

Mechanism of Zuojin Pill in the treatment of anxiety disorder and Major depressive disorder based on network pharmacology and molecular docking validation

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Author contributions

Pei-Xin Ge performed the data analyses and wrote the manuscript; Hong-Jie Cheng revised the paper and supervised; Wei-Fang Liu and Yan-Mei Cai contributed to the data analyses and manuscript preparation; Qiao-Yan Zhang and Shuang Dai visualised the results. Bo-Yi Jia designed the study and revised the paper.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

MDD, major depressive disorder; AD, anxiety disorder; ZJP, Zuojin pill; TCM, traditional Chinese medicine; PPI, protein-protein interaction; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; MF, molecular function; BP, biological process; CC, cell composition; 5-HT, serotonin; DA, dopamine; NA, norepinephrine; SSRI, selective serotonin reuptake inhibitor; SNRIs, selective serotonin-norepinephrine reuptake inhibitors

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Introduction

Major depressive disorder (MDD) is the leading mental health contributor to the Global Burden of Disease. About 4.7% of the world population suffer from depression in any 12-month time period. Patients may experience depressed mood, markedly diminished

Abstract

Background: Zuojin Pill (ZJP) is a classic Chinese herbal prescription with good efficacy in the treatment of Anxiety disorder (AD) and Major depressive disorder (MDD). Nevertheless, the potential mechanisms of ZJP remain unclear. Based on network pharmacology and molecular docking methods, this study aims to elucidate the possible mechanism of ZJP in the treatment of AD and MDD.

Methods: The components and targets of *Rhizoma Coptidis* and *Fructus Evodiae* were collected from TCMSP, ETCM, HERB, SWISSADME and STITCH databases. The disease targets related to MDD and AD were collected from DISGENET, GENECARDS and OMIM databases. Protein-protein interaction network was constructed by STRING database, GO and KEGG enrichment analysis was performed by METASCAPE database, and “drugs-components-targets network” was constructed by Cytoscape software. Molecular docking verification was performed by Sailvina2.0 software.

Results: ZJP may act on AKT1, IL6, TNF and other targets through caffeine, isorhamnetin, berberine and other components, regulating the Inflammatory mediator regulation of TRP channels, Serotonergic synapse, Dopaminergic synapse, PI3K/AKT signaling pathway, and other pathways. The results of molecular docking showed that berberine had the best binding activity with the core target.

Conclusion: ZJP can exert anti-anxiety and anti-depression effects through multiple components, multiple targets and multiple pathways.

Keywords: Network pharmacology; Zuojin Pill; Major depressive disorder; Anxiety disorder; Molecular docking; mechanism of action

interest or pleasure in activities, decreased ability to concentrate, beliefs of low self-esteem or inappropriate guilt, frequent thoughts of death, hopelessness about the future, psychomotor agitation or retardation, reduced energy, and fatigue [1]. Anxiety disorder (AD) forms the most common group of mental disorders. Core features include excessive fear and anxiety or avoidance of perceived threats that are persistent and impairing [2]. Traditional Chinese medicine

(TCM) has the characteristics of good effect, low toxicity and low side effects, no addiction, and no withdrawal reaction. Therefore, in China, TCM is widely used to treat MDD and AD. According to the TCM theory, the pathogenesis of the disease is qi stagnation and dysfunction of the zang-organs and fu-organs. The basic therapeutic principles of MDD and AD are regulating the qi and easing emotions. MDD and AD are both classified as “*yu zheng*” in TCM, and was first used in “*Yi Xue Zheng Chuan*” (Orthodox of Medicine), a famous medical book published in the *Ming* Dynasty.

