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Exploring the Mechanism of Eczema Action of Jinhuang Gao Based on Network Pharmacology and Molecular Docking

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Abstract: To analyze the mechanism of Jinhuang Gao in treating eczema by pharmacology. We screened active components and targets of Jinhuang Gao by TCMSP database (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform), and disease targets through GeneCards database. "Pathway-Component-Target" network was assembled via Cytoscape 3.9.1 software, and PPI network was formulated utilizing STRING database. GO function and KEGG pathway enrichment were analyzed to predict its mechanism. Additionally, SYBYL-X 2.1.1 and PyMOL software were used for molecular docking. 232 components, 122 therapeutic targets, 5835 GO items and 250 pathways were found. Among them, five key components (beta-sitosterol, quercetin, Licochalcone-A, naringenin, and formononetin) and five core targets (EGF, NFKBIA, IL4, MAPK14, and RELA) in the treatment of eczema were found. There are 20 key pathways, including PI3K-Akt signaling pathway, Pathways of neurodegeneration multiple diseases, Relaxin signaling pathway, Calcium signaling pathway, JAK-STAT signaling pathway, etc. Drug components were found to bind better to target proteins. This study preliminarily revealed active components, targets and pathways of Jinhuang Gao in treating eczema.

Keywords: Network pharmacology, Jinhuang Gao, Eczema

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1. Introduction

Eczema is a kind of allergic inflammatory reactive skin disease mainly characterized by polymorphic skin damage with an obvious oozing tendency, corresponding to the category of wet sores in Chinese medicine. Eczema lesions are often symmetrically distributed, accompanied by intense itching, recurrent and chronic, seriously affecting the quality of life of patients [1]. At present, Chinese medicine treatment mostly adopts the principles of clearing heat, strengthening the spleen, resolving dampness and activating. The clinical efficacy is very remarkable [2], with the advantages of multi-target, multi-level, small adverse reactions and low recurrence rate. The external treatment method of Chinese medicine has a long history, the origin can be traced back to the Han Dynasty "Huangdi Neijing", the Qing Dynasty Wu Shiji [3] carried out a systematic collation and theoretical discussion of external treatment prescriptions and medicines, and perfected the theory of external treatment, proposing that "the theory of external treatment is also the theory of internal treatment". With the development of medicine, many kinds of external treatment methods (wet compresses, medicated baths, etc.) and external treatment agents (gels, oils, ointments, creams, etc.) have become an effective way of treating dermatologic diseases in Traditional Chinese Medicine (TCM). Eczema is located in the skin, and the external treatment of traditional Chinese medicine is applied on the body surface, so the doctor can observe the patient's adaptation and tolerance at any time, and the medication is relatively safe. Jinhuang Gao is a homemade topical medicine for eczema made by Zunyi Hospital of Traditional Chinese Medicine, which has the effect of clearing heat and removing dampness, dispelling wind and relieving itching. Current research shows that Jinhuang Gao can significantly improve the symptoms of eczema patients' skin lesions and itching symptoms, and the recurrence rate is low [4]. However, due to a lack of the mechanism's elucidation of Jinhuang Gao in treating eczema based on the holistic concept of traditional Chinese medicine, the promotion and application of Jinhuang Gao have been greatly hindered in the clinical process. By integrating a large number of network database resources and utilizing bioinformatics technology, network pharmacology constructs the overall network relationship of drug-target-disease, to elucidate the regulatory mechanism of multi-pathway and multi-target treatment of disease by drugs from the micro to the macro level, which is in line with the holistic concept of TCM. In this study, we screened the active ingredients and targets of Jinhuang Gao for the treatment of eczema through network pharmacology and molecular docking, established a relevant network, analyzed the material basis and mechanism of action of Jinhuang Gao for the treatment of eczema, and provided references and bases for subsequent experimental studies and clinical applications.

2. Materials and Methods

2.1 Screened for Active Components and Targets of Jinhuang Gao

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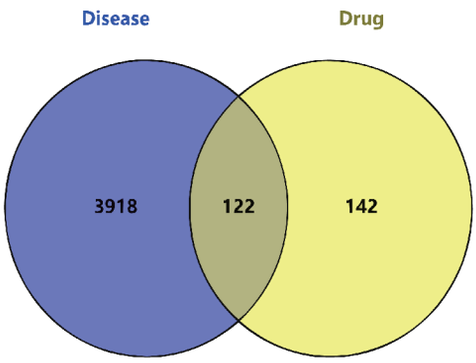

Figure 1. Venn diagram of intersection target of Jinhuang Gao and eczema

The active components and targets were screened with Baizhi, Bingpian, Cangzhu, Chenpi, Dahuang, Danggui, Gancao, Houpu, Huangbai, Huanglian, Kushen, Tianhuafen and Zhizi as keywords through TCMSP（http://tcmspw.com/tcmsp.php）, in which OB≥30% and DL≥0.18 (OB: oral bioavailability; DL: drug properties). TCMSP database is a comprehensive platform for researchers to study network pharmacology, with powerful filter functions, identification of drug targets, and the ability to quickly establish target-disease networks from pharmacological data and drug action patterns to meet the needs of researchers[5]. Then converted the target proteins to gene names through Uniprot (https://www.uniprot.org/).

