Elucidation of The Potential Mechanism of Guanxin Formula Against Traumatic Brain Injury, Depression and Functional Dyspepsia Through Network Pharmacology Strategy

Yawen Cai
Nanjing University of Chinese Medicine

Li Zhou
Nanjing University of Chinese Medicine

Li Chen
Nanjing University of Chinese Medicine

Xiaohang Zhang
Nanjing University of Chinese Medicine

Ping Ren
Jiangsu Province Hospital of Traditional Chinese Medicine

Xi Huang (✉️ 290606@njucm.edu.cn)
Nanjing University of Chinese Medicine

Research Article

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Abstract

With the aging of the world population trend intensifying, a growing number of individuals are suffering from two or more long-term diseases, namely multimorbidity. Traditional Chinese Medicine (TCM) medication is a unique treatment method in China. Traditional Chinese medicine formulae (FTCM) contain multiple herbs with multi-target, multichannel, and multi-link characteristics. Besides, FTCM is known for its multi-targeted pharmacological function and fewer side effects. Guanxin is a representative compound with powerful pharmacological effects, such as cardio-protection, anti-traumatic brain injury (TBI), anti-depression, and anti-functional dyspepsia (FD). Moreover, its mechanism is mainly through inhibiting the inflammatory response. However, due to the complex biological active ingredients of the herbal formula, its active ingredients and mechanism of action in the treatment of TBI, depression, and FD are not yet fully elucidated. Therefore, we first explored the main active ingredients and potential mechanisms of action of Guanxin in the treatment of TBI, depression, and FD by using network pharmacology methods. Nextly, principal component analysis (PCA) was used to validate the relationship between Guanxin and three commonly used drugs for these diseases. Subsequently, we docked the principal components (luteolin, quercetin, and β-sitosterol) with key targets (IL-6, IL-1β, and TNF) of Guanxin in the treatment of TBI, depression, and FD, by molecular docking methods, which further validated the above analysis results. In conclusion, Guanxin has therapeutic effects on TBI, depression, and FD, mainly by inhibiting the secretion of inflammatory cytokines from reducing the inflammatory response.

1. Introduction

Multimorbidity is usually defined as having two or more long-term diseases in the same individual [1]. It is estimated that about one-third of the people worldwide suffer from multimorbidity, among which 50 million people in the European Union [2]. The global prevalence of multimorbidity is increasing due to the aging population. Studies have found that England's prevalence of "complex multimorbidity" will increase from approximately 10% in 2015 to 17% in 2035 [3].

Traumatic brain injury (TBI) is a type of brain injury caused by severe trauma from external mechanical forces (sharp or blunt objects), resulting in permanent or temporary impairment of cognitive, physical, and psychosocial functions [4–6]. Moreover, it is featured in high morbidity, disability, and mortality [7]. The clinical manifestations of TBI patients usually are memory and attention deficits, depression, fatigue, and cognitive decline. TBI is among the leading causes of death and disability [8], and more than 50 million people worldwide are affected annually [9].

TBI mainly comprises primary brain injury and secondary brain injury. Bao et al. [10] proposed that secondary TBI development is usually caused by multiple mechanisms of damage and deterioration of the surrounding microenvironment diffusion-proinflammatory response, oxidative stress, local hypoxia/reoxygenation, and accumulation of neurotoxic substances. Among them, neuroinflammation is the primary pathological process of secondary reactions after TBI, and also is a crucial secondary injury
factor that can lead to sustained neuronal damage [11]. The inflammatory reaction to TBI was thought to occur solely through peripheral immune mediators entering a disturbed blood-brain barrier (BBB) [12]. Of note, TBI also can lead to BBB damage and leakage, leading to increased extravasation of immune cells (increased neuroinflammation). Therefore, the key to delaying the progression of secondary injury is to resist the toxic effect of neuroinflammatory reaction [13].

