CLINICAL SAFETY OF NATURAL PRODUCTS, AN EVIDENCE-BASED APPROACH

EDITED BY: Mojtaba Heydari, Abdur Rauf, Muthu Thiruvengadam and Xiao Chen

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CLINICAL SAFETY OF NATURAL PRODUCTS, AN EVIDENCE-BASED APPROACH

Topic Editors:
Mojtaba Heydari, Shiraz University of Medical Sciences, Iran
Abdur Rauf, University of Swabi, Pakistan
Muthu Thiruvengadam, Konkuk University, South Korea
Xiao Chen, Second Military Medical University, China

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EDITED BY
Javier Echeverria,
University of Santiago, Chile

REVIEWED BY
Dâmaris Silveira,
University of Brasilia, Brazil

*CORRESPONDENCE
Mojtaba Heydari,
mheydari@sums.ac.ir

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Editorial: Clinical safety of natural products, an evidence-based approach

Mojtaba Heydari[1]*, Abdur Rauf[2], Muthu Thiruvengadam[3], Xiao Chen[4] and Mohammad Hashem Hashempur[5]

[1]Persian Medicine Network (PMN), Universal Scientific Education and Research Network (USERN), Shiraz University of Medical Sciences, Shiraz, Iran., [2]Department of Chemistry, University of Swabi, Swabi, Pakistan, [3]Department of Crop Science, College of Sanghuh Life Science, Konkuk University, Seoul, South Korea, [4]Department of Orthopedic Trauma, Shanghai Changhai Hospital, Second Military Medical University, Shanghai, China, [5]Research Center for Traditional Medicine and History of Medicine, Department of Persian Medicine, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

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natural products, herbal medicine, Persian medicine, safety, side effect, toxicity

Editorial on the Research Topic
Clinical safety of natural products, an evidence-based approach

There is increasing use of natural products including medicinal plants, phytopharmaceuticals, nutraceuticals, vitamins, and nutritional supplements for different health purposes worldwide (Mosavat et al., 2018). It is generally believed that these products are safe (Lynch and Berry, 2007). However, there is growing evidence of safety concerns associated with these natural products (Haq, 2004). Nevertheless, little is known about the adverse events associated with these products (Bent, 2008).

There are different concerns regarding the safety of natural products. The complexity in the nature of these formulations is one of the sources of concern (Capasso et al., 2003). Not only compound herbal formulations, but also simple herbal drugs have many biologically active ingredients which may have toxic effects. The interaction of the multiple herbs in a formulation and multiple ingredients in one herb with each other, make the safety evaluation more difficult than in chemical medicines. Besides the potential intrinsic toxicity of the natural products, extrinsic toxicity is another source of concern. For example, heavy metals are found at higher than standard levels in many herbal formulations on the market (Keshvari et al., 2021). Inaccurate identification of the used medicinal plants and their use in wrong clinical indications are other source of the safety issues. And the last point is the drug interactions between the natural products and chemical medicines taken by the patients (Ghosh et al., 2018).

Different methods have been popularly used to evaluate the safety of natural products. *In-vitro* and *in vivo* studies evaluating the toxicities of different cells and organs are among these methods. However, applying these data in clinical practice faces multiple limitations. Besides these traditional methods, different types of research including clinical safety

studies and pharmacovigilance-based investigations can help us to have an evidence-based approach to the safety of these products (Raoufinejad et al., 2020). Information presented as associated adverse events of these products gathered from pharmacovigilance systems needs to be analyzed by scientific methods to determine the significance and potential causal relationship. The identification of adverse events associated with the use of natural products is challenging due to different reasons including impurities, batch-to-batch variability, misidentification and/or labeling, and different source of used production materials. There are also concerns about the suitable working of classic reporting systems in gathering reports on adverse events on unregulated medicines and supplements including so-called borderline products.

By collecting articles on this theme, we tried to develop information about the safety of natural products to enhance their proper use in the general population. In this regard, we focused on clinical, and epidemiologic studies in this field. Studies published in this research topic focused on the safety of medicinal use of multiple medicinal plants (*Tripterygium wilfordii, Entada abyssinica,* and *Brucea javanica*) and nutritional supplements (coffee and cinnamon) as natural products. We need to expand the scientific data on the clinical safety of natural products help us with evidence-based decision making on their proper use.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SYSTEMATIC REVIEW
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Efficacy and Safety of Tripterygium Wilfordii Hook. F for Connective Tissue Disease-Associated Interstitial Lung Disease:A Systematic Review and Meta-Analysis

Yehui Li[1,2†], Wen Zhu[3†], Hailang He[1,2], Yordan Angelov Garov[3], Le Bai[1,2], Li Zhang[1,2], Jing Wang[1,2], Jinghai Wang[1,2] and Xianmei Zhou[1,2*]*

[1]Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, China, [2]Jiangsu Provincial Hospital of Chinese Medicine, Nanjing, China, [3]Nanjing University of Chinese Medicine, Nanjing, China

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Edited by:
Mojtaba Heydari,
Shiraz University of Medical Sciences, Iran

Reviewed by:
Jingbo Zhai,
Tianjin University of Traditional Chinese Medicine, China
Lanping Guo,
China Academy of Chinese Medical Sciences, China

***Correspondence:**
Xianmei Zhou
zhouxianmeijs@aliyun.com
Hailang He
lyghehailang@163.com

[†]These authors have contributed equally to this work

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Background: Tripterygium wilfordii Hook. F (TwHF), a Chinese herbal medicine used to treat CTD-ILD patients in China, has been previously found to have immunoinhibitory, antifibrotic and anti inflammatory effects. It has also shown good results in treating autoimmune and inflammatory diseases.

Objectives: This systematic review and meta-analysis aims to evaluate the efficacy and safety of TwHF for CTD-ILD.

Methods: A systematic search was performed on PubMed, Embase, Cochrane Library, Web of Science, PsycINFO, Scopus, CNKI, Wanfang, VIP, and CBM databases up to May 2021. Randomized controlled trials (RCTs) comparing TwHF plus conventional therapy versus conventional therapy alone were included. We followed the PRISMA checklist, and applied Cochrane handbook 5.1.0 and RevMan 5.3 for data analysis and quality evaluation of the included studies.

Results: Based on Cochrane handbook 5.1.0, nine RCTs consisting 650 patients met the inclusion/exclusion criteria and were selected for further analysis. The obtained data showed significant improvement in lung function with TwHF plus conventional treatment compared with conventional treatment (post-treatment FVC% (MD= 8.68, 95%CI (5.10, 12.26), $p < 0.00001$), FEV1% (MD = 11.24, 95%CI (6.87, 15.61), $p < 0.00001$), TLC% (MD = 5.28, 95%CI (0.69, 9.87), $p = 0.02$)], but no significant difference in the post-treatment DLCO% [(MD = 4.40, 95%CI (–2.29, 11.09), $p = 0.20$)]. Moreover, the data showed that TwHF combined with conventional treatment significantly reduced the HRCT integral of patients [MD = -0.65, 95% (-1.01, -0.30), $p = 0.0003$], the level of erythrocyte sedimentation rate (MD = –9.52, 95%CI (–11.55, –7.49), $p < 0.00001$), c-reactive protein (CRP) (MD = –8.42, 95%CI (–12.47, –4.38), $p < 0.0001$), and rheumatoid factor (MD = –25.48, 95%CI (–29.36, –21.60), $p < 0.00001$). Compared to conventional therapy, TwHF combined with conventional therapy significantly improved clinical effects (RR = 1.33, 95%CI (1.17, 1.51), $p < 0.0001$), in five trials with 354 patients. In

terms of improvement of symptoms and signs, the TwHF group showed a more significant improvement than the conventional treatment group (Cough (MD = −0.96, 95%CI (−1.43, −0.50), $p < 0.0001$), velcro rales (MD = −0.32, 95%CI (−0.44, −0.20), $p < 0.00001$), shortness of breath (MD = −1.11, 95%CI (−1.67, −0.56), $p < 0.0001$)], but no statistical difference in dyspnea (MD = −0.66, 95%CI (−1.35, 0.03), $p = 0.06$). There was no statistical significance in the incidence of adverse reactions.

Conclusion: The performed meta-analysis indicated that TwHF combined with conventional treatment was more beneficial to patients for improving symptoms, lung function and laboratory indicators. As it included studies with relatively small sample size, the findings require confirmation by further rigorously well-designed RCTs.

Keywords: tripterygium wilfordii hook F, connective tissue disease-associated interstitial lung disease, efficacy, safety, meta-analysis

INTRODUCTION

Connective tissue disease (CTD) is a group of autoimmune diseases characterized by the damage of connective tissue components in various parts of the body (Zhang and Kang, 2009). Multiple organs and systems are involved, and the sites of related pulmonary lesions include respiratory tract, stroma, alveoli, blood vessels, pleura, and diaphragm (Zhao et al., 2018). Connective tissue disease-associated interstitial lung disease (CTD-ILD) can occur in a variety of connective tissue diseases, such as rheumatoid arthritis (RA), primary Sjogren's syndrome (pSS), systemic sclerosis (SSc), and so on. According to a population-based cohort study by Ng KH et al. (Ng et al., 2020), the risk of ILD in patients with the above connective tissue diseases is significantly increased, but the prevalence rates vary due to different detection methods. Different CTD-ILD can exhibit different types of clinical manifestations, imaging and pathological features (Rheumatology and Immunology branch of Chinese Medical Doctor Association, 2018), which presents different development abnormalities, leading to difficulties in early diagnosis and treatment. Clinically, it often requires respiratory, rheumatology, radiology and other disciplines to participate in the disease assessment.

One study showed that approximately forty percent of patients with ILD have associated CTD at the same time (Mira-Avendano et al., 2019). Some ILD patients can develop progressive pulmonary fibrosis, impeded lung function and, eventually, even respiratory failure (Fischer and Distler, 2019). CTD-ILD is now seriously affecting the quality of life in those patients, even life-threatening. Therefore, the early diagnosis of CTD-ILD patients, and select the most appropriate individualized treatment and formulate the corresponding follow-up plan according to the condition of the patients, so as to achieve the remission of CTD-ILD patients and the long-term stability of lung function, prolong the survival time of patients to the greatest extent, and improve the quality of life, are the urgent problems to be solved.

At present, the etiology and pathogenesis of CTD-ILD are still unclear, but immune-mediated pulmonary inflammation and subsequent fibrosis are key elements in the development of the condition (Atzeni et al., 2018). Therefore, glucocorticoid and immunosuppressive therapy are an essential choice of CTD-ILD (Mathai and Danoff, 2016). They can effectively prevent the progression and deterioration of ILD, and help maintain adequate lung function. For CTD-ILD patients with short course of disease, rapid progress and severe sympoms intensity, high-dose methylprednisolone shock therapy can be used at the beginning of treatment (Rheumatology and Immunology branch of Chinese Medical Doctor Association, 2018). Immunosuppressive drugs commonly used in clinical practice include cyclophosphamide, mycophenolate, azathioprine and tacrolimus (Jee and Corte, 2019). Moreover, the occurrence of adverse events requires further monitoring in multicenter trials. In addition, relevant studies about biology agent or antifibrotic drugs have shown a good efficacy and safety in the treatment of CTD-ILD, especially in NSIP mode (Duarte et al., 2019; Moran-Mendoza et al., 2019; Maher et al., 2020). The cost of treatment with those, however, is extremely high, leading to CTD-ILD patients being unable to follow up with the treatment for prolonged periods of time.

As a complementary and alternative therapy for CTD-ILD, Chinese herbal medicine has the advantages of being multi-targeting and safe, therefore it has gradually become a popular choice for managing the condition by patients. Our previous study found that certain traditional Chinese medicine compounds can interfere with the pulmonary fibrosis process by inhibiting the aging of fibroblasts, showing a good anti-fibrosis effect (Feng et al., 2019; Peng et al., 2021). Tripterygium wilfordii Hook. F (TwHF) is an important herb in traditional Chinese medicine. Data increasingly has been showing that the related extracts of TwHF have immunomodulatory, anti-inflammatory and anti-allergic effects, and is widely used for the treatment of rheumatoid arthritis in China (Bao and Dai, 2011; Bai et al., 2020; Hu et al., 2019). The results of mutiple clinical trials have demonstrated that TwHF monotherapy exhibits good efficacy in patients with active RA (Lv et al., 2015), SLE (Liu et al., 2014; Chen et al., 2020a), ankylosing spondylitis (Li et al., 2015; Ji et al., 2015), kidney disease (Ge et al., 2013; Wang, 2018)and some skin diseases, such as psoriasis (Lv et al., 2018). Zhang et al. found that TwHF can disrupt the process of CTD-ILD through multiple

targets and multiple pathways (Zhang et al., 2021). In another study (Yang et al., 2011), TwHF inhibited the excessive apoptosis of pulmonary epithelial cells and the accumulation of extracellular matrix (ECM) in lung tissue by regulating the expression of immune/inflammatory regulatory factors, thus preventing the progress of pulmonary fibrosis. Furthermore, studies have shown that the therapeutic effect of TwHF is related to the regulation of the proportion between CD4+and CD8+T cells, the immune balance of Th17 cells and Tregs, and the differentiation of dendritic cells (Chen et al., 2015; Luo et al., 2019).

There is a plethora of anecdotal evidence of TwHF use in traditional Chinese medicine as an immunosuppressant with an outstanding curative effect. However, there is no reliable evidence-based medical research that confirms its efficacy in the treatment of CTD-ILD. In order to fully prove its effectiveness and safety, it is necessary to evaluate its preparation clinically. Therefore, we conducted a systematic review and meta-analysis following the PRISMA checklist in order to comprehensively evaluate all relevant clinical randomized controlled trials and provide more consistent scientific evidence for the clinical application of TwHF for CTD-ILD patients.

METHODS

This systematic review and meta-analysis was performed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and readers can access the protocol of this systematic review in International Prospective Register of Systematic Reviews (PROSPERO) (CRD42020210690).

Literature Search Strategy

A comprehensive literature search was performed in the following electronic databases: PubMed, Embase, Cochrane Library, Web of Science, PsycINFO, Scopus, CNKI, WanFang, VIP and CBM database. All of the databases were searched to identify the relevant human clinical studies published until May 2021. Two reviewers (Yehui Li and Wen Zhu) conducted the literature search independently, the search strategy used was as follows: [("*Tripterygium wilfordii* Hook F") OR ("Tripterygium wilfordii") OR ("TwHF")] AND [("Connective tissue disease") OR ("CTD") OR ("Rheumatoid arthritis") OR ("Sjogren's syndrome") OR ("Systemic lupus erythematosus") OR ("Systemic sclerosis") OR ("Dermatomyositis")] AND [("Interstitial lung disease") OR ("ILD")].

Inclusion Criteria

We believed that the research included in the meta-analysis should meet the following criteria: 1) The study design was confined to RCT regardless of blinding. 2) According to the classification and diagnostic criteria of RA, SLE formulated by the American College of Rheumatology (Andonopoulos et al., 1987; Hochberg, 1997; Aletaha et al., 2010), Primary Sjogren syndrome formulated by the American-European Consensus Group (Vitali et al., 2002) and the diagnostic criteria for interstitial lung disease formulated by the Thoracic Association (Raghu et al., 2011). 3) The studies provided the experimental group with TwHF in combination with conventional therapy while the control group with conventional therapy alone. Conventional therapy of CTD-ILD mostly consisted of the suppression of inflammation with corticosteroid or immunosuppressive therapy including azathioprine, ciclosporin, cyclophosphamide, mycophenolate mofetil or tacrolimus (Wells and Denton, 2014).

Exclusion Criteria

Relevant clinical trials were manually removed if any of the following factors were identified: 1) duplicated articles, 2) inappropriate interventions, 3) incomplete data, 4) irrelevance to outcome indicators.

Outcome Measures

Primary outcome measures included pulmonary function related indicators such as forced expiratory rate of the 1st second (FEV1%), forced expiratory volume (FVC%), total lung volume (TLC%), carbon monoxide diffusing capacity (DLCO %); HRCT integral (Austin et al., 1996), commended by The Outcome Measures in Rheumatology (OMERACT) (Saketkoo et al., 2014).

Secondary outcome measures included clinical efficacy, dyspnea, cough, shortness of breath and Velcro rales, according to the efficacy standards of "CTD-ILD" issued by the Chinese Medical Doctor Association (Rheumatology and Immunology branch of Chinese Medical Doctor Association, 2018), and laboratory indicators included c-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) are also evaluated.

Safety outcome measures included occurrence of adverse events.

Data Extraction and Quality Assessment

Two reviewers (YL and WZ) independently searched, screened and selected the articles, then extracted, and examined all the data. The extracted data included: 1) basic information such as the name of lead author, publication year, and gender; 2) number of participants in total and in each group (experimental and control), and the average age; 3) details of interventions, treatments of control groups, and treatment duration; 4) outcomes from each study and adverse reactions. The two researchers (YL and WZ) also evaluated the methodological quality of all the RCTs based on the criteria in the Cochrane evaluation handbook of RCTs 5.1.0.

In terms of bias, the articles were divided into three grades: low risk, high risk and unclear risk according to the following quality items: randomization generation, allocation concealment, blinding of subjects, outcome assessment, incomplete outcome data and selective outcome reporting. In case of differing opinions, consensus was reached through consultation and discussion.

Statistical Analysis

According to the RevMan 5.3 software (Cochrane Collaboration), risk ratios (RR) with 95% confidence intervals (CI) for

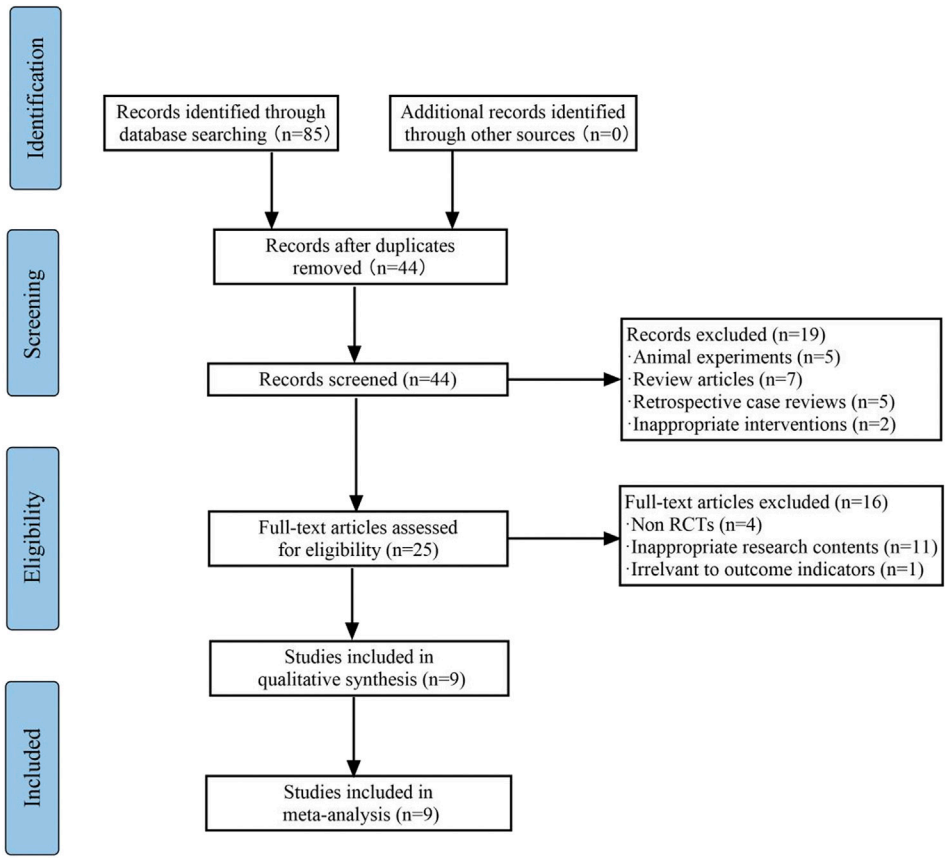

FIGURE 1 | PRISMA flow diagram of the literature search process.

dichotomous data and mean differences (MD) with 95% CIs for continuous data were reported. Heterogeneity was evaluated statistically using the I^2statistic. Meta-analysis was carried out using a random effects model if I^2>50%, otherwise if I^2<50%, the fixed effects model was selected. A sensitivity analysis of HRCT integral, laboratory indicators including CRP, ESR, RF and improvement of dyspnea, cough, shortness of breath and Velcro rales was performed by deleting each study in sequence. Then, meta-analysis was re-conducted for the remaining studies.

RESULTS

Literature Search Results

Based on the retrieval strategy, a total of 112 clinical studies were obtained. After screening according to the inclusion/exclusion criteria, nine articles were determined for further analysis (**Figure 1**).

Description of Studies

There were nine RCTs included in the meta-analysis, involving 650 patients with CTD-ILD (Hu, 2015; Li et al., 2017; Yang et al., 2017; Lin et al., 2017; Dong, 2017; Gao, 2020; Chen et al., 2020a; Fan and Bai Ma, 2020; Chen et al., 2020b). All of the studies were conducted in China and the year of publication was between 2015 and 2021. There were 324 cases in the experimental group and 326 cases in the control group. Characteristics of the included studies included lead author, publication year, sample size, average age, interventions, outcome indicators, period of treatment and adverse reactions were summarized in **Table 1**.

Description of Interventions

The control group was given glucocorticoid or immunosuppressive therapy. In addition to these conventional treatments in the control group, the experimental group was supplemented with TwHF.

Quality Evaluation of Literature

All of the included trials mentioned applying randomization methodology. Six studies (Li et al., 2017; Lin et al., 2017; Dong, 2017; Chen et al., 2020a; Gao, 2020; Chen et al., 2020b) mentioned specific randomization grouping methods. Four of them used random number table to generate a sequence (Li et al., 2017; Chen et al., 2020c; Gao, 2020; Chen et al., 2020a), one used a lottery method to group participants (Lin et al., 2017), and the other used dynamic randomization method (Dong, 2017). None

TABLE 1 | The characteristics of the included trials.

Study	No	Gender (M/F)		Average age(Y)		Interventions		Duration(M) T/C	Outcomes
	T/C	T	C	T	C	T	C	T/C	
Gao (2020)	25/30	16/9	19/11	52.95 ± 6.32	52.82 ± 6.41	TwHF + CTX	CTX	12/12	②⑦⑧⑪⑫⑬
Chen et al. (2020a)	50/50	22/28	20/30	45.42 ± 9.37	46.34 ± 9.65	TwHF + PAT	PAT	6/6	①②③④⑦⑧⑨⑪⑫⑬
Fan et al. (2020)	30/30	16/14	17/13	54.37 ± 3.52	55.18 ± 3.37	TwHF + MP	MP	6/6	①②⑧⑨⑩
Chen et al. (2020b)	20/20	7/13	8/12	53.58 ± 2.06	52.97 ± 1.05	TwHF + PAT	PAT	6/6	①⑤⑥
Li et al. (2017)	41/39	16/25	16/23	57.7 ± 10.3	56.9 ± 10.5	TwHF + CTX	CTX	6/6	①②③④⑤⑪⑫⑬
Yang et al. (2017)	30/30	2/28	2/28	50.3 ± 7.7	48.4 ± 8.1	TwHF + PAT	CTX + PAT	6/6	②⑥
Lin et al. (2017)	37/37	12/25	10/27	57.4 ± 7.5	56.3 ± 6.9	TwHF + PAT	PAT	6/6	①②⑤⑥⑧⑨⑩
Hu (2015)	31/30	17/44		56		TwHF + PAT + CTX	PAT + CTX	6/6	②⑦⑧
Dong (2017)	60/60	22/38	20/40	50 ± 3.43	51 ± 2.78	TwHF + DXM + CTX	DXM + CTX	7/7	⑦⑧

No., number of participants; T, treatment; C, control; M, male; F, female; Y, year; M, month; TwHF, Tripterygium wilfordii Hook. F; CTX, cyclophosphamide; DXM, dexamethasone; MP, methylprednisolone; PAT, prednisone acetate tablets. ①Clinical efficacy; ②HRCT score; ③FEV1%; ④FVC%,; ⑤TLC%,; ⑥DLCO%; ⑦improvement of dyspnea; ⑧improvement of cough; ⑨improvement of shortness of breath; ⑩improvement of Velcro rales; ⑪ CRP, ⑫ ESR, ⑬ RF.

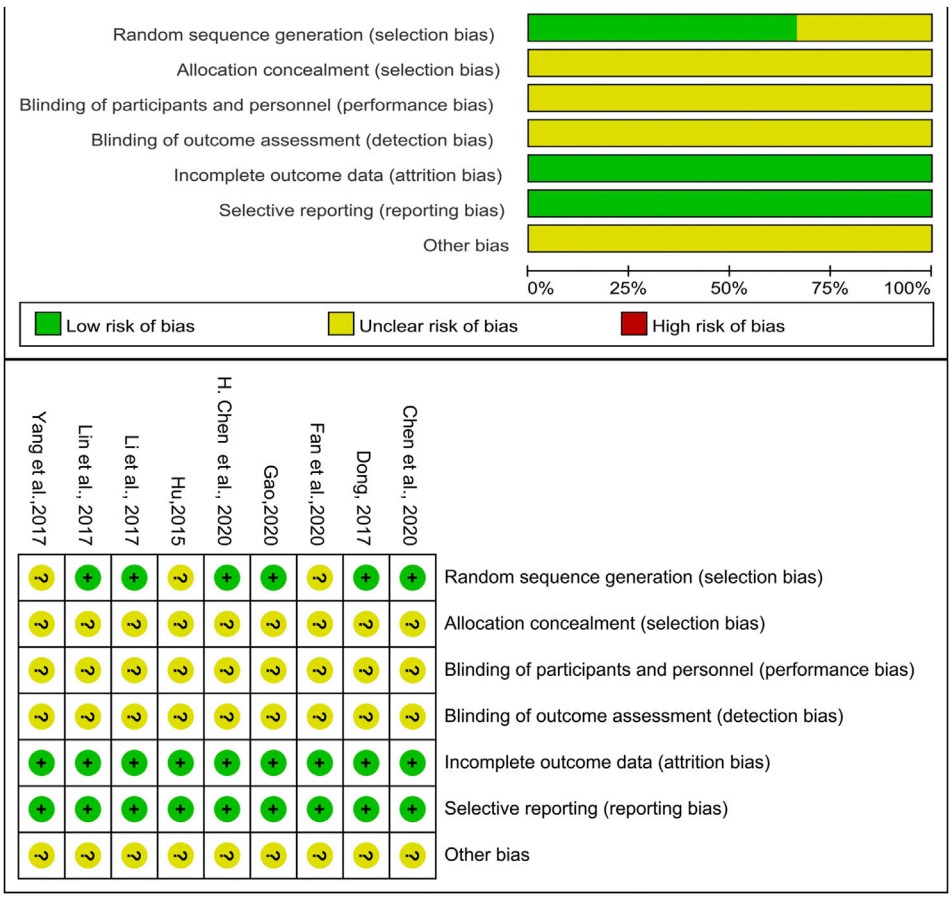

FIGURE 2 | | Risk of bias summary and risk of bias graph.

of the trials specified the methods of allocation concealment and the blinding procedures. This indicated that there were unclear risks of bias. No trials mentioned selective reporting, so a low risk of bias was chosen. Other biases were not determined. **Figure 2** shows detailed information about the studies' research methods quality.

Outcome Measures

Primary Outcomes

Changes in Lung Function

Lung function data was included in five trials (Li et al., 2017; Lin et al., 2017; Yang et al., 2017; Chen et al., 2020a; Chen et al., 2020b), which included 354 patients in total

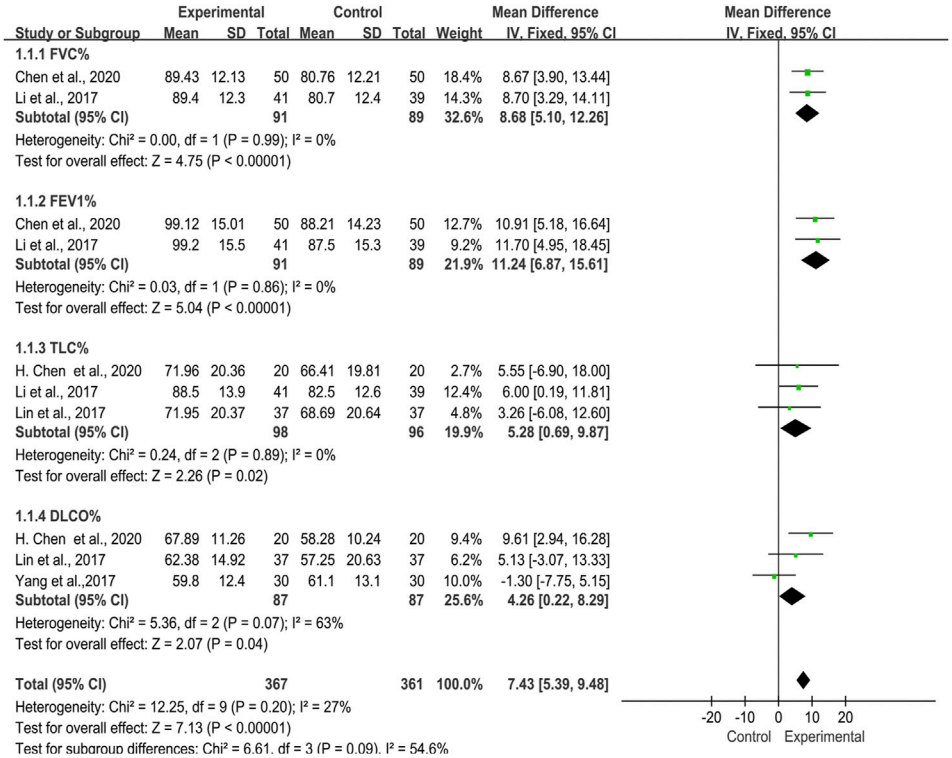

FIGURE 3 | Forest plot of improvement of lung function with TwHF combined with conventional treatment versus pure conventional treatment.

(**Figure 3**). As the heterogeneity test showed of DLCO% was high (I^2 = 63%, p = 0.07), a random-effects model was applied to calculate the MD and 95%Cl so as to ensure reliability of the results. The results demonstrated the TwHF group, compared with the control group could improve patients' lung function significantly (MD = 7.30, 95% (4.86, 9.74), p < 0.00001). Next, we separately described the lung function indexes such as FEV1%, FVC %, TLC% and DLCO%.

FEV1% and FVC%

Two trials (Li et al., 2017; Chen et al., 2020c), 180 patients, described changes of lung function in FEV1% and FVC%. The studies showed no significant heterogeneity in these two indicators [FEV1% (I^2 = 0%, p = 0.86); FVC% (I^2 = 0%, p = 0.99)]. The performed meta-analysis showed that there was a statistically significant difference between two groups in FEV1% [MD = 11.24, 95% (6.87, 15.61), p < 0.00001] and FVC% [MD = 8.68, 95% (5.10, 12.26), p < 0.00001], indicating that TwHF plus conventional treatment had advantages in improving FEV1% and FVC% compared with using conventional treatment alone.

TLC%

Three trials (Li et al., 2017; Lin et al., 2017; Chen et al., 2020a) reported 194 patients with TLC%. We noted no significant heterogeneity in two studies (I^2 = 0%, p = 0.89). According to the statistical difference of the results in TLC% [MD = 5.28, 95% (0.69, 9.87), p = 0.02], we concluded that the TwHF group could improve the TLC% of patients.

DLCO%

There were also three trials (Lin et al., 2017; Yang et al., 2017; Chen et al., 2020b) introduced DLCO%, which included 174 patients. The performed meta-analysis showed that the heterogeneity test was high (I^2 = 63%, p = 0.07), and there was no statistical significant difference between the TwHF group and control group [MD = 4.40, 95% (-2.29, 11.09), p = 0.20].

HRCT Integral Evaluation

In nine trials, seven trials (Hu, 2015; Li et al., 2017; Lin et al., 2017; Yang et al., 2017; Chen et al., 2020c; Fan and Bai Ma, 2020; Gao, 2020), including 490 cases, reported HRCT integral (**Figure 4**). The heterogeneity was high ($I2$ = 97%, p < 0.00001) and we applied the random-effects model in this meta-analysis. In the meta-analysis, a statistically difference [MD = -0.65, 95% (-1.01, -0.30), p = 0.0003] existed between TwHF combination group and control group, signifying that the combination of TwHF and conventional therapy could significantly reduce the HRCT integral of patients.

Secondary Outcomes

Clinical Efficacy Evaluation

Efficiency was evaluated based on interventions and controls in five trials (Li et al., 2017; Chen et al., 2020a; Chen et al., 2020b; Fan

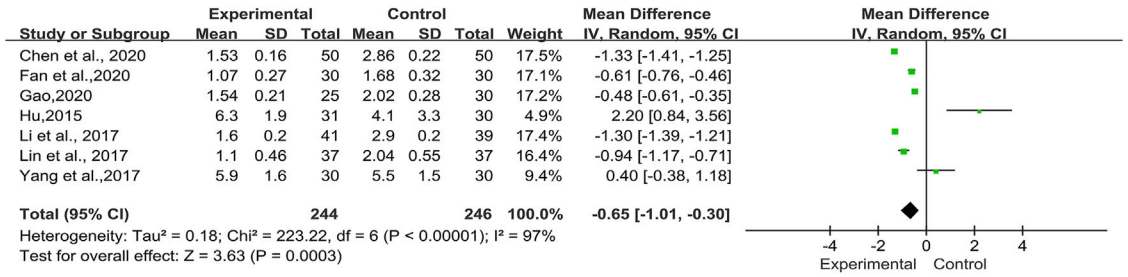

FIGURE 4 | Forest plot of HRCT integral with TwHF combined with conventional treatment versus pure conventional treatment.

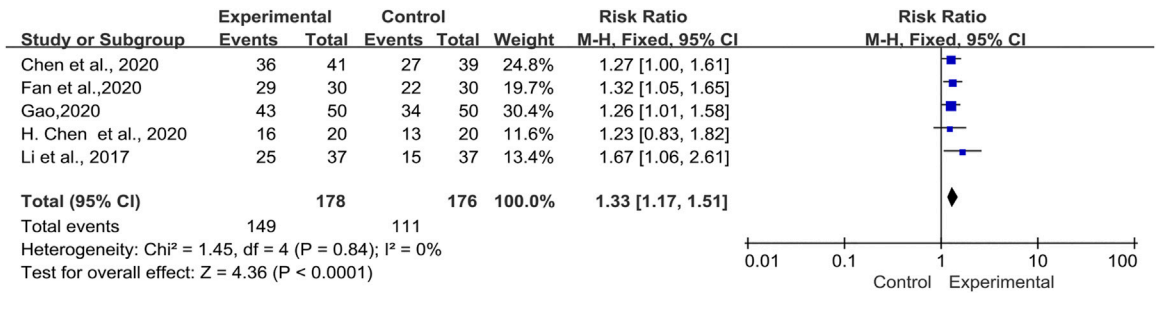

FIGURE 5 | Forest plot of clinical efficacy with TwHF combined with conventional treatment versus pure conventional treatment.

and Bai Ma, 2020; Gao, 2020) including 354 patients (**Figure 5**). The pooled results indicated that there was no significant heterogeneity (I^2 = 0%, p = 0.84). We chose the fixed-effects model for the analysis. The results showed that compared with western medicine alone, TwHF combined with conventional treatment can significantly improve the clinical efficiency of CTD-ILD patients [RR = 1.33, 95% (1.17, 1.51), p < 0.0001].

Assessment of Symptoms and Signs

A total of six trials (Hu, 2015; Dong, 2017; Lin et al., 2017; Chen et al., 2020c; Fan and Bai Ma, 2020; Gao, 2020) involving 150 patients reported improvement of various symptoms and signs. We applied a random-effects model according to the heterogeneity test results. (**Figure 6**).

Dyspnea

The improvement of dyspnea was reported in four trials (Hu, 2015; Dong, 2017; Chen et al., 2020b; Gao, 2020), which included 336 patients. High heterogeneity (I^2 = 94%, p < 0.00001) between the included studies was observed. The meta-analysis showed that there was no statistical significance between the TwHF group and the control group [MD = −0.66, 95% (−1.35, 0.03), p = 0.06].

Cough

Six trials (Hu, 2015; Dong, 2017; Lin et al., 2017; Chen et al., 2020a; Fan and Bai Ma, 2020; Gao, 2020), involving 470 patients, reported the improvement of cough. The data showed a high heterogeneity (I^2 = 78%, p = 0.0004) and a significant difference in the post-treatment of cough values favoring TwHF [MD = −0.96, 95% (−1.43, −0.50), p < 0.0001].

Shortness of Breath

Three trials (Lin et al., 2017; Chen et al., 2020b; Fan and Bai Ma, 2020) including 234 patients, reported improvement of shortness of breath. We noted a high heterogeneity (I^2 = 79%, p = 0.008) in the meta-analysis. The results were statistically significant, indicating that in the TwHF group the symptoms of shortness of breath were significantly improved compared with the control group [MD = −1.11, 95% (−1.67, −0.56), p < 0.0001].

Velcro Rales

Fan et al., 2020 and Lin et al., 2017 reported Velcro rales in 134 patients, which had no significant heterogeneity (I^2 = 0%, p = 0.65). The pooled results indicated that there was a significant statistical difference between two groups (MD = −0.32, 95% (−0.44, -0.20), p < 0.00001), indicating that Velcro rales in the combined group were more likely to improve in clinical practice.

Laboratory Indicators

Three trials (Li et al., 2017; Chen et al., 2020c; Gao, 2020) involving 235 patients, compared the TwHF group with a pure conventional treatment in the post-treatment of laboratory indicators including CRP, ESR, and RF. As illustrated in **Figure 7**, the results showed that there was no significant heterogeneity, aside from CRP (I^2 = 84%, p = 0.002). Thus, a random-effects model was applied for analysis. According to the statistical difference of the CRP [MD = −8.42, 95%Cl (−12.47,

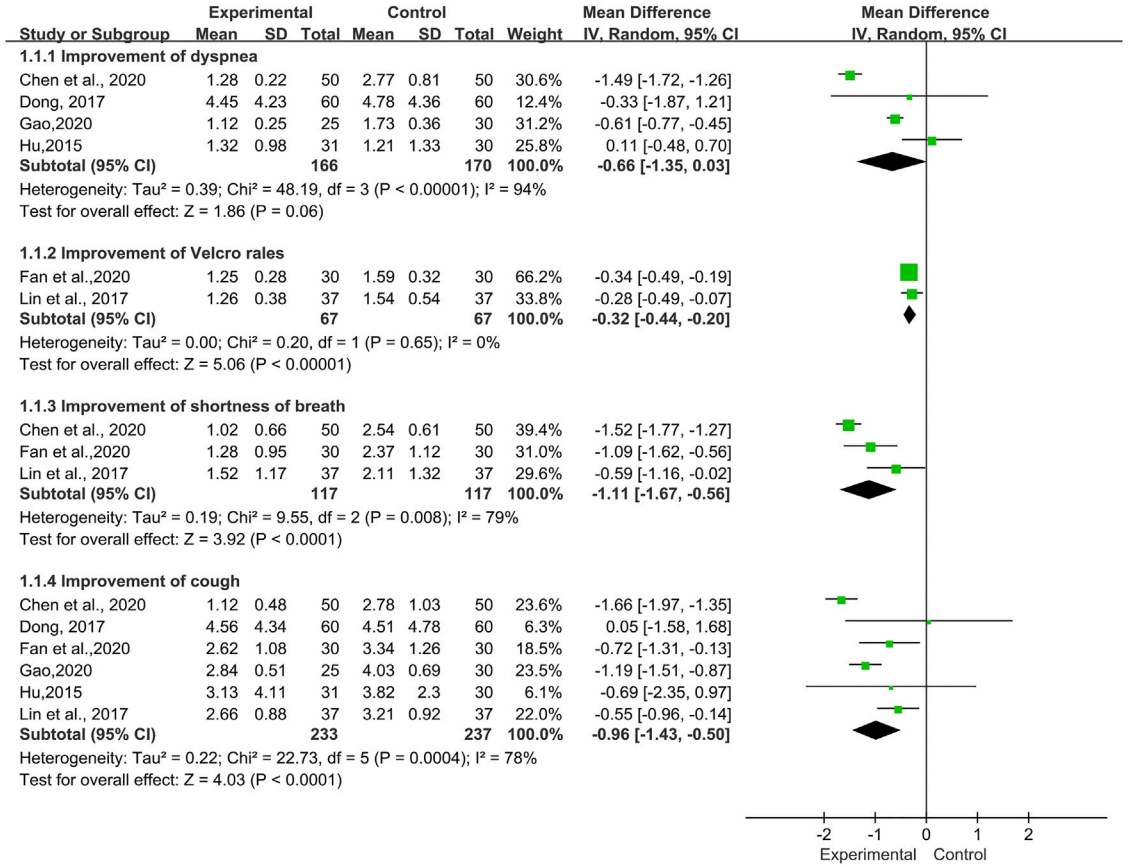

FIGURE 6 | Forest plot of improvement of symptoms and signs with TwHF combined with conventional treatment versus pure conventional treatment.

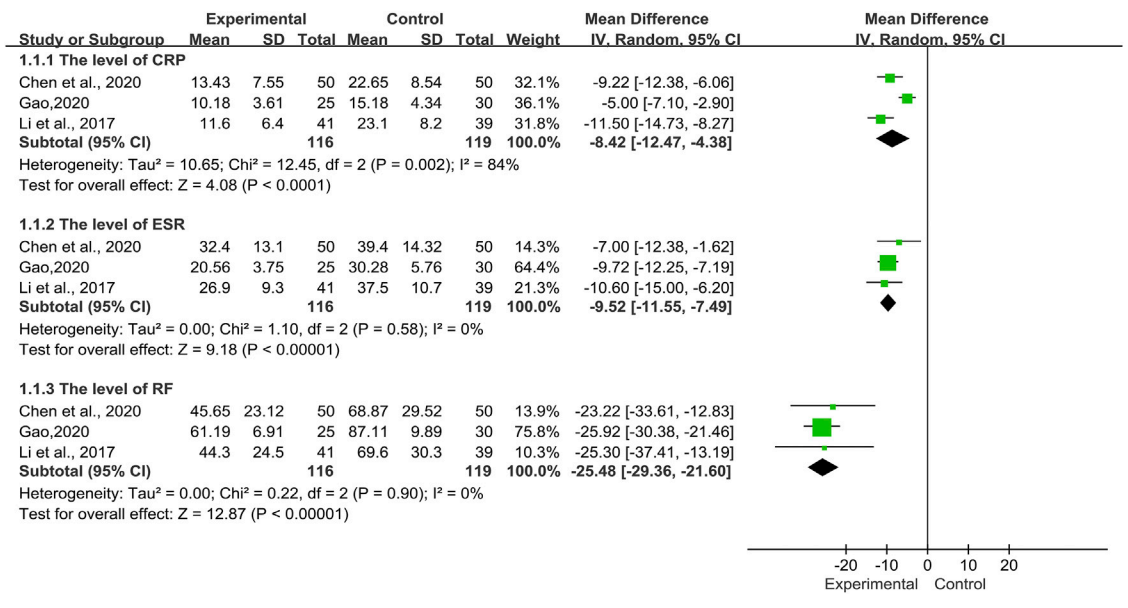

FIGURE 7 | Forest plot of the level of laboratory indicators with TwHF combined with conventional treatment versus pure conventional treatment.

TABLE 2 | Adverse events reported in the studies.

Study	Adverse events	
	Intervention	Control
Fan et al. (2020)	One alopecia; no hyperglycemia and infection; one transaminase increased	Three alopecia; two hyperglycemia, infections and transaminase increased
Chen et al. (2020a)	Three gastrointestinal symptoms; one headache	Three gastrointestinal symptoms and headache
Gao (2020)	Two gastrointestinal symptoms	Three gastrointestinal symptoms; one headache
Chen et al. (2020b)	One menstrual disorder and leukopenia; no hyperglycemia	Two menstrual disorders and hyperglycemia; no leukopenia
Li et al. (2017)	Five alopecia; six gastrointestinal symptoms; three mouth ulcers	Five alopecia and gastrointestinal symptoms; four mouth ulcers
Lin et al. (2017)	Four alopecia; eight menstrual disorders; five hyperglycemia, infections and transaminase increased; four leukopenia	One alopecia; four menstrual disorders; six hyperglycemia; three infections; two transaminase increased; one leukopenia
Yang et al. (2017)	No adverse events	Three infections and thrombocytopenia; two leukopenia and transaminase increased; one gastrointestinal symptoms
Hu (2015)	Five cushing syndrome; seven menstrual disorders; one hyperglycemia; no alopecia; ten gastrointestinal symptoms; two transaminase increased and infections; eight leukopenia	Nineteen cushing syndrome; three menstrual disorders; six hyperglycemia; three alopecia; sixteen gastrointestinal symptoms; three transaminase increased; four infections; nine leukopenia

From: *Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097.*
For more information, visit: www.prisma-statement.org.

−4.38), $p < 0.0001$], ESR [MD = −9.52, 95%Cl (−11.55, −7.49), $p < 0.00001$], and RF [MD = −25.48, 95%Cl (−29.36, −21.60), $p < 0.00001$], we thought that TwHF combined with conventional therapy could effectively reduce the level of laboratory indicators such as CRP, ESR, and RF.

Adverse Events

Eight trials reported AEs (Hu, 2015; Li et al., 2017; Yang et al., 2017; Lin et al., 2017; Dong, 2017; Gao, 2020; Chen et al., 2020a; Chen et al., 2020b; Fan and Bai Ma, 2020). AE details are shown in **Table 2** These studies clearly reported the accurate numbers of different symptoms of AEs including menstrual disorders, gastrointestinal symptoms, headache, transaminase increase, leukopenia, hyperglycemia, alopecia, and infection in TwHF group and control group. Furthermore, only one trial reported Cushing's syndrome alone, so a meta-analysis of this adverse event was not available (Hu, 2015). The pooled results showed that there was no statistically significant difference in the incidence of AEs between the TwHF combined treatment group and the conventional treatment group (**Figure 8**). However, the relationships between the AEs and the interventions were not further discussed in any study.

Sensitivity Analysis

The results of all studies in the fixed-effects model showed good consistency. For the continuous data, we eliminated the included studies one by one, and the others were reanalyzed by meta-analysis. The results showed that HRCT integral did not change substantially, but in DLCO% index, after excluding Yang et al., 2017, the heterogeneity test decreased from 63 to 0%, $p = 0.003$ (**Figure 9**). The data suggested that Yang et al., 2017 was the main reason for the heterogeneity in DLCO%, which may be related to the blowing state of the patients participating in the study during the lung function examination. In terms of symptoms improvement, after excluding Chen et al., 2020a, the heterogeneity test of dyspnea improvement decreased from 94 to 63%, $p = 0.25$, the heterogeneity test of shortness of breath decreased from 79 to 38%, $p = 0.0006$, and the heterogeneity test of cough improvement decreased from 78 to 48%, $p < 0.0001$ (**Figure 10**). In addition, after the deletion of Gao, 2020, the heterogeneity test of CRP level decreased from 84 to 0%, $p < 0.00001$ (**Figure 11**). These findings suggested that Chen et al., 2020a and Gao, 2020 were the reasons for the heterogeneity of symptom improvement and CRP level results.

Analysis of Publication Bias

We planned to use the total effective rate as the outcome index for Funnel plots analysis of the included studies, but only five studies met the requirements. Therefore, no publication bias analysis was conducted.

