

Meta-Analysis

## Meta-analysis of traditional herbal medicine in the treatment of nonalcoholic fatty liver disease

H. Peng<sup>1#</sup>, Y. He<sup>1#</sup>, G. Zheng<sup>1\*</sup>, W. Zhang<sup>2</sup>, Z. Yao<sup>1</sup>, W. Xie<sup>3</sup>

<sup>1</sup>Department of Traditional Chinese Medicine, Changhai Hospital, the Second Military Medical University, Shanghai, China

<sup>2</sup>Department of Ophthalmology, Changhai Hospital, the Second Military Medical University, Shanghai, China

<sup>3</sup>Jiujiang Hospital of Traditional Chinese Medicine, Jiujiang, China

**Abstract:** Traditional Chinese herbal medicine (TCM) has been found effective for nonalcoholic fatty liver disease (NAFLD) based on its unique theory system and substantial herb remedies. The aim of this meta-analysis was to evaluate the efficiency and safety of one of the TCM, Danshen in the treatment of NAFLD. Seven English and Chinese databases were searched from inceptions to December 2015. RCTs which compared Danshen with placebo in adult patients with NAFLD were included. Totally, 8 RCTs with a total of 800 patients were identified. The results showed that compared with placebo, Danshen had increased total effectiveness rate, lower level of ALT, AST, TC and TG, LDL and higher level of liver/spleen computed tomography ratio. The present meta-analysis suggests that Danshen may have positive effects on nonalcoholic fatty liver disease. Future multicenter large-sample randomized clinical trials are still needed to confirm the efficacy and safety of our study.

**Key words:** Non-alcoholic fatty liver disease, Traditional Chinese herbal medicine, Danshen, Meta-analysis.

### Introduction

Currently, non-alcoholic fatty liver disease (NAFLD) is defined as the accumulation of excessive fat in the liver in the absence of excessive drinking of alcohol and is recognized as the most common form of liver disease worldwide (1). NAFLD encompasses a range of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis, fibrosis, cirrhosis, hepatocellular carcinoma and is rapidly becoming the leading cause of liver failure and need for transplantation (2). It has a global estimated median prevalence of 20%, ranging from 6.3% to 33% depending on the population (3). The prevalence of NAFLD is increasing as the prevalence of metabolic syndrome including obesity, type 2 diabetes mellitus in Western and, more recently, in Asian populations (4).

The treatments of NAFLD generally include lifestyle interventions, such as weight reduction, exercise, and dietary changes. However, the curative effects of these non-pharmacotherapies are minimal, owing to high rates of non-compliance. In addition, pharmaceutical therapies, including statins, antihypertensive agents, and  $\beta$ -blockers, are also known to ameliorate NAFLD. Several of these interventions have been reported with improvement in liver enzyme activity and liver histology. However, western drugs are generally associated with adverse effects, including manifestations due to hepatotoxicity, increased risk of death, heart failure, or pro-arrhythmic potential.

Traditional Chinese medicine (TCM) has been used to treat liver disease in China for many years. Some systematic reviews and meta-analyses (5, 6) have been conducted to synthesize the effectiveness of TCM in NAFLD. As one of the best-known Chinese traditional herbs, Danshen, also known as *Salvia miltiorrhiza* Bunge, is a member of the genus *Salvia* of the mint family, Lamiaceae, which has been clinically used for

more than 2000 years. In recent years, some randomized controlled trials (RCTs) have been designed to investigate the efficacy and safety of Danshen in the treatment of NAFLD. However, the evidence is still controversial and there lacked a systematical review about the issue.

Therefore, we conducted a comprehensive systematic review and meta-analysis of all available RCTs to assess the clinical value of Danshen for patients with NAFLD.

