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Clinical efficacy and security of Glycyrrhizic acid preparation combined with conventional therapy in the treatment of COVID-19 with liver injury

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Clinical efficacy and security of Glycyrrhizic acid preparation combined with conventional therapy in the treatment of COVID-19 with liver injury
a protocol of systematic review and meta-analysis

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ABSTRACT

Introduction COVID-19 is a highly infectious acute pneumonia. GAP has been found to have hepatoprotective and antiviral effects, but there is no supporting evidence on efficacy and security for patients with COVID-19.

Methods and analysis The systematic review methods will be defined by PRISMA guidelines. A comprehensive electronic will be conducted with the search of Web of Science, PubMed, Ovid web, China National Knowledge Infrastructure(CNKI), Chinese Scientific and Journal Database (VIP) Database, Wanfang Database, and grey literature search, and manual search will be conducted to search literatures of randomized controlled trials, single-arm trials and retrospective studies about GAP combined with conventional therapy in the treatment of COVID-19 with liver injury from December 1, 2019 to July 1, 2021. There is no time of publication limitations and language will be restricted to Chinese and English. Retrieved studies will be independently screened by two researchers and relevant data will be extracted from studies. Interstudy heterogeneity will be assessed using the *I*² statistic and explored through meta regressions and subgroup analyses. Depending on data availability, we plan to conduct subgroup analyses by study population, geographical region and other selected clinical variables of interest. Quality assessment of the studies will be performed. Cochrane Handbook for Systematic Reviews of Interventions will be used to assess the risk of bias, and GRADE will be used to access the confidence in cumulative evidence.

Ethics and dissemination Ethical approval will not be required, for no primary data of individual patients will be collected. The final report will be shared with the scientific community through publication in a peer-reviewed journal, as well as with key stakeholders, including patients, healthcare professionals, and those working on COVID-19 research.

Strengths and limitations of this study We adopted strict inclusion and exclusion criteria and applied a recognized tool to evaluate the quality of the included studies. These ensured, to some extent, that our review could serve as an up-to-date and comprehensive summary of the published evidence on the topic of treatment for COVID-19 hepatocellular injury patients. Given the different measures of outcomes adopted in these studies, it was dimensional and interventional. Several limitations of this systematic review and meta-analysis should be mentioned below, yet. First, some of the negative results may not have been published and excluded, as a result, publishing bias is not applicable. Failure to report details of design methodology is also a potential source of increased heterogeneity in the included studies. Second, the sample size might not be large enough to estimate the accurate efficacy which there exists a problem that it is probably impossible to evaluate the therapeutic effect in multiple dimensions. Last, generally, nearly all the studies included focused on short-term outcomes only, and follow-up duration with long term was reported merely. So, further prognosis cannot be determined without adequate information.

Registration PROSPERO registration number(CRD42021234647)

Abbreviations GAP = glycyrrhizic acid preparation, COVID-19 = coronavirus disease 2019, CNKI = China National Knowledge Infrastructure, CBM=Chinese Biomedical Database, WHO=World Health Organization.

Keywords Glycyrrhizic acid preparation; COVID-19; systematic review and meta-analysis

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INTRODUCTION

Since the first outbreak of COVID-19 in Wuhan, China at the end of December 2019, the virus has rapidly spread around the world. More than 1 billion(112,902,746) people have been confirmed and over 2 million(2,508,679) have died as of 27 February 2021.[1] COVID-19 is a highly infectious acute pneumonia. Although the COVID-19 is controlled within China, it has been affecting other countries around the world since February 25, 2021, especially the United States, Iran, and Brazil. The WHO has declared the COVID-19 a global pandemic, moreover, Europe and the United States are now at the center of the pandemic.[2] Up to now, there are no effective pharmacological treatment regimens for COVID-19. It is urgently demanded safe and efficient drugs to treat the pandemic. Antiviral therapy becomes a routine treatment for COVID-19, however, these drugs can damage liver function of COVID-19 patients. In addition, although COVID-19 is highlighted by atypical pneumonia, some patients may present with abnormal liver function, which suggests that the disease can be with liver injury.[3-5] Therefore, COVID-19 patients are often combined with impairment of liver function due to the application of conventional antiviral drug therapy.

