Network Pharmacology Dissection of Multi-scale Mechanisms of Action of Tu-Xian Mixture for the Treatment of Diabetic Cognitive Impairment

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**Network pharmacology dissection of multi-scale
mechanisms of action of Tu-Xian mixture for the treatment
of diabetic cognitive impairment**

Running title: Network pharmacology to dissect the mechanisms of
traditional Chinese medicine

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Abstract

Background Traditional Chinese medicine (TCM) has long been used in the treatment of diabetic cognitive impairment (DCI), and Tu-Xian mixture is one of the prescriptions. This study used a network pharmacology method to predict the active ingredients, potential targets and pathways of Tu-Xian mixture in the treatment of DCI. **Methods** The potential active ingredients of Tu-xian mixture were obtained by querying databases such as TCMSP, drug targets were screened through Pharmmapper, ETCM, and UniProt databases, and disease target protein groups were collected using TTD, OMIM, and CTD databases. Therapeutic targets were obtained by comparative analysis. Then use WebGestalt to complete GO enrichment analysis and KEGG pathway analysis. **Results** A total of 51 active ingredients and 619 potential targets were obtained, among which there were 33 key targets for diabetes and 18 key targets for cognitive impairment. After GO analysis and KEGG pathway analysis, steroid hormone biosynthesis and γ -aminobutyric acid signaling pathway may be the important metabolic pathways for Tu-Xian mixture to perform therapeutic effects. **Conclusions** This work promoted the comprehension of Tu-Xian mixture in the treatment of DCI, and further experimental verification of the prediction results was still needed to support its clinical application.

Keywords

traditional Chinese medicine, network pharmacology, diabetic cognitive medicine, Tu-Xian mixture

1. Introduction

The 9th edition of the IDF Diabetes Atlas released by The International Diabetes Federation (IDF) in 2019 showed that there were about 463 million people with diabetes worldwide, of which about 116 million are in China [1]. Diabetes can cause a variety of chronic complications, and damage to the central nervous system is called diabetic cognitive impairment (DCI). Patients are mainly manifested in decreased ability to learn, memory, and reasoning. Study found that patients with diabetes were more likely to develop cognitive impairment than non-diabetics [2].

Traditional Chinese medicine (TCM) has a complete theoretical system and has unique advantages in the treatment of complex diseases. A systematic review has found that Chinese medicine herbs (CMH) had advantages over conventional western medicine alone in the treatment of DCI [3]. Kidney-reinforcing and blood-activating drugs were commonly used in the treatment of DCI by TCM. In previous work, we have selected some commonly used CMH based on clinical experience, researched their main extracts, and achieved some results.

Sustained hyperglycemia can cause significant atrophy of hippocampal volume [4, 5], which affects cognitive function in patients with diabetes [6]. Icariin, the main extract of *Epimedium brevicornu* Maxim (XLP), can reduce hippocampal neuron apoptosis in high glucose environment by upregulating phosphorylation of protein kinase B (Akt) protein [7]. Puerarin, the main extract of *Pueraria lobata* Ohwi (GG), can inhibit phosphorylation of p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) to reduce the apoptosis of hippocampal neuron

cultured in high glucose [8]. Therefore, based on long-term clinical experience and modern drug research [9], we combined a new prescription, namely Tu-Xian mixture. Tu-Xian mixture includes five kinds of CMH, which are *Cuscuta australis* R.Br (TSZ), XLP, *Ligustrum lucidum* Ait (NZZ), *Rhodiola crenulata* H. Ohba (HJT), GG.

Network pharmacology is an emerging subject. Through the overall analysis of the "drug-target-gene-disease" of the biological system, the interaction mechanism between the drug and the body is clarified. The systematic and holistic view of this research method is very similar to TCM theory, and network pharmacology has achieved some results in explaining the mechanism of action of TCM [10, 11].

At present, this study analyzed the mechanism of Tu-Xian mixture in the treatment of DCI through network pharmacology. We were expected to find the active ingredients in this prescription that play a therapeutic role and potential targets for treating the disease. The obtained results, we hope, may not only promote the comprehension of Tu-Xian mixture in the treatment of DCI, but also promote TCM as a more important alternative therapy in the treatment of complex diseases.

