Data Mining and Network Pharmacology-Based Exploration of the Mechanism of Action of Huangqin Qingre Chubi Capsule in Improving Immune Inflammation of Ankylosing Spondylitis

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Abstract

Objective. The goal of this research was to probe the mechanism of Huangqin Qingre Chubi (HQC) in the improvement of immune inflammation of Ankylosing spondylitis (AS).

Methods. Clinical data mining was implemented to assess the efficacy of HQC in AS therapy. The target network of HQC active ingredients for AS therapy was established by network pharmacology. The relationships between HQC active ingredients and major objectives of AS were forecasted by molecular docking technology. Finally, Cytological tests were performed for the evaluation of the effects of the drugs on the core targets.

Results. Clinical data mining indicated that HQC was highly related to the improvement of immunoinflammatory markers. Network pharmacology results demonstrated that the main targets of HQC, including Peroxisome proliferator-activated receptor gamma (PPARG), Prostaglandin G synthase 2 (PTGS2), recombinant catalase (CAT), Chemokine (C-X-C motif) ligand 8 (CXCL8), and Vascular endothelial growth factor (VEGFA), were closely associated with the signaling pathways of IL-17, NF-kappa B, TNF, T cell receptor, and Th1, and Th2 cell differentiation in AS therapy. In the molecular docking analysis of the HQC active components, 70% of the binding energy was under -4.25 kcal/mol, and 53% of the binding energy was no more than -5.0 kcal/mol, of which the binding energy of stigmasterol with PPARG, CXCL8, and CAT were under -7.50 kcal/mol, implying that the chemically active ingredients of HQC formula have good binding activity against inflammatory targets. In vitro cell experiment results suggested that HQC down-regulated the expression of PTGS2, and up-regulated the expression of PPARG and CAT.

Conclusion. HQC exerts functions in improving the immune inflammatory indicators in patients with AS for the reason that the active ingredient of Traditional Chinese medicine (TCM) in HQC act through oxygen species, oxidative stress, transcription factors, and apoptosis.

Introduction

Ankylosing spondylitis (AS) is a kind of disease which does not constitute infection and has long-term accumulation to form disease damage, progressive, and it mainly affects the spine and may involve the sacroiliac and surrounding joints to varying degrees, causing them to develop inflammatory reactions\[1\][2]. The lesions mainly involve sacroiliac joints, spinal joints, paravertebral soft tissues, peripheral joints, and even spinal deformity and ankylosis. The main manifestations of AS patients are low back pain, knee pain, and ankle pain, which are often accompanied by morning stiffness. AS is equivalent to the category of “joint pain” in Chinese medicine [3]. Arthralgia is a kind of disease syndrome mainly resulted from the sensation of wind, cold, dampness, and heat on the surface of human muscles and meridians, which contributes to pain, numbness, stress, unfavorable flexion and extension, or joint swelling and burning. Non-steroidal anti-inflammatory drugs, immunosuppressants, and biological inhibitors are the main approaches for the treatment of AS with acceptable overall curative effects but there are many side effects of these drugs. Recently, TCM treatment of AS has attracted more and more attention, and it can not only have a good curative effect but also reduce the adverse reactions of Western medicine, thereby improving the prognosis and reducing the recurrence of the disease.

HQC consisting of Scutellariae Radix, Gardeniae Fructus, Coicis Semen, Radix Clematidis, and Persicae Semen, is a hospital prescription of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. Among them, Scutellariae Radix and Gardeniae Fructus are sovereign drugs, Radix Clematidis is minister drug, and Coicis Semen and Persicae Semen are envoy drugs. HQC has the capabilities of clearing heat, eliminating dampness, and removing toxicity. According to previous clinical studies, HQC has good clinical efficacy in treating AS, however, the multi-target mechanism of HQC on AS remains to be elucidated. Network pharmacology is a new scientific method based on network data analysis and system biology, and the conjunction of which with TCM fills the gap between traditional medicine and modern medicine [4]. Therefore, this paper aims to unveil the immune-inflammatory latent targets of HQC in AS patients upon the combination of network pharmacology and molecular docking. Finally, the above results are simply verified by experiments.

Materials and Methods

Ethical approval
This work was ratified by the Ethics Committee of Anhui Provincial Hospital of Traditional Chinese Medicine (approval number: 2020AH-08). All the clinical experimenters signed the informed consent form.

