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To explore the mechanism of Erxian decoction in treating insomnia based on network pharmacology

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Abstract

Background: This paper aims to explore the mechanism of Erxian Decoction in treating insomnia by means of network pharmacology research method. **Methods:** The components and related targets of Erxian Decoction are screened by TCMSP database. Disease targets are obtained by OMIM and Gene Cards database, and the common targets of drugs and diseases are obtained. The network diagram of "drug-component-disease-target" is constructed and analyzed. STRING database constructs PPI network and finds the core target. GO and KEGG enrichment analysis are employed on intersection targets. **Results:** 84 effective components and 169 drug targets of Erxian Decoction as well as 2614 targets of insomnia are screened out. Seventy-two intersection targets are selected by Venn diagram, and the core targets include IL-6, TNF, VEGFA and L-1β. These intersection targets contain 404 GO processes and 67 KEGG pathways, including TOLL-like receptor signaling pathway, cyclic adenosine monophosphate (cAMP) signaling pathway and tumor necrosis factor (TNF) signaling pathway. **Conclusion:** Erxian Decoction may play a role in treating insomnia by regulating TOLL-like receptor signaling pathway, cyclic adenosine monophosphate (cAMP) signaling pathway and tumor necrosis factor (TNF) signaling pathway.

Keywords: erxian decoction; traditional Chinese medicine; insomnia; network pharmacology

***Competing interests*:**

The authors declare no conflicts of interest.

Data availability statement:

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Background

Insomnia is a relatively common clinical sleep disorders.According to the statistics of the World Health Organization (WHO), the global rate of sleep disorders is 27%, and more than 300 million people have sleep disorders, among which the incidence of adult insomnia is as high as 38.2%, and this figure is still rising year by year [1].Studies have shown that insomnia can increase the risk of cardiovascular metabolic diseases, including changes in blood pressure, impaired heart rate variability, and increased mortality [2]. It is an important risk factor for cardiovascular diseases, depression, and some cancers [3-5].At present, benzodiazepines are the most widely used drugs in the clinical treatment of insomnia in western medicine, but they have side effects such as central nervous system inhibition, movement disorders, memory impairment, etc., and long-term application can induce dependence and addiction, and the efficacy cannot be sustained after drug withdrawal [6].Therefore, it is an important task to explore safe and effective drugs for insomnia. Studies have shown that the treatment of insomnia with modified Erxian Decoction has a significantly better effect than the treatment group with western medicine alone [7], and can significantly improve the insomnia, dreaminess and other related diseases of patients. Previous studies have found that modern Erxian Decoction has complex ingredients and numerous targets, and its mechanism of action is still unclear. Therefore, this study used network pharmacology method to establish Erxian Decoction - component - target - insomnia interaction network, explain the possible mechanism of Erxian Decoction in the treatment of insomnia, and provide a new treatment method for insomnia prevention and treatment.

Materials and methods

Screening of active ingredients and target of Erxian decoction

Through the Chinese medicine pharmacology system database search and analysis platform TCMSP (http://ibts.hkbu.edu.hk/lsp/tcmsp.php), with "curculigo, epimedium, medicinal indianmulberry root, Chinese angelica, cortex phellodendri, rhizoma anemarrhenae" as a keyword query, get two fairy soup candidate components.Oral bioavailability (OB) OB≥ 30% and drug-like (DL) DL≥0.18 were set for screening to obtain the effective ingredients of Erxian Decoction.The MOLID of the component is used to query the relevant drug target.

Gene name annotation

The gene names of related targets obtained in 1.1 were normalized by Uniprot database (www. uniprot.org /).

Screening of disease targets

In the OMIM database (http://omim.org/) and Genecards database (https://www.genecards.org), enter the keywords "insomnia", search target disease.Using Venny platform (version 2.1, bioinfogp. Cnb.csic. es/tools/Venny), the target of Erxian decoction for treating Insomnia was intersected with the disease target, and the target of Erxian decoction for treating Insomnia was obtained.

