Quantitative and bioinformatics integrated strategy to investigate the pharmacological mechanism of Ermiao Wan against eczema

Ting Xia

Southern Medical University

Chang-Shun Liu

Southern Medical University

Xiao Liang

Southern Medical University

Yan-Nan Hu

Southern Medical University

Zhen-Ye Luo

Southern Medical University

Fei-Long Chen

Southern Medical University

Xiaomei Tan (✉ tannxm_smu@163.com)

Southern Medical University <https://orcid.org/0000-0002-5797-6763>

Research

Keywords: Ermiao Wan, Eczema, Quantification, Network pharmacology, Molecular docking

Posted Date: April 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-365252/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background

Ermiao Wan (EMW) is used to treat eczema in China. However, its underlying pharmacological mechanisms against eczema remain unclear.

Methods

In this study, the components of EMW were quantitatively analyzed using HPLC. The role of the components, targets, and signaling pathways were predicted by network pharmacology. Moreover, molecular docking was used to verify the binding forces of the components with the target proteins. Results

The results showed that the established HPLC method is simple and reliable, and can be used for the simultaneous determination of seven components in EMW. Moreover, 57 primary causal targets of EMW against eczema were identified. Among them, 10 hub targets were identified, including *EGFR*, *AKT1*, *STAT3*, *MMP9*, *ICAM1*, *MAPK8*, *JUN*, *MAPK1*, and *VCAM1*. The potential signaling pathways involved in the effect of EMW against eczema were identified, including ErbB, estrogen, and Epstein-Barr virus infection. Furthermore, palmatine, chlorogenic acid, and jatrorrhizine from EMW were shown to bind to the identified targets. Accordingly, *EGFR*, *AKT1*, and *PTGS2* had good binding forces with EMW components.

Conclusion

Our study revealed a possible pharmacological mechanism of EMW in treating eczema. This simple and effective method can help increase our understanding of the mechanisms of Chinese herbal formulations and further promote their research and development.

Highlights

- High-content compounds in EMW were screened and used to investigate their pharmacological mechanism on eczema by network pharmacology.
- EMW treats eczema mainly by regulating the key pathways of anti-inflammatory system, including ErbB signaling pathway, estrogen signaling pathway and Epstein - barr virus infection.
- Validated our predictions of nine candidate targets by performing docking studies with components of EMW.
- This method can help increase our understanding of the mechanisms of Chinese herbal formulas and promote their further research and development.

1. Introduction

Eczema is a common chronic, inflammatory skin disorder characterized by severe itching, with a complex pathogenesis and recurrent attacks [1]. It seriously affects the quality of life and economic outcomes of affected individuals, especially in those with moderate to severe eczema. In particular, with the outbreak of COVID-19, overzealous hand hygiene has caused an increased incidence of hand eczema among the general population [2]. Approximately 200 million people worldwide are affected by eczema, and the incidence is increasing, which can lead to tremendous medical and financial pressure [3]. The relapsing and remitting nature of eczema can make it difficult to control flare-ups. At present, although drugs for the treatment of eczema are effective in the short term, this disorder can easily occur repeatedly, accompanied by adverse reactions such as skin atrophy, hormone-dependent dermatitis, dry mouth, and dizziness [4]. Thus, the research and development of drugs that are effective against eczema in the long term has gained interest.

Although various treatments have achieved sustained control of eczema, their relative benefit remains unclear owing to the limited number of trials directly comparing the treatments. Traditional Chinese medicine (TCM) has the advantages of high safety and less side effects. In China, the TCM Er-miao Wan (EMW) is commonly used for the clinical treatment of eczema and included in the 2020 edition of the Chinese Pharmacopeia. The clinical application of EMW for treating eczema first appeared in the book *Danxi Xinfu*, an ancient clinical TCM book from the Ming Dynasty of China. Owing to its efficacy in eliminating heat and dampness, EMW has been used to treat red and swollen skin, fever, and itching, which are the symptoms of eczema [5].

EMW is composed of equal amounts of *Rhizoma Atractylodis* (RA) and *Cortex Phellodendri* (CP). The alkaloids in EMW have been reported to have good biological activity [6, 7], and the content of compounds in EMW is also an important factor affecting its efficacy. We believe that the main components of TCM should have high content and biological activity at the same time for its effective application. Although some components of EMW have been separated and shown to have pharmacological effects, the combined pharmacological action of the main compounds of EMW may be the main way to exert its efficacy [8]. However, the main components and pharmacological mechanisms of EMW in the treatment of eczema remain unclear. Moreover, owing to the multi-component and multi-target characteristics of TCM, it is difficult to clarify the action mechanism of EMW using conventional methods.

Network pharmacology is a novel discipline that integrates biology, pharmacology, and informatics [9]. According to this concept, multiple node targets of drugs in interrelated systems, rather than individual nodes, have been analyzed from a holistic point of view. The idea of network pharmacology is consistent with the action mechanism of TCM, which emphasizes synergistic effects and interconnection [10]. TCM formulations are composed of several herbs and compounds that exert synergistic effects by affecting multiple genes, proteins, and pathways [11]. Network pharmacology can elucidate the interaction between multiple compounds and disease targets, allowing the exploration of the actions of TCM. At present, network pharmacology has been widely used in the prediction of potential active components, targets, and action mechanism of TCM [12, 13].

In this study, we aimed to elucidate the mechanism of action of EMW in the treatment of eczema using a combined method of quantification of components and network pharmacology. The method established in this study can help increase our understanding of the mechanisms of Chinese herbal formulations and promote their further research and development. Figure 1 shows the workflow of the study.

