



A Randomized Trial of Silymarin for the Treatment of Nonalcoholic Steatohepatitis

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BACKGROUND & AIMS:

Silymarin is a complex mixture of 6 major flavonolignans and other minor polyphenolic compounds derived from the milk thistle plant *Silybum marianum*; it has shown antioxidant, anti-inflammatory and antifibrotic effects, and may be useful in patients with nonalcoholic fatty liver disease (NAFLD). We aimed to study the efficacy of silymarin in patients with nonalcoholic steatohepatitis (NASH)—the more severe form of NAFLD.

METHODS:

We performed a randomized, double-blind, placebo-controlled trial of consecutive adults with biopsy-proven NASH and a NAFLD activity score (NAS) of 4 or more at a tertiary care hospital in Kuala Lumpur, Malaysia, from November 2012 through August 2014. Patients were randomly assigned to groups given silymarin (700 mg; n = 49 patients) or placebo (n = 50 patients) 3 times daily for 48 weeks. After this 48-week period, liver biopsies were repeated. The primary efficacy outcome was a decrease of 30% or more in NAS; findings from 48-week liver biopsies were compared with those from the baseline biopsy. Secondary outcomes included changes in steatosis, lobular inflammation, hepatocyte ballooning, NAS and fibrosis score, and anthropometric measurements, as well as glycemic, lipid, and liver profiles and liver stiffness measurements.

RESULTS:

The percentage of patients achieving the primary efficacy outcome did not differ significantly between the groups (32.7% in the silymarin group vs 26.0% in the placebo group; $P = .467$). A significantly higher proportion of patients in the silymarin group had reductions in fibrosis based on histology (reductions of 1 point or more; 22.4%) than did the placebo group (6.0%; $P = .023$), and based on liver stiffness measurements (decrease of 30% or more; 24.2%) than did the placebo group (2.3%; $P = .002$). The silymarin group also had significant reductions in mean aspartate aminotransferase to platelet ratio index (reduction of 0.14, $P = .011$ compared with baseline), fibrosis-4 score (reduction of 0.20, $P = .041$ compared with baseline), and NAFLD fibrosis score (reduction of 0.30, $P < .001$ compared with baseline); these changes were not observed in the placebo group (reduction of 0.07, $P = .154$; increase of 0.18, $P = .389$; and reduction of 0.05, $P = .845$, respectively). There was no significant difference between groups in number of adverse events; adverse events that occurred were not attributed to silymarin.

CONCLUSIONS:

In a randomized trial of 99 patients, we found that silymarin (700 mg, given 3 times daily for 48 weeks) did not reduce NAS scores by 30% or more in a significantly larger proportion of patients with NASH than placebo. Silymarin may reduce liver fibrosis but this remains to be confirmed in a larger trial. It appears to be safe and well tolerated. [ClinicalTrials.gov: NCT02006498](https://clinicaltrials.gov/ct2/show/study/NCT02006498).

Keywords: FibroScan; Randomized Controlled Trial; Silybin; Treatment.

See editorial on page 1863.

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease and is estimated to affect up to 30% of the general population.¹ Nonalcoholic steatohepatitis (NASH), the more severe form of NAFLD, can progress to cirrhosis, and is associated with increased mortality from liver-related

Abbreviations used in this paper: ALT, alanine aminotransferase; AST, aspartate aminotransferase; FIB-4, fibrosis-4; GGT, gamma glutamyl transpeptidase; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis.

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complications.² NASH-related cirrhosis is one of the leading causes for liver transplantation, and is the only growing indication for liver transplantation in the United States.³ In a large study of patients with biopsy-proven NASH, weight loss $\geq 10\%$ through a comprehensive lifestyle program led to NASH resolution and fibrosis improvement in 90% and 45%, respectively. However, only 10% of patients were able to achieve the desired amount of weight loss. The majority (70%) of patients had $<5\%$ weight loss, and 21% of these patients had fibrosis progression during the 48-week study period.⁴ Lifestyle intervention is important but clearly not enough in the management of the majority of NASH patients.

In a study on nondiabetic patients with biopsy-proven NASH, vitamin E and pioglitazone significantly reduced steatosis, lobular inflammation, and hepatocyte ballooning, but not fibrosis, compared with placebo.⁵ However, the use of vitamin E has been limited by concern about increased risk of prostate cancer and conflicting reports on increased all-cause mortality. On the other hand, pioglitazone was associated with significant weight gain, which did not resolve after discontinuation of the drug, and congestive cardiac failure. The farnesoid X receptor agonist, obeticholic acid, was shown to result in significant improvement in steatosis, lobular inflammation, hepatocyte ballooning and even fibrosis compared with placebo, but was associated with an adverse lipid profile and pruritus.⁶ There are clearly unmet needs in the treatment for NASH patients.

Silymarin, which is derived from the milk thistle plant *Silybum marianum*, has been used for centuries as an herbal remedy for liver diseases. It is a complex mixture of 6 major flavonolignans (silybins A and B, isosilybins A and B, silychristin, and silydianin), as well as other minor polyphenolic compounds.⁷ There have been numerous in vitro and animal studies demonstrating the antioxidant, anti-inflammatory, and antifibrotic properties of silymarin.^{8–11} Several human clinical trials have also suggested that silymarin may be useful for the treatment of NAFLD. However, the trials were single armed, open labeled, or did not have histological endpoints.^{12–16} A randomized, double-blinded, placebo-controlled study looked at the use of a silybin phytosome complex coformulated with vitamin E for 12 months in NAFLD patients with or without chronic hepatitis C. However, the majority of the NAFLD patients in the study had simple steatosis and there appeared to be significant heterogeneity in the baseline histology between the treatment and placebo groups. Moreover, only 32.4% of the NAFLD patients had an end-of-treatment liver biopsy. Nevertheless, the authors found significant improvement in steatosis, inflammation, ballooning, and fibrosis in the treatment group, albeit without providing the results of statistical comparison of these changes with that in the placebo group.¹⁷ We conducted this study with the aim to provide more robust evidence on the use of silymarin for the treatment of NASH.

Methods

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and ethical approval was obtained from our institutional review board prior to the commencement of the study (Approval Date: May 25, 2011; Reference No.: 853.1). All subjects who participated in the study provided written informed consent. The study was registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT02006498). All authors had access to the study data and had reviewed and approved the final manuscript.

Consecutive adult NAFLD patients (>18 years old) seen at the Gastroenterology and Hepatology Clinic of this institution were considered for inclusion into the study. The diagnosis of NAFLD was based on ultrasonography findings of fatty liver and exclusion of significant alcohol intake, use of medications that can cause hepatic steatosis, viral hepatitis B and C infection, and other causes of chronic liver disease where indicated.¹⁸ NAFLD patients with serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels ≥ 40 international units/L were offered screening for the study, which included a liver biopsy. Screening was also offered when there were other reasons for NASH to be suspected (eg, significant liver fibrosis based on liver stiffness measurement, obese patients with metabolic syndrome). Patients who were on silymarin or other milk thistle preparations, vitamin C, vitamin E, glutathione, alpha-tocopherol, or nonprescribed complementary alternative medications must have stopped these preparations for at least 30 days prior to screening, and must have been willing to refrain from taking these preparations throughout the study period.

Liver Biopsy and Histological Assessment

Ultrasonography-guided percutaneous liver biopsy was performed by either one of 2 experienced operators (W.-K.C., S.M.) using 18 G Temno II semiautomatic biopsy needle (Cardinal Health, Dublin, OH). Liver biopsy specimens were processed using standard laboratory procedures. Liver biopsy slides were stained with H&E and Masson trichrome stain. Liver biopsy slides were examined by an experienced histopathologist (N.R.N.M.) who was blinded to the clinical data. NASH was diagnosed based on the presence of steatosis, lobular inflammation, and hepatocyte ballooning. Histopathological findings were reported according to the Nonalcoholic Steatohepatitis Clinical Research Network Scoring System.¹⁹ Fibrosis stages 1a, 1b, and 1c were considered stage 1 for the purpose of analysis. Patients with NASH and NAFLD activity score (NAS) of ≥ 4 were included into the study. Patients with cirrhosis were excluded.

Randomization and Treatment Groups

Patients who met the eligibility criteria were randomized within 1 month of the liver biopsy to receive

either silymarin 700 mg 3 times daily or placebo 3 times daily for 48 weeks. Both groups were given lifestyle advice by either one of the 2 physicians involved in the study (W.-K.C., S.M.). A computer-generated table of random numbers was utilized with a simple randomization method. The numbers were kept centrally at this institution's Clinical Investigation Center and released to a research assistant only after a subject was recruited into the study. Silymarin was in the form of 140 mg capsules (Legalon, Meda Group, Solna, Sweden). The capsules and similar-appearing placebo capsules were provided by Meda Group. Clinical investigators were not aware of the treatment group assignment as they were packaged in a similar manner.

