Int J Clin Exp Med 2018;11(12):12880-12888
www.ijcem.com /ISSN:1940-5901/IJCEM0073286

Review Article

The therapeutic effect of Chaihu-Shugan-San in fatty liver disease: a meta-analysis randomized controlled trials

Yong-Yong Zhang[1], San-Qiang Li[1], Ying Song[1], Wei-Qiang Qiao[2], Ping Wang[1], Xin-Juan Pan[1], Dong-Mei Wang[1], Rui-Fang Li[1], Yi-Xiang Chen[1]

[1]*The Molecular Medicine Key Laboratory of Liver Injury and Repair, Medical College, Henan University of Science and Technology, Luoyang 471003, Henan, China;* [2]*Department of Breast Surgery, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang 471003, Henan, China*

Received January 23, 2018; Accepted October 8, 2018; Epub December 15, 2018; Published December 30, 2018

Abstract: Subject: To assess the effect of Chaihu-Shugan-San (CSS) in the treatment of fatty liver disease. Method: A meta-analysis was conducted to examine the effect of CSS treating on fatty liver. Comprehensive literature searches were performed to search for randomized controlled trials up to August 2017. Result: 12 randomized controlled trials involving 1,049 patients were included in this study. The results showed that CSS had significant effect for the treatment of fatty liver patients in comparison with control groups (Cure OR = 2.47, 95% CI 1.77 to 3.45, P < 0.00001; Significant effect OR = 1.57, 95% CI 1.16 to 2.12, P = 0.004; Effective OR = 0.65, 95% CI 0.47 to 0.89, P = 0.008; Invalid OR = 0.23, 95% CI 0.15 to 0.36, P < 0.00001), and also revealed that CSS could significantly reduce the levels of TG, TC, AST and ALT in fatty liver patients group (TG mmol/L MD = -0.88, 95% CI -1.23 to -0.54: P < 0.00001; TC mmol/L MD = -1.29, 95% CI -1.92 to -0.66: P < 0.00001; AST U/L MD = -10.89, 95% CI -12.70 to -9.09: P < 0.00001; ALT U/L MD = -13.46, 95% CI -18.25 to -8.67: P < 0.00001), but no significant effect on HDL and LDL (HDL mmol/L MD = 0.07, 95% CI -0.12 to 0.27: P = 0.46; LDL mmol/L MD = -0.48, 95% CI -1.02 to -0.06: P = 0.08). Conclusion: CSS has positive effect on blood lipids, liver function and fatty liver condition of fatty liver patients. However, due to the limitation of number and quality of trials included, more clinical randomized controlled trials with high quality are needed for further verification.

Keywords: Chaihu-Shugan-San, meta-analysis, fatty liver

Introduction

Liver, as a vital organ of the human body, plays important roles in digestive and excretory functions, nutrient storage, metabolic functions, synthesis of new molecules, and purification of toxic chemicals [1]. The fatty liver disease is currently one of the world's major public health problems, and its pathologic processes include fatty liver, steatohepatitis and cirrhosis. Fatty liver is a clinical syndrome caused by various factors such as environmental, genetic and metabolic stress [2], and it contains alcoholic and nonalcoholic liver diseases. Around the world, the morbidity of nonalcoholic fatty liver disease (NAFLD) is 6.3%-33%, but in Europe and other western countries, the average prevalence rate in adults ranging from 20-33% [3], and a previous study showed a trend toward a gradual increase in the younger generations [4]. In particular, it should be considered that simple NAFLD can progress to non-alcoholic steatohepatitis (NASH) in about 20-25% of cases and nearly 20% of patients with NASH can develop fibrosis and cirrhosis [9]. However, its pathogenesis is not clear, and hepatic fibrosis may be a pathological pathway through which steatohepatitis develops toward cirrhosis [5, 6].

At present, there was no particularly effective Western medicine in the treatment of fatty liver through liver protection, anti-inflammatory, and lipid-lowering. Traditional Chinese medicine (TCM) has been clinically used in China for thousands of years for the treatment of

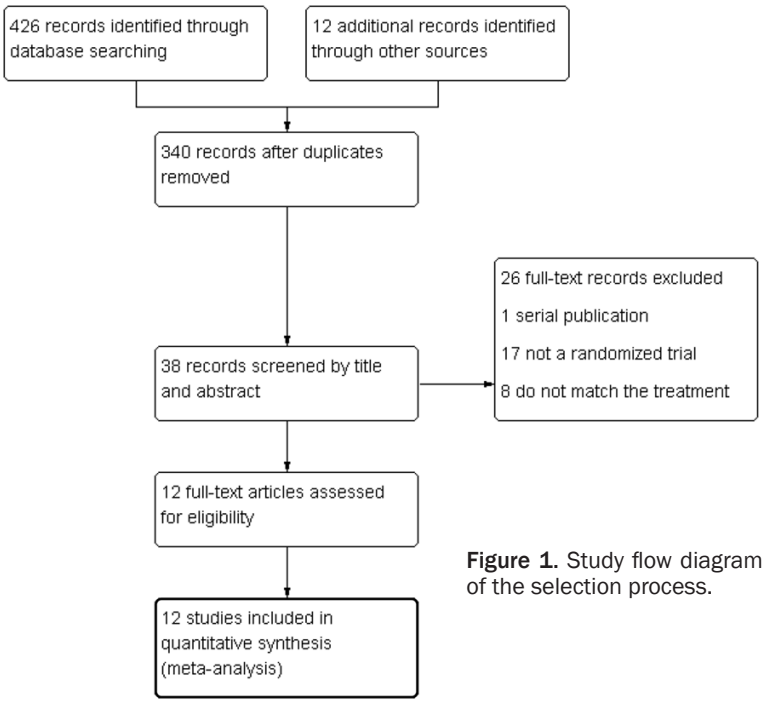

Figure 1. Study flow diagram of the selection process.