Protein interaction network and core target screening

Import the intersection target obtained in 1.3 into the String database [15] Protein-protein Interaction (PPI) was obtained, and the core targets were found by sorting them according to the connectivity of targets.

Gene Oncology (GO) biological process analysis

The intersection targets were imported into David database (HTTP: // www.david.niaid.nih.gov) for GO enrichment analysis of gene function.This database provides detailed annotation of gene function from three aspects: cellular component, molecular function and biological process.The threshold values $P < 0.05$ and $Q < 0.05$ were set for screening, and the function enrichment analysis of the intersection target was conducted.

Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) gene pathway

The intersection targets import KOBAS3.0 pathway analysis in the database (kobas.cbi.pku.edu.cn).Select "Gene Symbol", select "Homo Sapiens", check "KEGG", and set $P < 0.05$ for pathway enrichment analysis.

"drug-disease-component-target" network construction

Cytoscape3.7.2 was used to build the network diagram, and the "drug-disease-component-target" network table and the attribute table were made.Nodes of different colors and shapes represent drugs, ingredients, diseases, and targets.

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Results

Chemical composition collection results of Erxian Decoction

A total of 646 compounds were collected from the screening of active ingredients of Erxiandecoction, including 78 species of Xianmao, 130 species of Epimedium, 174 species of Morinda officinalis, 125 species of Angelica sinensis, 58 species of Phelhelia chinensis and 81 species of Anemarrhenae. If OB≥30% and DL≥ 0.18 were selected as conditions, a total of 84 active ingredients were found to meet the criteria, as shown in Table 1.

Target collection results of active ingredients

A total of 3758 targets were obtained by using the screened active ingredients to predict the target, and 169 targets were obtained after deconduplication.

Disease gene collection and core target screening

A total of 2614 targets related to insomnia were identified in OMIM and GeneCards databases.After crossing drug targets and disease targets, 72 targets that may be related to Erxian Decoction in the treatment of insomnia were finally obtained, as shown in Figure 1.

Table 1 The Active Ingredient of Er-Xian-Tang

MOL_ID	Molecule_Name	OB	DL
MOL000358	beta-sitosterol	36.91	0.75
MOL000449	Stigmasterol	43.83	0.76
MOL000622	Magnograndiolide	63.71	0.19
MOL000762	Palmidin A	35.36	0.65
MOL000785	palmatine	64.6	0.65
MOL000787	Fumarine	59.26	0.83
MOL000790	Isocorypalmine	35.77	0.59
MOL001454	berberine	36.86	0.78
MOL001458	coptisine	30.67	0.86
MOL002636	Kihadalactone A	34.21	0.82
MOL002641	Phellavin_qt	35.86	0.44
MOL002643	delta 7-stigmastenol	37.42	0.75
MOL002644	Phellopterin	40.19	0.28
MOL002651	Dehydrotanshinone II A	43.76	0.4
MOL002652	delta7-Dehydrosophoramine	54.45	0.25
MOL002656	dihydroniloticin	36.43	0.81
MOL002659	kihadanin A	31.6	0.7
MOL002660	niloticin	41.41	0.82
MOL002662	rutaecarpine	40.3	0.6
MOL002663	Skimmianin	40.14	0.2
MOL002666	Chelerythrine	34.18	0.78
MOL002668	Worenine	45.83	0.87
MOL002670	Cavidine	35.64	0.81
MOL002671	Candletoxin A	31.81	0.69
MOL002672	Hericenone H	39	0.63
MOL002673	Hispidone	36.18	0.83
MOL013352	Obacunone	43.29	0.77
MOL000422	kaempferol	41.88	0.24
MOL000483	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide	118.35	0.26
MOL000546	diosgenin	80.88	0.81
MOL000631	coumaroyltyramine	112.9	0.2
MOL001677	asperglaucide	58.02	0.52
MOL003773	Mangiferolic acid	36.16	0.84
MOL004373	Anhydroicaritin	45.41	0.44
MOL004489	Anemarsaponin F_qt	60.06	0.79
MOL004492	Chrysanthemaxanthin	38.72	0.58
MOL004497	Hippeastrine	51.65	0.62
MOL004514	Timosaponin B III_qt	35.26	0.87