2. Materials and methods

2.1. Construction of database and active ingredients screening

The ingredients of all herbs in Tu-Xian mixture came from related literature and databases, including Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://tcmspw.com/tcmsp.php>, accessed August 2019) [12], Traditional Chinese Medicines Integrated Database (TCMID,

<http://119.3.41.228:8000/tcmid/>, accessed August 2019) [13], a Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM, <http://bionet.ncpsb.org/batman-tcm/index.php/Home/Index/index>, accessed August 2019) [14], and Chemistry Database of Chinese Academy of Sciences (www.organchem.csdb.cn, accessed August 2019). Then the ingredients were screened according to oral bioavailability (OB) and drug-likeness (DL). Oral bioavailability is one of the most basic pharmacokinetic properties of oral drugs, which refers to the relative absorption of the drug into the blood circulation after oral administration [15]. When TCM decoction is taken orally, there may be hundreds of ingredients entering the body simultaneously, and only a part of which has a higher OB can play a therapeutic role. Drug-likeness is a qualitative analysis method to assess whether a molecule is suitable as a drug by evaluating the chemical properties [16]. According to the recommendation of the TCMSP database, ingredients that meet $OB \geq 30\%$ and $DL \geq 0.18$ were reserved for further analysis.

2.2. Drug targets prediction

Potential drug targets consisted of two parts. Firstly, retrieve the above potential ingredients through Pubchem server (<https://pubchem.ncbi.nlm.nih.gov/>, accessed August 2019), download their structural files, and upload them to the Phrammapper server (<http://www.lilab-ecust.cn/phrammapper/>, accessed August 2019) [17]. The Phrammapper server can perform target prediction through reverse pharmacophore matching. After obtaining the result, retain the targets with Norm Fit ≥ 0.7 , and

convert them from Uniprot code to Gene Symbol standard code. Secondly, retrieve the above potential ingredients through The Encyclopedia of Traditional Chinese Medicine (ETCM, <http://www.nrc.ac.cn:9090/ETCM/index.php/Home/Index/>, accessed August 2019), get the predicted targets directly provided by this server. The two parts were combined and analyzed to obtain all potential drug targets.

2.3. Known therapeutic targets of DCI

At present, the exact pathogenesis of DCI is unclear, but numerous studies have found that there were many of the same pathological changes between DCI and Alzheimer's disease (AD)[18-20]. Therefore, in the absence of DCI targets, we collected the targets of diabetes and AD as the disease targets of DCI. Then using "Diabetes" and "Alzheimer's Disease" as keywords, they were searched and screened in Therapeutic Target Database (TTD, <http://bidd.nus.edu.sg/group/cjttd/>, accessed September 2019)[21], Online Mendelian Inheritance in Man (OMIM, <https://omim.org/>, accessed September 2019)[22], and Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>, accessed September 2019)[23]. Integrate the target of diabetes and AD separately, and compare and analyze with the potential drug targets of the previous step. Thus, we obtained the relevant targets of DCI that Tu-Xian Mixture may act on.

2.4. Protein-protein interaction and network construction

Import the therapeutic targets into STRING (<https://string-db.org/>, accessed

October 2019)[24] and download the protein-protein interaction (PPI) file. Introduce the PPI file into the visual tool Cytoscape (version 3.7.1, Boston, MA, USA) to analyze the topological parameters of the PPI network. Node degree, a very important topological parameter, is the number of connections between nodes, which can reflect the importance of a node in the network. To improve the accuracy of the results, those with a node degree above the median were key targets.

2.5. GO enrichment analysis and KEGG pathway analysis

Complete the GO enrichment analysis and KEGG pathway analysis of the selected key targets through the WebGestalt (<http://www.webgestalt.org/>, accessed October 2019) [25] online server. The GO term in biological processes indicated the function of genes, and the KEGG term symbolized a specific function of a gene in a metabolic pathway. Those GO and KEGG terms with $p\text{-value} \leq 0.05$ and false discovery rate (FDR) ≤ 0.05 were significant, which represents the function of key targets and the metabolic pathways involved.