2.2 Screened Disease Targets

The GeneCards (https://www.genecards.org) database is a comprehensive, searchable database that provides comprehensive information on all annotated and

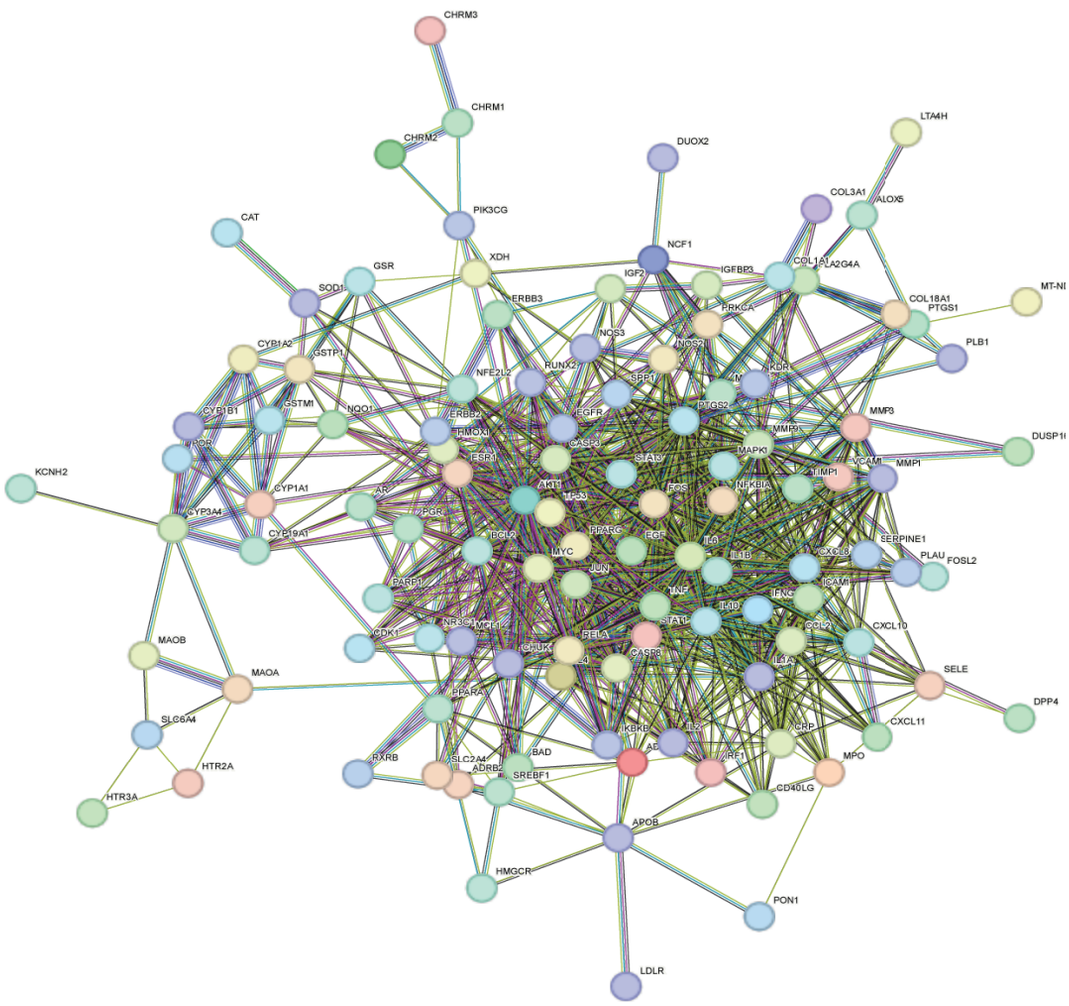

Figure 2. PPI Network of Jinhuang Gao in treating eczema.

Table 1. The basic information of some active components in Jinhuang Gao

Mol ID	Molecule Name	OB (%)	DL	source
MOL005807	sen-byakangelicol	58	0.61	baizhi
MOL001942	isoimperatorin	45.46	0.23	baizhi
MOL000449	Stigmasterol	43.83	0.76	baizhi
MOL013430	Prangenin	43.6	0.29	baizhi
MOL001749	ZINC03860434	43.59	0.35	baizhi
MOL006862	bronyl acetate	59.3	0.51	bingpian
MOL006865	dipterocarpol	41.71	0.76	bingpian
MOL006861	asiatic acid	41.38	0.71	bingpian
MOL000179	2-Hydroxyisoxypropyl-3-hydroxy-7-isopentene-2,3-dihydro-benzofuran-5-carboxylic	45.2	0.2	cangzhu
MOL000186	Stigmasterol 3-O-beta-D-glucopyranoside_qt	43.83	0.76	cangzhu
MOL000188	3β-acetoxyatractylone	40.57	0.22	cangzhu
MOL000184	NSC63551	39.25	0.76	cangzhu
MOL000085	beta-daucosterol_qt	36.91	0.75	cangzhu
MOL005815	Citromitin	86.9	0.51	chenpi
MOL005828	nobiletin	61.67	0.52	chenpi
MOL004328	naringenin	59.29	0.21	chenpi
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27	chenpi
MOL000359	sitosterol	36.91	0.75	chenpi
MOL000471	aloe-emodin	83.38	0.24	dahuang
MOL002293	Sennoside D_qt	61.06	0.61	dahuang
MOL002235	EUPATIN	50.8	0.41	dahuang
MOL002276	Sennoside E_qt	50.69	0.61	dahuang
MOL000096	(-)-catechin	49.68	0.24	dahuang
MOL000449	Stigmasterol	43.83	0.76	danggui
MOL000358	beta-sitosterol	36.91	0.75	danggui
MOL002311	Glycyrol	90.78	0.67	gancao
MOL004990	7,2',4'-trihydroxy − 5-methoxy-3 − arylcoumarin	83.71	0.27	gancao
MOL004904	licopyranocoumarin	80.36	0.65	gancao
MOL004891	shinpterocarpin	80.3	0.73	gancao