**Materials**

From May 2012 to December 2021, the inpatient data of 1,365 AS patients hospitalized in the Department of Rheumatology and Immunology of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine were collected. The information collected includes the use of HQC and immune inflammatory indicators, including Erythrocyte sedimentation rate (ESR), Complement component 4(C4), C-reactive protein(CRP), Immunoglobulin A(IgA), Immunoglobulin M(IgM) and Immunoglobulin G(IgG). The collected medical records were divided into a reference group (treatment of Western medicine only, \( n = 1,122 \)) and an experimental group (treatment of Western medicine and HQC, \( n = 243 \)).

To further verify this study, some core targets were further verified by experimental methods. Serum samples from 30 healthy individuals were harvested from the Health Examination Center of the First Affiliated Hospital of Anhui University of Traditional Chinese Medicine. Additionally, 30 AS patients were randomly selected from the Biobank of the hospital. All 30 patients were treated with HQC, and their serum was collected before and after treatment. Human cyclooxygenase 2 (COX-2) enzyme-linked immunosorbent assay (ELISA) kit (catalog number: JYM0940Hu), prostaglandin G synthase 2(PTGS2) ELISA kit and human peroxisome proliferator-activated receptor gamma (PPARG) ELISA Kit (catalog number: JYM1906Hu) are from Wuhan Meigen Biotechnology Co., Ltd. (Wuhan, China). Catalase assay (CAT) ELISA kit (catalog number: A007-1-1&202208016) is from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

**Methods**

**Propensity Score Methods** Propensity score matching (PSM) is a widely-used statistical method for the analysis of data from observational studies. There are many biases and confounding variables for different reasons. The PSM approach is designed to diminish the effects of the aforesaid biases and confounding variables in allocating subjects into the experimental and control groups [5]. Step 1: The "grouping" was used as the dependent variable, and confounding factors, including gender, age, length of stay, and comorbidities (hypertension, hyperlipidemia, diabetes, and hyperuricemia), were independent variables. Step 2: The propensity score of each subject was calculated referring to the regression operation results. Step 3: The subjects between the two groups were matched according to the propensity score to achieve the balance of confounding factors between groups. Finally, 243 cases were classified into the treatment group and the control group.

**Association Rules** An association rule [6] is in the form of \( X \rightarrow Y \) where \( X \) and \( Y \) are known as antecedent or left-hand-side (LHS) and consequent or right-hand-side (RHS) of association rules, respectively. Among them, support and trust are included in the association rule \( XY \). We analyzed the correlation between HQC and immunoinflammatory indicators using a prior module in IBM SPSS Modeler 18.0 software. The formula is shown below [7]:

\[
\text{support}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{N}
\]

\[
\text{confidence}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{N}
\]

\[
\text{lift}(X \rightarrow Y) = \text{confidence}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{N}
\]

**Random Walking** The formation of the random walking routes could reflect improved overall efficacy of the treatment when the patient reaches a certain level for the scale. The random walking model of the international BioMedimmune-inflammation indices was evaluated by the Oracle Developer Suite 10 g, then observe the improvement of laboratory indicators on drug compatibility. Here shows the formula [8]:

\[
...
Acquisition and Screening of Pharmaceutical Ingredients

Traditional Chinese Medicine Systems Pharmacology (TCMSP) is utilized for screening the effective chemical ingredients of TCM and their corresponding potential targets, which can capture the associations between drugs, targets, and diseases\[^9\]. Following the parameters of absorption, distribution, metabolism, and excretion (ADME), the effective active ingredients were screened by setting the screening threshold of oral bioavailability (OB) to be $\geq 30\%$ and the drug similarity (DL) to be $\geq 0.18$\[^10\].

Prediction of Drug Component Targets

HQC TCM components were input into TCMSP, and the species were defined as "homosapiens". The potential target genes related to TCM compounds were acquired, and Universal Protein Resource (UniProt, https://www.uniprot.org) correction was applied to establish a target gene database of drug active components. UniProt is an information resource, which can provide the scientific community with the advantages of integration and high quality, and is a free source of protein sequences and functions\[^11\]. The HQC chemical composition-target network of HQC was analyzed using the network analyzer properties in cytoscape3.9.1 software. Cytoscape\[^12\] can integrate biomolecular interaction networks into a unified conceptual framework with high throughput expression data and other molecular states.

