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Exploring the mechanism of Xiaopi Hewei capsule in treating functional dyspepsia based on network pharmacology

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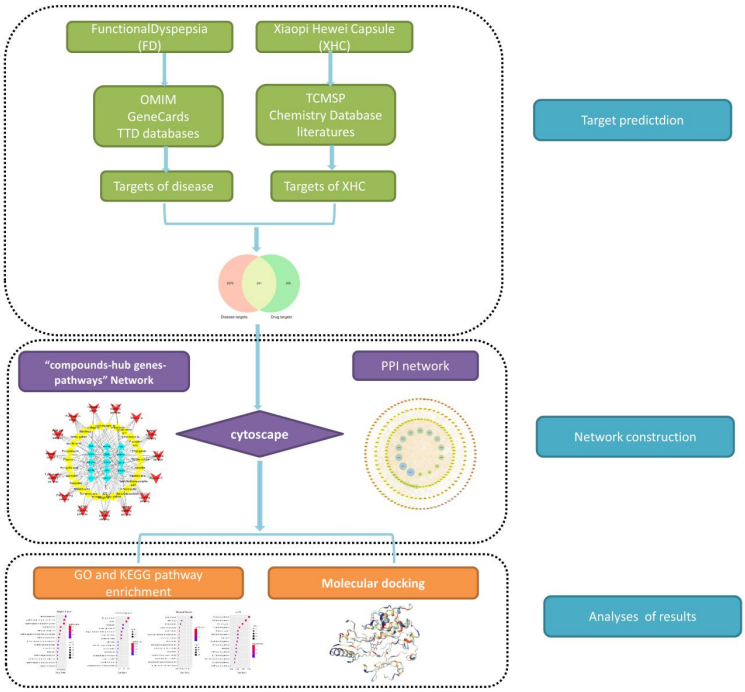
Highlights

The core targets and key pathways of Xiaopi Hewei capsule (XHC) in treating functional dyspepsia (FD) were explored by network pharmacology. XHC participated in the complex treating effects associated with anti-depression, inflammatory reaction and eradicating Helicobacter Pylori (HP).And, this study will provide the theoretical basis for further research of XHC in the later stage.

Traditionality

Xiaopi Hewei capsule (XHC) is a Miao medicine prescription medicine composed Geshanxiao (Cynanchum wilfordii (Maxim.) Hemsl.), cili (Rosa roxbunghii), Liuzhi (Salix babylonica L.) and Sanqi (Panax notoginseng (Burkill) in the rule of “Jun, Chen, Zuo and Shi”. Nowadays, XHC can be generally used to treat some diseases of the stomach. Recently, clinical trials have shown that XHC can effectively improve the clinical syndrome of Function dyspepsia (FD) by acting on alleviating epigastric pain and relaxing fullness via reducing the gastric emptying time.



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Abstract

Objective: Functional dyspepsia (FD) is a widely prevalent gastrointestinal disorder throughout the world, whereas the efficacy of current treatment in the Western countries is limited. The traditional Chinese herbal formula Xiaopi Hewei capsule (XHC) is a clinically validated remedy in treating FD, but there is no literature expounds the underlying therapeutic mechanism of XHC so far.

Methods: In the present study, the network pharmacology technology was used to explore the therapeutic mechanism of XHC in treating FD. We obtained relative compounds of XHC, potential targets of these compounds and FD-related targets by retrieving particular websites. Based on the matching results between XHC potential targets and disease targets, Protein-Protein Interaction (PPI) network was constructed to screen the hub targets by topology. Furthermore, DAVID bioinformatics resources were utilized for the enrichment analysis on GO and KEGG.

Results: A total of 62 active compounds and 547 putative identified targets were screened from XHC, of which 241 overlapped with the targets of FD and were considered potential therapeutic targets.14 hub genes were recognized as potential targets of treatments. Moreover, the results of DAVID enrichment analysis indicated that XHC participated in the complex treating effects associated with anti-depression, inflammatory reaction and eradicating Helicobacter Pylori (HP). Molecular docking stimulation results showed that most bioactive compounds of XHC had a strong binding efficiency with hub genes.

Conclusions: This study demonstrated that XHC has the characteristics of multi-compounds, multi-targets and multi-pathways in treating FD, which provides the theoretical basis for further research of XHC.

Key words: Xiaopi Hewei capsule, Function dyspepsia, Network Pharmacology

Abbreviations: XHC, Xiaopi Hewei capsule; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HP, Helicobacter Pylori; FD, Function dyspepsia; EPS, epigastric pain syndrome; PDS, postprandial distress syndrome; TCM, Traditional Chinese medicine; TTD, Therapeutic Target Database; OMIM, Online Mendelian Inheritance in Man; TCMSP, The traditional Chinese medicine system pharmacology database and analysis platform Database; ADME, absorption, distribution, metabolism, and excretion; RO5, Lipinski's rule of five; MW, molecular mass; HBD, hydrogen bond donors; LogP, fatty water partition coefficient; RB, rotatable Bonds; HBA, hydrogen bond acceptors; BC, betweenness centrality; CC, closeness centrality; DC, degree centrality; DAVID, The Database for Annotation, Visualization and Integrated Discovery; BP, biological process; CC, cell component; MF, molecular function.

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Competing interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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Background

Function dyspepsia (FD) is a clinical syndrome with epigastric pain and/or burning, early fullness and/or postprandial fullness according to the Rome IV criteria, which cannot be explained by organic or metabolic disease [1]. Patients are grouped and diagnosed according to symptoms, with those accompanying only epigastric pain or burning considered as epigastric pain syndrome (EPS), those having early fullness or postprandial fullness identified as postprandial distress syndrome (PDS) and those with both types of symptoms seemed as overlap of EPS and PDS [2]. According to the results of epidemiological investigation, the global prevalence rate of FD is 11.5%-14.5%, which not only affects people's quality of life, but also consumes a lot of medical resources [3]. Currently, there are many approaches to treat FD, but they do have pros and cons. Clinically, the treatment strategies for FD mainly focus on promoting gastrointestinal wriggle, applying central action drugs, regulating gastrointestinal immune function, and eradicating of HP, etc [4]. However, a single drug has the limitations of single target, large side effects and short course of treatment [5], so it is difficult to meet the treatment needs of its complex pathogenesis. Therefore, more effective therapeutic measures are urgently needed for FD treatment.