Zuojin Pill (ZJP) is from “*Dan Xi Xin Fa*” (Danxi’s Mastery of Medicine), a medical book of the *Yuan* Dynasty in China. It is a common prescription for treating MDD and AD with TCM, composed of “*Rhizoma Coptidis*” (Huang Lian in Chinese) (six taels) and “*Fructus Evodiae*” (Wu Zhu Yu in Chinese) (one tael). *Rhizoma Coptidis* has the effect of heat-clearing and damp-drying, purging fire and detoxifying; *Fructus Evodiae*, which enters the liver meridian, can disperse liver and regulating qi, and is the main medicine for treating pains associated with liver-cold and qi stagnation. The combination of the two has the function of clearing liver-fire, descending adverse qi and stopping vomiting, and is particularly suitable for those with the syndrome of qi depression transforming into fire.

This paper aims to clarify the molecular mechanism of the anti-anxiety and anti-depression effect of ZJP through network pharmacological methods, in order to provide new ideas for clinical treatment and further research.

Methods

Collection of components and targets of ZJP

With the keywords “Huang Lian” and “Wu Zhu Yu”, the components of ZJP were obtained from 3 databases: TCMSP (<https://tcmsp-e.com/>), HERB (<http://drug.ac.cn/>) and ETCM (<http://www.tcmip.cn/ETCM/>). Then combine the components and remove duplicates. The molecule smiles of all the components were collected and uploaded to the SWISSADME platform (<http://www.swissadme.ch/>) for screening. The screening criteria were: Gastrointestinal absorption (GI absorption) was “High” and two or more of the results of the Druglikeness (Lipinski, Ghose, Veber, Egan, Muegge) were “Yes”. The structural formulae of the smiles of the screened components were uploaded to the STITCH database (<http://stitch.embl.de>) to collect targets, with species set to human and confidence level selected > 0.400

Collection of targets related to MDD and AD

Targets related to MDD and AD were collected in the DisGeNet database (<https://www.disgenet.org/>), the Gene Cards database (<https://www.genecards.org>) and the OMIM database (<https://omim.org/>). Collect depression-related targets with the keywords as “depression”, “depressed mood”, “Major Depressive Disorder”, “Depressive disorder”, “depressed”, “depressive”, “melancholic depression”, and “Melancholia”; anxiety-related targets with “angst”, “anxiety”, “anxious”, “anxiety disorder”, and “anxiety state”; anxiety-depression co-morbid targets with “mixed anxiety and depressive disorder”, and “comorbid anxiety and depression”.

Obtainment of intersection targets among ZJP and MDD and AD and construction of protein-protein interaction (PPI) network

The disease genes of MDD, AD, and anxiety-depression co-morbidities collected in 1.2 were intersected with the targets of ZJP separately and then merged to obtain the intersection targets of drugs and diseases. A Venn diagram was plotted by Bioinformatics (<http://www.bioinformatics.com.cn>). The common targets were uploaded to the STRING database (<https://string-db.org>) to find potential relationships between the targets and construct PPI networks. Then the network was imported into Cytoscape (version 3.6.0) for topological analysis to predict potential key targets.

Drugs-components-targets network construction

Correspondence between ZJP and its components, components and its

targets was constructed by Excel, and imported into Cytoscape software to draw the drugs-components-targets network. Then analyze the topology of this network to predict the key therapeutic components of ZJP.

Enrichment analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

The intersection targets acquired in 1.3 were uploaded to the Metascape database (<https://metascape.org/gp/index.html#/main/step1>) for enrichment analysis of KEGG and GO and predicting ZJP’s potential action mechanism and pathways. Parameters were set to enrichment factor > 1.5, $P < 0.01$, minimum number of genes = 3, and the results were visualized with the help of the Bioinformatics.

Molecular docking

SDF files of the 3D structures of the core components of “1.4” were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The components were energy minimized with the aid of Sailvina 2.0 software and exported as “pdbqt” files. The crystal structures of the core target proteins attained in “1.5” were downloaded from the PDB database (<http://www.rcsb.org/>) and were dehydrated, hydrogenated and charged and exported as “pdbqt” files. Docking of compounds and target proteins was performed using Auto Dock Vina to obtain binding energies, and some of the 3D docking results were displayed by PyMol and the 2D docking results were visualized by Ligplus software.