Disease-related Target Acquisition

Based on the databases of Gene Cards (https://www.genecards.org/), PharmGkb (https://www.pharmgkb.org/), OMIM (https://omim.org/), Drugbank (https://go.drugbank.com/), and TTD (http://db.idrblab.net/ttd/), the disease-related targets were screened with "ankylosing spondylitis" as the keyword.

Construction of Protein-Protein Interaction (PPI) Network and Screening of Core Genes

First, Venn analysis was implemented on the common targets corresponding to the active components of HQC and the AS-related targets, and the intersection genes were obtained, which were considered to be the potential targets of HQC in AS therapy. Subsequently, the intersection target was input into the String database, and the "organization" was selected and set to "Homo sapiens" with a confidence score $\geq 0.4$. Next, the free target was excluded, and the target in the network was taken as the core target, followed by an analysis using the Cytoscape 3.9.1 software. The network was set by using "generate style from statistics" in tools. The node size and color gradient corresponded to the degree value, and the thickness of the edge represented the interaction intensity between proteins.

$$F^2(l) = \frac{[\Delta y(l) - \Delta y(l)]^2}{[\Delta y(l)]^2} = \frac{[\Delta y(l)]^2}{[\Delta y(l)]^2} - \frac{[\Delta y(l)]^2}{[\Delta y(l)]^2}$$

$$\Delta y(l) = y(l_0 + l) - y(l_0).$$

$$F^2(l) \sim l^\alpha, \text{where} \alpha \neq \frac{1}{2}.$$

$$F^2(l) \sim l^\alpha, \text{where} \alpha \neq \frac{1}{2}.$$
**Pathway Enrichment Analysis** DAVID (https://david.ncifcrf.gov/summary.jsp) was utilized for uploading the overlapping targets acquired from the PPI network \(^{[13]}\), followed by GO and KEGG Pathway Enrichment Analyses. The obtained results were plotted using bioinformatics (http://www.bioinformatics.com.cn/).

**Network Diagram for Component Target Pathway** The effective components of TCM were obtained from the core protein, and the first 20 pathways enriched by the KEGG pathway were selected to plot the "component target pathway" network diagram by Cytoscape 3.9.1 software.

**Molecular Docking of Core Components with High-Frequency Targets** The core targets screened from the "component target pathway" network diagram and the core components were paired. First, the structure of the core components was downloaded from the TCMSp and then saved it in mol 2 format. Next, the ligand structure of the core target was downloaded from the database of Protein Data Bank (PDB) (https://www.rcsb.org). AutoDock Tools1.5.7 was utilized to process and dock each ligand and receptor, and lastly, PyMOL software was utilized for the visualization of the docking results.

**Statistical Processing** SPSS v.22.0 statistical software was utilized for all data analysis. A nonparametric test was performed to analyze the data between the two groups. However, in the process of data analysis, this test was no longer applicable due to various reasons, and people often failed to make a simple assumption on the overall distribution shape. A nonparametric test was implemented to infer the overall distribution form with the sample data when the overall variance is unknown or almost unknown\(^{[14]}\). The results were shown in Table 2, and a \(p\)-value below 0.05 was statistically different.

**Experimental Validation** PTGS2, CAT, and PPARG, with a high moderate value of core targets, and whose bind energy was less than -7.0 kcal/mol, were selected as the detection indicators and detected by ELISA. The supematant was supplemented to the ELISA plate (100μL per well) and cultivated at 37°C for 1.5h. The operation was carried out strictly under the instructions of each kit. The results were plotted using GraphPad Prism 8.0.2.

**Results**

**Association Rule Analysis of HQC and Immune Inflammatory Indicators**

The minimum confidence and minimum support were set to 50% and 30%, respectively. The relationships between HQC and immune-inflammatory indicators were obtained by using the Apriori module. HQC improved CRP and ESR with a confidence of 65.85% and 50.62%, respectively, based on the association rule analysis. Besides, all the lifting degrees exceeded 1. These results indicate that HQC is closely correlated with improved immune-inflammatory indices of AS (Table 1).

