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# Clinical effectiveness of bicyclol for the treatment of nonalcoholic fatty liver diseases: a meta-analysis

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## ABSTACT

**Objective** Nonalcoholic fatty liver disease (NAFLD) is a global epidemic without admitted therapeutic agents in clinic. This meta-analysis aimed to assess the efficacy of the marketed hepatoprotective and anti-inflammatory drug bicyclol in the treatment of NAFLD.

**Design** Studies up to February 2020 were searched in Embase, Pubmed, Cochrane Library and several Chinese databases including CNKI, VIP and Wanfang database for randomized controlled trials (RCTs) using bicyclol to treat NAFLD. Supplemental researches were also hand-searched. The studies were screened by the corresponding inclusion and exclusion criteria and analyzed using Review Manager 5.3.

**Results** Twelve RCTs involving 1008 patients were included. No serious adverse events were reported in bicyclol-treated groups. The total effective rate of bicyclol treatment is significantly higher comparing with the control group. The efficacy of reducing serum AST, TBIL and TC in NAFLD patients by bicyclol treatment was significantly higher than in control group. When the substantial heterogeneity exists, subgroup analyses showed that bicyclol monotherapy significantly decreased the ALT level and was likely to decrease TG level. Correspondingly, bicyclol combination usage significantly decreased TG level and presented the tendency to decrease ALT.

**Conclusions** Bicyclol is safe and effective in the treatment of patients with NAFLD following improved liver function and reduced blood lipids. As liver dysfunction characterized by elevated aminotransferases and high blood lipids will substantially exacerbate NAFLD to advanced liver diseases, bicyclol might be an alternative available drug to be explored for NAFLD therapy in the future.


**KEYWORDS:** nonalcoholic fatty liver disease; meta-analysis; clinical practice; bicyclol

**Strengths and limitations of this study**

- This was the first systematic review to determine the clinical effectiveness and safety of bicyclol on nonalcoholic fatty liver diseases
- The significance of this study is to provide evidence-based clinical decision and alternative medicine for solving the current "no-cure" situation of NAFLD.
- The limitation of this meta-analysis was the low quality of the existing literatures.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common spectrum of liver diseases typically ranging from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH).[1] The benign and reversible NAFL merely characterized by excessive lipid droplets deposition in hepatocytes, while NASH is a more aggressive condition characterized by inflammatory infiltrates, visible cellular injury, and possible progression to or accompanied by fibrosis and cirrhosis.[2] NAFLD is closely related to the high incidence of metabolic syndrome, cardiovascular disease, type 2 diabetes, and advanced liver diseases.[1,3] Currently, the prevalence of NAFLD worldwide is up to 25%, with the highest prevalence of 32% in the Middle East and 31% in South America, and even the lowest prevalence in Africa was estimated to be 14%.[4] Worse still, the prevalence of NAFLD worldwide was thought to be on the rise. Though lifestyle changes targeted at weight loss through dietary interventions and exercise are the most effective treatment, patients tend to be lack of adherence to these important intervention targets.[5] Recently, only one dual PPAR-α/γ agonist saroglitazar magnesium has been approved for the treatment of NASH without cirrhosis in India.[6] However, numerous potential agents, such as farnesoid X receptor agonists, apoptosis signal-regulated kinase 1 inhibitors and C-C chemokine receptor type 2/5 inhibitors, had entered different phases in clinical trials but presented limited or even no benefits.[1,7,8] Therefore, new or complementary drugs for treating NAFLD are still urgently needed and this dilemma might persistent for a long time.

Bicyclol, a hepatoprotective and anti-inflammatory drug approved in China since 2004, was used to treat the elevation of aminotransferases caused by various chronic hepatitis mainly in Asian countries.[9] It was rather safe and suitable for long term (more than 6 months) oral administration.[9] Many preclinical animal experiments have proved its excellent therapeutic effect in chemical-, immunological-, fatty-, drug-induced liver injury, and bile duct ligation, dimethylnitrosamine, bovine serum albumin or carbon tetrachloride caused hepatic fibrosis.[9-11] Its detailed mechanisms involved in inhibiting hepatocyte apoptosis, stabilizing mitochondrial or hepatocyte membranes, scavenging free radicals, enhancing antioxidant gene expression and reducing lipid peroxides.[10,12] Although relevant clinical and

preclinical cases, especially in China, have been reported for its potential therapeutic role in NAFLD, [13,14] the effectiveness of bicyclol for NAFLD has not been precisely demonstrated due to insufficient sample size and low quality of literature researches.

Given the urgent need for drugs and the reported application of bicyclol in NAFLD, this meta-analysis evaluated its clinical therapeutic role in the treatment of patients with NAFLD to provide evidence-based clinical decision and alternative medicine.

## METHODS

The data used in this meta-analysis derived from previously published multicenter clinical researches. The study protocol was confirmed by all authors before data collection. We used analytical methods recommended in the Cochrane Handbook for Systematic Reviews of Interventions[15] and reported this study following the Preferred Reporting Items for Systematic Reviews and meta-Analyses (PRISMA) checklist.[16]

### Search Strategy

Studies up to February 2020 were searched in Pubmed, Embase, Cochrane Library and Chinese databases, including China National Knowledge Infrastructure database (CNKI), VIP-Chinese scientific and technological journal database, Wanfang digital periodical full-text database. Search terms were ((("Non alcoholic Fatty Liver Disease " OR " NAFLD" OR "nonalcoholic fatty liver" OR "non-alcoholic fatty liver" OR " Nonalcoholic Steatohepatitis" OR " Nonalcoholic Steatohepatitides")) AND ("bicyclol" OR "4,4'-bi-(1,3-benzodioxole)-5-carboxylic acid, 5'-(hydroxymethyl)-7,7'-dimethoxy-, methyl ester " OR "6-methoxycarbonyl-6'-hydroxymethyl-2,3,2',3'-bis(methylenedioxy)-4,4'-dimethoxybiphenyl") without other restrictions. Additional studies were hand-searched in Google Scholar and reference lists of relevant articles.

### Inclusion and exclusion criteria

Inclusion criteria fulfilled the following criteria: 1) being randomized controlled trial (RCT); 2) male and female patients were diagnosed as NAFLD with or without type 2 diabetes mellitus according to their corresponding guidelines; 3) the average baseline alanine

transaminase (ALT) level should be more than 90 U/L (2~3 times the upper limit of normal values[17]), while triglyceride (TG) level should be between 2.5 and 5 mmol/L; 4) being published English or Chinese articles. Exclusion criteria were 1) non-clinical studies, non-randomized controlled trials; 2) drug-, viral-, alcohol-, autoimmune-, primary biliary cholangitis, liver decompensation-, or malignancy- or genetic- caused liver injury; 3) studies enrolling fewer than 20 subjects in each group, or treatment time of the studies less than 4 weeks; 4) studies without enough experimental data, such as case reports, reviews, conference abstracts, or biochemical indicator missing.

**Intervention Measures**

Bicyclol monotherapy group (experimental group) was versus lifestyle intervention (LSI) or other drug monotherapy group (control group). Bicyclol combined with other medical treatment (experimental group) was versus the corresponding medicine group (control group). Other potential factors, such as lifestyle intervention had to be consistent between the two groups.

**Outcome Indicators**

Liver function indicators ALT, aspartate aminotransferases (AST), total bilirubin (TBIL) and blood lipid parameters TG, total cholesterol (TC) were recorded. Total effective rate, adverse events, and the anthropometric parameters body mass index (BMI) were also analyzed.

**Data Extraction and quality assessment**

The outcome indicators from all included articles were extracted from selected studies and checked by two authors to guarantee the accuracy of data. The quality of randomized controlled trials, which assigned as ‘high risk’, ‘low risk’ or ‘unclear risk’ to each item, was assessed independently by two reviewers according to the Cochrane risk of bias tool.[15] Any discrepancies were resolved through discussion.

**Data analysis**

Review Manager 5.3 was used to analyze the data.[18,19] Odds ratio (OR) and pooled mean difference (MD) with its corresponding 95% confidence interval (95% CI) were estimated for binary outcomes and continuous outcomes, respectively. Heterogeneities were evaluated using the chi-square ($\chi 2$) and $I^2$ statistics. When the outcome was homogeneous ($I^2 < 50\%$ and $P > 0.10$) and the fixed-effect model was used; when the outcome was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used. When the significant heterogeneity exists ($I^2 \geq 75\%$ and $P < 0.10$), subgroup analysis was conducted according to bicyclol monotherapy and combination usage, and if $I^2$ still over 75% in the subgroup, descriptive results were provided. The statistical significance between the experimental and control group was set at $P < 0.05$.

### Patient and public involvement

Patients and the public were not involved in this review.

## RESULTS

### Study selection

The whole flow chart of data selection was presented in Figure 1. Initially, 163 records were searched out and then 91 records were retained after duplicates exclusion. We then achieved 33 studies after screening by title and abstract, in which reviews, case reports, animal experiments, and studies with incongruent intervention measure and research orientation were excluded. After screening the full text, we excluded studies without appropriate samples, biochemical indicators, and baseline ALT and TG levels. One irrelevant study, which included alcoholic fatty liver, were also excluded. At last, 12 studies in Chinese were included.[20-31]

### Characteristics and quality evaluation of included studies

The characteristics of the included studies were clarified in Table 1. The publication year ranges from 2005 to 2017, and the sample size ranges from 50 to 152 (median is 81). The total sample size is 1008 with 523 patients in the treatment group and 485 counterparts in the

control group. The baselines of patients' outcome indicators are not different between the two groups.

The quality assessment of the included studies was shown in Figure 2, in which one study applied the random number table,[20] other studies used random but without detailed methods. All the studies did not report the blinding condition or the plan of allocation and concealment. Besides, all the studies had provided complete outcome data without other predictable bias.

**Bicyclol treatment is effective and safe for NAFLD patients**

The therapeutic effect and safety of bicyclol for NAFLD were firstly evaluated. As shown in Figure 3, the total effective rate and BMI indicated good homogeneity with $I^2$ of 42%, $P$ = 0.18 and 0%, $P$ = 0.75, respectively. 205 patients in 3 studies were reported for the total effective rate, while 456 patients in 4 studies were included in BMI analysis. The fixed-effects model revealed that the bicyclol group presented higher total effective rate (total effective rate: OR = 4.49; 95% CI 2.02 to 9.95; $P$ = 0.0002), but no significant effect for BMI (BMI: MD = −0.68; 95% CI −1.37 to 0.02; $P$ = 0.06) comparing with the control group. No gastrointestinal adverse events, such as nausea, vomiting, diarrhea, or headache were reported in the bicyclol treatment group in the included studies.

**Effect of bicyclol on liver function in NAFLD patients**

Serum ALT level was reported in 12 studies. These trials involved 1008 patients, with 523 patients in the treatment group and 485 patients in control group. There was a high statistical heterogeneity for ALT with $I^2$ of 95% and $P$ < 0.00001. Therefore, the subgroup analysis was conducted according to bicyclol monotherapy or combination treatment. The results revealed that bicyclol monotherapy significantly reduced ALT in NAFLD patients than other drugs or lifestyle interventions alone, which were analyzed by a random-effects model (ALT U/L: MD = −34.07; 95% CI −36.70 to −31.43; $P$ < 0.00001). However, there was significant heterogeneity in the bicyclol combination subgroup with $I^2$ of 95%, $P$ < 0.00001. Therefore, we just performed descriptive analysis where bicyclol was more likely to decrease the levels of ALT in all the seven studies when combination usage with other drugs (Figure 4A).

Serum AST was recorded in eight trials covered 658 patients, including 335 and 323 in treatment and control groups, respectively. There was a heterogeneity for AST with $I^2$ of 74% (Figure 4B). A random-effects model demonstrated that the reduction of AST in NAFLD patients treated by bicyclol was significant when monotherapy and combination usage (AST U/L: MD =−15.20; 95% CI −20.51 to −9.90; $P < 0.00001$).

Serum TBIL was detected in six trials, which involved 472 participants, with 255 and 217 patients in treatment and control groups, respectively (Figure 4C). There was an excellent homogeneity among the 6 studies with $I^2 = 0\%$ and $P = 0.60$, and the fixed-effect model demonstrated that bicyclol could significantly decrease the TBIL level in NAFLD patients (TBIL μmol/L: MD =−1.72; 95% CI −2.72 to −0.72; $P = 0.0008$).

**Effect of bicyclol on blood lipids in NAFLD patients**

Twelve studies reported the data of TG. These trials involved 1008 patients, with 523 patients in treatment groups and 485 patients in control groups. There was a high statistical heterogeneity for TG with $I^2$ of 90% and $P < 0.00001$, and thus the subgroup analysis was conducted. Bicyclol combination subgroup indicated well homogeneous with $I^2$ =0% and $P$ = 0.89, and it significantly decreased the TG level in NAFLD patients compared with other drug monotherapy, which was analyzed by a random-effects model (TG mmol/L: MD = −0.39; 95% CI −0.45 to −0.33; $P < 0.00001$). There was a substantial heterogeneity in the bicyclol monotherapy subgroup with $I^2$ of 95% and $P < 0.00001$. The descriptive analysis suggested that bicyclol monotherapy was more likely to decrease the levels of TG in all the five monotherapy studies (Figure 5A).

There were 11 studies that reported the data of TC. These trials involved 958 patients, with 498 and 460 patients respectively in the treatment and control group. There was a heterogeneity for TC with $I^2$ of 67% and $P = 0.0007$. A random-effects model demonstrated that the reduction of TC in NAFLD patients treated by bicyclol was significant (TC mmol/L: MD =−0.52; 95% CI −0.70 to −0.34; $P < 0.00001$) when monotherapy and combination usage (Figure 5B).

## DISCUSSION

Liver injury and inflammation were the main causes to deteriorate liver histology during NAFLD progression, and bicyclol was exactly an approved hepatoprotective and anti-inflammatory drug used for the treatment of liver injury in multiply hepatitis.[9] Though treating for NAFLD is its off-label use, the Chinese guidelines of prevention and treatment for nonalcoholic fatty liver disease updated in 2018 recommend that hepatoprotectants could be complementary treatment measures for NASH patients with elevated aminotransferases or liver injury.[32] A randomized, multicenter, vitamin E-controlled trial with 223 Chinese NAFLD patients also showed vitamin E at a dose of 300 mg/day for 24 weeks is not better than bicyclol plus metformin in the improvement of serum aminotransferase and hepatic histological changes.[14] In spite of this, systematic studies for the efficacy of bicyclol for NAFLD are still lacking.

With meta-analysis through 12 Chinese studies including 1008 patients, this review suggests that bicyclol, no matter monotherapy or combination with other drugs, has a positive effect on improving liver function (ALT, AST, TBIL) and blood lipids (TG, TC). Though bicyclol combination treatment for ALT and monotherapy for TG have considerable heterogeneity, each trial in these included studies showed excellent therapeutic effects, respectively. The total effective rate of bicyclol was also higher than that of the control group, though only three studies clarified this outcome. Clinically, bicyclol was recommended to oral administration for up to 6 months. In this meta-analysis, though adverse events, such as gastrointestinal intolerance or headache were sporadically reported in control group, no such mild discomforts were reported in bicyclol-treated group, which agreed with the extremely mild and rare incidence of adverse reactions demonstrated by long-term clinical practice.[9] Therefore, bicyclol was rather safe, well-tolerated, and more importantly, effective for NAFLD patients.

The pathogenesis of NAFLD is complex, including immune responses, oxidative stress, lipid peroxidation injury, abnormal fat metabolism, and environmental and genetic factors.[2] These multiply mechanisms also remind us that hepatoprotection therapy and the combination of multi-targeted drugs might represent a valid strategy to be used in the future. Correspondingly, the limited research data suggested the detailed mechanisms of bicyclol for

treating NAFLD were associated with ameliorating mitochondrial function, inhibition of oxidative stress, and lipid metabolism regulation.[13,33] However, explicit targets need to be further revealed especially in high fat and obesity related animal model, and we speculated the multiple targets related to immune, inflammation or lipid metabolism should be focused in the further.

Since NAFLD is tightly associated (over 76%) with type 2 diabetes mellitus (T2DM),[34] patients with or without T2DM were included in this review. Besides, the course of disease varies among these studies, and some studies did not report the patient's medical history, we thus limited the baseline ALT and TG level to ensure the consistency of the included patients as much as possible. We also defined the treatment duration as at least 4 weeks, for that NAFLD is a chronic disease, and bicyclol was suitable for long-term oral administration. Some limitations were accompanied by the analysis, which was reflected in the risk of bias assessment. For example, most studies included in this study did not provide specific methods of blind and random allocation concealments. In terms of the outcome indexes, most articles lack the blood glucose and insulin resistance index. Therefore, the results of the meta-analysis merely provide a reference based on the current evidence.

In conclusion, monotherapy or combination application of bicyclol can significantly improve liver function and blood lipids and is rather safe in patients with NAFLD. Therefore, bicyclol might be a new available choice for the treatment of NAFLD. However, the conclusions also need to be further verified by more well designed and implemented studies.

## Authors contribution

Conceptualization, analysis, writing original draft, visualization: Hu Li; Validation of data and formal analysis: Nan-Nan Liu; Supervision, validation and writing draft: Zong-Gen Peng; Approval of final manuscript: all authors.

## Conflicts of Interest

All authors declare that no conflicts of interest.

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**Competing interests**

None.

**Patient consent**

Not required.

**Data sharing statement**

All data are shown in the manuscript.