### Materials and Methods

This meta-analysis of randomized controlled trials (RCTs) was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

### Search strategy

Comprehensive literature search was performed in databases of Pubmed, EMBASE, The Cochrane Library, Web of Science, Chinese Biomedical Databases, Wanfang database and VIP database from their inceptions to December 2015. The search term that combined free textwords and MeSH terms was as follows: "Danshen", "*Salvia miltiorrhiza*", "non alcoholic fatty liver disease", "non alcoholic steatohepatitis" and "randomized controlled trial". The search was independently performed by two reviewers and no restriction were set for language or date. In addition, the reference lists of included studies and relevant reviews were also ma-

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\* **Corresponding author:** Guoyin Zheng, Department of Traditional Chinese Medicine, Changhai Hospital, the Second Military Medical University, 880, Xiangyin Road, Shanghai, China. Email: zhengguoyinshh@sina.com

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nually checked for possible studies.

### Eligibility criteria

Two authors screened for included studies independently and disagreements were solved after discussion with a third author. Studies were considered eligible for inclusion if they met the following criteria: (1) the patients have a definite diagnosis of NAFLD; (2) the study is a randomized controlled trial; (3) the patients of included study are divided into two groups, and the intervention group is Danshen, whereas the control group is placebo; (4) the patients included in the studies are adults aged more than 18.

Studies were excluded if they: (1) were animal studies or experimental studies; (2) focused on subjects aged below 18 or above 70 years; (3) were duplicated publications.

The outcomes evaluated in our meta-analysis were as follows: (1) total effectiveness rate; (2) serum indices of liver function: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin (TBil); (3) serum indices of blood lipid: total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL); (4) Liver/spleen computed tomography ratio; (5) adverse events.

### Data Extraction

The following data were collected independently by two authors: first author's name, year of publication, language, study design, the number of patients in each group, age, intervention medicines and reported outcomes. If the data were missing or conflicting, we tried to get further information by contacting the authors. The available information extracted was recorded using a data collection form and saved into electronic databases.

### Quality assessment

Two researchers assessed the methodological quality of included studies according to the Cochrane Handbook for Systematic Reviews of Interventions 5.1 for assessing risk of bias (7). The items assessed were as following: (1) details of randomized method, (2) allocation concealment, (3) blinding, (4) incomplete outcome data (5) selective outcome reporting and (6) other sources of bias.

### Statistical analysis

All statistical analysis and meta-analysis was performed with RevMan 5.3 software (Cochrane Collaboration, Oxford, UK). The mean difference (MD) with 95 % confidence interval (CI) between Danshen and control groups was pooled for continuous variable data (AST, ALT and TBil, TC, TG, HDL and LDL). The odds ratio (OR) with 95 % CI between the Danshen and control groups was calculated for dichotomous data (total effectiveness rate and adverse events). Assessment for heterogeneity among studies was calculated using the  $I^2$  statistic. When  $I^2 < 50\%$ , low heterogeneity is assumed. When  $I^2 > 50\%$ , heterogeneity is thought to exist, a random-effects meta-analysis weights the studies relatively more equally than a fixed-effect analysis. A probability of  $p < 0.05$  was considered to be statistically significant.

## Results

### Search results

The systematic literature search identified a total of 224 citations as potential eligible for inclusion into this meta-analysis. 43 studies were excluded because of duplicate studies and 18 studies were selected as being potentially by screening the title, reading the abstract. After reading the full-text articles, 10 of them were also excluded. Finally, 8 RCTs (8-15) that met our inclusion criteria were included in the present system review. No published trials were identified from the reference lists of included studies or review. The flowchart of studies included in the system review is shown in Figure 1.

### Characteristics of included studies

8 RCTs involving 800 patients were eligible for inclusion, with individual sample size ranging from 60 to 200 patients. About 416 patients were treated with Danshen and other 384 patients without Danshen. All included were conducted in China and published in Chinese. The mean duration of treatment reported varied from 14 to 90 days. The studies were published between 2008 and 2014. Demographic data at baseline were similar in the two treatment groups. The main characteristics of the 8 RCTs included in the meta-analysis are presented in Table 1.

### Quality assessment

Of the 8 studies, none of included studies described the detail of randomization procedures. No studies blinded the patients or the assessors. In addition, none of the studies documented concealment of randomization. Descriptions of patient dropouts and withdrawals did not appear in included reports. The details of results of methodological quality of the studies were listed in Figure 2.