<table>
<thead>
<tr>
<th>Items (LHS (\Rightarrow) RHS)</th>
<th>Support</th>
<th>Confidence</th>
<th>Lift</th>
</tr>
</thead>
<tbody>
<tr>
<td>{HQC} (\Rightarrow) {CPR↓}</td>
<td>32.92%</td>
<td>65.85%</td>
<td>1.15</td>
</tr>
<tr>
<td>{HQC} (\Rightarrow) {ESR↓}</td>
<td>25.31%</td>
<td>50.62%</td>
<td>1.15</td>
</tr>
<tr>
<td>{HQC} (\Rightarrow) {C4↓}</td>
<td>17.69%</td>
<td>35.40%</td>
<td>1.11</td>
</tr>
<tr>
<td>{HQC} (\Rightarrow) {IgA↓}</td>
<td>15.23%</td>
<td>30.45%</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Note: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; C4, complement C4; IgA, immunoglobulin A. Values are regarded to be % degrees of relevancy.

**Improvement of Immune Inflammatory Indices**

The clinical immune inflammatory indices, such as ESR, CRP, IgA, IgM, IgG, and C4, were selected for validation to observe the specific situations of AS patients in the two groups after treatment. The standards of the aforesaid indices in both groups were reduced after treatment in comparison to those before treatment (all \(p\leq 0.01\), Table 2), while no change was observed in the IgM level. Meanwhile, the application of HQC could better reduce the levels of ESR, CRP, IgM, and IgA in AS patients compared with healthy individuals(Table 2).
Table 2: Changes in immune-inflammatory indices in two groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>19.50(10.00,34.00)</td>
<td>18.00(9.50,30.00)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>15.67(4.79,36.80)</td>
<td>11.17(3.11,24.95)</td>
</tr>
<tr>
<td>IgA (g/L)</td>
<td>2.57(1.85,3.43)</td>
<td>2.53(1.85,3.38)</td>
</tr>
<tr>
<td></td>
<td>29(15.00,31.50)</td>
<td>22(12.00,47.00)</td>
</tr>
<tr>
<td>IgM (g/L)</td>
<td>1.00(0.75,1.34)</td>
<td>1.00(0.75,1.31)</td>
</tr>
<tr>
<td></td>
<td>2.44(1.93,3.23)</td>
<td>2.41(1.87,3.17)</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>11.79(9.77,14.43)</td>
<td>11.74(9.72,14.3)</td>
</tr>
<tr>
<td></td>
<td>12.7(10.04,15.33)</td>
<td>12.50(10.05,14.70)</td>
</tr>
<tr>
<td>C4 (g/L)</td>
<td>24.30(0.37,31.10)</td>
<td>24.00(0.36,29.70)</td>
</tr>
</tbody>
</table>

**Note:** ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IgA, M, and G, immunoglobulin A, M, and G; C4, complement C4.

P₂ value: Comparison of inflammatory and immune indices between the two groups post-treatment.

**Assessment of Immune Inflammation Indicators by the Random Walking Model**

For the indicator of ESR, there were 180 comprehensive evaluations recorded in the control group, and 280 comprehensive evaluations recorded in the treatment group. The improvement coefficient of the control and the treatment groups was 0.2944 and 0.3036, respectively. For each improvement of the patient's comprehensive indicators, 7.87 steps were needed for the clinical significance of the control group, while only 6.07 steps were needed for the treatment group. For the CRP index, the control group recorded 194 comprehensive evaluations, and the treatment group recorded 300 comprehensive evaluations. The improvement coefficient of the control and the treatment groups was 0.2629 and 0.4433, respectively. For each improvement of the patient's comprehensive indicators, 8.57 steps were needed for the clinical significance of the control group, while only 4.04 steps were needed for the treatment group. For the indicator of C4, 141 comprehensive evaluations were recorded in the control group, and 158 comprehensive evaluations were recorded in the treatment group. The improvement coefficient of the control and the treatment groups was 0.3901 and 0.4304, respectively. For each improvement of the patient's comprehensive indicators, 6.87 steps were needed for the clinical significance of the control group, while only 5.84 steps were needed for the treatment group. For the index of IgA, 141 comprehensive evaluations were recorded in the control group, and 158 comprehensive evaluations were recorded in the treatment group. The improvement coefficient of the control and the treatment groups was 0.2340 and 0.2405, respectively. For each improvement of the patient's comprehensive indicators, 11.45 steps were needed for the clinical significance of the control group, while only 10.45 steps were needed for the treatment group. For the index of IgG, 141 comprehensive evaluations were recorded in the control group and 158 comprehensive evaluations were recorded in the treatment group. The improvement coefficient of the control and the treatment groups was 0.1560 and 0.2025, respectively. For each improvement of the patient's comprehensive indices, 17.18 steps were needed for the clinical significance of the control group, while only 12.41 steps were needed for the treatment group. Collectively, we conclude that HQC can better improve patients' immune inflammatory indicators (Table 3, Figure 1).