Traditional Chinese medicine (TCM) has been playing an important part in curing disease and improving the people's wellbeing. In recent years, much more attention has been paid to the treatment of FD with TCM, with some superiority of shorting the duration of symptoms, reducing the recurrence rate and minimizing side effects [6]. XHC, a Miao medicine prescription medicine, has been proven to be effective in treating FD. Moreover, clinical trials have shown that XHC can effectively improve the EPS and PDS by acting on alleviating epigastric pain and relaxing fullness via reducing the gastric emptying time [7]. XHC is consisted of four herbs which corresponding to the rule of "Jun, Chen, Zuo and Shi". Specifically, Geshanxiao (Cynanchum wilfordii (Maxim.) Hemsl.) is defined as Jun herb in this prescription with the effects of immune regulation, digestion improvement, tumor inhibition, anti-oxidation and liver protection; Chen herb is cili (Rosa roxbunghii), which existing the effect of curing food retention and fullness; As Zuo herbs, Liuzhi (Salix babylonica L.) serves as assistant to alleviate pain and help digestion; Sanqi (Panax notoginseng (Burkill) F. H. Chen ex C. H.) is the Shi herb with function of hemostasis, dispersing blood and pain relief [8]. However, the molecular mechanism of XHC in treating FD has ever been addressed. Therefore, the study of the active components, targets and possible mechanisms of XHC will further reveal the scientific significance of XHC in treating FD and promote its clinical application.

On account of multiple components and multiple targets of TCM, it is a complicated task to identify potential bioactive molecules and mechanisms of combinations [9]. Network pharmacology is applied to the analysis of TCM increasingly, which is a promising approach to illustrate the mechanisms of TCM in treatment and elucidate the complex interactions between drugs and complex diseases [10, 11]. Hence, in this study, the network pharmacology was used to expound the potential mechanism of XHC in treating FD, which lays the foundation for further research of XHC.

Methods

Data preparation

Collection Targets of FD. Related targets of FD were collected with the help of the integration of multi-source databases. The following is databases resources used in our study: The GeneCards (https://www.genecards.org/) is a whole bioinformatics database including transcriptomics, genomics, proteomics and etc [12]. The Therapeutic Target Database (TTD, http://db.idrblab.net/ttd/) is known for providing information related to the explored targets of therapeutic protein and diseases [13]. Online Mendelian Inheritance in Man (OMIM, https://omim.org/) is a representative online database presenting continuously updated resources of genetic disorders and human genes. By searching these databases with the keyword 'functional dyspepsia', all the screened targets were list as FD-targets. And the names of targets were corrected to gene symbol through UniProt website (https://www.uniprot.org/), and removed duplicate genes meanwhile [14].

Collection Bioactive Compounds of XHC In this paper, chemical compounds of XHC were acquired from Chemistry Database, TCMSP and literature mining [15-21]. Chemistry Database (http://www.organchem.csdb.cn/) is one of the important scientific databases of the Chinese Academy of Sciences [17], which provides the retrieval service of chemical information for the whole scholars. The traditional Chinese medicine system pharmacology database and analysis platform Database (TCMSP, http://tcmspw.com/tcmsp.php.), a Chinese medicine pharmacology database, includes information of herbs used in TCM, and characteristics

of the individual compounds, and their targets, related diseases, and pathways [22].

ADME Screening of Bioactive Compounds Recently, early assessment of absorption, distribution, metabolism, and excretion (ADME) of bioactive compounds has become a popular choice to give priorities to those components having good pharmacokinetic properties. To ensure that we got meaningful results, OB, DL (RO5), GI absorption as ADME-related models were exploited to screen the active compounds from XHC in the present work.

The convergence of the ADME process is displayed by OB (%F), which elucidated the percentage of an orally administered dose of the chemical compounds that reaches the systemic circulation [9]. Compounds with OB ≧ 30% were selected as candidate components for next step.

The "drug-like" prospective of compounds was estimated by DL an established concept for drug design. Furthermore, Lipinski's rule of five (RO5) is applied to evaluate DL or determines if a chemical compound has chemical and physical properties [23]. The rule is composed of relative molecular mass (MW), number of hydrogen bond donors (HBD), fatty water partition coefficient (LogP), rotatable Bonds (RB) and number of hydrogen bond acceptors (HBA). Candidate compounds that accord with the RO5 tend to have an increased chance of reaching the market. In this process, compounds with MW ≤ 500, HBD ≤ 5, LogP ≤5, RB≤10 and HBA ≤ 10 were selected as the active compounds for next step.

GI absorption, a pharmacokinetic behavior of drugs, is significant to supervision at various stages of the drug discovery processes [24]. In this study, the GI absorption value of the components in XHC was obtained through SwissADME website (http://www.swissadme.ch/index.php) [25]. The screening criterion of GI absorption was defined as high.

Finally, herbal compounds were chosen as the candidate ingredients for further analysis when they meet three criteria.

Collection Predicted Targets of Bioactive Compounds The active compounds of drugs exert related biological functions via targets. Related targets were located by target collection according to aforementioned included active ingredients. After obtaining the micromolecular structure information about the active compounds of XHC by retrieving Pubchem database (https://pubchem.ncbi.nlm.nih.gov/), all the screened targets were collect as predicted targets based on Swiss Target Prediction (http://swisstargetprediction.ch/).

At last, the overlapping targets were chosen as the related targets of XHC in treating FD through matching the targets of bioactive compounds of XHC and the related targets of FD based on Draw Venn diagrams website (http://jvenn.toulouse.inra.fr/app/example.html) [26].

Construction of PPI Network and Topological Analysis

PPI network was completed based on String database (https://string-db.org/) and Cytoscape 3.7.1 platform. String database covered most of functional interactions between the proteins reported. The parameters were frequently set as follows during data preprocessing: Homo sapiens; minimum required interaction score: 0.9; display simplifications: hide disconnected nodes in the network [27]. Moreover, Cytoscape 3.7.1 platform, an open source bioinformatics software platform, was available for visualization and analysis of interconnection network. Three parameters, betweenness centrality (BC), closeness centrality (CC) and degree centrality (DC), were employed to evaluate topological features of PPI network. The corresponding median values of each parameter were deemed to be the threshold values of the hub genes in the network analysis [26].

