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Original Research Article

Network pharmacology approach to screen hypoglycemic extracts from *Coptidis rhizoma* and study their targets and pathways

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Abstract

Purpose: *To screen the hypoglycemic active ingredients of Coptidis rhizoma, and study their targets as well as signal pathways via network pharmacology.*

Methods: *Fifty-nine ingredients of Coptidis rhizoma were screened. Their targets were confirmed by comparing with the hypoglycemic targets in DrugBank databases. The relationship between ingredients and targets was revealed through String database. The ingredient-target-passageway network was constructed. Coptidis rhizoma was soaked in boiling water and concentrated. Rat models were rendered diabetic by the administration of streptozotocin (STZ) intraperitoneal injection. The hyperglycemic rats received Coptidis rhizoma extract (0.40 g/kg, once a day by gavage).*

Results: *After four weeks of treatment, the blood glucose levels (BG) of all treated hyperglycemic rats decreased ($p < 0.05$). Twenty-four hypoglycemic active compounds were obtained after screening the extract of Coptidis rhizome via network pharmacology. These active compounds activated 13 targets, including D (2) dopamine receptor (DRD2), Insulin-like growth factor 1 receptor (IGF1R), 5-hydroxytryptamine receptor 2C (HTR2C), 5-hydroxytryptamine receptor 3A (HTR3A) and sodium-dependent noradrenaline transporter (SLC6A2). These targets were involved in 141 pathways, e.g., cAMP signaling pathway, chemokine signaling pathway, Rap1 signaling pathway, estrogen signaling pathway, and apelin signaling pathway.*

Conclusion: *Coptidis rhizoma contains several active compounds that exhibit good hypoglycemic effects. Thus, there is a need for human studies on the hypoglycemic effects of Coptidis rhizome extracts.*

Keywords: *Coptidis rhizoma, Hypoglycemic effects, Network pharmacology, D (2) dopamine receptor (DRD2), Insulin-like growth factor 1 receptor*

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INTRODUCTION

Coptidis rhizoma is a traditional herbal medicine obtained from the family Ranunculacease, and it is called *Coptis chinensis* in France [1]. As a traditional Chinese medicine (TCM), *Coptidis rhizoma* is the dry rhizome of *Coptis ranunculaceae* [2]. As a shade-loving plant,

Coptidis usually grows in cool and damp valleys or shaded in dense forests at altitudes of 1000 - 1900 meters. Clinically, it has been widely used to treat gastroenteritis, dysentery, diabetes mellitus, and severe skin diseases [3-5].

Significantly, *Coptidis rhizoma* has good hypoglycemic effect [6-8]. However, the active compounds in *Coptidis rhizoma* and their targets as well as pathways need further study. In the study of herbs, the traditional research and development mode of drug-gene-disease is characterized by high costs, long cycle, and big risk, especially in clinical trials. These defects seriously hamper the progress of drug development. However, network pharmacology, as an important way to develop new drug molecular design model, was developed rapidly [9-11]. With the birth of network pharmacology, researchers improved and developed medicine development model with the aid of modern technologies, which is from the perspective of relationship between target and disease. With the development of network pharmacology, its characteristics of integrity and system are consistent with the holistic thought of TCM, and the principle of dialectical treatment. Therefore, it has attracted more and more attention in the research of TCM [12,13].

In this study, the hypoglycemic compounds of *Coptidis rhizoma* and their targets as well as signal pathways were analyzed with network pharmacology. The active ingredients in *Coptidis rhizoma* were screened out, and their hypoglycemic targets were confirmed with Batman-TCM database. In addition, these Food and Drug Administration (FDA) approved targets were found out in Drug Bank database. The detailed information on targets was obtained from the String database. Finally, the network among ingredient, target and pathway was built.

EXPERIMENTAL

Reagents

Streptozotocin (STZ, no. S110910) was purchased from Shanghai Aladdin Biochemical Technology Company. Metformin hydrochloride tablets (batch no. H31022081) were bought from Shanghai Shang-yao-xin-yi Pharmaceutical Factory. Glucometer and blood glucose test strip with non-regulated code were the products of Changsha Sano Biosensors Technology Company. Sodium citrate (Batch no. XK13-201-00147), citric acid (batch no. 20180213) and normal saline (NS, batch no. H20003438) were obtained from Tianda Chemical Reagent Factory, Dongli District, Tianjin, Tianjin-hengxing Chemical Reagent Manufacturing Company, and Henan Kelun Pharmaceutical Co. Ltd, respectively.

