Efficacy of different interventions for nonalcoholic fatty liver disease: A meta-analysis of life style modifications, silymarin, and medications

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ABSTRACT

Background and Objectives: To compare the effectiveness of silymarin or its combination with lifestyle modifications, Mediterranean hypocaloric diets, and medications for improving nonalcoholic fatty liver disease (NAFLD). **Methods and Study Design**: PubMed, Embase, Cochrane Library, Web of Science, and ClinicalTrails.gov were used to identify relevant studies. The treatment arm was silymarin or its combination with Mediterranean hypocaloric diets, medications, or lifestyle modifications. The comparators were placebo, Mediterranean hypocaloric diets, medications, and lifestyle modifications. **Results:** This meta-analysis included 25 studies with 2283 patients. Total cholesterol levels were reduced by silymarin+Mediterranean hypocaloric diets (SMD: -0.39 (-0.81, 0.03), p=0.072) or medications [SMD: -1.12(-1.67, -0.58), p<0.001]. Triglyceride levels were decreased by silymarin combined with the medication [SMD: -0.92(-1.98, 0.14), p=0.080]. Low-density lipoprotein cholesterol levels were reduced by silymarin alone [SMD: -0.25(-0.48, -0.03), p=0.027]. The combination of silymarin with Mediterranean hypocaloric diets [SMD: -0.47(-0.90, -0.04), p=0.031] or lifestyle modifications [SMD: -0.88(-1.09, -0.66), p<0.0001] decreased alanine aminotransferase levels. Aspartate aminotransferase levels were reduced by a combination of silymarin and lifestyle modifications [SMD: -0.72(-1.49, 0.05), p=0.061] or medications [SMD: -1.41(-2.24, -0.59), p=0.005]. Silymarin (2.5 times) or silymarin plus lifestyle modifications (39%) reduced the hepatic steatosis rate in patients with NAFLD. The silymarin use increased the rate of patients with adverse effects [RR:1.98 (1.11, 3.54)]; gastrointestinal problems were the most common adverse effects. **Conclusions**: Despite the overall advantages of therapies, different interventions showed different effects on markers in patients with NAFLD. These results highlight the need for more research to fully comprehend the features of the intervention.

Key words: **Lifestyle modification, silymarin, Nonalcoholic fatty liver disease, meta-analysis. Mediterranean hypocaloric diet.**

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) was first linked to excess liver fat in the absence of substantial alcohol consumption.[1] Then, the terms "nonalcoholic fatty liver disease (NAFLD)" and "metabolic associated fatty liver disease (MAFLD)" were developed to more accurately describe the disease process.[2,3] The term MAFLD considers metabolic dysregulatory variables[3,4], such as type 2 diabetes mellitus (T2DM), overweight/obesity, and elevated body mass index (BMI), that may be varied in relation to ethnic groups [BMI ≥25 kg/m^2 in Caucasians or BMI ≥23 kg/m^2 in Asians)].[4]

Nonalcoholic fatty liver disease affects 25.2% of the global population[5] and is defined as histologic evidence of hepatic steatosis alone. Obesity, dyslipidemia [high plasma triglyceride (TG) levels, low plasma concentrations of high-density lipoprotein cholesterol (HDL-C)], insulin resistance, glucose intolerance, T2DM, and hypertension are metabolic disorders that manifest hepatically as NAFLD.[6,7] Furthermore, elevated oxidative stress can cause NAFLD by increasing lipid peroxidation and reactive oxygen species formation in hepatocytes.[8] This, in turn, causes lipotoxicity and mitochondrial dysfunction.[8] The most prevalent biochemical anomaly in NAFLD is elevated blood levels of transaminases, such as aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT).[7] From basic steatosis to cirrhosis as the final stage of liver disease, the pathogenesis of NAFLD is varied and complex.[4] The liver undergoes metabolic-related alterations due to a variety of circumstances.[4] Overnutrition can cause dysbiosis in the gastrointestinal system, and pro-inflammatory reactions can also be triggered by the liver's transfer of microbial-associated molecular processes.[4] Genetic vulnerabilities to NAFLD development are increased by SNPs in PNPLA3 (rs738409, I148M).[9] Within hepatocytes, this protein is located close to lipid droplets.[10]

Effective medication therapy is currently lacking, although the prevalence of NAFLD is steadily growing.[6] In patients with NAFLD, the two main causes of death are cardiovascular disease and cancer.[11] Thus, the primary objective of treatment is to lower the risk of cardiovascular disease, cancer, hepatic steatosis, and inflammation. It is important to remember that most liver disease treatments are not yet approved. As an alternative, probiotics (Lactobacilli, Lactobacillus bulgaricus, and Streptococcus thermophilus) decrease liver enzymes but not liver steatosis in patients with NAFLD.[12] Moreover, Mediterranean diets and taking supplements

containing probiotics, antioxidants, polyphenols, or certain nutrients with hepatoprotective effects may help lower the progression of hepatic steatosis and improve liver enzyme levels in NAFLD.[6,13] Probiotics are not, however, typically advised for the treatment of patients with NAFLD.[4] Every patient with NAFLD must undergo lifestyle modification as an additional intervention. Within a year following a suggested lifestyle modification, Vilar-Gomez et al. demonstrated a decrease in hepatic steatosis and inflammation.[14] Obese people with NAFLD benefit most from lifestyle changes. [6,14,15] Hepatic fibrosis and inflammation can be reversed with a consistent weight decrease of approximately 7%[14,15]; however, there is no evidence to support the idea that weight loss helps lean patients.[6,14,15] In addition to physical activity to reduce liver fat, patients are advised to abstain from alcohol consumption and excessive fructose intake.[4] However, because patients require a change in behavioral patterns, regular physical activity and long-term weight loss are difficult.[4,14,15] Other drugs that may reduce liver fat include sodium–glucose transporter 2 (SGLT2) inhibitors, such as empagliflozin.[16,17] After receiving norursodeoxycholic acid for 12 weeks, a study found that ALT decreased.[16] As an agonist of the peroxisome proliferator-activated receptor-γ, pioglitazone possesses anti-inflammatory and antifibrotic activities and can mitigate liver fibrosis and NAFLD.[16,17] Another medication is phosphatidylcholine, which protects the liver from damage caused by oxidative stress.[18] Nevertheless, these effects can be reversed if drug use is stopped. Therefore, treating NAFLD, particularly incurable NASH, is an urgent medical need.

Silymarin has been found as a therapeutic option for NAFLD as an additional possible therapy.[6,19] Milk thistle (Silybum marianum) is a source of silymarin, a strong liver-tropic antioxidant.[19] Among its many hepatoprotective benefits are enhanced protein synthesis, cell regeneration, antioxidant activity, and anti-inflammatory and antifibrotic abilities.[6] By describing the effects of silymarin on blood biochemical indices, liver enzymes, fatty liver scores, and BMI, this meta-analysis examined the effectiveness of silymarin either alone or in conjunction with lifestyle changes or medications in patients with NAFLD.

MATERIALS AND METHODS

Methods

The Preferred Reporting Items for Systemic Reviews and Meta-Analysis (PRISMA) guidelines were followed in the conduct of this meta-analysis. This systematic review is registered as PROSPERO CRD42025633115. Ethical approval for this study was waived by the Ethics Committee of Philippine Women's University because this study was a meta-analysis without patient involvement.

Search strategy

PubMed, Embase, the Cochrane Library, Web of Science, clinicaltrails.gov, and the China National Knowledge Infrastructure were the sources from which pertinent articles were obtained. Records were retrieved from the databases using search terms pertaining to "silymarin", "nonalcoholic fatty liver disease", "lifestyle modifications", "mediterranean hypocaloric diet", and "anti-diabetic medication".

Study selection

Three researchers separately searched for and chose studies. After screening the titles and abstracts of the identified papers, the complete texts of possible studies were further evaluated in accordance with the eligibility requirements. Three authors compared the selected entire texts, and any differences or disagreements were discussed and settled.

Inclusion and exclusion criteria

Clinical trials were accepted if they met the following requirements: (1) Study design: RCTs; (2) Participants: NAFLD patients of any age, gender, or race; (3) Intervention: silymarin or silymarin complex, silymarin + lifestyle change, silymarin + Mediterranean hypocaloric diets, and silymarin + medicines; (4) Comparators: lifestyle modification, placebo, lifestyle modification, and medications (anti-diabetic drugs such as pioglitazone, diisopropylamine dichloroacetate (vitamin B15), polyene phosphatidylcholine (a more bioavailable form of phosphatidylcholine); (5) Outcomes: blood biochemical indicators [total cholesterol (TC), TG, HDL-C, low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), and

homeostatic model assessment of insulin resistance (HOMA- IR); liver injury indicators [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)]; liver histological indicators (hepatic steatosis grade); anthropometric indicator (body mass index, BMI); and (6) adverse effects (AEs), including headache, musculoskeletal disorders, fatigue, and difficulties with the heart, lungs, and gastrointestinal tract.