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**Table 1. Characteristics of included studies**

| Study | Sample size | | Intervention | | Dose of bicyclol | Duration | Outcomes |
|---|---|---|---|---|---|---|---|
| | Experimental | Control | Experimental | Control | | | |
| Liao 2011 | 30 | 30 | Bicyclol | Vitamin C | 50 mg, tid | 12 weeks | ①③④⑤⑦⑧ |
| Liang 2007 | 45 | 38 | Bicyclol | UDCA | 25~50 mg, tid | 24 weeks | ④⑥⑦⑧ |
| Zhu 2005 | 36 | 29 | Bicyclol | Silymarin | 25~50 mg, tid | 24 weeks | ①④⑥⑦⑧ |
| Yan 2017 | 30 | 30 | Bicyclol | DGEC | 50 mg, tid | 4 weeks | ④⑤⑦⑧ |
| Zhang 2012 | 60 | 60 | Bicyclol | LSI | 25 mg, tid | 24 weeks | ②④⑥⑦⑧ |
| Gao 2011 | 25 | 25 | Bicyclol + PPC | PPC | 25~50 mg, tid | 6 months | ③④⑤⑥⑦ |
| Ding 2009 | 42 | 30 | Bicyclol + PPC | PPC | 25~50 mg, tid | 6 months | ④⑤⑥⑦⑧ |
| He 2011 | 47 | 35 | Bicyclol + PPC | PPC | 25~50 mg, tid | 6 months | ③④⑥⑦⑧ |
| Li 2014 | 50 | 50 | Bicyclol + Metformin | Metformin | 25 mg, bid | 6 months | ②④⑤⑦⑧ |
| Zhang 2011 | 42 | 42 | Bicyclol + Metformin | Metformin | 25~50 mg, tid | 6 months | ②④⑤⑦⑧ |
| Sun 2015 | 76 | 76 | Bicyclol + Metformin | Metformin | 25 mg, bid | 6 months | ②④⑤⑦⑧ |
| Guan 2013 | 40 | 40 | Bicyclol + Silibinin | Silibinin | 50 mg, tid | 12 weeks | ①④⑤⑦⑧ |

①Total effective rate;②BMI;③Adverse Events;④ALT;⑤AST;⑥TBIL;⑦TG;⑧TC.

UDCA, ursodeoxycholic acid; DGEC, diammonium glycyrrhizinate enteric-coated capsule; LSI, lifestyle intervention; PPC, polyene phosphatidylcholine

## FIGURE LEGENDS

**FIGURE 1. Flow diagram of data selection process.**

**FIGURE 2. The quality assessment of the included studies.**

The quality of randomized controlled trials was assessed as 'high risk', 'low risk' or 'unclear risk' to each item independently by two reviewers according to the Cochrane risk of bias tool.

**FIGURE 3. The effect of bicyclol on total effective rate and BMI in NAFLD patients.**

Review Manager 5.3 was used to analyze the data. Odds ratio (OR) with its 95% confidence interval (95% CI) were estimated for total effective rate. Mean difference (MD) with its 95% CI were estimated for BMI. Heterogeneities were evaluated using the chi-square ($\chi 2$) and $I^2$ statistics. $I^2 < 50\%$ and $P > 0.10$ were deemed as homogeneous and the fixed-effect model was used. $P < 0.05$ were considered as statistically different between the experimental and control group.

**FIGURE 4. The effect of bicyclol on ALT, AST and TBIL in NAFLD patients.**

Review Manager 5.3 was used to analyze the data. Mean difference (MD) with its 95% CI were estimated for continuous outcomes. Heterogeneities were evaluated using the chi-square ($\chi 2$) and $I^2$ statistics. Among the studies, the ALT parameter was significantly heterogeneous ($I^2 \geq 75\%$ and $P < 0.10$) and subgroup analysis was conducted (A); the AST parameter was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used (B); the TBIL parameter was homogeneous ($I^2 < 50\%$ and $P > 0.10$) and the fixed-effect model was used (C). $P < 0.05$ were considered as statistically different between the experimental and control group.

**FIGURE 5. The effect of bicyclol on TG and TC in NAFLD patients.**

Review Manager 5.3 was used to analyze the data. Mean difference (MD) with its 95% CI were estimated for continuous outcomes. Heterogeneities were evaluated using the chi-square

($\chi$2) and $I^2$ statistics. Among the studies, the TG parameter was significantly heterogeneous ($I^2 \geq 75\%$ and $P < 0.10$) and subgroup analysis was conducted (A); the TC parameter was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used (B). $P < 0.05$ were considered as statistically different between the experimental and control group.

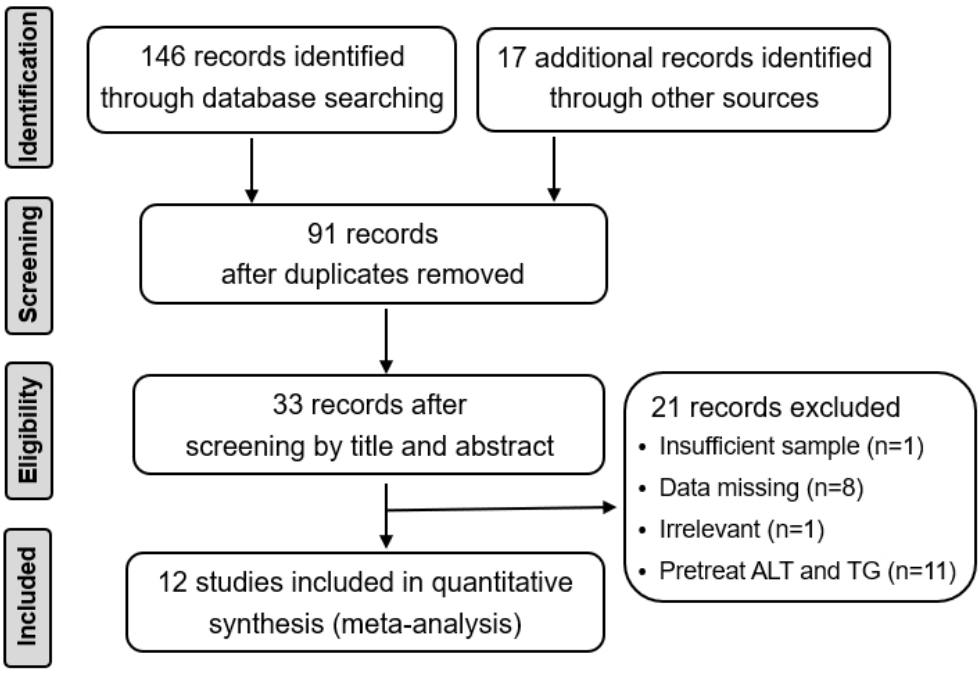


Figure 1

152x103mm (120 x 120 DPI)

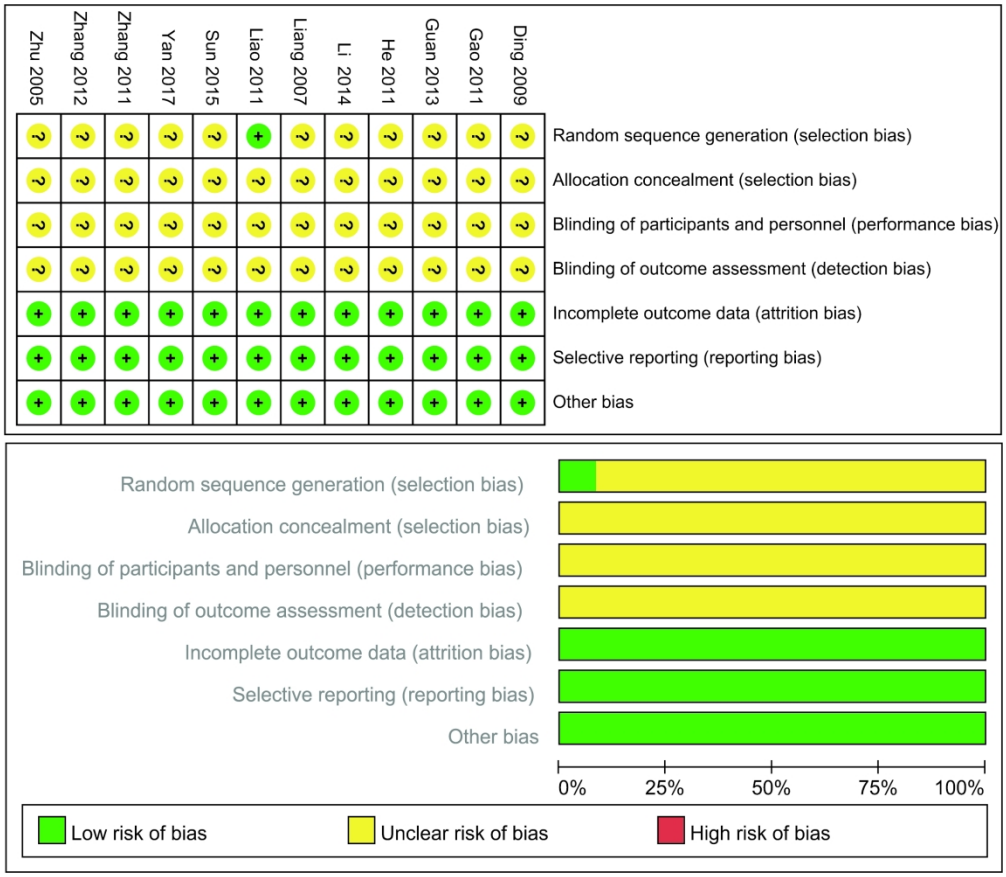


Figure 2

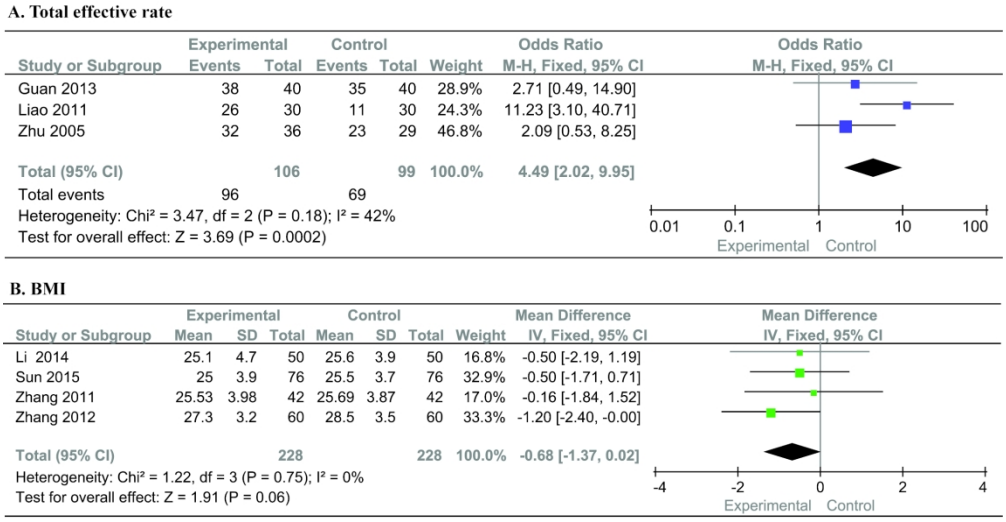


Figure 3

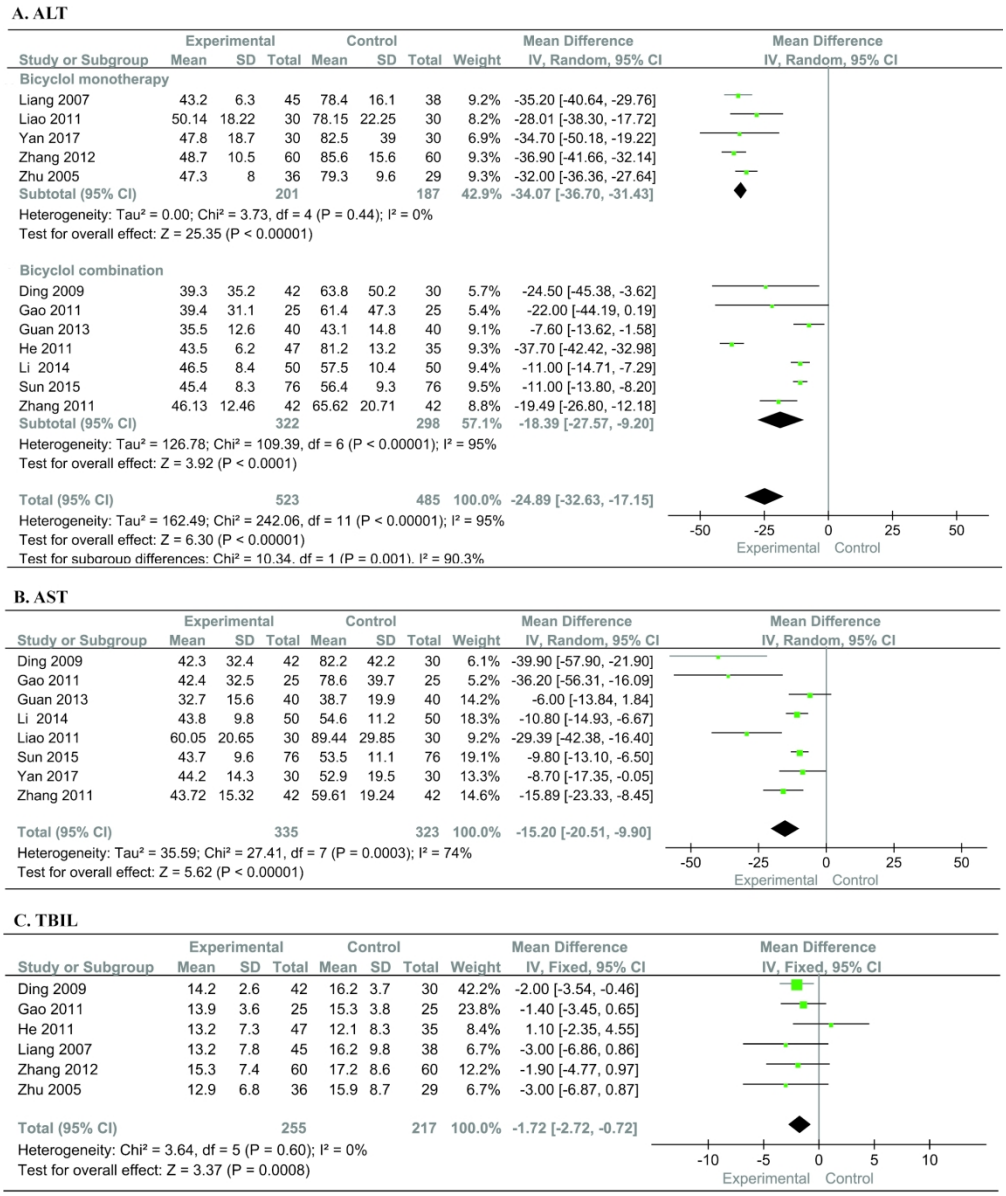


Figure 4

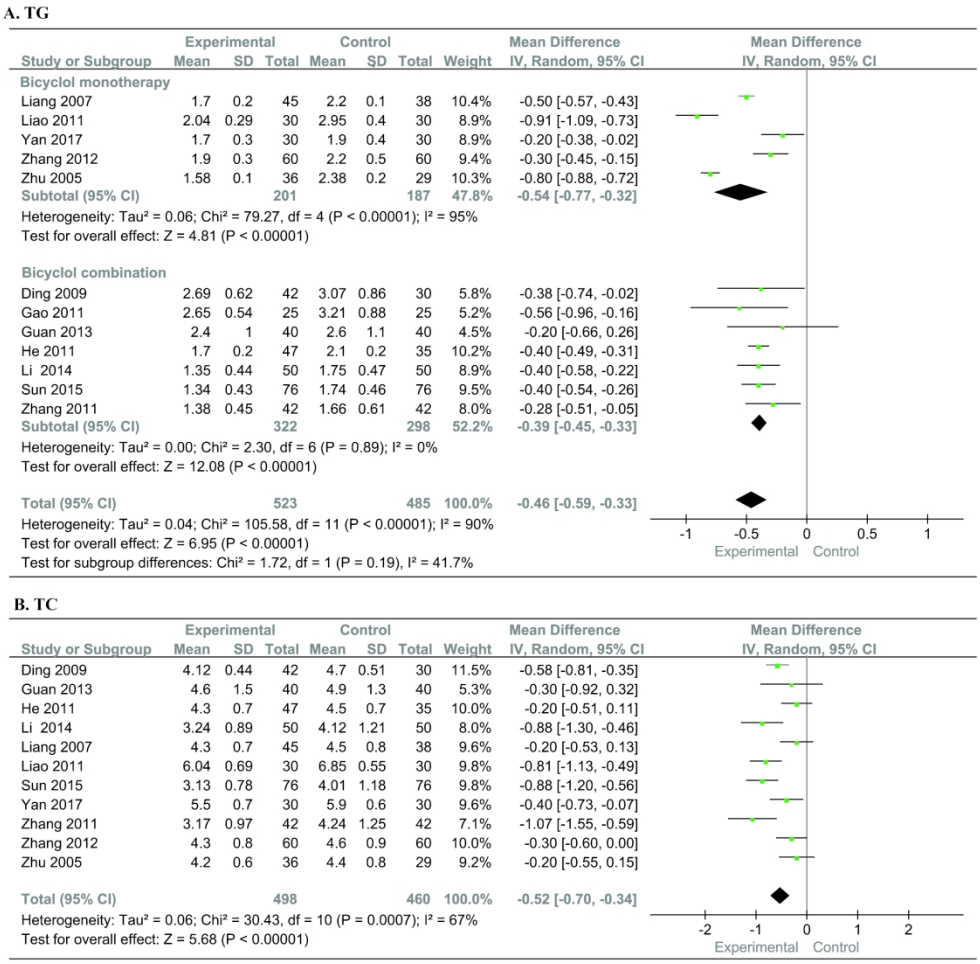


Figure 5

# PRISMA 2009 Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 4 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 5 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 5 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 5-6 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 5 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 5 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 5-6 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 6 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 6, Table1 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 6 |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 7 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 7 |

# PRISMA 2009 Checklist



| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 6, Figure 2 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 7 |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 7 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 7, Table1 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 7-8, Figure 2 |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 8-9 |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 8-9 |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 7-8, Figure 2 |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | 8-9 |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 10 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 11 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 11 |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 12 |

*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

BMJ Open

# The effect of bicyclol on blood biomarkers of NAFLD: a systematic review and meta-analysis



| | |
|---|---|
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| Secondary Subject Heading: | Complementary medicine, Gastroenterology and hepatology |
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| | |

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# The effect of bicyclol on blood biomarkers of NAFLD: a systematic review and meta-analysis

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## ABSTRACT

**Objective** Nonalcoholic fatty liver disease (NAFLD) is a global epidemic without admitted therapeutic agents in the clinic. This meta-analysis aimed to assess the efficacy of the marketed hepatoprotectant bicyclol for improving blood biomarkers in patients with NAFLD.