Construction of "compounds-hub genes-pathways" Network

The network construction, analysis and visualization were carried out using the Cytoscape 3.7.1 platform (https://cytoscape.org/) [28]. The pathway information of targets was obtained according to the result of KEGG pathway enrichment. In the network diagram, compounds, hub genes and pathways present in the form of nodes, and intermolecular interactions among them were indicated by edges. Meanwhile, "network analysis" was used to analyze the whole network.

GO and KEGG pathway enrichment analyses

The Database for Annotation, Visualization and Integrated Discovery [29](DAVID, https://david.ncifcrf.gov/) was usually preferred to accomplish GO and KEGG pathway enrichment analysis. Ordinarily, the composition of Go analysis includes three parts: biological process (BP), molecular function (MF) and cell component (CC). Moreover, the KEGG pathway enrichment analyses were used for identifying the biological functions and candidate targets. In this research, the results of GO and KEGG pathway enrichment analyses were processed with R package.

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Molecular docking stimulation

CB-Dock (http://cao.labshare.cn/cb-dock/), a friendly online molecular docking tool, predicts the binding sites of a given protein and calculates the center and size of the pocket, which was performed with AutoDock Vina. PDB files of the top 5 compounds in "compounds-hub genes-pathways" network and ligand files in SDF formats in the top 5 targets in PPI network were processed with CB-Dock to evaluate the binding behaviors. [30].

Identification of Active compounds

In the current work, a total of 232 chemical constituents were identified from Chemistry Database, TCMSP and literature mining. Even if any TCM formulation contains a variety of components, only a few components have the characteristics of pharmacodynamic and pharmacokinetic. Therefore, 62 active compounds were filtered out via three ADME-related models indicated above. The detail information was shown in Table 1 and the herbs-compounds network was built as Figure 1.

Results

Table 1 Pharmaceutical ingredients specific information.

NO.	Compound name	Herb	Compound CID	MW /(g/mol)	MF
1	3-hydroxy-4-methoxybenzoic acid	Geshanxiao	12575	168.15	C8H8O4
2	2, 5-dihydroxyacetophenone	Geshanxiao	10279	152.0473	C8H8O3
3	4-hydroxyacetophenone	Geshanxiao	7469	136.15	C8H8O2
4	2, 4-dihydroxyacetophenone	Geshanxiao	6990	152.15	C8H8O3
5	Scopoletin	Geshanxiao	5280460	192.17	C10H8O4
6	Shikimic acid	Geshanxiao	8742	174.15	C7H10O5
7	Gallic acid	Geshanxiao	370	170.12	C7H6O5
8	3, 4-Dihydroxybenzoic acid	Geshanxiao	72	154.12	C7H6O4
9	Methyl gallate	Geshanxiao	7428	184.152	C8H8O5
10	Ellagic acid	Geshanxiao	5281855	302.19	C14H6O8
11	Ethyl gallate	Geshanxiao	13250	198.17	C9H10O5
12	Isorhamnetin	Geshanxiao	5281654	316.26	C16H12O7
13	p-Cresol	Geshanxiao	2879	108.14	C7H8O
14	2', 5'-Dihydroxyacetophenone	Geshanxiao	10279	152.15	C8H8O3
15	baishouwubenzophenone	Geshanxiao	3082748	302.28	C16H14O6
16	Acetovanillone	Geshanxiao	2214	166.17	C9H10O3
17	Vanillin	Geshanxiao	1183	152.15	C8H8O3
18	caudatin	Geshanxiao	72948694	490.6	C28H42O7
19	Deacylmetaplexigenin	Geshanxiao	21633061	380.5	C21H30O6
20	Euscaphic acid	Cili	471426	488.7	C30H48O5
21	Kaempferide	Cili	5281666	300.2629	C16H12O6
22	Tormentic acid	Cili	73193	488.6991	C30H48O5
23	(E)-2-Decenal	Cili	5283345	154.25	C10H18O
24	(E, E)-2;(2e, 4e)-2, 4-decadienal	Cili	5283349	152.23	C10H16O
25	1, 8-Cineole	Cili	2758	154.25	C10H18O
26	Benzyl alcohol	Cili	244	108.14	C7H8O
27	1-Octanol	Cili	957	130.229	C8H18O
28	1, 2-Decanediol	Cili	2758	174.2805	C10H22O2
29	malic acid	Cili	525	134.0874	C4H6O5
30	2-Hydroxypropanoic acid	Cili	612	90.0779	C3H6O3
31	Oxalic acid	Cili	971	126.0654	C2H6O6
32	Succinic Acid	Cili	1110	118.088	C4H6O4
33	Linolenic acid	Cili	5280934	278.4296	C18H30O2
34	palmitic acid	Cili	985	256.4241	C16H32O2
35	Arjunolic acid	Cili	73641	488.6991	C30H48O5
36	Pyrogallic Acid	Cili	1057	126.11	C6H6O3
37	1beta-Hydroxyeuscaphic acid	Cili	14312996	504.7	C30H48O6
38	Ascorbic Acid	Cili	54670067	176.1241	C6H8O6
39	Phenylalanine	Cili	6140	165.19	C10H13NO2
40	Valine	Cili	6287	117.1463	C5H11NO2
41	Leucine	Cili	6160	131.17	C14H19NO4
42	Isoleucine	Cili	6306	131.1729	C6H13NO2

43	L-Threonine	Cili	6288	119.1192	C4H9NO3
44	Lysine	Cili	5962	146.1876	C6H14N2O2
45	DL-Methionine	Cili	876	149.2113	C5H11NO2S
46	Aspartic acid	Cili	5960	133.1027	C4H7NO4
47	glutamic acid	Cili	33032	147.1293	C3H7NO3
48	tyrosine	Cili	6057	181.19	C9H11NO3
49	histidine	Cili	6274	155.1546	C6H9N3O2
50	L-Arginine	Cili	6322	174.201	C6H14N4O2
51	Physcion	Cili	10639	284.2635	C16H12O5
52	Ethyl gallate	Cili	13250	198.1727	C9H10O5
53	Benzaldehyde	Cili	240	106.12	C7H6O
54	Arbutin	Liuzhi	440936	272.25	C12H16O7
55	Liquiritigenin	Sanqi	114829	256.25	C15H12O4
56	ginsenoside rh2	Sanqi	119307	622.9	C36H62O8
57	ginsenoside f2	Sanqi	9918692	785	C42H72O13
58	beta-sitosterol	Sanqi	222284	414.7	C29H50O
59	Mandenol	Sanqi	5282184	308.5	C20H36O2
60	Diop	Sanqi	33934	390.6	C24H38O4
61	Stigmasterol	Sanqi	5280794	412.7	C29H48O
62	quercetin	Sanqi	5280343	302.23	C15H10O7