Extraction

Coptidis rhizoma (no. 20161201) was obtained from Zhixin Zhongyao Yinpian Co., Ltd (Fuyang, Anhui province). *Coptidis rhizoma* (40 g) was soaked in 400 mL of boiling water for 2 h, and the extracting solution was filtered. Then, these *Coptidis rhizoma* was again extracted in another 300 mL boiling water for 1 h, and the extracting solution was also filtered. Finally, all extracting solutions were mixed and concentrated to 80 mL. The obtained concentrated solution contains 0.2 g dried medicinal herbs per milliliter (40 g/80 mL).

Rat handling and treatment

The experiment was approved by the Animal Ethics Committee of Wuhan No. 3 Hospital (No. Wu-San-Yi-Shi-Lun SY2020-028), and the animal research was conducted in accordance with the internationally accepted principles for laboratory animal use and care in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). Male Sprague Dawley (SD) rats (220 ± 20 g) were used. Forty SD rats (male) were fed in individual cages with standard feed for a week to adapt to the environment. Six rats were randomly selected as normal control. The rest of the rats were administered STZ (0.5 %, 45 mg/kg) by intraperitoneal injection after 12 h of fasting. The STZ solution was prepared in the citric acid - sodium citrate buffer solution (0.1 mol/L, pH 4.5). The blood glucose (BG) levels of the treated rats were monitored after 3 and 7 days, respectively. If their BG levels were over 11.1 mmol/L, the diabetic rat model was established. Then, rats were randomly divided into four groups of six rats per group. These include, normal control, negative control, positive control, and *Coptidis rhizoma* group, respectively. Rats of the positive control group were administrated with metformin hydrochloride by gavage (0.18 g/kg, once a day). Rats in *Coptidis rhizoma* group were treated with concentrated solution of *Coptidis rhizoma* (0.40 g/kg, once a day) by gavage. By contrast, rats in positive and normal control groups were give same volume of normal saline. The BG levels of all the rats were monitored after 4 weeks of treatment.

Network pharmacology

The active ingredients of *Coptidis rhizoma* were summarized from TCMSP database

(https://tcmspw.com/tcmsp.php). Chemical compounds (55) were separated from *Coptidis rhizoma*, and all chemical structures were drawn with ChemOffice software (Version 7.0, Cambridge Soft Corporation). Subsequently, these structures obtained from ChemOffice software were transferred into *inchi* format with an Open Babel GUI software (version 2.4.1). By uploading these structures to Batman-TCM database (http://bionet.ncpsb.org.cn/batman-tcm/), the targets of these compounds were obtained. Among these targets, some approved by the FDA were gathered from Drug Bank database (https://go.drugbank.com/). By comparing the potential targets of *Coptidis rhizoma* with those hypoglycemic targets showed in Drug Bank database, the potential hypoglycemic ingredients in *Coptidis rhizoma* and their active targets were screened out. The information on these targets was obtained from the String database (http://www.string-db.org/). Their biological processes, molecular functions, cellular ingredients, and KEGG pathways were also analyzed. Finally, their 'ingredient-target-pathway' network models were built with Cytoscape software (Version 3.5, the Cytoscape Consortium).

Statistical analysis

Significant differences were analyzed by one-way ANOVA (two-tailed). At $p < 0.05$, it was considered that there were significant differences among the data.

RESULTS

Blood glucose levels of rats

As listed in Table 1, the average BG levels of diabetic rats was 16.3 ± 1.4 mmol/L (n = 6) which was over 11.1 mmol/L. So, the diabetic rat models were built successfully. By contrast, the average BG of normal rats was 5.0 ± 1.0 mmol/L (n = 6). A significant difference was observed between both rat group rat BGs ($p < 0.05$). Importantly, the BG levels of rats in positive control and *Coptidis rhizoma* groups decreased significantly, after 4 weeks of treatment. By comparing with the negative control, significant differences were observed in these two groups ($p < 0.05$). These data suggested that *Coptidis rhizoma* had good hypoglycemic effect, which was in agreement with previous reports [141,5].

