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BIOMEDICAL SCIENCES

Traditional Chinese Medicine Strategy for Treating Major Depressive Disorder Based on a Famous Formulation-Baweixiaoyaosan

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Abstract: In this study, systematic pharmacological methods were used to reveal the potential pharmacological targets of baweixiaoyaosan in the treatment of major depressive disorder (MDD). We identified 133 potential active compounds through data mining and absorption, distribution, metabolism, and excretion evaluation systems. Then, the target of potential active compounds is predicted by a system model based on random forest and support vector machine methods. Next, construct herbal ingredient-target networks and target-disease networks for further analysis of multi-directional treatment methods. At the same time, we also performed gene ontology enrichment analysis, tissue location analysis, and pathway analysis on 76 potential targets. Finally, we conducted the Jun-Chen-Zuo-Shi compatibility analysis of the formula and scientifically explained the different functions of different herbs in the formula. In short, we found that the formula mainly exerts the effect of treating MDD through the four functional modules of inflammation inhibition, neuroprotection, monoamine neurotransmitter and liver. This research not only explores the mechanism of Traditional Chinese Medicine treatment of MDD from a multi-scale perspective, but also provides a reference for future research on BWXYS. It plays a role in promoting the widespread use of BWXYS.

Key words: Baweixiaoyaosan formula, depression, herbal medicine, molecular pathogenesis, systems pharmacology.

INTRODUCTION

As a common affective disorder, major depressive disorder (MDD) is mainly characterized by recurrent episodes of depression, loss of interest, unresponsiveness, difficulty sleeping, and disgust, or with physical disorders such as sluggishness, dizziness, headache, and loss of appetite (American Psychiatric Association 2013). MDD is a common psychiatric condition and leads to significant physical, psychological, and economic distress in individuals, families, and society (Aghamohammadi-Sereshki et al. 2021). The disease not only damages the health of patients, but also reduces the quality of life and causes a great burden on families and society, and thus has become a serious social and medical problem (Brambilla et al. 2010).

Modern medicine has elaborated the possible pathogenesis of MDD from different perspectives, but the full pathogenesis remains unexplained. With the advancement of research, it has been recognized that MDD is the result of a huge network of genetic, environmental, neuroendocrine, immune inflammatory, and intestinal flora factors acting together (Yin et al. 2022). Currently, most of the drugs on the market for the treatment of MDD are western drugs, and commonly used drugs include fluoxetine, paroxetine, venlafaxine, duloxetine, moclobemide, and toloxacin (Wang 2010). Some novel drugs are also being investigated

in recent years. For example, brexanolone for the GABAergic system, buprenorphine and naltrexone for the opioid system. In particular, ketamine, similar to imipramine, can target the glutamatergic system to exert antidepressant effects, which is an important breakthrough in the pharmacology of depression (Sanches et al. 2021).

Traditional Chinese medicine has a long history of treating MDD. It is recorded in the "*Internal Medicine Abstract*" written by Xue Ji in the Ming Dynasty, Bawei Xiaoyao San (BWXYS) has the effects of soothing the liver, strengthening the spleen, relieving depression, nourishing yin and clearing away heat, and is a classic prescription for treating MDD in ancient times (Luo et al. 2006). BWXYS is composed of eight kinds of herbs: Atractylodes Macrocephala Koidz, Radix Bupleuri, Angelicae Sinensis Radix, Poria cocos (Schw.) Wolf, Licorice, Cortex Moutan, Gardeniae Fructus, and Paeoniae Radix Alba. It is also called Jiawei Xiaoyao San and Danzhi Xiaoyao San. In fact, the prescription has also achieved modern clinical effect verification in the treatment of MDD (Wang et al. 2013). Nevertheless, the material basis and mechanism of action of BWXYS in the treatment of MDD are still unclear.

Systems pharmacology is an emerging discipline that studies the interaction between drugs and the body, its laws, and mechanisms of action at the system level. This method helps to reveal the mechanism of disease treatment and clarifies key issues such as the pharmacodynamics of traditional Chinese medicine and the basic theories of traditional Chinese medicine. In this study, we will use systems pharmacology to explore the mechanism of action of BWXYS in the treatment of MDD. First, we established a database of BWXYS ingredients and then determined the active ingredients through ADME parameter screening. Secondly, through target recognition to determine the target of the active ingredient, the obtained target protein is a clue to determine the biological process, pathway, organ and disease involved. Finally, we construct a compound-target network, a target-disease network, a target-organ network, and a pathway network to reveal the mechanism of action of BWXYS in treating MDD. Fig. 1 is the research flow chart of this study.

MATERIALS AND METHODS

Candidate compound screening

The ingredients of all herbs in BWXYS were data-mined from not only the TCM Systems Pharmacology Database (TCMSP, https://tcmsp-e.com/tcmsp.php) (Ru et al. 2014), but also all related literature. In the end, 1,064 chemicals were obtained. Since Chinese herbal medicines are rich in a variety of chemical components with different structures, only a small part of the chemical components has good ADME properties to reach the corresponding target location in the human body and exert their activity. Here, we used three ADME parameters for screening to obtain potential active ingredients, namely oral bioavailability (OB), drug-like (DL), and half-life (HL).

An in-house software OBioavail 1.1 is used to predict the OB of BWXYS ingredients, which is built based on a dataset of 805 structurally diverse drugs and drug-like molecules with evaluated OB values (Xu et al. 2012). In the present study, those BWXYS ingredients with OB≥30% are selected as the candidates.

The DL computation of BWXYS ingredients is carried out based on a Tanimoto coefficient (Clark & Pickett 2000). Specifically, the structural feature vector of the molecule is calculated firstly, and then compared with the average structural feature vector of all the drugs contained in DrugBank database based on a

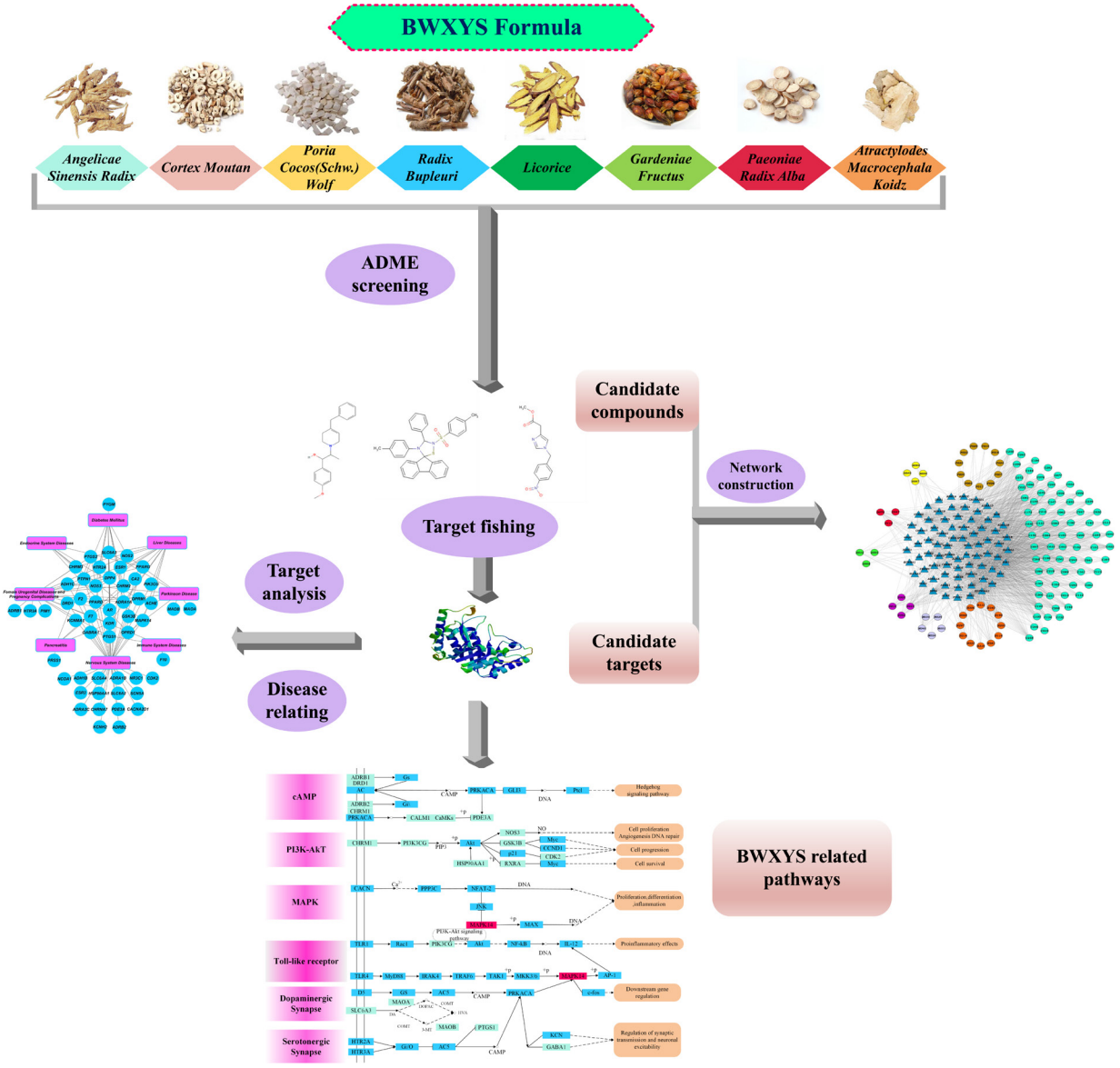

Figure 1. Systems pharmacology approach framework for the BWXYS study.

specifically designed mathematical program. During this process, DL≥0.18 (the average DL value of all drugs in DrugBank) is used as the threshold for screening possible candidates.

HL is the time it takes for the blood concentration of a drug in the body to decrease by half from its maximum value. We introduced a model (Madden 2010) to perform the calculation and selected the components with longer HL as candidate molecules.