Most TBI patients also have concomitants with a variety of mental complications. Depression, prevalence is about 60%, is the most common mental complication [14]. It is a common, debilitating, and potentially lethal disorder [15], and predominantly pathogenesis is closely related to the inflammatory response. On the one hand, depression can drive the inflammatory response; On the other hand, inflammation will also lead to depression [16]. It is worth noting that the increase in the inflammatory reaction can also lead to the pathogenesis of many diseases, including cardiovascular disease, diabetes, and functional dyspepsia [17–19].

Functional dyspepsia (FD) refers to a common functional gastrointestinal disease (FGID), and its symptoms are mainly triggered by psychological characteristics, states, or mental disorders [20]. Studies have shown that psychological factors are essential in gastrointestinal sensation and movement. Psychological factors such as anxiety and depression can affect gastrointestinal target organs through the brain-gut axis, resulting in changes in gastrointestinal movement, sensation, secretion, and immune functions. Therefore, depression plays a vital role in the pathogenesis of functional indigestion [21]. Furthermore, substantial evidence shows that impairment of intestinal mucosal barrier function triggers a series of inflammatory responses, which is the crucial cause for the development of FD [22].

In summary, the occurrence and development of TBI, depression, and FD are interrelated. Moreover, its pathogenesis is all related to inflammation, and clinical treatment drugs also cross. However, there are few reports on the common pathogenesis of these three diseases and the mechanism of drug treatment. Although modern medicine has a variety of drugs available for these diseases, the ideal drugs still need further looking. Based on the characteristics of solid pharmacological action, few adverse reactions and high safety of FTCM. This study will find reliable therapeutic drugs from FTCM and then provide new ideas for the treatment and drug research of TBI, depression, FD, and other diseases.

The active components of herbal drugs and substances are pleiotropic multi-ingredient compounds with multi-target properties, including anti-inflammatory effects. Anti-inflammatory effects may mean additional advantages in patients with multimorbidity or coronary heart disease [23]. Most importantly, it might be an effective way to treat multimorbidity by choosing FTCM [24].

The formula of Guanxin (Guanxin), a representative formula for the treatment of "chest paralysis" and "heart pain" formulated by the Beijing Collaborative Group for the Prevention and Treatment of Coronary Heart Disease in the 1970s [25], is consisted of Salvia miltiorriza, Chuanxiong Rhizoma, Safflower, Red Peony Root and Dalbergia odorifera. In addition, Guanxin has been widely used in China, Japan, and other Asian countries for a long history in treating cardiovascular diseases. Studies have confirmed that Guanxin has powerful pharmacological effects such as promoting blood circulation, removing blood
stasis, anti-inflammatory, anti-oxidation, and neuroprotection. Guanxin, powerful pharmacological activities, is often used clinically to treat acute heart disease, myocardial infarction, and congestive heart failure [26]. Moreover, previous studies identified that Guanxin could relieve TBI, depression, and FD by suppressed inflammatory response via inhibiting inflammatory cytokines. However, given the characteristics of FTCM, its components are complex, and its curative effect and mechanism are difficult to determine. We should investigate the underlying mechanism by which Guanxin acts in treating concomitant depression and FD after TBI.

As a new method, the network pharmacology method has been widely used in the discovery and development of drugs. So far, it has helped clarify the multi-target effects of traditional Chinese medicine on various diseases [27]. Recently, a large number of studies show that the network pharmacology method is widely used to study the "compound-protein/gene-disease" pathway, and then, from the perspective of network pharmacology, the complexity between biological systems, drugs, and diseases is clarified, which has a holistic philosophy similar to that of TCM [28].

Thus, in this study, we first assessed whether Guanxin could treat TBI, depression, and FD, and the potential mechanism of action, by network pharmacology approaches. Following, we validated the mechanism of Guanxin intervention in TBI, depression, and FD by molecular docking, mainly by inhibiting the production of pro-inflammatory cytokines (IL-6, IL-1β, and TNF) and then inhibiting inflammatory reactions. Eventually, playing a therapeutic role (Fig. 1).

