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Study on the mechanism of JiChuanJian in the treatment of slow-transmission constipation based on network pharmacology

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Author contributions

Hai-Zhao Liu and Lu Han proposed the research topic and wrote the paper; Yu-Hong Bian and Chen Xu provided guidance and funding support; Yu-Tong Jin, Yi-Yang Wang and Joseph kofi Abankwah revised the paper; Xue-Tao Dong collected and sorted out the data.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

JCJ, JiChuanJian; STC, slow transit constipation; PPI, protein-protein interaction; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; AKT, threonine protein kinase; 5-HT, serotonin; cAMP, cyclic adenosine monophosphate.

Citation

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Abstract

Background: Slow-transit constipation remains a functional colonic disorder with the most common gastrointestinal complaint. Although JiChuanJian (JCJ) is used for treatment, little is known about its mechanism of action. We aimed to investigate the mechanism of JCJ in treating slow-transmission constipation based on network pharmacology. **Methods:** The chemical components and action targets of the six herbs of JCJ were retrieved from the Traditional Chinese Medicine Systematic Pharmacology Analysis Platform. Protein targets of the chemical components were obtained from the UniProt database, whiles the disease targets were retrieved from the Gene Cards database, Disgenet, and TTD databases. A Wayne diagram was drawn and the intersected targets were imported onto the STRING website for analysis to construct protein-protein interaction target interactions. The Cytoscape software constructed protein-protein interaction to identify core targets and drug-active component-target interaction networks. The Kyoto Encyclopedia of Genes and Genomes and gene ontology enrichment analyses were performed through the DAVID analysis website. **Results:** A total of 55 potential drug-active components of JCJ, 233 drug-active ingredient targets, 3215 slow-transmission constipation-related targets, and 176 drug and disease targets were identified. The core drug-active ingredients included quercetin, beta-sitosterol, stigmasterol, kaempferol and wogonin. Through enrichment analysis in DAVID database, we obtained 236 biological processes, 31 cellular components and 41 molecular functions, mainly involving serotonin binding, G-protein coupled serotonin receptor activity, and neurotransmitter receptor activity, were identified. 118 pathway enrichment results were obtained after the Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. Therefore, we hypothesized that the active components in JCJ, such as quercetin, β-sitosterol, and stigmasterol, may treat slow transit constipation by regulating the serotonergic synapse and cyclic adenosine monophosphate signaling pathway. **Conclusion:** This study showed that JCJ could play a role in treating slow-transmission constipation through multiple components, targets and pathways.

Keywords: JiChuanJian; slow-transmission constipation; network pharmacology

Introduction

Slow transit constipation (STC) refers to the difficulty in defecation caused by the slow transfer of bowel contents due to the transmission dysfunction of the colon [1]. Domestic epidemiological surveys have shown that STC is more frequent in elderly, female, and pediatric patients. The prevalence of STC in the elderly is as high as 30%, with the spleen-kidney-yang deficiency type of constipation being more common [2]. The symptoms of the disease gradually worsen over time and could seriously affect patients' quality of life [3]. Studies have shown that STC significantly increases the incidence of related diseases such as colorectal polyps, an essential factor in inducing cardiovascular diseases such as heart failure and atherosclerosis [4]. Currently, laxatives and prokinetics are commonly used clinically to alleviate STC symptoms [5]. However, nearly half (47%) of patients are dissatisfied with the current treatment outcome due to the lack of efficacy and significant side effects [6]. Previous studies have reported that the pathogenesis of STC is closely related to the changes in intestinal nerve cells, neurotransmitters, and intestinal microbial ecological disorders [7]. But the exact mechanism is unclear. Therefore, there is an urgent need to find an effective treatment for STC.

