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Exploration of the Potential Mechanism of Oroxylum indicum in the Treatment of Chronic Gastritis Based on Network Pharmacology

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Abstract. Chronic gastritis is a common clinical digestive disease prone to recurrent attacks, and long-term persistent illness can lead to precancerous lesions. *Oroxylum indicum* is a traditional Chinese herbal medicine sweet in flavor and cool in nature, commonly used to treat epigastric pain and epigastric stuffiness caused by stomach heat and qi stagnation. In this study, network pharmacology was adopted to screen the common targets of active ingredients of *Oroxylum indicum* and chronic gastritis, construct a protein-protein interaction network, and conduct GO and KEGG enrichment analysis. The results showed that the targets of *Oroxylum indicum* were mainly concentrated in biological processes such as inflammatory response, cell proliferation and kinase regulation, and were significantly enriched in IL-17, VEGF, metabolism and tumor-related signaling pathways. This study elaborates the action characteristics of *Oroxylum indicum* in the treatment of chronic gastritis from the perspective of bioinformatics, and provides a theoretical basis for subsequent experimental research.

Keywords: *Oroxylum indicum*, chronic gastritis, network pharmacology

1. Introduction

Chronic gastritis is an extremely common digestive disease worldwide. Characterized by chronic inflammation of the gastric mucosa, chronic gastritis is an important precancerous condition of gastric cancer [1]. A meta-analysis of chronic gastritis treatment indicated that the traditional Chinese medicine (TCM) group was significantly superior to the conventional western medicine group in overall clinical efficacy (OR=4.65; 95%CI: 3.29-6.56; P<0.00001), and the incidence of adverse reactions in the TCM group was lower than that in the western medicine group [2]. Modern pharmacological studies have shown that *Oroxylum indicum* contains various active ingredients such as flavonoids and their glycosides, and volatile oils, and exerts multiple pharmacological effects including antibacterial, anti-inflammatory and anti-cancer activities. Its extract has shown significant gastroprotective effects in an ethanol-induced gastric mucosal injury model [3, 4]. However, existing studies lack the elucidation of the molecular mechanism of *Oroxylum indicum* in the treatment of chronic gastritis. Network pharmacology conforms to the holistic features of TCM with multi-component, multi-target and multi-pathway integrated regulation, and can well predict

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the action network of drugs [5]. Based on the research method of network pharmacology, this paper explores the core targets and signaling pathways of *Oroxylum indicum* in the treatment of chronic gastritis, so as to provide bioinformatics support for the research on its pharmacological mechanism.

2. Research methods

2.1. Screening of active ingredients and targets of *Oroxylum indicum*

The Traditional Chinese Medicine Systems Pharmacology Database (TCMSP) was used to retrieve all chemical components of *Oroxylum indicum*, and active ingredients were screened with oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. SwissTargetPrediction was applied to predict the potential targets of each active ingredient. After removing duplicate entries, all targets were uniformly converted into standard human gene names to establish a drug target dataset.

2.2. Acquisition of chronic gastritis disease targets

With "chronic gastritis" as the search term, disease databases including GeneCards, OMIM, Gastritis and Drugbank were retrieved. Targets related to disease pathogenesis and regulation were collected, and duplicate targets were deleted to establish a chronic gastritis target dataset.

2.3. Construction of intersection targets and protein-protein interaction network

A Venn diagram was used to obtain the common targets of *Oroxylum indicum* and chronic gastritis. The common targets were imported into the STRING database to construct a protein-protein interaction (PPI) network. Key hub genes in the network topology were screened with combined score > 0.4, and free nodes were excluded. Subsequently, Cytoscape 3.10.4 was used for visualization and structural analysis of the network.

2.4. GO and KEGG enrichment analysis

The DAVID database was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on the intersection targets. GO consists of three parts: biological process, cellular component and molecular function, and the top ten entries were selected according to count score. The exported enrichment results were uploaded to the Bioinformatics online analysis platform for visualization mapping, so as to elucidate the molecular action pathways of *Oroxylum indicum* in interfering with chronic gastritis.

2.5. Construction of herb-target-pathway-disease interaction network

Four types of node information, namely *Oroxylum indicum*, intersection targets of *Oroxylum indicum* and chronic gastritis, KEGG enriched pathways, and chronic gastritis disease, were sorted out and imported into Cytoscape 3.10.4 software to construct a herb-target-pathway-disease interaction network. Node styles were uniformly set to distinguish different levels, and gray lines were used to represent the interaction relationships between nodes, so as to visually present the multi-level regulatory relationship of *Oroxylum indicum* in interfering with chronic gastritis by regulating multiple signaling pathways through core targets.

