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# Mechanism of Luteolin in the Treatment of Primary Sjogren's Syndrome: a Study Based on Systems Biology and Cell Experiments

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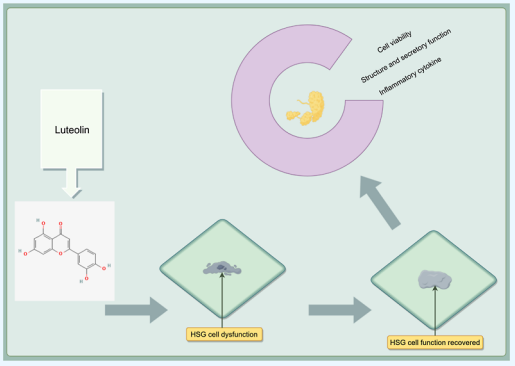
**ABSTRACT:** Introduction: Primary Sjogren's Syndrome (pSS) is a chronic inflammatory autoimmune disease that manifests as dry mouth and eyes. Luteolin can repress immuno-inflammation and improve the function of exocrine glands. Methods: Bibliometrics was used to visualize pSS-related key indicators. The efficacy of traditional Chinese medicines (TCMs) in pSS treatment was analyzed with the internal database containing the clinical records of pSS. Using the network pharmacology technology to identify involved pathways. Additionally, molecular docking and cell experiments were performed to screen and verify the therapeutic effect of luteolin on pSS. Results: Key indicators that were selected according to the bibliometrics were worse in pSS and had certain compatibility and correlation with laboratory and immunoinflammatory indicators. After treatment, pSS patients showed improvements in the above indicators. The results of risk analyses revealed that TCMs were protective factors for laboratory indicators and key indicators. The main effective TCMs for pSS treatment and TNF pathways were identified with network pharmacology. Cell experiments validated that luteolin indeed improved the secretion dysfunction and inflammation of modeled human submandibular gland epithelial cells through the TNF/NF-κB pathway. Conclusions: TCMs may effectively improve transcription factors and immuno-inflammatory markers in pSS patients. Moreover, we hypothesized and verified the potential mechanism of action of luteolin in HSG cells.




## 1. INTRODUCTION

Sjogren's syndrome (SS) is a chronic inflammatory autoimmune disease manifesting as dry mouth and eyes, which may be accompanied by pain and fatigue and involve several different systems or organs. This disease is characterized by the progressive infiltration of lymphocytes and atrophy of adenocytes and ductal cells.[1] SS has an overall prevalence rate varying from 0.1% to 4.8% and has a predilection for younger women in their 20s to 30s and postmenopausal women in their mid-50s.[2] Moreover, patients with the above symptoms can be definitively diagnosed with primary SS (pSS) through positive blood test results for inflammatory and autoimmune markers and by ruling out other connective tissue diseases that induce SS.[3] pSS is associated with several risk factors, including a family history of autoimmune diseases in first-degree relatives and negative stressful life events.[4] In addition, pSS is often accompanied by hypocomplementemia, which causes significant glucocorticoid exposure,[5] particularly in female pSS patients who present with more serious dryness, thrombocytopenia, positive autoantibodies, and hypocomplementemia.[6] Meanwhile, pSS increases the risk of pulmonary hypertension, cardiovascular disease, and overall tumors.[7−9]

Current treatments for pSS are mainly symptomatic, aimed primarily at relieving clinical symptoms and preventing organ damage caused by disease progression. The most common treatments are prednisone acetate, immunosuppressants, and artificial tears, which are selected based on clinician experience, expert opinion, and very limited clinical research. Nevertheless, these treatments, even relatively safe immunomodulators, may provoke many adverse reactions.[9] Traditional Chinese medicine (TCM) is an important auxiliary therapy with high efficacy and tolerability, which can repress immunoinflammation and improve the function of exocrine glands.[10] Importantly, prior studies elucidated that TCMs could alleviate anxiety and depression of various causes and prolong sleep time in most pSS patients.[11,12] TCM may be more effective and safer than traditional drugs when used alone or in combination with traditional drugs for the treatment of various diseases,[13,14] especially lacrimal and salivary gland dysfunction. Yet, there is extremely limited evidence on commonly used TCMs for the treatment of pSS and little evidence on available treatments for patients with no clinical manifestations. Accordingly, more research on TCM in the treatment of pSS

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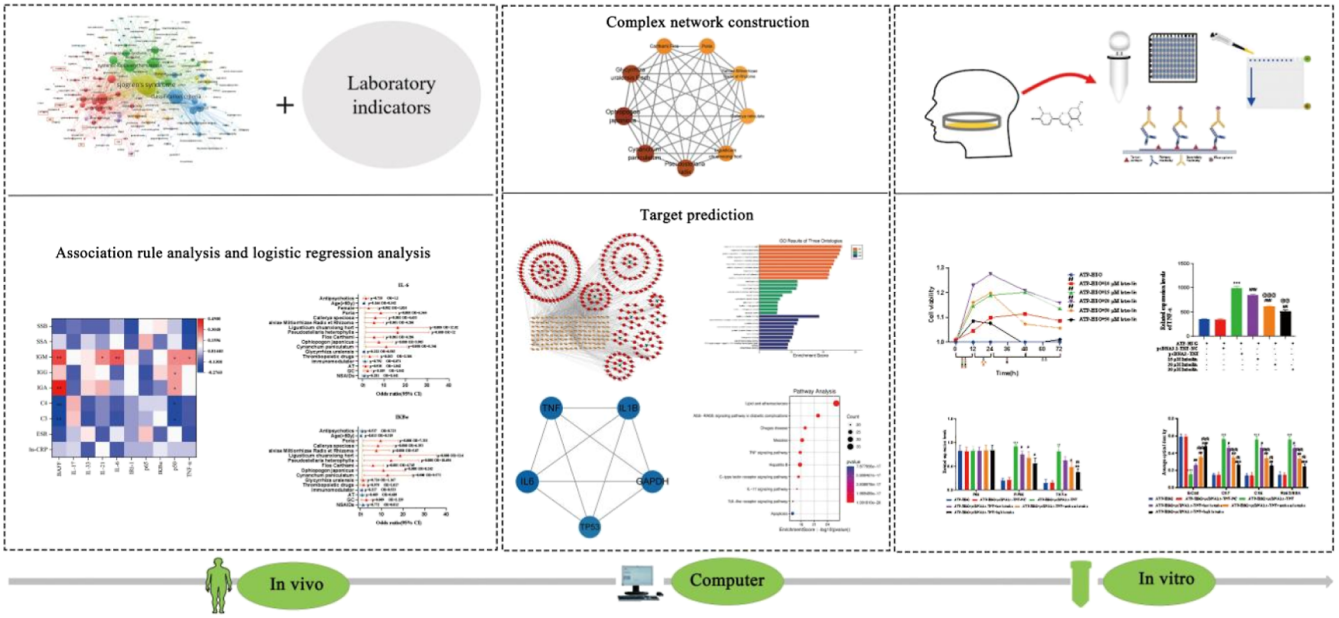


**Figure 1.** Flowchart.

is urgently needed for developing evidence-based effective TCM interventions.

A hallmark of pSS is acinar epithelial cell atrophy in secretory glands, whose cause remains largely unelucidated. Of note, a prior study investigated the role of acinar atrophy in the development of pSS by focusing on the metabolism of glandular epithelial cells and mitochondria, which unraveled that the levels of interleukin (IL)-6, interferon (IFN)-$\alpha$, and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) were high, and nuclear factor kappa-B (NF-$\kappa$B) and type I IFN-related pathways were markedly activated in epithelial cells under the high-lactate condition.[15] In pSS patients, epithelial cells are not only the target of autoimmune attack but also the predominant source of autoantigens during disease progression, and these cells express a variety of pro-inflammatory cytokines and chemokines.[16] Purinergic receptor type 2, family X, subunit 7 (P2 × 7) receptor (P2 × 7R) is a marker for salivary gland involvement in pSS[17] and may be associated with anxiety and depression.[18] A previous study elaborated that P2 × 7R activation contributed to salivary gland inflammation in vivo.[19] Additionally, it has been extensively demonstrated that P2 × 7R plays a key role in the development of pain by mediating inflammatory mediators. Specifically, P2 × 7R can be activated by ATP, which opens nonselective cation channels, activates multiple intracellular pathways, and releases many inflammatory cytokines, thus inducing pain. Therefore, targeted inhibition of P2 × 7R is anticipated to be a potential novel method for pain relief.[20] The agonist of P2 × 7 activates the receptor by interacting with the receptor at the ATP binding site.[21] In the P2X receptor family, P2 × 7R has the lowest affinity for ATP, causing the need for high concentrations of ATP to activate P2 × 7R.[22]

The appearance of scientific databases such as the Web of Science in recent years has enabled the ready availability of research data, thus encouraging the development of bibliometrics research.[23] Notably, bibliometrics is applicable to the analysis of studies related to pSS and inflammation because of its powerful analytical and visualization capabilities. "Inflammation" has been a hot research topic in recent years.[24] Nonetheless, few researchers have performed visualizations on studies related to pSS and inflammation. This study comprehensively analyzed references related to pSS and inflammation published between 1991 and 2023 and explored the co-occurrence network with VOSviewer. VOSviewer is a distance-based bibliometric tool focusing on the visualization of bibliometric networks. This tool assigns a set of closely related nodes into several clusters, where the same color indicates the high correlation between nodes, reflecting the connection among the key works of authors.[25] Bibliometrics analysis can be used to determine the research direction by identifying the relevant nodes and extracting useful information from a large amount of information.

Overall, this study evaluated the compatibility and clinical therapeutic effect of TCM in the treatment of pSS and probed the possible mechanism of its action with bibliometrics, network pharmacology, and cell experiments (Figure 1).

## 2. MATERIALS AND METHODS

**2.1. Data Acquisition and Search Strategy.** Web of Science[26] is a commonly used academic database containing over 12,000 influential journals[27] and is widely recognized as the most comprehensive and reliable database for bibliometric analysis. The relevant literature was searched in the Web of Science Core Collection database with the strategy of [TS = ("Primary Sjogren's syndrome") AND TS = ("immunoinflammation" OR "inflammation")] and exported on July 30, 2023.

The data mining system, which is independently developed by the Information Center of the Anhui Traditional Chinese Medicine Hospital, was used to collect the electronic medical record and disease-related laboratory indicators of 89 inpatients initially diagnosed with pSS from the structured query language (SQL) server database.[28,29] The laboratory indicators included inflammation markers such as high-sensitivity C-reactive protein (hs-CRP) and erythrocyte sedimentation rate (ESR), as well as immune indicators such as immunoglobulin A (IgA), IgG, IgM, complement C3 (C3), and C4. After serum samples of 89 pSS patients and 30 control


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participants were attained from the laboratory and the physical examination center, enzyme-linked immunosorbent assay (ELISA) was performed to measure serological indexes including the human B-cell activating factor (BAFF), human interleukin (IL)-17, human IL-33, human IL-21, human IL-6, human interferon regulatory factor-1 (IRF-1), human nuclear factor κB-p65 (p65), human inhibitory subunit of NF kappa B-α (IκB-α), human nuclear factor κB-p50 (p50), and human tumor necrosis factor α (TNF-α). The research was ratified by the Ethical Committee of the First Affiliated Hospital of the Anhui University of Traditional Chinese Medicine (2023AH-52). Patient informed consent was not required, as the serum specimens were derived from the secondary utilization of biological samples.