### Meta-analysis

#### Total effectiveness rate

The data of total effectiveness rate was reported in

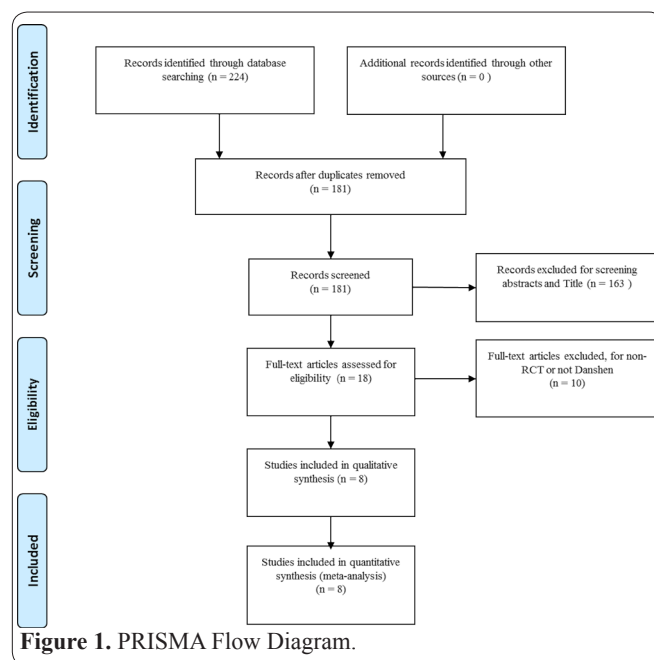


Figure 1. PRISMA Flow Diagram.

**Table 1.** Characteristics of included randomized controlled trials.

Included studies	Language	No. of Patients		Sex (male/female)		Mean Age (Years)		TCM treatment	Treatment Duration (days)	Response				
		Danshen group	Control group	Danshen group	Control group	Danshen group	Control group			Effectiveness rate	Liver function	blood lipids	liver/spleen CT ratio	adverse events
An YG 2011	Chinese	75	75	50/25	53/22	45	47	Danshen	90	Reported	improved	improved	improved	Reported
Chen LP 2014	Chinese	30	30	18/12	20/10	37.6	38.5	Danshen	28	NA	improved	improved	improved	Reported
Li XY 2008	Chinese	48	20	43/5	17/3	35.5	32.2	Danshen	28	NA	improved	improved	NA	Reported
Liu SJ 2014	Chinese	100	100	61/39	65/35	43.5	45.5	Danshen	30	Reported	improved	improved	NA	Reported
Liu ZH 2011	Chinese	50	50	28/22	30/20	42.2	41.1	Danshen	15	Reported	improved	improved	NA	Reported
Ma HX 2014	Chinese	38	38	NA	NA	NA	NA	Danshen	14	NA	improved	improved	NA	Reported
Zhang JH 2011	Chinese	45	41	26/19	22/19	43.1	42.8	Danshen	90	Reported	improved	improved	NA	Reported
Zou Y 2009	Chinese	30	30	20/10	18/12	38.5	39	Danshen	30	NA	improved	improved	NA	Reported

TCM, traditional Chinese medicine; NA, not reported.