many diseases. Chaihu-Shugan-San (CSS), an ancient classical formula from "Jingyue Quanshu", is composed of seven Chinese herbs: Bupleurum Chinese DC (also known as Chaihu in Chinese), Pericarpium Citri Reticulatae, Ligusticum chuanxiong Hort, Rhizoma Cyperi, Fructus Aurantii, Radix Paeonia Alba, and Glycyrrhiza uralensis Fisch with a traditional dose ratio of 6:6:5:5:5:5:3 [7]. Many studies have demonstrated that CSS protected against lipid peroxidation [8], liver fibrosis [9, 10] and insulin resistance. The major compounds from CSS such as saikosaponins [11] and total glucosides of peony have been demonstrated their potential protection on the liver. From the point of TCM theory, the basic pathogenesis of fatty liver was closely correlated to liver stagnation and spleen deficiency [12, 13], and evidence showed CSS could be used for the treatment of liver qi stagnation [7].

Liver stagnation is one of the major syndromes for fatty liver patients, and the application of CSS addition and subtraction treatment has proved to be effective. However, there is no published evidence based on randomized controlled trials (RCTs) to demonstrate that applying CSS is an effective treatment for fatty liver development. Based on the RCTs of CSS for the treatment of fatty liver, the quality and efficacy of these RCTs were systematically evaluated, and this could provide a basis for the treatment of fatty liver by CSS.

Materials and methods

Search strategy

The following databases were retrieved without any language restriction (date last searched: August 2017): the Cochrane Library, PubMed and EMBASE, the China National Knowledge Infrastructure (CNKI), VIP and the WangFang Database. The following terms were used in the search: "fatty liver or fatty or liver", "Chaihu or Shugan or powders" and "randomized controlled trial or randomized controlled trials". The reference lists of full-text articles were also screened to avoid omitted studies.

Inclusion and exclusion criteria

Literatures were considered eligible if they met the following criteria: (1) randomized controlled trials; (2) the participants are older than 18 years of age; (3) providing sufficient data to estimate the odds ratio (OR) and 95% confidence intervals (CIs). Studies were excluded if one of the following existed: (1) duplicate publications; (2) case reports, reviews, conference abstracts, letters and animal trials; (3) treatment time of the studies less than 1 month.

Data extraction

Included articles are searched by two authors (Yongyong Zhang and Ying Song) according to the inclusion and exclusion criteria listed above. The disagreement was resolved by discussion until consensus was reached. The extracted information contains the following: the first author's surname, publication year, country, size of sample, age and sex of subjects, intervention, treatment.

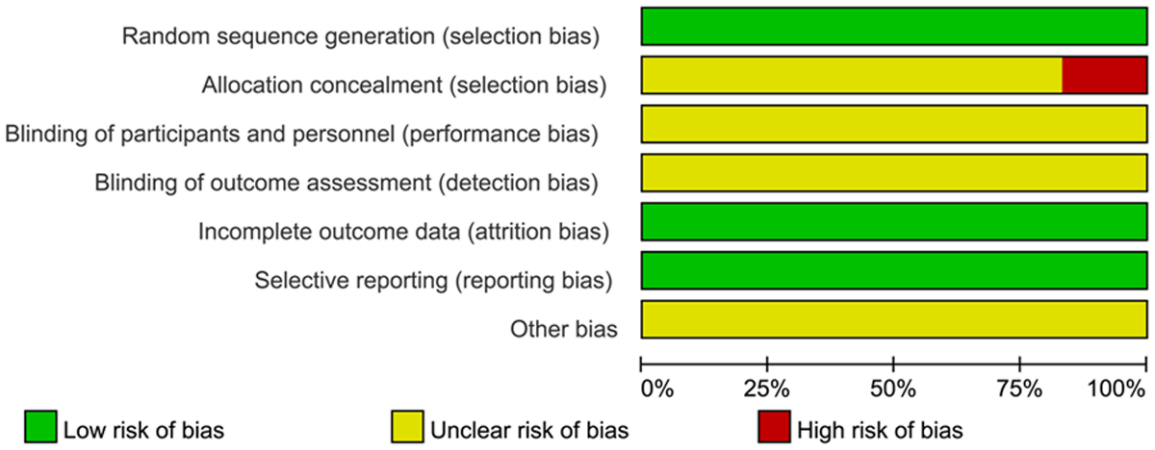

Figure 2. Risk of bias in included studies.

