Explore the mechanism of Shengma-Gegen drug pair on hepatitis B virus based on network pharmacology

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Research

Keywords: Chebulae Fructus, Radix Puerariae, Hepatitis B, Pharmacology, Herbal Medicine

Posted Date: August 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-59583/v1>

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Abstract

Background

Sheng-Ma-Ge-Gen-Tang (SMGGT; 升麻葛根汤) is a famous prescription of traditional Chinese medicine used against measles of children for many hundreds of years. And its anti-hepatitis B virus (HBV) activity has been justified in clinical, however, the function substances and the mechanisms have not been studied yet.

Methods

The latent active compounds of *Cimicifugae Rhizoma* (Shengma)-*Radix Puerariae* (Gegen) drug pair were searched, and excavated their related targets. Then seek the targets of HBV through three network databases. The drug-disease targets of protein-protein interaction (PPI) data was carried out. The drug-disease targets were enriched by gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Subsequently, anti-HepG2.2.15 cytotoxicity and anti-HBV experiments were performed on the aqueous and ethanol extracts of Shengma-Gegen *in vitro* to test and verify their anti-HBV activities.

Results

GO enrichment indicated biological processes (cellular response to lipid, cellular response to organic cyclic compound, etc.), cellular components (vesicle lumen, cytoplasmic vesicle lumen, etc.) and molecular function (kinase binding, steroid binding, etc.). Many cancer-related pathways were enriched in KEGG pathway analysis. Also got some virus and bacteria diseases. A KEGG-targets network showed that RAF1, CCND1, BCL2, and EGFR might be the core targets. Cytotoxicity of Shengma and Gegen to HepG2.2.15 are in proportion to their concentrations. Shengma and Gegen aqueous and ethanol extracts exhibited a curtain extant anti-HBV-DNA activity.

Conclusions

Shengma-Gegen showed not only anti-HBV activity but also anti-tumor and anti-viral activities, which need to be tested in the future.

Background

The World Health Organization (WHO) estimates that the global burden of HBV infection is 257 million people [1], and about 650,000 people die every year [2]. In China, a national survey conducted in 2014 showed that the total prevalence of hepatitis B surface antigen (HBsAg) among people aged from 1 to 59 years old was 7.18% [3, 4], which is equivalent to 93 million people infected with HBV [3]. Chronic

hepatitis B (CHB) is the main cause of chronic hepatitis, liver cirrhosis, and liver cancer. In China, 60% and 80% of liver cancer are caused by HBV infection [4].

At present, there is no specific treatment for hepatitis B worldwide. The main treatment methods for CHB include anti-viral and anti-liver fibrosis. The generally recognized effective anti-HBV drugs mainly include interferon and nucleoside drugs [5]. Although there are currently strong and highly resistant antiviral drugs, a complete cure for CHB cannot be achieved. In China, about 30–50% of the drugs used in the treatment of hepatitis B is low-cost and low-toxic Chinese herbal medicines. About 80% of patients with CHB rely on Chinese herbal medicines [6]. However, it is the focus and difficulty of current research to dig out the effective material basis of existing anti-HBV traditional Chinese medicine (TCM) and to find out its mechanism of function.

SMGGT is first derived from "Yan's Prescriptions for Children" in the Song Dynasty in China. It is originally used to treat measles of children. The prescription consists of four TCMs: *Cimicifugae Rhizoma*, *Radix Puerariae*, *Paeoniae Radix Alba* and licorice. In the prescription, *Cimicifugae Rhizoma* is used to relieve muscle, clear heat and detoxify; *Radix Puerariae* is externally opened and internally clears heat to produce body fluid. In the prescription, Shengma and Gegen are the drug pair, which promotes each other, and so, they are the monarch drugs in the prescription. SMGGT had been proven against measles virus [7]. Furthermore, it has been proven to be effective at inhibiting HRSV-induced plaque formation *in vitro* [8]. SMGGT has been clinically used to treat hepatitis B and has a significant effect [9]. Someone used SMGGT to treat patients with CHB in the immune eradication stage, then observed the symptoms and alanine transaminase (ALT), aspartate transaminase (AST), HBV DNA, etc. Results indicated that SMGGT can improve the clinical symptoms, signs and liver function, and can inhibit virus replication, furthermore, the effect is better than Shuanghu Qinggan Granules group [10].