Results

Collection of components and targets of ZJP

304 components related to ZJP were collected from the three databases. After screening and target prediction, 67 components, corresponding to 193 targets, were remained.

Collection of targets related to MDD and AD

A total of 9803 depression-related targets, 5572 anxiety-related targets and 2267 anxiety-depression co-morbidity targets were collected.

Obtainment of intersection targets among ZJP and MDD and AD and construction of PPI network

There were 155 intersecting targets of ZJP with MDD, 136 intersecting targets with AD and 86 intersecting targets with anxiety-depression co-morbidity, and a total of 159 targets were obtained by taking the three together. The Venn diagram is shown in Figure 1.

The PPI network of these 159 targets was built using the STRING database and imported into Cytoscape for topological analysis, which is shown in Figure 2. 12 targets with Closeness Centrality greater than the median, Betweenness Centrality and Degree greater than the triple median are shown in Table 1. The 12 may be the core targets of the ZJP for anti-anxiety and anti-depression.

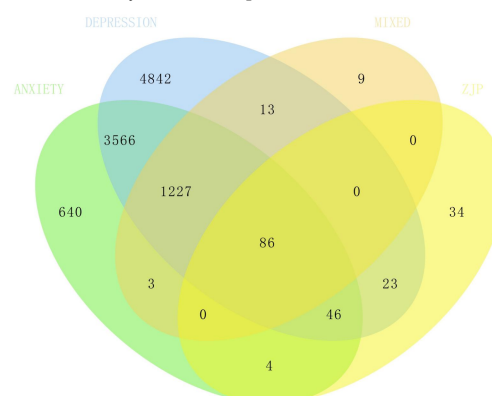


Figure 1 Venn diagram of target of ZJP and the target of the disease

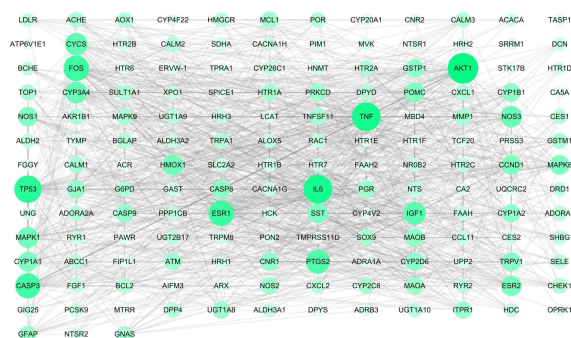


Figure 2 PPI network of ZJP and the diseases

Table 1 Core target parameters

Name	Degree	Betweenness Centrality	Closeness Centrality
AKT1	64	0.105995	0.610442
IL6	58	0.074576	0.59375
TNF	58	0.066248	0.584615
TP53	53	0.082864	0.571429
FOS	47	0.059105	0.546763
ESR1	47	0.041831	0.535211
CASP3	46	0.033731	0.548736
PTGS2	42	0.029426	0.542857
IGF1	38	0.018776	0.513514
CYCS	37	0.039677	0.524138
CYP3A4	35	0.051252	0.513514
NOS3	34	0.026438	0.503311

Drugs-components-targets network construction

The drugs-components-targets network, shown in Figure 3, was constructed using Cytoscape. Isorhamnetin, evoden, caffeine, berberine, and MeODMT are likely to be the core components with the highest connectivity.

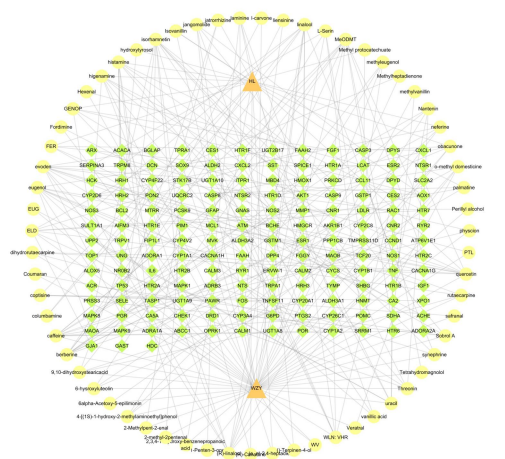


Figure 3 Drugs-compounds-targets network of ZJP (triangle represents drug, circle represents compound, diamond represents target)