2. Materials And Methods

2.1. Materials and reagents

EMW was purchased from Guangdong Traditional Chinese Medicine Co., Ltd. (Guangdong, China). Reference standards, including berberine, palmatine, jateorhizine, phellodendrine, magnoflorine, atractylodin, and chlorogenic acid, were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile was of high-performance liquid chromatography (HPLC) grade and purchased from Merck (Darmstadt, Germany). The water used was distilled water, and other reagents were of analytical grade.

2.2. Quantification of EMW components

EMW was ground to powder and weighed (1.0 g). Next, 50 mL of ethanol-water (1:1, v/v) was added to the powder and stirred. The samples were then placed in an ultrasound machine for 30 min for extraction, followed by centrifugation (2500r/min, 15min.) to obtain the supernatants. Subsequently, 5 μ L of the supernatant was injected into an HPLC system to determine the components of EMW.

The HPLC system composed of an Agilent 1260 liquid chromatograph and a DAD ultraviolet detector (Agilent Technologies Inc., CA, USA). Samples were separated on an Agilent ZORBAX SB-C18 (4.6 mm× 250 mm, 5 µm) column, with a mobile phase consisting of acetonitrile (A) and 0.1% phosphoric acid (B). The gradient elution was as follows: 0-30 min, 7-18% A; 30-45 min, 18-22.5% A; 45-50 min, 22.5-24% A; 50-55 min, 24-48% A; 55-65 min, 48-51% A; 65-85 min, 51-70% A; 85-90 min, 70-7% A. The wavelengths were as follows: at 0-65 min, 280 nm; at 65-90 min, 330 nm. The flow rate was 1.0 mL/min, and the column temperature was 25 °C.

The method was validated as follows. (1) The reference substances of chlorogenic acid, phellodendrine, magnoflorine, jatrorrhizine, palmatine, berberine, and atracylodine were precisely weighed, and 50% ethanol was added to prepare a series of concentrations of the reference solution. A standard curve was drawn according to the chromatographic conditions with the peak area (Y) and reference substance concentration (X), and a linear regression equation was obtained. (2) The same sample solution was injected continuously six times according to the chromatographic conditions, the chromatographic peak area was recorded, and the precision of the instrument was verified. (3) Six samples of test solution were prepared and used for the chromatographic separation, the content of the components was calculated, and repeatable experimental results were obtained. (4) The content of the components in the same solution was determined within 24 h to obtain experimental stability data. (5) Recovery was tested by comparing the peak responses of spiked analytes at pre-extraction with those at post-extraction.

2.3. Network pharmacology studies

All protein targets of the seven components of EMW were obtained from the TCMSP database (<http://tcmbspw.com/tcmbsp.php>). Eczema-related targets were obtained by using 'eczema' as the keyword in the DisGeNET (<https://www.disgenet.org/>) database. A Venn diagram of EMW active ingredient targets and eczema targets were drawn using the Venny 2.1 database (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>). The targets at the intersection were considered as the key targets of EMW in the treatment of eczema.

2.4. Protein-protein interaction network

The key targets were introduced into the STRING database (<https://string-db.org/>) to construct a protein-protein interaction (PPI) network model, with the protein species set as "*Homo sapiens*". The results were then imported into the Cytoscape 3.7.2 software to obtain the protein-protein interaction network.

2.5. Compound-target network

The active components and key targets were imported into the Cytoscape3.7.2 software to build a compound-target network. Simultaneously, visualization and network topology analyses were carried out to determine the critical degree of EMW targets.

2.6. Gene ontology (GO) and pathway enrichment analysis

To further elucidate the function of the screened target protein genes and their role in signaling pathways, the top 10 targets were introduced into the DAVID 6.8 database (<https://david.ncifcrf.gov/>). A list of target gene names was entered into the database, with the species set to "*Homo sapiens*". The names of all target genes were corrected to their official names (official gene symbol). Targets were searched and transformed in the above database, and the threshold was set to less than 0.5. Next, GO biological process enrichment analysis and KEGG signaling pathway enrichment analysis were carried out, and the top 10 items were determined. The R-language packages 'ClusterProfiler', 'ReactomePA', 'org.Hs.eg.Db', and 'GOplot' were used for analysis and visualization of the top 10 hub targets and top 10 terms. GO data were analyzed using 'org.Hs.eg.Db', with cutoffs for enrichment being $P < 0.05$ and $q < 0.05$. The outputs were bubble charts and Circos-circle charts. The 'pathview' package was used to create pathway diagrams for correlative targets of the enriched KEGG pathways; this step allowed for further analysis of the results [14, 15].

2.7. Molecular docking analysis

Molecular docking analysis was conducted to examine the binding of the seven components of EMW to the top 10 targets. Unfortunately, as there are no ligands in the protein structure of the 10th target, only nine targets were docked. The molecular structure of the seven components was drawn using ChemDraw and imported into Sybyl X2.1 for transformation of the three-dimensional (3D) structure, optimization of minimizing energy. The 3D structures of *EGFR* (ID:5GTY), *AKT1* (ID:6HHH), *PTGS2* (ID:5F1A), *STAT3* (ID:6SMB), *MMP9* (ID:2OW0), *ICAM1* (ID:3E2M), *MAPK8* (ID:3PZE), *JUN* (ID:2NO3), and *MAPK1* (ID:5NHF) were downloaded from the PDB database (<https://www.rcsb.org/>). After removing the water molecule residues, hydrogenation, and side chains, the Surflex-Dock program was used to dock and score the core compounds using the Sybyl X2.1 docking software. The higher the score, the more stable the binding between the ligand and the receptor.