Exclusion criteria

Studies that (1) reported alcoholic steatohepatitis, alcoholic fatty liver, cirrhosis, or liver cancer; (2) received additional medication(s) or had a genetic predisposition (single nucleotide polymorphisms); (3) underwent liver transplantation; and (4) were based on conference papers, abstracts, non-original research, case reports, and non-peer-reviewed articles (e.g., conference materials and thesis) were excluded.

Quality assessment

Two authors independently evaluated the titles and abstracts of the obtained records to avoid irrelevant research. The full texts of selected publications were individually evaluated based on the eligibility criteria. The risk of bias in eligible RCTs was assessed using the Quality in Prognostic Studies (QUIPS) tool.[20] According to the Cochrane Methods Prognosis Group, the QUIPS tool is useful for prognostic research because it tackles potential biases in studies.[20] The six domains of the QUIPS tool are study confounding, study participation, study attrition, statistical analysis and reporting, outcome measurement, and prognostic factor measurement.[20] Low bias rates are observed in studies that provide comprehensive descriptions of the type of device, method of administration, power analysis, use of suitable statistical analysis, consideration of confounding variables, and interpretation of results (including bias causes). The overall risk of bias in these studies is moderate when there is some potential for bias but not enough to cause ambiguous findings. Cases with considerable biases are marked by numerous biases and serious faults that can render the judgment erroneous. At least two major errors in the design, analysis, or reporting process should be noted in high-risk research.[20] Two team members screened each study and determined its level of bias, which was classified as low, moderate, or high. To settle any possible disputes, an extra author

was engaged in the review process. All studies, regardless of possible bias, were included.

Data synthesis and analysis

Meta-analyses were conducted using the Meta-mar tool. Given the various techniques used to quantify outcomes in each original investigation, continuous and categorical variables were aggregated using the standard mean difference (SMD) and odds ratio (OR), respectively. The statistical heterogeneity between studies was evaluated using the Cochran-Q test (I^2 statistic), which is the percentage of variance in the meta-analysis due to heterogeneity. The effects were estimated differently for each study, partly because of heterogeneity and random sampling errors.[21] When heterogeneity substantially surpasses within-study sampling and measurement variability, the I^2 statistic reaches its maximum value of 1. If the I^2 was less than 50%, a fixed-effects model was used; otherwise, a random-effects model was used. Subgroup analyses were conducted to identify the source of heterogeneity. All statistical tests were considered significant at $p<0.05$. A funnel plot was used to measure publication bias using Egger's regression test. Meta-regression analysis was also used to assess the heterogeneity (Q statistic) among the outcomes. The impact of each study on the total pooled effect estimate was assessed using a leave-one-out meta-analysis (OpenMeta-Analyst software). By investigating how the pooled effect estimate would have differed if each research were methodically eliminated, this methodology assessed the robustness of the meta-analysis findings.

RESULTS

Study selection

The search approach yielded a total of 1539 articles (until November 12, 2024), and the meta-analysis comprised 25 trials[7,22-40] (Figure 1).

Basic characteristics and quality assessment

The meta-analysis included 25 trials and 2283 patients. Supplementary Table 1 summarizes the features of the included studies. The QUIPS tool indicated that 21 out of 25 studies had a

medium to high overall risk (Supplementary Table 2). The most problematic domains were statistical analysis and reporting and study confounding.

Total cholesterol (TC) levels

In patients with NAFLD, the overall effect of therapies decreased the TC levels (SMD: -0.84 (-1.25, -0.44), p=0.0005; Figure 2). A significant difference between the subgroups was found by the test for subgroup differences (p<0.01), indicating that the subgroups contributed differently to the total effect. There was significant heterogeneity among the studies included in this meta-analysis (I^2=94%, p<0.01; Figure 2). Publication bias was not present in the studies according to the Funnel plot analysis using Egger's regression test (t=1.18, p=0.257, Supplementary Figure 1A). the meta-regression analysis showed high heterogeneity between outcomes (Q=185.291; p<0.0001; Supplementary Figure 1A).

Silymarin + medications (SMD: -1.12 (-1.67, -0.58), p<0.001) decreased TC, according to subgroup analysis. Total cholesterol levels were unaffected by using silymarin alone (SMD: -0.15 (-0.38, 0.07), p=0.183) or silymarin with lifestyle adjustment (SMD: -0.08 (-3.18, 1.56), p=0.280; Figure 2). Regarding the effect of therapies on TC, the sensitivity analysis indicated a moderate variability in the pooled effect estimate for the silymarin subgroup, revealing a moderate sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 4A). Regarding the silymarin + medication subgroup, there was no variability in the pooled effect estimate, indicating a lack of sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 4A). The leave-one-out meta-analysis for the silymarin + lifestyle modification subgroup was not conducted because only three studies were included in the meta-analysis.

Blood TG levels

The overall effect of therapies decreased the TG levels in patients with NAFLD (SMD: -0.69 (-1.25, -0.13), p=0.020; Figure 3). Subgroups did not have any impact on the total effect observed, according to the test for subgroup differences, which showed no significant difference between subgroups (p=0.23). The studies included in this meta-analysis showed a

significant degree of heterogeneity (I^2=97%, p<0.01; Figure 3). The studies had a modest publication bias, according to the Funnel plot analysis using Egger's regression test (t= -2.94, p<0.01; Supplementary Figure 1B). The meta-regression analysis showed high heterogeneity between outcomes (Q=503.505; p<0.0001; Supplementary Figure 1B).

According to subgroup analysis, silymarin + medication moderately decreased TG levels (SMD: -0.92 (-1.98, 0.14), p=0.081; Figure 3). The effects of silymarin (SMD: -0.20 (-0.79, 0.38), p=0.345) and silymarin + lifestyle modification (SMD: -0.69 (-1.87, 0.51), p=0.168) on TG levels were not significant (Figure 3). Regarding the effect of therapies on TGs, the sensitivity analysis indicated a variability in the pooled effect estimate for the silymarin and silymarin + medication subgroups, revealing the sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 4B).

Blood HDL-C levels

The HDL-C levels in patients with NAFLD were not affected by the overall effect of the therapies (SMD: 0.28 (-0.21, 0.76), p=0.212; Figure 4). There was no significant difference between the subgroups, according to the test for subgroup differences (p=0.34), indicating that the subgroups had no bearing on the result that was achieved. There was a substantial degree of heterogeneity among the studies that were part of this meta-analysis (I^2=69%, p<0.01; Figure 4). Egger's regression test, which was used to analyze the funnel plot, showed that there was no publication bias in the studies (t=0.42, p=0.691; Supplementary Figure 1C). Meta-regression analysis showed high heterogeneity between outcomes (Q=17.234; p=0.004; Supplementary Figure 1C).

Silymarin (SMD: 0.15 (-0.07, 0.38), p=0.188) and silymarin + lifestyle modification (SMD: 0.56 (-1.28, 2.39), p=0.320) did not affect HDL-C levels, according to subgroup analysis (Figure 4). Regarding the effect of therapies on HDL-C, the sensitivity analysis indicated a moderate variability in the pooled effect estimate for the silymarin subgroup, revealing a moderate sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 5A). The leave-one-out meta-analysis for the silymarin + lifestyle

modification subgroup was not conducted because only three studies were included in the meta-analysis.

Blood LDL-C levels

In patients with NAFLD, the overall effect of interventions had no effect on LDL-C levels (SMD: -1.75 (-5.45, 1.96), p=0.292; Figure 5). There were no significant differences between the subgroups according to the test for subgroup differences (p=0.31), indicating that the subgroups had no effect on the result achieved. There was significant heterogeneity among the studies included in this meta-analysis (I^2=93%, p<0.01; Figure 5). Egger's regression test-based Funnel plot analysis revealed that the studies had publication bias (t= -5.05, p=0.004; Supplementary Figure 1D). Meta-regression analysis showed high heterogeneity between outcomes (Q=84.597; p<0.0001; Supplementary Figure 1D).

According to subgroup analysis, silymarin decreased LDL-C levels (SMD: -0.25 (-0.47, -0.03), p=0.027). Silymarin plus lifestyle changes (SMD: -3.89 (-19.43, 11.66), p=0.395) did not affect LDL-C levels (Figure 5). Regarding the effect of therapies on LDL-C, the sensitivity analysis indicated a variability in the pooled effect estimate for the silymarin subgroup, revealing the sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 5B). The leave-one-out meta-analysis for the silymarin + lifestyle modification subgroup was not conducted because only three studies were included in the meta-analysis.

Fasting blood glucose (FBG) levels

The FBG levels in patients with NAFLD were unaffected by the overall effect of the therapies [SMD: -0.09 (-0.25, 0.08)], p=0.325; Figure 6). There was no significant difference between the subgroups, according to the test for subgroup differences (p=0.68), implying that the subgroups had no effect on the result obtained. Heterogeneity was low among the studies included in this meta-analysis (I^2=0.0%, p=0.92; Figure 6). There was no publication bias in the studies according to the Funnel plot analysis using Egger's regression

test (t= -0.59, p=0.582; Supplementary Figure 2A). The meta-regression analysis showed no heterogeneity between outcomes (Q=1.140; p=0.768; Supplementary Figure 2A).