GAP (e.g. monopotassium glycyrrhizinate, monoammonium glycyrrhizinate, compoundammonium glycyrrhizinate, compound glycyrrhizin, etc.) has long been found to have hepatoprotective effects and applied in the treatment of liver disease. In Japan, glycyrrhizic acid has been used for more than 40 years as treatment for liver diseases, in particular to treat chronic hepatitis.[6] It is an efficient hepatoprotective medication in patients with chronic hepatitis C [7] and more broadly to protect from a variety of hepatic diseases such as chronic viral hepatitis and drug or chemical-induced liver injury.[8] The drug is considered as medicine with a good safety and economical profile. It is increasingly using clinically. With certain pharmacological activities, glycyrrhetic acid (C₃₀H₄₆O₄, MW = 470.7) is main components in Glycyrrhizae Radix et Rhizome (Gan Cao). It can be against different viruses, including human and animal coronaviruses like SARS, and it has been shown to be an effective immunoactive anti-inflammatory drug [9]. Glycyrrhizic acid extract can selectively inhibit the synthesis of single negative RNA encapsulated viral protein [10], and some derivatives of glycyrrhizic acid have strong anti-SARS-CoV activity with fewer adverse reactions[11-12]. Glycyrrhetic acid is a non-hemolytic saponin which displays both cytoplasmic and membrane effects and an amphiphilic compound like a saponin could interfere with the virus entry into cells, owing to the well-known membrane effects of this class of compounds[13]. Glycyrrhizae Radix et Rhizome appears as one of the highest frequencies in *the Pneumonia Treatment Protocol for Novel Coronavirus Infection* and in the recommended Chinese herbal compound[14]. Hong Ding's study[15] reported that glycyrrhetic acid and its derivatives as potential alternative medicine to relieve symptoms in nonhospitalized COVID-19 patients. Diammonium glycyrrhizinate, one of the derivative of glycyrrhetic acid, can be metabolized into glycyrrhetic acid. With the similar chemical structure to corticosteroid, its functions just like glucocorticoid, helping provide immune system against cytokine storm and reduce inflammation, although might be less stringent than steroids[16]. It was reported that the active compounds of Glycyrrhizae Radix et Rhizome possess antiviral, antimicrobial, anti-inflammatory, and immunoregulatory functions, which can protect neuro, alimentary, respiratory, endocrine and cardiovascular systems[17]. Molecular docking technology has been used to screen ACE2 binding compounds in traditional Chinese medicine, and the results showed that glycyrrhetic acid can bind to ACE2, suggesting that glycyrrhetic acid is a potential anti-COVID-19 compound[18]. It was reported that glycyrrhizin and 18 β -glycyrrhetic acid can suppress the activation of proinflammatory cytokine cyclooxygenase-2 (COX-2), IL-6, IL-10, TGF-β ,and ABCA1[19-26] Additionally, glycyrrhizin, 18 β -glycyrrhetic acid, and licochalcone A presented immunoregulatory activity[27-32]. Glycyrrhizin revealed a fine immune stimulant and

antiviral effect against duck hepatitis virus (DHV) [33]. A combination of glutamyl-tryptophan and glycyrrhizin exerted a protective effect in reducing the death of H3N2 IAV-infected mice[34]. Studies have shown that these compounds can protect mice against disseminated candidiasis both in vivo and in clinic [35], relieve experimental autoimmune encephalomyelitis in mice[36], increase the endpoint serum antibody titers, and perform as an immune stimulant against DHV[37]. All these reports affirmed the antiviral effect and immunoregulatory activity of Glycyrrhizae Radix et Rhizome.

Therefore, the use of GAP is expected to improve the clinical efficacy in the treatment of COVID-19 with liver injury. The aim of this study is to provide a systematic review of clinical efficacy and security of GAP combined with conventional therapy in the treatment of COVID-19 with liver injury.

METHODS

The systematic review will be performed in accordance with the guideline of Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA- P) 2015[38]. The results of the review will be reported according to the recommendation of PRISMA[39]. The study has been registered on PROSPERO (CRD42021234647).

Inclusion criteria

Types of studies

We will include randomized controlled trials (RCTs) of GAP for COVID-19 with liver injury in the treatment groups. If multi-arm RCTs included, we will select the group which used GAP and another without GAP for analysis. In addition, single-arm trials and retrospective studies also will be included in this systematic review. Studies' language of English and Chinese will be included.

Types of participants

Patients suffering COVID-19 with liver injury (>18 years old) will be included. The conformation of COVID-19 is that the SARS-CoV-2 will be detected by real-time reverse transcription PCR [40]. Liver injury will be diagnosed by abnormal liver function tests [41]. We defined ALT and/or AST over 3× the upper limit unit of normal (ULN), ALP, GGT, and/or TBIL over 2× ULN as liver injury. Participants of any sex and ethnicity will be enrolled.

Types of interventions

The treatment group using GAP combined with conventional therapy, while the control group receiving treatment of conventional therapy combined with other liver-protective drugs, oral medication, acupuncture, Chinese herbal medication, etc., or even with no treatment will be included. There will be no restrictions on the types, dosage forms, doses, and methods of use of GAP.

Types of outcome measures

Primary outcome

Liver function will be tested with serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Improvement of clinical symptoms will be evaluated by TCM symptom scores or chest CT.

Secondary outcome

Mortality rate will be defined as the percentage of the death toll to total number after treatment. Blood test will be evaluated by CRP, PCT, and WBC count.

Safety outcome

Adverse events will be observed by liver and kidney function, blood test and clinical symptoms.

Exclusion criteria

1)GAP was not only in the experimental group but also in the control group. 2)The data cannot be extracted, and the correct data cannot be obtained by contacting authors and data calculation. 3)Repeatedly published studies, literature reviews, meta-analyses, case reports, etc.

Search Strategy

Electronic Searches

Main information resource databases, including PubMed, Web of Science, Ovidweb, China National Knowledge Infrastructure (CNKI), Chinese Biomedical Database(CBM), VIP Database, and Wanfang database. The search time limit is from the date of December 1, 2019 to July 1, 2021. The search terms are 'glycyrrh*', 'COVID-19 combined with liver injury', 'clinical efficacy', etc. The language is limited to Chinese and English. The detail search strategy of Web of Science is listed in table 1, and the search strategy will be modified according to other different databases.

4 #3 AND #2 AND #1

3 TS=(randomised controlled trial OR controlled clinical trial OR randomized OR single-arm trial OR retrospective study OR placebo OR drug therapy OR randomly OR trial OR groups)

# 2	TS=(COVID-19 OR Novel Coronavirus Pneumonia OR 2019-nCoV OR SARS-CoV-2)
# 1	TS=(glycyrrh* OR glycyrrhizic acid OR monopotassium glycyrrhizinate OR monoammonium glycyrrhizinate OR compoundammonium glycyrrhizinate OR compound glycyrrhizin OR compoundmonoammonium glycyrrhizinate OR diammonium glycyrrhizinate OR glycyrrhizic acid preparation OR magnesium isoglycyrrhizinate)

Table 1 Search strategy of WOS

Other search strategies

Manual search, including conference papers, searched literature references. In addition, preprinted website including *arXiv* (<http://arxiv.org/>), *BioRxiv* (<https://www.biorxiv.org/>), *F1000* (<https://f1000.com/>), and *PeerJ Preprints* (<https://peerj.com/preprints/>) will also be searched to find out more unpublished papers.