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3. Results

3.1. Screening results of drug and disease targets

After screening by TCMSP, a total of 12 active compounds of *Oroxylum indicum* were obtained (Table 1). 318 potential action targets were obtained after prediction by SwissTargetPrediction; 1935 chronic gastritis-related disease targets were retrieved from disease databases.

Table 1. Screening of active compounds of *Oroxylum indicum*

Mol ID	Molecule Name	OB(%)	Caco-2	DL	FASA-	HL
MOL012101	Mosloflavone	34.04	0.86	0.26	0.28	16.76
MOL012108	Negletein	41.16	0.85	0.23	0.3	15.9
MOL013058	2,5-dihydroxy-6,7-dimethoxyflavone	41.52	0.33	0.28	0.28	16.07
MOL013059	3,7,3',5'-tetramethoxy-2hydrochroxyflavone	53.26	0.05	0.42	0.22	15.13
MOL000173	wogonin	30.68	0.79	0.23	0.32	17.75
MOL001735	Dinatin	30.97	0.48	0.27	0.28	16.44
MOL002714	baicalein	33.52	0.63	0.21	0.36	16.25
MOL002928	oroxylin a	41.37	0.76	0.23	0.29	17.15
MOL003044	Chryseriol	35.85	0.39	0.27	0.32	16.31
MOL004004	6-OH-Luteolin	46.93	0.14	0.28	0.35	14.62
MOL000471	aloe-emodin	83.38	0.12	0.24	0	31.49
MOL000098	quercetin	46.43	0.05	0.28	0.38	14.4

3.2. Screening results of drug-disease intersection targets

As shown in the Venn diagram, 116 common regulatory genes were obtained by intersecting drug and disease targets, which were taken as the common target set of *Oroxylum indicum* in interfering with chronic gastritis (Figure 1).

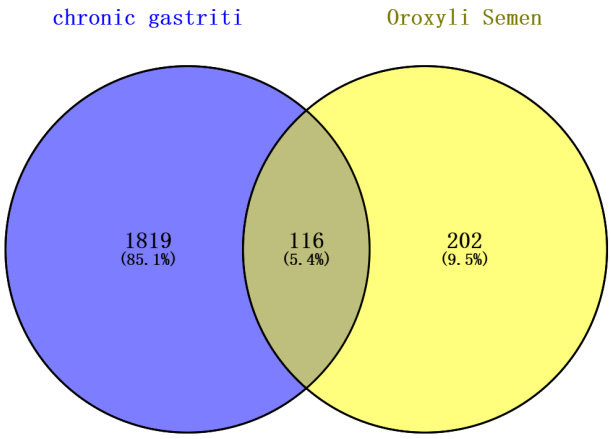

Figure 1. Intersection target genes of "*Oroxylum indicum* - chronic gastritis"

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3.3. GO functional enrichment analysis

GO enrichment is divided into three categories: BP (biological process), CC (cellular component) and MF (molecular function). For biological processes, the top-ranked entries include regulation of cell proliferation, negative regulation of apoptotic process, inflammatory response to lipopolysaccharide, protein phosphorylation, and response to oxidative stress, suggesting that *Oroxylum indicum* may protect the gastric mucosa by inhibiting inflammation, regulating cell growth and repairing oxidative damage. For cellular components, target proteins are mainly distributed in cytoplasm, protein complexes, nucleus, mitochondria and cell membrane adhesion regions, indicating that the action sites are mainly intracellular signal transduction structures. For molecular functions, the core enriched entries are protein kinase activity, enzyme binding, ATP binding and transferase activity, suggesting that the active substances of the drug may exert pharmacological effects by regulating kinase signals and energy metabolism (Figure 2).

