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# Silymarin decreases liver stiffness associated with gut microbiota in patients with metabolic dysfunction-associated steatotic liver disease: a randomized, double-blind, placebo-controlled trial

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## Abstract

**Background** Despite centuries of traditional use of silymarin for hepatoprotection, current randomized controlled trial (RCT) studies on the effectiveness of silymarin in managing metabolic dysfunction-associated steatotic liver disease (MASLD) are limited and inconclusive, particularly when it is administered alone. The low bioavailability of silymarin highlights the possible influence of gut microbiota on the effectiveness of silymarin; however, no human studies have investigated this aspect.

**Objective** To determine the potential efficacy of silymarin in improving MASLD indicators and to investigate the underlying mechanisms related to gut microbiota.

**Method** In this 24-week randomized, double-blind, placebo-controlled trial, 83 patients with MASLD were randomized to either placebo ($n=41$) or silymarin (103.2 mg/d, $n=42$). At 0, 12, and 24 weeks, liver stiffness and hepatic steatosis were assessed using FibroScan, and blood samples were gathered for biochemical detection, while faecal samples were collected at 0 and 24 weeks for 16S rRNA sequencing.

**Results** Silymarin supplementation significantly reduced liver stiffness (LSM, -0.21 ± 0.17 *vs.* 0.41 ± 0.17, $P=0.015$) and serum levels of γ-glutamyl transpeptidase (GGT, -8.21 ± 3.01 *vs.* 1.23 ± 3.16, $P=0.042$) and ApoB (-0.02 ± 0.03 *vs.* 0.07 ± 0.03, $P=0.023$) but had no significant effect on the controlled attenuation parameter (CAP), other biochemical indicators (aminotransferases, total bilirubin, glucose and lipid parameters, hsCRP, SOD, and UA), physical measurements (DBP, SBP, BMI, WHR, BF%, and BMR), or APRI and FIB-4 indices. Gut microbiota analysis revealed increased species diversity and enrichment of *Oscillospiraceae* in the silymarin group.

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**Conclusion** These findings suggest that silymarin supplementation could improve liver stiffness in MASLD patients, possibly by modulating the gut microbiota.

**Trial registration** The trial was registered at the Chinese Clinical Trial Registry (ChiCTR2200059043).

**Keywords** Metabolic dysfunction-associated steatotic liver disease, Silymarin, Liver stiffness, Gut microbiota, Randomized controlled study, Flavonoids, Phytochemicals

## Background

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most recent terminology for steatotic liver disease that is associated with metabolic syndrome [1]. It is categorized into steatotic liver disease (SLD) and metabolic dysfunction-associated steatohepatitis (MASH), and the latter may further develop into liver fibrosis and cirrhosis [2]. MASLD is not only a primary cause of cirrhosis and hepatocellular carcinoma (HCC) but is also strongly and reciprocally correlated with metabolic syndrome, type 2 diabetes and cardiovascular diseases [3, 4]. Its high prevalence (approximately 25% ~ 30%) has posed a significant public health burden worldwide [3, 5]. No specific medicine has been approved for MASLD treatment by regulatory agencies. Weight loss and lifestyle modification are still the cornerstone strategies; however, maintaining them is challenging because it is difficult to change ingrained behaviours. Several synthetic drugs, particularly those that target metabolism and fibrosis, are currently under intense investigation, but their efficacy and potential side effects remain a subject of concern and debate [6–9]. On the other hand, some natural bioactive constituents and herbal and botanical agents have attracted considerable attention and have been identified as promising candidates for MASLD management due to their significant benefits, multitarget mechanisms and relatively high safety [10, 11].

Silymarin is primarily a complex of flavonolignans derived from milk thistle (Silybum marianum), a traditional herbal medicine for liver disorders [12, 13]. The hepatoprotective effect of silymarin has been verified in many experimental studies and a few clinical trials, involving viral hepatitis, cirrhosis, alcoholic and nonalcoholic fatty liver disease, and toxic liver injury [14–22]. In addition, multiple biological activities or pharmacological actions relevant to liver abnormalities, such as anti-inflammatory, antioxidant, antifibrotic, antiviral, insulin-sensitization, immunoregulatory, and inhibition of hepatic cholesterol synthesis and lipogenesis, have been proposed [13, 23]. Milk thistle is currently used in clinical practice in India and China, and the US FDA has approved phase 4 clinical trials of silymarin. However, the efficacy and clinical prospects of silymarin in MASLD remain controversial due to the limited available trial data and inconsistent results, as well as its very low bioavailability [11, 12]. Furthermore, in intervention trials evaluating the efficiency of silymarin in treating MASLD patients, greater attention has been given to evaluating the combined effect of silymarin and other ingredients or exploring the formulation to increase its bioavailability. However, there is a lack of information regarding the effectiveness of silymarin when used alone at a moderate dose.

The gut microbiota is considered the largest 'hidden organ', and its homeostasis is closely associated with human disease [24]. Observational and microbiota manipulation data from both animal and human studies have provided substantial evidence that dysbiosis may intervene in the pathogenesis and progression of MASLD [24–26]. Gut microbiota not only mediate the health-promoting or pathogenic effects of certain dietary components, dietary patterns and dietary quantity but also influence the transformation, absorption and efficacy of some drugs, nutraceuticals, traditional medicines and medical food homologues [26–29]. Many plant/herbal extracts and naturally derived bioactive compounds, particularly those with low bioavailability, such as polyphenols, terpenoids and alkaloids, have been shown to protect against metabolic diseases through mechanisms related to or dependent on gut microbiota [29–32]. However, despite the long history of traditional use and the ongoing popular research topic of silymarin treatment in liver disease, its influence on the gut microbiome and the possible mediation of these microbiota in silymarin hepatoprotection have rarely been reported. Only a few animal experiments have provided some suggestive evidence [33–37], while human studies are lacking.

To evaluate the efficacy of silymarin in MASLD management and to preliminarily explore the underlying mechanisms related to gut microbiota, a double-blind, randomized controlled trial (RCT) was conducted here using a combination of vibration-controlled transient elastography (VCTE; FibroScan) and 16S rRNA sequencing. The findings could provide important evidence for the efficacy and safety of silymarin in treating MASLD, as well as new insights into the mechanistic implications of gut microbiota.

## Materials and methods

### Study design and participants

This study employed a randomized, double-blind, placebo-controlled design, and the total intervention period was 24 weeks. Volunteers were recruited from June 2022 to February 2023 at The First People's Hospital of Shunde in Foshan, China, through advertisements, on-site presentations, community advocacy, and doctor recommendations. The inclusion criteria were as follows: (1) Foshan residents aged between 18 and 85 years; (2) met the diagnostic criteria for MASLD through the FibroScan test (CAP ≥ 238 dB/m or LSM ≥ 7.3 kPa) [38]; (3) habitual alcohol consumption ≤ 140 g/week for males and ≤ 70 g/week for females; (4) were willing to sustain a consistent dietary routine and physical exercise regimen throughout the duration of the trial; and (5) agreed to sign the informed consent form. The exclusion criteria included: (1) serum alanine aminotransferase (ALT) levels > 80 U/L, aspartate aminotransferase (AST) > 80 U/L, or γ-glutamyl transpeptidase (GGT) > 100 U/L; (2) history of acute or chronic infectious diseases, autoimmune diseases, cancer; clinically diagnosed as viral hepatitis, autoimmune liver disease, cirrhosis, cardiovascular, cerebrovascular diseases, other serious chronic diseases; liver and kidney insufficiency, trauma or surgery within the past month; (3) use of any drugs for the treatment of MASLD hepatic fibrosis or lipid-lowering, related health products, or phytochemicals within 3 months prior to baseline examination; and (4) lactating or pregnant females. The clinical investigations in our study were conducted in accordance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee of Biomedical Research, School of Public Health, Sun Yat-Sen University. Furthermore, the trial protocol has been registered at the Chinese Clinical Trial Registry (ChiCTR2200059043). Written informed consent was obtained from all participants before enrolment.