Potential target prediction

Firstly, the Systems Drug Targeting (SysDT) algorithm, a pharmacophore modeling approach, was employed to predict the possible treatment targets based on the collected candidate compounds. This model involves protein and ligand encoding vectors. It takes Random Forest (RF) and Support Vector Machine (SVM) as the major ensemble-based methods and incorporates the chemical, genomic, and pharmacological information into an integrated

framework using the DrugBank database. RF score≥0.8 and SVM score≥0.7 were set as the thresholds to screen potential targets. Next, we used the Retrieve/ID mapping tool in the UniProt database (https://www.uniprot.org/uploadlists/) to standardize the target-related genes and screen genes for Homo sapiens. Secondly, all the targets were mapped to the BioGPS database (http://biogps.org/), and the distribution of the 76 targets in different levels of human tissues was studied. Thirdly, all resulting targets were sent to the Therapeutic Targets Database (TTD, http://bidd.nus.edu.sg/group/ttd/) and PharmGKB (http://www.pharmgkb.org) to determine corresponding diseases.

GO analysis and analysis of KEGG signaling pathway enrichment

The functional enrichment analysis of the targets was performed by DAVID (https://david.ncifcrf.gov/) (Huang et al. 2009). Later, the gene ontology (GO) biological process (BP) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were enriched based on the adjusted P-value of <0.05.

Network construction

In order to explore the multiple mechanisms of action of BWXYS formula for MDD, we analyzed the relationships between candidate molecules, diseases, and potential targets by constructing the candidate compound-target (C-T) network and candidate target-disease (T-D) network. The network was generated by Cytoscape 3.2.1 (Smoot et al. 2012), a standard tool for integrated analysis and visualization of biological networks.

Toxicity prediction

ADMETlab 2.0 (Xiong et al. 2021) was used to predict the toxicological properties of the active ingredients. A total of 27 parameters such as hERG, H-HT, DILI and AMES Toxicity were included.

RESULTS

Candidate compounds and target of BWXYS

Through the method of data mining, for BWXYS formula, 1,064 ingredients were identified, which constitutes a BWXYS component database for further analysis. However, though traditional Chinese medicines (TCM) are rich in the quantity and structures of their ingredient components, only a small part of these components, i.e., the real active compounds, have ADME properties proper to reach corresponding target location in human body and exert their activity after oral administration. Thus, presently, to identify the potential active compounds from the BWXYS component database as mentioned above, we used OB≥40%, DL≥0.2, and HL≥4 as the screening criteria, and finally obtained 133 active molecules, which account for 13% of all ingredients. According to structures, it is found that these active molecules belong to nine categories: flavonoids, terpenes, organic acids, coumarins, volatile oils, amino acids, glycosides, alkaloids, and others. Among them, flavonoids, which contain five sub-classes, i.e., flavones (26%), flavonols (10%), isoflavones (7%), chalcones (6%), and isoflavanes (3%), are identified as the richest in the present study, accounting for more than half of all active molecules (Fig. 2). In this study, the 133 candidate compounds of BWXYS can act on 76 target proteins in the human body, and many of these targets are key targets for the treatment of MDD, such as sodium-dependent dopamine transporter (SLC6A3), prostaglandin G/H synthase 2 (PTGS2), glucocorticoid receptor (NR3C1) and mineralocorticoid receptor (NR3C2).

The basic information of the candidate compounds is located in Supplementary Material - Table SI and the toxicological properties are located in Table SII. Drugs can have multiple effects on the human body, therefore 27 toxicological properties have been predicted.

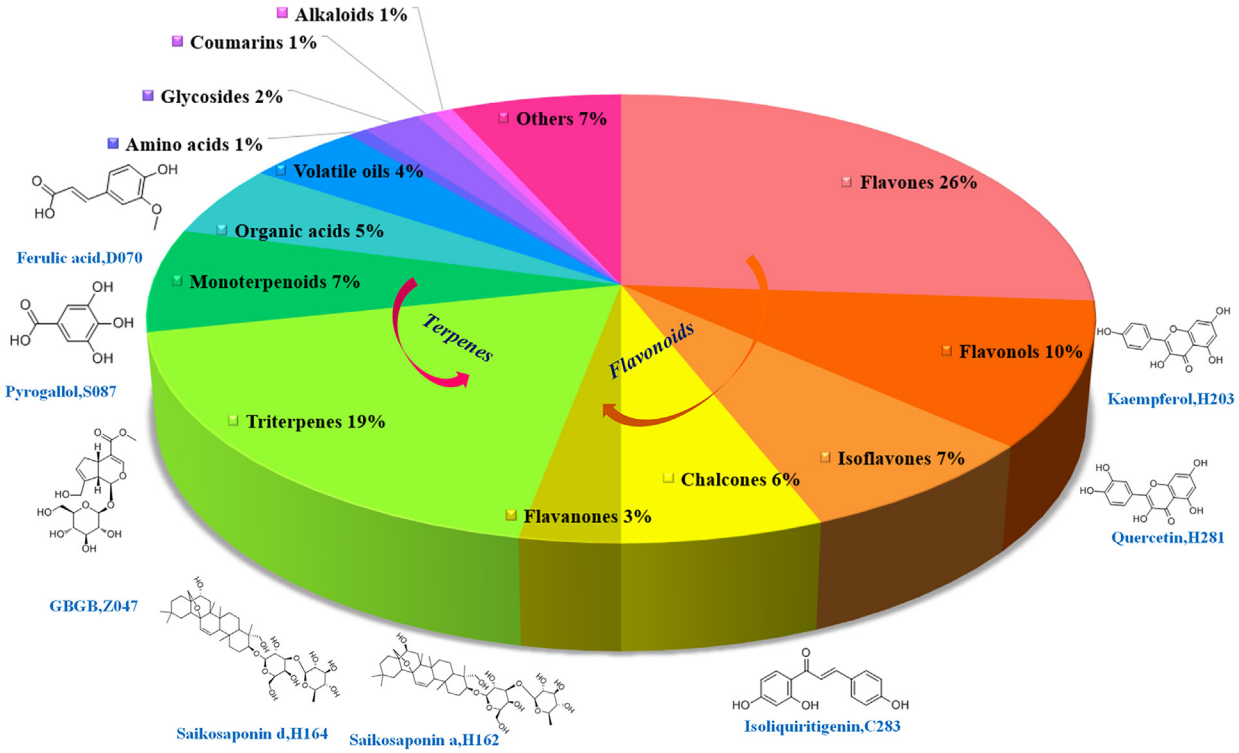

Figure 2. The structural classifications of the active compounds of BWXYS formula. The arrow marks two major categories: flavonoids and terpenes. The molecular structures surrounding them are the compounds mentioned in the text.

These properties are divided into three main parts, the first of which includes hERG, H-HT, DILI, AMES Toxicity, Rat Oral Acute Toxicity, FDAMDD, Skin Sensitization, Carcinogenicity, Eye Corrosion, Eye Irritation, and Respiratory Toxicity. the second part is Environmental Toxicity, which includes Bioconcentration Factors, IGC50, LC50, and LC50DM. the third part is Tox21 Pathway, which includes NR- AR, NR-AR-LBD, NR-AhR, NR-Aromatase, NR-ER, NR-ER-LBD, NR-PPAR-gamma, SR-ARE SR-ATAD5, SR-HSE, SR-MMP, and SR-p53. the output values of the first and third parts are the probability of being actives within the range of 0 to 1. From the results it can be seen that some components do have some toxicity, for example Medicarpin (C022) has H-HT and DILI values of 0.751 and 0.89 respectively, which indicates that it has a higher probability of being toxic in terms of liver damage. However, these results are not experimentally verified and are only for reference in future studies of BWXYS.

Network pharmacology analysis

To study the complex mechanism of BWXYS prescriptions in the treatment of MDD, we firstly constructed a C-T network. Through network pharmacological analysis, it is found that the target of BWXYS is mainly related to neuroprotective, anti-inflammatory, and regulation of monoamine neurotransmitters. Therefore, we constructed a C-T network and three sub-C-T networks for BWXYS formula and studied the effects of the formula on depression from three aspects: neuroprotective, anti-inflammatory, and regulation of monoamine neurotransmitters. In addition, the distribution of all targets of the formula in tissues of human body was also investigated to further explain the mechanism of action of BWXYS in the treatment of MDD from the tissue and organ level.

Table I. Compatibility of BWXYS and the number of herbal ingredients.

Function of the herbs	Herbs name	Number of all ingredients	Number of candidate compounds
Monarch	Radix Bupleuri	349	16
Minister	Gardeniae Fructus	97	10
	Paeoniae Radix Alba	87	11
	Angelicae Sinensis Radix	124	3
Assistant	Poria Cocos (Schw.) Wolf	33	12
	Cortex Moutan	54	5
	Atractylodes Macrocephala Koidz	54	5
Guide	Licorice	288	83

C-T network

In order to explore the complex interaction between the active ingredients and the targets of BWXYS, we constructed a C-T network using all targets and corresponding active ingredients of the formula. As shown in Fig. 3, this C-T network includes 209 nodes (133 active compounds and 76 targets) and 1,789 edges, where the circle represents the active ingredient, and the triangle represents the target.