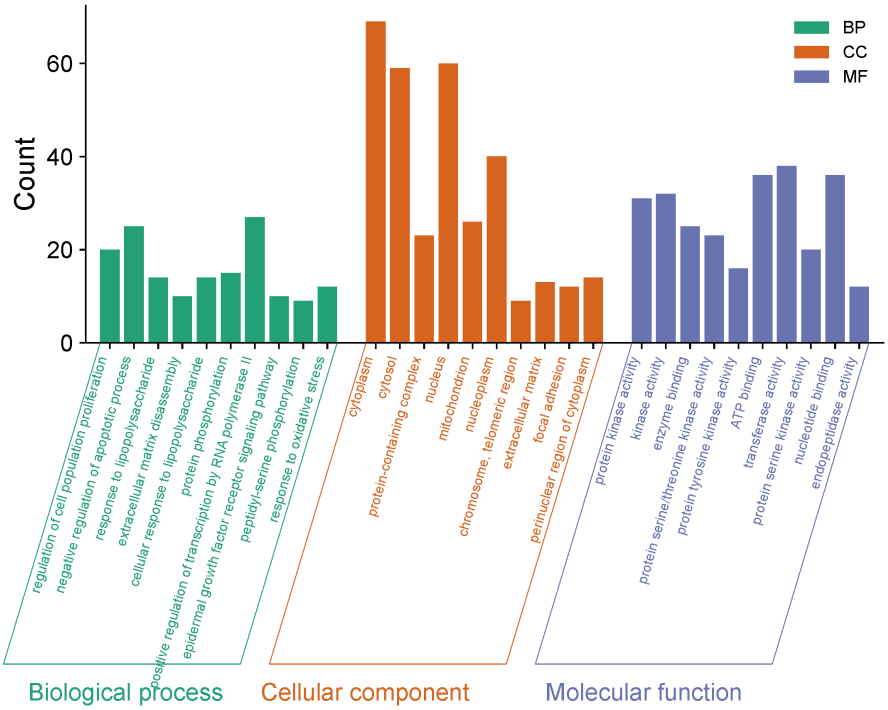

Figure 2. GO enrichment analysis

3.4. KEGG signaling pathway enrichment analysis

According to the biological functions of pathways, the significantly enriched pathways can be divided into three categories: The first category is inflammation-related pathways, including IL-17 signaling pathway, VEGF signaling pathway and Prolactin signaling pathway, which are mainly involved in regulating inflammatory cell infiltration in gastric mucosa and the process of angiogenesis and repair of damaged mucosa. After binding to receptors, the IL-17 signaling pathway can sequentially activate downstream cascade signals, and is widely involved in the body's inflammatory response, autoimmune injury and tumor occurrence and development. The second category is tumor-related regulatory pathways, covering Bladder cancer, Prostate cancer, and PD-L1 expression and PD-1 checkpoint pathway in cancer, suggesting that *Oroxylum indicum* can block the progression of chronic gastritis to gastric precancerous lesions by regulating such pathways. The

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third category is metabolic regulation pathways, including Caffeine metabolism and Central carbon metabolism in cancer, which are responsible for maintaining the energy metabolism homeostasis of gastric tissue cells. Among them, the IL-17 signaling pathway and VEGF signaling pathway have the highest number of enriched genes and the highest enrichment significance among all pathways, and are the core regulatory pathways of *Oroxylum indicum* screened in this study (Figure 3).