**2.2. Data Analysis.** *2.2.1. Association Rule Analysis and Correlation Analysis.* Association rule analysis and correlation analysis were conducted to assess the compatibility and correlation among the components. The use of any TCM was assigned 1, otherwise 0, while any improvement in the laboratory indicators was also assigned 1, otherwise 0. The Apriori module in IBM SPSS Modeler 18.0 (IBM Canada Ltd., Markham, Ontario, Canada) can analyze relationships between items, which was utilized to determine the correlation between the TCM and laboratory indicators. The minimum support rate was set at 30%, and the confidence level was set at 50%. Individual drugs and indicators were used as variables. The following formula was used[30]

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

$$\text{confidence}\ (X \rightarrow Y) = \sigma\frac{(X \cup Y)}{\sigma(X)}$$

$$\text{lift}\ (X \rightarrow Y) = \text{confidence}\ \frac{(X \rightarrow Y)}{\sigma(Y)}$$

Equation 1. Association rule analysis equation.

As an essential statistical index reflecting the close relationship between variables, the Pearson correlation coefficient in IBM SPSS Statistics 23 software was calculated to assess the correlation between immuno-inflammatory indicators in 89 pSS patients. The following formula was used[31]

$$r = \frac{\sum (X - \overline{X})(Y - \overline{Y})}{\sqrt{\sum_{2} (X - \overline{X}) \sum_{2} (Y - \overline{Y})}} = \frac{{}^{XY}_{l}}{\sqrt{{}^{XXYY}_{l\ l}}}$$

Equation 2. correlation analysis equation.

*2.2.2. Logistic Regression Analysis.* Forest maps of multivariate logistic regression were plotted with GraphPad Prism 9 for observational risk analysis. Binary logistic regression analysis was conducted with IBM SPSS statistics 23 software to identify risk and protective factors of laboratory and serological indicators. The specific formula was as follows[32]

$$\log itP = \ln\left[\frac{P}{1 - P}\right] = a + {}^{1}_{b}{}^{1}_{x} + {}^{2}_{b}{}^{2}_{x} + \cdots + {}^{n}_{b}{}^{n}_{x}$$

$$\text{OR} = \frac{[{}^{1}_{P}/(1 - {}^{1}_{P})]}{[{}^{0}_{P}/(1 - {}^{0}_{P})]}$$

Equation 3. Logistic regression analysis equation.

*2.2.3. Complex Network Construction.* The association rule flow of the TCM prescriptions in a standard format was acquired by importing the prescriptions into SPSS Modeler 18.0 to determine the node and weight of TCM. The association rule network of effective TCMs in the treatment of pSS was constructed by importing the association rule flow into Cytoscape 3.8.2 (http://www.cytoscape.org/), which is an open-source network visualization software for identifying and analyzing the core nodes of TCM in complex networks. The importance of TCM nodes was evaluated based on the degree value in Cytoscape.

**2.3. Network Pharmacology and Molecular Docking.** The components of the core TCM prescriptions were searched in the traditional Chinese medicine systems pharmacology database and analysis platform (TCMSP). The effective active ingredients and potential targets of the prescriptions were screened according to absorption, distribution, metabolism, and excretion, oral bioavailability, and similarity of drugs and corrected with Uniprot. pSS-related target genes were screened in GeneCards, DrugBank, OMIM, and TTD and deduplicated. The target genes of the drug and disease were intersected. Next, Cytoscape 3.9.1 software was utilized to construct the "active component-potential target-disease" network of the core prescriptions and the protein−protein interaction (PPI) network. After that, the core targets of the core prescriptions involved in the treatment of pSS were subjected to gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Finally, the structures of the ligands and target proteins were downloaded from PubChem and PDB, respectively, and the active box was created with AutoDockTools-1.5.7 software. The binding energy was analyzed by running the CMD command code, and the molecular docking results were visualized with PyMOL.

**2.4. Reagents, Cell Culture, and Cell Transfection.** Primary human submandibular gland epithelial (HSG) cells and the primary epithelial cell medium were purchased from iCell Bioscience (Shanghai, China). BzATP was purchased from MedChemExpress (Monmouth Junction, NJ, USA). Luteolin was provided by Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). The cell proliferation-toxicity kit, cell counting kit-8 (CCK-8), was purchased from Biosharp (Anhui, China), and pcDNA3.1-TNF overexpression plasmids were acquired from GenePharma (Shanghai, China).

HSG cells in the logarithmic growth stage were detached with 1 mL of trypsin in a 25T culture bottle and subjected to 5 min of centrifugation at 1500 rpm. The cell precipitate was resuspended with 5 mL of medium to a concentration of $3.6 \times 10^5$ cells/mL. Cell suspensions (1 mL) were completely mixed with 11 mL of medium and added into four 96-well plates (8 groups × 3 biological replicates, a total of 24 wells), with 3000 cells/well and 100 μL medium/well. Additionally, the remaining wells were added to phosphate-buffered saline to avoid edge effects. Next, cell culture was conducted for 4 h at 37 °C. After cell attachment to the wall, cells in the experimental wells were stimulated with the agonist BzATP (100 μM) for 24 h and then added with 5, 10, 15, 20, 30, and 50 μM luteolin, respectively. The CCK-8 assay was performed at 12, 24, 48, and 72 h after the addition of the drug. Optical density (OD) values were detected to observe changes in the cell viability.



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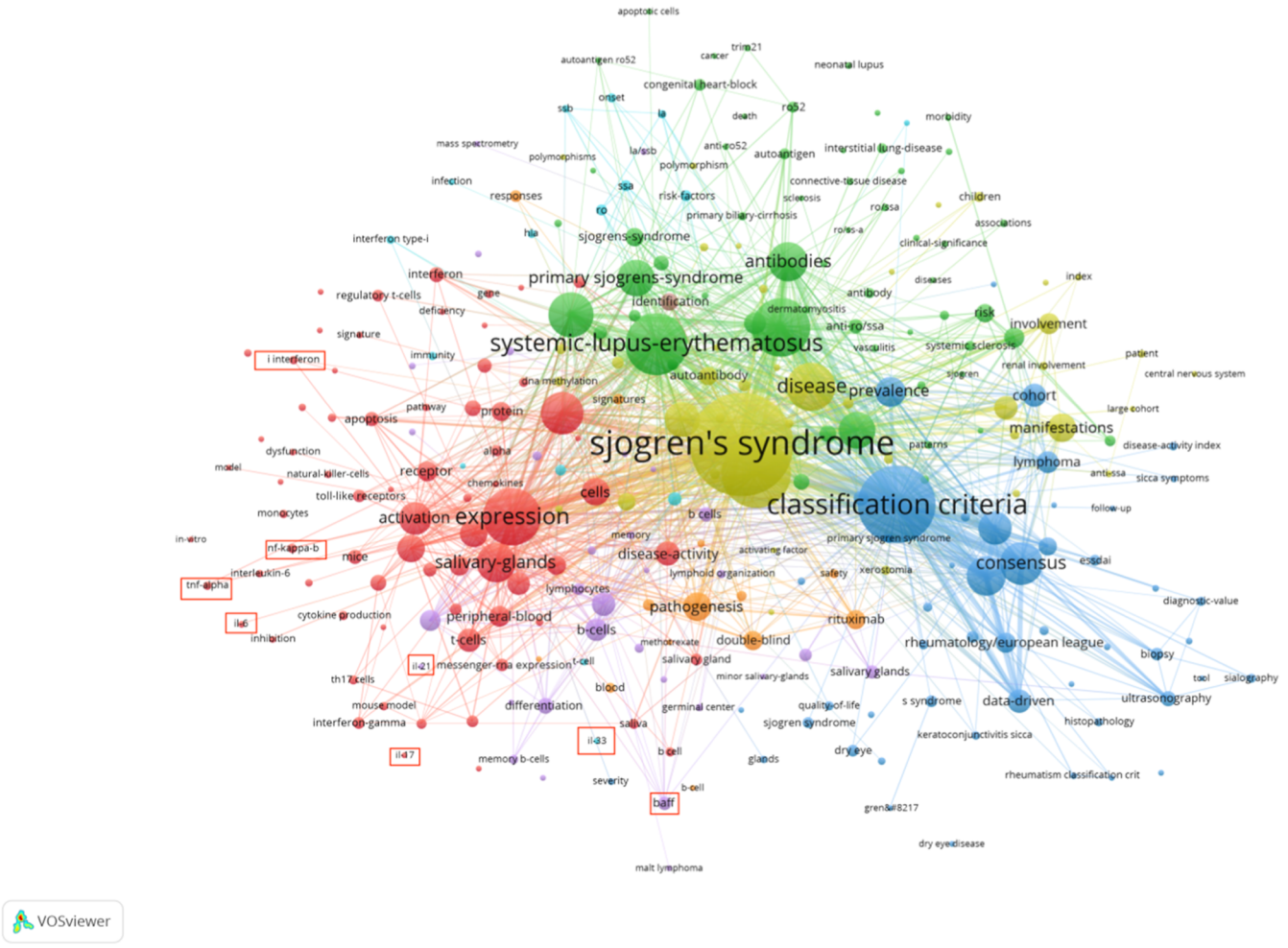


**Figure 2.** Visualization of keywords.

For transfection, the above steps were repeated. After 24 h of cell stimulation with BzATP, transfection was initiated. Specifically, 0.5 $\mu$g of pcDNA3.1-TNF or PCDNA3.1-TNF-negative control (NC) was diluted with 25 $\mu$L of reduced-serum optimal-minimal essential medium (MEM) and gently mixed. After the mixture was gently shaken, 0.5 $\mu$L of Lipofectamine 2000 was diluted in 25 $\mu$L of Opti-MEM and cultured at room temperature for 5 min. After 25 min, the above-diluted plasmids were gently mixed with Lipofectamine 2000 (the total volume was 500 $\mu$L) and left at room temperature for 20 min (the solution might become turbid). Next, 50 $\mu$L of the above mixture was added to each well and gently shaken, and the medium was renewed after transfection for 4−6 h. Following cell culture at 37 °C for 48 h, cell viability was detected with the CCK-8 assay. In addition, cells were collected for Western blotting, reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and ELISA. One of the 6-well plate slides was utilized for the immunofluorescence (IF) assay.

**2.5. CCK-8 Assay.** Cell viability was measured with a CCK-8 assay kit. In detail, 10 mL of CCK-8 solutions were added into each experimental well, followed by 1−4 h of cell culture at 37 °C. The viability of ATP-HSG cells was determined by measuring OD values at 450 nm.

**2.6. IF Assay.** The cell slides were fixed with 4% paraformaldehyde solutions for 20 min and washed with phosphate-buffered saline with Tween 20 (PBST) three times (5 min each time). The fixed cells were capped at 37 °C with 0.5% Triton X-100 for 30 min and blocked with normal goat blocking serum at room temperature for 1 h, followed by incubation with antibodies against E-cadherin (E-Cad), cytokeratin 7 (CK7), anti-CK5, and RO52/SSA for 1 h and with fluorescein isothiocyanate-coupled goat antirabbit IgG (1:400) for 30 min. Fluorescence slices were scanned with a digital microtome scanner (Pannoramic MIDI, Budapest, Hungary).

**2.7. RT-qPCR.** Total RNA was isolated from ATP-HSG cells with TRIzol for reverse transcription and amplification. Agarose-gel electrophoresis was performed, followed by semiquantitation of PCR products with Gelpro32 gel image analysis software. Relative quantitative analysis was performed using the $2^{-\Delta\Delta Ct}$ method, with $\beta$-actin as an internal reference. All primers used were as follows: TNF, 5′-CGAGTCTGGGCAGGTCTA-3′ (forward) and 5′-GAAGTGGTGGTCTTGTTGC-3′ (reverse); $\beta$-actin, 5′-CCCTGGAGAAGAGCTACGAG-3′ (forward) and 5′-GGAAGGAAGGCTGGAAGAGT-3′ (reverse).