3. Results

3.1. Fingerprint and quantification of major components in EMW

HPLC fingerprinting was performed to identify the main chemical compounds in EMW. Seven chemical constituents of EMW were identified according to the spectrograms and retention times of the standard substances (Fig.2A). Fingerprints of EMW were identified from six batches, with satisfactory similarity (Fig.2B), which suggested product stability. The seven components of EMW were measured quantitatively using HPLC, and their chemical structures are shown in Fig.2C.

The concentrations of major EMW components were as follows: chlorogenic acid, 1.52 ± 0.48 mg/mL; phellodendrine, 7.62 ± 0.81 mg/mL; magnoflorine, 0.62 ± 0.34 mg/mL; jateorrhizine, 0.42 ± 0.26 mg/mL; palmatine, 0.02 ± 0.004 mg/mL; berberine, 49.68 ± 1.38 mg/mL; and atractydin, 38.43 ± 0.92 mg/mL. The compounds present at high concentrations in EMW originated mainly from CP, including chlorogenic acid, phellodendrine, magnoflorine, jateorrhizine, palmatine, and berberine, whereas atractydin originated from RA. Alkaloids constituted the highest composition, followed by organic acids. The seven compounds represented the major bioactive components of EMW, and were therefore used for subsequent network pharmacology research in this study.

3.2. Method validation

(1) The linear regression analysis results, correlation coefficient ($R^2 > 0.991$), and linear range indicated that the established calibration curves could be quantified (Table.1). (2) After six consecutive injections, the relative standard deviation (RSD) % of chlorogenic acid, phellodendrine, magnolamine, jatrorrhizine, palmatine, berberine, and atractylodin was 0.56%, 0.24%, 0.90%, 0.67%, 0.88%, 0.17%, and 0.19%, respectively. This shows that the instrument has good precision. (3) Repeated experiment results showed that the content of chlorogenic acid, phellodendrine, magnolamine, jatrorrhizine, palmatine, berberine, and atractylodin was 1.31, 7.74, 0.61, 0.40, 0.02, 49.37, and 38.24 mg/mL, respectively. The RSD% of the content was 0.63%, 0.28%, 0.75%, 0.54%, 0.86%, 0.19%, and 0.18%, respectively, indicating that this method was reproducible. (4) The stability experiment showed that the average content of chlorogenic acid, phellodendrine, magnolamine, jatrorrhizine, palmatine, berberine, and atractylodin was 1.32, 7.69, 0.60, 0.39, 0.02, 49.28, and 38.34 mg/mL, respectively. The RSD% of the content was 0.32%, 1.24%, 1.15%, 0.32%, 1.43%, 0.22%, and 0.50%, respectively. The above results show that the components of EMW were stable within 24 h. (5) The recovery range of each component in EMW was 95.0-105.0%, which indicates that the method has good accuracy (Table.2).

Table 1 The linear regression equation and linear range of compounds in the EMW

Compound	Linear Regression Equation	R ²	Linear Range (mg/mL)
Chlorogenic acid	Y=7030.2X-18.292	0.999	0.040-3.500
Phellodendrine	Y=4248X+2.7625	1.000	0.071-7.521
Magnolamine	Y=18208X+6.8479	0.999	0.050-1.312
Jatrorrhizine	Y=14844X-1.5417	0.995	0.040-0.094
Palmitine	Y=11545X-5.9875	0.992	0.002-0.051
Berberine	Y=16162X-58.504	0.998	0.502-100.210
Atractydin	Y=16813X-60.708	0.998	0.407-70.105

Table 2 The Recoveries of each component in EMW (n=6)

Components	Amount of Standard Substance (mg)	Recoveries ± SD %	RSD%
Chlorogenic acid	0.65	101.88 ± 1.70	1.82
Phellodendrine	3.35	100.71 ± 1.60	1.74
Magnolamine	0.30	102.00 ± 1.75	1.88
Jatrorrhizine	0.20	101.70 ± 1.75	1.88
Palmitine	0.01	100.61 ± 1.40	1.52
Berberine	24.71	100.82 ± 1.28	1.39
Atractydin	19.10	101.29 ± 0.74	0.80

3.3. PPI network

The chemical components and eczema-related targets were screened according to a previous study [16]. A total of 788 and 295 targets were acquired for eczema and EMW, respectively, and 57 common targets were screened ($P < 0.001$, according to Fisher's exact test) and identified as the potential targets of EMW in treating eczema (Fig.3A). A PPI network was constructed to scientifically summarize the interfaces of EMW targets associated with eczema treatment. The network showed 57 possible protein target nodes connected by 318 edges, with an average node degree of 11.2. and an average local clustering coefficient of 0.548. The P -value of PPI enrichment was $< 1.0 \times 10^{-16}$ (Fig.3B). These results suggest that the key proteins are closely related to each other. The top 10 predicted hub genes included *EGFR*, *AKT1*, *PTGS2*, *STAT3*, *MMP9*, *ICAM1*, *MAPK8*, *JUN*, *MAPK1*, and *VCAM1*. As shown in Fig.3B, the darker the color of the node, the larger the size, which indicates that the protein may be more crucial for the treatment of eczema.

3.4. Compound-target network

Based on the results of target interaction analysis, a compound-target network was built as described in Fig.4. The network showed that most inflammation-related targets and immune-related targets were interrelated, indicating that the action mechanism of EMW was associated with inflammation and immune mechanisms. Furthermore, in terms of the compound structures, alkaloids corresponded to more targets than other compounds.