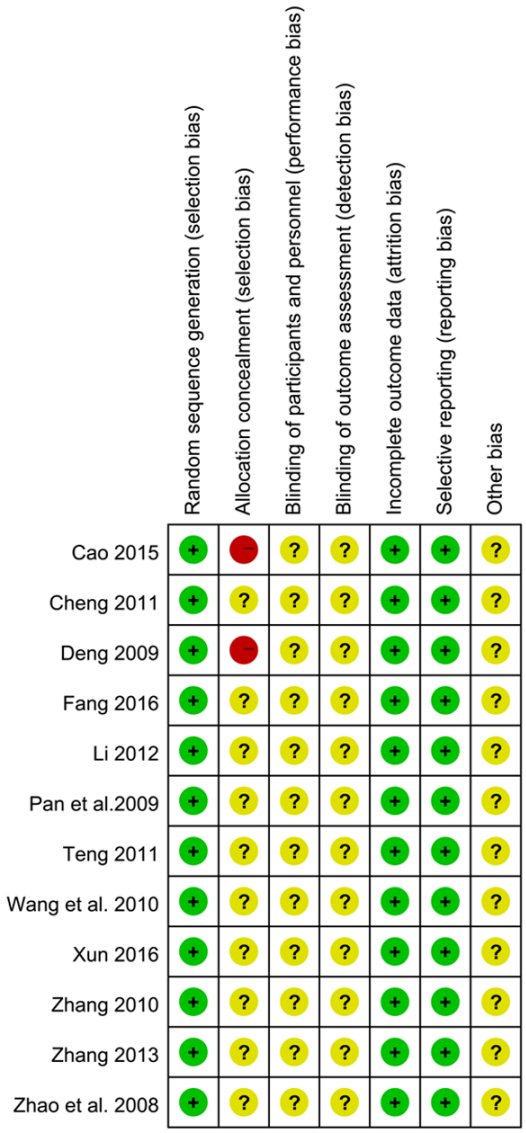

Figure 3. Summary of the risk of bias in included studies.

Outcome indicators

Effective, aspartate transaminase (AST), alanine transaminase (ALT), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

Quality assessment

The RCT methodology was evaluated according to the Cochrane Handbook for systematic review of interventions. Risk of bias for assessing the methodological quality of RCTs mainly included six items: random sequence generation (selection bias), allocation concealment (selection bias), blind subjects and test personnel (implementation bias), blindness Outcome evaluation (measurement bias), incomplete data (reporting bias), selection of publication (publication bias), and other bias.

Statistical analysis

We estimate the mean difference (MD) with 95% CIs for continuous outcomes and ORs with 95% CIs for dichotomous outcomes. Heterogeneities were estimated using the I^2 statistics. When $I^2 < 50\%$ and $P > 0.10$, the results were considered homogeneous and the fixed-effect model was used; when $50\% \leq I^2 < 75\%$, the results were considered heterogeneous and the random-effect model was used. Subgroup analysis was conducted to identify the causes of the heterogeneity if $I^2 \geq 75\%$. Sensitivity analysis was carried out to evaluate the effect of each individual study on the pooled ORs by excluding studies one-by-one and recalculating the ORs and 95% CIs. A funnel plot was

Table 1. Characteristics of included studies

Study	Sample size N		Intervention	Control	Duration	Outcomes
	T (M/F)	C (M/F)				
Cao 2015	36 (23/13)	36 (22/14)	CSS	Ro	6 months	1, 2, 3, 4
Cheng 2011	42	42	CSS	Ro	3 months	1, 2, 3, 4, 5, 6
Deng 2009	46 (30/16)	46 (32/14)	CSS + Con	Ro	3 months	1, 2, 3, 4
Fang 2016	63 (46/17)	63 (45/18)	CSS + Con	Ro	1 month	4, 5, 6
Li 2012	30 (23/7)	30 (25/5)	CSS	Ro	2 months	2, 3, 4
Pan et al. 2009	41 (29/12)	41 (26/15)	CSS	Dongbao liver Thai	3 months	1, 2, 3, 4, 5, 6
Teng 2011	30 (20/10)	30 (19/11)	CSS + Con	Polyene Phosphatidyl choline	3 months	1, 2, 3, 4, 5, 6
Wang et al. 2010	54 (29/25)	54 (27/27)	CSS	FS	3 months	3, 4, 5, 6
Xun et al. 2010	51 (24/27)	50 (27/23)	CSS	Ro	14 days	1, 2, 3, 4
Zhang 2010	65 (42/23)	65 (41/24)	CSS	Ro	2 months	1, 2, 3, 4
Zhang 2013	30 (18/12)	30 (16/14)	CSS	Dongbao liver Thai	3 months	1, 2, 3, 4, 5, 6
Zhao et al. 2008	38 (28/10)	36 (27/9)	CSS + Con	Zhibituo Jujuepian	3 months	1, 2, 3, 4

M: man, F: female, Con: Control group medication, FS: Fenofibrate Sustained Release Capsules, Ro: routine medicine, 1: AST, 2: ALT, 3:TC, 4: TG, 5: HDL, 6: LDL.

used to evaluate publication bias. All statistical analysis was performed using Review Manager Version 5.3 (The Cochrane Collaboration, Software Update, Oxford, UK). All *P* values were two-sided, and a $P < 0.05$ was considered as statistically significant.