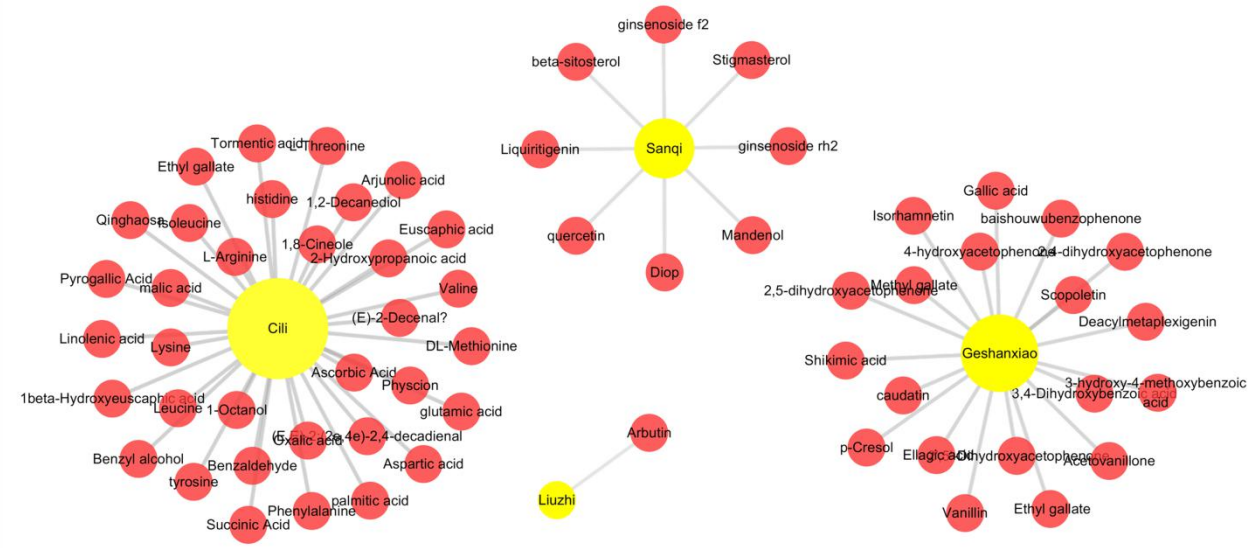

Figure 1 Herbs-Compounds network. The yellow nodes represent herbs in XHC, and the red nodes represent active compounds. The edges represent the relationship between them.

PPI Network for XHC in Treating FD

2817 targets associated with FD coming from the OMIM, GeneCards and TTD databases, and 547 related targets of the 62 chemical components were selected for preparation of 241 common targets (see the supplement for more information), which were regarded as the related targets of XHC in treating FD (see Figure 2).

241 common targets were early screened by string website with 233 nodes and 1711 edges remained. Subsequently, PPI network of those 233 nodes established in STRING database was later processed to screen key targets based on three major parameters of BC, CC and DC. The third screening ended up with 14 big hub nodes and 72 edges (Figure 3), which included AKT1, MAPK1, STAT3, EGFR, IL6, MAPK3, VEGFA, SRC, MAPK8, PIK3R1, PIK3CA, JUN, TNF and CCND1 (Table 2). When the 14 hub nodes and the other 219 nodes are sorted in descending order and viewed in the network, AKT1 (degree=43), MAPK1 (degree=42), STAT3 (degree=41), EGFR (degree=40), IL6 (degree=39), MAPK3 (degree=37), VEGFA (degree=37) and SRC (degree=36) were key targets in this network (Figure 4).

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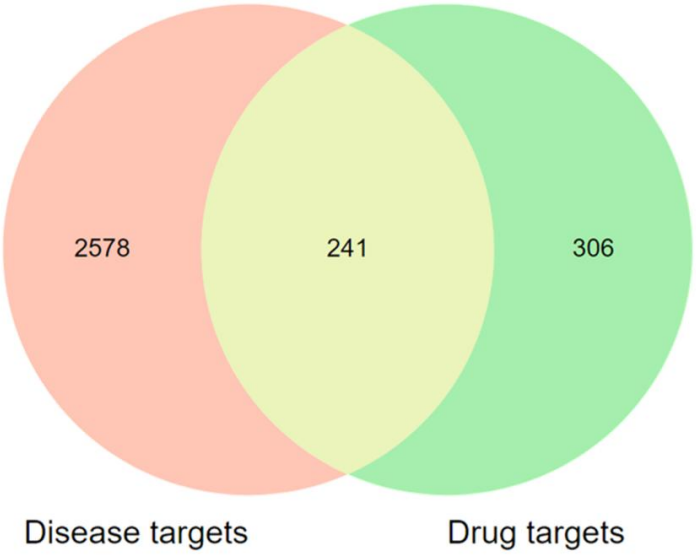

Figure 2 The venn diagram of the targets both in functional dyspepsia targets and Xiaopi Hewei Capsule targets.

