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ORIGINAL RESEARCH

Exploring the Mechanism of Qinzhuliangxue Mixture for Treating Skin Lesions in Atopic Dermatitis: Insights from Network Pharmacology and Experimental Validation

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Background: Atopic dermatitis (AD) is a chronic, recurrent inflammatory skin disorder that causes systemic skin lesions. The hospital preparation-Qinzhuliangxue mixture (QZLX) has shown potential in alleviating skin lesions in AD patients; however, its underlying mechanisms remain largely unexplored.

Methods: QZLX's key compounds and their targets in AD skin lesions were screened by network pharmacology, and gene enrichment analysis was performed. Mouse model of AD induced by 2,4-dinitrofluorobenzene (DNFB) was established. Then animals were divided into control (Con), model (Model), dexamethasone (DSMS), and QZLX group. The DSMS group and QZLX group was orally administrated DSMS (10 mg/kg/day) and QZLX (18.27 g/kg/day) for 14 days respectively, after the DNFB was first injected intradermally into the abdomen of mice. Phenotypic changes in mice after treatment were evaluated by skin SCORAD score, hematoxylin-eosin (HE) staining, ELISA assays, and Western Blot (WB).

Results: According to the findings of enrichment analysis based on GO and KEGG, QZLX may exert its effects at the top position via the JAK-STAT pathway. Subsequently the experimental validation showed that the skin score and HE staining of the QZLX group was significantly improved compared to the Model group ($P < 0.05$). Moreover, the levels of serum IgE, TSLP, and IL-4 in the QZLX group were notably lower than those in the Model group ($P < 0.05$). Furthermore, the expression of P-JAK2 and P-STAT3 detected by WB in the skin of the QZLX group was obviously higher than that in the Model group ($P < 0.05$).

Conclusion: QZLX demonstrated a significant ability to mitigate AD-like skin lesions and effectively reduced serum levels of IgE, TSLP, and IL-4 in AD mice. The underlying mechanism of action may involve modulation via the JAK2/STAT3 pathway.

Keywords: qinzhuliangxue mixture, atopic dermatitis, skin lesions, network pharmacology, molecular docking, JAK2/STAT3 pathway

Introduction

Atopic Dermatitis (AD) is a prevalent chronic inflammatory skin disorder that affects about 15% to 30% of children and 10% to 15% of adults worldwide.[1] This disorder manifests as skin lesions, including dry skin and eczema-like rashes, which can considerably impact both the patient's appearance and psychological well-being.[2] AD is an autoimmune skin disorder characterized by immune dysfunction, skin lesions, and alterations in the microbial environment of the skin. The

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primary goal in managing AD is to address the skin lesions.[3] Skin damage could facilitate the activation of Th2-type immune responses and enhances levels of Th2-related cytokines such as IL-4, IL-6, TNF-α and IgE in the bloodstream.[4]

Current research indicates that Janus kinase (JAK) inhibitors represent an up-and-coming therapeutics for treating AD, as they have been shown to remarkably ameliorate skin lesions and other symptoms associated with AD.[5] Nowdays there are two JAK inhibitors approved for AD treatment in China, namely Upadacitinib and Abrocitinib, with Delgocitinib (JTE-032), Ruxolitinib, and Tofacitinib soon to be launched. These inhibitors are both expensive and prone to adverse reactions, and different JAK inhibitors have different selectivity, which affects efficacy and safety.[6–9] However, long-term use of JAK inhibitors may increase the risk of malignancy and cardiovascular events, and disease resistance may occur.[10]

Traditional Chinese medicine (TCM) is characterized by multi-component composition, cross-organ efficacy, and multi-target action. These properties enable them to exert a coordinated systemic effect through various targets.[11] QZLX mixture was developed by the Professor Xia Han, a renowned practitioner of traditional Chinese medicine. This QZLX has been utilized clinically for over thirty years and has demonstrated remarkable efficacy in treating pruritic disorders.[12] Thereby, there is a great need to investigate the mechanisms behind it.

QZLX consists of ten medicinal herbs (Table 1),[13] and the quality control standards of it have been established.[14] Clinical studies have demonstrated that QZLX is effective in alleviating AD skin lesions,[15] however, the underlying mechanism remains unclear. As is well-known, AD-like skin lesions can exacerbate inflammatory responses, leading to increased symptoms such as redness, swelling, and itching. These manifestations of skin inflammation further contribute to the deterioration of the existing skin lesions.[16] Therefore, mitigating skin lesions in patients with AD is of paramount importance in the overall management of AD. The present study primarily employed a network pharmacology approach to predict the targets and pathways through which QZLX may contribute to the amelioration of AD-like skin lesions.

Although network pharmacology has been extensively applied in elucidating the mechanisms of TCM, the inherent complexity of network construction and analysis may introduce errors in interaction predictions, potentially compromising the accuracy of the results.[17] To address this limitation, the present study incorporated both negative control experiments (Supplementary Materials; Figures S1–S3) and animal experiments to validate the findings derived from network pharmacology. This research aims to provide a more comprehensive understanding of QZLX's pharmacological mechanisms in the treatment of AD.

Materials and Methods

QZLX and Drugs

QZLX is a hospital-prepared formulation developed by Shanghai Yueyang Hospital of Integrative Medicine affiliated to Shanghai University of Traditional Chinese Medicine. This preparation (Batch No.: 2309001) has been commissioned for manufacturing by the Pharmacy Department of the hospital and produced by Shanghai Baolong Pharmaceuticals Co.,

Table 1 The Compositions of the Qinzhuliangxue Mixture (QZLX)

Chinese Name	Latin Name	English Name	Number of Compounds
Zhen Zhumu	Margaritifera Concha	Nacre	0
Zi Cao	Arnebiae Radix	Radix Arnebiae	113
Xu Changqing	Cynanchi Paniculati Radix et Rhizoma	Radix Cynanchi Paniculati	86
Mu Li	Ostreae Concha	Oyster Shell	0
Fo Er Cao	Herba Gnaphalii Affinis	Cudweed	117
Gan Cao	Glycyrrhizae Radix et Rhizoma	Radix Glycyrrhizae	540
Ci Shi	Magnetitum	Magnetite	0
Fang Feng	Saposhnikoviae Radix	Radix Sapoahnikoviae	261
Yi Yiren	Coicis Semen	Jobstears Seed	133
Huang Qin	Scutellariae Radix	Radix Scutellariae	402
	Total		1652

Ltd. (Shanghai, China). The quality standards for this product are strictly controlled and regulated by the Shanghai Municipal Drug Administration.

2,4-Ditrofluorobenzene (DNFB, Lot No. 20230501107), Olive oil (Batch No.: 20221209), and acetone (Batch No.: 20220308) were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China;). Dexamethasone was obtained from Shanghai Pharmaceutical Xinyi Pharmaceutical Co., Ltd. (Shanghai, China; State Drug License No. H31020793), while the physiological saline solution was purchased from Sichuan Kelun Pharmaceutical Liquid Co., Ltd. (Batch No: L223022209).

Animals

Specific pathogen-free female BALB/c mice (6–8 weeks, 16–20 g) provided by Shanghai Bikai Keji Biotechnology Co., Ltd. (Shanghai, China) were used in this study. The mice were given standard food and water in an air-conditioned environment with a room temperature (20 −24 °C), relative humidity (40–60%), and a standard 12h/12h light/dark cycle. The experimental scheme was approved by the Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine and animal experiment under approval number PZSHUTCM2310260002. The UK Animals (Scientific Procedures) Act, 1986, and related regulations, EU Directive 2010/63/EU for animal studies, and the National Research Council's Guide for the Care and Use of Laboratory Animals were all followed in the conduct of this study, which conforms with the ARRIVE criteria.