3.5. GO and pathway enrichment analysis

GO analysis results revealed 76 terms of biological processes, and the core terms of EMW targets against eczema were mainly involved in the negative regulation of apoptotic process, positive regulation of vasoconstriction, positive regulation of nitric oxide biosynthetic process, positive regulation of smooth muscle cell proliferation, cellular response to mechanical stimulus, aging, response to drug, positive regulation of transcription from RNA polymerase II promoter, signal transduction, and regulation of sequence-specific DNA binding transcription factor activity (Fig.5A and 5 B and Table.3). The 71 enriched KEGG pathways ($P<0.05$) included the *TNF* signaling pathway, pathways in cancer, hepatitis B, pancreatic cancer, ErbB signaling pathway, estrogen signaling pathway, choline metabolism in cancer, Epstein-Barr virus infection, hepatitis C, and FoxO signaling pathways (Fig.5C and 5D and Table.4).

Table 3. Top 10 Biological Processes of EMW against Eczema

ID	Description	Gene symbol	Number of gene	P-Value
Term1	negative regulation of apoptotic process	<i>MAPK8, STAT3, AKT1, MMP9, EGFR</i>	5	6.02 e ⁻⁵
Term2	positive regulation of vasoconstriction	<i>AKT1, PTGS2, EGFR, ICAM1</i>	4	6.24 e ⁻⁰⁷
Term3	positive regulation of nitric oxide biosynthetic process	<i>AKT1, PTGS2, EGFR, ICAM1</i>	4	1.30 e ⁻⁰⁶
Term4	positive regulation of smooth muscle cell proliferation	<i>JUN, AKT1, PTGS2, EGFR</i>	4	3.59 e ⁻⁰⁶
Term5	cellular response to mechanical stimulus	<i>MAPK8, AKT1, PTGS2, EGFR</i>	4	5.97 e ⁻⁰⁶
Term6	aging	<i>JUN, VCAM1, STAT3, AKT1</i>	4	7.49 e ⁻⁰⁵
Term7	response to drug	<i>JUN, STAT3, PTGS2, ICAM1</i>	4	4.55 e ⁻⁰⁴
Term8	positive regulation of transcription from RNA polymerase II promoter	<i>JUN, STAT3, AKT1, EGFR</i>	4	1.28 e ⁻⁰²
Term9	signal transduction	<i>STAT3, MAPK1, AKT1, EGFR</i>	4	2.02 e ⁻⁰²
Term10	regulation of sequence-specific DNA binding transcription factor activity	<i>JUN, MAPK8, MAPK1</i>	4	7.61 e ⁻⁰⁵

Table 4. Top 10 KEGG Pathway of EMW Against Eczema

ID	Description	Gene symbol	Number of gene	P-Value
Term1	TNF signaling pathway	<i>JUN, MAPK8, VCAM1, MAPK1, AKT1, PTGS2, MMP9, ICAM1</i>	8	6.35 e ⁻¹²
Term2	Pathways in cancer	<i>JUN, MAPK8, STAT3, MAPK1, AKT1, PTGS2, MMP9, EGFR</i>	8	6.15 e ⁻⁰⁸
Term3	Hepatitis B	<i>JUN, MAPK8, STAT3, MAPK1, AKT1, MMP9</i>	6	4.57 e ⁻⁰⁷
Term4	Pancreatic cancer	<i>MAPK8, STAT3, MAPK1, AKT1, EGFR</i>	5	8.83 e ⁻⁰⁷
Term5	ErbB signaling pathway	<i>JUN, MAPK8, MAPK1, AKT1, EGFR</i>	5	2.87 e ⁻⁰⁶
Term6	Estrogen signaling pathway	<i>JUN, MAPK1, AKT1, MMP9, EGFR</i>	5	4.81 e ⁻⁰⁶
Term7	Choline metabolism in cancer	<i>JUN, MAPK8, MAPK1, AKT1, EGFR</i>	5	5.21 e ⁻⁰⁶
Term8	Epstein-Barr virus infection	<i>JUN, MAPK8, STAT3, AKT1, ICAM1</i>	5	1.11 e ⁻⁰⁵
Term9	Hepatitis C	<i>MAPK8, STAT3, MAPK1, AKT1, EGFR</i>	5	1.56 e ⁻⁰⁵
Term10	FoxO signaling pathway	<i>MAPK8, STAT3, MAPK1, AKT1, EGFR</i>	5	1.61 e ⁻⁰⁵

3.6. Compound-target-pathway network

On the basis of the compound-target network and pathway enrichment analysis results, we created a compound-target-pathway network consisting of the top 10 KEGG pathways (Fig.6A). The network showed that EMW and eczema shared 10 KEGG pathways, representing their combined anti-eczema targets. The ErbB signaling pathway, estrogen signaling

pathway, and Epstein-Barr virus infection were the three pathways connected with the key targets and associated with the effect of EMW on eczema. Hence, their network relationships were isolated and further analyzed through annotation of the KEGG pathway. The network highlighted the hub genes *EGFR*, *AKT1*, *MMP9*, *ICAM1*, *MAPK8*, *JUN*, and *MAPK1* (Fig.6B).

3.7. Molecular Docking

EMW components were chosen for molecular docking analysis based on their high content and network pharmacology results. Moreover, the importance of the top 10 hub genes in eczema treatment was validated through network pharmacology analysis. The docking results showed that *EGFR*, *AKT1*, and *PTGS2* bound well with all components. Palmatine, phellodendrine, chlorogenic acid, and jatrorrhizine bound well with all docking targets (Fig.7 and Table.5). The Surflex-Dock scores (total scores) represent binding affinities. Generally, a docking score greater than 4.25 indicates certain binding activity, whereas a docking score greater than 7.00 indicates strong binding activity [17]. The comparable binding score between the compounds may be due to their similar molecular structures, which is consistent with a previous finding [18]. However, among the seven chemical components of EMW, atractylodin only exhibited 3 (33.33%) binding efficiencies with the docking targets. Among the nine targets, *ICAM1* only exhibited 2 (28.57%) binding efficiencies with the components of EMW. These findings imply that not every component has a good binding efficiency with every target, but the interaction between each component and each target is consistent with the multi-component and multi-target characteristics of TCM [19].

