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Molecular mechanism of Mimenghua Granules in treating dry eye

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Abstract

Objective: To explore the molecular mechanism of Mimenghua Granules in treating dry eye based on network pharmacology and bioinformatics methods. **Methods:** Screening and prediction of possible blood-inducing active ingredients and action target of Mimenghua Granules through Traditional Chinese Medicine Systems Pharmacology database and analysis platform; mining dry eye-related diseases through disease gene database, gene target; use the functional protein combined network database STRING to draw the component-target and disease-target PPI networks, and extract the intersection of these two networks; use DAVID database analysis to screen key targets and analyze the mechanism of action. **Results**: A total of 593 active ingredients related to Mimenghua Granules were retrieved from the Traditional Chinese Medicine Systems Pharmacology database, and 59 blood active ingredients were obtained by screening based on pharmacokinetic parameters, and 680 targets related to these ingredients were retrieved; from disease genes, the database searches for 47 genes directly related to dry eye; 3 key genes (ICAM1, IFNG, and IL-6) were obtained after the intersection of the component target and disease target PPI network; these genes are mainly involved in natural killer cell-mediated cytotoxicity, Jak-STAT signaling pathway, and Cytokine-cytokine receptor interaction. **Conclusion:** The mechanism of Mimenghua Granules in treating dry eye is related to the interaction of cytotoxic pathway, Jak-STAT signal pathway, and cytokines. The key gene targets are ICAM1, IFNG, and IL-6.

Key words: Mimenghua Granules, *Buddlejae Flos*, *Lycii Fructus*, *Chrysanthemi Flos*, Dry eye, Network pharmacology, Bioinformatics.

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***Abbreviations*:**

TCMSP, Traditional Chinese Medicine Systems Pharmacology; TTD, Therapeutic Target Database; Genetic Association Database; GAD, Genetic Association Database; Pharm GKB, pharmacogenetics and pharmacogenomics knowledge-based; OB, oral bioavailability; DL, drug likeness; BP, biological process; CC, cellular component; MF, molecular function; PPI, protein-protein-interaction.

***Competing interests*:**

The authors declare that they have no conflict of interest.

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Background

The International Dry Eye Working Group redefined dry eye in 2017. Dry eye is not only a multifactorial disease of tear and ocular surface but also can cause subjective discomfort, visual disturbance, and tear film instability. [1] Xerophthalmia incidence rate has a rising trend year by year [2, 3], because of its subjective discomfort symptoms to patients with greater pain. The pathogenesis of dry eye is more, with immune response, inflammatory response, hormone levels, tear osmotic pressure, and other factors related [4, 5]. The previous study of our group found that Milkvetch Monkshood Granules (according to the mass ratio of Buddleja Officinalis: Medlar: Chrysanthemum = 1: 1), can inhibit inflammatory reaction and apoptosis of lacrimal gland cells through multiple targets, and has a good application prospect. However, the active ingredients of the granules are not clear nor are the targets of these active ingredients. It greatly limits the further research and development of the Mimenghua Granules and the development of innovative new Chinese medicine drugs. In this paper, the main components of Mimenghua Granules and its mechanism of action in treating Xerophthalmia were analyzed. This study aims to offer some theoretical support for clinical pharmacology research and to provide a basis for further research and development of the granules.

Information and methods

Cytoscape (version 3.6.1); Systematic Pharmacology Database and Analysis Platform (Traditional Chinese Medicine Systems Pharmacology database and analysis platform, TCMSP); Functional Protein Connectivity Network database STRING (version 10.5); annotating, visualizing, and integrating discovery databases. The database for annotation, visualization, and integrated discovery, DAVID 6.8); Drug Bank; Online Human Mendelian Genetic Database (Online Mendelian Inheritance in Man, OMIM); Therapeutic Target Database (TTD); Genetic Linkage Database (the Genetic Association Database, GAD); Pharmacognosy and pharmacogenomics knowledge-based, (pharmacogenetics and pharmacogenomics knowledge-based, Pharm GKB).