DISCUSSION

Based on the current lack of high-level scientific evidence, the management and treatment of CTD-ILD patients are relying on case reports and clinical experience. It is worth noting that many disease remission anti-rheumatic drugs (DMARDs) for CTD treatment have different degrees of adverse reactions, including pulmonary infection, interstitial pneumonia and pulmonary sarcoidosis, of which interstitial pneumonia is the main type. The most common drugs involved include methotrexate and leflunomide (Skeoch et al., 2018; Gao and Moua, 2020). With the development of modern medicine, the treatment of CTD-ILD is diversified, but there is still a considerable number of patients with end-stage respiratory failure, prompting people to consider anti-fibrosis, hematopoietic stem cell transplantation, lung transplantation and other treatments (De Cruz and Ross, 2013; Crespo et al.,

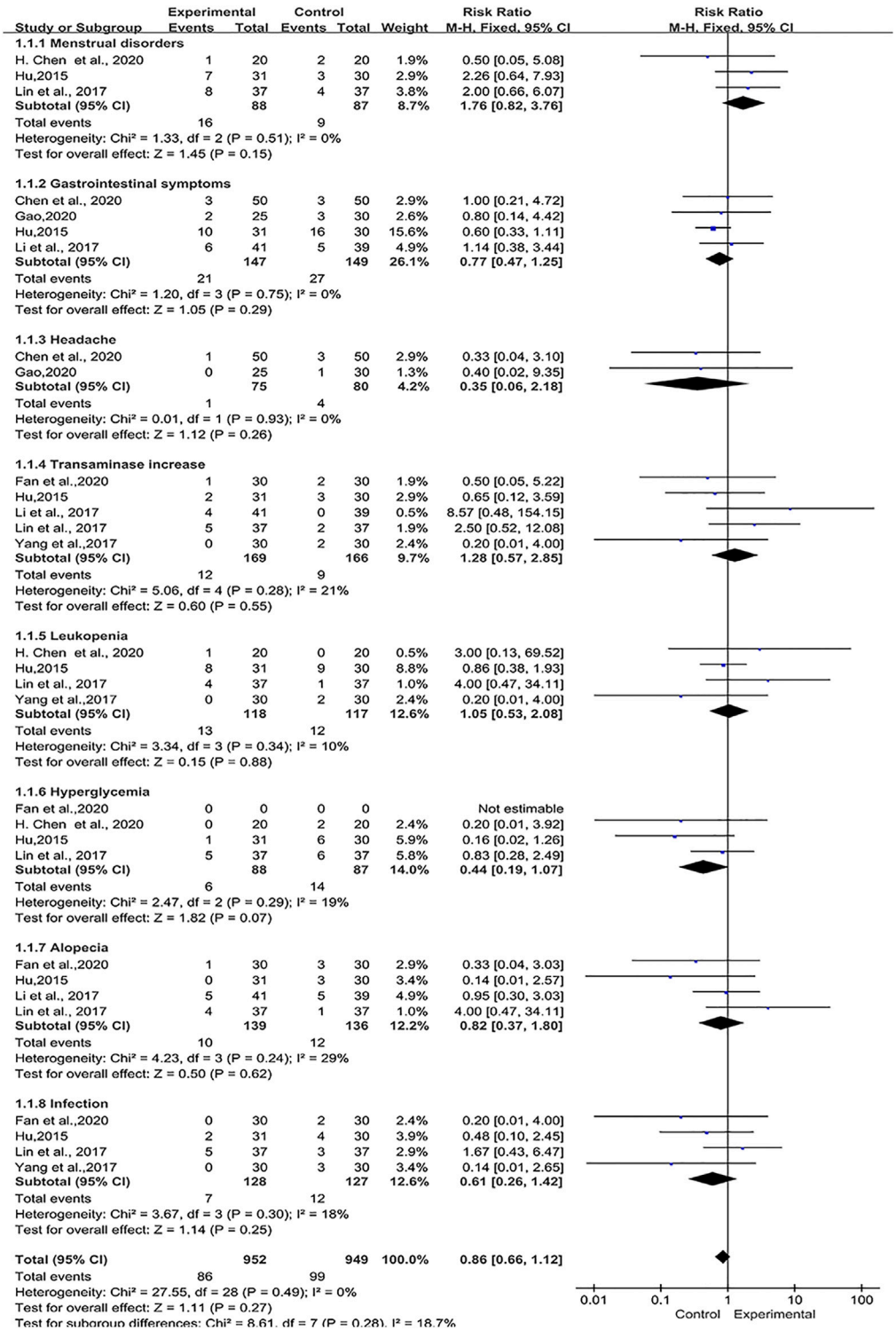

FIGURE 8 | Forest plot of adverse events with TwHF combined with conventional treatment versus pure conventional treatment.

2016; Pradère et al., 2018). Therefore, new treatment methods or standardized therapeutic measures are urgently needed.

Recent research has increasingly been focusing on the role of alternative therapies such as traditional Chinese medicine for the treatment of rheumatic diseases. Tripterygium wilfordii Hook. F (TwHF) is a Chinese herb also known as Lei Gong Teng (thunder god vine). It has been found to contain more than 70 chemical components (Chen et al., 2018) and has been confirmed to have

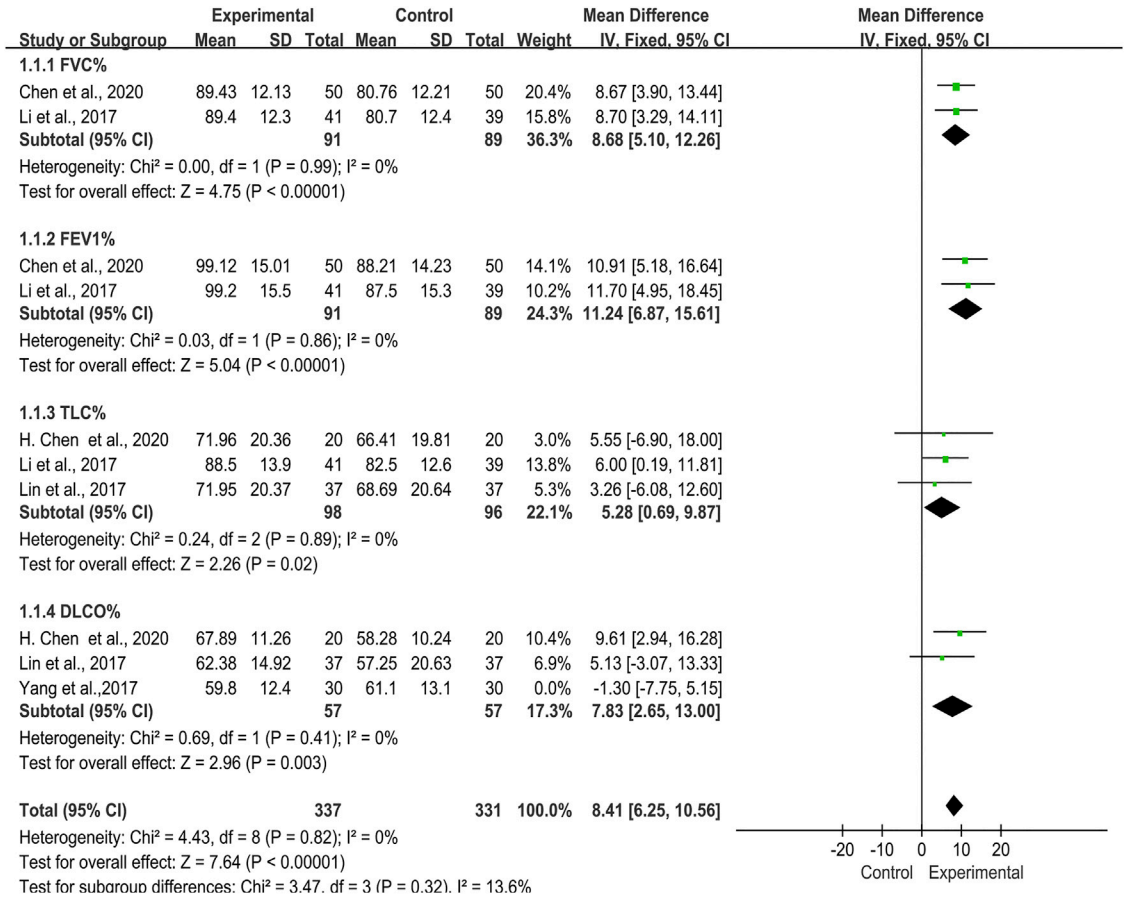

FIGURE 9 | Forest plot of sensitivity analysis of lung function with TwHF combined with conventional treatment versus pure conventional treatment.

definite anti-inflammatory, immunomodulatory effects, in addition to being effective for improving the symptoms in many CTDs. For examble, Zhou YY et al. provided more scientific and convincing evidence for the treatment of rheumatoid arthritis by TwHF in a meta-analysis (Zhou et al., 2018). The main active components of TwHF can reduce capillary permeability, inhibit inflammatory cytokines or chemokines, and regulate the expression of various inflammatory mediators, thereby reducing lung injury (Law et al., 2011; Wei and Huang, 2014).

Triptolide improved the pulmonary function by inhibiting myofibroblast activation and collagen deposition in lung tissues. Triptolide also mitigated pulmonary fibrosis partly by downregulating nicotinamide adenine dinucleotide phosphateoxidase 2 (NOX2) through the NF-κB pathway (Yuan et al., 2019) and increase the alveolar space (Hoyle et al., 2010). TwHF may upregulate CD4+CD25+regulatory T cells and improve immunity, which has been found to be beneficial for the improvement of lung function in another study (Lei and Jian, 2012). However, there is no clinical evidence-based medicine summary of TwHF for the treatment of CTD-ILD.

Conducting a systematic review and meta-analysis of the efficacy and safety in the treatment of CTD-ILD utilised TwHF has never been attempted before. This study reviewd nine RCTs consisting of 650 patients. Based on the meta-analysis results, we found that TwHF combined with western medicine was superior to pure western medicine in terms of improving lung function, HRCT integral, velcro rales, cough, and shortness of breath. Moreover, as ESR and CRP are representative inflammatory indexes in many CTD, we also conducted corresponding analysis. As a result, TwHF could significantly reduce the level of CRP, ESR and other inflammatory factors indicators, with good clinical efficacy.

Nevertheless, adverse events are always a focus of concern. TwHF may be harmful to the liver, kidneys, reproductive tissues, and immune tissues. In this meta-analysis, eight studies (Hu, 2015; Li et al., 2017; Yang et al., 2017; Lin et al., 2017; Gao, 2020; Chen et al., 2020a; Fan and Bai Ma, 2020; Chen et al., 2020b) mentioned the occurrence of adverse events including menstrual disorders, gastrointestinal symptoms, headache, transaminase increase, leukopenia, hyperglycemia, alopecia, and infection, but none of them dropped out of the study. The diversity of the components makes it difficult to analyze the safety of TwHF extracts. Subgroup analysis was not performed due to the different preparations of Tripterygium wilfordii and the limited data. Through the analysis of adverse events, it showed

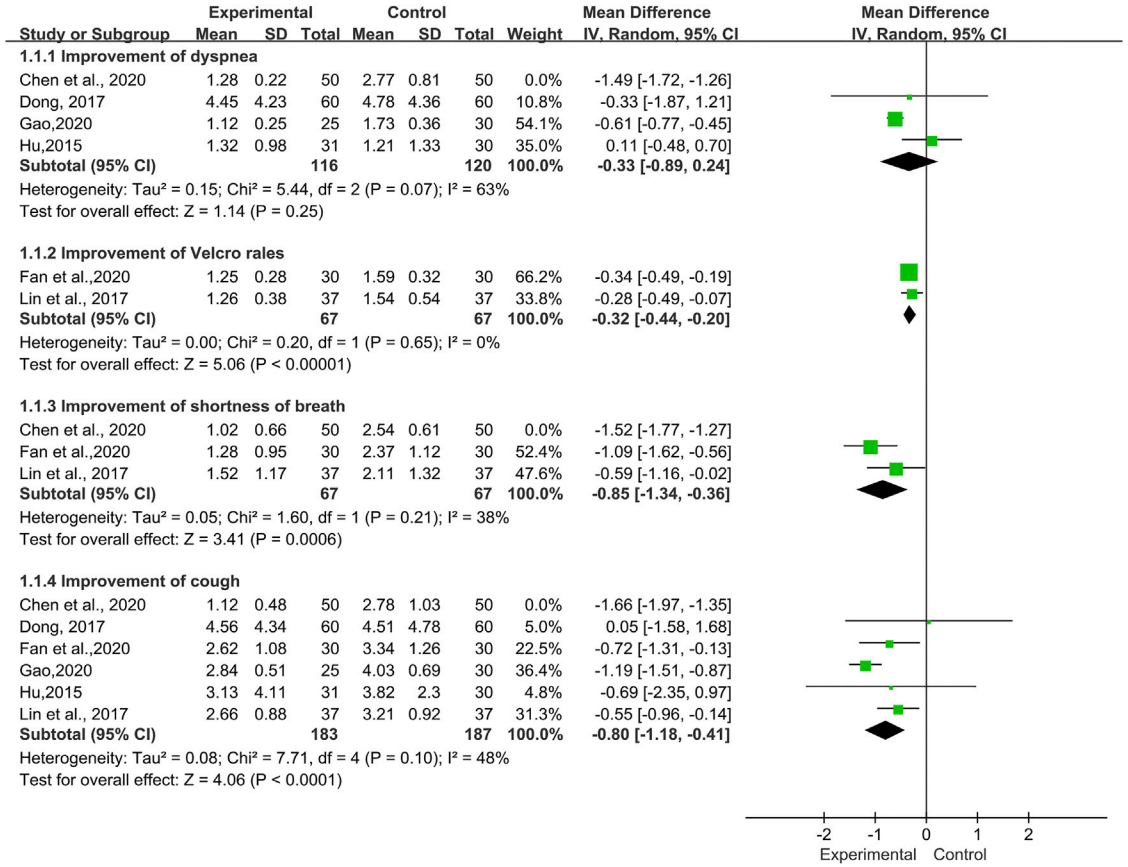

FIGURE 10 | Forest plot of sensitivity analysis of symptoms and signs improvement with TwHF combined with conventional treatment versus pure conventional treatment.

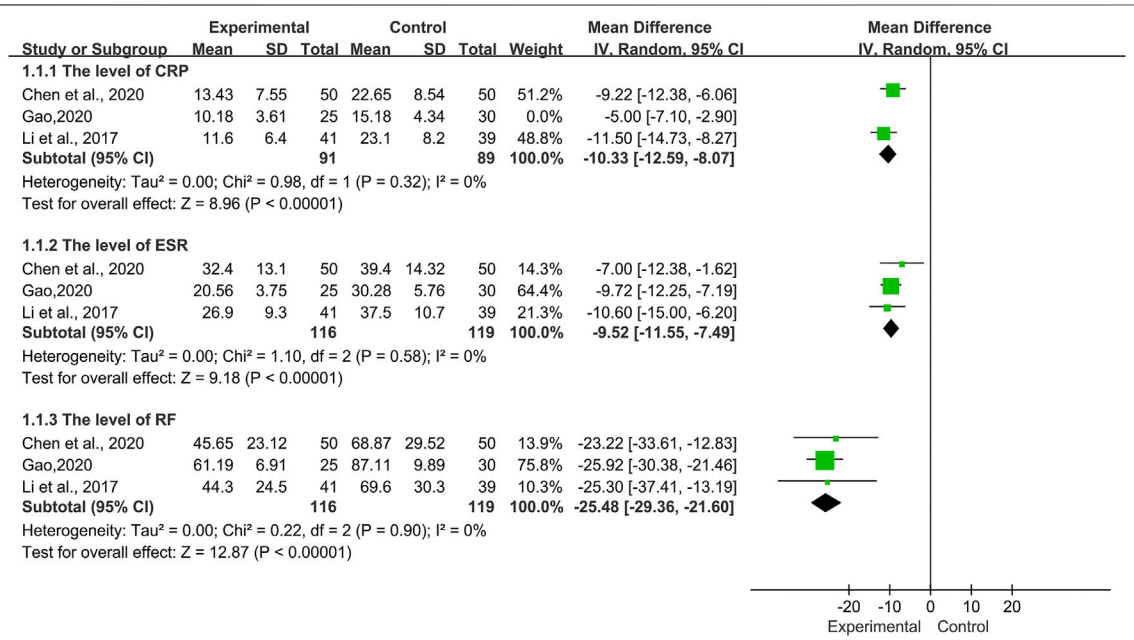

FIGURE 11 | Forest plot of sensitivity analysis of laboratory indicators with TwHF combined with conventional treatment versus pure conventional treatment.

that there was no statistical significance between the TwHF combined treatment group and the control group. Although this study's findings showed that the adverse events caused by TwHF were not significantly different from those caused by immunosuppressive agents, there is a clear need for improving prevention and management of patients' tolerance for TwHF. Once adverse events appear, the patient should discontinue medication immediately and the clinician should take steps to manage the adverse event if necessary.

There are a number of limitations of this study. Although we have searched the basic Chinese and English databases, there are few randomized controlled trials on TwHF in the treatment of CTD-ILD or ILD. In addition, 100% of the trials included in this study were conducted in China, which may be viewed as certain ethical bias. Furthermore, there are few included studies, small sample capacity and lack of long-term follow-up. Although all the nine included trials mentioned the randomized grouping method, only six of them mentioned a specific random grouping method, among which 4 trials used random number table to generate a sequence (Li et al., 2017; Chen et al., 2020b; Gao, 2020; Chen et al., 2020c), one used a lottery method to group (Lin et al., 2017), and the other used dynamic randomization method (Dong, 2017). None of the studies included in this meta-analysis included blinding or allocation information. Furthermore, the quality of literature was found to be low, which reduced the credibility and reliability of its findings. Besides, as the course of medication were not uniform across the studies, there were some differences in the use of immunosuppressive agents in various studies, which may be the reason for the heterogeneity of related indicators. However, the treatment duration and the choice of immunosuppressive agents largely depend on the severity of the disease and individual tolerance to drugs. According to the nine included trials, we noted that T. wilfordii tgpolyglycoside (TWP) was used in six trials (Li et al., 2017; Lin et al., 2017; Chen et al., 2020a; Gao, 2020; Chen et al., 2020a; Fan et al., 2020) and Glucosidorum Tripterygll Totorum (GTT) was used in three trials (Hu, 2015; Dong, 2017; Yang et al., 2017). Different kinds of Tripterygium preparations may produce a certain heterogeneity, thus influencing the results and reducing the robustness of the conclusion. As a result, the findings of this study only served as a reference for the clinical application of TwHF in the treatment of CTD-ILD. Based on the published data of TwHF in the treatment of CTD-ILD, it is difficult to judge its potential in the treatment of CTD-ILD. It is suggested that in the design of clinical studies in the future, we should choose objective, international and universal therapeutic indexes as far as possible. At the same time, it is important that large-scale, multicenter and high-quality randomized controlled trials are conducted. We are looking forward to stronger evidence to confirm or refute the results reported in this study.

CONCLUSION

In conclusion, evidence of this paper was found to support the fact that TwHF combined with conventional therapy provided statistically significant and clinically important improvement in CTD-ILD. To further support the conclusion, stronger scientific evidence is needed.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

YL and WZ designed this study and performed the online database search. LB, JW, LZ, and JW all contributed to the data collection, data extraction and data analysis. YL and WZ prepared the original draft. HH and XZ finished the revision of the manuscript. YG polished up the whole article. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.691031/full#supplementary-material

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REVIEW
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Association of Dietary Cholesterol Intake With Risk of Gastric Cancer: A Systematic Review and Meta-Analysis of Observational Studies

Peng Miao[1] *and Lin Guan*[2]*

[1] General Surgery Department, The First Hospital of China Medical University, Shenyang, China, [2] Gastroenterology Department, The First Hospital of China Medical University, Shenyang, China

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Mojtaba Heydari, Shiraz University of Medical Sciences, Iran

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***Correspondence:**
Lin Guan
guanlin1985@163.com

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Background: Many case–control studies have investigated the association between dietary cholesterol and gastric cancer, yielding inconsistent findings. We carried out a systematic review and meta-analysis of observational studies to assess the relationship between dietary cholesterol intake and gastric cancer among adults.

Methods: PubMed, Scopus, and Google Scholar were systematically searched to identify articles that evaluated the association of dietary cholesterol with gastric cancer up to May 2021. Pooled odds ratio (ORs) and 95% confidence intervals (CIs) were computed using random-effects models. Dose–response analysis was used to explore the shape and strength of the association.

Results: Fourteen case–control studies with 6,490 gastric cancer patients and 17,793 controls met our inclusion criteria. In the meta-analysis of the highest vs. the lowest dietary cholesterol categories, a significantly higher (∼35%) risk of gastric cancer was observed in association with high cholesterol consumption (pooled OR: 1.35, 95% CI: 1.29–1.62, I^2 = 68%; 95%CI: 45–81%). Subgroup analysis also showed this positive relationship in population-based case–control studies, those conducted on non-US countries, those with a higher number of cases and high-quality studies, those that collected dietary data via interviews, studies not adjusted for *Helicobacter pylori* infection, and studies where the body mass index was controlled. Besides, a non-linear dose–response association was also identified (P = 0.03).

Conclusion: This study demonstrated that dietary cholesterol intake could significantly augment the risk of gastric cancer in case–control studies. Prospective cohort studies with large sample sizes and long durations of follow-up are required to verify our results.

Keywords: dietary cholesterol, gastric cancer, meta-analysis, dose-response, systematic review, diet

INTRODUCTION

Gastric cancer (GC) represents the fifth most common cancer and the third leading cause of cancer deaths in males and females worldwide, with nearly one million new cases and 723,100 deaths from GC every year (1). Given the increasing prevalence of GC and its mortality, new strategies are necessary to minimize the disease burden. *Helicobacter pylori* infection, high alcohol consumption, obesity, smoking, and dietary factors are the main risk factors of GC (2, 3). Numerous studies have shown the association between nutritional factors and GC (3, 4). In fact, one meta-analysis found that the total dietary fat was positively associated with GC (5).

Cholesterol is a common nutrient in the human diet, with eggs, red meat, dairy products, fish, and poultry representing its major sources (6). It has been indicated that dietary cholesterol can increase serum cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol concentrations (7). Hypercholesterolemia may be involved in cancer development via a rise in the level of inflammatory markers (8).

Some meta-analyses demonstrated that high dietary cholesterol intake increases the risk of ovarian, breast, pancreatic, and esophageal cancers (9–12). However, the association between dietary cholesterol intake and GC risk remains controversial. Some case–control studies have indicated a positive relationship (13, 14), while others showed no association (15, 16). Based on our knowledge, there is no systematic review and meta-analysis to summarize the findings regarding dietary cholesterol intake and GC.

Therefore, considering the conflicting results and increasing incidence of GC worldwide, we carried out a systematic review and meta-analysis to provide a quantitative synthesis of the existing data on the association between dietary cholesterol intake and the risk of GC in adults. Furthermore, we aimed to assess the shape and strength of the dose–response association between dietary cholesterol intake and GC.

METHODS

The framework of this review was structured according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement [(17); **Supplementary Table 1**].

Search Strategy

An advanced systematic search of PubMed, Scopus, and Google Scholar was performed without any restrictions (including language) using Medical Subject Heading (MeSH) and related keywords to discover relevant articles published until May 2021. The search terms were:[("cholesterol*" OR "dietary cholesterol" OR "cholesterol intake" OR "cholesterol consumption" OR "fat intake" OR "dietary fat") AND ("gastrointestinal cancer" OR "gastrointestinal carcinoma" OR "gastrointestinal neoplasm" OR "gastrointestinal adenocarcinoma" OR "gastrointestinal tumor" OR "gastric cancer" OR "gastric carcinoma" OR "gastric neoplasm" OR "gastric adenocarcinoma" OR "gastric tumor" OR "stomach cancer" OR "stomach carcinoma" OR "stomach neoplasm" OR "stomach adenocarcinoma" OR "stomach tumor")]. Besides, the reference lists of the relevant articles and reviews were manually inspected in order to complete the search. The protocol of this investigation was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42021255008).

Inclusion Criteria

Studies with the following criteria were included: (1) a prospective cohort or case–control design; (2) participants were aged ≥18 years; (3) provided risk estimates, including relative risk (RR), hazard ratios (HRs), and odds ratios (ORs) with 95% confidence intervals (CIs) to evaluate the association between dietary cholesterol intake and GC. When several studies used one dataset, we selected the one with the greatest number of cases. Two independent authors reviewed articles according to the mentioned items. If they encountered any controversy, the principal investigator resolved the issue.

Exclusion Criteria

Unpublished papers, abstracts, ecological studies, reviews, letters, and comments were excluded. Furthermore, studies that considered another cancer along with GC and articles that used population-attributable risks to assess the association were removed.

Data Extraction

The following items were extracted from each included study: name of the first author, publication year, study location, study design, gender, age (mean/range), the total number of participants, cases, controls, median/range of cholesterol intake in each category, most adjusted RRs, HRs, or ORs and 95% CIs, dietary assessment method, outcome assessment approach, and adjustments. Two authors extracted the data independently, and the corresponding author resolved any disagreements.

Risk of Bias Assessment

The risk of bias for each study was determined using the Newcastle–Ottawa scale (18). Each study received an overall score between 0 and 9 according to the selection of case and control groups, comparability, and ascertainment of exposure and outcome. A total score of ≥7 was representative of a high-quality study.

Statistical Methods

We used a random-effects model to compute summary risk estimates and 95% CIs for the associations between dietary cholesterol intake (highest vs. lowest categories) and GC. Between-study heterogeneity was assessed using the I^2 index and its CI (19). In terms of between-study heterogeneity, I^2-values of 25–50%, 50–75%, and >75% were considered as low, moderate, and high heterogeneity, respectively (20). To discover potential sources of heterogeneity, subgroup and meta-regression analyses were conducted based on study design (population-based case–control studies, hospital-based case–control studies), number of cases, study quality, exposure reporting method, and adjustments

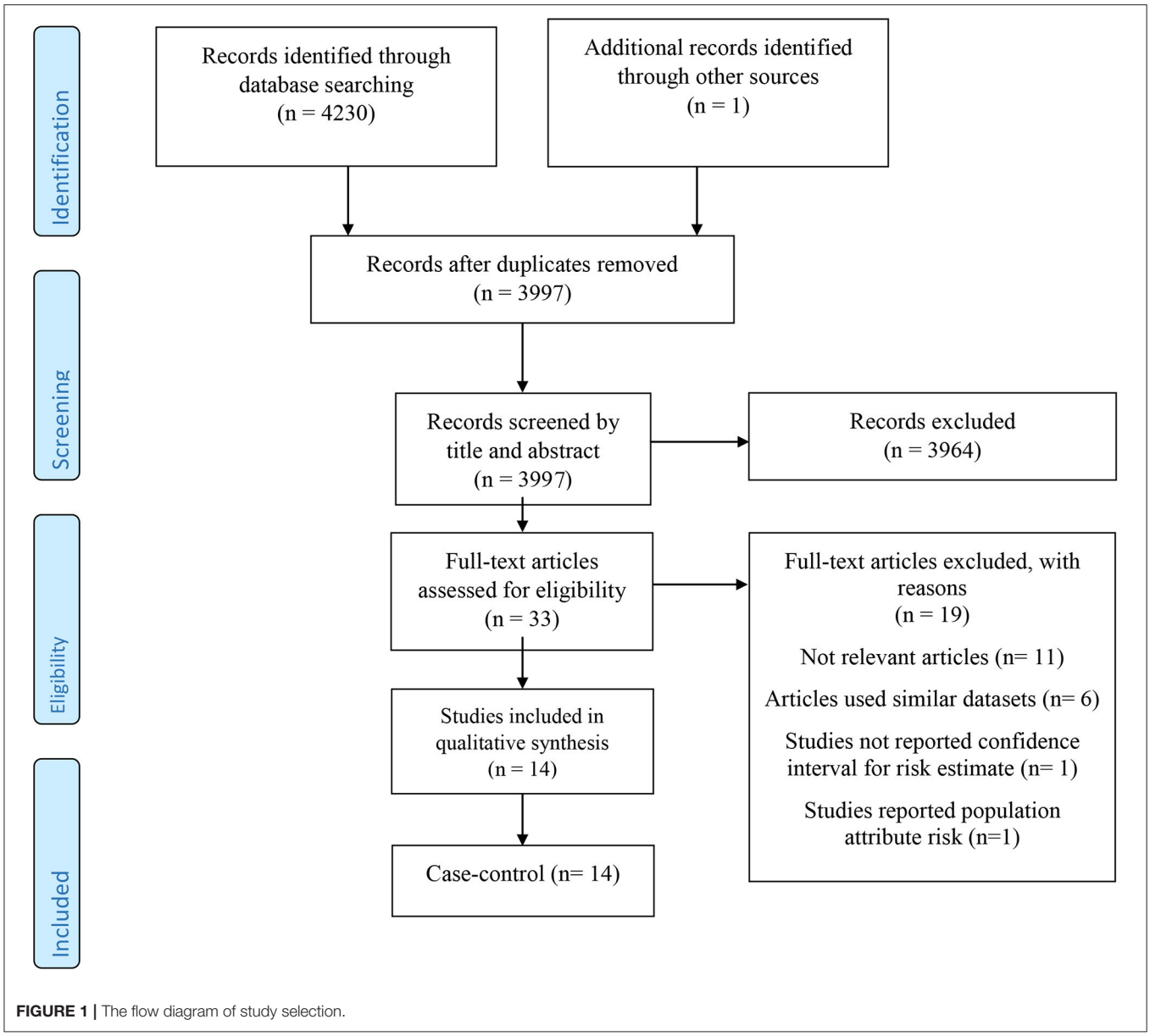

FIGURE 1 | The flow diagram of study selection.

(yes/no) for *H. pylori* infection, energy intake, and body mass index (BMI). In studies that reported the separate risk estimates for each gender, we first combined the risk estimates using a fixed model and then entered them into the final analysis.

We used the generalized least-squares trend estimation method to conduct a linear dose–response analysis (21, 22). Estimated study-specific slope lines were combined to create an average slope using a random-effects model. Studies that reported the number of cases and controls, the mean/median intake of cholesterol, and the RRs with a 95% CI for at least three exposure categories were eligible for dose–response analysis. For studies that only reported the total number of cases and controls, we estimated the number of cases and controls in each category by dividing the total number by the number of categories.

In non-linear dose–response analysis, exposures were modeled using restricted cubic splines with three knots at percentiles of 10, 50, and 90% of the distribution. The correlation within each set of provided risk estimates was taken into account, and the study-specific estimates were combined using a one-stage linear mixed-effects meta-analysis. The significance for non-linearity was determined by null hypothesis testing, where the coefficient of the second spline was considered equal to zero.

Publication bias was identified using Egger's linear regression test and funnel plot inspection (23). Sensitivity analysis was done using a random-effects model to assess the impact of each study on the overall risk estimate. This analysis was carried out by excluding each study and reanalyzing the data. All analyses were done using STATA version 16.0, and $P < 0.05$ was considered statistically significant for all tests.

TABLE 1 | Characteristics of included studies on the association between cholesterol intake and gastric cancer in adults aged >18 years in case-control studies.

References	Country	Age*	Age (cases)	Age (controls)	Cases *n*	Control *n*	Exposure assessment	Median/cutoff point	OR (95%CI)	Adjustment
Buiatti et al. (24)	Italy	<70	NR	NR	M/F:1,016	M/F:1,159	FFQ/interview	142 mg/d 199 mg/d 242 mg/d 300 mg/d 434 mg/d	1 0.9 (0.7–1.2) (0.8–1.4) 1.3 (0.9–1.7) 1.2 (0.8–1.6)	Age, sex, area, place of residence, migration from south, socioeconomic status, familial GC history, and BMI
Hu et al. (26)	Canada	20–76	61.9	56.8	M:802 F:379	M:2,547 F:2,492	FFQ/self-report	≤966.26 mg/wk 966.26–1412.75 mg/wk 1412.75–1880.26 mg/wk ≥1880.26 mg/wk	1 1.10 (0.87–1.39) 1.41 (1.10–1.80) 1.60 (1.21–2.13)	Sex, age group, province, education, body mass index, alcohol drinking, pack year smoking, total of vegetable and fruit intake, saturated fat, and total energy intake
Kim et al. (27)	South Korea	57.2 ± 0.84	57.2 ± 1.19	57.2 ± 1.20	M:92 F:44	M/F:136	M:92 F:44	Cases: 123 mg/d 174 mg/d 240 mg/d Controls: 140 mg/d 185 mg/d 248 mg/d	1 0.62 (0.34–1.13) 0.51 (0.25–1.05)	Age, sex, socioeconomic status, family history, refrigerator use, and *Helicobacter pylori* infection.
Lazarevic et al. (28)	Serbia	NR	NR	NR	M/F:102	M/F:204	FFQ/interview	NR	1 0.92 (0.86–3.59) 0.79 (0.37–2.28)	Age, sex, residence, education, physical activity, total energy intake, tobacco smoking, and history of cancer in the first degree
Lissowska et al. (16)	Poland	NR	NR	NR	M:175 F:99	M:304 F:159	FFQ/interview	<144.6 mg/d 144.6–167.9 mg/d 168–196.1 mg/d >196.1 mg/d	1 1.08 (0.71–1.64) 0.94 (0.61–1.43) 1.57 (0.89–2.78)	Age, sex, education, smoking, and calories from foods
López-Carrillo et al. (29)	Mexico	>20	24–88	20–98	M:121 F:99	M:301 F:451	FFQ/interview	≤190.5 mg/d 190.51–264.03 mg/d 264.04–359.51 mg/d ≥359.52	1 1.58 (0.87–2.87) 1.77 (0.96–3.24) 2.39 (1.23–4.64)	Age, gender, total calories, chili-pepper consumption, socio-economic status, cigarette smoking, salt consumption, history of peptic ulcer, type of interview, duration of interview, place of interview
Lucenteforte et al. (15)	Italy	22–80	22–80	22–80	M:143 F:87	M:286 F:261	FFQ/interview	NR Per 105 mg/d	1 0.97 (0.64–1.47) 1.27 (0.86–1.89) Continuous: 1.11 (0.94–1.32)	Age and sex, year of interview, education, physical activity, body mass index, tobacco smoking, family history of stomach cancer and total energy intake
Mayne et al. (13)	US	30–79	64.2	61.8	M:467 F:140	M:543 F:145	FFQ/interview	NR	Gastric cardia adenocarcinoma 1 1.50 (1.19–1.90) Non-cardia gastric cancer 1 1.68 (1.35–2.09)	Sex; site; age; race; proxy status; income; education; usual body mass index; cigarettes/day; years of consuming beer, wine, and liquor; and energy intake.

(Continued)

TABLE 1 | Continued

References	Country	Age*	Age (cases)	Age (controls)	Cases *n*	Control *n*	Exposure assessment	Median/cutoff point	OR (95%CI)	Adjustment
Qiu et al. (30)	China	NR	30–85	28–82	M:81 F:22	M:95 F:38	FFQ/interview	NR	Males: 1.0 1.08 (0.40–2.87) 2.53 (0.99–6.44) 2.76 (1.01–7.53) Females: 1.0 6.05 (0.53–69.17) 5.31 (0.44–63.44) 11.9 (0.97–146.53)	Age, present residence, education, economic status, smoking, alcoholics, and total calories intake
Tan et al. (31)	US	40–80	NR	NR	M:411 F:12	M:1,796 F:1,630	FFQ/self-report	NR	1 1.10 (0.88–1.37) 0.88 (0.67–1.16)	Age, gender, race/ethnicity, smoking status, alcohol status, body mass index, *H. pylori* infection, and total energy intake
Toorang et al. (32)	Iran	≥40	64.3 ± 12.2	53.9 ± 11.6	M:158 F:59	M:132 F:55	FFQ/interview	NR Cases: Per 249 mg/d Controls: Per 246 mg/d	1 1.88 (1.09–3.2) 2.22 (1.28, 3.85) Continuous: (1.00, 1.01)	Age, gender, energy, education, smoking, and body mass index
Zhu et al. (14)	China	NR	64.1 ± 10.8	64.0 ± 11.3	M:1,401 F:499	M:4,713 F:1,819	FFQ/interview	<107.24 mg/d 107.24–207.21 mg/d 207.21–352.09 mg/d >352.09 mg/d Per 250 mg/d	1 1.06 (0.87, 1.29) 1.32 (1.08, 1.61) 1.57 (1.26, 1.96) Continuous 1.13 (1.06, 1.22)	Study area, age, gender, education level, income 10 years ago, smoking, alcohol consumption, family history of stomach cancer, H. pylori infection, BMI, exercise 10 years ago, dietary sodium intake, and total energy intake
Harrison et al. (25)	US	NR	62 + 11.7	54.2 + 13.5	M:24 F:67	M:62 F:70	FFQ/self-report	NR	Intestinal: 1 (0.7–1.4) Diffuse: 1 1.3 (0.9–1.8)	Age, gender, calorie intake, race, education, smoking, alcohol drinking, BMI
Wu et al. (33)	US	30–74	NR	NR	M/F:192	M/F:343	FFQ/interview	NR	Gastric cardia: 1 1.73 (0.8–3.9) 1.71 (0.8–3.8) 1.83 (0.8–4.0) Distal gastric 1 2.16 (0.95–4.9) 2.20 (0.98–4.9) 2.90 (1.3–6.3)	Age, sex, race, birthplace, education, smoking, body size, reflux, use of vitamins and total calories

OR, Odds Ratio; CI, confidence interval; GC, gastric cancer; M, male; F, female; FFQ, food frequency questionnaire; BMI, body mass index; US, United States; NR, not-reported; wk, week.
**Presented as mean or rang.*

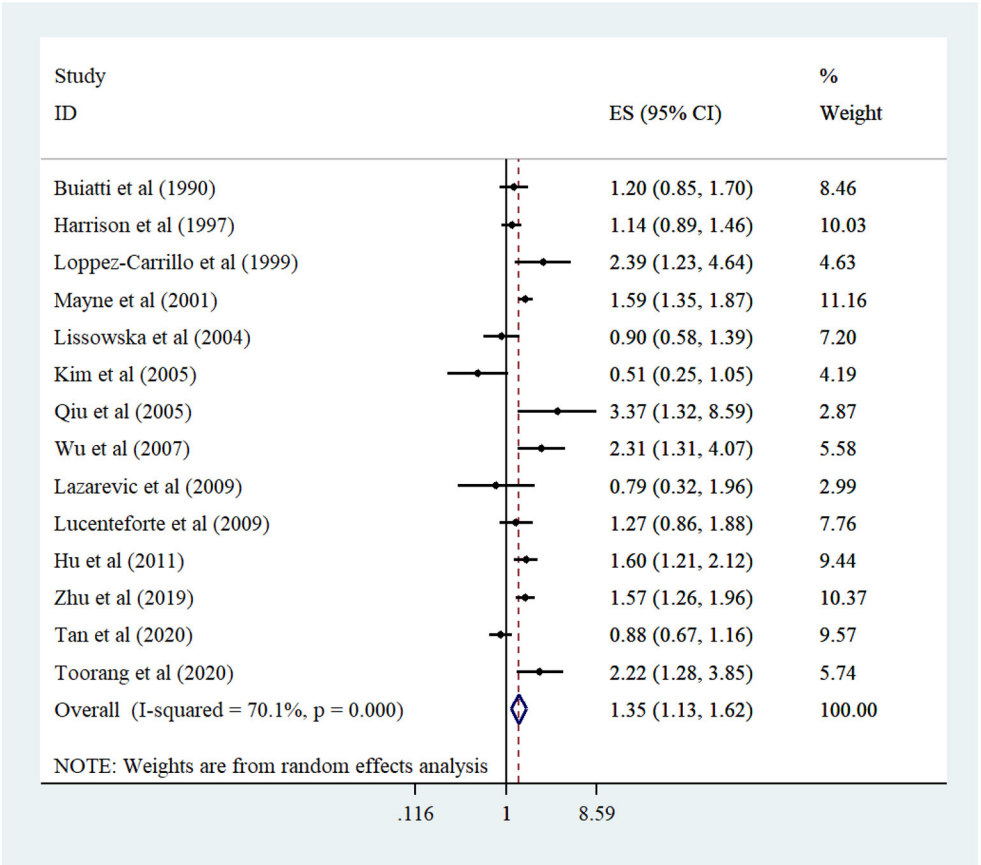

FIGURE 2 | Forest plot derived from random-effects meta-analysis of studies investigating the association between high vs. low intake of dietary cholesterol and gastric cancer in adults. CI, confidence interval; ES, effect size.

RESULTS

After removing 234 duplicate articles from a total of 4,231 papers identified through the initial search, 3,997 papers remained for reviewing the title and abstract. At this stage, 3,964 publications were excluded, and the full texts of 33 remaining articles were checked. Among 19 studies that were eliminated in this step, six used similar datasets, one did not report the CI, 11 were irrelevant, and one reported the population-attributable risk. Finally, 14 case–control studies were eligible for our systematic review and meta-analysis [(13–16, 24–33); **Figure 1**].

Study-specific characteristics are illustrated in **Table 1**. Nine population-based case–control (13, 14, 16, 24, 26, 29–31, 33) and five hospital-based case–control studies (15, 25, 27, 28, 32) published from 1990 to 2020 met our criteria. In total, 6,490 GC patients and 17,793 controls aged between 20 and 98 years were included. Studies were conducted in United States ($n = 4$) (13, 25, 31, 33), Italy ($n = 2$) (15, 24), China ($n = 2$) (14, 30), Canada ($n = 1$) (26), Mexico ($n = 1$) (29), Poland ($n = 1$) (16), South Korea ($n = 1$) (27), Serbia ($n = 1$) (28), and Iran ($n = 1$) (32). All studies were conducted on both genders and used food frequency questionnaires (FFQs) for dietary assessment. In 11 case–control studies, matching for age and gender was carried out between the case and control groups (13–16, 24, 26–30, 33). Furthermore, some important covariates, including *H. pylori* infection ($n = 4$) (14, 27, 31, 33), total energy intake ($n = 13$) (13–16, 24–26, 28–33), BMI ($n = 8$) (13–15, 24–26, 31, 32), alcohol consumption ($n = 6$) (13, 14, 25, 26, 30, 31), and smoking ($n = 12$) (13–16, 25, 26, 28–33), were adjusted in the analysis. According to quality assessment findings, nine studies were classified as high-quality (score of ≥7) studies [(13, 15, 16, 24, 26, 29, 30, 32, 33); **Supplementary Table 2**].

Meta-Analysis

In total, 14 case–control studies (13–16, 24–33) were included in the analysis of the highest vs. the lowest dietary cholesterol intake and risk of GC. The meta-analysis indicated an increased risk of GC among participants who consumed the greatest amount of cholesterol compared to participants with the lowest cholesterol intake (pooled OR: 1.35, 95% CI: 1.29–1.62, $I^2 = 68\%$; 95% CI: 45–81%) (**Figure 2**). Subgroup analysis and meta-regression failed to detect potential sources of heterogeneity. Furthermore, subgroup analysis indicated a positive relationship between dietary cholesterol and GC in population-based case–control studies, studies conducted in non-US countries, those with a higher number of GC

TABLE 2 | Summary risk estimates for the association between cholesterol intake and risk of gastric cancer in adults aged ≥18 years in case-control studies[a].

	#RR[b]	Pooled RR (95% CI)[c]	I^2 (%)[d]	*P*-heterogeneity[e]	Meta-regression
The highest vs. lowest comparison					
Dietary cholesterol intake					
Overall	14	1.35 (1.129–1.62)	70.1	<0.001	–
Subgroup analysis					
Study location					
US	4	1.32 (0.94–1.84)	83.8	<0.001	0.898
Non-US	10	1.37 (1.08–1.74)	63.3	0.004	
Study design					
Population-based case-control study	9	1.45 (1.17–1.79)	72	<0.001	0.309
Hospital-based case-control study	5	1.13 (0.78–1.64)	64.5	0.024	
Study quality					
High quality	9	1.56 (1.28–1.89)	53.1	0.029	0.146
Low quality	5	1.01 (0.73–1.41)	76.3	0.002	
Number of cases					
<400	9	1.37 (1.00–1.89)	69.6	0.001	0.664
≥400	5	1.35 (1.08–1.68)	74.9	0.003	
Exposure reporting					
Interview	11	1.43 (1.16–1.77)	64	0.002	0.441
Self-report	3	1.16 (0.84–1.61)	77.5	0.012	
Adjustment for *H. pylori*					
Yes	4	1.17 (0.71–1.91)	85.6	<0.001	0.471
No	10	1.41 (1.17–1.70)	56.9	0.013	
Adjustment for BMI					
Yes	8	1.35 (1.14–1.61)	67.6	0.003	0.947
No	6	1.35 (0.77–2.39)	76.9	0.001	
Dietary cholesterol intake (per 100 mg/d increase)					
Overall	8	1.05 (0.99–1.12)	83.5	<0.001	
Subgroup analysis					
Study design					
Population-based case-control study	5	1.10 (1.04–1.16)	39.7	0.156	0.153
Hospital-based case-control study	3	0.96 (0.83–1.12)	72.8	0.025	
Study quality					
High quality	6	1.07 (0.99–1.15)	74.8	0.001	0.222
Low quality	2	0.93 (0.64–1.35)	90.7	0.001	
Number of cases					
<400	5	1.01 (0.90–1.13)	71.2	0.008	0.335
≥400	3	1.10 (1.05–1.15)	30	0.24	
Adjustment for *H. pylori*					
Yes	2	0.93 (0.64–1.35)	90.7	0.001	0.407
No	6	1.07 (0.99–1.15)	74.8	0.001	
Adjustment for BMI					
Yes	5	1.07 (1.00–1.15)	86.7	<0.001	0.421
No	3	0.97 (0.74–1.26)	83.8	0.002	

[a] *BMI, body mass index; CI, confidence interval; RR, Relative Risk; FFQ, food frequency questionnaire; US, United States.*
[b] *Number of risk estimates.*
[c] *Obtained from the random-effects model.*
[d] *Inconsistency- the percentage of variation across studies due to heterogeneity.*
[e] *Obtained from the Q-test.*

patients (≥400), high-quality studies, those that collected dietary data through interviews, studies not adjusted for *H. pylori* infection, and studies where the BMI was controlled (**Table 2**). In addition, sensitivity analysis did not show evidence for the impact of each study on the overall risk estimate (**Supplementary Figure 1**). No evidence of publication bias was observed through the Egger test ($P = 0.83$) and funnel plot (**Supplementary Figure 2**).

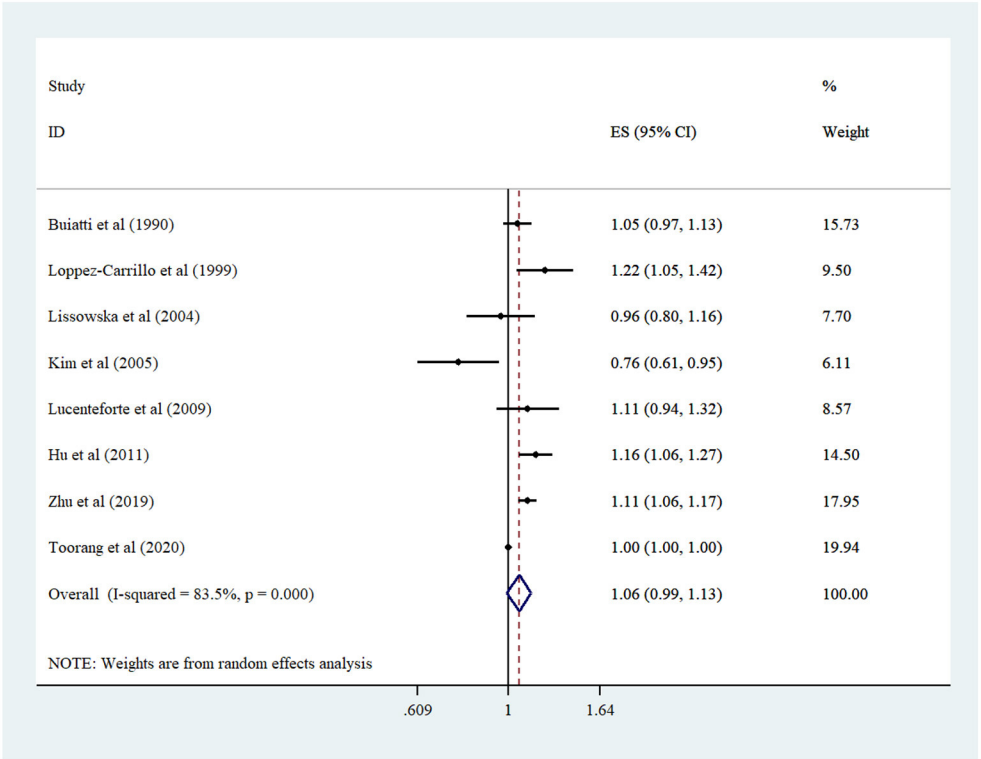

FIGURE 3 | Forest plot derived from random-effects meta-analysis of studies investigating the association between 100 mg/d increment in cholesterol intake and gastric cancer in adults. CI, confidence interval; ES, effect size.