Table.5 Total score of 7 chemical composition of EMW and hub genes molecule docking.

Compound	Palmatine	Phellodendrine	Chlorogenic acid	Magnoflorine	Berberine	Jateorhizine	Atractylodin
<i>EGFR</i>	5.8814	4.9264	6.6037	4.5828	6.6112	5.2378	4.4279
<i>AKT1</i>	5.1865	6.6481	6.4127	5.6517	5.6987	5.4850	4.2639
<i>PTGS2</i>	5.3339	4.9394	6.9139	4.4581	6.9586	6.3229	4.7931
<i>STAT3</i>	5.4003	4.9290	7.6658	4.6498	5.0630	5.0233	3.9572
<i>MMP9</i>	5.4053	4.8698	6.9478	4.7065	4.5353	5.1131	4.0299
<i>ICAM1</i>	5.0165	4.2205	4.6740	3.4962	3.9165	5.1307	3.3362
<i>MAPK8</i>	4.8426	5.5351	7.2720	4.4886	5.0827	5.3900	4.1246
<i>JUN</i>	6.4617	5.3219	5.1704	4.3920	5.9145	6.2663	3.6751
<i>MAPK1</i>	6.4342	6.8556	5.9204	4.5037	5.0003	6.2831	3.7213

The 3D mode and schematic 2D representation of chlorogenic acid in the active site of *MAPK8* are shown in Fig.8A. Chlorogenic acid showed 6H-bond interactions with ARGs 69, 34, 109, 111, 156, and 153. Other interactions included P- π conjugation and π - π conjugation. The 3D mode and schematic 2D dimensional representation of chlorogenic acid in the active site of *MMP9* are shown in Fig.8B. Chlorogenic acid showed 9H-bond interactions, three of which were amidogen with GLY 186, LEU 188, and ALA 189. ARG 424, GLN 402, HIS 401, and TRY 420. Other interactions, including Pi-Sigma and Pi-Anion, were connected to ALA 417 and HIS 411. The 3D mode and schematic 2D representation of chlorogenic acid in the active site of *STAT3* are shown in Fig.8C. Chlorogenic acid showed 5H-bond interactions, which were GLU 883, SER 963, GLY 1020, GLU 996, and GLU 957. Other interactions included Pi-Sigma, Pi-Alkyl, and Pi-Pi. The 3D mode and schematic 2D dimensional representation of berberine in the active site of *PTGS2* are shown in Fig.8D. Berberine showed

6H-bond interactions, including hydroxyls with ARGs 69, 34, 109, 111, 156, and 153. Other interactions included Pi-sigma and π - π conjugation.

4. Discussion

EMW is often used to treat eczema in clinical setting, but its specific mechanism is unclear. Thus, in this study, we aimed to elucidate the mechanism of EMW in the treatment of eczema. The fingerprints of EMW were obtained by HPLC, and seven components with high content and excellent activity were quantitatively analyzed. Owing to its simplicity and reliability, the established method can provide a reference for evaluating the quality of EMW. Subsequently, compounds with high content in EMW were used for predicting eczema-related targets by network pharmacology. The results revealed the complex mechanism of EMW in treating eczema, which involves the interrelation of multiple components, protein targets, and pathways. Further, molecular docking analysis was performed to verify the binding force of the drug with the target proteins.

The pathogenesis of eczema is determined by many factors, such as poverty, environment, infection, and immunity. In fact, recent research has revealed a close relationship between eczema and inflammatory factors. *EGFR*, *TH-17*, *IL-17*, *TNF- α* , *MMP*, and other inflammatory factors interact with each other, forming a large and complex network [20, 21]. Thus, owing to the complex pathogenesis of eczema, it is difficult for single-target drugs to achieve a good curative effect. Therefore, multi-component and multi-target drugs have become a trend for the treatment of eczema. Pharmacological studies have shown that EMW has anti-inflammatory and anti-allergic effects [22, 23]. EMW can relieve the symptoms of eczema in rats by stimulating the inactivation of NF- κ B and mitogen-activated protein kinases (*MAPKs*) in cells and reducing the level of I κ B α [24]. Moreover, EMW significantly inhibits NO and prostaglandin E₂ production as well as inducible NO synthase and *COX-2* expression in cells to regulate immunity and inflammation [25]. The immunomodulatory, anti-inflammatory, and anti-allergic effects of EMW are similar to the current treatment strategies for eczema.

Some evidence indicates that chlorogenic acid can inhibit the levels of pro-inflammatory cytokines (*TNF- α* and *IL-2*) and elevate the expression levels of anti-inflammatory cytokines (*IL-4* and *IL-13*). Thus, chlorogenic acid can reduce the production of inflammatory factors and effectively inhibit the occurrence and development of inflammation [26]. In addition, magnoflorine has a considerable anti-inflammatory effect; it can inhibit inflammation induced by excess production of NO, and the possible mechanisms are associated with the inhibition of Toll-like receptor 4-mediated activation of the NF- κ B and *MAPK* signaling pathways. Moreover, magnoflorine exerts antifungal and immunomodulatory activities by promoting the phosphorylation of *JNK*, *ERK*, *p38 MAPK*, and *AKT* [27]. Berberine, palmatine, and jatrorrhizine elevate the production of inflammatory factors mediated by *TNF- α* and *IL-1 β* [28, 29]. Furthermore, berberine has been widely used to treat eczema in China [30]. Atractylodin can also treat eczema through its anti-inflammatory and immunomodulatory effects [31]. The findings of these pharmacological studies are consistent with those of our current study.