Analysis of Network Pharmacology

Construction of Compounds Contained in QZLX and Their Targets

Utilizing the Python 3.8.1 program, search for the chemical constituents and target molecules associated with ten herbs, namely Zhen Zhumu, Xinjiang Zicao, Xu Changqing, Mu li, Fo erCao, Gan Cao, Ci Shi, Fang Feng, Yi Yiren, and Huang Qin. This investigation was carried out by the SymMap database (http://www.symmap.org/), ETCM database (http://www.tcmip.cn/ETCM/index.php/), TCMSP database (https://old.tcmsp-e.com/tcmsp.php), along with other relevant databases. Additionally, perform literature searches concerning these herbs through platforms such as CNKI (https://www.cnki.net/), PubMed (https://pubmed.ncbi.nlm.nih.gov/), and Web of Science in order to supplement any missing information regarding their chemical components and target molecules from the aforementioned databases. Using the pandas software package and the Spider module in Python 3.8.1, we can identify potential action targets for the compounds listed in SwissTargetPrediction (http://www.Swisstarget-prediction.ch/), TargetNet (http://targetnet.scbdd.com/), and the SEA platform (https://sea.bkslab.org/). After sorting and removing duplicate targets, we can obtain standardized gene names through the Batch Entrez platform. (https://www.ncbi.nlm.nih.gov/sites/batchentrez).

Collection of AD-Like Skin Lesions Related Targets

Using "Atopic Dermatitis" and "Skin Lesions" as keywords, search for related protein targets in the DisGeNet database (https://www.disgenet.org/) and the GeneCards database (https://www.genecards.org/), respectively. Subsequently, consolidate and eliminate duplicate targets collected from both sources. Utilizing the Batch Entrez platform to obtain standardized gene names, which can be proposed as potential therapeutic targets.

Acquisition of Key Targets for QZLX in the Treatment of AD-Like Skin Lesions

Using the Venn Diagram package in R language,[18] we identified and visualized the common targets by intersecting the potential targets of active compounds for the treatment of AD skin lesions with those specifically associated with this condition. A Venn diagram was employed to illustrate these shared targets effectively.

Establishment of a Protein-Protein Interaction (PPI) Network and Identification of Key Targets

We entered the key target genes related to QZLX for treating AD skin lesions into the Multiple Proteins module of the STRING 11.5 platform (https://version-11-5.string-db.org/). We selected "Homo sapiens" as the protein species, configured the network type as the full STRING network, set the required score to high confidence (0.700), and adjusted the FDR level to medium (5%). Following a comprehensive comparison of all genes, we constructed a PPI network. After

downloading the protein interaction network file in TSV format, we imported it into Cytoscape version 3.10.0. Subsequently, selecting the core targets based on their degree (Degree) values and visualizing them effectively.

GO Functional Annotation and KEGG Pathway Enrichment Analyses

Utilizing the "GO and Pathway Online Analysis Module" on the MicrobiomeX platform (https://www.bioinformatics.com.cn/basic_local_go_pathway_enrichment_analysis_122) to perform GO analysis and KEGG signaling pathway enrichment analysis on the key target genes associated with QZLX's treatment for skin lesions related to AD. Additionally, the ggplot2 software package in R was employed to create a bubble chart illustrating the enrichment results.

Molecular Docking Validation

Based on the compound-target database established in this study, we selected the top ten compounds that exhibited the highest number of targets. The SDF format files for these compounds were obtained from the PubChem database (https://www.ncbi.nlm.nih.gov/pccompound). Based on the findings from PPI network analysis and KEGG enrichment analysis, along with a review of existing literature, the core targets JAK2 and STAT3 of QZLX were identified. Download high-resolution PDB format files of core target proteins with superior accuracy from the PDB database (https://www.rcsb.org/) and the UniProt database (http://www.uni-prot.org/). The aforementioned SDF format file and protein information were input into AutoDock Vina 2.0 (The Scripps Research Institute, Molecular Biology, CA, USA) software for molecular docking. The docking scores are presented as a heat map; a lower binding energy indicates enhanced stability between the receptor and ligand. A benchmark for assessing the binding efficiency of the receptor-ligand complex has been established at −6.0 kcal/mol.[19] The 3D and 2D representations of the docking results-the six with the strongest binding force were visualized utilizing Pymol 3.0 (https://pymol.org/) and Discovery Studio (https://www.3ds.com/products/biovia/discovery-studio) software.

Experimental Verification

DNFB-Induced Atopic Dermatitis-Like Skin Lesions in BALB/c Mice and Grouping

The mice were divided into four groups (n=5): control group (Con, without gavage), model group (Model, DNFB only), dexamethasone-treated (DSMS, DNFB+10 mg/kg DSMS) group,[20] and the QZLX-treated group (QZLX, DNFB+18.27 g/kg QZLX).

The abdominal hair of the mice was removed two days before the experiment. Subsequently, 50 μL of 0.5% DNFB (dissolved in olive oil/acetone at a ratio of 1:4) was injected intradermally into the abdominal skin, and then an additional 100 μL of 0.5% DNFB was applied around the injection site to sensitize the immune system. After five days, depilatory cream was used on the back of each mouse's neck to remove hair from an area of approximately 1×1 cm^2. This depilated area underwent stimulation with 50 μL of 0.2% DNFB (olive oil/acetone at a ratio of 1:4), with stimulation occurring every other day for five applications. Following the initial stimulation of the neck and back, skin conditions were documented using a camera. This process continued until modeling completion, after which samples were collected.[21,22]

Skin Lesion Scoring

Videos and photos were taken on the back of mice on the second day after 0.2% DNFB stimulation, and then the pictures and videos were sent to 3 independent individuals for scoring according to the below scoring criteria.

According to the SCORAD, EASI, and IGA scoring systems, we classified the six primary symptoms observed on the mouse back—edema, erythema, exudation, dryness, crusting, and lichenification—into four indicators for evaluating the severity of skin lesions.[23] Each indicator was assigned a score ranging from 0 to 3: 0 indicated no symptoms; 1 represented mild symptoms; 2 denoted moderate symptoms; and 3 signified severe symptoms. The total score for each sample was calculated by summing the scores of the four indicators, resulting in a possible range from 0 to 12. Data were manually recorded by three individuals and subsequently averaged after organization. Finally, statistical analysis was performed on the collected data.

HE Staining

Mouse skin samples were fixed in 4% paraformaldehyde for 24 hours to facilitate histological examination. Subsequently, the samples were embedded in paraffin wax and sectioned. The resulting sections were then stained with HE (Wuhan service Biotechnology Co., Ltd.) following standard protocols. Finally, the stained sections were examined using a pathological slide scanner (Shanghai Leica Instruments Co., Ltd. Shanghai, China).

Determination of Inflammatory Factors in Mice Serum Using Elisa Assay

After anesthetizing the mice, blood was collected from their eyeballs and allowed to coagulate at 37°C for 2 hours. Subsequently, the supernatant was centrifuged at 4000 revolutions per minute (rpm) for 15 minutes. This process was followed by a second centrifugation of the supernatant at the same speed (4000 rpm) for an additional 15 minutes. The levels of IgE, TSLP, and IL-4 in mouse serum were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Jiangsu Enzyme Immunoassay Co., Ltd. Yancheng, Jiangsu Province), following the manufacturer's instructions meticulously.