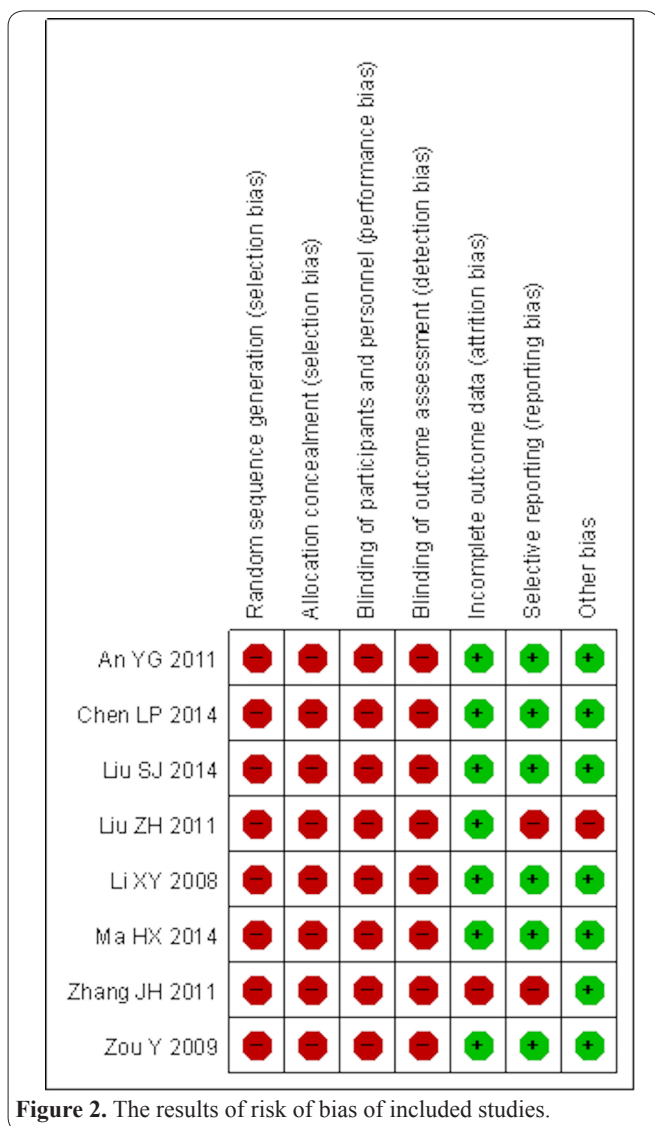


Figure 2. The results of risk of bias of included studies.

four studies (8, 11, 13, 15) with a total of 536 patients (270 patients in the Danshen group and 266 patients in the control group). There was no evidence of significant heterogeneity when data for total effectiveness rate were pooled ( $I^2 = 0\%$ ), therefore, a fix-effect model was used (Figure 3A). The results of meta-analysis demonstrated that patients with Danshen treatment had significantly higher effectiveness rate than patients in the control group [OR = 4.35, 95%CI (2.68, 7.06),  $p < 0.05$ ].

**Liver function outcomes**

**ALT level.** Six RCTs (8, 10-13, 15) involving 698 patients reported the result of ALT at prior treatment and post-treatment. No significant difference was found in the level of ALT at prior treatment between Danshen and control groups [MD = 1.01, 95%CI (-1.65, 3.67),  $p = 0.46$ ]. There was significant heterogeneity when data for ALT level at post-treatment was pooled ( $I^2 = 93\%$ ), therefore, a random-effect model was used. The results of meta-analysis demonstrated that patients with Danshen treatment had significantly lower level of ALT at post-treatment than patients in the control group [MD = -7.63, 95%CI (-14.82, -0.44),  $p < 0.05$ ] (Figure 3B).

**AST level.** Four studies (8, 11, 13, 15) with a total of 536 patients reported the result of AST at prior treatment and post-treatment. No significant difference was found in the level of AST at prior treatment between Danshen and control groups [MD = 0.31, 95%CI (-3.04, 3.67),

$p = 0.86$ ]. There was significant heterogeneity when data for AST level at post-treatment was pooled ( $I^2 = 80\%$ ), therefore, a random-effect model was used. The results of meta-analysis showed that patients with Danshen treatment had significantly lower level of AST at post-treatment than patients in the control group [MD = -14.31, 95%CI (-18.83, -9.79),  $p < 0.05$ ] (Figure 3C).

**TBil level.** The result of TBil was available in three RCTs (8, 9, 12) involving 294 patients (161 patients in the Danshen group and 133 patients in the control group). No significant difference was found in the level of TBil at prior treatment between Danshen and control groups [MD = 0.03, 95%CI (-2.48, 2.55),  $p = 0.98$ ]. There was no evidence of significant heterogeneity when data for TBil level at post-treatment was pooled ( $I^2 = 0\%$ ), therefore, a fix-effect model was used. The results of meta-analysis revealed that there was no significant differences in TBil at post-treatment between Danshen and control group [MD = -0.04, 95%CI (-1.60, 1.53),  $p = 0.96$ ] (Figure 3D).