Screening of active constituents of Buddleja Officinalis Granula and prediction of its target

Search the TCMSP database for all the chemical composition data of Herba Milkvetch Granules, according to the principle of oral bioavailability (OB) ≥ 30% and drug likeness (DL) ≥ 0.18, the possible active ingredients were screened. At the same time, the TCMSP database was used to predict the target of the active components in the blood, and the molecule and target protein were represented by "node". The active component-target network of Corydalis Officinalis Granules was constructed by the software Cytoscape.

Search for genes known to be specifically associated with dry eye

Using "dry eye" as the keyword, we searched DrugBank, OMIM, TTD, GAD, PharmGKB databases for current known gene targets associated with dry eye.

Gene target analysis for dry eye treated by Mimenghua Granule

The active ingredient target and Xerophthalmia disease target were introduced into STRING (Version 10.5) to draw a protein-protein-interaction (PPI) network, using Cytoscape 3.6.1 software to extract the intersection of the two PPI networks and get the direct or indirect target of Mycronia Monkshood Granules in treating dry eye.

Gene ontology and pathway enrichment analysis for treating xerophthalmia treated by Corydalis Officinalis Granules

Gene ontology enrichment analysis can be divided into three parts: biological process (BP), cellular component (CC), and molecular function (MF). Through the analysis of GO enrichment, the biological function, pathway, or cell location of drug targets can be identified, which can help researchers explore new research directions. KEGG enrichment analysis can be used to analyze the signal pathway of drug target, to understand the signal pathways that change significantly in the course of the disease. This study uses DAVID Bioinformatics Resources 6.8 The GO and KEGG pathway enrichment analysis of direct or indirect targets for dry eye treated with Mimenghua Granules was conducted.

Results

Analysis of chemical composition of flower Granules

A total of 593 chemical constituents were identified, of which 46 were Mimenghua (*Buddlejae Flos*). 8 chemical components were satisfying OB ≥ 30%, accounting for 17.39% of all drug components, and 31 chemical constituents satisfying DL ≥ 0.18. The proportion of all drug components was 67.39%, and the OB ≥ 30% and DL ≥ 0.18 were 4, accounting for 8.70%. A total of 188 Gouqizi (*Lycii Fructus*), 93 chemical components satisfying OB ≥ 30%. It accounted for 49.47% of all drug components, 96 chemical components satisfying DL ≥ 0.18. The proportion of all drug components was 51.06%, and 45 chemical components of OB ≥ 30% and DL ≥ 0.18, accounting for 23.94% of all drugs; Chrysanthemum morifolium (Juhua, *Chrysanthemi Flos*) has a total of 359, 171 chemical components satisfying OB ≥ 30%.

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It accounted for 47.63% of all drug components, 101 chemical components satisfying DL ≥ 0.18. The proportion of all drug components was 28.13%, and 20 chemical components with OB ≥ 30% and DL ≥ 0.18, accounted for 5.57% of all drugs. After eliminating the duplicated components, 59 chemical components with OB ≥ 30% and DL ≥ 0.18 were obtained. The corresponding target genes were 680, as shown in Table 1.