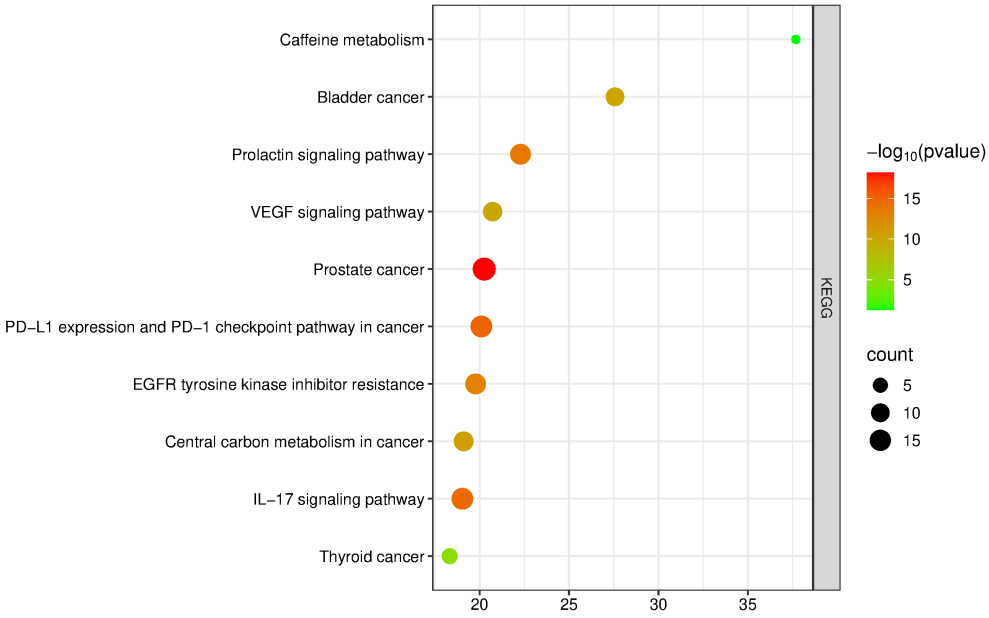

Figure 3. KEGG pathway enrichment analysis

3.5. Core genes of protein-protein interaction network

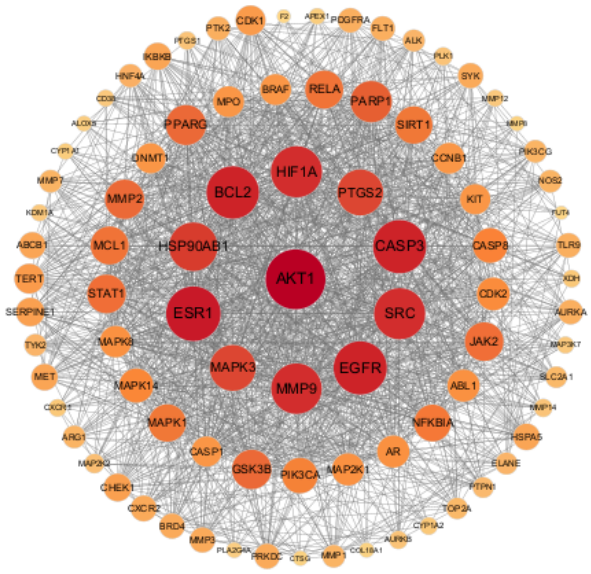

Figure 4. PPI network of "drug-disease" target genes

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Topological analysis of the PPI network screened out hub core genes such as AKT1, TNF, IL6, VEGF and TP53, all of which are involved in key pathophysiological processes such as gastric mucosal inflammatory response, cell apoptosis and vascular remodeling. The analysis conclusions are mutually confirmed with the previous GO and KEGG enrichment analysis results, further verifying the reliability of target regulatory pathways (Figure 4).

3.6. Analysis of herb-target-pathway-disease interaction network

Four types of nodes were integrated to construct a multi-level "herb-target-pathway-disease" interaction network. The network contains four types of differentiated nodes: the pink circular node on the left represents the single TCM herb *Oroxylum indicum*, the pink circular node on the right represents chronic gastritis disease, the green square nodes are the common potential therapeutic targets of *Oroxylum indicum* and chronic gastritis, and the yellow diamond nodes are the key signaling pathways obtained by KEGG enrichment. Gray lines between nodes represent molecular regulatory interactions. A single herb *Oroxylum indicum* can simultaneously target hundreds of disease intersection targets, and a single target can cross-participate in the regulation of multiple KEGG signaling pathways, which intuitively presents the holistic action characteristics of *Oroxylum indicum* with "multi-component, multi-target and multi-pathway" in the intervention of chronic gastritis, and fully elucidates its multi-level molecular regulatory mechanism (Figure 5).