In traditional Chinese medicine, STC is considered a deficiency of essence and fluid, a deficiency of kidney Yang. Its treatment is mainly to nourish the essence, warm the kidney, moisten the intestine, and facilitate colonic transit. JCJ is a commonly used oral prescription for the clinical treatment of STC. It is made from a mixture of six herbs: *Cistanche deserticola*, *Angelica sinensis*, *Achyranthes bidentata*, *Cimicifuga rhizoma*, *Fructus aurantii*, *and Alismatis rhizoma*. *Cistanche deserticola,* as a principal herb, is characterized by its sweet and salty flavor, which can warm the kidney, invigorate the vital essence, and moisten the intestine, consequently facilitating bowel movements.

Angelica sinensis tonifies blood and relaxes the bowel, whiles *Achyranthes bidentata* vitalizes the kidney and strengthens tendons. *Fructus aurantii* facilitates the downward movement of stagnated Qi in the intestine, facilitating bowel movement.

Alismatis rhizoma plays a role in removing dampness and turbidity, whiles *Cimicifuga rhizoma* raises Yang and reduces turbidity. Hence, JCJ drugs warm the kidney, enrich the essence, replenish the blood, and moisten the intestine to facilitate defecation.

Since ancient times, Chinese medicine has had the principles of "ruler, subject, and adjuvant" and contraindications. It did not extensively explore the characteristics of multi-components, multi-targets, multi-pathways, and synergistic treatment, which help improve patients' symptoms. As an emerging discipline, network pharmacology, based on computer technology and systems biology, analyzes the relationship between each node in the network and related drugs through a drug-active ingredient-target interaction network, emphasizing multiple pathways to regulate signaling pathways, thus improving drug efficacy [8]. Observing drugs' interventional effects and regulatory mechanisms on the disease through multiple targets and perspectives also provides avenues and ideas for developing new Chinese medicines. It could help promote the inheritance and development of Chinese medicine compounding. In this study, we constructed a drug-active component-target interaction network based on the molecular components of JCJ through a network pharmacology approach to analyze the molecular mechanism of JCJ in regulating STC as a whole and provide a specific theoretical basis for subsequent research.

Materials and methods

Screening of active components and targets of JCJ

We used the Traditional Chinese Medicine Systematic Pharmacology Analysis Platform database (http://www.tcmspw.com/tcmsp.php) to search for the constituent drugs of JCJ (*Cistanche deserticola*, *Angelica sinensis*, *Achyranthes bidentata*, *Cimicifuga rhizoma*, *Fructus aurantii*, and *Alismatis rhizoma*) and their corresponding chemical and pharmacological data. Oral bioavailability and drug-likeness quality were selected as parameters for screening the collected compounds. As an essential pharmacokinetic parameter, oral bioavailability indicated the percentage of unchanged drugs reaching systemic circulation after oral administration. The drug-likeness quality index was used to select drug properties based on their solubility and chemical stability of a potentially effective component. Here, we used oral bioavailability ≥ 30 and drug-likeness quality ≥ 0.18 as criteria for screening biologically active compounds [9]. Further, Traditional Chinese Medicine Systematic Pharmacology Analysis Platform was used to screen the targets of the active components of JCJ, and the drug targets were corrected using Uniprot (https://www.uniprot.org/) [9].

Construction of drug-active component-target interaction network

The obtained active components and cross-targets were sorted using the Microsoft Excel worksheet. After importing the data into Cytoscape 3.7.0 software, a "drug-active component-target" network model was constructed. The nodes represented the herbs, components, and targets, and the edges represented the relationship roles between the three nodes. We calculated the "degree" value based on the number of associations between each node.

Screening of potential STC targets

We used the term "slow transit constipation" as the keyword in the screening of potential targets from the Genecards (https://www.genecards.org), Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/ group/ttd/ttd.asp), the Online Human Mendelian Inheritance (OMIM, https://www.genecards.org) and DisGeNET (https://www.disgenet.org/home/) databases. Potential targets related to STC were obtained after eliminating duplicate targets. In order to determine the intersection point of the JCJ and STC targets, a Venn diagram was drawn.