According to the subgroup analysis, silymarin (SMD: -0.05 (-0.30, 0.20), p=0.714) and silymarin + other therapies (Mediterranean hypocaloric diet, lifestyle modification, or medications) (SMD: -0.12 (-0.35, 0.11), p=0.672) had no effect on FBG (Figure 6). Regarding the effect of silymarin + other therapies on FBG, there was no variability in the pooled effect estimate, indicating a lack of sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 5C). The leave-one-out meta-analysis for the silymarin subgroup was not conducted because only three studies were included in the meta-analysis.

Homeostatic model assessment of insulin resistance (HOMA-IR)

The HOMA-IR in patients with NAFLD was not affected by the overall effect of the therapies (silymarin, silymarin + Mediterranean hypocaloric diet, or Silymarin + medications) (SMD: -0.36 (-1.02, 0.29), p=0.174; Figure 7). The studies included in this meta-analysis showed a high degree of heterogeneity (I^2=64%, p=0.04; Figure 7). Meta-regression analysis showed a lack of heterogeneity between outcomes (Q=0.083; p=0.773; Supplementary Figure2B). Regarding the effect of therapies on HOMA-IR, the sensitivity analysis indicated a variability in the pooled effect estimate, revealing the sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 5D).

Body mass index (BMI)

The overall effect of the interventions decreased BMI in patients with NAFLD (SMD: -0.19 (-0.39, 0.00), p<0.05; Figure 7). Subgroups did not affect the overall effect achieved (p=0.78). There was low heterogeneity among the studies included in this meta-analysis (I^2=33%, p=0.18; Figure 7). Publication bias was not present in the studies, according to the Funnel plot analysis using Egger's regression test (t= -0.20, p=0.848; Supplementary Figure 2C). The meta-regression analysis showed no heterogeneity between outcomes (Q=0.412; p=0.938; Supplementary Figure 2C).

Silymarin + lifestyle modification (SMD: -0.16 (-0.48, 0.16), p=0.331) and other therapies (silymarin, silymarin + Mediterranean hypocaloric diet, or silymarin + medication) had no effect on BMI (SMD: -0.21 (-0.46, 0.03), p=0.343; Figure 7). Regarding the effect of therapies on BMI, there was no variability in the pooled effect estimate, indicating a lack of sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 5E). The leave-one-out meta-analysis for the silymarin + lifestyle modification subgroup was not conducted because only three studies were included in the meta-analysis.

Alanine transaminase (ALT) levels

The overall effect of the therapies decreased the ALT levels in patients with NAFLD (SMD: -0.73 (-1.25, -0.22), p=0.008; Figure 8). According to subgroup analysis, subgroups had no effect on the total effect observed (p=0.77; Figure 8). The studies included in this meta-analysis showed a significant degree of heterogeneity (I^2=97%, p<0.01; Figure 8). Publication bias was not present in the studies, according to the Funnel plot analysis using Egger's regression test (t= -1.07, p=0.297; Supplementary Figure 2D). The meta-regression analysis showed heterogeneity between outcomes (Q=637.683; p<0.0001; Supplementary Figure 2D).

Silymarin + lifestyle modification (SMD: -0.88 (-1.09, -0.66), p<0.0001) or silymarin (SMD: -0.64 (-1.55, 0.26), p=0.120) had lower ALT levels than the comparator group, according to the subgroup analysis (Figure 8). The ALT levels in patients with NAFLD were unaffected by silymarin + medication (SMD: -0.69 (-1.91, 0.519), p=0.223; Figure 8). Regarding the effects of silymarin, silymarin + lifestyle modification, and silymarin + medication subgroups on ALT, the sensitivity analysis indicated a variability in the pooled effect estimate, revealing the sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 6).

Aspartate aminotransferase (AST) levels

The AST levels in patients with NAFLD were decreased by the overall effect of the therapies (SMD: -1.06 (-1.54, -0.58), p=0.0002; Figure 9). The subgroup analysis showed no significant differences (p=0.32), indicating that the subgroups did not affect the overall effect (Figure 9). The studies included in this meta-analysis showed a significant degree of heterogeneity (I^2=95%, p<0.01; Figure 9). Publication bias was not present in the studies according to the Funnel plot analysis using Egger's regression test (t=0.71, p=0.487; Supplementary Figure 3A). The meta-regression analysis showed heterogeneity between outcomes (Q=360.475; p<0.0001; Supplementary Figure 3A).

Silymarin + medication (SMD: -1.41 (-2.24, -0.59), p=0.005) or silymarin + lifestyle change (SMD: -0.72 (-1.49, -0.05), p=0.061) decreased AST levels compared with the comparator group (Figure 9). The AST levels were unaffected by silymarin (SMD: -0.88 (-2.45, 0.68), p=0.193; Figure 9). Regarding the effects of silymarin, silymarin + lifestyle modification, and silymarin + medication on AST, the sensitivity analysis indicated a variability in the pooled effect estimate, revealing the sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 7A).

Fatty liver score

The fatty liver score in patients with NAFLD was moderately decreased by the overall effect of the therapies (Silymarin + medication, Mediterranean hypocaloric diet, or Silymarin) (SMD: -0.59 (-1.51, 0.33), p=0.133; Figure 10). The studies included in this meta-analysis showed a high degree of heterogeneity (I^2=76%, p<0.01; Figure 10). Meta-regression analysis showed heterogeneity between outcomes (Q=8.48; p=0.004; Supplementary Figure 3B). Regarding the effects of therapies on fatty liver score, the sensitivity analysis indicated a variability in the pooled effect estimate, revealing the sensitivity of the meta-analysis to the exclusion of individual studies (Supplementary Figure 7B).

Hepatic steatosis rate

The overall effect of the therapies demonstrated a 55% decrease in the fatty liver rate in patients with NAFLD (SMD: 1.55 (1.08, 2.23), p=0.018; Figure 10). There was no difference between the subgroups according to the subgroup differences (p=0.58; Figure 10). This implies that the subgroups had no effect on the results achieved. There was low heterogeneity across the studies included in this meta-analysis (I^2=0.0%, p=0.84; Figure 10). There was no publication bias according to the Funnel plot analysis using Egger's regression test (t=0.37, p=0.729; Supplementary Figure 3C). The meta-regression analysis showed lack of heterogeneity between outcomes (Q=1.235; p=0.872; Supplementary Figure 3C).

Silymarin + lifestyle modification (RR: 1.39 (0.91, 2.14), p=0.130) moderately decreased the hepatic steatosis rate by 39% (Figure 10). Other therapies, including silymarin or silymarin + Mediterranean hypocaloric diet, decreased the rate of hepatic steatosis by 103% (RR: 2.03 (1.03, 4.00); Figure 10).

Adverse effects

The use of silymarin increased the rate of patients with AEs by 98%, according to the meta-analysis (RR: 1.98 (1.11, 3.54), p=0.002; Figure 11A). However, 10 studies found no AEs among patients receiving medication, and only two trials documented AEs related to silymarin use. Among the silymarin group, gastrointestinal problems were the most frequent AEs (Figure 11B).

DISCUSSION

This meta-analysis demonstrated that interventions improved biochemical marker levels and hepatic steatosis rates in patients with NAFLD. However, subgroup analysis found that individual therapies, such as silymarin alone or in combination with lifestyle changes, Mediterranean hypocaloric diet and medications, had no consistent effect on patients with NAFLD.

This meta-analysis found that therapies including silymarin lowered BMI, biochemical indices (e.g., TC, TG, LDL-C, ALT, and AST), and improved HDL-C and hepatic steatosis rates in patients with NAFLD, consistent with earlier research.[41] Dyslipidemia (high TC, TG, and LDL-C levels) is a significant risk factor for NAFLD.[42] Furthermore, higher serum AST and ALT levels are the most common biochemical abnormalities in NAFLD.[42,43] Currently, no specific medications have been licensed for NAFLD/NASH therapy; additionally, pharmacological treatments are only used in advanced NASH patients with fibrosis.[7] In recent years, there has been an increasing focus on the therapeutic effects of numerous nutraceuticals for treating NAFLD.[7] In this meta-analysis, we found that all therapies, including silymarin, improved the aforementioned metrics in patients with NAFLD. Silymarin is a liver-tropic antioxidant derived from milk thistle (Silybum marianum). This extract contains many antioxidants, the most abundant of which are silibin A and B and the flavonoid taxifolin.[19] Silibinin reduces oxidative stress and inhibits PARP activation, thereby replenishing the NAD^+ pool.[44] A mouse model demonstrated that silymarin reduced hepatic steatosis by regulating lipid metabolism and oxidative stress while simultaneously benefiting the circulatory system.[45] Silymarin's hepatoprotective effects include antioxidant activity, cell regeneration, enhanced protein synthesis, and anti-inflammatory and antifibrotic characteristics.[46] Furthermore, silymarin reduces the mRNA expression of enzymes in charge of de novo lipogenesis, such as sterol-regulatory element binding protein (SREBP1c), fatty acid synthetase, and acetyl-CoA carboxylase 1, which phosphorylates AMP-activated protein kinases in obese mice with diabetes and NAFLD.[6] As a result, silymarin can enhance lipid metabolism and may be used as a therapy for NAFLD.[6]

This meta-analysis found that therapies (all trials included silymarin) had no effect on FBG levels or HOMA-IR in patients with NAFLD. The initial stage of NAFLD is fat deposition in the liver, which causes insulin resistance.[47] Insulin resistance and glucose metabolic dysfunction are common clinical signs of NAFLD, with increased blood glucose levels present in 70-80% of patients with NAFLD.[6] Silymarin has been demonstrated to lower blood glucose, insulin, and HOMA-IR, although the precise mechanism by which it affects glucose levels is unknown.[48] Because silymarin is a potent antioxidant, its influence on glucose levels could be mediated by reducing lipid peroxidation.[6] Silymarin was found to be beneficial in reducing insulin resistance in NAFLD, mostly by lowering visceral fat,

increasing lipolysis, and suppressing gluconeogenesis.[49] However, we found that treatment with silymarin did not affect FBG levels or HOMA-IR. This finding highlights the need for additional studies into the effects of silymarin on FBG levels and insulin resistance in patients with NAFLD.