**2.8. ELISA.** The supernatant of ATP-HSG cells was collected and centrifuged at 2500 rpm for 20 min with the precipitate discarded. The supernatant was added to the ELISA plate (50 $\mu$L per well) and cultured at 37 °C for 30 min after the plate was sealed with a sealing membrane. TNF-$\alpha$, IL-1$\beta$, and IL-6 levels were measured as instructed in the manuals of the corresponding ELISA kits.


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Table 1. Clinical Characteristics of pSS Patients and Control Participants

| variables | | pSS ($n$ = 89) | reference range | $p$ value |
|---|---|---|---|---|
| laboratory indicators | SSB | 40 | negative | NA |
| | SSA | 62 | negative | NA |
| | hs-CRP (mg/L) | 1.51 (0.57, 4.05) | <1 | NA |
| | IGA (g/L) | 2.52 (1.83, 3.43) | 1−4.2 | NA |
| | IGM (g/L) | 1.25 (0.91, 1.69) | 0.5−2.8 | NA |
| | IGG (g/L) | 15.01 ± 4.86 | 8.6−17.4 | NA |
| | C3 (g/L) | 1.11 (0.94, 1.30) | 0.7−1.4 | NA |
| | C4 (g/L) | 0.27 (0.21, 0.34) | 0.1−0.4 | NA |
| | ESR (mm/h) | 16.00 (6.50, 30.00) | 0−12 | NA |
| | ALT (U/L) | 17.00 (12.00, 31.50) | 7−40 | NA |
| | AST (U/L) | 23.40 (18.70, 31.00) | 13−35 | NA |
| | ALP (U/L) | 74.00 (61.00, 103.00) | 35−100 | NA |
| | GGT (U/L) | 21.00 (14.00, 43.00) | 7−45 | NA |
| | BUN (mmol/L) | 4.53 (3.58, 5.14) | 2.6−7.5 | NA |
| | Cr ($\mu$mol/L) | 56.30 (50.05, 64.00) | 41−73 | NA |
| | UA ($\mu$mol/L) | 279.00 (232.00, 327.50) | 154.7−357 | NA |
| | WBC ($\times 10^9$) | 5.00 (4.09, 6.33) | 3.5−9.5 | NA |
| | RBC ($\times 10^{12}$) | 4.06 (3.79, 4.37) | 3.8−5.1 | NA |
| | HGB (g/L) | 122.00 (113.00, 130.00) | 115−150 | NA |
| | PLT ($\times$109) | 178.24 ± 6.35 | 125−350 | NA |
| variables | | pSS ($n$ = 89) | control ($n$ = 30) | $p$ value |
| baseline characteristics | age (year) | 56.48 ± 12.43 | 54.12 ± 9.11 | 0.23[a] |
| | females | 85 | 26 | 0.41 |
| | disease course (year) | 5(2, 8) | | |
| | interstitial lung disease | 7 | | |
| | autoimmune liver disease | 5 | | |
| | pulmonary hypertension | 3 | | |
| key indicators | BAFF (pg/mL) | 2361.78 ± 165.11 | 609.26 ± 72.38 | 0.002[a] |
| | IL-17 (pg/mL) | 597.35 (551.16, 639.01) | 99.21 (84.65, 126.49) | 0.001 |
| | IL-33 (pg/mL) | 557.36 ± 44.68* | 187.63 ± 25.11 | 0.01[a] |
| | IL-21 (pg/mL) | 463.74 (447.44, 485.91) | 122.43 (110.00, 132.59) | 0.001 |
| | IL-6 (pg/mL) | 133.90 (129.78, 143.12) | 37.99 (33.85, 42.12) | 0.003 |
| | IRF-1 (pg/mL) | 8037.89 ± 692.34 | 2122.84 ± 264.86 | 0.001[a] |
| | p65 (pg/mL) | 10868.58 (9953.07, 11390.01) | 4275.50 (4102.27, 4688.89) | 0.002 |
| | IκB-$\alpha$ (pg/mL) | 1305.36 ± 249.25 | 5716.87 ± 387.47 | 0.001[a] |
| | p50 (pg/mL) | 18.78 (16.05, 20.33) | 3.59 (2.93, 3.87) | 0.001 |
| | TNF-$\alpha$ (pg/mL) | 876.59 (816.26, 922.80) | 301.47 (290.25, 318.41) | 0.001 |

[a]Note: NA, Not applicable; hs-CRP, hyper-sensitivity C-reactive protein; C3, complement C3; C4, complement C4; IgA, G, M, immunoglobulin A, G, M; ESR, erythrocyte sedimentation rate; SSA, Anti-SSA antibody; SSB, Anti-SSB antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; BUN, blood urea nitrogen; Cr, serum creatinine; UA, uric acid; WBC, white blood cell count; HGB, hemoglobin; RBC, red blood cell; PLT, platelet count; BAFF, B-cell activating factor; IL-17, interleukin 17; IL-33, interleukin 33; IL-21, interleukin 21; IL-6, interleukin 6; IRF-1, interferon regulatory factor 1; p65, RelA; IκB-$\alpha$, I-kappa-B-alpha; p50, NF-$\kappa$B1; TNF-$\alpha$, tumor necrosis factor-alpha. **$p$ < 0.01; *$p$ < 0.05. a: Student's $t$-test; b: Wilcoxon test; c: Chi-square test.

**2.9. Western Blotting.** Cells were lysed and centrifuged at 12,000 rpm for 10 min to extract total proteins. A sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel preparation kit (Beyotime, Shanghai, China) was utilized. Protein samples were obtained by adding 5 × SDS-PAGE loading buffer into cells at a ratio of 1:4, heated for 10 min in a boiling-water bath, and added into SDS-PAGE gel wells (5−10 $\mu$L/well). The filter paper and the polyvinylidene fluoride membrane were cut, soaked in methanol for 2−3 min in advance, and then soaked in rotating buffer for 5 min. After the proteins were transferred into the membranes, the membranes were cooled and washed with the prepared Western washing solution for 5 min to remove the transfer solution. Thereafter, the membranes were sealed with 5% skim milk powder for 2 h and then washed with PBST three times, 10 min each time, followed by incubation with primary and secondary antibodies. p65, phosphorylation (P)-p65, and TNF-$\alpha$ expression was calculated after electrogenerated chemiluminescence coloring, exposure in the darkroom, fixation, and photographing.

## 3. STATISTICAL ANALYSIS

The Spearman correlation analysis was carried out to determine the correlation of cytokines, stimulators, and transcription factors with immunoinflammatory indicators (suitable for non-normally distributed data). The Apriori algorithm in IBM SPSS Modeler 18.0 was utilized to analyze the association rules among indicators, herbs, and indicator improvements. GraphPad Prism 8.2 (GraphPad Software, La Jolla, CA, USA) was used to capture the images. Data between groups were compared with the Student's $t$-test (normally distributed data) or the Kruskal−Wallis nonparametric test



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(non-normally distributed data). The Student's *t*-test, Wilcoxon test, and Chi-square test were used to analyze the clinical characteristics of pSS patients and control participants. Continuous variables with normal distribution were compared with the Student's *t*-test, and those without normal distribution were compared with the Wilcoxon test. Categorical variables were compared with those in the Chi-square test. Data were presented as the mean ± standard deviation or median (quartile range). A difference was significant at $p < 0.05$.

## 4. RESULTS

**4.1. Key Indicator Analysis.** According to the bibliometrics theory, keywords are representative of hotspots and trends in the field of research, and key indicators should be analyzed since they reflect the focus of a study.[33] In the web visualization of key indicators, the size of nodes reflects the appearance frequency of keywords, and the distance between the two nodes indicates the strength of their association. Key indicators relatively close to each other were grouped into the same category, roughly reflecting the main concerns in the research fields of pSS and inflammation. The most relevant node was pSS, which was the same as our research topic, including four main areas with complex relationships, namely related activated factors, research methods, standards and consensus, and related to autoimmune diseases. Cluster 1 was labeled in red, and the main key indicators are centered on the activated factors in pSS and related factors. Therefore, BAFF, IL-6, TNF-α, IL-17, IRF-1, p65, IκB-α, p50, IL-21, and IL-33 were selected as key indicators for detection (Figure 2).

**4.2. Clinical Characteristics of pSS Patients and Control Participants.** A total of 119 participants were enrolled, including 30 control participants (4 males and 26 females; age of 54.12 ± 9.11 years) and 89 pSS patients (4 males and 85 females; age of 56.48 ± 12.43 years). There were no statistically significant differences in age and sex distribution but significant differences in the levels of BAFF, IL-6, TNF-α, IL-17, IRF-1, p65, IκB-α, p50, IL-21, and IL-33 ($p < 0.05$ or $p < 0.01$; Student's *t*-test, Wilcoxon test, and Chi-square test) between the two groups (Table 1).

**4.3. Association Rule Analysis between Transcription Factors, Inflammatory Factors, and Laboratory Indicators.** To further ascertain the relationship among transcription factors, inflammatory factors, and laboratory indicators, association rule analysis was performed to determine the confidence and support. The results displayed a strong correlation of decreased IκB-α levels with positive SSA and a close correlation of elevated p65 levels with positive SSA, positive SSB, advancing age, and females. In addition, increased levels of IL-33, IL-17, and IL-21 were tightly correlated with advancing age, disease course, and females, respectively, while elevated IL-6 levels were strongly correlated with positive SSA and positive SSB. The elevation in TNF-α levels was closely correlated with advancing age, disease course, and IgA levels, and the increase in p50 levels was strongly correlated with positive SSA and females. These correlations all had a support degree of greater than 40% and a confidence level of over 50% (Table 2).

**4.4. Correlation Analysis Among Transcription Factors, Cytokines, and Laboratory Indicators.** Significant correlations among the aforementioned transcription factors and cytokines were confirmed with Spearman correlation analysis (Table 3, Figure 3). BAFF levels were negatively correlated with C3 and C4 levels and positively correlated with IgA and IgM levels. IL-21 levels had a positive correlation with IgM levels. p50 levels exhibited a negative correlation with C3 and C4 levels and a positive correlation with IgA, IgG, and IgM levels. A positive correlation was observed between TNF-α and IgM levels. These results were statistically significant ($p < 0.01$ or $p < 0.05$; Spearman test), and the heat map of these correlations was plotted based on the results.

**Table 2. Analysis of Association Rules between Transcription Factors, Inflammatory Factors, and Laboratory Indicators**[a]

| consequence | preceding paragraph | support degree | confidence degree | lift degree |
|---|---|---|---|---|
| SSA (+) | IκB-α ↓ | 56.18 | 74.00 | 1.06 |
| SSB (+) | p65↑ | 50.56 | 53.33 | 1.19 |
| age↑ | IL-33↑ | 43.82 | 58.97 | 1.17 |
| SSA (+) | p65↑ | 50.56 | 80.00 | 1.15 |
| SSB (+) | IL-6↑ | 41.57 | 51.35 | 1.14 |
| SSA (+) | IL-6↑ | 41.57 | 78.38 | 1.13 |
| IgA↑ | TNF-α↑ | 60.67 | 50.00 | 1.11 |
| age↑ | TNF-α↑ | 60.67 | 55.56 | 1.1 |
| SSA (+) | p50↑ | 52.81 | 72.34 | 1.04 |
| age↑ | p65↑ | 50.56 | 51.11 | 1.01 |
| disease course↑ | IL-17↑ | 47.19 | 50.0 | 1.06 |
| disease course↑ | TNF-α↑ | 60.67 | 50.0 | 1.06 |
| females | IL-21↑ | 47.19 | 97.62 | 1.02 |
| females | p50↑ | 52.81 | 95.75 | 1.01 |
| females | p65↑ | 50.56 | 95.56 | 1.01 |

[a]Note: NA, Not applicable; IgA, immunoglobulin A; SSA, anti-SSA antibody; SSB, anti-SSB antibody; IL-33, interleukin 33; IL-6, interleukin 6; p65, RelA; IκB-α, I-kappa-B-alpha; p50, NF-κB1; and TNF-α, tumor necrosis factor-alpha.