**Design** Studies up to August 2020 were searched in electronic databases for randomized controlled trials (RCTs) using bicyclol to treat NAFLD. The risk of bias, quality of evidence, and publication bias were evaluated. Blood biomarkers including alanine transaminase (ALT), aspartate aminotransferases (AST), total bilirubin (TBIL), triglyceride (TG) and total cholesterol (TC) were analyzed using Review Manager 5.3 software. Outcomes with significant heterogeneity ($I^2 \geq 75\%$) were divided into bicyclol monotherapy subgroup and combination treatment subgroup.

**Results** Twelve RCTs involving 1008 patients were finally included. No serious adverse events were reported in bicyclol-treated groups. The total effective rate of bicyclol intervention for the fatty liver was significantly higher than that of the control group. The decreased levels of AST (MD=−15.20; 95% CI −20.51 to −9.90; $I^2$ = 74%), TBIL (MD =−1.72; 95% CI −2.72 to −0.72; $I^2$ = 0%) and TC (MD=−0.52; 95% CI −0.70 to −0.34; $I^2$ =67%) by bicyclol was significantly higher than in control group. When the high heterogeneity existed ($I^2 \geq 75\%$), subgroup analyses were conducted and showed that ALT level (MD = −34.07; 95% CI −36.70 to −31.43; $I^2$ =0%) was significantly decreased merely in bicyclol monotherapy subgroup, while TG level (MD = −0.39; 95% CI −0.45 to −0.33; $I^2$ =0%) decreased in bicyclol combination therapy subgroup.

**Conclusions** The study presents the evidence of bicyclol monotherapy and/or combination therapy for improving liver function and blood lipid biomarkers in NAFLD patients. This preliminary study predicts that bicyclol might be an alternative available drug to be explored for NAFLD therapy in the future.


**KEYWORDS:** nonalcoholic fatty liver disease; meta-analysis; bicyclol; blood biomarkers

**Strengths and limitations of this study**

- This was the first systematic review to determine the effect of bicyclol on blood biomarkers of nonalcoholic fatty liver disease.
- The significance of this study is to provide preliminary evidence that bicyclol might be efficacious for NAFLD.
- The limitation of this meta-analysis was the low quality of the existing literature.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common spectrum of liver diseases typically ranging from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH).[1] The benign and reversible NAFL is merely characterized by excessive lipid droplets deposition in hepatocytes, while NASH is a more aggressive condition characterized by inflammatory infiltrates, visible cellular injury, and possible progression to or accompanied by fibrosis and cirrhosis.[2] NAFLD is closely related to the high incidence of metabolic syndrome, cardiovascular disease, type 2 diabetes, and advanced liver diseases.[1,3] Currently, the prevalence of NAFLD worldwide is up to 25%, with the highest prevalence of 32% in the Middle East and 31% in South America, and even the lowest prevalence in Africa was estimated to be 14%.[4] Worse still, the prevalence of NAFLD worldwide was thought to be on the rise.[5] There are no admitted therapeutic agents from international societies for treating NAFLD except for lifestyle changes.[6-8] However, patients tend to be lack of adherence to this important intervention style.[9] Recently, only one dual PPAR-α/γ agonist saroglitazar magnesium has been approved for the treatment of NASH without cirrhosis in India.[10] However, numerous potential agents, such as farnesoid X receptor agonists, apoptosis signal-regulated kinase 1 inhibitors, and C-C chemokine receptor type 2/5 inhibitors, have entered different phases in clinical trials but presented limited or even no benefits.[1,11,12] Therefore, new or complementary drugs for treating NAFLD are still urgently needed and this dilemma might be persistent for a long time.

Bicyclol, a hepatoprotective and anti-inflammatory drug approved in China since 2004, was used to treat the elevation of aminotransferases caused by various forms of chronic hepatitis mainly in Asian countries and partially in Russia, while it has not been approved in Australia and America.[13] It was rather safe and suitable for long term (more than 6 months) oral administration.[13] Many preclinical animal experiments have proven its therapeutic effect in chemical-, immunological-, fatty-, drug-induced liver injury, and bile duct ligation, dimethylnitrosamine, bovine serum albumin or carbon tetrachloride caused hepatic fibrosis.[13-15] The detailed mechanisms of bicyclol involved in inhibiting hepatocyte apoptosis, stabilizing mitochondrial or hepatocyte membranes, scavenging free radicals, enhancing

antioxidant gene expression, and reducing lipid peroxides.[14,16] Although liver histology and magnetic resonance imaging (MRI) have high accuracy for evaluating liver fat[17], liver function and blood lipid biomarkers, which mainly include alanine transaminase (ALT), aspartate aminotransferases (AST), total bilirubin (TBIL), triglyceride (TG) and total cholesterol (TC), are commonly used for evaluating the severity of NAFLD and the subsequent abnormal metabolism.[18,19] Relevant clinical and preclinical cases have been reported for the potential therapeutic role of bicyclol in NAFLD,[20,21] however, its effect on non-invasive blood biomarkers in NAFLD has not been precisely demonstrated due to insufficient sample size and low quality of literature researches. Hence, this meta-analysis aimed to evidence the effect of bicyclol for blood biomarkers in NAFLD patients through synthesize the clinical data using bicyclol monotherapy and/or combination to treat NAFLD, and preliminarily predict its clinical practice in the future.

## METHODS

The data used in this meta-analysis were derived from previously published clinical studies, all of which were derived from China. The study protocol was confirmed by all authors before data collection. Our protocol has been registered on the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY). The registration number was INPLASY202080017 (DOI number is 10.37766/inplasy2020.8.0017, https://inplasy.com/inplasy-2020-8-0017/). We used analytical methods recommended in the Cochrane Handbook for Systematic Reviews of Interventions[22] and reported this study following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.[23]

### Search Strategy

Studies up to August 2020 were searched in PubMed, Embase, Cochrane Library, and Chinese databases, including the China National Knowledge Infrastructure database (CNKI), VIP-Chinese scientific and technological journal database, Wanfang digital periodical full-text database. Search terms were (("Non alcoholic Fatty Liver Disease " OR " NAFLD" OR "nonalcoholic fatty liver" OR "non-alcoholic fatty liver" OR " Nonalcoholic

Steatohepatitis" OR " Nonalcoholic Steatohepatitides")) AND ("bicyclol" OR "4,4'-bi-(1,3-benzodioxole)-5-carboxylic acid, 5'-(hydroxymethyl)-7,7'-dimethoxy-, methyl ester " OR "6-methoxycarbonyl-6'-hydroxymethyl-2,3,2',3'-bis(methylenedioxy)-4,4'-dimethoxybiphenyl") without other restrictions (online supplementary Methods). Additional studies were hand-searched in Google Scholar and the reference lists of relevant articles.

**Inclusion and exclusion criteria**

The inclusion criteria were as follows: 1) being randomized controlled trial (RCT); 2) male and female patients were diagnosed as NAFLD with or without type 2 diabetes mellitus according to their corresponding guidelines; 3) the average baseline alanine transaminase (ALT) level should be more than 90 U/L (2~3 times the upper limit of normal values[24]), while triglyceride (TG) level should be between 2.5 and 5 mmol/L; and 4) being published English or Chinese articles. The exclusion criteria were 1) non-clinical studies, non-randomized controlled trials; 2) drug-, viral-, alcohol-, autoimmune-, primary biliary cholangitis, liver decompensation-, or malignancy- or genetic- caused liver injury; 3) studies enrolling fewer than 20 subjects in each group, or the treatment time of the study was less than 4 weeks; and 4) studies without enough experimental data, such as case reports, reviews, conference abstracts, or biochemical indicator missing.

**Intervention measures**

The bicyclol monotherapy group (experimental group) was versus lifestyle intervention (LSI) or other drug monotherapy group (control group). Bicyclol combined with other medical treatment (experimental group) was versus the corresponding medicine group (control group). Other potential factors, such as lifestyle intervention had to be consistent between the two groups.

**Outcome indicators**

Liver function indicators (ALT, AST, and TBIL) and blood lipid parameters (TG and TC) were recorded. Adverse events, the anthropometric parameters body mass index (BMI), and

the total effective rate of bicyclol intervention for the fatty liver which is based on blood biomarkers and B-model ultrasonography results, were also analyzed.

**Data extraction and quality assessment**

The outcome indicators from all included studies were independently extracted and checked by two authors (Hu Li and Nan-nan Liu) to guarantee the accuracy of the data. The quality of randomized controlled trials, which was assigned as 'high risk', 'low risk', or 'some concerns' to each item, was also assessed independently by two reviewers according to the revised Cochrane risk of bias tool.[25] Any discrepancies were resolved through discussion.

**Data analysis**

Review Manager 5.3 was used to analyze the data.[26,27] Odds ratio (OR) and pooled mean difference (MD) with the corresponding 95% confidence interval (95% CI) were estimated for binary outcomes and continuous outcomes, respectively. Heterogeneities were evaluated using the chi-square ($\chi 2$) and $I^2$ statistics.[26] When the outcome was homogeneous ($I^2 < 50\%$ and $P > 0.10$), the fixed-effect model was used; when the outcome was considered heterogeneous ($50\% \leq I^2 < 75\%$), the random-effect model was used. When significant heterogeneity existed ($I^2 \geq 75\%$), subgroup analysis was conducted according to bicyclol monotherapy and combination usage, and if $I^2$ in subgroup was still over 75% in the subgroup, descriptive results were provided without pooling estimates. The statistical significance between the experimental and control group was set at $P < 0.05$. Publication bias was assessed only for comparisons with at least five studies using the funnel plot and its symmetry evaluation was performed by Egger's regression tests through Stata 12.0 software. Significant publication bias was defined as $P < 0.10$.[28] Grading of the evidence for the key comparisons was carried out using the grades of recommendation, assessment, development and evaluation (GRADE) working group approach.[22]

**Patient and public involvement**

Patients and the public were not involved in this review.

## RESULTS

### Study selection

The whole flow chart of data selection is presented in Figure 1. Initially, 166 records were searched out, and 94 records were retained after duplicates exclusion. We then achieved 34 studies after screening by title and abstract, in which reviews, case reports, animal experiments, and studies with incongruent intervention measures and research orientation were excluded. After screening the full text, we excluded studies without appropriate samples, biochemical indicators, and baseline ALT and TG levels. One irrelevant study, which included alcoholic fatty liver, was also excluded. At last, 12 studies in Chinese were included.[29-40]

### Characteristics, quality evaluation and publication bias of the included studies

The characteristics of the included studies were clarified in Table 1. All the studies are from China with publication year ranging from 2005 to 2017, and the sample size ranging from 50 to 152 (median is 81). The total sample size is 1008 with 523 patients in the treatment group and 485 counterparts in the control group. The baselines of patient outcome indicators are not different between the two groups.

The quality assessment of the included studies was shown in Figure 2 according to the latest revised Cochrane risk-of-bias tool (online supplementary Table S1), in which one study applied the random number table,[29] other studies used randomization but did not provide detailed methods. All the studies did not report the blinding condition or the plan of allocation and concealment. Besides, all the studies had provided complete outcome data without other predictable bias.

The Egger's tests of funnel plots (online supplementary Figure S1) for primary outcomes showed that there was no significant publication bias among the blood biomarkers of ALT (8 studies, $P$=0.964), TC (11 studies, $P$=0.567), and TBIL (6 studies, $P$=0.485). However, ALT (12 studies, $P$=0.027) and TG (12 studies, $P$=0.004) showed significant publication bias, we speculated the heterogeneity in studies was the main determining factor and subgroup analysis was conducted.

**Effect and safety of bicyclol intervention for patients with NAFLD**

The therapeutic effect and safety of bicyclol for NAFLD were firstly evaluated. As shown in Figure 3, changes in BMI and the total effective rate for improving fatty liver indicated no heterogeneity with $I^2$ of 0%, $P$ = 0.75, and $I^2$ of 42%, $P$ = 0.18, respectively. A total of 205 patients in 3 studies were reported for the total effective rate, while 456 patients in 4 studies were included in BMI analysis. The fixed-effects model revealed that the bicyclol group presented a higher total effective rate (total effective rate: OR = 4.49; 95% CI 2.02 to 9.95; $P$ = 0.0002), but no significant effect for BMI (BMI: MD = −0.68; 95% CI −1.37 to 0.02; $P$ = 0.06) comparing with the control group. No gastrointestinal adverse events, such as nausea, vomiting, diarrhea, or headache were reported in the bicyclol treatment group in the included studies (Table 1).

**Effect of bicyclol on liver function biomarkers in patients with NAFLD**

Serum ALT levels were reported in 12 studies. These trials involved 1008 patients, with 523 patients in the treatment group and 485 patients in the control group. There was a high statistical heterogeneity for ALT with $I^2$ of 95% and $P$ < 0.00001. Therefore, we further divided these studies into bicyclol monotherapy subgroup and bicyclol combination treatment subgroup according to the drug regimen used in the experimental group. The results revealed that ALT in bicyclol monotherapy subgroup, which was analyzed by a random-effects model, was significantly decreased compared with the corresponding control group (ALT U/L: MD = −34.07; 95% CI −36.70 to −31.43; $P$ < 0.00001). However, there was significant heterogeneity in the bicyclol combination subgroup with $I^2$ of 95% and $P$ < 0.00001. Therefore, we performed a descriptive analysis in which bicyclol was more likely to decrease the levels of ALT in all the seven studies when combined with other drugs (Figure 4A).

Serum AST was recorded in eight trials covering 658 patients, including 335 and 323 in the treatment and control groups, respectively. There was a heterogeneity for AST with $I^2$ of 74% (Figure 4B). A random-effects model demonstrated that the reduction of AST in NAFLD

patients treated by bicyclol was significant when monotherapy and combination usage (AST U/L: MD =−15.20; 95% CI −20.51 to −9.90; $P < 0.00001$).

Serum TBIL was detected in six trials, which involved 472 participants, with 255 and 217 patients in treatment and control groups, respectively (Figure 4C). There was an excellent homogeneity among the 6 studies with $I^2 = 0\%$ and $P = 0.60$, and the fixed-effect model demonstrated that bicyclol could significantly decrease the TBIL level in NAFLD patients (TBIL μmol/L: MD =−1.72; 95% CI −2.72 to −0.72; $P = 0.0008$).

**Effect of bicyclol on blood lipid biomarkers in patients with NAFLD**

Twelve studies reported the data of TG. These trials involved 1008 patients, with 523 patients in treatment groups and 485 patients in control groups. There was a high statistical heterogeneity for TG with $I^2$ of 90% and $P < 0.00001$, and thus the subgroup analysis was conducted. Bicyclol combination subgroup indicated no heterogeneity with $I^2$ =0% and $P$ = 0.89, and it significantly decreased the TG level in NAFLD patients compared with other drug monotherapy, which was analyzed by a random-effects model (TG mmol/L: MD = −0.39; 95% CI −0.45 to −0.33; $P < 0.00001$). There was a substantial heterogeneity in the bicyclol monotherapy subgroup with $I^2$ of 95% and $P < 0.00001$. The descriptive analysis showed that bicyclol monotherapy was more likely to decrease the levels of TG in all the five monotherapy studies (Figure 5A).

There were 11 studies that reported the data of TC. These trials involved 958 patients, with 498 and 460 patients in the treatment and control group, respectively. The $I^2$ of TC was 67%, and therefore, the random-effects model was conducted and showed that the reduction of TC in NAFLD patients treated by bicyclol was significant (TC mmol/L: MD =−0.52; 95% CI −0.70 to −0.34; $P < 0.00001$) (Figure 5B).

**Grading the evidence**

Grading of the evidence was carried out for the key outcomes based on the limitations of precision, publication bias, risk of bias, and heterogeneity. The quality of evidence was observed to be either low or very low (Table 2).

## DISCUSSION

With a meta-analysis of 12 Chinese studies including 1008 patients, this review evidenced that bicyclol, regardless of monotherapy or combination with other drugs, has a positive effect on improving liver function (ALT, AST, and TBIL) and blood lipids (TG and TC). Although bicyclol combination treatment for ALT and monotherapy for TG have considerable heterogeneity, each trial in these included studies showed promising therapeutic effects for abnormal blood biomarkers.