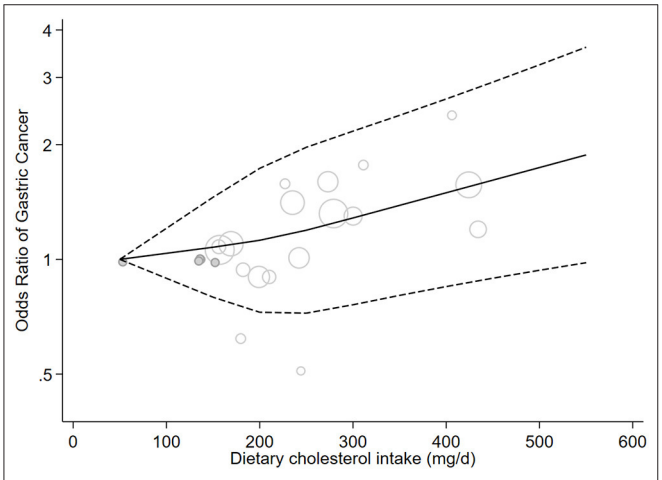

FIGURE 4 | Non-linear dose-response meta-analysis of case-control studies investigating the association between cholesterol consumption and risk of gastric cancer in adults ($P = 0.03$).

Findings from linear dose–response analysis demonstrated that a 100 mg/d increment in cholesterol intake was not associated with the risk of GC (pooled OR: 1.05, 95% CI: 0.99–1.12, $I^2 = 84\%$; 95% CI: 69–91%) (**Figure 3**). Sensitivity analysis was done to assess the effect of each study on the overall effect size (**Supplementary Figure 3**). Because the study of Toorang et al. had a major effect on the main analysis, we repeated the analysis once without it. Here, a marginally significant association was identified between a 100 mg/d increment in cholesterol intake and GC (pooled OR: 1.07, 95% CI: 1.00–1.15, $I^2 = 65\%$; 95% CI: 22–85%). The study design and the number of cases were sources of heterogeneity in the subgroup analysis. Besides, a positive association was seen in population-based case–control studies, studies with higher cases, and studies adjusted for BMI (**Table 2**). Moreover, there was no evidence of publication bias in the Egger test ($P = 0.18$) and funnel plot (**Supplementary Figure 4**).

A non-linear dose–response association was observed between dietary cholesterol intake and the risk of GC ($P = 0.03$; **Figure 4**).

DISCUSSION

In this systematic review and meta-analysis of 14 case–control studies, we found that higher intakes of dietary cholesterol were associated with a 35% greater risk of GC among adults. In addition, a non-linear dose–response relationship was observed. This study is the first systematic review and meta-analysis to examine the relationship between cholesterol intake and the risk of GC.

Cholesterol plays a vital role in maintaining cellular homeostasis in the body (34). Major dietary sources of cholesterol

include red meat, processed meat, egg yolks, dairies, fish, butter, cheese, shrimp, and poultry (35). Considering that a high-cholesterol diet might represent an unhealthy dietary pattern and lead to chronic diseases such as cancer and cardiovascular diseases (36, 37), the relationship between dietary cholesterol and the risk of cancer has received much attention (11, 12). This meta-analysis suggests that high dietary cholesterol intake may elevate the odds of GC. In line with our finding, one hospital-based case–control study in Spain found a positive relationship between cholesterol consumption and GC (38). Jung et al. (39) also expressed that high serum cholesterol was linked to the incidence of GC. Furthermore, some meta-analyses found a significant positive association between dietary cholesterol intake and cancers of the ovaries, breasts, pancreas, esophagus, and lungs (9–12, 34).

In contrast, in two meta-analyses, intake of red meat and eggs (rich sources of cholesterol) was not associated with the risk of GC (40, 41). Given that cholesterol is consumed in combination with other compounds such as salt, nitrates, multivitamins, minerals, and high-quality protein, the interaction between different nutrients prevents us from understanding the individual effect of cholesterol. We know that cholesterol is found in animal foods and high-cholesterol diets are poor sources of plant foods, including fruits and vegetables. Evidence indicates that people who consume high amounts of vegetables and fruits have a lower risk of GC (42, 43). This effect might be due to the presence of many antioxidants (particularly vitamin C, vitamin E, and carotenoids) in fruits and vegetables, which possess anticarcinogenic properties (44). In addition, an inverse association was seen between serum cholesterol concentrations and the occurrence of GC in some cohort studies (45, 46). The amount of cholesterol in cancer cells is higher than the normal cells, and cholesterol helps in cancer promotion (47). It is still ambiguous whether low serum cholesterol is a cause or effect in relation to GC, and this issue needs to be examined. Therefore, it is likely that dietary cholesterol increases the risk of cancer without augmenting blood cholesterol levels.

The inconsistencies among studies may be explained by variations in study design, geographic regions, adjustments, reporting of dietary data, quality of studies, and/or the number of cases. It has been shown that *H. pylori* infection, smoking, alcohol consumption, obesity, salt-rich diet, nitrites, and hot meals are the determinants of GC (48, 49). High dietary cholesterol intake may take part in GC initiation or progression by supporting *H. pylori* infection. *H. pylori* infection leads to gastric atrophy and hypochlorhydria, which promote the colonization of acid-intolerant bacteria (50) and elevate the occurrence of GC (51). Our findings indicated no association between dietary cholesterol intake and GC after adjusting our results for *H. pylori* infection. Furthermore, most of the included studies were adjusted for smoking and energy intake, which are the critical risk factors of GC. Besides, we found a significant positive association between cholesterol intake and GC in studies adjusted for BMI.

There are some potential mechanisms regarding the relationship between cholesterol and GC. Dietary cholesterol might play a role in cancer development via changes in lipid metabolism, which are related to cellular inflammation (52). An increase in total cholesterol and LDL as well as a decrease in HDL could induce the production of inflammatory biomarkers such as interleukin-6 and tumor necrosis factor-α (53).

This study possessed some strengths. First, linear and non-linear dose–response analyses help us to reveal the shape and strength of probable association. Second, most of included studies applied an interview-administered questionnaire. Self-reported questionnaires for cholesterol intake assessment might inevitably lead to some misclassification of participants in terms of exposure. Third, most studies took into account a wide range of important confounding factors, including energy intake, smoking, alcohol consumption, and BMI. Finally, publication bias was not detected. Nonetheless, our study had some limitations. First, based on our knowledge, there was no cohort study to examine the association between dietary cholesterol and GC. Because case–control studies have diverse kinds of bias, including selection bias, recall bias, and measurement bias, the case–control nature of included studies prevented us from reaching a decisive conclusion. Second, some fundamental residual confounders such as *H. pylori* infection, dietary factors (salt, nitrates, etc.), and lipid-lowering medications (especially statin use) were ignored in the adjustments of most studies. Third, although we tried to detect the sources of heterogeneity among studies, we could not find them through subgroup analysis and meta-regression. Due to a limited number of studies, we could not perform subgroup analysis for other potential relevant factors. Finally, measurement errors are unavoidable in estimates of dietary cholesterol intake.

In conclusion, this review illustrated an association between high dietary cholesterol intake and GC development in case–control studies. This study suggests the importance of dietary cholesterol modification in the prevention of GC. Considering that all of the included studies had case–control designs prone to biases, these results warrant cohort investigations. Large, long-duration, prospective cohort studies that consider the important dietary and non-dietary covariates are obligatory to achieve a comprehensive understanding of this matter.

AUTHOR CONTRIBUTIONS

PM and LG designed the work, extracted the data, analyzed the data, and critically reviewed the manuscript. LG wrote the first draft of the manuscript. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.722450/full#supplementary-material

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Sub-Acute Toxicity Effects of Methanolic Stem Bark Extract of *Entada abyssinica* on Biochemical, Haematological and Histopathological Parameters in Wistar Albino Rats

Samuel Baker Obakiro[1,2,3]*, *Ambrose Kiprop*[2,3], *Elizabeth Kigondu*[4], *Isaac K'owino*[3,5], *Kenedy Kiyimba*[1,6], *Charles Drago Kato*[7] and *Yahaya Gavamukulya*[8]

[1]Department of Pharmacology and Therapeutics, Faculty of Health Sciences, Busitema University, Mbale, Uganda, [2]Department of Chemistry and Biochemistry, School of Sciences and Aerospace Studies, Moi University, Eldoret, Kenya, [3]Africa Centre of Excellence II in Phytochemicals, Textile and Renewable Energy (ACE II PTRE), Moi University, Eldoret, Kenya, [4]Centre of Traditional Medicine and Drug Research, Kenya Medical Research Institute, Nairobi, Kenya, [5]Department of Pure and Applied Chemistry, Faculty of Science, Masinde-Muliro University, Kakamega, Kenya, [6]Department of Pharmacology and Toxicology, School of Pharmacy, Kampala International University, Bushenyi, Uganda, [7]Department of Biotechnical and Diagnostic Sciences, College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda, [8]Department of Biochemistry and Molecular Biology, Faculty of Health Sciences, Busitema University, Mbale, Uganda

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***Correspondence:**
Samuel Baker Obakiro
sobakiro@gmail.com

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Background: Whereas the efficacy of *Entada abyssinica* (fabaceae) extracts against various ailments has been scientifically validated, its safety has not been established. This study was undertaken to evaluate the toxicity effects of methanolic stem bark extract of *E. abyssinica* on biochemical, haematological and histological parameters of Wistar albino rats following repeated oral administration.

Methods: Wistar albino rats of both sexes were randomized into groups and orally administered daily with determined doses (150, 300 and 600 mg/kg) of *E. abyssinica* methanolic extract using 1% tween 80 in distilled water as a control for 28 days. On the 29th day, all the animals were sacrificed and dissected to collect blood and selected organs. The serum and whole blood were assayed for biochemical and haematological parameters respectively while selected organs were examined for histopathological lesions. Numerical data was analyzed using graph pad prism and expressed as mean ± standard error of mean. The differences between the treatment and control groups were tested for statistical significance using one-way analysis of variance and/or Student's *t*-test.

Results: In repeated daily oral doses (150, 300 and 600 mg/kg), the methanolic stem bark extract of *E. abyssinica* did not cause significant alteration in majority of the biochemical and hematological indices. However, the extract significantly elevated the level of uric acid (all doses), aspartate aminotransferase (300 and 600 mg/kg), low density lipoproteins (150 mg/kg) and mean corpuscular heamoglobin concentration (all doses). On the other hand, the extracts reduced high density lipoproteins (150 and 300 mg/kg), mean corpuscular volume (all doses), haematocrit (150 and 600 mg/kg), mean platelet volume (150 and 600 mg/kg) and procalcitonin (150 mg/kg). In the vital organs, there

were no significant lesions observed except at the highest dose (600 mg/kg) where there was mild evidence of lymphocyte infiltration in the liver and focal interstitial nephritis.

Conclusion: The methanolic stem bark extract of *E. abyssinica* is relatively safe in Wistar albino rats when repetitively administered orally in small doses for a prolonged period of time. We recommend more chronic toxicity studies and clinical trials on herbal remedies containing this plant to ensure that its use is free of potential toxicity to humans.

Keywords: toxicity, fabaceae, traditional medicine, *Entada abyssinica*, biochemical, haematological, histopathalogical, wistar albina rats

INTRODUCTION

Globally, approximately 80% of the world's population depend on nonconventional therapies for primary health care with herbal products being the most widely utilized (WHO Report on Traditional Medicine, 2019). This is because plants contain abundant secondary metabolites (phytochemicals) with potential pharmacological activity against various diseases. Therefore, the use of herbal medicines in management of several ailments continues to gain momentum in several communities due to their availability, affordability, perceived effectiveness and safety (Omara et al., 2020; Schultz et al., 2020; Tugume et al., 2016). Their use in management of infectious diseases and cancer is even expected to increase due to increasing development of resistance to the available chemotherapeutic agents (Obakiro et al., 2020; Omara et al., 2020)

Toxicity of herbal remedies remains a huge challenge that limits their use despite the general public belief that they are safe and devoid of potential toxicities (WHO Report on Traditional Medicine, 2019). The common toxicities are hepatotoxicity, nephrotoxicity, neurotoxicity, pulmonary toxicity, cardiac toxicity, adult respiratory distress syndrome, seizures, and acute eosinophilic pneumonia (Ko, 2004; Obakiro et al., 2018). The cause of toxicity may be due to presence of inherent toxic secondary metabolites, preparation procedure of the herbal product, variability in active and/or toxic ingredients due to growth conditions and soil chemistry, misidentification of herbs during harvesting, contamination by pathogenic fungi during storage and transport, and adulteration (Anywar et al., 2021; Selamoglu, 2021). Therefore, the World Health Organization (WHO) recommends that herbal remedies undergo rigorous scientific testing for both efficacy and safety so as to protect the public against exposure to poisonous phytochemicals.

E. abyssinica (fabaceae) is a deciduous tree with limited branching, spreading flat crown and grows to a height of about 7–10 m tall mainly in East and central Africa. Its stem bark is grey to reddish and the leaves are alternate, pinnate with a round to slightly obtuse apex. The inflorescence has creamy white or fading yellowish, sweet scented flowers. Its fruits are large, flat legumes which splits open to release oval and flat seeds. In several communities, *E. abyssinica* is grown for ornamental and cultural purposes but also harvested from the wild for fibre and wood (Kakudidi, 2004). Traditionally, the stem bark of *E. abyssinica* is harvested and used in preparation of herbal remedies for symptoms of tuberculosis, ulcers, abortion, asthma, cancers, bacterial, and fungal infections (Bunalema et al., 2014; Tibiri et al., 2007; Wagate et al., 2010). Several scientific studies have validated the various pharmacological potential of this plant and reported significant findings. These include; antimycobacterial (Magadula et al., 2012; Mariita et al., 2010), anti-inflammatory (Olajide and Alada, 2001), antibacterial, antifungal, and antioxidant activities (Dzoyem et al., 2017; Tchindaa et al., 2007). Despite the sufficient evidence for its efficacy, there was little scientific evidence to support its safety with regards to its use in herbal medicines. Since there is widespread use of the stem bark of this plant in preparation of herbal remedies for management for tuberculosis and other chronic illnesses, it is necessary to evaluate the toxicity effects of this plant following prolonged repetitive administration. This study was therefore undertaken to evaluate the effect of methanolic stem bark extract of *E. abyssinica* on biochemical, haematological and histological parameters of Wistar albino rats following daily oral administration of the extracts for 28 days.

MATERIALS AND METHODS

Sample Collection, Authentication and Processing

Samples *of E. abyssinica* were collected from their natural habitats in Siaya and Kisumu counties, Western Kenya during the month of January 2020 with the help of a plant taxonomist. The stem barks of the plant were carefully harvested from mature healthy plants with minimal injury to the plant. Leaves, branches and fruits were used to prepare a voucher specimen (OSB/01/2020/001) which was deposited at the University of Eldoret Herbarium, Botany department for correct botanical authentication and reference purposes. The harvested stem barks were packed in sacks and transported to the Chemistry laboratory at Moi University for drying and pulverization. The samples were chopped into small pieces and air-dried under shade at room temperature (25.0 ± 2.0°C) for 4 weeks until a constant mass was obtained. The samples were then pulverized using an electric grinder (NutriBullet® 600 Series), packed and stored in clean labelled paper envelopes at room temperature until extraction.

Extraction

Methanol of analytical grade was purchased from Merck-Sigma Aldrich and used to extract phytochemicals from the samples. A

sample (300 g of powder) was macerated in 1,000 ml of methanol for 3 days with occasional shaking. The mixture was first filtered using cotton wool and then through Whatman's filter paper No. 1 (pore size 11 μm) to obtain the crude extract. The extract was concentrated to a minimum volume using a rotary evaporator (Hahnvapor HS-2005S) at 40°C and reduced pressure. The concentrated crude extracts were dried in a desiccator over anhydrous copper (II) sulphate to constant weight at room temperature. The concentrated crude extract was stored in clean labeled bottles at 4°C in a refrigerator until further use.

Experimental Animals and Handling

Mature healthy inbred Wistar albino rats (100–200 g) about 8–10 weeks old were purchased from the animal facility at the College of veterinary medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda. Three animals of the same sex were housed in standard wooden cages (15 × 21 × 29 cm) bedded with wood chips and equipped with continuous flow nipple watering devices. The animals were fed *ad libitum* on standard feeds, allowed free access to water and cage beddings changed every 2 days. The animals were maintained in clean animal facility at 23–27°C, with a 12-h light and darkness cycle. The animals were acclimatized to the housing conditions for 2 weeks prior to commencement of the study and were handled in conformity with guidelines for handling laboratory animals (Kilkenny et al., 2010). Animals which died before the end of the experiment and those sacrificed were pooled in a bio-hazard container and stored at −20°C (for approximately 24 h) before being incinerated. All animals at the end of the experiment were sacrificed under general anesthesia using an overdose of pentobarbitone sodium solution.

Preparation of Extract Solutions

The concentrated extract (1000 mg) were dissolved in 10 ml of 1% tween 80 in distilled water at room temperature (25.0 ± 2.0°C) to make an extract suspension of 100 mg/ml. The suspension was vortexed (Analog Vortex mixer OHAUS) for 20 min and after digitally shaken (VWR–digital shaker) for 2 h to allow maximum dissolution. The prepared extract solution was then poured in clean labelled flask for administration to the animals. All the extract solutions were freshly prepared every day for use.

Randomization, Dose Determination and Administration

The OECD 407 guidelines on oral repeated toxicity testing of chemicals in rodents were adopted (Olayode et al., 2019). Wistar Albino rats (24) of both sexes were randomized basing on their body weight into four groups (A–D). Each group consisted of six rats (three males and three females) which were housed in different cages. The administered doses (150, 300, 600 mg/kg) were determined based on the median lethal dose (4183 mg/kg) that was predetermined using the Lorke's method. Three groups (A, B and C) were orally administered different doses of the extract (150, 300, 600 mg/kg) respectively while group D received 1% tween 80 in distilled water (control) depending on their body weight daily for 28 days. The volume of the extract administered was calculated using the equation below.

$$\textit{Volume given to each animal}\ (\text{ml}) = \frac{\text{Body weight of the animal}\ (\text{kg}) \times \text{Dose}\ (\text{mg/kg})}{\text{Concentration of the extract}\ (\text{mg/mL})} \quad (1)$$

Observations of any toxic symptoms including death manifested were noted and recorded systematically daily up to the end of the experiment. Body weights of the rats were taken on day 0, 7, 14, 21, and 28. On the 29th day, the surviving rats were sacrificed under general anesthesia using anesthetic ether solution. The rats were dissected to obtain blood and vital organs (liver, kidney, heart, and spleen) for biochemical, hematological and histopathological analyses. The weights of the vital organs were measured using a digital analytical balance.

Biochemical Analysis

Blood (2 ml) was collected by cardiac puncture from each rat into non-heparinized vacutainers using syringes. Blood was centrifuged at 3,000 rpm for 5 min to obtain serum which was assayed using an automated chemistry analyzer (HumaStar 200) for levels of different biochemical parameters. Test kits for measurement of different parameters were purchased from Sigma-Aldrich and used according to the manufacturer's instructions. The parameters assayed included creatinine, urea, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), serum proteins, bilirubin, triglycerides, total cholesterol, Low density lipoproteins (LDL), high density lipoprotein (HDL) and serum electrolytes (Na^+, K^+, Ca^{2+}, Cl^-, H^+).

Hematological Analysis

Blood (2 ml) was collected by cardiac puncture from each rat into heparinized vacutainers using syringes and analyzed using an analyzer (Sysmex 1000i) for hematological counts of different parameters. These included White blood cell (WBC), neutrophils (NEUT), lymphocytes (LYMP), monocytes (MONO), eosinophil (EO), basophils (BASO), red blood cells (RBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet count (PCH).

Histopathological Evaluation

The obtained vital organs (liver, heart, spleen, and kidney) were grossly examined for the observable histomorphological changes and the weight of each organ measured using a digital weighing scale. The isolated organs were fixed in 10% (v/v) buffered formalin labeled bottles for 72 h. After fixation, the tissues were trimmed and loaded in cassettes for processing using an automated tissue processer (Leica 40). They were first dehydrated by placing them in tissue cassettes with graded alcohol concentration (70, 80, 90, and 96%, v/v) and then removed and placed into xylene solution baths to clear off the alcohol. They were then impregnated with molten wax and allowed to dry. The tissues were then sectioned by use of

TABLE 1 | Mean body weights of the rats at different days of the experiment.

Dose (mg/kg)	Mean body weights (g) at different days				
	0	7	14	21	28
150	117.4	130.3 ± 6.62	129.9 ± 5.45	140.2 ± 6.43	149.9 ± 5.5
300	124.4	152.2 ± 4.95[a]	156.8 ± 4.71[a]	155.9 ± 4.43	157.0 ± 4.26[a]
600	108.2	104.6±± 6.7	113.2 ± 4.9	110.5 ± 5.77	115.3 ± 4.68
1% tween 80 in distilled water	110.1	112.4 ± 2.3	117.1 ± 2.56	125.3 ± 3.14	129.9 ± 2.84

Data were expressed as mean ± SEM, n = *6.*
[a]*significant at p < 0.05.*

TABLE 2 | Mean levels of biochemical parameters after 28 days at different doses of the extract.

Biochemical parameters	Mean levels of biochemical parameters			
	150 mg/kg	300 mg/kg	600 mg/kg	1% tween 80 in distilled water
Alb (g/dl)	3.52 ± 0.05	3.37 ± 0.05[a]	3.21 ± 0.10[a]	3.56 ± 0.07
Total Protein (g/dl)	6.28 ± 0.13	6.65 ± 0.48	6.617 ± 0.16	6.89 ± 0.23
ALP (U/L)	399.20 ± 69.08	330.50 ± 35.73	447.00 ± 100.10	255.70 ± 43.84
AST (U/L)	145.70 ± 6.47	169.70 ± 16.27[a]	202.3 ± 11.68[a]	119.70 ± 12.93
ALT (U/L)	100.20 ± 7.12	80.33 ± 3.02	84.50 ± 4.5	91.67 ± 6.77
Bilirubin total (mg/dl)	0.16 ± 0.02	0.18 ± 0.02	0.27 ± 0.06	0.26 ± 0.04
Bilirubin direct (mg/dl)	0.25 ± 0.09	0.13 ± 0.01	0.16 ± 0.02	0.06 ± 0.01
Creatinine (mg/dl	0.51 ± 0.04	0.61 ± 0.04	0.60 ± 0.02	0.62 ± 0.01
Urea (mg/dl)	36.12 ± 4.73	32.72 ± 2.95	25.53 ± 1.38	42.85 ± 1.56
Uric acid (mg/dl)	2.55 ± 0.25[a]	2.80 ± 0.27[a]	2.76 ± 0.32[a]	1.66 ± 0.11
Cholesterol (mg/dl)	35.6 ± 58.79	43.00 ± 3.32	46.50 ± 2.63	46.17 ± 3.93
LDL (mg/dl)	11.08 ± 1.68[a]	6.95 ± 0.87	8.45 ± 0.82	4.86 ± 1.11
Trig (mg/dl)	66.85 ± 17.94	72.83 ± 7.30	55.52 ± 10.45	84.00 ± 5.79
HDL (mg/dl)	12.19 ± 4.60[a]	16.43 ± 2.44[a]	31.65 ± 6.67	38.88 ± 4.80

Data were expressed as mean ± SEM, n = *6.*
[a]*significant at p < 0.05.*

Rotary microtome (at 5 μm thickness), and then stained with hematoxylin and eosin (H & E). Slides were prepared and then examined using a research light microscope connected to computerized camera (Lieca LB_2–image analyzer). Photomicrographs were captured and then examined for histopathological changes by two independent pathologists who were not aware of the biochemical and hematological data.

Statistical Analysis

Quantitative data was entered in Microsoft excel version 2013 and its means and standard error of mean calculated. The results were presented as means ± standard error of mean. Statistically significant differences were determined using one-way analysis of variance (ANOVA) and/or Student's *t*-test followed by Dunnett's post hoc test using Graph Pad Prism version 5.01 (Graph Pad software, San Diego, California, United States). Differences were considered statistically significant at $p < 0.05$.

Ethical Approval

The stud was approved and registered by the Scientific and Ethics Research Unit of Kenya Medical Research Institute (KEMR/SERU/CTMDR/CSCP085/4067).

RESULTS

Effect of the Extract on Body Weight

All the animals in the treatment and control groups increased in body weight over the 28-day period except for those administered with the highest dose (600 mg/kg). Over the 28-day period, the increase in body weight was only significant ($p < 0.05$) for animals dosed with 300 mg/kg of the extract. There was no statistically significant differences ($p > 0.05$) in the body weight between animals in the treatment and control group (**Table 1**).

Effect of the Extract on Biochemical Parameters

Majority of the biochemical parameters were not significantly altered by administration of the extracts except for serum albumin, AST, Uric acid, LDL and HDL (**Table 2**). All doses of the extract significantly increased the uric acid levels. The extracts (at 300 and 600 mg/kg) significantly increased ($p < 0.05$) the level of AST but significantly decreased serum albumin ($p > 0.05$). At doses of 150 and 300 mg/kg, the extract significantly decreased the level of HDL. At 150 mg/kg of the extract, the extract significantly increased the level of LDL. All these differences are in comparison with the control (1% tween 80 in distilled water).

TABLE 3 | Mean levels of electrolytes after 28 days at different doses of the extract.

Dose (mg/kg)	Mean concentration of electrolytes					
	K+ (mmol/L)	Na+ (mmol/L)	Cl– (mmol/L	ICa2+ (mmol/L)	TCa2+ (mmo/L)	pH
150	4.8 ± 0.11	143.4 ± 0.37	104.2 ± 0.65	0.22 ± 0.07	0.42 ± 0.15	7.018 ± 0.02[a]
300	5.0 ± 0.27**	144 ± 0.47	106.2 ± 0.44	0.51 ± 0.15	0.99 ± 0.29	7.01 ± 0.02[a]
600	4.52 ± 0.05	142.6 ± 0.39	105.5 ± 0.8	0.67 ± 007	1.31 ± 0.14	6.97 ± 0.01[a]
1% tween 80 in distilled water	4.29 ± 0.03	144.3 ± 0.67	104.6 ± 0.65	0.56 ± 0.06	1.06 ± 0.08	7.38 ± 0.17

Data were expressed as mean ± SEM, n = *6, Intracellular calcium (ICa^{2+}), Total calcium (TCa^{2+}).*
***Significant elevation of Potassium ions.*
[a]significant at $p < 0.05$.

TABLE 4 | Mean levels of hematological parameters after 28 days at different doses of the extract.

Haematological parameters	Mean levels of haematological parameters			
	150 mg/kg	300 mg/kg	600 mg/kg	1% tween 80 in distilled water
WBC (10*3/UL)	8.30 ± 1.62	9.45 ± 1.17	10.52 ± 1.01	12.3 ± 1.37
NEUT (10*3/uL)	1.03 ± 0.16	1.19 ± 0.17	0.795 ± 0.10	1.58 ± 0.28
LYMPH (10*3/uL)	6.37 ± 1.34	7.35 ± 0.96	8.67 ± 0.88	9.73 ± 1.17
MONO (10*3/uL)	0.78 ± 0.18	0.70 ± 0.10	0.90 ± 0.08	0.56 ± 0.11
EO (10*3/uL)	0.11 ± 0.03	0.20 ± 0.05	0.14 ± 0.03	0.19 ± 0.05
BASO (10*3/uL)	0.18 ± 0.03	0.49 ± 0.32	0.15 ± 0.03	0.26 ± 0.05
RBC (10*6/Ul)	7.30 ± 0.43	6.83 ± 1.24	7.25 ± 0.25	7.01 ± 0.31
HGB (g/dL)	13.17 ± 0.69	14.53 ± 0.46	13.28 ± 0.44	13.47 ± 0.22
HCT (%)	45.12 ± 2.09[a]	48.1 ± 1.55	44.93 ± 1.61[a]	52.83 ± 1.32
MCV (FL)	62.05 ± 1.08[a]	58.92 ± 0.29[a]	61.95 ± 0.47[a]	69.92 ± 3.45
MCH (pg)	18.08 ± 0.14	17.82 ± 0.05	18.33 ± 0.18	19.38 ± 0.87
MCHC (g/dl)	29.12 ± 0.28[a]	30.23 ± 0.14[a]	29.60 ± 0.28[a]	27.78 ± 0.22
RDW-SD (fL)	33.25 ± 0.89	33.88 ± 1.02	36.65 ± 2.51	35.23 ± 4.13
RDW-CV (%)	16.65 ± 0.80	18.35 ± 0.81[a]	17.48 ± 1.18	14.68 ± 0.69
PLT (10*3/uL)	544.00 ± 79.23	636.70 ± 64.24	663.30 ± 77.53	774.50 ± 28.42
PDW (fL)	7.83 ± 0.29	7.90 ± 0.14	8.10 ± 0.28	8.57 ± 0.15
MPV (fL)	7.53 ± 018[a]	7.38 ± 0.11[a]	7.57 ± 0.19	8.15 ± 0.11
P-LCR (%)	8.38 ± 1.16	7.28 ± 0.67	8.82 ± 1.25	10.98 ± 0.76
PCT (%)	0.41 ± 0.06[a]	0.47 ± 0.05	0.50 ± 0.05	0.63 ± 0.02

Data were expressed as mean ± SEM, (n = *6).*
[a]significant at $p < 0.05$.

Effect of the Extract on Electrolytes

Extract administration at all doses did not significantly alter the concentration of sodium, potassium, chloride, and calcium ions except at 300 mg/kg where it elevated the potassium ions (**Table 3**). On the other hand, all doses significantly elevated the hydrogen ion concentration (lowered the pH).

Effect of the Extract on Hematological Parameters

The extract did not have a significant effect ($p > 0.05$) on the white blood cells and its differentials at all doses (**Table 4**). However, it significantly ($p < 0.05$) altered some red blood differentials (HCT, MCV, MCHC, and RDW-CV) and Platelet differentials (MPV and PCT). At all doses the extract significantly reduced the MCV and HCT while increased the MCHC. MPV was significantly reduced at 150 and 300 mg/kg of extract while PCT at 150 mg/kg only.

Effect of the Extract on the Weight of Vital Organs After 28 days

The extract did not significantly alter the weight of the various organs as compared to the control group except for the liver and kidney which were significantly reduced ($p < 0.05$) at 600 mg/kg dose of the extract (**Table 5**).

Effect of Repeated Doses of the Extract on Histology of the Liver, Kidney, Heart, and Spleen

No significant organ lesions were associated with administration of the extract in the heart, liver, kidney and spleen (**Figures 1A–D**). However, at the 600 mg/kg dose, the extract caused mild to moderate multifocal parenchymal hepatocytes necrosis (**Figure 1A**), periportal mononuclear inflammatory cell infiltration and focal interstitial nephritis (**Figure 1C**). In all the treatment and control groups, the spleen exhibited

TABLE 5 | Mean weight of vital organs after 28 days at different doses of the extracts.

Dose of extract (mg/kg)	Mean organ weights (g)			
	Kidney	Liver	Heart	Spleen
150	1.03 ± 0.04	5.80 ± 0.30	0.65 ± 0.03	0.90 ± 0.08
300	1.25 ± 0.06	5.80 ± 0.20	0.67 ± 0.03	0.65 ± 0.04
600	0.80 ± 0.03[a]	4.80 ± 0.18[a]	0.55 ± 0.04	0.82 ± 0.07
1% tween 80 in distilled water	1.15 ± 0.06	5.97 ± 0.30	0.63 ± 0.02	0.93 ± 0.09

Data were expressed as mean ± SEM, n = 6.
[a]*p < 0.05.*

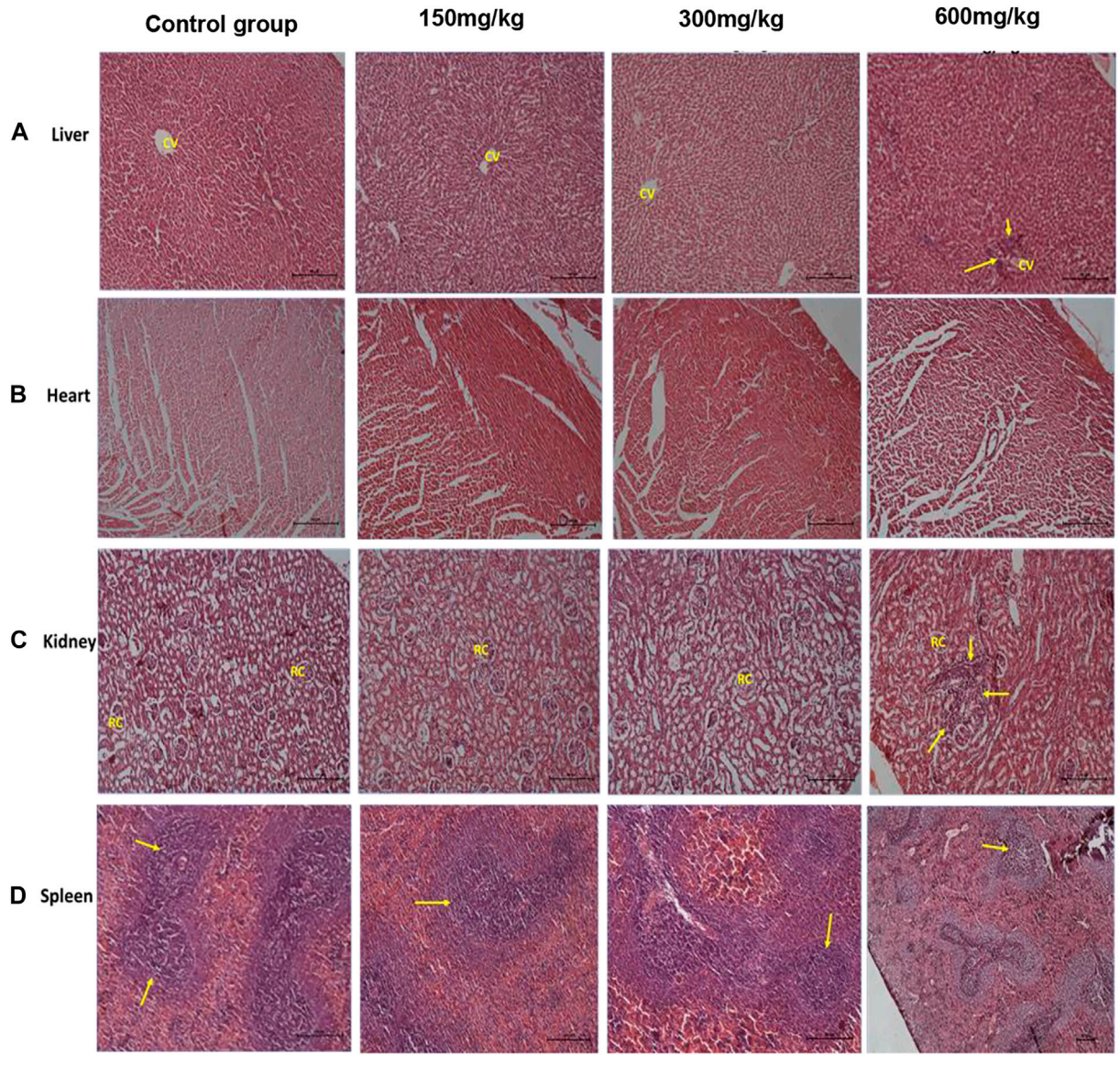

FIGURE 1 | H&E rat organ sections. Panel **(A)** shows normal liver architecture at lower doses with clear central vain, at 600 mg/kg, cellular infiltration is seen (arrow heads). Panel **(B)** shows normal heart section with clear cardiac fibers. Panel **(C)** shows normal kidney architecture at lower doses with clear renal capsules, at 600 mg/kg focal interstitial nephritis is seen (arrow heads). Panel **(D)** shows spleen white pulp hyperplasia due to non-specific immunostimulation at all levels (arrow heads). Each photomicrograph enlargement is 100 μm.

TABLE 6 | Summary of organ specific histopathology.

Organ	Histopathology in the different treatment groups			
	1% tween 80 in distilled water	150 mg/kg	300 mg/kg	600 mg/kg
Liver	*No significant lesions*	*No significant lesions*	*No significant lesions*	Mild to moderate multifocal parenchymal hepatocytes necrosis and periportal mononuclear inflammatory cells infiltration
Heart	*No significant lesions*	*No significant lesion*	*No significant lesion*	No significant lesions
Kidney	*No significant lesions*	*No significant lesion*	*Non-significant lesion*	Focal interstitial nephritis
Spleen	*Mild to moderate diffuse lymphoid hyperplasia in the follicles (non-specific immunostimulation)*	*Mild to moderate diffuse lymphoid hyperplasia in the follicles (non-specific immunostimulation)*	*Mild to moderate diffuse follicular lymphoid hyperplasia (non-specific immunostimulation)*	Mild to moderate diffuse follicular lymphoid hyperplasia (non-specific immunostimulation)

nonspecific immunostimulation as indicated by mild to moderate diffuse lymphoid hyperplasia within the white pulp (**Figure 1D**). A summary of the organ specific findings is indicated in **Table 6**.

DISCUSSION

In repeated daily oral doses for 28 days, the extract did not significantly increase the body weight over time except for the dose of 300 mg/kg. In the latter group, it is plausible that the extracts were in optimum concentration to stimulate the conversion of nutrients into body tissues. The elevated levels of uric acid in the treatment group indicate a disturbance in the nitrogen metabolism which could probably be due to presence of phytochemicals in the extracts that interact with enzymes or upset the nitrogen metabolism. Additionally, it could as well be due to the extract interfering with renal excretion of uric acid as observed from some histopathologies on the Kidney. Therefore, there is a likely risk of hyperuricemia and gout developing in patients who chronically use herbal remedies that contain this medicinal plant.

The significant increase in AST at higher doses indicated that the extracts could have caused injury to the liver, lungs, heart and kidney. But AST is a non-specific enzyme whose activity/concentration in serum could be due to injury to various vital organs in the body (Akanmu et al., 2020; Mujahid et al., 2017). Histopathological findings revealed that this increase could have been probably due to the mild damage that was observed in the liver and kidney tissues. Changes in LDL and HDL indicated dysregulation of lipid metabolism which could be due to interfering with the process of lipolysis and mobilization of free fatty acids from the peripheral depots (Oluwatoyin et al., 2008; Arunsi et al., 2020; Sowunmi et al., 2020).

The blood indices (white blood cells, red blood cells, platelets and their differentials) serve as an indicator of physiological and pathological status of the body and significant changes imply that the administered chemical is either protective or toxic to the haemopoietic tissue. Findings from our study report non-significant effects on most of the important blood indices by the methanolic extract of *E. abyssinica*. The major functions of WBCs and its differential are to provide immunity and defend the body against invasion by pathogens or toxins. Therefore, the non-significant difference in WBC count and its differentials between the treatment and control groups suggested that the administered doses did not interfere with the differentiation of haemopoietic stem cells into these parameters. The significant effect on red blood cell differentials indicated that the extract affected the process of erythropoiesis probably by the phytochemicals interfering with the secretion and/or activity of erythropoietin (Awe and Banjoko, 2013; Mugisha et al., 2014; Zaruwa et al., 2016). Diminished levels of mean platelet volume and procalcitonin could probably be due to presence of toxic phytochemicals that interfere with the functioning of thrombopoietin or cause inflammation of the bowel (Mwale et al., 2014). The significant lowering of the pH indicated the potential of the extract to cause acidosis probably by stimulating the secretion of hydrogen ions into blood and/or inhibiting their renal excretion.

The low extract dose levels (150 mg/kg, 300 mg/kg) exhibited no significant effect on the histomorphology and gross anatomy of the vital organs. However, at a high dose (600 mg/kg, the extract exhibited a significant decrease in the weight of the liver and kidney in comparison with the control. Histopathological assessment further revealed mild to moderate multifocal parenchymal hepatocytes necrosis and periportal mononuclear inflammatory cells infiltration as well as focal interstitial nephritis. These findings are indicative of infectious or inflammatory lesions although we could not ascertain or propose their actual causes (Singh et al., 2013). These results are in agreement with those reported from methanolic stem bark extract *Entada africana* (a related species) which also did not show significant effect on many biochemical and hematological parameters except a significant increase in the triglycerides and a decrease in Alanine aminotransferase (Tibiri et al., 2007). The later finding indicates the hepatoprotective effects of the extract while the former its risk of hyperlipidemia.

Phytochemical analysis of the methanolic extracts of *E. abyssinica* and *E. africana* revealed presence of alkaloids, tannins, triterpenes, flavonoids, steroid glycosides and coumarins as dominant secondary metabolites (Dzoyem et al.,

2017; Yusuf and Abdullahi, 2019). Using ThermoFinnigan LCQ-Duo ion trap mass spectrometer with an ESI source, 28 secondary metabolites were identified from the methanolic stem bark extract of *E. abyssinica*. Majority of these compounds were tannins and gallic acid derivatives. Among the compounds identified was dimethyl caffeoyl galloylglucose, with a retention time of 37.86 min and showed a molecular ion peak at (M–H)– m/z 521 with three daughter ions at 331, 271, and 169. The other compounds were cinnamoyl-O-galloylglucose, p-coumaroyl pyrogalloylgalloylglucose, quercetin galloylglucose, catechin gallate, kaempferol syringyl gallate, gentisic acid dipentoside among others (Sobeh et al., 2020). A new peltogynoid, entadanin and monoglyceride, 1′,26′-bis-[(S)-2,3-dihydroxypropyl]hexacosanedioate along with eight known compounds, were isolated from the stem bark of *E. abyssinica*. The compounds were characterized using 1D and 2D NMR spectra, in combination with high-resolution mass spectrometry, and by comparison with related data from the literature. The other compounds isolated included ursolic acid, quercetin-3-O-β-D-glucosyl (1→4)-α-l-rhamnoside, quercetin-3-O-α-l-rhamnoside (quercitrin), 13,14,15,16-tetranor-3-clerodene-12,18-dioic acid, (8S)-kolavic acid 15-methyl ester, methyl gallate (Melong et al., 2014). These chemical compounds have been reported to possess cytotoxicity, antioxidant and antimicrobial activities (Sobeh et al., 2020; Dzoyem et al., 2017). Eight compounds isolated from this plant had low cytotoxicity with cytotoxic concentrations ranging between 20 and 80 μg/ml (Dzoyem et al., 2017). Therefore these phytochemicals have good pharmacological potential and elicit no-toxicity within possible therapeutic doses (Dzoyem et al., 2017). These *in vitro* findings resonate with our *in vivo* findings which report that the methanolic stem bark of *E. abyssinica* is relatively nontoxic on many biochemical, haematological and histological indices.

CONCLUSION

The methanolic stem bark extract of *E. abyssinica* is relatively safe in Wistar albino rats when repetitively administered orally in small doses for a prolonged period of time. We recommend more chronic toxicity studies in animal and clinical trials on herbal remedies containing this plant to ensure that its use is free of potential toxicity to humans.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Scientific and Ethics Research Unit of Kenya Medical Research Institute.

AUTHOR CONTRIBUTIONS

SO conceptualized the study and applied for funding under the supervision of AK, EK, and IK'. SO conducted the laboratory experiments, data curation and analysis with technical support from KK, YG, and CD. SO, and KK drafted the manuscript which was reviewed by AK, IK', EK, YG, and CD. All Authors read and approved the final manuscript.

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frontiers in Nutrition

REVIEW
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Consumption of Coffee and Risk of Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis of Observational Studies

Jiaying Ni[1†], *Ping Wang*[2†], *Tao Zheng*[1], *Long Lv*[3] *and Hao Peng*[4*]

[1] Department of Obstetrics and Gynecology, Xinhua Hospital Affiliated to Shanghai JiaoTong University School Medicine, Shanghai, China, [2] Department of Internal Medicine, Nanjing Gaochun People's Hospital, Nanjing, China, [3] Department of General Surgery, Nanjing Gaochun People's Hospital, Nanjing, China, [4] Department of Obstetrics and Gynecology, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Edong Healthcare Group, Huangshi, China

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Mojtaba Heydari,
Shiraz University of Medical Sciences, Iran

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Elnaz Daneshzad,
Tehran University of Medical Sciences, Iran

***Correspondence:**
Hao Peng
penghao19861024@sina.com

[†]These authors share first authorship

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Objective: The results from epidemiologic studies on the relationship between intake of coffee and the risk of gestational diabetes mellitus (GDM) remain inconclusive. A meta-analysis was performed to achieve a comprehensive finding regarding the association between intake of coffee and the risk of GDM.

Methods: PubMed, Scopus, ISI Web of Science, and Google Scholar were searched to find articles published up to August 2021. Observational studies that reported risk estimates [risk ratios (RRs), hazard ratios (HRs), and odds ratios (ORs)] for the association of consumption of coffee with the risk of GDM in pregnant women were included. Random effects model was applied to calculate summarized risk estimate and 95% CIs for the highest vs. lowest categories of intake of coffee.

Results: Seven observational studies (three cohort, two case-control, and two cross-sectional studies) with 75,607 participants and 1,625 women with GDM met the inclusion criteria. The meta-analysis of comparing the highest vs. lowest intake of coffee categories showed no significant association between intake of coffee and risk of GDM (summarized risk estimate: 0.89; 95% CI: 0.76, 1.05; $I^2 = 63.4\%$). Subgroup analysis showed that consumption of coffee had an inverse relationship with GDM in studies conducted in non-Asia countries (summarized risk estimate: 0.75; 95% CI: 0.58, 0.97; $I^2 = 6\%$).

Conclusion: This study has shown that high consumption of coffee did not decrease the risk of GDM. Furthermore, large-scale cohort studies are required to confirm our findings.

Keywords: coffee, gestational diabetes mellitus, meta-analysis, systematic review, safety

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disease in which pregnant women who did not have diabetes before pregnancy develops glucose intolerance because of the interference of pregnancy hormones with the action of insulin (1, 2). It has been estimated that roughly 15 to 20% of pregnant women were affected by GDM (3). High gestational age, obesity, polycystic ovary syndrome, ethnicity, glycosuria, family history of diabetes, and previous history of GDM are underlying risk factors of GDM (4). Approximately 70% of women with GDM will be affected by type 2 diabetes mellitus (T2DM) later in their life (5). Infants born to mothers with GDM are at increased risk of macrosomia, hypoglycemia, jaundice, and epigenetic changes (6). In the long term, they are prone to being obese or diabetic in childhood (7). Therefore, the identification of preventive strategies to reduce GDM has great importance.

Diet and physical activity are fundamental lifestyle interventions to control GDM (7). Using foods with a low glycemic index, high antioxidants, and also decrease in intake of energy, distribution of carbohydrates, and intake of fat or protein modification share some dietary recommendations for women with GDM (8–11).

Coffee is commonly drunk among women aged 20–50 years, and thus their possible effects on GDM absorb many interests (12). A meta-analysis of prospective cohort studies exhibited both the caffeinated and decaffeinated coffee was associated with reduced diabetes risk (13). It appears that the influence of intake of coffee on GDM may vary from T2DM in non-pregnant women. Metabolism of caffeine as the main phytochemical in coffee decreases during pregnancy (14), and subsequently, a high amount of caffeine levels in blood was related to elevating insulin resistance (15). It seems that the favorable effects of coffee are not linked to caffeine and its metabolites during pregnancy, and originated from antioxidants and prebiotic compounds, including phenolic components and micronutrients, which can improve insulin sensitivity and glycemic response (16).

The findings of studies regarding the association of intake of coffee with GDM are conflicting. A double-blind, randomized crossover study indicated that acute caffeine ingestion impairs insulin sensitivity in women with GDM (17). Furthermore, some observational studies displayed no relationship between intake of coffee and GDM (18–21), while two studies showed inverse associations (22, 23).

Based on our knowledge, there is no systematic review and meta-analysis to clarify the association between the consumption of coffee and the risk of GDM. Therefore, the purpose of this study is to achieve a solid response to this question: Is consumption of coffee associated with the risk of GDM in pregnant women?

METHODS

The protocol of this study has been established based on Preferred Reporting Items of Systematic Reviews and Meta-Analysis (PRISMA) criteria (24).

Search Strategy

Two independent authors undertook a systematic search in PubMed, Scopus, ISI Web of Sciences, and Google Scholar to determine the pertinent articles with publication dates until August 2021. The search was performed using medical subject heading (MeSH) and related keywords including: coffee, caffeine diabetes, gestational, gestational diabetes, GDM or diabetes, and gestational (Mesh). No restrictions such as language were taken into account when the search was conducted. The citations of selected articles and retrieved reviews were manually checked to avoid missing any papers.