Data Collection and Analysis

Literature screening

Import all search results into Endnote X9 for classification and sorting, and duplicate studies will be excluded. Two researchers (X.T. and W.F.G.) will independently screen the titles and abstracts of the literatures, and screen the literatures based on the inclusion criteria. For any potential related research, we will download and read the full text. If there is disagreement during the screening process, discuss with the third researcher (Y.S.N.) to make a decision. A research flow chart will be drawn to show the whole process of research selection (Fig 1).

Data extraction

According to the inclusion criteria, the results of the included studies and all valuable information will be correctly extracted. Two researchers (X.T. and W.F.G.) will complete this work independently and review with each other. Data extraction includes 5 aspects, basic research information (e.g. title, journal, research ID number, author, and contact information, etc.), research method (e.g. research design, random unit, random method, etc.), observation object (e.g. age, gender, sample size, etc.), intervention measures (e.g. treatment course information, etc.), measurement indicators (e.g. measurement indicators and time points for judgment, judgment indicators, measurement units, etc.). If the data is missing, we will try to contact with the original author. If data is unavailable, we will exclude the study. Similarly, if the data is disputed, it will be solved by discussing with the third researcher (Y.S.N.). Once the extraction is completed, the 2 researchers (X.T. and W.F.G.) will check with each other to ensure the accuracy of the data.

Assessment of risk of bias in included studies

All the included studies will be evaluated based on the guidelines of Cochrane Handbook 5.2.0 for Systematic Reviews of Interventions [42-43]. Different risk of bias assessment tools will be used according to different types of studies. There are 7 domains, and each item should be judged as “low risk”, “high risk” and “unclear” deviations according to the quality classification standards. If there is a difference, it will be decided through collective consultation. The risk of bias of RCTs will be conducted using version 2 of the Cochrane risk-of-bias tool. The risk of bias in non-randomized studies of interventions tool will be used to assess the risk of bias of non-RCTs according to Cochrane Handbook.

Assessment of heterogeneity

The heterogeneity of the included studies is measured by Q -test and I^2 statistic. When I^2 is more than or equal to 50%, the heterogeneity will be considered large. For this reason, we will use a random effects model to analyze the data. If I^2 is less than 50%, it can be considered the included studies are homogenous, and the fixed effects model can be used to analyze the data.

Data synthesis

The meta-analysis will be performed using RevMan 5.3 software provided by the Cochrane Collaboration Center. Binary variables and continuous variables are included in the results of interest. If the statistical heterogeneity is low ($P>0.1$, or $I^2<50\%$), we will use the fixed- effect model to combine the data, while if the statistical heterogeneity is high ($P<0.1$, or $I^2>50\%$), we will use the random- effect model. However, if the heterogeneity level much significant, a descriptive analysis will be performed.

Subgroup analysis and investigation of heterogeneity

Subgroup analysis is to explore whether the results of the study are different due to the existence of some factors that may affect the prognosis. These factors include the dosage of conventional therapy, course of treatment, intervention measures, etc. If there are enough data, we will conduct subgroup analysis to explore the source of heterogeneity.

Sensitivity analysis

To determine the stability and reliability of the summary results, a sensitivity analysis will be performed. We will remove some studies of high risk of bias, or check up processing method of missing data.

Assessment of reporting bias

If more than 10 studies are included, we will make a funnel chart based on the data of the included studies to assess the deviation. When the funnel chart is asymmetrical, indicating that there may be

publication bias, we will discuss the source and explain the possible causes of the bias.

Grading the quality of evidence

In order to rate the quality of evidence, understand the actual situation of the evidence rating and analyze the possible problems, the Grading of Recommendations Assessment, Development and Evaluation[44] will be used to evaluate the evidence quality. According to the recommendations of the GRADE working group, the evaluation of the evidence quality of the key outcome indicators can be divided into 4 levels: high (+++), moderate (++), low (+), and very low (+).

Amendments

We will show all the amendments with detailed description and rationale in the amendments of this study.

Ethics and dissemination

Meta-analysis is an analysis of previous research data and uses data that existed in studies published, so this review doesn't require ethical approval. The results of this review will be published in peer-reviewed journals.

DISCUSSION

In view of the serious epidemiological situation of COVID-19, there is no effective treatment at present. One study[45] explored the possibilities for the treatment of COVID-19 by systematically reviewing evidence on the efficacy and safety of GAPs for SARS and MERS. Based on the available evidence related to GAPs for the treatment of SARS and MERS, Li H[45] postulated that compound glycyrrhizin could be an optional strategy of treatment for SARS-CoV-2 infections, especially those with complex liver injury. Recently, a study[46] found that glycyrrhizic acid showed significant inhibition towards a variety types of viruses and considered glycyrrhizic acid as a potential drug for the treatment of COVID-19. Therefore, there is a strong case for conducting this review. In this review, we aim to assess the available evidence on the clinical efficacy and safety of GAP for the treatment of COVID-19.

The results of this study may have valuable practical implications for patients, healthcare professionals, and those working on COVID-19 research. Our findings will be expected to provide validated clinical decision support for COVID-19. It can also be used to guide healthcare professionals in the treatment of COVID-19.

The results of this review will be published in a peer-reviewed journal, and we believe the results will benefit clinicians, patients and guideline makers.

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Competing interests None declared.