Construction of protein-protein interaction (PPI) network and screening of key targets

A PPI network using the STRING database (https://string-db.org/) was constructed to elucidate the functional interactions between the screened potential proteins. The PPI network first imported protein interaction data from the STRING database into Cytoscape 3.7.0 software. Then we used CytoNCA, a plug-in in the software, to select critical targets according to the degree value. Further, we used the nodes with "degree" values greater than twice the median as the basis for screening the key targets and finally obtained the critical targets of JCJ for STC.

Enrichment analysis of STC-related target gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of JCJ

Import the selected key targets into the DAVID 6.8 database. GO enrichment and KEGG pathway analysis were performed using the DAVID 6.8 database (http://david.ncifcrf.gov). For functional annotation clustering, a threshold of Count ≥ 2 and an EASE (Expression Analysis Systematic Explorer) score of ≤ 0.05 were selected for the terms.

Results

This study used a comprehensive approach of network pharmacology, functional gene pathway analysis and network analysis to identify the active compounds related to JCJ, key therapeutic targets and the molecular mechanism of JCJ in STC treatment.

Screening of active components of JCJ and its targets for the treatment of STC

There were 2, 24, 6, 10, 17, and 5 key chemical constituents in *Angelica sinensis*, *Achyranthes bidentata*, *Cistanche deserticola*, *Alismatis rhizoma*, *Cimicifuga rhizoma*, *Aurantii fructus*, respectively as shown in Table 1. After removing duplicate entries, 55 active and 233

Table 1 Active components of JCJ

Drug	Mol ID	Molecule Name	OB (%)	DL
Angelica sinensis	MOL000358	beta-sitosterol	36.91	0.75
	MOL000449	Stigmasterol	43.83	0.76
Cistanche deserticola	MOL000098	Quercetin	46.43	0.28
	MOL000358	beta-sitosterol	36.91	0.75
	MOL005320	Arachidonate	45.57	0.2
	MOL005384	Suchilactone	57.52	0.56
	MOL007563	Yangambin	57.53	0.81
	MOL008871	Marckine	37.05	0.69
Achyranthes bidentata	MOL000085	beta-daucosterol_qt	36.91	0.75
	MOL000098	Quercetin	46.43	0.28
	MOL000173	Wogonin	30.68	0.23
	MOL000358	beta-sitosterol	36.91	0.75
	MOL000422	Kaempferol	41.88	0.24
	MOL000449	Stigmasterol	43.83	0.76
	MOL000785	Palmatine	64.6	0.65
	MOL001006	poriferasta-7,22E-dien-3beta-ol	42.98	0.76
	MOL001454	Berberine	36.86	0.78
	MOL001458	Coptisine	30.67	0.86
	MOL002643	delta 7-stigmastenol	37.42	0.75
	MOL002714	Baicalein	33.52	0.21
	MOL002776	Baicalin	40.12	0.75
	MOL002897	Epiberberine	43.09	0.78
	MOL003847	Inophyllum E	38.81	0.85
	MOL004355	Spinasterol	42.98	0.76
	MOL012461	28-norolean-17-en-3-ol	35.93	0.78
	MOL012505	bidentatoside,ii_qt	31.76	0.59
	MOL012537	Spinoside A	41.75	0.4
	MOL012542	β-ecdysterone	44.23	0.82
	MOL000098	Quercetin	46.43	0.28
	MOL000358	beta-sitosterol	36.91	0.75
	MOL012286	Betavulgarin	68.75	0.39
	MOL012298	Rubrosterone	32.69	0.47
Alismatis Rhizoma	MOL000359	Sitosterol	36.91	0.75
	MOL000830	Alisol B	34.47	0.82
	MOL000831	Alisol B monoacetate	35.58	0.81
	MOL000832	alisol,b,23-acetate	32.52	0.82
	MOL000849	16β-methoxyalisol B monoacetate	32.43	0.77
	MOL000853	alisol B	36.76	0.82
	MOL000854	alisol C	32.7	0.82
	MOL000856	alisol C monoacetate	33.06	0.83

Table 1 Active components of JCJ (*continued*)