### Randomization and blinding

A total of 83 participants who met all eligibility criteria were allocated to either the silymarin group ($n=42$) or the placebo group ($n=41$) using complete randomization. Statistical software was used by experts to generate random codes based on the experimental design. Once the coding process was complete, the data were stored securely in a file, sealed, and entrusted to a nonparticipating individual for safekeeping. The trial was carried out as a double-blinded study, signifying that the patients and researchers were unaware of the treatment group. The treatments were masked by packaging them in bottles with identical appearance and packaging, and the tablets themselves were indistinguishable.

### Intervention

The tablets were provided by BYHEALTH Co., Ltd. (Guangzhou, China). Participants were instructed to take 4 tablets daily (2 with breakfast and 2 with dinner). The intervention group was administered a total daily dose of 103.2 mg of silybin, while the control group was administered placebo tablets, with dextrin as the main ingredient. The entire intervention period lasted for 24 weeks. Throughout the intervention period, compliance was monitored every two weeks through mobile devices or on-site visits to assess adverse events and to ensure compliance based on the remaining quantity of tablets (compliance = the number of tablets used/the number of those expected to use × 100%).

### Baseline and follow-up visits

Study visits were conducted at baseline, 12 weeks, and 24 weeks after intervention initiation. At weeks 0, 12, and 24, liver stiffness and hepatic steatosis were tested by FibroScan; anthropometric parameters and the basal metabolic rate (BMR) were measured; overnight fasting blood samples were collected, and the serum was separated and subsequently stored at -80 °C. Dietary data were collected using a 3-day 24-h dietary recall method and converted to nutrient intakes using a computer-aided nutritional analysis program (China Food Composition Table). The smoking status of participants was categorized into two distinct groups: current smokers and nonsmokers. Nonsmokers were defined as individuals who had never smoked or who were ex-smokers (having quit smoking for at least 6 months); physical activity and other lifestyles and sociodemographic information were collected via structured questionnaires. The metabolic equivalent of task (MET) was used to assess physical activity [39] according to the following formula: total physical activity MET-min/week = Moderate + Vigorous MET-min/week, Moderate MET-min/week = 4.0 × moderate-intensity activity minutes × moderate days. Vigorous MET-min/week = 8.0 × vigorous-intensity activity minutes × vigorous-intensity days. Faecal samples were also collected and stored at − 80 °C at baseline and 24 weeks.

### Primary and secondary outcomes

The primary outcome was liver health, including liver stiffness, hepatic steatosis, and liver function. Metabolic risk factors, including body composition, blood pressure, glucose and lipid profiles, inflammation, and antioxidant capacity, served as secondary outcomes.

### Assessment of hepatic steatosis and liver stiffness

In this study, FibroScan (Echosens, Paris, France) was employed, which has a sensitivity and specificity of approximately 83% and 89%, respectively [40].

FibroScan is recommended by the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) [41, 42]. M probe VCTE was employed to assess steatosis and stiffness in patients with suspected MASLD. An expert physician conducted the FibroScan. The aim was to obtain 10 acceptable measurements, and the maximum number of attempts was set to 20 [43]. FibroScan offers a noninvasive method to assess hepatic steatosis by precisely detecting the ultrasonic attenuation of echo waves, which is referred to as the controlled attenuation parameter (CAP). Additionally, FibroScan can be used to estimate the severity of liver fibrosis via an indicator called liver stiffness measurement (LSM), which precisely determines the velocity of a mechanically generated shear wave within the liver tissue [40, 44, 45]. The aspartate aminotransferase-to-platelet ratio index (APRI) was calculated as AST (IU/L)/upper limit of normal (ULN) × 100/platelet count (PLT) ($10^9$/L). The fibrosis index based on 4 factors (FIB-4) was calculated as $[\text{Age (years)} \times \text{AST (U/L)}]/\left[\text{PLT } (10^9/\text{L}) \times \sqrt{\text{ALT(U/L)}}\right]$.

#### Anthropometric data

Anthropometric measurements were commonly conducted by a trained researcher using standard machines. Body weight and height were measured and recorded to the nearest 0.1 kg and 0.1 cm, respectively, ensuring that participants wore lightweight clothing and were barefoot during the assessment. Body mass index (BMI) and the waist–hip ratio (WHR) were calculated with the following formulas: weight (kg)/height$^2$ (m); waist circumference (cm)/hip circumference (cm). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were tested after resting for at least 15 min. Body composition was analysed using a body composition analyser (JAWON IOI535, JAWON, Kungsang Bukdo, Korea) and expressed as body fat percentage (BF%). All measured parameters were obtained using standardized procedures and regularly calibrated equipment.

#### Laboratory measurements

At 0, 12, and 24 weeks, after overnight fasting for at least 12 h, blood samples were collected via venipuncture conducted by trained nurses the following morning. Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), Apolipoprotein B (ApoB), high-density lipoprotein-cholesterol (HDL-C), Apolipoprotein A1 (ApoA1), glucose, high-sensitivity C-reactive protein (hsCRP), uric acid (UA), and superoxide dismutase (SOD) concentrations were analysed using a Cobas c702 automatic biochemical analyser (Roche Diagnostics, Basel, Switzerland). Total bilirubin levels were assayed using the 2–4 and 2–5 diazotised dichloroaniline method (Roche Diagnostics, Basel, Switzerland). Fasting insulin levels were measured using a Cobas e602 automatic biochemical analyser (Roche Diagnostics, Basel, Switzerland). The other serum biochemical variables, including ALT, AST, and GGT concentrations, were determined using an automated chemistry analyser (Hitachi 747 autoanalyser, Hitachi Ltd., Tokyo, Japan). The homeostatic model assessment of insulin resistance (HOMA-IR) was determined using the following formula: HOMA-IR = [glucose (mmol/L) × insulin (μU/mL)]/22.5.

#### Faecal sample collection and 16S rRNA gene sequencing

Before physical examination (at 0 and 24 weeks), faecal samples were self-collected from the well-instructed participants in a sterile container and then stored at -80 °C within two hours of collection. If collected at home, the samples were stored at -20 °C until delivery to the hospital, where they were promptly transferred to -80 °C storage. 16S rRNA sequencing analysis was applied to assess the diversity and abundance of the microbial communities.

Total genomic DNA was extracted using the CTAB method. Subsequently, the concentration and purity of the sample were estimated through 1% agarose gel electrophoresis. Next, the sample was diluted to 1 ng/L with sterile water. The V3-V4 region of the 16S rRNA gene was amplified using the specific 341F/806R primer pair (341F: 5'-CCTACGGGRBGCASCAG-3'; 806R: 5'-GGACTACNNGGGTATCTAAT-3'). The PCRs were conducted in a 30 μL reaction mixture containing 15 μL of 2 × Phusion HF PCR Master Mix (New England Biolabs, Ipswich, USA), 0.2 μL of forwards and reverse primers (1 μM), and 10 ng of template DNA. The PCR cycling parameters were: (1) initial denaturation at 98 °C for 1 min; (2) 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s; and (3) postelongation at 72 °C for 5 min. For the purification of the amplified products, the PCR products were electrophoresed and visualized on 2% agarose gels and then cut using a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany).