Inclusion and Exclusion Criteria

Studies with the following criteria were eligible for this review: observational studies (cohort, cross-sectional, or case-control studies), those carried out on pregnant women, studies reported risk estimates [relative risk (RR), odds ratio (OR), and hazard ratio (HR) with corresponding 95% CIs] for the association between consumption of coffee and the risk of GDM. Articles were included whether they considered total coffee or caffeinated coffee and decaffeinated coffee separately.

We excluded one study that assessed the association between coffee and tea and the risk of GDM (25). Tea has a different nutritional composition as compared with coffee, and therefore, the compounds in tea are likely to have different effects, and assessment of a combination of coffee and tea unable us to find a pure effect of coffee. Furthermore, irrelevant papers, abstracts, unpublished essays, review articles, commentary, editorial, or letters were removed.

Quality Assessment

Newcastle-Ottawa Scale (NOS) was used to determine the risk of bias of each article included (26). If one study acquires a score of $\geq$7, it is contemplated as high quality. Two researchers evaluated the methodological quality of each study independently. If they could not reach any consensus, a third party (Principal investigator) decided by a discussion with them.

Data Extraction and Abstraction

Two investigators exploited separately the desired information using prespecified forms, and in case when they faced disagreements, they discussed it with the third author to reach a firm opinion. The following data were extracted: surname of the first author, date of publication, study design, geographic region, age, gender, follow-up duration (in prospective cohort studies), sample size, number of cases, number of controls in case-control studies, categories of intake of coffee, estimated risk (RR, HR, OR), diagnostic criteria for GDM, dietary measurement method, and adjusted variables. In the terms of estimated risk with different adjustment models, we choose that one controlled the greatest number of main covariates. If one study reported a separate risk estimate for caffeinated and decaffeinated coffee rather than the total consumption of coffee, we included a risk estimate for caffeinated coffee in the principal analysis.

Statistical Analysis

In the meta-analysis comparing high vs. low intake categories of coffee, we used a random effects model to combine risk estimates (including RRs, HRs, and ORs) and 95% CIs of GDM. To assess the weight of each study, the standard error for the log RR/HR/OR of each study was regarded as the estimated variance of the log RR, using inverse variance methods (27). Cochrane *Q*-test and I^2-test were used to assess heterogeneity among pertinent studies. Cochrane *Q* test, with $P < 0.1$ indicating significant between-study heterogeneity. The values I^2 of 25–50, 50–75, and >75% were considered as low, moderate, and high heterogeneity, respectively (28). Subgroup analysis was implemented to identify sources of heterogeneity according to the relevant variables: geographic region, study design, number of cases, sample size, diagnostic criteria for GDM, dietary assessment method, adjustment to dietary energy intake, and body mass index (BMI), and quality of studies. Inspection of the funnel plots for asymmetry and Egger test ($P < 0.10$) were employed to detect publication bias (29). A sensitivity analysis was carried out to investigate the dependency of overall effect size to each study by leaving one study and repeating the analysis. All statistical analyses were performed using STATA software version 15.1 (Stata Corporation, College Station, Texas, USA). The $P > 0.05$ was speculated as significant.

RESULTS

The flowchart of study selection was displayed in **Figure 1**. The title and abstract of 162 records identified through the initial search were screened following inclusion and exclusion criteria. After deleting unrelated papers, the full-texts of a total of 17 remained articles were checked. In this stage, 10 articles were omitted because of the following reasons: irrelevant articles ($n = 9$) and consideration of both tea and coffee as exposure ($n = 1$). Finally, seven epidemiologic studies possessed eligibility to this study (18–23, 30).

The brief information of each selected study has been described in **Table 1**. Three cohort studies (18, 19, 30), two case-control studies (20, 22), and two cross-sectional studies (21, 23) were imported to meta-analysis. The studies were published between 2007 and 2021, and these were conducted in the US (18), Denmark (19), Indonesia (22), Oman (20), Spain (21), Malaysia (30), and Ethiopia (23). Women with GDM were aged between 13 and 49 years. A total of 75,607 pregnant women participated,

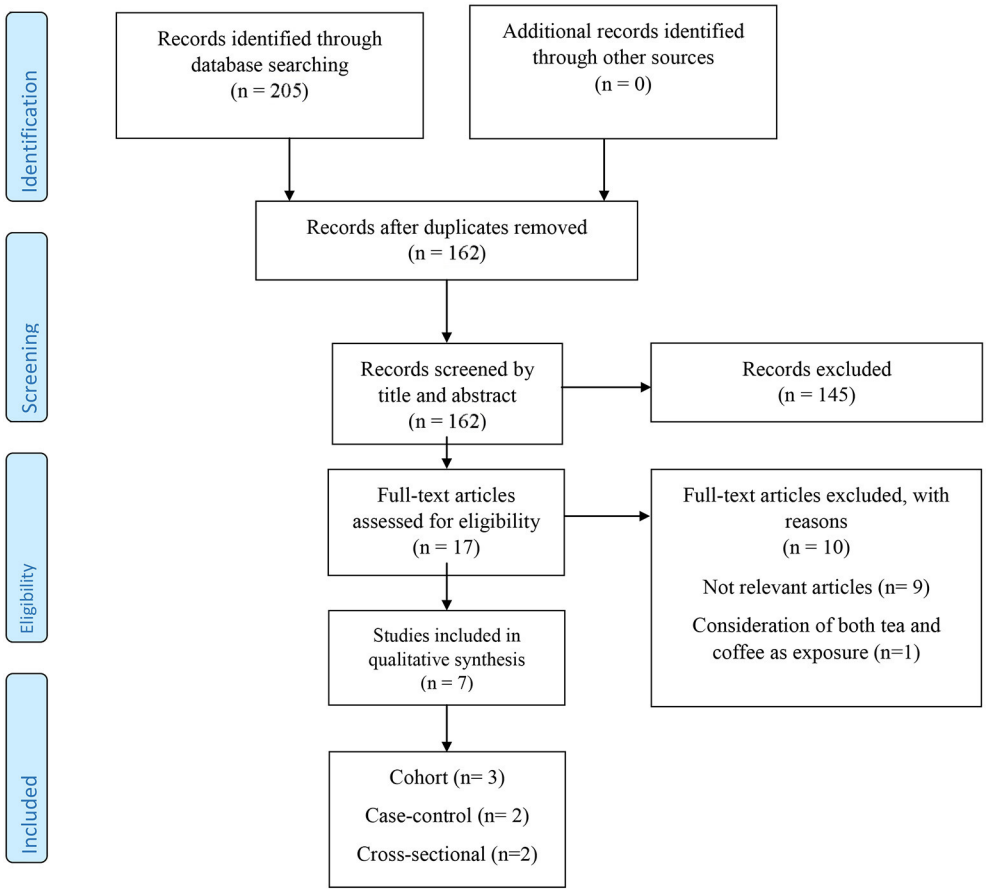

FIGURE 1 | The flow diagram of study selection.

TABLE 1 | Characteristics of included studies on the association between coffee intake and gestational diabetes mellitus.

Author	Country	Study design	Age*	Sample size	Follow up (years)	Cases	Outcome assessment	Exposure assessment	Median/cutoff point	RR (95%CI)	Quality score	Adjustment
Adeney et al. (18)	US	Cohort	32.1 ± 0.1	576	6	23	3-h OGTT	FFQ/interview	NR	1 0.64 (0.37_1.10)	7	Maternal age, smoking during pregnancy, and regular alcohol use before pregnancy, maternal race, pre-pregnancy BMI, and chronic hypertension
Hinkle et al. (19)	Denmark	Cohort	16–48	71,239	6	912	Discharge Register or self-reported on either of the DNBC interviews	Dietary questionnaire/ interview	0 0.5–3 cups/day 4–7 cups/day ≥8 cups/day	1 0.97 (0.84-1.13) 0.81 (0.64-1.02) 0.89 (0.64-1.25)	7	Coffee or tea consumption, age, socio-occupational status, parity, pre-pregnancy body mass index, smoking, and calorie intake
Amiruddin et al. (22)	Indonesia	Case-Control	NR	135	NA	45	Medical record	Dietary history questionnaire/ interview	NR	2.40 (1.10–5.25)	4	No
Chitme et al. (20)	Oman	Case-Control	15–49	591	NA	291	Literature-Based questionnaire	Literature-Based questionnaire/ interview	NR	1 1.12 (0.98–1.29)	2	No
Ramos-Levi et al. (21)	Spain	Cross-Sectional	13–47	2,194	NA	213	OS +OGTT	FFQ/self-report	0–1 cup/day >3 cup/day	1 0.7 (0.4–1.05)	5	No
Larebo et al. (23)	Ethiopia	Cross-Sectional	18–49	420	NA	110	OGTT	24 h food recall/ interview	NR	2.70 (1.04–7.00) 1	9	NR
Yong et al. (30)	Malaysia	Cohort	30.01 ± 4.48	452	NR	31	OGTT	FFQ	NR	1 0.99 (0.98–1.04)	6	Age, parity, total energy, pre-pregnancy BMI, and total gestational weight gain

RR, Relative Risk; CI, confidence interval; FFQ, food frequency questionnaire; US, United States; NR, not-reported; NA, Not applicable; wk, week; OS, O'Sullivan test; OGTT, Oral glucose tolerance test.
**Presented as mean or range.*

1,625 of whom were diagnosed with GDM. Three studies used a food frequency questionnaire (FFQ) (18, 21, 30), one 24-h food recall (23), and others used a dietary questionnaire (19, 20, 22). The method of GDM diagnosis was oral glucose tolerance test (OGTT) for four studies (18, 21, 23, 30) and medical records (22) or questionnaire (19, 20) for the others. Four studies controlled the covariates (18, 19, 23, 30), and others did not adjust. Three studies had high-methodological quality (score $\geq$ 7) (18, 19, 23) (**Table 1**).

Meta-Analysis

Three cohorts, two case-control, and two cross-sectional studies were included in the meta-analysis (18–23, 30). When extreme categories were compared, no significant association was detected between intake of coffee and risk of GDM (summarized risk estimate: 0.89; 95% CI: 0.76, 1.05; I^2 = 63.4%) (**Figure 2**). Subgroup analysis found study design, study location, sample size, dietary assessment tool, study quality, adjustment, and controlling for energy and BMI as sources of heterogeneity (**Table 2**). Furthermore, subgroup analysis showed that consumption of coffee had an inverse relationship with GDM in studies conducted in non-Asia countries (**Table 2**). Sensitivity analysis was performed to assess the effect of each study on overall effect size (**Supplementary Figure 1**). A study performed by Amiruddin et al. (22) considered both GDM and prediabetes as the outcome. Therefore, we excluded it and carried out the analysis. No significant association was found (summarized risk estimate: 0.95; 95% CI: 0.83, 1.09; I^2 = 53.6%). Furthermore, it seems that studies of Chitme et al. (20) and Yong et al. (30) had an influence on overall effect size. After removing these studies step by step and reanalyzing, we observed that high-coffee intake significantly decreased GDM (summarized risk estimate: 0.75; 95% CI: 0.57, 0.98; I^2 = 62.1%), and marginally decline risk of GDM (summarized risk estimate: 0.73; 95% CI: 0.53, 1.02; I^2 = 71.6%) compared to women with low intake, respectively. We did not identify the evidence of publication bias by Egger test (P = 0.116) and inspection of funnel plot (**Supplementary Figure 2**).

DISCUSSION

This meta-analysis of seven observational studies demonstrated that intake of coffee is not linked with GDM. However, a

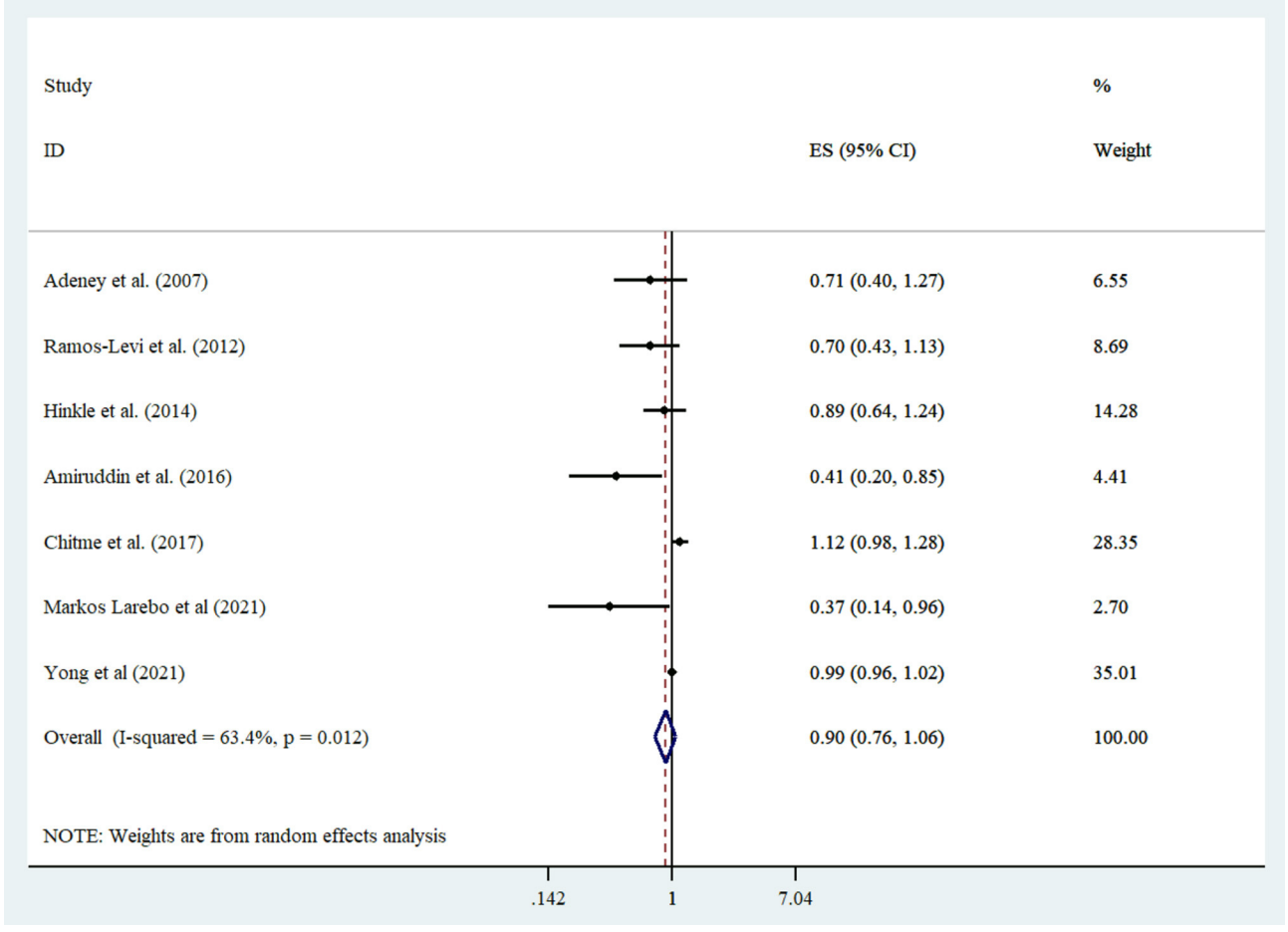

FIGURE 2 | Forest plot derived from random effects meta-analysis of studies investigating the association between high vs. low intake of consumption of coffee and gestational diabetes mellitus. CI, confidence interval; ES, effect size.

TABLE 2 | Summary risk estimates for the association between coffee consumption and risk gestational diabetes mellitus in subgroup analysis[a].

	#RR[b]	Pooled RR (95% CI)[c]	I^2 (%)[d]	*P*-heterogeneity[e]
The highest vs. lowest comparison				
Coffee intake				
Overall	7	0.89 (0.76–1.05)	63.4	0.012
Subgroup analysis				
Study location				
Asia	3	0.99 (0.82–1.19)	76.8	0.013
Non-Asia	4	0.75 (0.58–0.97)	6	0.363
Study design				
Cohort	3	0.98 (0.96–1.01)	0	0.439
Case-control	2	0.72 (0.27–1.92)	85.8	0.008
Cross-sectional	2	0.58 (0.33–1.02)	26.4	0.244
Sample size				
≤1,000	5	0.63 (0.35–1.14)	79.8	0.002
>1,000	2	0.82 (0.62–1.08)	0	0.423
Number of cases				
≤200	4	0.64 (0.39–1.06)	75.2	0.012
>200	3	0.95 (0.73–1.23)	55.5	0.106
Dietary assessment tool				
FFQ	3	0.88 (0.69–1.12)	38.2	0.199
Non-FFQ	4	0.74 (0.48–1.15)	76.3	0.005
Outcome assessment				
OGTT	4	0.77 (0.54–1.08)	58.7	0.064
Medical record/questionnaire	3	0.85 (0.57–1.27)	75.5	0.017
Adjustment				
Yes	4	0.87 (0.68–1.10)	47.3	0.128
No	3	0.74 (0.43–1.30)	79.9	0.007
Adjustment for energy intake				
Yes	3	0.98 (0.96–1.01)	0	0.439
No	3	0.74 (0.43–1.30)	79.9	0.007
NR	1	0.37 (0.14–0.96)	–	–
Adjustment for BMI				
Yes	3	0.98 (0.96–1.01)	2.1	0.312
No	3	0.74 (0.43–1.30)	79.9	0.007
NR	1	0.37 (0.14–0.96)	–	–
Study quality				
High	3	0.73 (0.49–1.08)	33.9	0.220
Low	4	0.95 (0.79–1.14)	71.8	0.014

[a] *BMI, body mass index; CI, confidence interval; RR, Relative Risk; FFQ, food frequency questionnaire; OGTT, oral glucose tolerance test.*
[b] *Number of risk estimates.*
[c] *Obtained from the random-effects model.*
[d] *Inconsistency, the percentage of variation across studies due to heterogeneity.*
[e] *Obtained from the Q-test.*

significant inverse association was indicated between intake of coffee and GDM in studies conducted in non-Asia countries. To date, no systematic review and meta-analysis addressing a correlation between intake of coffee and GDM.

Coffee is a common drink all over the world and consists of a combination of antioxidants and micronutrients, which possess favorable effects on cardiovascular disease (31, 32). In this review, we saw no significant reduced risk of GDM among high-coffee consumers compare to those with low intake. Inline with our finding, a cross-sectional study conducted on 785 adult pregnant women in São Paulo illustrated no association between consumption of coffee and tea and GDM (25). In 2018, one meta-analysis investigated the association of poly-phenol-rich foods and the risk of gestational diabetes. This study captured no connection between non-alcoholic beverages (coffee, tea, and juice) and GDM (33). On the other hand, the evidence shows a relationship between GDM and T2DM, and women with GDM

are prone to T2D in the future (7). A recent meta-analysis of 30 cohort studies depicts that the risk of T2D lowered by 6% for each cup-per-day increment in consumption of coffee (34). Furthermore, one cup increase of caffeinated and decaffeinated consumption of coffee in a day leads to a 7 and 6% reduced risk of diabetes, respectively (13). Moreover, a clinical trial study found that intake of caffeine was related to impaired insulin sensitivity in women with GDM early in their third trimester but not in controls without GDM (17).

Some potential reasons can be considered for these inconsistencies. First, intake of caffeine during pregnancy has been related to adverse outcomes including low birth weight, elevated risk of delivering, impaired fetal length growth, and an infant with small-for-gestational-age (35–37). According to the guidelines of the American College of Obstetrics and Gynecology (ACOG), daily intake of up to two cups of moderate-strength coffee may be safe for pregnant women (38). It has been demonstrated that pregnant women significantly decline their intakes of caffeine, particularly coffee during pregnancy (39, 40). The low intake of coffee among pregnant women in the most included studies may explain finding any significant association. This study showed an inverse association between intake of coffee and risk of GDM among pregnant women who lived in the western countries. People in the western countries consume higher coffee as compared with Asia countries. In the United States, about 25% of women aged between 20 and 29 years, and 46% of women aged between 30 and 39 years, drink coffee daily (41).

Furthermore, studies reported the amount of coffee used as "cups" without expressing the volume of the cup. Therefore, the lack of standardization in the measurement of coffee must be taken into account. Besides, coffee is commonly drunk along with sugar, creamer, or milk, which attenuates the beneficial impact of coffee on GDM since fructose worsens hepatic insulin resistance (42). Finally, the degree of roasting and the method of preparation of coffee including coffee-grind setting and brew type may be different in varied populations.

Caffeine and its metabolites such as paraxanthine accumulate in the blood during pregnancy because of a decrease in the metabolism of caffeine (14). High concentrations of coffee and its metabolites have been associated with elevated insulin resistance during pregnancy (15). Therefore, it appears that the favorable effect of coffee on GDM may originate from components of non-caffeine, including micronutrients and phenolic compounds, which have antioxidant and prebiotic traits (16). The evidence indicated that coffee constitutes 60%, or nearly 11.1 mmol, of the total intake of daily antioxidant (43). Chlorogenic acid is the main polyphenol in coffee and is assumed to be the main antioxidant of coffee (44). Coffee also contains potassium, magnesium, niacin, and other antioxidants, which may have beneficial effects on glucose metabolism and insulin resistance (45). Furthermore, habitual intake of coffee declines subclinical inflammation and augments adiponectin levels (46, 47) that may improve insulin sensitivity (48).

This study has some limitations that should be considered once we interpret the findings. First, the small number of studies and a moderate amount of heterogeneity among them are the main limitations. We tried to find the sources of heterogeneity through subgroup analysis. Second, four of the seven included studies have case-control or cross-sectional designs. These kinds of studies possess some biases such as recall and selection biases. Third, measurement errors may occur when estimating consumption of coffee, particularly for those with low intake. Fourth, studies applied different methods for the diagnosis of GDM. Fifth, some studies did not control covariates, or they may ignore to adjust some underlying residual confounding. For example, only three studies controlled energy intake, which is an important risk factor for GDM. Moreover, most studies did not have any information on the intake of coffee of women before pregnancy. Finally, a lack of control for these dietary factors could disable us to identify a firm finding.

In conclusion, this systematic review and meta-analysis of seven observational studies have depicted no relationship between extreme intake of coffee and the risk of GDM. Furthermore, prospective cohort studies with a large sample size are obligatory to understand the relationship between coffee and GDM. Given that, the circulating level of caffeine and its metabolites are different in patients with GDM, future studies are required to examine biomarkers of coffee and its metabolites, including serum caffeine and paraxanthine.

AUTHOR CONTRIBUTIONS

JN and PW designed the work and extracted the data. TZ and LL analyzed the data. HP wrote the first draft of the manuscript. All authors critically read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.739359/full#supplementary-material

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REVIEW
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Efficacy and Safety of *Brucea javanica* Oil Emulsion Injection in the Treatment of Gastric Cancer: A Systematic Review and Meta-Analysis

Xinmiao Wang[1†], *Heping Wang*[1†], *Luchang Cao*[1†], *Jingyuan Wu*[1,2], *Taicheng Lu*[1,2], *Shixin Li*[1,2] *and Jie Li*[1*]

[1] *Department of Oncology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China,* [2] *Graduate College, Beijing University of Traditional Chinese Medicine, Beijing, China*

Background: Gastric cancer (GC) is one of the most common digestive tract cancers and ranks fifth in the incidence of malignant tumors worldwide. *Brucea javanica* oil emulsion injection (BJOEI), a Chinese patent medicine extracted from *Brucea javanica* (Yadanzi in Chinese Pinyin), is widely used as an adjuvant treatment for GC in China. This systematic review and meta-analysis aimed to evaluate the available data on the efficacy and safety of BJOEI in the treatment of GC and assess the quality of the synthesized evidence.

Methods: A comprehensive search was performed on PubMed, EMBASE, CENTRAL, Web of Science, Chinese Biomedical Literature Database (CBM), China National Knowledge Infrastructure (CNKI), Wanfang database and Chinese Scientific Journals Database (VIP database), and other potential resources, such as the Chinese Clinical Trial Registry (ChiCTR) and ClinicalTrials.gov from their inception to July 31, 2021. Randomized controlled trials (RCTs) comparing the therapeutic effects of BJOEI combined with conventional therapy to those of conventional therapy alone were included. We used RevMan 5.3 for data analysis and quality evaluation of the included studies and assessed the evidence quality based on the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria.

Results: Eighteen RCTs involving 1,210 patients were included, and the meta-analysis results demonstrated that compared with the control group (conventional therapy), the experimental group (BJOEI combined with conventional therapy) showed a significantly improved overall response rate (ORR) (risk ratio [RR] = 1.52, 95% CI: 1.36–1.69, $P < 0.00001$), clinical benefit rate (CBR) (RR = 1.17, 95% CI: 1.11–1.23, $P < 0.00001$), performance status (RR = 1.72, 95% CI: 1.46–2.01, $P < 0.00001$), and reduced incidence of the following adverse drug reactions (ADRs): neutropenia, leukopenia, nausea and vomiting, diarrhea, liver damage, hand-foot syndrome, and peripheral sensory nerve toxicity. Subgroup analysis showed that the BJOEI intervention could significantly improve the ORR and CBR in patients with GC when combined with FOLFOX4, XELOX, and other chemotherapeutics.

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***Correspondence:**
Jie Li
qfm2020jieli@yeah.net

[†]*These authors have contributed equally to this work*

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Conclusion: The evidence presented in this study supports the fact that BJOEI combined with conventional chemotherapy provides a statistically significant and clinically important effect in the improvement of ORR, CBR, performance status, and ADR reduction in patients with GC. To further support this conclusion, more rigorously designed, large-scale, and multicenter RCTs are needed in the future.

Keywords: *Brucea javanica* oil emulsion injection, gastric cancer, efficacy, safety, meta-analysis

INTRODUCTION

Gastric cancer (GC) is one of the most common malignant tumors of the digestive tract and ranks fifth in incidence worldwide (1). There were ∼1.089 million new cases of GC worldwide, of which 43.9% were reported in China, in 2020 (2). Due to the lack of specific symptoms in early GC, the diagnosis is often made at an advanced disease stage, and the mortality rate is high (3). At present, radical resection is still the main GC treatment, but most patients experience recurrence within 3 years after surgery. The postoperative recurrence rate of patients with locally advanced GC is as high as 50–80%. Once patients experience recurrence and metastasis after the operation, even if palliative chemotherapy is administered again, the 5-year survival rate remains low (4–7). Moreover, molecularly targeted therapy and immunotherapy of GC lag behind those of many other tumor types, and better survival benefits are still being explored (8).

Traditional Chinese Medicine (TCM) has a long historical tradition and currently attracts extensive attention because of its potential treatment benefits in the field of oncology. Our team has been committed to investigating the preventive and therapeutic values of TCM for many years (9–11). *Brucea javanica* oil emulsion injection (BJOEI) is a Chinese patent medicine extracted from *Brucea javanica* (*Yadanzi* in Chinese *Pinyin*). Its main active component is quassinoid sand fatty acids, which exert anticancer effects through multiple mechanisms (12). Studies have shown the synergistic effects of BJOEI combined with chemoradiotherapy on tumor attenuation, such as reversal of chemotherapy resistance, reduction of the recurrence and metastasis rates, and improvement of the quality of life (13–16). Although several existing systematic reviews have been conducted to evaluate the clinical efficacy of BJOEI in GC, none of them assessed the quality of the synthesized evidence and arrived at definitive conclusions (13, 17–19). The most recent one was reported by Wu et al. in 2018, in which the retrieval deadline was January 2017 (17). With the growing number of studies on the value of BJOEI in GC treatment, more randomized controlled trials (RCTs) have been published in recent years (20–23). Therefore, we conducted a systematic review to evaluate all available evidence of the efficacy and safety of BJOEI in the treatment of GC and assessed the quality of the synthesized evidence.

METHODS

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guidelines, and readers can access the protocol of this systematic review in the International Prospective Register of Systematic Reviews (CRD42021265646).

Inclusion Criteria

Studies that met the following criteria were included: (1) the study design was limited to RCTs, whether it was blinding or not; (2) the studies needed to meet the diagnostic criteria for GC by biopsy or postoperative pathological examination; and (3) studies provided the experimental group with BJOEI in combination with the same interventions provided to the control group.

Exclusion Criteria

Studies were excluded if any of the following reasons were involved: (1) duplicate studies; (2) inappropriate interventions; (3) incomplete data; and (4) irrelevance to outcome indicators.

Outcome Measures

Primary outcome measures included the overall response rate (ORR) and clinical benefit rate (CBR). The secondary outcome measure was the performance status. Safety outcome measures included the occurrence of adverse drug reactions (ADRs).

Literature Search Strategy

We searched the following relevant databases from inception to July 31, 2021: PubMed, EMBASE, CENTRAL, Web of Science, the Chinese Biomedical Literature Database (CBM), the China National Knowledge Infrastructure (CNKI), Wanfang database, and Chinese Scientific Journals Database (VIP database), and other potential resources, such as the Chinese Clinical Trial Registry (ChiCTR) and ClinicalTrials.gov for more study records. The combination of MeSH terms and text words was applied to study retrieval. "Stomach Neoplasms" was regarded as the MeSH term. All the strategies were adapted from different databases. The search strategies used in PubMed were as follows:

#1 "Stomach Neoplasms" [MeSH]
#2 "Stomach Neoplasms∗" [Title/Abstract] OR "Gastric Cancer∗" [Title/Abstract] OR "Gastric Carcinoma" [Title/Abstract] OR "Gastric Neoplasm∗" [Title/Abstract] OR "Cancer of Stomach" [Title/Abstract] OR "Stomach Cancer∗" [Title/Abstract]
#3 #1 OR #2
#4 "Javanica oil emulsion injection" [Title/Abstract] OR "Yadanzi" [Title/Abstract] OR "Brucea javanica oil emulsion" [Title/Abstract] OR "Brucea javanica" [Title/Abstract]
#5 #3 AND #4

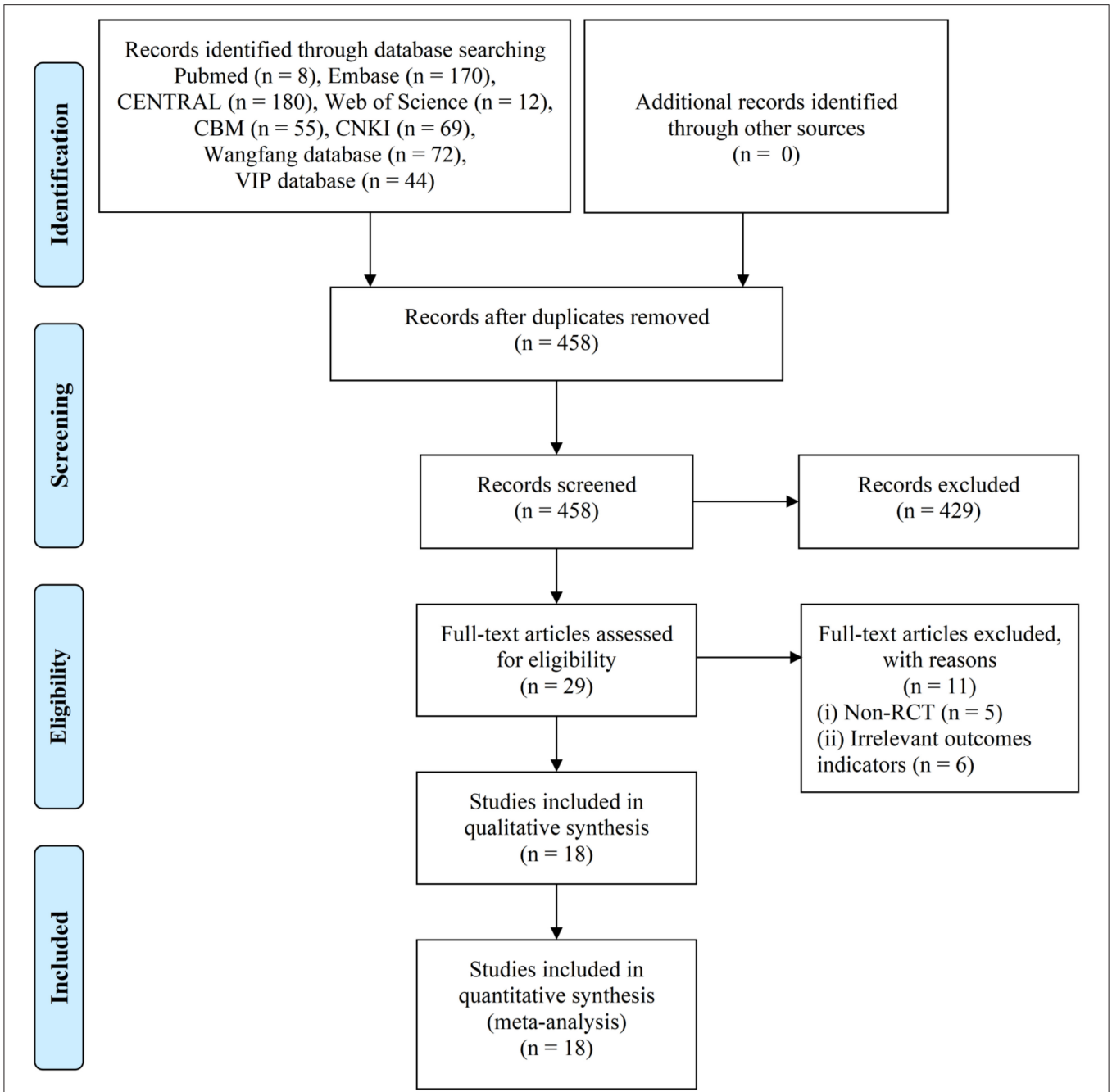

FIGURE 1 | PRISMA flow diagram of the literature search process. PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

Study Selection

The search results were imported into Excel 2003. After removing duplicates, the titles and abstracts were screened for potential studies. Then, the full articles were checked to determine whether the studies met the inclusion criteria. The study selection process was independently performed by two investigators.

Data Extraction and Quality Assessment

All data were independently extracted by two investigators, and any discrepancies between the reviewers were resolved by the intercessor (JL) until consensus was reached. Data retrieved from the publications included author name, year of publication, number of patients, average age, gender, details about dosage and course of treatment, and outcome data. When necessary and feasible, the corresponding authors of the selected studies were contacted to obtain missing or incomplete data.

In terms of bias, the articles were evaluated as low risk, high risk, and unclear risk according to the following quality items: randomization generation, allocation concealment, subject

TABLE 1 | The characteristics of the included trials.

References	No	Gender (M/F)		Age (year)		Interventions		Course (week)	Outcomes
	T/C	T	C	T	C	T	C	T/C	
Cui (20)	60/60	40/20	36/24	51.43 ± 9.86	50.76 ± 10.63	BJOEI 30 ml+ FOLFOX4	FOLFOX4	4/4	①②③
Deng et al. (25)	21/21	29/13		39–81 (mean 60.2)		BJOEI 30 ml + DDP+MMC+VP-16	DDP+MMC+VP-16	–	①②③
Fan et al. (26)	24/18	14/10	13/5	70–85	70–85	BJOEI 30 ml + mFOLFOX4	mFOLFOX4	12/12	①②③
Gao (27)	26/26	14/12	15/11	32–79	35–75	BJOEI 30 ml+ MC/CF	MC/CF	4/4	①③
Jiang et al. (28)	32/32	21/11	20/12	36–64	32/63	BJOEI 30 ml+XELOX	XELOX	6/6	①②③
Li et al. (29)	40/40	22/18	21/19	64.5 ± 4.1	63.7 ± 3.4	BJOEI 30–50 ml+ XELOX	XELOX	12/12	①②③
Liu et al. (30)	40/38	30/10	26/12	29–71	34–68	BJOEI 30 ml + DX	DX	6/6	①②③
Ma et al. (31)	58/50	46/12	42/8	46.52 ± 5.13	47.13± 5.42	BJOEI 20 ml + XELOX	XELOX	12/12	①②③
Tan and Zhang (21)	20/20	11/9	12/8	51.53 ± 2.98	53.42 ± 3.22	BJOEI 20 ml+ DP	DP	6/6	②③
Tong and Hu (22)	42/42	30/12	28/14	54.69 ±8.42	54.41 ± 8.25	BJOEI 30 ml + SOX	L-OHP+TS-1	6/6	①③
Wang et al. (33)	31/31	17/14	16/15	29–63 (mean 50.2)		BJOEI 30 ml+ XELOPAC	XELOPAC	12/12	①③
Wang and Yang (34)	24/23	13/11	13/10	31–75	32–74	BJOEI 30 ml+ FOLFOX4	FOLFOX4	8/8	①②③
Wang (35)	38/30	23/15	19/11	32–71	35/69	BJOEI 30 ml + 5-FU+HCPT+CF+RT	5-FU+HCPT+CF+RT	9–12.86/9–12	①②③
Wang (36)	31/29	20/11	20/9	52.3 ± 12.71	51.6 ± 12.39	BJOEI 30 ml+ FOLFOX4	FOLFOX4	12.86/12	①②③
Wu et al. (37)	50/50	38/12	33/17	34–78	31–82	BJOEI 30 ml + FOLFOX4	FOLFOX4	4/4	①③
You et al. (23)	19/23	15/4	14/9	28–75	36–71	BJOEI 20–40 ml + TX	TX	6/6	①③
Zhang et al. (38)	41/41	28/13	26/15	68.8 ± 3.8	68.6 ± 5.2	BJOEI 30 ml + XELOX	XELOX	9/9	①②③
Wang et al. (32)	22/21	25/18		70–85		BJOEI 30 ml + UFT+FA	UFT+FA	16.57–24.86/16.57–24.86	①②③

No, number of participants; T, treatment; C, control; M, male; F, female; Y, year; W, week; BJOEI, Brucea javanica Oil Emulsion injection; FOLFOX4, 5-FU+L-OHP+CF/THFA; mFOLFOX, 5-FU+L-OHP+CF; MC/CF, MMC+CF+5-FU; XELOX, L-OHP+CAP; DX, DXT+CAP; DP, DXT+DDP; SOX, L-OHP+TS-1; XELOPAC, PTX+CAP; TX, PTX/DXT+CAP; 5-FU, 5-Fluorouracil; L-OHP, Oxaliplatin; CF, Calcium Folinate; DDP, Cisplatin; MMC, Mitomycin-C; VP-16, Etoposide; CAP, Capecitabine; DTX, Docetaxel; TS-1, Tegafur; PTX, Paclitaxel; HCPT, Hydroxycamptothecin; RT, Radiotherapy; THFA, Tetrahydrogen folic acid; UFT, Tegafur-Uracil; FA, Folic acid. (1) Clinical total effective rate; (2) performance status; (3) adverse drug reactions; (4) adverse events; (5) withdrawals for any reason.

blinding, outcome assessment, incomplete outcome data, and selective outcome reporting.

Statistical Analysis

Quantitative synthesis was conducted for outcomes reported in more than one homogeneous RCT. The systematic review was performed using the RevMan 5.3 software. Random-effects or fixed-effects models were chosen based on the analysis of heterogeneity. Randomized individuals were considered as unit-of-analysis issues. If a meta-analysis was not appropriate because of clinical/methodological issues or statistical heterogeneity, a narrative summary of the findings or relevant subgroup analyses were used. The RR was used to evaluate dichotomous outcomes, while the mean difference (MD) was used to assess continuous variables. Each outcome numerical value was presented with 95% CIs. Funnel plots were used to test the risk of publication bias. The heterogeneity between RCTs was analyzed using the chi-square test and estimated using I^2. Results of $P \geq 0.1$ and $I^2 \leq 50\%$ suggested a lack of significant heterogeneity, and a fixed-effects model was used accordingly; otherwise, the random-effects model was used. When conducting the meta-analysis, several subgroup analyses were performed to identify subpopulations that might be associated with differences in efficacy. The results of the sensitivity analysis were reported.

Quality of the Synthesized Evidence

Quality assessment of the synthesized evidence was performed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (24). This assessment of evidence quality includes the risk of bias, heterogeneity, indirectness, imprecision, and publication bias. The quality of the evidence was classified as high, moderate, low, or very low.

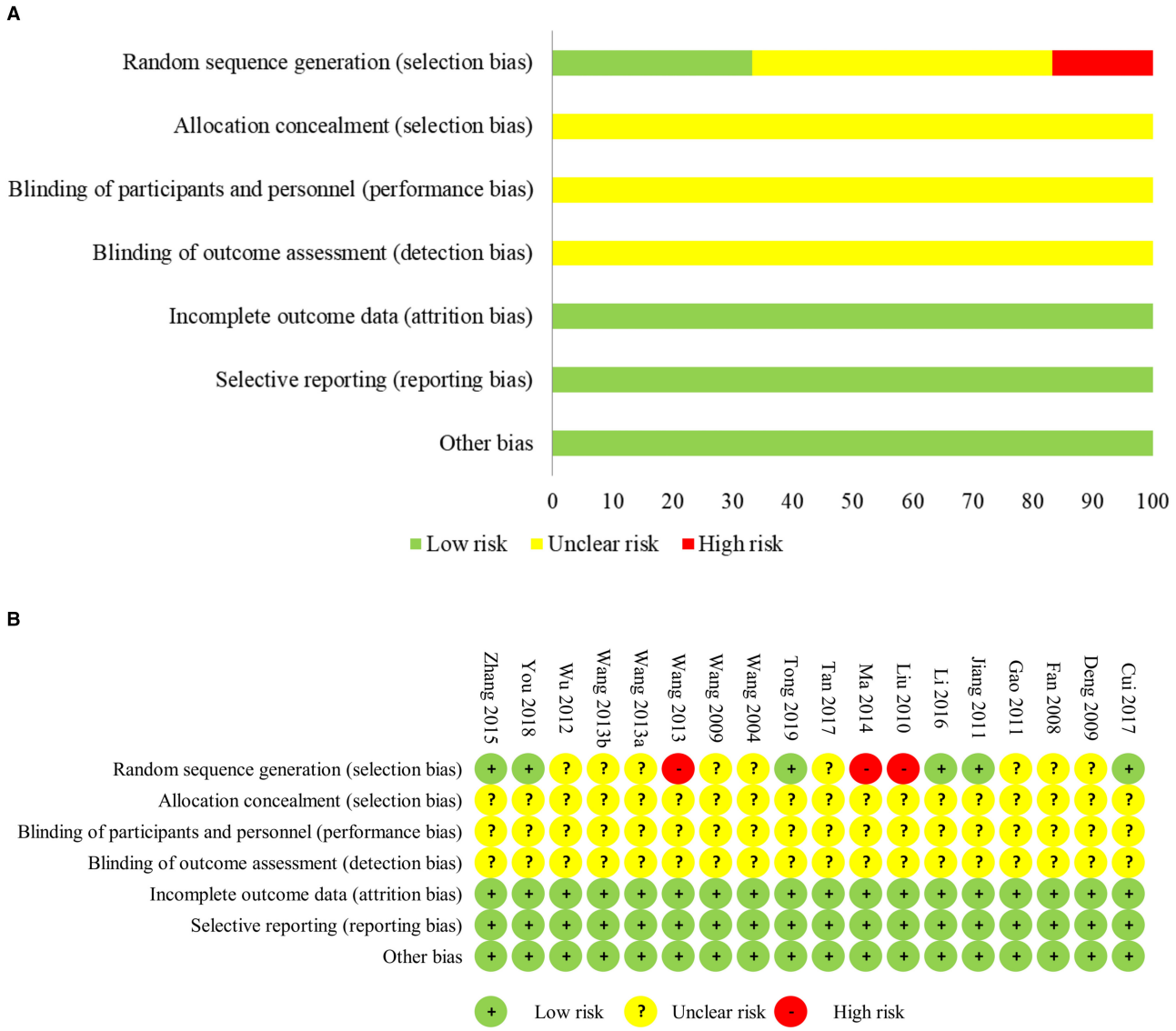

FIGURE 2 | Risk of bias of included study. **(A)** Risk of bias summary, **(B)** risk of bias graph.

RESULTS

Literature Search Results

A total of 458 clinical studies were identified based on the retrieval strategy. After screening based on the inclusion/exclusion criteria, 18 articles were selected for further analysis (**Figure 1**).

Study Description

Eighteen RCTs (20–23, 25–38) were included in this study, involving 1,210 patients with 618 cases in the experimental group and 592 cases in the control group. Furthermore, a total of four RCTs (20, 34, 36, 37) adopted BJOEI + FOLFOX4, and four RCTs (28, 29, 31, 38) employed BJOEI + XELOX. Due to the diverse combination therapy of BJOEI, subgroup analysis was considered. Additional details are summarized in **Table 1**.

Quality Evaluation of the Literature

As shown in **Figure 2**, in terms of random sequence generation, six RCTs (20, 22, 23, 28, 29, 38) were considered to have a low bias risk by applying a random number table or random envelope. Three RCTs (30, 31, 36) were marked as "high risk" because they divided patients according to hospitalization period, ID, and postoperative chemotherapy, respectively. The other nine RCTs (21, 25–27, 32–35, 37) did not describe the specific randomized method and were evaluated as "uncertain risk." None of the trials reported the methods of allocation concealment and blinding procedures, which indicated that there were unclear bias risks.

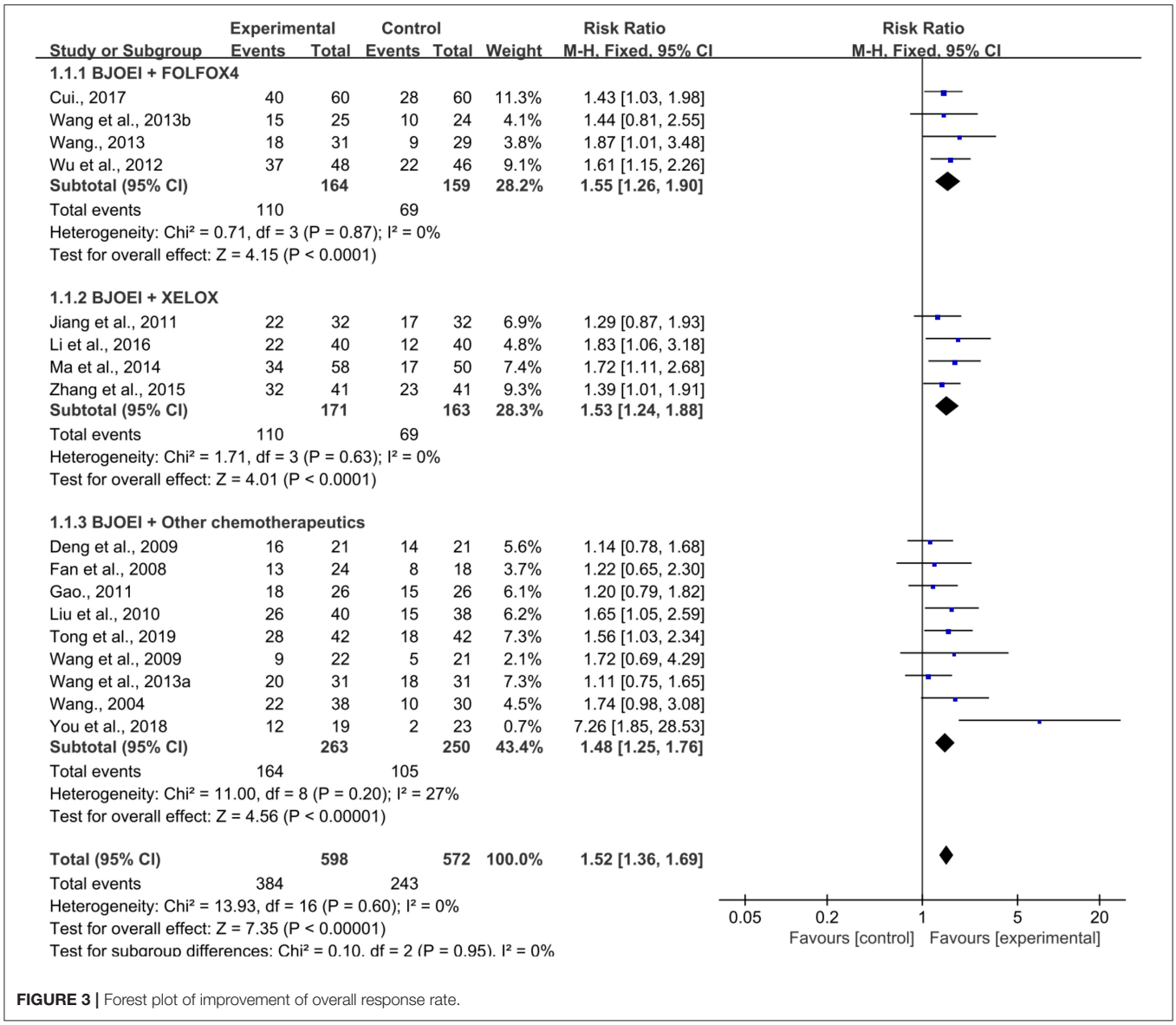

FIGURE 3 | Forest plot of improvement of overall response rate.