Result

Study characteristics

The detailed manuscript screening processes are shown in **Figure 1**. Using the search terms described above, a total of 426 published records were identified and checking the reference lists, we identified 12 additional publications. After screening the title and abstracts, 38 publications met the crude inclusion criteria and were selected for further assessment. Among them, 1 was excluded for serial publications, 17 not randomized trials and 8 do not match the treatment. Ultimately, 12 studies [14-25] were included in the quantitative synthesis.

All trials had two parallel groups and were described as randomized, and allocation concealment was not reported in any of the Chinese studies. The blinding process was described in only 1 study. The risk of bias, as assessed using the tool from the Cochrane Collaboration, is shown in **Figures 2** and **3**.

Among the 12 studies, the total number of patients is 1,049 with 526 patients in the treatment group and 523 patients in the control group. The maximum amount of a single RCT sample is 130 cases, and a minimum of 60 cases. The characteristics of studies included are shown in **Table 1**. For all the studies included, there was no difference between control and treatment groups before these patients were randomly divided into two groups.

Many of the RCT test groups and control groups included routine treatment, which included health education, alcohol consumption, diet control, sports and other basic treatment, and only 1 RCT reported adverse outcomes, including skin itching, nausea, dizziness.

Meta-analysis results

Ten trials reported the data to be effective. We chose a fixed-effects model for the analysis of effective, because of the heterogeneity (Cure $I^2 = 0\%$, Marked effect $I^2 = 45\%$, effective $I^2 = 0\%$, Invalid $I^2 = 0\%$). The results showed that CSS has a significant effect for fatty liver patients compare with control groups (Cure OR = 2.47, 95% CI 1.77 to 3.45, $P < 0.00001$; Marked effect OR = 1.57, 95% CI 1.16 to 2.12, $P = 0.004$; Effect OR = 0.65, 95% CI 0.47 to 0.89, $P = 0.008$; Invalid OR = 0.23, 95% CI 0.15 to 0.36, $P < 0.00001$) (**Figure 4**).

Ten trials reported the data on liver function. The heterogeneity of ALT ($I^2 = 76\%$) is large, so