Baseline and Follow-Up Visits

Demographic, clinical, anthropometric, and laboratory data were obtained using a standard protocol at baseline and at Weeks 12, 24, 36, and 48. In addition, patients were seen every 4 weeks for assessment of compliance, adverse events and concomitant medications. Pill count was performed for assessment of compliance. Weight and height were measured using standard equipment. Obesity was defined as body mass index ≥ 25.0 kg/m².²⁰ Waist circumference was measured at the midpoint between the lowest margin of the least palpable rib and the top of the iliac crest in the standing position. Central obesity was defined as waist circumference >90 cm for men and >80 cm for women.²¹ Venous blood was drawn after an overnight fast for complete blood count, blood glucose, glycated hemoglobin (HbA1c), lipid profile, and liver profile. Biochemical measurements were performed using standard laboratory procedures. In addition, serum insulin level was measured at baseline and at weeks 24 and 48. Insulin resistance was estimated using the Homeostatic Model Assessment of Insulin Resistance. Patients who were on insulin therapy were not included into all Homeostatic Model Assessment of Insulin Resistance analyses.

Transient elastography was performed by either one of 2 experienced operators (W.-K.C., S.M.) using FibroScan 502 Touch with M probe (EchoSens, Paris, France) at baseline and at weeks 24 and 48. Ten measurements were obtained for each patient. Adequate pressure of the probe on the skin surface, good layering on time-motion mode and a straight imaginary line on A mode were ensured for each measurement. An examination was considered successful when valid measurements were $\geq 80\%$ and interquartile range and median for liver stiffness measurement was $<30\%$. A liver biopsy was repeated at week 48.

Primary and Secondary Outcomes

The primary efficacy outcome was $\geq 30\%$ improvement in NAS in the repeat liver biopsy compared with

baseline. This criterion was an average rate of improvement based on previous pharmacological studies in NAFLD.^{22,23} Secondary outcomes included changes in steatosis, lobular inflammation, hepatocyte ballooning, NAS and fibrosis, anthropometric measurements, glycaemic, lipid and liver profiles, and FibroScan measurements. Improvement in steatosis, lobular inflammation, hepatocyte ballooning, NAS, and fibrosis was defined as ≥ 1 point improvement in the corresponding histological component. In addition, the outcome of resolution of steatohepatitis without worsening of fibrosis was included in the post hoc analysis in view that this outcome has become an important outcome in clinical trials. Resolution of steatohepatitis was defined as disappearance of ballooning, and disappearance or persistence of mild lobular inflammation only, while worsening of fibrosis was defined as any stage increase in fibrosis.²⁴ Safety was assessed based on the occurrence of adverse events and serious adverse events during the study period.

Sample Size Calculation and Statistical Analysis

The percentage of subjects achieving the primary efficacy outcome in the placebo and silymarin groups was approximated based on previous pharmacological studies on NAFLD.^{5,22} A sample size of 88 patients would be needed to have a 90% chance of detecting, as significant at the 5% level, an increase in the primary efficacy outcome from 15% in the placebo group to 45% in the silymarin group.²⁵ Assuming a dropout rate of 10%, a total of 97 patients were needed. Data were analyzed using a standard statistical software program (SPSS 15.0, SPSS Inc, Chicago, IL). Continuous variables were expressed as means with standard deviation or medians with interquartile ranges, and analyzed using *t* test or Mann-Whitney test, where appropriate. Categorical variables were expressed as percentages and analyzed using chi-square test or Fisher exact test, where appropriate. Analyses were based on intention to treat unless stated otherwise.

Results

Study Subjects and Compliance

Screening began in November 2012 and ended in August 2014 when a sufficient number of subjects have been enrolled. Of the 148 subjects who underwent screening, 99 subjects were eligible for the study and underwent randomization. There were 49 subjects in the silymarin group and 50 subjects in the placebo group (Supplementary Figure 1). The baseline characteristics were comparable between the groups except the serum triglyceride and total cholesterol levels, and the controlled attenuation parameter, which were higher in

the silymarin group (Table 1). Overall, there was excellent compliance to the medication dispensed with an average compliance rate of 97.6% (ranging from 86.7% to 100%). A total of 89 subjects (89.9%) underwent the end-of-treatment liver biopsy. The median (interquartile range) of liver biopsy length in the silymarin group and placebo group at the end of treatment was 15 (12–16) mm and 14 (12–15) mm, respectively ($P = .065$). The

number of portal tracts was 7 (5–9) and 7 (5–10), respectively ($P = .769$).

Primary Efficacy Outcome and Other Changes in Histologic Features

The results on primary efficacy outcome and other changes in histologic features are summarized in Table 2.

Table 1. Baseline Patient Characteristics

	Overall (n = 99)	Silymarin (n = 49)	Placebo (n = 50)	P value
Age, y	49.9 ± 11.4	49.6 ± 12.7	50.1 ± 10.2	.820
Male, %	46.5	49.0	44.0	.619
Body mass index, kg/m ²	30.5 ± 4.3	30.0 ± 4.0	31.0 ± 4.6	.233
Obesity, %	90.9	89.8	92.0	.741
Waist circumference, cm	99 ± 11	99 ± 11	101 ± 11	.487
Central obesity, %	98.0	98.0	98.0	1.000
History of diabetes mellitus, %	53.5	46.9	60.0	.193
History of dyslipidemia, %	76.8	83.7	70.0	.107
History of hypertension, %	62.6	61.2	64.0	.775
Fasting blood sugar, mmol/L	5.6 (4.9–7.2)	5.6 (4.9–7.2)	5.9 (4.9–7.2)	.936
HbA1c, %	6.5 (5.7–7.8)	6.6 (5.7–7.9)	6.4 (5.7–7.8)	.866
HOMA-IR ^a	6.2 (3.9–9.9)	5.8 (3.6–10.0)	6.3 (4.3–10.0)	.506
Triglyceride, mmol/L	1.70 (1.20–2.10)	1.70 (1.50–2.10)	1.30 (1.10–1.93)	.027
Total cholesterol, mmol/L	4.90 (4.20–5.70)	5.10 (4.60–6.05)	4.60 (4.18–5.43)	.032
HDL, mmol/L	1.14 (0.98–1.34)	1.18 (0.99–1.34)	1.13 (0.96–1.36)	.607
LDL, mmol/L	2.95 (2.39–3.72)	3.15 (2.48–4.08)	2.81 (2.34–3.57)	.121
ALT, U/L	79 (55–122)	88 (60–132)	73 (47–121)	.156
AST, U/L	50 (32–74)	52 (36–78)	46 (31–70)	.120
GGT, U/L	87 (53–138)	97 (64–135)	78 (45–140)	.073
Liver stiffness measurement, kPa ^b	8.8 (6.4–12.0)	8.8 (6.7–12.0)	8.7 (6.1–12.1)	.826
Liver stiffness measurement, %	12 (9–17)	13 (9–18)	12 (9–17)	.345
Controlled attenuation parameter, dB/m	325 (303–351)	329 (312–362)	315 (285–350)	.043
Liver biopsy length, mm	15 (12–17)	15 (13–17)	15 (12–17)	.822
Number of portal tracts	8 (7–11)	8 (7–11)	8 (7–11)	.434
Steatosis grade				.913
0	2 (2–3)	2 (2–3)	2 (2–3)	
1	1 (1.0)	0 (0)	1 (2.0)	.463
2	15 (15.2)	6 (12.2)	9 (18.0)	
3	54 (54.5)	30 (61.2)	24 (48.0)	
4	29 (29.3)	13 (26.5)	16 (32.0)	
Inflammation grade				.849
0	2 (1–2)	2 (1–2)	2 (1–2)	
1	1 (1.0)	1 (2.0)	0 (0)	.719
2	34 (34.3)	17 (34.7)	17 (34.0)	
3	59 (59.6)	28 (57.1)	31 (62.0)	
4	5 (5.1)	3 (6.1)	2 (4.0)	
Ballooning grade				.615
0	1 (1–2)	1 (1–2)	1 (1–2)	
1	6 (6.1)	3 (6.1)	3 (6.0)	.854
2	58 (58.6)	30 (61.2)	28 (56.0)	
3	35 (35.4)	16 (32.7)	19 (38.0)	
NAFLD activity score				.854
0	5 (4–6)	5 (4–6)	5 (4–6)	
1	32 (32.3)	17 (34.7)	15 (30.0)	.618
2	67 (67.7)	32 (65.3)	35 (70.0)	
Fibrosis stage				.310
0	1 (1–2)	1 (1–3)	1 (0–2)	
1	23 (23.2)	10 (20.4)	13 (26.0)	.768
2	44 (44.4)	21 (42.9)	23 (46.0)	
3	8 (8.1)	4 (8.2)	4 (8.0)	
4	24 (24.2)	14 (28.6)	10 (20.0)	

NOTE. Values are mean ± SD, median (interquartile range), or n (%), unless otherwise indicated. The P values were calculated using independent t test or Mann-Whitney test, where appropriate, for continuous variables, and chi-square test or Fisher exact test, where appropriate, for categorical variables.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NAFLD, nonalcoholic fatty liver disease; U, units.