Chinese formula usually consists of many drugs, select the monarch drug or drug pair in a prescription for study can make the work more concise and clear. In this study, we used a network pharmacology strategy to explore the mechanism and molecular targets of Shengma-Gegen drug pair in SMGGT on hepatitis B infection. GO enrichment and KEGG pathway analysis about the drug-disease targets were carried out and comprehensively analyzed. Subsequently, the bioactivities of Shengma-Gegen water and ethanol extracts on HBV were experimentally verified *in vitro*.

Methods

Materials

Cimicifugae Rhizoma and *Radix Puerariae* were purchased from Tongrentang, Yancheng, Jiangsu Province, China. They were identified by Professor JP Song of Jiangsu Vocational College of Medicine. Diagnostic kits for HBsAg and HBeAg were purchased from Abbott Trading Shanghai Co. Ltd. (Shanghai, China), and a diagnostic kit for HBV DNA was purchased from Hunan Shengxiang Jiancheng

Biotechnology Co. Ltd. (Hunan, China). All other analytical chemicals were purchased from Shanghai Chemical Reagents Co. Ltd (Shanghai, China).

Active ingredient screening

We found 85 ingredients of Shengma and 14 ingredients of Gegen from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>) [11]. Some researchers then screened the compounds based on oral bioavailability (OB) $\geq 30\%$ and drug similarity (DL) ≥ 0.18 [12], but many TCM ingredients were bio-transformed to real active ones in vivo after oral administration [13], furthermore, [puerarin](#) as the main ingredient with many bioactivities would be excluded for its OB = 24.03, and so, all the ingredients were used for later analysis. There is one common ingredient both in Shengma and Gegen, so a total of 98 ingredients were found.

Potential drug-related targets

For the 98 ingredients obtained, the TCMSP system was also used to search for the ingredients related targets, and one platform could make the results consistent.

Hepatitis B related targets

For disease-related targets, the Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>) database was searched by Hepatitis B and Hepatitis B chronic; GeneCard (<http://www.genecards.org>) and OMIM (<http://omim.org/>) searched by Hepatitis B; Searching results were filtered as follows: CTD results set Inference Score ≥ 5 for screening, got 1556 targets; GeneCard with the median degree ≥ 15 for screening, got 818 targets; and OMIM got 195 approved targets. After combining the three database targets for deduplication, a total of 1631 disease targets were obtained.

Venn diagram of drug-disease targets

A total of 131 drug-targets were merged with the 1631 disease-targets to obtain an intersection of 71 drug-disease targets. Calculate and draw custom Venn diagram website (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was used to draw a Venn diagram for visualization.

Drug-targets network construction

Cytoscape 3.7.2 software was used to construct and visualize the ingredient-disease target network of Shengma and Gegen. Firstly, the documents of network and type were established with R Studio Version 1.2.5033 and Perl software, then the ingredient-target network was established with Cytoscape for visualization, calculating the Degree Centrality (DC). Cytoscape software was also used to visualize the network of Shengma and Gegen candidate targets and enriched KEGG pathways of Hepatitis B.

Bioinformatics analysis

Metascape (<https://metascape.org/>) was used to perform GO enrichment analysis on the identified 71 drug-disease targets, and accumulative hypergeometric *p*-values and enrichment factors were calculated

and used for filtering. GO enrichment analysis includes three terms: biological process, cell composition, and molecular function. Also, MCODE (App of Cytoscape) was used to find clusters (highly interconnected regions) in the PPI network, then GO enrichment analysis was applied to each MCODE network to assign “meanings” to the network component, and the top three best *p*-value terms were retained and visualized by Cytoscape. KEGG pathway enrichment analysis was compared with Metascape (<https://metascape.org/>), STRING and the ClusterProfiler package of the R language.

HepG2.2.15 cell activity experiment [14]

Shengma and Gegen powders were extracted twice in a 10-fold volume of water and ethanol-water (70:30, v/v) under reflux for two hours each time. The extracted solutions were first concentrated by rotary evaporator and then dried under vacuum heating to prepare the water and ethanol extract powder for the use of subsequent experiments.