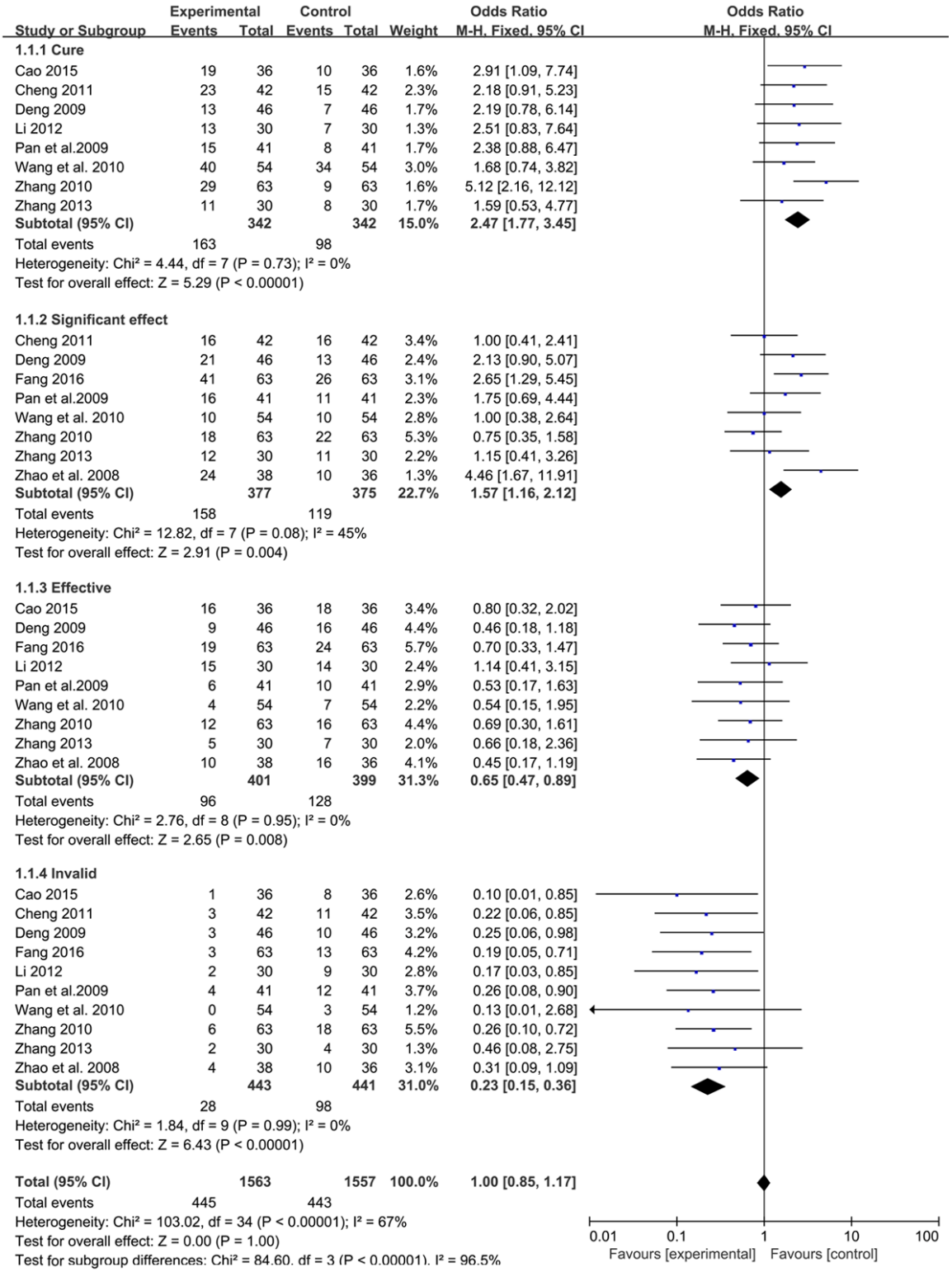

Figure 4. The effective of CSS in patients.

we chose a random model for liver function. The results indicated that the levels of AST and ALT reduced in fatty liver patients who were treated with CSS than those received other drugs or lifestyle intervention (AST U/L MD = -10.89, 95% CI -12.70 to -9.09: P < 0.00001;

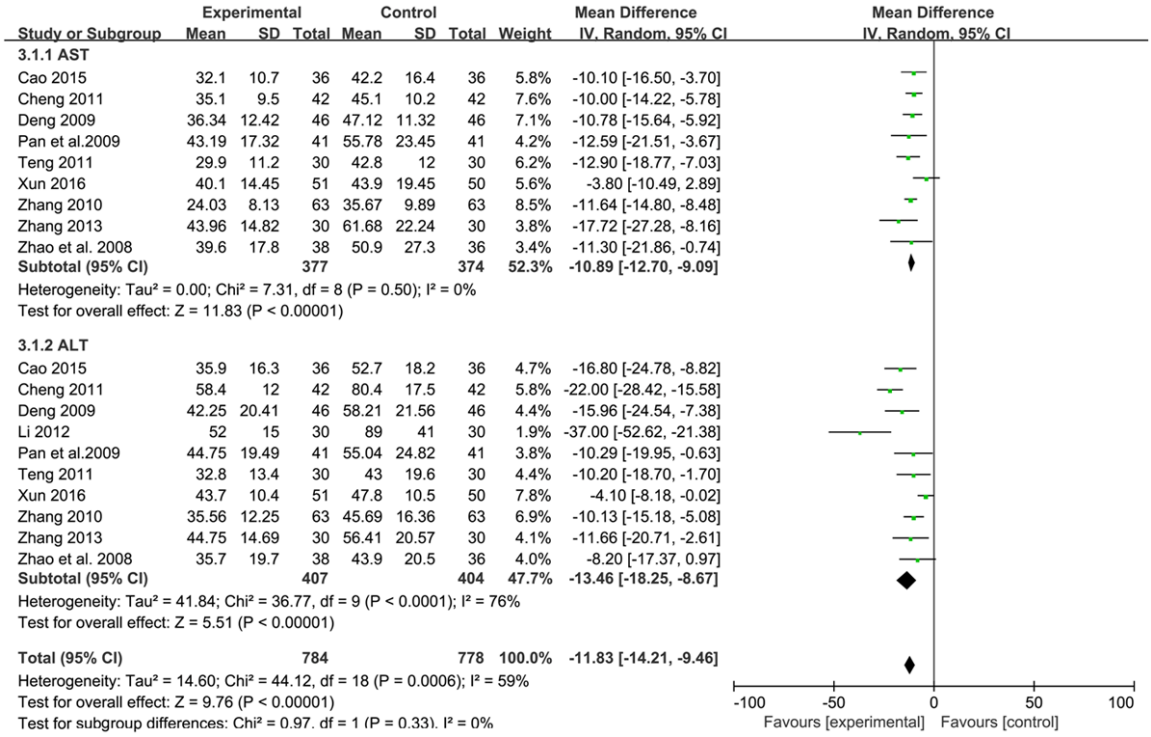

Figure 5. The liver function of CSS in patients.

ALT U/L MD = -13.46, 95% CI -18.25 to -8.67: P < 0.00001) (**Figure 5**).

All trials reported the data of blood lipids. There was a large heterogeneity in the studies (TG I^2 = 95%, TC I^2 = 97%, HDL I^2 = 92%, LDL I^2 = 98%), so random model was used for liver function. The results showed that CSS significantly reduced the level of TG and TC in fatty liver patients when compared with those who use other drugs (TG mmol/L MD = -0.88, 95% CI -1.23 to -0.54: P < 0.00001; TC mmol/L MD = -1.29, 95% CI -1.92 to -0.66: P < 0.00001). However, there was no statistically significant in the levels of HDL and LDL in the two groups (HDL mmol/L MD = 0.07, 95% CI -0.12 to 0.27: P = 0.46; LDL mmol/L MD = -0.48, 95% CI -1.02 to -0.06: P = 0.08) (**Figure 6**).