2.6. Workflow

The specific workflow is shown in Figure 1. Firstly, the active ingredients of Tu-Xian mixture were screened by searching the databases, and they needed to satisfy the screening requirements, $OB \geq 30\%$ and $DL \geq 0.18$. Secondly, the databases were also used to screen potential therapeutic targets of active ingredients and disease targets of DCI, respectively. Subsequently, construct a network model and complete

the enrichment GO analysis and KEGG pathway analysis.

Figure 1 Workflow of the approach

3. Results

3.1. Active ingredients screening

By searching the above databases, we demonstrated that the 5 herbs in Tu-Xian mixture contained a total of 407 known chemical ingredients (Table 1). Among them, 48 ingredients satisfied the screening requirements, $OB \geq 30\%$ and $DL \geq 0.18$. In addition, several ingredients, such as icaraside II, puerarin and salidroside were the main compounds of the herbs, were also considered as active ingredients. The detailed information about the 51 ingredients all listed in Supplementary Table S1, and Table 2 displays part of them as examples.

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Table 1 Basic information about the ingredients of Tu-Xian mixture

No.	Latin name	Chinese pinyin	Abbreviation	Ingredients
1	<i>Cuscuta australis</i> R.Br	Tu si zi	TSZ	35
2	<i>Epimedium brevicornu</i> Maxim	Xian ling pi	XLP	139
3	<i>Ligustrum lucidum</i> Ait	Nv zhen zi	NZZ	153
4	<i>Rhodiola crenulata</i> H. Ohba	Hong jing tian	HJT	26
5	<i>Pueraria lobata</i> Ohwi	Ge gen	GG	54

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Table 2 Detailed information about the ingredients of Tu-Xian mixture

Molecule ID	Ingredient name	OB (%)	DL	Structure
MOL001558	sesamin	56.55	0.83
MOL000184	NSC63551	39.25	0.76
MOL005440	Isofucosterol	43.78	0.76

MOL000358	beta-sitosterol	36.91	0.75
MOL000359	sitosterol	36.91	0.75
MOL012254	campesterol	37.58	0.71
MOL005043	campest-5-en-3beta-ol	37.58	0.71
MOL000953	CLR	37.87	0.68

MOL006649	sophranol	55.42	0.28
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MOL005944	matrine	63.77	0.25
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173 Molecule ID was provided by TCMSP; OB, oral bioavailability; DL, drug-likeness.

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175 3.2. Potential therapeutic targets.

176 A total of 619 potential drug targets of the above 51 active ingredients were
177 obtained through Pharmmapper and the ETCM databases. Detailed information about
178 these targets was provided in Supplementary Table S2. In total, 1158 diabetes targets
179 and 437 AD targets were obtained from TTD, OMIM and CTD databases (Table S3).
180 After a comparative analysis of potential drug targets and disease targets, we
181 demonstrated that Tu-Xian mixture might affect 64 diabetes-related targets and 32
182 AD-related targets (Table S4).

183

184 3.3. Network and visual analysis

185 Upload the above potential therapeutic targets of diabetes and AD to the STRING
186 database, and the obtained PPI relationship was introduced into Cytoscape for visual

analysis (Figure 2). The nodes with degree above the median were selected as the key targets, and 33 key targets for diabetes and 18 key targets for AD were obtained (Table S5).

Figure 2 The PPI between potential therapeutic targets of diabetes and AD

AD, Alzheimer's disease. Circles represent diseases or targets. The circle size of targets is proportional to its degree. The green, blue, and yellow lines indicate the target-to-target, diabetes-to-target, and AD-to-target links, respectively.

3.4. GO enrichment analysis and KEGG pathway analysis

To explore the biological processes and metabolic pathways involved in the above key targets, we further conducted GO analysis and KEGG pathway analysis. Our results were presented in two parts, diabetes-key-target and AD-key-target.

3.4.1 Diabetes-key-target

The results of GO enrichment analysis suggested that the biological processes of the diabetes key targets mainly included DNA-templated transcription, initiation, hormone-mediated signaling pathway, steroid metabolic process, and responses to steroid hormone. And KEGG pathway analysis suggested that the signal pathways involved in these key genes mainly included three functional modules, namely endocrine metabolism (steroid hormone biosynthesis), cancer, and infection (Figure 3, with their p-value and FDR shown in Supplementary Table S6). Among them, the biosynthesis of steroid hormones has a high enrichment rate in both GO analysis and KEGG pathway analysis, which indicated that Tu-Xian mixture may play a role in treating diabetes by regulating steroid hormones.