In terms of active compounds, the average degree of all active ingredients is 15.73. In fact, in all candidate compounds, more than half of the active ingredients have a degree higher than the average value, and 9 active ingredients interact with more than 30 targets, which shows that BWXYS active ingredients have a typical "multi-target" pharmacological characteristic. It can be seen from Fig. 4a that the three active ingredients with the highest degree are 7-methoxy-2-methylisoflavonoid (C035), medicarpin (C022), and shinpterocarpin (C140). In addition, although the degrees of ferulic acid (D070), paeonol (M045), and gallic acid (S087) are not high, they are highly betweenness. Therefore, the above-mentioned ingredients may be the key active ingredients of BWXYS in the treatment of MDD, and the biological activities of some compounds have been reported. For example, experiments have confirmed that M045 has a positive therapeutic effect on depression of liver-qi stagnation syndrome (Wu et al. 2019). Actually, it inhibits the activation of TLR4 and NF-κB signaling pathways, thereby inhibiting the expression of p65, IκBα, IKKβ, and NLRP3, further inhibiting the pro-inflammatory factors Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and promoting the production of anti-inflammatory factors IL10 to relieve depression. In addition, C035 interacts with 43 targets such as LTA4H, F2, and SLC6A3, and has a significant impact on the central nervous system (Li 2004). Besides, D070 is a kind of phenolic acid, which exists widely in plants in nature. It has strong antioxidant properties, scavenges hydrogen peroxide and hydroxyl free radicals, and also has neuroprotective, immunomodulatory (Yu et al. 2006, Zhang et al. 2008, Liao et al. 2010), antibacterial, anti-inflammatory and inhibition of liver damage. In short, we speculate that BWXYS treats MDD through these key active ingredients.

As for the targets, average degree of all the targets is 23.75. As shown in the C-T network, 66 of the 76 targets are linked to at least two different herbal ingredients (Fig. 3), which indicates that

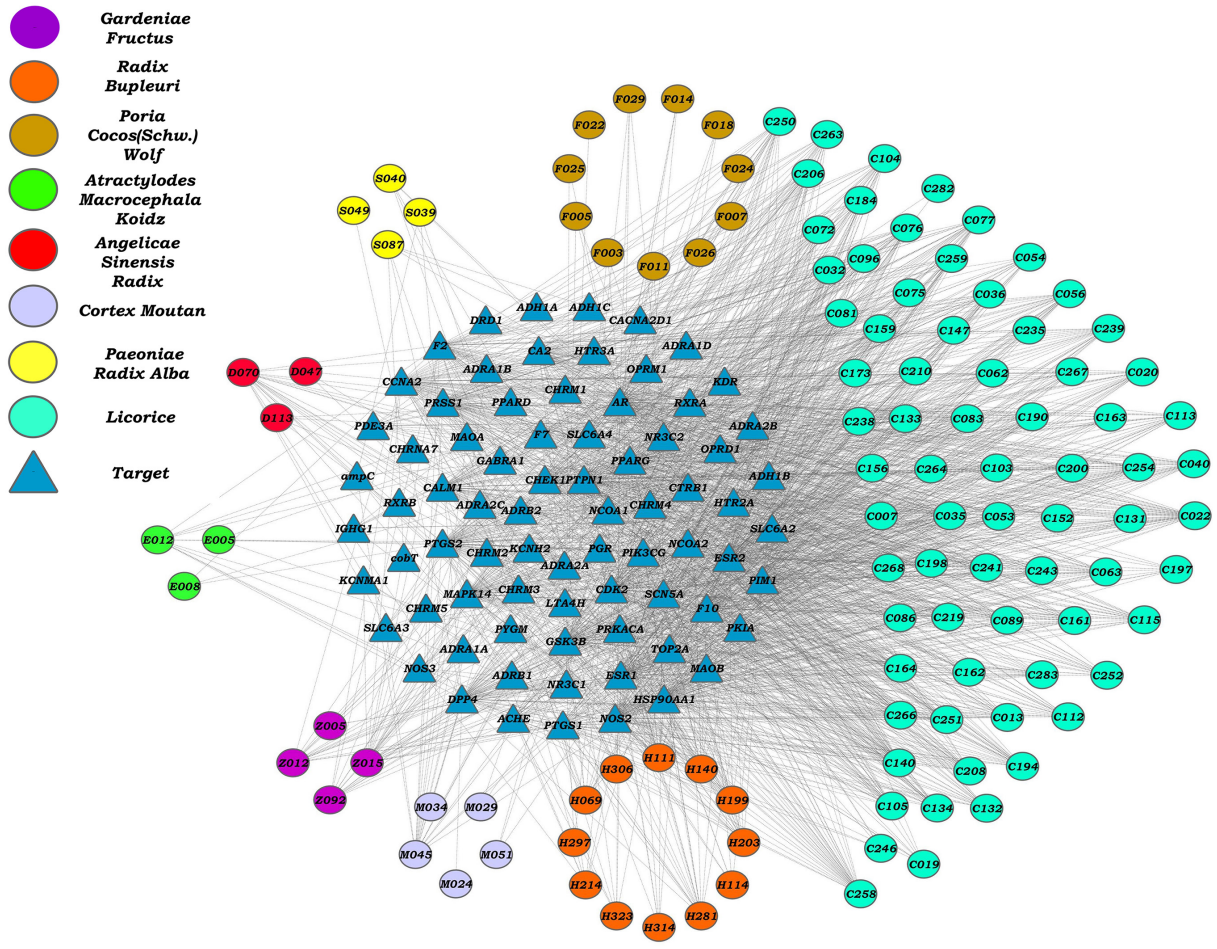

Figure 3. C-T network. The blue triangles represent target proteins while the purple, orange, brown, green, red, grey, yellow, light blue circles represent candidate compounds contained in Gardeniae Fructus, Radix Bupleuri, Poria Cocos (Schw.) Wolf, Atractylodes Macrocephala Koidz, Angelicae Sinensis Radix, Cortex Moutan, Paeoniae Radix Alba, Licorice, respectively, while each edge represents the interaction between targets and compounds.

BWXYS has the typical characteristics of "multi-component, multi-target" Chinese medicine. For example, PTGS2, the degree ranked 3rd target, has 80 active ingredients acting on it, such as H323 (Troxerutin), E012 (8β ethoxy atractylenolide III), Z015 (Corymbosin), and D070 (ferulic acid). PTGS2 is the target of most of the currently widely used non-steroidal anti-inflammatory drugs (non-steroidal anti-inflammatory drugs such as aspirin and ibuprofen). In fact, their anti-inflammatory function is closely related to the production of prostaglandin E2, which is a member of the eicosanoid family and a lipid regulator that participates in the regulation of different stages of inflammatory response. PTGS2 increases the level of prostaglandin E2, so inhibiting PTGS2 will lead to a decrease in the level of prostaglandin E2 and ultimately produce anti-inflammatory effects.

Among all the targets (76 in total) of BWXYS, 43% are the main targets namely, 33 proteins, of BWXYS in the treatment of MDD (Figure 5). Among these 33 targets, 45%, including 15 targets such as ADRA1B, ADRA1D and ADRA2A, are related to the lack of monoamine transmitter in the synaptic cleft. About one-third are related to the protection of the nervous system, such as MAPK14, NOS2 and NR3C1. The rest of the

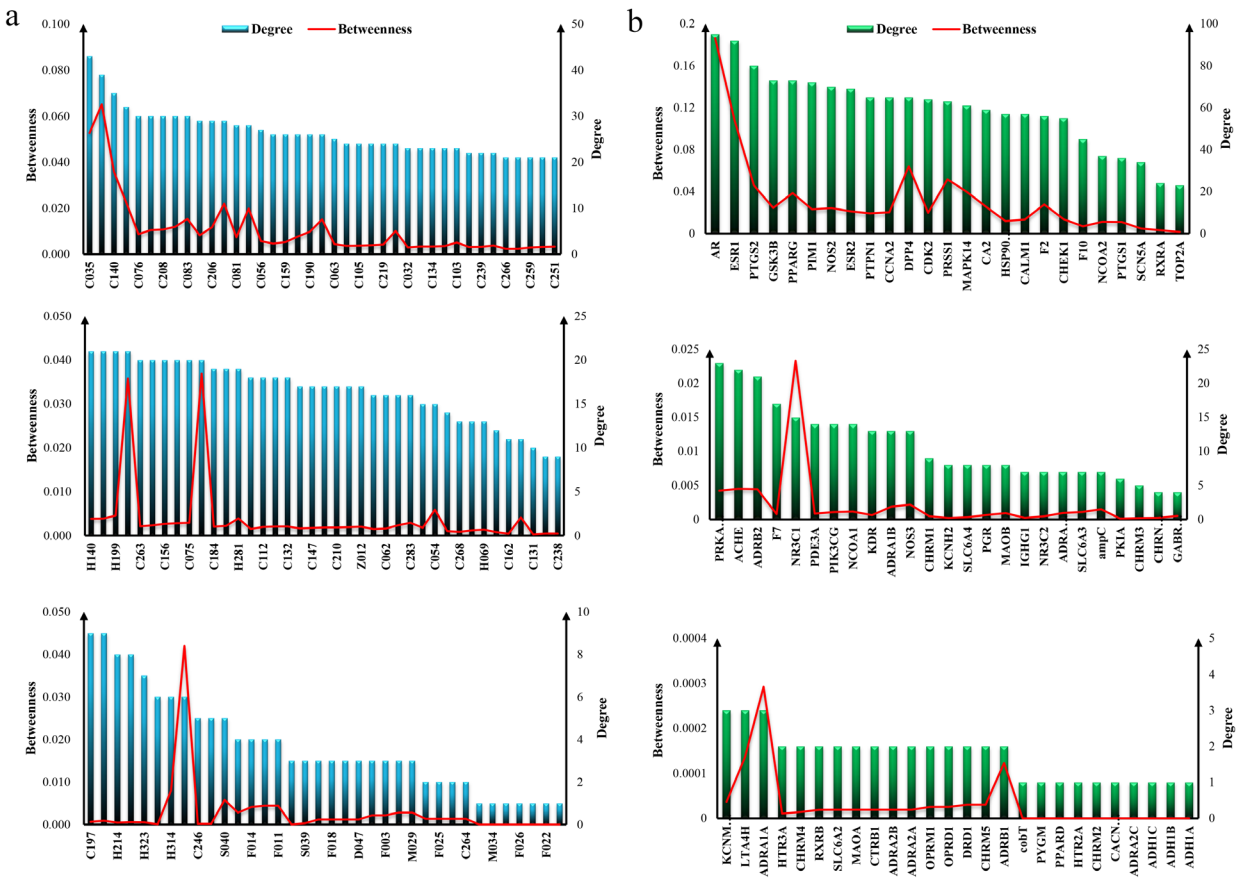

Figure 4. The betweenness and degree of all candidate compounds (graph a) and targets (graph b) of BWXYS formula. In graphs a and b, the left and right y-axes represent the betweenness and degree of candidate compounds and target proteins of the BWXYS formula, respectively.

targets (about 24%) are related to the damage of human inflammatory factors to nerve cells, including 8 targets including PTGS1, PTGS2 and LTA4H. The above indicates that the treatment of MDD by BWXYS is the result of a multi-mechanism synergistic effect, especially the three mechanisms of lacking monoamine transmitter, protection of the nervous system, and anti-inflammatory.