Table 3: Assessment of immune inflammation factors by the random walking model.
<table>
<thead>
<tr>
<th>Index</th>
<th>Group</th>
<th>Maximum random Fluctuation</th>
<th>Walking positive growth rate</th>
<th>Random fluctuation power law value</th>
<th>Improvement coefficient</th>
<th>Comprehensive evaluation records</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>Control Group</td>
<td>53</td>
<td>0.1271</td>
<td>0.2472±0.1117</td>
<td>0.2944</td>
<td>180</td>
<td>7.87</td>
</tr>
<tr>
<td></td>
<td>Treatment Group</td>
<td>85</td>
<td>0.1647</td>
<td>0.2826±0.1331</td>
<td>0.3036</td>
<td>280</td>
<td>6.07</td>
</tr>
<tr>
<td>CRP</td>
<td>Control Group</td>
<td>51</td>
<td>0.1167</td>
<td>0.3493±0.1517</td>
<td>0.2629</td>
<td>194</td>
<td>8.57</td>
</tr>
<tr>
<td></td>
<td>Treatment Group</td>
<td>133</td>
<td>0.2477</td>
<td>0.2931±0.1184</td>
<td>0.4433</td>
<td>300</td>
<td>4.04</td>
</tr>
<tr>
<td>C4</td>
<td>Control Group</td>
<td>55</td>
<td>0.1455</td>
<td>0.4297±0.2025</td>
<td>0.3901</td>
<td>141</td>
<td>6.87</td>
</tr>
<tr>
<td></td>
<td>Treatment Group</td>
<td>68</td>
<td>0.1713</td>
<td>0.1947±0.1067</td>
<td>0.4304</td>
<td>158</td>
<td>5.84</td>
</tr>
<tr>
<td>IgA</td>
<td>Control Group</td>
<td>33</td>
<td>0.0873</td>
<td>0.2899±0.1495</td>
<td>0.2405</td>
<td>141</td>
<td>11.45</td>
</tr>
<tr>
<td></td>
<td>Treatment Group</td>
<td>38</td>
<td>0.0957</td>
<td>0.2769±0.1181</td>
<td>0.2340</td>
<td>158</td>
<td>10.45</td>
</tr>
<tr>
<td>IgG</td>
<td>Control Group</td>
<td>22</td>
<td>0.0582</td>
<td>0.1682±0.0950</td>
<td>0.1560</td>
<td>141</td>
<td>17.18</td>
</tr>
<tr>
<td></td>
<td>Treatment Group</td>
<td>32</td>
<td>0.0806</td>
<td>0.2640±0.1168</td>
<td>0.2025</td>
<td>158</td>
<td>12.41</td>
</tr>
</tbody>
</table>

Note: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; IgA, M, and G, immunoglobulin A, M, and G; C4, complement C4.

**Potential Active Ingredients of HQC Drugs**

Finally, 90 kinds of active ingredients of HQC were obtained, including 36 kinds of Scutellariae Radix, 15 kinds of Gardeniae Fructus, 23 kinds of Persicae Semen, 7 kinds of Radix Clematidis, and 9 kinds of Coicis Semen. Two kinds of ingredients were shared by more than two kinds of TCM. As there are many active ingredients in HQC, only 25 items are listed, as shown in Table 4.