2. Material And Methods

2.1. Collection of Chemical Ingredients and Potential Targets in Guanxin

The Traditional Chinese Medicine Systematic Pharmacology database (TCMSP, https://old.tcmsp-e.com/tcmsp.php) provides comprehensive information about herbal components such as chemical structure, oral bioavailability, intestinal epithelial permeability, water solubility, drug-like properties, drug targets and their relationship with diseases [29]. The active ingredients and targets in the Guanxin compounds, including Salvia miltiorriza, Chuanxiong Rhizoma, Safflower, Red Peony Root, and Dalbergia odorifera, were retrieved by the TCMSP database. Meanwhile, the oral bioavailability (OB) ≥ 30% and drug-like properties(DL) ≥ 0.18 as criteria to screen the chemical components of Guanxin. Besides, to facilitate subsequent analysis, the relative molecular weight (MW), octanol-water ratio (AlogP), blood-brain barrier (BBB), and Caco-2 Permeability (Caco-2) parameters of the candidate active ingredients were recorded. Finally, the targets of the obtained active ingredients were converted into gene names through the UniProt database (https://www.uniprot.org/).

2.2. Candidate Diseases Targets Prediction

TCMSP database, the GeneCards database (https://www.genecards.org/) [30], the Online Human Mendelian Inheritance (OMIM) database (https://www.omim.org/) [31], the Targeted Drug Database
(TTD) (http://db.ldrblab.net/ttd) [32], and DisGeNET (http://www.disgenet.org/) databases were used to collected diseases targets. Next, we take “Traumatic brain injury,” “Depression,” and “Functional dyspepsia” as the keywords, respectively search the targets of TBI, depression, and FD in this database.

2.3. Candidate Chemical Components Principal Component Analysis (PCA)

The DrugBank (https://www.Drugbank.Ca/) database was used to find commonly used drugs for TBI, depression, and FD [33], and recorded the parameters of each drug, namely MW, AlogP, BBB, and Caco-2. And then, the principal component analysis (PCA) was used to evaluate the drug-like properties of the Guanxin formula.

2.4. Active Ingredients and Key Targets Screening

Cytoscape 3.7.1 software was applied to construct a “single drug-active ingredient-target” visualization network of Guanxin. In addition, we take the median of degree ≥ 2 times as the screening standard, obtained the most promising active ingredients in Guanxin. Afterward, VENNY2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) [34] was used to visualize the targets of active ingredients in Guanxin with the targets of TBI, depression, and FD. The intersection part is the common target of disease and drug action.

2.5. PPI Network Construction

The intersection part of the Venn diagram, namely common targets of the active ingredient of Guanxin with TBI, depression, and FD, was imported to the Search Tool for Interacting Genes/Proteins (STRING) database (https://www.string-db.org/) [35] In the STRING database, we set the threshold value “interaction threshold” as “high confidence > 0.9” [36], and the rest of the parameters were left as default. And then, the protein-protein interaction (PPI) network maps were obtained. Next, Cytoscape software was used to analyze the PPI network topological properties [37]. Besides, to evaluate the centrality properties of nodes in the network, we calculated three topological parameters, namely degree centrality (DC), intermediate centrality (BC), and proximity centrality (CC). At last, choose “DC > 2 × medians” as the screening criteria to obtain the key targets.

2.6. Gene Ontology (GO) and KEGG Pathway Enrichment

The (DAVID) v6.8 (https://david.ncifcrf.gov/) database [38] was used for KEGG pathway enrichment and GO enrichment analysis. We firstly imported the common targets of Guanxin treatment TBI, depression, and FD, and the restricted species was human to perform KEGG pathway enrichment and GO enrichment analysis. Following, the threshold of P < 0.05 is standard for screening the top-ranked biological processes or pathways [39]. GO enrichment included biological processes, cellular components, and molecular function.