MOL005017	Phaseol	78.77	0.58	gancao
MOL005970	Eucalyptol	60.62	0.32	houpu
MOL005980	Neohesperidin	57.44	0.27	houpu
MOL000785	palmatine	64.6	0.65	huangbai
MOL000622	Magnograndiolide	63.71	0.19	huangbai
MOL000787	Fumarine	59.26	0.83	huangbai
MOL001131	phellamurin_qt	56.6	0.39	huangbai
MOL002652	delta7-Dehydrosophoramine	54.45	0.25	huangbai
MOL002907	Corchoroside A_qt	104.95	0.78	huanglian
MOL008647	Moupinamide	86.71	0.26	huanglian
MOL000785	palmatine	64.6	0.65	huanglian
MOL000622	Magnograndiolide	63.71	0.19	huanglian
MOL002903	(R)-Canadine	55.37	0.77	huanglian
MOL006596	Glyceollin	97.27	0.76	kushen
MOL000456	Phaseolin	78.2	0.73	kushen
MOL001484	Inermine	75.18	0.54	kushen
MOL004941	(2R)-7-hydroxy-2-(4-hydroxyphenyl)chroman-4-one	71.12	0.18	kushen
MOL000392	formononetin	69.67	0.21	kushen
MOL004355	Spinasterol	42.98	0.76	tianhuafen
MOL006756	Schottenol	37.42	0.75	tianhuafen
MOL004561	Sudan III	84.07	0.59	zhizi
MOL007245	3-Methylkempferol	60.16	0.26	zhizi
MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone	51.96	0.41	zhizi
MOL000098	quercetin	46.43	0.28	zhizi
MOL009038	GBGB	45.58	0.83	zhizi

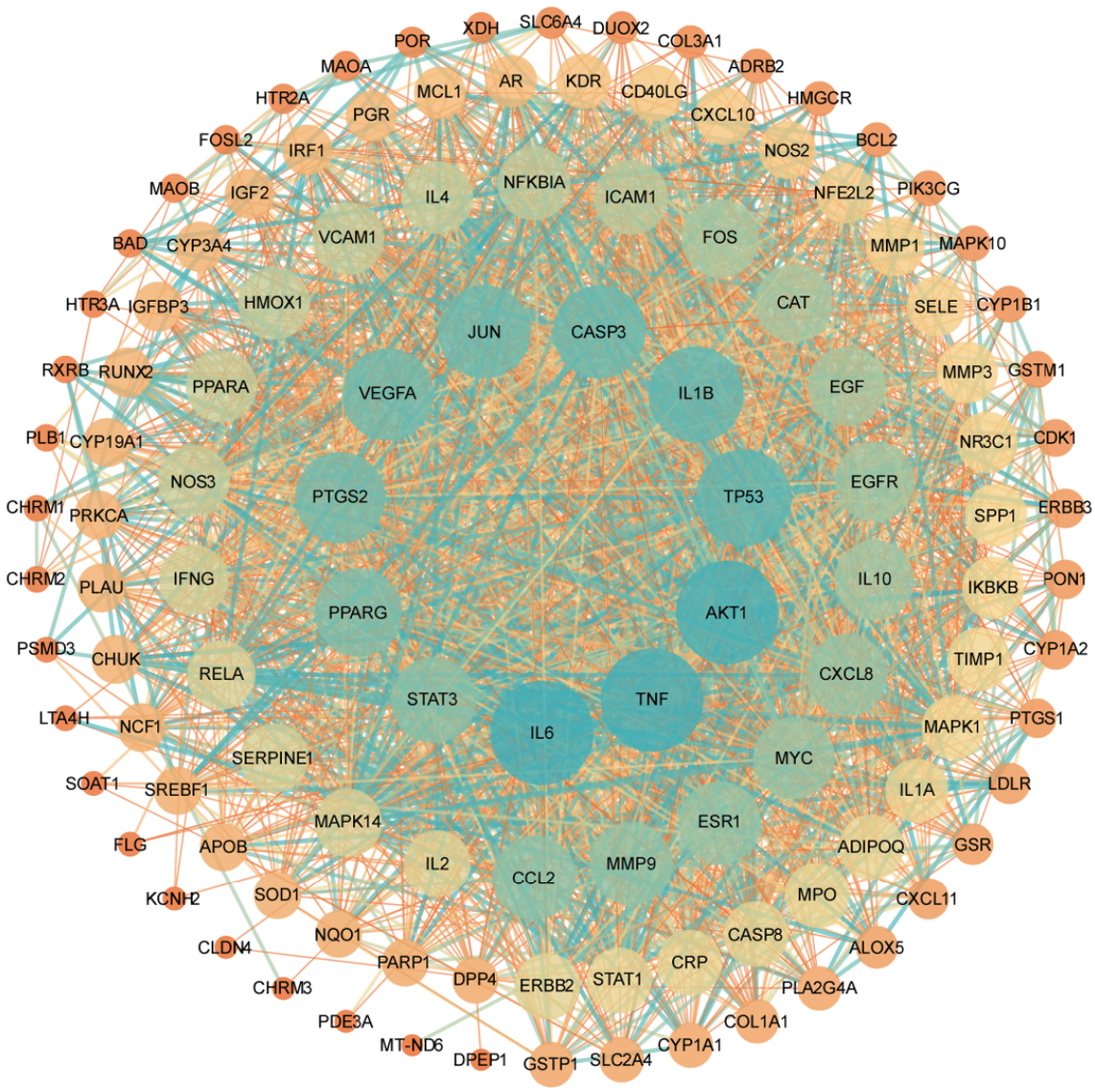

Figure 3. Visualization of PPI Network

predicted human genes, allowing efficient analysis of gene-disease associations. [6]. Therefore, we searched for disease targets with "eczema" as the keyword through it.

2.3 Constructed PPI Network

The active component targets of Jinhuang Gao and the disease targets of eczema were introduced into Venny analysis platform(https://bioinfogp.cnb.csic.es/tools/venny/) to obtain the therapeutic targets (intersection part) of Jinhuang Gao in treating eczema. Imported intersection targets into STRING (https://cn.string-db.org/), the species was limited to "Homo sapiens", the isolated targets were removed, and the PPI network was constructed with a confidence of 0.4 as the screening condition.

