contribution to a shared function that is both significant and specific. Network statistics: nodal: 30, border: 143, mean nodal: 9.53, with a local clustering coefficient of 0.683, the expected border is 30, and the PPI-enriched p value is <1.0e-16. (D) Central themes affected by intertwined issues.

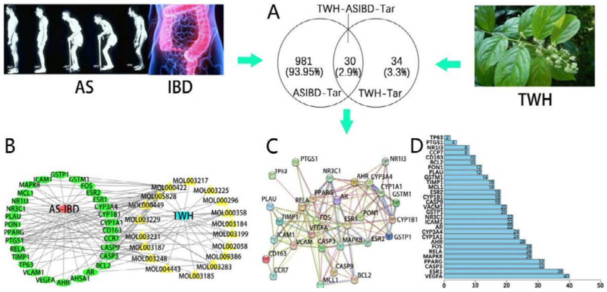

Figure 1: Therapeutic Targets Linking AS, IBD, TWH.

Based on the OB and DL values, 51 TWH active components and 64 relevant targets were selected by TCMSP. Next, the database containing AS-IBD 1011 targets was obtained from GeneCards and DisGeNET. After mapping the targets associated with TWH and AS-IBD were used in Venn diagrams, resulting in 30 targets that overlapped and included related target-related bioactivity components (Figure1(A)). Finally, Cytoscape visualization revealed 19 bioactive compounds (Table 1) and 30 overlapping as targets for TWG in AS-IBD therapy and constructed a Drug Target-Drug Interaction Plot (Figure1(B)). Furthermore, we obtained primary active components of TWH activity from the “TWH-Active Component-ASIBD-Target” network parameters. In accordance with the degree value, the top seven are triptolide, kaempferol, nobiletin, (2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol, beta-sitosterol,Isoxanthohumol,5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3) -coumarin.

Construction of PPI Network and Identification of Core Targets for AS-IBD Therapy

To investigate the instrument of TWH, we established a “PPI network.” A STRING database was constructed by entering 30 overlying targets. There were 30 nodes in the target network. The network was refined with 29 interconnected nodes and 143 edges from unconnected nodes, yielding an average node degree of 9.53 (Figure1 (C)). PPI network data were constructed in STRING and then the platform was importe d into Cytoscape to select the Cytohubba plug-in step-down to get to the core target. From the scoring, VEGFA, ESR1, PARG, CASP3, RELA, and MAPK8 may play a critical role in the therapy of AS-IBD (Figure1 (D)).

Gene Ontology (GO) Annotation and KEGG Pathway Analysis

Table 1: The Top 10 of GO Analysis of the Common Core Targets of TWH and AS-IBD.

BP	GO:0032496	Response to lipopolysaccharide	5.79E-08 7	7
BP	GO:0030518	Intracellular steroid hormone receptor signaling pathway	3.60E-07	4
BP	GO:0034097	Response to cytokine	7.55E-07	5
BP	GO:1902895	Positive regulation of pri-miRNA transcription from RNA	1.05E-06	5
BP	GO:0009636	Polymerase II promoter response to toxic substance	4.84E-06	5
BP	GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	5.62E-06	11
BP	GO:0043065	Positive regulation of apoptotic process	7.60E-06	7
BP	GO:0043066	Negative regulation of apoptotic process	9.06E-06	8
BP	GO:0006805	Xenobiotic metabolic process	2.18E-05	5
BP	GO:0000122	Negative regulation of transcription from RNA polymerase II promoter	7.10E-05	9
BP	GO:0000785	Chromatin	9.23E-06	10
BP	GO:0043231	Intracellular membrane-bounded organelle	3.10E-04	8
BP	GO:0032991	Macromolecular complex	3.23E-04	7
BP	GO:0005737	Cytoplasm	3.96E-04	18
BP	GO:0005739	Mitochondrion	6.39E-04	9
BP	GO:0005829	Cytosol	0.0011371	17
BP	GO:0005654	Nucleoplasm	0.0014810	14
BP	GO:0005634	Nucleus	0.0029077	17