Figure 3 GO enrichment analysis and KEGG pathway analysis of diabetes key targets

The y-axis represents the type of biological process or signaling pathway, and the x-axis

represents the enrichment ratio of these items ($p\text{-value} \leq 0.05$ and $FDR \leq 0.05$).

3.4.2 AD-key-target

Similar steps as above, from the results of GO enrichment analysis, it could be seen that key genes were mainly involved in the γ -aminobutyric acid signaling pathway, synaptic transmission, GABAergic, steroid metabolic process, regulation of neurotransmitter levels, and neuronal death. And KEGG pathway analysis results suggested that the enriched signaling pathways mainly included neural signaling (GABAergic synapse, retrograde endocannabinoid signaling, neuroactive ligand-receptor interaction), drug addiction and endocrine metabolism (Figure 4, with their p -value and FDR shown in Supplementary Table S7). We found that the γ -aminobutyric acid signaling pathway has a higher enrichment rate in both GO analysis and KEGG pathway analysis. Therefore, Tu-Xian mixture might improve cognitive function mainly by regulating the γ -aminobutyric acid signaling pathway.

Figure 4 GO enrichment analysis and KEGG pathway analysis of AD key targets

The y-axis represents the type of biological process or signaling pathway, and the x-axis represents the enrichment ratio of these items ($p\text{-value} \leq 0.05$ and $FDR \leq 0.05$).

4. Discussion

Previous studies have found that the five herbs or their ingredients in Tu-Xian mixture have certain effects in the treatment of diabetes and cognitive impairment. *Cuscuta australis* R.Br (TSZ) can improve memory function in rats with cognitive impairment induced by scopolamine by restoring cholinergic function, reducing oxidative stress and neuroinflammation [26]. *Ligustrum lucidum* Ait (NZZ) can reduce fasting blood glucose in diabetic rats and protect the islet β cells[27]. Multiple extracts of XLP can improve diabetes complications [28, 29], and icariside II can reduce cognitive deficits in rats with cognitive impairment induced by neurotoxins [30]. Puerarin has hypoglycemic effect and can improve insulin resistance [31] and

diabetes complications [32] in diabetic mice, and it can reduce cognitive impairment in precursor protein/presenilin-1 double transgenic model mice [33]. Salidroside has been found to reduce fasting blood glucose and blood lipid levels in diabetic rats, and to prevent cognitive impairment caused by diabetes by regulating the Rho/Rho-associated kinase (ROCK)/sirtuin 1 (SIRT1)/nuclear factor (NF)- κ B pathway[9].

By searching multiple databases, the number of targets that Tu-Xian mixture can affect diabetes and AD was 33 and 18, respectively. After GO analysis and KEGG pathway analysis, the results suggested that Tu-Xian mixture mainly works through three functional pathways in the treatment of diabetes: steroid hormone biosynthesis, cancer and infection. And the key functional pathway is steroid hormone biosynthesis. Similarly, when treating cognitive impairment, there were three functional pathways: neural signal transduction, drug addiction, and endocrine metabolism. Obviously, neural signal transduction played a leading role.

Steroid hormone biosynthesis is closely related to the occurrence and development of diabetes. A health survey report showed that low testosterone and high estradiol levels were associated with obesity in men and increased insulin resistance and risk of diabetes [34]. Prospective studies found low levels of testosterone as risk factor for diabetes in men [35, 36], and men with low levels of testosterone had 2.3 times more diabetes than normal men[37]. Therefore, low levels of testosterone were closely related to the occurrence and development of disease in male diabetic patients. The possible mechanism is that testosterone can inhibit

lipoprotein lipase activity, increase the breakdown and utilization of fat and after testosterone levels decrease, fat deposition increases and insulin sensitivity decreases [38]. And low testosterone levels could inhibit glycogen synthesis leading to muscle-level insulin resistance [39]. For women, the imbalance of steroid hormones was also closely related to the occurrence and development of diabetes [40, 41]. Among them, sex hormone binding globulin was negatively correlated with the onset of diabetes, and estradiol is positively correlated with the onset of diabetes. Animal experiments indicated that increased muscle steroid hormones can increase insulin sensitivity in type 2 diabetic rats [42], which may be achieved by activating the Akt / PKC- ζ / λ -GLUT-4 signal pathway [43]. Therefore, the application of adjuvant hormone therapy for the treatment of diabetes has a broad prospect. This may also be an important way for Tu-Xian mixture to treat diabetes.