Combined with network topology analysis and related literature reports, this study predicted that *EGFR*, *AKT1*, *PTGS2*, *STAT3*, *MMP9*, *ICAM1*, *MAPK8*, *JUN*, *MAPK1*, and *VCAM1* may be the key targets of EMW in the treatment of eczema. *EGFR*, *AKT1*, *PTGS2*, and *JUN* have anti-inflammatory pharmacological effects, and certain nonsteroidal anti-inflammatory drugs modulate cellular glycosaminoglycan synthesis by affecting *EGFR* and *PI3K* signaling pathways [32, 33]. The allergic inflammatory response has been associated mainly with the activation of *MAPKs*, which include the extracellular signal-regulated kinase, c-*Jun* N-terminal kinase, and *p38 MAPK*. *MAPKs* are involved in the activation of the transcription of inflammatory and allergy-related mediators. Therefore, regulation of the *MAPK* pathway is considered vital for eczema prevention [34]. Accumulating evidence has shown that the skin barrier function is regulated via the IL-13/IL-4–JAK–STAT6/STAT3 axis; furthermore, the regulation of *STAT3* can repair the skin barrier function and reduce allergic inflammation, thereby playing a therapeutic role in eczema [35]. *MMP9* may be directly related to the pathogenesis and development of eczema, as it may affect the susceptibility to eczema by degrading *COL5A3* [36]. Moreover, the

downregulation of *ICAM-1* and *AKT* expression via activation of *MAPK8* and *JNK* has been shown as one of the mechanisms in the treatment of eczema [37]. These targets play an important role in the pathogenesis of eczema; thus, drug interventions targeting these genes can be effective for eczema treatment. This finding suggests that EMW may alleviate the anaphylactic reaction and immunologic and inflammatory responses by adjusting the network at different nodes, which exerts a synergistic effect on eczema [38].

Pathway enrichment analysis results showed that the effect of EMW in treating eczema may be related to the ErbB signaling pathway, estrogen signaling pathway, and Epstein-Barr virus infection. *ErbB* family members represent important biomarkers and drug targets for modern precision therapy, and they play a pro-inflammatory role by activating the MMP, PTGS2, and MAPK signaling pathways [39, 40]. The components of EMW may regulate the ErbB signaling pathway to treat eczema by acting on its key target proteins, such as *MMP*, *PTGS2*, and *MAPK*. Estrogen affects the expression of *MMP9* and *EGFR* through the classical endoplasmic reticulum pathway, which plays important anti-inflammatory role and regulates cell survival and cell cycle [41]. In the treatment of eczema, EMW regulates the levels of *MMP9* and *EGFR* proteins by acting on the estrogen signaling pathway, which plays an anti-inflammatory and anti-allergic role. Epstein-Barr virus (*EBV*) infection is associated with various human cancers. The expression of *EGFR*, *AKT1*, *JUN*, and *MAPK* can be inhibited by inhibiting the expression of *EBV* [42]. The occurrence and development of eczema is closely related to these pathways. The enrichment analysis results showed that each pathway contained key targets of the active components of EMW, and each pathway played a complex role in the treatment of eczema, suggesting that these pathways have potential application in future research. But the mechanism of EMW treat in T2DM is complicated, network pharmacology only shows that EMW has hypoglycemic effect in theory, we will verify the results of this research in the future stage.

5. Conclusion

In this study, seven components with the highest content in EMW were quantitatively determined. Network pharmacology revealed 57 crossover genes between eczema and these seven components. EMW was shown to effectively treat eczema by regulating the key pathways of the anti-inflammatory system, including the ErbB signaling, estrogen signaling, and Epstein-Barr virus infection pathways, which involve *EGFR*, *AKT1*, *STAT3*, *MMP9*, *ICAM1*, *MAPK8*, *JUN*, and *MAPK1*. These findings were supported by molecular docking results, which provide insights into the mechanism of EMW against eczema. We successfully applied an integrated strategy of network pharmacology and content determination to explore the pharmacological mechanism of the major components of EMW in eczema treatment. The method established in this study can help increase our understanding of the mechanisms of Chinese herbal formulations and promote their further research and development.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no conflict of interest.

Funding

This work is supported by grants from the National Key R&D Program of China (2018YFC1704500; 2018YFC1704503).

Authors' contributions

XT designed the study and wrote the manuscript. LCS and LX revised the manuscript. HYN and LZY collected the data. CFL provided help for quantitative analysis. TXM provided the concept and ideas support. All data were generated inhouse, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

