Original Article

The effect of silymarin on liver enzymes and serum lipid profiles in Iranian patients with non-alcoholic fatty liver disease: A double-blind randomized controlled trial

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of silymarin on liver enzyme levels and serum lipid profiles in patients with Non-Alcoholic Fatty Liver Disease (NAFLD), the most common chronic liver disease worldwide.

Methods: This randomized double-blinded clinical trial included 80 NAFLD patients referred to the gastrointestinal clinic of Kowsar Hospital in Semnan. Forty of these patients were supplemented with 150 mg of silymarin twice a day for two months, while the other 40 received a placebo. Both groups were advised to follow a hypertriglyceridemia correction and lifestyle modification. Evaluation of serum lipid profiles and liver enzymes in both groups were performed at the baseline and after two months.

Results: The results showed that silymarin use significantly reduced ALT (U/L) (58.72± 32.16 vs 42.2 ± 20.2, p = 0.003) and AST (U/L) (36.62± 13.46 vs 30.3 ± 9.7, p = 0.036) levels compared with the placebo group. Additionally, a statistically significant reducing effect of silymarin on triglycerides (mg/dL) (189.5 ± 65.5 vs 164.6 ± 91.3, p = 0.026), total cholesterol (mg/dL) (192.8 ± 40.3 vs 174.07± 34.5, p = 0.027), and LDL-cholesterol (mg/dL) (114.6±33.9 vs 95.6± 26.5, p = 0.012) levels was found, with no significant statistical difference for HDL-C (mg/dL) (41.5 ± 6.8 vs 43.5± 9.2, p = 0.44).

Conclusion: Silymarin, at a dose of 150 mg twice daily, significantly reduced liver enzymes and some lipid markers in patients with NAFLD, suggesting that this compound could be a novel therapy for NAFLD.

Keywords: Silymarin, Fatty liver, Non-alcoholic Fatty Liver Disease, NAFLD

Abbreviations: NAFLD: Non-alcoholic fatty liver disease; TG: Triglyceride; TC: Total cholesterol; LDL: Low density lipoprotein; HDL: High density lipoprotein; AST: Aminotransferase; ALT: Alanine aminotransferase; UDCA: Ursodeoxycholic acid; US: Ultrasonography; ROS: Reactive oxygen species; NASH: Non-alcoholic steatohepatitis; PPARγ: Peroxisome proliferator-activated receptor γ; SREBP-1c: Sterol regulatory element binding protein.

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Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most common chronic liver diseases, with a global prevalence of 25% and is the third leading cause of liver transplantation in the United States (1). NAFLD has become a significant health challenge worldwide due to its metabolic complications (2). This disease encompasses a range of conditions from simple fatty liver (steatosis) to cirrhosis development. It affects 5-20% of healthy individuals and may be present in up to 70% of patients with diabetes (3). NAFLD is usually asymptomatic, but when symptomatic, it is associated with abdominal pain, fatigue, malaise, and an enlarged liver. Patients often have elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (4).

The pathogenesis of NAFLD is not fully understood, but obesity, oxidative stress, and insulin resistance are recognized as significant factors (5, 6). In addition to genetic factors, lifestyle characteristics such as dietary habits play an important role in the pathogenesis of NAFLD. High triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) levels are prevalent in patients with NAFLD. The prevalence of NAFLD in people with dyslipidemia is estimated to be 50% (7). Age, sex, and ethnicity are also associated with the prevalence of NAFLD. Recent studies have reported that males are at risk for fatty liver disease (8). Older NAFLD patients are more likely to progress to advanced fibrosis or even death (9). Hypothyroidism, pituitary insufficiency, hypogonadism, sleep apnea, and obesity are other risk factors for NAFLD (10).

Treatment of NAFLD patients includes addressing liver disease and related metabolic complications such as obesity, hypertension, insulin resistance, and type 2 diabetes (11). Weight loss generally reduces hepatic steatosis with a low-calorie diet alone or in combination with increased physical activity. Losing at least 3-5% of body weight seems necessary to improve steatosis, but more weight loss (more than 10%) is needed to improve inflammation and necrosis (12). Ursodeoxycholic acid (UDCA) is not recommended for the treatment of nonalcoholic steatohepatitis (NASH) or NAFLD. Metformin also is not recommended as a specific treatment for liver disease in adults with NASH and pioglitazone can be used to treat steatohepatitis in biopsy-proven patients with NASH (13).