T-D network

In the long history of traditional Chinese medicine, there has been an interesting phenomenon that one herbal compound can treat multiple diseases. In this work, we established a target-disease network (Fig. 6) to study the protein interactions and to understand the mechanism of action of multi-target and multi-effect TCM. From this network, we found 29 targets implicated in two or more types of diseases, such as neurological diseases, pancreatic diseases, immune system diseases, and liver diseases. The most connected targets are Peroxisome proliferator-activated receptor gamma (PPARG, degree = 5), Vascular endothelial growth factor receptor 2 (KDR, degree = 5), Nitric oxide synthase (NOS2, degree = 5), all of which are linked to five diseases, are pivotal proteins in the disease network and may affect the pathophysiological processes and treatment of multiple diseases if used as drug targets,

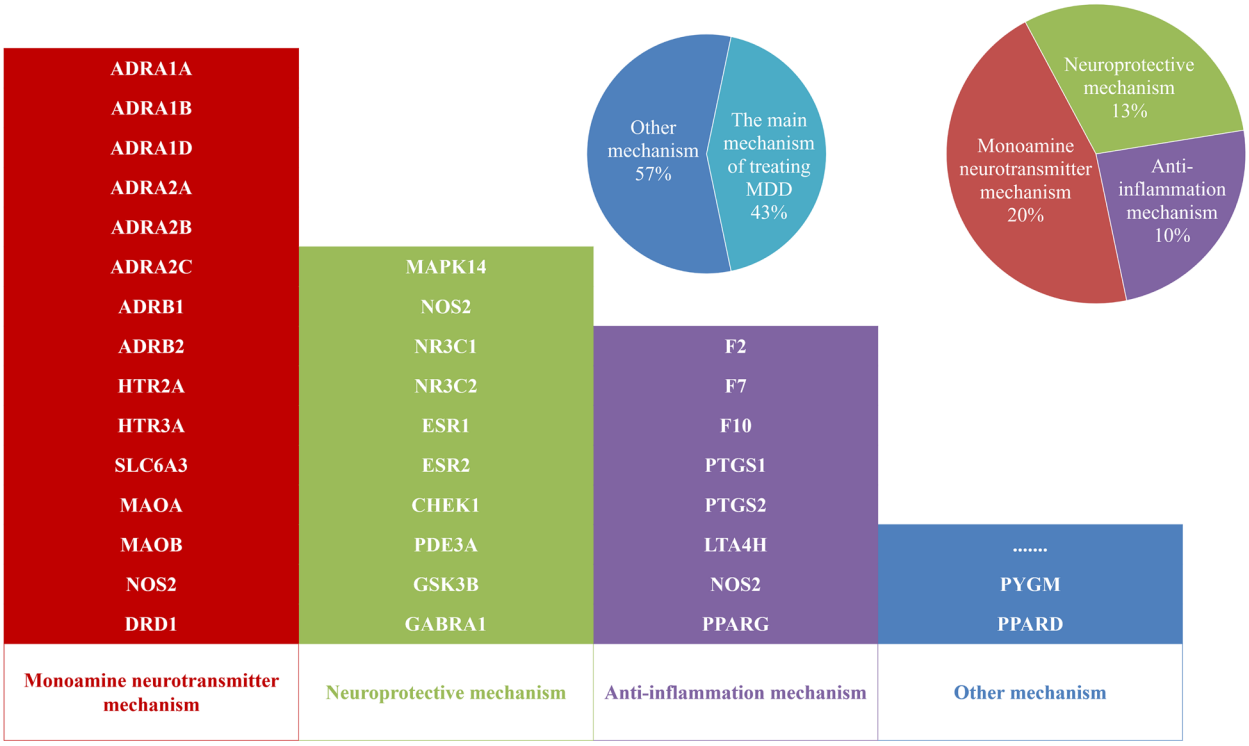

Figure 5. The target mechanism of BWXYS in the treatment of MDD.

facilitating the screening and discovery of novel drug targets.

Target tissue location

All targets were mapped to the BioGPS database (http://biogps.org/) to study the distribution of these targets in human tissues at different levels. We plotted the tissue distribution network of each target based on its expression pattern (as shown in Fig. 7), where the targets are found mostly distributed across five tissues, including brain, liver, lymph, heart, and whole blood.

Firstly, as up to 21% of the targets are located in the brain, we speculate that BWXYS has a direct anti-depression effect by acting on these targets. Taking protein HTR2A as an example, HTR2A encodes one of the receptors for serotonin, which is a neurotransmitter with multiple functions. Low serotonin levels can cause depression. Mutations in this gene are associated with susceptibility to schizophrenia and obsessive-compulsive disorder and are also associated with response to the antidepressant citalopram in patients with MDD. BWXYS down-regulates the expression of the HTR2A gene, thereby alleviating the symptoms of MDD patients. Besides, muscarinic receptors (CHRM3) affect the various effects of acetylcholine in the central nervous system and peripheral nervous system. By regulating the cholinergic nervous system, BWXYS inhibits the activation of the hypothalamic-pituitary-adrenal axis and reduces the level of cortisol, thereby antagonizing the production of depression. Therefore, the active ingredients of BWXYS act on many targets distributed in the brain to regulate the nervous system and thereby treat MDD.

Secondly, the analysis of factors related to the immune response research of depression patients showed that compared with the healthy group, the immune indicators of depression patients were lower, including the activity of NK cells, the number of lymphatic B cells, and T cells (Irwin et al. 1992). The phenomenon of low NK

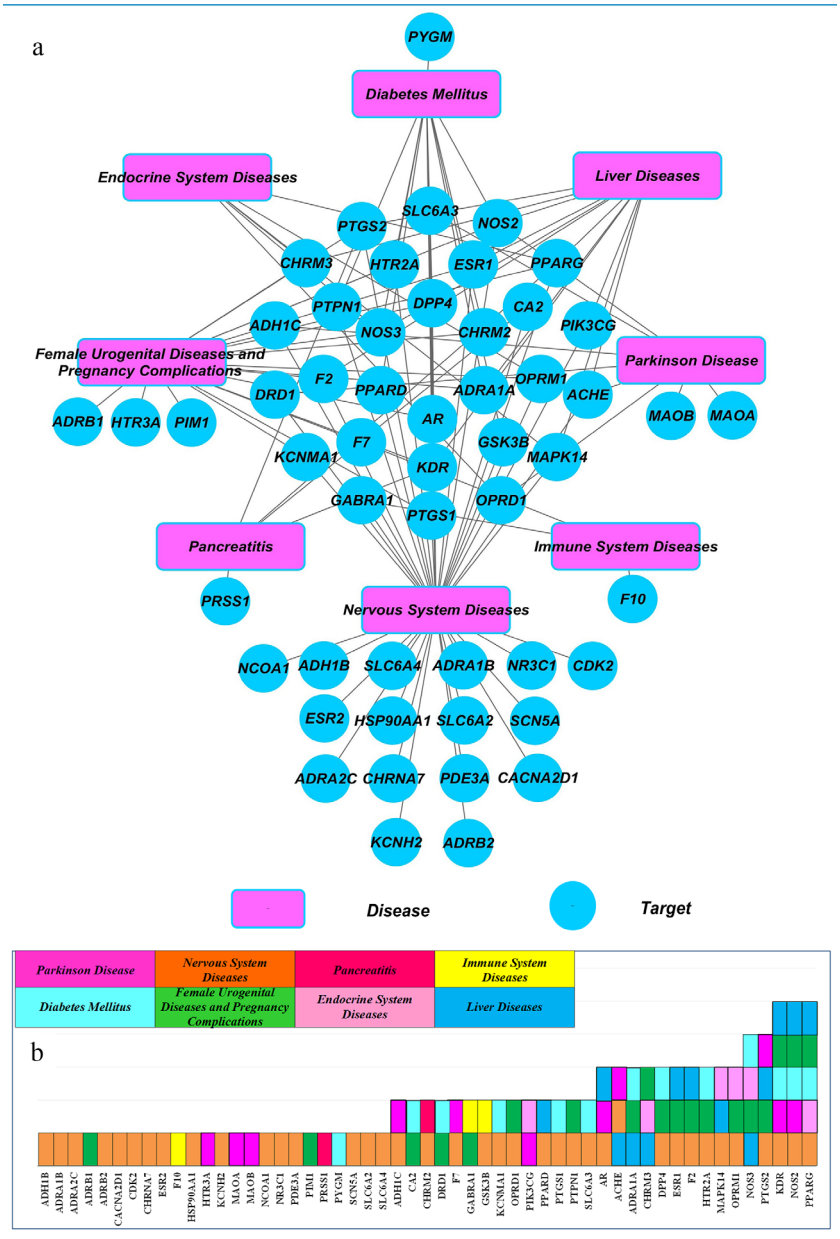

Figure 6. A. In the T-D network, the blue circle represents the target, the pink rectangle represents the disease, and the edge represents the interaction between them. B. Targets related to different diseases.

cell activity after depression is cured no longer exists. As shown in Fig. 7, 19.7% of the targets are distributed in the lymph. BWXYS candidate compounds promote the transformation of lymphocytes by acting on these targets to enhance immune function (Liu 2017). PIK3CG can participate in the migration of T lymphocytes, regulate the proliferation of T lymphocytes and induce the production of cytokines. Autophagy-related target GSK3B can promote the production of inflammatory factors (IL-2, IL-6, etc.), and inhibiting the activity of GSK3B can achieve anti-inflammatory effects.