HepG2.2.15 cells were derived from American-type culture sets (atcc, Manassas, USA). All cells were stored in Dulbecco's Modified Eagle Media (DMEM) containing 10% FBS (fetal bovine serum, Hy clone, Logan, UT) and cultured at 37 °C (5% CO₂, 95% relative humidity). The Cell Counting Kit-8 (CCK-8) method was used to analyze the cytotoxicity. Briefly, a volume of 200 ml adherent cells was seeded into a 96-well plate and lasted for 24 h before adding drugs at an initial density of 1.0×10^5 cells/ml. The tumor cell line was exposed to Shengma and Gegen water and ethanol extract at the concentrations of 500, 250, 125, and 60 µg/ml (DMEM with 0.1% dimethyl sulfoxide, DMSO) for three times within 48 hours. The control group was treated with the same solvent. After 48 hours, the medium and test drugs were replaced with CCK-8 solution and incubated for 4 hours in the dark. The formed formazan crystals were dissolved in dimethyl sulfoxide. The absorbance was measured with a microplate reader at 450 nm. The cell survival rate (%) was calculated as sample/control $\times$ 100%.

Determination of anti-hepatitis B virus activity [14]

HepG2.2.15 cells were stored in DMEM containing 10% PBS and cultured at 37 °C (5% CO₂, 95% relative humidity) for anti-HBV cell activity detection. First, a volume of 500 ml cells was inoculated into a 24-well cell culture plate at an initial density of 3×10^5 cells/ml. The cell supernatant was collected every three days. Then 250 µg/ml drug solutions were added, and DMSO and entecavir (ETV) were added as negative control and the positive control respectively at the concentration of 50 µg/ml. The experiment was terminated on the ninth day. The cell supernatants were collected for the analysis of the level of HBsAg, HBeAg, and HBV DNA using the following methods. The levels of HBsAg and HBeAg were measured by enzyme-linked immunosorbent assay (ELISA) method. Detection of HBV DNA was used luminescence method. The relative level is calculated as follows: relative level (%) = $[(A_{\text{TEST}} - A_{\text{Control}})/A_{\text{Control}}] \times 100$ (where A is the level of HBsAg or HBeAg or HBV DNA), and the subscripts “test” and “control” represent the drugs and control group, respectively.

Statistical analysis

All data were expressed as mean $\pm$ SD. At least three independent experiments were performed, five times each. One-way analysis of variance was used for data analysis (ANOVA) with GraphPad Prism 8.0.1 software. Statistical significance was analyzed, and the Dunnett test was used for statistical analysis. $p < 0.05$ was considered statistically significant.

Results

Ingredients related target network analysis

Finally, 85 kinds of Shengma ingredients and 14 kinds of Gegen ingredients were selected as candidate compounds, there was one uniform ingredient of Shengma and Gegen, and the Mol ID (in TCMSP) and molecule name were shown in **Supplementary Table 1**. The drug-related targets were searched from the TCMSP database, and a total of 131 drug-related targets were identified.

The compound-disease target network of Shengma and Gegen was constructed between the talented active ingredients and their related targets, as shown in Fig. 1. The network contains 121 nodes and 245 edges including 10 ingredients of Gegen and 38 ingredients of Shengma, and 71 compound-related targets. Component genistein, daidzein, puerarin, and eugenol connected with a degree of 30, 24, 22, and 10, respectively. Therefore, they might be the key active compounds of Shengma and Gegen.

Targets Of Hepatitis B

The CTD database was used to search Hepatitis B and Hepatitis B chronic disease for the targets, then GeneCard and OMIM were also searched for Hepatitis B. CTD results were screened with Inference Score ≥ 5 , and 1556 targets were obtained. GeneCard set Relevance score ≥ 15 , got 818 targets, and all 195 approved targets of OMIM were obtained. After merging the three database targets to remove duplicates, a total of 1631 hepatitis B related targets were obtained.

Venn Synergy Target

To explore the mechanism of the function of Shengma-Gegen drug pair on hepatitis B, the 131 targets of drug ingredients were intersected with 1,631 disease-targets to determine candidate drug-targets for Hepatitis B. Finally, 71 common targets for drugs and diseases were obtained. The Venn diagram was shown in Fig. 2.

PPI Network Of Shengma And Gegen Against Hepatitis B

As shown in Fig. 3, a new PPI network of 71 drug-disease targets was constructed in the STRING database. The gene symbol of 71 targets was shown in **Supplementary Table 2**.