Table 2 Information of 14 hub targrts

Uniprot ID	Gene symbol	Protein name	Degree
Q96B36	AKT1	Proline-rich AKT1 substrate 1	43
P28482	MAPK1	Mitogen-activated protein kinase 1	42
P40763	STAT3	Signal transducer and activator of transcription 3	41
P00533	EGFR	Epidermal growth factor receptor	40
P05231	IL6	Interleukin-6	39
P27361	MAPK3	Mitogen-activated protein kinase 3	37
P15692	VEGFA	Vascular endothelial growth factor A	37
P12931	SRC	Proto-oncogene tyrosine-protein kinase Src	36
P45983	MAPK8	Mitogen-activated protein kinase 8	34
P27986	PIK3R1	PI3-kinase regulatory subunit alpha	32
P42336	PIK3CA	PI3-kinase subunit alpha	32
P05412	JUN	Transcription factor AP-1	32
P01375	TNF	Tumor necrosis factor	29
P24385	CCND1	G1/S-specific cyclin-D1	29

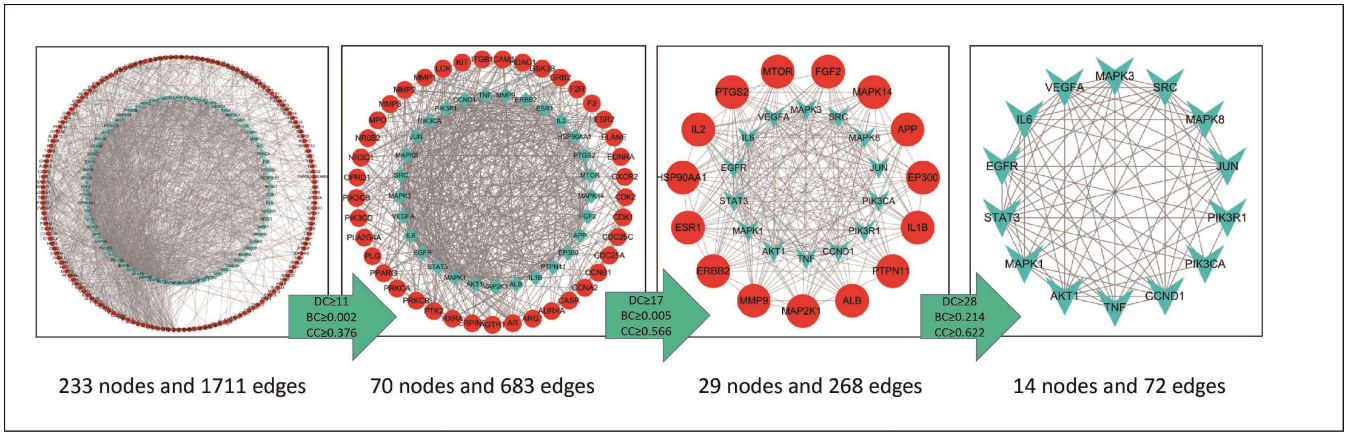

Figure 3 The process of topological screening for the PPI network

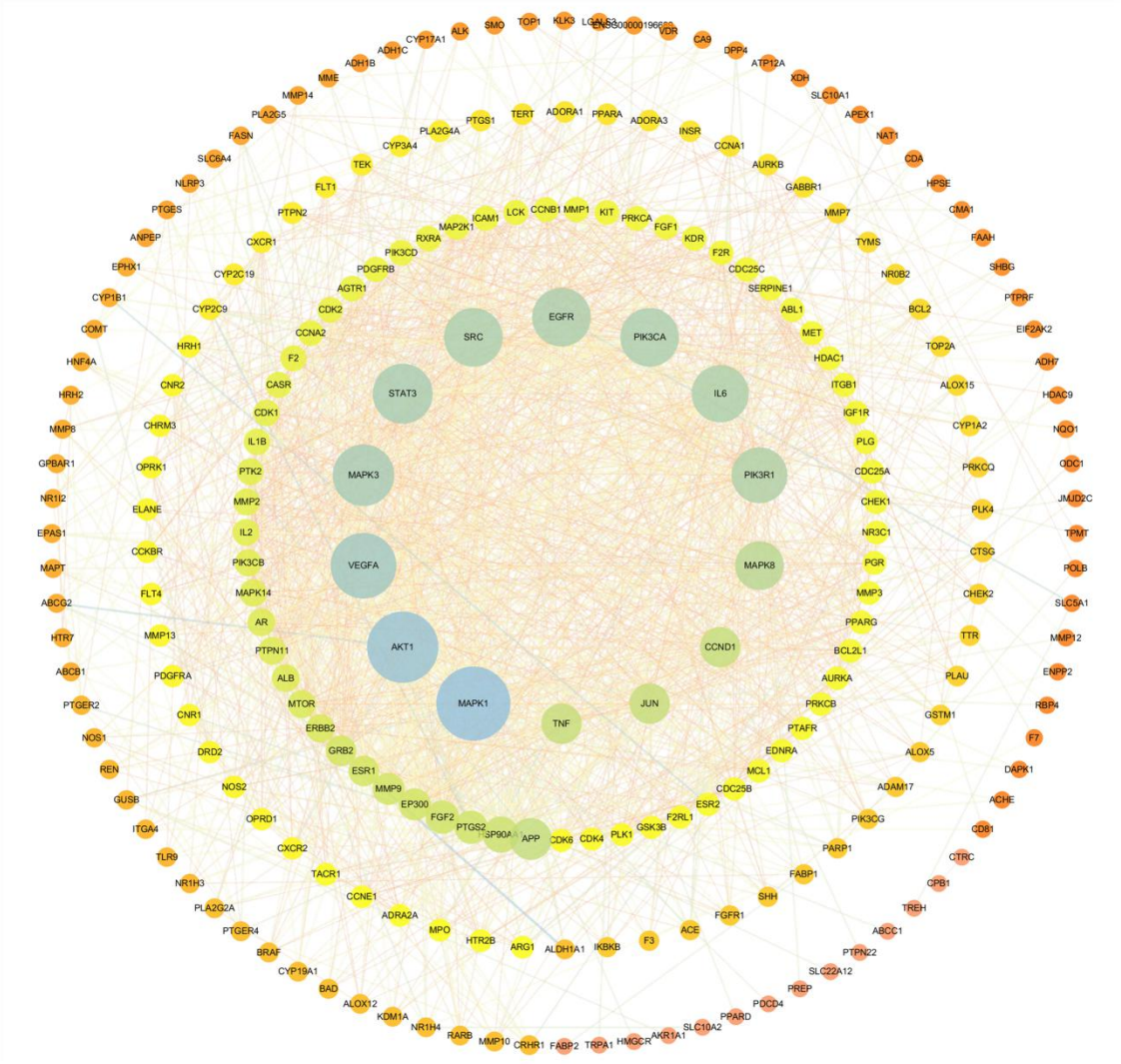

Figure 4 The PPI network. The node color changes from orange to blue reflect the degree value changes from low to high in the network.