In addition, six targets were found in the liver. From the perspective of traditional Chinese medicine, the pathogenesis of MDD is mostly liver depression and stagnation, liver depression and fire, liver depression and spleen deficiency, etc., and the treatment of traditional Chinese medicine is based on liver treatment or liver and

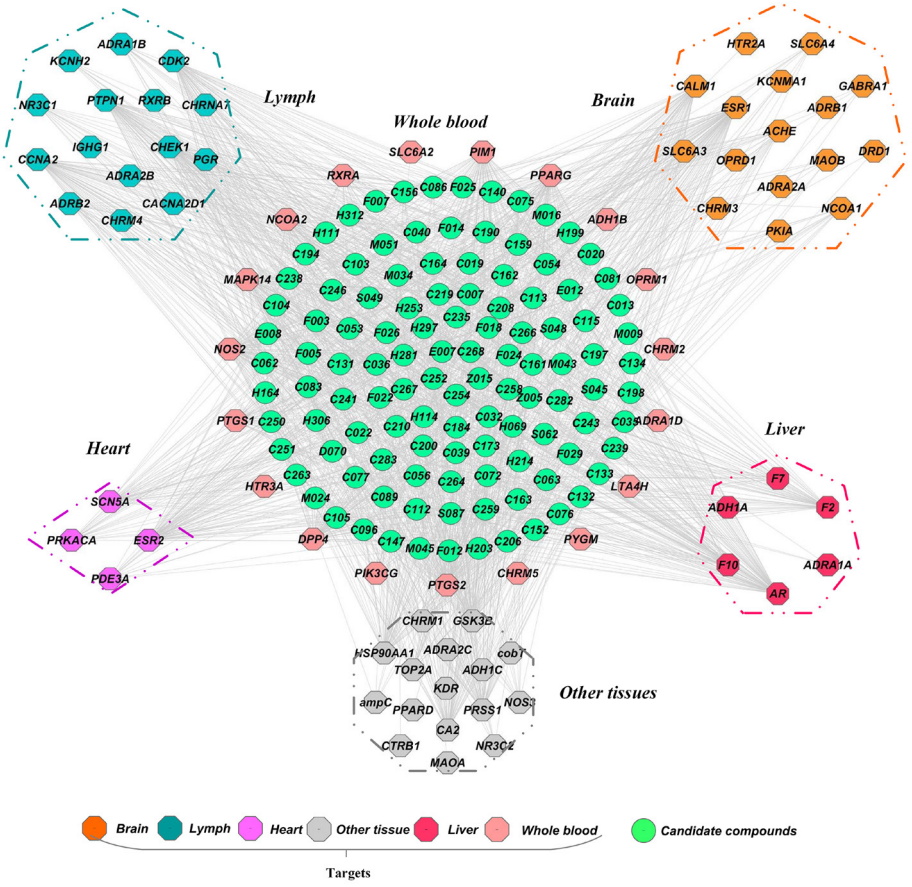

Figure 7. Organ location map of BWXYS targets. Among the targets, orange, blue, pink, grey, red and flesh pink hexagons represent the targets in brain, lymph, heart, other tissues, liver and whole blood, respectively. The green circle represents the candidate compounds of BWXYS formula.

brain treatment. The liver acts on the intestine through the liver-gut axis to intervene in intestinal microecological disorders. Abnormal liver function developed to a certain stage can affect the intestinal barrier function, such as cirrhosis which decreases bile acid secretion and indirectly leads to imbalance of bacterial growth in the intestine, which in turn leads to dysbiosis in the intestinal flora (Gunnarsdottir et al. 2003). It leads to intestinal barrier dysfunction and increased bacterial translocation, which in turn activates circulating immune cells, leading to cytokine production and systemic inflammation (Kronsten et al. 2022). Inflammation is also widespread in depression. Intestinal flora can act on the central nervous system, causing psycho-behavioral changes in the host, leading to the onset or exacerbation of depression (Zheng et al. 2016). Clinical data (Chen 2008) has shown that the BWXYS formula significantly reduces cholesterol and triglycerides in patients with liver disease. This further explains that BWXYS can have a therapeutic effect on liver lesions. Androgen receptors have high mRNA expression in the liver. Experiments have confirmed that androgen receptors enhance the production of intracellular oxidative stress during the process of liver cancer and inhibit the progression of hepatocellular carcinoma (Maes et al. 1993).

Moreover, 25% of the targets are located in whole blood, so they may act as a bridge connecting all body tissues through whole blood to help exert the pharmaceutic effects of the formula. Therefore, the target organ distribution network demonstrates that the active ingredients of the BWXYS formula, through the blood circulation, act on multiple organs

or systems such as the brain, heart, liver, and lymph, and systematically treat MDD as a whole.

Pathway enrichment

The activation, enhancement or inhibition of many pathways are closely related to the occurrence of multiple diseases. To gain insight into the therapeutic mechanism of MDD in BWXYS, we mapped the targets to the KEGG database (www.genome.jp/kegg) and obtained 159 pathways corresponding to the targets, and found that the following pathways were the most abundant and statistically significant in the enrichment analysis, namely PI3K-Akt, cGMP-PKG, MAPK Therefore, we analyzed the above pathways and constructed a pathway network diagram (Fig. 9). The different pathways are classified into modules: inflammatory module, neuroprotective module, monoamine neurotransmitter modulation module, and liver function module depending on the biological processes they are implicated in.

GO analysis

To investigate the microscopic biological processes involved in the targets, we also performed gene ontology (GO) analysis. Fig. 8 shows the most enriched 14 GO terms. The results show that most of the targets are closely related to multiple biological processes, such as anti-inflammation, affecting neuroplasticity, drug metabolism, and regulating neurotransmitter receptor activity. Most biological processes are associated with the onset of MDD, such as the "Adenylate cyclase-activating adrenergic receptor signaling pathway" associated with neuroplasticity, "Synaptic transmission, cholinergic" associated with neurotransmitters. Among them, CALM1, MAPK14, GSK3B, PRKACA are involved in the regulation of dopamine and serotonin; The "Inflammatory response" is related to inflammation and immune response, and targets such as PPARγ and ADRB2 are involved in regulating the acute inflammatory response; "Drug metabolism", "Regulation of liver cell" is related to the regulation of liver function, where there are targets such as ACHE, ADRA1A,

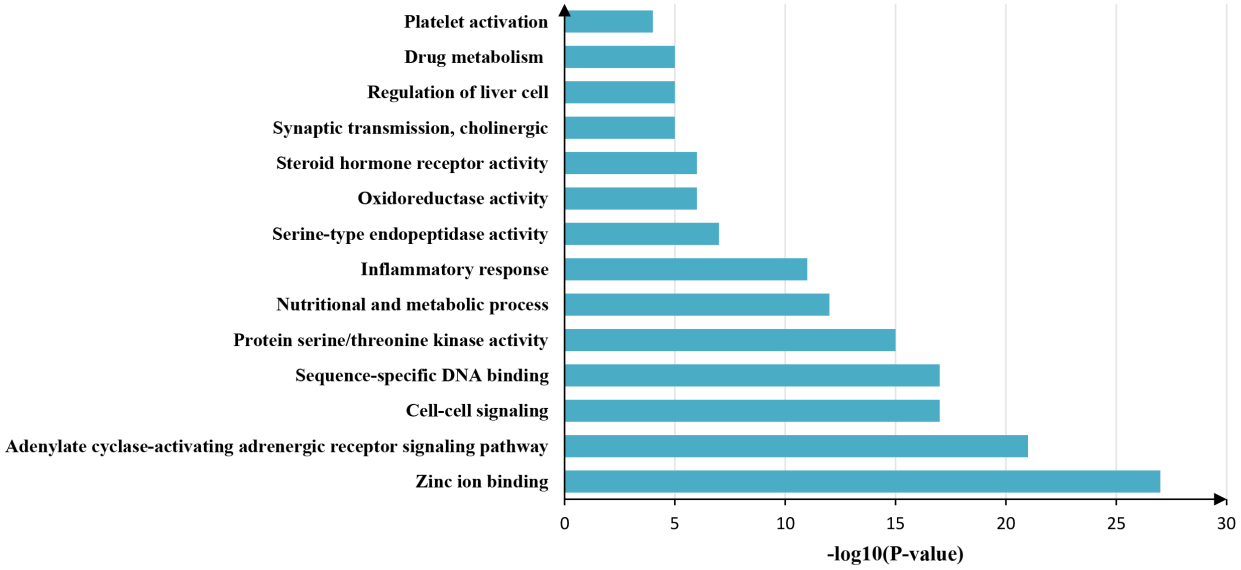

Figure 8. Gene ontology analysis. The y-axis is a category of "biological processes" that are significantly enriched in go relative to the target gene.

CHRM3, ESR1, F2, and KDR affect the processes of liver inflammatory cell accumulation, liver fibrosis, and vascular remodeling. Therefore, the active ingredients of this formula may also be involved in the regulation of a variety of related biological processes and exert antidepressant effects.