Patient and Public Involvement No patient involved.

Figure Legend Figure 1. Flow diagram of study selection

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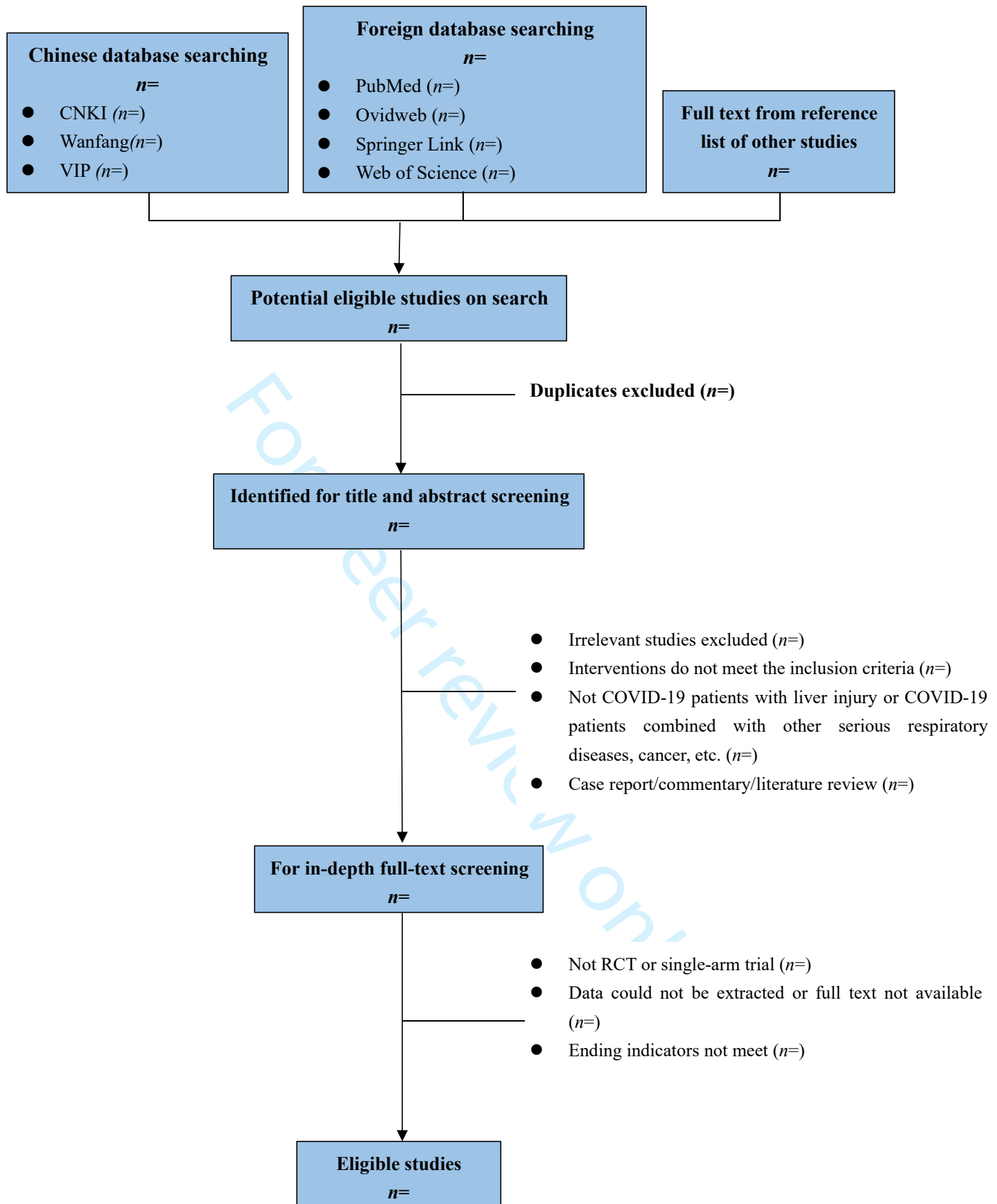
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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item
ADMINISTRATIVE INFORMATION		
Title:		
Identification	1a	Identify the report as a protocol of a systematic review
Update	1b	If the protocol is for an update of a previous systematic review, identify as such
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number
Authors:		
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments
Support:		
Sources	5a	Indicate sources of financial or other support for the review
Sponsor	5b	Provide name for the review funder and/or sponsor
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol
INTRODUCTION		
Rationale	6	Describe the rationale for the review in the context of what is already known
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)
METHODS		
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated
Study records:		
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review

Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms done independently, in duplicate), any processes for obtaining and confirming data from investigators
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2 , Kendall's τ)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)

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Clinical efficacy and security of glycyrrhizic acid preparation in the treatment of anti-SARS-CoV-2 drug-induced liver injury: a protocol of systematic review and meta-analysis

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ABSTRACT

Introduction COVID-19 is a highly infectious acute pneumonia. GAP has been found to have hepatoprotective and antiviral effects, but there is no supporting evidence on efficacy and security for patients with COVID-19.

Methods and analysis The systematic review methods will be defined by PRISMA guidelines. This study will start in July 1, 2021 and end in October 31, 2021. A comprehensive electronic will be conducted with the search of Web of Science, PubMed, Ovid web, China National Knowledge Infrastructure(CNKI), Chinese Scientific and Journal Database (VIP) Database, Wanfang Database, and grey literature search, and manual search will be conducted to search literatures of randomized controlled trials, single-arm trials and retrospective studies about GAP combined with conventional therapy in the treatment of anti-SARS-CoV-2 drug-induced liver injury from December 1, 2019 to July 1, 2021. There is no time of publication limitations and language will be restricted to Chinese and English. Retrieved studies will be independently screened by two researchers and relevant data will be extracted from studies. Interstudy heterogeneity will be assessed using the I^2 statistic and explored through meta regressions and subgroup analyses. Depending on data availability, we plan to conduct subgroup analyses by study population, geographical region and other selected clinical variables of interest. Quality assessment of the studies will be performed. Cochrane Handbook for Systematic Reviews of Interventions will be used to assess the risk of bias, and GRADE will be used to access the confidence in cumulative evidence.