The relationship between diabetes and cancer is extremely close. A meta-analysis of a prospective cohort study involving 900,000 participants showed that prediabetes was closely related to the occurrence of cancer [44], and the common molecular mechanism might be the bridge between them [45]. Diabetes is also closely related to infection. Studies showed that people with diabetes had a significantly higher risk of infection than ordinary people [46]. Therefore, Tu-Xian mixture may achieve the therapeutic effect on diabetes and its complications by regulating cancer and infection related pathways.

γ -aminobutyric acid is an important neurotransmitter with various physiological functions. Its neurons mainly exist in the cerebral cortex, hippocampus, and striatum.

γ -aminobutyric acid can inhibit the activity of excitatory neurons such as the cortex and hippocampus, and thus maintain normal neural network activity in the brain. Brain excitement suppresses imbalance induced by γ -aminobutyric acid system dysfunction is an important mechanism for cognitive impairment [47, 48]. The levels of γ -aminobutyric acid in the brain of patients with opioid addiction were significantly reduced, which was positively related to cognitive impairment [49]. Alpha-asarone, the major ingredient isolated from the Chinese medicinal herb *Acorus tatarinowii* Schott, can improve the cognitive function of AD rats by enhancing the γ -aminobutyric acid system, inhibiting the overexcitation of hippocampal neurons, and reducing the deposition of amyloid β -protein (A β)[50]. Therefore, γ -aminobutyric acid is involved in the development of cognitive impairment, and its specific mechanism in DCI needs further study. This research lays the theoretical foundation for the next experimental verification.

5. Conclusion

In this study, a network pharmacology research method was used to reveal the chemical basis of Tu-Xian mixture and explore the mechanism of Tu-Xian Mixture in the treatment of DCI. Total 51 active ingredients were selected from Tu-Xian mixture, and 619 potential drug targets were identified. Among them, there were 33 targets for diabetes treatment and 18 targets for cognitive impairment treatment, which play a therapeutic role by regulating steroid hormone biosynthesis and γ -aminobutyric acid signaling pathway. This work provided a theoretical basis for the study of Tu-Xian

mixture on the pharmacological mechanism of DCI. Further experimental verification of the prediction results is still needed to support its clinical application.

List of abbreviations

AD: Alzheimer's disease; BATMAN-TCM: a Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine; CMH: Chinese medicine herbs; CTD: Comparative Toxicogenomics Database; DCI: diabetic cognitive impairment; DL: drug-likeness; ETCM: Encyclopedia of Traditional Chinese Medicine; GG: Ge gen; HJT: Hong jing tian; IDF: International Diabetes Federation; NZZ: Nv zhen zi; OB: oral bioavailability; OMIM: Online Mendelian Inheritance in Man; TCM: Traditional Chinese medicine; TCMID: Traditional Chinese Medicines Integrated Database; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; TSZ: Tu si zi; TTD: Therapeutic Target Database; XLP: Xian ling pi.

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared no conflict of interests.

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Authors' contributions

Bin Yan conceived and designed the experiment; Guoqing Tian guided the experimental design; Bin Yan, Qing Sun and Jianhong Sun performed the experiment and analyzed the data; Bin Yan wrote the original draft; Guoqing Tian edited the paper. All authors reviewed the manuscript.

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Figures

Figure 1

Workflow of the approach

Figure 2

The PPI between potential therapeutic targets of diabetes and AD

Figure 3

GO enrichment analysis and KEGG pathway analysis of diabetes key targets

Figure 4

GO enrichment analysis and KEGG pathway analysis of AD key targets

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