Table 1 Bioactive constituents of Buddleja Miltiorrhiza granules

Component code	Ingredient name	OB value	DL value	Target number
MOL000006	Luteolin	36.16	0.25	57
MOL001689	Acacetin	34.97	0.24	26
MOL001790	Linarin	39.84	0.71	1
MOL006673	butyrospermyl acetate	46.04	0.83	0
MOL000098	Quercetin	46.43	0.28	154
MOL000354	Isorhamnetin	49.60	0.31	37
MOL000358	beta-sitosterol	36.91	0.75	38
MOL000422	Kaempferol	41.88	0.24	63
MOL001506	Supraene	33.55	0.42	0
MOL001733	EUPATORIN	30.23	0.37	14
MOL001755	24-Ethylcholest-4-en-3-one	36.08	0.76	2
MOL001771	Poriferast-5-en-3beta-ol	36.91	0.75	2
MOL002881	Diosmetin	31.14	0.27	10
MOL003044	Chryseriol	35.85	0.27	18
MOL004328	Naringenin	59.29	0.21	37
MOL005100	5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)chroman-4-one	47.74	0.27	10
MOL005229	Artemetin	49.55	0.48	23
MOL007326	Cynarin(e)	31.76	0.68	0
MOL011319	Truflex OBP	43.74	0.24	7
MOL011802	(24r)-saringosterol	39.36	0.79	1
MOL011816	[(1S,5S,7S)-7-acetoxy-5-isopropenyl-2,8-dimethylene-cyclodecyl] acetate	37.02	0.19	8
MOL000449	Stigmasterol	43.83	0.76	31
MOL000953	CLR	37.87	0.68	4
MOL001323	Sitosterol alpha1	43.28	0.78	6
MOL001494	Mandenol	42.00	0.19	3
MOL001495	Ethyl linolenate	46.10	0.20	2
MOL001979	LAN	42.12	0.75	3
MOL003578	Cycloartenol	38.69	0.78	1
MOL005406	Atropine	45.97	0.19	25
MOL005438	Campesterol	37.58	0.71	1
MOL006209	Cyanin	47.42	0.76	2

Component code	Ingredient name	OB value	DL value	Target number
MOL007449	24-methylidenelophenol	44.19	0.75	3
MOL008173	Daucosterol_qt	36.91	0.75	2
MOL008400	Glycitein	50.48	0.24	23
MOL009604	14b-pregnane	34.78	0.34	3
MOL009612	(24R)-4alpha-Methyl-24-ethylcholesta-7,25-dien-3 beta-ylacetate	46.36	0.84	0
MOL009615	24-Methylenecycloartan-3beta,21-diol	37.32	0.80	0
MOL009617	24-ethylcholest-22-enol	37.09	0.75	1
MOL009618	24-ethylcholesta-5,22-dienol	43.83	0.76	2
MOL009620	24-methyl-31-norlanost-9(11)-enol	38.00	0.75	1
MOL009621	24-methylenelanost-8-enol	42.37	0.77	1
MOL009622	Fucosterol	43.78	0.76	3
MOL009631	31-Norcyclolaudenol	38.68	0.81	0
MOL009633	31-norlanost-9(11)-enol	38.35	0.72	1
MOL009634	31-norlanosterol	42.20	0.73	3
MOL009635	4,24-methyllophenol	37.83	0.75	1
MOL009639	Lophenol	38.13	0.71	1
MOL009640	4alpha,14alpha,24-trimethylcholesta-8,24-dienol	38.91	0.76	1
MOL009641	4alpha,24-dimethylcholesta-7,24-dienol	42.65	0.75	3
MOL009642	4alpha-methyl-24-ethylcholesta-7,24-dienol	42.30	0.78	1
MOL009644	6-Fluoroindole-7-Dehydrocholesterol	43.73	0.72	3
MOL009646	7-O-Methylluteolin-6-C-beta-glucoside_qt	40.77	0.30	5
MOL009650	Atropine	42.16	0.19	27
MOL009651	Cryptoxanthin monoepoxide	46.95	0.56	0
MOL009653	Cycloeucalenol	39.73	0.79	0
MOL009656	(E,E)-1-ethyl octadeca-3,13-dienoate	42.00	0.19	1
MOL009660	methyl(1R,4aS,7R,7aS)-4a,7-dihydroxy-7-methyl-1-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxy methyl)oxan-2-yl]oxy-1,5,6,7a-tetrahydrocyclopen ta[d]pyran-4-carboxylate	39.43	0.47	0
MOL009662	Lantadene A	38.68	0.57	0
MOL009664	Physalin A	91.71	0.27	0
MOL009665	Physcion-8-O-beta-D-gentiobioside	43.90	0.62	1
MOL009677	Lanost-8-en-3beta-ol	34.23	0.74	2
MOL009678	Lanost-8-enol	34.23	0.74	3
MOL009681	Obtusifoliol	42.55	0.76	3
MOL010234	Delta-Carotene	31.80	0.55	0

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Target Prediction of Buddleja Officinalis Granules

After excluding the repeated gene target of active components in blood, the Cytoscape 3.6.1 software was used to predict the active components of Corydalis officinalis. A total of 350 nodes, an average of 3.88 targets per target.