GO and KEGG Pathway Enrichment Analyses

GO and KEGG pathway enrichment analysis of selected 233 targets were carry out in the DAVID system, which end up with 893 GO terms (BP: 652; CC: 75; MF: 166) and 131 KEGG pathways. The results were visualized by R package (Figure 5). Biological process (BP) mainly included oxidation-reduction process, inflammatory response, protein phosphorylation, positive regulation of ERK1 and ERK2 cascade, etc. Cell composition (CC) was composed of plasma membrane, cytosol, integral component of plasma membrane, extracellular exosome, cell membrane, etc. The results of molecular function (MF) suggested these targets were mostly involved receptor activity, kinase activity, RNA polymerase II transcription factor activity, protein binding, drug binding, etc.

In addition, KEGG enrichment analysis has identified much pathways of potential target genes, such as, FOXO signaling pathway, ErbB signaling pathway, MAPK signaling pathway, cAMP signaling pathway and Epithelial cell signaling in Helicobacter pylori infection. Meanwhile, 15 pathways were screened out based on the threshold of FDR<0.05 to establish the "compounds-hub genes-pathways" network.

"compounds-hub genes-pathways" Network

The "compounds-hub genes-pathways" network map of XHC was established using Cytoscape, which shows the relationship among 28 active components, 14 hub nodes and 15 key pathways (Figure 6). The top five compounds were caudatin, kaempferide, quercetin, isorhamnetin, and ellagic acid in descending order of degree, indicating the crucial roles of these components in treating FD.

Docking stimulation verification

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The top 5 compounds in "compounds-hub genes-pathways" network were docked with the respective top 5 targets in PPI network, respectively. Ligands and proteins were represented by licorices and a cartoon chains respectively. It is generally assumed that the highest cavities' size and the lowest Vina score indicate a strong binding ability between a protein and a compound. Thus, the Vina scores and cavities' sizes from CB-Dock were selected as the group representative (Table 3 and Table 4). According to the Figure 7, all the bioactive components of XHC showed a good binding with the hub genes, suggesting that the process of treating FD with XHC may be achieved by these hub genes, namely, AKT1, MAPK1, STAT3, EGFR and IL-6.

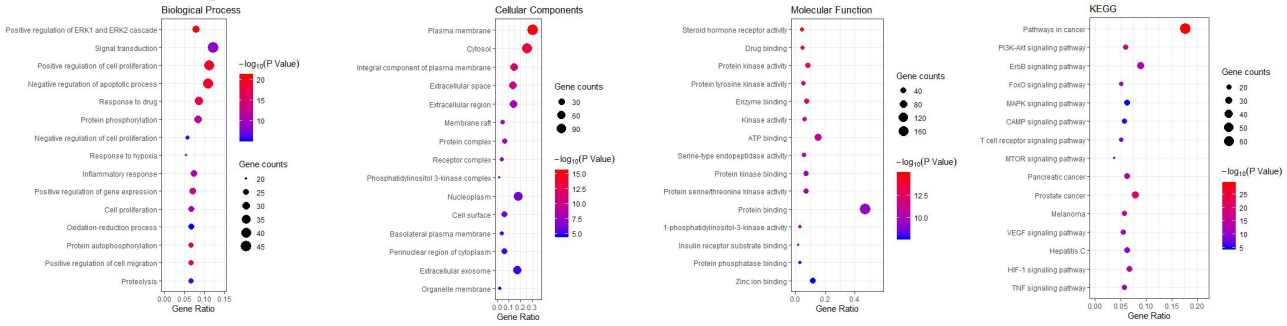

Figure 5 GO and KEGG pathway enrichment analyses.