Ethics and dissemination Ethical approval will not be required, for no primary data of individual patients will be collected. The final report will be shared with the scientific community through publication in a peer-reviewed journal, as well as with key stakeholders, including patients, healthcare professionals, and those working on COVID-19 research.

Registration PROSPERO registration number(CRD42021234647)

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STRENGTHS AND LIMITATIONS

- We adopted strict inclusion and exclusion criteria and applied a recognized tool to evaluate the quality of the included studies. These ensured, to some extent, that our review could serve as an up-to-date and comprehensive summary of the published evidence on the topic of treatment for COVID-19 hepatocellular injury patients. Given the different measures of outcomes adopted in these studies, it was dimensional and interventional.
- Some of the negative results may not have been published and excluded, as a result, publishing bias is not applicable. Failure to report details of design methodology is also a potential source of increased heterogeneity in the included studies.
- The sample size might not be large enough to estimate the accurate efficacy which there exists a problem that it is probably impossible to evaluate the therapeutic effect in multiple dimensions.
- Nearly all the studies included focused on short-term outcomes only, and follow-up duration with long term was reported merely. So, further prognosis cannot be determined without adequate information.

Abbreviations GAP = glycyrrhizic acid preparation, COVID-19 = coronavirus disease 2019, SARS-CoV-2 = severe acute respiratory syndrome - coronavirus - 2, CNKI = China National Knowledge Infrastructure, CBM=Chinese Biomedical Database, WHO =World Health Organization.

Keywords Glycyrrhizic acid preparation; COVID-19; protocol of systematic review and meta-analysis

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INTRODUCTION

Since the outbreak of COVID-19 reported in Wuhan, China at the end of December 2019, the virus has rapidly spread around the world. More than 1 billion (165,772,430) people have been confirmed and over 3 million (3,437,545) have died as of 27 May 2021. COVID-19 is a highly infectious acute pneumonia. Although the COVID-19 is controlled within China, it has been affecting other countries around the world since February 25, 2021, especially the United States of America, India and Brazil.[1] The WHO has declared the COVID-19 a global pandemic, moreover, Europe and the United States are now at the center of the pandemic.[2] Up to now, some countries have developed a variety of vaccines and anti-COVID-19 drugs, and some of them have entered the clinical observation stage. It found that some vaccines and anti-COVID-19 drugs are effective against COVID-19 after clinical studies, but the efficacy and safety are still to be improved. So, it is urgently demanded safe and efficient vaccines and anti-COVID-19 drugs to fight the pandemic.

Antiviral therapy becomes a routine treatment for COVID-19, however, these drugs can damage liver function of COVID-19 patients. In addition, although COVID-19 is highlighted by atypical pneumonia, some patients may present with abnormal liver function, which suggests that the disease can be with liver injury.[3-5] Therefore, COVID-19 patients are often combined with impairment of liver function due to the application of conventional antivirals therapy.

GAP (e.g. monopotassium glycyrrhizinate, monoammonium glycyrrhizinate, compoundammonium glycyrrhizinate, compound glycyrrhizin, etc.) has long been found to have hepatoprotective effects and applied in the treatment of liver disease. In Japan, glycyrrhizic acid has been used for more than 40 years as treatment for liver diseases, in particular to treat chronic hepatitis.[6] It is an efficient hepatoprotective medication in patients with chronic hepatitis C [7] and more broadly to protect from a variety of hepatic diseases such as chronic viral hepatitis and drug or chemical-induced liver injury.[8] The drug is considered as medicine with a good safety and economical profile. It is increasingly using clinically. With certain pharmacological activities, glycyrrhetic acid (C₃₀H₄₆O₄, MW = 470.7) is main components in Glycyrrhizae Radix et