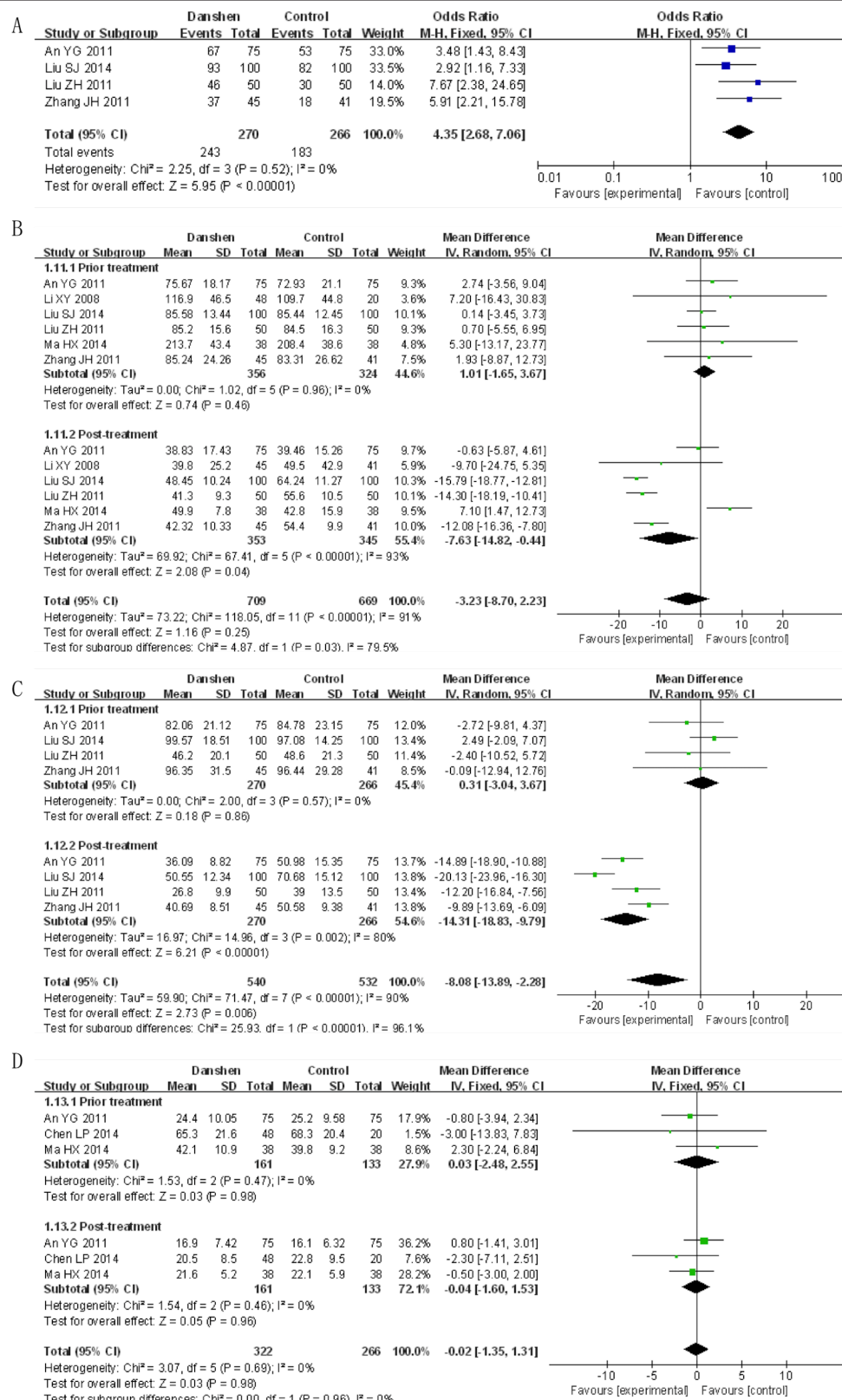
**Blood lipid**

**TG.** Seven studies (8-13, 15) with a total of 740 patients provided the data of TG level at prior treatment and post-treatment. No significant difference was found in the level of TG at prior treatment between Danshen and control groups [MD = 0.01, 95%CI (-0.19, 0.22),  $p = 0.89$ ]. There was significant heterogeneity when data for TBil level at post-treatment was pooled ( $I^2 = 97\%$ ), therefore, a random-effect model was used. The results of meta-analysis demonstrated that Danshen treatment had significantly lower level of TG at post-treatment than patients in the control group [MD = -0.52, 95%CI (-0.97, -0.06),  $p < 0.05$ ] (Figure 4A).

**TC.** Seven studies (8-13, 15) with a total of 740 patients provided the data of TC level at prior treatment and post-treatment. No significant difference was found in the level of TC at prior treatment between Danshen and control groups [MD = 0.06, 95%CI (-0.07, 0.19),  $p = 0.40$ ]. There was significant heterogeneity when data for TBil level at post-treatment was pooled ( $I^2 = 75\%$ ), therefore, a random-effect model was used. The results of meta-analysis revealed that Danshen treatment had significantly lower level of TC at post-treatment than patients in the control group [MD = -0.97, 95%CI (-1.25, -0.68),  $p < 0.05$ ] (Figure 4B).

**LDL.** Three RCTs (8, 10, 15) involving 418 patients reported the outcome of LDL level at prior treatment and post-treatment. No significant difference was found in the level of LDL at prior treatment between Danshen and control groups [MD = -0.04, 95%CI (-0.19, 0.12),  $p = 0.63$ ]. There was significant heterogeneity when data for LDL level at post-treatment was pooled ( $I^2 = 77\%$ ), therefore, a random-effect model was used. The results of meta-analysis showed that Danshen treatment had significantly lower level of LDL at post-treatment than patients in the control group [MD = -0.50, 95%CI (-0.88, -0.12),  $p < 0.05$ ] (Figure 4C).

**HDL.** Three RCTs (8, 10, 15) with a total of 418 patients provided the results of HDL level at prior treatment and post-treatment. No significant difference was found in the level of HDL at prior treatment between Danshen and control groups [MD = 0.04, 95%CI (-0.01, 0.09),  $p = 0.14$ ]. There was significant heterogeneity when data