Publication bias

Publication bias was evaluated by funnel plot. It can be seen from the funnel diagram that most of the literature appears at the top of the funnel and is distributed near the mean effect value, which suggests that the bias of the publication used in meta-analysis is very small (**Figure 7**).

Discussion

Fatty liver is a common chronic liver disease, and it is one of the important factors in leading to hepatocirrhosis and liver cancer [26, 27]. Modern pharmacological studies have shown that CSS might work as a significant anti-inflammatory effect in Kupffer cells, and it can also scavenge oxygen free radicals, reduce enzymes released by liver injury, inhibit lipid peroxidation, and have the ability to improve the liver function of damaged hepatocytes [28]. Bupleurum, pericarpium citri reticulatae can decrease the content of TC in hepatocytes and inhibit fiber proliferation and promote fiber absorption [29]; ligusticum wallichii could inhibit TGF-β1 secretion and has the role of anti-hepatic fibrosis [30]; rhizoma cyperi anti-inflammatory could enhance blood circulation; fructus aurantii could promote the digestion of fat food and lower blood fat; Chinese herbaceous peony could improve microcirculation, increase liver blood flow, liver and reduce enzymes and promote liver cell regeneration [31]; licorice could stabilize the liver cell membrane, antagonize or scavenge free radicals, and prevent HSC activation.

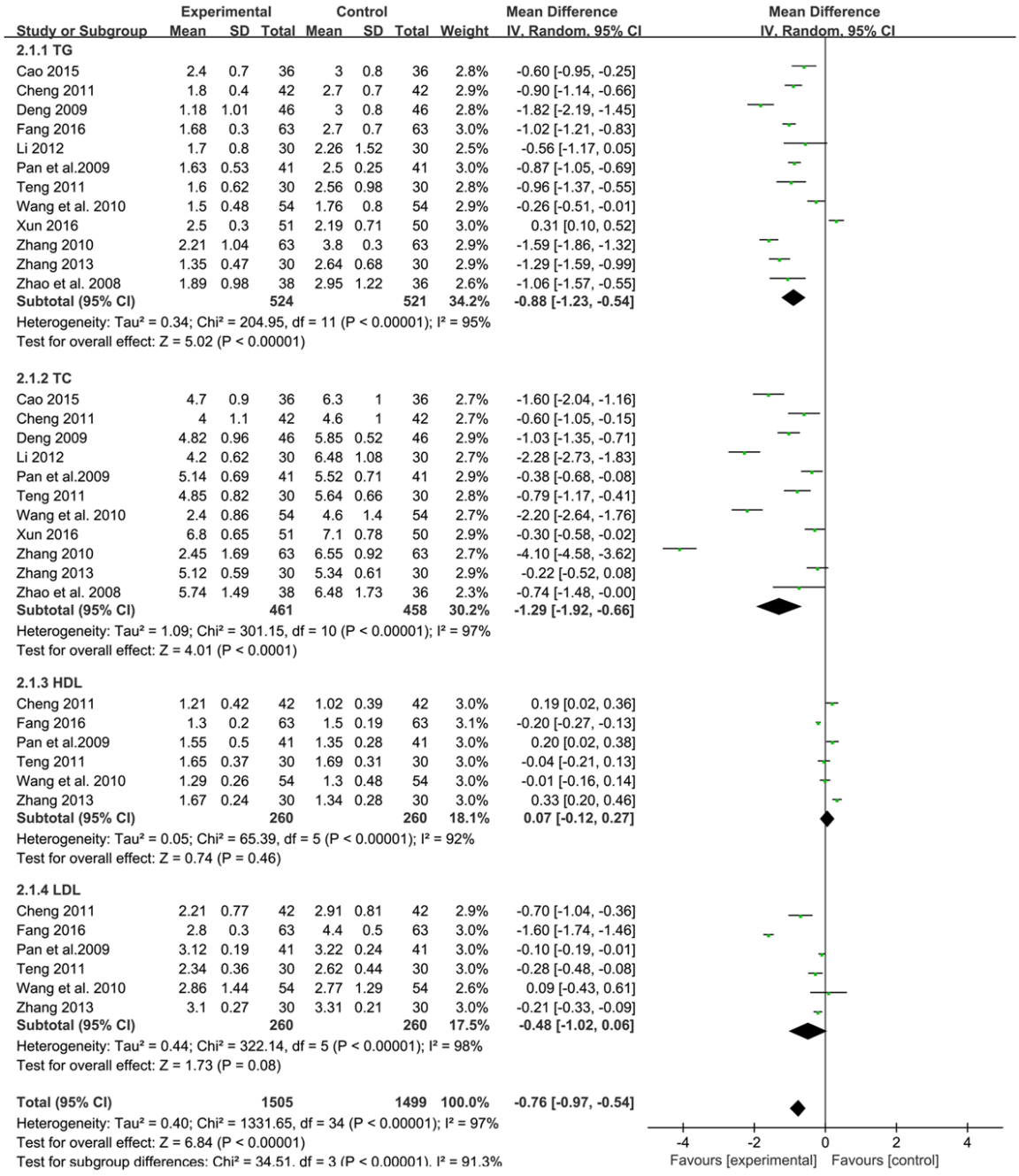

Figure 6. The blood lipids of CSS in patients.