Outcome Measures

Primary Outcomes

ORR

In total, 17 RCTs (20, 22, 23, 25–38) with 1,170 patients presented ORR data. To explore the potential effect differences in ORR, we conducted a subgroup analysis according to the different combination therapies of BJOEI, namely, BJOEI + FOLFOX4, BJOEI + XELOX, and BJOEI + other chemotherapeutics. As shown in **Figure 3**, the results demonstrated that compared with the control group, the experimental group of patients with GC exhibited a significantly improved ORR (RR = 1.52, 95% CI: 1.36–1.69, Z = 7.35, $P < 0.00001$). Furthermore, subgroup analysis showed that there were statistically significant differences in ORR between the BJOEI intervention and control groups in patients who received BJOEI combined with FOLFOX4 (RR = 1.55, 95% CI: 1.26–1.90, Z = 4.15, $P < 0.0001$), XELOX (RR = 1.53, 95% CI: 1.24–1.88, Z = 4.01, $P < 0.0001$), and other chemotherapeutics (RR = 1.48, 95% CI: 1.25–1.76, Z = 4.56, $P < 0.00001$).

CBR

In total, 17 RCTs (10, 20, 22, 23, 25–33, 35–38) recorded CBR data. We conducted a subgroup analysis according to the different combination therapies of BJOEI, namely, BJOEI + FOLFOX4, BJOEI + XELOX, and BJOEI + other chemotherapeutics. As shown in **Figure 4**, the results demonstrated that, compared with the control group, the experimental group of patients with GC exhibited significantly improved CBR (RR = 1.17, 95% CI: 1.11–1.23, Z = 5.70, $P < 0.00001$). Subgroup analysis showed that there were

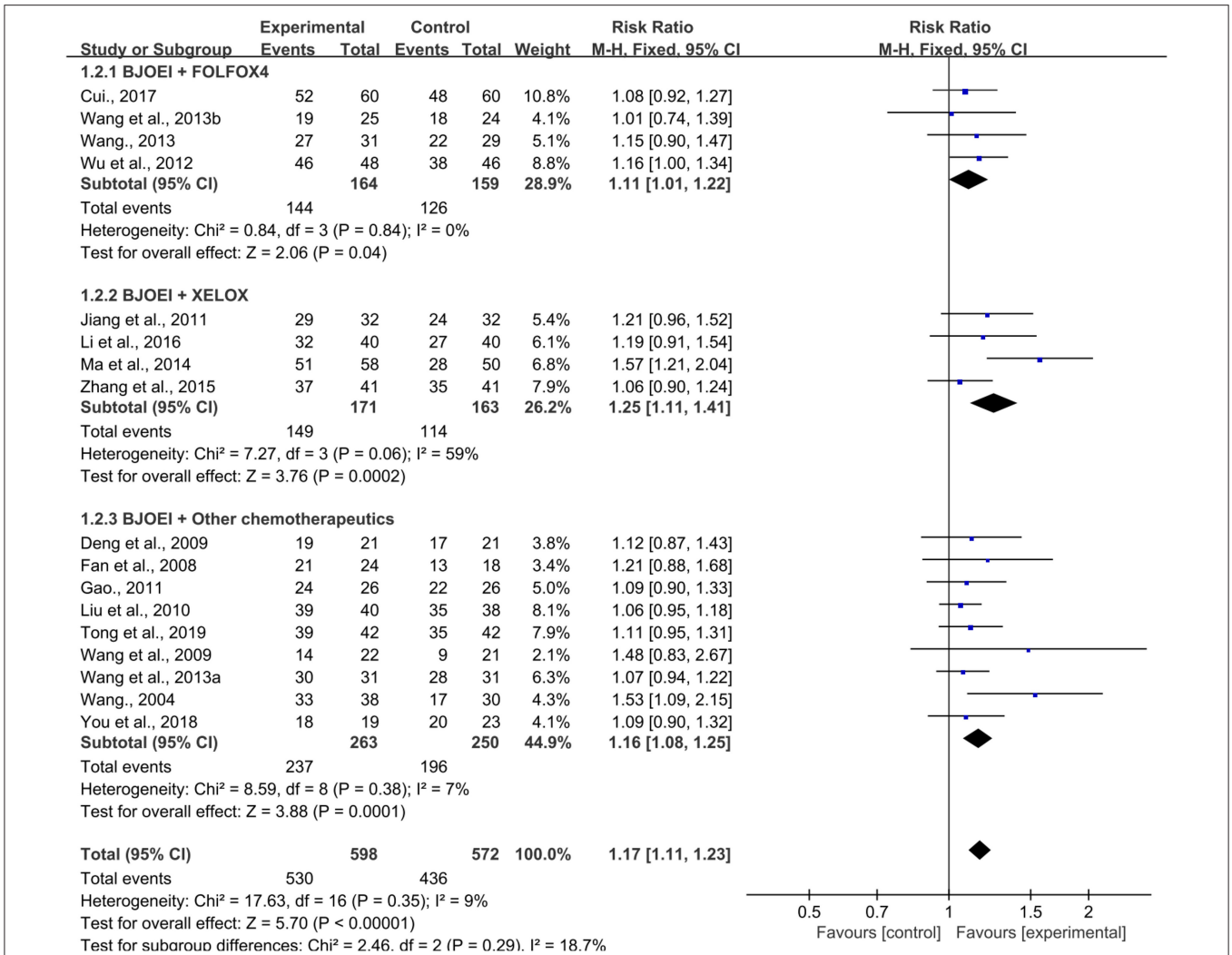

FIGURE 4 | Forest plot of improvement of clinical benefit rate.

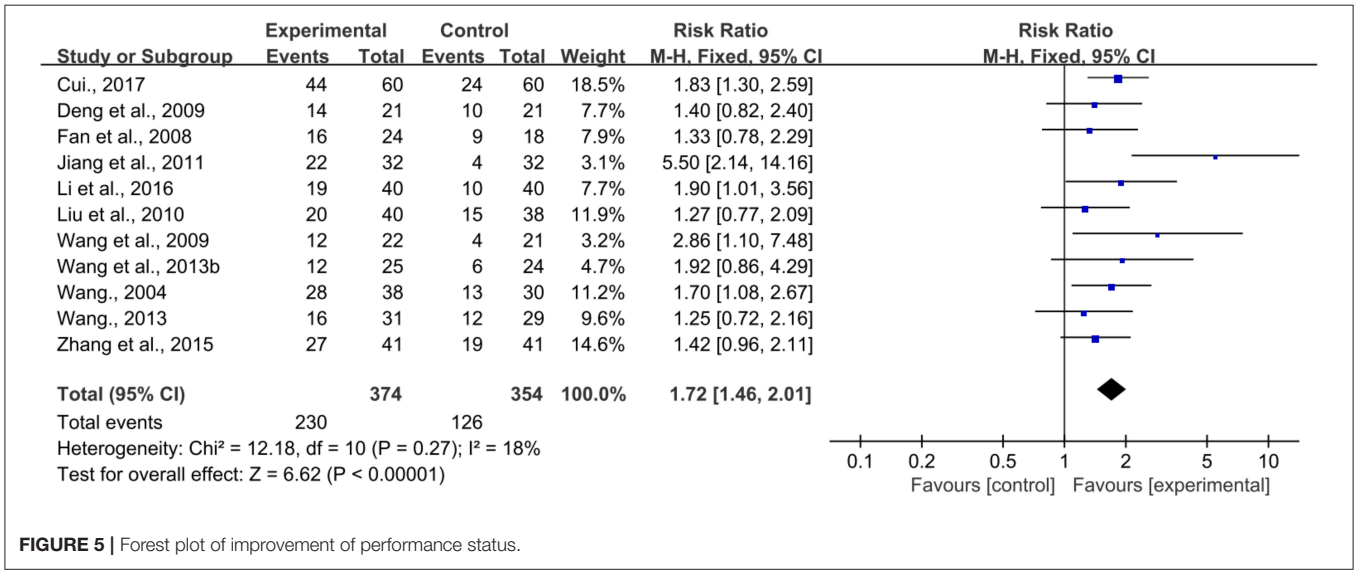

FIGURE 5 | Forest plot of improvement of performance status.

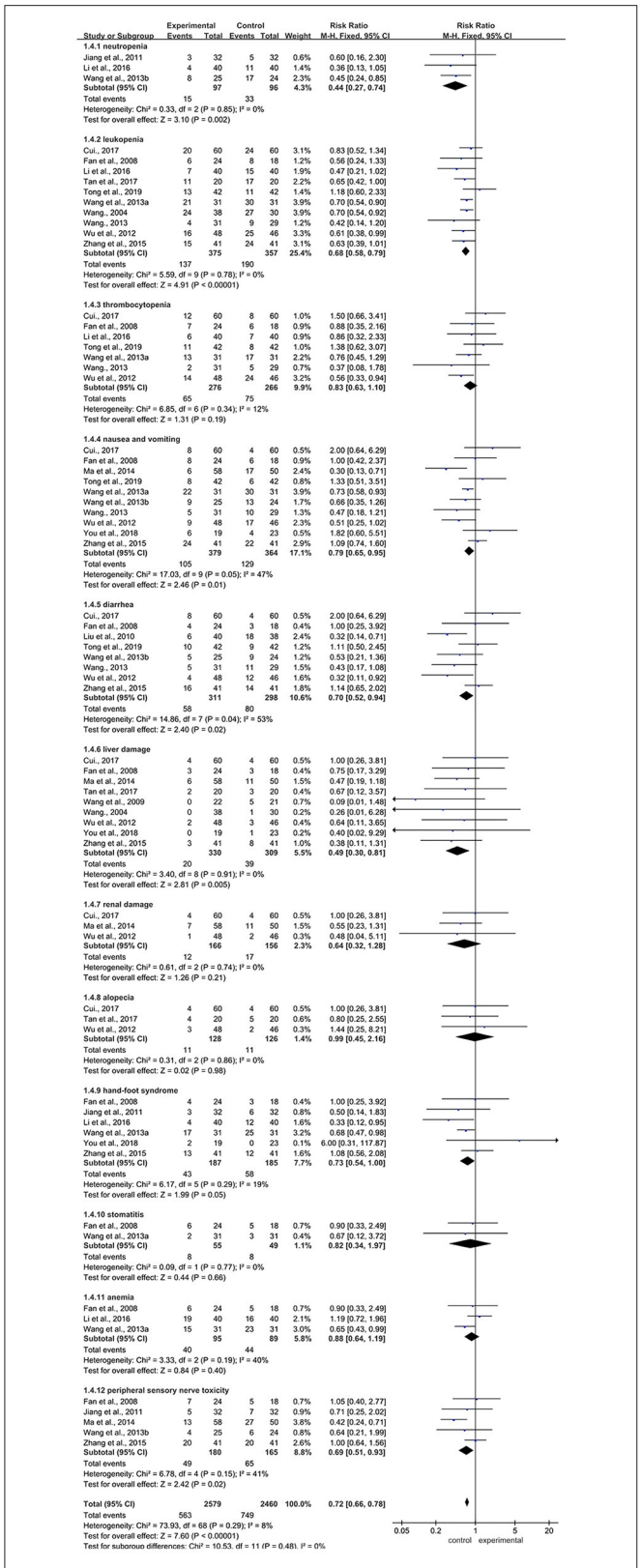

FIGURE 6 | Forest plot of ADRs. ADRs, adverse drug reactions.

statistically significant differences in CBR between the BJOEI intervention and control groups in patients who received BJOEI combined with FOLFOX4 (RR = 1.11, 95% CI: 1.01–1.22, Z = 2.06, $P = 0.04$), XELOX (RR = 1.25, 95% CI: 1.11–1.41, Z = 3.76, $P = 0.0002$), and other chemotherapeutics (RR = 1.16, 95% CI: 1.08–1.25, Z = 3.88, $P = 0.0001$).

Secondary Outcomes

Performance Status

As shown in **Figure 5**, 11 RCTs (20, 25, 26, 28–30, 32, 34–36, 38) reported the performance status data of the BJOEI and control groups with a slight heterogeneity ($P = 0.27$, $I^2 = 18\%$ <50%). A meta-analysis demonstrated that the BJOEI group experienced ∼72% superiority in terms of this outcome compared with the control group, and the difference was statistically significant (RR = 1.72, 95% CI: 1.46–2.01, Z = 6.62, $P < 0.00001$).

ADRs

Sixteen RCTs referred to this outcome. The main ADRs were neutropenia (3 RCTs) (28, 29, 34), leukopenia (10 RCTs) (26, 29, 33, 35–38), thrombocytopenia (7 RCTs) (20, 22, 26, 29, 33, 36, 37), nausea and vomiting (10 RCTs) (20, 22, 23, 26, 31, 33, 34, 36–38), diarrhea (8 RCTs) (20, 22, 26, 30, 34, 36–38), liver damage (9 RCTs) (20, 21, 23, 26, 31, 32, 35, 37, 38), renal damage (3 RCTs) (20, 31, 37), alopecia (3 RCTs) (20, 21, 37), hand-foot syndrome (6 RCTs) (23, 26, 28, 29, 33, 38), stomatitis (2 RCTs) (26, 33), anemia (3 RCTs) (26, 29, 33), and peripheral sensory nerve toxicity (5 RCTs) (26, 28, 31, 34, 38). Meta-analysis showed that there was a statistically significant difference between the two groups (RR = 0.72, 95% CI: 0.66–0.78, Z = 7.60, $P < 0.00001$). Compared with the control group, the BJOEI group exhibited fewer of the following ADRs: neutropenia (RR = 0.44, 95% CI: 0.27–0.74, Z = 3.10, $P = 0.002$), leukopenia (RR = 0.68, 95% CI: 0.58–0.79, Z = 4.91, $P < 0.00001$), nausea and vomiting (RR = 0.79, 95% CI: 0.65–0.95, $Z = 2.46$, $P = 0.01$), diarrhea (RR = 0.70, 95% CI: 0.52–0.94, $Z = 2.40$, $P = 0.02$), liver damage (RR = 0.49, 95% CI: 0.30–0.81, $Z = 2.81$, P =0.005), hand-foot syndrome (RR = 0.73, 95% CI: 0.54–1.00, $Z = 1.99$, $P = 0.05$), and peripheral sensory nerve toxicity (RR = 0.69, 95% CI: 0.51–0.93, $Z = 2.42$, $P = 0.02$). However, no statistically significant differences were detected in the occurrence of thrombocytopenia, renal damage, alopecia, stomatitis, and anemia. The results of ADR were shown in **Figure 6**.

Sensitivity Analysis

According to the *Cochrane Handbook for Systematic Reviews of Interventions* (39), I^2 values between 0 and 40% indicated that heterogeneity might not be important. Therefore, we eliminated the included studies with $I^2 \geq 40\%$ one by one and then conducted a meta-analysis. The results showed that in the CBR of BJOEI + XELOX, after excluding Zhang et al. (38), the heterogeneity was decreased from 59 to 32% ($P = 0.0002$; **Figure 7**). After excluding Ma et al. (31), the heterogeneity was decreased from 59 to 0% ($P = 0.04$; **Figure 8**). The data suggested

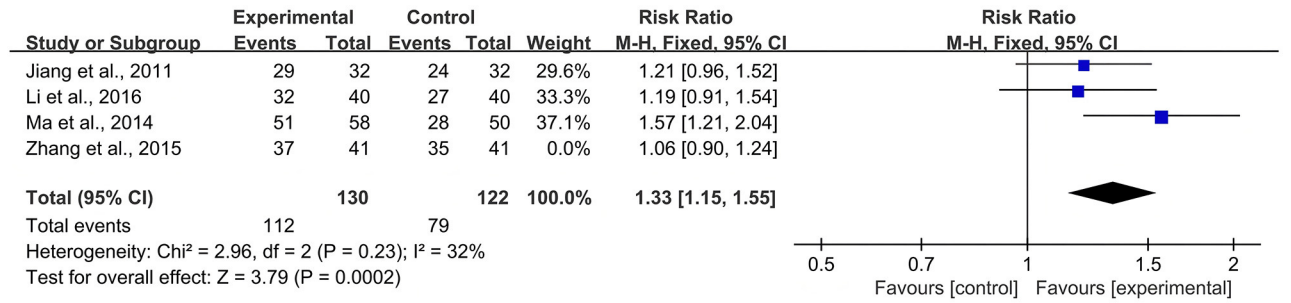

FIGURE 7 | Forest plot of sensitivity analysis of CBR with BJOEI combined with XELOX treatment vs. pure XELOX treatment (a). CBR, clinical benefit rate; BJOEI, *Brucea javanica* oil emulsion injection.

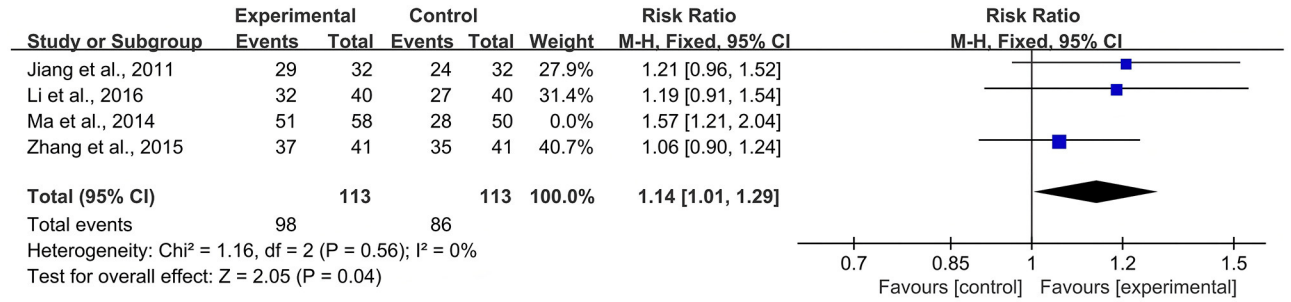

FIGURE 8 | Forest plot of sensitivity analysis of CBR with BJOEI combined with XELOX treatment vs. pure XELOX treatment (b). CBR, clinical benefit rate; BJOEI, *Brucea javanica* oil emulsion injection.

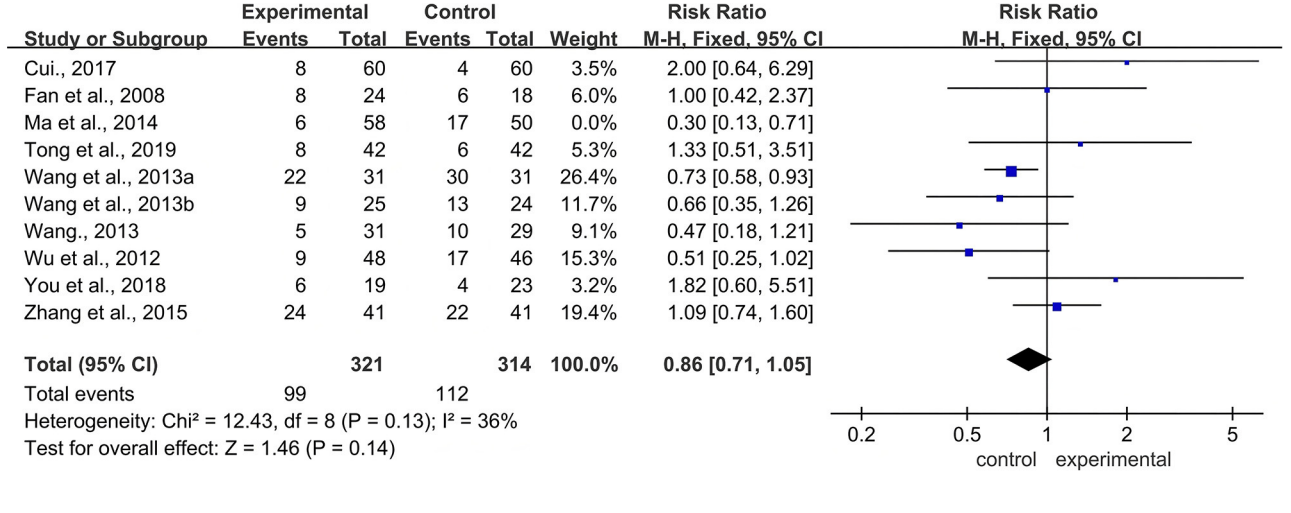

FIGURE 9 | Forest plot of sensitivity analysis of ADRs of nausea and vomiting. ADRs, adverse drug reactions.

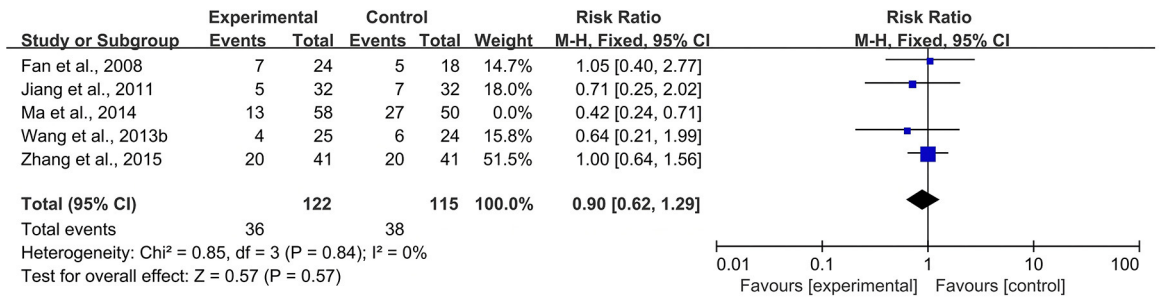

FIGURE 10 | Forest plot of sensitivity analysis of ADRs of peripheral sensory nerve toxicity (a), ADRs, adverse drug reactions.

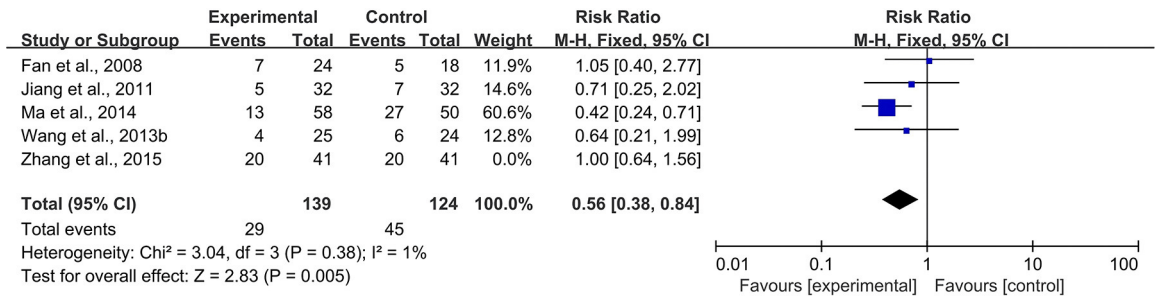

FIGURE 11 | Forest plot of sensitivity analysis of ADRs of peripheral sensory nerve toxicity (b). ADRs, adverse drug reactions.

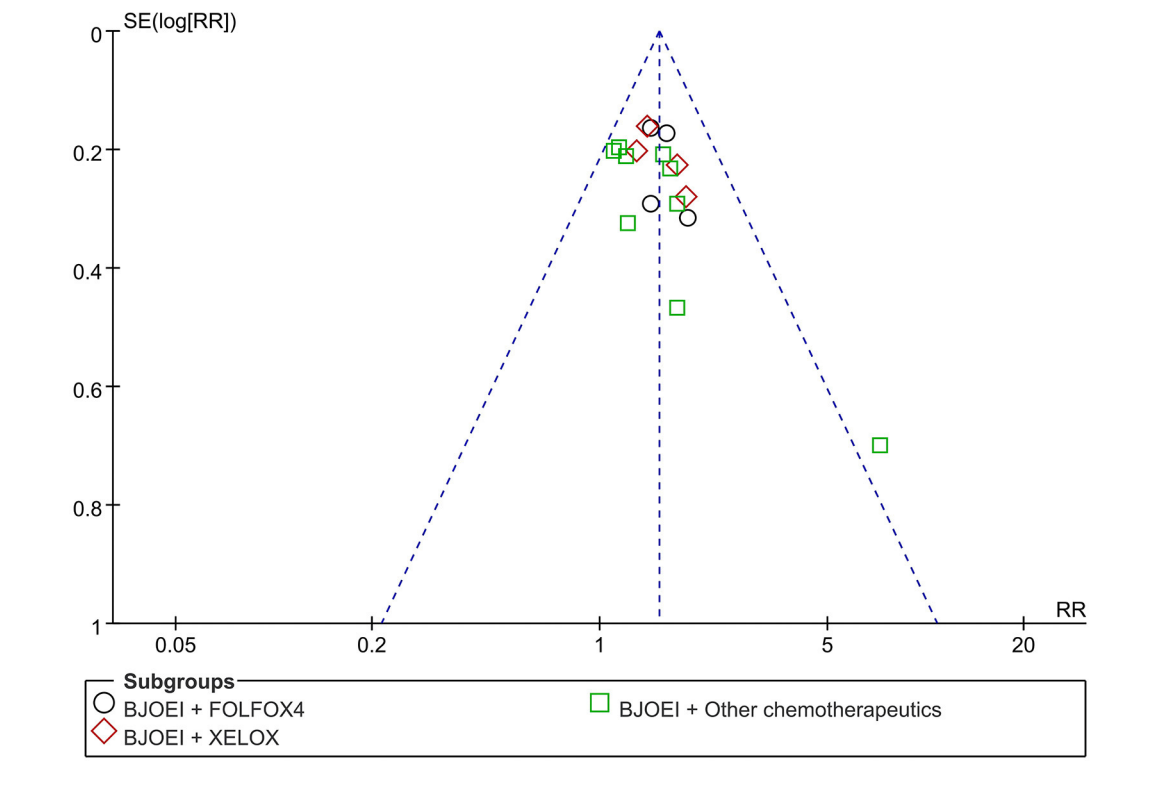

FIGURE 12 | Funnel plot of ORR.

TABLE 2 | Quality of evidence of primary outcomes.

***Brucea javanica* oil emulsion injection plus chemotherapy compared to chemotherapy for gastric cancer**

Patient or population: **gastric cancer**
Setting: **Randomized trials**
Intervention: **Brucea javanica Oil Emulsion Injection plus chemotherapy**
Comparison: chemotherapy

Outcome No of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty
		Risk without BJOEI	Risk with BJOEI	Difference	
Overall response rate No of participants: 1170 (17 RCTs)	RR 1.52 (1.36–1.69)	42.5%	64.6% (57.8–71.8)	22.1% more (15.3 more to 29.3 more)	⊕⊕◯◯ LOW[a,b]
Overall response rate - FOLFOX4 No of participants: 323 (4 RCTs)	RR 1.55 (1.26–1.90)	43.4%	67.3% (54.7–82.5)	23.9% more (11.3 more to 39.1 more)	⊕⊕◯◯ LOW [a,c]
Overall response rate - XELOX No of participants: 334 (4 RCTs)	RR 1.53 (1.24–1.88)	42.3%	64.8% (52.5–79.6)	22.4% more (10.2 more to 37.3 more)	⊕⊕◯◯ LOW [a,c]
Overall response rate - Other chemotherapeutics No of participants: 513 (9 RCTs)	RR 1.48 (1.25–1.76)	42.0%	62.2% (52.5–73.9)	20.2% more (10.5 more to 31.9 more)	⊕◯◯◯ VERY LOW [a,b,c]
Clinical benefit rate No of participants: 1170 (17 RCTs)	RR 1.17 (1.11–1.23)	76.2%	89.2% (84.6–93.8)	13.0% more (8.4 more to 17.5 more)	⊕⊕◯◯ LOW [a,b]
Clinical benefit rate - FOLFOX4 No of participants: 323 (4 RCTs)	RR 1.11 (1.01–1.22)	79.2%	88.0% (80–96.7)	8.7% more (0.8 more to 17.4 more)	⊕⊕◯◯ LOW [a,c]
Clinical benefit rate - XELOX No of participants: 334 (4 RCTs)	RR 1.25 (1.11–1.41)	69.9%	87.4% (77.6–98.6)	17.5% more (7.7 more to 28.7 more)	⊕◯◯◯ VERY LOW [a,c,d]
Clinical benefit rate - Other chemotherapeutics No of participants: 513 (9 RCTs)	RR 1.16 (1.08–1.25)	78.4%	90.9% (84.7–98)	12.5% more (6.3 more to 19.6 more)	⊕◯◯◯ VERY LOW [a,b,c]

**The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).*
RR, Risk ratio; BJOEI, Brucea javanica oil emulsion injection; [a]Most information is from studies at unclear risk of bias; [b]Clinical heterogeneity exists due to the different chemotherapy; [c]Small sample size; [d]Statistical heterogeneity exists.

that Zhang et al. (38) and Ma et al. (31) were the main reasons for the heterogeneity in the CBR of BJOEI + XELOX. In terms of ADRs, after excluding Ma et al. (31), the heterogeneity of nausea and vomiting decreased was from 47 to 36% ($P = 0.14$; **Figure 9**), and the heterogeneity of peripheral sensory nerve toxicity was decreased from 41 to 0% ($P = 0.57$; **Figure 10**). In addition, after deleting Zhang et al. (38), the heterogeneity of peripheral sensory nerve toxicity was decreased from 41% to 1% ($P = 0.005$; **Figure 11**). These findings suggest that Zhang et al. (38) and Ma et al. (31) might explain the heterogeneity in ORR and ADRs.

Analysis of Publication Bias

A funnel plot of publication bias for ORR is displayed in **Figure 12**, which indicates that there was no evidence of significant publication bias.

Quality of Evidence Assessment

Based on the GRADE criteria, the ORR, CBR, performance status, and ADRs were all assessed as low-quality evidence, owing to the existence of clinical heterogeneity and low participant numbers in most studies (**Tables 2**, **3**).

DISCUSSION

Despite advances in disease screening and modern technology, GC remains one of the most common malignant tumors. Its metastasis, morbidity, and mortality rates are all on the rise, while the cure, radical resection, and 5-year postoperative survival rates of patients with advanced GC are low (40). In recent years, TCM has made great progress in anti-tumor therapy, and the manufacturing technologies of Chinese medicine compounds, Chinese patent medicine, Chinese medicine extract, and Chinese medicine monomers have developed more rapidly. BJOEI is a Chinese patent medicine that is widely used in the treatment of various cancers, such as lung (41) and several gastrointestinal cancers (42, 43). Previous studies have shown that its antitumor effects might be related to the following mechanisms: 1) inhibition of DNA synthesis in tumor cells (44, 45); 2) induction of tumor cell apoptosis and differentiation (46–48); 3) anti-angiogenesis (49); and 4) reversion of drug resistance (50).

In this study, we searched as many RCTs as we could and conducted a meta-analysis to evaluate the treatment efficacy and safety of BJOEI in patients with GC. All available data from the collected trials were applied without intentional selection. The results showed that BJOEI combined with chemotherapy

TABLE 3 | Quality of evidence of secondary outcomes.

***Brucea javanica* oil emulsion injection plus chemotherapy compared to chemotherapy for gastric cancer**

Patient or population: gastric cancer
Setting: Randomized trials
Intervention: *Brucea javanica* Oil Emulsion Injection plus chemotherapy
Comparison: chemotherapy

Outcome No of participants (studies)	Relative effect (95% CI)	Anticipated absolute effects (95% CI)			Certainty
		Risk without BJOEI	Risk with BJOEI	Difference	
performance status No of participants: 728 (11 RCTs)	RR 1.72 (1.46–2.01)	35.6%	61.2% (52–71.5)	25.6% more (16.4 more to 35.9 more)	⊕⊕◯◯ LOW [a,b]
ADRs No of participants: 5039 (16 RCTs)	RR 0.72 (0.66–0.78)	30.4%	21.9% (20.1–23.7)	8.5% fewer (10.4 fewer to 6.7 fewer)	⊕⊕◯◯ LOW [a,b]
ADRs - neutropenia No of participants: 193 (3 RCTs)	RR 0.44 (0.27–0.74)	34.4%	15.1% (9.3–25.4)	19.3% fewer (25.1 fewer to 8.9 fewer)	⊕◯◯◯ VERY LOW [a,b,c]
ADRs - leukopenia No of participants: 732 (10 RCTs)	RR 0.68 (0.58–0.79)	53.2%	36.2% (30.9–42)	17.0% fewer (22.4 fewer to 11.2 fewer)	⊕⊕◯◯ LOW [a,b]
ADRs - thrombocytopenia No of participants: 542 (7 RCTs)	RR 0.83 (0.63–1.10)	28.2%	23.4% (17.8–31)	4.8% fewer (10.4 fewer to 2.8 more)	⊕⊕◯◯ LOW [a,b]
ADRs - nausea and vomiting No of participants: 743 (10 RCTs)	RR 0.79 (0.65–0.95)	35.4%	28.0% (23–33.7)	7.4% fewer (12.4 fewer to 1.8 fewer)	⊕⊕◯◯ LOW [a,b]
ADRs - diarrhea No of participants: 609 (8 RCTs)	RR 0.70 (0.52–0.94)	26.8%	18.8% (14–25.2)	8.1% fewer (12.9 fewer to 1.6 fewer)	⊕◯◯◯ VERY LOW [a,b,d]
ADRs - liver damage No of participants: 639 (9 RCTs)	RR 0.49 (0.30–0.81)	12.6%	6.2% (3.8–10.2)	6.4% fewer (8.8 fewer to 2.4 fewer)	⊕⊕◯◯ LOW [a,b]
ADRs - renal damage No of participants: 322 (3 RCTs)	RR 0.64 (0.32–1.28)	10.9%	7.0% (3.5–13.9)	3.9% fewer (7.4 fewer to 3.1 more)	⊕⊕◯◯ VERY LOW [a,b,c]
ADRs - alopecia No of participants: 254 (3 RCTs)	RR 0.99 (0.45–2.16)	8.7%	8.6% (3.9–18.9)	0.1% fewer (4.8 fewer to 10.1 more)	⊕◯◯◯ VERY LOW [a,b,c]
ADRs - hand-foot syndrome No of participants: 372 (6 studies)	RR 0.73 (0.54–1.00)	31.4%	22.9% (16.9–31.4)	8.5% fewer (14.4 fewer to 0 fewer)	⊕◯◯◯ VERY LOW [a,b,c]
ADRs - stomatitis No of participants: 104 (2 RCTs)	RR 0.82 (0.34–1.97)	16.3%	13.4% (5.6–32.2)	2.9% fewer (10.8 fewer to 15.8 more)	⊕◯◯◯ VERY LOW [a,b,c]
ADRs - anemia No of participants: 184 (3 RCTs)	RR 0.88 (0.64–1.19)	49.4%	43.5% (31.6–58.8)	5.9% fewer (17.8 fewer to 9.4 more)	⊕◯◯◯ VERY LOW [a,b,c]
ADRs - peripheral sensory nerve toxicity No of participants: 345 (5 RCTs)	RR 0.69 (0.51–0.93)	39.4%	27.2% (20.1–36.6)	12.2% fewer (19.3 fewer to 2.8 fewer)	⊕◯◯◯ VERY LOW [a,b,c]

**The risk in the intervention group (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).*
RR, Risk ratio; BJOEI, Brucea javanica oil emulsion injection; [a]Most information is from studies at unclear risk of bias; [b]Clinical heterogeneity exists due to the different chemotherapy; [c]Small sample size; [d]Statistical heterogeneity exists.

was superior to single chemotherapy in improving ORR, CBR, and performance status. Considering that the different patient regimens might lead to high outcome heterogeneity, to obtain a more convincing conclusion, we conducted a subgroup analysis according to chemotherapeutic regimens. The results showed that for each BJOEI + FOLFOX4 and BJOEI + XELOX subgroup, the ORR and CBR were significantly improved by the addition of the BJOEI intervention. Furthermore, we have paid special attention to neutropenia, leukopenia, nausea and vomiting, diarrhea, liver damage, hand-foot syndrome, and peripheral sensory nerve toxicity, which are common symptoms of chemotherapy-associated ADRs. The meta-analysis showed that the BJOEI group had fewer symptoms related to the above ADRs. However, more RCTs are needed to further demonstrate the positive effect of BJOEI in ameliorating chemotherapy-associated toxicities.

Although we strictly conducted this meta-analysis according to the review procedure released by the Cochrane Collaboration, this study has several limitations. First, the duration of the intervention is an important factor in the evaluation of efficacy. The observation time of the included studies was mainly concentrated at 12 and 6 W, and the longest was 24 W (in only one RCT). Furthermore, the long-term effects of BJOEI in the treatment of GC remain unknown. Moreover, high-quality

original studies were scarce in this study. The problems in most RCTs included unexplained randomization methods, insufficient attention to allocation concealment, low utilization rate of blinding, and unreported lost follow-up cases. Finally, recent advances have renewed the hope that immune and targeted agents can be leveraged to improve patient survival (51, 52). Although chemotherapy is still the backbone of therapy against GC, studies should also investigate the efficacy of BJOEI combined with immunotherapy or targeted therapy.

Due to the limitations associated with the poor quality of pooled studies, it is difficult to draw a definitive conclusion. Nevertheless, our study suggests the positive effect of BJOEI in facilitating the management of ORR, CBR, performance status, and ADRs in patients with GC. More prospectively designed, large-sample, and multicenter RCTs are expected to offer persuasive evidence to demonstrate the efficacy and safety of BJOEI.

AUTHOR CONTRIBUTIONS

JL designed this study. XW and HW performed the online database search. LC, JW, TL, and SL contributed to the data collection, extraction, and analysis. XW, HW, and LC prepared the original draft and finished the revision of the manuscript. All authors have read and approved the final manuscript.

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REVIEW
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Safety Evaluation of Natural Drugs in Chronic Skeletal Disorders: A Literature Review of Clinical Trials in the Past 20 years

Dongyang Zhou[1,2,3,4†], *Hao Zhang*[1,2†], *Xu Xue*[1,2,4†], *Yali Tao*[1,2], *Sicheng Wang*[1,2*], *Xiaoxiang Ren*[1,2*] *and Jiacan Su*[1,2*]

[1]*Institute of Translational Medicine, Shanghai University, Shanghai, China,* [2]*Institute of Advanced Interdisciplinary Materials Science, Shanghai University, Shanghai, China,* [3]*College of Medicine, Shanghai University, Shanghai, China,* [4]*College of Environmental and Chemical Engineering, Shanghai University, Shanghai, China*

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Xiao Chen,
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***Correspondence:**
Sicheng Wang
464716070@qq.com
Xiaoxiang Ren
rxx1991917@hotmail.com
Jiacan Su
jiacansu@shu.edu.cn

†These authors have contributed equally to this work

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Chronic skeletal disorders (CSDs), including degenerative diseases such as osteoporosis (OP) and autoimmune disorders, have become a leading cause of disability in an ageing society, with natural drugs being indispensable therapeutic options. The clinical safety evaluation (CSE) of natural drugs in CSDs has been given priority and has been intensively studied. To provide fundamental evidence for the clinical application of natural drugs in the elderly population, clinical studies of natural drugs in CSDs included in this review were selected from CNKI, Web of Science, PubMed, Science Direct and Google Scholar since 2001. Seventeen randomized controlled trials (RCTs) met our inclusion criteria: four articles were on OP, seven on osteoarthritis (OA), four on rheumatoid arthritis (RA) and two on gout. Common natural drugs used for the treatment of OP include *Epimedium brevicornu Maxim* [Berberidaceae], *Dipsacus asper Wall ex DC* [Caprifoliaceae] root, and *Phalaenopsis cornu-cervi (Breda) Blume & Rchb. f*[Orchidaceae], which have been linked to several mild adverse reactions, such as skin rash, gastric dysfunction, abnormal urine, constipation and irritability. The safety of *Hedera helix L* [Araliaceae] extract, *Boswellia serrata Roxb* [Burseraceae] extract and extract from perna canaliculus was evaluated in OA and upper abdominal pain, and unstable movements were obsrerved as major side effects. Adverse events, including pneumonia, vomiting, diarrhoea and upper respiratory tract infection, were reported when RA was treated with *Tripterygium wilfordii, Hook. F* [Celastraceae][TwHF] polyglycosides and quercetin (*Capsella bursa-pastoris (L.) Medik* [Brassicaceae]). The present review aimed to summarize the CSE results of natural drugs in CSDs and could provide evidence-based information for clinicians.

Keywords: natural drugs, chronic skeletal disorders, ageing, clinical trial, safety evaluation

Abbreviations: CSDs, Chronic skeletal disorders; CSE, clinical safety evaluation; RCTs, randomized controlled trials; OP, Osteoporosis; OA, osteoarthritis; RA, Rheumatoid arthritis; GLM, Green Lipped Mussel; BSRE, *Boswellia serrata Roxb* extract; TwHf, *Tripterygium wilfordii Hook.f*; *MTX*, methotrexate; G2013, guluronic acid.

INTRODUCTION

The skeletal system has pivotal physiological functions, such as maintaining body shape (Gordon and Gordon, 2020), participating in limb movement, protecting important organs and detoxification (Londzin et al., 2020). The difficulty of healing with ageing is a main feature of CSDs, which has become a growing worldwide public health concern (Peng et al., 2021; Jaschke et al., 2021). The incidence of these chronic diseases is also impacted by the improvement in living standards and lack of exercise (Linbi et al., 2019). Age-related bone loss and joint degeneration are common causes of CSDs, such as OP and arthritis (Sebastian et al., 2020; Ju et al., 2021). Chemical drugs play a predominant role in the clinical treatment of these CSDs due to their rapid effects (Rodan and Martin, 2000; Gordon and Gordon, 2020). However, it has been observed that chemical drugs have many adverse effects (Lien et al., 2021). For example, Gupta, et al. (Gupta, et al., 2019) reported bisphosphonates are used to treat OP, resulting in adverse reactions such as o oily skin, fluid retention, nausea, long-term toxicity, and even prostate cancer in males; Christian et al. (Christian et al., 2021) reported NSAIDs would lead to cardiovascular, renal and gastrointestinal events in the elderly. Low metabolism and healing rates amplify the side effects of chemical drugs, which could subsrequently engender serious secondary injury, especially in elderly patients (Molina et al., 2014; Colón et al., 2018). The search for and study of drugs for CSDs with fewer side effects have been a lifelong focus of many researchers.

Natural drugs are those obtained from animals, plants and minerals that have certain proven pharmacological activity by modern pharmaceutical systems (Ma et al., 2019; Chang et al., 2020). Natural drugs are increasingly favoured by researchers and clinicians for their lower side effects and adverse reactions (Zeng and Jiang, 2010). Some natural drugs have been used to treat chronic skeletal disease since ancient times (Chen et al., 2016; Li et al., 2021). Natural drugs can be classified according to the different CSDs they treat: 1) OP, for instance, lcariin (Zhai et al., 2013; Xu et al., 2016; Wang Z. et al., 2017), *D. asper Wall ex DC* root (Zhan et al., 2009; Su et al., 2018), resveratrol (Wang X. et al., 2017; Feng et al., 2017; Yang et al., 2019), quercetin (*C. bursa-pastoris (L.) Medik*) (Zhen et al., 2018), ginsenoside (Yang et al., 2020) and genistein (Chen et al., 2018); and 2) OA, for instance, curcumin (Sun et al., 2017), capsaicin (Persson e t al., 2018), berberine (Wong et al., 2019), and *TwHF* polyglycosides (Lindler et al., 2020). Recent developments in CSDs research have heightened the demand for natural drugs.

To maximize the benefits and minimize the toxicity of natural drugs, safety evaluation has become an indispensable component of natural drug research (Si et al., 2017; Silva and Pogacnik, 2020). This article not only provides the CSEs of natural drugs in treating CSDs but also provides a comprehensive reference for natural drug research in skeletal disease.

Natural Drugs for Osteoporosis

Osteoporosis (OP) is a systemic bone disease characterized by decreased bone density and bone mass due to different causes, and this disruption of bone microstructure increases bone fragility (Jarvinen and Kannus, 2019; Wang et al., 2020), such as that seen in postmenopausal OP, senile OP and idiopathic OP (Ann et al., 2019; Jarvinen and Kannus, 2019). As ageing is aggravated in developed countries and in China, the incidence of OP is increasing sharply and has become an assignable health problem (Cui et al., 2019; Fan and Xia, 2019).

Lu Min et al. (Lu et al., 2013) designed an RCTs in patients from different regions to evaluate the clinical safety of the total flavones in *E. brevicornu Maxim.* capsules in treating primary OP. A random sample of 480 patients with primary OP was recruited and divided into two groups. The experimental group with 360 cases was prescribed total progesterone 0.7 g three times per day, while the control group with 120 cases was prescribed Gusongbao capsule 1 g three times per day. After 2 years of follow-up, the results revealed that the total efficacy of the experimental group and control group was 90.83 and 75.00%, respectively. The safety evaluation revealed that 1) no significant abnormalities occurred in noticeable parameters such as blood pressure, heart rate and body temperature after medication; 2) during the trial, no significant abnormalities were found in routine blood, urine and stool tests, including blood ALT, BUN, and Cr tests and electrocardiogram; and 3) twenty-four adverse events happened in the group receiving the total flavones from *E. brevicornu Maxim* capsules with an incidence of 6.67%. These adverse events were rash, dizziness, constipation, diarrhoea, palpitations, tinnitus, abdominal pain, sore stomatitis, ulcerative stomatitis, gastric dysfunction, pharyngitis, reduced sweating, and abnormal urine. Moreover, the control group had six adverse events with an incidence of 5.00%; these events included constipation, stomatitis, and ulcerative stomatitis.

In a multicentre, randomized, double-blind, parallel controlled clinical trial, Zhan Hongsheng et al. (Zhan et al., 2009) divided 600 volunteers into *D. asper Wall ex DC* capsule group (total saponin extract of *D. asper Wall ex DC* 0.28 g/tablet) and two other control groups (experimental group) at a ratio of 3: 1:1. The total effective rate of experimental group was 86.82%. The safety analysis results after 6 months indicated that 12 adverse events occurred within the trial group, with an incidence rate of only 3.33%; among them, there were 6 cases of abdominal discomfort with an incidence rate of 1.67%; 3 cases of constipation with an incidence rate of 0.83%; and 1 case of dysphoria, 1 case of swollen gums and 1 case of elevated blood ALT levels, each with an incidence rate of 0.28%. All of the above symptoms were mild adverse reactions.

Wang Hong et al. (Wang et al., 2004) reported clinical efficacy obserervations of *P. cornu-cervi* (The young horns of a male deer are not ossified and densely hairy) capsule in treating primary OP. Sixty OP patients were divided into two groups based on different treatments: *P. cornu-cervi* capsule (A group) or Gushukang capsule (B group) for 12 months. The total effective rate of group A was 82.14%, while the total effective rate of group B was 67.86%. Drug safety analysis showed that there was no heart, liver, or renal function damage, and electrocardiography of four cases that experienced myocardial strain returned to normal after *P. cornu-cervi* capsule treatment. Interestingly, two patients with abnormal routine urinary tests became normal after *P. cornu-cervi* capsule treatment.

Nevertheless, one patient had abnormal counts of leukocytes in the routine urinary test but returned to normal after norfloxacin treatment. No adverse effects of this drug on routine blood, stool or electrolyte tests were found during treatment. Interestingly, the WBC count of one case in routine blood tests rose from 2,700 to 4,600. Except for one case in the treatment group who withdrew from the experiment due to severe stomach pain, the majority of the volunteers tolerated the treatment very well.