Libraries for sequencing were produced using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, USA), adhering strictly to the manufacturer's guidelines, and indexed with appropriate codes. The quality of the library was appraised on a Qubit 2.0 fluorometer (Thermo Scientific, Carlsbad, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, USA). In the end, the library was sequenced on a NovaSeq 6000 platform, and 250 bp paired-end reads were produced.

Sequences with an identity of not less than 97% were allocated to the same operational taxonomic units (OTUs). The α diversity was calculated for the OTU table using QIIME software (version 1.9.1) and displayed using R software (version 4.2.2). Principal coordinate analysis (PCoA) based on Bray–Curtis distance and permutational multivariate analysis of variance (PERMANOVA) were employed to compare the genus-level composition of the gut microbiota before and after the intervention within each group. The linear discriminant analysis (LDA) threshold was set to 3 for LDA effect size (LEfSe) analysis, and the Kruskal–Wallis rank-sum test was used to compare the difference. The microbial taxa that significantly differ in relative abundance between the groups were presented as a histogram.

### Sample size estimation

The sample size was calculated using PASS software (version 15.0, NCSS, Inc.). It was reported that [15], compared with the placebo group, 2100 mg/d silymarin for 48 weeks led to a 27 dB/m decrease in CAP. Based on the reference value, 37 participants were needed per group, assuming $\alpha = 0.05$ and power = 90%. However, in view of a potential dropout rate of 10%, the minimum number of volunteers per group was set at 40.

#### *Statistical analysis*

Intention-to-treat (ITT) analyses were performed in this study. For continuous variables, the mean ± standard deviation (SD) is typically expressed if the data follow a normal distribution. Conversely, for nonnormally distributed data, the median and interquartile range (IQR) were used. Categorical variables are presented as frequencies and percentages. For comparing normally distributed variables, the t test was utilized, whereas the Mann–Whitney U test was employed for nonnormally distributed variables. For categorical variables, the Pearson chi-square test or Fisher's exact test was used for analysis. One-way analysis of covariance (ANCOVA) was used to compare the changes in the primary and secondary outcomes from baseline between groups. Baseline characteristics that were not balanced, such as age and SOD levels, were included as covariates.

All of the statistical analyses were conducted using R software (version 4.2.2). The *P* values were two-tailed, and a *P* value < 0.05 was considered to indicate statistical significance.

## Results

### Compliance and tolerability

Recruitment started in June 2022 and ended in February 2023. During this period, 463 participants were assessed with FibroScan for inclusion, 113 of whom did not meet the inclusion criteria, 225 of whom did not respond, and 42 of whom refused to participate. A total of 83 participants were included in the study and randomly allocated to two groups: the placebo group ($n = 41$) and the silymarin group ($n = 42$). Of the 83 participants, 4 were lost to follow-up (1 in the control group and 3 in the treatment group). All participants who were lost to follow-up discontinued tablet use for personal reasons. The flow chart depicting this trial is presented in Fig. 1. Both groups have more than 80% of compliance with daily tablet dosing (89% in the placebo group and 88% in the silymarin group). The tolerability was good for each group, with no serious side effects observed or reported during the experiment.

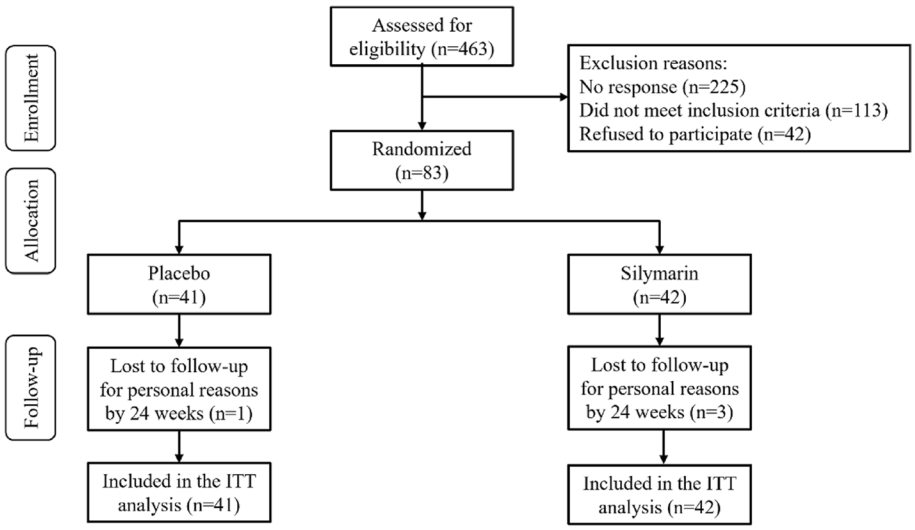


**Fig. 1** Flow chart of participant selection

### Baseline characteristics and dietary monitoring

Most baseline characteristics of participants in the ITT analyses were balanced between the two groups, except for age and AST/ALT, ApoA1, and SOD concentrations, with values for the first three indicators being slightly greater and SOD levels being slightly lower in the silymarin group (Table 1). The average ages at baseline in the silymarin and placebo groups were 45.4 ± 10.7 years and 40.4 ± 10.2 years, respectively ($P = 0.032$), and the SOD levels were 159.31 ± 13.03 U/mL and 167.93 ± 13.18 U/mL, respectively ($P = 0.012$). The average daily nutrient intake and weekly physical activity before and after intervention are displayed in Table 2. No significant differences were found in total energy or essential nutrient intake between groups at pre-, mid-, and post-intervention ($P > 0.05$).

### Effects of silymarin treatment on liver indicators

After adjustment for age and AST/ALT, ApoA1 and SOD concentrations, the participants in the silymarin group at 24 weeks showed a significant decrease in LSM from baseline (with a change in the mean ± SD of -0.21 ± 0.17 kPa), while those in the placebo group tended to increase (0.41 ± 0.17 kPa), and a significant difference was found between the two groups ($P = 0.015$). Additionally, a significant difference in the change in serum GGT