With meta-analysis through 12 studies including 1,049 patients, it demonstrates that CSS has a positive effect on the treatment of fatty liver patients. CSS could improve blood lipid (TG, TC), liver function (AST, ALT) and decrease the TC, TG, ALT and AST level in patients with fatty liver, and the difference is statistically significant compared with other drugs. The effective rate of the experimental group increased significantly when compared with the control group. Some articles refer to the improvement of HDL and LDL in CSS, but there is no difference in this study. The reason may be that the literature is too small and the quality of the literature is low. There are only 12 studies included in this analysis and all of them are Chinese literature. Moreover, the course of treatment is shorter in these studies, of which the longest is

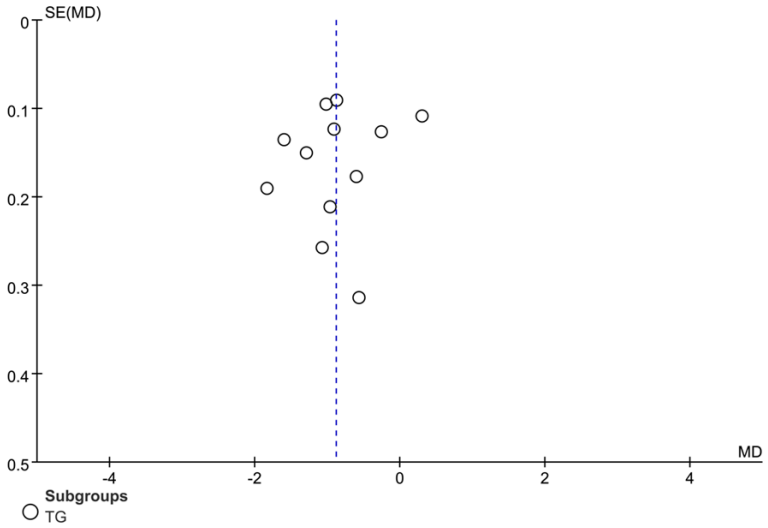

Figure 7. Funnel plot of blood lipids of CSS in patients.

only 3 months, which cannot respond to the long-term use of Chaihu Shugan Scattered for the treatment of fatty liver. All the RCTs are designed for research, assigning implicit and other aspects of the description of the relevant information are inadequate. There was no uniform standard in the related components, quality control, dosage and usage of Bupleurum and Radix Recipe in RCT, which affected the comparability between clinical trials. In addition, articles included in this study did not provide the methods of blind and allocation concealment, and the random allocation concealments of most studies were not described in detail. So we should be cautious about the results of the analysis.

In conclusion, based on the current meta-analysis, CSS has obvious advantages in reducing fatty liver patients with blood lipid (TC, TG) and improve liver function (AST, ALT). However, as the study included in this meta-analysis are all Chinese literature, and limited by the number and quality of articles, the conclusions need to be further validated by more strictly designed multi-centered RCTs with high quality and large scale.

Acknowledgements

This work was supported by program for Science & Technology Innovation teams in Universities of Henan Province (#18IRTSTHN026), Outstanding Youth of Science and Technology Innovation in Henan Province (#184100510006), the innovation team of Henan University of Science and Technology (#2015XTD003) and Henan province's Key Project of tackle key problems of science and technology (#172102310693). The authors thank all the members in the laboratory when this work was carried out. The experiments comply with the current laws of China.

Disclosure of conflict of interest

None.

Address correspondence to: San-Qiang Li, The Molecular Medicine Key Laboratory of Liver Injury and Repair, Medical College, Henan University of Science and Technology, Luoyang 471003, Henan, China. Tel: +86-0379-64820863; Fax: +86-0379-64830346; E-mail: sanqiangli2001@163.com

References

[1] Guerra S, Mamede AC, Carvalho MJ, Laranjo M, Tralhão JG, Abrantes AM, Maia CJ and Botelho MF. Liver diseases: what is known so far about the therapy with human amniotic membrane? Cell Tissue Bank 2016; 17: 653-663.

[2] Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M and Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. Hepatology 2012; 55: 1592-1609.

[3] Farrell GC and Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006; 43: S99-S112.

[4] Fan JG and Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. J Hepatol 2009; 50: 204-210.

[5] Teli MR, James OF, Burt AD, Bennett MK and Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. Hepatology 1995; 22: 1714-1719.

[6] Bertot LC and Adams LA. The natural course of non-alcoholic fatty liver disease. Int J Mol Sci 2016; 17: 774.