Clinically, bicyclol was recommended for oral administration for up to 6 months. In this meta-analysis, although adverse events, such as gastrointestinal intolerance were sporadically reported in the control group (Table 1), no such mild discomforts were reported in bicyclol-treated group, which agreed with the extremely mild and rare incidence of adverse reactions demonstrated by long-term clinical practice.[13] Moreover, only three of the included studies concluded that there was a higher total effective rate of bicyclol intervention for fatty liver, which was mainly based on blood biomarkers and B-model ultrasonography results. We thus evaluated the liver function and blood lipid biomarkers as the primary outcome, though liver histology is the gold standard and MRI has higher accuracy for assessing fatty liver.[17]

The pathogenesis of NAFLD is complex and is tightly associated (over 76%) with type 2 diabetes mellitus (T2DM),[41-43] patients with or without T2DM were thus included in this review. Besides, the course of the disease varies among these studies, and some studies did not report the patient's medical history, we thus limited the baseline ALT and TG level to ensure the consistency of the included patients as much as possible. We also defined the treatment duration as at least 4 weeks, for that NAFLD is a chronic disease, and bicyclol was suitable for long-term oral administration. Though treating for NAFLD is its off-label use, the Chinese guidelines of prevention and treatment for nonalcoholic fatty liver disease updated in 2018 recommend that hepatoprotectants could be complementary treatment measures for NASH patients with elevated aminotransferases or liver injury.[44] Compared with the intervention in the control group, including lifestyle changes and other drug treatments, the alleviation for abnormal blood biomarkers by bicyclol is evident and is consistent with its

clinical practice.[21] It is worth noting that, subgroup analyses for ALT and TG, which were conducted when the significant heterogeneity existed, also provided substantial evidence for its effect.

This review has to interpret the limitations of the low quality of the included studies, publication bias, and low grading of evidence. All the included studies were only reported in China and many of them did not provide specific methods of blinding and random allocation concealments. In terms of the outcome indicators, most articles lack the blood glucose and insulin resistance index, which leads to the results of the meta-analysis merely provide the effect of bicyclol on liver function and blood lipid indicators. Though the biomarker AST，TC，TBIL showed no publication bias, ALT and TG showed significant publication bias. We speculated that the heterogeneity and a language bias contributed to it, and subgroup analysis was conducted. Also, when the degree of heterogeneity was large, Egger's tests did not have good properties.[45] Similarly, the low grading of evidence was mainly derived from the publication bias, risk of bias, and heterogeneity. Therefore, the results of the meta-analysis merely provide a reference based on the current evidence.

In conclusion, the present evidence presents the effect of bicyclol monotherapy and/or combination therapy for reducing liver function and blood lipid biomarkers in NAFLD patients. This preliminary study predicts that bicyclol might be an alternative available drug to be explored for NAFLD therapy in the future. However, the conclusion also needs to be further verified by more well designed and implemented studies.

## Authors contribution

Conceptualization, analysis, writing original draft, visualization: Hu Li; Validation of data and analysis: Nan-Nan Liu; Supervision, validation and writing draft: Zong-Gen Peng; Approval of final manuscript: all authors.

## Conflicts of Interest

All authors declare that no conflicts of interest.

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**Competing interests**

None.

**Patient consent**

Not required.

**Data sharing statement**

All data are shown in the manuscript.

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**Table 1. Characteristics of the included studies**

| Study | Sample size | | Intervention | | Dose of bicyclol | Duration | Outcomes | Adverse events | |
|---|---|---|---|---|---|---|---|---|---|
| | Experimental | Control | Experimental | Control | | | | Experimental | Control |
| Liao 2011 | 30 | 30 | Bicyclol | Vitamin C | 50 mg, tid | 12 weeks | ①③④⑤⑥⑦⑧ | None | None |
| Liang 2007 | 45 | 38 | Bicyclol | UDCA | 25~50 mg, tid | 24 weeks | ④⑥⑦⑧ | - | - |
| Zhu 2005 | 36 | 29 | Bicyclol | Silymarin | 25~50 mg, tid | 24 weeks | ①④⑥⑦ | - | - |
| Yan 2017 | 30 | 30 | Bicyclol | DGEC | 50 mg, tid | 4 weeks | ④⑤⑦⑧ | None | None |
| Zhang 2012 | 60 | 60 | Bicyclol | LSI | 25 mg, tid | 24 weeks | ②④⑥⑦ | - | - |
| Gao 2011 | 25 | 25 | Bicyclol + PPC | PPC | 25~50 mg, bid | 6 months | ③④⑤⑥⑦⑧ | Weight loss | None |
| Ding 2009 | 42 | 30 | Bicyclol + PPC | PPC | 25~50 mg, bid | 6 months | ④⑤⑥⑦⑧ | Weight loss | None |
| He 2011 | 47 | 35 | Bicyclol + PPC | PPC | 25~50 mg, tid | 6 months | ③④⑥⑦⑧ | None | None |
| Li 2014 | 50 | 50 | Bicyclol + Metformin | Metformin | 25 mg, bid | 6 months | ②④⑤⑦⑧ | - | - |
| Zhang 2011 | 42 | 42 | Bicyclol + Metformin | Metformin | 25~50 mg, bid | 6 months | ②④⑤⑦⑧ | None | Nausea, poor appetite |
| Sun 2015 | 76 | 76 | Bicyclol + Metformin | Metformin | 25 mg, bid | 6 months | ②④⑤⑦⑧ | - | - |
| Guan 2013 | 40 | 40 | Bicyclol + Silibinin | Silibinin | 50 mg, tid | 12 weeks | ①④⑤⑦⑧ | None | None |

①Total effective rate;②BMI;③Adverse Events;④ALT;⑤AST;⑥TBIL;⑦TG;⑧TC.

UDCA, ursodeoxycholic acid; DGEC, diammonium glycyrrhizinate enteric-coated capsule; LSI, lifestyle intervention; PPC, polyene phosphatidylcholine

**Table 2. Grading the evidence for key comparisons**

| Outcomes | Corresponding risk (95% CI) * | No. of Participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|
| ALT | The mean ALT in the intervention groups was 24.89 standard deviations lower (32.63 to 17.15 lower) | 1008 (12 studies) | ⊕⊖⊖⊖ very low [1,2] | SMD -24.89 (-32.63 to -17.15) |
| AST | The mean AST in the intervention groups was 15.2 standard deviations lower (20.51 to 9.9 lower) | 658 (8 studies) | ⊕⊕⊖⊖ low [2] | SMD -15.2 (-20.51 to -9.9) |
| TBIL | The mean TBIL in the intervention groups was 1.72 standard deviations lower (2.72 to 0.72 lower) | 472 (6 studies) | ⊕⊕⊖⊖ low [2] | SMD -1.72 (-2.72 to -0.72) |
| TG | The mean TG in the intervention groups was 0.46 standard deviations lower (0.59 to 0.33 lower) | 1008 (12 studies) | ⊕⊖⊖⊖ very low [1,2] | SMD -0.46 (-0.59 to -0.33) |
| TC | The mean TC in the intervention groups was 0.52 standard deviations lower (0.7 to 0.34 lower) | 958 (11 studies) | ⊕⊕⊖⊖ low [2] | SMD -0.52 (-0.7 to -0.34) |

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

* The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and its 95% CI.

[1] Downgraded one level due to serious limitations in publication bias.

[2] Downgraded one level for including studies with high risk of bias

## FIGURE LEGENDS

**Figure 1. Flow diagram of data selection process.**

**Figure 2. The quality assessment of the included studies.**

The quality of randomized controlled trials was assessed as 'high risk', 'low risk' or 'some concerns' to each item independently by two reviewers according to the revised Cochrane risk of bias tool.

**Figure 3. The effect of bicyclol on total effective rate and BMI in patients with NAFLD.**

Review Manager 5.3 was used to analyze the data. Odds ratio (OR) with its 95% confidence interval (95% CI) were estimated for total effective rate. Mean difference (MD) with its 95% CI were estimated for BMI. Heterogeneities were evaluated using the chi-square ($\chi 2$) and $I^2$ statistics. $I^2 < 50\%$ and $P > 0.10$ were deemed as homogeneous and the fixed-effect model was used. $P < 0.05$ were considered as statistically different between the experimental and control group.

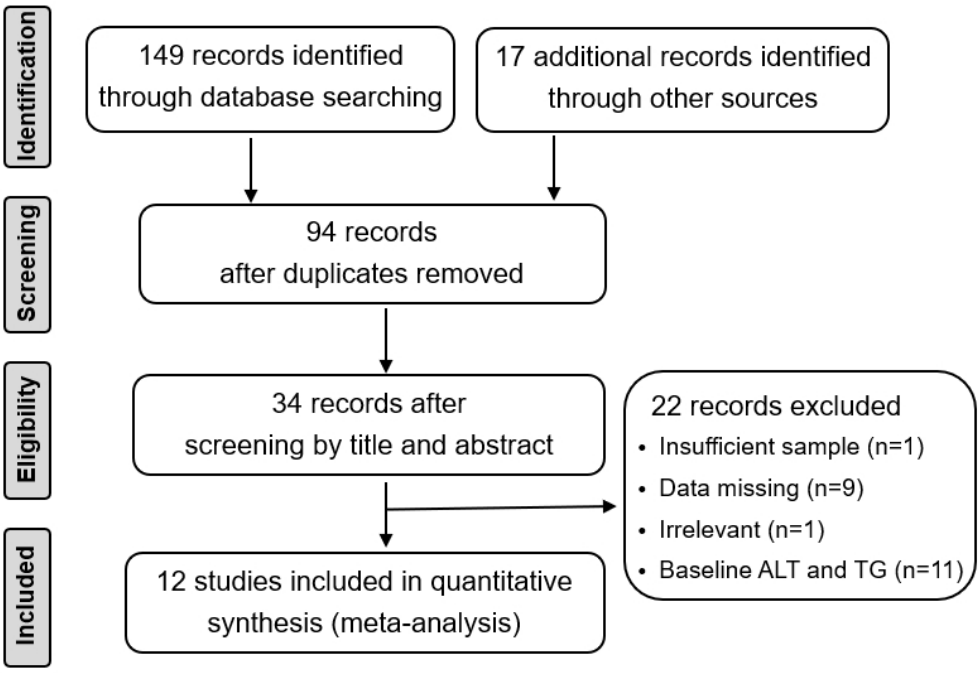
**Figure 4. The effect of bicyclol on ALT, AST and TBIL in patients with NAFLD.**

Review Manager 5.3 was used to analyze the data. Mean difference (MD) with its 95% CI were estimated for continuous outcomes. Heterogeneities were evaluated using the chi-square ($\chi 2$) and $I^2$ statistics. ALT parameter was significantly heterogeneous ($I^2 \geq 75\%$ and $P < 0.10$) and subgroup analysis was conducted (A); the AST parameter was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used (B); the TBIL parameter was homogeneous ($I^2 < 50\%$ and $P > 0.10$) and the fixed-effect model was used (C). $P < 0.05$ were considered as statistically different between the experimental and control group.

**Figure 5. The effect of bicyclol on TG and TC in patients with NAFLD.**

Review Manager 5.3 was used to analyze the data. Mean difference (MD) with its 95% CI were estimated for continuous outcomes. Heterogeneities were evaluated using the chi-square

($\chi$2) and $I^2$ statistics. The TG parameter was significantly heterogeneous ($I^2 \geq 75\%$) and subgroup analysis was conducted (A); the TC parameter was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used (B). $P < 0.05$ were considered as statistically different between the experimental and control group.

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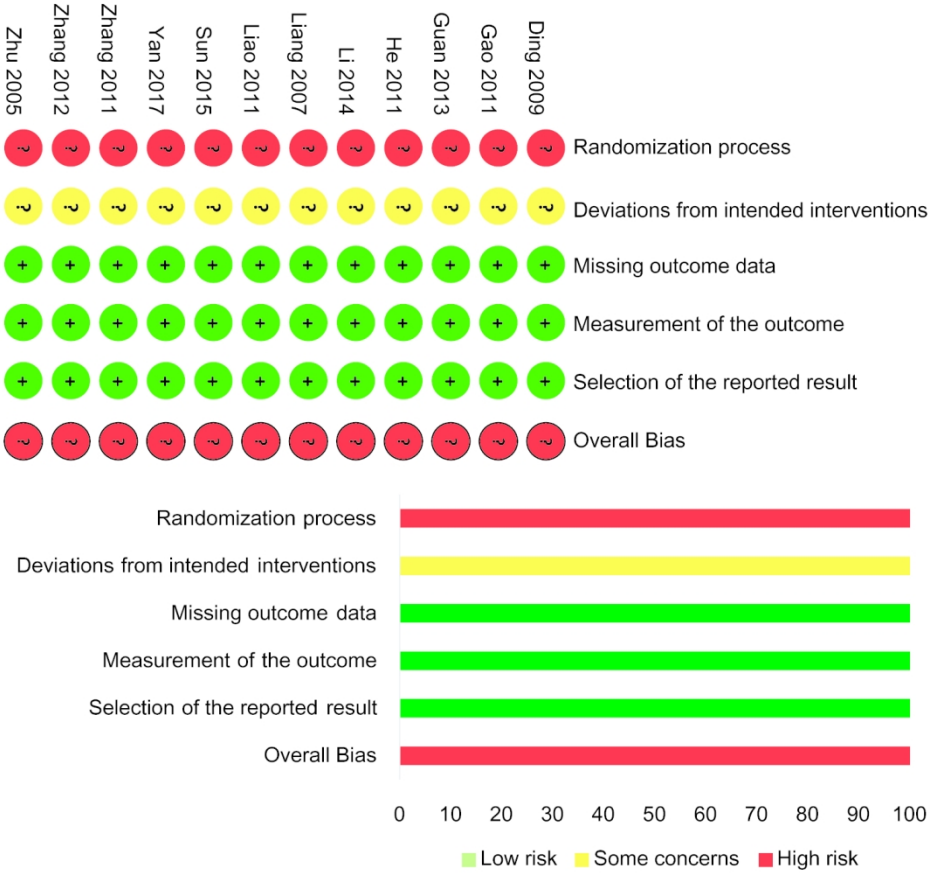
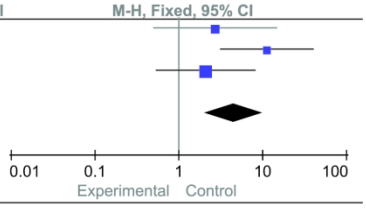


**A. Total effective rate**

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Guan 2013 | 38 | 40 | 35 | 40 | 28.9% | 2.71 [0.49, 14.90] |
| Liao 2011 | 26 | 30 | 11 | 30 | 24.3% | 11.23 [3.10, 40.71] |
| Zhu 2005 | 32 | 36 | 23 | 29 | 46.8% | 2.09 [0.53, 8.25] |
| **Total (95% CI)** | | **106** | | **99** | **100.0%** | **4.49 [2.02, 9.95]** |
| Total events | 96 | | 69 | | | |

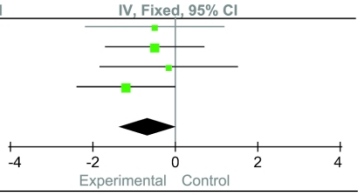
Heterogeneity: Chi² = 3.47, df = 2 (P = 0.18); I² = 42%
Test for overall effect: Z = 3.69 (P = 0.0002)

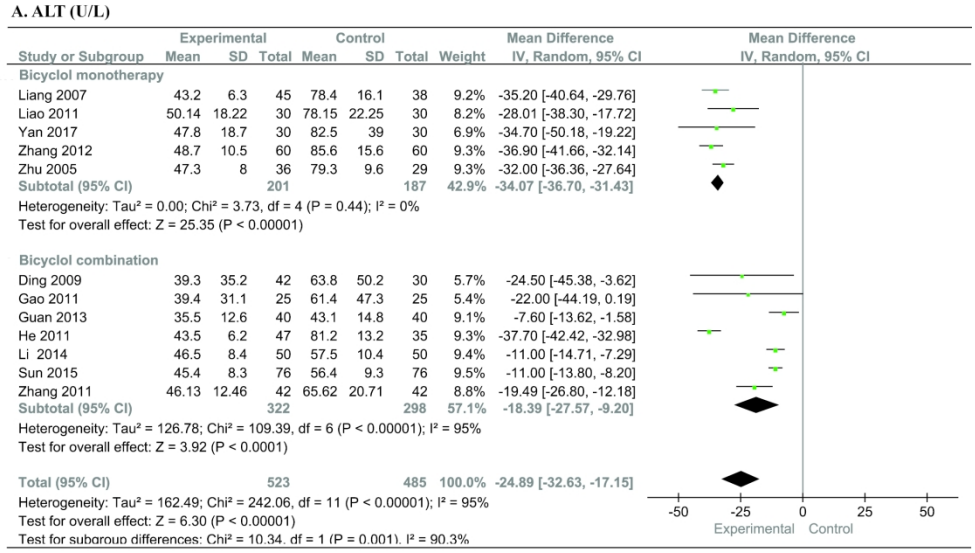


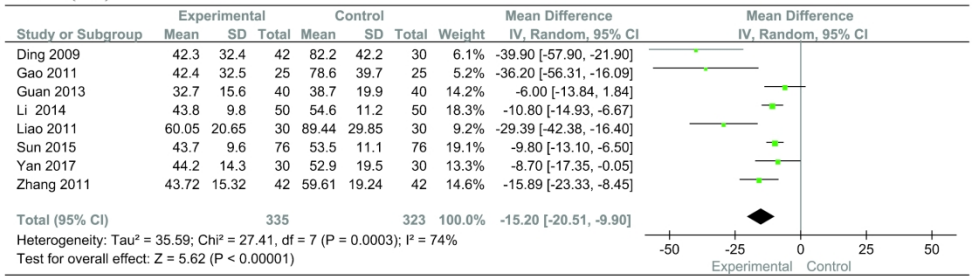

**B. BMI**

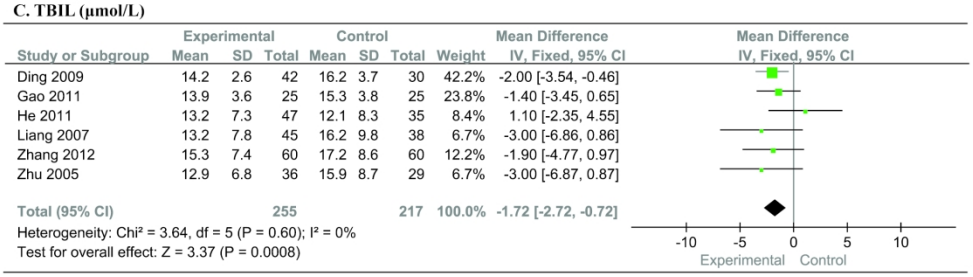
| Study or Subgroup | Experimental Mean | Experimental SD | Experimental Total | Control Mean | Control SD | Control Total | Weight | Mean Difference IV, Fixed, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Li 2014 | 25.1 | 4.7 | 50 | 25.6 | 3.9 | 50 | 16.8% | -0.50 [-2.19, 1.19] |
| Sun 2015 | 25 | 3.9 | 76 | 25.5 | 3.7 | 76 | 32.9% | -0.50 [-1.71, 0.71] |
| Zhang 2011 | 25.53 | 3.98 | 42 | 25.69 | 3.87 | 42 | 17.0% | -0.16 [-1.84, 1.52] |
| Zhang 2012 | 27.3 | 3.2 | 60 | 28.5 | 3.5 | 60 | 33.3% | -1.20 [-2.40, -0.00] |
| **Total (95% CI)** | | | **228** | | | **228** | **100.0%** | **-0.68 [-1.37, 0.02]** |