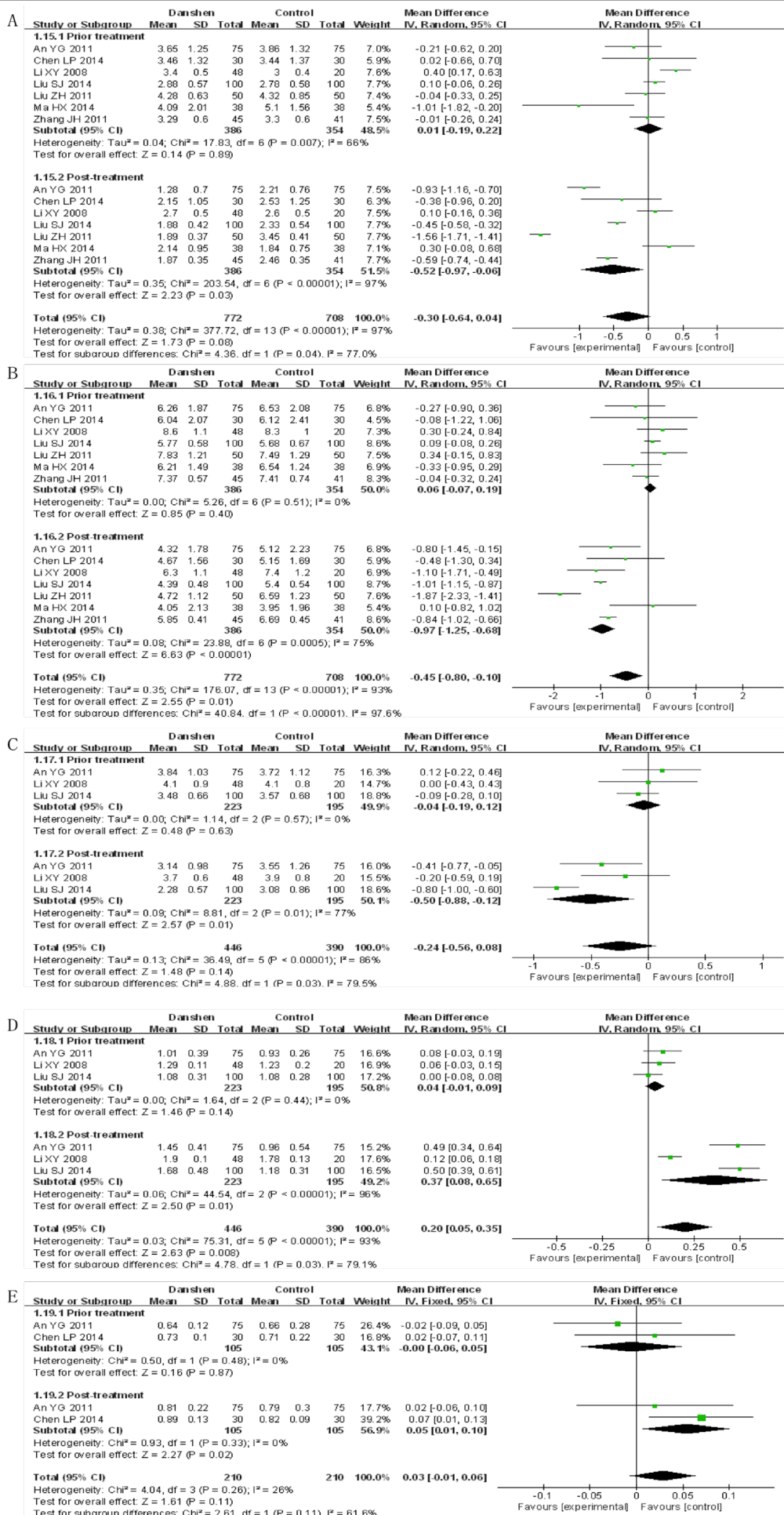
**Figure 3.** Forest plot analysis of (A) total effectiveness rate, and (B) ALT level (prior- and post-treatment, respectively), (C) AST level (prior- and post-treatment, respectively), and (D) TBil level (prior- and post-treatment, respectively).

for HDL level at post-treatment was pooled ( $I^2 = 96\%$ ), therefore, a random-effect model was used. The results of meta-analysis demonstrated that Danshen treatment had significantly higher level of HDL at post-treatment than patients in the control group [MD = 0.37, 95%CI (0.08, 0.65),  $p < 0.05$ ] (Figure 4D).

### Liver/spleen computed tomography ratio

The outcome of liver/spleen computed tomography ratio was presented in two studies (8, 9) at prior treatment and post-treatment. No significant difference was

found in liver/spleen computed tomography ratio at prior treatment between Danshen and control groups [MD = 0.00, 95%CI (-0.06, 0.05),  $p = 0.87$ ]. There was no evidence of significant heterogeneity when data for liver/spleen computed tomography ratio at post-treatment was pooled ( $I^2 = 0\%$ ), therefore, a fix-effect model was used. The results of meta-analysis showed that Danshen treatment had significantly higher level of liver/spleen computed tomography ratio at post-treatment than patients in the control group [MD = 0.05, 95%CI (0.01, 0.10),  $p < 0.05$ ] (Figure 4E).



**Figure 3.** Forest plot analysis of (A) TG level (prior- and post-treatment, respectively), (B) TC level (prior- and post-treatment, respectively), (C) LDL level (prior- and post-treatment, respectively), (D) HDL level (prior- and post-treatment, respectively), and (E) Liver/spleen computed tomography ratio (prior- and post-treatment, respectively).