In the following stage, we aimed to determine the effect of silymarin (monotherapy) and its combination with other therapies on NAFLD to better understand potential interactions or synergies between silymarin and other treatments. We found that silymarin monotherapy did not reduce TC, TG, or AST levels, whereas its combination with certain medications (including diisopropylamine dichloroacetate, polyene phosphatidylcholine, lovastatin, pioglitazone, or atorvastatin) significantly reduced TC, TG, and AST levels when compared with the comparator group that received these medications alone. Furthermore, silymarin alone did not reduce ALT levels, but when combined with Mediterranean diet or lifestyle modification, ALT levels were lower than in the group that received only Mediterranean diet or lifestyle modification. A randomized trial of 99 individuals found that silymarin administered 700 mg three times a day for 48 weeks did not diminish NAFLD.[50] NAFLD is a multisystem illness; thus, a combination therapy may be advantageous for patients with NAFLD.[6] A randomized clinical trial found that silymarin, vitamin E, and a low-calorie diet decreased liver enzyme levels and non-invasive NAFLD.[51] A study using overweight/obese patients with NAFLD that were treated with a Mediterranean-hypocaloric diet combined with nutraceutical supplementation (Vitamin E, L-glutathione, silymarin, and hepato-active compounds) showed that the use of specific supplements can improve the efficacy of interventions.[7] These data suggest that the combination of silymarin and other therapies, such as Mediterranean diet, lifestyle modification, and medication, may affect the success of NAFLD treatment.

We also found that silymarin monotherapy, silymarin plus Mediterranean diet, and silymarin plus lifestyle modification decreased hepatic steatosis rates by 153% (two studies), 77% (one trial), and 39% (three studies), respectively. As previously stated, silymarin may reduce hepatic steatosis by regulating lipid metabolism and oxidative stress while also benefiting the circulatory system.[52] Silymarin's hepatoprotective effects include antioxidant activity, cell regeneration, enhanced protein synthesis, and anti-inflammatory and antifibrotic characteristics.[6] In addition, silymarin reduces the mRNA expression of enzymes

involved in de novo lipogenesis.[53] These findings suggest that silymarin could be a viable treatment to reduce hepatic steatosis rates.

To date, first-line therapy for NAFLD has been based on modifications to lifestyle (such as calorie restriction and physical activity) that result in weight loss.[54] However, our findings imply that combining lifestyle modification or medication with silymarin may enhance metabolism-related indices and reduce hepatic steatosis rates to a greater extent in patients with NAFLD. As previously stated, silymarin combined with lifestyle modification decreased the incidence of hepatic steatosis. The development of NAFLD is strongly linked to lifestyle variables, including high caloric consumption combined with low physical activity and exercise.[55] Global urbanization and modernization during the 20th and 21st centuries have been connected to unfavorable lifestyle changes.[55] Therefore, the mean worldwide BMI and obesity prevalence, which are the pathophysiological causes of NAFLD, have increased significantly during the previous three decades.[55] This is highlighted by the significant rise in the prevalence of NAFLD in Asia over the last 15 years, which is linked to urbanization and the use of "Western" foods.[56] Studies have shown that lifestyle management in patients with NAFLD reduces body weight, improves hepatic lipid content, and improves the NAFLD activity score (a composite of steatosis, inflammation, and hepatocyte ballooning).[55,57] Of note, lifestyle modifications may be less effective for NAFLD resolution if the patient is severely obese (BMI $\geq$35 kg/m^2), has T2DM, or has severe NAFLD.[58] Furthermore, persistent lifestyle modifications and weight loss are difficult to achieve, and lifestyle modifications alone are not effective in every case.

Although mild, this meta-analysis showed that a higher number of patients receiving silymarin developed AEs than those receiving alternative therapies. However, there is not much evidence of causality, and milk thistle is linked to some relatively mild AEs.[59] In a previous animal study, silymarin was orally administered to pregnant female mice.[60] At the conclusion of the trial, the fetal weight was lower than that of the control group, and silymarin exhibited teratogenic effects.[60] Abnormalities were noted in the skull, face, and vertebrae.[60] Because there is currently little evidence on silymarin's teratogenicity in pregnant humans and animals, more research is needed. Our results are consistent with those of other studies that indicated that silymarin was safe in humans at therapeutic dosages, with some trials reporting only temporary AEs, including gastrointestinal discomfort; however, further prenatal trials are

required. Furthermore, silymarin must be used carefully when taking medications with a limited therapeutic window because of possible interactions. To examine other medications, ingredients, or contaminants as possible causes, concurrent use of other medications should also be mentioned when AEs are noted.

Limitations

There were several limitations in this meta-analysis. Our understanding of the chronic nature of NAFLD may be limited because most of the included studies were cross-sectional. The inclusion of biased studies was another limitation of this meta-analysis. The majority of the studies included in this analysis had a moderate to high risk of bias, namely in the areas of statistical analysis, reporting, and study confounding. The majority of these studies lacked adequate data presentation to evaluate the suitability of the analytical approach, and statistical analysis was inappropriate in these investigations. Furthermore, insufficient information was available to determine whether the selected statistical model was suitable for the study design. Important potential confounders like sex, age, income, marital status, comorbidities, and educational attainment were not considered in the study design or analysis. Because the connections between the interventions and these potential confounders were not corrected, the result was probably misleading by confusing factors. Consequently, values that do not accurately represent the relationship are produced. In this situation, it is possible to make a false positive (Type I) error or draw the incorrect conclusion that the dependent variables and the independent variables are causally associated.[61] Therefore, the validity of causal inference (internal validity) is seriously damaged by confounding.[61] The small number of studies in each group and the high level of heterogeneity were additional limitations of this study. The high heterogeneity in this meta-analysis may be explained by the use of different interventions in the comparator group, including different hypocaloric Mediterranean diets, broad lifestyle changes (e.g., alcohol consumption, high fructose intake, and physical activity), or different medications (e.g., phosphatidylcholine or pioglitazone), in addition to different doses of silymarin use (e.g., 70, 94, 140, or 285 mg/day).

Conclusions

This meta-analysis demonstrated that silymarin-based therapies can relieve NAFLD. However, subgroup analysis found that individual therapies, such as silymarin alone or in combination with lifestyle changes, a hypocaloric Mediterranean diet, and medication, had no consistent effect on NAFLD. The optimal strategy for silymarin combination with other treatments should be determined in future investigations.

DATA AVAILABILITY STATEMENT

The data supporting the results of this study are available upon request from the corresponding author.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this article.