2.7. Molecular Docking
Molecular docking is a way to study receptor-ligand interactions and recognition and is usually used to study intermolecular interactions and predict their binding patterns and affinities [40]. We selected the top 3 components (luteolin, quercetin, and β-sitosterol) in the “single drug-active ingredient-common target” network for molecular docking with the corresponding targets (IL-6, IL-1β, and TNF) in the PPI network. Firstly, the chemical structures of the drugs were downloaded from the PubChem database, and the 3D structures of the targets were downloaded from the Protein Data Bank (PDB) in PDB format. Subsequently, Vina was used for molecular docking. The binding activity between the component and the target was also assessed using the magnitude of the binding energy (affinity) and analyzed and mapped using Pymol [41].

3. Results

3.1. Potential Active Components and Targets of Guanxin Against TBI, Depression and FD

The result initially shows that a total of 797 activity components were identified. Subsequently, we conducted the OB ≥ 30% and DL ≥ 0.18 as the screening criteria. 160 components were screened in which, including Salvia miltiorriza (65), Chuanxiong Rhizoma (7), Safflower (22), Red Peony Root (29), and Dalbergia odorifera (37). (Supplementary Table S1)

After target fishing using the TCMSP database, 531 potential targets were predicted to be regulated by the five active ingredients of Guanxin. These included 131 Salvia miltiorriza, 28 Chuanxiong Rhizoma, 206 Safflower, 89 Red Peony Root, and 77 Dalbergia odorifera targets (Fig. 2a).

3.2. Principal Component Analysis (PCA) of Guanxin

To further evaluate the reliability of Guanxin in treating TBI, depression, and FD [42], we collected the physical parameters (MW, AlogP, BBB, and Caco-2) of the commonly used therapeutic drugs for the diseases mentioned above using the Drugbank database. We analyzed the Guanxin ingredients harvested and collected treatment drugs for PCA analysis. The PCA results are shown in Fig. 2b. We can see that most of the active ingredients of Guanxin clustered with the frequently used drugs for the clinical treatment of TBI, depression, and FD. It indicating that these active ingredients in Guanxin have physicochemical properties similar to those of these treatment drugs. Potentially, Guanxin could be used to treat TBI, Depression, and FD. Thus, it may have a chance to be developed as a therapeutic agent against above mentioned multimorbidity [43].

3.3. Disease Target Prediction

We retrieved 1017 TBI targets, 1044 depression targets and 624 FD related targets from the five databases (TCMS, GeneCards, OMIM, TTD and DisGeNet). Subsequently, we obtain a total of 978 TBI targets, 891 depression targets and 622 FD targets after deleting repetitions (Fig. 3a).

3.4. PPI Network Construction
The active ingredient targets in Guanxin were co-imported into VENNY with TBI, depression, and FD targets to find the intersection (Fig. 3b), and 17 common targets of drugs and diseases were obtained eventually. Following this, the STRING database was used to analyze drug-disease interactions. We firstly imported 17 common targets into the STRING database, selected the species as human, and set the interaction score > 0. 9 to construct the PPI network (Fig. 4a).

And then, we exported the PPI network diagram in PNG format and Cytoscape format for further analysis. Eventually, Cytoscape software was used to beautify the PPI network graph. Besides, the plug-in CytoNCA was used to calculate DC in the PPI network. Nine key targets, IL-6, IL-1β, TNF, IL10, CXCL8, IFNG, PTGS2, CRP, and AKT1, were screened according to Degree ≥ 2-fold median as condition (Table 1). Through this network, we obtained three core targets: IL-6, IL-1β, and TNF. These targets may be considered as the main action targets for Guanxin treatment of TBI, depression, and FD, and their identification suggests that Guanxin treatment of TBI, depression, and FD through multiple potential targets. Figure 4b shows the PPI network with colored and vary in size nodes; the color and size of each node denotes the degree, from deep red and shape prominent (highest) to light red and small (lowest), as the node degree decreases.