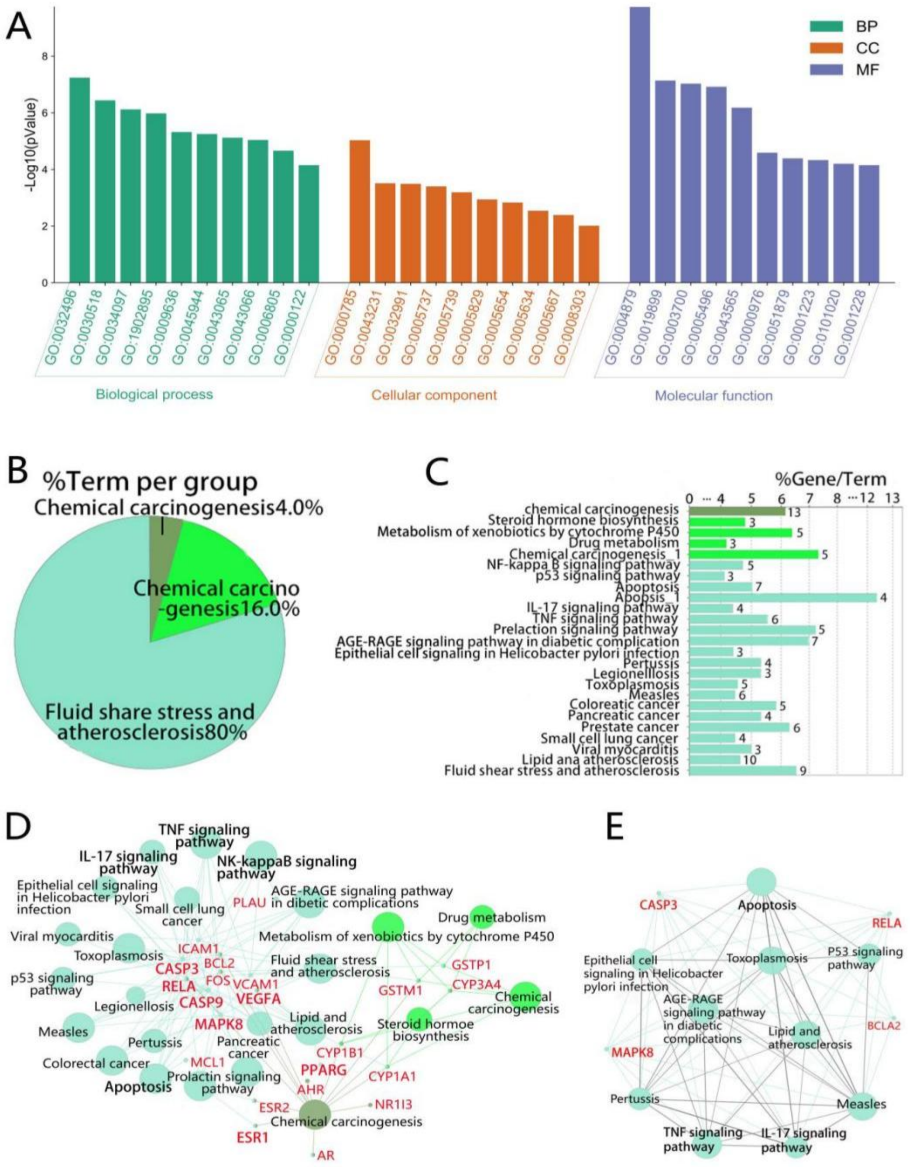

Figure 2: Gene Ontology and Pathway Enrichment Analysis.

I conducted enrichment analyses to research the shared pathways and interactions between AS-IBD and TWH therapies. DAVID database was employed to perform a GO analysis and KEGG pathway enrichment on the common core targets of TWH and AS-IBD. There were 206 GO terms found within this study and 140 annotated for BP, while 16 had an annotation of CC, and 50 annotated for MF. In Figure 2(A), the graphic illustrates the top 10 terms that are overrepresented for BP, CC, and MF. The primary BP terms relate to lipopolysaccharide response, intracellular steroid hormone receptor signalling, cytokine responses, positive and negative regulation of apoptosis, and various other biological processes. The CC term is very highly enriched in chromatin, intracellular membrane-bound organelles, macromolecule complexes, cytoplasm, mitochondria, cytosol, nucleoplasm, nucleus, transcription factor complexes, and caspase complexes. The MF terms are essentially identified as RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, enzyme binding, transcription factor activity, sequence-specific DNA binding, transcription regulatory region-specific DNA binding, Hsp90 protein binding, transcription coactivator binding, and oestrogen 16a-hydroxylase activity (Figure 2(A), Table 2). Through KEGG pathway enrichment analysis, 59 pathways were found of which were deemed significant. Those potential TWH anti-AS-IBD targets: fluid shear stress and atherosclerosis, lipid metabolism, the AGE-RAGE signalling pathway in diabetic complications, apoptosis, TNF signalling, IL-17 signalling, Th17 cell differentiation, osteoblast differentiation, NF-kappa B signalling, Toll-like receptor signalling, and MAPK signalling (Figure 2(B, C)). Findings of the present study indicate that the innate immune system, cytokine signalling, and immune responses are connected to the development of AS-IBD, with the TWH playing a modifying role in these immune functions.