In recent years, compounds of plant origin for treating various diseases have attracted special attention among researchers (14, 15). Among these, milk thistle (or *Silybum marianum*), an annual herb native to the Mediterranean and North African regions, has been known to strengthen the liver for centuries (16).

Silymarin ($C_{25}H_{22}O_{10}$), the active ingredient of Silybum marianum, is a naturally occurring bioflavonoid with antioxidant properties that is beneficial in the treatment of several diseases (17). Silymarin is a well-known hepatoprotective agent whose effects are mediated through antioxidant (18), immunomodulatory, anti-proliferative (19), anti-fibrotic (20), anti-inflammatory (21), and anti-viral (22) functions.

Silymarin consists of an isomeric mixture of seven flavonolignans: silybin A, silybin B, isosilybin A, isosilybin B, silychristin A, silychristin B, and silydianin, along with one flavonoid taxifolin. A mixture of silybin A and silybin B (Silibinin) has been reported to be responsible for the hepatoprotective effect of silymarin (23).

Most studies on silymarin are directed toward liver-related diseases. Silymarin is one of the most well-studied plant extracts with a known mechanism of function for the treatment of liver disorders (24-27). Silibinin has long been used to treat chronic liver disease without confirmed pathogenesis, which protects liver cells from damage by reducing and eliminating free radicals (28).

Due to the high prevalence of NAFLD and the lack of a globally accepted standard treatment for this disease, this randomized controlled trial aimed to evaluate the effect of silymarin on serum lipid profiles and liver enzymes in an Iranian population with NAFLD.

Methods

Patients' characteristics

This study was a double-blind randomized clinical trial approved by the Ethics Committee of Semnan University of Medical Sciences (Ethical code: IRCT2015031721502N1). All patients who participated were volunteers and informed consent was obtained from all of them. The study was conducted on clinically suspected cases of NAFLD referred to the Gastroenterology Clinic of Kowsar Hospital in Semnan (Semnan province).

The primary inclusion criterion was evidence of fatty liver with increased liver enzymes. Additionally, the study included patients with the following features:

- Age range between 20 to 60 years old
- Increased liver enzyme (ALT> 40)
- Evidence of fatty liver on ultrasonography (US)

The exclusion criteria included:

- Cirrhosis
- Recognition of viral hepatitis, alcoholic hepatitis, autoimmune hepatitis, Wilson's disease, and hemochromatosis
- Chronic liver disease
- Pharmacological causes of fatty liver (anabolic steroids, chlorpromazine, etc.)
- Drug allergy and drug side effects

Patients were also allowed to withdraw from the study at any stage if they wished.

Study design

In this study, 80 patients were included and randomly divided into intervention or placebo groups. The randomization method employed was stratified randomization. During the screening process, eligible patients were stratified by gender (male and female) and age, and assigned into one of the two arms of the study (A or B). The randomization sequence was created using Winpepi software (version 11.6). The allocation sequence was concealed using the sealed envelopes mechanism.

Participants were evaluated for liver enzymes (AST and ALT), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and HDL-C levels using a Pars Azmoon kit. Medical history, demographic characteristics including height, weight, body mass index (BMI), and diet habits of each patient were recorded using a self-administered questionnaire at the beginning of the study. All relevant experiments were performed in a single laboratory.

Similar treatment methods and nutrient habits were advised for all participants. Then, the participants in the intervention group were treated with silymarin (150 mg/twice a day) as an oral tablet and the other group received a placebo. The intervention period was 8 weeks. Since the standard treatment for fatty liver disease is currently based on dietary modifications and weight loss, the use of silymarin in the intervention group did not disrupt the therapeutic process in the control group.

Prior to the study initiation, patients were reassured that all information would remain confidential. Venous blood in a fasting state was collected in the morning in evacuated tubes and followed by centrifugation at 3000 g for 10 min at 4 °C. The supernatant serum was aliquoted and stored at-70 °C until analysis. At the end of the intervention period, liver enzyme levels including ALT, and AST, and lipid profile including TC, TG, LDL-C, and HDL-C were also determined.

Statistical analysis

The sample size for this study was calculated based on the changes in serum TG level as the primary outcome of treatment. According to a previous study, silymarin decreased the mean serum TG level from 254.1 to 239.09 (mg/dL) with a standard deviation (SD) of 52.7 (29). The minimal important difference (MID) of the TG level was estimated to be 37.2 (mg/dL) using $SD*\sqrt{1-r}$ ($r = 0.5$) (the distributional-based method) (30). With a study power of 80%, an alpha level of 0.05%, and MID of 37.2, the required sample size was calculated to be 40 patients in each arm using G-power software (version 3.1.9.2).