2.4 Gene Enrichment Analysis

GO functional enrichment analysis and KEGG pathway enrichment analysis were performed on R4.3.1 software for the main components of Jinhuang Gao (P Value cutoff=0.01). The top 20 biological processes and pathways were selected.

Table 2. Amino acid residues and hydrogen bonding in some docking results.

Docking Results	Amino Acid Residue	Num of Hydrogen Bond	Distance of Hydrogen Bond(Å)
MOL000392-EGF	SER-9	2	2.0, 2.8
	TYR-29	1	2.0
MOL000392-IL4	ARG-75	2	2.1, 2.0
MOL000392-MAPK14	MET-109	2	1.8, 2.1
MOL000098-NFKBIA	TRP-66	1	2.1
MOL000392-RELA	ARG-131	1	2.0

Table 3. Amino acid residues and Hydrophobic Interaction in some docking results.

Docking Results	Amino Acid Residue	Num of Hydrophobic Interaction	Distance of Hydrophobic Interaction (Å)
MOL000392-EGF	PRO-7	1	3.5
	LEU-8	1	3.6
MOL000392-IL4	GLN-20	1	3.8
	GLN-71	1	3.9
	HIS-214	1	3.3
MOL000392-MAPK14	TYR-35	1	2.9
	VAL-38	1	3.9
	ALA-51	1	3.5
	LYS-53	1	3.5
	LEU-108	1	3.3
	PHE-169	2	3.6, 3.8
MOL000098-NFKBIA	TRP-66	3	3.8, 3.5, 3.8
	LYS-67	1	3.4
MOL000392-RELA	ARG-131	1	3.4

2.5 Constructed Component-Target-Pathway Network

In order to better elucidate the relationship between components, targets and pathways, we constructed a "component-target-pathway" network using Cytoscape 3.9.1. Through this network, we can visualize the active substances in Jinhuang Gao for the treatment of eczema and their possible mechanisms of action.

2.6 Molecular Docking

On the basis of the above active ingredient and core target screening results, the key drug active ingredient was selected as the ligand and the core target in the PPI network was taken as the receptor for molecular docking. The steps are as follows: download the 3D structure information of the drug active ingredient in TCMSP database (http://tcmspw.com/tcmsp.php), import it into the software SYBYL-X for optimization, set the value of max iterations to 1000, and then set Charges in Energy to Gasteiger-Huckel after modification. download the 3D of the core target proteins in the RCSB Protein Data Bank (PDB, https://www.rcsb.org/) database structure, separated small molecule ligands and removed water molecules using the software SYBYL-X. Procs was set to 4 and the rest of the parameters were set to default values during docking, and the results were imported into PyMol for structure preprocessing and output of the 3D structure picture.

The 3D molecular structure of active ingredients was downloaded from TCMSP database (http://tcmspw.com/tcmsp.php). The crystal structure of target proteins was obtained from the molecular docking of ligands with receptors was conducted by SYBYL-X2.1.1 software. After removing residual water molecules and adding hydrogen, docking was performed through the Surflex-Dock program using SYBYL-X2.1.1 docking software.

3.Results

3.1 Active Components and Disease Targets

In total, 280 ingredients of Jinhuang Gao were selected based on the screening criteria(OB(oral bioavailability)≥30% and DL(drug properties)≥0.18), including 23 Baizhi, 3 Bingpian, 9 Cangzhu, 5 Chenpi, 27 Dahuang, 2 Danggui, 92 Gancao, 2 Houpu, 37 Huangbai, 14 Huanglian, 45 Kushen, 5 Tianhuafen, 15

Table 4. Amino acid residues and π-Cation Interaction in some docking results.

Docking Results	Amino Acid Residue	Num of π-Cation Interaction	Distance of π-Cation Interaction (Å)
MOL000392-EGF	NONE	NONE	NONE
MOL000392-IL4	ARG-75	1	3.2
MOL000392-MAPK14	NONE	NONE	NONE
MOL000098-NFKBIA	NONE	NONE	NONE
MOL000392-RELA	ARG-131	1	3.3

Table 5. Amino acid residues and π-Stacking (parallel) in some docking results.

Docking Results	Amino Acid Residue	Num of π-Stacking (parallel)	Distance of π-Stacking (parallel) (Å)
MOL000392-EGF	NONE	NONE	NONE
MOL000392-IL4	HIS-214	1	3.4
MOL000392-MAPK14	NONE	NONE	NONE
MOL000098-NFKBIA	TRP-66	3	3.2, 3.3, 3.2
MOL000392-RELA	NONE	NONE	NONE

Zhizi. The basic information of some active components was as follows (See Table 1, which lists only the top 5 compounds in terms of OB (oral bioavailability) for each medicine). After excluding corresponding targets and duplicates, 232 components and their corresponding 264 targets were finally collected from the TCMSP database for further analyses. of Jinhuang Gao by TCMSP platform.