Rhizome (Gan Cao). It can be against different viruses, including human and animal coronaviruses like SARS, and it has been shown to be an effective immunoactive anti-inflammatory drug [9]. Glycyrrhizic acid extract can selectively inhibit the synthesis of single negative RNA encapsulated viral protein [10], and some derivatives of glycyrrhizic acid have strong anti-SARS-CoV activity with fewer adverse reactions [11-12]. Glycyrrhetic acid is a non-hemolytic saponin which displays both cytoplasmic and membrane effects and an amphiphilic compound like a saponin could interfere with the virus entry into cells, owing to the well-known membrane effects of this class of compounds [13]. Glycyrrhizae Radix et Rhizome appears as one of the highest frequencies in the *Pneumonia Treatment Protocol for Novel Coronavirus Infection* and in the recommended Chinese herbal compound [14]. Hong Ding's study [15] reported that glycyrrhetic acid and its derivatives as potential alternative medicine to relieve symptoms in nonhospitalized COVID-19 patients. Diammonium glycyrrhizinate, one of the derivative of glycyrrhetic acid, can be metabolized into glycyrrhetic acid. With the similar chemical structure to corticosteroid, its functions just like glucocorticoid, helping provide immune system against cytokine storm and reduce inflammation, although might be less stringent than steroids [16]. It was reported that the active compounds of Glycyrrhizae Radix et Rhizome possess antiviral, antimicrobial, anti-inflammatory, and immunoregulatory functions, which can protect neuro, alimentary, respiratory, endocrine and cardiovascular systems [17]. Molecular docking technology has been used to screen ACE2 binding compounds in traditional Chinese medicine, and the results showed that glycyrrhetic acid can bind to ACE2, suggesting that glycyrrhetic acid is a potential anti-SARS-CoV-2 compound [18]. Seshu Vardhan found that the GAP is a potential phytochemical against COVID-19 by a good deal of computation work, because glycyrrhizic acid binds at the active site of all the five protein targets of SARS-CoV-2. [19] It was reported that glycyrrhizin and 18 β -glycyrrhetic acid can suppress the activation of proinflammatory cytokine cyclooxygenase-2 (COX-2), IL-6, IL-10, TGF- β , and ABCA1 [20-27]. Additionally, glycyrrhizin, 18 β -glycyrrhetic acid, and licochalcone A presented immunoregulatory activity [28-33]. Glycyrrhizin revealed a fine immune stimulant and antiviral effect against duck hepatitis virus (DHV) [34]. A combination of glutamyl-tryptophan and glycyrrhizin exerted a protective effect in reducing the death of H3N2 IAV-infected mice [35]. Studies have shown that these compounds can protect mice against disseminated candidiasis both in vivo and in clinic [36], relieve experimental autoimmune encephalomyelitis in mice [37], increase the endpoint serum antibody titers, and perform as an immune stimulant against DHV [38]. All of these reports affirmed the antiviral effect and immunoregulatory activity of Glycyrrhizae Radix et Rhizome.

Therefore, GAP is expected to be an alternative or complementary drug to anti-SARS-CoV-2 drug and improve anti-SARS-CoV-2 drug-induced liver injury. The aim of this study is to provide a systematic review of clinical efficacy and security of GAP in the treatment of anti-SARS-CoV-2 drug-induced liver injury.

METHODS

The systematic review will be performed in accordance with the guideline of Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA- P) 2015 [39]. The results of the review will be reported according to the recommendation of PRISMA [40]. The study has been registered on PROSPERO (CRD42021234647), and will start in July 1, 2021 and end in October 31, 2021.

Inclusion criteria

Types of studies

We will include randomized controlled trials (RCTs) of GAP for anti-SARS-CoV-2 drug-induced liver injury in the experimental groups. If multi-arm RCTs included, we will select the group which used GAP and another without GAP for analysis. In addition, single-arm trials and retrospective studies also will be included in this systematic review. Studies' language of English and Chinese will be included.

Types of participants

Patients suffering anti-SARS-CoV-2 drug-induced liver injury with COVID-19 will be included. Because the population is generally susceptible to SARS-CoV-2, there is no restriction on the age of patients with COVID-19. The conformation of COVID-19 is that the SARS-CoV-2 will be detected by real-time reverse transcription PCR [41]. Liver injury will be diagnosed by abnormal liver function tests [42]. We defined ALT and/or AST over 3 \times the upper limit unit of normal (ULN), ALP, GGT, and/or TBIL over 2 \times ULN as liver injury. Participants of any sex and ethnicity will be enrolled.

Types of interventions

The experimental group using GAP alone or combined with conventional therapy, while the control group receiving conventional therapy will be included. There will be no restrictions on the types, dosage forms, doses, and methods of use of GAP.

Types of outcome measures

Primary outcome

Liver function will be tested with serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Length of stay will be evaluated by days of hospitalization.

Secondary outcome

1 Mortality rate will be defined as the percentage of the deaths to total number after treatment. Blood test will be evaluated by CRP, PCT, and WBC count.

2

3 **Safety outcome**

4 Incidence of adverse reactions will be observed by kidney function, bilirubin level, gastrointestinal symptoms(e.g. nausea, vomiting, abdominal pain, diarrhea) and rash.

6

7 **Exclusion criteria**

8 1) GAP was not only in the experimental group but also in the control group. 2) The patients with pre-existing liver disease (e.g., cirrhosis, hepatocellular carcinoma,
9 non-alcoholic fatty liver disease, autoimmune liver diseases or liver transplant). 3) Liver injury resulting from toxicity from commonly used drugs. 4) The data cannot be
10 extracted, and the correct data cannot be obtained by contacting authors and data calculation. 5) Repeatedly published studies, literature reviews, meta-analyses, case reports,
11 etc.

14

15 **Search Strategy**

16 **Electronic Searches**

17 Main information resource databases, including PubMed, Web of Science, Ovidweb, China National Knowledge Infrastructure (CNKI), Chinese Biomedical
18 Database(CBM), VIP Database, and Wanfang database. The search time limit is from the date of December 1, 2019 to July 1, 2021. The search terms are ‘glycyrrh*’,
19 ‘COVID-19 combined with liver injury’, ‘clinical efficacy’, etc. The language is limited to Chinese and English. The detail search strategy of Web of Science is listed in table
20 1, and the search strategy will be modified according to other different databases.

# 4	#3 AND #2 AND #1
# 3	TS=(randomised controlled trial OR controlled clinical trial OR randomized OR single-arm trial OR retrospective study OR placebo OR drug therapy OR randomly OR trial OR groups)
# 2	TS=(COVID-19 OR Novel Coronavirus Pneumonia OR 2019-nCoV OR SARS-CoV-2)
# 1	TS=(glycyrrh* OR glycyrrhizic acid OR monopotassium glycyrrhizinate OR monoammonium glycyrrhizinate OR compoundammonium glycyrrhizinate OR compound glycyrrhizin OR compoundmonoammonium glycyrrhizinate OR diammonium glycyrrhizinate OR glycyrrhizic acid preparation OR magnesium isoglycyrrhizinate)

35 Table 1 Search strategy of WOS

36 **Other search strategies**

37 Manual search, including conference papers, searched literature references. In addition, preprinted website including *arXiv* (<http://arxiv.org/>), *BioRxiv*
38 (<https://www.biorxiv.org/>), *F1000* (<https://f1000.com/>), and *PeerJ Preprints* (<https://peerj.com/preprints/>) will also be searched to find out more unpublished papers.