Enrichment Analysis Of GO And KEGG

The above 71 drug-disease targets were analyzed with Metascape software for GO enrichment and KEGG pathway enrichment, and the ClusterProfiler package in R language was also used for comparing the KEGG pathway analysis. Based on biological processes, cell composition, and molecular function, the GO of candidate targets was analyzed. Enrichment parameters were set as follows: Min Overlap: 3; *p*-Value Cutoff: 0.01; Min Enrichment: 1.5. A total of 2,204 GO terms were significantly enriched, and there were 2000 terms in biological processes, 78 terms in cellular components, and 126 terms in molecular functions (in **Supplementary Table 3**). The top 20 terms enriched by each GO type were shown in Fig. 4. Biological process (cellular response to lipid, cellular response to organic cyclic compound, response to steroid hormone, apoptotic signaling pathway, cytokine-mediated signaling pathway), cellular components (vesicle lumen, cytoplasmic vesicle lumen, receptor complex, secretory granule lumen, membrane raft, membrane microdomain, membrane region, early endosome), and molecular function (kinase binding, steroid binding, protein kinase binding, peptide binding, amide binding, ubiquitin-like protein ligase binding, proximal promoter sequence-specific DNA binding, lipid binding) were the primary enriched GO terms.

MCODE was then applied to the PPI network to identify neighborhoods where proteins were densely connected. Each MCODE network was assigned a unique color (red, blue, and green). GO enrichment analysis was applied to each MCODE network to assign “meanings” to the network component, and the top three best *p*-value terms were retained. The top three clusters were shown in Fig. 5, and the GO enrichment of each network was shown in Table 1.

Table 1
GO core functional groups with MCODE

Network	Annotation
MCODE-ALL	GO:0048545 response to steroid hormone -17.2;GO:0071407 cellular response to organic cyclic compound -16.8;GO:0097190 apoptotic signaling pathway -16.1
MCODE-1	GO:0009612 response to mechanical stimulus -13.5;GO:0035994 response to muscle stretch -9.8;GO:0043281 regulation of cysteine-type endopeptidase activity involved in apoptotic process -9.1
MCODE-2	GO:0001085 RNA polymerase II transcription factor binding -7.7;GO:0071407 cellular response to organic cyclic compound -5.4;GO:0097190 apoptotic signaling pathway -5.3
MCODE-3	GO:0008395 steroid hydroxylase activity -14.2;GO:0004497 monooxygenase activity -12.0;GO:0020037 heme binding -11.3

The KEGG pathway analysis with Metascape, STRING and R method identified the pathways in which the components of Shengma and Gegen targets may significantly participate. A total of 132 significant KEGG pathways were enriched by Metascape with *p*-Value Cutoff: 0.01; 128 KEGG pathways by R language with *p*-value ≤ 0.05 and q-value ≤ 0.05; 105 KEGG pathways by STRING with false discovery rate ≤ 0.05 (in **Supplementary Table 4**). The first top 30 pathways enriched in Metascape, STRING, and R

were shown in Table 2. As we can see, the pathways enriched in the top by Metascape were most in common with STRING and R method, and so, we select Metascape as KEGG enrichment analysis method. The analysis results showed that multiple signaling pathways were associated with cancer, such as Pathways in cancer, Prostate cancer, Proteoglycans in cancer, Colorectal cancer, MicroRNAs in cancer, Pancreatic cancer, Bladder cancer, p53 signaling pathway, Apoptosis, PI3K-Akt signaling pathway, TNF signaling pathway, and NF-kappa B signaling pathway, and also included some virus and bacteria diseases such as Influenza A, African trypanosomiasis, Toxoplasmosis, Pertussis, Measles, HTLV-I infection, Legionellosis. Diabetic disease: AGE-RAGE signaling pathway in diabetic complications, FoxO signaling pathway. Hepatitis B was one of the most important pathways enriched in three platforms mentioned above, it was the second in Metascape, the third in STRING, and the sixth in R. For comparison, the bubble diagram of KEGG analysis with R for Shengma and Gegen on the target of hepatitis B was shown in Fig. 6, the size of the spot represented the count of genes and color represented p adjust value.