Detection of JAK2/STAT3 Expression in Skin Tissues Using Western Blot

The skin tissue from each group of experimental animals was added to 10 mL of RIPA tissue lysis buffer. To the lysate, 200 μL of protease inhibitor, 200 μL of phosphatase inhibitors, and 100 μL of PMSF were incorporated. Additionally, for every 20 mg of skin tissue, 300 μL of the tissue lysate was added. Each test tube received two magnetic beads. The samples were then placed in a tissue grinder operating at 60 hz and ground five times. The mixture was left on ice for half an hour before being centrifuged at 12,000 g for ten minutes; the supernatant was retained.

The total protein content was measured using a BCA protein quantification kit (Beyotime Biotechnology Co., Ltd. Shanghai, China). A 10% separating gel and a 5% stacking gel were prepared for electrophoresis. After loading the samples into the gels, electrophoresis was conducted at a constant voltage of 80 V for thirty minutes and then increased to a constant voltage of 120 V until bromophenol blue migrated below the platinum wire in the electrophoresis tank.

After transferring the samples to a PVDF membrane, it was blocked with 5% skim milk and incubated with anti-JAK2 [D2E12] (Cell Signaling Technology, 3230R, 1:1000), anti-Phospho-JAK2 (Cell Signaling Technology, 3776R, 1:1000), anti-STAT3 [SY24-08] (HUABIO, Ab 3069762, 1:1000), anti-Phospho-STAT3 [SZ43-01] (HUABIO, Ab 3069682, 1:1000), and β-actin (Proteintech, Ab 2687938, 1:20000) for detection.

After washing the membrane, the enhanced chemiluminescence (ECL) method (New Cell & Molecular Biotechnology Co., Ltd. Suzhou, Jiangsu Province) was employed, and the relative gray values of the proteins were quantified using Image J software. The experiment was conducted in triplicate, and the results were averaged for analysis.

Statistical Analysis

Data were analyzed utilizing GraphPad Prism 9 software and SPSS 26.0 software, and results are presented as mean ± SD. One-way ANOVA was employed to compare data among the experimental groups. The *t*-test was applied for comparisons between two groups. Two-way Repeated Measures ANOVA was used to analyze the subgroup, time, and subgroup by time interaction effects of Skin Lesions Scores at each time point across intervention groups. One-way ANOVA was used to compare skin lesions scores between intervention groups at the same time points, and One-Way Repeated Measures ANOVA was used to compare skin lesions scores at different time points within the groups, and two-by-two comparisons were made using the Bonferroni method. A difference was deemed significant when $p < 0.05$.

Results

Natural Chemical Compounds of QZLX Mixture

A total of 1652 natural chemical compounds in QZLX mixture were acquired from the database (TCMSP, ETCM, SymMap) and literature review, including 113 for Radix Arnebiae, 86 for Radix Cynanchi Paniculati, 117 for Cudweed, 540 for Radix Glycyrrhizae, 261 for Radix Sapoahnikoviae, 133 for Jobstears Seed, and 402 for Radix Scutellariae. Among them, Nacre, Oyster Shell and Magnetite are mineral medicines and do not contain natural compounds (Table 1).

Analysis of Drug Targets and Disease Targets

A total of 4780 QZLX-related targets were identified,1614 AD-related potential targets were identified, and 565 skin lesion-related potential targets were identified. The Venn diagram shows that a total of 188 drug-disease intersection targets were identified. A compound-core target network for the 188 targets and related compounds associated with topical treatment of AD-like skin lesions was constructed (Figure 1).

PPI Network Analysis

A total of 188 targets were screened, and the top 30 targets were visualized based on their Degree values. The top 10 targets were selected as key targets, they were TNF, IL6, IL1B, AKT1, STAT3, IL10, VEGFA, TP53, IL4, and EGFR (Figure 2).

GO and KEGG Analysis

GO functional analysis yielded a total of 4913 entries ($P < 0.05$), comprising 4175 entries related to biological processes (BP), 285 entries pertaining to cellular components (CC), and 453 entries associated with molecular functions (MF). The top ten enriched entries were selected based on their significance levels as indicated by the P values, and a bar chart was created to illustrate the results of the GO functional analysis (Figure 3).

Furthermore, KEGG pathway enrichment analysis identified 30 pathways ($P < 0.05$), from which the top 10 signaling pathways were chosen based on both P value outcomes and the number of enriched genes (Figure 4). Conspicuously, the results indicated that the JAK-STAT pathway ranked first among these pathways.

JAK2 and STAT3, Exhibit Strong Binding Affinity with the Ten Core Components of QZLX

Based on the compound-target database established in this study, the ten herbal active ingredients identified with the highest number of targets are as follows: Quercetin, Curcumin, Baicalein, Naringin, Ginsenoside, Baicalin, Berberine, Emodin, Naringenin, and Genistein. The core targets of the QZLX that are set for validation include JAK2 and STAT3. The molecular docking results indicate that the binding energies of JAK2 and STAT3 with the ten core components are all below −6.0 kcal/mol, suggesting effective binding interactions (Figure 5).

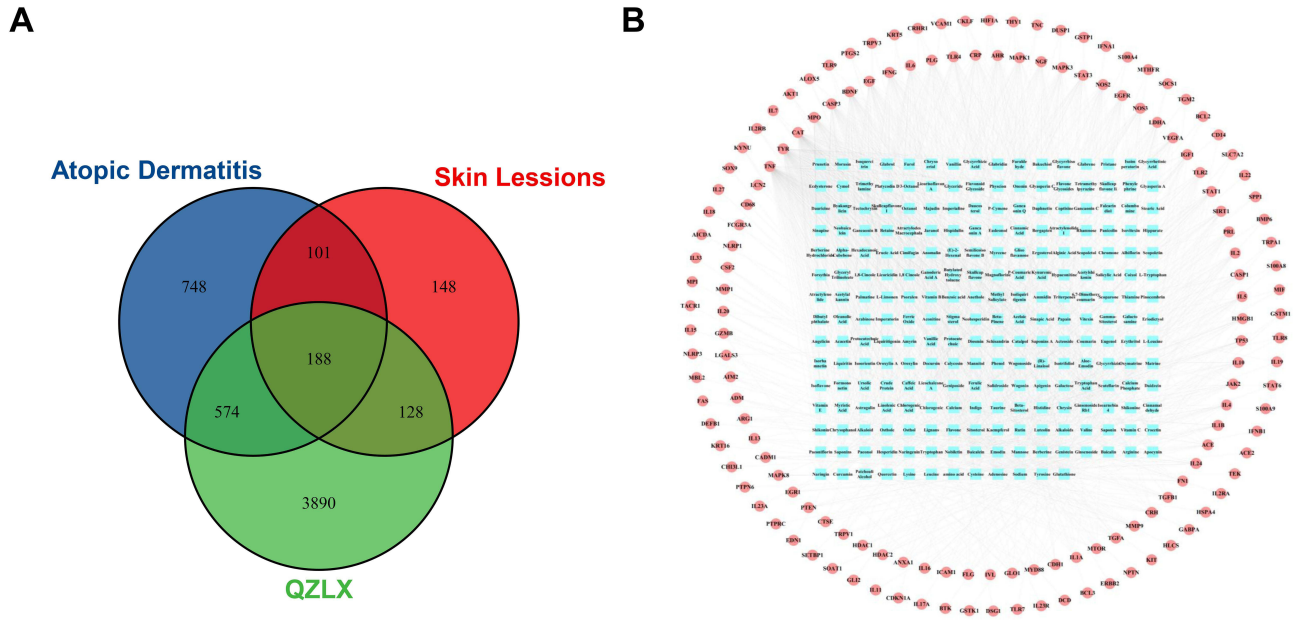

Figure 1 Common targets and common targets-active ingredients network. (**A**) 188 Common Targets of QZLX and AD Skin Lesions (Veen Diagram). (**B**) Common targets–active ingredients network.