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Figure 1

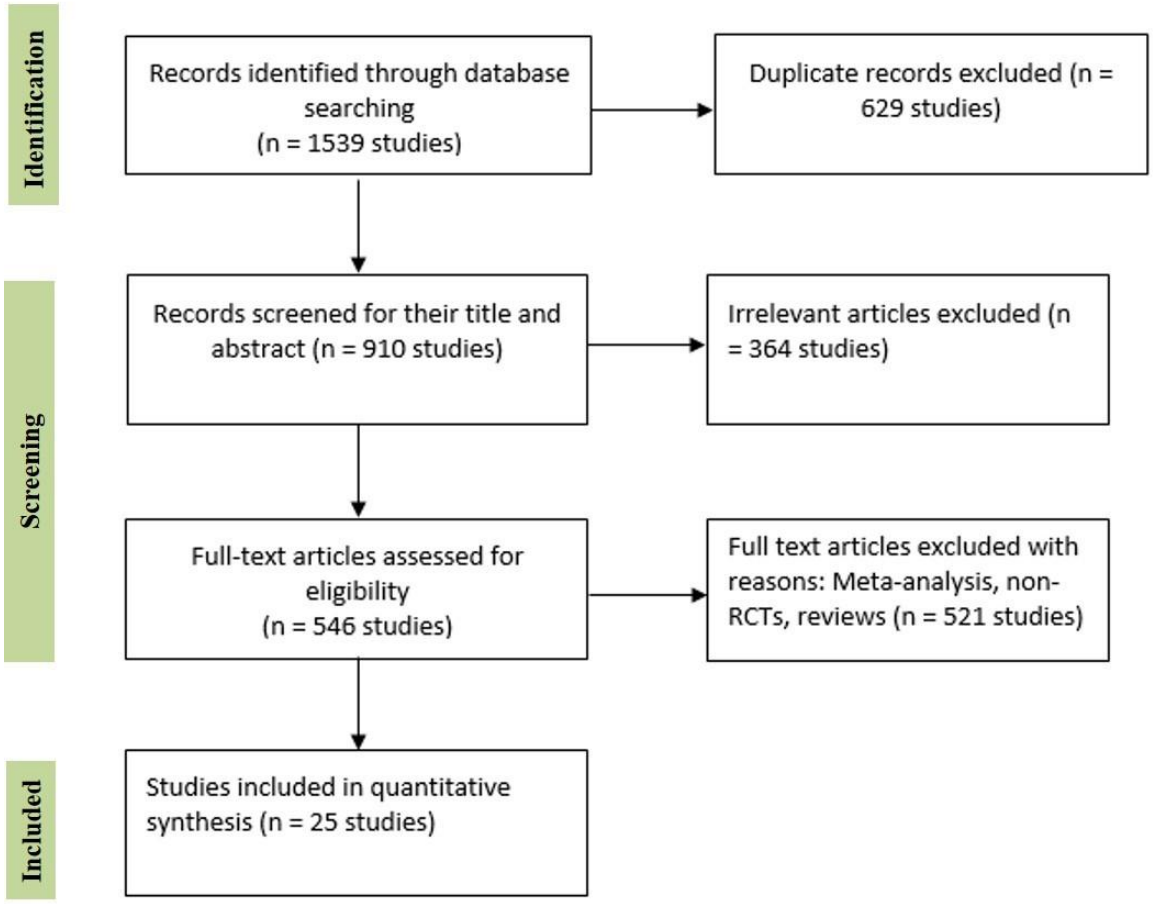

Figure 1. PRISMA flow chart of the studies included in the meta-analysis.

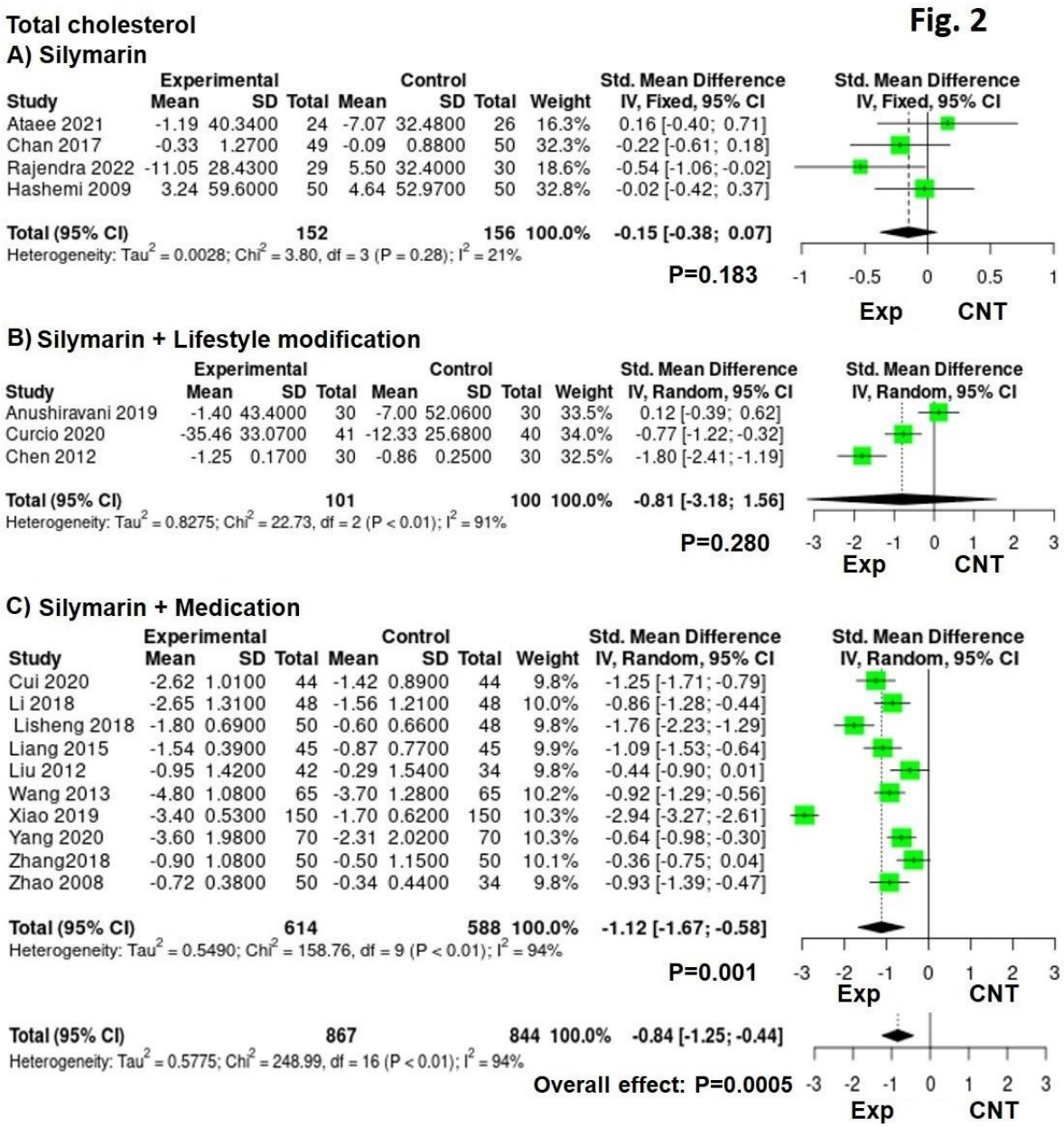

Figure 2. The forest plot shows the effect of various treatments (Exp) on total cholesterol levels in blood compared with the comparator control (CNT) in patients with NAFLD.

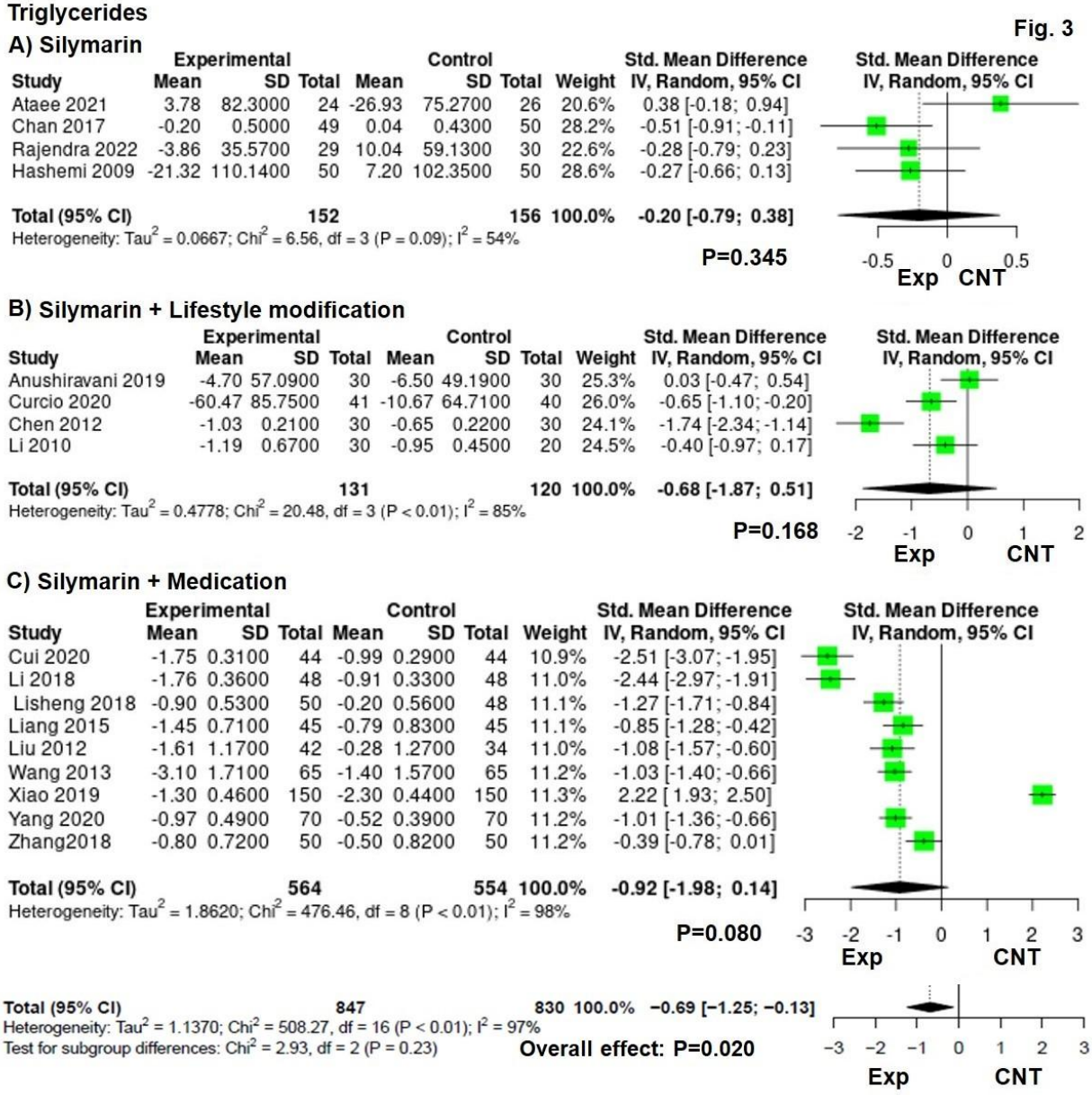

Figure 3. The forest plot shows the effect of various treatments (Exp) on blood triglyceride levels compared with the comparator control (CNT) in patients with NAFLD.