## Adverse events

All studies reported that no adverse events were found in Danshen or control group.

## Discussion

### Summary of findings

We included 8 randomised clinical trials, which involved 800 patients with NAFLD. The most important findings of this meta-analysis were that compared with control group, Danshen group had significant increased total effectiveness rate, decreased level of ALT, AST, TBil, TC, TG and LDL, and higher level of HDL and liver/spleen computed tomography ratio.

During the past two decades, although some cellular processes of NAFLD have been extensively studied, the pathogenesis of this common liver disease has not been better understood. Until now, there has not been a specific drug or effective treatment for NAFLD (16). In recent decades, herbal medicines with the multi-targeted and less toxic features have attracted more attention in the prevention of NAFLD, including Lycii fructus, Garlic and Danshen (17).

The Chinese herb Danshen is the dried root of *Salvia miltiorrhiza*, which is one of the most versatile Chinese herbal drugs that have been used for hundreds of years in the treatment of numerous ailments in Chinese clinical practice. Danshen was first recorded in Shennong's Herbal, which was an ancient traditional Chinese medicine (TCM) code written in the period of the Qin and Han dynasties (221 BC to 220 AD) (18). In the past decade, Danshen has been described in the Chinese Pharmacopoeia since 1963 and is reported to promote blood circulation, end blood stagnation (Huoxue Huayu), nourish the blood, and tranquilize the mind (Yangxue Anshen) (18).

Meta-analysis from animal models have showed that compared with placebo, Danshen significantly decreased the level of ALT, AST after rat liver injury induced by carbon tetrachloride (19). Evidence from clinical studies also found that in patients with liver cirrhosis, Danshen increased the level of serum albumin, decreased the serum TBil, alanine transaminase, type III procollagen, hyaluronic acid, laminin, and type-IV collagen (20). Many clinical studies (8-15) have suggested that treatment with Danshen effectively prevents the development of fatty liver diseases, especially for NAFLD (5).

Total effectiveness rate was one of main outcomes in our meta-analysis. Four included studies all reported increased total effectiveness rate in Danshen group. Totally, the total effectiveness rate was 90% (243/270) in the Danshen group, which was higher than control group (69%, 183/266). Liver function outcomes and blood lipid were also the main results in our study. Chen *et al* (9) randomized 60 patients into Danshen group and control group, and revealed that Danshen group had significantly lower level of ALT and AST. Besides, in another RCT of 200 patients, Liu *et al* demonstrated that the level of ALT, AST, TC and LDL were significant decreased compared with control group (15). Regarding the adverse events, there was no reporting on adverse events in the included trials, and we cannot make to firm conclusions about the safety of Danshen.

### Strength and Limitation

Previously, some systematic reviews (5, 6, 16) have investigated the efficacy and safety of traditional Chinese herbal medicines for NAFLD, however, no study focused on the Danshen in the treatment of NAFLD. Our study is the first meta-analysis of randomized controlled trials to investigate the efficacy and safety of Danshen for NAFLD. Besides, our meta-analysis was reported under the guidance from the PRISMA statement.

But, it must be noted that this meta-analysis had some limitations. First, all identified 8 studies were conducted in China and none of them were published in English, which may cause language bias and location bias, but we could not exclude potential publication bias. Second, the studies included were of high risk of bias in terms of their methodology. They provided only limited descriptions of study randomization, allocation concealment, and baseline data. Third, no data with longer term follow-up was reported in the included trials. Therefore, the long-term effects and safety of the tested Danshen needs to be tested in the future. Finally, the data about toxicity of Danshen was limited in our meta-analysis. Studies needs to be further examined the safety of Danshen in well-designed clinical trials.

The present systematic review suggests that Danshen may have positive effects on nonalcoholic fatty liver disease, especially on reducing AST, ALT, TC, TG and improving total effectiveness rate. In the future, large sample, multicentred, high quality trials should be carried out to give more reliable evidence on the effects of Chinese herbal medicines on nonalcoholic fatty liver disease.

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