DISCUSSION

As a matter of fact, flavonoids are a class of polyphenolic compounds that exist in nature with multiple pharmacological activities. Numerous studies have demonstrated that flavonoids have various pharmacological activities, such as liver injury protection, antibacterial, antiviral, vasodilator, hypoglycemic, and anti-tumor effects (Zhang et al. 2001). In recent years, it has also been that flavonoids have a significant effect on central nervous system, such as antidepressant, anti-anxiety, central depression, neuroprotection, and other effects. In addition, it can be seen from Fig. 2 that terpenes are a type of natural hydrocarbon that is widely found in plants. The content of this type of active ingredient in BWXYS is as high as 26%, making it the second major contributor to the efficacy of BWXYS. In fact, many pharmacological effects of terpenoids have been verified, such as anti-tumor, anti-cancer, anti-inflammatory and

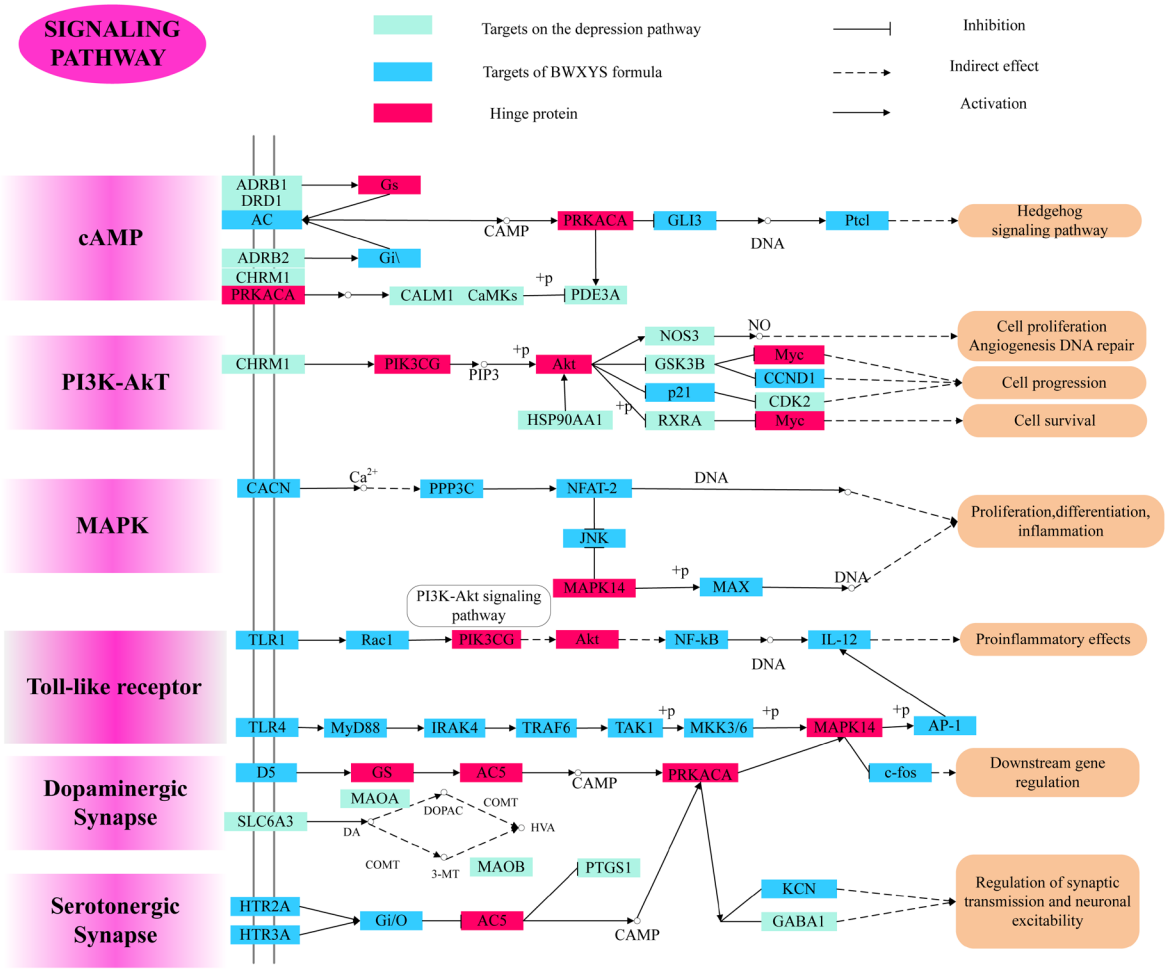

Figure 9. The depression pathway and therapeutic targets of BWXYS formula.

anti-spasmodic effects (Fu et al. 2003, Dong & Zhang 2004).

Both NR3C1 and NR3C2 are receptors for corticosteroids. Long-term abnormal corticosteroid levels not only affect hippocampal cell response, but also affect cell survival (Liu et al. 2011). In addition, corticosteroids and their receptor systems have an important regulatory effect on the hypothalamus-pituitary-adrenal axis (HPA axis). The HPA axis is an important endocrinological axis (Lupien & Mcewen 1997). Abnormal endocrine system function is one of the factors that triggers MDD. The imbalance of corticosteroids and their receptors can cause depression. Therefore, we speculate that the active ingredients of BWXYS act on these targets to achieve the treatment of MDD.

T-D network analysis

In terms of the degree of disease connectivity, the largest degree was for neurological diseases (degree = 38). As seen in this figure, 8 targets are associated with Parkinson's disease (PD). In fact, Parkinson's disease is a neurodegenerative disease characterized by neurodegenerative lesions of the nigrostriatal pathway (Zhang et al. 2016), and sodium-dependent dopamine transporter (SLC6A3) is the target of the drug benzatropine mesylate for Parkinson's disease treatment.

The degree of liver disease is 12. Androgen receptor (AR), alpha-1A adrenergic receptor (ADRA1A), muscarinic acetylcholine receptor M3 (CHRM3), thrombospondin (F2), vascular endothelial growth factor receptor 2 (KDR) influence the processes of liver inflammatory cell accumulation, liver fibrosis, and vascular remodeling. It was confirmed that AR enhances cellular oxidative stress and increases DNA damage in the process of liver tissue carcinogenesis and that targeting curcumin derivative ASC-J9 to modulate AR inhibits the process of hepatocellular carcinogenesis (Ma et al. 2008). From this, we conclude that liver disease and depression have a mutually reinforcing effect and that the formula is effective in both diseases One of its mechanisms for treating MDD is by enhancing and taking care of the liver function.

In addition to liver diseases, several common diseases such as female genitourinary disorders, pregnancy complications, diabetes mellitus, and abnormal endocrine system function can also cause depression, which is aptly confirmed by this T-D network diagram.

BWXYS has been experimentally shown to have a better therapeutic effect in women with urinary tract infections and intrahepatic cholestasis during pregnancy (Cao & Wei 1998, Xu & Gao 2009). In fact, MDD during pregnancy is a type of depression with a high incidence. The biological basis for the development of the disorder is the great changes in the endocrine environment of pregnant women during pregnancy and delivery, with dramatic changes in the hormone levels in the body. In addition, the somatic factors of pregnant women should not be ignored, as pregnant women with somatic diseases during pregnancy are more likely to suffer from depression, and there are also environmental and genetic causes (Szpunar 2020, Chen & Zhong 2000). Moreover, the occurrence of MDD during pregnancy may increase the incidence of pregnancy complications, such as habitual abortion, gestational hypertension, preterm labor, and prolonged labor (Xia et al. 2011). This explains why the BWXYS also has efficacy in treating this kind of MDD during pregnancy.

Another complication of depression is diabetes, and diabetic patients are more likely to suffer from depression than general population (Kou 2009). Modern medicine has also reported that BWXYS has better efficacy in diabetes (Wang

et al. 2013), which undoubtedly broadens the therapeutic scope of this formula. As seen in Fig. 6, there are as many as eight common targets for neurological disorders and diabetes, among which 5-hydroxytryptamine receptor 2A (HTR2A) is both a target for drug treatment of MDD and a target for diabetes treatment. SSRIs produce antidepressant efficacy by acting on serotonin receptors (Zhang 2007), Paroxetine, an SSRI, was found to both increase insulin sensitivity and decrease serum hydrocortisone levels in diabetic patients and to alleviate depressive symptoms such as anxiety (Xu & Wang 2007).

Abnormalities in the function of the endocrine system also play a very important role in the development of MDD, of which the hypothalamic-pituitary-adrenal axis (HPA) is an important endocrine axis (Liu et al. 2011). Since the 1970s, it has been discovered that the HPA axis is involved in the pathological mechanisms of MDD and has a key role in regulating the stress response. Patients with depression often show hyperactivity of the HPA axis, resulting in overproduction of cortisol, which in turn damages hippocampal neurons and leads to cognitive decline (Zuo & Xu 2001). This suggests a close relationship between abnormal endocrine function and depression, and it is hypothesized that modulation of endocrine function may be efficacious in depression.

It was found that depressive symptoms occurred in patients treated with the inflammatory cytokine interferon and were diminished at the end of treatment. In depressed patients with systemic inflammatory disease, plasma concentrations of inflammatory cytokines and other acute phase proteins such as IL-1, IL-2, IL-6, tumor necrosis factor (TNF), human C-reactive protein (CRP), and α1 macroglobulin (α1MG) were increased (Karaoulanis et al. 2014). During acute episodes of depression, TNF-α levels are elevated. These peripheral immune processes may be associated with microglia activation in localized areas of the anterior cingulate cortex (Steiner et al. 2011). Changes in the immune system may be one of the ways in which antidepressant therapy works. Many pharmacological antidepressants reduce inflammatory activation in immune cells and decrease circulating inflammatory cytokine levels (Lee & Giuliani 2019).