References

1. Sawangjit R, Dilokthornsakul P, Lloyd-Lavery A, et al. Systemic treatments for eczema: a network meta-analysis. *Cochrane Database Syst Rev*. 2020;9:D13206. DOI:10.1002/14651858.CD013206.pub2.
2. Singh M, Pawar M, Bothra A, et al. Overzealous hand hygiene during the COVID 19 pandemic causing an increased incidence of hand eczema among general population, *JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY* 2020;83: e37-e41.
3. Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. *ALLERGY*. 2014;69:3–16.
4. Chong M, Fonacier L. Treatment of Eczema: Corticosteroids and Beyond. *Clin Rev Allergy Immunol*. 2016;51:249–62.
5. Chen Y, Xian Y, Lai Z, et al. Anti-inflammatory and anti-allergic effects and underlying mechanisms of Huang-Lian-Jie-Du extract: Implication for atopic dermatitis treatment. *JOURNAL OF ETHNOPHARMACOLOGY*. 2016;185:41–52.
6. Zhang H, Zhang S, Wang W, et al. Characterizing metabolites and potential metabolic pathways changes to understanding the mechanism of medicinal plant *Phellodendri Amurensis* cortex against doxorubicin-induced nephritis rats using UPLC-Q/TOF-MS metabolomics. *J Pharm Biomed Anal*. 2020;188:113336.
7. Huang J, Guo W, Cheung F, et al. Integrating Network Pharmacology and Experimental Models to Investigate the Efficacy of Coptidis and Scutellaria Containing Huanglian Jiedu Decoction on Hepatocellular Carcinoma. *Am J Chin Med*. 2020;48:161–82.
8. Zhu C, Cai T, Jin Y, et al. Artificial intelligence and network pharmacology based investigation of pharmacological mechanism and substance basis of Xiaokewan in treating diabetes. *PHARMACOLOGICAL RESEARCH*. 2020;159:104935.
9. Li R, Wu K, Li Y, et al. Integrative pharmacological mechanism of vitamin C combined with glycyrrhizic acid against COVID-19: findings of bioinformatics analyses, *BRIEFINGS IN BIOINFORMATICS* 2020. DOI: 10.1093/bib/bbaa141.
10. Liu CS, Xia T, Luo ZY, et al. Network pharmacology and pharmacokinetics integrated strategy to investigate the pharmacological mechanism of Xianglian pill on ulcerative colitis. *PHYTOMEDICINE*. 2021;82:153458.
11. Li S, Zhang B. Traditional Chinese medicine network pharmacology: theory, methodology and application. *Chin J Nat Med*. 2013;11:110–20.
12. Wang Y, Sun YW, Wang YM, et al. Virtual screening of active compounds from *Artemisia argyi* and potential targets against gastric ulcer based on Network pharmacology. *BIOORGANIC CHEMISTRY*. 2019;88:102924.
13. Xu L, Zhang Y, Zhang P, et al. Integrated Metabolomics and Network Pharmacology Strategy-Driven Active Traditional Chinese Medicine Ingredients Discovery for the Alleviation of Cisplatin Nephrotoxicity. *CHEMICAL RESEARCH IN TOXICOLOGY*. 2019;32:2411–21.
14. Li R, Ma X, Song Y, et al. Anti-colorectal cancer targets of resveratrol and biological molecular mechanism: Analyses of network pharmacology, human and experimental data, *JOURNAL OF CELLULAR BIOCHEMISTRY* 2019.
15. Zhou R, Wu K, Su M, et al. Bioinformatic and experimental data decipher the pharmacological targets and mechanisms of plumbagin against hepatocellular carcinoma. *Environ Toxicol Pharmacol*. 2019;70:103200.

16. Li R, Guo C, Li Y, et al. Therapeutic targets and signaling mechanisms of vitamin C activity against sepsis: a bioinformatics study, BRIEFINGS IN BIOINFORMATICS 2020.
17. Gu L, Lu J, Li Q, et al. A network-based analysis of key pharmacological pathways of *Andrographis paniculata* acting on Alzheimer's disease and experimental validation. JOURNAL OF ETHNOPHARMACOLOGY. 2020;251:112488.
18. Liu CS, Xia T, Luo ZY, et al. Network pharmacology and pharmacokinetics integrated strategy to investigate the pharmacological mechanism of Xianglian pill on ulcerative colitis. PHYTOMEDICINE. 2021;82:153458.
19. Zhai B, Zhang N, Han X, et al. Molecular targets of beta-elemene, a herbal extract used in traditional Chinese medicine, and its potential role in cancer therapy: A review. BIOMEDICINE PHARMACOTHERAPY. 2019;114:108812.
20. Marenholz I, Esparza-Gordillo J, Lee YA. Shared genetic determinants between eczema and other immune-related diseases. Curr Opin Allergy Clin Immunol. 2013;13:478–86.
21. Eyerich K, Eyerich S. Immune response patterns in non-communicable inflammatory skin diseases. J Eur Acad Dermatol Venereol. 2018;32:692–703.
22. Chen G, Li KK, Fung CH, et al. Er-Miao-San, a traditional herbal formula containing *Rhizoma Atractylodis* and *Cortex Phellodendri* inhibits inflammatory mediators in LPS-stimulated RAW264.7 macrophages through inhibition of NF-kappaB pathway and MAPKs activation. JOURNAL OF ETHNOPHARMACOLOGY. 2014;154:711–8.
23. Zhang C, Su T, Yu D, et al. Revealing active ingredients, potential targets, and action mechanism of Er-miao fang for treating endometritis based on network pharmacology strategy. JOURNAL OF ETHNOPHARMACOLOGY. 2020;260:113051.
24. Tan L, Wang Y, Ai G, et al. Dihydroberberine, a hydrogenated derivative of berberine firstly identified in *Phellodendri* Chinese Cortex, exerts anti-inflammatory effect via dual modulation of NF-kappaB and MAPK signaling pathways. INTERNATIONAL IMMUNOPHARMACOLOGY. 2019;75:105802.
25. Chen LG, Jan YS, Tsai PW, et al. Anti-inflammatory and Antinociceptive Constituents of *Atractylodes japonica* Koidzumi. J Agric Food Chem. 2016;64:2254–62.
26. Lee TK, Kang IJ, Kim B, et al. Experimental Pretreatment with Chlorogenic Acid Prevents Transient Ischemia-Induced Cognitive Decline and Neuronal Damage in the Hippocampus through Anti-Oxidative and Anti-Inflammatory Effects, MOLECULES 2020;25. DOI: 10.3390/molecules25163578.
27. Xu T, Kuang T, Du H, et al. Magnoflorine: A review of its pharmacology, pharmacokinetics and toxicity. PHARMACOLOGICAL RESEARCH. 2020;152:104632.
28. Kalmarzi RN, Naleini SN, Ashtary-Larky D, et al. Anti-Inflammatory and Immunomodulatory Effects of Barberry (*Berberis vulgaris*) and Its Main Compounds. Oxidative Medicine Cellular Longevity. 2019;2019:6183965.
29. Liu B, Piao X, Niu W, et al. Kuijieyuan Decoction Improved Intestinal Barrier Injury of Ulcerative Colitis by Affecting TLR4-Dependent PI3K/AKT/NF-kappa B Oxidative and Inflammatory Signaling and Gut Microbiota. Front Pharmacol. 2020;11:1036.
30. Wang J, Wang L, Lou GH, et al. *Coptidis Rhizoma*: a comprehensive review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. PHARMACEUTICAL BIOLOGY. 2019;57:193–225.
31. Wan HL, Chen HZ, Shi XQ. Study on effect of Traditional Chinese Medicine Jianpi Chushi decoction and ointment on chronic eczema. Asian Pacific Journal of Tropical Medicine. 2016;9:920–3.
32. Shin JW, Lee HS, Na JI, et al. Resveratrol Inhibits Particulate Matter-Induced Inflammatory Responses in Human Keratinocytes, INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 2020;21. DOI:10.3390/ijms21103446.
33. Mozolewski P, Moskot M, Jakobkiewicz-Banecka J, et al. Nonsteroidal anti-inflammatory drugs modulate cellular glycosaminoglycan synthesis by affecting EGFR and PI3K signaling pathways. Sci Rep. 2017;7:43154.
34. Lorz LR, Kim MY, Cho JY. Medicinal potential of *Panax ginseng* and its ginsenosides in atopic dermatitis treatment. Journal of Ginseng Research. 2020;44:8–13.