Enrichment analysis of GO enrichment and KEGG pathway

Metascape database was used for GO and KEGG enrichment analysis of the 159 targets. GO enrichment analysis consists of 3 parts: molecular function (MF), biological process (BP) and cell composition (CC). The results of GO analysis showed a total of 1505 gene function-related information. Among them, 1275 biological processes were enriched by BP, 150 biological processes by MF, and 80 biological processes by CC. The top ten p-value functions were visualized in bubble diagrams, shown in Figure 4. 20 groups of 191 pathways were enriched by KEGG, shown in Figure 5.

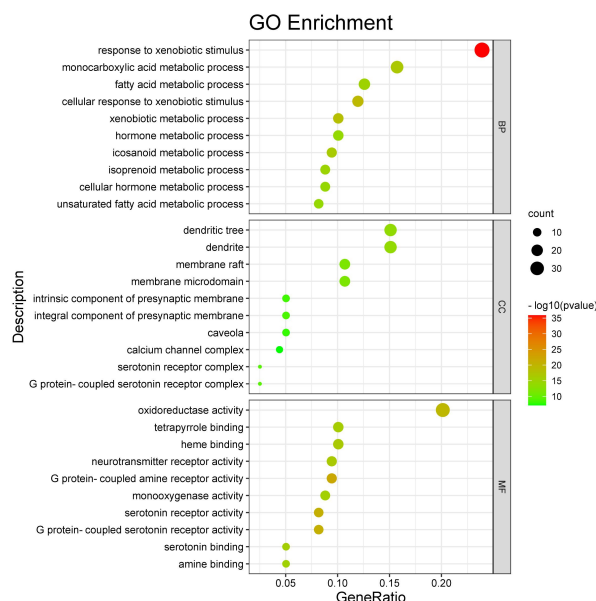


Figure 4 Results of GO enrichment

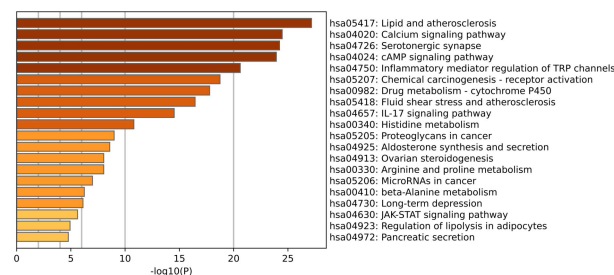


Figure 5 Results of KEGG enrichment

Molecular docking

Select the core target berberine, caffeine, isorhamnetin, MeODMT and 12 core targets to conduct molecular docking. The results are shown in Figure 6. It is generally accepted that compounds bind spontaneously to target proteins at binding energies less than 0, the compound binds spontaneously to the target protein; less than -5.0 kJ/mol the two are well bound; less than -7.0 kJ/mol, the docked two have strong binding activity. It can be seen that the binding energies of the core targets and the core components are all less than or equal to 0, with good binding activity. Among them, berberine had the lowest binding energy to the target sites, and the four lowest targets were taken for visualization, as shown in Figure 7.

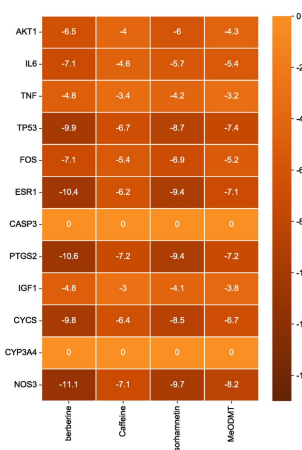


Figure 6 Results of Core component-target molecular docking

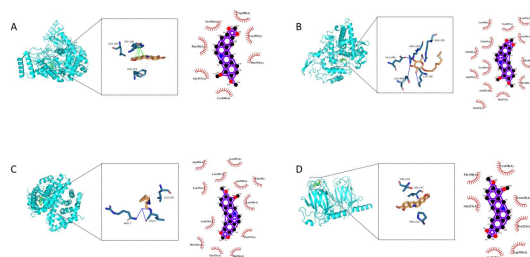


Figure 7 Molecular docking results (A) Berberine and NOS3. (B) Berberine and PTGS2. (C) Berberine and ESR1. (D) Berberine and TP53