Constructed PPI Network

A search for "eczema" in the GeneCards database yielded 4040 disease targets, and the intersections with 264 potential active ingredient targets were plotted by Venn analysis, resulting in 122 targets for the treatment of eczema by Jinhuang Gao (see Figure 1). These 122 intersected targets were imported into the STRING database to construct a PPI network (see

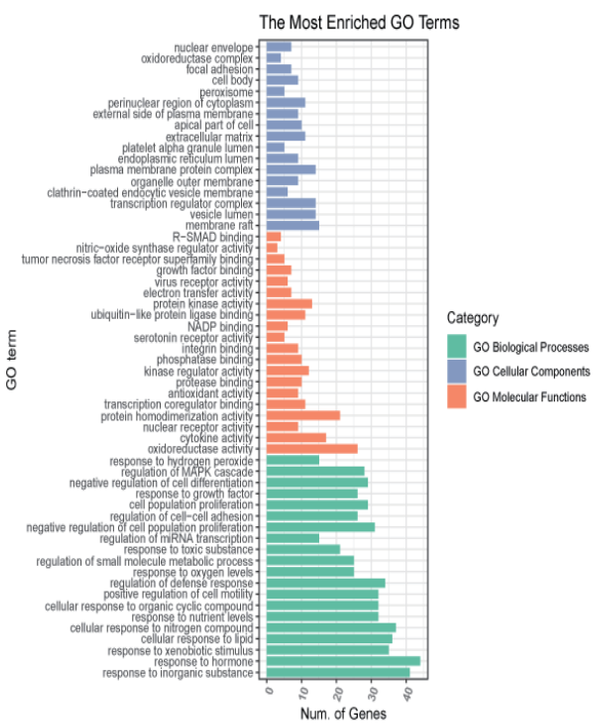

Figure 4 GO enrichment analysis of Jinhuang Gao.

Figure 5. KEGG pathway enrichment analysis of Jinhuang Gao.

Table 6. Amino acid residues and interactions in some docking results

Docking Results	Total Score
MOL000098-RELA	1.09
MOL000497-RELA	1.2947
MOL000358-RELA	1.7937
MOL000392-RELA	1.7192
MOL004328-RELA	1.6145
GOL-RELA	2.9901
MOL000497-MAPK14	6.2852
MOL000358-MAPK14	6.096
MOL000098-MAPK14	5.827
MOL004328-MAPK14	5.6558
MOL000392-MAPK14	4.9354
O8W-MAPK14	4.2434
MOL000358-EGF	3.418
MOL004328-EGF	2.8761
MOL000098-EGF	1.9288
MOL000497-EGF	1.9118
MOL000392-EGF	1.7448
2PE-EGF	-0.7072
MOL000392-IL4	3.9713
MOL000497-IL4	3.6999
MOL000358-IL4	3.4392
MOL004328-IL4	3.168
MOL000098-IL4	3.0434
NAG-IL4	2.7425
MOL000098-NFKBIA	2.9546
MOL000392-NFKBIA	2.1269
MOL000497-NFKBIA	2.757
MOL004328-NFKBIA	2.7662
MOL000358-NFKBIA	2.6098
GOL-NFKBIA	3.2684

Figure 2), saved in tsv format, and then the file in this format was imported into Cytoscape for visualization, and the results are shown in Figure 3. Then, based on the data imported into Excel, the 122 targets were filtered according to the value greater than or equal to twice the respective median of Degree, Betweenness, LAC, Neighborhood connectivity, Closeness, etc., and 104 targets were obtained and analyzed by using the cytoNCA plug-in in Cytoscape. After filtering the median value, 14 targets were obtained. Then, these 14 targets were screened according to their betweenness value and closeness value (greater than or equal to twice the median, respectively), and five core targets were finally obtained, i.e., IL4, EGF, NFKBIA, MAPK14 and RELA.

3.2 GO Enrichment Analysis and KEGG Enrichment Analysis

The common targets were enriched in 5835 GO terms, including 4744 for biological process (BP) terms, 703 for molecular functions (MF) terms and 388 for cellular components (CC) terms ($p<0.01$). The top 20 GO terms for each category are shown in the bar chart (Figure 4). Specifically, the three highest ranked items of BP in the bar chart were Response to hormone, Response to inorganic substance and Cellular response to nitrogen compound. The top 3 CC terms of the bar chart included Membrane raft, Vesicle lumen, Transcription regulator complex and Plasma membrane protein complex (The figures for the last three were the same).

The top 3 MF terms were Oxidoreductase activity, Protein homodimerization activity and Cytokine activity.

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Table 7. PDB ID for the target proteins

Targets	PDB ID
EGF	3NJP
IL4	3QB7
MAPK14	6Y4T
NFKNIA	6Y1J
RELA	6QHL

Meanwhile, KEGG enrichment analysis indicated that the 122 common targets were significantly enriched in 250 pathways ($p<0.01$). The results of the enrichment analysis of the first 20 KEGG pathways showed that the main pathways included Pertussis, Allograft rejection, Platelet activation, etc. (Figure 5)

3.3 Constructed "Component-Target-Pathway" Network

By integrating the active ingredients, protein interactions in section 3.1, direct targets in 3.2, the top 20 GO functions and the correspondence between KEGG pathways of eczema in Jinhuang Gao in 3.3, and visualizing them with Cytoscape 3.9.1 software, we can obtain the network of "Component-Target-Pathway" network. Then we used the Analyze Network module of Cytoscape 3.9.1 to analyze the network and obtain the characteristic parameters of the network nodes: Degree was used to screen the important targets and effective components by the median value. the target signaling pathway network of anti-eczema medicinal ingredients was drawn by selecting ingredients greater than 2 and targets greater than 5 using the DEGREE value as a filtering condition (Figure 6). The top five highest-ranked components were selected as core components based on the DEGREE value. We obtained specific information on these components and targets to further reveal the core components, targets and mechanism of action of Jinhuang Gao in the treatment of eczema.

3.4 Molecular Docking

SYBYL-X software was used to validate the molecular docking of the five core drug components obtained in 3.2 and the 5 core targets obtained in 3.4 above, to obtain the docking scores of the molecules and targets, and then the file of docking results were imported into PyMOL2.5 for visualization, and the results are shown in Figure 8. The docked amino acid residues and the interactions formed by each component are shown in Table 2. We additionally compared the docking of the protein's original ligands and found that the total scores of the core components were all higher than or approximated those of the original ligands (Table 3 and Figure 7), with the total scores of the docking results of the components beta-sitosterol, quercetin, Licochalcone-A, and naringenin with MAPK14 being higher than 5, indicating that the binding is stable [7,8]. The target proteins with corresponding PDB designations are shown in Table 4. One of the most stable docking results was the binding of Licochalcone-A to the MAPK14 target (6.2852 points), indicating that the golden cream has a direct therapeutic effect on eczema.