### 3.5. Screening of Major Active Ingredients

To construct a “single drug-active ingredient-common target” network of Guanxin (Fig. 5), we improved the active ingredient targets of Guanxin into Cytoscape software. At the same time, calculated the DC of the PPI network was by CytoNCA plug-in [47]. Finally, nine key active ingredients were screened based on Degree ≥ 2 times the median. These active ingredients including: luteolin (MOL000006), quercetin (MOL000098), beta-sitosterol (MOL000358), baicalein (MOL002714), Stigmasterol (MOL000449), Myricanone (MOL002135), ellagic acid (MOL001002), Medicarpin (MOL002565), tanshinone a (MOL007154), 1,2,5,6-tetrahydrotanshinone (MOL001601) (Table 2).

### 3.6. KEGG Pathway Enrichment of Candidate Targets for Guanxin Treating TBI, depression and FD

The above-obtained targets of Guanxin treatment TBI, depression, and FD were imported into the DAVID database for KEGG pathway analysis and GO enrichment analysis. First, we take P < 0.05 as a screened standard to obtain 66 relevant signaling pathways in KEGG pathway enrichment. KEGG results revealed that targets were enriched mainly in the AGE-RAGE signaling pathway in diabetic complications, Amoebiasis, Inflammatory bowel disease, Tuberculosis, IL-17 signaling pathway, C-type lectin receptor signaling pathway, Cytokine-cytokine receptor interaction, Lipid and atherosclerosis, Toll-like receptor signaling pathway, Human cytomegalovirus infection, and TNF signaling pathway (Fig. 6). Notably, the signal mentioned above is all associated with inflammation, indicating that the mechanism of Guanxin in treating TBI, depression, and FD diseases is mainly through intervened the potentially inflammation-related signal pathways.
3.7. GO Biological Process analysis of Candidate Targets for Guanxin – Treating TBI, depression and FD

Subsequently, GO enrichment analysis is performed around three main aspects, namely biological process (BP), cellular component (CC), and molecular function (MF) (Fig. 7). First, the result of the BP term is mainly an inflammation response. Next, the main CC terms were extracellular space, extracellular region, and macromolecular complex; Finally, the main MF terms showed cytokine activity, identical protein binding, and enzyme binding.

3.8. Molecular Docking and Analysis

The binding modes of IL-6, IL-1β, and TNF to luteolin, quercetin, and beta-sitosterol were verified using molecular docking. As shown in Fig. 8a-b, luteolin formed 3 hydrogen bonds with CYS-104, TYR-59, and ASP-221 in IL6. In addition, one hydrogen bond was formed between luteolin and IL-1β (ASP-142) (Fig. 8c-d), and five hydrogen bonds were formed between luteolin and TNF, as shown in Fig. 8e-f (THR-26, HIS-181, TYR-186, ARG-142, and ARG-233). And then, quercetin formed four hydrogen bonds in IL-6 with CYS-104, SER-101, HIS-223, and PRO-222 (Fig. 9a-b). Besides, three hydrogen bonds, namely PRO-87, LYS-65, and ASN-7, were formed between quercetin and IL-1β (Fig. 9c-d), and four hydrogen bonds were formed between quercetin and TNF (ARG-142, HIS-181, ARG-233, and GLN-100), as shown in Fig. 9e-f. At the same time, beta-sitosterol formed 1 hydrogen bond with ASN-77 in IL-6 (Fig. 10a-b). Moreover, 1 hydrogen bond (ASP-142), was formed between beta-sitosterol and IL-1β (Fig. 10c-d), and 1 hydrogen bond was formed between beta-sitosterol and TNF (HIS-181), as shown in Fig. 10e-f. The docking binding energy of molecules is an important index to evaluate the formation of a stable conformation between the active ingredient and its target—usually, the lower the binding energy, the more stable the conformation [44].