Potential active ingredients and their targets

These fifty-nine active ingredients found in *Coptidis rhizoma* were associated with 1417 target compound involving 561 unrepeatable ones. All the potential targets of each ingredient was sorted from high to low according to their Z'-score. If the Z'-score was ≥ 23, this target was compared with the hypoglycemic target which was shown in Drug Bank database. As listed in Table 2, twenty-four potential hypoglycemic ingredients were screened out from *Coptidis rhizoma*, and these compounds were associated with thirteen hypoglycemic targets.

Gene ontology (GO) enrichment analysis

In string database, the biological process, molecular function, and cellular ingredients were analyzed with the GO enrichment analysis method. These compounds were associated with 707 biological processes (1135 frequencies). Figure 1 A showed 25 biological processes (*e.g.,* regulation of biological quality, response to oxygen-containing compound, and chemical synaptic transmission). These processes' occurrence frequencies were ≥ 5. Figure 1 B indicates 137 relevant molecular functions (191 frequencies). Ten main molecular functions were given, because their occurrence frequencies were ≥ 3. These functions included drug binding, g protein-coupled receptor binding, neurotransmitter receptor activity, and signaling receptor binding. The relative cellular ingredients were shown in figure 1 C. One hundred cellular ingredients with 170 frequencies were analyzed. Among them, sixteen were important, *e.g.,* neuron projection, integral ingredients of plasma membrane, and plasma membrane. Their occurrence frequencies were more than three.

Pathway analysis and 'ingredient-target-pathway' network

The relationships among targets were studied with KEGG Pathways analysis. Figure 2 gave 141 pathways with a frequency of 277. Among these pathways, 21 pathways had occurrence frequency of over 3. The 'ingredient-target-pathway' network of the hypoglycemic compounds in *Coptidis rhizoma* was built with the Cytosacape software (as shown in Figure 3). In this network diagram, the node represented active ingredients, targets, and pathway, respectively. The lines were applied to correlate the active ingredients, targets and pathways. This network diagram showed the relationships among the active ingredients, their targets, and the pathways. The network would provide the basis for new drug research and development.

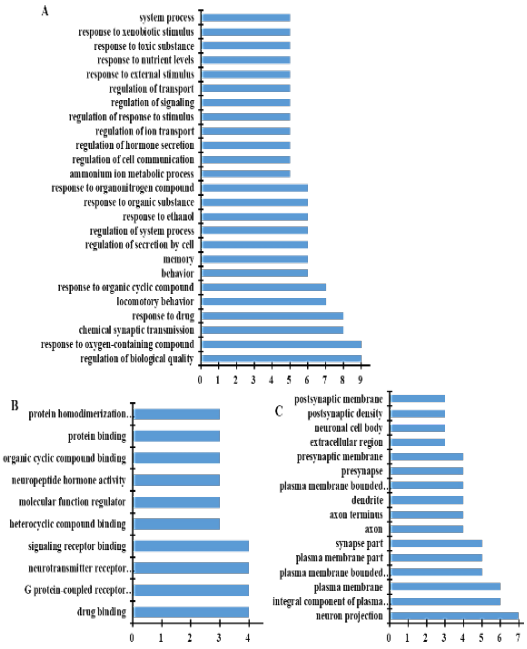

Figure 1: GO enrichment analysis results of 13 hypoglycemic targets (A) Biological process, (B) Molecular function, (C) Cellular component

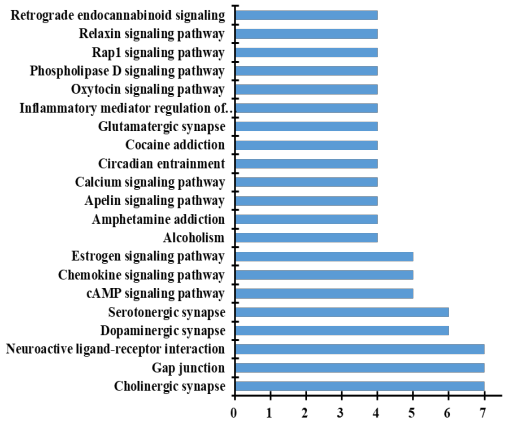

Figure 2: The analysis results of KEGG pathways on the hypoglycemic effect of *Coptidis rhizome*