[7] Yang QH, Xu YJ, Liu YZ, Liang YJ, Feng GF, Zhang YP, Xing HJ, Yan HZ and Li YY. Effects of Chaihu-Shugan-San and Shen-Ling-Bai-Zhu-

San on p38 MAPK pathway in Kupffer cells of nonalcoholic steatohepatitis. Evid Based Complement Alternat Med 2014; 2014: 1-8.
[8] Li SQ, Su ZH, Peng JB, Zou ZM and Yu CY. In vitro and in vivo antioxidant effects and the possible relationship between the antidepression efficacy of traditional Chinese medicine formulation Chaihu Shugan San. Chin J Nat Med 2010; 8: 353-361.
[9] Decai F, Shizhong Y, Xiangfu S and Yi X. Effect of Chaihu Shugan San in treating liver fibrosis. J Gerontol 2007; 27: 1146-1148.
[10] Feng MP, De XZ and Jiang RH. Effect of Chaihu Shugan San on B ultrasonic and the index of liver fibrosis in nonalcoholic fatty liver disease patient. Journal of Sichuan of Traditional Chinese Medicine 2009; 27: 66-67.
[11] Chiang LC, Ng LT, Liu LT, Shieh DE and Lin CC. Cytotoxicity and anti-hepatitis B virus activities of saikosaponins from Bupleurum species. Planta Med 2003; 69: 705-709.
[12] Meng MJ, Yang QH, Wang Q, Chen XM, Wang FZ, Wang YP, Tang HL, Cheng SB, Ling JS and Wen CY. Effects of different therapeutic methods and typical recipes on activation of ERK1/2 in Kupffer cells of rats with fatty liver. Chinese Journal of Pathophysiology 2007; 23: 1551-1555.
[13] Yang QH, Hu SP, Zhang YP, Ping HH, Yang HW, Chen TY and Liu HT. Effects of different therapeutic methods and typical recipes of Chinese medicine on activation of c-Jun N-terminal Kinase in Kupffer cells of rats with fatty liver disease. Chinese Journal of Integrative Medicine 2012; 18: 769-774.
[14] Fuling C. Observation on therapeutic effect of Chaihu Shugan San in treating nonalcoholic fatty liver disease. Guangming Journal of Chinese Medicine 2015; 20: 746-749.
[15] Zehui C. Clinical investigation of Shuganjiangzhi Fang in the treatment of nonalcohol. China Modern Doctor 2011; 48: 83-84.
[16] Zhihong D. Clinical observation on 46 cases of fatty liver treated with Chaihu Shugan San. Hunan Journal of Traditional Chinese Medicine 2009; 25: 14-15.
[17] Qixiaoxiao F. Clinical observation on treatment of non - alcoholic fatty liver stagnation syndrome with integrated traditional Chinese and Western medicine. Journal of Practical Traditional Chinese Medicine 2016; 32: 344-345.
[18] Yuanming Z and Wenli F. Treatment of 38 cases of fatty liver with Chaihu Shugan powder. Strait Pharm J 2008; 20: 82-83.
[19] Lin Z. Clinical observation of Chaihu Shugan powder in treating non-alcoholic fatty liver. Chinese Medicine Modern Distance Education of China 2013; 11: 90-91.
[20] Hongbing Z. Clinical observation on treatment of 65 cases of fatty liver with Chaihu Shugan powder. China Modern Doctor 2010; 48: 45-46.
[21] Lei X and Bingjiu L. Analysis of clinical effect by soothing liver and strengthening spleen combined with dissolving turbidity and removing stasis for non-alcoholic fatty liver disease patients. Chin J M od Drug Appl 2016; 10: 12-13.
[22] Wang X, Wang D and Wan Z. Clinical investigation of Chaihushugansan on nonalcoholic fatty liver. Hebei J TCM 2010; 32: 1129-1131.
[23] Xiaosheng T. Efficacy of combined treatment of traditional Chinese medicine and western medicine for non-alcoholic fatty Hver disease. Chinese Journal of Primary Medicine and Pharmacy 2011; 18: 1299-1301.
[24] Fengman P and Jiangrong H. Clinical observation on 82 cases of non-alcoholic fatty liver treated with Chaihu Shugan powder. Lishizhen Medicine and Materia Medica Research 2009; 20: 2010-2011.
[25] Jinghe L. Self-made Chaihu Shugan Jiangzhi soup decoction for treating 50 cases of fatty liver. China's Naturopathy 2012; 20: 30-31.
[26] Cohen JC, Horton JD and Hobbs HH. Human fatty liver disease: old questions and new insights. Science 2011; 332: 1519-1523.
[27] Fuchs M. Non-alcoholic fatty liver disease: the bile acid-activated farnesoid x receptor as an emerging treatment target. J Lipids 2012; 2012: 934396.
[28] Xiaohong Z, Shuyan F and Lei Y. Therapeutic effect of Chaihu Shugan powder on liver fibrosis. Acta Chinese Medicine and Pharmacology 2003; 3: 38-39.
[29] Xuemei Y. Research progress on the pharmacological effects of Ligustrazine. Chin J Biochem Pharm 2010; 31: 215-216.
[30] Youqing T and Ping D. Research progress and developmental prospect on volatile oils from cyperus rotundus. China Pharmaceuticals 2010; 19: 1-2.
[31] Abenavoli L and Beaugrand M. Transient elastography in non-alcoholic fatty liver disease. Ann Hepatol 2012; 11: 172-178.