Table 1 The Active Ingredient of Er-Xian-Tang

MOL_ID	Molecule_Name	OB	DL
MOL004528	Icariin I	41.58	0.61
MOL004540	Anemarsaponin C_qt	35.5	0.87
MOL004542	Anemarsaponin E_qt	30.67	0.86
MOL000359	sitosterol	36.91	0.75
MOL001506	Supraene	33.55	0.42
MOL002879	Diop	43.59	0.39
MOL002883	Ethyl oleate (NF)	32.4	0.19
MOL006147	Alizarin-2-methylether	32.81	0.21
MOL009495	2 - hydroxy - 1, 5 - dimethoxy - 6 - (methoxymethyl) - 9, 10 - anthraquinone	95.85	0.37
MOL009496	1, 5, 7 - trihydroxy - 6 - methoxy - 2 - methoxymethylanthracenequinone	80.42	0.38
MOL009500	1, 6 - dihydroxy - 5 - methoxy - 2 - (methoxymethyl) - 9, 10 - anthraquinone	104.54	0.34
MOL009503	1 - hydroxy - 3 - methoxy - 9, 10 - anthraquinone	104.33	0.21
MOL009504	1-hydroxy-6-hydroxymethylanthracenequinone	81.77	0.21
MOL009513	2 - hydroxy - 1, 8 - dimethoxy - 7 - methoxymethylanthracenequinone	112.3	0.37
MOL009519	(2R,3S)-(+)-3',5-Dihydroxy-4 ,7-dimethoxydihydroflavonol	77.24	0.33
MOL009524	3beta,20(R),5-alkenyl-stigmastol	36.91	0.75
MOL009525	3beta-24S(R)-butyl-5-alkenyl-cholestol	35.35	0.82
MOL009537	americanin A	46.71	0.35
MOL009541	Asperuloside tetraacetate	45.47	0.82
MOL009551	isoprincepin	49.12	0.77
MOL009558	2-hydroxyethyl 5-hydroxy-2-(2-hydroxybenzoyl)-4-(hydroxymethyl)benzoate	62.32	0.26
MOL009562	Ohioensin-A	38.13	0.76
MOL000006	luteolin	36.16	0.25
MOL000098	quercetin	46.43	0.28
MOL001510	24-epicampesterol	37.58	0.71
MOL001645	Linoleyl acetate	42.1	0.2
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
MOL001792	DFV	32.76	0.18
MOL003044	Chryseriol	35.85	0.27
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL004367	olivil	62.23	0.41
MOL004380	C - Homoerythrinan, 1, 6 - didehydro - 3,15,16 - trimethoxy -, - (3. Beta.)	39.14	0.49
MOL004382	Yinyanghuo A	56.96	0.77
MOL004384	Yinyanghuo C	45.67	0.5
MOL004386	Yinyanghuo E	51.63	0.55
MOL004388	6 to 11, 12 - hydroxy - dimethoxy - 2, 2 - dimethyl - 1, 8 - dioxo,3,4,8 - tetrahydro - 2-1 h - isochromeno [3, 4 - h] isoquinolin - 2 -ium	60.64	0.66
MOL004391	8-(3-methylbut-2-enyl)-2-phenyl-chromone	48.54	0.25
MOL004394	Anhydroicaritin-3-O-alpha-L-rhamnoside	41.58	0.61
MOL004396	1, 2 - bis (4 - hydroxy - 3 - methoxyphenyl) propan - 1, 3 - diol	52.31	0.22
MOL004425	Icariin	41.58	0.61
MOL004427	Icariside A7	31.91	0.86
MOL001607	ZINC03982454	36.91	0.76
MOL003578	Cycloartenol	38.69	0.78
MOL004114	3, 2 ', 4 ', 6 '- Tetrahydroxy - 4, 3' - dimethoxy chalcone	52.69	0.28
MOL004125	Curculigoside B_qt	83.36	0.19
MOL004146	curculigosaponin C	39.31	0.19