Heterogeneity: Chi² = 1.22, df = 3 (P = 0.75); I² = 0%
Test for overall effect: Z = 1.91 (P = 0.06)



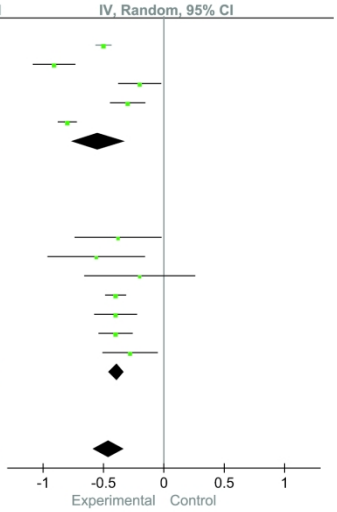


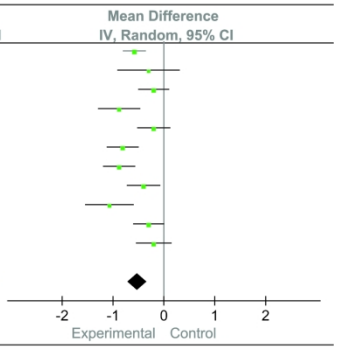
**A. TG (mmol/L)**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Bicyclol monotherapy | | | | | | | | |
| Liang 2007 | 1.7 | 0.2 | 45 | 2.2 | 0.1 | 38 | 10.4% | -0.50 [-0.57, -0.43] |
| Liao 2011 | 2.04 | 0.29 | 30 | 2.95 | 0.4 | 30 | 8.9% | -0.91 [-1.09, -0.73] |
| Yan 2017 | 1.7 | 0.3 | 30 | 1.9 | 0.4 | 30 | 8.9% | -0.20 [-0.38, -0.02] |
| Zhang 2012 | 1.9 | 0.3 | 60 | 2.2 | 0.5 | 60 | 9.4% | -0.30 [-0.45, -0.15] |
| Zhu 2005 | 1.58 | 0.1 | 36 | 2.38 | 0.2 | 29 | 10.3% | -0.80 [-0.88, -0.72] |
| Subtotal (95% CI) | | | 201 | | | 187 | 47.8% | -0.54 [-0.77, -0.32] |
| Heterogeneity: Tau² = 0.06; Chi² = 79.27, df = 4 (P < 0.00001); I² = 95% | | | | | | | | |
| Test for overall effect: Z = 4.81 (P < 0.00001) | | | | | | | | |
| Bicyclol combination | | | | | | | | |
| Ding 2009 | 2.69 | 0.62 | 42 | 3.07 | 0.86 | 30 | 5.8% | -0.38 [-0.74, -0.02] |
| Gao 2011 | 2.65 | 0.54 | 25 | 3.21 | 0.88 | 25 | 5.2% | -0.56 [-0.96, -0.16] |
| Guan 2013 | 2.4 | 1 | 40 | 2.6 | 1.1 | 40 | 4.5% | -0.20 [-0.66, 0.26] |
| He 2011 | 1.7 | 0.2 | 47 | 2.1 | 0.2 | 35 | 10.2% | -0.40 [-0.49, -0.31] |
| Li 2014 | 1.35 | 0.44 | 50 | 1.75 | 0.47 | 50 | 8.9% | -0.40 [-0.58, -0.22] |
| Sun 2015 | 1.34 | 0.43 | 76 | 1.74 | 0.46 | 76 | 9.5% | -0.40 [-0.54, -0.26] |
| Zhang 2011 | 1.38 | 0.45 | 42 | 1.66 | 0.61 | 42 | 8.0% | -0.28 [-0.51, -0.05] |
| Subtotal (95% CI) | | | 322 | | | 298 | 52.2% | -0.39 [-0.45, -0.33] |
| Heterogeneity: Tau² = 0.00; Chi² = 2.30, df = 6 (P = 0.89); I² = 0% | | | | | | | | |
| Test for overall effect: Z = 12.08 (P < 0.00001) | | | | | | | | |
| Total (95% CI) | | | 523 | | | 485 | 100.0% | -0.46 [-0.59, -0.33] |
| Heterogeneity: Tau² = 0.04; Chi² = 105.58, df = 11 (P < 0.00001); I² = 90% | | | | | | | | |
| Test for overall effect: Z = 6.95 (P < 0.00001) | | | | | | | | |
| Test for subgroup differences: Chi² = 1.72, df = 1 (P = 0.19), I² = 41.7% | | | | | | | | |




**B. TC (mmol/L)**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Ding 2009 | 4.12 | 0.44 | 42 | 4.7 | 0.51 | 30 | 11.5% | -0.58 [-0.81, -0.35] |
| Guan 2013 | 4.6 | 1.5 | 40 | 4.9 | 1.3 | 40 | 5.3% | -0.30 [-0.92, 0.32] |
| He 2011 | 4.3 | 0.7 | 47 | 4.5 | 0.7 | 35 | 10.0% | -0.20 [-0.51, 0.11] |
| Li 2014 | 3.24 | 0.89 | 50 | 4.12 | 1.21 | 50 | 8.0% | -0.88 [-1.30, -0.46] |
| Liang 2007 | 4.3 | 0.7 | 45 | 4.5 | 0.8 | 38 | 9.6% | -0.20 [-0.53, 0.13] |
| Liao 2011 | 6.04 | 0.69 | 30 | 6.85 | 0.55 | 30 | 9.8% | -0.81 [-1.13, -0.49] |
| Sun 2015 | 3.13 | 0.78 | 76 | 4.01 | 1.18 | 76 | 9.8% | -0.88 [-1.20, -0.56] |
| Yan 2017 | 5.5 | 0.7 | 30 | 5.9 | 0.6 | 30 | 9.6% | -0.40 [-0.73, -0.07] |
| Zhang 2011 | 3.17 | 0.97 | 42 | 4.24 | 1.25 | 42 | 7.1% | -1.07 [-1.55, -0.59] |
| Zhang 2012 | 4.3 | 0.8 | 60 | 4.6 | 0.9 | 60 | 10.0% | -0.30 [-0.60, 0.00] |
| Zhu 2005 | 4.2 | 0.6 | 36 | 4.4 | 0.8 | 29 | 9.2% | -0.20 [-0.55, 0.15] |
| Total (95% CI) | | | 498 | | | 460 | 100.0% | -0.52 [-0.70, -0.34] |
| Heterogeneity: Tau² = 0.06; Chi² = 30.43, df = 10 (P = 0.0007); I² = 67% | | | | | | | | |
| Test for overall effect: Z = 5.68 (P < 0.00001) | | | | | | | | |



# Online supplementary materials

# The effect of bicyclol on blood biomarkers of NAFLD: a systematic review and meta-analysis

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## Online supplementary materials

## Online supplementary Methods: Search Strategy

### Pubmed

| No. | Query |
|---|---|
| 1 | Non alcoholic Fatty Liver Disease |
| 2 | NAFLD |
| 3 | nonalcoholic fatty liver |
| 4 | non-alcoholic fatty liver |
| 5 | Nonalcoholic Steatohepatitis |
| 6 | Nonalcoholic Steatohepatitides |
| 7 | 1 or 2 or 3 or 4 or 5 or 6 |
| 8 | bicyclol |
| 9 | 4,4'-bi-(1,3-benzodioxole)-5-carboxylic acid, 5'-(hydroxymethyl)-7,7'-dimethoxy-, methyl ester |
| 10 | 6-methoxycarbonyl-6'-hydroxymethyl-2,3,2',3'-bis(methylenedioxy)-4,4'- dimethoxybiphenyl |
| 11 | 8 or 9 or 10 |
| 12 | 10 and 11 |

### Embase

| No. | Query |
|---|---|
| #1 | non AND alcoholic AND fatty AND liver AND disease |
| #2 | nafld |
| #3 | nonalcoholic AND fatty AND liver |
| #4 | 'non alcoholic' AND fatty AND liver |
| #5 | nonalcoholic AND steatohepatitis |
| #6 | nonalcoholic AND steatohepatitides |
| #7 | #1 or #2 or #3 or #4 or #5 or #6 |
| #8 | bicyclol |
| #9 | #7 AND #8 |

### Cochrane Library

| ID | Search |
|---|---|
| #1 | MeSH descriptor: [Non-alcoholic Fatty Liver Disease] explode all trees |
| #2 | ((nonalcoholic or non-alcoholic) near (fatty liver or steatohepatitis)):ti,ab,kw or fatty liver or steatohepatitis:ti or nafld or nash:ti,ab,kw (Word variations have been searched) |
| #3 | #1 or #2 |
| #4 | bicyclol:ti,ab,kw (Word variations have been searched) |
| #5 | #3 and #4 |

**China National Knowledge Infrastructure database (CNKI)**

( ( (主题=非酒精性脂肪性肝病 或者 题名=非酒精性脂肪性肝病 或者 v_subject=中英文扩展(非酒精性脂肪性肝病) 或者 title=中英文扩展(非酒精性脂肪性肝病)) 或者 (主题=非酒精性脂肪性肝炎 或者 题名=非酒精性脂肪性肝炎 或者 v_subject=中英文扩展(非酒精性脂肪性肝炎) 或者 title=中英文扩展(非酒精性脂肪性肝炎)) ) 并且 (主题=双环醇 或者 题名=双环醇 或者 v_subject=中英文扩展(双环醇) 或者 title=中英文扩展(双环醇)) ) (模糊匹配),专辑导航：全部; 数据库：文献 跨库检索

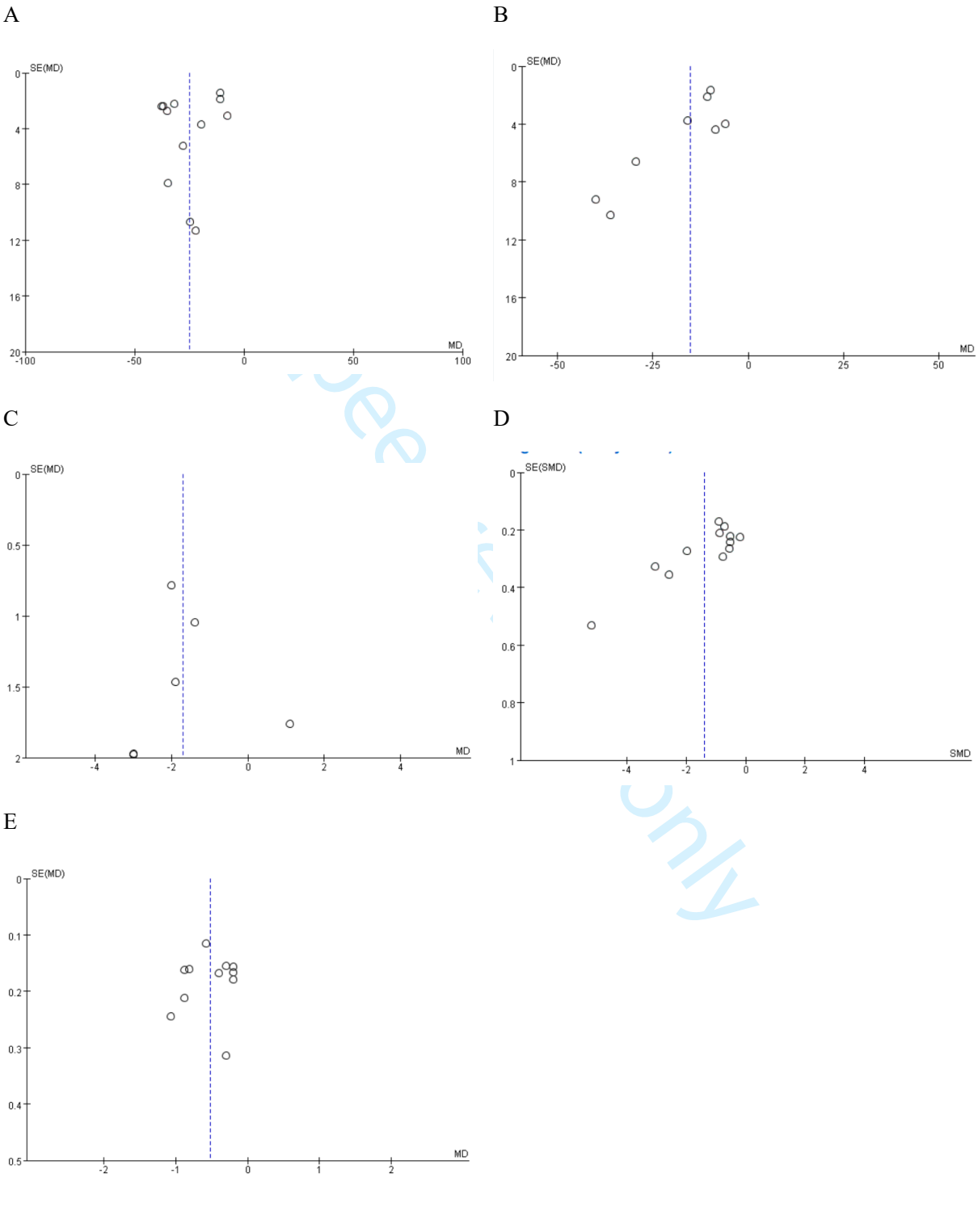
**VIP-Chinese scientific and technological journal database**

((题名或关键词=非酒精性脂肪性肝病 OR 题名或关键词=非酒精性脂肪性肝炎) AND 题名或关键词=双环醇)

**Wanfang digital periodical full-text database**

主题词扩展&中英文扩展：(主题:(非酒精性脂肪性肝病)+主题:(非酒精性脂肪性肝炎) )* *主题:(双环醇)

Figure S1. Funnel plot of alanine aminotransferase (A), aspartate transaminase (B), total bilirubin(C), triglyceride (D) and total cholesterol (E).



## Table S1. Support table for risk of bias judgement

| | Randomization process # | | | Deviations from intended interventions | | | | | | | Missing outcome data | | | | Measurement of the outcome | | | | | Selection of the reported result | | | Overall Bias * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study \ Unique ID | 1.1 | 1.2 | 1.3 | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 2.6 | 2.7 | 3.1 | 3.2 | 3.3 | 3.4 | 4.1 | 4.2 | 4.3 | 4.4 | 4.5 | 5.1 | 5.2 | 5.3 | |
| Ding 2009 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Gao 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Guan 2013 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| He 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Li 2014 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Liang 2007 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Liao 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Sun 2015 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Yan 2017 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Zhang 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Zhang 2012 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Zhu 2005 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |

# comments of Randomization process: study "Liao 2011": Random number table method, no information about allocation concealment; others: Random number table method, no information about allocation concealment

***** Y/PY/PN/N/NI means Yes/Probably yes/Probably no/No/No information; Overall judgement for the result will be 'High' if one of the domains is judged at 'High' risk of bias.

1.1 Was the allocation sequence random?

1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?

1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?

2.1 Were participants aware of their assigned intervention during the trial?

2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?

2.3 If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?

2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?

2.5 Was an appropriate analysis used to estimate the effect of assignment to intervention?

3.1 Were data for this outcome available for all, or nearly all, participants randomized?

4.1 Was the method of measuring the outcome inappropriate?

4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?

4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?

4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?

5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?

5.2 Is the numerical result being assessed likely to have been selected, on the basis of the results, from multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?

5.3 ... multiple eligible analyses of the data?

# PRISMA 2009 Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 4-5 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 5 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 5 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 6 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 5-6 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 5-6 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 5-6 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 7 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 6-7 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 7 |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 7 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 7 |

# PRISMA 2009 Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 7 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 7 |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 8 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 8, Table1 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 8, Figure 2 |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. . | 8-10 |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 8-10 |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 8, Figure 2 |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | 9-10 |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 11 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 12 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 12 |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 13 |

*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

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BMJ Open

# The effect of bicyclol on blood biomarkers of NAFLD: a systematic review and meta-analysis



| | |
|---|---|
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| | |

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# The effect of bicyclol on blood biomarkers of NAFLD: a systematic review and meta-analysis

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## ABSTRACT

**Objective** Nonalcoholic fatty liver disease (NAFLD) is a global epidemic without effective therapeutic agents in the clinic. This meta-analysis aimed to assess the efficacy of the marketed hepatoprotectant bicyclol at improving blood biomarkers in patients with NAFLD.