**Table 1** Baseline characteristics

| | Placebo ($n = 41$) | Silymarin ($n = 42$) | *P* value |
|---|---|---|---|
| Male n (%) | 23 (56.10%) | 26 (61.90%) | 0.753 |
| Age (year) | 40.4 ± 10.2 | 45.4 ± 10.7 | 0.032 |
| current smoker n (%) | 6 (14.7%) | 7 (16.7%) | 0.801 |
| DBP (mmHg) | 81 ± 11 | 82 ± 10 | 0.969 |
| SBP (mmHg) | 130 ± 15 | 130 ± 14 | 0.719 |
| BMI (kg/m$^2$) | 27.6 ± 4.1 | 27.0 ± 4.1 | 0.493 |
| WHR | 0.92 ± 0.06 | 0.95 ± 0.15 | 0.281 |
| BF (%) | 29.38 ± 5.52 | 28.76 ± 5.35 | 0.604 |
| BMR (kcal) | 1315.85 ± 188.80 | 1308.19 ± 170.07 | 0.848 |
| CAP (dB/m) | 278.95 [258.00, 294.00] | 278.00 [259.25, 296.75] | 0.891 |
| LSM (kPa) | 4.50 [4.30, 5.10] | 5.10 [4.35, 5.60] | 0.068 |
| ALT (U/L) | 33.00 [18.25, 49.00] | 20.00 [16.00, 35.00] | 0.066 |
| AST (U/L) | 22.00 [17.75, 30.00] | 20.00 [17.00, 22.75] | 0.155 |
| GGT (U/L) | 30.50 [21.00, 57.25] | 28.50 [20.00, 44.50] | 0.603 |
| AST/ALT | 0.82 ± 0.31 | 1.01 ± 0.48 | 0.037 |
| Total bilirubin (μmol/L) | 8.69 ± 4.54 | 7.84 ± 3.20 | 0.331 |
| APRI | 0.26 ± 0.11 | 0.25 ± 0.21 | 0.866 |
| FIB-4 | 0.67 ± 0.23 | 0.75 ± 0.33 | 0.251 |
| TG (mmol/L) | 1.58 [1.31, 2.74] | 2.08 [1.52, 3.06] | 0.112 |
| TC (mmol/L) | 4.87 [4.39, 5.41] | 4.98 [4.50, 5.72] | 0.179 |
| HDL-C (mmol/L) | 1.09 [0.94, 1.26] | 1.17 [0.98, 1.31] | 0.376 |
| LDL-C (mmol/L) | 3.14 ± 0.83 | 3.07 ± 0.78 | 0.871 |
| ApoA1 (g/L) | 1.30 ± 0.17 | 1.39 ± 0.21 | 0.033 |
| ApoB (g/L) | 1.09 ± 0.28 | 1.06 ± 0.22 | 0.614 |
| ApoA1/ApoB | 1.26 ± 0.35 | 1.36 ± 0.36 | 0.194 |
| FPG (mmol/L) | 5.38 ± 1.13 | 5.94 ± 2.45 | 0.187 |
| Insulin (μU/mL) | 13.61 ± 8.85 | 12.56 ± 7.13 | 0.555 |
| HOMA-IR | 3.41 ± 2.69 | 3.34 ± 2.26 | 0.911 |
| UA (μmol/L) | 404.50 [349.25, 485.50] | 415.00 [350.00, 488.00] | 0.489 |
| hsCRP (mg/L) | 1.25 [0.78, 1.87] | 1.29 [0.78, 2.93] | 0.707 |
| SOD (U/mL) | 167.93 ± 13.18 | 159.31 ± 13.03 | 0.012 |

Data are presented as the mean ± SD, median (IQR), or number (%), as appropriate

*Abbreviations*: *SD* standard deviation, *IQR* interquartile range, *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *BMI* body mass index, *WHR* waist-hip ratio, *BF* body fat, *BMR* basal metabolic rate, *CAP* controlled attenuation parameter, *LSM* liver stiffness measurement, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* γ-glutamyl transpeptidase, *APRI* aspartate aminotransferase-to-platelet ratio index, *FIB-4* fibrosis index based on 4 factors, *TG* triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *ApoA1* apolipoprotein A1, *ApoB* apolipoprotein B, *FPG* fasting plasma glucose, *HOMA-IR* homeostasis model of insulin resistance, *UA* uric acid, *hsCRP* high-sensitivity C-reactive protein, *SOD* superoxide dismutase

**Table 2** Daily dietary intakes of total energy, nutrients and physical activities at 0, 12 and 24 weeks

| | Placebo (n = 41) | Silymarin (n = 42) | *P* value |
|---|---|---|---|
| Physical activities (MET-min/week) | | | |
| 0 week | 4942.07 ± 6825.17 | 4743.57 ± 7079.22 | 0.915 |
| 12 weeks | 4802.94 ± 4963.32 | 3745.25 ± 4558.61 | 0.370 |
| 24 weeks | 4386.67 ± 4889.21 | 3998.12 ± 7604.25 | 0.814 |
| Total energy (kcal/day) | | | |
| 0 week | 1701.37 [1328.08, 2306.14] | 1781.20 [1119.33, 2488.77] | 0.881 |
| 12 weeks | 1672.83 [1142.71, 2259.20] | 1420.22 [890.58, 2301.13] | 0.386 |
| 24 weeks | 1513.68 [1051.53, 2229.83] | 1721.25 [1247.00, 2434.10] | 0.215 |
| Protein (g/day) | | | |
| 0 week | 82.15 ± 35.87 | 75.07 ± 30.25 | 0.343 |
| 12 weeks | 78.42 ± 37.27 | 65.47 ± 37.94 | 0.135 |
| 24 weeks | 72.53 ± 31.75 | 86.92 ± 44.33 | 0.124 |
| Fat (g/day) | | | |
| 0 week | 49.77 [32.09, 76.72] | 47.61 [33.11, 69.29] | 0.740 |
| 12 weeks | 49.31 [26.95, 77.40] | 37.06 [29.05, 71.10] | 0.336 |
| 24 weeks | 47.44 [34.30, 70.12] | 53.92 [34.38, 82.92] | 0.408 |
| Carbohydrate (g/day) | | | |
| 0 week | 237.62 [163.44, 321.74] | 229.18 [158.34, 375.92] | 0.996 |
| 12 weeks | 186.54 [153.93, 334.85] | 193.05 [131.08, 326.20] | 0.935 |
| 24 weeks | 198.22 [125.82, 316.18] | 222.45 [137.56, 358.68] | 0.277 |
| Dietary fibre (g/day) | | | |
| 0 week | 7.18 [3.78, 13.64] | 6.63 [3.55, 11.97] | 0.456 |
| 12 weeks | 5.05 [2.70, 10.33] | 4.58 [2.46, 8.12] | 0.775 |
| 24 weeks | 4.55 [3.16, 6.09] | 4.80 [3.37, 7.93] | 0.573 |
| Cholesterol (mg/day) | | | |
| 0 week | 335.97 [222.38, 547.44] | 313.03 [195.91, 437.15] | 0.416 |
| 12 weeks | 345.00 [158.93, 536.75] | 257.95 [161.21, 368.25] | 0.237 |
| 24 weeks | 338.00 [233.35, 595.58] | 354.17 [262.83, 517.75] | 0.655 |
| Vitamin A (μg retinol equivalent/day) | | | |
| 0 week | 490.57 [359.22, 667.77] | 510.33 [269.59, 886.28] | 0.806 |
| 12 weeks | 458.50 [241.17, 807.93] | 517.26 [222.80, 766.22] | 0.984 |
| 24 weeks | 470.80 [194.38, 716.54] | 559.63 [277.30, 903.78] | 0.262 |
| Vitamin B1 (mg/day) | | | |
| 0 week | 0.75 [0.50, 1.17] | 0.77 [0.45, 1.07] | 0.613 |
| 12 weeks | 0.71 [0.49, 1.01] | 0.55 [0.37, 1.07] | 0.250 |
| 24 weeks | 0.55 [0.42, 0.89] | 0.73 [0.45, 0.95] | 0.333 |
| Vitamin B2 (mg/day) | | | |
| 0 week | 0.85 [0.61, 1.05] | 0.80 [0.48, 1.05] | 0.258 |
| 12 weeks | 0.73 [0.57, 1.10] | 0.69 [0.38, 0.96] | 0.151 |
| 24 weeks | 0.84 [0.57, 0.99] | 0.72 [0.53, 1.02] | 0.703 |
| Vitamin B3 (mg/day) | | | |
| 0 week | 17.28 ± 8.47 | 15.20 ± 7.26 | 0.242 |
| 12 weeks | 16.37 ± 6.37 | 14.25 ± 8.14 | 0.209 |
| 24 weeks | 16.33 ± 8.61 | 18.88 ± 11.17 | 0.290 |
| Vitamin E (mg/day) | | | |
| 0 week | 11.99 [7.07, 21.22] | 10.27 [5.49, 16.48] | 0.279 |
| 12 weeks | 10.86 [5.10, 16.16] | 8.38 [4.72, 11.91] | 0.221 |
| 24 weeks | 7.76 [4.60, 10.02] | 7.77 [4.19, 10.92] | 0.981 |
| Vitamin C (mg/day) | | | |
| 0 week | 66.83 [35.00, 90.44] | 71.23 [28.73, 112.17] | 0.560 |
| 12 weeks | 51.00 [32.82, 97.95] | 63.42 [32.85, 101.14] | 0.733 |
| 24 weeks | 54.00 [35.67, 81.80] | 62.00 [23.83, 106.92] | 0.756 |

Data are presented as the mean ± SD or median (IQR), as appropriate. T tests were used to compare differences between the groups at different times

levels was found between the two groups at 24 weeks. The change was -8.21 ± 3.01 U/L in the silymarin group and 1.23 ± 3.16 U/L in the placebo group ($P = 0.042$). There were no significant changes in the mean CAP, serum ALT and AST levels, AST/ALT ratio, total bilirubin concentrations, or the APRI or FIB-4 (Table 3).