41 **Data Collection and Analysis**

42 **Literature screening**

43 Import all search results into Endnote X9 for classification and sorting, and duplicate studies will be excluded. Two researchers (X.T. and W.F.G.) will independently screen
44 the titles and abstracts of the literatures, and screen the literatures based on the inclusion criteria. For any potential related research, we will download and read the full text. If
45 there is disagreement during the screening process, discuss with the third researcher (Y.S.N.) to make a decision. A research flow chart will be drawn to show the whole
46 process of research selection (Fig 1).

49

50 **Data extraction**

51 According to the inclusion criteria, the results of the included studies and all valuable information will be correctly extracted. Two researchers (X.T. and W.F.G.) will
52 complete this work independently and review with each other. Data extraction includes 5 aspects, basic research information (e.g. title, journal, research ID number, author,
53 and contact information, etc.), research method (e.g. research design, random unit, random method, etc.), observation object (e.g. age, gender, sample size, etc.), intervention
54 measures (e.g. treatment course information, etc.), measurement indicators (e.g. measurement indicators and time points for judgment, judgment indicators, measurement
55 units, etc.). If the data is missing, we will try to contact with the original author. If data is unavailable, we will exclude the study. Similarly, if the data is disputed, it will be
56 solved by discussing with the third researcher (Y.S.N.). Once the extraction is completed, the 2 researchers (X.T. and W.F.G.) will check with each other to ensure the
57 accuracy of the data.

59

60 **Assessment of risk of bias in included studies**

All the included studies will be evaluated based on the guidelines of Cochrane Handbook 5.2.0 for Systematic Reviews of Interventions [43-44]. Different risk of bias assessment tools will be used according to different types of studies. There are 7 domains, and each item should be judged as “low risk”, “high risk” and “unclear” deviations according to the quality classification standards. If there is a difference, it will be decided through collective consultation. The risk of bias of RCTs will be conducted using version 2 of the Cochrane risk-of-bias tool. The risk of bias in non-randomized studies of interventions tool will be used to assess the risk of bias of non-RCTs according to Cochrane Handbook.

Assessment of heterogeneity

The heterogeneity of the included studies is measured by Q -test and I^2 statistic. When I^2 is more than or equal to 50%, the heterogeneity will be considered large. For this reason, we will use a random effects model to analyze the data. If I^2 is less than 50%, it can be considered the included studies are homogenous, and the fixed effects model can be used to analyze the data.

Data synthesis

The meta-analysis will be performed using RevMan 5.3 software provided by the Cochrane Collaboration Center. Binary variables and continuous variables are included in the results of interest. If the statistical heterogeneity is low ($P>0.1$, or $I^2<50\%$), we will use the fixed- effect model to combine the data, while if the statistical heterogeneity is high ($P<0.1$, or $I^2>50\%$), we will use the random- effect model. However, if the heterogeneity level much significant, a descriptive analysis will be performed.

Subgroup analysis and investigation of heterogeneity

Subgroup analysis is to explore whether the results of the study are different due to the existence of some factors that may affect the prognosis. These factors include the dosage of conventional therapy, course of treatment, intervention measures, etc. If there are enough data, we will conduct subgroup analysis to explore the source of heterogeneity.

Sensitivity analysis

To determine the stability and reliability of the summary results, a sensitivity analysis will be performed. We will remove some studies of high risk of bias, or check up processing method of missing data.

Assessment of reporting bias

If more than 10 studies are included, we will make a funnel chart based on the data of the included studies to assess the deviation. When the funnel chart is asymmetrical, indicating that there may be publication bias, we will discuss the source and explain the possible causes of the bias.

Grading the quality of evidence

In order to rate the quality of evidence, understand the actual situation of the evidence rating and analyze the possible problems, the Grading of Recommendations Assessment, Development and Evaluation[45] will be used to evaluate the evidence quality. According to the recommendations of the GRADE working group, the evaluation of the evidence quality of the key outcome indicators can be divided into 4 levels: high (+++), moderate (++), low (+), and very low (+).

Amendments

We will show all the amendments with detailed description and rationale in the amendments of this study.

Ethics and dissemination

Meta-analysis is an analysis of previous research data and uses data that existed in studies published, so this review doesn't require ethical approval. The results of this review will be published in peer-reviewed journals.

DISCUSSION

COVID-19 is in the global pandemic. The vaccine is the key to the prevention and control of this disease. Current clinical studies confirmed that the vaccine is safe and effective with few adverse reactions, but the vaccine can only reduce the risk of SARS-CoV-2 infection. There is insufficient clinical evidence to confirm that anti-SARS-CoV-2 drugs(e.g. alpha-interferon, lopinavir/ritonavir, ribavirin, and chloroquine phosphate) are effective in suppressing the virus, which also cause a variety of adverse effects such as diarrhea, elevated transaminases, and skin rash.[46-48] One study consolidated and reviewed the available clinical and preclinical relevant results, showing mixed results in terms of efficacy within the framework of current clinical protocols.[49] Therefore, it is necessary to find an effective and safe scheme for the treatment of anti-SARS-CoV-2 drug-induced liver injury.

One study explored the possibilities for the treatment of COVID-19 by systematically reviewing evidence on the efficacy and safety of GAPs for SARS and MERS [50].