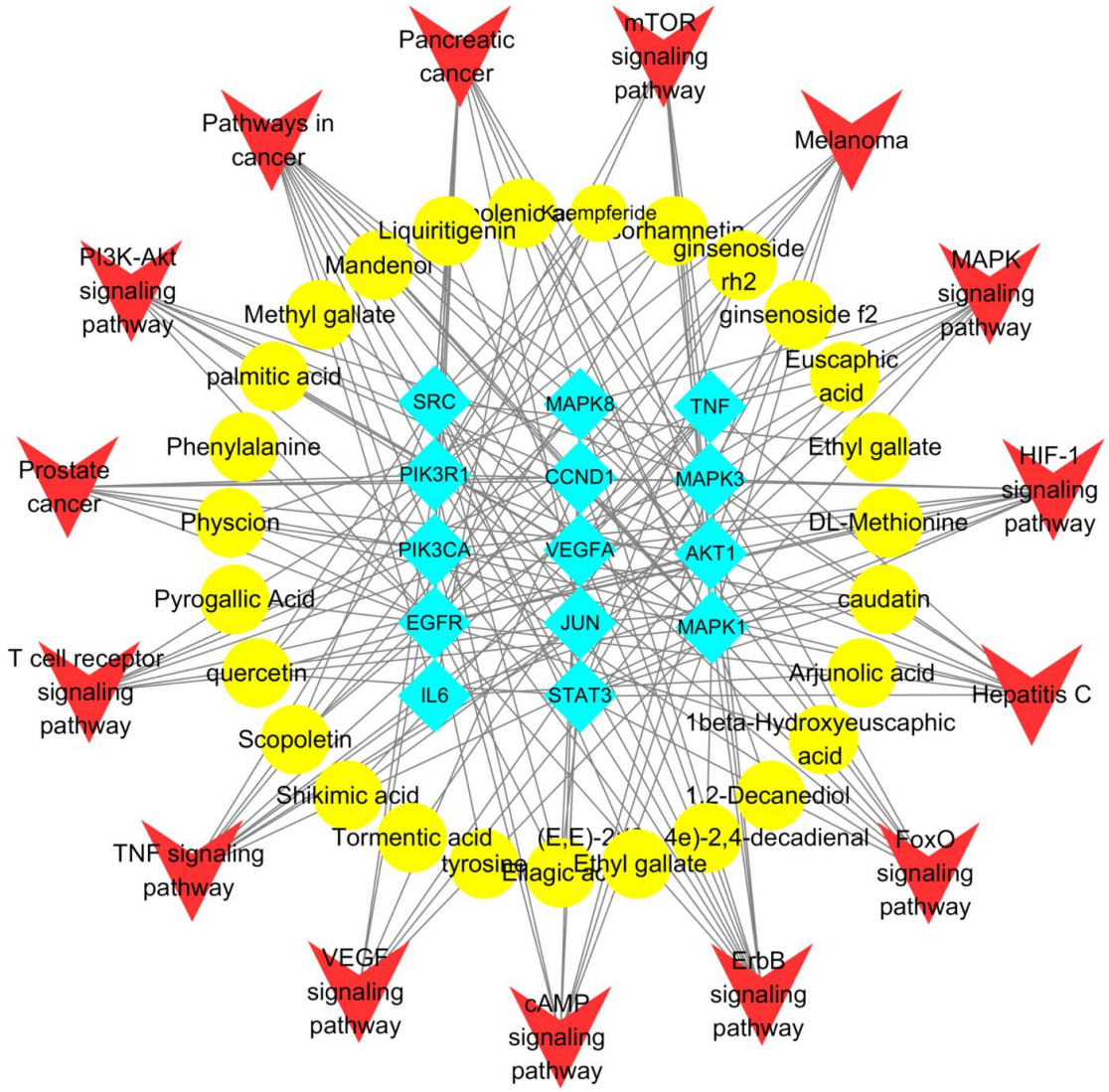

Figure 6 The "compounds-hub genes-pathways" network. The blue diamonds represent the hub genes, the yellow round nodes represent the active compounds, and the red nodes represent the related pathways

Table 3 Vina scores of molecular docking studies

Compounds	AKT1 (PDB:6HHF)	MAPK1 (PDB:6RQ4)	STAT3 (PDB:1BJ1)	EGFR (PDB:1XKK)	IL-6 (PDB:1ALU)
Caudatin	-7.7	-7.4	-7.8	-6.1	-5.6
Kaempferide	-6.5	-6.8	-7	-5.3	-6.5
Quercetin	-6.9	-7.1	-6.5	5.8	-7.2
Isorhamnetin	-7	-6.6	-6	-5.4	-6.8
Ellagic acid	-7	-6.4	-7.1	-5.5	-7.2

Table 4 Cavities' sizes of molecular docking studies

Compounds	AKT1 (PDB:6HHF)	MAPK1 (PDB:6RQ4)	STAT3 (PDB:1BJ1)	EGFR (PDB:1XKK)	IL-6 (PDB:1ALU)
Caudatin	393	413	384	228	230
Kaempferide	206	307	317	228	227
Quercetin	206	307	317	228	227
Isorhamnetin	206	307	317	228	227
Ellagic acid	206	307	317	228	227

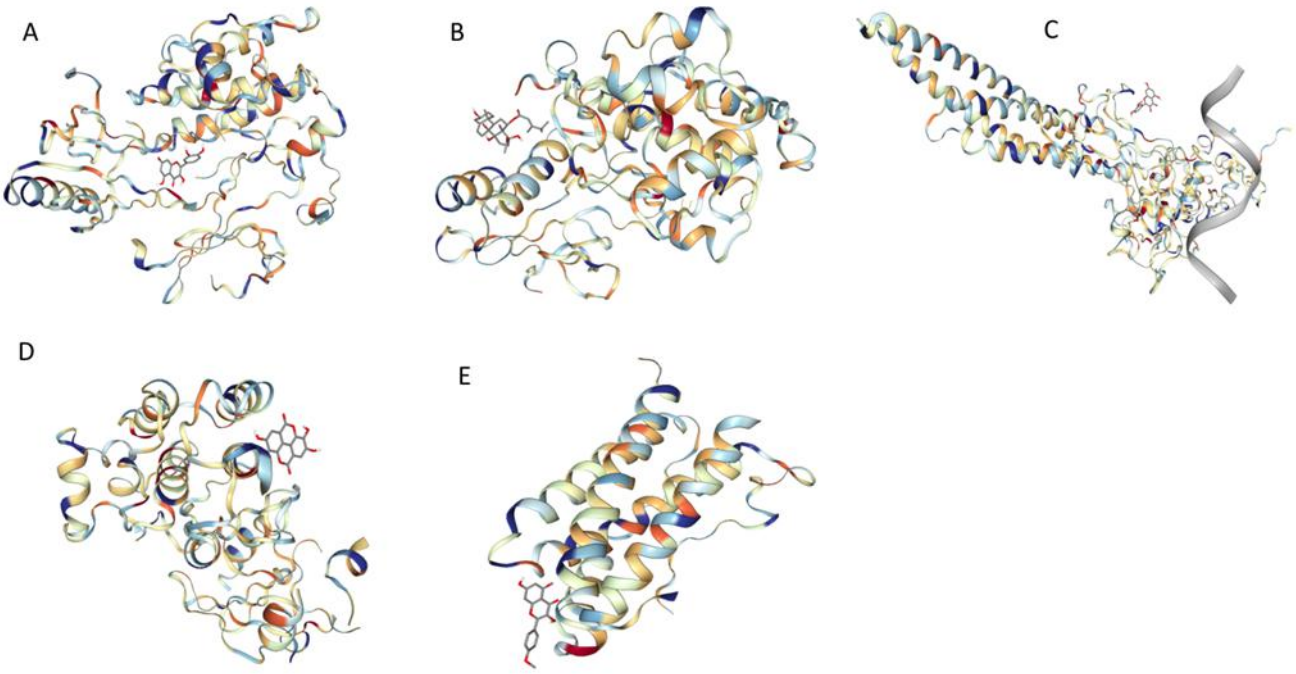

Figure 7 Molecular docking stimulation of active compound-hub gene.(A) Quercetin to AKT1; (B) caudatin to MAPK1; (C) isorhamnetin to STAT3; (D) ellagic acid to EGFR; (E) kaempferide to IL-6.

Discussion

At present, the incidence of FD is increasing year by year, which seriously threatens people's health and quality of life [31]. XHC has been used in the treatment of FD, but its mechanism is still unclear. Therefore, it is of great significance to study the molecular mechanism of XHC in the treatment of FD.