Table 4: Some active ingredients of Huangqin Qingre Chubi Capsule (HQC).
<table>
<thead>
<tr>
<th>Herb Name</th>
<th>Mol ID</th>
<th>Molecule Name</th>
<th>OB</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scutellariae Radix</td>
<td>MOL001689</td>
<td>acacetin</td>
<td>34.97</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>MOL000173</td>
<td>wogonin</td>
<td>30.68</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>MOL000228</td>
<td>(2R)-7-hydroxy-5-methoxy-2-phenylchroman-4-one</td>
<td>55.23</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>MOL002714</td>
<td>baicalein</td>
<td>33.52</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>MOL002909</td>
<td>5,7,2,5-tetrahydroxy-8,6-dimethoxyflavone</td>
<td>33.82</td>
<td>0.45</td>
</tr>
<tr>
<td>Gardeniae Fructus</td>
<td>MOL001406</td>
<td>crocetin</td>
<td>35.3</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>MOL001941</td>
<td>Ammidin</td>
<td>34.55</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>MOL004561</td>
<td>Sudan III</td>
<td>84.07</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>MOL000098</td>
<td>quercetin</td>
<td>46.43</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>MOL000358</td>
<td>beta-sitosterol</td>
<td>36.91</td>
<td>0.75</td>
</tr>
<tr>
<td>Radix Clematidis</td>
<td>MOL005235</td>
<td>Embelin</td>
<td>37.72</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>MOL000449</td>
<td>Stigmasterol</td>
<td>43.83</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>MOL005603</td>
<td>Heptyl phthalate</td>
<td>42.26</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>MOL000263</td>
<td>oleanolic acid</td>
<td>29.02</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>MOL005592</td>
<td>Clematisprosapogenin,Cp7a_qt</td>
<td>12.62</td>
<td>0.76</td>
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<tr>
<td>Coicis Semen</td>
<td>MOL001323</td>
<td>Sitosterol alpha1</td>
<td>43.28</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>MOL001494</td>
<td>Mandenol</td>
<td>42</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>MOL002372</td>
<td>(6Z,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene</td>
<td>33.55</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>MOL002882</td>
<td>[(2R)-2,3-dihydroxypropyl] (Z)-octadec-9-enoate</td>
<td>34.13</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>MOL000359</td>
<td>sitosterol</td>
<td>36.91</td>
<td>0.75</td>
</tr>
<tr>
<td>Persicae Semen</td>
<td>MOL001323</td>
<td>Sitosterol alpha1</td>
<td>43.28</td>
<td>0.78</td>
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<tr>
<td></td>
<td>MOL001328</td>
<td>2,3-didehydro GA70</td>
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<td>0.5</td>
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<tr>
<td></td>
<td>MOL001329</td>
<td>2,3-didehydro GA77</td>
<td>88.08</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>MOL001340</td>
<td>GA120</td>
<td>84.85</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>MOL001342</td>
<td>GA121-isolactone</td>
<td>72.7</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**Prediction of Drug Ingredients and Disease-associated Targets**

A total of 1,312 target genes were acquired by searching the targets corresponding to HQC active components in TCMSP. Finally, 179 target genes were obtained by removing the duplicate targets and standardized names using UniProt. The larger node reflected the greater degree value of a node (Figure 2). With “ankylosing spondylitis” as the keyword, the disease-associated targets were screened based on the GeneCards, OMIM, Drugbank, PharmGkb, and TTD databases, and 1,979 genes were finally obtained by deleting the duplicate values after summarizing.

**Analysis of PPI Network Topology and Screening of Core Targets**

First, the Venn analysis of 179 common action targets corresponding to HQC active components and 1,979 AS-related targets showed that 47 intersection genes were obtained, which are the potential action targets of HQC in AS therapy (Figure 3). Then, input the cross target into the String database and import it into the Cytoscape 3.9.1 software for analysis. It could be observed that
VEGFA, CXCL8, PTGS2, PPARG, Intercellular cell adhesion molecule-1 (ICAM1), Myeloperoxidase (MPO) and FOS may be the most important key targets (Figure 4).

**Pathway Enrichment Results**

From the findings of the GO functional enrichment analysis, we observed that there were 107 GO annotation items. Biological processes (BP) mainly involve aging, cellular response to hypoxia, negative modulation of the apoptotic process, as well as positive regulation of transcription from RNA polymerase II promoter and phosphatidylinositol 3-kinase signaling. Cellular component (CC) mainly involves the extracellular region, extracellular space, intracellular membrane-bounded organelle, and chromatin. Molecular function (MF) mainly involves transcription factor activity, growth factor activity, sequence-specific DNA binding, enzyme binding, chromatin binding, RNA polymerase II sequence-specific DNA binding, and transcription factor binding (Figure 5).