4. Discussion

Eczema is one of the most common skin diseases in clinical practice, and its etiology and pathogenesis are still unclear. At present, the etiology of eczema is not clear and is the result of a combination of factors, pathogenesis and immune dysfunction, free radicals, infection and other factors. There are also studies showing that it may be related to internal causes such as heredity, endocrine, nerve, spirit, blood circulation, and external environments such as food, biological environment, chemistry, animal hair, etc. [9,10], and glucocorticoids are mostly used for local treatment [11,12]. However, local topical drug therapy has potential adverse reactions, such as induced infection, burning sensation, skin atrophy, capillary dilation, etc., and easy to form hormone dependence, symptoms recur after stopping the drug [13]. Bi Zhigang [14] believes that the presence of staphylococcus in the skin lesions, general oral antihistamines and external hormone creams play a role in relieving the symptoms, and cannot cure the disease.

Chinese medicine believes that although eczema symptoms are manifested in the skin, the root cause is in the body, and its onset of internal organs, qi and blood dysfunction related to spleen dampness, heart fire, liver and gallbladder dampness and heat [15]. Zhang Zhili [16] summarized the disease mechanism as "this originates from dampness, and then from heat and wind, wind, dampness and heat are intertwined in the skin, or the dryness injury to the yin", which can be seen that dampness is the key to cause eczema, so the dampness is the main method of treatment of eczema. There is a thought in "Neijing" that "dampness and edema in the

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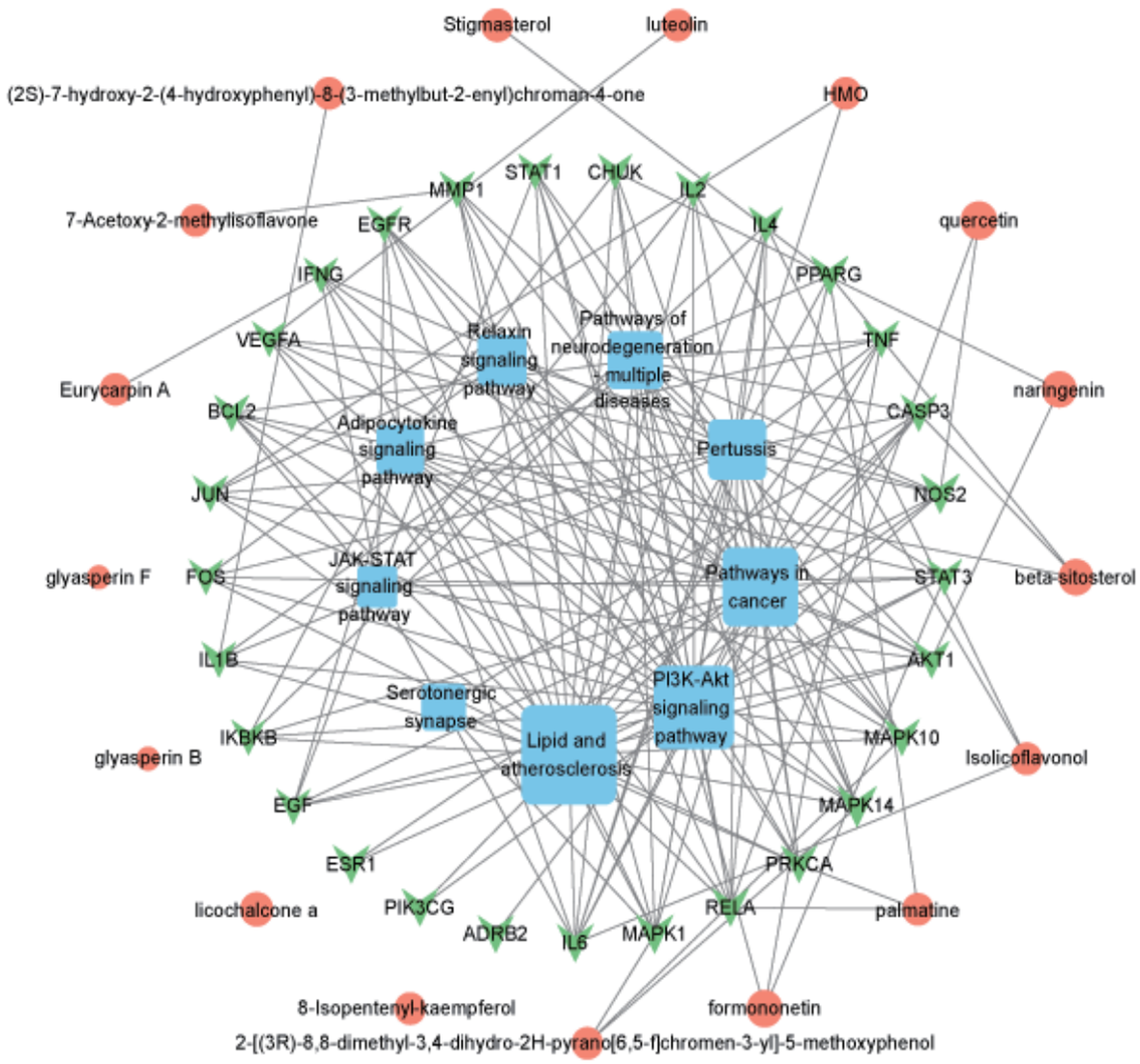

Figure 6 Component-Target-Pathway network of Jinhuang Gao.