Search for targets associated with the development of dry eye

4, 0, 42, 0, and 12 targets were retrieved from TTD, PharmGKB, OMIM, DrugBank and GAD databases, respectively. After removing duplicated genes, 47 target genes related to dry eye were obtained (Table 2 and Figure 1).

Construction of PPI network for the effect of Herba Mimenghua Granules on dry eye

A PPI network was constructed by using STRING database for the active components of Buddleja officinalis granules. There were 188 nodes in the network, and the relationship between the target was 3,347. The PPI network was constructed for the gene targets related to the occurrence and development of dry eye. There were 46 nodes in the network, and the relationship between targets was 65. The intersection networks were extracted from the above two PPI network graphs by using Cytoscape software. According to the topological properties of network nodes, three gene targets were identified which were ICAM1, IFNG and IL-6. (Figures 2 and Figure 3).

Table 2 Target information related to dry eye

Serial number	Target name	Serial number	Target name
1	ICAM1	25	NLRP3
2	ITGAL	26	RPGR
3	ERG11	27	OPN5
4	P2RY2	28	TP63
5	CCR5	29	EDA2R
6	FGF10	30	GPR135
7	AQP5	31	CHRM3
8	HLA-DRA	32	TLR3
9	SCN9A	33	ABCC11
10	ESR1	34	AQP3
11	ESR2	35	ELOVL4
12	IFNG	36	ATP5F1D
13	NFKB1	37	RHO
14	MUC16	38	SRPX
15	EYA1	39	SOX3
16	EYA2	40	SHOX
17	EYA3	41	NRCAM
18	EYA4	42	CTCFL
19	EYS	43	CORIN
20	LMOD1	44	PIEZO2
21	HPS5	45	TRPV3
22	HPS6	46	MUC1
23	ADA2	47	IL-6
24	CECR2		

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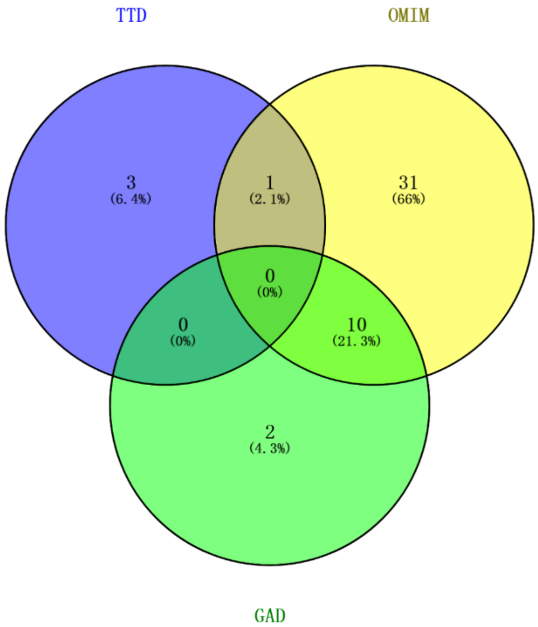