The enrichment analysis of KEGG pathway showed that HQC may play an anti-inflammatory role by regulating IL-17, Th1 and Th2 cell differentiation, NF kappa B, TNF, T-cell receptor, c-type lectin receptor, toll like receptor, pathogenic E. coli infection, human T-cell leukemia virus 1 infection and Th17 cell differentiation (Figure 6).

**Component-Target-Pathway Network Diagram**

Through network analysis, it was found that the top 5 targets were PTGS2, FOS, RELA, PPARG, and VEGFA based on the order of degree value. Following the degree values of active ingredients, the top 5 active ingredients were beta-sitosterol, Stigmasterol, quercetin, Baicalein, and wogonin. It is suggested that these components and targets may be important components and targets for HQC in the treatment of AS (Figure 7).

**Molecular Docking results**

The binding energy of the components, the targets, and the number of hydrogen bonds formed are important indicators to evaluate the binding ability. The increase in the number of hydrogen bonds reflects a more stable binding. According to the data, 70% of the binding energy was under -4.25 kcal/mol, and 53% of the binding energy was -5.0 kcal/mol below, of which the binding energy of stigmasterol with PPARG, CXCL8, and CAT were under -7.50 kcal/mol. This indicated that the chemical active ingredients in HQC formula had high binding activity with inflammatory targets (Figure 8-9).

**Experimental Validation Results**

The experimental results revealed that increased serum PTGS2 levels and reduced serum levels of PPARG and CAT were witnessed in AS patients before treatment compared with healthy individuals ($P < 0.01$). HQC can make PTGS2 low expression and PPARG and CAT high expression ($P < 0.01$; Figure 10).

**Discussion**

From the analysis of its etiology from the theory of TCM, spleen deficiency is the basis of AS, spleen deficiency and stasis blood are the key factors of AS, and wind, cold, dampness, heat, and blood stasis are also the key factors of AS. HQC is beneficial for AS therapy. Nevertheless, the mechanism of action of TCM in AS is still unclear owing to a large number of chemically active components in TCM and the insufficiency of molecular research. In this study, We discussed the active components, potential targets and approaches of HQC in the treatment of AS, and further studied its molecular mechanism. Stigmasterol is the common active ingredient of Scutellariae Radix, Persicae Semen, Radix Clematidis, and Gardeniae Fructus, which can regulate inflammatory factors$^{[15][16][17]}$. Kaempferol is the active ingredient of Scutellariae Radix, and it can reduce oxidative stress, inhibit cell apoptosis and inflammatory response$^{[18][19][20]}$. Baicalein and wogonin are both important active components in Scutellariae Radix, and they have been found to reduce oxidative stress, and inhibit inflammation and apoptosis$^{[21][22][23]}$. Beta-sitosterol, the common active ingredient of Scutellariae Radix, Persicae Semen, Radix Clematidis, and Gardeniae Fructus, has been demonstrated to inhibit the formation of osteoclasts$^{[24]}$ and reduce inflammatory reaction by suppressing the NF-κB pathway$^{[25]}$. Quercetin, the common active ingredient of Gardeniae Fructus, has anti-inflammatory, antioxidant, and anticancer functions$^{[26][27]}$. In general, the effective components of HQC have good anti-inflammatory, anti-oxidation, and anti-immune effects, thereby improving joint inflammation.
Our results indicated that HQC alleviated the immune inflammatory indicators of AS, and HQC acted on PTGS2, CAT, PPARG, RELA, VEGFA, CXCL8, IL-2, ICAM, and FOS through the above chemical components in AS therapy. Additionally, HQC may play a multi-component, multi-target, and multi-network anti-inflammatory pharmacological impacts on AS through orchestrating the aging, cellular response to hypoxia, negative modulation of the apoptotic process, positive modulation of transcription from RNA polymerase II promoter, transcription factor activity, growth factor activity, and transcription factor binding by modulating the signaling pathways of IL-17, NF-kappa B, TNF, T cell receptor, C-type lectin receptor, Toll-like receptor, pathogenic Escherichia coli infection, human T-cell leukemia virus 1 infection, Th1, and Th2 cell differentiation, and Th17 cell differentiation.