body are related to the spleen", which shows that eczema is related to the impaired function of the spleen. It is recorded in "Ling Shu - Carbuncles and Gangrene" that "when Ying Qi is retained in the meridians, the blood will be stagnant and unable to flow freely, and Wei Qi will also be blocked and unable to flow freely, thus generating heat; If the body is overheated, it will cause the muscles to rot and turn into pus". The "Treatise on the Origins and Currents of All Diseases" written by Chao Yuanfang contains "immersion sores, which are caused by wind-heat in the heart, with symptoms appearing on the skin." And he also mentioned that "with its gradual growth, and are therefore called immersion." Chinese medicines usually consist of multiple components with a wide range of pharmacological activities and multiple targets and pathways [17]. However, the characteristics of traditional Chinese medicine may make it difficult to further study the inherent mechanism of disease treatment. According to the pathogenesis and clinical manifestations of subacute eczema, external treatments such as clearing heat and removing dampness, nourishing blood and moistening skin, dispelling wind and relieving itching were determined. In this formula,

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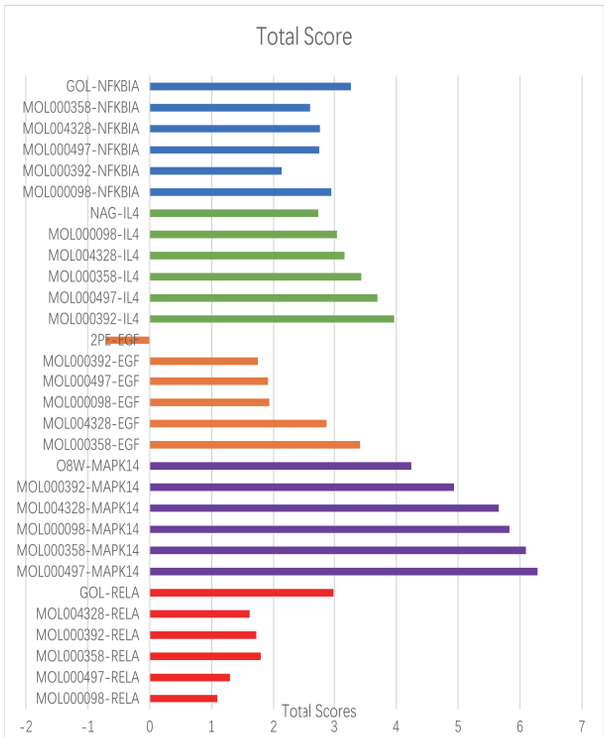

Figure 7. Total Scores of Docking Results.

Zhizi, Dahuang diarrhea and detoxification; Cangzhu, Huangbai and Huanglian clear heat and drying dampness; all the drugs are to help clear the heat and diarrhea, cool the blood and detoxification, dry dampness and stop itching effect. In addition, according to modern medical analysis, the above herbs can play the role of antibacterial and anti-inflammatory, immune regulation, anti-allergy, scavenging free radicals and promoting drug absorption respectively [18-33]. Zhang Jun et al. [4] used Jinhuang Gao to treat eczema, and the total effective rate was higher than that of dan phenol ointment, while the total score improvement was more obvious. Network pharmacology is an organic combination of system biology and histology, which can provide direction for the mechanical research of complex traditional Chinese medicine [34]. In this study, we utilized this method to elucidate the pharmacological mechanism of Jinhuang Gao for the treatment of eczema. First, the compounds in Jinhuang Gao were predicted as eczema-related targets. The results showed that the mechanism of Jinhuang Gao for eczema treatment was complex, involving the interrelationships of multiple components, protein targets and pathways. In addition, molecular docking analysis was performed to verify the binding of the drug to the target proteins. KEGG pathway enrichment mainly involves pathways of neurodegeneration-multiple diseases, Calcium signaling pathway, transcriptional misregulation in cancer, cAMP signaling pathway, adipocytokine signaling pathway, serotonergic synapse, etc. Five core active ingredients were screened in the ingredient-target-pathway map of Golden Cream for the treatment of eczema. The five core active ingredients were verified by molecular docking to have good binding effects with the core target proteins. Quercetin, β-sitosterol, Licochalcone-A, naringenin, and formononetin showed strong binding ability with the targets of NFKBIA, MAPK14, IL4, EGF and RELA, which reflected the characteristics of the multi-component and multi-target synergistic therapeutic effects on eczema of the Jinhuang Gao. Among them, quercetin can effectively inhibit the release of inflammatory mediators, reduce the inflammatory response, and has a variety of pharmacological effects such as antioxidant free radicals, anti-allergy, vasodilatation, etc. [35]. Licochalcone-A can be anti-inflammatory, antibacterial, and rapid absorption and metabolism[36]. Naringenin has antibacterial and anti-inflammatory effects and scavenges oxygen-free radicals [37]. Formononetin has antibacterial and immune-boosting properties [38]. β-Sitosterol has antimicrobial and free radical scavenging effects, in addition to reducing the secretion and expression levels of inflammatory cytokines by inhibiting the activation of inflammatory signaling-related pathways, such as TLR4/NF-KB, NLRP3, STAT and mitogen-activated protein kinases, MAPK [39]. Through the PPI network and molecular docking validation, we obtained 5 core genes with the strongest correlation for the treatment of eczema by Jinhuang Gao, which are NFKBIA, MAPK14, IL4, EGF and RELA, NFKBIA, the most common protein of the IκB family, acts as a repressor of the inhibitor-regulated transcription factor NF-κB and can participate in pathological processes such as inflammation, immune response, and apoptosis, as well as physiological processes such as cell growth and differentiation in vivo by binding NF-κB. [40].Abnormal expression of MAPK14 can lead to physiological dysfunction of the body, triggering dysfunction of the body's immune function, which is closely related to the inflammatory response [41]. IL4 is an important inflammatory mediator that mediates the pathogenesis of atopic dermatitis. [42]. EGF can promote epidermal cell regeneration by stimulating keratinization, proliferation and migration of formation cells, accelerating the renewal and replacement of senescent cells, migrating cells to damaged areas, and accelerating granulation neogenesis. [43-44]. RELA mainly acts on the NF-kB pathway, a signaling system responsible for regulating the inflammatory response [45]. GO functional enrichment analysis indicated that the treatment of eczema with golden ointment may be mainly through