Figure 1 Venn diagram of target information related to dry eye

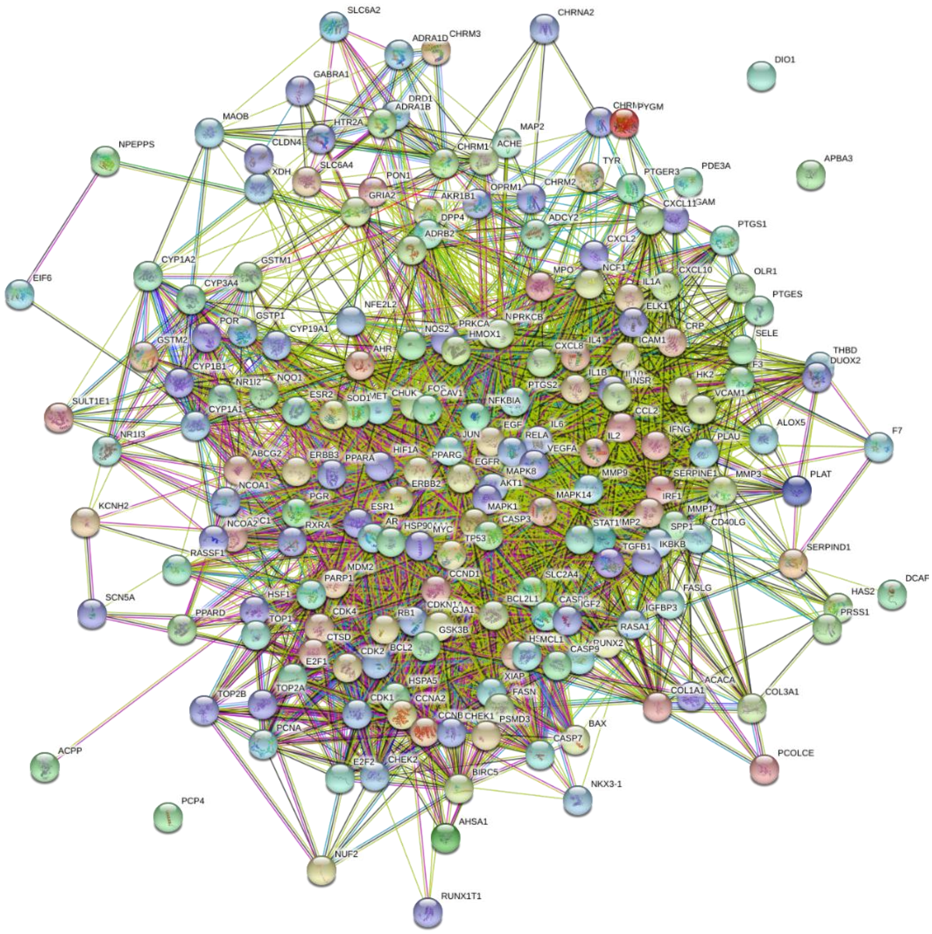

Figure 2 PPI network of gene target of blood component of Milkvetch Monkshood granules

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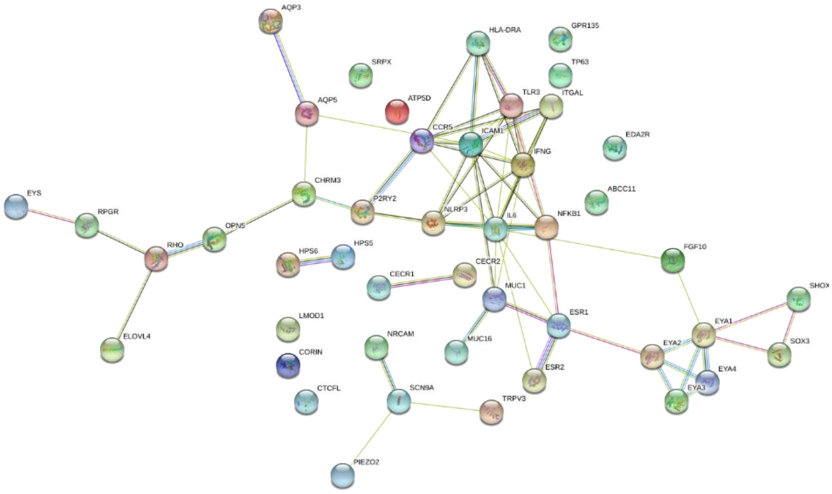

Figure 3 PPI network of dry eye related gene targets

Table 3 Enrichment analysis results of KEGG pathway

Project	Term	Count	P value
KEGG pathway	Natural killer cell mediated cytotoxicity	2	4.5
	Jak-STAT signaling pathway	2	5.8
	Cytokine-cytokine receptor interaction	2	8.0

Mechanism analysis on treatment of dry eye with Mimenghua Granule

GO analysis showed that BP, CC and MF were not enriched by DAVID database analysis. The results of KEGG pathway enrichment analysis showed that the mechanism of MG in treating dry eye mainly involved the cytotoxicity pathway mediated by natural killer cells (Natural killer cell mediated cytotoxicity), Jak-STAT signaling pathway, cytokine-cytokine receptor interaction (Table 3).