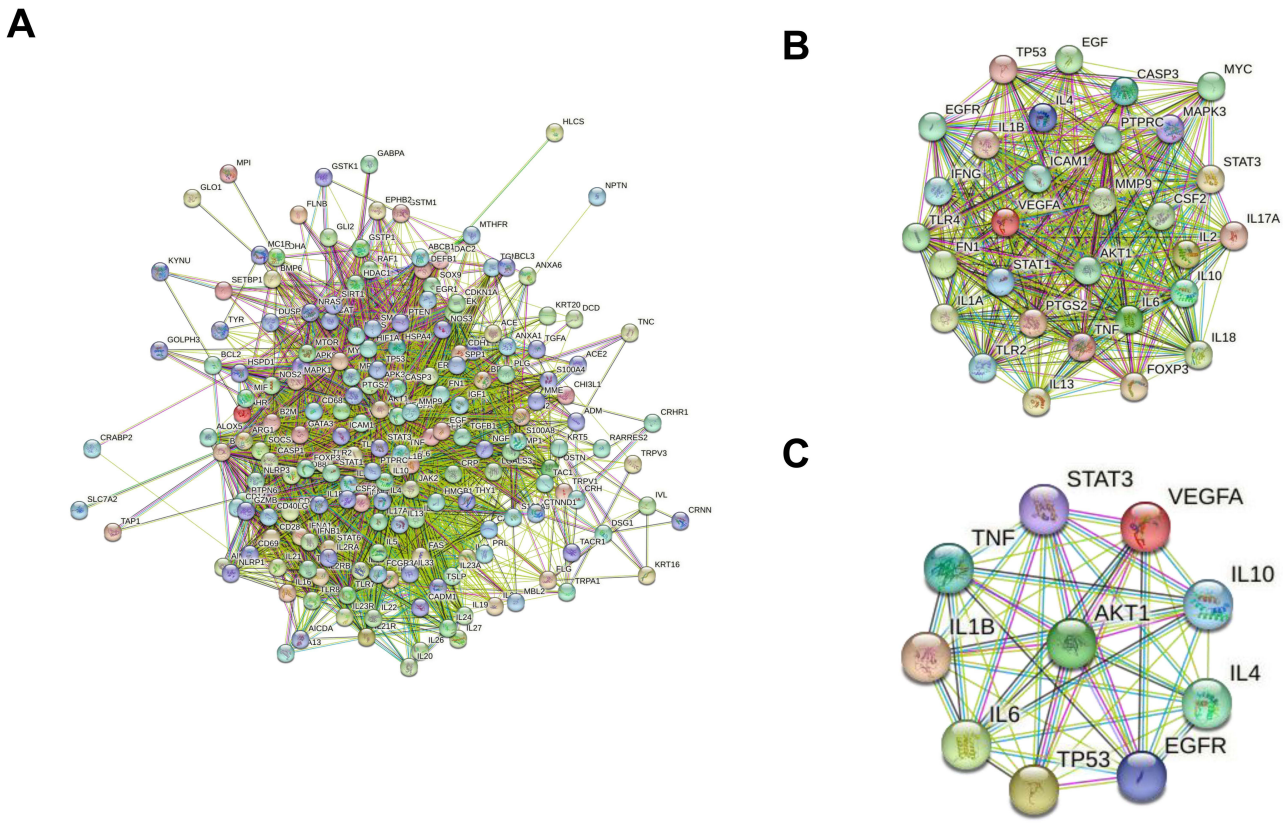

Figure 2 The protein-protein interaction (PPI) network of the QZLX targets treating AD skin lesions. (**A**) All Targets. (**B**) Top 30 Targets. (**C**) Top 10 Targets.

QZLX Markedly Improves Skin Lesions in DNFB-Induced Mice

By systematically documenting and analyzing the skin conditions observed on the dorsal surfaces of mice, along with conducting a comprehensive skin score assessment, QZLX demonstrates a significant enhancement in the dermatological condition of DNFB-induced mice. As described in Figure 6, the skin lesions score of the model group was significantly higher than that of the normal group (P<0.05), and it was most serious on day 8. And the QZLX could significantly reduce the skin lesions of the AD mice. Statistical analysis of skin lesion scores among different groups at each time point is shown in Table 2. It's obvious that there was a statistically significant difference in skin lesion scores among different intervention groups at different time points, subgroups, and in the $F_{\text{time*group}}$ (P<0.05). What's more, It's illustrated that the skin lesion scores at all time points were significantly higher in the Model, DSMS, and QZLX groups than that in the Control group (P<0.05). Last but not least, It's interestingly that he skin lesion scores at 6 and 10 days were significantly higher than those at 4 days in the Model group (P<0.05), while that at 10 days were significantly higher than those at 2, 4, 14 days in the QZLX group (P<0.05).

QZLX Suppresses Epidermal Thickening in DNFB-Induced Mice

After the skin tissue sections embedded in paraffin were stained with HE, the alterations in the skin thickness of AD model mice following QZLX treatment were observed using a 10x magnifying glass. Statistical analysis was subsequently performed. The results indicated that QZLX significantly inhibited the increase in skin thickness among AD model mice ($p < 0.05$) (Figure 7).

QZLX Dramatically Diminished Serum Concentrations of IgE, TSLP, and IL-4 in AD Models

The blood was collected from the mouse eyeball after 14 days. Next, the blood was allowed to stand for 1h at 4°C, centrifuged at 3500 rpm for 10 min. Finally, the supernatant was used to evaluate the content of IgE, TSLP, IL-4 and IL-