Based on the available evidence related to GAPS for the treatment of SARS and MERS, Li H postulated that compound glycyrrhizin could be an optional strategy of treatment for SARS-CoV-2 infections, especially those with complex liver injury [50]. Recently, a study found that glycyrrhizic acid showed significant inhibition towards a variety types of viruses and considered glycyrrhizic acid as a potential drug for the treatment of COVID-19 [51]. Therefore, there is a strong case for conducting this review. In this review, we aim to assess the available evidence on the clinical efficacy and safety of GAP for the treatment of anti-SARS-CoV-2 drug-induced liver injury.

The results of this study may have valuable practical implications for patients, healthcare professionals, and those working on COVID-19 research. Our findings will be expected to provide validated clinical decision support for COVID-19. It can also be used to guide healthcare professionals in the treatment of COVID-19.

The results of this review will be published in a peer-reviewed journal, and we believe the results will benefit clinicians, patients and guideline makers.

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Competing interests None declared.

Patient and Public Involvement No patient involved.

Figure Legend Figure 1. Flow diagram of study selection

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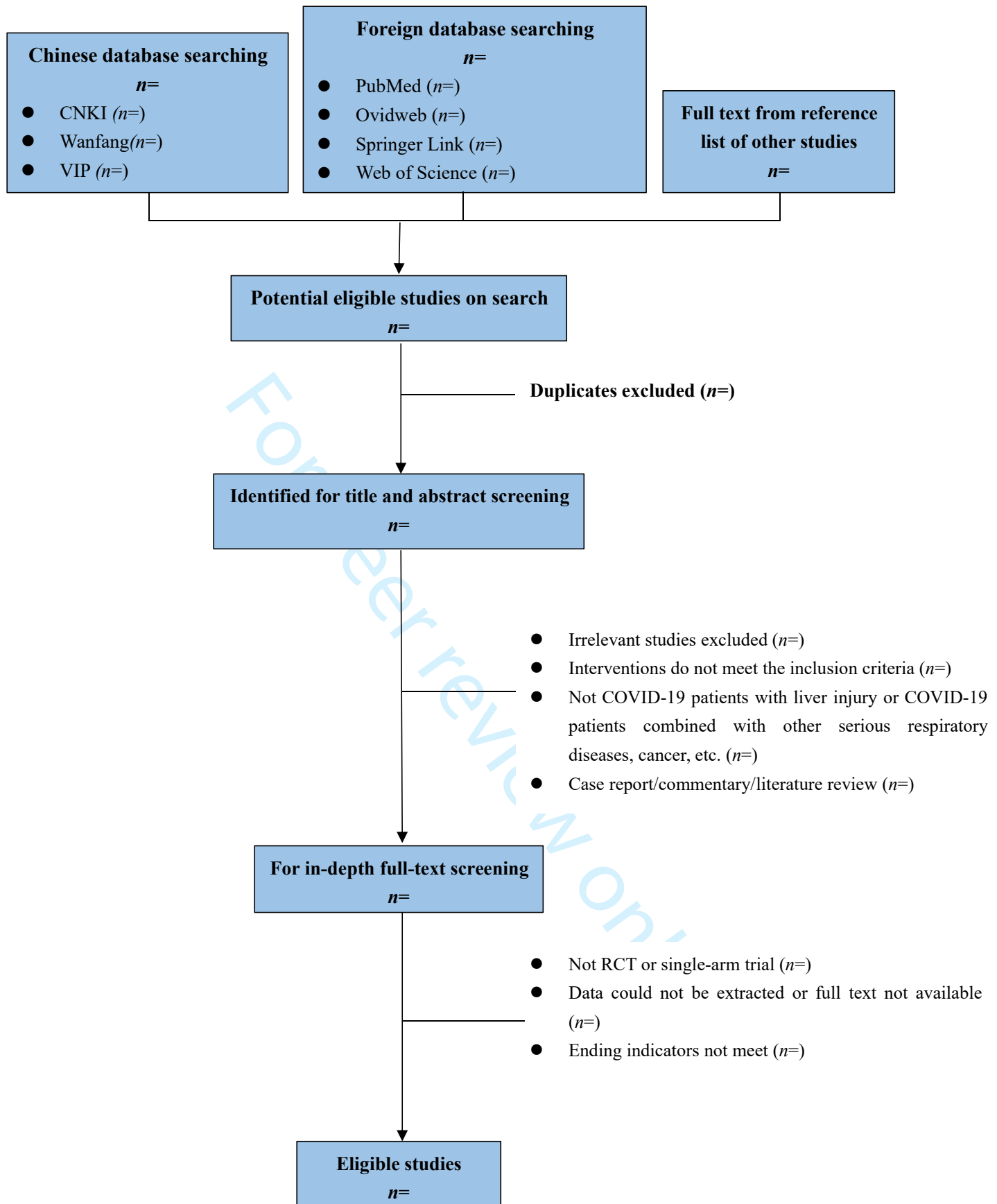
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PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item
ADMINISTRATIVE INFORMATION		
Title:		
Identification	1a	Identify the report as a protocol of a systematic review
Update	1b	If the protocol is for an update of a previous systematic review, identify as such
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number
Authors:		
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments
Support:		
Sources	5a	Indicate sources of financial or other support for the review
Sponsor	5b	Provide name for the review funder and/or sponsor
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol
INTRODUCTION		
Rationale	6	Describe the rationale for the review in the context of what is already known
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)
METHODS		
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated
Study records:		
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review

Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms done independently, in duplicate), any processes for obtaining and confirming data from investigators
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2 , Kendall's τ)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

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