The causes of FD mainly include dyspepsia, Helicobacter pylori infection, depression, etc [32], which can generate inflammation, gastrointestinal movement dysfunction, etc. According the analyses of biological process, we found the oxidation-reduction process has previously been shown to correlate with

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the pathogenesis of depression [33] and inflammatory diseases of the gastrointestinal tract (such as H. pylori infection and IBD) [34]. Moreover, the role of inflammatory response in FD is extensive, such as anti-depression [35], eradicating HP infection and improving dyspepsia [36]. The abnormalities of ERK1/2 signaling may be crucial to the vulnerability of depression, moreover, the ERK activity continuously or transiently may serve as a negative regulator of vascular inflammation by suppressing endothelial NF-κB activation, and play an anti-inflammatory role [37].

Based on the results of KEGG pathway enrichment analyses, we known MAPK signaling pathway and cAMP signaling pathway were closely related to emotional regulation. ErbB signaling pathway plays a crucial role in the inflammatory reaction process, and epithelial cell signaling in Helicobacter pylori infection was related to HP infection and therapy.

According the "compounds-hub genes-pathways" network, five active compounds with the highest degree value were screened out. Among them, caudatin exhibits antioxidants, neuroprotective [38], immunoregulation and inhibition of angiogenesis actions [39]. Quercetin has anti-depression [40], anti-inflammatory effect [41]. Kaempferide exhibits antioxidants and neuroprotective activities [42]. Ellagic acid exhibits strong activity at eradication of HP, protection of stomach and reduction of the secretion of gastric acid [43]. Isorhamnetin plays a role in anti-inflammatory and antibacterial [44]. To conclude, these active ingredients are the material basis of XHC in treating functional dyspepsia.

Therefore, the results certificated that the potential mechanism of treating functional dyspepsia of XHC is probably connected to its participant in anti-depression, inflammatory reaction and eradicating HP infection.

Anti-depression

Previous research showed that psychiatric factors may increase the likelihood of FD, and eighty percent of patients with FD have anxiety and depressive mood [45]. MAPK1, mitogen activated protein kinase 1, is an important molecule in MAPK signaling pathway [46]. Experimental verification showed that the occurrence of depression may be concerned with activation of MAPK signaling pathway, and MAPK1 pathway is closely related to the pathological mechanism of depression [47]. Furthermore, inflammatory cytokines play an important role in the development of depression [48]. Clinical studies also showed that the expression of IL-1β and IL-6 was up-regulated in patients with depression [49]. Besides, AKT1 gene is associated with antidepressant treatment response in patients with depressive disorders [50].

XHC's regulative effect on depression is possibly achieved by the inhibitory effect of caudatin, quercetin and kaempferide. For instance, caudatin participates in MAPK signaling pathway and down-regulate MAPK1 directly or indirectly, so as to achieve the purpose of antidepressant. Also, quercetin and kaempferol inhibit the expression of AKT1 to affect downstream targets, which further regulate the PI3K-Akt signaling pathway. Besides, quercetin is capable of intervening RAS -MAPK pathway via acting on MAPK1, further participating in regulation of anti-depression [51, 52].

Inflammation reaction

In the pathogenesis of FD, inflammatory cells will proliferate and differentiate on account of the damage of gut barrier function. The research suggested that SRC may promote the occurrence of intestinal inflammation by mediating the release of inflammatory factors, inducing the homing and activation of inflammatory cells, and angiogenesis [53]. Meanwhile, VEGFA contributes significantly in the pathogenesis of FD, and overexpression of VEGFA will mediate inflammation and promote angiogenesis [54]. A further investigation showed that SRC can increased VEGFA expression in a mechanism that implicates the EGFR/ErbB signal pathway [55]. Jun participates in inflammation reaction through ErbB signal pathway by regulating angiogenesis [56]. It is suggested that stimulation of Jun gene may consequently activate the expression of VEGFA [57]. Furthermore, AKT1 plays an important role in cell survival, growth and proliferation, which was controled by PI3K directly through PI3K-Akt signaling pathway that participates in the release of inflammatory mediators and the proliferation of inflammatory cells [58].

These results suggest that targets could produce a combination effect on inflammation reaction. It is possible that the formula of XHC can exert the effect on inflammatory of FD by inhibiting the expression level of SRC and JUN and then regulating negatively the activation of VEGFA. Also, quercetin and isorhamnetin inhibit the expression of AKT1, thus realize the alleviation of inflammatory reaction.

Eradicating HP infection

HP was overexpressed in the gastric mucosa of patients with FD [59]. Several studies have demonstrated that the changes of gastric motility and sensory function caused by HP infection are one of the crucial pathological foundations of FD. EGF

(epidermal growth factor) can promote the proliferation and migration of epithelial cells, which plays a functional role in the reconstruction of the basal surface of ulcer and the filling of mucosal defects [60]. Moreover, EGFR, also known as receptor tyrosine-protein kinase (RPTK), has the activity of tyrosine-protein kinase (PTK). Vacuolating cytotoxin A (VacA), an exocrine protein of HP, can in some degrees stop the up-regulation effect of EGF on the EGFR expression, and affect the proliferation and repair of gastric mucosal cells [61].

In XHC, compounds that act on EGFR and EGF include: kaempferide, ellagic acid, isorhamnetin, phenylalanine and tyrosine. Among them, ellagic acid and isorhamnetin can not only act on inflammatory, but also the eradicator of HP. Phenylalanine and tyrosine, as aromatic amino acids, can regulate the proliferation and differentiation of intestinal epithelial cells and promate the secretion of gastrointestinal hormones [62]. Hence, bioactive compounds of XHC participate in treating FD by up-regulating of the EGFR via Epithelial cell signaling in Helicobacter pylori infection, and then promoting the proliferation and repairing of gastric mucosal cells [63].

Conclusion

In summary, the study highlights that XHC has significant effects on FD via various bioactive targets and compounds. The mechanism of XHC in treating FD connecting to the process of anti-depression, inflammatory reaction and HP eradication was revealed by the approach of network pharmacology and molecular docking. It has a significant value to provide theoretical basis for clinical treatment of FD and promote the development of XHC. Regrettably, Liuzhi (Salix babylonica L.), as an adjuvant of prescription, has few active ingredients and been excluded by molecular docking, which may be due to limited literatures and complicated screening conditions and will be discussed further.

Supporting Information

Additional supporting information will be found online in the Supporting Information section.

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RHL and YKS conceived and designed the whole study and obtained funding. YS, STN, JQW, HW and CNZ performed the data analysis. RHL and YXQ wrote the manuscript. All authors read and approved the final manuscript.

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