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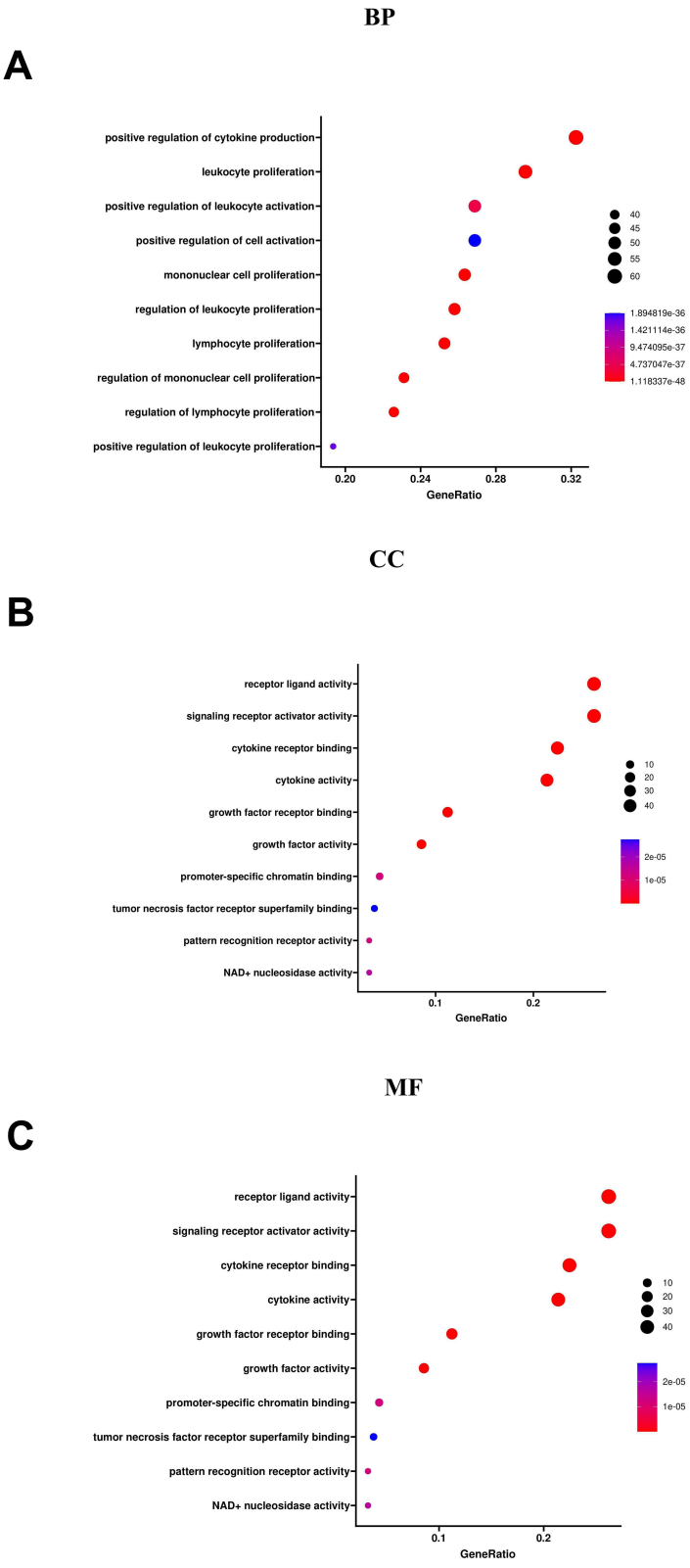

Figure 3 GO functional enrichment analysis results of QZLX in treating AD. (**A**) BP, biological process. (**B**) CC, cellular components. (**C**) MF, molecular function.

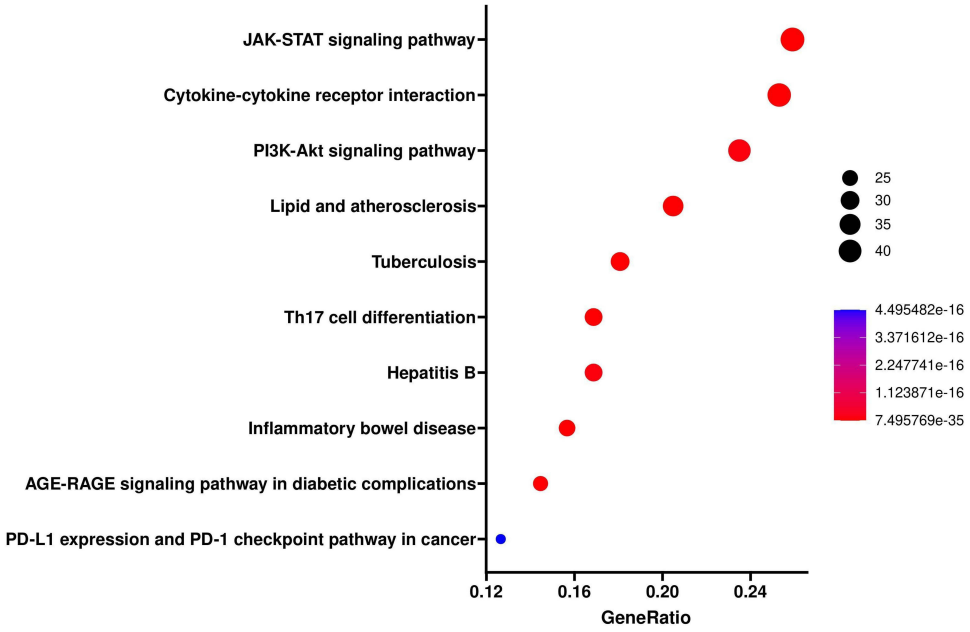

Figure 4 KEGG signaling pathway analysis results of QZLX in the treatment of AD.

31 according to the ELISA kit instructions. The results indicate that QZLX can markedly diminish the levels of inflammation in the serum of AD models, as shown in Figure 8, QZLX could extraordinarily downregulate the concentrations of IgE, TSLP, and IL-4.

Effect of QZLX on JAK2/STAT3 Signaling Pathways

The results from the WB analysis indicate a strong association between the JAK2/STAT3 signaling pathway and skin inflammation in AD mice. Notably, the expression levels of phosphorylated JAK2 (P-JAK2) and phosphorylated STAT3 (P-STAT3) in the skin of QZLX –treated group were significantly elevated (Figure 9). This upregulation of the signaling pathway plays a crucial role in mitigating skin inflammation observed in AD mice.[24]

Discussion

AD is characterized by the impairment of skin barrier function, which results in a diminished capacity of the skin to protect itself against external stimuli and allergens.[25] Consequently, the body experiences an allergic and inflammatory response, leading to a cyclical pattern characterized by "dryness, itching, and dryness"[26] In cases of AD, the integrity of the skin's barrier function is compromised or destroyed. The keratinocyte layer of the skin undergoes shedding while abnormalities arise in the interstitial lipids located between keratinocyte cells.[27] Additionally, there is a reduction in ceramide content, which ultimately contributes to increased skin dryness. This reduction heightens the susceptibility to microbial infections and skin inflammation, potentially leading to or exacerbating skin lesions and itching.[28] Therefore, the initial step in the management of AD is to ameliorate symptoms, restore skin barrier function, enhance skin hydration, mitigate skin inflammation, and interrupt the cycle of "dryness and itching".

QZLX was developed by the late Professor Xia Han, a renowned practitioner of traditional Chinese medicine in Shanghai and an heir to the Xia's Surgery tradition. This combination has been utilized clinically for over thirty years and has demonstrated remarkable efficacy in treating pruritic disorders. The majority of contemporary research regarding its mechanisms has concentrated on the therapeutic approaches for psoriasis.[29–32] There is a limited number of studies that have employed QZLX in the treatment of AD.[13,29,33] In order to further investigate the potential of the QZLX in the treatment of AD and to expand its applicability, we conducted a detailed exploration of the mechanisms underlying its effects on AD.