^aEight patients in the placebo group and 3 patients in the silymarin group were on insulin therapy and were excluded in the HOMA-IR analyses.

^bThree patients in the placebo group and 2 patients in the silymarin group had invalid or unreliable FibroScan measurements at baseline.

Table 2. Primary Efficacy Outcome and Other Changes in Histologic Features, Intention-to-Treat Analysis

	Silymarin (n = 49)	Placebo (n = 50)	P between groups
Primary efficacy outcome ^a	16 (32.7)	13 (26.0)	.467
Steatosis			
Improvement ^b	9 (18.4)	13 (26.0)	.361
Change within group	−0.102	−0.200	.387
P for change within group	.168	.024	
Lobular inflammation			
Improvement ^b	16 (32.7)	15 (30.0)	.776
Change within group	−0.225	−0.240	.896
P for change within group	.015	.004	
Hepatocyte ballooning			
Improvement ^b	20 (40.8)	17 (34.0)	.483
Change within group	−0.347	−0.280	.610
P for change within group	.001	.003	
NAS			
Improvement ^b	26 (53.1)	27 (54.0)	.925
Change within group	−0.674	−0.720	.857
P for change within group	.001	<.001	
Resolution of steatohepatitis without worsening of fibrosis ^c	14 (28.6)	11 (22.0)	.452
Fibrosis			
Improvement ^b	11 (22.4)	3 (6.0)	.023
Change within group	−0.184	+0.100	.026
P for change within group	.071	.200	
Resolution of fibrosis ^d	5/39 (12.8)	3/37 (8.1)	.712
Development of cirrhosis	1 (2.0)	3 (6.0)	.617

NOTE. Values are n (%) or n/n (%), unless otherwise indicated. The P values were calculated using paired t test for change within group and independent t test for change between groups for continuous variables, and chi-square test or Fisher exact test, where appropriate, for categorical variables.

NAS, nonalcoholic fatty liver disease activity score.

^aPrimary efficacy outcome was defined as $\geq 30\%$ improvement in the NAS.

^bImprovement was defined as ≥ 1 point improvement in the corresponding histological component.

^cResolution of steatohepatitis was defined as disappearance of ballooning, and disappearance or persistence of mild lobular inflammation only, and worsening of fibrosis was defined as any stage increase in fibrosis.

^dResolution of fibrosis was defined as absence of fibrosis on end-of-treatment liver biopsy in patients who had at least stage 1 fibrosis on baseline liver biopsy.

There was no significant difference in the percentage of subjects who achieved the primary efficacy outcome in the silymarin group compared with the placebo group (32.7% vs 26.0%; $P = .467$). There was significant reduction in lobular inflammation, hepatocyte ballooning and the NAS in both groups. However, these reductions were not significantly different between the groups. The proportion of subjects who had improvement in steatosis, lobular inflammation, hepatocyte ballooning, and the NAS was also not significantly different between the silymarin group and the placebo group. There was reduction in fibrosis in the silymarin group, which was not seen in the placebo group, and the changes were significant between the groups. The percentage of subjects who had improvement in fibrosis was also significantly higher in the silymarin group compared with the placebo group (22.4% vs 6.0%; $P = .023$). In addition, a symmetric direction of improvement in fibrosis was observed (ie, a numerically larger proportion of patients had resolution of fibrosis and a lower proportion of patients had progression to cirrhosis in the silymarin group compared with the placebo group). These findings were similar on per protocol analysis (Supplementary Table 1). In the subgroup analysis of patients with $\leq 2\%$ weight change (Supplementary Table 2), there was

a greater difference in the proportion of patients with fibrosis improvement in the silymarin group compared with the placebo group (32.1% vs 0%; $P = .002$). The results of subgroup analyses of patients with liver biopsy length ≥ 15 mm at baseline and at the end of treatment, and of patients with liver biopsy length < 15 mm either at baseline or at the end of treatment, are presented in Supplementary Tables 3 and 4.

Further Analyses on Fibrosis Improvement Based on Histologic Data

The end-of-treatment fibrosis stage for each of the patients, stratified according to their baseline fibrosis stage, is presented in Table 3. All the patients with fibrosis improvement had improvement in the NAS except for 2 patients in the silymarin group (the NAS remained unchanged in 1 of the patients and increased by 1 point in the other) (Supplementary Figure 2). There was no significant difference in the mean NAS change in patients with fibrosis improvement between the silymarin and the placebo groups (−1.455 vs −2.333; $P = .226$). However, patients with fibrosis improvement had greater improvement in mean NAS compared with those who did not have fibrosis improvement in both the

Table 3. End-of-Treatment Fibrosis Stage Stratified According to Baseline Fibrosis Stage

		End of treatment				
		F0	F1	F2	F3	F4
		Total				
Silymarin	Baseline					
	F0	6 ^a	4 ^b	0 ^b	0 ^b	0 ^b
	F1	5 ^c	16 ^a	0 ^b	0 ^b	0 ^b
	F2	0 ^c	2 ^c	2 ^a	0 ^b	0 ^b
	F3	0 ^c	3 ^c	1 ^c	9 ^a	1 ^b
	Total	11	25	3	9	1
Placebo	Baseline					
	F0	12 ^a	0 ^b	1 ^b	0 ^b	0 ^b
	F1	3 ^c	19 ^a	0 ^b	1 ^b	0 ^b
	F2	0 ^c	0 ^c	3 ^a	1 ^b	0 ^b
	F3	0 ^c	0 ^c	0 ^c	7 ^a	3 ^b
	Total	15	19	4	9	3

NOTE. Patients who did not undergo end-of-treatment liver biopsy were assumed to have unchanged fibrosis stage. The number of patients in the silymarin group who did not undergo end-of-treatment liver biopsy, according to baseline fibrosis stages, is as follows: F0, 1; F1, 3; F2, 0; F3, 1. The number of patients in the placebo group who did not undergo end-of-treatment liver biopsy, according to baseline fibrosis stages, is as follows: F0, 2; F1, 3; F2, 0; F3, 0.

^aUnchanged.

^bWorsened.

^cImproved.

groups (−1.455 vs −0.447, $P = .023$ in the silymarin group; −2.333 vs −0.617, $P = .019$ in the placebo group). There was no significant difference in the mean weight change between patients who had fibrosis improvement compared with those who did not (−0.12 kg vs −0.44 kg; $P = .718$). The use of silymarin remained an independent factor associated with fibrosis improvement after adjusting for age, gender, the baseline NAS and fibrosis stage, and changes in serum HbA1c and LDL levels (Supplementary Table 5).

Changes in Noninvasive Measures of Hepatic Fibrosis

There was a reduction in liver stiffness measurement in the silymarin group, which was not seen in the placebo group, and there was a trend toward significance in the changes when compared between the groups (Table 4). The findings were similar on per protocol analysis (Supplementary Table 6). A significantly higher percentage of subjects in the silymarin group had $\geq 30\%$ improvement in liver stiffness measurement compared with the placebo group (24.2% vs 2.3%; $P = .002$). In view of the observed improvement in fibrosis based on histology and liver stiffness measurement, further analyses were performed using other noninvasive measures of hepatic fibrosis, namely the AST to platelet ratio index, fibrosis-4 (FIB-4) score, and the NAFLD fibrosis scores. All these scores showed significant decline in the silymarin group but not in the placebo group. In addition, there was a trend toward significance in the changes in

the FIB-4 score when compared between the groups (Table 4). The findings were similar on per protocol analysis (Supplementary Table 6). Line charts illustrating the changes in the AST to platelet ratio index, FIB-4 score, and NAFLD fibrosis scores in the silymarin group and in the placebo group are presented in Supplementary Figure 3.

Changes in Weight, and Glycemic, Lipid and Liver Profiles

There was no significant change in mean weight in the silymarin group and the placebo group (Table 4). There was significant reduction in the serum HbA1c, triglyceride, ALT, AST, and GGT levels, and increase in the serum HDL level in the silymarin group. On the other hand, there was significant increase in the fasting blood glucose and reduction in the serum ALT level in the placebo group. However, the only significant change between the groups was in the serum triglyceride levels. The findings were similar on per protocol analysis (Supplementary Table 6). Line charts illustrating the changes in the serum ALT and AST levels in the silymarin group and in the placebo group are presented in Supplementary Figure 4.