The above data showed that the binding energies of IL-6 to luteolin, quercetin, and beta-sitosterol were −9.3, -7, and −8.9 kcal/mol, respectively. The binding energies between IL-1β and luteolin, quercetin, and beta-sitosterol were −8.7, -7.3, and −8.9 kcal/mol, respectively. Besides, as summarized in Table 3, the binding energies between TNF and luteolin, quercetin, and beta-sitosterol were, respectively, -7.4, -7.3, and −7.2 kcal/mol. The results indicate that the components can bind to the active site of the target.

4. Discussion

The inflammatory reaction is a double-edged sword. On the one hand, protective inflammation is a critical component of host defense, which helps the body to clear away pathogens and tumors. However, the persistent, uncontrollable inflammatory reaction or loss of sensitivity of the anti-inflammatory mechanism becomes out of balance, leading to sepsis and the development of many chronic diseases [45]. Dysregulated inflammatory response may cause various conditions, including sepsis, acute intestinal inflammation, rheumatoid arthritis, diabetes, and cancer [46]. Fabbri et al. [47] proposed that higher levels of IL-6 in older adults with multimorbidity in a study of older adults in the Chianti region of
Italy (InCHIANTI). Notably, the same study included several follow-up assessments over 9 years, and longitudinal analyses displayed a higher rate of chronic disease growth in those with higher baseline levels of IL-6, which increased more rapidly over time [47]. The above studies revealed that inflammation had become a significant risk factor for multimorbidity. Thus, suppressing IL-6 mediated inflammation response may be an effective way to treat multimorbidity. Unfortunately, fewer studies have investigated the link between inflammation and multimorbidity [48].

Due to the safety and efficacy of herbal formulas, TCM is often used clinically to alleviate multimorbidity. As previous studies mentioned, Guanxin has various pharmacological activities, such as cardio-protection, anti-oxidant and anti-inflammatory. Guanxin is a representative formula for treating coronary heart disease; however, there are few studies on its anti-TBI, depression, and FD effects and mechanisms. Thus, in the present studies, we focus on the FTCM, choose Guanxin as a starting point, and explore the action and mechanism of Guanxin in treating TBI, depression, and FD. With the expectation of providing a reference for clinical prevention and treatment of multimorbidity.

In this study, we combined network pharmacology and molecular docking to investigate the mechanism of Guanxin in treating TBI, depression, and FD. Firstly, 797 chemical constituents and 531 compounds related targets in Guanxin were collected by network pharmacological analysis. Among these targets, 17 are shared between compound-related targets and targets of TBI, depression, and FD, indicating that Guanxin may have anti-TBI, depression, and FD effects. Subsequently, we constructed a PPI network and further analyzed it by Cytoscape. The result displayed that luteolin (MOL 000006), quercetin (MOL 000098), beta-sitosterol (MOL 000358), maybe the main active compounds of Guanxin in TBI, depression, and FD treatment as they affect most of the compounds. The study found that luteolin, a flavone subclass of flavonoids, commonly regulates glucose and lipid metabolism, prevents cardiovascular diseases and has various pharmacological effects such as anti-inflammatory, anti-oxidant, and anti-tumor [49]. Aziz et al. [50] found that luteolin exerts potent anti-inflammation effects. It can inhibit the production of interleukin (IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, TNF-α, IFN-β) and granulocyte-macrophage colony-stimulating factor (all pro-inflammatory cytokines). At the same time, luteolin also can increase the secretion of IL-10 (an anti-inflammatory cytokine). Besides, quercetin is a flavonoid compound with various pharmacological activities, such as anti-tumor, anti-oxidant, anti-inflammatory, anti-viral, and immunomodulatory [51]. It is reported that quercetin is a persistent anti-inflammatory substance with a solid anti-inflammatory ability [52]. Endale et al. [53] found that quercetin could inhibit LPS-induced inflammation, thus limiting the activation of a downstream signaling pathway in RAW 264.7 cells. Furthermore, beta-sitosterol belongs to the tetracycline triterpenoids, which have hypolipidemic, anti-oxidant, anti-anti-inflammatory, anti-apoptotic, and other physiological activities [54]. Sun et al. [55] found that beta-sitosterol treatment decreased the expression of an inflammatory mediator (IL-6), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), and cyclooxygenase – 2 (COX-2)) induced by LPS. Above mentioned is consistent with our research that Guanxin plays a vital role in inhibiting the production of pro-inflammatory cytokines (such as IL-6, IL-1β, and TNF). These studies demonstrate that luteolin, quercetin, and beta-sitosterol have the ability and potential to become good anti-inflammatory agents.
In order to verify the drug-like characteristics of these active ingredients, we collected physical parameters (MW, AlogP, BBB, and Caco-2) of these active ingredients and commonly used drugs as therapeutic agents for TBI, depression, and FD. Following, these active ingredient parameters were compared with these commonly used drugs. PCA analysis showed that the parameters of the main ingredients of Guanxin are similar to the commonly used drugs for TBI, depression, and FD. Guanxin may have treatment effects in TBI, depression, and FD. Thus, the potential pharmacological effects of Guanxin deserve further investigation.