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Screening of core targets

The protein interaction PPI network constructed by String database contains 28 target protein nodes, and there are 227 interaction lines in total, with an average degree of 16.2. Among them, APP, IL6, NOS3, TNF, CAT, AChE, VEGFA, SLC6A4, IL1B, MAOA and so on have large degree values (Figure 2 and Figure 3).

Results of GO gene function enrichment analysis

Key targets of the intersection of diseases and drugs were input into David database, and 404 GO items were screened out according to P ≤0.05 and Q ≤0.05. Among them, 299 were Biological processes (BP), accounting for 74%;39 Cellular components (CC), accounting for 10%;66 Molecular functions (MF), accounting for 16%, as shown in Figure 4.

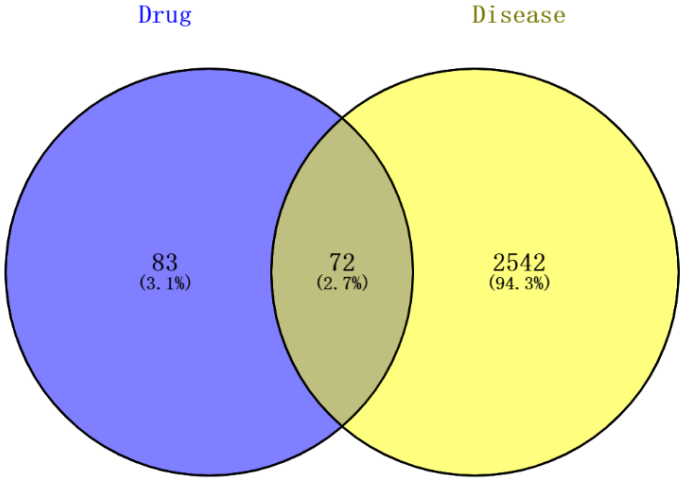

Figure 1 Venn plot of drug target - disease target