**4.5. Levels of Transcription Factors, Cytokines, and Laboratory Indicators in pSS Patients before and after Treatment.** The levels of hs-CRP, IgA, IgG, and ESR after treatment were statistically lower than the levels before treatment. The levels of indicators for liver and kidney function were in the normal range before and after treatment ($p < 0.01$ or $p < 0.05$; Wilcoxon test). Meanwhile, the levels of BAFF, IL-6, TNF-α, IL-17, IRF-1, p65, IκB-α, p50, IL-21, and IL-33 were improved in pSS patients after treatment ($p < 0.01$; Wilcoxon test) (Table 4).

**4.6. Drug Use.** The core nodes of TCM in the complex network were analyzed with Cytoscape 3.9.1, and the importance of the core nodes was assessed with a degree value. The results demonstrated that the core prescriptions encompassed Ophiopogon japonicus, Glycyrrhiza uralensis Fisch, Carthami Flos, Poria, Salviae Miltiorrhizae Radix et Rhizoma, Callerya reticulata, Ligusticum chuanxiong Hort, Pseudostellaria radix, and Cynanchum paniculatum (Figure 4). The western medicines used in the treatment of pSS patients were also retrieved with the SQL system (Table 5).

**4.7. Binary Logistic Regression Model Analysis of Transcription Factors, Cytokines, and Laboratory Indicators.** The binary logistic regression analysis was performed to screen independent risk and protective factors of transcription factors, cytokines, and laboratory indicators (Table 6; Figure S1), and Figure S1 includes visualizations of the binary logistic regression analysis, which can be found in Supporting Information. The results showed that age (>60


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Table 3. Correlation Analysis among Transcription Factors, Cytokines, and Laboratory Indicators[a]

| variables | BAFF | | IL-17 | | IL-33 | | IL-21 | | IL-6 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p | r | p |
| hs-CRP | −0.002 | 0.987 | 0.029 | 0.79 | −0.152 | 0.156 | 0.063 | 0.559 | −0.134 | 0.211 |
| ESR | −0.171 | 0.112 | −0.005 | 0.964 | 0.113 | 0.295 | −0.131 | 0.224 | 0.056 | 0.606 |
| C3 | −0.273** | 0.01 | 0.171 | 0.109 | −0.176 | 0.1 | −0.076 | 0.48 | −0.16 | 0.135 |
| C4 | −0.276** | 0.009 | 0.186 | 0.081 | −0.155 | 0.147 | −0.072 | 0.503 | −0.148 | 0.165 |
| IgA | 0.449** | 0 | −0.129 | 0.228 | 0.143 | 0.182 | 0.188 | 0.077 | −0.014 | 0.895 |
| IgG | 0.162 | 0.128 | −0.134 | 0.211 | 0.124 | 0.246 | 0.087 | 0.42 | −0.128 | 0.231 |
| IgM | 0.329** | 0.002 | 0.102 | 0.34 | 0.112 | 0.297 | 0.234* | 0.027 | 0.327** | 0.002 |
| SSA | 0.031 | 0.774 | −0.13 | 0.226 | 0.032 | 0.769 | −0.047 | 0.659 | 0.085 | 0.428 |
| SSB | −0.146 | 0.172 | −0.094 | 0.379 | −0.109 | 0.31 | −0.119 | 0.265 | −0.018 | 0.867 |
| variables | IRF-1 | | p65 | | IκB-α | | p50 | | TNF-α | |
| | r | p | r | p | r | p | r | p | r | p |
| hs-CRP | −0.033 | 0.758 | −0.03 | 0.779 | 0.043 | 0.69 | −0.009 | 0.936 | −0.001 | 0.995 |
| ESR | −0.043 | 0.692 | 0.165 | 0.125 | −0.115 | 0.288 | 0.063 | 0.559 | −0.168 | 0.119 |
| C3 | −0.058 | 0.588 | −0.02 | 0.853 | 0.073 | 0.499 | −0.213* | 0.045 | −0.151 | 0.157 |
| C4 | −0.047 | 0.661 | 0.032 | 0.769 | 0.021 | 0.842 | −0.209* | 0.049 | −0.152 | 0.154 |
| IgA | 0.095 | 0.377 | −0.135 | 0.206 | −0.076 | 0.477 | 0.214* | 0.044 | 0.105 | 0.33 |
| IgG | 0.068 | 0.526 | −0.166 | 0.12 | 0.158 | 0.139 | 0.221* | 0.038 | 0.104 | 0.332 |
| IgM | 0.112 | 0.297 | −0.102 | 0.34 | 0.07 | 0.516 | 0.234* | 0.028 | 0.234* | 0.028 |
| SSA | −0.15 | 0.161 | 0.198 | 0.063 | −0.099 | 0.356 | 0.044 | 0.68 | −0.182 | 0.088 |
| SSB | −0.185 | 0.083 | 0.108 | 0.314 | 0.116 | 0.279 | 0.007 | 0.95 | −0.235 | 0.057 |

[a]Note: hs-CRP, hyper-sensitivity C-reactive protein; C3, complement C3; C4, complement C4; IgA, G, M, immunoglobulin A, G, M; ESR, erythrocyte sedimentation rate; SSA, anti-SSA antibody; SSB, anti-SSB antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; BUN, blood urea nitrogen; Cr, serum creatinine; UA, uric acid; WBC, white blood cell count; HGB, hemoglobin; RBC, red blood cell; PLT, platelet count; BAFF, B-cell activating factor; IL-17, interleukin 17; IL-33, interleukin 33; IL-21, interleukin 21; IL-6, interleukin 6; IRF-1, interferon regulatory factor 1; p65, RelA; IκB-α, I-kappa-B-alpha; p50, NF-κB1; TNF-α, tumor necrosis factor-alpha. **$p < 0.01$; *$p < 0.05$. The Spearman test was used.

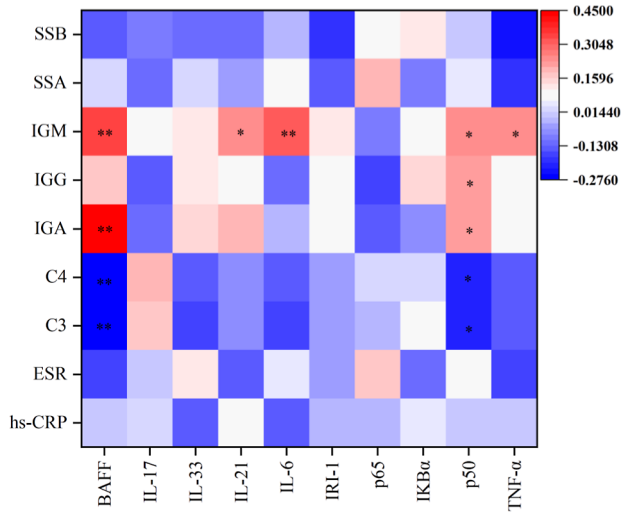


Figure 3. Heat map for correlations. **$p < 0.01$; *$p < 0.05$.

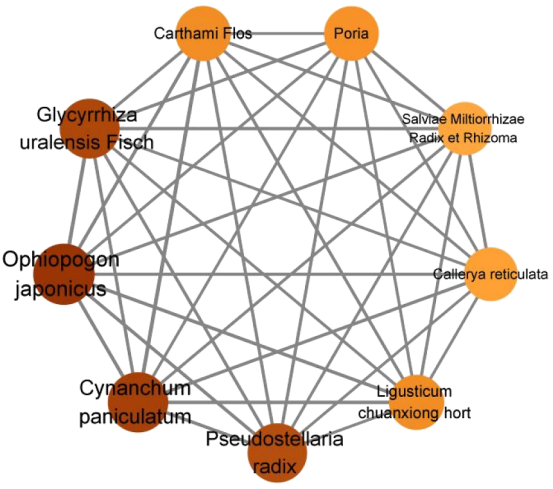


Figure 4. Complex network of the core prescriptions.

years) was a risk factor for IgA [odds ratio (OR) = 0.335, 95% confidence interval (95% CI) = 0.125−0.899, $p = 0.030$; likelihood ratio test], IκB-α (OR = 0.319, 95% CI = 0.13−0.783, $p = 0.013$; likelihood ratio test), and TNF-α (OR = 0.364, 95% CI = 0.15−0.882, $p = 0.025$; likelihood ratio test), the elder patients had higher chances of increased levels of IgA, IκB-α, and TNF-α. Antipsychotics were a protective factor for hs-CRP (OR = 2.878, 95% CI = 1.019−8.133, $p = 0.046$; likelihood ratio test); the use of antipsychotics mitigated anxiety and depression and potentially reduced hs-CRP levels in pSS patients. Interestingly, the core prescription utilized in this study was the only protective factor for all indicators, which improved transcription factors, cytokines, and laboratory indicators in pSS patients.

**4.8. Association Rule Analysis between the Core Prescriptions and Indicators.** Association rule analysis was conducted again to further delve into the relationship between the core prescriptions and indicators. The results unveiled that Poria, *O. japonicus*, chuanxiong Hort, and Pseudostellaria radix were all tightly correlated with decreases in IL-17 and TNF-α levels, all with a support degree of over 45%, a confidence degree of above 55%, and a lift degree of greater than 1 (Table 7).