In this study, the adequacy of randomization was first checked by comparing the independent contextual-confounding variables in the two groups through a preliminary analysis. The levels of enzymes before and after the intervention in the two groups were evaluated. The Chi-square, Fisher test, and paired t-test were used for analyses. Differences with $P\text{-}values < 0.05$ were considered significant. All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) version 18.

Results

Clinical and demographic characteristics of participants

The clinical and demographic characteristics of all participants are shown in Table 1. The study included 80 patients, with 40 patients receiving silymarin (intervention group) and 40 patients receiving a placebo. During the study period, 7 patients from the intervention group and 9 patients from the placebo group were excluded from the study (Fig 1).

Among the 40 patients studied in the intervention group, 29 (72.5%) were male and 11 (27.5%) were female. In the placebo group, 30 (75%) were male

Table 1: Summary of the characteristics of participants in the studies groups.

Parameters	Placebo	Silymarin	P value after adjustment for age and gender
Age (years)	41.02±10.35	38.67±10.25	0.209
Gender (M/F)	29/11	30/10	0.799
BMI (Kg/m^2)	28.48±1.97	27.09±3.33	0.148
FBS	91.47±11.85	96.8±17.41	0.106
ALT	62.84±24.46	68.9±43.54	0.639
AST	38.77±13.59	42.3±27.12	0.219
TG	214.05±85.73	200.47±118.72	0.530
TC	204±43.005	188.2±45.40	0.114
HDL-C	40.92±7.65	41.62±7.56	0.248
LDL-C	117.72±35.44	107.22±36.006	0.192

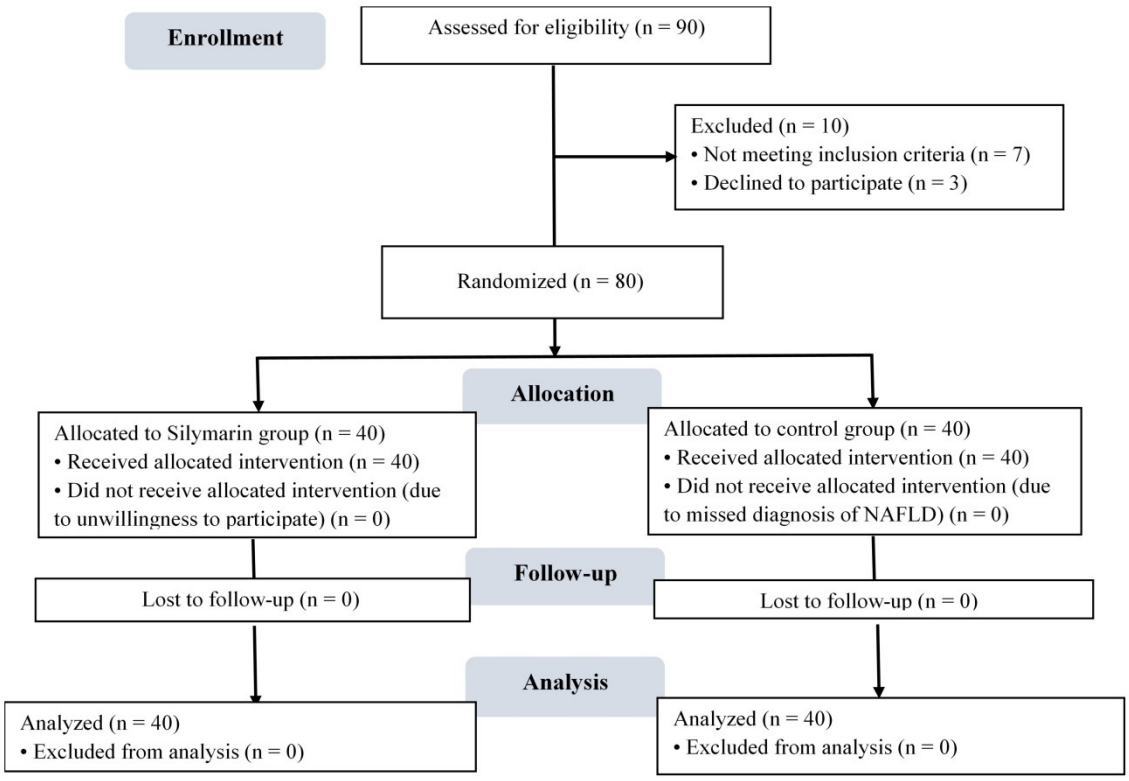

Figure 1: Flow chart of protocol. Patients with NAFLD were evaluated for protocol eligibility.