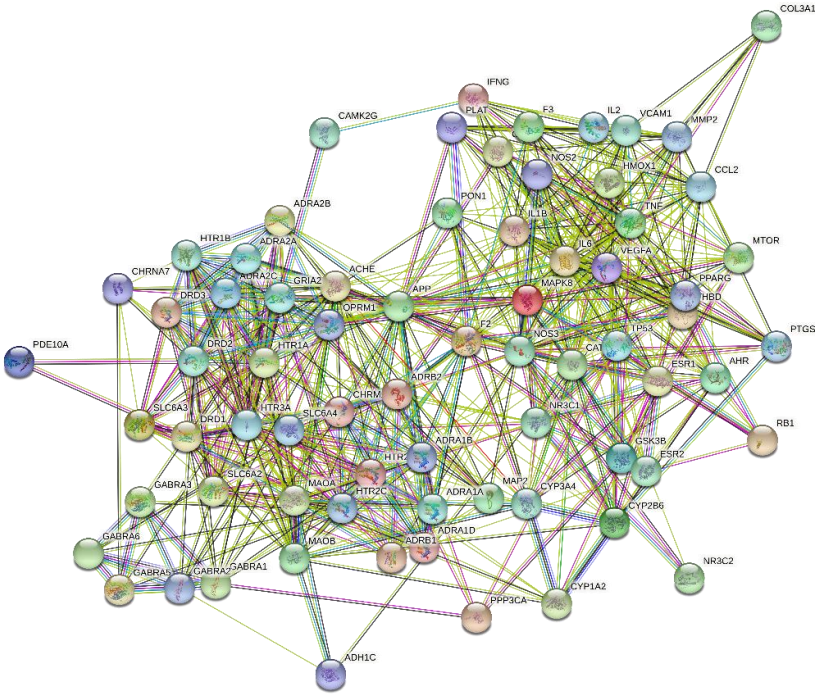

Figure 2 PPI network of Erxian Decoction in the treatment of insomnia

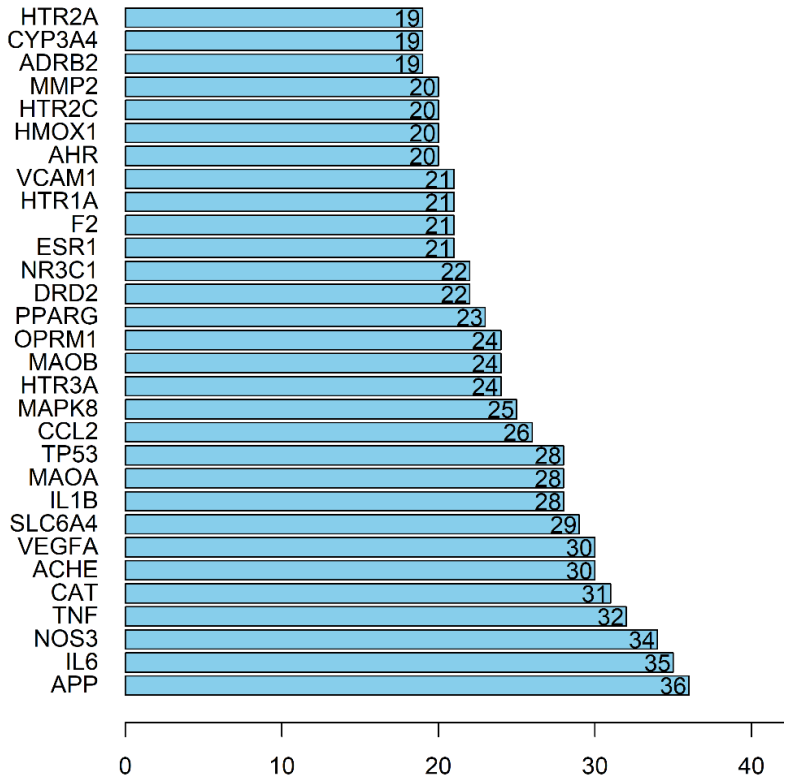

Figure 3 Core genes of Erxian Decoction in the treatment of insomnia

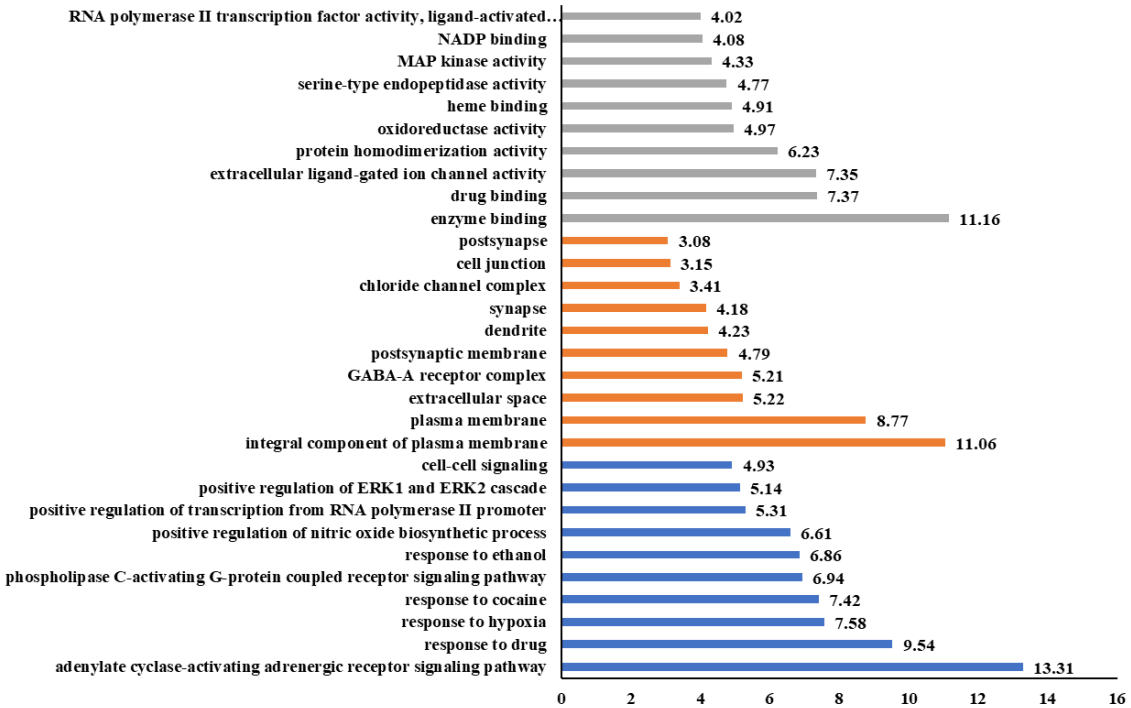

Figure 4 Functional enrichment analysis of GO gene

Results of KEGG gene pathway enrichment analysis

Key targets of the intersection of disease and drug were input into KOBAS 3.0 database, and a total of 67 pathways were enriched. Select the top 15 pathways according to the P value.Analysis showed that targets were significantly enriched in Toll-like receptor signaling pathway, TNF signaling pathway, cAMP signaling pathway and other pathways (Table 2).

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Table2 KEGG enrichment analysis of the targets of Er-Xian-Tang