Drug	Mol ID	Molecule Name	OB (%)	DL
	MOL000862	[(1S,3R)-1-[(2R)-3,3-dimethyloxiran-2-yl]-3-[(5R,8S,9S,10S,11S,14R)-11-hydroxy-4,4,8,10,14-pentamethyl-3-oxo-1,2,5,6,7,9,11,12,15,16-decahydrocyclopenta[a]phenanthren-17-yl]butyl] acetate	35.58	0.81
	MOL002464	1-Monolinolein	37.18	0.3
Cimicifuga Rhizoma	MOL000359	Sitosterol	36.91	0.75
	MOL000449	Stigmasterol	43.83	0.76
	MOL000483	(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl)ethyl]acrylamide	118.35	0.26
	MOL001924	Paeoniflorin	53.87	0.79
	MOL001925	paeoniflorin_qt	68.18	0.4
	MOL011991	23-epi-26-deoxyactein_qt	47.64	0.35
	MOL011999	24-epi-acerinol	31.31	0.42
	MOL012011	25-o-acetylcimigenol-3-o-beta-d-glc(1-2)beta-d-xylopyranoside_qt	30.04	0.32
	MOL012023	7,8-didehydrocimigenol	36.79	0.4
	MOL012040	Norkhelloside	31.31	0.84
	MOL012052	Tuberosine A	102.67	0.34
	MOL012053	cimicifugic acid	83.02	0.45
	MOL012055	cimicifugoside_qt	33.84	0.74
	MOL012062	Cimigenol	37.19	0.4
	MOL012073	methylcimicifugoside_qt	30.19	0.24
	MOL012078	Visamminol	50.01	0.23
	MOL012081	(20r,24r)-24,25-epoxy-3-beta-(beta-d-xylopyranosyloxy)-9,19-cyclolanost-7-ene-16,23-dione_qt	40.1	0.76
Aurantii Fructus	MOL000358	beta-sitosterol	36.91	0.75
	MOL002341	Hesperetin	70.31	0.27
	MOL004328	Naringenin	59.29	0.21
	MOL005828	Nobiletin	61.67	0.52
	MOL013381	Marmin	38.23	0.31

JCJ, JiChuanJian; OB, oral bioavailability; DL, drug-likeness.

component action targets were obtained. 3215 targets relevant to STC treatment were obtained from four databases, namely Genecards, OMIM, DisGeNET and TTD. Through the online Venn diagram editing website (http://jvenn.toulouse.inra.fr/app/example.html), 176 potential target genes of JCJ for STC treatment were identified by importing the potential targets of STC and the targets of JCJ active components (Figure 1).

Construction of drug-active component-target network

We used Cytoscape 3.7.0 software to construct the drug-active component-target network graph. It included 280 nodes and 922 edges (Figure 2). Through the drug-active component-target network, the Network Analyzer plug-in was used for topology analysis and filtering according to a degree. The top five compounds were quercetin, beta-sitosterol, stigmasterol, kaempferol and wogonin, which could be important for treating STC.

PPI network analysis and critical targets screening

A PPI network was constructed to obtain the critical proteins of JCJ for the treatment of STC based on the string database (Figure 3). Based

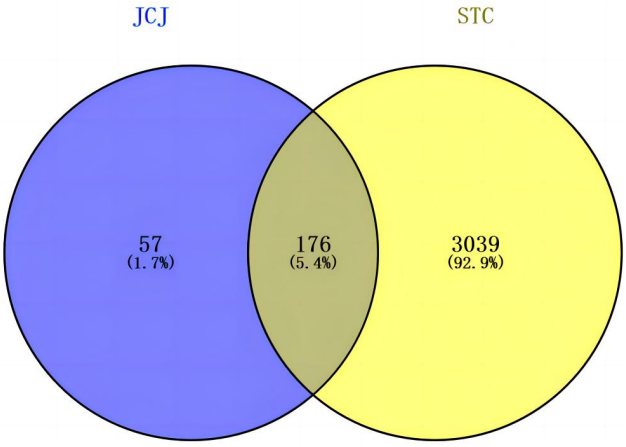

Figure 1 Venn diagram of JCJ targets and STC targets. JCJ, JiChuanJian; STC, slow transit constipation.