**Design** Electronic databases were searched for randomized controlled trials (RCTs) published up to August 2020 using bicyclol to treat NAFLD. The risk of bias, quality of evidence, and publication bias were evaluated. Blood biomarkers, including alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), triglyceride (TG) and total cholesterol (TC), were analyzed using Review Manager 5.3 software. Outcomes with significant heterogeneity ($I^2 \geq 75\%$) were divided into bicyclol monotherapy subgroup and combination treatment subgroup.

**Results** Twelve RCTs involving 1008 patients were finally included. No serious adverse events were reported in bicyclol-treated groups. The total effective rate of bicyclol intervention for NAFLD was significantly higher than that of the control group. The decreases in the levels of AST (mean difference (MD) = −15.20; 95% confidence interval (CI) −20.51 to −9.90; $I^2$ = 74%), TBIL (MD = −1.72; 95% CI −2.72 to −0.72; $I^2$ = 0%) and TC (MD = −0.52; 95% CI −0.70 to −0.34; $I^2$ = 67%) treated by bicyclol were significantly higher than those in control group. When a high heterogeneity existed ($I^2 \geq 75\%$), subgroup analyses were conducted and revealed significantly decreased ALT levels (MD = −34.07; 95% CI −36.70 to −31.43; $I^2$ = 0%) merely in bicyclol monotherapy subgroup, while TG level (MD = −0.39; 95% CI −0.45 to −0.33; $I^2$ = 0%) was decreased in bicyclol combination therapy subgroup.

**Conclusions** The study presents the evidence of bicyclol monotherapy and/or combination therapy for improving liver function and blood lipid biomarkers in NAFLD patients. This preliminary study predicts that bicyclol might be an alternative drug for NAFLD therapy in the future.


**KEYWORDS:** nonalcoholic fatty liver disease; meta-analysis; bicyclol; blood biomarkers

**Strengths and limitations of this study**

- This systematic review is the first to determine the effect of bicyclol on blood biomarkers of patients with NAFLD.
- This study provides preliminary evidence that bicyclol might be efficacious for treatment of patients with NAFLD.
- The limitation of this meta-analysis is the low quality of the existing studies, and the results of this study only apply to China due to bicyclol has not been approved in Europe and North America.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common spectrum of liver diseases typically ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH).[1] The benign and reversible NAFL is merely characterized by excessive lipid droplet deposition in hepatocytes, while NASH is a more aggressive condition characterized by inflammatory infiltrates, visible cellular injury, and possible progression to or accompanied by fibrosis and cirrhosis.[2] NAFLD is closely related to the high incidence of metabolic syndrome, cardiovascular disease, type 2 diabetes, and advanced liver diseases.[1,3] Currently, the prevalence of NAFLD worldwide is up to 25%, with the highest prevalence of 32% reported in the Middle East and 31% in South America, and even the lowest prevalence in Africa was estimated to be 14%.[4] Worse still, the prevalence of NAFLD worldwide is presumed to be increasing.[5] There are no admitted therapeutic agents from international societies for treating NAFLD, except for lifestyle changes.[6-8] However, patients tend to exhibit poor adherence to this important intervention.[9] Recently, only one dual peroxisome proliferator-activated receptor (PPAR)-α/γ agonist saroglitazar magnesium has been approved for the treatment of NASH without cirrhosis in India.[10] However, numerous potential agents, such as farnesoid X receptor agonists, apoptosis signal-regulated kinase 1 inhibitors, and C-C chemokine receptor type 2/5 inhibitors, have entered different phases in clinical trials but presented limited or even no benefits.[1,11,12] Therefore, new or complementary drugs for treating NAFLD are still urgently needed and this dilemma might persist for a long time.

Bicyclol, a hepatoprotective and anti-inflammatory drug that has been approved in China since 2004, was used to treat increased levels of aminotransferases caused by various forms of chronic hepatitis mainly in Asian countries, while it has not been approved in Europe and North America.[13] It is rather safe and suitable for long-term (more than 6 months) oral administration.[13] Many preclinical animal experiments have confirmed its therapeutic effect in chemical-, immunological-, fatty-, and drug- induced liver injury, as well as hepatic fibrosis caused by bile duct ligation, dimethylnitrosamine, bovine serum albumin or carbon tetrachloride.[13-15] The detailed mechanisms of bicyclol involved in the inhibition of hepatocyte apoptosis, stabilization of mitochondrial or hepatocyte membranes, scavenging

free radicals, increasing the expression of antioxidant genes, and reducing lipid peroxide levels.[14,16] Although liver histology and magnetic resonance imaging (MRI) have high accuracy for evaluating the liver fat content, [17] liver function and blood lipid biomarkers, which mainly include alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), triglyceride (TG) and total cholesterol (TC), are commonly used to evaluate the severity of NAFLD and the subsequent abnormal metabolism.[18,19] Relevant clinical and preclinical studies have reported the potential therapeutic role of bicyclol in NAFLD,[20,21] however, its effect on non-invasive blood biomarkers in patients with NAFLD has not been precisely confirmed due to insufficient sample sizes and the low quality of studies. Hence, this meta-analysis aimed to evidence the effect of bicyclol on blood biomarker levels in patients with NAFLD through synthesizing the clinical data using bicyclol monotherapy alone or in combination with other drugs to treat NAFLD, and to preliminarily predict its clinical efficacy in the future.

## METHODS

The data included in this meta-analysis were derived from previously published clinical studies, all of which were conducted in China. The study protocol was confirmed by all authors before data collection. Our protocol has been registered on the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY). The registration number was INPLASY202080017 (DOI number is 10.37766/inplasy2020.8.0017, https://inplasy.com/inplasy-2020-8-0017/). We used analytical methods recommended in the Cochrane Handbook for Systematic Reviews of Interventions[22] and reported this study following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.[23]

### Search strategy

Studies up to August 2020 were searched in PubMed, Embase, Cochrane Library, and Chinese databases, including the China National Knowledge Infrastructure database (CNKI), VIP-Chinese scientific and technological journal database, and Wanfang digital periodical full-text database. Search terms were (("Non alcoholic Fatty Liver Disease" OR "NAFLD"

OR "nonalcoholic fatty liver" OR "non-alcoholic fatty liver" OR "Nonalcoholic Steatohepatitis" OR "Nonalcoholic Steatohepatitides")) AND ("bicyclol" OR "4,4'-bi-(1,3-benzodioxole)-5-carboxylic acid, 5'-(hydroxymethyl)-7,7'-dimethoxy-, methyl ester" OR "6-methoxycarbonyl-6'-hydroxymethyl-2,3,2',3'-bis(methylenedioxy)-4,4'-dimethoxybiphenyl") without other restrictions (online supplementary Methods). Additional studies were hand-searched in Google Scholar and the reference lists of relevant articles.

**Inclusion and exclusion criteria**

The inclusion criteria were as follows: 1) randomized controlled trials (RCTs); 2) male and female patients diagnosed as NAFLD complicated with or without type 2 diabetes mellitus according to the corresponding guidelines; 3) an average baseline ALT level greater than 90 U/L (2 ~ 3 times the upper limit of normal values[24]), while a TG level ranging from 2.5 to 5 mmol/L; and 4) articles published in the English or Chinese language. The exclusion criteria were 1) non-clinical studies, non-randomized controlled trials; 2) studies examining patients with liver injury induced by drugs, viruses, alcohol, autoimmunity, primary biliary cholangitis, liver decompensation, malignancy or genetics; 3) studies enrolling fewer than 20 subjects in each group, or the treatment time of less than 4 weeks; and 4) studies without sufficient experimental data, such as case reports, reviews, conference abstracts, or a lack of sufficient biochemical indicators.

**Intervention measures**

The bicyclol monotherapy group (experimental group) was compared with groups treated with a lifestyle intervention (LSI) or another drug as a monotherapy (control group). Bicyclol combined with another medical treatment (experimental group) was compared with the corresponding medicine (control group). Other potential factors, such as lifestyle interventions were required to be consistent between the two groups.

**Outcome indicators**

Liver function indicators (ALT, AST, and TBIL levels) and blood lipid parameters (TG and TC levels) were recorded. Adverse events, the anthropometric parameter body mass index

(BMI), and the total effective rate, which was defined as the ratio of participants who have achieved significant decreases in blood biomarker levels (the decreased level of TC with greater than 10% and TG with greater than 20%) and parameters of liver fat reduction under B-model ultrasonography among the included participants in the corresponding studies, were also analyzed.

**Data extraction and quality assessment**

The outcome indicators from all included studies were independently extracted and checked by two authors (Hu Li and Nan-nan Liu) to guarantee the accuracy of the data. The quality of RCTs, which was assigned as a 'high risk', 'low risk', or 'some concerns' for each item, was also assessed independently by two reviewers using the revised Cochrane risk of bias tool.[25] Any discrepancies were resolved through discussion.

**Data analysis**

Review Manager 5.3 software was used to analyze the data.[26,27] Odds ratio (OR) and pooled mean difference (MD) with the corresponding 95% confidence interval (95% CI) were estimated for binary outcomes and continuous outcomes, respectively. Heterogeneities were evaluated using the chi-square ($\chi^2$) and $I^2$ statistics.[26] When the outcome was homogeneous ($I^2 < 50\%$ and $P > 0.10$), the fixed-effect model was used, and the random-effect model was used when the outcome was considered heterogeneous ($50\% \leq I^2 < 75\%$). When significant heterogeneity was observed ($I^2 \geq 75\%$), a subgroup analysis was conducted according to bicyclol monotherapy and combination therapy, and if $I^2$ of the subgroup was still over than 75%, descriptive results were provided without pooling estimates. The statistical significance of differences between the experimental and control group was set at $P < 0.05$. Publication bias was assessed only for comparisons with at least five studies using the funnel plot and its symmetry was evaluated using Egger's regression tests through Stata 12.0 software. Significant publication bias was defined as $P < 0.10$.[28] Grading of the evidence for the key comparisons was performed using the approach described by the grades of recommendation, assessment, development and evaluation (GRADE) working group.[22]

## Patient and public involvement

Patients and the public were not involved in this review.

## RESULTS

### Study selection

The whole flow chart of the data selection process is presented in Figure 1. Initially, 166 records were searched out, and 94 records were retained after duplicate exclusion. We then achieved 34 studies after screening the title and abstract, in which reviews, case reports, animal experiments, and studies with incongruent intervention measures and research orientation were excluded. After screening the full text, we excluded studies without appropriate samples, biochemical indicators, and baseline ALT and TG levels. One irrelevant study, which included patients with alcoholic fatty liver, was also excluded. Finally, 12 studies published in Chinese were included.[29-40]

### Characteristics, quality evaluation and publication bias of the included studies

The characteristics of the included studies are presented in Table 1. All the studies were conducted in China and published from 2005 to 2017, and the sample size ranged from 50 to 152 (median of 81). The total sample size is 1008 with 523 patients in the treatment group and 485 participants in the control group. The baseline values of patient outcome indicators were not different between the two groups.

The quality assessment of the included studies was shown in Figure 2 according to the most recently revised Cochrane risk of bias tool (online supplementary Table S1), in which one study applied the random number table,[29] and other studies used randomization but did not provide detailed methods. None of the studies reported the blinding condition or the plan of allocation and concealment. Additionally, all the studies had provided complete outcome data, without other predictable sources of bias.

The Egger's tests of funnel plots (online supplementary Figure S1) for primary outcomes did not reveal significant publication bias among the blood biomarkers of AST (8 studies, $P$ = 0.964), TC (11 studies, $P$ = 0.567), and TBIL (6 studies, $P$ = 0.485). However, ALT (12

studies, $P = 0.027$) and TG (12 studies, $P = 0.004$) showed significant publication bias. We speculated that the heterogeneity in studies was the main determining factor, and a subgroup analysis was conducted.

**Effect and safety of the bicyclol intervention for patients with NAFLD**

The therapeutic effect and safety of bicyclol for NAFLD were first evaluated. As shown in Figure 3, changes in BMI and the total effective rate at improving fatty liver indicated no heterogeneity, with $I^2$ of 0%, $P = 0.75$, and $I^2$ of 42%, $P = 0.18$, respectively. Two hundred and five patients in 3 studies were included in the analysis of the total effective rate, while 456 patients in 4 studies were included in the BMI analysis. The fixed-effects model revealed an increased total effective rate (total effective rate: OR = 4.49; 95% CI 2.02 to 9.95; $P = 0.0002$) but no significant effect on BMI (BMI: MD = −0.68; 95% CI −1.37 to 0.02; $P = 0.06$) in the bicyclol group compared with the control group. No gastrointestinal adverse events, such as nausea, vomiting, and diarrhea, or headache were reported in the bicyclol treatment group in the included studies (Table 1).

**Effect of bicyclol on liver function biomarkers in patients with NAFLD**

Serum ALT levels were reported in 12 studies. These trials involved 1008 patients, with 523 patients in the treatment group and 485 patients in the control group. A high level of statistical heterogeneity for ALT levels was observed, with $I^2$ of 95% and $P < 0.00001$. Therefore, we further divided these studies into a bicyclol monotherapy subgroup and bicyclol combination treatment subgroup according to the drug regimen used in the experimental group. ALT levels in the bicyclol monotherapy subgroup, which were analyzed using a random-effects model, were significantly decreased compared with those of the corresponding control group (ALT U/L: MD = −34.07; 95% CI −36.70 to −31.43; $P < 0.00001$). However, significant heterogeneity was observed in the bicyclol combination subgroup with $I^2$ of 95% and $P < 0.00001$. Therefore, we performed a descriptive analysis and showed that bicyclol was more likely to decrease the levels of ALT in all seven studies when administered in combination with other drugs (Figure 4A).

Serum AST levels were recorded in eight trials covering 658 patients, including 335 and 323 participants in the treatment and control groups, respectively. Heterogeneity was observed for AST levels, with $I^2$ of 74% (Figure 4B). The random-effects model demonstrated that the reduction of AST levels was significant in NAFLD patients treated by bicyclol as a monotherapy and combination therapy (AST U/L: MD = −15.20; 95% CI −20.51 to −9.90; $P < 0.00001$).

Serum TBIL levels were detected in six trials, involving 472 participants, with 255 and 217 patients in the treatment and control groups, respectively (Figure 4C). There was an excellent homogeneity among the 6 studies, with $I^2 = 0\%$ and $P = 0.60$, and the fixed-effect model indicated that bicyclol significantly decreased the TBIL level in NAFLD patients (TBIL μmol/L: MD = −1.72; 95% CI −2.72 to −0.72; $P = 0.0008$).

**Effect of bicyclol on blood lipid biomarkers in patients with NAFLD**

Twelve studies reported the TG levels. These trials involved 1008 patients, with 523 patients in the treatment groups and 485 patients in the control groups. A high level of statistical heterogeneity was observed for TG levels, with $I^2$ of 90% and $P < 0.00001$, and thus the subgroup analysis was conducted. The bicyclol combination subgroup did not display heterogeneity, with $I^2$ =0% and $P = 0.89$, and it significantly decreased the TG level in patients with NAFLD compared with patients receiving monotherapy with other drugs, which was analyzed by a random-effects model (TG mmol/L: MD = −0.39; 95% CI −0.45 to −0.33; $P < 0.00001$). Substantial heterogeneity was observed in the bicyclol monotherapy subgroup, with $I^2$ of 95% and $P < 0.00001$. The descriptive analysis showed that bicyclol monotherapy was more likely to decrease the levels of TG in all the five monotherapy studies (Figure 5A).

Eleven studies reported the TC levels. These trials involved 958 patients, with 498 and 460 patients in the treatment and control groups, respectively. The $I^2$ of TC was 67%, and therefore, the random-effects model was conducted and showed that the reduction of TC levels in NAFLD patients treated by bicyclol was significant (TC mmol/L: MD = −0.52; 95% CI −0.70 to −0.34; $P < 0.00001$) (Figure 5B).

### Grading the evidence

The evidence for the key outcomes was graded based on the limitations of precision, publication bias, risk of bias, and heterogeneity. The quality of evidence was either low or very low (Table 2).

## DISCUSSION

By performing a meta-analysis of 12 Chinese studies including 1008 patients, this review provided evidence that bicyclol, regardless of its application as a monotherapy or in combination with other drugs, exerts a positive effect on improving liver function (ALT, AST, and TBIL) and blood lipid levels (TG and TC). Although the bicyclol combination treatment for ALT levels and monotherapy for TG levels showed considerable heterogeneity, each trial among the included studies reported promising therapeutic effects on abnormal blood biomarker levels.