### Effects of silymarin treatment on biochemical and physical parameters

According to the serum lipid profile analysis, after adjustment for age, and AST/ALT, ApoA1, and SOD concentrations, ApoB levels significantly improved after treatment with silymarin for 24 weeks compared to those in the placebo control group (-0.02 ± 0.03 g/L *vs.* 0.07 ± 0.03 g/L, $P = 0.023$). The changes in TC, TG, HDL-C, LDL-C, and ApoA1 concentrations, and the ratio of ApoA1/ApoB did not significantly differ between the groups. Additionally, the changes in fasting blood glucose and insulin concentrations, HOMA-IR, and UA, SOD, and hsCRP concentrations before and after intervention have no significant differences between the two groups (Table 4).

In addition to biochemical parameters, changes in all physical parameters (DBP, SBP, BMI, WHR, BF%, and BMR) revealed no statistically significant differences between groups during the 24-week intervention period, although the mean values of SBP and the WHR showed a downward trend in the silymarin group (Table 5).

### Effects of silymarin treatment on the gut microbial composition

16S rRNA sequencing was carried out on faecal samples gathered at 0 and 24 weeks in both groups. As evaluated by the observed species, silymarin intervention increased the α diversity (Fig. 2A). A Venn diagram was used to analyse the distribution of OTU abundances across the placebo and silymarin baseline and intervention groups. A total of 172 OTUs were common to all four groups, with 22, 26, 6, and 58 OTUs found only in the placebo baseline, silymarin baseline, placebo intervention, and silymarin intervention groups, respectively (Fig. 2B), and the

**Table 3** Changes in liver indicators from baseline between groups

| Outcome | Placebo ($n=41$) | Silymarin ($n=42$) | *P* value |
|---|---|---|---|
| CAP (dB/m) | | | |
| 12 weeks | 2.28 ± 6.15 | 8.78 ± 5.98 | 0.471 |
| 24 weeks | -0.80 ± 6.42 | 1.84 ± 6.25 | 0.780 |
| LSM (kPa) | | | |
| 12 weeks | 0.43 ± 0.18 | -0.09 ± 0.17 | 0.049 |
| 24 weeks | 0.41 ± 0.17 | -0.21 ± 0.17 | 0.015 |
| ALT (U/L) | | | |
| 12 weeks | 6.48 ± 4.30 | -2.52 ± 4.24 | 0.159 |
| 24 weeks | -4.29 ± 4.44 | -3.45 ± 4.23 | 0.896 |
| AST (U/L) | | | |
| 12 weeks | 2.30 ± 2.14 | -1.85 ± 2.11 | 0.191 |
| 24 weeks | -2.20 ± 2.44 | -3.02 ± 2.32 | 0.816 |
| GGT (U/L) | | | |
| 12 weeks | 6.88 ± 2.76 | -2.47 ± 2.73 | 0.024 |
| 24 weeks | 1.23 ± 3.16 | -8.21 ± 3.01 | 0.042 |
| AST/ALT | | | |
| 12 weeks | -0.06 ± 0.04 | 0.00 ± 0.04 | 0.915 |
| 24 weeks | -0.03 ± 0.04 | 0.04 ± 0.04 | 0.335 |
| Total bilirubin (μmol/L) | | | |
| 12 weeks | -0.11 ± 0.56 | 0.74 ± 0.56 | 0.308 |
| 24 weeks | 1.23 ± 0.74 | 1.19 ± 0.70 | 0.968 |
| APRI | | | |
| 12 weeks | 0.0004 ± 0.0004 | -0.0004 ± 0.0004 | 0.169 |
| 24 weeks | -0.0004 ± 0.0004 | -0.0006 ± 0.0004 | 0.677 |
| FIB-4 | | | |
| 12 weeks | -0.02 ± 0.04 | -0.02 ± 0.04 | 0.711 |
| 24 weeks | -0.07 ± 0.03 | -0.07 ± 0.03 | 0.969 |

Data are presented as adjusted means ± SEs. One-way ANCOVA was used to compare the difference in changes from baseline after 12 and 24 weeks of intervention between the groups with adjustment for baseline age and AST/ALT, ApoA1 and SOD concentrations

*Abbreviations*: *CAP* controlled attenuation parameter, *LSM* liver stiffness measurement, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* γ-glutamyl transpeptidase, *TG* triglycerides, *APRI* aspartate aminotransferase-to-platelet ratio index, *FIB-4* fibrosis index based on 4 factors

**Table 4** Changes in other biochemical parameters from baseline between groups

| Outcome | Placebo ($n=41$) | Silymarin ($n=42$) | *P* value |
|---|---|---|---|
| TG (mmol/L) | | | |
| 12 weeks | 0.07 ± 0.28 | 0.29 ± 0.28 | 0.601 |
| 24 weeks | 0.06 ± 0.41 | 0.29 ± 0.39 | 0.692 |
| TC (mmol/L) | | | |
| 12 weeks | 0.39 ± 0.13 | 0.52 ± 0.13 | 0.223 |
| 24 weeks | 0.32 ± 0.12 | -0.01 ± 0.11 | 0.059 |
| HDL-C (mmol/L) | | | |
| 12 weeks | 0.10 ± 0.02 | 0.08 ± 0.02 | 0.566 |
| 24 weeks | 0.11 ± 0.03 | 0.06 ± 0.03 | 0.290 |
| LDL-C (mmol/L) | | | |
| 12 weeks | 0.26 ± 0.10 | 0.30 ± 0.10 | 0.799 |
| 24 weeks | 0.38 ± 0.12 | 0.08 ± 0.12 | 0.096 |
| ApoA1 (g/L) | | | |
| 12 weeks | 0.08 ± 0.02 | 0.06 ± 0.02 | 0.637 |
| 24 weeks | 0.09 ± 0.03 | 0.03 ± 0.02 | 0.157 |
| ApoB (g/L) | | | |
| 12 weeks | 0.07 ± 0.02 | 0.08 ± 0.02 | 0.848 |
| 24 weeks | 0.07 ± 0.03 | -0.02 ± 0.03 | 0.023 |
| ApoA1/ApoB | | | |
| 12 weeks | -0.01 ± 0.03 | -0.02 ± 0.03 | 0.776 |
| 24 weeks | 0.01 ± 0.04 | 0.07 ± 0.03 | 0.252 |
| FPG (mmol/L) | | | |
| 12 weeks | 0.02 ± 0.11 | -0.07 ± 0.10 | 0.570 |
| 24 weeks | 0.08 ± 0.18 | -0.10 ± 0.18 | 0.507 |
| Insulin (μU/mL) | | | |
| 12 weeks | 5.82 ± 1.68 | 1.92 ± 1.65 | 0.117 |
| 24 weeks | 3.48 ± 1.63 | -0.23 ± 1.55 | 0.119 |
| HOMA-IR | | | |
| 12 weeks | 1.36 ± 0.47 | 0.54 ± 0.47 | 0.239 |
| 24 weeks | 0.82 ± 0.47 | -0.17 ± 0.45 | 0.149 |
| UA (μmol/L) | | | |
| 12 weeks | 8.58 ± 14.80 | 1.21 ± 14.60 | 0.736 |
| 24 weeks | -35.3 ± 13.80 | -42.50 ± 13.20 | 0.719 |
| SOD (U/mL) | | | |
| 12 weeks | 4.22 ± 1.74 | 3.41 ± 1.72 | 0.754 |
| 24 weeks | 2.13 ± 1.95 | 2.32 ± 2.03 | 0.951 |
| hsCRP (mg/L) | | | |
| 12 weeks | 0.31 ± 0.34 | 0.17 ± 0.33 | 0.778 |
| 24 weeks | 2.35 ± 1.87 | 0.09 ± 1.78 | 0.406 |