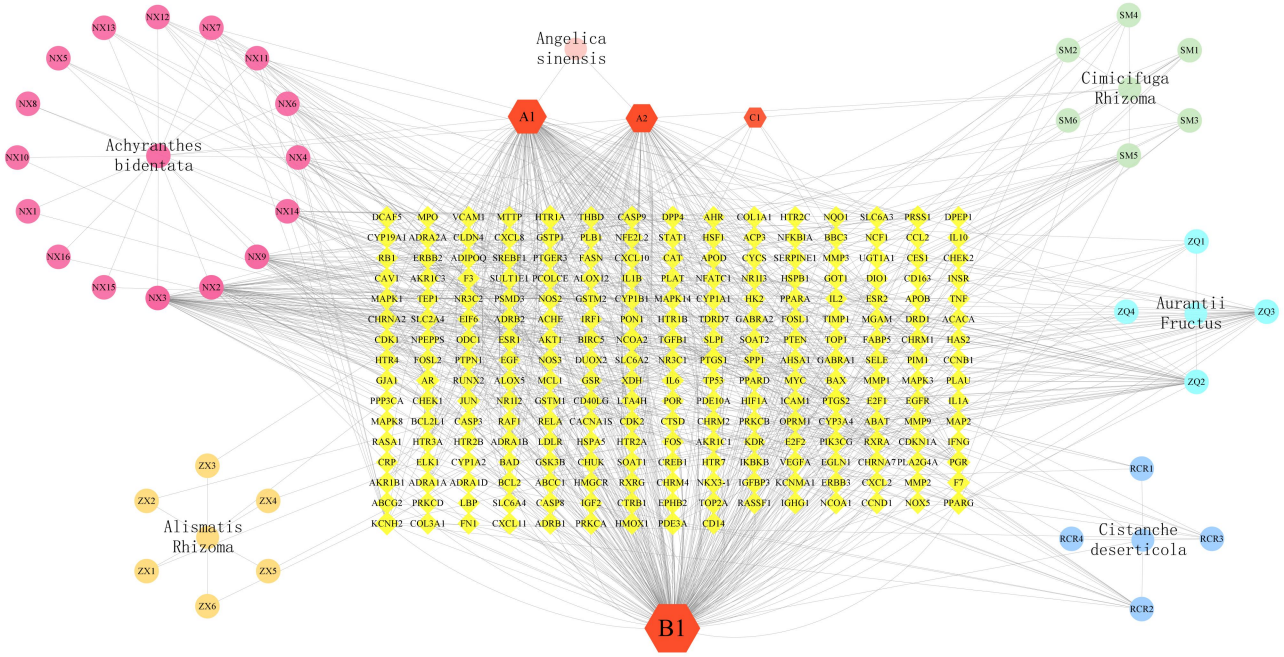

Figure 2 Drug-active component-target network diagram

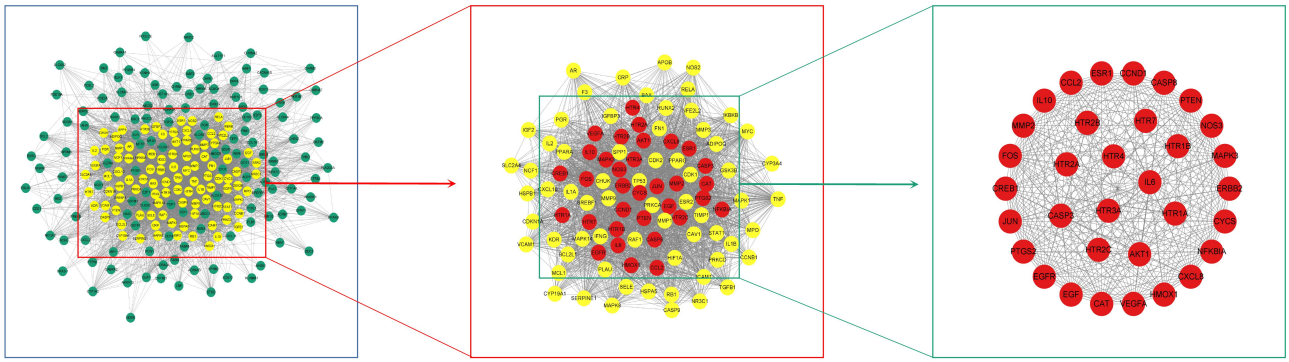

Figure 3 Screening process of key targets

on the "degree" values of topological parameters calculated by CytoNCA, 33 core targets (such as AKT1, HTR4, HTR3A, IL6 and CASP3) may be the core targets of JCJ for STC treatment were screened.

GO enrichment analysis and key target KEGG pathway analysis

We performed a GO enrichment analysis of 33 key targets using the DAVID database to identify the biological functions associated with JCJ for STC. This analysis revealed 236 biological processes, 31 cellular components and 41 molecular functions. As shown in Figure 4, the top 10 terms in the biological processes, cellular components, and molecular functions categories that are significantly enriched are demonstrated. Biological processes was found to be mainly related to drug response, response to xenobiotic stimulus, aging, angiogenesis, G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger, among others. Cellular components was mainly related to G-protein coupled serotonin receptor