35. Furue M. Regulation of Skin Barrier Function via Competition between AHR Axis versus IL-13/IL-4JAKSTAT6/STAT3 Axis: Pathogenic and Therapeutic Implications in Atopic Dermatitis, *Journal of Clinical Medicine* 2020;9. DOI:10.3390/jcm9113741.
36. Margaritte-Jeannin P, Babron MC, Laprise C, et al. The COL5A3 and MMP9 genes interact in eczema susceptibility. *CLINICAL AND EXPERIMENTAL ALLERGY*. 2018;48:297–305.
37. Gao CJ, Ding PJ, Yang LL, et al. Oxymatrine Sensitizes the HaCaT Cells to the IFN-gamma Pathway and Downregulates MDC, ICAM-1, and SOCS1 by Activating p38, JNK, and Akt, *INFLAMMATION* 2018;41:606–613.
38. Wang Z, Wang ZZ, Geliebter J, et al. Traditional Chinese medicine for food allergy and eczema, *Ann Allergy Asthma Immunol* 2020.
39. Jacobi N, Seeboeck R, Hofmann E, et al. ErbB Family Signalling: A Paradigm for Oncogene Addiction and Personalized Oncology, *Cancers (Basel)* 2017;9.
40. Zhang Y, Li Y, Li H, et al. Clostridium difficile toxin B recombinant protein inhibits tumor growth and induces apoptosis through inhibiting Bcl-2 expression, triggering inflammatory responses and activating C-erbB-2 and Cox-2 expression in breast cancer mouse model. *BIOMEDICINE PHARMACOTHERAPY*. 2018;101:391–8.
41. Zhao MY, Zhao T, Meng QY, et al. Estrogen and estrogen receptor affects MMP2 and MMP9 expression through classical ER pathway and promotes migration of lower venous vascular smooth muscle cells. *Eur Rev Med Pharmacol Sci*. 2020;24:1460–7. DOI:10.26355/eurev_202002_20205.
42. Talwar P, Gupta R, Kushwaha S, et al. Viral Induced Oxidative and Inflammatory Response in Alzheimer's Disease Pathogenesis with Identification of Potential Drug Candidates: A Systematic Review using Systems Biology Approach. *Curr Neuroparmacol*. 2019;17:352–65.

Figures

The workflow of this study.

Representative HPLC-based chemoprofiles of EMW samples. (A) Ingredient identify of the EMW by HPLC fingerprint and (B) Repeatability analysis. (C) The structures of analytes which labels as follow: 1, chlorogenic acid, 2, phellodendrine, 3, magnoflorine, 4, jateorhizine, 5, Palmatine, 6, Berberine, and 7, atractylodin.

Screening of targets for EMW in the treatment of eczema. (A) Targets attribution among the EMW and eczema. (B) Protein-protein interaction network of crossover targets. Node, target protein.

Figure 5

Biological processes and molecular pathways associated with core targets of EMW against Eczema. (A) Core biotargets of EMW against Eczema were related to the top 10 enriched GO terms in Circos diagrams. (B) Biological processes were presented by bubble diagrams generated through count algorithms and $-\log(\text{P-Value})$ calculation. (C) Core biotargets of EMW against Eczema were linked to the top 10 enriched KEGG terms in Circos diagrams. (D) Molecular pathways (from KEGG analysis) were presented by bubble diagrams based on count algorithms and $-\log(\text{P-Value})$.

Figure 6

Enriched network and pathway analysis of EMW. (A) Compound-target-pathway network of EMW and Eczema. Labels: green squares, ingredients, pink diamonds, protein targets, Cyan triangle, pathways.

Figure 7

Heat map of molecular docking result.