Discussion

The pathogenesis of MDD is complex, and common theories currently include the neurotransmitter theory, inflammatory, structural changes in the brain, gene-environment interaction theory, neuroendocrine changes, intestinal dysbacteriosis, and mitochondrial dysfunction, etc. Common theories for the pathogenesis of AD include the hypotheses of neurotransmitter, the neuroendocrine hypothesis, self-regulatory disorders, genetic factors, and psychosocial factors, etc [3]. Anxiety and depression have similarities in pathogenesis, endocrine aspects, and other aspects. The Venn diagram shows that MDD and AD have 4925 common targets, also suggesting that the two disorders can affect each other. Clinically, *Rhizoma Coptidis* and *Fructus Evodiae* are commonly used as anti-anxiety and anti-depressant couplet medicines in TCM. Experiments have shown that ZJP has the effect of anti-inflammatory and anti-depressant by elevating the concentration serotonin (5-HT) and dopamine (DA) in the brain and inhibiting the expression of multiple inflammatory factors, for instance, TNF- α , IL-6, and IL-1 β [4]. *Rhizoma Coptidis* can increase the percentage of times mice enter the open arm and stay duration during the total period in the elevated cross maze and increase the level of GABA in the brain, exerting an anti-anxiety effect [5]. The ethanolic extract of *Rhizoma Coptidis* showed antidepressant effects in both physiological and diabetic mice [6]. With the method of network pharmacology, this paper explored and predicted the possible mechanisms of the anti-anxiety and antidepressant effects of ZJP at three levels: composition, target, and signaling pathway, combined with molecular docking validation.

Components

"Drugs-compounds-targets network of ZJP" involves 63 effective components. Among them, caffeine, isorhamnetin, berberine, MeODMT, and evoden may be the core components of the anti-anxiety and anti-depression effects of ZJP. Research have shown that caffeine can act on G protein-coupled adenosine receptors (A1, A2A, A2B, A3), DARPP-32 and other targets to improve anxiety and depression through a variety of ways such as anti-inflammation, stimulating BDNF expression and reducing oxidative stress [7, 8]. Isorhamnetin inhibits the release of inflammatory cytokines IL6, TNF- α and IL-1 β , and upregulates the expression of anti-inflammatory cytokines IL-10 to exert anti-inflammatory effects [9]. Berberine can exert anxiolytic and depressive effects by inhibiting inflammatory cytokines, improving the energy metabolism of hippocampal tissue, and reducing the uptake and degradation of neurotransmitters such as NE, 5-HT, and DA in the brain [10, 11]. MeODMT, also known as N, N-dimethyl-5-methoxytryptamine, has a similar chemical structure to 5-HT and is a classical 5-HT-energetic hallucinogen. Many studies have shown that MeODMT can treat mood disorders such as MDD, AD, post-traumatic stress disorder, and drug addiction [12, 13], with 80% of depressed patients and 79% of anxiety patients improving [14]. At present, evoden has been found to have enhanced gastrointestinal

digestive function [15], with less research on anxiety and depression.