complex, caveola, synapse, plasma membrane, dendrite and many more. Molecular functions mainly included enzyme binding, identical protein binding, transcription factor binding, RNA polymerase II sequence-specific DNA binding, transcription factor binding, and transcriptional region. Other molecular functions included serotonin binding, G-protein coupled serotonin receptor activity,

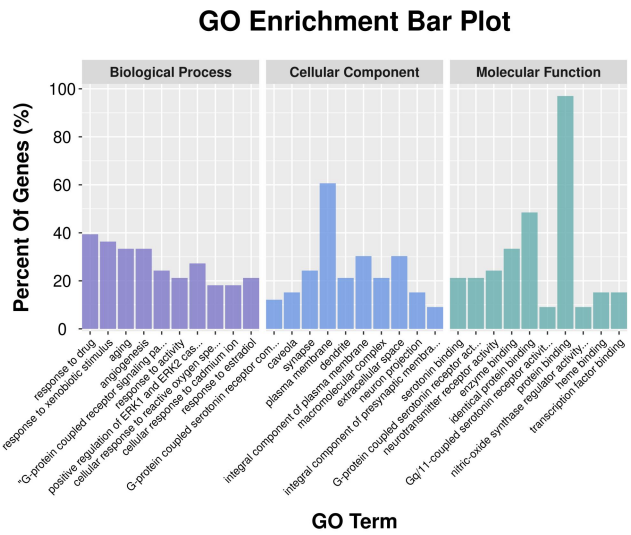

Figure 4 GO enrichment analysis. GO, gene ontology.

neurotransmitter receptor activity, enzyme binding, and identical protein binding. In order to identify the potential pathways of JCJ for STC, we performed KEGG pathway enrichment analysis and identified 118 signaling pathways associated with JCJ. A total of 20 pathways related to STC were screened, mainly including the serotonergic synapse, cyclic adenosine monophosphate (cAMP) signaling pathway, relaxin signaling pathway, p53 signaling pathway, MAPK signaling pathway and PI3K-Akt signaling pathway. The enrichment pathways were visible according to the p-value size (Figure 5). It is suggested that JCJ may exert its therapeutic effect on STC through the above pathways.