In the clinic, bicyclol is recommended for oral administration for up to 6 months. Although adverse events, such as gastrointestinal intolerance were sporadically reported in the control group in this meta-analysis (Table 1), these mild discomforts were not reported in the bicyclol-treated group, which agreed with the extremely mild and rare incidence of adverse reactions observed in long-term clinical practice.[13] Moreover, only three of the included studies concluded that the bicyclol intervention produced a higher total effective rate for fatty liver, which was mainly based on blood biomarker levels and B-model ultrasonography results. We thus evaluated the liver function and blood lipid biomarkers as the primary outcome, although liver histology is the gold standard and MRI has higher accuracy for assessing fatty liver.[17]

The pathogenesis of NAFLD is complex and is strongly associated (over 76%) with type 2 diabetes mellitus (T2DM),[41-43] patients with or without T2DM were thus included in this review. Additionally, the course of the disease varied among the included studies, and some studies did not report the patient's medical history; therefore, we limited the baseline ALT and TG levels to ensure the consistency of the included patients as much as possible. We also defined the treatment duration as at least 4 weeks, because NAFLD is a chronic disease and bicyclol is suitable for long-term oral administration. Although the use of bicyclol to treat

NAFLD is an off-label use, the Chinese guidelines of prevention and treatment for nonalcoholic fatty liver disease updated in 2018 recommend that hepatoprotectants are potentially complementary treatment measures for NASH patients with elevated aminotransferase levels or liver injury.[44] Compared with the intervention in the control group, including lifestyle changes and other drug treatments, the alleviation of abnormal blood biomarker levels by bicyclol is evident and consistent with its clinical practice.[21] Notably, subgroup analyses for ALT and TG levels, which were conducted when significant heterogeneity existed, also provided substantial evidence for its effect.

This review has to interpret the limitations of the low quality of the included studies, publication bias, and low grading of evidence. All the included studies were conducted in China, and many of them did not provide a description of specific methods of blinding and random allocation concealments. In terms of the outcome indicators, most articles lacked information on the blood glucose levels and insulin resistance index, and thus the results of the meta-analysis merely provide the effect of bicyclol on liver function and blood lipid indicators. Though the biomarkers AST, TC, and TBIL showed no publication bias, ALT and TG showed significant publication bias. We speculated that the heterogeneity and language bias contributed to this publication bias, and subgroup analysis was conducted. Additionally, when the degree of heterogeneity was large, Egger's tests did not have good properties.[45] Similarly, the low grading of evidence was mainly derived from the publication bias, risk of bias, and heterogeneity. Therefore, the results of the meta-analysis merely provide a reference based on the current evidence.

In conclusion, the present study presents the effectiveness of bicyclol monotherapy and/or combination therapy at ameliorating the altered liver function and blood lipid biomarkers in patients with NAFLD. This preliminary study predicts that bicyclol might be an alternative available drug to be explored for NAFLD therapy in the future. However, the conclusion also needs to be further verified in more well designed and implemented studies.

**Authors contribution**

Conceptualization, analysis, writing original draft, visualization: Hu Li; Validation of data and analysis: Nan-Nan Liu; Supervision, validation and writing draft: Zong-Gen Peng; Approval of final manuscript: all authors.

## Conflicts of Interest

All authors declare that no conflicts of interest.

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## Competing interests

None.

## Patient consent

Not required.

## Data sharing statement

All data are shown in the manuscript.

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**Table 1. Characteristics of the included studies**

| Study | Sample size | | Intervention | | Dose of bicyclol | Duration | Outcomes | Adverse events | |
|---|---|---|---|---|---|---|---|---|---|
| | Experimental | Control | Experimental | Control | | | | Experimental | Control |
| Liao 2011 | 30 | 30 | Bicyclol | Vitamin C | 50 mg, tid | 12 weeks | ①③④⑤⑥⑦⑧ | None | None |
| Liang 2007 | 45 | 38 | Bicyclol | UDCA | 25~50 mg, tid | 24 weeks | ④⑥⑦⑧ | - | - |
| Zhu 2005 | 36 | 29 | Bicyclol | Silymarin | 25~50 mg, tid | 24 weeks | ①④⑥⑦ | - | - |
| Yan 2017 | 30 | 30 | Bicyclol | DGEC | 50 mg, tid | 4 weeks | ④⑤⑦⑧ | None | None |
| Zhang 2012 | 60 | 60 | Bicyclol | LSI | 25 mg, tid | 24 weeks | ②④⑥⑦ | - | - |
| Gao 2011 | 25 | 25 | Bicyclol + PPC | PPC | 25~50 mg, bid | 6 months | ③④⑤⑥⑦⑧ | Weight loss | None |
| Ding 2009 | 42 | 30 | Bicyclol + PPC | PPC | 25~50 mg, bid | 6 months | ④⑤⑥⑦⑧ | Weight loss | None |
| He 2011 | 47 | 35 | Bicyclol + PPC | PPC | 25~50 mg, tid | 6 months | ③④⑥⑦⑧ | None | None |
| Li 2014 | 50 | 50 | Bicyclol + Metformin | Metformin | 25 mg, bid | 6 months | ②④⑤⑦⑧ | - | - |
| Zhang 2011 | 42 | 42 | Bicyclol + Metformin | Metformin | 25~50 mg, bid | 6 months | ②④⑤⑦⑧ | None | Nausea, poor appetite |
| Sun 2015 | 76 | 76 | Bicyclol + Metformin | Metformin | 25 mg, bid | 6 months | ②④⑤⑦⑧ | - | - |
| Guan 2013 | 40 | 40 | Bicyclol + Silibinin | Silibinin | 50 mg, tid | 12 weeks | ①④⑤⑦⑧ | None | None |

①Total effective rate;②BMI;③Adverse Events;④ALT;⑤AST;⑥TBIL;⑦TG;⑧TC.

UDCA, ursodeoxycholic acid; DGEC, diammonium glycyrrhizinate enteric-coated capsule; LSI, lifestyle intervention; PPC, polyene phosphatidylcholine

**Table 2. Grading the evidence for key comparisons**

| Outcomes | Corresponding risk (95% CI) * | No. of Participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|
| ALT | The mean ALT in the intervention groups was 24.89 standard deviations lower (32.63 to 17.15 lower) | 1008 (12 studies) | ⊕⊖⊖⊖ very low [1,2] | SMD -24.89 (-32.63 to -17.15) |
| AST | The mean AST in the intervention groups was 15.2 standard deviations lower (20.51 to 9.9 lower) | 658 (8 studies) | ⊕⊕⊖⊖ low [2] | SMD -15.2 (-20.51 to -9.9) |
| TBIL | The mean TBIL in the intervention groups was 1.72 standard deviations lower (2.72 to 0.72 lower) | 472 (6 studies) | ⊕⊕⊖⊖ low [2] | SMD -1.72 (-2.72 to -0.72) |
| TG | The mean TG in the intervention groups was 0.46 standard deviations lower (0.59 to 0.33 lower) | 1008 (12 studies) | ⊕⊖⊖⊖ very low [1,2] | SMD -0.46 (-0.59 to -0.33) |
| TC | The mean TC in the intervention groups was 0.52 standard deviations lower (0.7 to 0.34 lower) | 958 (11 studies) | ⊕⊕⊖⊖ low [2] | SMD -0.52 (-0.7 to -0.34) |

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

* The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and its 95% CI.

[1] Downgraded one level due to serious limitations in publication bias.

[2] Downgraded one level for including studies with high risk of bias

## FIGURE LEGENDS

**Figure 1. Flow diagram of data selection process.**

**Figure 2. The quality assessment of the included studies.**

The quality of randomized controlled trials was assessed as a 'high risk', 'low risk' or 'some concerns' to each item independently by two reviewers according to the most recently revised Cochrane risk of bias tool.

**Figure 3. The effect of bicyclol on total effective rate and BMI in patients with NAFLD.**

Review Manager 5.3 software was used to analyze the data. The odds ratio (OR) with its 95% confidence interval (95% CI) were estimated for total effective rate. Mean difference (MD) with its 95% CI was estimated for BMI. Heterogeneities were evaluated using the chi-square ($\chi^2$) and $I^2$ statistics. $I^2 < 50\%$ and $P > 0.10$ were deemed as homogeneous and the fixed-effect model was used. $P < 0.05$ was considered as statistically different between the experimental and control group.

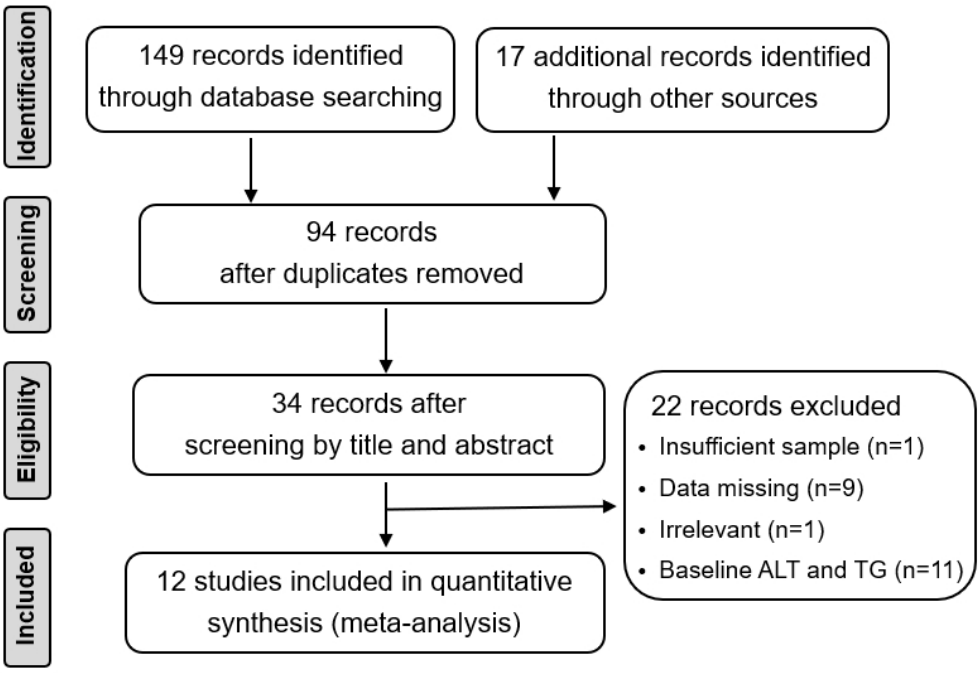
**Figure 4. The effect of bicyclol on ALT, AST and TBIL levels in patients with NAFLD.**

Review Manager 5.3 software was used to analyze the data. Mean difference (MD) with its 95% CI was estimated for continuous outcomes. Heterogeneities were evaluated using the chi-square ($\chi^2$) and $I^2$ statistics. ALT parameter was significantly heterogeneous ($I^2 \geq 75\%$ and $P < 0.10$) and subgroup analysis was conducted (A); the AST parameter was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used (B); the TBIL parameter was homogeneous ($I^2 < 50\%$ and $P > 0.10$) and the fixed-effect model was used (C). $P < 0.05$ was considered as statistically different between the experimental and control group.

**Figure 5. The effect of bicyclol on TG and TC levels in patients with NAFLD.**

Review Manager 5.3 software was used to analyze the data. Mean difference (MD) with its 95% CI was estimated for continuous outcomes. Heterogeneities were evaluated using the

chi-square ($\chi^2$) and $I^2$ statistics. The TG parameter was significantly heterogeneous ($I^2 \geq 75\%$) and subgroup analysis was conducted (A); the TC parameter was considered heterogeneous ($50\% \leq I^2 < 75\%$) and the random-effect model was used (B). $P < 0.05$ was considered as statistically different between the experimental and control group.

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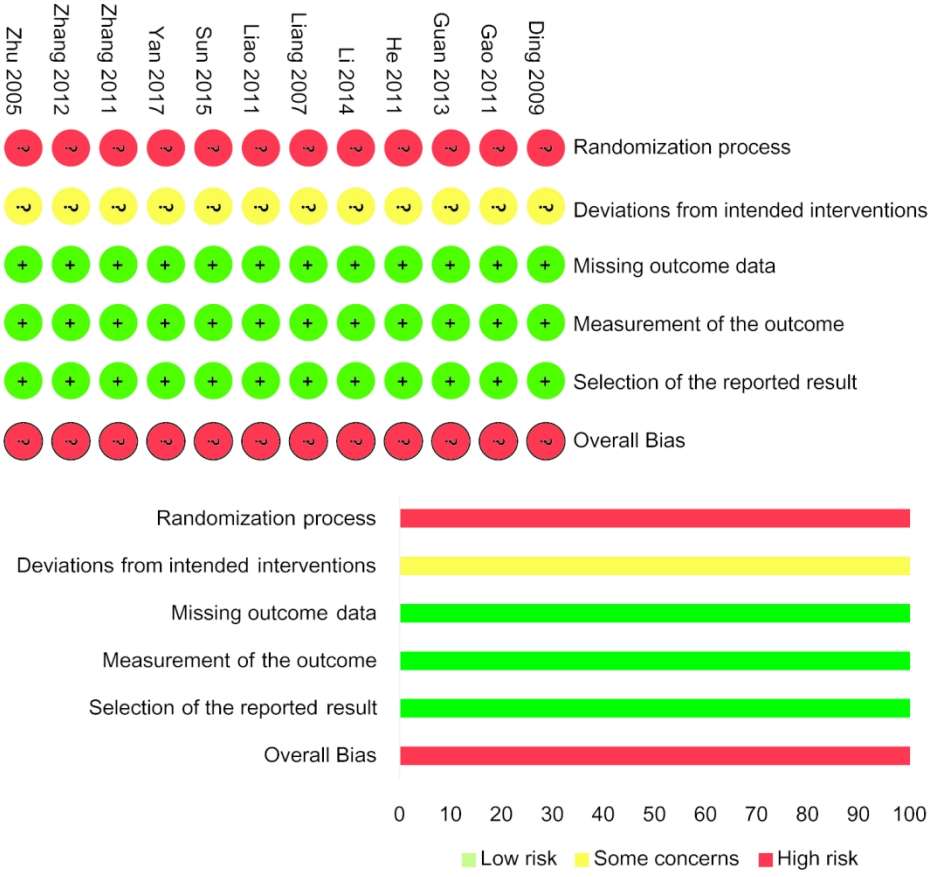
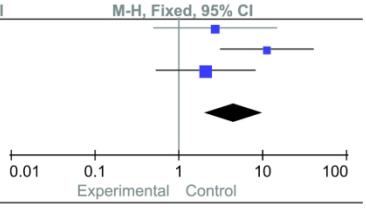


**A. Total effective rate**

| Study or Subgroup | Experimental Events | Experimental Total | Control Events | Control Total | Weight | Odds Ratio M-H, Fixed, 95% CI |
|---|---|---|---|---|---|---|
| Guan 2013 | 38 | 40 | 35 | 40 | 28.9% | 2.71 [0.49, 14.90] |
| Liao 2011 | 26 | 30 | 11 | 30 | 24.3% | 11.23 [3.10, 40.71] |
| Zhu 2005 | 32 | 36 | 23 | 29 | 46.8% | 2.09 [0.53, 8.25] |
| **Total (95% CI)** | | **106** | | **99** | **100.0%** | **4.49 [2.02, 9.95]** |
| Total events | 96 | | 69 | | | |

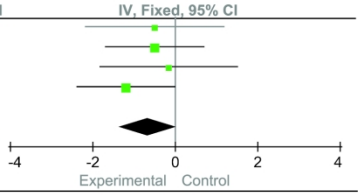
Heterogeneity: Chi² = 3.47, df = 2 (P = 0.18); I² = 42%
Test for overall effect: Z = 3.69 (P = 0.0002)

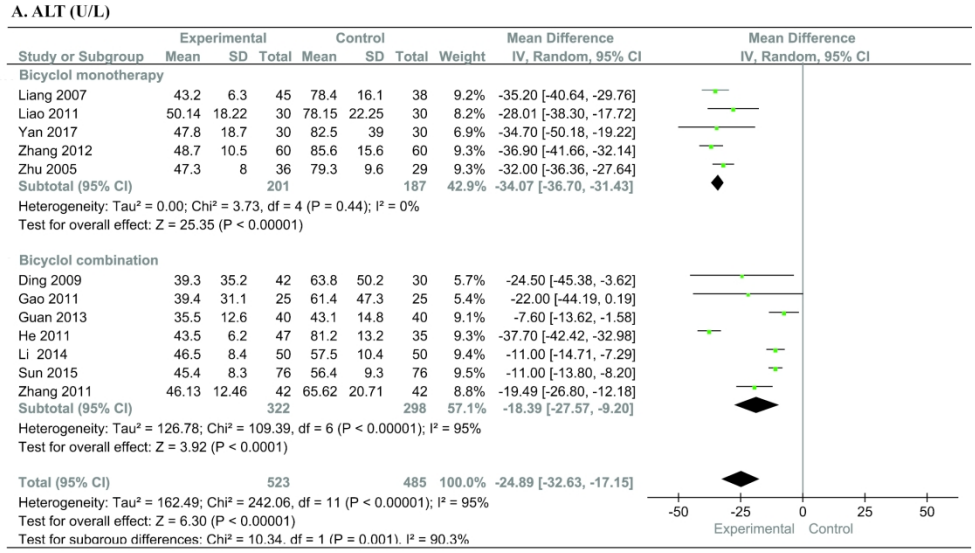


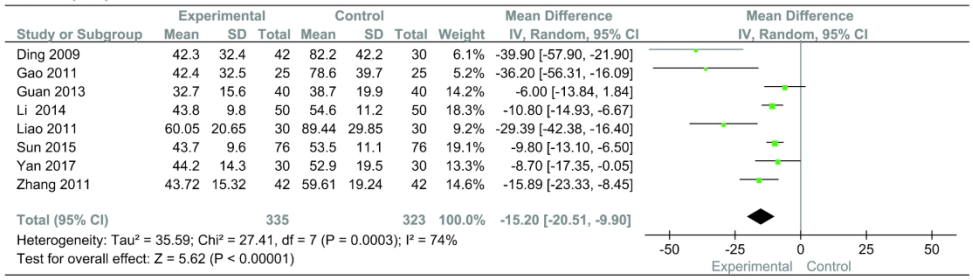

**B. BMI**

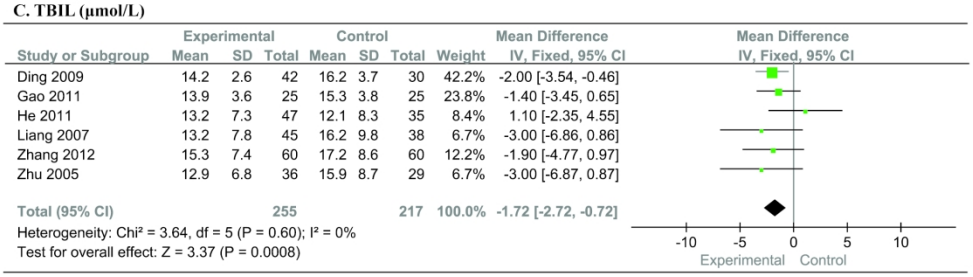
| Study or Subgroup | Experimental Mean | Experimental SD | Experimental Total | Control Mean | Control SD | Control Total | Weight | Mean Difference IV, Fixed, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Li 2014 | 25.1 | 4.7 | 50 | 25.6 | 3.9 | 50 | 16.8% | -0.50 [-2.19, 1.19] |
| Sun 2015 | 25 | 3.9 | 76 | 25.5 | 3.7 | 76 | 32.9% | -0.50 [-1.71, 0.71] |
| Zhang 2011 | 25.53 | 3.98 | 42 | 25.69 | 3.87 | 42 | 17.0% | -0.16 [-1.84, 1.52] |
| Zhang 2012 | 27.3 | 3.2 | 60 | 28.5 | 3.5 | 60 | 33.3% | -1.20 [-2.40, -0.00] |
| **Total (95% CI)** | | | **228** | | | **228** | **100.0%** | **-0.68 [-1.37, 0.02]** |