Discuss

The pathogenesis of dry eye involves many cellular and molecular processes [6−8]. These include the increase of inflammatory cytokines, the high expression of metalloproteinases, and the change of tear osmolality. The high osmotic pressure of tears changes the physical environment of the ocular surface. Oxidative mitochondrial DNA activates mitochondrial apoptotic pathway and induces lacrimal gland cell apoptosis, which is an important cause of Xerophthalmia [9, 10]. Suzanne Hagan et al. [11] thought that dry eye is a debilitating condition characterized by chronic inflammation, although the initial ocular surface damage in dry eye may be multifactorial (low tear volume, poor tear quality, contact lens use, etc.). But for the most part, the vicious cycle of chronic disease progression seems similar - innate immune pathways are activated, then trigger the adaptive immune response, such as T cell activation, the release of some cytokines, eventually lead to lacrimal gland cell damage, tear secretion deficiency, tear secretory system disorder can reduce the stability of the tear film, forming a vicious cycle. The loss of lacrimal gland function leads to destruction of tear film integrity, which is the main pathogenic factor of dry eye. The main reason for tear film dysfunction is the change of tear mucin and lipid composition. Androgen deficiency is the promoting factor of tear composition change [12].

The research team based on years of clinical experience combined with the pathogenesis of dry eye to create Mimenghua Granules. Sweet taste, slightly cold nature, into the liver, with clearing heat and purging fire nourishing liver, eyesight retreat Yi effect. [13] Modern research has found that flavonoids in the flower have androgen-like effects [14] such as Linarin, Buddletin, Robinia pseudoacacia, etc. play an androgenic role that can inhibit the inflammation of lacrimal gland tissue [15] reduce the death rate of lacrimal gland cells, thereby treating dry eye. Chinese wolfberry sweet, into the liver and kidney, can nourish liver and renal which benefit fine eyesight. Chrysanthemum taste sweet and bitter into the lung and liver, there is wind cooling, liver eyesight. The Seeking Truth of Materia Medica says: “Where the wind and heat incites the eyes and causes loss of nourishment, a covering of the eyes, and dizziness on the head. In the case of dampness and rheumatism arthralgia syndrome, if one takes this sweet and mild agent, he can make fire by leveling the eyes, nourishing the lungs and nourishing the kidneys. When the wood is flat, the wind will cease; when the fire drops, the heat will be removed; and the disease will not heal.” All drugs are used to nourish the liver and kidney, nourish yin and moisten the lungs, and dispel wind and heat. Previous experimental studies found that Mimenghua Granules can inhibit the inflammation of dry eye, and improve the basic tear secretion [16, 17].

In this study, a total of 593 activities related to the granules were retrieved. Among them, 59 active components and 680 related targets were injected into blood. There were many active components of Mycorrhizae Officinalis Granules, which could be used to treat dry eye. The number of targets of 59 active components varied greatly, and quercetin was the most. Up to 154, quercetin is also a kind of flavonoids and has the role of inhibiting inflammation; Kaempferol (63 target sites) is second only to quercetin and is also a flavonoid. Luteolin (luteolin) is a flavonoid with 57 targets. Flavonoids are the main active components in the granules of Corydalis officinalis. These flavonoids may be the major substances in treating dry eye.

47 genes directly related to dry eye were retrieved from the disease gene database. The PPI network analysis showed that the target of treating dry eye mainly involved inflammatory reaction. The key target genes are ICAM1, IFNG and IL-6, which have been identified by our previous experiments [8, 18]. GO enrichment analysis found that the mechanism of MG in the treatment of dry eye is complex, involving cytotoxicity pathway, Jak-STAT signaling pathway, cytokine interaction and so on, which can be verified by experiments in the future. In addition, because the database of traditional Chinese medicine and the disease database is not perfect, there may still be a key target gene in treating dry eye. It is possible to find a new target for dry eye in future experiments.

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