Pancreatitis is a common clinical condition. Studies have shown that the intraserum inflammatory factor IL-6 can respond to the severity of acute pancreatitis and that TNF-α levels rise at the onset of pancreatitis (Farrell et al. 2021). In addition, chronic pancreatitis increases the risk of developing pancreatic cancer (Ahmed et al. 2018), and approximately 43% of pancreatic cancer patients develop depression after diagnosis (Barnes et al. 2018), and some studies have shown that the incidence of pancreatic cancer-related depression ranks first in the incidence of tumor-related depression (Dengsø et al. 2020). Patients with pancreatic cancer have significantly higher levels of IL-6, which may explain the high rate of depression in this population (Breitbart et al. 2013).

Inflammation module

First, PI3K is a key component of the PI3K-Akt signaling pathway, a leukocyte-rich lipid kinase that regulates a variety of cellular processes, including cell growth, migration, and proliferation.

Indeed, the PI3K-Akt signaling pathway can suppress inflammation by negatively regulating the expression of TNF-α and TF and is involved in TREM-2-mediated immune regulation. Upon activation of this pathway by the corresponding receptor, PI3K catalyzes the production of PIP3 (Phosphatidylinositol-3,4,5-triphosphate) at the cell membrane, which acts as a second messenger to activate Akt and regulate a series of intracellular processes, including apoptosis,

protein production, metabolism, and cellular recycling (Li & Zhou 2014). In the BWXYS formula, up to seven targets are involved in the PI3K-Akt pathway as important regulators of the immune system, including CHRM1, PIK3CG, HSP90AA1, GSK3B, NOS3, RXRA, and CDK2 (as shown in Fig. 9). Among them, GSK3B, a downstream target of Akt, can produce inflammatory cytokines and participate in the immune system against invading pathogens, and in this way, control cell survival and cell cycle progression (Jayapalan & Natarajan 2013). It is thus clear that the PI3K-Akt pathway is an important regulatory pathway for the anti-inflammatory effects of BWXYS prescription drugs.

Another pathway of this module, the Mitogen-activated protein kinases (MAPK) signaling pathway, is an important intracellular signaling and inflammatory regulatory pathway that regulates gene expression, cell proliferation, differentiation, mitosis, apoptosis, etc (Li et al. 2006). This pathway consists of MAP kinase, MAPK kinase, and MEK kinase. Through the sequential phosphorylation of these three types of proteins, signals are transmitted from upstream to downstream response molecules to generate the corresponding response. The target of this pathway in BWXYS is MAPK14, which plays an important role in regulating other processes such as gene expression, cell proliferation, differentiation, and mitotic differentiation. A report showed that paeoniflorin (M024) was able to regulate the abnormal Ras-MAPKs signaling pathway by inhibiting the elevated phosphorylated expression of Raf-1 protein and MEK-1 protein in rat glioma cells (Guan et al. 2014), which is consistent with our prediction. This shows that the MAPK pathway is an important regulatory channel for the anti-inflammatory module of BWXYS formula.

Toll-like receptors (TLRs) were originally discovered in Drosophila as a signal transduction protein with important roles in development and immune response. As research progressed, it was discovered that Toll-like receptor proteins also exist in mammals, and these proteins were unified into the TLR family. They are able to bind to specific molecular structures shared by some pathogens or their products for recognition, and can also recognize the related molecular structures of different pathogens and then trigger a series of signal transduction that leads to the release of inflammatory mediators and ultimately activates the acquired immune response (Chen et al. 2008). Experiments have confirmed that the active ingredients in herbal medicines are able to act on multiple target proteins of the TLR pathway, thereby inhibiting the inflammatory response and reducing the release of inflammatory mediators. In the present work, we identified three targets involved in the regulation of the Toll-like receptor signaling pathway, namely PIK3CG, MAPK14, and GSK3B. The active ingredient H281 (quercetin) acts on them, which is consistent with the experimental findings that H281 exerts its antioxidant and anti-inflammatory properties by inhibiting Toll-like receptor signaling (Han et al. 2016). Taken together, this pathway is an important one for this module.

Neuroprotective module

MDD in humans is associated with reduced expression and function of brain-derived neurotrophic factors (BDNF), which are found in a variety of neurons in the central and peripheral nervous systems, with the highest levels in the hippocampus and cortex. Clinical studies have demonstrated that the PI3K-Akt pathway is associated with both the development and regulation of inflammation and BDNF. This pathway not only regulates the growth and proliferation of neurons in the hippocampus, but also regulates stress-induced depression

and pharmacological antidepressant responses (Zeng et al. 2017).

Glycogen synthase kinase 3B (GSK3B) in the PI3K-Akt pathway, a class of signaling molecules that regulate neuronal functions such as gene expression, neurogenesis, synaptic plasticity, and neuronal survival and apoptosis, is widely expressed in the brain. The active ingredient kaempferol (H203) in the compound can increase the activity of GSK3B, prevent apoptosis and promote neuronal survival by modifying the activity of PIK3CG, another target in this pathway (Marfe et al. 2009), which is a potential mechanism for the antidepressant of this signaling pathway.

Another important pathway in this module is the cAMP/PKA signaling pathway. cAMP is an important intracellular second messenger that plays a mediating role in translating extracellular stimulus signals into various intracellular physiological activities. It phosphorylates the protein CREB mainly by activating PRKACA, which then turns on gene expression and synthesizes related proteins, and therefore has an important significance in cellular life activities, participating in mediating many cellular metabolisms, gene expression, cell growth and division (Schaeffer & Weber 1999). Increasing the expression level of cAMP can upregulate the function of the cAMP-CREB pathway and increase the expression of BDNF mRNA in the hippocampus, which plays an antidepressant role.

Monoamine neurotransmitters module

The first important pathway in this part is the Dopaminergic Synapse signaling pathway. Through the supply of Na^+/K^+-ATPase in the cell membrane, dopamine is released from neurons and quickly reuptaken by the dopamine receptor (DRD1), which regulates the effective concentration of dopamine in the synaptic gap and enables time-dependent agonism of dopamine to presynaptic and postsynaptic receptors (Li & Zhao 2011). The previous study obtained eight active molecules targeting dopamine receptors (DRD1), such as liquiritigenin (C013) and medicarpin (C022). Among them, C013 is a natural flavonoid, while C022 has the effect of killing human hepatocellular carcinoma cells (Li et al. 2001), both of which are natural substances with neuroprotective effects as active components of licorice.

5-HT receptors play an important role in the formation of depression and the development of therapeutic drugs (Li & Zhao 2011). Another important pathway is the serotonergic synapse signaling pathway. In the present work, four targets, namely PTGS1, PRKACA, HTR2A, and HTR3A, are involved in the regulation of this pathway. Interestingly, in the formulation of BWXYS, the active ingredients inermine (C007), medicarpin (C022), and shinpterocarpin (C140) act on these four targets, which is consistent with the experimental results that SSRIs (Selective Serotonin Reuptake Inhibitors) exert antidepressant effects by enhancing 5-HTergic neurotransmission (Qian et al. 2012).

Liver function module

Depression, as a type of affective mental disorder, is a common and frequent disease in modern society. The pathogenesis of this disease is mostly liver depression and stagnation, liver depression and fire, liver depression and spleen deficiency, etc., and the treatment of traditional Chinese medicine is based on liver treatment or liver and brain treatment. BWXYS is mainly used to treat liver depression and is often selected clinically for the treatment of MDD (Feng et al. 2005), so the treatment module in the liver is also quite important. An important pathway in this part is the Toll-like receptor signaling pathway, which is closely associated with the inflammatory response and cellular matrix

accumulation that occurs in liver tissue following acute liver injury (Aoyama et al. 2010). Following pathogen infection, Toll-like receptors (TLR) are expressed on the surface of antigen-presenting cells, such as macrophages and dendritic cells, initiating a signaling pathway that stimulates the body's defense through the induction of reactive oxygen species and reactive nitrogen intermediates. Among the targets of this remedy, there are MAPK14 and PIK3CG involved in this signaling pathway, which together form a cellular signaling communication network that mediates the long pathological process of chronic liver injury to liver fibrosis. Kaempferol (H203) and isorhamnetin (H199) both target PIK3CG, both of which have been shown to have significant antioxidant activity as active components of the Chinese medicine Radix Bupleuri (Xiao et al. 2012), and have a significant inhibitory effect on the proliferation of human hepatocellular carcinoma cells.

Biology crosstalk analysis

Biological crosstalk refers to a situation in which one or more components of one signal transduction pathway affect another pathway (Kunkel & Brooks 2002). Crosstalk is often present in various signaling pathways due to the presence of overlapping hubs that connect pathways into adaptable and complex networks. In terms of target and pathway connectivity, 82% of target proteins were mapped to at least two more pathways, indicating that these targets can regulate interactions between multiple pathways.

In Fig. 9, we can see that multiple pathways produce multiple interactions by regulating multiple hinge proteins, such as PRKACA, PIK3CG, and MAPK14. Here we take the target mitogen-activated protein kinase 14 (MAPK14) as an example to explain the biological crosstalk effect in multiple signaling pathways of the hinge protein. As previously mentioned, MAPK14 is a member of the MAPK family, an important anti-inflammatory target, which is mainly involved in stress-dependent apoptosis and inflammatory responses and can be activated by stressors such as ischemia and hypoxia, inflammatory factors, endotoxins, and ultraviolet rays (Schaeffer & Weber 1999). Despite its relatively low degree of connectivity, it deserves more experimental studies because of its key position in the pathway network and because it is an essential target for the treatment of MDD. It can be seen from Fig. 9 that MAPK14 participates in the three pathways of Dopaminergic synapse, Toll-like receptor, and MAPK simultaneously. These pathways are combined by mediating the intracellular signaling cascade to regulate MAPK14 activity. In the BWXYS formula, MAPK14 is regulated by 61 active molecules such as H281 (quercetin) and C283 (isoliquiritigenin). H281, a natural bioflavonoid with a wide range of physiological effects, has been shown to scavenge liver tissue free radicals and protect hepatocyte integrity, in addition to its anti-inflammatory effects (Xin et al. 2008). C283 is an aldose reductase inhibitor, which inhibits the activity of cyclooxygenase, lipoxygenase, and peroxidase enzymes to prevent platelet agglutination and provide anti-inflammatory effects (Li et al. 2010).