Unfer et al. (Unfer et al., 2004) conducted a long-term randomized, double-blind, placebo-controlled trial to estimate the clinical safety of Phytoestrogens from *Glycine* max *(L.) Merr [*Fabaceae*]* in the treatment of postmenopausal OP. Three hundred seventy-six healthy postmenopausal women with intact uteri participated. One hundred seventy-nine patients in Group A received soy isoflavones (150 mg/d), and 197 patients in Group B received placebo for 5 years. However, There was no report on the effect of treatment, the safety results showed that among 298 female patients who completed 5 years of treatment, no malignant pathological changes were detected at the time of biopsy. Seventy percent of women receiving Phytoestrogens from *G.* max had atrophic or unevaluable changes in the endometrium, compared to 81% in the placebo group. The incidence of endometrial hyperplasia was higher in Group A than in Group B (3.37 vs 0%). Long-term administration of Phytoestrogens from *G.* max (up to 5 years) is associated with an increased incidence of endometrial hyperplasia. The above results suggest that the long-term impact and safety of phytoestrogens on the endometrium of postmenopausal women with OP should be considered.

From these clinical trials, it was found that the probability of adverse events of several natural drugs (*E. brevicornu Maxim.*), Himalayan teamel root; *P. cornu-cervi.* and Phytoestrogens from *G.* max was lower than that of the control group, indicating that the safety of those drugs was likely higher. Taken together, these results suggest that natural drugs could become a potential choice in the treatment of OP.

Natural Medications for Arthritis

Arthritis also belongs to the CSDs that affect load-bearing joints (knee, hip, foot and spine), joint synovium, periarticular bone and adjacent supporting connective tissue. The incidence of arthritis has become increasingly difficult to ignore in the elderly population and is expected to be a leading cause of worldwide population disability by 2030 (Glyn-Jones et al., 2015). The safety assessment of natural medicines for the treatment of OA, RA and gout is arranged in here to describe.

Osteoarthritis

Primary Osteoarthritis (OA) is widespread in middle-aged and elderly individuals, affects load-bearing joints and is characterized by articular cartilage degeneration, subchondral bone sclerosis, osteophyte formation and bone marrow oedema (Hunter and Bierma-Zeinstra, 2019). The clinical symptoms are mainly joint stiffness, joint pain, limited range of joint movement and joint deformity (Hunter and Bierma-Zeinstra, 2019; Glyn-Jones et al., 2015). The pathogenesis of OA is so unclear that the main treatment methods are simply aimed at relieving joint pain and at postponing joint replacement. Acesodyne, such as some nonsteroidal anti-inflammatory drugs, have side effects in the gastrointestinal, cardiovascular, and renal systems (Glyn-Jones et al., 2015). Clinical research on natural drugs has brought novel and safe therapeutic targets for the treatment of OA.

Pagosid is a pure natural plant preparation extracted from the massive roots of a plant called "devil's claw" (*Harpagophytum procumbens (Burch.) DC. ex Meisn* [Pedaliaceae]) growing in the Kalahari desert of Southwest Africa and the grassland of Namibia (Qiyun, 2001). It is mainly used for the treatment of rheumatic diseases and all kinds of pain. Liao Qiande et al. (Liao, et al., 2011) designed a clinical trial study to obsrerve the safety of pagosid and selected 268 patients with primary knee OA. Oral pagosid (3 × 820 mg/d, Swiss SmithKline pharmaceutical factory) treatment was administered for 8 weeks to observe knee tenderness, activity pain, joint swelling and daily activity at 4 and 8 weeks, as well as 4 weeks after drug withdrawal. The results indicated that the total efficacy was 65.8% at week 4, 80.8% at week 8, and 72.4% at 4 weeks after drug withdrawal. The incidence of adverse reactions, including digestive system, central nervous system and circulatory system events, was only 6.4% after 8 weeks of treatment. Specifically, there were 4 cases of dizziness, 6 cases of abdominal distension, 5 cases of nausea and vomiting, 1 case of giddiness, and 3 cases of abdominal discomfort. This research showed low adverse reactions and side effects of pagosid.

Morteza Dehghan et al. (Dehghan et al., 2020) published a comparison of the therapeutic effects of *H. helix L.* extract gel and diclofenac gel on knee OA. One hundred fifty (150) patients with primary OA were randomly assigned into three groups, namely, the 1% *H. helix L.* extract gel treatment group, 1% diclofenac gel treatment group, and placebo treatment group. Drugs were applied orally times a day, for 3–5 min each time, during a trial period of 6 weeks. All patients were allowed to take celecoxib capsules daily. The efficacy results revealed that the groups treated with 1% *H. helix L.* extract gel or 1% diclofenac gel had a significantly higher easement of pain than the placebo group. The safety analysis showed that the 1% *H. helix L* extract gel-treated group did not show any allergic reactions or adverse reactions compared to those of the 1% diclofenac gel-treated group. However, the 1% diclofenac gel-treated group exhibited a significant decrease in body function.

Marzieh Alazadeh et al. (Alazadeh et al., 2020) conducted an RCTs to obsrerve the effect of *Foeniculum vulgare Mill [*Apiaceae*]* (commonly known as Fennel) seed extract capsules in relieving knee OA pain. Sixty-six 66) patients were randomized to the sweet fennel seed extract capsule group and the placebo group. Treatment consisted of oral intervention of fennel extracts (4 capsules containing 800 mg of dry fennel extract from 28 g of fennel seeds) or placebo twice a day for 2 weeks. Oral fennel can reduce Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain by about 36%, Visual Analog Scale (VAS) pain by about 32%, and the placebo group's pain reduction rates were 15 and 13%, respectively. These results indicate that fennel is effective in controlling the symptoms of knee OA. The safety analysis found that no serious side effects occurred, but a transient effect on the breast appeared in the drug intervention group. Heartburn was another side effect

TABLE 1 | Adverse reactions to curcuma extracts in knee OA and ibuprofen patients.

	Curcuma domestica extract group (n = 48)	Ibuprofen group (n = 52)	p -value
Total No. of patients with an AE	16	23	0.35
Alimentary system	15	21	
Dizziness	5	2	
Dry mouth	0	2	
Rash	0	1	
Fatigue	0	1	

reported by three patients in the placebo group and two patients in the fennel group; these subjects were subsequently excluded from the study.

B. Grube et al. (Grube et al., 2007) reported the therapeutic effect of a comfrey root (*Symphytum officinale L [*Boraginaceae*]*) extract ointment on OA pain. This trial adopted a double-blind, double-centre, placebo-controlled randomized trial that recruited 220 patients with knee OA at an average age of 57.9 years. Patients were randomized into two groups with Kytta Salbe containing purple grassroots liquid extract or the control group. Both of the groups took drugs orally three times daily, 2 g each time for 3 weeks. The treatment effect was evaluated by the total score of VAS and the total scores of the WOMAC. The results suggest that a comfrey root extract ointment can reduce pain and improve mobility in the treatment of knee osteoarthritis. The safety evaluation depicted that 22 adverse reactions (10.0%) were reported, including 7 cases in the trial group (6.4%) and 15 cases in the control group (13.6%). All of the adverse reactions were mild symptoms, and no significant difference was found between the two groups. The authors considered that there were no obvious adverse reactions to the drug. Patients and doctors were questioned four times during the trial to comprehensively assess drug tolerance. Responses were classified as 'very good', 'good', 'moderate' or 'bad'. Among them, 'very good' was the response for 73.6% (doctors and patient) in the trial group.

Vilai Kuptniratsaikul, M.D. et al. (Kuptniratsaikul et al., 2009) reported the treatment effect and safety analysis of Curcuma domestica (*Curcuma longa L [*Zingiberaceae*]*) extract on the alteration of pain and joint function in knee OA patients. One hundred seven patients (107) with OA were randomized into 55 controls who took ibuprofen 800 mg a day, and 52 patients in the *C. domestica L.* extract group who took curcumin 2 g per day for 6 weeks. The time of walking 100 m, going up and down stairs was used to evaluate the improvement of horizontal walking pain, stair pain and knee function. The therapeutic effect of *C. domestica L.* extracts is almost consistent with that of ibuprofen for the treatment of keen OA. Then, the safety analysis results are shown in **Table 1**. Sixteen adverse reactions (33.3%) occurred in the curcumin group, while only three occurred in the ibuprofen group.

As we can see from **Table 1**, the adverse reactions included indigestion, dizziness, nausea and vomiting, and loose stool. No significant changes were found in the blood tests at 0 weeks or 6 weeks. The tolerance of the ibuprofen group was better than that of the *C. domestica L.* extract group (90.1 vs 82.8%, p = 0.001), which may be because of the odour of the *C. domestica L.* extract.

N. Kimmatkar et al. (Kimmatkar et al., 2003) reported the efficacy and tolerance of *B. serrata Roxb* extract (BSRE) in knee OA using RCTs. Thirty (30) knee OA patients were divided into the BSRE and placebo groups. All patients took capsules of the same appearance and weight three times per day, one capsule each time for 8 weeks, and the BSRE group capsules contained 333 mg of BSRE. The clinical efficacy suggest that compared with the placebo group, the reduction of pain and swelling severity and the improvement of functional loss in the active drug group were clinically and statistically significant ($p < 0.001$). Safety analysis revealed that adverse reactions included one instability of gait and one upper abdominal pain with nausea, and neither of them quit the trial due to adverse reactions. The overall compliance of patients to BSRE was acceptable.

Simon Stebbings et al. (Stebbings et al., 2017) reported the efficacy and safety of extract from the BioLex®-Green Lipped Mussel (BioLex®-GLM) (*perna canaliculus*) for treating pain in the hip and knee OA. This trial selected 80 patients with moderate to severe hip or knee OA pain and randomized them to two groups treated with 600 mg of BioLex®-GLM per day or placebo for 12 weeks. This biolex®-GLM extract treatment results only showed less acetaminophen than the placebo group, because biolex ®- GLM extract can reduce pain in patients with moderate and severe arthritis. In addition, the treatment group also significantly reduced the degree of stiffness. Safety analysis showed that three adverse events occurred in the placebo group: 1) systemic pain and flu-like symptoms 7 weeks after treatment with placebo, 2) a bilateral pulmonary embolism after 24 days of placebo administration, and 3) extensive generalized pruritus rash after 62 days of placebo, with the rash lasting for 3 weeks. Only one adverse event occurred in the Biolex®-GLM treatment group but required hospitalization because of abdominal pain that occurred 8 days after treatment started. The author found that the patient had a chronic history of stomach disease, which means that the adverse event may not have been caused by Biolex®-GLM. All of the above volunteers withdrew from the study after the adverse reactions occurred.

All the results of clinical trials indicated that natural drugs have good safety profiles and obvious treatment effects and are expected to become alternative drugs for the treatment of OA.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease in which the immune system is activated abnormally and attacks healthy joints (Brennan et al., 2019; Edmonds et al., 2019; Mutru et al., 2019) and affects over 21 million people worldwide (Aletaha and Smolen, 2018). Hyperplasia of synovial tissue invades and

TABLE 2 | Comparison of adverse reaction events between TwHf and sulfasalazine in the treatment of RA (Goldbach-Mansky et al., 2009), Copyright 2009, ACP.

Adverse events	TwHf all patients (n = 60)	Sulfasalazine group all patients (n = 61)	*p* Value
Total events, n (%)			
Any event	53 (88.3)	55 (90.2)	0.78
Related to study drug	34 (56.7)	37 (60.7)	0.71
Serious adverse events‡	3 (5)	7 (11.5)	0.32
Most frequent adverse events, n (%)			
Nausea	13 (22)	21 (34)	0.157
Vomiting	9 (15)	9 (15)	1.00
Diarrhoea	15 (25)	11 (18)	0.38
Constipation	5 (8)	8 (13)	0.56
Dyspepsia	13 (22)	15 (8)	0.044
Abdominal distention	6 (10)	2 (3)	0.163
Abdominal pain	11 (18)	6 (10)	0.20
Infectious adverse events			
Upper respiratory tract infection	11 (18)	6 (10)	0.20
Influenza	2 (3)	4 (7)	0.68
	1 (2)	0 (0)	0.50
Urinary tract infection	2 (3)	6 (10)	0.27
Pneumonia Other infections	2 (3)	7 (11)	0.163

damages articular cartilage and is the main characteristic of RA (Rosa et al., 2020). Symptoms of RA include weakness, fever, fatigue, weight loss and myalgia (Aletaha and Smolen, 2018). Females are more vulnerable to RA than males (Alpizar-Rodriguez et al., 2017).

(Goldbach-Mansky et al., 2009; Raphaela Goldbach-Mansky et al., 2021) compared the effect and safety of TwHf extracts and sulfasalazine on RA by randomized controlled trials. One hundred twenty-one 121) patients with active RA were selected, and more than six patients had joint pain and swelling. Participants took 3 × 60 mg/d TwHf in the TwHf group or sulfasalazine 1 g twice per day in the control group. After 2 weeks of treatment, a significantly greater improvement was found in the TwHF group compared with the sulfasalazine group at baseline, and this improvement continued throughout the assessment of health, ESR and CRP levels. From 8 weeks of treatment, the improvement in the number of swollen and tender joints in TwHF group was statistically significantly greater than that in sulfasalazine group. Adverse events are illustrated in **Table 2**; approximately 60% of all patients and volunteers had gastrointestinal symptoms. Fifteen serious adverse events were reported in 10 patients, three of whom received TwHf treatment and seven of whom received sulfapyridine. Seventeen patients in the sulfadiazine group dropped out due to adverse events, while only eight patients in the TwHf group dropped out. Adverse events that led to seceding from the study included six gastrointestinal events, one thrombus reduction, one severe adverse event and one femur fracture. It is obvious that patients in the sulfasalazine group had moderate to severe adverse events compared to those of the TwHf group.

Qian-wen Lv et al. (Lv et al., 2015) conducted a clinical trial to compare the safety between methotrexate (MTX) and TwHf in treating active RA (TRIFRA). This was a multicentre, open label and randomized controlled trial. Two hundred twenty-seven 227) active RA patients were recruited and were assigned randomly and equally to three groups to receive MTX 12.5 mg once weekly, TwHf 20 mg three times a day or a combination of both treatments in 2 years. After treatment, the erythrocyte sedimentation rate (ESR) in TwHF group and combination group decreased significantly at week 12, while ESR in MTX group did not decrease significantly until week 24. In the fourth week, the TwHF group had a greater improvement in ESR changes than the MTX group (p = 0.04). Then, the safety evaluation results are summarized in **Table 3**. The analysis revealed that 52.7% of the patients had adverse events in all groups. The incidences of adverse events were 46.4, 62.3 and 49.3% in the TwHf group, MTX group and TwHf + MTX group, respectively. Gastrointestinal tract adverse effects, as the most common adverse reactions, appeared in 29.0, 43.5 and 34.8% of the TwHf group, MTX group and TwHf + MTX group (p = 0.202), respectively. One patient in the TwHf group, three patients in the MTX group and three patients in the TwHf + MTX group dropped out due to severe adverse events, but there was no statistically significant difference in the quit rate among the treatment groups (p = 0.554). Patients in the TwHf group dropped out due to elevated blood alanine transaminase (ALT 92 U/L). In the combination group, tuberculous pleurisy, elevated blood ALT (ALT 89 U/L) and a gastrointestinal event resulted in withdrawal. In the 24 weeks of the trial, 15 (8.8%) female patients (n = 170, including 69 premenopausal women) had menstrual irregularities, including seven patients in the TwHf group, three patients in the MTX group and five patients in the combined group (TwHf group vs MTX group, p = 0.216).

According to the TRIFRA study, using TwHf alone is not inferior to the MTX + TwHf combination treatment, but combination treatment is better than MTX monotherapy in patients with active RA. These efficacy results confirmed that TwHf combined with methotrexate is a safer and effective treatment for active RA.

TABLE 3 | Clinical adverse reaction events of *Tripterygium wilfordii* Hook F Multiglycosides (TwHf) and methotrexate (MTX) in the treatment of motor-active rheumatoid arthritis (Lv et al., 2015), Copyright 2014, Ltd (and EULAR).

Adverse events	MTX (n = 69)	TwHf (n = 69)	MTX + TwHf (n = 69)	Total events (n = 207)
All	43 (62.3)	32 (46.4)	34 (49.3)	109 (52.7)
Gastrointestinal, n (%)	30 (43.5)	20 (29.0)	24 (34.8)	74 (35.7)
Nausea	7	6	10	23
Vomiting	1	2	1	4
Loss of appetite	10	1	6	17
Diarrhoea	3	2	1	6
Abdominal distention	2	2	3	7
Abdominal discomfort	11	7	8	26
ALT elevation	11	4	6	21
Infection, n (%)	10 (14.5)	3 (4.3)	7 (10.1)	20 (9.7)
Upper respiratory tract infection	3	1	0	4
Skin and mucous event, n (%)	14 (20.3)	7 (10.1)	14 (20.3)	35 (16.9)
Irregular menstruation, n (%)*	3 (5.1)	7 (12.5)	5 (9.1)	15 (8.8)
Skin and mucous event, n (%)	14 (20.3)	7 (10.1)	14 (20.3)	35 (16.9)
Other adverse events, n (%)	16 (23.2)	5 (7.2)	13 (18.8)	34 (16.4)
Fatigue	5	2	1	8

Shahin et al. (Khadem Azarian et al., 2019) reported the efficacy and safety of guluronic acid (G2013) from gulose (*Tinospora cordifolia (Willd.) Hook. f. and Thomson [*Menispermaceae*]*) through a randomized, double-blind clinical trial that recruited 52 R A patients. Twenty-six 26) patients were in the G2013 group, and the rest of the patients were in the control group. Both treatments significantly improved most primary efficacy endpoints within 12 weeks of G2013 and conventional drug treatment, and the difference between baseline and endpoint measurements of all efficacy endpoints of G2013 was significantly greater than that of conventional treatment subjects, which implies that the therapeutic effect of G2013 is significantly better than that of the traditional drug group. The outcome demonstrated a good tolerance of G2013 treatment. The adverse event incidence of the control group was higher (76.9%) than that of the G2013 group (15.3%). None of the patients receiving G2013 dropped out due to adverse events, while two patients quit in the control group. In addition, most patients reported a significant change in mood after G2013 treatment. There was no statistically meaningful dissimilarity in the mean haematologic and biochemical values at 0, 4 and 12 weeks after trial initiation, indicating the good safety profile of G2013.

Fatemeh et al. (Javadi et al., 2017) reported that quercetin (*C. bursa-pastoris (L.) Medik*) could change the clinical symptoms and inflammatory factors of females with RA via RCTs. Fifty women with RA were recruited and treated for 8 weeks with 500 mg/d quercetin or placebo. During the study period, 10 patients quit the trial due to the need to increase the drug volume or switch to other drugs. Eventually, 20 people in each group completed the trial. The evaluation of therapeutic effect showed that the clinical symptoms, disease activity, HS-TNFa and health assessment questionnaire of patients with rheumatoid arthritis were significantly improved after supplementing 500 mg quercetin every day for 8 weeks. And no side effects were reported except for one stomach ache from a patient in the placebo group who was excluded from the study.

Gout

Gout, a painful inflammatory arthritis, is caused by monosodium urate crystals being deposited in synovial fluid and other body tissues (Martinon et al., 2006). Hyperuricaemia has been identified as the primary cause of gout (Zimmet et al., 2019; Richette and Bardin, 2021). In Western countries, the prevalence of gout has increased over the past few decades, affecting approximately 1–2% of adult males (Dehlin et al., 2020). The main method for the treatment of gout and hyperuricaemia is to reduce serum uric acid levels (Solomon et al., 2018). Allopurinol (Crisp, 2021; Drugs, 2021) and febuxostat inhibitors are commonly used drugs to reduce the level of circulating urate. However, the side effects and adverse events of these drugs often reduce patients' willingness to receive drug therapy (Crisp, 2021), so it is worth paying attention to the safety of natural drugs in gout treatment.

Colchicine is a botanical alkaloid originally extracted from the seeds and bulbs of *Colchicum autumnale L [*Colchicaceae*]* (McKenzie et al., 2021). The therapeutic effect of high-dose and low-dose oral colchicine on early acute gout attacks was reported by Robert A et al. (Terkeltaub et al., 2010) in a multicentre RCTs. One hundred eighty-four 184) patients were recruited. Therapeutic effects were reported in 28 patients (37.8%), 17 patients (32.7%) and 9 (15.5%) patients in the low-dose group, the high-dose group and the placebo group, respectively. Twenty-three patients (31.1%) in the high-dose group, 18 patients (34.6%) in the low-dose group and 29 patients (39.7%) in the placebo group took rescue drugs within the first 24 h after the trial began. The results show that the effect of low dose is not as good as that of high dose. Adverse events in the low-dose group were similar to those in the placebo group. High doses of colchicine were related to diarrhoea, vomiting and other AEs compared with low doses of colchicine or placebo. After using a high dose of colchicine, 40 patients (76.9%) developed diarrhoea, 10 patients (19.2%) developed severe diarrhoea, and nine patients (17.3%) developed vomiting.

Meanwhile, only 23.0% of patients treated with low doses of colchicine developed diarrhoea.

Kubomura, D et al. (Kubomura et al., 2016) conducted 4-weeks RCTs to investigate the efficacy and safety of tuna (belongs to the *Thunnini* tribe and is a subgroup of the Scombridae family) extracts containing imidazole compounds in anti-hyperuricaemia treatment. Forty-eight males without gout but with slightly high levels of serum uric acid were randomized into the low-dose (supplement dose of 238.6 mg/d) and high-dose (supplement dose of 477.1 mg/d) tuna extract groups or the placebo group. In the process of treatment, the uric acid levels in the trial supplement (low-dose and high-dose tuna extract) group decreased significantly at weeks two and four compared with the placebo group. In addition, in the second week after the intervention, the uric acid level of the high-dose tuna extract test supplement group was significantly lower than other groups. However, the safety results revealed 44 adverse events, such as cold symptoms, stomach pain and headaches, in all groups. Since those adverse events were generally mild, none of them were inferred to be due to the study treatment. Furthermore, no physical or cardiovascular symptoms or haematologic abnormalities were found during the intervention and follow-up periods.

DISCUSSION AND OUTLOOK

Skeletal diseases have become the leading cause of disability in China, and the high quality of life demanded by elderly patients promotes the innovation of drugs for treating CSDs (Zhou M, et al., 2019). Chemical drugs have explicit pharmacological mechanisms and effects, and the side effects on other important systems or organs should not be ignored. The safety of drugs in CSDs therapy is the major concern because those diseases are not normally lethal. Severe adverse effects that may influence the circulatory system or central nervous system restrict the application of chemical drugs. Natural drugs are relatively safer than artificially synthesized drugs because of their natural sources but still need to be verified by the clinical results of evidence-based medicine.

In this review, we collected high evidence-level clinical results of natural drug application in CSDs, especially in OP and OA. The safety evaluation results of natural drugs in human clinical trials were summarized. This manuscript collected a total of 17 clinical trials on skeletal diseases using the RCTs method. Among them, there were four articles on the treatment of OP, seven articles on the treatment of OA, four articles on the treatment of RA and two articles on gout. This evidence supports the conclusion that natural drugs have better tolerance and fewer side effects in CSDs.

On the premise of ensuring the effectiveness of natural drugs, adverse events can almost be ignored in the report of clinical trials for the treatment of OP. For instance, 360 cases used total flavones of *E. brevicornu Maxim* capsule in a 2-years follow-up, and only 6.67% had adverse reactions, including rash, dizziness, constipation, and diarrhoea. In a clinical trial study of total saponin extract of *D. asper Wall ex DC* roots, it was found that only 12 events of 300 cases in the experimental group (total saponin extract 0.28 g/tablet) had adverse reaction events (abdominal discomfort and constipation) at the 6-month follow-up. After 12 months of the treatment with *P. cornu-cervi* for OP (30 cases), only one case had abnormal urine routine leukocyte count and returned to normal after norfloxacin treatment and one case of stomach pain were reported. We screened three natural drugs with the lowest incidence of adverse events. These natural drugs had enough safety for long-term administration. Among them, *P. cornu-cervi* had the best safety and has potential utilization value in the treatment of OP. On the other hand, the clinical reports discribing the treatment of natural drugs for osteoporosis were already 10 years ago. The clinical trials in recent years have not paid enough attention to the therapeutic effect of natural drugs, which hinders the development of natural drugs. In the future, more efforts should be made to the research on the use of natural drugs in the treatment of osteoporosis.

There are many studies on natural drugs for the treatment of OA. Here, we selected three natural drugs with high safety profiles, according to the number of adverse events from the seven natural drugs mentioned in this paper. After treatment with 1% *H. helix L.* extract for 6 weeks, 75 patients of all 1% *H. helix L* group did not report any adverse events. After 8 weeks of application of *B. serrata* extract (contained 333 mg/d of BSRE), there was only one case of instability of gait and one case of upper abdominal pain. Among 80 patients with OA treated with 600 mg of BioLex®-GLM per day or placebo for 12 weeks, only one case of abdominal pain occurred within 12 weeks due to a long-term history of stomach disease in that patient. In conclusion, the above three natural drugs for the treatment of OA have excellent safety and could be potential drugs for OA. By comparing these natural drugs, we found that few reports on adverse reaction events of these three natural drugs. Therefore, we have a hypothesis whether these drugs can still increase the dose (within a reasonable dose) to amplify the therapeutic effect. In addition, some natural drugs anti-inflammatory and pain-relieving effects can also be used for the clinical treatment of arthritis, because they are very corresponding to the symptoms of OA.

According to the statistics of clinical trials for the therapeutic effect of natural drugs in RA, the adverse event rate of *TwHF* polyglycosides (60 mg/d) accounted for 46% in 2 years. The incidence of adverse events in the G2013 group was 15.3% within 12 weeks, while there were no adverse events in the quercetin group for 8 weeks. Therefore, quercetin (*C. bursa-pastoris (L.) Medik*) is attractive for the treatment of RA because of the lack of adverse events. Many natural drugs had to stop clinical trials in view of serious adverse reactions, even they had good therapeutic effects. Therefore, there is a reliable inference that it may be difficult to find natural drugs with good therapeutic effect and low adverse side effects due to the complex pathological symptoms of RA.

According to the literature, there are few clinical trials of natural drugs for the treatment of gout, and only two RCTs were retrieved. Low-dose colchicine treatment is more suitable for gout patients. Using high-dose colchicine, 40 patients (76.9%) developed diarrhoea, 10 patients (19.2%) developed severe diarrhoea, and nine patients (17.3%) developed vomiting. Using low-dose colchicine, 23.0% of patients developed

diarrhoea, and no severe diarrhoea or vomiting occurred. Tuna extracts containing imidazole compounds in anti-hyperuricaemia treatment caused seven adverse events in the low-dose group, 10 in the high-dose group and five in the placebo group. Adverse reactions caused by these two natural drugs should not be ignored. Patients with gout should be given detailed instructions and suggestions for clinical medication. Research on natural drugs for gout is significantly less than that for other skeletal diseases, indicating that there is still enough space for developing new drugs with research value.

Many bone-related diseases require long-term medication. The safety evaluation of natural drugs can provide a detailed medication reference for clinicians, especially in the treatment of ageing-related CSDs. The development and safety evaluation of natural drugs have become a prominent topic in drug research on CSDs. Therefore, because clinical safety evaluation also requires long-term follow-up observation, we have screened out natural medicines that have reported few adverse events, but found that the evaluation time is often very short (<2 years). In order to evaluate their safety more rigorously, a long-term safety evaluation is still needed to verify, and we will continue to pay attention to reports on the safety of these drugs.

CONCLUSION

This review summarizes the clinical safety evaluation of multiple natural drugs in the treatment of OP, OA, RA and gout, and lists the clinical trial design, clinical experimental methods and adverse reaction events of patients treated with different natural drugs. Systematic analysis and rational evaluation of natural drugs in clinical adverse event and side effect were carried out to provide evidence for further clinical drug research in CSDs. In addition, clinical safety evaluations of natural drugs in the treatment of CSDs are exiguous. Using traditional literature search channels, it is difficult to obtain more comprehensive clinical trial data of natural drugs for CSDs, because of the following reasons: the complex structure requires more complex production technology, leading to an expensive production cost; the development of natural plants with therapeutic potential to treat CSD are limited by the existing scientific research level and technology; the active components in these natural plants are difficult to determine; The results of clinical trials of some natural drugs are not disclosed. But These results indicate that more clinical trials should be conducted to explore more natural drugs with good pharmacodynamic performance.

AUTHOR CONTRIBUTIONS

DZ, HZ and XX contributed equally to this work. DZ fixed the subject of the manuscript, searched the literature, sorted out the article structure, and wrote the full text, HZ carry out logical sorting and writing correction, XX designed this review frame, YT performed the online database search, and SW, XR and JS guided and revised the manuscript of this review.

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SYSTEMATIC REVIEW
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Safety of Cinnamon: An Umbrella Review of Meta-Analyses and Systematic Reviews of Randomized Clinical Trials

*Dan-Tong Gu[1†], Tao-Hsin Tung[2†], Zhu Liduzi Jiesisibieke[3], Ching-Wen Chien[3] and Wen-Yi Liu[3,4,5]**

[1]Institute of Otolaryngology, Clinical Research Center, Fudan University Affiliated Eye and ENT Hospital, Shanghai, China, [2]Evidence-based Medicine Center, Taizhou Hospital of Zhejiang Province Affiliated with Wenzhou Medical University, Linhai, China, [3]Institute for Hospital Management, Tsing Hua University, Shenzhen, China, [4]Department of Health Policy and Management, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [5]Shanghai Bluecross Medical Science Institute, Shanghai, China

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Xiao Chen,
Second Military Medical University, China

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***Correspondence:**
Wen-Yi Liu
66886908@qq.com

[†]These authors have contributed equally to this work

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Purpose: Many evidence-based studies have indicated that cinnamon has therapeutic effects. However, it may not be entirely safe and its adverse effects may be ignored. The present umbrella review was conducted to elucidate the safety of cinnamon.

Methods: Pertinent meta-analyses and systematic reviews of randomized controlled trials on cinnamon use in humans were identified by searching PubMed, EMBASE, and the Cochrane Library from their inception to September 15, 2021. All meta-analyses and systematic reviews on the safety or adverse effects of cinnamon were considered. PRISMA 2020 was used as the standard of reporting (PRISMA registration ID: 286746).

Results: We identified three meta-analyses and one systematic review that described the safety of cinnamon. The quality of the meta-analysis and systematic reviews was evaluated using "Assessing the Methodological Quality of Systematic Reviews." Their quality was rated as low in two (50%) instances and moderate in two (50%). There were no significant toxic- or side effects between cinnamon group and placebo group regardless of dose and duration.

Conclusion: There is evidence to support that the use of cinnamon has no adverse reactions. It can improve the health status of patients as an adjuvant treatment. Future studies exploring better profile risks and protective factors for cinnamon use-related adverse effect are needed, in order that preventive approaches can be developed.

Keywords: safety, cinnamon, umbrella review, systematic reviews, meta-analyses

Abbreviations: Hba1c, glycated hemoglobin; RCT, randomized clinical trial; AMSTAR 2, Assessing the Methodological Quality of Systematic Reviews; SXBXP, Shexiang Baoxin Pill; HOMA-IR, homeostasis model assessment of insulin resistance.

1 INTRODUCTION

Cinnamon obtained from *Cinnamomum* verum J. Presl (family Lauraceae) is a common spice used worldwide and a tropical medicine. It contains manganese, iron, dietary fiber, calcium, their derivatives, and other related compounds (Abraham et al., 2010). Cinnamon is a popular ingredient in cooking, medicine, forage, and is used in many industries (Michiels et al., 2007; Santos and da Silva, 2018). From a clinical viewpoint, it is often used in diabetes treatment because of its hypoglycemic and lipid-lowering potential (Santos and da Silva, 2018; Zare et al., 2019). It has also been found to be useful in reducing glycated hemoglobin (HbA1c) and fasting blood glucose levels in patients with type 2 diabetes (Pauline and Maddox, 2017). In addition, cinnamon has antimicrobial and antioxidant properties, and its application has been recommended singly or as a supplement in the treatment of cancers such as promyelocytic leukemia (Jayaprakasha and Rao, 2011; Assadollahi et al., 2013). Cinnamon bark, cinnamon twig, and shaved cinnamon bark differ in their compound compositions, and these differences could be used to achieve quality control when using cinnamon (Chen et al., 2016). Cinnamon bark contains natural antioxidants, that could reduce the risk of cancer, and signs of aging (Ghosh et al., 2015). Cinnamon twigs are commonly used for treating inflammatory diseases and amenorrhea in China (Ghosh et al., 2015). Shaved cinnamon bark together with other traditional Chinese medicines could delay the process of the deterioration of some heart diseases such as congestive heart failure (Huang, 2014).

Despite the several clinical benefits afforded by cinnamon, concerns about its safety persist (Kort and Lobo, 2014; Deyno et al., 2019). The results of some studies have indicated that the safety of cinnamon is related to parameters such as fasting blood glucose, serum insulin, and alanine aminotransferase levels (Blevins et al., 2007; Akilen et al., 2012; Shishehbor et al., 2018). With respect to adverse effects, while some studies reported no adverse effects in individuals treated with cinnamon, indicating the safety of this traditional medicine (Mang et al., 2006; Suppapitiporn et al., 2006), whereas others reported dermatological problems (Altschuler et al., 2007; Crawford, 2009). Studies of different dosages of cinnamon have mostly been conducted in animals. Thus, the evidence for the safety of cinnamon remains limited and controversial, and the potential safety problems remain unknown. The present umbrella review was conducted to elucidate the safety of cinnamon based on the existing systematic reviews and meta-analyses of randomized clinical trials (RCTs), which may facilitate a better understanding of the side effects of cinnamon among healthcare workers and policy makers.

2 METHODS

2.1 Search Strategy and Eligibility Criteria

This umbrella review involved an evaluation of pertinent systematic reviews and meta-analyses, so as to draw more reliable conclusions (Smith et al., 2011; Aromataris, 2014). PubMed, EMBASE, Cochrane Library, and the Web of Science were systematically searched from their inception to September 15, 2021, to identify systematic reviews and meta-analyses of RCTs examining the safety of cinnamon. The search string used was "[(cinnamo* OR cinnamic) AND (safety OR security) AND (efficacy OR efficiency OR effect*) AND (hepa* OR liver)]" without a language restriction (**Table 1**). The protocol for this systematic review was recorded in PROSPERO with the identification number 286746.

The exclusion criteria were as follows: 1) studies without safety evaluation; 2) studies on pharmacokinetics that involved *in vivo* experiments; and 3) animal studies. The search was not limited by the dosage of cinnamon or the length of treatment. Only meta-analyses and RCTs were considered. The entire selection process was conducted by Wen-Yi Liu and Zhu Liduzi Jiesisibieke

TABLE 1 | Search strategy until August 10, 2021.

		PubMed	Embase	Cochrane	Web of science
#1	Cinnamo*	6,537	3	523	13,913
#2	Cinnamic	28,049	9,996	45	8,123
#3	Safety	720,387	1,404,766	261,301	958,659
#4	Security	124,523	104,253	3,436	413,579
#5	Efficacy	942,083	1,868,531	385,327	1,128,079
#6	Efficiency	1,100,052	574,856	19,035	1,954,432
#7	Effect*	10,087,377	6,113,819	1,039,614	13,376,402
#8	Hepa*	1,083,168	3,792	69,829	961,359
#9	Liver	1,197,424	1,788,028	64,507	949,464
#10	#1 or #2	33,921	9,999	560	21,500
#11	#3 or #4	833,939	1,496,295	263,913	1,348,452
#12	#5 or #6 or #7	11,034,060	7,662,872	1,176,084	15,198,353
#13	#8 or #9	1,716,602	1,790,406	103,091	1,533,755
#14	#10 and #11	888	384	110	446
#15	#10 and #12	21,806	3,097	505	8,614
#16	#10 and #13	3,336	483	79	1,128
#17	#10 and #11 and #12	717	180	108	338
#18	#10 and #12 and #13	2,458	237	78	636
#19	#17 and #18	101	24	31	50

TABLE 2 | Characteristics of the included studies.

Reference	Outcome investigated	Patients	Selection as most comprehensive	RCTs included	Prospective studies included	Retrospective studies included	Study quality (AMSTAR rating)
Mousavi et al. (2021)	Alanine aminotransferase Aspartate aminotransferase Alkaline phosphatase	236 222 53	✓ ✓	9	0	0	Low
Zhou et al. (2016)	Gastrointestinal symptoms Headache or/and dizziness Feeling of numbness in the mouth and tongue Palpitation Adverse events	641 281 641 200 641	✓	11	0	0	Low
Leach et al. (2012)	Adverse events Fasting blood glucose level Postprandial blood glucose level Glycosylated hemoglobin A1c (HbA1c) Serum insulin Insulin sensitivity (CHO/unit insulin) Insulin sensitivity (HOMA-IR)	264 304 40 405 81 48 25	 ✓ ✓ ✓	10	0	0	Moderate
Deyno et al. (2019)	Fasting blood glucose Insulin resistance (HOMA-IR) Insulin HbA1c level Low-density lipoprotein (LDL) level (mmol/L) HDL level (mmol/L) TC level (mmol/L) TG level (mmol/L) BMI (kg/m^2)	1,098	✓ ✓	16	0	0	Moderate

independently, and any disagreements were settled by discussion with a third principal author, Tao-Hsin Tung.

2.2 Data Extraction and Quality Assessment

For each selected meta-analysis, we focused on the following items: level of comparison, random-effects summary, I^2, and small-study effects, a. k.a.,/excess significance bias. The following information was obtained from the selected meta-analyses: author and year, outcome, number of patients, number of study types, and AMSTAR 2 (Assessing the Methodological Quality of Systematic Reviews) assessment scores.

2.3 Assessment of Methodological Quality

The methodological quality of the works included was evaluated using the AMSTAR 2 guidelines, which includes16 items that systematically score evidence-based medicine studies (Shea et al., 2009; Poole et al., 2017). It is not intended to provide an overall score based on the evaluation results of each item, as a high score may mask some very serious methodological deficiencies and provide a high-quality evaluation (Shea et al., 2017) (**Table 2**). AMSTAR 2 is considered a reliable and valid tool for evaluating the quality of systematic reviews and meta-analyses of interventional and observational research (Shea et al., 2009; Pieper et al., 2014; Poole et al., 2017). It includes ratings for the quality of academic studies, statistical analyses, and openness of meta-analyses. Regarding the rating items for the methodological quality of meta-analyses, the fixed-effects model for the summary estimate was downgraded compared to the random-effects model. This means that the random-effects model was considered the most suitable to be used for pooled estimates due to the heterogeneity in study samples, study designs, methods of cinnamon preparation, and duration. A single real effect size was not considered relevant to all selected studies.

2.4 Assessment of Epidemiological Credibility

Relationships that had the highest evidence and no hints of major heterogeneity or bias were determined (Bellou et al., 2018). We considered persuading the relationships that met all the following criteria were persuasive: statistical significance per random-effects model at a p-value of <0.000001 with more than 1,000 cases, no high heterogeneity among selected studies (I^2 < 50%), 95% prediction interval (excluding the null value), and no evidence of small-study effects and significant bias. Associations with more than 1,000 cases, a p-value of <0.000001, and most studies indicating a significant effect were viewed as highly recommended. The associations supported by more than 1,000 cases and significant effects at a

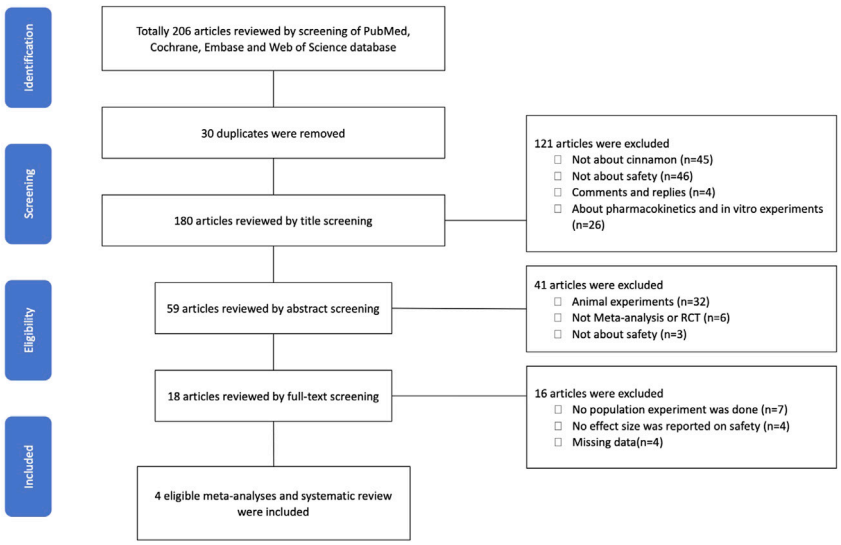

FIGURE 1 | PRISMA flow diagram of the study.

p-value of <0.001 were graded as "recommended." Nominally significant associations ($p < 0.05$) were considered weak evidence. Evidence obtained from fewer than 1,000 samples was graded as poor.

3 RESULTS

A total of 206 articles and 180 articles were reviewed using title screening (**Figure 1**). We finally included four studies that fulfilled the eligibility criteria. Of these four studies, the study by Mousavi et al. (2021) included alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels as outcomes that would indicate the safety of cinnamon supplementation with respect to liver enzymes. The authors found that cinnamon improved serum levels of hepatic enzymes in patients with type 2 diabetes. The clinical benefits of cinnamon described Zhou et al. (2016) included gastrointestinal symptoms, headache and/or dizziness, sensation of numbness in the mouth and speech difficulties, palpitations, and adverse events as outcomes that would indicate the effectiveness and safety of the Shexiang Baoxin Pill (SXBXP, a patented Chinese medicine). The SXBXP is composed of Cortex Cinnamomic and other seven medical materials or extracts, and there was no significant difference in adverse events between the SXBXP and control groups in the study. Leach and Kumar (Leach and Kumar, 2012) included adverse events; fasting blood glucose, postprandial blood glucose, HbA1c, and serum insulin levels; and insulin sensitivity (CHO/unit insulin, homeostasis model assessment of insulin resistance [HOMA-IR]) measured the efficacy and safety of cinnamon for the management of diabetes mellitus. The authors found no significant differences in adverse events, regardless of dosage and treatment duration, between the cinnamon and placebo groups. In a study by Deyno et al. (Deyno et al., 2019), no significant difference in safety was found between cinnamon and placebo. Furthermore, the authors concluded that cinnamon could help reduce the fasting blood glucose level and HOMA-IR values.

In terms of publication bias, Mousavi et al. (Mousavi et al., 2021) evaluated this bias using a funnel plot analysis and conducted Egger's test. They found no small-study effects and no excessive significance bias. However, the number of trials included in some subgroups was relatively low. Zhou et al. (2016) did not conduct a funnel plot analysis or Egger's test since less than 10 studies were included. In the study by Deyno et al. (2019), most of the included studies did not contain safety data, and the included studies had high heterogeneity. Thus, the results should be interpreted conservatively. Leach and Kumar, (2012) also did not conduct a funnel plot analysis. There were several explanations for asymmetry in their study, with publication bias being one of the possibilities.

4 DISCUSSION

4.1 Pathogenesis

Cinnamic acid, syringic acid and choline are also included in the chemical composition of cinnamon, which includes volatile components, polysaccharides, sesquiterpenoids, and their glycosides and flavonoids. The main biological effects of cinnamon are derived from the phytochemicals themselves and their interactions.

4.2 Clinical Implications

To our knowledge, this is the first umbrella review of the safety of cinnamon. It has received attention not only for its health benefits but also for its potential adverse effects. In the three included studies, there was no significant difference in adverse events between cinnamon and placebo.

4.2.1 Impact of Cinnamon on the Diabetes

Diabetes mellitus is concurrent with high morbidity and mortality and its prevalence is cumulative globally (Xiang et al., 2004). Cinnamon has been used as a supplement in the treatment of diabetes, cancer, and primary dysmenorrhea (Jahangirifar et al., 2018; Sadeghi et al., 2019; Careyva et al., 2020). A four-month double blinded, placebo-controlled trial using an aqueous extract of cinnamon in 22 subjects with impaired FBG reported a decline in fasting glucose as well as a decline in malondialdehyde, and plasma antioxidant markers were increased at the same time (Roussel et al., 2009). Cinnamon supplementation could improve glycemic management and alleviate oxidative stress in patients with diabetes and could thus decrease the release of liver enzymes (Allen et al., 2013; Deyno et al., 2019). Previous studies have shown that conventional antidiabetic agents have some side effects (Hanefeld, 2007; Inzucchi et al., 2012), whereas a study investigated the effect of combined polyherbal dietary supplement cinnamon, purple onion, and tea, and found that tea could lower lowering blood glucose. It was proved to be beneficial at the same time. (Weng et al., 2021). The possible hypoglycemic pathway exerts its action by enhancing the activities of bioenzymes such as hexokinase and pyruvate kinase during glycolysis by increasing the content of liver glycogen and inhibit the gene expression of glucose-6-phosphatase and phosphoenolpyruvate carboxylase, which are key enzymes in the process of liver gluconeogenesis, and to maintain insulin resistance (Prasath and Subramanian, 2011; Prasath et al., 2014).

4.2.2 Impact of Cinnamon on Bacterial Infections and Tumor

Cinnamon is also a source of antibiotics, particularly in the context of multidrug-resistant bacterial infections (Vasconcelos et al., 2018). Nanoparticle-based cinnamon oil gel is very effective for the treatment of burn wound infection (Wen et al., 2021). The essential oils of cinnamon also exert potent antiviral effects against influenza type A virus (Wani et al., 2021). Cinnamon extracts, essential oils and their compounds have been proven to inhibit bacteria through damage to the cell membrane; alteration of the lipid profile; and inhibition of ATPases, cell division, membrane porins, motility, and biofilm formation; and via anti-quorum sensing effects (Vasconcelos et al., 2018). Cinnamon bark has also been proven effective in the treatment of methicillin-resistant Staphylococcus aureus (MRSA) (Zouhir et al., 2016). Cinnamon bark essential oil is may also apply in combinatory therapies so as to act on a par with synergistic interactions (Yang et al., 2017).

Schoene NW et al. (Schoene et al., 2005; Schoene et al., 2009) found that cinnamon total polyphenols can inhibit the proliferation of acute lymphoblastic leukemia cells. The possible mechanism is that two signaling proteins, p38MAPK and cyclin B1, are regulated to disrupt the phosphorylation/dephosphorylation of G2/M phase and impede the G2/M phase of the cell cycle. Assadollahi V et al. (Assadollahi et al., 2013) found that cinnamon polyphenols could inhibit the proliferation of the HL-60 cell line on the basis of Schoene NW's study, indicating that the antitumor effect of the extract was correlated with concentration and time, and this process was associated with the fact that cinnamon water extract promoted tumor cell apoptosis and stopped G1 phase of the cell cycle. Other studies (Koppikar et al., 2010) found that cinnamon water extract could effectively inhibit the proliferation of cervical cancer SiHa cells and further induce the apoptosis of SiHa cells. The mechanism could be that it downregulates the expression of MMP-2 and Her-2 proteins, enhances intracellular calcium channel signals, eliminates the correlation of mitochondrial membrane potential, and thus inhibits the metastasis of malignant tumor cells.

4.2.3 Potential Side Effects of Cinnamon

The aforementioned clinical benefits have contributed to cinnamon consumption; however, there is evidence that cinnamon may have adverse effects. Coumarin, one of the main components of cinnamon, has been indicated to have hepatotoxic, and carcinogenic effects (Brancheau et al., 2015; Mousavi et al., 2021). The dose and duration of cinnamon supplementation in a meta-analysis included in this review did not exceed the daily tolerable coumarin intake. Higher cinnamon intake may lead to hepatotoxicity (Brancheau et al., 2015). Behar et al. (2016) showed that the cinnamaldehyde in electronic cigarettes may interfere with homeostasis in the respiratory tract. Clouet et al. (2019) found that cinnamaldehyde may result in skin sensitization. The tolerable daily intake of cinnamon has been determined to be 0.1 mg/kg/day and employed in Europe to ensure safe use (Abraham et al., 2010). Hossein, (2013) found that stomatitis, perioral dermatitis, gingivitis, contact dermatitis, and other hypersensitivity reactions can occur after exposure to cinnamic acid, especially as the toxicity of benzyl cinnamate is higher. Allyl cinnamyl ester is also irritating to human skin. Cinnamaldehyde and cinnamol are more toxic than cinnamic acid. A mere 1% of cinnamaldehyde can cause mild hepatic cell edema in animal experiments, but few studies have been conducted on humans. Cinnamaldehyde and cinnamol are strong skin sensitizers that can easily cause contact dermatitis.