Molecular Docking Verification

Table 3: Prediction of the Ability of Molecular Compounds to Bind to Target Genes.

Bioactive Compounds	VEGFA	ESR1	CASP3	PPARG	MAPK8	RELA
Triptolide	-6.8	-9.4	-7.4	-8.6	-7.4	-9.2
Kaempferol	-5.7	-7.9	-7.2	-7.5	-8.4	-9.2
Nobiletin	-5.7	-5.8	-6.8	-7.5	-6.4	-6.9
(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	-5.5	-5.9	-6.8	-6.4	-6.3	-7.1
Beta-Sitosterol	-5.8	-6.7	-8.0	-7.6	-8.4	-8.1
Isoxanthohumol	-6.0	-7.1	-7.8	-8.0	-7.0	-9.1
5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin	-6.7	-8.6	-7.6	-8.3	-9.5	-9.9

Table 4: Demonstration of the Binding of the Compounds to Molecular Target.

Ligands	Receptor	PBD ID	Residue Involved in H Binding	H-bond Length (Å) length(Å)	Docking Score(kcal/mol)
triptolide	RELA	3QXY	TYR223;ASN251	3.08;3.13	-9.2
kaempferol	VEGFA	5DN2	GLU327;ARG408;ARG410	2.23;2.08;2.03	-5.7
nobiletin	ESR1	1XPC	ARG515;SER518;TYR526	2.17;3.48;3.27	-5.8
(2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol	PPARG	3U9Q	GLN545;LYS457;MET463	2.26;1.94;2.45	-6.4
beta-sitosterol	CASP3	6BFJ	PHE143;GLY145	2.93;2.67	-8.0
Isoxanthohumol	RELA	3QXY	TYR223	2.94	-9.1
5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin	MAPK8	2XRW	ALA36;GLN37;LYS55;LYS153	2.35;2.21;1.89;3.18	-9.5

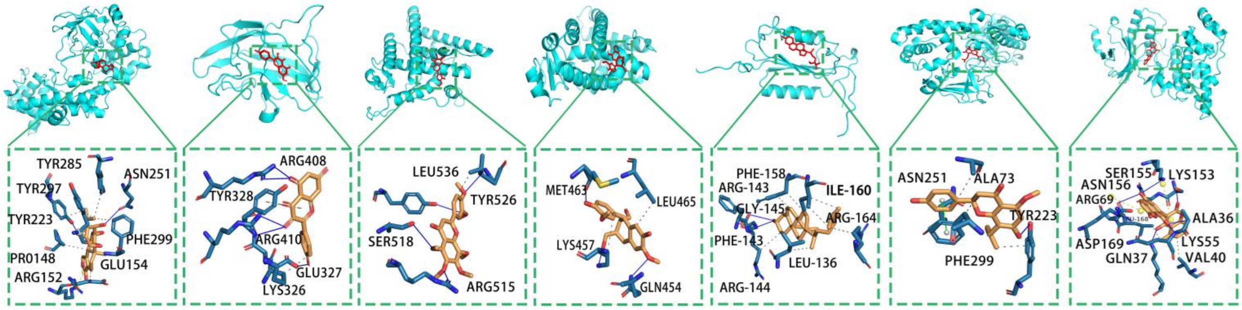

Figure 3: The Significant Molecular Docking. From left to right (triptolide,RELA,-9.2kcal/mol), (kaempferol,VEGFA,-5.7kcal/mol),(nobiletin,ESR1,-5.8kcal/mol),((2R,3R,4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3-dimethylol-tetralin-6-ol,PPARG,-6.4kcal/mol),(beta-sitosterol,CASP3,-8.0kcal/mol),(Isoxanthohumol,RELA,-9.1kcal/mol),(5,8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin, MAPK8, -9.5kcal/mol).

Molecular docking technology is a virtual screening approach that makes important use of the computer-aided design. The drug-drug interactions are analyzed using molecular docking. Results were obtained through molecular docking that VEGFA, ESR1, CASP3, PPARG, MAPK3, and RELA molecules can bind with stronger affinity to the TWH active components. Thus, the small molecular ligands directly bind into the target proteins in spontaneous and efficient manners (Figure 3, Tables 3–4).