Targets

AKT1, IL6, TNF, TP53, FOS, ESR1, CASP3, PTGS2, IGF1, CYCS, CYP3A4, and NOS3 are potential core targets. AKT1 is the most important of the three subtypes of AKT [16] and has an important bearing on varieties of psychiatric and neurological disorders. It is known that antidepressants, antipsychotics and mood stabilizers can act on AKT and AKT can also facilitate the action of antidepressants by enhancing the function of hippocampal stem cells [16]. The IL6 gene is a susceptibility gene for depression [17], and IL6 is also a biological marker of depression, significantly increased in the serum of depressed patients and positively correlated with the severity of depressive episodes [18]. In the depressive rats induced by chronic unpredictable stress, the level of TNF and other inflammatory cytokines are elevated, leading to increased expression of CASP3, and reduced anti-apoptotic proteins [19, 20]. TP53, closely related to apoptosis, can regulate astrocyte autophagy and neuronal apoptosis, and promote anti-depressant effects of fluoxetine [21]. FOS is a regulator of cell proliferation, differentiation, and transformation, and the expression of this gene is significantly increased in the brains of mice with anxiety and depression caused by hypoxia in the highlands [22]. ESR1 is an estrogen receptor, which can regulate the hypothalamus-pituitary-adrenal axis (HPA), oxytocin, and serotonin to play an anti-anxiety role [23]. It can also act as an antidepressant by regulating estrogen signaling and increasing the concentration of 5-HT, DA, and other neurotransmitters in the synapse [24]. PTGS2, which is referred to as COX-2, is associated with inflammation and cellular mitosis. COX-2 in depressed mice is significantly increased, and the increase of this gene will enhance the oxidative stress response [25]. CYP3A4 encodes one of the cytochrome P450 superfamily of enzymes that metabolize most psychoactive substances and psychotropic drugs such as lurasidone [26, 27]. The endothelial nitric oxide synthase NOS3 is a subtype of nitric oxide synthase. It can affect depression by regulating the release of various neurotransmitters such as 5-HT, DA, and NA, the activity of neurons and glial cells, and the synthesis of NO, which is neuroprotective [28]. IGF1 regulates cell growth, division, and differentiation, and is associated with cellular neuroplasticity, neuronal excitation, and neurotransmitter release [29]. The decrease of IGF-1 will disrupt cellular neuroplasticity and activate the inflammatory pathway in the brain, resulting in the morphological deterioration of the brain area responsible for emotional and cognitive processing, eventually leading to depression [30]. The decrease can increase the serum IGF1 concentration through the regulation of the "brain-liver" axis [31]. CYCS promotes the apoptosis of hippocampal neurons to affect their functions, and it increases in the hippocampus of chronically stressed rats and decreases with Fluoxetine, a Selective Serotonin Reuptake Inhibitor (SSRI) [32].

Signaling pathways

KEGG enrichment analysis showed that the therapeutic effect of ZJP on MDD and AD was mainly mediated through Serotonergic synapse, Pathways in cancer, p53 signaling pathway, Calcium signaling pathway, Neurotrophin signaling pathway, cAMP signaling pathway, Dopaminergic synapse, Neuroactive ligand-receptor interaction, Apoptosis, MAPK signaling pathway, Inflammatory mediator regulation of TRP channels, and other pathways.

Studies show that many Chinese herbs and formulas can act upon the same pathways as ZJP to disperse liver and regulate qi, and to clear stagnated heat. For example, Shugan Jieyu Capsule, dispersing stagnated liver qi for relieving qi stagnation, can increase the expression of Neurotrophic factor (NTF). The famous formula Danzhixiaoyao Powder can up-regulate protein phosphatase 2A (PP2A) and down-regulate α -synuclein and corticosterone to exert anxiolytic effects in the treatment of yu zheng with syndrome of liver depression transforming into fire [33]. Yueju Pill, known for resolving qi, fire, food, blood, damp, and phlegm stagnation, can increase CREB and NMDA activation and upregulate PKA/CREB/BDNF signaling

pathway, improving neuroplasticity and depression [34]. Sini Powder, which has the effect of dispersing liver and regulating qi, and clearing stagnated heat could reverse the reduction of 5-HT, NA, DA and other monoamine neurotransmitters [35]. These studies suggest the pathways that ZJP affects seem to be related to the mechanism of TCM treatment of “*yu zheng*”.