**4.9. Results of Network Pharmacology and Molecular Docking.** According to data mining results, the core


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**Table 4. Comparison of Transcription Factors, Cytokines, and Laboratory Indicators in pSS Patients before and after Treatment[a]**

| | variables | before treatment | after treatment | *p* value |
|---|---|---|---|---|
| laboratory index | hs-CRP (mg/dL) | 1.51 (0.57, 4.05) | 1.09 (0.54, 2.19)** | 0.008 |
| | IgA (g/L) | 2.52 (1.83, 3.43) | 2.48 (1.84, 3.39)** | 0.002 |
| | IgM (g/L) | 1.25 (0.91, 1.69) | 1.24 (0.93, 1.68) | 0.09 |
| | IgG(g/L) | 14.15 (11.30, 17.36) | 14.13 (11.33, 16.65)** | 0.002 |
| | C3 (g/L) | 1.11 (0.94, 1.30) | 1.11 (0.98, 1.28) | 0.776 |
| | C4 (g/L) | 0.27 (0.21, 0.34) | 0.27 (0.21, 0.33) | 0.284 |
| | ESR (mm/h) | 16.00 (6.50, 30.00) | 14.00 (6.00, 23.50)* | 0.012 |
| | SSB | 40 | 41 | 0.88 |
| | SSA | 62 | 63 | 0.87 |
| | ALT (U/L) | 17.00 (12.00, 31.50) | 20.30 (12.00, 37.45) | 0.78 |
| | AST (U/L) | 23.40 (18.70, 31.00) | 22.40 (18.00, 32.00) | 0.11 |
| | ALP (U/L) | 74.00 (61.00, 103.00) | 74.00 (60.00, 100.00) | 0.29 |
| | GGT (U/L) | 21.00 (14.00, 43.00) | 22.00 (15.50, 44.50) | 0.95 |
| | BUN (mmol/L) | 4.53 (3.58, 5.14) | 4.35 (3.54, 5.15) | 0.71 |
| | Cr ($\mu$mol/L) | 56.30 (50.05, 64.00) | 56.40 (49.55, 63.85) | 0.85 |
| | UA ($\mu$mol/L) | 279.00 (232.00, 327.50) | 251.00 (210.50, 299.50)** | 0.001 |
| | WBC ($\times 10^9$) | 5.00 (4.09, 6.33) | 5.65 (4.43, 7.66) | 0.06 |
| | RBC ($\times 10^{12}$) | 4.06 (3.79, 4.37) | 4.03 (3.76, 4.30) | 0.38 |
| | HGB (g/L) | 122.00 (113.00, 130.00) | 120.00 (111.00, 130.00) | 0.08 |
| | PLT ($\times 10^9$) | 179.00 (138.00, 219.00) | 194.00 (149.00, 241.50) | 0.85 |
| key index | BAFF (pg/mL) | 2367.96 (2233.51, 2466.09) | 1268.04 (1204.00, 1482.54)** | 0.001 |
| | IL-17 (pg/mL) | 597.35 (551.16, 639.01) | 297.81 (269.81, 311.80)** | 0.001 |
| | IL-33 (pg/mL) | 551.42 (531.07, 579.16) | 349.15 (311.57, 370.57)** | 0.001 |
| | IL-21 (pg/mL) | 463.74 (447.44, 485.91) | 265.88 (235.13, 283.50)** | 0.001 |
| | IL-6 (pg/mL) | 133.90 (129.78, 143.12) | 68.18 (63.69, 78.87)** | 0.001 |
| | IRF-1 (pg/mL) | 7730.06 (7549.60, 8572.19) | 4096.85 (3713.67, 4442.12)** | 0.001 |
| | p65 (pg/mL) | 10868.58 (9953.07, 11390.01) | 7435.17 (6751.29, 8025.79)** | 0.001 |
| | IκB-$\alpha$ (pg/mL) | 1292.80 (1101.02, 1499.73) | 4018.18 (3919.26, 4125.94)** | 0.001 |
| | p50 (pg/mL) | 18.78 (16.05, 20.33) | 8.43 (8.02, 9.03)** | 0.001 |
| | TNF-$\alpha$ (pg/mL) | 876.59 (816.26, 922.80) | 493.51 (466.59, 521.24)** | 0.001 |

[a]Note: hs-CRP, hyper-sensitivity C-reactive protein; C3, complement C3; C4, complement C4; IgA, G, M, immunoglobulin A, G, M; ESR, erythrocyte sedimentation rate; SSA, anti-SSA antibody; SSB, anti-SSB antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl-transferase; BUN, blood urea nitrogen; Cr, serum creatinine; UA, uric acid; WBC, white blood cell count; HGB, hemoglobin; RBC, red blood cell; PLT, platelet count; BAFF, B-cell activating factor; IL-17, interleukin 17; IL-33, interleukin 33; IL-21, interleukin 21; IL-6, interleukin 6; IRF-1, Interferon regulatory factor 1; p65, RelA; IκB-$\alpha$, I-kappa-B-alpha; p50, NF-κB1; TNF-$\alpha$, tumor necrosis factor-alpha. **$p < 0.01$; *$p < 0.05$. The Wilcoxon test was used.

**Table 5. Used Western Medicines in the Treatment of pSS Patients[a]**

| pharmacodynamic classification | drug name | usage | N (%) |
|---|---|---|---|
| NSAIDs | celecoxib | oral | 10 (11.24%) |
| | etoricoxib | oral | |
| | lornoxicam | oral | |
| | meloxicam | oral | |
| GC | methylprednisolone | oral | 39 (43.82%) |
| | prednisone | oral | |
| | hydrocortisone sodium succinate | intravenous drip | |
| | methylprednisolone sodium succinate | intravenous drip | |
| | dexamethasone sodium phosphate | intravenous drip | |
| AT | sodi umHya1uronate eye drops | eye-dropping | |
| immunomodulator | tripterygium glycosides | oral | 67 (75.28%) |
| | hydroxychloroquine sulfate tablets | oral | |
| | leflunomide | oral | |
| | mycophenolate mofetil | oral | |
| | total glucosides of paeony capsules | oral | |
| | methotrexate tablets | oral | |
| thrombopoietic drugs | human interleukin-11 | subcutaneous injection | 12 (13.48%) |
| | recombinant human interleukin-11 (I) | subcutaneous injection | |
| antipsychotics | clonazepam | oral | 22 (24.72%) |
| | flupentixol and melitracen tablets | oral | |
| | mirtazapine tablets | oral | |
| | olanzapine | oral | |

[a]Note: NSAIDs, nonsteroidal anti-inflammatory drugs; GC, glucocorticosteroid; AT, artificial tear.

prescriptions were closely related to improvements in transcription factors, cytokines, and laboratory indicators of pSS patients. Network pharmacology analysis was carried out to further probe the specific mechanism of the core prescriptions



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Table 6. Binary Logistic Regression Model Analysis of Transcription Factors, Cytokines, and Laboratory Indicators[a]

| OR (95% CI) | B | *Y* | *X* | *Y* | B | OR (95% CI) |
|---|---|---|---|---|---|---|
| 6.767(2.645, 17.312)*** | 1.912 | ESR | poria | IRF-1 | 1.882 | 6.564(2.603, 16.555)*** |
| 3.239(1.346, 7.795)** | 1.175 | hs-CRP | | IL-6 | 1.882 | 6.564(2.603, 16.555)*** |
| 2.576(1.045, 6.351)* | 0.946 | IgA | | p65 | 1.882 | 6.564(2.603, 16.555)*** |
| 5.28(2.138, 13.043)*** | 1.664 | TNF-$\alpha$ | | IL-33 | 1.881 | 6.558(2.6, 16.54)*** |
| 8.25(3.191, 21.328)*** | 2.11 | p50 | | IL-17 | 1.664 | 5.28(2.138, 13.043)*** |
| 7.333(2.875, 18.704)*** | 1.992 | IKB$\alpha$ | | BAFF | 1.882 | 6.564(2.603, 16.555)*** |
| 2.382(1.017, 5.579)* | 0.868 | IL-21 | | | | |
| 8(3.06, 20.913)*** | 2.079 | ESR | callerya speciosa | IRF-1 | 1.537 | 4.653(1.853, 11.679)** |
| 8.883(3.359, 23.492)*** | 2.184 | hs-CRP | | IL-6 | 1.537 | 4.653(1.853, 11.679)** |
| 7.031(2.59, 19.091)*** | 1.95 | IgM | | IL-21 | 1.114 | 3.048(1.258, 7.384)* |
| 7.412(2.796, 19.644)*** | 2.003 | TNF-$\alpha$ | | IL-33 | 2.446 | 11.541(4.134, 32.22)*** |
| 7.412(2.796, 19.644)*** | 2.003 | p50 | | IL-17 | 1.322 | 3.75(1.523, 9.23)** |
| 6.353(2.458, 16.421)*** | 1.849 | IKB$\alpha$ | | BAFF | 2.003 | 7.412(2.796, 19.644)*** |
| 4.653(1.853, 11.679)** | 1.537 | p65 | | C3 | 2.262 | 9.6(3.104, 29.695)*** |
| 4.8(1.516, 15.199)** | 1.569 | C4 | | | | |
| 8.571(3.258, 22.554)*** | 2.148 | ESR | salviae miltiorrhizae radix et rhizoma | BAFF | 2.11 | 8.25(3.191, 21.328)*** |
| 7.733(2.96, 20.202)*** | 2.046 | hs-CRP | | p65 | 1.455 | 4.286(1.765, 10.404)** |
| 6.5(2.29, 18.446)*** | 1.872 | IgM | | IRF-1 | 1.882 | 6.564(2.603, 16.555)*** |
| 10.509(3.946, 27.986)*** | 2.353 | TNF-$\alpha$ | | IL-6 | 1.455 | 4.286(1.765, 10.404)** |
| 6.564(2.603, 16.555)*** | 1.882 | p50 | | IL-21 | 1.882 | 6.564(2.603, 16.555)*** |
| 5.87(2.354, 14.637)*** | 1.77 | IKB$\alpha$ | | IL-33 | 1.881 | 6.558(2.6, 16.54)*** |
| 3.504(1.465, 8.384)** | 1.254 | IL-17 | | C3 | 2.977 | 19.63(4.224, 91.224)*** |
| 6.533(1.724, 24.754)** | 1.877 | C4 | | | | |
| 16.208(5.634, 46.626)*** | 2.785 | ESR | ligusticum chuanxiong hort | p65 | 2.235 | 9.35(3.558, 24.573)*** |
| 14.393(5.064, 40.911)*** | 2.667 | hs-CRP | | IRF-1 | 2.235 | 9.35(3.558, 24.573)*** |
| 13.263(4.038, 43.56)*** | 2.585 | IgM | | IL-6 | 2.487 | 12.02(4.425, 32.655)*** |
| 5.462(2.071, 14.406)** | 1.698 | IgA | | IL-21 | 1.776 | 5.905(2.363, 14.753)*** |
| 15.75(5.569, 44.543)*** | 2.757 | TNF-$\alpha$ | | IL-33 | 2.475 | 11.88(4.389, 32.154)*** |
| 21.143(7.115, 62.828)*** | 3.051 | p50 | | BAFF | 3.377 | 29.292(9.263, 92.623)*** |
| 13.6(4.928, 37.535)*** | 2.61 | IKB$\alpha$ | | IL-17 | 2.235 | 9.35(3.558, 24.573)*** |
| 47.143(5.95, 373.55)*** | 3.853 | C3 | | C4 | 2.467 | 11.786(2.502, 55.507)** |
| 10.667(3.857, 29.498)*** | 2.367 | ESR | pseudostellaria heterophylla | p65 | 1.998 | 7.371(2.882, 18.849)**** |
| 12.8(4.436, 36.937)*** | 2.549 | hs-CRP | | IRF-1 | 1.998 | 7.371(2.882, 18.849)**** |
| 14.773(3.999, 54.566)*** | 2.693 | IgM | | IL-6 | 2.485 | 12(4.416, 32.606)**** |
| 4.073(1.561, 10.63)** | 1.404 | IgA | | IL-21 | 1.998 | 7.371(2.882, 18.849)*** |
| 12(4.416, 32.606)*** | 2.485 | TNF-$\alpha$ | | IL-33 | 2.021 | 7.543(2.929, 19.427)*** |
| 15.725(5.559, 44.48)*** | 2.755 | p50 | | IL-17 | 1.998 | 7.371(2.882, 18.849)*** |
| 10.694(3.988, 28.68)*** | 2.37 | IKB$\alpha$ | | BAFF | 3.376 | 29.25(9.249, 92.503)*** |
| 36.08(4.576, 284.446)** | 3.586 | C3 | | C4 | 3.052 | 21.161(2.661, 168.278)** |
| 6.767(2.645, 17.312)*** | 1.912 | ESR | flos carthami | p65 | 1.664 | 5.28(2.138, 13.043)**** |
| 7.733(2.96, 20.202)*** | 2.046 | hs-CRP | | IRF-1 | 1.882 | 6.564(2.603, 16.555)**** |
| 6.5(2.29, 18.446)*** | 1.872 | IgM | | IL-6 | 1.455 | 4.286(1.765, 10.404)*** |
| 6.564(2.603, 16.555)*** | 1.882 | BAFF | | IL-21 | 1.254 | 3.504(1.465, 8.384)** |
| 5.28(2.138, 13.043)*** | 1.664 | TNF-$\alpha$ | | IL-33 | 1.881 | 6.558(2.6, 16.54)*** |
| 6.564(2.603, 16.555)*** | 1.882 | p50 | | IL-17 | 1.455 | 4.286(1.765, 10.404)** |
| 4.745(1.939, 11.611)** | 1.557 | IKB$\alpha$ | | C3 | 2.053 | 7.79(2.376, 25.54)** |
| 6.533(1.724, 24.754)** | 1.877 | C4 | | | | |
| 9.487(3.572, 25.198)*** | 2.25 | ESR | ophiopogon japonicus | p65 | 1.562 | 4.769(1.946, 11.688)*** |
| 8.516(3.234, 22.425)*** | 2.142 | hs-CRP | | IRF-1 | 1.776 | 5.905(2.363, 14.753)**** |
| 6.984(2.454, 19.876)*** | 1.944 | IgM | | IL-6 | 1.776 | 5.905(2.363, 14.753)**** |
| 3.436(1.368, 8.632)** | 1.234 | IgA | | IL-21 | 1.999 | 7.385(2.888, 18.88)*** |
| 12.02(4.425, 32.655)*** | 2.487 | TNF-$\alpha$ | | IL-33 | 2.475 | 11.88(4.389, 32.154)*** |
| 9.35(3.558, 24.573)*** | 2.235 | p50 | | IL-17 | 1.357 | 3.884(1.61, 9.367)** |
| 8.242(3.188, 21.311)*** | 2.109 | IKB$\alpha$ | | BAFF | 1.999 | 7.385(2.888, 18.88)*** |
| 21(4.511, 97.765)*** | 3.045 | C3 | | C4 | 1.934 | 6.92(1.825, 26.24)** |
| 4.857(1.803, 13.083)** | 1.58 | IgA | cynanchum paniculatum | IL-21 | 1.46 | 4.308(1.768, 10.494)** |
| 9.714(3.535, 26.695)*** | 2.274 | ESR | | p65 | 1.891 | 6.623(2.614, 16.785)**** |
| 11.667(4.068, 33.46)*** | 2.457 | hs-CRP | | IRF-1 | 2.367 | 10.667(3.976, 28.614)**** |
| 13.768(3.735, 50.758)*** | 2.622 | IgM | | IL-6 | 2.122 | 8.346(3.21, 21.704)**** |
| 9.573(3.608, 25.398)*** | 2.259 | IKB$\alpha$ | | BAFF | 2.63 | 13.875(4.981, 38.652)*** |
| 8.346(3.21, 21.704)*** | 2.122 | TNF-$\alpha$ | | IL-33 | 2.156 | 8.635(3.285, 22.7)*** |