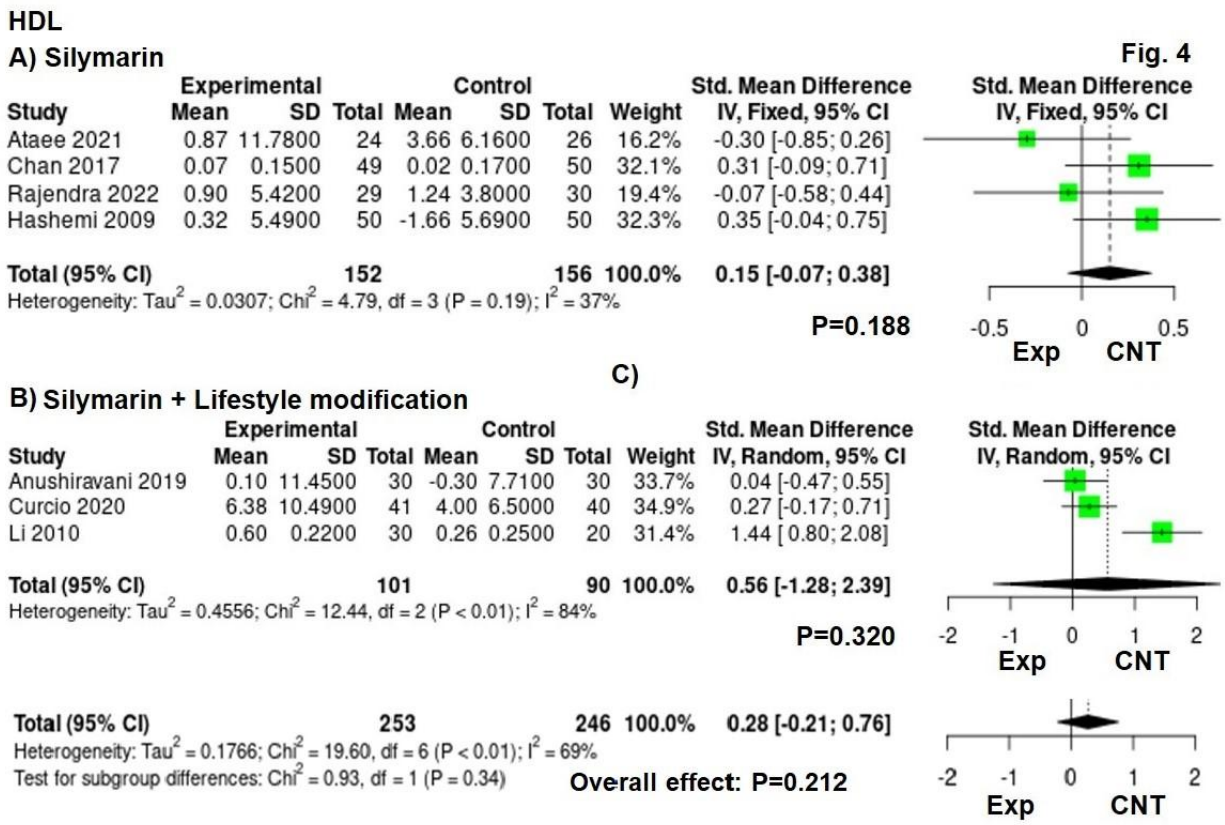

Figure 4. The forest plot shows the effect of various treatments (Exp) on HDL-C levels compared with the comparator control (CNT) in patients with NAFLD.

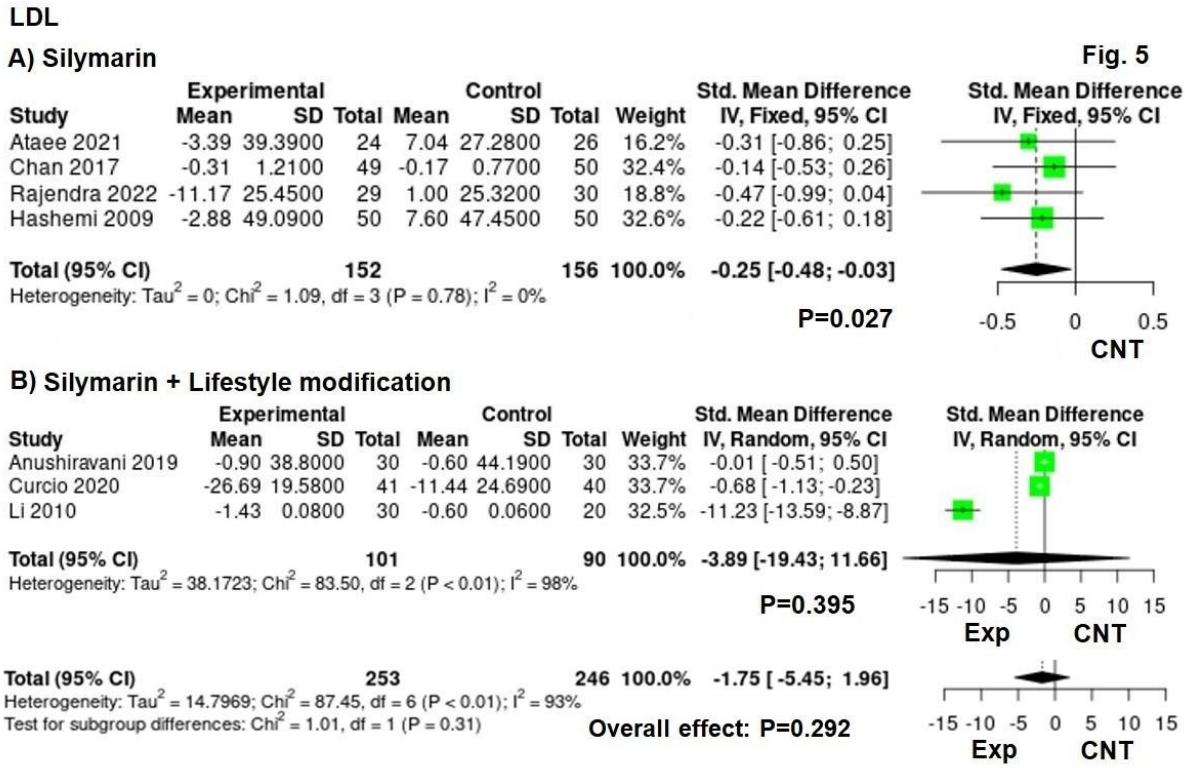

Figure 5. The forest plot shows the effect of various treatments (Exp) on LDL-C levels compared with the comparator control (CNT) in patients with NAFLD.

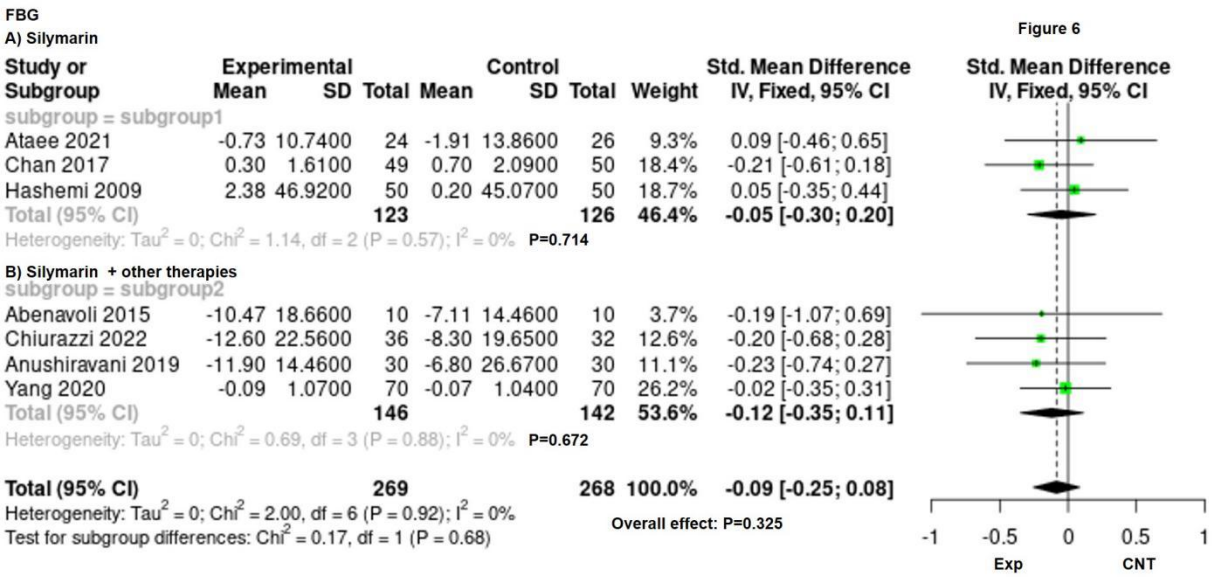

Figure 6. The forest plot shows the effect of various treatments (Exp) on fasting blood glucose (FBG) levels compared with the comparator control (CNT) in patients with NAFLD.