In conclusion, the PI3K-Akt signaling pathway and MAPK signaling pathway are involved in inflammation, and the regulation of these two pathways can help treat MDD from a neuroinflammatory perspective. Dopaminergic synapse and serotonergic synapse pathway are mainly involved in the regulation of monoamine neurotransmitters. PI3K-Akt is also related to the regulation of neurotrophic factors, as is the cAMP pathway. As for the Toll-like receptor pathway, it is mainly involved in liver inflammation and liver fibrosis processes. In summary, it is the complex network of these pathways and the

cross-talk between them that gives BWXYS its complementary antidepressant functions.

Compatibility analysis of BWXYS

Traditional Chinese prescriptions are usually composed of a variety of herbs, which are combined according to certain compatibility theories of traditional Chinese medicine, such as seven methods in compatibility of ingredients, symptomatologic modification, and "Jun-Chen-Zuo-Shi". Through the analysis of the pharmacological action of the active ingredient and the target, we found that the herbal combination in BWXYS conforms to the compatibility rule of "Jun-Chen-Zuo-Shi". We list the number of ingredients of the herbs in BWXYS and their function in the prescription in Table I.

Monarch is a drug that has a principal effect on treating the major syndrome in the prescription. Radix Bupleuri is the monarch drug in BWXYS. As can be seen from Table I, 16 compounds of Radix Bupleuri were identified as candidate compounds, more than half of which could play a direct part in the treatment of MDD. For example, the experiment confirmed that the effective component quercetin has a significant protective effect on PC12 cells damaged by corticosterone, and thus has a direct therapeutic effect on depression (Chen & Gan 2009). Besides, the triterpenoid component saikosaponin A (H162) and saikosaponin D (H164) has been shown to significantly reverse the reduction of monoamine neurotransmitters in the rat brain caused by depression (Ge et al. 2008). saikosaponin A (H162) has been verified to inhibit cholinesterase, and in this way, further regulates the digestive and nervous systems (Wang & Chen 2000) so as to soothe the liver and relieve depression.

The minister is a drug that assists or strengthens the efficacy of the monarch drug. Gardeniae Fructus, Paeoniae Radix Alba and Angelicae Sinensis Radix are the minister drugs in BWXYS. Terpenoids are rich in Gardeniae Fructus, which are actually almost the mostly active ingredients in the herb with outstanding pharmacological effects. For example, the active ingredient Genipin 1-Gentiobioside (Z047) has good anti-inflammatory and free radical scavenging effects. Another herb, Paeoniae Radix Alba, is the peeled and dried root of the genus Paeoniaceae, which is slightly cold and has a bitter acid. It is commonly used in the private sector to treat spontaneous night sweats, yin deficiency fever, and irregular menstruation, and many of these diseases are complications of depression. As for Angelica, it is the dried root of the Angelica plant in the umbelliferae. Angelica has the functions of nourishing blood, anti-hypoxia, regulating the body's immune function, promoting blood circulation, bacteriostasis, anti-arteriosclerosis, and so on (Institute of Materia Medica Chinese Academy of Medical Science 1984). In summary, these three herbs not only have curative effects on depression through an anti-inflammatory mechanism but also are responsible for the treatment of certain complications of MDD (such as pregnancy complications), so they are all major drugs in BWXYS formula.

Assistant drugs are used to treat concurrent symptoms or eliminate the side effects of the monarch drug. Patients with MDD tend to have liver qi stagnation and abnormal liver function. Atractylodes Macrocephala Koidz, Cortex Moutan, and Poria Cocos (Schw.) Wolf all have the function of protecting the liver and improving the body's immunity, and are used to improve the effect of the minister drugs on the treatment of MDD in the concurrent disease. On one hand, Atractylodes Macrocephala Koidz accelerates the glucose metabolism in the body, and on the other hand prevents the breakdown of liver glycogen[24] (Duan et al. 2008). In

addition, it also increases the conversion rate of lymphocytes, promotes cellular immune function, strengthens the spleen and stomach, and improves the body's disease resistance (Mao et al. 1996). Actually, volatile oils (like 8β-ethoxy atractylenolide III, E012), one important pharmacodynamic substance contained in Atractylodes Macrocephala Koidz, have pretty strong concentration-dependent bacteriostatic activities against bacteria and fungi (Tang et al. 2008). Helicobacter pylori (HP) causes an inflammatory response that can activate inflammatory factors including IL-1, IL-6, and TNF-α, thus HP infection substantially increases lymphocyte homing and hepatocytotoxicity in patients with chronic hepatitis C (Esmat et al. 2012). In addition, Nisimova et al. 2014 reported that blood ammonia concentrations in patients with hepatic encephalopathy infected with HP were significantly higher than those in patients without HP infection, suggesting that HP infection is a risk factor for the development of hepatic encephalopathy and should be given high clinical priority. Paeonol (M045), the active ingredient in Cortex Moutan, has been experimentally proven to relieve the liver toxicity caused by the anti-tuberculosis drug rifampicin (Wang et al. 1999). Poria cocos polysaccharides has a good inhibitory effect on liver viruses, and in fact, its therapeutic index is even higher than acyclovir (Duan et al. 2005). At the same time, Poria Cocos (Schw.) Wolf could improve the body's non-specific immunity and reduce hormonal side effects (Jiang et al. 2021). To sum up, Atractylodes macrocephala Koidz, Cortex Moutan, and Poria Cocos (Schw.) Wolf all make the effect of improving liver function abnormalities and are considered to be assistant drugs in BWXYS prescription.

The Guide drug directs medicines to reach the disease site or medicines that have the effect of reconciling various medicines. Licorice is the root of licorice, a legume plant, and contains many active ingredients. It has the functions of nourishing the spleen and nourishing qi, moistening lungs and cough, relieving pain, relieving viruses, and reconciling various medicines. (Abe et al. 2003) confirmed in animal experiments that glycyrrhizic acid can increase the amount of interleukin-10 (IL-10) produced by mouse liver dendritic cells in mouse liver induced by Concanavalin A (conA) and downregulate the liver immune response. In addition, many active ingredients in Licorice have important protective effects on nerve regeneration in the hippocampus, such as isoliquiritigenin (C283) and isoliquiritin (C200) can inhibit monoamine oxidase (MAO) (Hatano et al. 1991), and play the same role as the antidepressant monoamine oxidase inhibitor. In summary, licorice, as a guide drug, has detoxification and reconciliation effects and is widely used in traditional Chinese medicine prescriptions.

LIMITATIONS

We acknowledge that the present study still has some limitations. The first point is that the chemical composition of BWXYS was not obtained by our own experimental analysis, so some ingredients may have been missed. The second point is that we delineated the criteria in screening the active ingredients, which may also result in some of the potent ingredients not being listed as active ingredients. The third point is that although the findings of this study are consistent with the results of previous studies, some conclusions still need to be verified experimentally. Additional information can be obtained from the author upon request.

CONCLUSION

In this study, we used a systems pharmacology-based approach to explore the multi-compound, multi-target, and multi-pathway properties of the BWXYS formulation and the pathogenesis of MDD from the molecular to the pathway level. The main results are as follows.

Our results showed that 1064 components were obtained from the eight herbs of the BWXYS formulation, and the screening yielded 133 active ingredients in nine categories: flavonoids, terpenes, organic acids, coumarins, volatile oils, amino acids, glycosides, alkaloids and others, and identified 76 potential targets distributed in human brain, liver, lymph, heart and whole blood. The network pharmacological analysis showed that BWXYS formulas have typical "multi-component, multi-target and multi-pathway" characteristics, and the therapeutic effects are achieved through the synergistic effects of multiple active molecules.

By constructing a T-D network diagram for analysis, we found that BWXYS formula can also produce therapeutic effects on neurological diseases, pancreatic diseases, immune system diseases, and liver diseases in addition to the treatment of MDD, reflecting the characteristics of the traditional Chinese medicine prescription "one medicine with many diseases". At the same time, we also found that depression is a complex disease through GO analysis and target tissue location. Its causes are multi-faceted, and it is of great significance to treat MDD from multiple angles.

The mechanism of action of BWXYS formula in the treatment of MDD is achieved through its three functional modules, namely neuroprotective, anti-inflammation, regulating monoamine neurotransmitters and liver. And the implementation of these functions depends on the smooth operation of the complex biological pathway network, which specifically includes six pathways, namely PI3K-Akt, cGMP-PKG, MAPK, Dopaminergic synapse, Serotonergic synapse, and Toll-like receptors (TLRs) signaling pathway.

Starting with the research on BWXYS, which is a formula for relieving the liver and relieving depression, after GO analysis, target tissue location, T-D network analysis, and mechanism analysis, we found that depression and abnormal liver function are closely linked in terms of etiology, pathological characteristics, and clinical symptoms.

Jun-Chen-Zuo-Shi theory can scientifically explain the role of different herbs in BWXYS formula. After analyzing the active molecules in the herbal medicine and their interaction with the target, the conclusion is drawn: Radix Bupleuri is the monarch drug; angelica, Gardeniae Fructus, and white peony are the minister drug; Atractylodes, Poria and Cortex Moutan are assistant drug; and licorice is the guide drug.

In summary, MDD is a complex disease caused by multiple factors. This article uses BWXYS as an example to illustrate the advantages of TCM monolithic drugs in the multi-level and multi-modal treatment of molecule-tissue-organ-human body. This study is conducive to the development of botanical medicine in modern medicine and provides a modern interpretation of the traditional Chinese medicine theory "Jun-Chen-Zuo-Shi", which at the same time explains the relationship between abnormal liver function and depression.

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SUPPLEMENTARY MATERIAL

Tables SI-SII

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