In addition, we also found that IL6, IL1β, TNF, IL10, CXCL8, IFNG, PTGS2, CRP, and AKT1 are the targets of action of Guanxin II in the treatment of TBI, depression, and FD, of which IL6, IL1β, and TNF maybe the core targets. These target genes significantly enriched in inflammation-related pathways, namely the AGE-RAGE signaling pathway in diabetic complications, IL-17 signaling pathway, Toll-like receptor signaling pathway, and TNF signaling pathway. This result is consistent with studies on the pathogenesis of TBI, depression, and functional dyspepsia [56–58].

Moreover, to further verify the network, as mentioned earlier pharmacology results, we performed molecular docking to study the anti-inflammation activity of luteolin, quercetin, and β-sitosterol. The results showed their appreciable binding affinity with IL-6, IL-1β, and TNF, suggesting that the mechanism of action of Guanxin is related to inhibiting the production of inflammatory factors from reducing the inflammatory reaction. However, the specific pathway of the multifunctional role of Guanxin II in the treatment of multimorbidity needs further research and confirmation.

5. Conclusion

In this study, using a network pharmacology approach, we first clarify the multifunctional effect of Guanxin in treating TBI on depression and FD associated with TBI. At the same time, the above analysis was further verified by the molecular docking method. It is worth mentioning that the multiple active ingredients and multi-target mechanism of action of the TCM compound in treating diseases were demonstrated. It provides a theoretical basis for researching modern traditional Chinese medicine compounds. Studying the pathogenesis of various diseases and treating multimorbidity is also of great significance and value.

Abbreviations
Guanxin  compound

TCM  Traditional Chinese medicine

FTCM  Formulae of traditional Chinese medicine

TBI  Traumatic brain injury

FD  Functional dyspepsia

IL-6  Interleukin 6

IL-1β  Interleukin 1β

TCMSP  Traditional Chinese medicine systems pharmacology database and analysis platform

TTD  Targeted Drug Database

OMIM  Online mendelian inheritance in man

PDB  Protein data bank

DAVID  Database for Annotation Visualization and Integrated Discovery

STRING  Search Tool for the Retrieval of Interacting Genes/Proteins

PPI  Protein-protein interaction

PCA  principal component analysis

GO  Gene Ontology

KEGG  Kyoto Encyclopedia of Genes and Genomes

OB  oral bioavailability

DL  drug similarity

AlogP  An Octanol-Water Partition Coefficient log P

Caco-2  Caco-2 Permeability

MW  molecular weight

BBB  blood-brain barrier

References


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association, and inhibits MAPK/AP-1 and IKK/NF-κB-induced inflammatory mediators production in RAW 264.7 cells. Immunobiology 218(12): 1452-67.10.1016/j.imbio.2013.04.019


Tables

Table 1. Top nine targets information of PPI network.