DISCUSSION

It has been mentioned that in the clinical scenario, a percentage of 5-10% of patients suffering from AS are diagnosed with severe IBD and that an even greater number of AS patients have subclinical bowel inflammation which would be detected by colonoscopy.[3] Incidence and pathogenesis of AS and IBD are closely comparable according to recent studies. Thus, an inexpensive treatment for AS complicated with IBD is essentially important for identification.[28,29] Tripterygium wilfordii Hook (TWH) has been used to treat different inflammatory conditions, including redness and puffiness, in traditional medicine for centuries. Triptolide, one of the major active components of TWH, has been shown in vitro to inhibit collagen synthesis, alkaline phosphatase and calcium nodules.[30] These facts indicate that triptolide may be a potential treatment for AS. Previously, it was shown that triptolide can treat IBD symptoms both in mouse models and patients.[31] Of late, there has been increasing interest in finding out how TWH acts through its mechanisms in AS-IBD.

Using the data of the GeneCards and DisGeNET databases, we identified pathogenic genes associated with AS complicated by IBD in the present study. Based on the structure of TWH, we used computation of system pharmacology, bioinformatics, and molecular “Docking Techniques” to determine some of the targets and signaling pathways intricate in TWH for treatment with AS-IBD. Our study found some of the key chemical compounds of TWH, kaempferol, triptolide, nobiletin, (2R, 3R, 4S-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2, 3-dimethyltetralin-6-ol, beta-sitosterol, and 5, 8-dihydroxy-7-(4-hydroxy-5-methylcoumarin-3)-coumarin, isoxanthohumol. These compounds were targeted to core genes, especially VEGFA, ESR1, CASP3, PPARG, and MAPK8, and through multiple pathways, for example, TNF signaling, Th17 cells, IL-17 signaling, Th1, Th2 cells, NF-kB, as well as gut microbiota regulation.

Angiogenesis plays a key role in the onset of chronic inflammatory disease, and overexpression of VEGF may further contribute to its overexpression in rheumatological conditions. In addition, VEGF is involved in bone formation, and circulating plasma VEGF levels are reported to be elevated in patients with AS.[31,32] VEGF-A has also been shown to be overexpressed in IBD patients, although it remains unclear what this functionally implies. Moreover, the levels of VEGF-An mRNA are hugely upregulated in the animals with chronic experimental colitis.[33] Therefore, VEGF emerges as a potential therapeutic target in AS-IBD.[34] Thus, VEGF headed the list of core genes that served to validate the interaction between triptolide and kaempferol with VEGF during molecular docking analysis. In addition, these results suggest that VEGF is a potential site of action for TWH during treatment of AS-IBD.

Further studies, by us and other researchers, have shown that also ESR1, CASP3, PPARG, MAPK8, and RELA may be critical targets for AS-IBD therapy. Estrogen is one of the crucial sex hormones and has a role in the regulation of inflammation and immune responses by binding to the nuclear hormone receptor encoded by ESR1. RELA is a member of the NF-kB family and is involved in the NF-kB signaling pathways which include both RELA-dependent canonical and the RELB-dependent non-canonical pathways, which are significant in the modulation of immune response.[35] The CASP3 or caspase-3 is an enzyme with a role in apoptosis, which must have an active function in cell death processes, such as atrophy of epithelial and villous cells in the case of celiac disease.[36] The biological functions of TWH in AS-IBD have been studied in particular regarding estrogen 16-alpha-hydroxylase activity, NF-kB signaling, and apoptosis pathways.[37]

In KEGG analysis, the most important pathway that was associated with this data set was found to be TNF signaling. Here, TNF-α is the most powerful pro-inflammatory cytokine, which is a central regulator of inflammation and leads to cytokine production, increased expression of adhesion molecules, and activation of pro-inflammatory cells. The pathogenesis of several autoimmune conditions, including AS and IBD is associated with the presence of TNF-α in inflammatory sites.[38] TNF-α inhibitors can neutralize the signal transduction initiated by TNF-α. This implies that anti-TNF-α can inhibit the downstream effects of TNF-α through a reduction in levels of TNF-α production and promotion of apoptosis in immune cells that subsequently reduces other inflammatory factors produced by additional immune cells.

In our research, the active substances of TWH were aligned to the established targets of AS-IBD, thereby showing that these shared targets might be associated with the therapeutic mechanisms behind TWH's anti-inflammatory activities against AS-IBD. Bioinformatics analysis by KEGG supported the involvement of TNF signaling in the treatment of AS-IBD by TWH.[39] The treatments of ankylosing spondylitis of rats by TWH are shown to decrease inflammation through downregulated mRNA levels of TNF-α. We will therefore further clarify this subject on the basis of our results and in agreement with such findings that will thereby support the hypothesis that TWH thwarts therapeutic action through alteration of TNF signalling pathways in AS-IBD patients.