Fig. 7

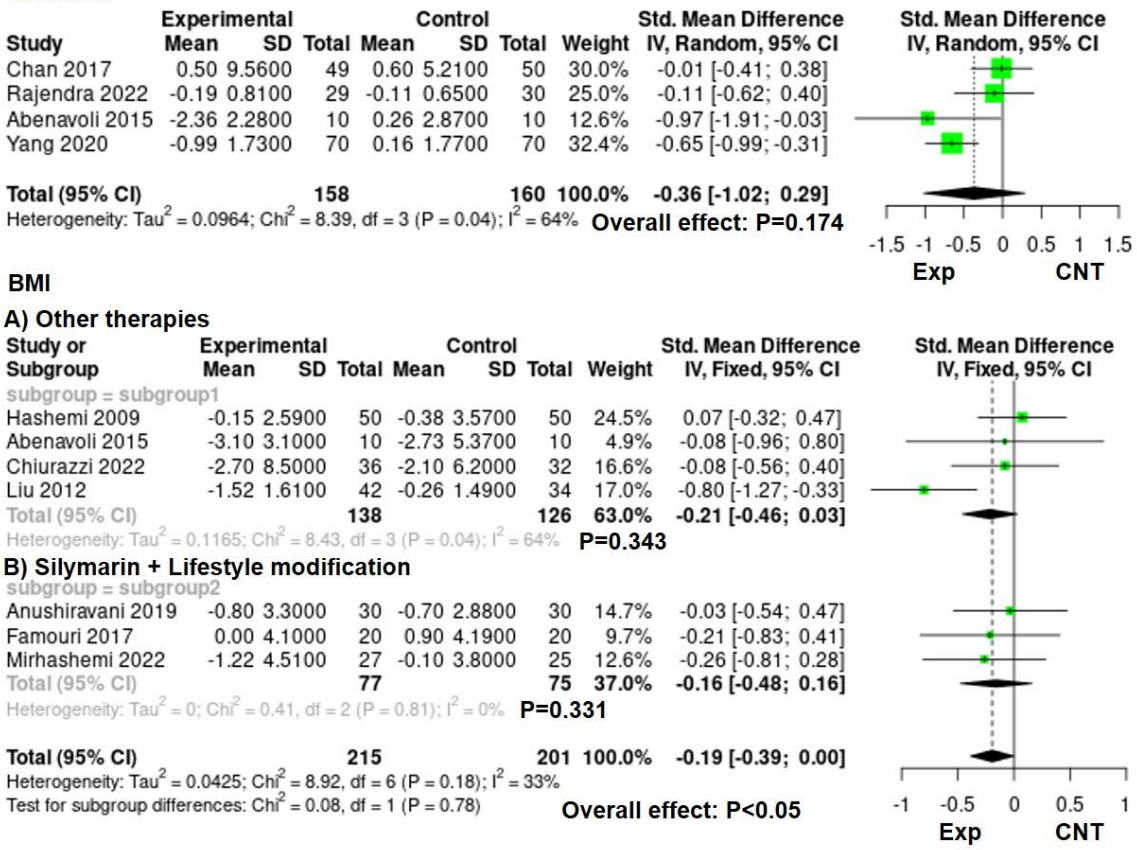

Figure 7. The forest plot shows the effect of various treatments (Exp) on the homeostatic model assessment of insulin resistance (HOMA-IR) and body mass index (BMI) compared with the comparator control (CNT) in patients with NAFLD.

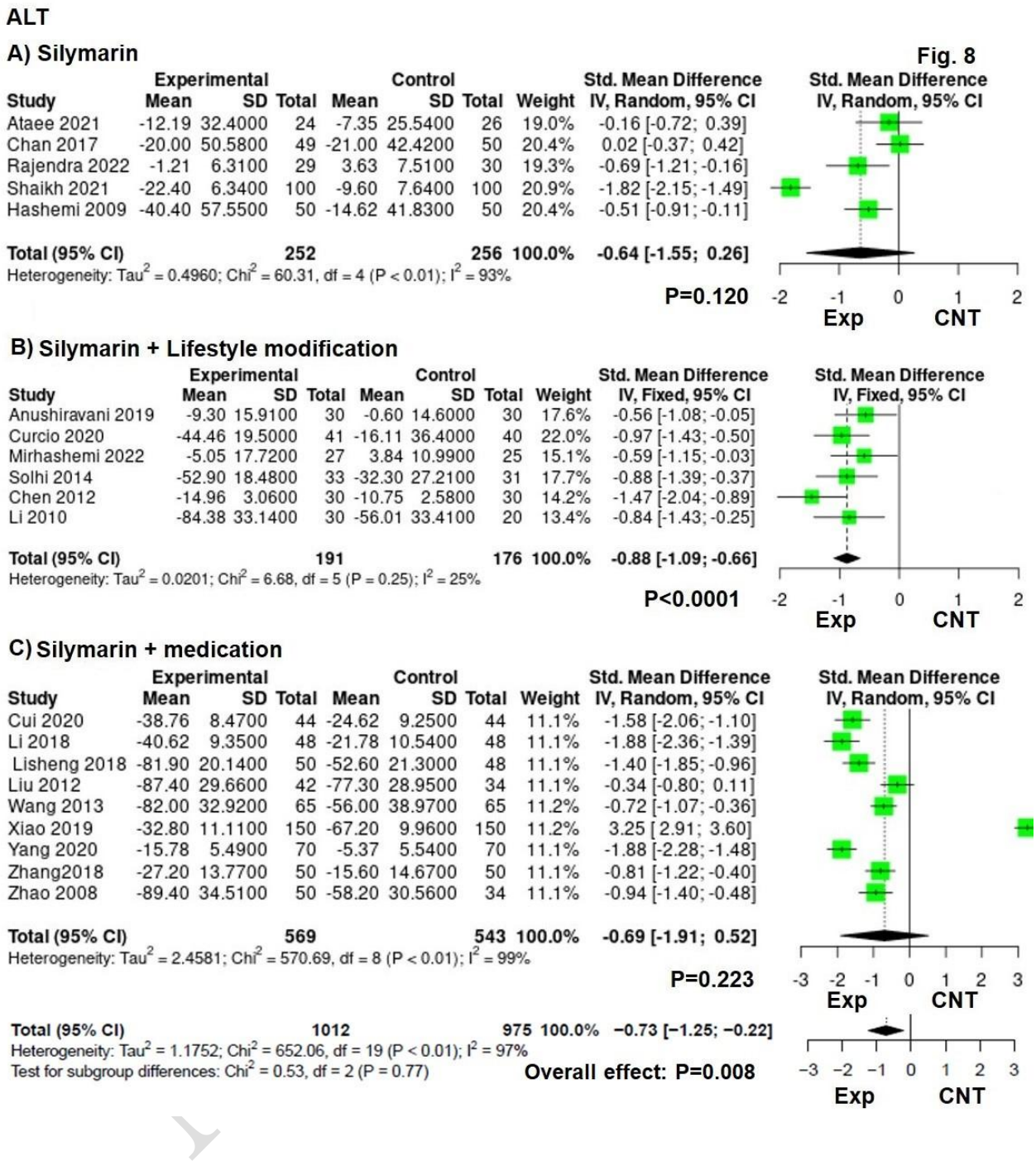

Figure 8. The forest plot shows the effect of various treatments (Exp) on alanine-aminotransferase (ALT) levels compared with the comparator control (CNT) in patients with NAFLD.