Adverse Events and Discontinuations

Five patients (5.1%) had serious adverse events, 3 in the silymarin group and 2 in the placebo group. There were 22 adverse events occurring in 19 patients. Thirteen adverse events were in patients receiving silymarin while 9 were in patients receiving placebo (Table 5). Nine patients (9.1%) discontinued the study, 4 in the silymarin group and 5 in the placebo group. Two were due to serious adverse events (ureteric calculi and myocardial infarction in the silymarin group and the placebo group, respectively). Reasons for discontinuation among the other subjects included lost to or declined follow up (3 in the silymarin group and 1 in the placebo group), and noncompliance (3 in the placebo group). Overall, there was no significant difference in serious adverse events, adverse events, and discontinuations in the silymarin and placebo groups. None of the serious adverse events and adverse events was deemed related to the study drug.

Discussion

This is the first study on the use of silymarin for the treatment of NASH that utilized paired liver biopsies and provides histological confirmation of previous observations that silymarin may be useful for the treatment of NAFLD. Furthermore, the randomized, double-blinded, placebo-controlled design eliminates the potential of confounding by lifestyle modifications and the potential biases that may be seen in open-labeled, uncontrolled

Table 4. Changes in Weight, Glycemic, Lipid and Liver Profile, FibroScan Measurements, and Other Noninvasive Indices for Fibrosis, Intention-to-Treat Analysis

	Silymarin (n = 49)	Placebo (n = 50)	P between groups
Weight			
Change, kg	−0.7	−0.1	.414
P for change within group	.221	.653	
Fasting blood glucose			
Change, mmol/L	+0.3	+0.7	.201
P for change within group	.212	.022	
HbA1c			
Change, %	−0.4	0	.133
P for change within group	.030	.970	
HOMA-IR ^a			
Change	+0.5	+0.6	.952
P for change within group	.716	.420	
Triglyceride			
Change, mmol/L	−0.20	+0.04	.017
P for change within group	.008	.522	
Total cholesterol			
Change, mmol/L	−0.33	−0.09	.265
p for change within group	.077	.473	
HDL cholesterol			
Change, mmol/L	+0.07	+0.02	.152
P for change within group	.003	.425	
LDL cholesterol			
Change, mmol/L	−0.31	−0.17	.477
P for change within group	.081	.125	
ALT			
Change, mmol/L	−20	−21	.904
P for change within group	.008	.001	
AST			
Change, mmol/L	−13	−6	.314
p for change within group	.020	.109	
GGT			
Change, mmol/L	−15	−8	.501
P for change within group	.048	.214	
Controlled attenuation parameter ^b			
Change, dB/m	−7	+6	.198
P for change within group	.297	.426	
Liver stiffness measurement ^b			
Change, kPa	−0.7	+1.1	.086
P for change within group	.311	.164	
APRI			
Change	−0.14	−0.07	.273
p for change within group	.011	.154	
FIB-4			
Change	−0.20	+0.18	.102
P for change within group	.041	.389	
NAFLD fibrosis score			
Change	−0.30	−0.05	.329
P for change within group	<.001	.845	

NOTE. The P values were calculated using paired t test for change within group and independent t test for change between groups.

ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis 4; GGT, gamma glutamyl transpeptidase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease; U, units.

^aEight patients in the placebo group and 3 patients in the silymarin group were on insulin therapy and were excluded in the HOMA-IR analyses. In addition, 1 patient in the placebo group was started on insulin during the study and was not included in the HOMA-IR analyses.

^bThree patients in the placebo group and 2 patients in the silymarin group had invalid or unreliable FibroScan measurements at baseline. Four patients in the placebo group and 5 patients in the silymarin group had invalid or unreliable FibroScan measurements at month 12. One of the patients in the placebo group had invalid FibroScan measurements at baseline and at month 12. Hence, there were a total of 44 patients in the placebo group and 42 patients in the silymarin group with valid and reliable measurements at baseline and at month 12. APRI = AST level (U/L) / AST upper limit of normal (U/L) / Platelet count ($10^9/L$) \times 100 FIB-4 = age (y) \times AST level (U/L) / Platelet count ($10^9/L$) / ALT (U/L)^{1/2}; NAFLD fibrosis score = $-1.675 + 0.037 \times \text{age (y)} + 0.094 \times \text{body mass index (kg/m}^2\text{)} + 1.13 \times \text{impaired fasting glucose/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelet (}10^9/L\text{)} - 0.66 \times \text{albumin (g/dL)}$.

Table 5. Serious Adverse Events and Adverse Events in the Silymarin and Placebo Groups

Silymarin	Placebo
<p>Serious adverse events:</p> <ul style="list-style-type: none"> • Hospitalization for further management of ureteric calculi^a • Hospitalization for hemolytic anemia in a patient with underlying thalassemia following an episode of upper respiratory tract infection • Hospitalization for percutaneous coronary intervention in a patient with coronary artery disease^b <p>Adverse events:</p> <ul style="list-style-type: none"> • Dyspepsia • Gastroesophageal reflux disease • Transient ischemic attack and soft tissue injury in the same patient • Upper respiratory tract infection (twice in the same patient) • Viral fever • Gastroenteritis • Dry skin • Backache • Musculoskeletal pain • Soft tissue injury • Ankle swelling 	<p>Serious adverse events:</p> <ul style="list-style-type: none"> • Uncontrolled diabetes mellitus • Myocardial infarction^a <p>Adverse events:</p> <ul style="list-style-type: none"> • Acute gouty arthritis and worsening kidney function in the same patient • Urinary tract infection • Upper respiratory tract infection • Gastroesophageal reflux disease in 2 patients • Palpitation • Abdominal cramp • Anemia due to menorrhagia

NOTE. Patients with serious adverse events and adverse events completed the study unless stated otherwise.

^aDiscontinued the study.

^bCompleted the study but did not undergo end-of-treatment liver biopsy as the patient was on dual antiplatelet therapy.

studies. Treatment with silymarin for 48 weeks did not achieve the primary efficacy outcome of this study but was found to be associated with significantly greater fibrosis improvement compared with placebo based on histology, and supported by liver stiffness measurements by FibroScan as well as other noninvasive measures of hepatic fibrosis. This improvement in fibrosis, without a corresponding difference in the NAS between the silymarin group and the placebo group appeared to be contradictory. However, the improvement in fibrosis was associated with an improvement in the NAS in the majority of subjects. It is also possible that limitation of histological assessment for necroinflammatory activity and ballooning contributed to this discrepancy.²⁶ From a biochemical marker perspective, no significant differences were observed in the change in serum ALT and AST levels between patients on silymarin and those on placebo. However, this is not entirely surprising as several reports have consistently shown that serum aminotransferase levels do not correlate well with NASH severity.^{27,28} It is possible that silymarin has mainly antifibrotic properties, as is seen with compounds in recent clinical trials such as the apoptosis-regulating signal kinase 1 inhibitor selonsertib²⁹ and the dual C-C chemokine receptor inhibitor cenicriviroc.³⁰

The mechanism of action of silymarin is not entirely clear. There have been numerous *in vitro* and animal studies demonstrating the antioxidant, anti-inflammatory, and antifibrotic properties of silymarin.⁸ In a study using an established *in vitro* model of human hepatic fibrogenesis, silybin demonstrated direct and indirect antifibrotic properties by reducing platelet-derived growth factor-induced cell proliferation and migration, by reducing transforming growth

factor- β -induced *de novo* synthesis of collagen type I.¹¹ Recently, an *in vitro* study found that silybin stimulated the farnesoid X receptor in a dose-dependent manner, and the obeticholic acid binding site of the farnesoid X receptor was validated as the binding pocket of silybin.³¹

Low bioavailability of silybins A and B has been associated with customary doses of silymarin but this may be overcome with increased doses of silymarin of up to 2.1 g daily, which has been shown to be safe and well-tolerated without any observed drug-related adverse events.³² The dosage of 700 mg 3 times daily was chosen to provide the highest likelihood of finding a therapeutic effect in this study, similar to the strategy employed in a large, multi-centre study on the use of silymarin for chronic hepatitis C.³³ The previous study on the use of silymarin for chronic hepatitis C utilized serum ALT and hepatitis C virus RNA levels as efficacy outcomes and did not look into changes in fibrosis.³³ Whether a similar effect on fibrosis improvement can be observed with the use of silymarin in other chronic liver diseases deserves further study. In an observational study on patients with advanced liver fibrosis due to chronic hepatitis C, an inverse relationship was noted between silymarin use and the progression from fibrosis to cirrhosis.³⁴ The findings from our study support this previous observation.