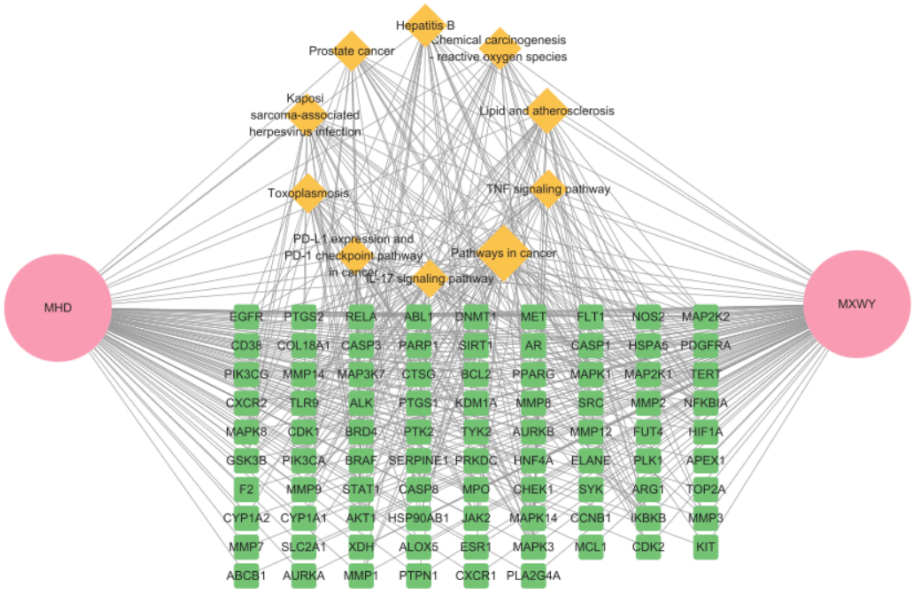

Figure 5. Network diagram of "drug-target gene-key signaling pathway-disease" interaction

4. Discussion

In TCM theory, *Oroxylum indicum* has various pharmacological effects such as anti-inflammation, anti-oxidation, anti-cancer and hypoglycemic activities [6]. A large number of inflammation and oxidative stress-related entries were enriched by KEGG in this study, which provides theoretical support for the pharmacological effect of *Oroxylum indicum* in the treatment of chronic gastritis [7].

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Chronic gastritis has now been recognized as a group of chronic diseases, with morphological characteristics of persistent inflammatory infiltration and impaired cell renewal, accompanied by the development of intestinal metaplasia, atrophy and gastric mucosal epithelial dysplasia [8]. In this study, GO enrichment obtained entries such as inflammatory response, response to oxidative stress, regulation of cell proliferation and protein kinase activity, which are consistent with existing conclusions of molecular pathology research on chronic gastritis. Continuous gastric mucosal injury can trigger lipopolysaccharide-mediated inflammatory response, and a large amount of oxidative stress products accumulate, breaking the homeostasis of proliferation and apoptosis of gastric epithelial cells, which is an important molecular basis for promoting mucosal atrophy and intestinal metaplasia [9, 10]. The results of cellular components suggest that most targets are distributed in mitochondria, cytoplasm and cell membrane adhesion regions. Mitochondria are the main sites of oxidative stress injury in gastric epithelium. Phosphorylation cascade conduction of inflammatory signals is completed in the cytoplasm, and adhesion molecules are involved in maintaining the integrity of epithelial barrier. The above subcellular localizations are all consistent with the pathological process of gastric mucosal injury repair [11]. Molecular function enrichment is mainly focused on protein kinase activity and ATP binding. Abnormal activation of kinase pathways in chronic gastritis will continuously amplify inflammatory signals, and inflammatory stress will in turn disrupt the energy metabolism of epithelial cells [12], suggesting that *Oroxylum indicum* can achieve gastric mucosal protection by regulating kinase activity and improving cellular energy status.

The IL-17 signaling pathway is a key pathogenic pathway of chronic gastritis, especially atrophic gastritis. *Helicobacter pylori* infection can drive Th17 cells to secrete IL-17, activate downstream pro-inflammatory cascade reactions such as NF-κB, recruit neutrophils to infiltrate the gastric mucosa, induce parietal cell apoptosis, aggravate gland atrophy, and participate in the process of inflammation-cancer transformation of gastric mucosa [13]. The VEGF signaling pathway is mainly involved in angiogenesis and tissue repair after gastric mucosal injury. When mucosal erosion and damage occur, VEGF expression is up-regulated to promote local neovascularization and provide a microenvironment for epithelial repair. The activation level of this pathway directly affects the repair efficiency of gastric mucosal injury [14]. This suggests that *Oroxylum indicum* most likely achieves a bidirectional regulatory effect on chronic gastritis by inhibiting IL-17-mediated inflammatory injury and simultaneously regulating VEGF-mediated tissue repair.

This study is only a bioinformatics prediction analysis with limitations. In the follow-up, in vitro gastric epithelial cell experiments can be carried out around the core pathways of IL-17 and VEGF to verify the anti-inflammatory and mucosal protective effects of the active ingredients of *Oroxylum indicum*.

5. Conclusion

This study confirmed through network pharmacology methods that multiple active ingredients of *Oroxylum indicum* can act on multiple core genes of chronic gastritis, regulate key signaling pathways such as IL-17 and VEGF, exert anti-inflammatory, anti-oxidative, gastric mucosal repair and lesion progression blocking effects, and preliminarily reveal its multi-target holistic action mechanism, providing a bioinformatics theoretical basis for subsequent pharmacological experiments of *Oroxylum indicum* in the treatment of chronic gastritis.

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