ZJP for MDD-related pathways

Studies have shown that the decrease in 5-HT, Ach, NE and DA may be the biological basis of depression in TCM [35, 36]. In enrichment results, Serotonergic synapse and Dopaminergic synapse are closely related to the secretion and transmission of these neurotransmitters. The hypothesis of monoamine neurotransmitter believes that a decrease in monoamine neurotransmitters for instance 5-HT, DA and NA leads to anxiety and depression. It is the first hypothesis proposed for depression and is the basis for most antidepressants today [37]. The monoamine neurotransmitter receptor hypothesis also occupies an important place. Related drugs such as Selective Serotonin-norepinephrine Reuptake Inhibitors (SNRIs), SSRIs are the current first-line drugs for MDD and AD [38]. There are several hypotheses for the pathogenesis of depression, all of which involve disturbances in serotonergic synapse [39]. 5-HT play a major part in mood, sleep, pain, learning and memory, endocrine secretion and motor function. If the concentration of 5-HT in the synaptic cleft is relatively or absolutely insufficient, it will depress the function of psychological and mental activity [40]. 5-HT receptors, divided into seven families (5-HT₁₋₇), can exhibit antidepressant and anxiolytic effects when they were stimulated or inhibited [41].

Dopaminergic synapse can influence mood by affecting the reward system. DA regulates the neuronal activity and synaptic plasticity and is the most important neurotransmitter in the reward system, being associated with autonomic activity, motivation and reward, learning and memory, and endocrine regulation [42]. DA receptors are divided into two main groups: D1-like receptors (D1Rs), which activate adenylyl cyclase and increase intracellular cAMP concentrations, including D1 and D5 receptors; and D2-like receptors (D2Rs), which exert inhibitory effects and reduce neuro excitability and inhibit the release of dopaminergic neurons, including D2, D3, and D4 receptors. Wang Q-S et al. [43] found that the ethanolic extract of ZJP significantly increased the levels of NE and 5-HT in the hippocampus and the levels of NE, 5-HT, and DA in the striatum of mice, thus exerting an antidepressant effect, which is in accord with the predicted outcomes of this study.

Inflammatory cytokines have a vital part in the pathogenesis of MDD by reducing neurotransmitter concentrations. In the brain, it can enhance the reuptake of monoamine neurotransmitters and inhibit the 5-HT synthesis mentioned above. Overexpression of inflammatory cytokines can also cause excessive activation of the hypothalamic-pituitary-adrenal axis (HPA), exacerbating depression-like behavior in rats [44]. The NF- κ B signaling pathway, a hot pathway involved in the inflammation, is involved in the regulation of immunity, inflammation and cell survival. NF- κ B is an important mediator of the blood-brain barrier, conveying peripheral inflammatory signals to the central nervous system [45]. In the hippocampus of depressed rats, NF- κ B p65, p-NF- κ B p65, and p-I κ B α levels were increased, NF- κ B p65 DNA binding activity was also increased, and the NF- κ B signaling pathway showed hyperactivation [46]. Wang Q-S et al. [47] demonstrated that ZJP prevented nuclear translocation of NF- κ B p50 and p65 subunits, thereby inhibiting IL-6, IL-1 β , and TNF- α and other inflammatory cytokines, achieving anti-inflammatory efficacy. This suggests that ZJP may achieve anti-inflammatory effects through modulating NF- κ B signaling pathway, and thus treat depression.

The neurotrophic factor signaling pathway is closely related to the neurotrophic factor hypothesis and the neuroplasticity hypothesis, of which the neuroplasticity hypothesis is one of the most groundbreaking hypotheses for depression [37]. NTF is a family of trophic factors involved in the differentiation and survival of nerve cells. They nourish the key brain regions involved in the regulation of emotional behavior in the central nervous system [44]. The decrease

in NTF level can lead to the atrophy of the hippocampus. NTF is composed of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophic factor 3 (NT-3), and neurotrophic factor 4 (NT-4), among which the BDNF is the most studied. NTF transmits signals through the intracellular signaling cascade to the MAPK signaling pathway, the PI3K signaling pathway, PLC pathway, and apoptosis pathway.