The beneficial effect of silymarin on components of the metabolic syndrome has been observed in previous studies,^{35–37} but its mechanism of action remains poorly understood. A randomized, placebo-controlled study found that treatment with silymarin was associated with significantly increased superoxide dismutase activity, glutathione peroxidase activity and total antioxidant

capacity in patients with diabetes mellitus, suggesting that the effects of silymarin may also be related to its antioxidant property.³⁸ With the increasing understanding of the intricate relationship between NAFLD and the metabolic syndrome where oxidative stress appears to play an important role,³⁹ and the limited therapeutic options targeting the liver in the background of increasing prevalence of these conditions, the potential role of silymarin should be further explored and defined. An effective supplement that can be used for long term without side effects and as an adjunct to lifestyle control would certainly be attractive for lifestyle diseases such as NAFLD and the metabolic syndrome. In this study, we observed a trend toward some improvement in metabolic factors in the silymarin group, compared with the control group. However, as these may have been influenced by lifestyle factors not measured in the study, it would be difficult to attribute the metabolic changes in the silymarin group entirely to the drug.

Our study utilized a well-characterized silymarin preparation, included a well-characterized study population, and had a high compliance rate to the study drug and to the end-of-treatment repeat liver biopsy. However, despite our best effort, this study had several limitations. First of all, patients were not enrolled in a comprehensive lifestyle program involving dietitians and sports medicine specialists. They were provided with regular advice on dietary intake and physical activity by either one of the 2 senior physicians involved in the study. This actually reflects the real-life practice in many centers where such specialized services are not available. In fact, 55.5% of the patients in our study had weight gain and 12.1% had unchanged weight compared with only 12.5% and 6.1%, respectively, in a study that enrolled patients in a comprehensive lifestyle program.⁴ The lack of effectiveness of lifestyle advice that is reflected by the patients' weight change may explain the relatively low rate of fibrosis improvement in this study compared with other studies.^{6,30} Secondly, it was difficult to adjust for the effect of the changes in types and dosages of other medications during the study period. It is unethical to disallow the addition or the adjustment of the dosage of medications during the study period when it was necessary to do so. Last but not least, while a liver biopsy is the current best standard for assessment of the severity of liver disease in NAFLD, it only represents 1 in 50,000 of the total liver volume and may be limited by sampling variability. We acknowledge that the number of portal tracts of less than 11 may be associated with incorrect grading and staging.⁴⁰ The addition of liver stiffness measurement which evaluates an area 200 times greater than that of a liver biopsy and the consistent finding of fibrosis improvement using this modality as well as other noninvasive measures of hepatic fibrosis is reassuring. Perhaps a modality that could evaluate the degree of steatosis and fibrosis of the entire liver, such as magnetic resonance spectroscopy and elastography, will be preferred for future studies.

In conclusion, silymarin 700 mg 3 times daily for 48 weeks did not achieve the primary efficacy outcome of this study, but was found to be associated with significantly greater fibrosis improvement compared with placebo, and appeared to be safe and well tolerated. The findings from this study suggest that silymarin may be useful for fibrosis improvement in biopsy-proven NASH patients and should prompt further study in larger number of patients in a multicenter setting, especially to better define the optimum dosage and duration of treatment.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at <http://dx.doi.org/10.1016/j.cgh.2017.04.016>.

References

1. Wah-Kheong C, Khean-Lee G. Epidemiology of a fast emerging disease in the Asia-Pacific region: non-alcoholic fatty liver disease. *Hepatol Int* 2013;7:65–71.
2. Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011;43:617–649.
3. Zazos P, Renner EL. Liver transplantation and non-alcoholic fatty liver disease. *World J Gastroenterol* 2014;20:15532–15538.
4. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology* 2015; 149:367–378.e5, quiz e14–15.
5. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675–1685.
6. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand oseticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015;385:956–965.
7. Flora K, Hahn M, Rosen H, et al. Milk thistle (*Silybum marianum*) for the therapy of liver disease. *Am J Gastroenterol* 1998; 93:139–143.
8. Loguercio C, Festi D. Silybin and the liver: from basic research to clinical practice. *World J Gastroenterol* 2011;17:2288–2301.
9. Bosisio E, Benelli C, Pirola O. Effect of the flavanolignans of *Silybum marianum* L. on lipid peroxidation in rat liver microsomes and freshly isolated hepatocytes. *Pharmacol Res* 1992; 25:147–154.
10. Schumann J, Prockl J, Kierner AK, et al. Silibinin protects mice from T cell-dependent liver injury. *J Hepatol* 2003;39:333–340.
11. Trappoliere M, Caligiuri A, Schmid M, et al. Silybin, a component of silymarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. *J Hepatol* 2009;50:1102–1111.
12. Cacciapuoti F, Scognamiglio A, Palumbo R, et al. Silymarin in non alcoholic fatty liver disease. *World J Hepatol* 2013; 5:109–113.
13. Hashemi SJ, Hajiani E, Sardabi EH. A placebo-controlled trial of silymarin in patients with nonalcoholic fatty liver disease. *Hepat Mon* 2009;9:265–270.

14. Hajiaghamohammadi AA, Ziaee A, Oveisi S, et al. Effects of metformin, pioglitazone, and silymarin treatment on non-alcoholic fatty liver disease: a randomized controlled pilot study. *Hepat Mon* 2012;12:e6099.
15. Solhi H, Ghahremani R, Kazemifar AM, et al. Silymarin in treatment of non-alcoholic steatohepatitis: A randomized clinical trial. *Caspian J Intern Med* 2014;5:9–12.
16. Aller R, Izaola O, Gomez S, et al. Effect of silymarin plus vitamin E in patients with non-alcoholic fatty liver disease. A randomized clinical pilot study. *Eur Rev Med Pharmacol Sci* 2015; 19:3118–3124.
17. Loguercio C, Andreone P, Brisc C, et al. Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: a randomized controlled trial. *Free Radic Biol Med* 2012;52:1658–1665.
18. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; 55:2005–2023.
19. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;41:1313–1321.
20. Anuurad E, Shiwaku K, Nogi A, et al. The new BMI criteria for asians by the regional office for the western pacific region of WHO are suitable for screening of overweight to prevent metabolic syndrome in elder Japanese workers. *J Occup Health* 2003;45:335–343.
21. Alberti KG, Zimmet P, Shaw J, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. *Lancet* 2005;366:1059–1062.
22. Zein CO, Yerian LM, Gogate P, et al. Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *Hepatology* 2011;54:1610–1619.
23. Promrat K, Kleiner DE, Niemeier HM, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010;51:121–129.
24. Ratzliff V, Harrison SA, Francque S, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor- α and - δ , induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology* 2016; 150:1147–1159 e1145.
25. Sealed Envelope. Power calculator for binary outcome superiority trial. 2012. Available at: <https://www.sealedenvelope.com/power/binary-superiority/>. Accessed October 1, 2012.
26. Wieckowska A, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Semin Liver Dis* 2008; 28:386–395.
27. Charatcharoenwittaya P, Lindor KD, Angulo P. The spontaneous course of liver enzymes and its correlation in nonalcoholic fatty liver disease. *Dig Dis Sci* 2012;57:1925–1931.
28. Wong VW, Wong GL, Tsang SW, et al. Metabolic and histological features of non-alcoholic fatty liver disease patients with different serum alanine aminotransferase levels. *Aliment Pharmacol Ther* 2009;29:387–396.
29. Loomba R, Lawitz E, Mantry PS, et al. GS-4997, an inhibitor of apoptosis signal-regulating kinase (ASK1), alone or in combination with simvastatin for the treatment of nonalcoholic steatohepatitis (NASH): a randomized phase 2 trial. *Hepatology* 2016;64:1119A–1120A.
30. Sanyal AJ, Ratzliff V, Harrison S, et al. Cenicriviroc vs placebo for the treatment of non-alcoholic steatohepatitis with liver fibrosis: results from the Year 1 primary analysis of the Phase 2b CENTAUR trial. *Hepatology* 2016;64:1118A.
31. Gu M, Zhao P, Huang J, et al. Silymarin ameliorates metabolic dysfunction associated with diet-induced obesity via activation of farnesyl X receptor. *Front Pharmacol* 2016;7:345.
32. Hawke RL, Schrieber SJ, Soule TA, et al. Silymarin ascending multiple oral dosing phase I study in noncirrhotic patients with chronic hepatitis C. *J Clin Pharmacol* 2010;50:434–449.
33. Fried MW, Navarro VJ, Afdhal N, et al. Effect of silymarin (milk thistle) on liver disease in patients with chronic hepatitis C unsuccessfully treated with interferon therapy: a randomized controlled trial. *JAMA* 2012;308:274–282.
34. Freedman ND, Curto TM, Morishima C, et al. Silymarin use and liver disease progression in the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis trial. *Aliment Pharmacol Ther* 2011; 33:127–137.
35. Velussi M, Cernigoi AM, De Monte A, et al. Long-term (12 months) treatment with an anti-oxidant drug (silymarin) is effective on hyperinsulinemia, exogenous insulin need and malondialdehyde levels in cirrhotic diabetic patients. *J Hepatol* 1997;26:871–879.
36. Huseini HF, Larjani B, Heshmat R, et al. The efficacy of Silybum marianum (L.) Gaertn. (silymarin) in the treatment of type II diabetes: a randomized, double-blind, placebo-controlled, clinical trial. *Phytother Res* 2006;20:1036–1039.
37. Hussain SA. Silymarin as an adjunct to glibenclamide therapy improves long-term and postprandial glycemic control and body mass index in type 2 diabetes. *J Med Food* 2007;10:543–547.
38. Ebrahimpour Koujan S, Gargari BP, Mobasser M, et al. Effects of Silybum marianum (L.) Gaertn. (silymarin) extract supplementation on antioxidant status and hs-CRP in patients with type 2 diabetes mellitus: a randomized, triple-blind, placebo-controlled clinical trial. *Phytomedicine* 2015;22:290–296.
39. Cusi K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. *Gastroenterology* 2012;142:711–725 e716.
40. Rockey DC, Caldwell SH, Goodman ZD, et al; American Association for the Study of Liver Diseases. Liver biopsy. *Hepatology* 2009;49:1017–1044.