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Table 6. continued

| OR (95% CI) | B | Y | X | Y | B | OR (95% CI) |
|---|---|---|---|---|---|---|
| 13.875(4.981, 38.652)*** | 2.63 | p50 | | IL-17 | 1.891 | 6.623(2.614, 16.785)*** |
| 33.846(4.297, 266.605)** | 3.522 | C3 | | C4 | 2.182 | 8.864(1.888, 41.616)** |
| 0.319(0.13, 0.783)* | −1.143 | IKB$\alpha$ | age (>60y) | IgA | −1.094 | 0.335(0.125, 0.899)* |
| 0.364(0.15, 0.882)* | −1.012 | TNF-$\alpha$ | | | | |
| 2.878(1.019, 8.133)* | 1.057 | hs-CRP | antipsychotics | | | |

[a]Note: Binary logistic regression model analysis results of transcription factors, cytokines, and laboratory indicators. The likelihood ratio test was utilized. hs-CRP, hyper-sensitivity C-reactive protein; C3, complement C3; C4, complement C4; IgA, G, M, immunoglobulin A, G, M; ESR, erythrocyte sedimentation rate; BAFF, B-cell Activating Factor; IL-17, interleukin 17; IL-33, interleukin 33; IL-21, interleukin 21; IL-6, interleukin 6; IRF-1, Interferon regulatory factor 1; p65, RelA; I$\kappa$B-$\alpha$, I-kappa-B-alpha; p50, NF-$\kappa$B1; and TNF-$\alpha$, Tumor Necrosis Factor-alpha. *:$p < 0.05$; **:$p < 0.01$; ***:$p < 0.001$.

Table 7. Analysis of Association Rules between the Core Prescriptions and Indicators[a]

| consequence | preceding paragraph | support degree | confidence degree | lift degree |
|---|---|---|---|---|
| IL-17↓ | poria | 49.438 | 89 | 1.011 |
| TNF-$\alpha$↓ | poria | 49.438 | 76 | 1.011 |
| IL-17↓ | ophiopogon japonicus | 48.315 | 90 | 1.011 |
| TNF-$\alpha$↓ | ophiopogon japonicus | 48.315 | 77 | 1.011 |
| IL-17↓ | ligusticum chuanxiong Hort | 48.315 | 58 | 1.011 |
| TNF-$\alpha$↓ | ligusticum chuanxiong Hort | 48.315 | 63 | 1.011 |
| IL-17↓ | pseudostellaria radix | 52.809 | 70 | 1.011 |
| TNF-$\alpha$↓ | pseudostellaria radix | 52.809 | 82 | 1.011 |

[a]Note: IL-17, interleukin 17; TNF-$\alpha$, Tumor Necrosis Factor-alpha.

in the treatment of pSS. The core prescriptions were searched in TCMSP, and then active ingredients were screened with the criteria of oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. The target gene names of all active ingredients were corrected with Uniprot. After removing duplicate core prescriptions and disease target genes, respectively, cross-genes between them were obtained, and the active ingredient-potential target-disease network of core prescriptions was constructed using Cytoscape 3.9.1 (Figure 5A,B). The intersected target genes were input into the String database to attain the TSV file of PPI, which was then imported into Cytoscape 3.9.1 software to screen out the top 5 core target genes in terms of degree values, eventually creating the core target genes network of the core prescriptions (Figure 5C).

Core target genes were further subjected to gene annotation and pathway analysis to determine the energy that they represented. Metascape online software was used to analyze cellular components(CC), biological processes(BP), molecular functions(MF), and KEGG pathways of the core targets in the treatment of pSS. The results unveiled that GO enrichment analysis of BP showed that GO items that responded to lipopolysaccharides, positively regulated cytokine production, and cell response to chemical stress, oxygen species, and oxidative stress were enriched. The results of CC showed that graphene oxide items such as membrane raft, membrane microdomain, membrane region, cytoplasmic vesicle lumen, transcriptional regulatory complex, and receptor complex were enriched. The results of MF showed that graphene oxide items such as cytokine receptor binding, protein tyrosine kinase activity, and transcription factor binding were enriched(Figure 5D). These targets were mainly enriched in TNF pathways, and corresponding graphs were drawn (Figure 5E).

We screened out the top 5 core active components and the top 5 core targets of TCM involved in the treatment of pSS (Figure 6A,B). Subsequently, the two-dimensional structure chart of these active components was downloaded from TCMSP. Molecular docking was performed on pathway proteins TNF and p65 and core target IL-6, respectively. The lower binding energy between the component and the target protein receptor was associated with higher binding activity (Figure 6C). Given the lack of effective hydrogen bonding in stigmasterol, the active component with the lowest binding energy to the target protein, luteolin, with the second lowest binding energy, was selected for analysis. The results revealed that luteolin had the lowest binding energy with TNF (−8.0 kcal/mol), p65 (−6.9 kcal/mol), and IL-6 (−7.7 kcal/mol) (Figure 6D).

**4.10. Luteolin Improved Glandular Secretion Function and Inflammation in HSG Cells via the TNF/NF-$\kappa$B Pathway.** Furthermore, in vitro experiments were carried out to verify the mechanism underlying improvements in pSS predicted based on network pharmacology analysis. Compared with cell viability at 12 h, cell viability at 24 h was increased in almost all groups except for the ATP-HSG + 50 $\mu$M luteolin group ($p < 0.05$ or $p < 0.01$; the Kruskal−Wallis nonparametric test). After treatment for 24 h, cell viability was diminished in almost all groups. Hence, 24 h was selected as the optimal treatment time. After 24 h of treatment, almost all groups, except for the ATP-HSG +50 $\mu$M luteolin group, exhibited an enhancement in cell viability ($p < 0.01$; the Kruskal−Wallis nonparametric test). Therefore, HSG cells were treated with 15 $\mu$M, 20 $\mu$M, and 30 $\mu$M luteolin for subsequent experiments (Figure 7A). After pcDNA3.1-TNF treatment, TNF-$\alpha$ expression was augmented in modeled HSG cells ($p < 0.001$ or $p < 0.01$; the Kruskal−Wallis nonparametric test), accompanied by elevated P-p65 levels ($p < 0.001$; the Kruskal−Wallis nonparametric test), lowered cell viability and IL-6 levels ($p < 0.01$; the Kruskal−Wallis nonparametric test), and enhanced TNF-$\alpha$ and IL-1$\beta$ levels ($p < 0.001$ or $p < 0.01$; the Kruskal−Wallis nonparametric test), which were nullified by luteolin at 15, 20, and 30 $\mu$M in a dose-dependent manner ($p < 0.05$ or $p < 0.01$; the Kruskal−Wallis nonparametric test) (Figure 7A−H). In addition, CK5 and CK7 are a kind of marker for the duct epithelium. E-Cad is a