Table 2
Information on the main pathways of KEGG in three platforms

No.	STRING	Metascape	R language
1	Pathways in cancer	Pathways in cancer	Prostate cancer
2	Prostate cancer	Hepatitis B	Kaposi sarcoma-associated herpesvirus infection
3	Hepatitis B	Prostate cancer	Human cytomegalovirus infection
4	Kaposi's sarcoma-associated herpesvirus infection	Proteoglycans in cancer	Hepatitis B
5	Proteoglycans in cancer	HIF-1 signaling pathway	Measles
6	MicroRNAs in cancer	Platinum drug resistance	Epstein-Barr virus infection
7	HIF-1 signaling pathway	AGE-RAGE signaling pathway in diabetic complications	Proteoglycans in cancer
8	PI3K-Akt signaling pathway	Apoptosis	Platinum drug resistance
9	AGE-RAGE signaling pathway in diabetic complications	p53 signaling pathway	p53 signaling pathway
10	p53 signaling pathway	PI3K-Akt signaling pathway	HIF-1 signaling pathway
11	Platinum drug resistance	Colorectal cancer	Colorectal cancer
12	Human papillomavirus infection	MicroRNAs in cancer	Hepatitis C
13	Colorectal cancer	Endocrine resistance	AGE-RAGE signaling pathway in diabetic complications
14	Fluid shear stress and atherosclerosis	Fluid shear stress and atherosclerosis	Apoptosis
15	Endocrine resistance	TNF signaling pathway	Human immunodeficiency virus 1 infection
16	Apoptosis	Influenza A	TNF signaling pathway
17	TNF signaling pathway	FoxO signaling pathway	PI3K-Akt signaling pathway
18	Pancreatic cancer	African trypanosomiasis	Endocrine resistance
19	Influenza A	Pancreatic cancer	Pancreatic cancer
20	FoxO signaling pathway	Bladder cancer	Influenza A

No.	STRING	Metascape	R language
21	Small cell lung cancer	Prolactin signaling pathway	Fluid shear stress and atherosclerosis
22	African trypanosomiasis	Toxoplasmosis	African trypanosomiasis
23	Non-small cell lung cancer	Pertussis	Bladder cancer
24	Breast cancer	EGFR tyrosine kinase inhibitor resistance	Small cell lung cancer
25	Prolactin signaling pathway	Cell cycle	Human papillomavirus infection
26	Bladder cancer	Measles	Non-small cell lung cancer
27	Cellular senescence	HTLV-I infection	Prolactin signaling pathway
28	Toxoplasmosis	Legionellosis	FoxO signaling pathway
29	Pertussis	Focal adhesion	NF-kappa B signaling pathway
30	Hepatocellular carcinoma	NF-kappa B signaling pathway	Pertussis

Gene-pathway Network Analysis

Based on the significantly enriched top 20 pathways and 91 genes that participated in these pathways, a gene-pathway network was constructed, as shown in Fig. 7. The V shape represents the pathways, and the oval shape represents the targets of the network. The size of the shape reflects the degree value, and the transparency indicates the KEGG enriched *p*-value. The network diagram showed that pathways in cancer, hepatitis B and TNF signaling pathway are the top three pathways enriched in KEGG; RAF1, CCND1, BCL2, and EGFR got the largest DC, speculated to be the core targets. The CASP9, CDKN1B, MDM2, PRKCA and RELA genes also had a larger DC value, and they might be the key targets for Shengma and Gegen function on hepatitis B.

Cytotoxic Activity And Anti-HBV Activity Results

First, the anti-HepG2.2.15 activity of water and ethanol extracts of Shengma and Gegen were studied. As shown in Fig. 8, the cytotoxic activities of Shengma and Gegen on HepG2.2.15 were in proportion to their concentrations, and all the tested drugs exhibited good cytotoxic activities at the concentration of 500 µg/ml. Considering the application, we selected the concentration of 250 µg/ml for the later test, at which only the ethanol extract of Gegen showed no cell activity.

Subsequently, we selected the concentration of 250 µg/ml of Shengma and Gegen ethanol and water extract for anti-HBV tests, as shown in Fig. 9, compared with the positive control Entecavir (ETV) group, SH, GH, GC and SGH groups showed similar anti-HBeAg effects ($p > 0.05$), except SC and SGC group, had weaker anti-HBsAg effects ($p < 0.05$). SH and SGH exhibited similar anti-HBeAg effects ($p > 0.05$), but all the tested drugs show weaker activity on HBV-DNA ($p < 0.05$).

Discussion

In this study, compounds of Shengma and Gegen and their corresponding 71 compound-disease targets were used to construct a compound-target network. Results showed that most Shengma and Gegen compounds affected multiple targets, such as genistein, daidzein, puerarin in Gegen and eugenol in Shengma, of which genistein had the most related targets of 30. Therefore, they were likely to be the key active compounds of Shengma and Gegen.