Reprint requests

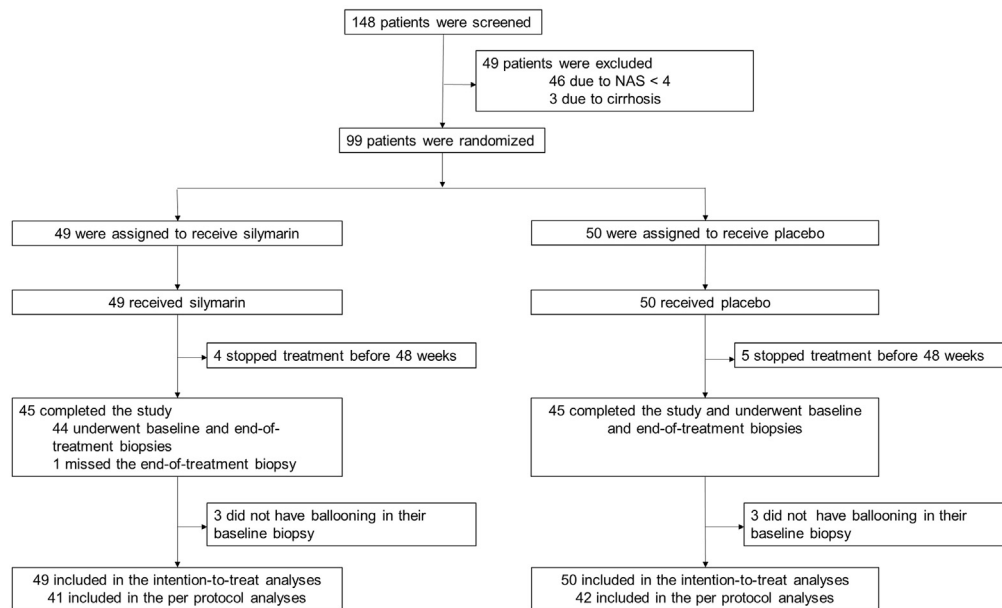
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Conflicts of interest

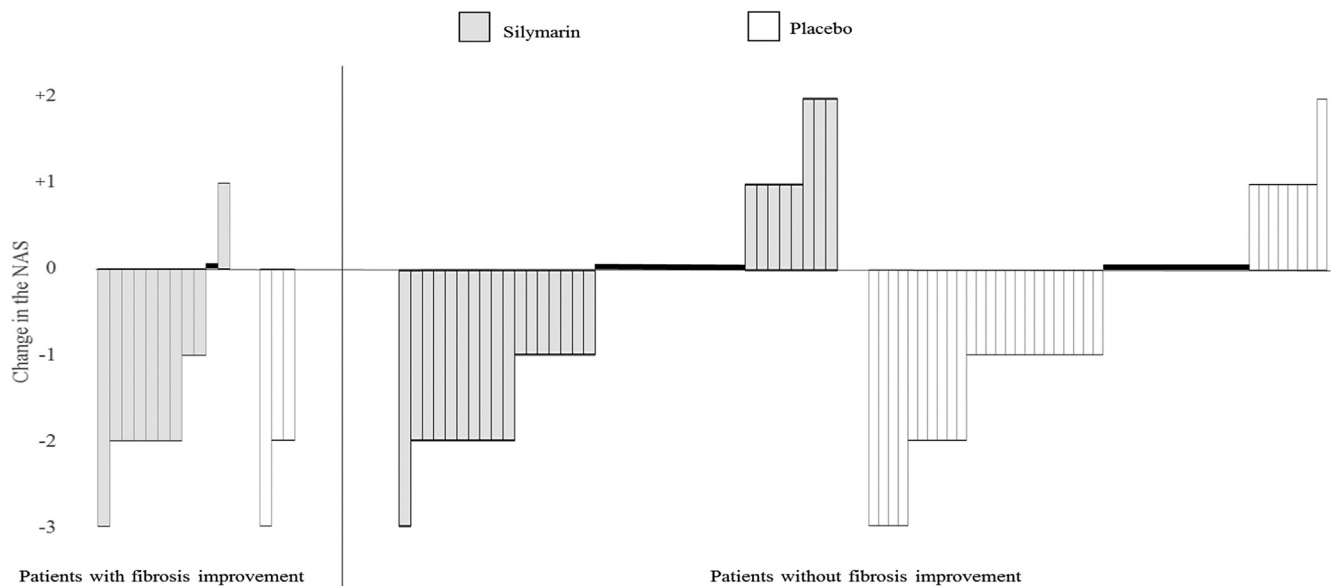
The authors disclose no conflicts.

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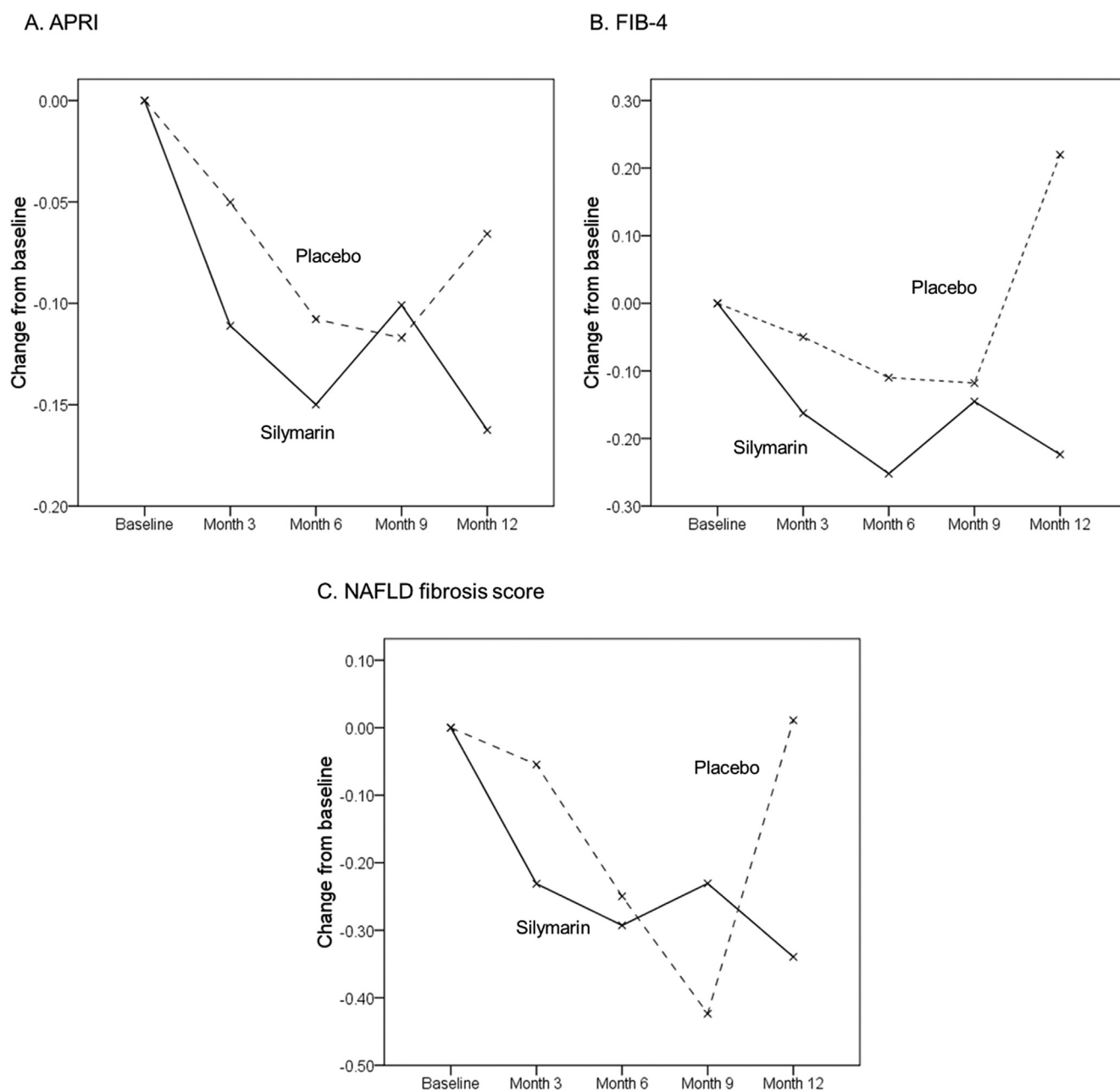
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Supplementary Figure 1. Trial profile. NAS, nonalcoholic fatty liver disease activity score.