Data are presented as adjusted means ± SEs. One-way ANCOVA was used to compare the difference in changes from baseline after 12 and 24 weeks of intervention between the groups with adjustment for baseline age and AST/ALT, ApoA1 and SOD concentrations

*Abbreviations*: *TG* triglycerides, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *ApoA* apolipoprotein A, *ApoB* apolipoprotein B, *FPG* fasting plasma glucose, *HOMA-IR* homeostasis model of insulin resistance, *hsCRP* high-sensitivity C-reactive protein; SOD, superoxide dismutase

richness of the bacterial species increased after silymarin intervention. Moreover, PCoA was utilized to assess the β diversity of the two groups at pre- and post-intervention. The data showed a significant difference ($P=0.001$) within the silymarin group, but not in the placebo group ($P=0.067$) (Fig. 2C and Fig. 2D). Furthermore, the relative abundances of major taxonomic groups before and after intervention were compared between the two groups (Fig. 2E). The most common bacteria at the family level in MASLD patients were *Lachnospiraceae*, *Bacteroidaceae*, and *Enterobacteriaceae*. Notably, the abundance of *Selenomonadaceae* in the silymarin group decreased after the intervention, while it showed little change in the

**Table 5** Changes in anthropometric parameters from baseline between groups

| Outcome | Placebo ($n=41$) | Silymarin ($n=42$) | *P* value |
|---|---|---|---|
| DBP (mmHg) | | | |
| 12 weeks | -4.88 ± 3.40 | 4.67 ± 3.15 | 0.054 |
| 24 weeks | 0.92 ± 2.33 | 1.08 ± 2.29 | 0.962 |
| SBP (mmHg) | | | |
| 12 weeks | 0.62 ± 1.72 | -0.62 ± 1.60 | 0.619 |
| 24 weeks | 2.47 ± 1.61 | -0.08 ± 1.58 | 0.279 |
| BMI (kg/m$^2$) | | | |
| 12 weeks | -0.16 ± 0.22 | -0.07 ± 0.21 | 0.796 |
| 24 weeks | -0.57 ± 0.24 | -0.42 ± 0.24 | 0.653 |
| WHR | | | |
| 12 weeks | 0.02 ± 0.02 | -0.02 ± 0.02 | 0.141 |
| 24 weeks | 0.03 ± 0.02 | -0.03 ± 0.02 | 0.081 |
| BF (%) | | | |
| 12 weeks | -0.11 ± 0.29 | -0.60 ± 0.28 | 0.257 |
| 24 weeks | -0.29 ± 0.35 | -0.08 ± 0.35 | 0.675 |
| BMR (kcal) | | | |
| 12 weeks | -5.74 ± 5.57 | 2.50 ± 5.41 | 0.314 |
| 24 weeks | -16.00 ± 5.51 | -13.10 ± 5.51 | 0.724 |

Data are presented as adjusted means ± SEs. One-way ANCOVA was used to compare the difference in changes from baseline after 12 and 24 weeks of intervention between the groups with adjustment for baseline age and AST/ALT, ApoA1 and SOD concentrations

*Abbreviations*: *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *BMI* body mass index, *WHR* waist-hip ratio, *BF* body fat, *BMR* basal metabolic rate

placebo group. LEfSe analysis indicated that the bacterial family that was the most differentially enriched in the silymarin group after 24 weeks of intervention was *Oscillospiraceae* (Fig. 2F).

## Discussion

The present study, using FibroScan, provides evidence that supplementation with silymarin equivalent to 103.2 mg/d silybin for 24 weeks has a significant protective effect on liver stiffness in patients with MASLD, despite the absence of a notable impact on steatosis. The observed hepatoprotective effect was accompanied by significant improvements (i.e., decreases) in serum GGT and ApoB levels, but there were no significant changes in other serum biochemical parameters (ALT, AST, AST/ALT, total bilirubin, TG, TC, HDL-C, LDL-C, ApoA1, ApoA1/ApoB, insulin, glucose, HOMA-IR, SOD, hsCRP, and UA), physical measurements (DBP, SBP, BMI, WHR, BF%, and BMR), or the APRI or FIB-4. The gut microbiota analysis revealed that silymarin supplementation effectively modulated the composition and abundance of microbial populations, resulting in a significant increase in diversity and differential abundance among multiple microbes at the family level. Specifically, *Oscillospiraceae* was greatly enriched, while *Selenomonadaceae* exhibited a decrease.

The compound silymarin is a naturally occurring flavonolignans extracted from an herbal medicine milk thistle. The hepatoprotective effects of silymarin have been extensively investigated in alcoholic liver diseases, MASLD, viral hepatitis, and chemical- or mycotoxin-induced liver injuries [12, 46–50]. Silymarin administration has also been shown to reduce the mortality of patients with cirrhosis [51]. Furthermore, a meta-analysis indicated that silymarin may have liver-protective effects in patients with MASLD [52]. However, the available data from RCTs on the efficacy of silymarin in MASLD management remain limited and inconsistent, with the majority focusing on the effectiveness of silymarin combination therapy and/or evaluation indicators usually confined to biochemical markers [15–22]. Our study demonstrated that treatment with silymarin equivalent to 103.2 mg/d of silybin for 24 weeks may lead to a significant improvement in liver stiffness (LSM), despite having no significant impact on steatosis (CAP), serum ALT or AST levels, or the AST/ALT ratio (Table 3). Notably, in MASH patients, silymarin administration (2100 mg/d, for 48 weeks) has been reported to effectively reduce liver fibrosis, according to both histology and the LSM and APRI, but does not affect AST, ALT, or GGT concentrations or serial glycaemic or lipidaemic parameters except for TG concentrations [15]. In addition, treatment with Realsil, a silybin + vitamin E + phosphatidylcholine complex (equivalent to approximately 94 mg/d silybin, for 12 months), was found to improve plasma levels of aminotransferases, HOMA-IR, and liver histology in patients with MASLD [16]. In addition, by using ultrasound-liver-steatosis (ULS) grade, silymarin-enriched nutraceutical supplementation (7 ingredients, equivalent to approximately 286 mg/d silymarin, 3 months) has been suggested to enhance the efficacy of a Mediterranean hypocaloric diet treatment in overweight/obese patients with MASLD [17]. However, another study on MASH yielded inconclusive results regarding the antifibrotic role of silymarin (420 and 700 mg/d, respectively, for 48 weeks) due to a certain defect in participant inclusion based on histological analysis [18]. Different interventions and different outcome indicators and methodologies may affect the results. Biochemical markers and fibrosis scores are preferred but have relatively low sensitivity and specificity, especially for detecting more advanced disease stages. Histology is the diagnostic gold standard but is infrequently used because of its invasive features, and this approach also exhibits limitations in terms of potential sampling bias and the presence of inter- and intra-rater variations [8, 53]. In the present study, by using FibroScan, a