Through GO enrichment analysis, the targets of Shengma and Gegen against hepatitis B were enriched in biological processes, cellular components, and molecular functions. The results showed that Shengma and Gegen regulated certain biological processes (cellular response to lipid, cellular response to organic cyclic compound, response to steroid hormone, etc.), cellular components (vesicle lumen, cytoplasmic vesicle lumen, receptor complex, secretory granule lumen, etc.) and molecular function (kinase binding, steroid binding, protein kinase binding, peptide binding, amide binding, etc.). Hepatitis B, as invasive disease, was mainly bounded by cell membrane ligand receptors, thereby producing a series of cytokines and carrying out cell signals. This process may include the process of both the pathogen invading the host and the drugs functioning on pathogen in vivo.

In this study, KEGG enrichment analysis was compared in three platforms, they are Metascape, STRING and R language, as shown in Table 2, hepatitis B was enriched in the top sixth in each method, testified that the targets we excavated were reasonable. Many cancer-related pathways were enriched, such as Pathways in cancer, Prostate cancer, Proteoglycans in cancer, Colorectal cancer, MicroRNAs in cancer, Pancreatic cancer, Bladder cancer, p53 signaling pathway, etc., indicated that hepatitis B may lead to liver cancer in many cases [4], and studies about cancer are still the hot tissue for scholars. 7,8-didehydrocimigenol, as an ingredient in Shengma, can inhibit NF-κB activity of TNF-α-activated EC by upregulation of PPAR-γ [15]. Enrichment also got some virus and bacteria diseases such as Influenza A, African trypanosomiasis, Toxoplasmosis, Pertussis, Measles, HTLV-I infection, and Legionellosis. It is well known that Chinese medicine has multi-components, multi-targets, and multi-pathways. Shengma and Gegen contain different kinds of chemical ingredients and has similar but different characteristics, which means that Shengma and Gegen can treat HBV through multiple routes, meanwhile, different diseases can be treated with Chinese medicine in the same way (同病异治), if their mechanism is similar. Studies have found that Shengma and Gegen and their components were active on many species of virus, such as human respiratory syncytial virus (HRSV), Enterovirus 71, and measles virus, etc. [7, 15–17].

As shown in Table 1, GO was clustered by MCODE. We can speculate that drugs function on hepatitis B may first cause the cellular response to organic cyclic compound reaction, then stimulate some enzymes, such as steroid hydroxylase, monooxygenase, cysteine-type endopeptidase, RNA polymerase II, heme binding, following related signal pathways and resulting apoptotic signaling pathway.

A KEGG-targets network was constructed to study the talented biomarker targets of Shengma and Gegen against hepatitis B. Results showed that RAF1, CCND1, BCL2, and EGFR had the largest DC, regarded as the core targets.

Hepatitis B pathway was one of the top three enriched KEGG pathways. The targets that participated in hepatitis B were marked in red color, as shown in Fig. 10. Experiments were conducted to verify the efficacy of Shengma and Gegen on hepatitis B *in vitro*, and it was found that cytotoxicity of Shengma and Gegen to HepG2.2.15 are in proportion to their concentrations. As shown in Fig. 9, both Shengma and Gegen ethanol extracts and aqueous extracts exhibited a certain extent anti-HBV-DNA activity. The drug aqueous extracts showed a better anti-HBsAg and anti-HBeAg activity than ethanol extracts, although there was no statistical difference between tested drugs and ETV group ($p > 0.05$). Chinese herbal formula is usually extracted with water, which is in accordance with this result. HBsAg, HBeAg, and HBV DNA were selected as the indicators of drugs function on hepatitis B. For example, the presence of a positive HBsAg lasted for over 6 months indicated a CHB virus infection. HBeAg has long been seen as an indicator of viral replication and infectivity, and the HBeAg level can reflect a patient's phase in the history of chronic HBV infection [18, 19]. HBV DNA can be a marker of viral replication and is the main target of antiviral therapy [18, 20–21]. Studies have found that cohosh, Pueraria lobata root and their main components have the effect of treating hepatitis and anti-Hepatitis B. Cohosh can not only resist the elevation of ALT and AST caused by chronic hepatitis [22], but also reduce the degeneration and necrosis of liver cells, and improve liver tissue damage [23]. It can be seen that it is better than challenge and detoxification. Cimicifuga total phenolic acid can significantly reduce HepG2-2.2.15 [24]. ADCX, a natural naphthenic triterpenoid compound in Cimicifugae Rhizoma, inhibits autophagy degradation in multidrug-resistant liver cancer HepG2/ADM cells [25]. Puerarin injection can improve liver function and regulate the immune function of CHB patients, which is beneficial to the treatment of CHB [26]. Water extract of Pueraria Lobata could also inhibit HRSV-induced plaque formation [27]. Therefore, SMGGT is presumed to have a wider antiviral spectrum. Although this study chose Shengma-Gegen drug pair for anti-hepatitis B network pharmacology research, however, licorice and its main ingredient glycyrrhizin in SMGGT have obvious antiviral activity and cannot be ignored [28–30].