Table 2: Comparison of changes in biochemical parameters during the study period

Parameters	Placebo group		*P-value*	Silymarin group		*P-value*
	Before	After 2 months		Before	After 2 months	
ALT	63.60± 24.03	86.90± 43.54	0.004	58.72± 32.16	42.42± 20.27	0.003
AST	38.77± 13.59	52.30± 27.12	0.013	36.62± 13.46	30.32± 9.79	0.036
TG, mg/dL	214.05±85.73	200.4±118.7	0.218	189.5 ± 65.5	164.6 ± 91.3	0.026
TC ,mg/dL	204.00±43.00	188.2±45.4	0.082	192.8 ± 40.3	174.07± 34.5	0.027
LDL-C, mg/dL	117.7±35.44	107.22±36.00	0.116	114.6±33.9	95.6 ± 26.5	0.012
HDL-C, mg/dL	40.92±7.65	44.62±7.56	0.017	41.5 ± 6.8	43.5 ± 9.2	0.440

and 10 (25%) were female. There was no significant difference in gender distribution between the two groups (*P = 0.799*).

The mean age of the subjects in the intervention group was 41.02±10.35 years and in the placebo group was 38.67±10.25 years, with no significant difference between the two groups (*P = 0.209)*. The mean BMI before treatment was 27.40±3.47 in the intervention group and 28.92±7.65 in the placebo group (*P = 0.295*). At the end of the study, the mean BMI of patients in the intervention group was 27.09±3.33 and in the placebo group was 28.48±1.97 (P = 0.148), indicating no statistically significant difference between the end-BMI of treatment in the two groups.

According to Fisher's exact test, there was no significant difference in the distribution of diabetes and impaired fasting blood sugar (FBS) among participants in both groups (P value: 0.746).

At the end of the trial, individuals in the silymarin group showed a statistically significant decrease in TG (189.5 ± 65.5 versus 164.6 ± 91.3, p = 0.026), total Cholesterol (192.8 ± 40.3 versus 174.07±34.5, p = 0.027) and LDL-C (114.6±33.9 versus 95.6 ±26.5, p = 0.012) levels compared with the placebo group, with no significant statistical difference for HDL-C (41.5 ±6.8 versus 43.5 ±9.2, p =0.44).

The liver enzymes levels were significantly reduced after silymarin consumption compared to placebo group: ALT level at the beginning and end of treatment was 58.7±32.1 and 42.4±20.2, respectively (P =0.003), and AST level at baseline was 36.6±13.4, and at end of treatment was 30.3±9.7 (P =0.03) (Table 2). Importantly, all measured parameters did not decrease after two months in the placebo group.

Discussion

NAFLD is the most common form of chronic liver disease and a significant health issue worldwide (31). It is characterized by the aggregation of triglycerides in the liver, ranging from steatosis to steatohepatitis, fibrosis, and rarely to cirrhosis or hepatocellular carcinoma (32, 33). Although initially characterized as a benign state, it is now considered a major cause of morbidity and mortality associated with the liver. Several clinical studies have reported pharmacological treatment results; however, there is still no specific therapy for NAFLD. According to previous findings, the only effective treatment for NAFLD is weight loss. However, continuous lifestyle modification is a challenging task, and therefore the identification of novel alternative therapies seems necessary (34). Herbal medicine is a potential therapeutic option for NAFLD, which has attracted a lot of attention among researchers. In this study, the effect of silymarin on liver enzyme levels and lipid profile in patients with NAFLD was evaluated.

Statistical analysis of the data from this study demonstrates that ALT and AST levels were significantly lower in patients with NAFLD receiving silymarin (dose at 150 mg/kg) compared to controls. Several studies have previously shown that high ALT and AST levels can be biomarkers for liver injury in NAFLD (35, 36). Thus, the mild to moderate elevation of serum aminotransferase (ALT, AST) found in our patients at baseline represents the most common abnormality found in patients with NAFLD. In this study, the significant reduction of ALT and AST levels following silymarin treatment seems to be due to the antioxidant and anti-inflammatory effects of silymarin. Silymarin has been reported to play a protective role against oxidative damage by intercepting reactive oxygen species (ROS) produced from hepatic metabolism of toxic substances. The excess production of ROS in the liver can lead to activation of hepatic stellate and macrophage cells leading to induction of pro-inflammatory and fibrotic conditions. Therefore, the proposed mechanism for silymarin function in decreasing liver enzymes can be suggested to be through an increase in total antioxidant capacity in serum and liver tissue of the patients (37, 38).