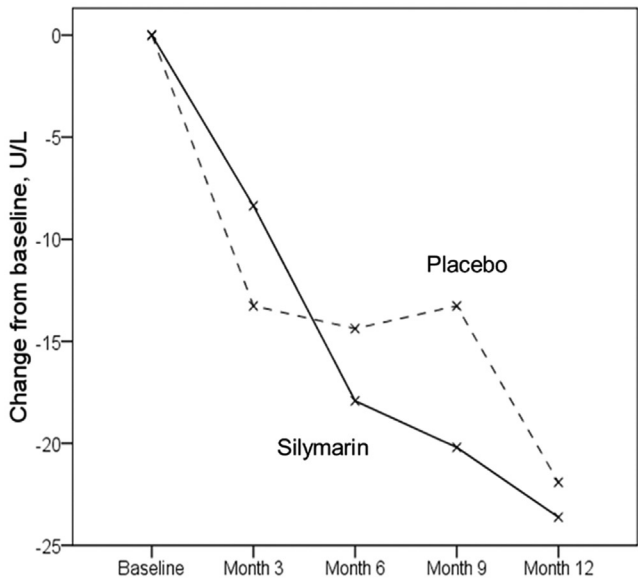


Supplementary Figure 2. Changes in the nonalcoholic fatty liver disease activity score (NAS) of individual patients. Mean change in the NAS in the silymarin group vs the placebo group for patients with fibrosis improvement (-1.455 vs -2.333 ; $P = .226$). Mean change in the NAS in the silymarin group vs the placebo group for patients without fibrosis improvement (-0.447 vs -0.617 ; $P = .654$). Mean change in the NAS in patients with fibrosis improvement vs without fibrosis improvement in the silymarin group (-1.455 vs -0.447 ; $P = .023$). Mean change in the NAS in patients with fibrosis improvement vs without fibrosis improvement in the placebo group (-2.333 vs -0.617 ; $P = .019$).

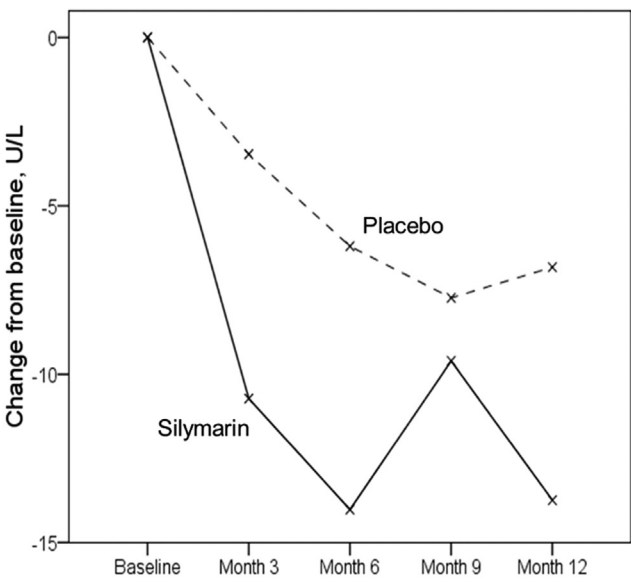


Supplementary Figure 3. Line charts illustrating the changes in the (A) aspartate aminotransferase to platelet ratio index (APRI), (B) fibrosis-4 (FIB-4) score, and (C) nonalcoholic fatty liver disease (NAFLD) fibrosis scores in the silymarin group and in the placebo group.

A. Alanine aminotransferase



B. Aspartate aminotransferase



Supplementary Figure 4. Line charts illustrating the changes in the (A) alanine aminotransferase and (B) aspartate aminotransferase in the silymarin group and in the placebo group.

Supplementary Table 1. Primary Efficacy Outcome and Other Changes in Histologic Features, per Protocol Analysis

	Silymarin (n = 41)	Placebo (n = 42)	P between groups
Primary efficacy outcome ^a	16 (39.0)	12 (28.6)	.314
Steatosis			
Improvement ^b	9 (22.0)	11 (26.2)	.652
Change within group	-0.122	-0.167	.721
P for change within group	.168	.070	
Lobular inflammation			
Improvement ^b	15 (36.6)	15 (35.7)	.934
Change within group	-0.268	-0.310	.755
P for change within group	.010	.001	
Hepatocyte ballooning			
Improvement ^b	20 (48.8)	17 (40.5)	.447
Change within group	-0.415	-0.333	.596
P for change within group	.001	.003	
NAS			
Improvement ^b	25 (61.0)	25 (59.5)	.893
Change within group	-0.805	-0.810	.987
P for change within group	.001	.000	
Resolution of steatohepatitis without worsening of fibrosis ^c	12 (29.3)	9 (21.4)	.411
Fibrosis			
Improvement ^b	11 (26.8)	3 (7.1)	.020
Change within group	-0.220	+0.119	.026
P for change within group	.071	.200	
Resolution of fibrosis ^d	5/39 (12.8)	3/35 (8.6)	.714
Development of cirrhosis	1 (2.4)	3 (7.1)	.616

NOTE. Values are n (%) or n/n (%), unless otherwise indicated. The P values were calculated using paired t test for change within group and independent t test for change between groups for continuous variables, and chi-square test or Fisher exact test, where appropriate, for categorical variables.

NAS, nonalcoholic fatty liver disease activity score.

^aPrimary efficacy outcome was defined as $\geq 30\%$ improvement in the NAS.

^bImprovement was defined as ≥ 1 point improvement(s) in the corresponding histological component.

^cResolution of steatohepatitis was defined as disappearance of ballooning, and disappearance or persistence of mild lobular inflammation only, and worsening of fibrosis was defined as any stage increase in fibrosis.

^dResolution of fibrosis was defined as absence of fibrosis on end-of-treatment liver biopsy in patients who had at least stage 1 fibrosis on baseline liver biopsy.

Supplementary Table 2. Primary Efficacy Outcome and Other Changes in Histologic Features, Intention-to-Treat Analysis, for Patients With $\leq 2\%$ Weight Change

	Silymarin (n = 28)	Placebo (n = 27)	P between groups
Primary efficacy outcome ^a	9 (32.1)	6 (22.2)	.409
Steatosis			
Improvement ^b	4 (14.3)	5 (18.5)	.729
Change within group	-0.125	-0.136	.940
P for change within group	.185	.266	
Lobular inflammation			
Improvement ^b	9 (32.1)	9 (33.3)	.925
Change within group	-0.250	-0.409	.374
P for change within group	.083	.001	
Hepatocyte ballooning			
Improvement ^b	11 (39.3)	9 (33.3)	.646
Change within group	-0.458	-0.409	.802
P for change within group	.002	.009	
NAS			
Improvement ^b	14 (50.0)	14 (51.9)	.891
Change within group	-0.833	-0.955	.743
P for change within group	.005	.001	
Resolution of steatohepatitis without worsening of fibrosis ^c	9 (32.1)	7 (25.9)	.612
Fibrosis			
Improvement ^b	9 (32.1)	0 (0)	.002
Change within group	-0.292	+0.091	.030
P for change within group	.070	.162	
Resolution of fibrosis ^d	5/23 (21.7)	0/20 (0)	.051
Development of cirrhosis	1 (3.6)	1 (3.7)	1.000

NOTE. Values are n (%) or n/n (%), unless otherwise indicated. The P values were calculated using paired t test for change within group and independent t test for change between groups for continuous variables, and chi-square test or Fisher exact test, where appropriate, for categorical variables.

NAS, nonalcoholic fatty liver disease activity score.

^aPrimary efficacy outcome was defined as $\geq 30\%$ improvement in the NAS.

^bImprovement was defined as ≥ 1 point improvement(s) in the corresponding histological component.

^cResolution of steatohepatitis was defined as disappearance of ballooning, and disappearance or persistence of mild lobular inflammation only, and worsening of fibrosis was defined as any stage increase in fibrosis.

^dResolution of fibrosis was defined as absence of fibrosis on end-of-treatment liver biopsy in patients who had at least stage 1 fibrosis on baseline liver biopsy.