No.	Term	Pathways	Number of genes	-Log(p)
1	hsa04620	Toll-like receptor signaling pathway	26	17.8041
2	hsa04020	Calcium signaling pathway	14	7.917215
3	hsa04668	TNF signaling pathway	11	6.452225
4	hsa04726	Serotonergic synapse	10	5.978811
5	hsa05033	Nicotine addiction	7	5.696804
6	hsa04024	cAMP signaling pathway	10	4.712198
7	hsa05032	Morphine addiction	8	4.59176
8	hsa04066	HIF-1 signaling pathway	8	4.440093
9	hsa05031	Amphetamine addiction	7	4.407823
10	hsa05142	Chagas disease (American trypanosomiasis)	8	4.215383
11	hsa05144	Malaria	6	3.987163
12	hsa05030	Cocaine addiction	6	3.987163
13	hsa05143	African trypanosomiasis	5	3.565431
14	hsa05332	Graft-versus-host disease	5	3.565431
15	hsa05152	Tuberculosis	9	3.533132

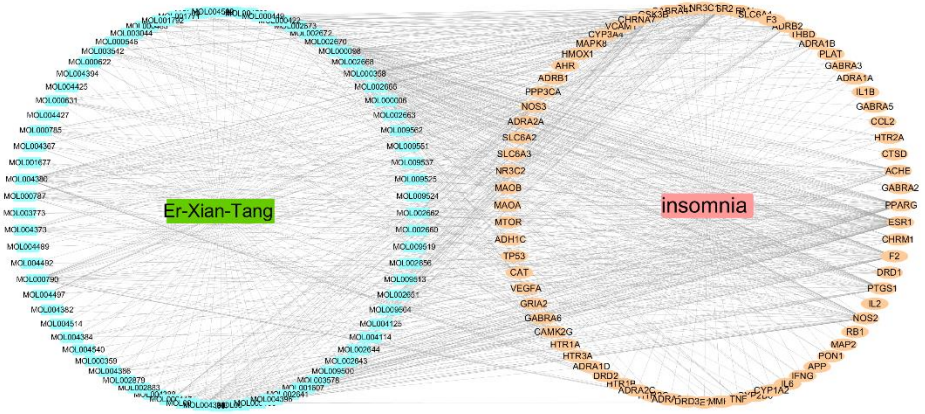

Figure 5 Network diagram of "component - target - pathway" of Insomnia treated by Erxian decoction