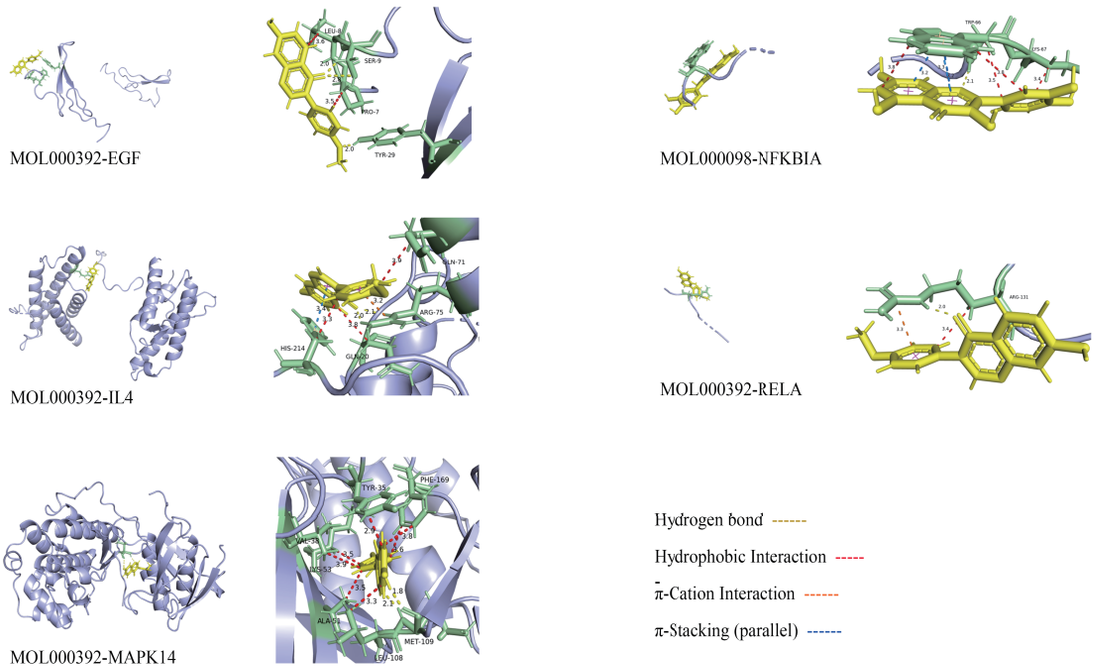

Figure 8. Visualization of docking results.

membrane raft, vesicle lumen, transcription regulator complex, plasma membrane protein complex, perinuclear region of the membrane protein complex, perinuclear region of cytoplasm, extracellular matrix, cell body, etc., regulating molecular functions such as oxidoreductase activity, protein homodimerization activity, cytokine activity, protein kinase activity, etc. kinase activity, thereby affecting response to hormone, response to inorganic substance, cellular response to nitrogen compound, regulation of defense response, negative regulation of cell population proliferation, cellular response to lipid, response to nutrient levels, etc. The results of KEGG pathway enrichment analysis showed that the golden paste The signaling pathways interfering with eczema mainly involved PI3K-Akt signaling pathway, pathways of neurodegeneration-multiple diseases, Relaxin signaling pathway, Calcium signaling pathway, JAK-STAT signaling pathway, Transcriptional misregulation in cancer and so on. Among them, the PI3K-Akt signaling pathway can be activated by many types of cellular stimuli or toxic injuries and regulates basic cellular functions such as transcription, translation, proliferation, growth and survival. It has been well documented that AKT, once activated, can control key cellular processes by phosphorylating substrates involved in apoptosis, protein synthesis, metabolism, and the cell cycle [46], and, in addition, inhibition of the JAK-STAT signaling pathway reduces inflammation and improves cellular homeostasis [47]. The calcium signaling pathway treats eczema by affecting mast cell degranulation [48]. In this study, through network pharmacological analysis and molecular docking validation, we found that beta-sitosterol, quercetin, Licochalcone-A, naringenin, and formononetin were the main active ingredients in the treatment of eczema with Jinhuang Gao and NFKBIA, MAPK14, IL4, EGF and RELA, etc., are the main targets, which involve multiple biological processes such as response to hormone, response to inorganic substance, cellular response to nitrogen compound, etc., and through the regulation of PI3K-Akt signaling pathway, Calcium signaling pathway, JAK-STAT signaling pathway and other pathways to treat eczema. Moreover, molecular docking showed that the core components of Jinhuang Gao have a strong binding ability to the core target proteins.

5. Conclusion

This study used network pharmacology to analyze the mechanism of action of Jinhuang Gao in treating eczema, confirming the complex mechanism of action of Jinhuang Gao with multiple components, multiple targets, and multiple pathways, laying the foundation for its in-depth research. We found that the main core components in Jinhuang Gao, such as beta-sitosterol, quercetin, Licochalcone-A, naringenin and formononetin, may be associated with the modulation

of targets such as NFKBIA, MAPK14, IL4, EGF and RELA, which play a role in the treatment of eczema through the processes of anti-inflammation and immune regulation. In summary, the present study revealed the material basis and possible mechanism of action of Jinhuang Gao for the treatment of eczema and unearthed multiple potential therapeutic targets. In the follow-up study, the effective drug components in Jinhuang Gao can be identified by liquid chromatography and mass spectrometry, and the key targets and pathways screened by network pharmacology can be validated, laying the foundation for clinical application.

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