Supplementary Table 3. Primary Efficacy Outcome and Other Changes in Histologic Features, Intention-to-Treat Analysis, in Patients With Liver Biopsy Length ≥ 1.5 cm at Baseline and at the End of Treatment

	Silymarin (n = 24)	Placebo (n = 14)	P between groups
Primary efficacy outcome ^a	5 (20.8)	2 (14.3)	1.000
Steatosis			
Improvement ^b	4 (16.7)	2 (14.3)	1.000
Change within group	-0.125	-0.071	.730
P for change within group	.185	.583	
Lobular inflammation			
Improvement ^b	6 (25.0)	1 (7.1)	.227
Change within group	-0.083	+0.143	.280
P for change within group	.539	.336	
Hepatocyte ballooning			
Improvement ^b	7 (29.2)	3 (21.4)	.715
Change within group	-0.208	0	.326
P for change within group	.096	1.000	
NAS			
Improvement ^b	11 (45.8)	4 (28.6)	.329
Change within group	-0.417	+0.071	.247
P for change within group	.116	.828	
Resolution of steatohepatitis without worsening of fibrosis ^c	5 (20.8)	3 (21.4)	1.000
Fibrosis			
Improvement ^b	5 (20.8)	0 (0)	.137
Change within group	-0.208	+0.286	.019
P for change within group	.096	.104	
Resolution of fibrosis ^d	2/19 (10.5)	0/10 (0)	1.000
Development of cirrhosis	0 (0)	2 (14.3)	.129

NOTE. Values are n (%) or n/n (%), unless otherwise indicated. The P values were calculated using paired t test for change within group and independent t test for change between groups for continuous variables, and chi-square test or Fisher exact test, where appropriate, for categorical variables.

NAS, nonalcoholic fatty liver disease activity score.

^aPrimary efficacy outcome was defined as $\geq 30\%$ improvement in the NAS.

^bImprovement was defined as ≥ 1 point improvement(s) in the corresponding histological component.

^cResolution of steatohepatitis was defined as disappearance of ballooning, and disappearance or persistence of mild lobular inflammation only, and worsening of fibrosis was defined as any stage increase in fibrosis.

^dResolution of fibrosis was defined as absence of fibrosis on end-of-treatment liver biopsy in patients who had at least stage 1 fibrosis on baseline liver biopsy.

Supplementary Table 4. Primary Efficacy Outcome and Other Changes in Histologic Features, Intention-to-Treat Analysis, in Patients With Liver Biopsy Length < 1.5 cm at Baseline or at the End of Treatment

	Silymarin (n = 25)	Placebo (n = 36)	P between groups
Primary efficacy outcome ^a	11 (44.0)	11 (30.6)	.282
Steatosis			
Improvement ^b	5 (20.0)	11 (30.6)	.393
Change within group	-0.080	-0.250	.296
P for change within group	.491	.027	
Lobular inflammation			
Improvement ^b	10 (40.0)	14 (38.9)	.930
Change within group	-0.360	-0.389	.834
P for change within group	.004	.000	
Hepatocyte ballooning			
Improvement ^b	13 (52.0)	14 (38.9)	.311
Change within group	-0.480	-0.389	.591
P for change within group	.003	.000	
NAS			
Improvement ^b	15 (60.0)	23 (63.9)	.758
Change within group	-0.920	-1.028	.737
P for change within group	.002	.000	
Resolution of steatohepatitis without worsening of fibrosis ^c	9 (36.0)	8 (22.2)	.238
Fibrosis			
Improvement ^b	6 (24.0)	3 (8.3)	.142
Change within group	-0.160	+0.028	.266
P for change within group	.327	.744	
Resolution of fibrosis ^d	3/20 (15.0)	3/27 (11.1)	1.000
Development of cirrhosis	1 (4.0)	1 (2.8)	1.000

NOTE. Values are n (%) or n/n (%), unless otherwise indicated. The P values were calculated using paired t test for change within group and independent t test for change between groups for continuous variables, and chi-square test or Fisher exact test, where appropriate, for categorical variables.

NAS, nonalcoholic fatty liver disease activity score.

^aPrimary efficacy outcome was defined as $\geq 30\%$ improvement in the NAS.

^bImprovement was defined as ≥ 1 point improvement(s) in the corresponding histological component.

^cResolution of steatohepatitis was defined as disappearance of ballooning, and disappearance or persistence of mild lobular inflammation only, and worsening of fibrosis was defined as any stage increase in fibrosis.

^dResolution of fibrosis was defined as absence of fibrosis on end-of-treatment liver biopsy in patients who had at least stage 1 fibrosis on baseline liver biopsy.

Supplementary Table 5. Univariate and Multivariate Analyses of Factors Associated With Fibrosis Improvement

	β	Unadjusted OR (95% CI)	P	β	Adjusted OR* (95% CI)	P
Age	-0.05	0.96 (0.91-1.00)	.073	-0.02	0.98 (0.91-1.06)	.613
Male	1.23	3.40 (0.99-11.7)	.052	1.53	4.62 (0.70-30.6)	.113
Baseline NAS	0.43	1.53 (0.84-2.80)	.164	0.01	1.01 (0.44-2.31)	.991
Baseline fibrosis stage	0.37	1.44 (0.86-2.41)	.161	0.35	1.42 (0.64-3.16)	.389
Percentage weight change	0.02	1.02 (0.87-1.21)	.803	—	—	—
Silymarin	1.51	4.54 (1.18-17.4)	.028	1.80	6.03 (1.11-32.7)	.037
HbA1c change	-0.45	0.64 (0.37-1.10)	.108	-0.42	0.66 (0.33-1.30)	.228
TG change	-0.22	0.80 (0.25-2.55)	.710	—	—	—
HDL change	-1.43	0.24 (0.01-8.43)	.432	—	—	—
LDL change	-0.51	0.60 (0.34-1.04)	.069	-0.34	0.71 (0.38-1.34)	.291

CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAS, nonalcoholic fatty liver disease activity score; OR, odds ratio; TG, triglyceride.

Supplementary Table 6. Changes in Weight, Glycemic, Lipid and Liver Profile, and FibroScan Measurements, per Protocol Analysis

	Silymarin (n = 42)	Placebo (n = 42)	P between groups
Weight			
Change, kg	−0.9	−0.2	.336
P for change within group	.159	.627	
Fasting blood glucose			
Change	+0.3	+0.8	.210
P for change within group	.239	.028	
HbA1c			
Change	−0.5	0	.139
P for change within group	.025	.903	
HOMA-IR ^a			
Change	+0.5	+0.6	.984
P for change within group	.732	.514	
Triglyceride			
Change	−0.22	+0.05	.021
P for change within group	.009	.542	
Total cholesterol			
Change	−0.43	−0.10	.166
P for change within group	.025	.461	
HDL cholesterol			
Change	+0.07	+0.02	.201
P for change within group	.004	.437	
LDL cholesterol			
Change	−0.41	−0.20	.334
P for change within group	.026	.125	
ALT			
Change	−23	−25	.827
P for change within group	.009	.001	
AST			
Change	−14	−8	.405
P for change within group	.025	.081	
GGT			
Change	−16	−10	.566
P for change within group	.064	.224	
Controlled attenuation parameter ^b			
Change	−9	+7	.186
P for change within group	.245	.452	
Liver stiffness measurement ^b			
Change	−0.8	+1.4	.091
P for change within group	.345	.159	
APRI			
Change	−0.17	−0.08	.322
P for change within group	.015	.141	
FIB-4			
Change	−0.24	+0.21	.113
P for change within group	.045	.408	
NAFLD fibrosis score			
Change	−0.33	−0.05	.381
P for change within group	.001	.861	

NOTE. The P values were calculated using paired t test for change within group and independent t test for change between groups.

ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; FIB-4, fibrosis-4; GGT, gamma glutamyl transpeptidase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; LDL, low-density lipoprotein; NAFLD, nonalcoholic fatty liver disease.

^aEight patients in the placebo group and three patients in the silymarin group were on insulin therapy and were excluded in the HOMA-IR analyses. In addition, one patient in the placebo group was started on insulin during the study and was not included in the HOMA-IR analyses.

^bThree patients in the placebo group and two patients in the silymarin group had invalid or unreliable Fibroscan measurements at baseline. Four patients in the placebo group and five patients in the silymarin group had invalid or unreliable Fibroscan measurements at Month 12. One of the patients in the placebo group had invalid Fibroscan measurements at baseline and at Month 12. Hence, there were a total of 36 patients in the placebo group and 35 patients in the silymarin group with valid and reliable measurements at baseline and at Month 12. APRI = AST level (U/L) / AST upper limit of normal (U/L) / Platelet count (10⁹/L) × 100/FIB-4 = age (years) × AST level (U/L) / Platelet count (10⁹/L) / ALT (U/L) 1/2; NAFLD fibrosis score = −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m²) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet (10⁹/L) − 0.66 × albumin (g/dL).