Table 1: Blood glucose levels (BG) of rats before and after treatment by gavage (n = 6)

Group	Dosage (g/kg)	BG_{AT} (mmol/L)
Normal Control		5.0±1.0*
Negative control		16.3±1.4
Positive control	0.18	8.0±1.4*
Coptidis rhizoma	0.40	9.8±0.9*

P.S. (1) BG_{AT}: the blood glucose levels of diabetic rats after treatment by gavage; (2) Compared with negative control, the BG of rats in Normal control, Positive control, and *Coptidis rhizoma* showed significant difference (*$p < 0.05$)

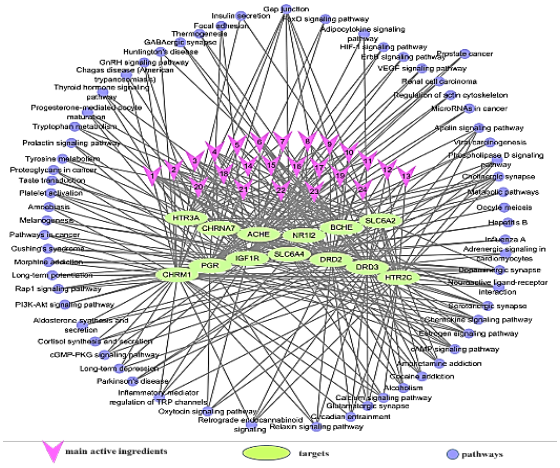

Figure 3: The network model of "hypoglycemic component-target-pathway" for *Coptidis rhizoma* (components 1-24 are shown in Table 2)

DISCUSSION

Twenty-four potential hypoglycemic compounds were screened from *Coptidis rhizoma*, and these ingredients were associated with thirteen hypoglycemic targets, *e.g.*, Insulin-like growth factor 1 receptor (IGF1R), D(2) dopamine receptor (DRD2), 5-hydroxytryptamine receptor 2C (HTR2C), 5-hydroxytryptamine receptor 3A (HTR3A), Sodium-dependent noradrenaline transporter (SLC6A2), Sodium-dependent serotonin transporter (SLC6A4), Acetylcholinesterase (ACHE), Cholinesterase (BCHE), Neuronal acetylcholine receptor subunit alpha-7 (CHRNA7), D(3) dopamine receptor(DRD3), Muscarinic acetylcholine receptor M1 (CHRM1), Nuclear receptor subfamily 1 group I member 2 (NR1I2), and Progesterone receptor (PGR).

Insulin-like growth factor 1 receptor (IGF1R), showed good clinical future of diabetes treatment. It was well known that IGF1R could promote the metabolism of glucose with the mediation of IR. Therefore, IGF1R usually show good curative effects on the treatment of Type II diabetes. Similarly, 5-HTR2C is another important target which is associated with diabetes. The association of schizophrenia (SCZ) as comorbidity with type 2 diabetes (T2DM) with the single nucleotide polymorphism (rs498177) of 5-HTR2C was studied previously [16]. Study found that the single nucleotide polymorphism (rs498177) of 5-HTR2C in the patients with schizophrenia was associated with comorbidity of T2DM. This phenomenon could be due to a susceptibility gene of this comorbidity in Han Chinese population [16]. All targets mentioned above had close relationship to T2DM.