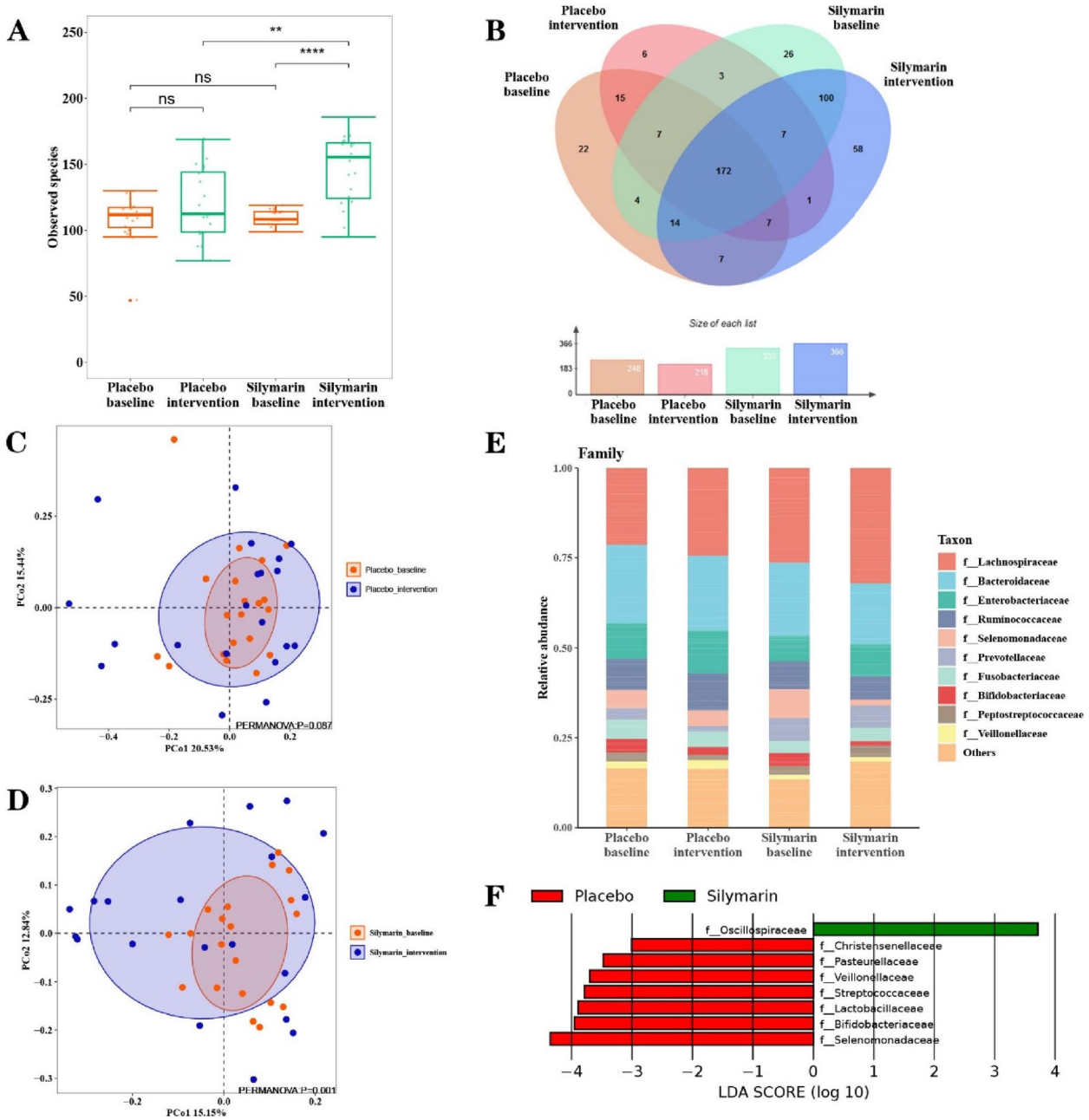


**Fig. 2** The effects of silymarin treatment on gut microbiota. A Boxplots showing the α diversity measured for the observed species between the placebo and silymarin groups. **B** A Venn diagram was used to analyse the distribution of abundances in operational taxonomic units (OTUs) across the placebo and silymarin groups. **C**, **D** PERMANOVA (PCoA) of the gut microbiota in the placebo and silymarin groups. **E** The abundance of major taxonomic groups before and after intervention in the two groups. **F** LDA effect size (LEfSe) analysis indicating differentially abundant bacterial families between the two groups. $P < 0.05$ and LDA scores > 3 were considered significant

noninvasive and reliable way to assess both steatosis and fibrosis, we provide direct evidence that moderate doses of silymarin supplementation alone may confer certain protection against liver stiffness but have no significant effect on steatosis in patients with MASLD.

Liver diseases or dysfunctions are often reflected by biochemical abnormalities such as elevated liver enzymes and bilirubin [54, 55]. Moreover, abnormal blood glucose and lipid profiles, abnormal anthropometric measurements, increased oxidative stress and increased inflammatory markers are the main risk factors for MASLD.

Hence, we determined the serum levels of AST, ALT, GGT, total bilirubin, TC, TG, HDL-C, ApoA1, LDL-C, ApoB, glucose, insulin, hsCRP, SOD, and UA, calculated the AST/ALT, ApoA1/ApoB, and HOMA-IR, and measured DBP, SBP, BMI, WHR, BF% and BMR. The results indicated that the silymarin-treated patients showed a significant improvement (i.e., decreases) in serum GGT and ApoB concentrations but no significant difference in changes in other indicators compared to the placebo-treated patients (Tables 4 and 5).

The available information on the role of silymarin in blood biochemical and physical measurements is inconsistent [15–22]. The inclusion/exclusion criteria, baseline characteristics, and intervention details (including dose, duration, and formulation) can be crucial determinants. Notably, several RCTs using silymarin at doses much greater than those we used revealed no significant effect on ALT or AST concentrations in MASLD patients, regardless of whether silymarin treatment was provided alone or in combination [15, 18, 19]. Due to their easy availability and clinical significance, ALT and/or AST concentrations are commonly used as the primary variables for calculating fibrosis scores including the FIB-4 and APRI. However, the findings from this trial indicated that there was no significant difference in the change in either score between the two groups (Table 3), suggesting that their performance in terms of sensitivity for evaluating fibrosis is inferior to that of FibroScan in this population. Currently, elevated GGT levels are also considered a marker of liver damage and are correlated with hepatic fibrosis and steatosis in patients with MASLD [54, 56–58]. Moreover, data from a clinical trial in children with MASLD suggested that dynamic alterations in serum GGT and ALT levels may serve as powerful markers for histologic response [59]. Elevated GGT has also been shown to be related to an increased risk of mortality in men, and this relationship was even stronger in men who had hepatic steatosis [60]. In summary, silymarin supplementation may lead to improvements (i.e., decreases) in serum GGT and ApoB levels. These improvements, along with the reduced LSM, validate the efficacy of silymarin in managing MASLD.