As the research progresses, it is evident that Th17 cells are at the center of the inflammation process involving AS. These cells release other inflammatory cytokines like IL-17, IL-6, IL-22, IL-26, and TNF-α that functionally take part in the process of mobilizing, recruiting, and activating neutrophils, thereby resulting in pro-inflammatory responses and acute inflammation. IL-17, the major effector cytokine of Th17 cells, by binding to IL-17 receptors (IL-17R) on various cells, stimulates the production of pro-inflammatory mediators and chemokines that initiate multiple inflammatory pathways.[40] Therefore, IL-17 is considered the significant inflammation mediator in AS. The activities of "IL-17" and "IL-23" are tissue-specific, and elevated stages of "IL-17" and "IL-23" were observed in serum and synovium of patients in several studies. Studies also speculate that the IL-23 might be translocated from the intestines to the affected musculoskeletal tissues though such a mechanism is yet not clearly elucidated.[41] The differentiation of Th17 cells is mediated mainly by IL-6 and TGF-β with a further stabilization by IL-23 and IL-1β. Strober and Fuss[42] found, in IBD research, that both Th1 (such as TNF-α and IFN-γ) and Th17 (such as IL-17A) cytokines are overproduced in the inflamed intestinal mucosa of patients with Crohn's disease.[3] Maxwell *et al.*[43] and Lee *et al.*[44] had demonstrated through various IBD mouse models that with antibodies that block IL-17RA signaling, intestinal permeability was increased and colitis was exacerbated, with an increased mortality rate.[45] It would thus signify that although IL-17 is a pro-inflammatory cytokine, the more crucial function of the molecule, preventing inflammation and additional damage to the barrier, may be to maintain the barrier and control the presence of microbes.

In the study conducted here, we found that the active compounds in Tripterygium wilfordii treat AS-IBD by modulating the pathways of Th17 and IL-17. However, from these studies, it seems as if the compound specifically acts on a few major, well-known pathways responsible for the treatment of AS-IBD. Further research is required to elucidate more about the mechanisms involved in the regulation of the pathways.

Toll-like receptors are a type I transmembrane glycoprotein and non-catalytic receptor belonging to the TLR protein family.[46] They have been associated with immune responses, mainly those of both innate and adaptive immunity, against infectious agents, as is predominantly the case for TLR2 and TLR4.[47] These have been associated with Ankylosing Spondylitis (AS) among others. Initiation of inflammatory responses in IBD is primarily through TLR4, and genetic polymorphisms of TLR4 have an effect on the rate of incidence in the disease.[48] TLR4 mediates excessive activation of the NF-kB signaling pathway resulting in overproduction of inflammatory cytokines and accumulation of neutrophils, macrophages, and lymphocytes in the diseased sites. The overproduction of cytokines results in extreme tissue damage, and high levels of cytokines within the intestinal mucosa result in disruption to tight junctions, through which microorganisms can penetrate the epithelial barrier.[49] Loss of the integrity of the intestinal mucosal layer and altered gut microflora imbalances are two key events that contribute to the pathology of both AS and IBD.

We found, in our study, that it is the active constituents of Tripterygium wilfordii Hook (TWH) responsible for the process. The mucosa of the intestine co-evolves with the host and plays a vital role in several physiological activities, such as metabolism and immunity. Costello *et al.*[50] using the technique of 16S rRNA sequencing, reported that only a few bacterial families, such as Trichospiridae, Veronicaceae, Porphyromonadaceae, and Bacteroidaceae, are highly enriched in intestines of AS patients, whereas Lachnospiraceae and Prevolaceae are closely associated with AS as well as IBD.[51] These studies indicate higher evidence suggesting that gut microbiota dysregulation is also very prevalent among the AS patients. Additionally, depletion of the mucin layer results in an IBD-like phenotype and endoplasmic reticulum stress that could be driving the production of IL-23.

This delicate interplay between IECs, microbes, and local immune cells maintains homeostasis in the gut but opens the door to disease with disruption. In IBD, breaks in tight junctions mean there is increased permeability between IECs; thus, "intestinal leakage" occurs.[52] In first-degree relatives of patients with AS, intestinal permeability is also increased, indicating a genetic contribution to the disease that affects the intestinal tract.

Our study indicated that TWH's active component is part of Toll-like receptor signaling pathway and, as well as being involved in pathways of bacterial infection and, therefore might exhibit potential anti-inflammatory activity through the modulation of the NF-kB signaling pathway.[53] Therefore, we hypothesize that AS-IBD can be treated by preventing damage to the intestinal mucosa barrier and inhibition of the Toll-like and other signaling pathways.[54] However, further work needs to be performed in order to completely understand mechanisms behind these processes.

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Conflicts of Interest

The authors declare no conflicts of interest

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