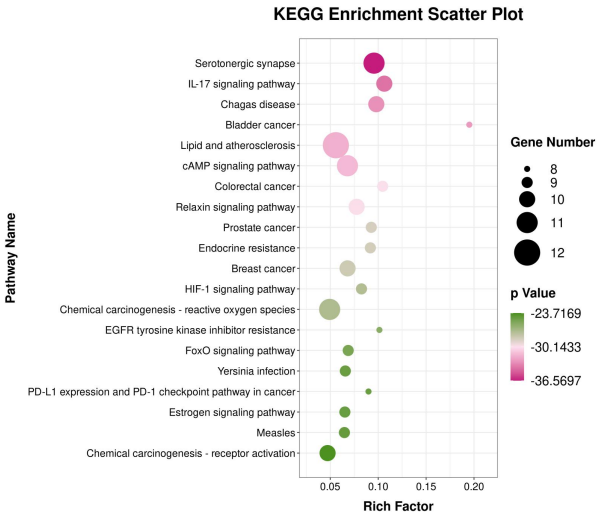

Figure 5 KEGG enrichment analysis. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Discussion

STC is a subtype of primary constipation, primarily studied in epidemiology, yet relatively lacks large-scale independent epidemiological studies [10]. A retrospective study in the United States showed that the prevalence of STC accounted for 10.3–45.5% of primary constipation and was more prevalent compared to normal transmission constipation, lousy bowel movement constipation and mixed constipation [11]. It is well known that China is currently the most populous country in the world, and the aging of the population structure is one of the social problems faced. Likewise, the incidence of STC is positively correlated to the increase in age.

Among them, spleen-kidney-yang deficiency is the most common type [12]. Although the location of the disease is in the large intestine, it can be caused by the dysfunction of the significant intestine conduction or by the failure of the transport function of the spleen and the propulsion function of the kidney, resulting in the metabolic disorders of the body, which in turn affect the abnormalities of the significant intestine conduction function leading to STC symptoms [13].

The network pharmacological analysis of JCJ revealed a total of 64 active components, and preliminary screening revealed that the active components of JCJ for STC regulation are mainly quercetin, beta-sitosterol, stigmasterol, kaempferol and wogonin. Among them, quercetin is a flavonoid with anti-inflammatory, antioxidant and anticancer pharmacological activities, which could improve the symptoms of STC model rats. The mechanism may act through binding to acetylcholine receptors to regulate the peristaltic function of gastrointestinal smooth muscle cells and promote mucin secretion, thus alleviating the symptoms of STC [14]. Studies have shown that quercetin could effectively restore the intestinal microbiota in mice after antibiotic treatment, lower intestinal pH and thus stimulate gastrointestinal motility, and could be used as a prebiotic for treating intestinal flora dysbiosis [15, 16]. Kaempferol, also known as kaempferol-3, is a natural flavonol compound with anti-inflammatory, antioxidant, and anti-tumor functions. Kaempferol can improve the integrity of the intestinal barrier, inhibit intestinal inflammation, reduce the disturbance of the intestinal environment by inflammation, and regulate the intestinal microbiota in mice on a high-fat diet [17]. Also, it attenuates cellular damage caused by inflammatory mediators and tumor necrosis factors and improves intestinal homeostasis. Beta-sitosterol can reduce the levels of TNF-α, IL-6 and IL-1β in the intestinal tissue of colitis mice in a concentration-dependent manner and significantly increase the expression of antimicrobial peptides in intestinal epithelial cells, reduce the survival rate of pathogenic bacteria of intestinal inflammation, and reduce the interference of inflammation on the intestinal environment [18].

The AKT1, HTR4, HTR3A, IL6 and CASP3 were the core targets from the active ingredient-disease fundamental target network diagram. Threonine protein kinase 1 (AKT1) is associated with immune response. AKT1 regulates cell metabolism and proliferation, and it was found that reducing the AKT gene and its protein expression in the colon of STC rats could alleviate constipation symptoms [19]. Furthermore, HTR4 is one of the most important and well-studied subtypes of the serotonin (5-HT) receptor in the gastrointestinal tract. HTR4 is found in many gastrointestinal tract cells, including enterochromaffin cells, smooth muscle cells, secretory cells, and nerve cells. Studies have found that HTR4 is mainly distributed in the colon's mucosal, submucosal, and muscular layers, among which the mucosal layer and the intermuscular plexus are the most apparent [20]. The HTR4 receptor binds to 5-HT secreted by enterochromaffin cells on the intestinal mucosa, and regulates the release of neurotransmitters in the cholinergic system, stimulating secretion and peristaltic reflex, consequently promoting intestinal peristalsis. The HTR3A is a ligand-gated anion channel, a postsynaptic receptor, found on neurons in the intermuscular intestinal plexus. Activation of HTR3A leads to depolarization of cell membrane, thus increasing the concentration of intracellular calcium ions, regulating the excitability of central and peripheral neurons, releasing more neurotransmitters, causing more acetylcholine to be released from parasympathetic nerve endings, leading to gastrointestinal functional abnormalities such as visceral hypersensitivity and abdominal discomfort [21].