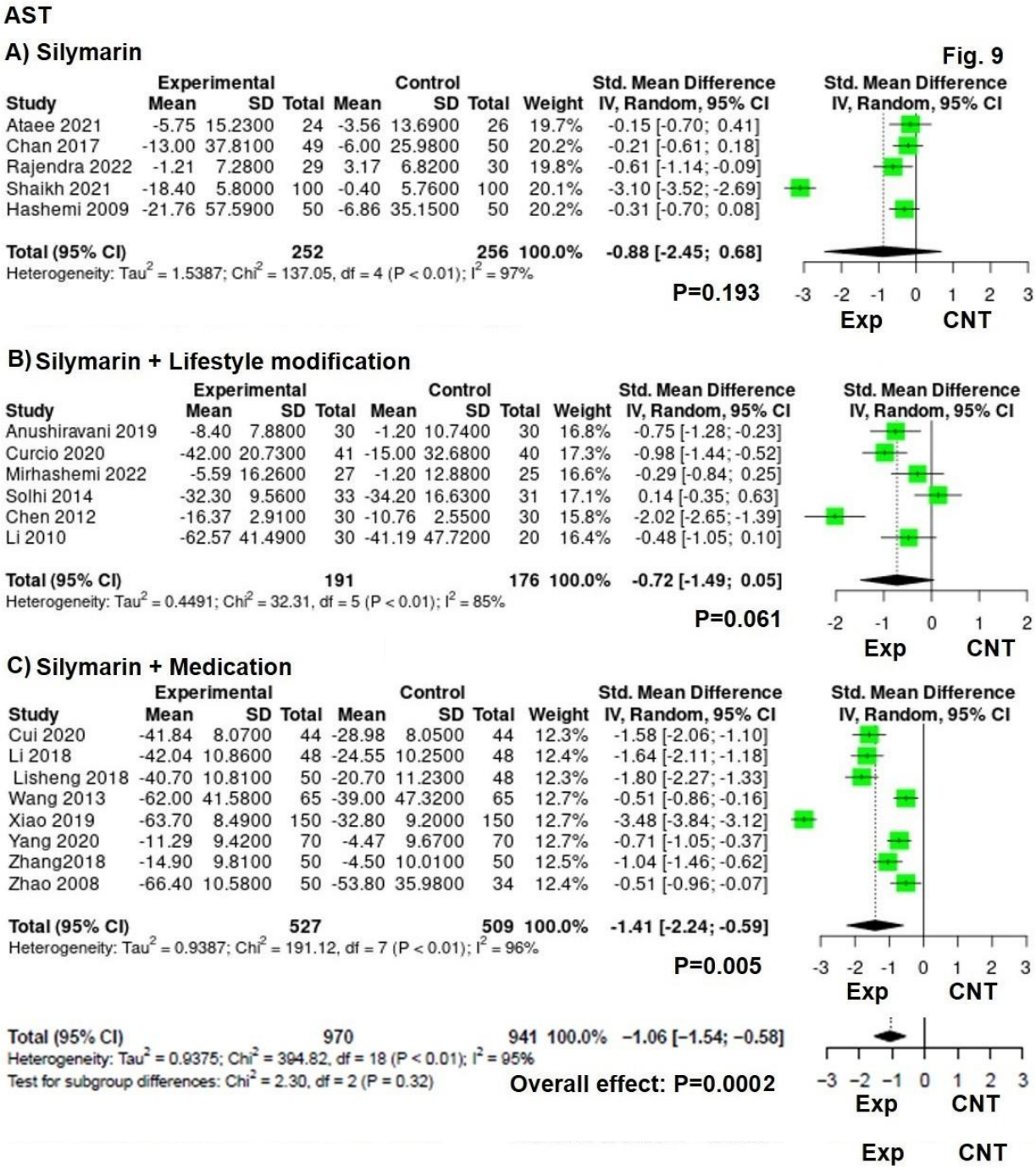

Figure 9. The forest plot shows the effect of various treatments (Exp) on aspartate-aminotransferase (AST) levels compared with the comparator control (CNT) in patients with NAFLD.

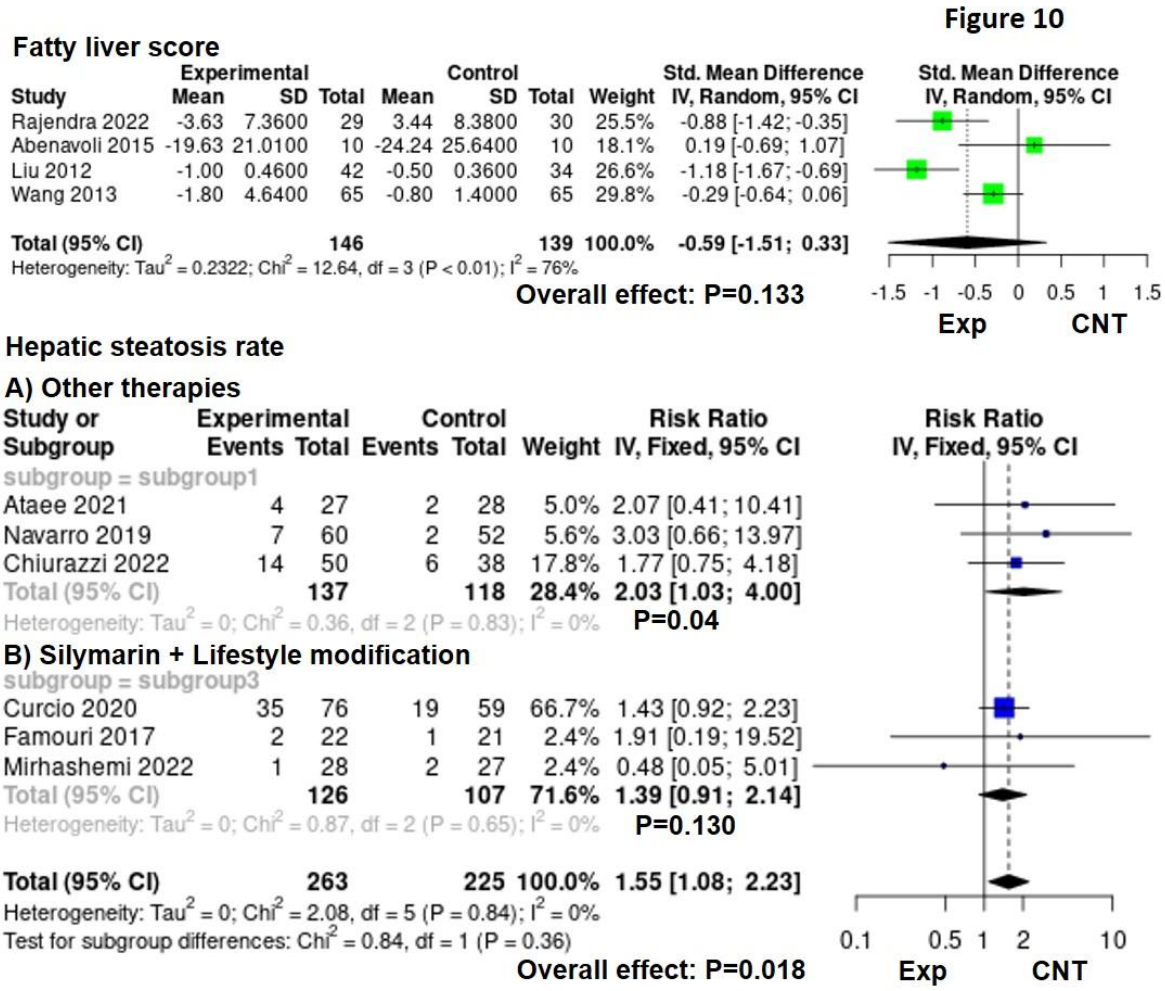

Figure 10. The forest plot shows the effect of various treatments (Exp) on fatty liver score and hepatic steatosis rate compared with the comparator control (CNT) in patients with NAFLD.

Fig. 11

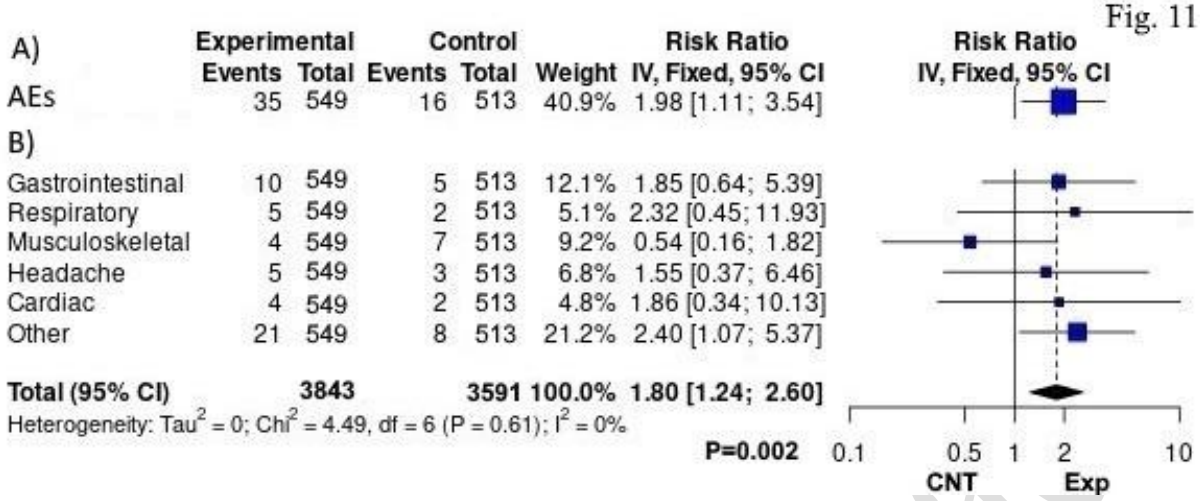

Figure 11. The forest plot shows (A) the proportion of patients with adverse effects (AEs) and (B) the proportion of AEs incidence in patients with NAFLD. Exp: silymarin group; CNT: the comparator control.