The cAMP signaling pathway is an intracellular signaling pathway associated with neurogenesis of neural stem cells and learning and memory capacity. cAMP is one of the most common second messengers and acts directly on the three main targets of this pathway, PKA, Epac and CNGCs. The cAMP-PKA cascade plays an important role in depression, regulating synaptic plasticity by phosphorylating AMPAR and Yotiao proteins on the NMDAR; mediating the NF- κ B/NLRP3 pathway to suppress neuroinflammation and reduce the production of inflammatory cytokines; reducing neurological damage caused by HPA axis hyperactivity via promoting the transcription of related genes and the synthesis of BDNF by phosphorylating CREB [48]. Berberine, one of the common components of *Rhizoma Coptidis* and *Fructus Evodiae*, can act on the cAMP/PKA/CERB pathway in a dose-dependent manner [49]. It seems that cAMP signaling pathway may be the main action pathway of ZJP in treating depression.

ZJP for AD-related pathways

The exhaustion of neurotransmitters and the abnormality of receptors are also important reasons for AD. The mechanism is similar to the depression mentioned above, so it will not be repeated.

Anxiety and inflammation are also closely related. Patients with higher levels of inflammatory markers also have higher levels of anxiety [50]. Take inflammatory mediator regulation of TRP channels for example. It involves the TRP family of TRPA1, TRPV1-4, and TRPM8. Deleting or inhibiting the expression of TRPA1, anxiety will be reduced [51]. TRPV1 can signal to p-JNK and p-p38MAPK signaling pathways. When it is inhibited, it can reduce cell damage in the amygdala and brain vascular damage, thereby reducing anxiety-like behavior in mice [52]. TRPV2 promotes Ca²⁺ influx and phosphorylation of MEK1/2, and is a mediator of the anxiolytic effects of oxytocin [53]. TRPV4 stimulates the activation of astrocytes and microglia and increases the expression of NLRP3 inflammatory vesicles, leading to depression-like and anxiety-like behaviors [54].

PI3K/Akt signaling pathway, which is associated with transcription, translation, proliferation, differentiation, and apoptosis, can modulate AD in a variety of ways. It improves mitochondrial function, regulates glucose and lipid metabolism, promotes cerebral angiogenesis, and also regulates apoptosis in hippocampal neuronal cells by generating cascade responses with apoptosis and p53 signaling pathway. Besides, this pathway has a circulating pathway between BDNF and glutamatergic system, regulating the expression of BDNF and the uptake and transport of glutamate (GLU) [16]. AKT can also upregulate the expression of mTOR and GSK-3 β , and activate the MEK/ERK pathway, leading to AD [55]. Zhou B-G et al. [56] found that ZJP could inhibit PI3K/Akt signaling pathway, regulate intestinal flora and Treg cells to treat ulcerative colitis, and ZJP may treat MDD and AD in a similar way.

Molecular docking

Molecular docking showed that the binding energy of the core targets to the core components was low, with berberine showing the best binding activity, which may be the key component of the therapeutic effect of ZJP. As the 3D structure of evoden was not retrieved, this component was not included in the molecular docking.

Conclusion

This study predicted and analyzed the mechanism of ZJP in treating MDD and AD based on the network pharmacology and molecular docking technology. The anxiolytic and antidepressant effects of ZJP may be achieved through 63 components such as isorhamnetin, berberine, MeODMT and 159 targets including AKT1, IL6, TNF, TP53,

FOS, ESRI, CASP3, PTGS2, as well as 191 signaling pathways, for instance, Serotonergic synapse, Dopaminergic synapse, p53 signaling pathway, and Calcium signaling pathway. To sum up, ZJP can exert anti-anxiety and anti-depression effects through multiple components, multiple targets and multiple pathways.

This study has some limitations. Network pharmacology has the function of prediction, which can give a certain direction to explain the mechanism of TCM. However, this study only stays at the theoretical level. The data is collected from multiple databases, and the accuracy and the screening process of the components and core targets can be further improved, which still needs further verification by experiments.

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