To verify our research results, core indicators were selected for experimental research. The experimental results demonstrated that increased serum PTGS2 levels and reduced serum levels of PPARG and CAT were witnessed in AS patients before treatment compared with healthy individuals. HQC down-regulated the expression of PTGS2 and up-regulated the expression of PPARG and CAT. PPARG exerts important biological functions in cell differentiation and metabolism regulation\cite{28}, which can regulate glucose and lipid metabolism, and is involved in the body’s inflammatory reaction, and cell apoptosis\cite{29}. A previous study has indicated that HQC may inhibit immune-inflammatory response and improve oxidative stress in patients with AS by activating PPARG, thus improving clinical symptoms of patients\cite{30}. PTGS2 is the essential enzyme in prostaglandin biosynthesis, and it plays a special role in the mechanism of inflammatory response\cite{31}. In recent years\cite{32,33}, some scholars have found that oxidative stress factors are relevant to the pathogenesis of AS, which is mainly manifested by the high expression of oxidative stress. CAT is one of the main antioxidant enzymes widely existing in plants, animals, and microorganisms. It mainly catalyzes the decomposition of intracellular hydrogen peroxide and has the function of protecting the antioxidant enzyme system.

Conclusions

To sum up, this study shows the complex molecular network relationship of HQC in AS therapy through network pharmacology and molecular docking technology, which reflects the biological processes of a multi-component, multi-channel, and multi-target treatment of TCM compounds, HQC might function through aging, cellular response to hypoxia, negative modulation of the apoptotic process, positive modulation of transcription from RNA polymerase II promoter, transcription factor activity, growth factor activity, and transcription factor binding, and can also act on the signaling pathways of IL-17, NF kappa B, TNF, T cell receptor, Toll-like receptor, Th1, Th2 and Th17 cell differentiation all play a part in regulating immune inflammatory response, cell apoptosis and oxidative stress response.

In recent years, In the treatment of AS, the perfect combination of traditional Chinese medicine and western medicine can better improve the symptoms of patients. However, there are also some objective problems in treating AS with traditional Chinese medicine, such as the small number of experimental samples, the lack of long-term follow-up and retrospective research, and the lack of mechanism research. These are all problems that we need to solve in the future.

**Declarations**

**Ethics approval and consent to participate**

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of the Ethics Committee of Anhui Provincial Hospital of Traditional Chinese Medicine (approval number: 2020AH-08). Written informed consent was obtained from individual or guardian participants.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Competing interests**
The authors declare that they have no competing interests.

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**Authors’ Contributions**

Fan Zhang performed the data analysis and drafted the manuscript. Jian Liu contributed to the design of research ideas and modified the paper. Ling Xin and Yanyan Fang contributed to the data collection. Qiao Zhou, Jinchen Guo, Yanqiu Sun and Fanfan Wang contributed to the specimen collection and the experiment design. All authors read and approved the final manuscript.

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Not applicable.

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**References**


**Figures**

**Figure 1**

The assessment of immune-inflammatory factors in the treatment and the control groups by the random walking model.

Note: The elevation of the horizontal line length corresponds to the walking step number, and the increase of the vertical line height corresponds to the intervention efficacy and response.
Figure 2

Active components of Huangqin Qingre Chubi Capsule (HQC) target network diagram.

Note: ▲ Predicted targets of HQC.
〇 HQC active ingredients.
〇 Active ingredients are shared by drugs.
Figure 3

HQC targets and disease-associated targets by the Venn diagram.
Figure 4

PPI network for treating AS with HQC

Note: Color depth is positively correlated with degree value
Figure 5

The GO function enrichment analysis.
Figure 6

The KEGG pathway enrichment analysis.
Figure 7

Component-target-pathway network.
Figure 8

Binding energy of the core target protein to the chemically active ingredient prescribed by HQC.

Note: The darker color reflects the stronger binding activity.
Figure 9

The docking diagram of the core target and the active ingredient.

Note:

I: kaempferol (MOL000422) and CXCL8 (4xds).
II: quercetin (MOL000098) and IL-2 (1m49).
III: wogonin (MOL000173) and PPARG (2fhp).
IV: Stigmasterol (MOL000449) and CXCL8 (4xds).
V: Stigmasterol (MOL000449) and PPARG (2fhp).
VI: beta-sitosterol (MOL000358) and VEGFA (1mkg).
Figure 10

Influences of HQC on the expression levels of PTGS2 (I), PPARG (II), and CAT (III)

$P < 0.01$, vs the normal group;  $P < 0.01$, vs the AS + HQC group.