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<th>Name</th>
<th>Degree</th>
<th>Betweenness Centrality</th>
<th>Closeness Centrality</th>
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<tr>
<td>IL-6</td>
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<td>0.14</td>
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<tr>
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Table 2 The top 10 active components of Guanxin in degree value.
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<th>Components</th>
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<td>beta-sitosterol</td>
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<td>baicalein</td>
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<tr>
<td>MOL000449</td>
<td>Stigmasterol</td>
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<td>MOL002135</td>
<td>Myricanone</td>
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MOL001002  Ellagic acid  5281855

MOL002565  Medicarpin  336327

MOL007154  Tanshinone a  164676
**Table 3** Binding energy of active components of Guanxin and core targets.

<table>
<thead>
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<td>IL-1β</td>
<td>TNF</td>
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**Figures**
Figure 1

Flow chart of experimental design of this study, draw by Figdraw (www.figdraw.com). Firstly, the active ingredients and targets of the active ingredients in Guanxin were acquired using TCMSP. Subsequently, disease databases (TCMSP, GeneCards, TTD, OMIM, DisGeNET) were used to obtain TBI, depression, and FD disease targets. Final verification by molecular docking above results.
Figure 2

(a) The active components targets of Guanxin. (b) PCA score plot of the components of Guanxin and the available drugs for treating TBI, depression, and FD. Crosses represent the components of Guanxin, Boxes represent drugs for TBI, circles represent drugs for DP, and diamond represents drugs for FD.
Figure 3

Targets screening involved in Guanxin for treating TBI, depression, and FD. (A) Venn diagram of TBI, depression, and FD targets. (B) Venn diagram of potential targets of Guanxin for treating TBI, depression, and FD.

Figure 4

(a) PPI network of 17 intersecting targets constructed with STRING. (b) The PPI network was constructed using Cytoscape.
Figure 5

Active ingredients-Target Network diagram. The blue diamond represents the target site. Green circles represent *Salvia miltiorriza* (DS), *Chuanxiong Rhizoma* (CX), *Safflower* (HH), *Red Peony Root* (CS), and *Dalbergia odorifera* (JX). The skin-pink hexagon represents the active ingredients in *Salvia miltiorriza* (DS01-DS24). Yellow hexagons represent the active ingredients in *Chuanxiong Rhizoma* (CX01-CX05). The purple hexagon represents the active ingredient in *Safflower* (HH01-HH04). The pink hexagon represents the active ingredient in *Red Peony Root* (CS01-CS10). The blue-green hexagon represents the active ingredient in *Dalbergia odorifera* (JX01-JX14). A1-F1 represent their common components.
**Figure 6**

The top 20 enriched KEGG pathways of 17 intersecting targets involved in Guanxin’s anti-TBI, depression, and FD therapeutic effects.
Figure 7

GO enrichment analysis of the 17 intersecting targets for the treatment of TBI, depression, and FD with Guanxin.
Figure 8

The molecular docking results of *luteolin*. (a,b) *luteolin* binds with IL-6. (c,d) *luteolin* binds with IL-1β. (e,f) *luteolin* binds with TNF.
Figure 9

The molecular docking results of quercetin. (a,b) quercetin binds with IL-6. (c,d) quercetin binds with IL-1β. (e,f) quercetin binds with TNF.
Figure 10

The molecular docking results of beta-sitosterol. (a,b) beta-sitosterol binds with IL-6. (c,d) beta-sitosterol binds with IL-1β. (e,f) beta-sitosterol binds with TNF.

Supplementary Files
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- SupplementaryTableS1.docx