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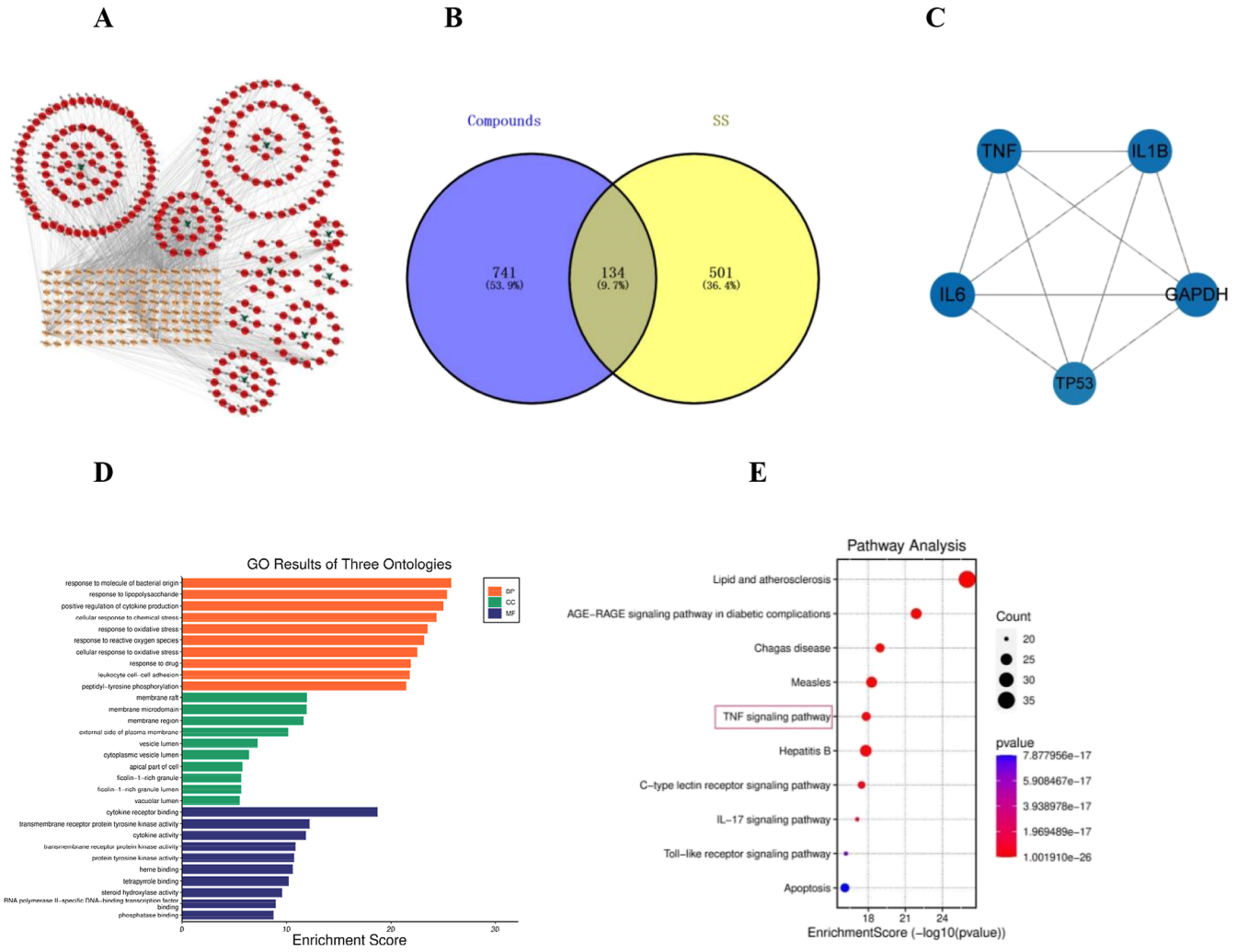


**Figure 5.** (A) Diagram of the active ingredient-potential target-disease network of the core prescriptions; (B) Venn diagram; (C) Core target genes network; (D,E) GO and KEGG analyses. Maidong, *Ophiopogon japonicus*; Gancao, *Glycyrrhiza uralensis* Fisch; Honghua, Carthami Flos; Fuling, Poria; Danshen, Salviae Miltiorrhizae Radix et Rhizoma; Ji Xueteng, *Callerya reticulata*; Chuanxiong, Ligusticum chuanxiong Hort; Tai Zishen, Pseudostellaria radix; and Xu Changqin, Cynanchum paniculatum.

transmembrane glycoprotein mainly expressed on the epithelial cell membrane, which is vital for intercellular adhesion and is highly expressed in normal epithelial tissues. Immunohistochemical results exhibited higher RO-52/SSA, CK5, and CK7 expression ($p < 0.001$; the Kruskal–Wallis nonparametric test) and lower E-Cad expression ($p < 0.001$; the Kruskal–Wallis nonparametric test) in the pcDNA3.1-TNF group than in the model group. In HSG cells treated with ATP + pcDNA3.1-TNF, luteolin at 15, 20, and 30 $\mu$M reduced CK5, CK7, and RO-52/SSA expression and elevated E-Cad expression in a dose-dependent manner ($p < 0.05$ or $p < 0.01$; the Kruskal–Wallis nonparametric test) (Figure 7I).

## 5. DISCUSSION

When initially identified, pSS may be attributed to chronic inflammation in exocrine glands, which impairs the secretion of glands and causes dryness symptoms, particularly in the mouth and eyes, thus bringing huge economic burdens to individuals and society.[34] pSS occurs more commonly in women, with an estimated male-to-female ratio of 14 to 1.[35] Over the past few years, increasing efforts have been made to deepen the understanding of pSS and its potential therapeutic targets for achieving earlier diagnosis and more precise therapeutic interventions.[36] Unfortunately, the etiology of pSS remains incompletely understood, and effective treatments are lacking. Meanwhile, systemic immunosuppression is largely ineffective in pSS patients compared to patients with other autoimmune diseases.[37] Clinically, pSS patients are often given symptomatic treatments and, in severe cases, even hormones to control the condition. Therefore, it is imperative to develop new potential therapeutic targets and drugs for the treatment of pSS.

TCM has been increasingly valued and recognized in clinical practice for its guaranteed safety and efficacy.[38] Accordingly, it is necessary to observe the clinical efficacy of TCM in the treatment of pSS and explore its potential targets and optimal treatment regimen since there is no clear treatment protocol for pSS in the current guidelines.[39] Our study first analyzed the efficacy of TCM in the treatment of pSS through keyword visualization, association rule analysis, correlation analysis, and data mining, investigated the possible targets of TCM involved in the treatment of pSS with network pharmacology and molecular docking, and validated the targets with cell experiments.


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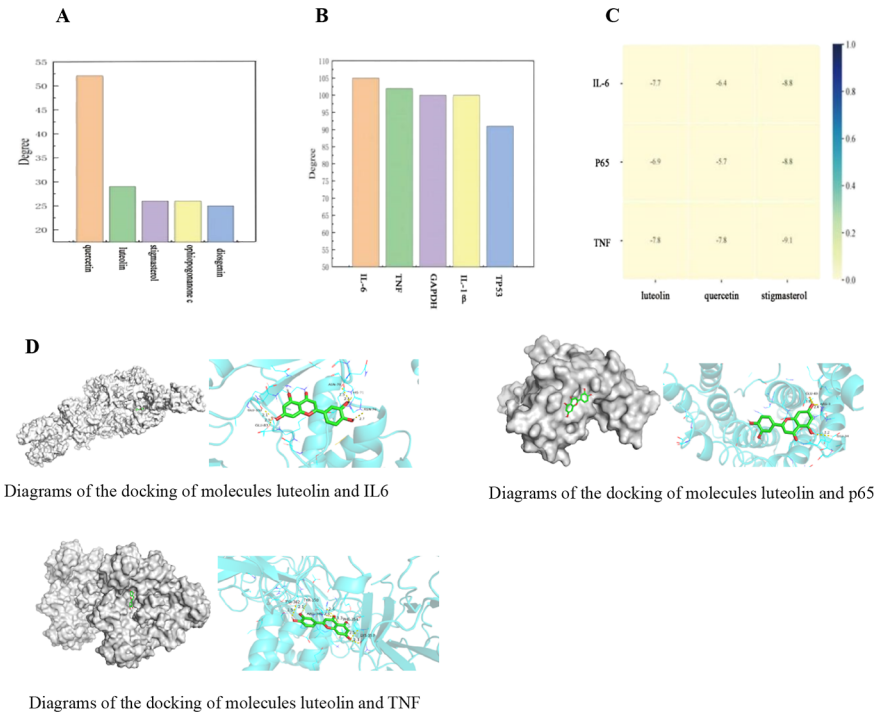


**Figure 6.** (A) Degree of the top 10 core active components of TCM involved in the treatment of pSS, with quercetin (MOL000098) from *Glycyrrhiza uralensis* Fisch, luteolin (MOL000006) from Carthami Flos, Salviae Miltiorrhizae Radix et Rhizoma, *Callerya reticulata*, and Pseudostellaria radix, and stigmasterol (CID5280794) from *Ophiopogon japonicus*; (B) degree of the top 5 core targets of TCM involved in the treatment of pSS. TNF, tumor necrosis factor; IL-6, interleukin-6; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TP53, cellular tumor antigen p53; (C) binding energy of core active components to core target; and (D) molecular docking results.

First, our findings demonstrated that the levels of BAFF, IL-6, TNF-$\alpha$, IL-17, IRF-1, p65, p50, IL-21, and IL-33 were significantly higher, and I$\kappa$B-$\alpha$ levels were markedly poorer in pSS patients than in control participants ($p < 0.01$, $p < 0.05$). Through association rule analysis and correlation analysis, it was found that some transcription factors, cytokines were correlated with age, laboratory indicators, and sex. Additionally, TCM treatment improved transcription factors, cytokines, and some laboratory indicators to varying degrees ($p < 0.05$), without affecting liver and kidney function indicators. Then, the core prescriptions were obtained and included in the logistic regression model analysis along with the use of conventional Western drugs. The results unraveled that age (>60 years) was a risk factor for IgA, I$\kappa$B-$\alpha$, and TNF-$\alpha$. Consistently, previous immunohistochemical results also displayed some differences in the synthesis of IgG, IgM, and IgA among pSS patients of different ages, particularly patients aged over 60 years.[40,41] Moreover, aging has also been reported to facilitate immunoinflammation in pSS.[42,43] In addition, our findings revealed that antipsychotics were a protective factor for hs-CRP, which can be explained by the fact that the mood of patients with anxiety and depression is closely associated with immunoinflammatory indicators.[44,45] Of note, logistic regression model analysis and association rule analysis elucidated that TCM used in our study was the only protective factor for all indicators and improved the levels of transcription factors, cytokines, and laboratory indicators. A prior study reported that TCM at high concentrations protected joints more quickly than immunomodulators such as methotrexate, thus delaying the course of the disease.[46] As a result, it can be speculated that the protective effect of the core prescriptions on the indicators may be associated with their dose and speed of action. However, this speculation requires further pharmacodynamic and pharmacokinetic proof, which will be the subject of our next research program. Overall, TCM plays a leading and important role in the treatment of pSS since it decreases the levels of transcription factors and cytokines and represses inflammation through multitargets and multipathways according to network pharmacology analysis. In our study, binding energies, molecular docking, and KEGG enrichment analysis speculated that luteolin might ameliorate inflammation in pSS by regulating the TNF/NF-$\kappa$B pathway. The ATP-HSG cell model, under normal circumstances, has extracellular ATP (eATP) levels that are tightly regulated by ecto-ATPase; however, high concentrations of eATP are able to activate most members of the purinergic receptor family, inducing salivary gland inflammation and associated salivary insufficiency.[47] Prior research reported that the abnormal expression of inflammatory factors increased exocrine gland epithelial cell apoptosis in the pSS model, triggering secretion dysfunction.[48] pSS is primarily characterized pathologically by lymphocyte infiltration in salivary and lacrimal glands and focal infiltration around the catheter.[49−52] Therefore, in addition to changes in inflammatory factors, ductal epithelial cell-related markers CK5 and CK7, and normal epithelial cell membrane protein E-Cad were also examined in ATP-HSG cells after luteolin treatment and TNF overexpression. Our data disclosed that luteolin not only lowered the levels of pro-inflammatory factors and marker antibodies in ATP-HSG cells but also improved duct infiltration of cells, which might be achieved by the TNF/NF-$\kappa$B pathway. TNF overexpression enhanced the levels of pro-inflammatory cytokines (IL-6, TNF-$\alpha$, and IL-1$\beta$), CK5 protein, CK7 protein, and Ro52/SSA protein, declined E-Cad levels in ATP-HSG cells, facilitated