Given the low bioavailability of silymarin [61] and the presence of the gut-liver axis [24], it can be speculated that the health-promoting effect of silymarin may involve gut microbiota, similar to our previous findings on anthocyanin and resveratrol [62, 63]. Indeed, a few studies have demonstrated the modulatory role of silymarin in the composition and abundance of gut bacterial communities in rodent models of alcoholic fatty liver disease (AFLD), MASLD, and Alzheimer's disease [33–37]. Hence, we further detected and analysed faecal microbiota in this RCT population. The results indicated that silymarin supplementation favoured the diversity of the faecal microbiota and regulated its distribution. At the family level, multiple differentially abundant taxa were identified. Specifically, LEfSe analysis revealed an enrichment of *Oscillospiraceae* after silymarin treatment; however, *Selenomonadaceae* exhibited considerable abundance at baseline in both groups but showed a marked reduction after silymarin treatment (Fig. 2). To the best of our knowledge, this is the first population-based study that unveils the modulatory effect of silymarin on the gut microbiota in patients with MASLD.

The proportion and significance of *Selenomonadaceae* in faeces or colon contents under various pathophysiological conditions remain unclear. Intriguingly, Mo et al. reported a decrease in *Selenomonadaceae* levels following *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 supplementation in overweight individuals, suggesting that this shift may represent one of the mechanisms by which probiotics regulate the characteristics and β diversity of gut microbiota [64]. Animal experiments have demonstrated that *Oscillospiraceae* exhibits a reduction in obesity and obesity-related diseases, including MASLD. This phenomenon can be mitigated by various plant extracts and bioactive substances [65–69]. In particular, the increased abundance of *Oscillospiraceae* has been involved in the amelioration of hepatic fibrosis induced by a combination of sodium alginate and oxymatrine in a $CCl_4$ mouse model [70]. *Oscillospiraceae* has the ability to ferment complex carbohydrates [71], and its increased abundance was found to be significantly associated with the generation of short-chain fatty acids (SCFAs), which are well-established metabolites linking dietary nutrition, gut microbiota and host metabolic health. Similarly, Li et al. reported that silymarin supplementation changes the composition of gut microbiota and increases caecal concentrations of SCFAs in high-fat diet (HFD)-fed mice [72], which may be responsible for improving MASLD. The biotransformation of silymarin mediated by human gut microorganisms has been monitored ex vivo, and several types of biotransformation products have been identified [73, 74]; however, their absorbability and bioactivity remain poorly understood. Furthermore, a recent study in HFD-fed rats revealed that silymarin significantly induced an improvement in liver lipid metabolism, which was closely relevant to the gut microbiota and B12 production [75]. Overall, we speculate that the modulation of gut microbiota by silymarin and the possible interactions between them may constitute a potential mechanism underlying the favourable outcomes observed in this RCT, but the specific mediating effect of the gut microbiota and the main mediators or metabolites deserve further in-depth investigation.

### Strengths and limitations

To our knowledge, this is the first RCT to evaluate the role of silymarin in the gut microbiota and its potential mediation of MASLD. In addition, efficacy was assessed using a relatively low-dose and individual intervention approach. Furthermore, practical and convincing endpoints were established, namely, quantitative analysis of steatosis and stiffness. However, several limitations need to be taken into consideration. First, some baseline characteristics were not well balanced between the two groups after randomization. We adjusted for these factors to avoid confounding by using ANCOVA. Second, MASLD is a heterogeneous disease, but patients with severe liver dysfunction and chronic diseases, including clinically diagnosed cardiovascular diseases, were excluded from the trial for possible medication interference. Moreover, this was a single-centre RCT with a small sample size and only one intervention dose, which may affect the generalizability of our findings. Third, the bioavailability and gut microbiota metabolites of silymarin were not determined. Finally, liver stiffness and steatosis diagnosed by FibroScan need to be validated by histology.

## Conclusions

In conclusion, our findings suggest that silymarin supplementation alone could have a modest role in improving liver stiffness through mechanisms involving gut microbiota, but contribute little to existing steatosis in MASLD patients without severe liver dysfunction, hyperglycaemia, and dyslipidaemia. The results warrant further validation through large-scale and long-term trials, particularly conducted in specific patient populations given the high heterogeneity of MASLD. Taking evidence from our and previous studies together, silymarin may hold the potential to be a complementary medicine or nutraceutical for managing MASLD.

#### Abbreviations

| | |
|---|---|
| MASLD | Metabolic dysfunction-associated steatotic liver disease |
| SLD | Steatotic liver disease |
| MASH | Metabolic dysfunction-associated steatohepatitis |
| HCC | Hepatocellular carcinoma |
| RCT | Randomized controlled trial |
| VCTE | Vibration-controlled transient elastography |
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| GGT | γ-Glutamyl transpeptidase |
| BMR | Basal metabolic rate |
| MET | Metabolic equivalent of task |
| EASL | European Association for the Study of the Liver |
| AASLD | American Association for the Study of Liver Diseases |
| CAP | Controlled attenuation parameter |
| LSM | Liver stiffness measurement |
| APRI | Aspartate aminotransferase-to-platelet ratio index |
| ULN | Upper limit of normal |
| PLT | Platelet count |
| FIB-4 | Fibrosis index based on 4 factors |
| FPG | Fasting plasma glucose |
| BMI | Body mass index |
| WHR | Waist-hip ratio |
| SBP | Systolic blood pressure |
| DBP | Diastolic blood pressure |
| BF% | Body fat percentage |
| TC | Total cholesterol |
| TG | Triglyceride |
| LDL-C | Low-density lipoprotein cholesterol |
| HDL-C | High-density lipoprotein cholesterol |
| ApoB | Apolipoprotein B |
| ApoA1 | Apolipoprotein A1 |
| hsCRP | High-sensitivity C-reactive protein |
| UA | Uric acid |
| SOD | Superoxide dismutase |
| HOMA-IR | Homeostatic model assessment of insulin resistance |
| OTUs | Operational taxonomic units |
| PCoA | Principal coordinate analysis |
| PERMANOVA | Permutational multivariate analysis of variance |
| LDA | Linear discriminant analysis |
| LEfSe | Linear discriminant analysis effect size |
| ITT | Intention-to-treat |
| SD | Standard deviation |
| IQR | Interquartile range |
| ANCOVA | Analysis of covariance |
| ULS | Ultrasound-liver-steatosis |
| AFLD | Alcoholic fatty liver disease |
| SCFAs | Short-chain fatty acids |
| HFD | High-fat diet |

#### Authors' contributions

Y.J. contributed to the analysis, collected the data, led the investigation, and prepared the original draft of the manuscript. X.W. collected the data, contributed to the formal analysis, prepared the original draft of the manuscript, and contributed to data visualization. K.C. collected the data, contributed to data visualization, and prepared the original draft of the manuscript. Y.C. collected the data, prepared the original draft of the manuscript, and contributed to data visualization. L.Z. collected the data and contributed to data visualization. Y.Z. (Yupeng Znegn), Y.Z. (Yuqing Zhou), and Z.P. curated the data and contributed to data visualization. D.W. and Z.L. contributed to the writing, review, and editing of the manuscript. Y.L. contributed to the conception, design and administration of the project, as well as the writing, review, and editing of the manuscript. W.L. and D.L. contributed to the conception, design and administration of the project and funding acquisition. All authors reviewed the manuscript.

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#### Availability of data and materials

The data presented in this study are available on request from the corresponding author.

## Declarations

#### Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Biomedical Research, School of Public Health, Sun Yat-Sen University. (protocol code: 2022–068 and date of approval: 2022–03-23).

#### Consent for publication

Informed consent was obtained from all volunteers involved in the study.

#### Competing interests

Zhongxia Li, Di Wang. are employed by BYHEALTH Institute of Nutrition & Health. The other authors declare no conflict of interest.

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