Construction of drug-disease-component-target network

The network relationship table of "drug-disease-component-target" was made, which was imported into Cytoscape3.7.2 software, and the drug-disease-component-target network diagram of Erxian Decoction for treating Insomnia was constructed.As shown, each active ingredient acts on multiple targets (Figure 5).

Discussion

Erxian Decoction is an empirical recipe developed by Professor Zhang Bone in the 1950s. It has the effects of warming and tonifying the spleen and kidney, filling the blood and nourishing the body, and harmonizing and invigorating the body [8]. Studies have shown that it can treat insomnia [7], but the specific mechanism of action remains to be studied. In this study, network pharmacological methods were used to explore the potential pharmacodynamic components, target and signal pathway of Erxian Decoction in the treatment of insomnia.

Two Immortals Decoction is composed of Xianmao, Epimedium, Morinda officinalis, Cypress Phelobus, Anemarrhenae and Angelica Angelica. Through TCMSP database, 84 active ingredients of Erxien Decoction were obtained, including Coumaroyl tyramine, diosgenin, β-sitosterol, Stigmasterol and other active ingredients. Coumaroyl tyramine is a phenolic substance, which may play a calming and tranquilizing role through anti-thrombus and other pathways [9]. Diosgenin mediated targeting of high-mobility group protein 1(HMGB-1)/TLR4 and NF-κB signaling has been shown to change the expression of pro-inflammatory cytokines in the case of brain injury [10] and play a calming and calming role by inhibiting the

inflammatory response. Moringa oleiformis oil rich in β-Sitosterol and Stigmasterol can enhance pentobarbitural-induced sleep behavior in mice through the GABA-ergic system [18].

According to PPI network, we found that the key targets of Erxian Decoction in the treatment of insomnia were IL-6, TNF, VEGFA and IL-1β.TNF is recognized as one of cytokines to damage neurons, probably by promoting brain serotonin (5-HT) synthesis and regulating sleep [11], also can through collaborative other inflammatory factors such as beta, IL - 6, IL - 1 to change neuroendocrine activity, cause genetic activation in different brain regions, to maintain the human activities such as learning and memory and sleep [12]. Many studies have found that insomnia is related to the regulation of the expression of certain inflammatory genes, such as TNF-α, IL-1β and IL-6 [13].Insomnia is associated with changes in vascular endothelial growth factor (VEGF) [14]. The levels of VEGF in blood circulation are increased in patients with sleep apnea [15].

KEGG enrichment analysis showed that Er-xian decoction in the treatment of insomnia is related to the cAMP signaling pathway[16], TNF signaling pathway[11], and toll-like receptor signaling pathway[17].TNF signaling pathway is an important and inflammation-related pathway in the pathogenesis of insomnia [2].Studies have shown that cAMP can participate in sleep and circadian rhythm regulation through PKA, causing prolonged slow-wave sleep and total sleep time, and shortening the latency period to fall asleep, with significant sleep-promoting effects [19].Toll-like receptor 4 (TLR4 signaling pathway) in the Toll-like receptor signaling pathway activates an inflammatory signaling cascade associated with both endogenous and pathogen-associated ligands associated with sleep loss [2].

Conclusion

In conclusion, this study preliminarily explored the active ingredients, potential targets and potential action pathways of Erxian Decoction in the treatment of insomnia, which provided the pharmacologic substance basis and research direction for clinical application, and also provided a new idea for the treatment of insomnia with traditional Chinese medicine. However, its specific mechanism of action needs to be further verified by experiments.

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