Numerous studies have reported the advantages of cinnamon when used safely (Ghosh et al., 2015; Pauline and Maddox, 2017; Santos and da Silva, 2018; Zare et al., 2019), for example, in the treatment of diabetes and as a natural antioxidant in foods. Furthermore, the active ingredients of cinnamon have been proven helpful in controlling and preventing the complications of coronavirus disease 2019 (Prasanth et al., 2021; Zareie et al., 2021). Cinnamon provided whole or as an aqueous extract contains a different number of active agents with various antihyperglycemic actions, since hypoglycemic activity appears in both aqueous extract, and powdered bark (Anderson et al., 2004; Verspohl et al., 2005; Kim et al., 2006).

The results of umbrella systematic review indicated that cinnamon intake within the daily intake range did not have significant adverse

effects. However, it is essential to discuss that while we evaluated the safety of cinnamon supplementation, it was not quantitatively analyzed in this review. Due to a lack of strict rules, the nutraceutical manufacturers must check the safety of a marketed good products that is applied at a lower dosage than in the pharmaceutical setting. It should be noted that systematic reviews and meta-analyses are at the top of the hierarchy of clinical practice.

4.3 Methodological Considerations

The main strength of the present umbrella systematic review is that it included systematic reviews, meta-analyses, and an evaluation of the overall evidence. However, there are some limitations of this work. First, the sample count was relatively low; hence, more in-depth studies involving larger samples are needed in the future. Second, the number of relevant meta-analyses and systematic reviews was relatively small; therefore, further investigation into the safety of cinnamon is necessary. Third, a study of the different dosages and durations of treatment is necessary. Fourth, the included systematic reviews or meta-analyses were of relatively low methodologic quality, thus reducing the significance, and reliability of the clinical evidence for cinnamon safety in this umbrella review. For example, there was no comparator or placebo; therefore, placebo evidences could not be entirely excluded. Finally, due to a lack of comprehensive information about the above safety profile, it is difficult to assess the effects of cinnamon on other biomarkers relevant to safety. This needs to be explored in future studies as well.

5 CONCLUSION

This study summarized existing evidence on the safety of cinnamon, showing that cinnamon dose not cause obviously increased adverse effects when used on a large scale. It also has benefits in the treatment of a variety of diseases, such as type 2 diabetes and cancer. In other words, while cinnamon is effective for many of its benefits, it does not increase the risk of injury or mortality. The results of this study implied that cinnamon can be used as an adjunctive drug in the clinic field in future years, and its safety can be guaranteed.

DATA AVAILABILITY STATEMENT

The study data and materials are in the custody of the corresponding author and can be made available on reasonable request.

AUTHOR CONTRIBUTIONS

D-TG, T-HT, ZLJ, CC, and W-YL conducted the study and drafted the article. D-TG, T-HT, and ZLJ participated in the design of the study and performed statistical analyses. CC and W-YL conceived the study, and participated in its design and coordination. All of the authors read and approved the final article.

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A Potential Mechanism of Kidney-Tonifying Herbs Treating Unexplained Recurrent Spontaneous Abortion: Clinical Evidence From the Homogeneity of Embryo Implantation and Tumor Invasion

Hang Zhou[1,2†], *Yi Yang*[2†], *Linwen Deng*[2*], *Yongqing Yao*[2] *and Xin Liao*[3]

[1]School of Basic Medical Sciences, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Department of Gynecology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]Department of Information, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China

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***Correspondence:**
Linwen Deng
dlwen1982@163.com

[†]These authors have contributed equally to this work and share first authorship

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Background: Kidney-tonifying herbs (KTHs) are widely used to treat unexplained recurrent spontaneous abortion (URSA) based on the theory of traditional Chinese medicine (TCM). However, there is still a lack of systematic evaluation and mechanistic explanation for these treatments.

Objective: The purpose of this study was to assess the clinical efficacy, and to investigate the potential mechanisms, of KTH based on TCM for the treatment of URSA.

Methods: A systematic literature search was conducted within PubMed, Embase, China Biomedical Literature database, Web of Science (WOS), China National Knowledge Infrastructure (CNKI) database, and the Wanfang database to find articles reporting on the Chinese herbal formula based around KTH for treating URSA, which were published between January 2010 and June 2021. A full bibliometric analysis was carried out; in addition, randomized controlled trial (RCT) articles were selected for systematic evaluation and meta-analysis. The drugs with the highest frequency of KTHs were screened for meta-analysis. Finally, network analysis and molecular docking were used to study the key components and potential pathway of KTHs in the treatment of URSA.

Results: The meta-analysis included nine RCTs involving 1,054 subjects. Compared with the control groups, the clinical efficacy of TCM-based KTHs in the treatment of URSA patients significantly improved outcomes. Additionally, a component target pathway network was identified, which included 32 potential blood activating components and 113 main targets. Japonine, sopranol, lysine, and matrine were considered the most important bioactive molecules for KTHs. The key potential therapeutic pathway for URSA was a tumor-related signaling pathway. The target genes for URSA regulated by KTHs were highly similar to tumor biological processes such as the regulation of apoptotic signaling pathways, inflammatory responses, angiogenesis, and epithelial metabolic transition.

Conclusion: KTH has great potential for treating URSA. Because the maintenance of pregnancy has a high similarity with tumor invasion, the research relating to tumor mechanisms should also be followed up as it may lead to new ideas and breakthroughs for research into URSA. At the same time, embryonic and decidual cells share a high degree of cellular heterogeneity and spatial structural complexity with tumor cells, and a single cell combined with spatial omics may be the best future approach for validating KTH mechanisms.

Keywords: unexplained recurrent spontaneous abortion, tumor invasion, clinical trials and validation experiments, kidney-tonifying herbs, embryo implantation

INTRODUCTION

Recurrent spontaneous abortion (RSA) is challenging to diagnose and treat. Its classic definition is two or more clinical continuous abortions before 20 weeks of pregnancy in fertile couples (Practice Committee of the American Society for Reproductive Medicine, 2012; Colley et al., 2019). The etiology of RSA includes genetic abnormalities, endocrine disorders, anatomical abnormalities, infectious, prothrombotic state, and immune factors (Li et al., 2002; Qian et al., 2018). Its incidence rate ranges approximately from 1% to 5% in women during their childbearing years (Nigro et al., 2011; Ewington et al., 2019; Homer 2019). However, about 50% of RSAs are still undiagnosed and/or untreated. This condition is often referred to as unexplained recurrent spontaneous abortion (URSA) after exclusion of diagnosis and is considered an early spontaneous abortion within the first 12 weeks of pregnancy (Pereza et al., 2017; ESHRE Guideline Group on RPL et al., 2018). The disease has serious physical and mental impacts on the patients and their families (Cao et al., 2017; Tavoli et al., 2018). Therefore, it is necessary to study effective treatment methods to reduce pregnancy loss and to help maintain pregnancy in URSA patients (Zhang et al., 2021). The current therapeutic options for URSA mainly include preimplantation genetic screening (PGS), suppression of alloimmunity, and anticoagulant therapy (Rey et al., 2003; Qin et al., 2016; Zhao et al., 2017; Ding et al., 2019; Feng, 2019; Qin et al., 2020; Zhang et al., 2021). Cyclosporine A (Zhou et al., 2007), intravenous immunoglobulin (Wang et al., 2016; Muyayalo et al., 2018), lymphocyte active immunity, and glucocorticoids are the main regimens used to suppress alloimmunity, but the effectiveness and safety of these regimens have not been fully validated using large sample clinical studies. Therefore, until now, there has been a lack of unified diagnostic criteria and efficient treatments for URSA (Mekinian et al., 2016; Li et al., 2020).

In recent years, traditional Chinese medicine (TCM) has been accepted as a mainstream of medical care, and has become a popular supplement to Western medicine for the treatment of URSA (Fujii et al., 2000; Li et al., 2014; Yang et al., 2018). Kidney-tonifying herbs (KTHs) are the most commonly used prescription for TCM-based treatment (Li et al., 2012; Li et al., 2016). In the past few years, the data accumulated from personal clinical experience, case reports, noncontrolled trials, animal experiments, and randomized controlled trials (RCT) show that when treating URSA, KTHs alone and KTHs combined with Western medicine have similar effects regarding the improvement of pregnancy outcomes and symptoms (Liu et al., 2009; Zhao et al., 2018; Feng 2019; Zhang et al., 2019). Although KTHs are widely used in patients with URSA, it is difficult for KTHs to be recognized internationally due to the complexity of its ingredients and a lack of pharmacological mechanisms.

At present, it is known that embryo implantation is highly similar to cancer invasion. The phenotypes of apoptosis, inflammation, proliferation, invasion, adhesion, and angiogenesis in the interactions between embryonic trophoblast cells and endometrial epithelial cells at the maternal–fetal interface are highly similar to cancer processes (Murray and Lessey, 1999; Hannan and Salamonsen, 2008; Perry et al., 2009; Zhang et al., 2020). According to previous studies, we hypothesized that the mechanism of TCM for tocolysis may be similar to that observed in tumor activation. A key difference between them lies in the gene targets and biological process affected by the medicine. The goal of this study was to test this hypothesis. Specifically, bibliometric analysis, meta-analysis, network pharmacology, and molecular docking were conducted to examine the efficacy of the published TCM-based KTHs preparations and the control groups for treating URSA. The research also identified the potential pathways of TCM kidney-tonifying prescriptions in the treatment of URSA, and provided evidence-based scientific support for URSA treatment in clinical practice (**Figure 1**).

DATA AND METHODS

Bliometric Analysis of Unexplained Recurrent Spontaneous Abortion Treated by Traditional Chinese Medicine in the Last 10 Years

We conducted a systematic literature search in the following clinical research databases: PubMed, Embase, Cochrane Library, Web of Science (WOS), China National Knowledge Infrastructure (CNKI), Wanfang Database, China Biomedical Database (CBM), and the China Science Journal Database (VIP), from the establishment of each database through June, 2021. We developed a search formula for the treatment of URSA

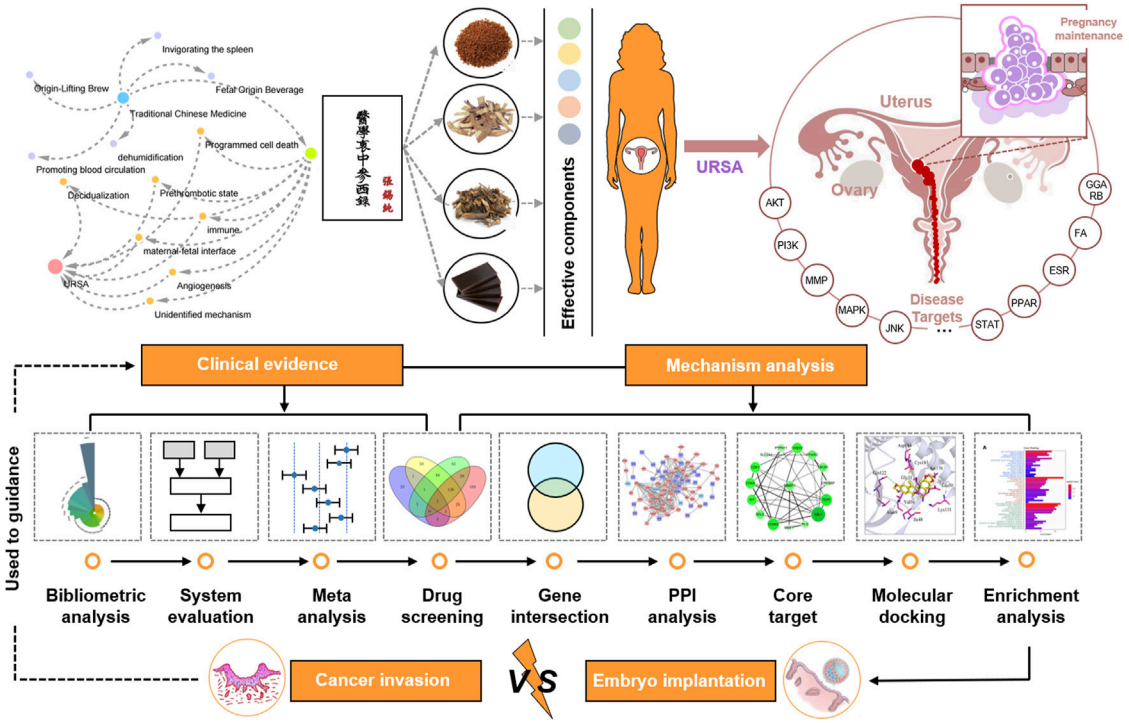

FIGURE 1 | Clinical evidence and underlying mechanisms of kidney-tonifying herbs (KTHs) for treating unexplained recurrent spontaneous abortion (USRA).

by KTHs based on the PICOS strategy (Detailed strategies are provided in **Supplementary Data Sheet S1**), and then collated the screened literature using ENDNOTE software.

We also established a bibliometric analysis strategy for the CNKI and WOS databases (**Supplementary Data Sheet S2**). Between ("2011-01-01," "2021-06-01") and (literature classification was limited to traditional Chinese medical science, Chinese herb and integrated traditional Chinese and Western medicine); Search scope: general database. After downloading the refworks format, the synonyms were converted into Chinese and English, and the synonyms were clustered uniformly, including KTHs. The drug was defined as "KTH"; the selection used a modified g-index in each slice:

$$g^2 \leq k \sum i_{\leq g} c_i \, k \in Z^+.$$

To include more or fewer nodes, increase or decrease the scale factor $k = 25$. The keywords co-occurrence analysis, cluster analysis, mutation term analysis, as well as time line graph analysis were performed using CiteSpace (Version 5.8.R1).

Clinical Evidence of Kidney-Tonifying Herbs in the Treatment of Unexplained Recurrent Spontaneous Abortion

The systematic review and meta-analysis were conducted in accordance with the PRISMA guidelines (Moher et al., 2009; McInnes et al., 2017). The detailed research process is illustrated in the Supplementary Materials. All randomized controlled trials (RCTs) were included to study the efficacy of KTHs alone or combined with modern drugs, in the treatment of URSA. Studies reported in languages besides Chinese and English were excluded. Nonrandomized controlled trials or animal experiments were excluded.

Participant Inclusion

Patients included were in their first 3 months of pregnancy, had a pregnancy confirmed by serum human chorionic gonadotropin (hCG) or ultrasound, and had a history of URSA diagnosis, which was defined as two or more spontaneous abortions. The following four causes were excluded: infection, abnormal parental karyotype, endocrine imbalance, and anatomical abnormalities, regardless of the maternal or gestational age, race or nationality, educational level, or economic status. The inclusion criteria also ensured that no treatment was received before pregnancy or before entering the trial. Trials with recurrent spontaneous abortion and nonpregnant URSA participants with a definite etiology were also excluded.

Types of Intervention

The treatment of interest was KTHs, regardless of dose, administration methods, administration time, or whether KTHs were used in combination with Western medicine. The KTH group was compared with a treatment using only Western medicine. A randomized controlled trial was excluded if the Western medicine changed within the control group. Studies using only bed rest and/or psychological support were also excluded.

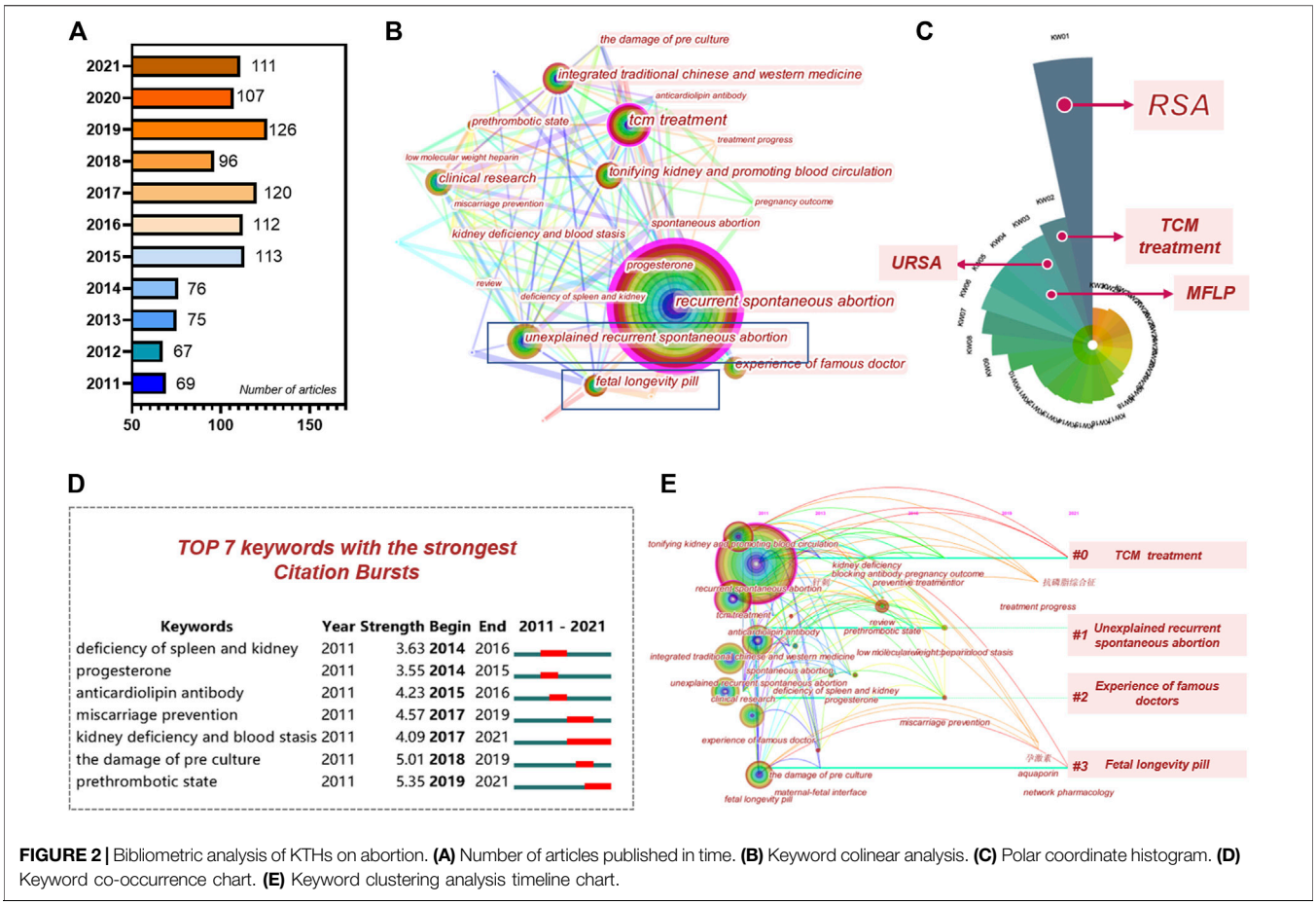

FIGURE 2 | Bibliometric analysis of KTHs on abortion. **(A)** Number of articles published in time. **(B)** Keyword colinear analysis. **(C)** Polar coordinate histogram. **(D)** Keyword co-occurrence chart. **(E)** Keyword clustering analysis timeline chart.

Measurement of Treatment Outcomes

The primary outcome was the clinical response rate and pregnancy outcome reported in the trials. The secondary outcomes included reported hormone levels, serum immunological parameters, and incidence of adverse events during treatment.

Search Strategy

The search strategy was the same as the bibliometric analysis. The reference list of all identified articles was also manually searched to find possible related studies to supplement the relevant literature.

Data Extraction and Quality Assessment

Two reviewers (Hang Zhou and Yi Yang) extracted the general information from eligible studies through a predesigned standardized data extraction table: first author, year of publication, TCM syndrome difference, sample size, age, abortion frequency, definition of abortion and live birth, intervention time, treatment intervention and control group, treatment time, and results. Any inconsistencies were resolved by a third reviewer (Yongqing Yao). The methodological quality of each individual study was independently evaluated by two researchers (Yi Yi and Hang Zhou) referring to the Cochrane Handbook (Cumpston et al., 2019; Propadalo et al., 2019) for systematic review of interventions. We used the following criteria for evaluation: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome evaluation, incomplete outcome data, selective reporting, and other biases. Each study was classified as either low-risk, high-risk, or unclear. If there were differences in opinion, the third researcher was referred to (Yongqing Yao).

Statistical Analysis

A meta-analysis was conducted using the Review Manager (Revman) (computer program; version 5.3, Copenhagen: Nordic Cochrane Center, Cochrane Collaboration, 2014). The relative risk (RR) of 95% confidence interval (CI) was used for binary variables, while weighted mean variance (WMD) and 95% confidence intervals were used for continuous variables. Cochrane's *p*-value and I2 were used to test the heterogeneity of the study.

Network Pharmacological Mechanism of Kidney-Tonifying Herbs in the Prevention and Treatment of Unexplained Recurrent Spontaneous Abortion

Drug Composition and Target Screening

Drug screening was carried out for the selected literature, and the results were recorded in the tcmsp database (http://tcmspw.com/

TABLE 1 | The basic characteristics of the included studies.

References	Sample size	Age (years)	Abortion time (days)	Times of abortions	Intervention time	Intervention measures	Duration of intervention	Main outcomes
He (2020)	T:60	T: 30.12 ± 3.63	NR	T: 3.35 ± 0.62	NR	T:KTHs (1/dose/day) + C	12 weeks	①③⑥
	C:60	C: 29.67 ± 3.42		C: 2.98 ± 0.56		C: dydrogesterone (10 mg, bid, po)		
Niu and Hao (2020)	T:60	T: 28.76 ± 2.51	T: 68.92 ± 5.12	T: 4.12 ± 0.16	3 days after ovulation	T:KTHs (1/dose/day) + C	Until the 12th week of pregnancy	①②③④⑤⑥
	C:60	C: 29.02 ± 2.47	C: 69.13 ± 5.07	C: 4.15 ± 0.23		C: dydrogesterone (10 mg, qd, po)		
Feng et al. (2020)	T:40	T: 32.33 ± 4.74	NR	NR	NR	T: modified STP (1/dose/day) + C	4 weeks	①④⑥⑦
	C:40	C: 30.75 ± 4.02				C: aspirin (25 mg, tid, po)		
Xu et al. (2020)	T:45	T: 29.43 ± 5.42	T: 60.02 ± 12.41	T: 2.53 ± 0.69	After diagnosis	T:KTHs (1/dose/day) + C	Until 3 months of pregnancy	①②④⑤⑦
	C:45	C: 29.14 ± 5.13	C: 59.77 ± 11.36	C: 2.47 ± 0.66		C: aspirin (25 mg, tid, po) + metacortandracin (5 mg, po, qn) + dydrogesterone (10 mg, bid, po)		
Han et al. (2019)	T1:86	T1: 27.7 ± 2.8	NR	T1: 2.81 ± 0.57	NR	T1:KTHs (1/dose/day)	Until the 12th week of pregnancy	①②④⑥⑦
	T2:87	T2: 28.1 ± 2.6		T2: 2.88 ± 0.53		T2:KTHs (1/dose/day) + C		
	C:88	C:27.6 ± 2.5		C: 2.72 ± 0.48		C: dydrogesterone (10 mg, bid, po)		
Feng (2019)	T1:79	T1: 28.6 ± 3.1	NR	T1: 2.67 ± 0.47	NR	T1:KTHs (1/dose/day)	12 weeks	①②④⑦
	T2:84	T2: 29.1 ± 2.5		T2: 2.79 ± 0.45		T2:KTHs (1/dose/day) + C		
	C:44	C:28.2 ± 2.7		C: 2.65 ± 0.44		C: dydrogesterone (10 mg, bid, po)		
Feng et al. (2019)	T:30	T:30.5 ± 2.7	T:47.6 ± 5.6	T: 30.5 ± 2.7	NR	T:KTHs (1/dose/day) + C	4 weeks	①④⑤
	C:30	C:28.6 ± 2.9	C:48.3 ± 5.6	C: 28.6 ± 2.9		C: dydrogesterone (10 mg, bid, po)		
Xu et al. (2018)	T:28 C:28	T:28.5 ± 3.5 C:29.3 ± 3.0	T:55.3 ± 16.8 C:56.7 ± 15.4	T:3.2 ± 0.6 C:3.6 ± 0.4	NR	T:KTHs (1/dose/day) + C C:Active immunotherapy (intradermal injection every 3 weeks, two times for a course of treatment)	Until the 20th week of pregnancy	①②③④⑥⑦
Liu et al. (2015)	T:30	T: 28.73 ± 4.29	NR	T: 2.4 ± 0.62	3 months before planned pregnancy	T: KTHs (1/dose/day) + C	Until the 12th week of pregnancy	①③⑥
	C:30	C: 30.77 ± 5.70		C: 2.60 ± 0.72		C: progesterone capsule/ progesterone injection (20 ~ 40 mg po/im qd)		

Note. T, trial group; C, control group; NR, not reported; ①, clinical response rate; ②, syndrome integral; ③, pregnancy outcome; ④, immune function-related outcome indicators; ⑤, coagulation function test; ⑥, reproductive hormone test; ⑦, adverse event report.

tcmsp.php) and the Batman database (http://bionet.ncpsb.org/batman-tcm/). The structures of the above components were obtained from the PubChem database and imported into the Swiss target prediction database (http://www.swisstargetprediction.ch/). The targets with prediction scores greater than 0 were selected as drug targets. OMIM (https://omim.org/) and the Genecards database (https://www.genecards.org/) were also searched with the keywords "unexplained recurrent spontaneous abortion" and the disease targets were obtained. The drug targets and disease targets were integrated, and then gene intersection was performed.

PPI Network Construction and Core Target Analysis

We searched the above drug disease common targets using the string database (https://string-db.org) (Von Mering et al., 2003). The protein type was set as "*Homo sapiens*." The minimum interaction threshold was set at 0.4. After the construction of the PPI network of protein interaction, Mcode module was used to analyze gene clusters and screen core targets.

Molecular Docking

According to the CAS number of small molecules, the 3D structure of small molecules in SDF format was downloaded

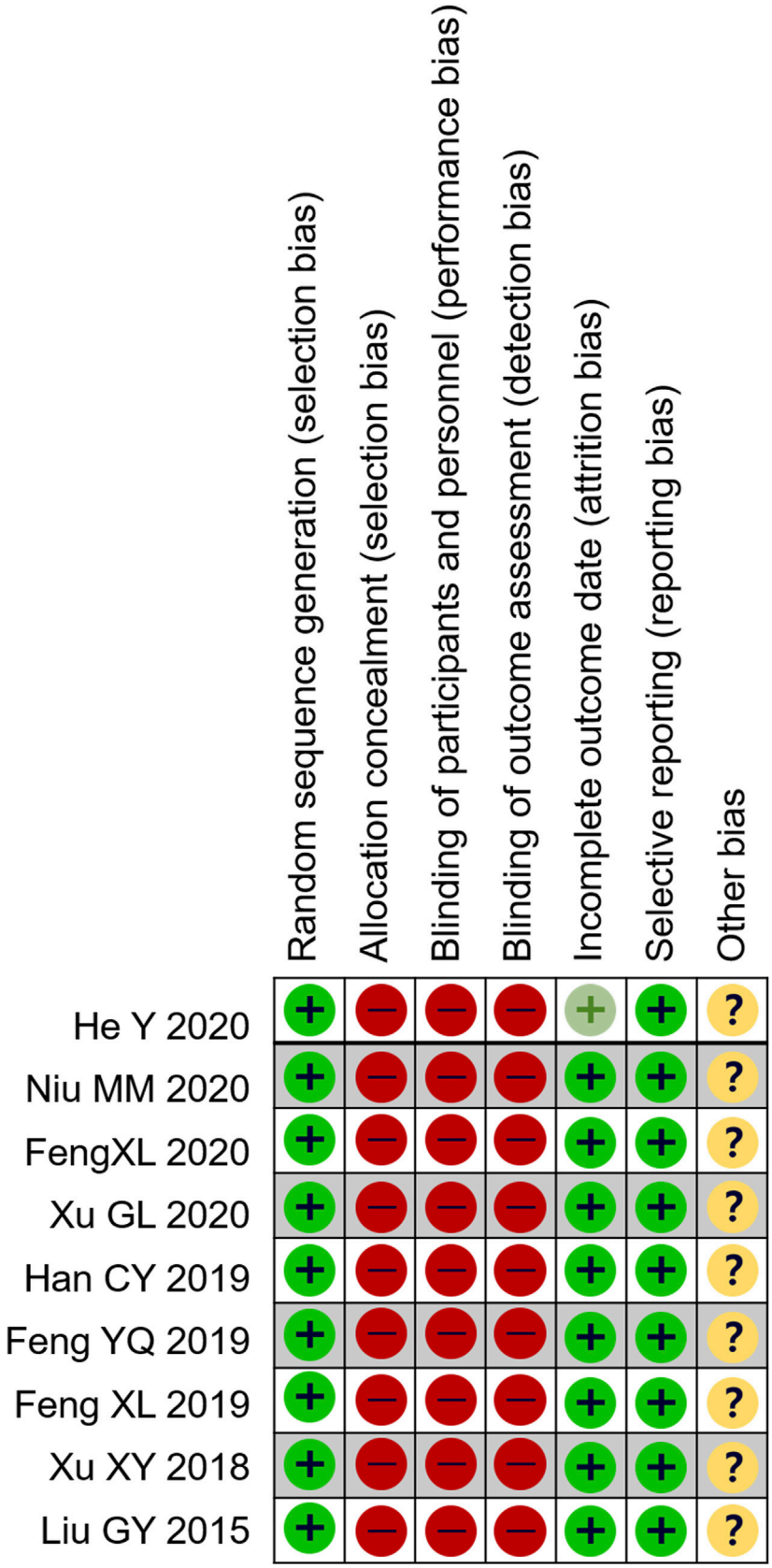

FIGURE 3 | Risks of bias assessment.

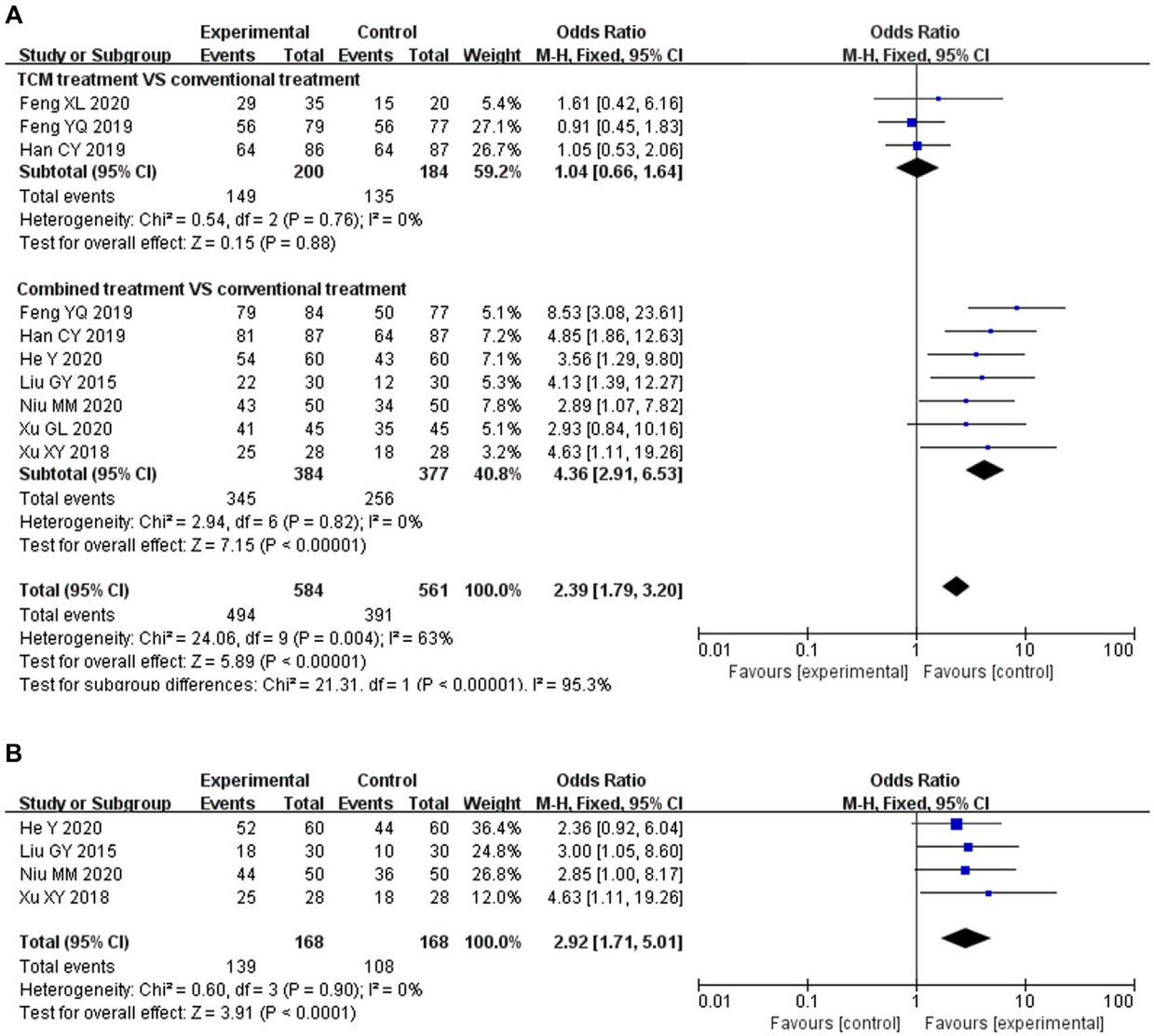

FIGURE 4 | Forest plot of experimental treatment vs. control treatment for the main outcomes. **(A)** Forest plot of clinical response rate of KTHs treated URSA (combined treatment vs. conventional treatment or pure Western medicine vs. KTHs). **(B)** Forest plot of pregnancy outcomes of KTHs treated URSA.

from the PubChem database, imported into chembio3d ultra 14.0 for energy minimization, and autodock tools-1.5.6 for hydrogenation, charge calculation, charge distribution, and rotatable key setting. The key target proteins were downloaded from the database PDB (http://www.rcsb.org/), and the crystal water and original ligands were removed using pymol2.3.0. They were then hydrogenated, the charge was calculated and distributed, and the atomic type was specified. AutoDock Vina1.1.2 was used for molecular docking, and PyMOL2.3.0 was used to analyze the interaction mode of the docking results.

Enrichment Analysis of GO and KEGG

In metascape and R software, the Bioconductor bioinformatics software package was used to analyze the function enrichment of key target genes GO and KEGG with a p-value <0.05 and a Q-value <0.05, the results were output in the form of bar and bubble charts. A heatmap was plotted using http://www.bioinformatics.com.cn, an online platform for data analysis and visualization. According to the results of the enrichment analysis, the network diagram for traditional Chinese medicine–components–targets–pathways–phenotypes–diseases was constructed using Cytoscape.

RESULTS

Results of Bibliometric Analysis

A total of 1,012 articles were obtained through the literature searches, and the number of published articles increased annually (**Figure 2A**). Additionally, Citespace was used for keyword

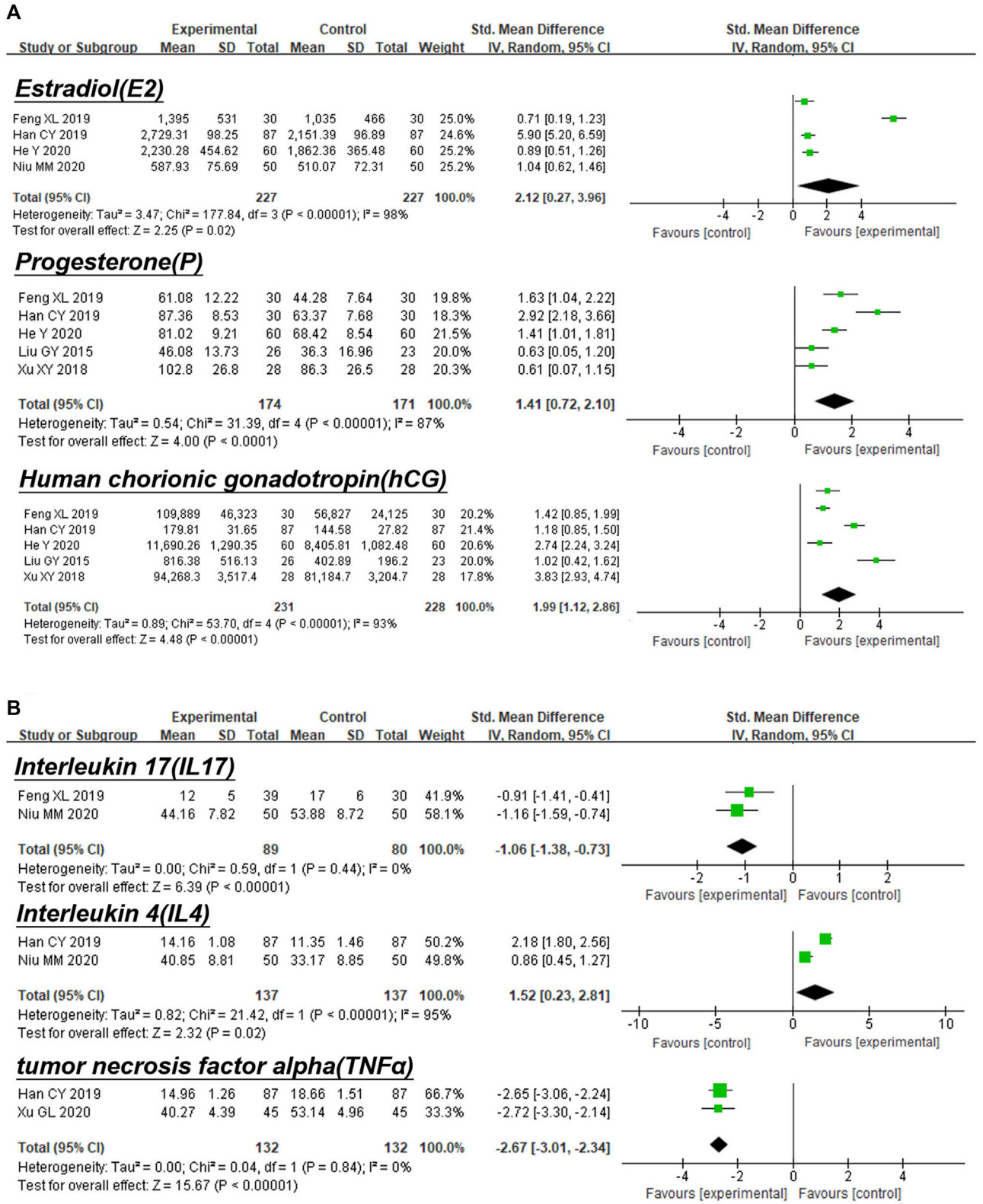

FIGURE 5 | Forest plot of experimental treatment vs. control treatment in secondary outcomes. **(A)** Forest plot of hormone index. **(B)** Forest plot of serum immunological indexes.

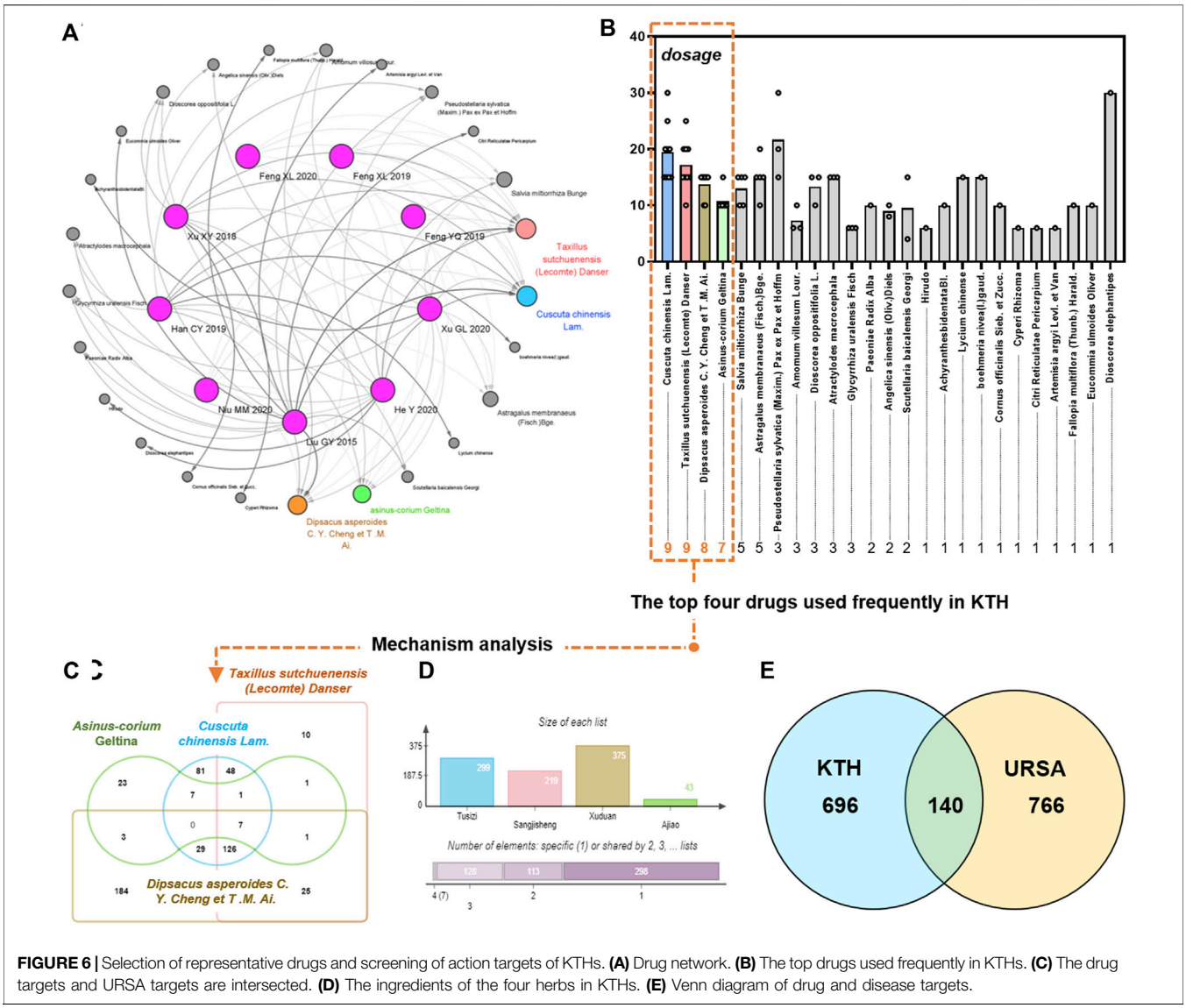

FIGURE 6 | Selection of representative drugs and screening of action targets of KTHs. **(A)** Drug network. **(B)** The top drugs used frequently in KTHs. **(C)** The drug targets and URSA targets are intersected. **(D)** The ingredients of the four herbs in KTHs. **(E)** Venn diagram of drug and disease targets.

colinear analysis (**Figure 2B**), and the polar coordinate histogram was calculated according to the count value (**Figure 2C**). Analysis from the publications over the past 10 years showed that the research hotspots for traditional Chinese medicine in the treatment of RSA mainly focused on the following key words: recurrent spontaneous abortion, TCM treatment, tonifying kidneys, and promoting blood circulation, KTH, integrated traditional Chinese and Western medicine, clinical research, experience of famous doctor, prethrombotic state, progesterone, kidney deficiency and blood stasis, the damage of pre culture, and deficiency of spleen and kidney (Yang et al., 2013; Obstetrics; Subgroup et al., 2019; Cao et al., 2021; Li et al., 2021). The formula was used to show the keyword saliency map (**Figure 2D**), with a total of seven keywords. Finally, a clustering time line chart was constructed. The results showed that the main related research areas were divided into one of four categories: TCM treatment, USRA, KTH, and famous doctor experience (**Figure 2E**).

Clinical Evidence Search Results

After excluding duplicate studies, 562 studies were examined according to their abstracts and titles, resulting in 41 articles in the final evaluation. Finally, nine studies (Liu et al., 2015; Xu et al., 2018; Feng, 2019; Feng et al., 2019; Han et al., 2019; Feng et al., 2020; He, 2020; Niu and Hao, 2020; Xu et al., 2020) were included in the present systematic review.

Research Characteristics

Table 1 summarizes the basic information pertaining to the randomized controlled trials included. All of these trials were published in China. These studies included 1,054 URSA patients, of which 629 participants were assigned to the treatment group, and 425 participants were assigned to the control group. There was no significant difference in the baseline parameters of all trials.

The patients in the treatment group were treated with KTHs or combined with Western medicine, while the patients in the control group were treated with Western medicine alone. Of

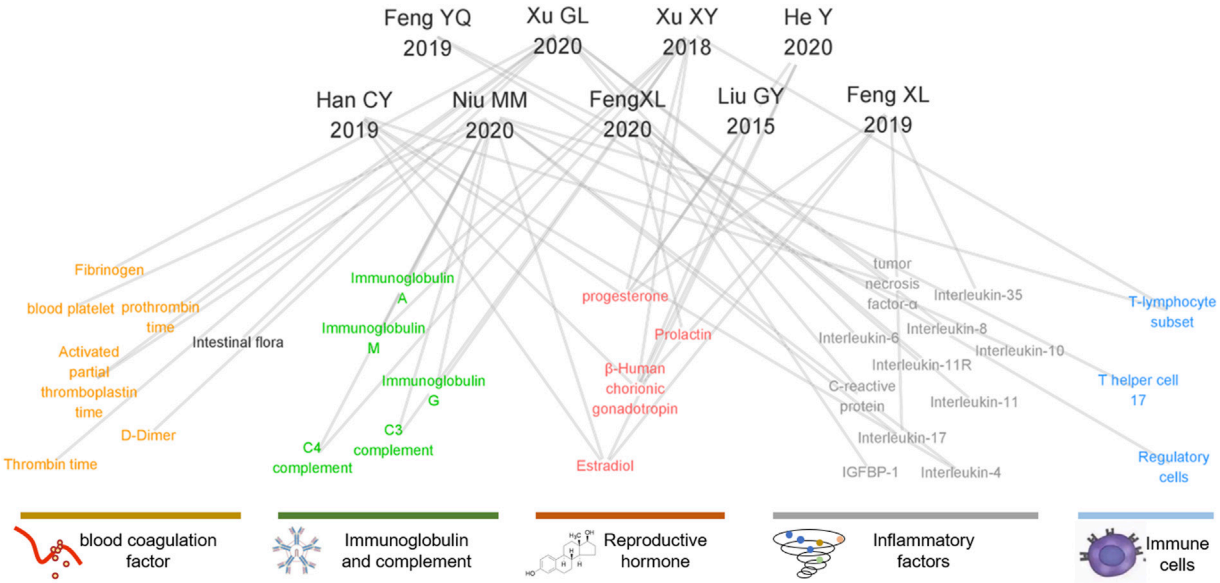

FIGURE 7 | Network diagram of screening literature research indicators.

those, six randomized controlled trials used natural progesterone, including injection, capsule, or desgesterone (Liu et al., 2015; Feng, 2019; Feng et al., 2019; Han et al., 2019; He, 2020; Niu and Hao, 2020). One study used natural progesterone in combination with other treatments (Mekinian et al., 2016), and one used active immunotherapy (Xu et al., 2018). The treatment duration ranged from 4 to 20 weeks of gestation.

Bias Risk Assessment

Overall, the methodological quality of the included trials was poor. All nine studies were designed with two arms and were declared randomized controlled trials. None of the trials reported any details relating to the blinding of patients and researchers. No trials showed the number and reasons of dropouts. The evaluation results are shown in **Figure 3**.

Outcome Analysis of Clinical Research

Main Outcomes

Nine studies (Liu et al., 2015; Xu et al., 2018; Feng, 2019; Feng et al., 2019; Han et al., 2019; Feng et al., 2020; He, 2020; Niu and Hao, 2020; Xu et al., 2020) reported the clinical response rate. The pregnancy outcome was reported in four trials (Liu et al., 2015; Xu et al., 2018; He, 2020; Niu and Hao, 2020). Compared with the pure Western medicine treatment group, the pregnancy success was higher for the KTHs/KTHs with Western medicine combined group (RR: 2.92; 95% CI: 1.71–5.01: $p < 0:01$, $I^2 = 0\%$). Meta-analysis showed that the incidence of early pregnancy loss in the KTHs group was significantly lower than that observed in the control group (**Figure 4B**). Subgroup analysis (**Figure 4A**) showed that there was no difference in the efficacy between pure Chinese medicine and Western medicine (RR: 1.04; 95% CI: 0.66–1.64; $p = 0.88$, $I^2 = 0\%$), however, the combination of KTHs and modern drug therapy showed better effects (RR: 4.36; 95% CI: 2.91–6.53; $p < 0:01$, $I^2 = 0\%$).