Conclusions

The mechanism of Shengma-Gegen drug pair function on hepatitis B was systematically studied based on network pharmacology strategy with experimental verification. Shengma-Gegen drug pair may have anti-tumor activities, which need to be tested in the future. Some other viral diseases and bacterial diseases may also be treated with Shengma-Gegen drug pair.

Abbreviations

ALT: alanine transaminase; **AST**: [aspartate transaminase](#); **CCK-8**: Cell Counting Kit-8; **CHB**: Chronic hepatitis B; **CTD**: Comparative Toxicogenomics Database; **DC**: Degree Centrality; **DL**: Drug:like:ness; **DMEM**: Dulbecco's Modified Eagle Media; **DMSO**: Dimethyl sulfoxide; **ETV**: entecavir; **GO**: Gene ontology; **FBS**: Fetal bovine serum; **HBV**: hepatitis B virus; **KEGG**: Kyoto Encyclopedia of Genes and Genomes; **OB**: Oral bioavailability; **PPAR-c**: Peroxisome proliferator activated-receptor c; **PPI**: Protein:protein interaction; **SMGGT**: Sheng-Ma-Ge-Gen-Tang; **TCM**: Traditional Chinese medicine; **TCMSP**: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

Project supported by the Research Fund for the National Natural Science Foundation of China Youth Fund Project, China (No. 81702422); Project supported by the 2018 Jiangsu Commission of Health foundation from Jiangsu province, China (No. Z2017017).

Authors Contribution

Guozhe Zhang conceived, designed the experiments, analyzed the data and wrote the paper. Chunmiao Chen and Yiyu Qin helped with the data analysis. Jianwei Ji contributed reagents, materials on the anti-HBV experiments. All authors approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

Figure 2

Venn of drug and disease targets.

Figure 3

PPI network of 71 drug-disease targets in the STRING.

Figure 4

Mythological gene ontology terms for candidate targets of hepatitis B. The top 20 GO function categories were selected.

Figure 5

Clusters of drug-disease targets by MCODE.

Figure 6

The bubble diagram of KEGG analysis of shengma and gegen on hepatitis B targets. Size of the spot represents the count number of genes and color represents p adjust value.

Figure 7

Network of Shengma Gegen candidate targets and enriched KEGG pathway of Hepatitis B. Top 20 pathways with significant changes of p-value were shown. The size of the dot represents the degree value, and the transparency represents the p-value of the pathway.

Figure 8

Cytotoxic activity result of Shengma and Gegen on HepG2.2.15 cell (at 60, 125, 250, and 500 µg/mL). Control- DMSO, SH-Shengma aqueous, SC-Shengma ethanol, GH-Gegen aqueous, GC-Gegen ethanol extract, SGH-Shengma-Gegen aqueous, SGC-Shengma-Gegen ethanol extract.

Figure 9

Anti-HBV activity results of Shengma and Gegen at 250 $\mu\text{g/ml}$, DMSO-negative control, ETV (50 $\mu\text{g/ml}$)-positive control, SH-Shengma aqueous, SC-Shengma ethanol, GH-Gegen aqueous, GC-Gegen ethanol extract, SGH-Shengma-Gegen aqueous, SGC-Shengma-Gegen ethanol extract. Results are expressed as the mean $\pm$ SD (n = 3). * p < 0.0332, ** p < 0.0021, *** p < 0.0002, ****p < 0.0001 compared with ETV group.

- [TableS4.xlsx](#)
- [TableS3.xlsx](#)
- [TableS2.xlsx](#)
- [TableS1.xlsx](#)