Heterogeneity: Chi² = 1.22, df = 3 (P = 0.75); I² = 0%
Test for overall effect: Z = 1.91 (P = 0.06)



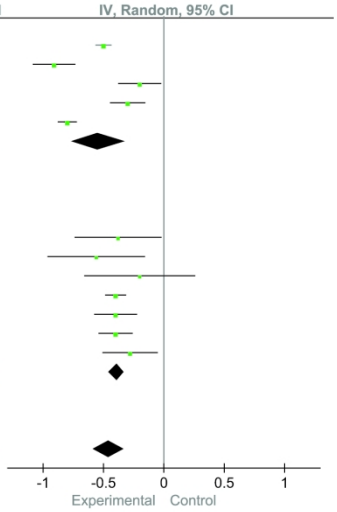


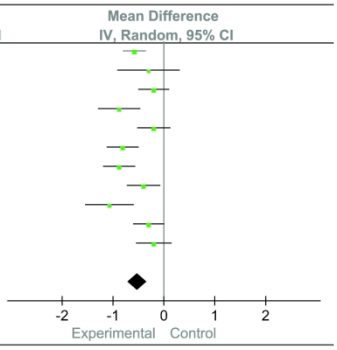
**A. TG (mmol/L)**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Bicyclol monotherapy | | | | | | | | |
| Liang 2007 | 1.7 | 0.2 | 45 | 2.2 | 0.1 | 38 | 10.4% | -0.50 [-0.57, -0.43] |
| Liao 2011 | 2.04 | 0.29 | 30 | 2.95 | 0.4 | 30 | 8.9% | -0.91 [-1.09, -0.73] |
| Yan 2017 | 1.7 | 0.3 | 30 | 1.9 | 0.4 | 30 | 8.9% | -0.20 [-0.38, -0.02] |
| Zhang 2012 | 1.9 | 0.3 | 60 | 2.2 | 0.5 | 60 | 9.4% | -0.30 [-0.45, -0.15] |
| Zhu 2005 | 1.58 | 0.1 | 36 | 2.38 | 0.2 | 29 | 10.3% | -0.80 [-0.88, -0.72] |
| Subtotal (95% CI) | | | 201 | | | 187 | 47.8% | -0.54 [-0.77, -0.32] |
| Heterogeneity: Tau² = 0.06; Chi² = 79.27, df = 4 (P < 0.00001); I² = 95% | | | | | | | | |
| Test for overall effect: Z = 4.81 (P < 0.00001) | | | | | | | | |
| Bicyclol combination | | | | | | | | |
| Ding 2009 | 2.69 | 0.62 | 42 | 3.07 | 0.86 | 30 | 5.8% | -0.38 [-0.74, -0.02] |
| Gao 2011 | 2.65 | 0.54 | 25 | 3.21 | 0.88 | 25 | 5.2% | -0.56 [-0.96, -0.16] |
| Guan 2013 | 2.4 | 1 | 40 | 2.6 | 1.1 | 40 | 4.5% | -0.20 [-0.66, 0.26] |
| He 2011 | 1.7 | 0.2 | 47 | 2.1 | 0.2 | 35 | 10.2% | -0.40 [-0.49, -0.31] |
| Li 2014 | 1.35 | 0.44 | 50 | 1.75 | 0.47 | 50 | 8.9% | -0.40 [-0.58, -0.22] |
| Sun 2015 | 1.34 | 0.43 | 76 | 1.74 | 0.46 | 76 | 9.5% | -0.40 [-0.54, -0.26] |
| Zhang 2011 | 1.38 | 0.45 | 42 | 1.66 | 0.61 | 42 | 8.0% | -0.28 [-0.51, -0.05] |
| Subtotal (95% CI) | | | 322 | | | 298 | 52.2% | -0.39 [-0.45, -0.33] |
| Heterogeneity: Tau² = 0.00; Chi² = 2.30, df = 6 (P = 0.89); I² = 0% | | | | | | | | |
| Test for overall effect: Z = 12.08 (P < 0.00001) | | | | | | | | |
| Total (95% CI) | | | 523 | | | 485 | 100.0% | -0.46 [-0.59, -0.33] |
| Heterogeneity: Tau² = 0.04; Chi² = 105.58, df = 11 (P < 0.00001); I² = 90% | | | | | | | | |
| Test for overall effect: Z = 6.95 (P < 0.00001) | | | | | | | | |
| Test for subgroup differences: Chi² = 1.72, df = 1 (P = 0.19), I² = 41.7% | | | | | | | | |




**B. TC (mmol/L)**

| Study or Subgroup | Experimental Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| Ding 2009 | 4.12 | 0.44 | 42 | 4.7 | 0.51 | 30 | 11.5% | -0.58 [-0.81, -0.35] |
| Guan 2013 | 4.6 | 1.5 | 40 | 4.9 | 1.3 | 40 | 5.3% | -0.30 [-0.92, 0.32] |
| He 2011 | 4.3 | 0.7 | 47 | 4.5 | 0.7 | 35 | 10.0% | -0.20 [-0.51, 0.11] |
| Li 2014 | 3.24 | 0.89 | 50 | 4.12 | 1.21 | 50 | 8.0% | -0.88 [-1.30, -0.46] |
| Liang 2007 | 4.3 | 0.7 | 45 | 4.5 | 0.8 | 38 | 9.6% | -0.20 [-0.53, 0.13] |
| Liao 2011 | 6.04 | 0.69 | 30 | 6.85 | 0.55 | 30 | 9.8% | -0.81 [-1.13, -0.49] |
| Sun 2015 | 3.13 | 0.78 | 76 | 4.01 | 1.18 | 76 | 9.8% | -0.88 [-1.20, -0.56] |
| Yan 2017 | 5.5 | 0.7 | 30 | 5.9 | 0.6 | 30 | 9.6% | -0.40 [-0.73, -0.07] |
| Zhang 2011 | 3.17 | 0.97 | 42 | 4.24 | 1.25 | 42 | 7.1% | -1.07 [-1.55, -0.59] |
| Zhang 2012 | 4.3 | 0.8 | 60 | 4.6 | 0.9 | 60 | 10.0% | -0.30 [-0.60, 0.00] |
| Zhu 2005 | 4.2 | 0.6 | 36 | 4.4 | 0.8 | 29 | 9.2% | -0.20 [-0.55, 0.15] |
| Total (95% CI) | | | 498 | | | 460 | 100.0% | -0.52 [-0.70, -0.34] |
| Heterogeneity: Tau² = 0.06; Chi² = 30.43, df = 10 (P = 0.0007); I² = 67% | | | | | | | | |
| Test for overall effect: Z = 5.68 (P < 0.00001) | | | | | | | | |



# Online supplementary materials

# The effect of bicyclol on blood biomarkers of NAFLD: a systematic review and meta-analysis

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## Online supplementary materials

## Online supplementary Methods: Search Strategy

### Pubmed

| No. | Query |
|---|---|
| 1 | Non alcoholic Fatty Liver Disease |
| 2 | NAFLD |
| 3 | nonalcoholic fatty liver |
| 4 | non-alcoholic fatty liver |
| 5 | Nonalcoholic Steatohepatitis |
| 6 | Nonalcoholic Steatohepatitides |
| 7 | 1 or 2 or 3 or 4 or 5 or 6 |
| 8 | bicyclol |
| 9 | 4,4'-bi-(1,3-benzodioxole)-5-carboxylic acid, 5'-(hydroxymethyl)-7,7'-dimethoxy-, methyl ester |
| 10 | 6-methoxycarbonyl-6'-hydroxymethyl-2,3,2',3'-bis(methylenedioxy)-4,4'- dimethoxybiphenyl |
| 11 | 8 or 9 or 10 |
| 12 | 10 and 11 |

### Embase

| No. | Query |
|---|---|
| #1 | non AND alcoholic AND fatty AND liver AND disease |
| #2 | nafld |
| #3 | nonalcoholic AND fatty AND liver |
| #4 | 'non alcoholic' AND fatty AND liver |
| #5 | nonalcoholic AND steatohepatitis |
| #6 | nonalcoholic AND steatohepatitides |
| #7 | #1 or #2 or #3 or #4 or #5 or #6 |
| #8 | bicyclol |
| #9 | #7 AND #8 |

### Cochrane Library

| ID | Search |
|---|---|
| #1 | MeSH descriptor: [Non-alcoholic Fatty Liver Disease] explode all trees |
| #2 | ((nonalcoholic or non-alcoholic) near (fatty liver or steatohepatitis)):ti,ab,kw or fatty liver or steatohepatitis:ti or nafld or nash:ti,ab,kw (Word variations have been searched) |
| #3 | #1 or #2 |
| #4 | bicyclol:ti,ab,kw (Word variations have been searched) |
| #5 | #3 and #4 |

**China National Knowledge Infrastructure database (CNKI)**

( ( (主题=非酒精性脂肪性肝病 或者 题名=非酒精性脂肪性肝病 或者 v_subject=中英文扩展(非酒精性脂肪性肝病) 或者 title=中英文扩展(非酒精性脂肪性肝病)) 或者 (主题=非酒精性脂肪性肝炎 或者 题名=非酒精性脂肪性肝炎 或者 v_subject=中英文扩展(非酒精性脂肪性肝炎) 或者 title=中英文扩展(非酒精性脂肪性肝炎)) ) 并且 (主题=双环醇 或者 题名=双环醇 或者 v_subject=中英文扩展(双环醇) 或者 title=中英文扩展(双环醇)) ) (模糊匹配),专辑导航：全部; 数据库：文献 跨库检索

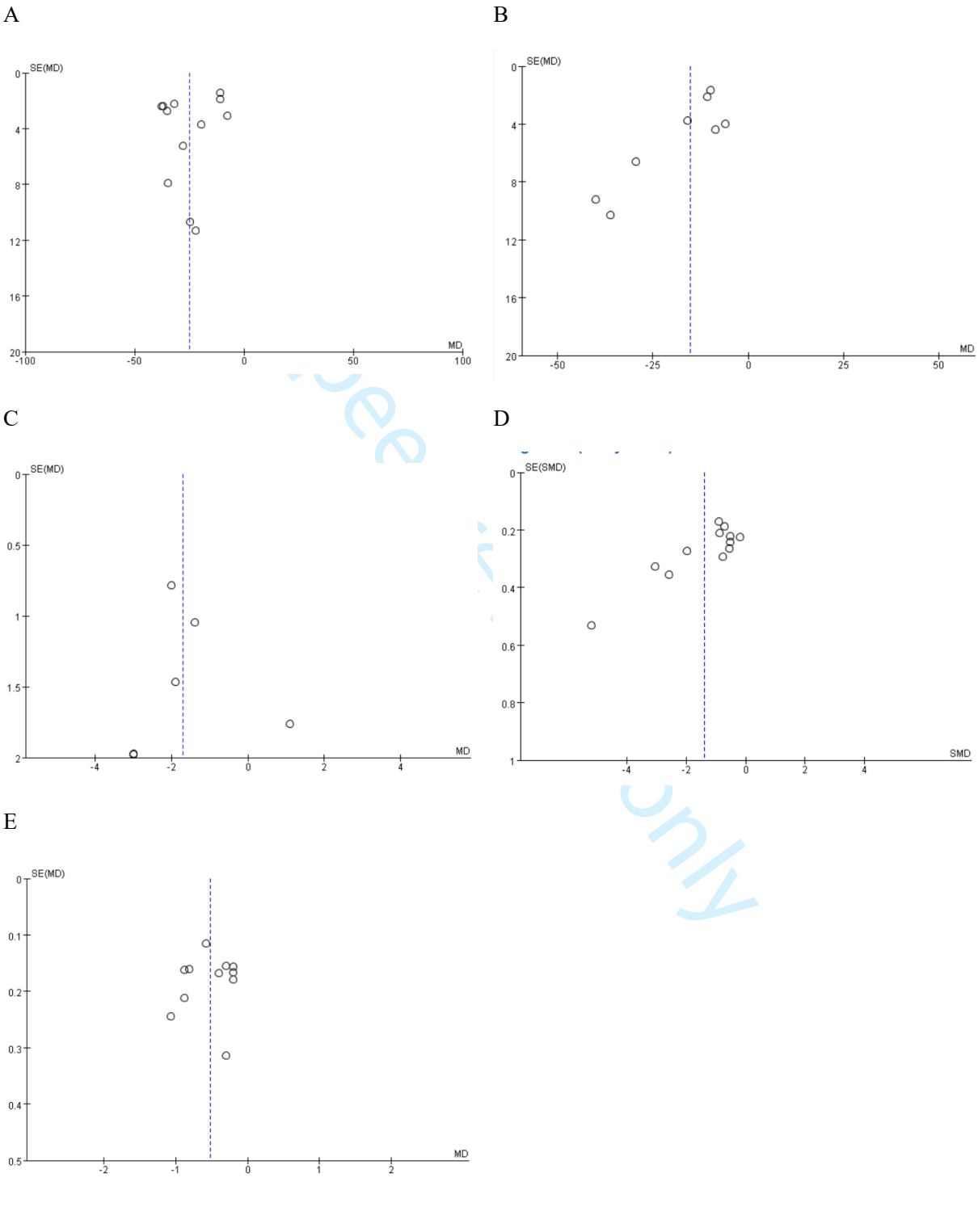
**VIP-Chinese scientific and technological journal database**

((题名或关键词=非酒精性脂肪性肝病 OR 题名或关键词=非酒精性脂肪性肝炎) AND 题名或关键词=双环醇)

**Wanfang digital periodical full-text database**

主题词扩展&中英文扩展：(主题:(非酒精性脂肪性肝病)+主题:(非酒精性脂肪性肝炎) )* *主题:(双环醇)

Figure S1. Funnel plot of alanine aminotransferase (A), aspartate transaminase (B), total bilirubin(C), triglyceride (D) and total cholesterol (E).



## Table S1. Support table for risk of bias judgement

| | Randomization process # | | | Deviations from intended interventions | | | | | | | Missing outcome data | | | | Measurement of the outcome | | | | | Selection of the reported result | | | Overall Bias * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study \ Unique ID | 1.1 | 1.2 | 1.3 | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 2.6 | 2.7 | 3.1 | 3.2 | 3.3 | 3.4 | 4.1 | 4.2 | 4.3 | 4.4 | 4.5 | 5.1 | 5.2 | 5.3 | |
| Ding 2009 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Gao 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Guan 2013 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| He 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Li 2014 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Liang 2007 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Liao 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Sun 2015 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Yan 2017 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Zhang 2011 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Zhang 2012 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |
| Zhu 2005 | Y | NI | Y | NI | NI | Y | N | NA | Y | NA | Y | NA | NA | NA | N | N | NI | N | NA | Y | N | N | High |

# comments of Randomization process: study "Liao 2011": Random number table method, no information about allocation concealment; others: Random number table method, no information about allocation concealment

***** Y/PY/PN/N/NI means Yes/Probably yes/Probably no/No/No information; Overall judgement for the result will be 'High' if one of the domains is judged at 'High' risk of bias.

1.1 Was the allocation sequence random?

1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?

1.3 Did baseline differences between intervention groups suggest a problem with the randomization process?

2.1 Were participants aware of their assigned intervention during the trial?

2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?

2.3 If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?

2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?

2.5 Was an appropriate analysis used to estimate the effect of assignment to intervention?

3.1 Were data for this outcome available for all, or nearly all, participants randomized?

4.1 Was the method of measuring the outcome inappropriate?

4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?

4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?

4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?

5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?

5.2 Is the numerical result being assessed likely to have been selected, on the basis of the results, from multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?

5.3 ... multiple eligible analyses of the data?

# PRISMA 2009 Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 4-5 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 5 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 5 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 6 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 5-6 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 5-6 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 5-6 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 7 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 6-7 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 7 |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 7 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 7 |

# PRISMA 2009 Checklist



| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 7 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 7 |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 8 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 8, Table1 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 8, Figure 2 |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 8-10 |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | 8-10 |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | 8, Figure 2 |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | 9-10 |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 11 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 12 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 12 |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 13 |

*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

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