The findings of this study align with the results of several other studies. For instance, Hashemi et al. conducted a study on 100 patients with NAFLD, who received 140 mg of silymarin daily for 6 months. The mean weight before and after the treatment was not statistically significant. However, the decrease in AST and ALT levels were significant in the intervention group compared to the control group. Moreover, the authors did not find a significant difference in the levels of TG, TC, LDL-C, and HDL-C before and after the intervention (39).

Similar to our results, Hajaghamohammadi et al. performed a study titled "The effect of silymarin on the reduction of liver enzymes in patients with NAFLD" in 2008 in Qazvin. In this study, patients treated with 140 mg silymarin for two months had a significantly lower AST and ALT levels in comparison with the placebo group (40).

The results of the study by Masoudi et al. suggested that the consumption of silymarin significantly decreased ALT and AST levels in one-hundred patients with non-alcoholic steatohepatitis (NASH) (41). A study in subjects with NAFLD with oral consumption of twice daily of silymarin for 3 months demonstrated a significant decrease of ALT, AST, GGT and brightness level of hepatorenal as a hepatic steatosis index, while no significant difference for TG, TC, LDL-C and HDL-C levels before and after the intervention (42). Furthermore, consumption of 140 mg silymarin twice daily for 6 months led to a significant reduction in the levels of liver enzymes in NAFLD patients (43).

In the next step, we found that the silymarin consumption for two months has reducing impact on serum levels of TG, TC, LDL-C in NAFLD subjects. Similar to our data, there are a few human studies about silymarin effects on lipids profile in NAFLD patients. Of these, a randomized clinical trial in subjects with NAFLD with oral consumption of three 140 mg tablets per day of silymarin for 45 days demonstrated a significant decrease in the TG, TC and LDL-C levels and a significant increase in HDL-C levels compared to the control group (44). In addition, consumption of 200 mg/3 tablets of silymarin every day for 4 months led to a significant reduction in the levels of TG, TC and LDL-C and an increase in HDL-C levels (45).

The molecular mechanisms underlying the anti-lipogenic effects of silymarin have been examined in some studies. It has been suggested that silymarin reduces the serum lipid levels through inhibition of fatty acid synthesis, decrease of de-novo synthesis of cholesterol, suppresses cholesterol absorption and reduces the level of cytochrome p450 CYP2E1 activity in liver cells (46, 47). Silymarin also increases fatty acid oxidation through induction of the peroxisome proliferator-activated receptor α (PPARα) gene expression (47, 48). Furthermore, silymarin could down-regulate the expression of genes involved in hepatic lipogenesis such as PPAR-γ coactivator 1-β (PGC-1β), sterol regulatory element binding protein (SREBP-1c) (49).

This study had several limitations: first, the relatively small number of cases in studies groups; secondly, given the high prevalence of NAFLD in different countries and variations in dietary habits used across these countries, we cannot generalize the obtained results from this study to other populations. Furthermore, further intervention studies with larger sample sizes are needed to establish the beneficial impact of silymarin in prevention of NAFLD.

Conclusion

The findings of this study suggest that silymarin, at a dose of 150 mg twice per day, could improve liver function as evidenced by a reduction in the levels of transaminases in patients with NAFLD. Therefore, silymarin could potentially be an effective treatment for NAFLD. However, more studies with larger sample sizes and appropriate controls are needed to elucidate the precise mechanism of silymarin's effect on NAFLD.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Availability of data and materials

The data used to support the findings of this study are included within the article. Additional information can be requested by contacting the corresponding author.

Ethics approval

This study is a double blind randomized clinical trial, which was approved by the Ethics Committee of Semnan University of Medical Sciences (Ethical code: IRCT2015031721502N1).

Authors' Contributions

Somayeh Chahkandi: Scientific investigation, Writing – original draft, Data curation, Visualization, Methodology. Reza Dabiri: Conceptualization, Supervision, Project administration, Methodology. Majid Mirmohammadkhani: Writing-original draft, Visualization, Software. Nasrin Amiri-Dashatan: Scientific investigation, Writing-original draft. Mehdi Koushki: Scientific investigation, Writing-original draft.

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