The GO functional enrichment results showed that the potential process of JCJ for STC involves response to the drug, response to xenobiotic stimulus, G-protein coupled serotonin receptor complex, serotonin binding, and G-protein coupled serotonin receptor activity. The results of KEGG pathway enrichment showed that JCJ could treat STC through the serotonergic synapse, cAMP signaling pathway, relaxin signaling pathway, p53 signaling pathway, MAPK signaling pathway and PI3K-Akt signaling pathway. The 5-HT, secreted mainly by enterochromaffin cells and distributed throughout the digestive tract mucosa, has about 95% of 5-HT originating from the gastrointestinal tract, and the rest is distributed to the central 5-HT-potent neurons [22]. The 5-HT can regulate intestinal peristalsis and secretion and affect gut sensitivity. Low levels of 5-HT in the central system and gastrointestinal tract could lead to intestinal motility disorders. Excessive damage of 5-HT to the regulation function of the vagus nerve could lead to increased intestinal compliance and enhanced intestinal motility under intense stress [23].

In summary, the 5-HT signaling pathway leads to constipation through various mechanisms such as the enteric-brain axis, changes in gut sensitivity, and stress response and influences gastrointestinal peristalsis. cAMP is present in the cell. It regulates the cell's functional activity by binding to the binding subunit of cAMP-dependent protein kinase A, exerting the corresponding physiological effects [24]. cAMP-dependent protein kinase A signaling pathway activation leads to a corresponding decrease in Ca^{2+} inward flow and smooth muscle diastole in the gastrointestinal tract, resulting in constipation [25, 26]. During the contraction-diastole of smooth muscle cells, phosphorylation modifications of proteins play a regulatory role, such as altered phosphorylation of MAPK signaling pathway and AKT

leading the contraction-diastole of smooth muscle cells [27, 28].

Furthermore, blocking the MAPK signaling pathways could downregulate the expression of water channel proteins AQP3 and AQP4 in rat colon tissue, effectively inhibiting water reabsorption in the intestine and reducing the symptoms of constipation [29]. AKT1 is an isoform of AKT, which can be activated by PI3K, the PI3K/AKT signaling pathway, and activation of the PI3K/AKT signaling pathway could promote smooth muscle cell proliferation [30, 31]. The phosphorylation levels of PI3K and AKT were significantly upregulated in the colon tissue of a rat model of constipation, suggesting that the PI3K/AKT signaling pathway is involved in the development of constipation [32, 33]. It is thus predicted that the active components in JCJ, such as quercetin, beta-sitosterol, stigmasterol, kaempferol and wogonin, may, in turn, regulate the serotonergic synapse, cAMP signaling pathway, relaxin signaling pathway, p53 signaling pathway, MAPK signaling pathway and PI3K-Akt signaling pathway for the treatment of STC.

Conclusion

In conclusion, this study investigated the mechanism of JCJ in the treatment of STC using network pharmacology and found that JCJ achieved its therapeutic purpose through multiple components, multiple targets, multiple biological pathways and multiple pathways, and provides a theoretical basis for further extraction of practical active components from JCJ in the treatment of STC. Given the limitations of network pharmacology, further rigorous animal experiments and clinical trials should be designed to verify the mechanism of action.

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