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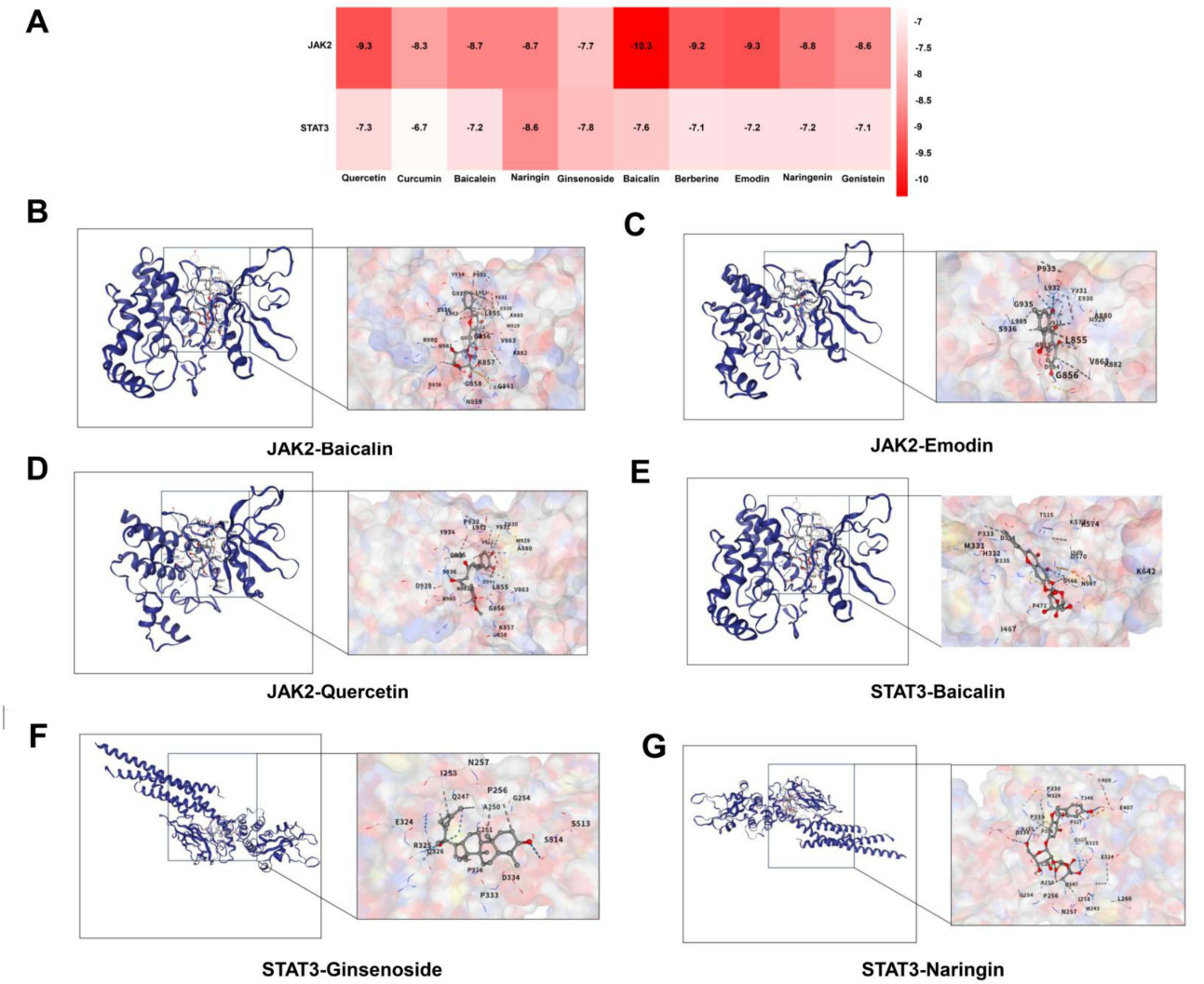

Figure 5 The molecular docking analysis of the ten core components of QZLX with JAK2 and STAT3. (**A**) Evaluation of the Docking and Binding Capacities of Ten Potential Active Compounds to Each Core Target Protein. (**B**) JAK2 and Baicalin. (**C**) JAK2 and Emodin. (**D**) JAK2 and Quercetin. (**E**) STAT3 and Baicalin. (**F**) STAT3 and Ginsenoside. (**G**) STAT3 and Naringin.

Based on the analysis of the GO and KEGG analysis, we identified that the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway may represent a crucial mechanism through which QZLX exerts its effects. The JAK/STAT pathway is a critical signaling mechanism implicated in immunosuppression and plays a significant role in the inflammatory response. Among the JAKs, JAK2 is paramount for the transduction of various cytokines. The interaction between JAK2 and STAT relies on activated STAT transcription factors, with the JAK2/STAT3 complex being particularly vital in this process.[34] According to the existing literature, the polarization of M2 macrophages can be enhanced and the release of inflammatory factors can be inhibited through the activation of the JAK2/STAT3 signaling pathway. This approach has been shown to significantly ameliorate skin lesions in mice with AD. The results of the PPI topology analysis indicated that STAT3 was ranked fifth among the 181 predicted targets. This ranking is one of the key reasons why this study has chosen to validate the JAK2/STAT3 signaling pathway.

AD is an immune-mediated disease characterized by IgE-mediated hypersensitivity reactions upon exposure to allergens. This condition arises from an imbalance of Th2 cell responses, resulting in damage to the skin lesions and excessive production of IgE. Clinically, serum IgE levels are commonly utilized as a key criterion for diagnosing AD and serve as one of the parameters for evaluating treatment outcomes and prognoses.[35,36] A study that statistically analyzed clinical data from 169 patients with AD revealed that higher baseline levels of IgE were associated with an increased