Table 2: Hypoglycemic active compounds of *Coptidis rhizome*

Ingredient	Molecular formula	CAS No	Target gene (Z'-score)
D-tetrahydropalmatine	$C_{21}H_{25}NO_4$	3520-14-7	CHRM1; CHRNA7; DRD2; HTR2C; HTR3A; ACHE; BCHE; SLC6A2; SLC6A4
corydaline	$C_{22}H_{27}NO_4$	518-69-4	CHRM1; CHRNA7; DRD2; HTR2C; HTR3A; ACHE; BCHE; SLC6A2; SLC6A4
cavidine	$C_{21}H_{23}NO_4$	32728-75-9	CHRNA7; DRD2; HTR3A; ACHE; BCHE; SLC6A2; SLC6A4
(R)-Canadine	$C_{20}H_{21}NO_4$	522-97-4	CHRNA7; DRD2; HTR3A; ACHE; BCHE; SLC6A2; SLC6A4
stylopine	$C_{19}H_{17}NO_4$	4312-32-7	CHRM1; CHRNA7; DRD2; HTR2C; HTR3A; BCHE
(S)-Scoulerine	$C_{19}H_{21}NO_4$	6451-72-5	DRD2; DRD3; HTR2C; HTR3A; ACHE; BCHE
oxyacanthine	$C_{37}H_{40}N_2O_6$	548-40-3	DRD2; HTR3A; ACHE; BCHE
Secoisolariciresinol	$C_{20}H_{26}O_6$	29388-59-8	HTR2C; SLC6A2; SLC6A4; NR1I2
tetrahydrocolumbamine	$C_{20}H_{23}NO_4$	483-34-1	DRD2; HTR3A; ACHE; BCHE
cheilanthifoline	$C_{19}H_{19}NO_4$	483-44-3	DRD2; HTR3A; ACHE; BCHE
beramine	$C_{37}H_{40}N_2O_6$	478-61-5	DRD2; HTR3A; ACHE; BCHE
isocorydine	$C_{20}H_{23}NO_4$	475-67-2	DRD2; DRD3; HTR2C
corydine	$C_{20}H_{23}NO_4$	476-69-7	DRD2; DRD3; HTR2C
allocryptopine	$C_{21}H_{23}NO_5$	485-91-6	DRD2; ACHE
danshensu	$C_9H_{10}O_5$	76822-21-4	DRD3; DRD2
oxyepiberberine	$C_{20}H_{17}NO_5$	19716-60-0	IGF1R
8-Oxocoptisine	$C_{19}H_{13}NO_5$	19716-61-1	IGF1R
epiberberine	$C_{20}H_{18}NO_4^+$	6873-9-2	IGF1R
columbamine	$C_{20}H_{20}NO_4^+$	3621-36-1	NR1I2
groenlandicine	$C_{19}H_{16}NO_4^+$	38691-95-1	IGF1R
betulin	$C_{30}H_{50}O_2$	473-98-3	PGR
cycloeucalenol	$C_{30}H_{50}O$	469-39-6	PGR
coptisine	$C_{19}H_{14}NO_4^+$	3486-66-6	IGF1R
jatrorrhizine	$C_{20}H_{20}NO_4^+$	3621-38-3	IGF1R

Twenty-one pathways, including cAMP signaling pathway, Rap1 signaling pathway, Chemokine signaling pathway, Estrogen signaling pathway, and Apelin signaling pathway, had occurrence frequency of over 3. The cAMP signaling pathway, also called protein kinase A system, is a kind of cyclic nucleotide system. In this system, the extracellular signals bind to relative receptors. This signal pathway triggers a reaction by adjusting the level of the second messenger cAMP in the cell. This pathway is composed of five ingredients which were on the plasma membrane, *i.e.,* activated receptor (RS), inhibitor receptor (Ri), activated and inhibited regulating G proteins (Gs and Gi), and Adenylate cyclase (AC). Furthermore, previous study reported that the cAMP signaling pathway could adjust insulin secretion from the β cells of the pancreas. Therefore, this pathway showed close association with diabetes. Another study reported that inflammatory reaction could promote the progression of diabetic nephropathy by activating relative Chemokine signaling pathway [17]. In general, all these signal pathways mentioned above are related to diabetes.

CONCLUSION

In this study, *Coptidis rhizoma* extract show good hypoglycemic effect. The potential hypoglycemic active ingredients in *Coptidis rhizoma* screened are twenty-four, and their relative targets analyzed with network pharmacology are thirteen. This study lay a good foundation for further study on the hypoglycemic effects of *Coptidis rhizoma*.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

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

Conflict of interest

No conflict of interest is associated with this

work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ming Wei, Liu Xiao and Yi Li conceived and designed the study. Liu Xiao and Yi Li contributed equally to this work, and they are co-first authors. Ming Wei are co-corresponding author, Ming Wei, Liu Xiao, Yi Li, Xin-guo Liu, Quan-wei Yang, Lei Yang, He-gui Huang and Lei Huang collected and analysed the data. Ming Wei, Liu Xiao and Yi Li wrote the manuscript.

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