Secondary Outcomes

Six of the nine studies enrolled (Liu et al., 2015; Xu et al., 2018; Han et al., 2019; Feng et al., 2020; He, 2020; Niu and Hao, 2020) reported estradiol and progestins before and after KTH treatment in the URSA patients, as detailed in **Figure 5**. The conclusions were consistent across the studies.

Estradiol

Combination therapy of KTHs and Western medicine could result in an increase in E2 compared with Western medicine alone (RR: 2.12; 95% Cl 0.27 to 3.96; $p < 0.00001$; $I^2 = 98\%$; random-effects model; four studies; very low-certainty evidence) (**Figure 5A**) (Feng, 2019; Han et al., 2019; He, 2020; Niu and Hao, 2020).

Progesterone

Combination therapy of KTHs and Western medicine could result in an increase in P compared with Western medicine alone (RR:1.41; 95% Cl 0.72 to 2.10; $p < 0.00001$; I2 = 87%; random-effects model; five studies; very low-certainty evidence) (**Figure 5A**) (Liu et al., 2015; Xu et al., 2018; Feng, 2019; Han et al., 2019; He et al., 2020).

Human Chorionic Gonadotropin

Combination therapy of KTHs and Western medicine could result in an increase in hCG compared with Western medicine alone (RR:1.99; 95% Cl 1.12 to 2.86; $p < 0.00001$; $I^2 = 93\%$; random-effects model; five studies; very low-certainty evidence) (**Figure 5A**) (Liu et al., 2015; Xu et al., 2018; Feng, 2019; Han et al., 2019; He et al., 2020). Seven studies (Xu et al., 2018; Feng, 2019; Feng et al., 2019; Han et al., 2019; Feng et al., 2020; Niu and Hao, 2020; Xu et al., 2020) reported changes in immune function-related outcome measures in URSA patients treated with KTHs, mainly comprising interleukin family

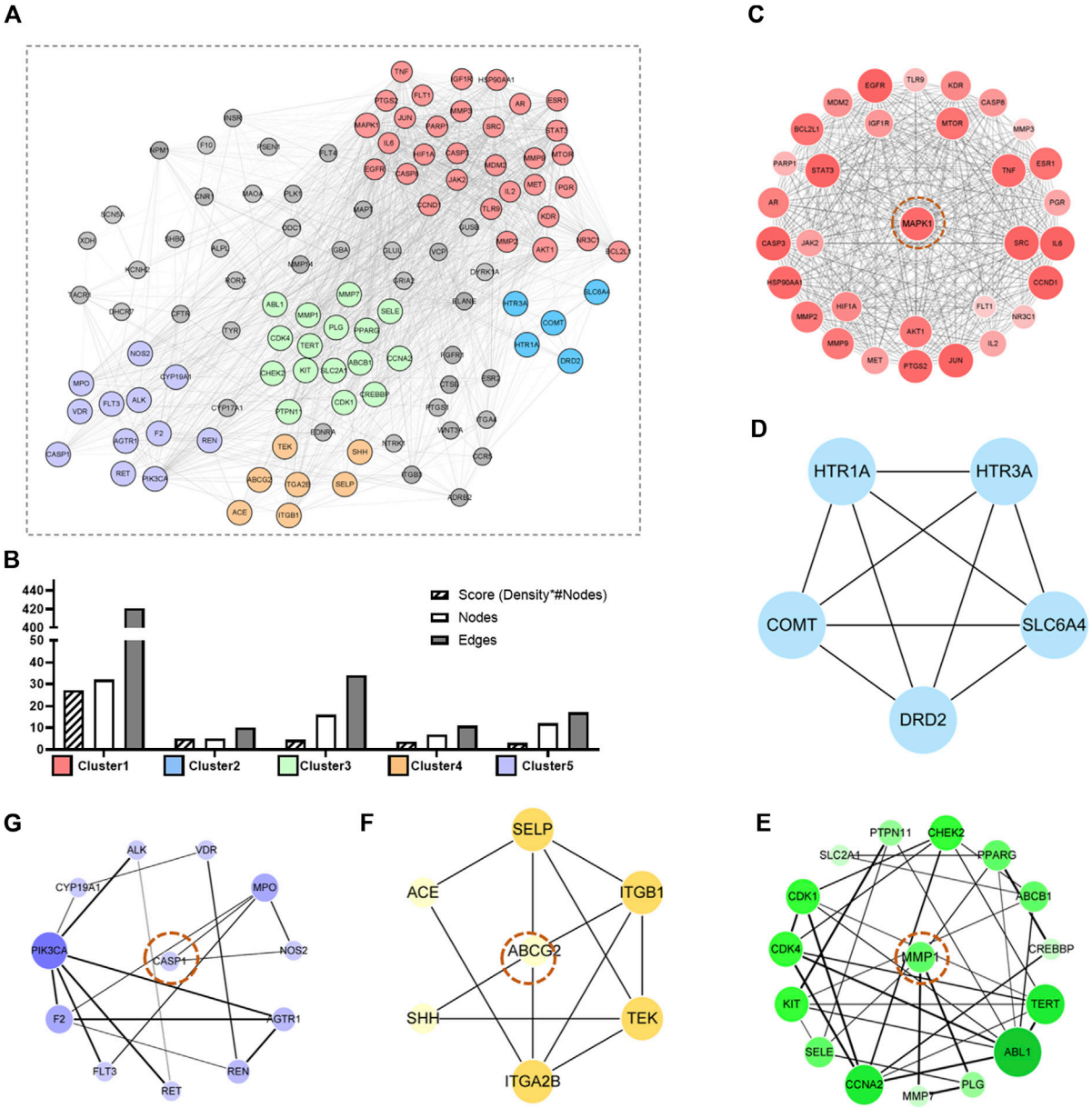

FIGURE 8 | PPI analysis and core gene screening. **(A)** Protein interaction network of intersecting genes. **(B)** The top five DEGs with the score (density * # nodes), nodes, and edges of each cluster were expressed. **(C–G)** The network graph of five foundation clusters.

expression and T-cell subset proportion distribution, with generally consistent expression trends after treatment, as detailed in **Figure 5**.

Interleukin 17

Combination therapy of KTHs and Western medicine could decrease the level of IL17 compared with Western medicine alone (RR: 1.06; 95% CI −1.38 to −0.73; $p = 0.44$; $I^2 = 0\%$; random-effects model; two studies; very low-certainty evidence) (**Figure 5B**) (Feng, 2019; Niu and Hao, 2020).

Interleukin 4

Combination therapy of KTHs and Western medicine could result in an increase in IL4 compared with Western medicine alone (RR:1.52; 95% CI 0.23 to 2.81; $p < 0.0001$; $I^2 = 95\%$; random-effects model; two studies; very low-certainty evidence) (**Figure 5B**) (Han et al., 2019; Niu and Hao, 2020).

Tumor Necrosis Factor Alpha

Combination therapy of KTHs and Western medicine could decrease the level of TNFa compared with Western medicine

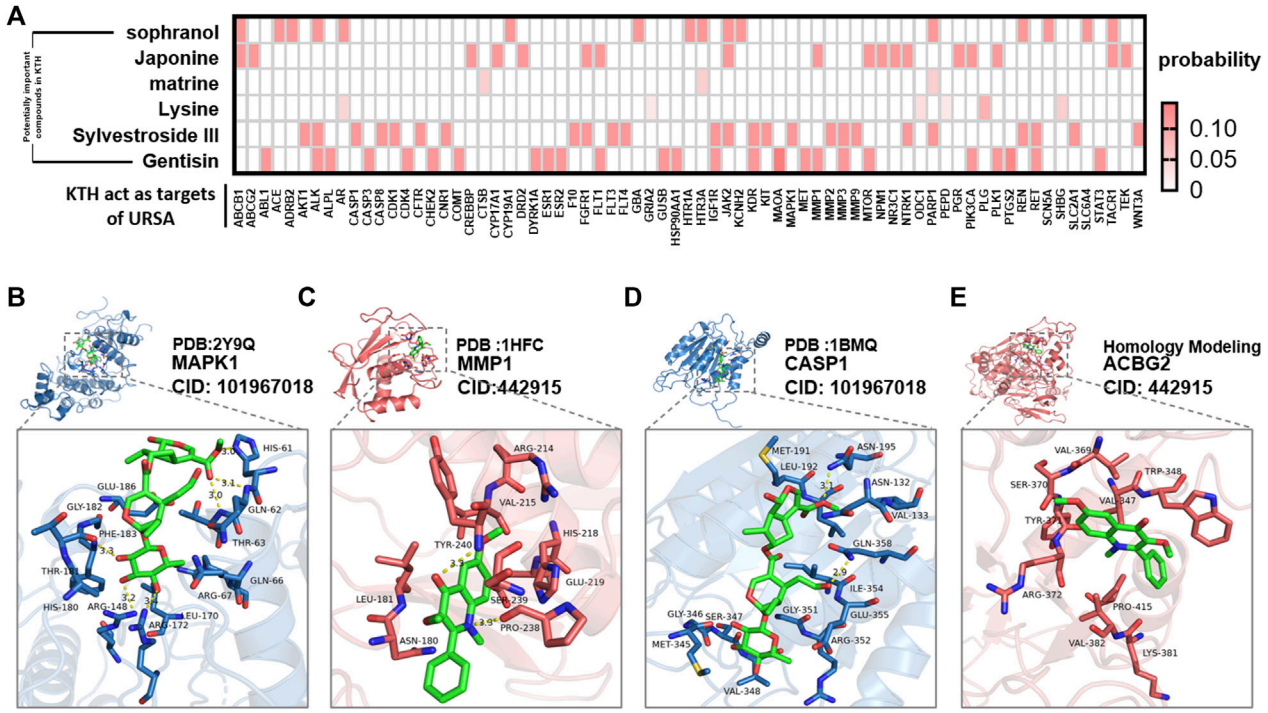

FIGURE 9 | Molecular docking results of active components for KTH and URSA targets. **(A)** The heatmap of docking possibilities of major effective compounds and target proteins of KTHs. **(B–E)** The best conformation of the most effective compound and its corresponding protein receptor in KTHs.

alone (RR: −2.67; 95% CI −3.01 to −2.34; $p = 0.84$; $I^2 = 0\%$; random-effects model; two studies; very low-certainty evidence) (**Figure 5B**) (Han et al., 2019; Xu et al., 2020).

Publication Bias and Sensitivity Analysis

Although the funnel plot of early pregnancy loss rate was asymmetrically distributed, Egger's test analysis showed only marginally significant publication bias ($p = 0.09$). The sensitivity analysis of early pregnancy loss rates showed that the effect evaluation remained unchanged, indicating no strong publication bias of the combined results.

Coagulation- and Anticoagulation-Related Outcome Indicators

Three studies (Feng et al., 2019; Niu and Hao, 2020; Xu et al., 2020) included changes in indicators related to coagulation and anticoagulation, such as PT, APTT, TT FIB, t-PA, and PAI-I in URSA patients before and after treatment with KTHs. Unfortunately, due to the large variations in detection methods, modes of intervention, and modes of comparison among the studies, a quantitative analysis of this data was not possible. The wide variation in results across the studies precludes formation of a consistent conclusion. Finally, we developed a grade assessment for the meta-analysis results based on the Cochrane recommendation (**Supplementary Data Sheet S3**), but the quality of clinical evidence and grade of recommendation for these indicators are of concern.

Drug Screening and Data Intersection

A drug network was constructed for the nine articles screened (**Figure 6A**). Statistics were also performed on the drug doses and frequencies used (**Figure 6B**). The drugs with the top four tastants of frequency, which were set at ob ≥ 30% and DL ≥ 0.18 in the tcmsp database, were selected to screen the active ingredients of the screened drugs acanthopanax, *Sambucus parasitica*, and *Cuscuta cuspida* sequestration. Additionally, the ingredients of the four herbs in KTHs were also searched in the Batman database (**Figure 6D**), resulting in a total of 32 potential active ingredients after removal of any duplicates from both databases. Furthermore, 546 drug targets were screened using the Swiss target prediction database with strict criteria of the species as "human" and a probability greater than 0.6. A total of 708 disease targets for URSA were obtained after dereplication. Finally, 546 drug targets and 708 disease targets were established, and a Venn diagram was drawn using the venny2.1 online software mapping tool platform, and 113 drug disease common targets were obtained after intersection of the two sets (**Figure 6E**).

PPI Network Construction and Core Target Analysis

The 113 intersection targets were brought into the string database to construct the PPI network conditional on the species "human" and a confidence score ≥0.4. The results

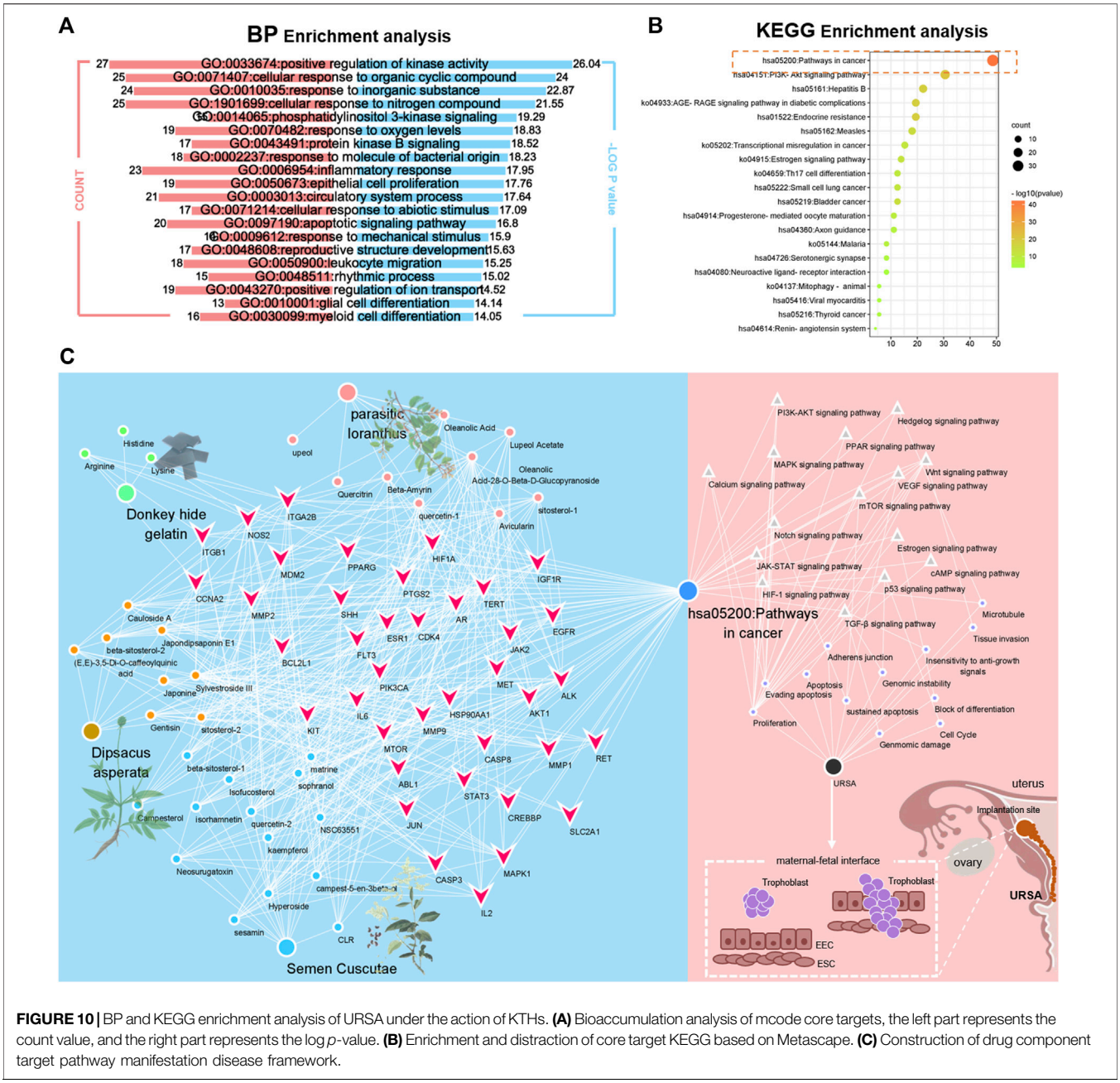

FIGURE 10 | BP and KEGG enrichment analysis of URSA under the action of KTHs. **(A)** Bioaccumulation analysis of mcode core targets, the left part represents the count value, and the right part represents the log *p*-value. **(B)** Enrichment and distraction of core target KEGG based on Metascape. **(C)** Construction of drug component target pathway manifestation disease framework.

were imported into Cytoscape. **Figure 7** shows the intersection target PPI network. The larger the node, the larger the degree value was. Next, the PPI network of URSA was further analyzed by mcode module with score cutoff = 0.2 and K core = 2. The clustering analysis was performed conditional on maximum depth = 100 and degree cutoff = 2. A total of 16 DEGs were obtained, and the top five DEGs with the highest score were taken (**Figure 8B**). In total, five DEGs (**Figures 8C–G**) and four core genes were obtained, and these five foundation clusters were scored. After screening, the four core genes obtained were mitogen-activated protein kinase (MAP1), matrix metalloprotinase1 (MMP1) (Zhang et al., 2021), ATP-binding cassette subfamily G member 2 (ABCG2), and recombinant caspase 1 (CASP1).

Molecular Docking Verification

Using different drug complexes in KTH as active compounds, the core proteins analyzed by mcode were used as targets for the molecular docking validation (**Figure 9A**), and the best binding compounds were selected to construct the docking map. The binding energy of Sylvestroside III small molecules with MAPK1 protein was −7.6 kcal/mol, which proved to have a good binding interaction (**Figure 9B**). Sylvestroside III small molecules interact with the MAPK1 protein, mainly

TABLE 2 | Core components and targets of KTHs.

Pubchem CID	Key components	Molecular structure	Weight g/mol	Possible target of URSA
101967018	Sylvestroside III	$C_{27}H_{36}O_{14}$	584.60	AKT1, ALK, CASP1, CASP8, CDK1, CFTR, CNR1, F10, FGFR1, FLT3, FLT4, IGF1R, JAK2, KDR, KIT, MAPK1, MMP2, MMP3, MMP9, NTRK1, PARP1, REN, RET, SLC2A1, and WNT3A
442915	Japonine	$C_{18}H_{17}NO_3$	295.30	ABCB1, ABCG2, CREBBP, CYP17A1, DRD2, FGFR1, FLT1, JAK2, MMP1, MTOR, NPM1, NR3C1, NTRK1, PGR, PIK3CA, PLK1, TACR1, and TEK
12442899	sophranol	$C_{15}H_{24}N_2O_2$	264.36	ABCB1, ACE, ADRB2, ALK, AR, CYP19A1, GBA, HTR1A, HTR3A, JAK2, KCNH2, PARP1, REN, SCN5A, SLC6A4, and TACR1
5281636	Gentisin	$C_{14}H_{10}O_5$	258.23	MAOA, PTGS2, ABL1, ALK, ALPL, CASP3, CDK4, CHEK2, COMT, DYRK1A, ESR1, ESR2, FLT1, GUSB, HSP90AA1, IGF1R, KDR, MET, MMP1, MMP3, MTOR, PIK3CA, PLK1, RET, and STAT3
5962	Lysine	$C_6H_{14}N_2O_2$	146.19	PLG, SHBG, AR, ODC1, GRIA2, and PEPD
91466	Matrine	$C_{15}H_{24}N_2O$	248.36	CTSB, HTR3A, and PARP1

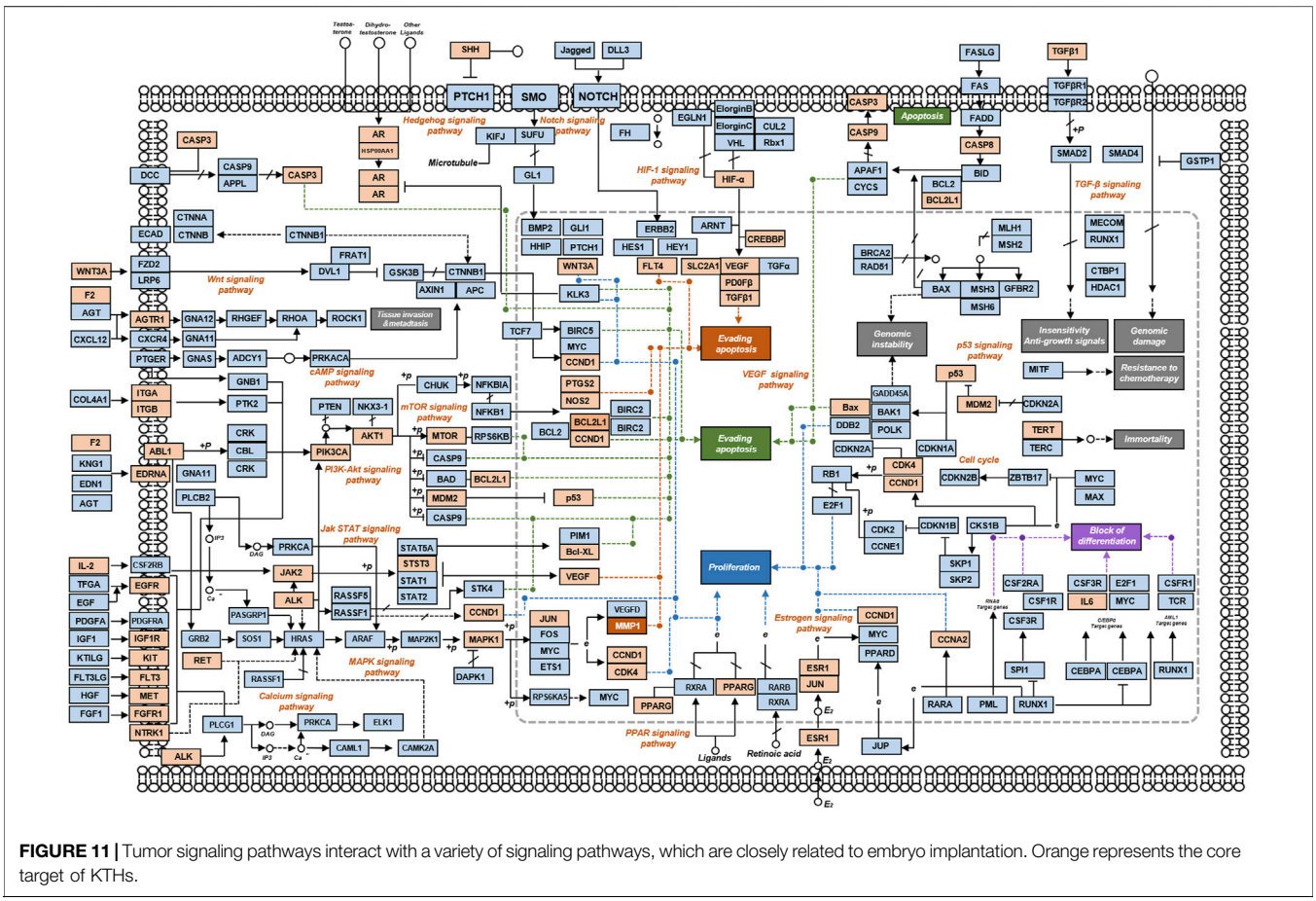

FIGURE 11 | Tumor signaling pathways interact with a variety of signaling pathways, which are closely related to embryo implantation. Orange represents the core target of KTHs.

through the formation of hydrogen bonds as well as hydrophobic forces. Six hydrogen bonds formed with ARG-148, ARG-172, THR-63, GLN-62, HIS-61, and PHE-183, respectively, with hydrogen bond lengths of 3.2, 3.4, 3.0, 3.1, 3.0, and 3.3 Å. Sylvestroside III has hydrophobic interactions with LEU-170, ARG-67, GLN-66, GLU-186, GLY-182, THR-181, and HIS-180.

Japonine small molecules, on the other hand, also bind well to the MMP1 protein, with a binding energy of −6.3 kcal/mol (**Figure 9C**). Avicularin small molecules interact with the MMP1 protein, mainly through the formation of hydrogen bonds as well as hydrophobic forces, resulting in seven hydrogen bonds with PRO-238 and TYR-240 with a hydrogen bond of 3.3 and 3.3 Å, respectively. Japonine has hydrophobic interactions with ASN-180, LEU-181, VAL-215, ARG-214, HIS-218, GLU-219, and SER-239. Molecular docking results confirmed the results of the network analysis relating to the effect of KTHs when treating URSA.

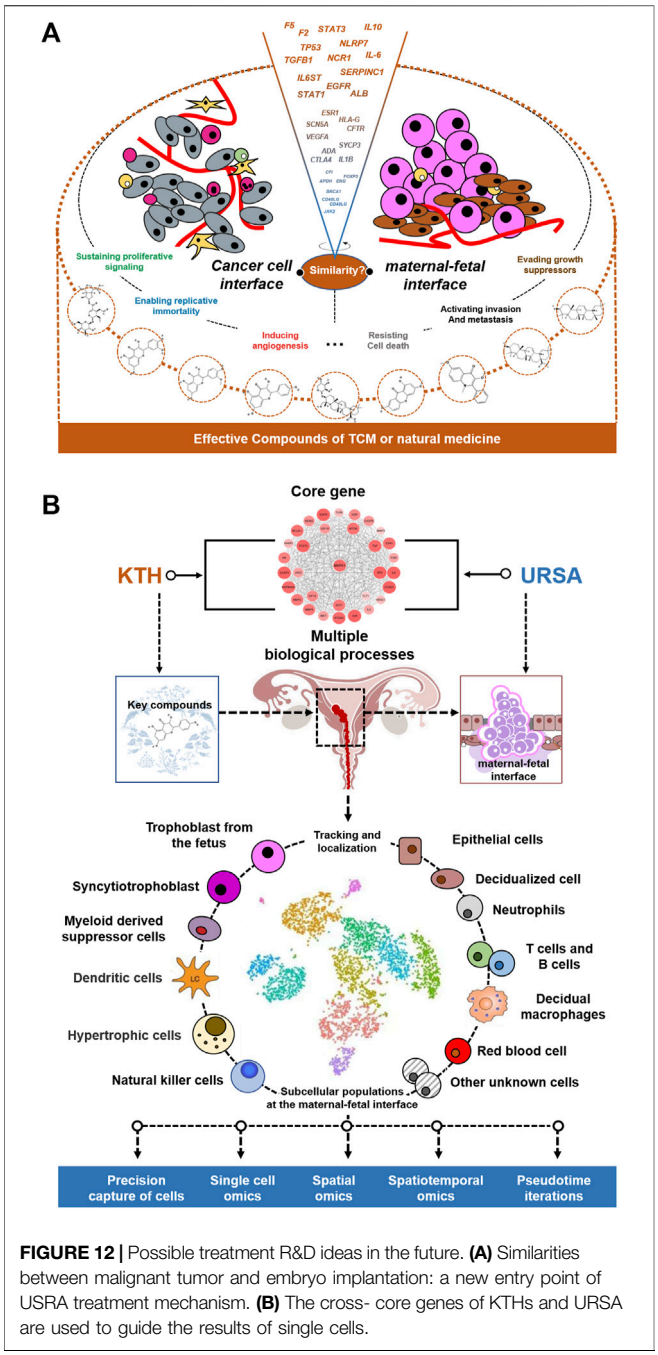

FIGURE 12 | Possible treatment R&D ideas in the future. **(A)** Similarities between malignant tumor and embryo implantation: a new entry point of USRA treatment mechanism. **(B)** The cross- core genes of KTHs and URSA are used to guide the results of single cells.

Enrichment Analysis of BP and KEGG

After running mcoed core clustering through Metascape, GO analysis selected biological processes for analysis. The BP results showed that the intersection gene set was enriched to 2,045 biological process pathways (**Figure 10A**), mainly including: positive regulation of kinase activity, cellular responses to organic cyclic compound, responses to inorganic substance, cellular responses to nitrogen compound, phosphatidylinositol 3-kinase signaling, responses to oxygen levels, protein kinase B signaling, responses to molecules of bacterial origin, inflammatory responses, epithelial cell proliferation, and others. Core genes were selected by Metascape, with parameters min overlap = 3, p-value cutoff = 0.01, and min enrichment = 1.5. Twenty KEGG pathways were screened out, and the results of the top 20 formed a bar graph of KEGG functional enrichment (**Figure 10B**). Padjust represented the significance of enrichment; the more the red the color is, the more significant. Finally, based on the core target screening and molecular docking, together with the results of BP and KEGG enrichment analyses, the 32 potentially active ingredients from the lifespan pill were the inputs with the 113 drug disease cotargets into Cytoscape software to remove isolated ingredients with no intersection with targets. A network diagram of "drug ingredient target disease" interactions was drawn (**Figure 10C**). Using the score of 30 potentially active ingredients, and the average of the degrees as the screening criterion, we selected six compounds including Sylvestroside III, Japonine, sophranol, Gentisin, Lysine, and Matrine as core ingredients that were either large at the network nodes or showed high binding to protein targets (The screening process and criteria are detailed in **Supplementary Data Sheet S4**). The most important active compounds that were selected are listed in **Table 2**.

DISCUSSION

At present, there is a lack of targeted diagnostic and treatment options for URSA, all of which provide suboptimal clinical outcomes (Pierce, 1983; Youssef et al., 2019; Li et al., 2020). The question on how to find better interventions to achieve good pregnancy outcomes for more URSA patients is currently a hot topic and a challenging point in the field of reproductive medicine.

Although progesterone was used as the primary method of treatment in most of the studies included in this research, it has limited clinical efficacy and can cause side effects such as dizziness, nausea, and vomiting (Yan et al., 2013; Coomarasamy et al., 2020a; Coomarasamy et al., 2020b). Potential risks include allergies, infectious diseases, and bleeding, as well as an ever-increasing financial burden on individuals and society. In the future, new methods for treating URSA will be necessary. As a supplement, traditional Chinese medicine, based on four main diagnostic methods, which regulate the human body as a whole using an inspection method, auscultation, olfaction method, and pulse diagnosis method, looks promising (Zhou et al., 2007). In TCM, KTHs are considered as a treatment, which pays more attention to the syndrome of patients and infers localized treatment from the whole, which may be the result of multichannel and multitarget approaches. However, modern medicine emphasizes the specific etiological treatment affecting URSA, such as focusing in on improving the uterine blood supply and endometrial immune status of the patient. Although KTH has a complex composition and a presently unclear mechanism of action, its comprehensive effects on diseases, especially on health care and prevention, are

TABLE 3 | The main biological processes of cancer progression and embryo implantation, and the targets of related components in traditional Chinese medicine.

Cell biological processes	The significance in embryonic development	The significance in cancer progression	The related genes obtained in this study	Components of KTHs
Regulation of epithelial cell proliferation	Promote the uterus into the receptive state, decidualization of uterine stromal cells and the occurrence of placenta (Zhu et al., 2019)	It can make tumor proliferate rapidly, compress tissue, and promote angiogenesis (Zhong et al., 2020)	AR, TNF, XDH, PPARG, PGR, VDR, STAT3, JUN, MTOR, EGFR, KDR, AKT1, SHH, ITGB3, SCN5A, HIF1A, AGTR1, ITGA4, GLUL, FLT1, FGFR1, TEK, FLT4, WNT3A, and CCND1	(a), (b), (d), €, and (f)
Epithelial cell migration	It can promote the mutual recognition and interaction between blastotrophoblast cells and endometrial cells, and promote the balance of maternal–fetal interface (Zhu et al., 2019)	Cell migration plays an important role in the early occurrence and development of various cancers, especially before primary tumor cells develop into invasive lesions (Hsieh and Wu, 2020)	PTGS2, TNF, PPARG, PTPN11, JUN, MTOR, PIK3CA, SRC, KDR, MMP9, MET, AKT1, ITGB3, HIF1A, ITGB1, GLUL, ABL1, FGFR1, TEK, FLT4, and KIT	(b), (e), and (f)
Regulation of tissue remodeling	Embryo implantation and development involve degradation and remodeling of extracellular matrix, placental villous vasculogenesis, and reconstruction of uterine spiral artery (Zhang et al., 2021)	Tumor destroys normal tissue and makes it remodel to change its original biological function (Zhong et al., 2020)	PLG, IL2, VDR, MDM2, MMP2, MMP14, EGFR, SRC, ITGB3, HIF1A, ADRB2, ACE, IL6, and FLT4	(a), (d), and (e)
Response to oxidative stress	Excessive ROS can cause mitochondrial damage, DNA damage, lipid peroxidation, and even cell apoptosis in embryos, and then lead to embryonic development arrest (Ivanov et al., 2021)	High level and long-term oxidative stress can directly damage tissues through this redox system, and also lead to oxidative modification of amino acid residues and DNA mutation, thus promoting the occurrence of tumor (Shrivastava et al., 2021)	PTGS2, TNF, PTGS1, MDM2, JUN, MMP3, MMP2, MMP14, EGFR, MPO, SRC, CDK1, MMP9, MET, AKT1, MAPT, PARP1, HIF1A, JAK2, CASP3, IL6, ABL1, CCNA2, MAPK1, and PSEN1	(a), (b), (c), (e), and (f)
Regulation of mitochondrion organization	In the process of embryo implantation, the number, distribution, and activity of mitochondria are regulated strictly and orderly, and affect the embryo implantation potential at the same time (Moher et al., 2009)	The malignant phenotypes of tumor cells, such as unlimited proliferation, abnormal metabolism, inhibition of apoptosis, strong invasion, and easy metastasis, are also closely related to mitochondrial dysfunction (Roth et al., 2020; Suldina et al., 2018)	BCL2L1, KDR, MMP9, AKT1, MAPT, HIF1A, GBA, and CASP8	(a), (e), and (f)
Response to lipopolysaccharides	Inflammation and infection result in LPS affecting decidual differentiation through toll-like receptor 4, which leads to stress injury and thrombosis of trophoblast (Guo et al., 2019)	LPS can promote tumor survival by activating upregulated inflammatory signaling pathway, and can also increase the expression of adhesion factors of tumor cells to endothelial cells by activating neutrophils (Shetab et al., 2019)	NOS2, PTGS2, TNF, CNR1, MPO, SRC, AKT1, CCR5, REN, JAK2, ELANE, SELE, SELP, CASP3, CASP8, CASP1, IL6, CDK4, ALPL, ABL1, COMT, and MAPK1	(a), (b), (e), and (f)
Regulation of autophagy	Autophagy can affect embryo delayed implantation, abnormal decidua, and reduce the expression of autophagy in endometrial receptive period to ensure the success of embryo implantation (Su et al., 2020)	Moderate autophagy can make the damaged tumor cells survive, while excessive autophagy can accelerate the death of tumor cells (Wang et al., 2019)	STAT3, MTOR, PIK3CA, KDR, MET, AKT1, MAPT, HIF1A, GBA, ADRB2, CASP3, and ABL1	(a), (b), (e), and (f)
Regulation of apoptotic signaling pathway	Proapoptotic factors and antiapoptotic factors play a key role in regulating the survival and apoptosis of embryonic cells, and the balance between them determines the survival or death of embryos (Zhang et al., 2021)	Imbalance in the ratio of proliferation and apoptosis of tumor cells is the key factor in tumorigenesis and progression (Mohamed et al., 2017)	AR, PTGS2, TNF, TERT, MDM2, BCL2L1, SRC, MMP9, AKT1, PARP1, HIF1A, JAK2, CASP8, RET, FGFR1, and PSEN1	(a), (b), (c), (d), (e), and (f)
Regulation of inflammatory response	The balance of pro-inflammatory factors and anti-inflammatory factors promotes endometrial receptivity, and appropriate inflammatory environment promotes embryo implantation and pregnancy maintenance (Quirke et al., 2021)	The infiltration of inflammatory cells and the production of ROS are necessary and sufficient conditions to accelerate the carcinogenesis (Rossi et al., 2021)	NOS2, PTGS2, TNF, IL2, ESR1, CYP19A1, PPARG, CNR1, F2, TLR9, STAT3, MMP3, EGFR, MMP9, GBA, JAK2, ELANE, SELE, CASP1, AGTR1, IL6, and TEK	(a), (b), (e), and (f)
Regulation of angiogenesis	On the basis of the original blood vessels, the formation of blood vessels through the process of endothelial cell proliferation, and migration is conducive to the development and infiltration of embryonic cells (Barrientos et al., 2013)	It can promote the secretion of tumor cells, promote angiogenic factors, promote the proliferation of endothelial cells, and chemotaxis the migration of endothelial cells (Li et al., 2019)	PTGS2, TNF, TERT, PPARG, STAT3, KDR, HIF1A, ITGB1, AGTR1, IL6, GLUL, FLT1, ABL1, and TEK	(b), (e), and (f)

(Continued on following page)

TABLE 3 | (*Continued*) The main biological processes of cancer progression and embryo implantation, and the targets of related components in traditional Chinese medicine.

Cell biological processes	The significance in embryonic development	The significance in cancer progression	The related genes obtained in this study	Components of KTHs
Epithelial–mesenchymal transition, EMT	It can make endometrial epithelial and stromal cells more invasive and mobile, promote embryonic organ formation, embryonic differentiation, and nervous system differentiation (Ran et al., 2020)	EMT plays an important role in the invasion and metastasis of tumor *in situ* and the formation of new metastasis. It is an important way of invasion and metastasis of epithelial cell carcinoma, which accounts for more than 90% of malignant tumors in adults (Thiery, 2002)	MMP2, MMP7, NOS2, MET, CDK1, CYP19A1, SHH, JAK2, CCNA2, MMP1, MMP3, MDM2, EGFR, ABL1, CHEK2, PGR, RET, ABCB1, STAT3, IL6, CASP3, VDR, ABCG2, PIK3CA, IGF1R, HIF1A, TLR9, JUN, AR, TNF, ITGB1, KIT, F2, PTGS2, CCND1, ESR1, CDK4, SLC2A1, FLT1, TERT, SRC, REN, PTPN11, ALK, CASP8, PPARG, NR3C1, MTOR, DRD2, AGTR1, HSP90AA1, PARP1, KDR, BCL2L1, and MMP9	(a), (b), (c), (d), (e), and (f)

Note. The main active components in KTHs: (a) Sophranol; (b) Japonine; (c) Matrine; (d) Lysine; (e) Sylvestroside III; and (f) Gentisin.

temporarily irreplaceable by modern medicine (Li et al., 2016; Luo et al., 2012; Liu et al., 2009).

Oncology is an important crossover area for reproduction. Based on the evidence from this meta-analysis, a network pharmacological approach was used to investigate which targets are important, and how these targets and signaling pathways play a role in RSA. Since the concept of an embryonal origin of cancer was proposed in 1982 by Lobstein, the resemblance between the biological behaviors of embryo implantation and tumor invasion and metastasis has been increasingly recognized (**Figure 11**) (Murray and Lessey, 1999; Perry et al., 2009). Especially given that "pseudo malignant" blastocyst trophoblast cells and malignant cells (Soundararajan and Rao, 2004; Fest et al., 2008) exhibit defects in cell proliferation and differentiation, invasion signaling pathways, vascular erosion, and neovascularization. There are additional striking similarities in many aspects of both processes, such as immune escape and apoptosis. The most fundamental biological process during embryo implantation is the invasive properties of trophoblast cells (Hanahan and Weinberg, 2011), which is regulated by a network of extracellular matrix, matrix degrading enzyme, cell adhesion molecules, and growth factors (**Figure 12A**).

The results of this study suggest that the main signaling pathway of KTHs when used to treat URSA is a cancer signaling pathway, which confirms our conjecture. This signaling pathway dominates many biological processes. Therefore, we identified several main biological processes and related targets, which are regulated by KTH active components, and compared this biological process between embryo implantation and cancer progression, as shown in **Table 3**, in order to obtain more insights and beneficial information.

In conclusion, tumor cells and early embryos show similar mechanisms to each other, which gives us enlightenment as to how the microenvironment of embryo growth can guide and activate the potential of tumor development and reverse its phenotype. The process of embryonic development and differentiation is almost a reversion of tumor differentiation. Further study on the regulation mechanism and important regulation steps during embryonic development can provide important indicators and cues for the study of tumor differentiation reversal.

The combination of embryo implantation and tumor invasion and metastasis not only helps understand the internal mechanism of complex life phenomena but also may provide new concepts for clinical treatment. Antitumor invasion and metastasis therapy may ultimately find the answer from embryo implantation, and repeated embryo implantation failure or repeated abortion may also provide new evidence-based answers toward understanding and treating tumors.

LIMITATION AND OUTLOOK

Although we have undertaken a comprehensive analysis and evaluation of all of the published studies, this research still has some limitations, which are worthy of recognition. The quality of the included studies was low due primarily to unclear allocation concealment, selective bias, consumption bias, and the blinding methods, or lack of thereof. The one major limitation within all of the included studies was that they were all conducted in China and have major methodological flaws, which greatly reduced the reliability and validity of study results as a whole.

The approach taken helps map cell types, cell subpopulations, and even cell states in a spatial context. The similarity of pregnancy maintenance to cancer invasion allows us to recognize the high complexity of subcellular species and spatial architecture at the maternal–fetal interface (Srivatsan et al., 2021). This complexity is often accompanied by spatiotemporal dynamic changes, so the study of KTHs for URSA cannot be satisfied by employing traditional single *ex vivo* experiments with animal experiments (**Figure 12B**). Therefore, how KTHs could map the cell types, cell subsets, and even cell states and gene expression profiles of each class of cells regulated at the maternal–fetal interface of URSA patients in a spatial context, is a very

urgent matter, which provides an important area of future research.

It is widely known that single-cell RNA sequencing (scRNA-seq) identifies cell subpopulations within tissue but does not capture their spatial distribution nor reveal local networks of intercellular communication acting *in situ*. Fortunately, a suite of recently developed techniques that localizes RNA within tissues, including multiplexed *in situ* hybridization and *in situ* sequencing (here defined as high-plex RNA imaging) and spatial barcoding, could help address this issue (Longo et al., 2021). We believe that this method of single-cell sequencing, combined with spatial transcription, will bring about a more profound insight into how KTHs acts on URSA.

CONCLUSION

In conclusion, the TCM-based KTH formula provides good efficacy and safety for the treatment of URSA, and has great potential. According to the results of the network pharmacology analysis, we predicted that the main pathway of the KTH effective component in URSA is a tumor signaling pathway. With the in-depth study of the mechanisms of tumor formation, as well as single-cell spatial transcriptome in the future, will bring forth breakthroughs in the pathogenesis research and treatment of USRA.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

AUTHOR CONTRIBUTIONS

LD was responsible for the overall research framework of this project. HZ was responsible for the clinical data analysis and all figure designs. YiY and YoY were responsible for the data collection. XL provided evidence-based idea guidance.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.775245/full#supplementary-material

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EDITED BY
Javier Echeverria,
University of Santiago, Chile

REVIEWED BY
Luca Rastrelli,
University of Salerno, Italy

*CORRESPONDENCE
Abdur Rauf,
mashaljcs@yahoo.com

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Potential therapeutics against neurological disorders: Natural products-based drugs

Abdur Rauf[1]* and Md. Mominur Rahman[2]

[1]Department of Chemistry, University of Swabi, Swabi, KPK, Pakistan, [2]Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka, Bangladesh

Neurodegenerative disorders, which are defined by the breakdown of neurons over time, are affecting an increasing number of people. Stroke, Alzheimer's, Parkinson's, Multiple Sclerosis, Migraine, and Amyotrophic Lateral Sclerosis are just a few examples of brain disorders that have no cure. Besides, there is a huge demand for drugs that can cure the diseases mentioned above because the majority of the medications we use to treat them only alleviate diseases. Different neurological disorders have responded satisfactorily to the pharmacological effects of medicinal plants. Despite the numerous multiple types of plants in the world, only a small number of them have been investigated for neurological disorders. As a result, there are many opportunities in this area for further research on plants and their bioactive chemicals. The search for natural therapeutic alternatives that promote faster healing and adverse effects avoidance has gained popularity in recent years. The aim of this mini-review is to explore some natural products that have strong therapeutic effects on neurodegenerative disorders such as Stroke, Alzheimer's Disease, Parkinson's Disease, Multiple Sclerosis, Migraine, Amyotrophic Lateral Sclerosis, and others. We have also shown the safety of natural products to improve their appropriate usage in neurological disorders from recent literature.

KEYWORDS

neurological disorders, natural products, potential therapeutics, drugs, Malnutrition

Introduction

The prevalence of neurological disorders is on the rise and poses a significant public health problem. Parkinson's disease, dementia, neuro infections, epilepsy, neurological diseases linked with malnutrition, pain associated with neurodevelopmental problems such as stroke, headache, multiple sclerosis, and traumatic brain injuries are all examples of neurological disorders. There are around 450 million people in the world who suffer from various types of mental diseases. Approximately 50 million individuals suffer from epilepsy, and this figure is rising every day (Rahman et al., 2022b; Sorboni et al., 2022). Alzheimer's and other forms of dementia are expected to double in population in the next 20 years. More than 322 million individuals suffer from serious depression at any given time (Clevenger et al., 2017) and this figure is expected to continue rising. Neurological disorders constitute over 6% of the global burden of disease (Ferrari, 2022). Many

low- and middle-income nations bear a disproportionate share of this cost. As a result of the multiple pathogenic pathways linked with neurodegenerative diseases, techniques such as neuroprotection, which includes avoiding cell death and restoring function to injured neurons, may be promising for the prevention and treatment of these disorders (Hartman et al., 2020).

Natural products, such as medicinal plants, phytopharmaceuticals, nutraceuticals, vitamins, and nutritional supplements, are being used more frequently around the world for various health conditions. These products are generally considered to be secure. We can have an evidence-based approach to the safety of these products with the use of several research methods, such as clinical safety studies and pharmacovigilance-based investigations. We have acquired additional knowledge about the safety of natural products to improve their appropriate usage in neurological disorders by compiling papers on this topic from recent literature. An essential overview of the knowledge that is available today on these issues were provided by certain systematic reviews and meta-analyses. The use of natural substances in complementary and alternative medicine can lead to the discovery of new medication lead compounds. To combat neurodegenerative diseases, the use of natural substances has recently emerged as a new sector (Bhattacharya et al., 2022).

Both *in vitro* and *in vivo* studies have indicated that the use of natural ingredients can improve pre-clinical models of neurodegenerative diseases. The phytoconstituents like polyphenolic antioxidants that may be found in herbs, nuts, fruits, and vegetables as well as freshwater and marine flora are among the many natural items that can be used to treat various health problems. These phytoconstituents may be able to reduce neurodegeneration and improve cognitive and memory functioning in the brain. Several neurodegenerative diseases, such as Alzheimer's disease Parkinson's disease, epilepsy and other degenerative disorders of the nervous system may benefit from their use (Rahman et al., 2020). Researchers have shown that natural products can have widespread neuro-pharmacological effects by suppressing the production of inflammatory mediators or increasing levels of specific cell survival proteins (Rahman et al., 2021). Opioids, galantamine, and anticholinesterases like physostigmine and neostigmine were all isolated from plants as proof of the relevance of plant-derived bioactive substances in the treatment of neurological disease. Seven of the 26 natural medicines licensed in the recent decade were for the treatment of neurological disease, including three for Parkinson's disease (Karim et al., 2018; Rahman et al., 2022a).

Even just a small number of plants from across the world have been studied for their potential to cure neurological disorders, therefore there are several directions in which this area of study might be expanded. In recent years, there is a growing interest in the quest for alternative treatments based on natural products, offering better recovery and the avoidance of side effects. This special topic was a platform for relevant experts in the field of ethnopharmacology and neuropharmacology to share cutting-edge research and emerging literature-based reviews related to neurological disorders. The primary goal of this study was to examine research and reviews connected to the creation of novel medications derived from plant sources against neurological disorders and created interest in the prevention and treatment of neurodegenerative diseases as well as confirmed the safety of natural products to enhance their proper use.

In conclusion, medicinal plants are a vital source of a wide range of bioactive chemicals. Ethnopharmacology studies can lead to the development of more effective multi-target drugs for therapeutic approaches to various diseases, such as neurological disorders, in addition to providing a scientific basis for the optimal dose and possible toxicological effects on the local community.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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