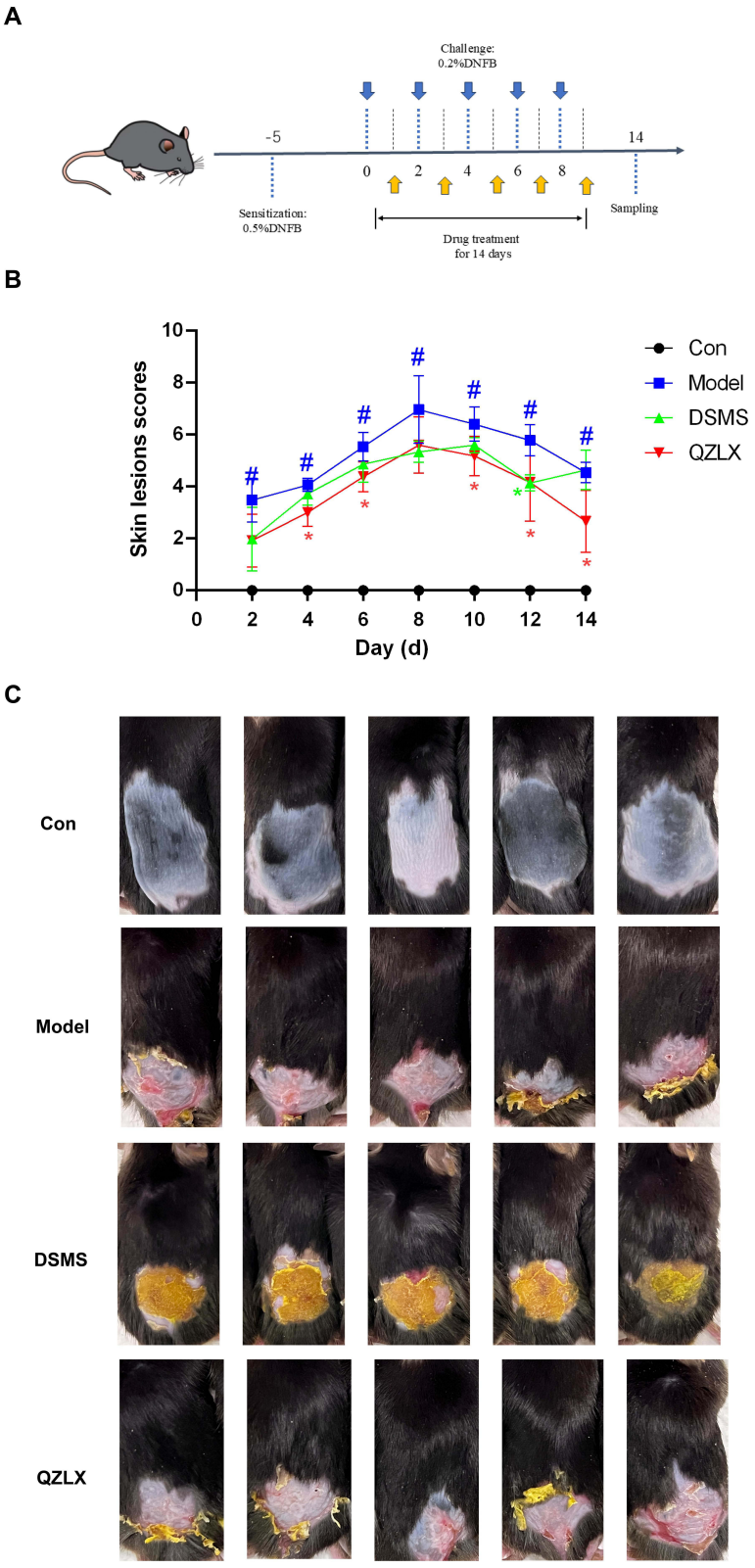

Figure 6 QZLX can alleviate skin lesions induced by DNFB in AD mice. (**A**) Methodology for the Creation of AD Model Mice. (**B**) Skin lesion scores at different times for mice in different groups. (**C**) The skin condition of mice in different groups on Day 14.

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Table 2 Statistical Analysis of Skin Lesions Scores Among Different Groups at Each Time Point

Time	Control (n=5)	Model (n=5)	DSMS (n=5)	QZLX (n=5)	*F*	*P*
Day2	0.00±0.00	3.47±0.84$^{\#}$	2.00±1.25$^{\#}$	1.93±1.01$^{\#}$	12.337	<0.001
Day4	0.00±0.00	4.07±0.28$^{\#}$	3.73±0.43$^{\#}$	3.00±0.53$^{\#*\&}$	126.313	<0.001
Day6	0.00±0.00	5.53±0.56$^{\#b}$	4.87±0.69$^{\#}$	4.40±0.60$^{\#*}$	110.110	<0.001
Day8	0.00±0.00	6.93±1.30$^{\#}$	5.33±0.41$^{\#a}$	5.60±1.12$^{\#}$	60.368	<0.001
Day10	0.00±0.00	6.40±0.64$^{\#b}$	5.60±0.28$^{\#ab}$	5.20±0.77$^{\#*ab}$	157.113	<0.001
Day12	0.00±0.00	5.80±0.61$^{\#}$	4.13±0.30$^{\#*}$	4.13±1.50$^{\#*}$	45.101	<0.001
Day14	0.00±0.00	4.53±0.38$^{\#}$	4.67±0.75$^{\#}$	2.67±1.20$^{\#*\&e}$	44.256	<0.001
F	–	15.529	18.209	15.009	–	–
P	–	0.001	0.001	0.001	–	–

Notes: F_{time}=44.435, P<0.001; F_{group}=156.441, *P*<0.001; $F_{time*group}$=6.559, *P*<0.001. ^{a}*P*<0.05 versus Day2, ^{b}*P*<0.05 versus Day4, ^{e}*P*<0.05 versus Day10. $^{\#}$*P*<0.05 versus control, **P*<0.05 versus model, $^{\&}$*P*<0.05 versus DSMS.

likelihood of worse long-term prognosis in AD patients. Additionally, serum total IgE has the potential to serve as a simple and effective method for predicting the prognosis of AD.[37] Therefore, in this study, the serum concentration of IgE in mice was assessed. It was found that the IgE levels in the serum of mice administered with QZLX were significantly lower compared to those in the model group. This finding indicates that treatment with QZLX may considerably enhance the prognosis of AD.

Thymic stromal lymphopoietin (TSLP) is a cytokine similar to interleukin 7, primarily produced by leukocytes, it is known to trigger the Th2 inflammatory response and plays a critical role in the pathogenesis of AD.[38] It is produced by keratinocytes; when AD occurs, TSLP is activated. Following skin lesions, TSLP is released into the systemic circulation, resulting in elevated serum levels of TSLP.[39] TSLP has the capability to produce factors such as IL-4 through the mediation of cell polarization,[40] it also increases the sensitivity of cells to IL-4.[41] IL-4 plays a critical role in the pathogenesis of AD and serves as an essential link in stimulating the Th2 immune response, it also regulates skin inflammation associated with AD.[42,43] Combined with the top 10 targets identified through Network Pharmacology PPI analysis, this study evaluated the serum levels of TSLP and IL-4 in mice treated with QZLX. The results indicated a significant reduction in the serum levels of both TSLP and IL-4 in mice receiving QZLX treatment.

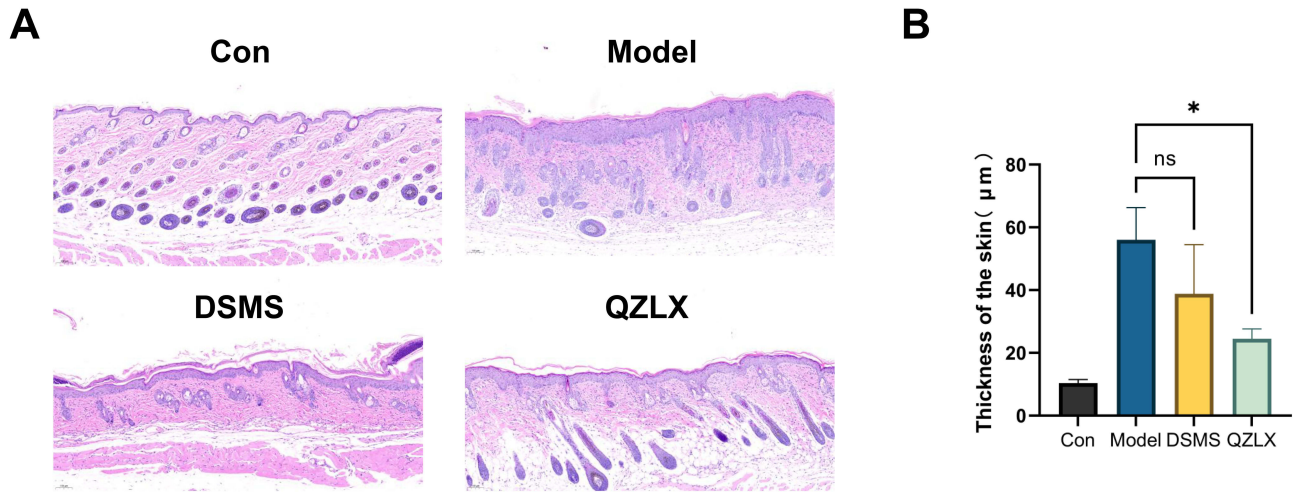

Figure 7 QZLX examined the histological changes in the skin tissue of AD mice induced by DNFB. Skin tissue sections were stained with HE (**A**), and epidermal thickness was analyzed (**B**). *p < 0.05 compared with the M group.
Abbreviation: N.S., no significant difference.