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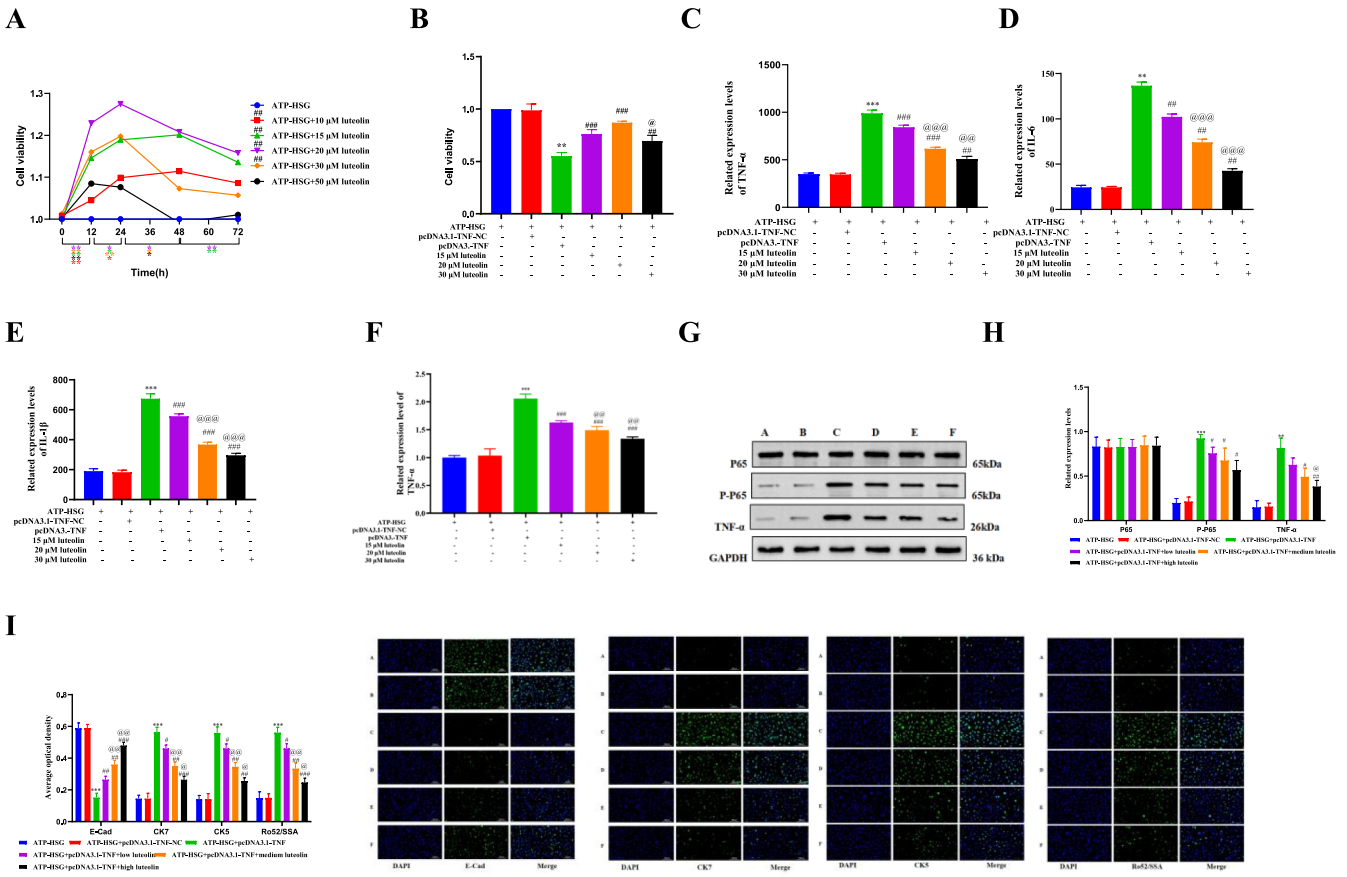


**Figure 7.** (A) Optimal action time and low, medium, and high treatment concentrations of luteolin screened with CCK-8. (B) CCK-8 conducted to determine cell viability. (C) Expression of TNF detected with RT-qPCR. (D−F) Levels of pro-inflammatory factors IL-6, TNF-$\alpha$, and IL-1$\beta$ measured with ELISA. (G,H) Western blotting conducted to test the levels of TNF-$\alpha$ and P-p65. (I) Immunofluorescence used to determine CK5, CK7, RO-52/SSA, and E-Cad expression. A, ATP-HSG; B, ATP-HSG + pcDNA3.1-NC; C, ATP-HSG + pcDNA3.1-TNF; D, ATP-HSG + pcDNA3.1-TNF +15 $\mu$M luteolin; E, ATP-HSG + pcDNA3.1-TNF +20 $\mu$M luteolin; and F: ATP-HSG + pcDNA3.1-TNF +30 $\mu$M luteolin. All cell experiments were repeated three times, and results are expressed as mean ± standard deviation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to the ATP-HSG group (the Kruskal−Wallis nonparametric test); #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared with the ATP-HSG + pcDNA3.1-TNF group (the Kruskal−Wallis nonparametric test); @ $p < 0.05$, @@ $p < 0.01$,$p < 0.001$ compared to the previous group (the Kruskal−Wallis nonparametric test).

the translocation of p65 into the nucleus, and activated the NF-$\kappa$B pathway, which could be abrogated by luteolin treatment. As a group of transcription factors, the NF-$\kappa$B family can activate numerous downstream inflammatory targets, including pro-inflammatory cytokines. NF-$\kappa$B over-activation has been found in salivary gland epithelial cells of pSS patients.[53,54] TNF, also named TNF-$\alpha$, is a cell signaling protein implicated in systemic inflammation and is one of the cytokines evoking the acute phase reaction. The TNF family activates transcription factors, such as NF-$\kappa$B, to favor cell survival, differentiation, immunity, and inflammation. The localization of TNF in mitochondria is also associated with the activation of NF-$\kappa$B.[55] Luteolin, a member of the flavonoid family, has been confirmed to have anti-inflammatory and immunomodulatory activities.[56] More interestingly, luteolin functioned as an inhibitor of cytokines, such as TNF-$\alpha$, and all experiments demonstrated that luteolin and the TNF-$\alpha$ inhibitor had synergistic inhibitory effects on TNF-$\alpha$. Additionally, prior studies showed that inhibition of NF-$\kappa$B and other related pathways decreased the incidence of arthritis,[57,58] partially consistent with our results.

## 6. CONCLUSIONS

Conclusively, TCM can markedly improve transcription factors, cytokines, and laboratory immuno-inflammatory markers in pSS patients. According to the results of network pharmacology and molecular docking, luteolin, the active ingredient with the best potential energy for molecular docking, was selected for in vitro cell experiments. Finally, we found that the effects of luteolin in blocking the TNF/NF-$\kappa$B pathway, reducing the levels of pro-inflammatory factors, and improving the secretion function and morphological integrity of HSG cells (Figure 8). Our study provides evidence for the use of TCM in the clinical treatment of pSS and indicates TCM as a promising alternative therapy for pSS. However, there are limitations to this study: 1. Although we have confirmed the possible role and mechanism of luteolin in HSG cells, this is only a database-based prediction and does not directly represent the efficacy of the TCM prescription. Therefore, it is very necessary to identify the active ingredients of the TCM prescription by targeting or nontargeting chemical or pharmacological analysis; 2. Luteolin was chosen based on computational predictions. There is a wide range of active ingredients in TCM prescriptions. In the next step, based on the identification of active ingredients, we will extend the study


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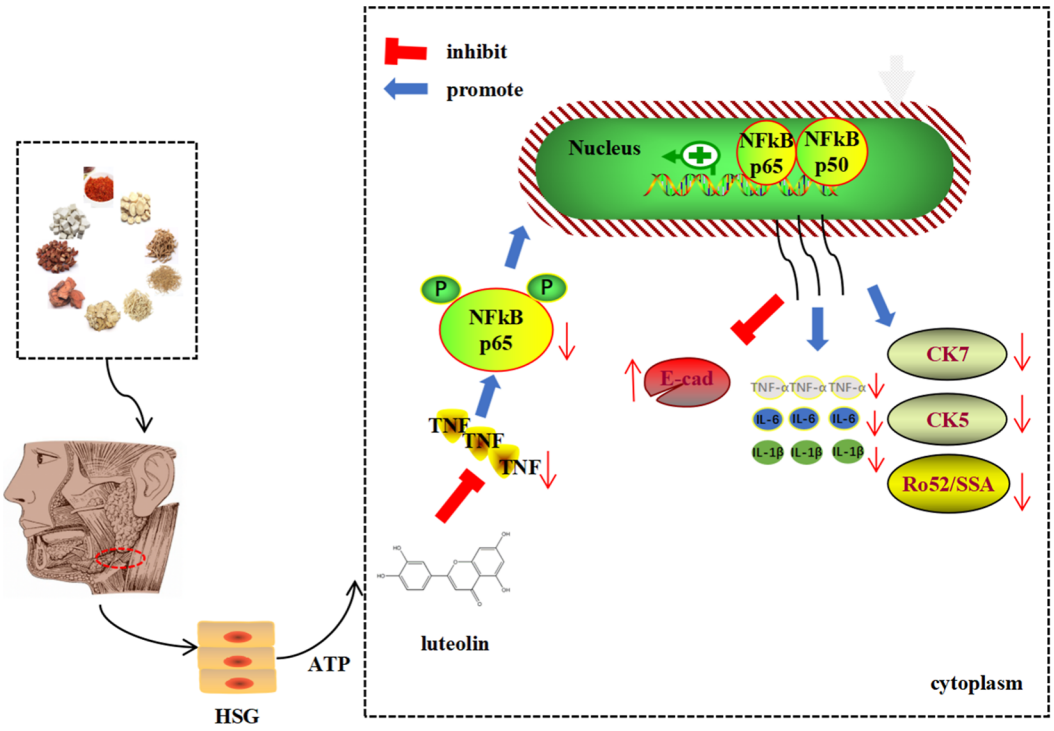


**Figure 8.** Schematic diagram of the mechanism.

to all the active ingredients identified and analyze the priority of their roles.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

The data that support the findings of this study are available from the information center of Anhui Hospital of Traditional Chinese Medicine. It obtains the electronic medical record information on effective pSS inpatients from the SQL Server database, but restrictions apply to the availability of these data, which were used under license for the current study and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from the information center.

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c10653.

Visualization of the binary logistic regression analysis (PDF)

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### Author Contributions

CXL and LJ contributed to the study design. CXL contributed to data analysis, wrote the first draft, and revised the manuscript. ZXH and DX contributed to the specimen and data collection. CXLsupervised the project and contributed to the manuscript revision. All authors reviewed and accepted the content of the final manuscript.


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### Notes

The authors declare no competing financial interest.

## ■ LIST OF ABBREVIATIONS

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; AT, artificial tear; BAFF, B-cell activating factor; BP, biological process; BUN, blood urea nitrogen; CC, cellular components; C3, complement C3; C4, complement C4; Cr, serum creatinine; ESR, erythrocyte

sedimentation rate; GC, glucocorticosteroid; GGT, gamma-glutamyltransferase; GO, gene ontology; hs-CRP, hypersensitive C-reactive protein; HGB, hemoglobin; HSG, human submandibular gland epithelial cells; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IκB-&alpha, I-kappa-B-alpha; IL-6, interleukin 6; IL-17, interleukin 17; IL-21, interleukin 21; IL-33, interleukin 33; IRF-1, interferon regulatory factor 1; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function; NA, not applicable; NSAIDs, nonsteroidal antiinflammatory drugs; p50, NF-κB1; p65, RelA; PPI, protein−protein Interaction network; PLT, platelet count; pSS, primary Sjogren's Syndrome; RBC, red blood cell; SQL, structured query language; SSA, anti-SSA antibody; SSB, anti-SSB antibody; TCM, traditional Chinese medicine; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; TNF-&alpha, tumor necrosis factor-alpha; UA, uric acid; WBC, white blood cell count

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