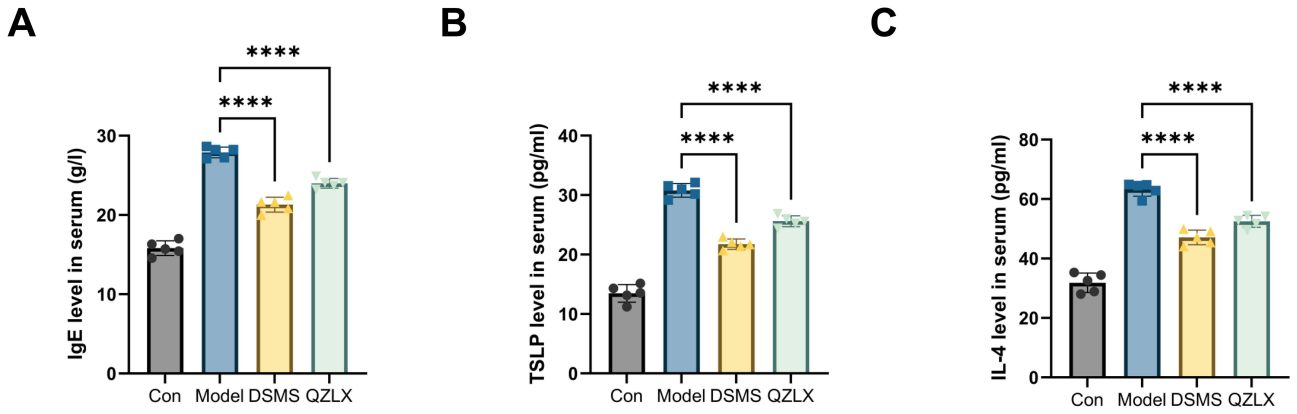

Figure 8 The effect of QZLX on the levels of inflammatory cytokines in the serum of AD mice induced by DNFB was investigated. The levels of IgE (**A**), TSLP (**B**), and IL-4 (**C**) in the serum of mice were measured by ELISA kit. Compared with the M group, ****p < 0.0001.

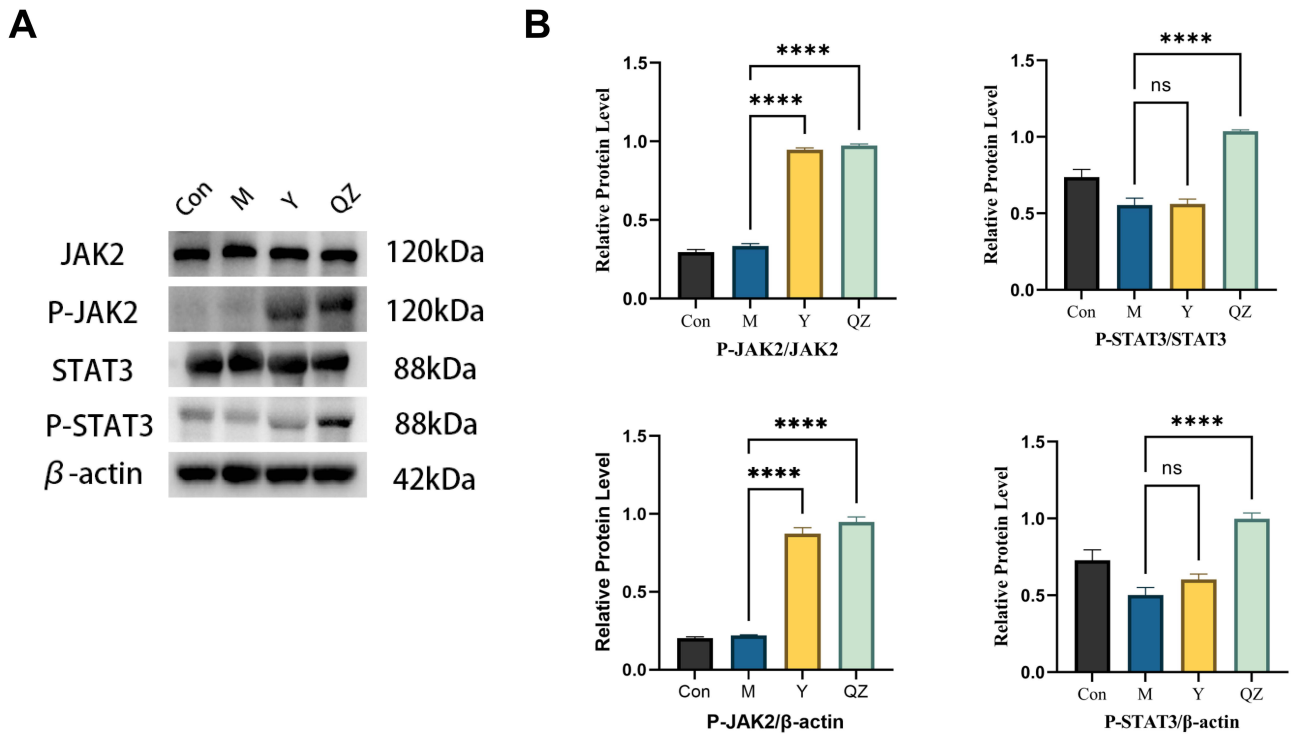

Figure 9 QZLX affects the JAK2/STAT3 signaling pathway in the skin tissue of AD-like mice induced by DNFB (**A**). The protein expression levels of JAK2, P-JAK2, STAT 3, P-STAT 3 (**B**), in the ear tissue of each group, were quantitatively analyzed by ImageJ software. Compared with the M group, ****p < 0.0001.
Abbreviation: N.S., no significant difference.

Conclusion

Network pharmacology elucidates the mechanism underlying the therapeutic efficacy of QZLX in treating dermatitis associated with AD. Topological characterization, coupled with enrichment analysis, suggests that the mode of action for QZLX in addressing AD skin lesions may involve down-regulation of inflammatory mediators such as IgE, TSLP, and IL-4, alongside modulation of the JAK2/STAT3 signaling pathway. Furthermore, both in vitro and in vivo experimental validation has provided robust evidence demonstrating that QZLX exerts its effects on AD by targeting the JAK2/STAT3 signaling pathway and ameliorating skin conditions induced by AD.

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Abbreviations

AD, Atopic dermatitis; QZLX, Qinzhuliangxue mixture; DNFB, 2, 4-dinitrofluorobenzene; Con, Control; DSMS, Dexamethasone; HE, Hematoxylin-Eosin; WB, Western blotting; JAK, Janus kinase; IL-4, interleukin-4; IgE, Immunoglobulin E; TSLP, Thymic stromal lymphopoietin; PPI, Protein-Protein Interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; Elisa, Enzyme-linked immunosorbent assay; rpm, revolutions per minute; RIPA, Radio-Immunoprecipitation Assay Lysis Buffer; PMSF, Phenylmethanesulfonyl fluoride; ECL, enhanced chemiluminescence; SD, standard deviation; ANOVA, One-way analysis of variance.

Ethics Approval

There was no need for extra ethical clearance for this study because the data used from this research had been approved by the relevant ethics committees. The Animal Ethics Committee at Shanghai University of Traditional Chinese Medicine thoroughly examined and authorized the animal research.

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Disclosure

The authors declare no conflicts of interest in this work.

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