

Effect of silymarin plus vitamin E in patients with non-alcoholic fatty liver disease. A randomized clinical pilot study

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Abstract. – OBJECTIVE: Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized health problem. Various treatment strategies such as thiazolidinediones, metformin, lipid-lowering agents and antioxidants have been evaluated. So far, no single intervention has convincingly improved liver histology. Experience of using silymarin alone or in combination with other agents in patients with NAFLD is limited in the medical literature. The present study was conducted to evaluate the efficacy of silymarin plus vitamin E in the treatment of NAFLD.

PATIENTS AND METHODS: A sample of 36 patients was enrolled. The diagnosis of NAFLD was confirmed by percutaneous liver biopsy. All patients were randomized to one of the following intervention groups: group I: treated with 2 tablets per day of silymarin plus vitamin E (Eurosil 85®, MEDAS SL) and a lifestyle modification program consisting of hypocaloric diet (1520 kcal, 52% of carbohydrates, 25% of lipids and 23% of proteins) and exercise for 3 months and group II (only with the hypocaloric diet). Anthropometric variables as waist circumference, weight, body mass index (BMI) were measured. Biochemical parameters: Glucose, triglycerides, AST, ALT, GGt levels and insulin resistance (HOMA-IR) were determined under fasting conditions. Non-invasive NAFLD-index were applied before and after the treatments: Fatty liver index (FLI), liver accumulation product (LAP) and NAFLD-Fibrosis score (FS).

RESULTS: The mean age was 47.4 ± 11.2 years old (range 18-67); 22 men and 14 women. In group I, 11 patients (61%) have a NAS-score > 5 and 10 (55.5%) in the group II (NS). Anthropometric parameters decreased after treatment in both groups. Patients in both groups showed a decrease in GGt levels after treatment (group I: 68 IU/L vs. $46.2 \pm 27 \text{ IU/L}$; $p < 0.05$ and group II $80.5 \pm 46 \text{ IU/L}$ vs. $50.3 \pm 27 \text{ IU/L}$; $p < 0.05$). Only in group II we observed a significant decrease in AST and ALT levels. In both groups, we observed a decrease in:

FLI index (group I: 86.2 ± 19 vs. 76.9 ± 20 ; $p < 0.05$ and in group II: 85.2 ± 18 vs. 77.5 ± 23 ; $p < 0.05$), and NAFLD-FS index (group I: -1.6 ± 1.8 vs. -2.1 ± 1.5 ; $p < 0.05$ and in group II -1 ± 1.9 vs. -1.5 ± 2.1 ; $p < 0.05$). Patients in group I who did not get a 5% loss of weight also displayed decreased GGt levels, and in the FLI and NAFLD-FS indexes; whereas patients in group II without decrease of 5% by weight showed no improvement in any of the analyzed parameters.

CONCLUSIONS: Treatment with silymarin plus vitamin E and a hypocaloric diet ameliorate function hepatic test, and non-invasive NAFLD index. Silymarin can be an alternative valid therapeutic option particularly when other drugs are not indicated or have failed or as a complementary treatment associated with other therapeutic programs.

Key Words:

Silimaryne, Steatosis, NAFLD.

Abbreviations

NAFLD = Non-alcoholic fatty liver disease; NASH = non alcoholic steatohepatitis; FLI = Fatty liver index; LAP = liver accumulation product; NAFLD-FS = NAFLD-Fibrosis score; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGt = gamma-glutamyl transpeptidase; LDL-Chol = low density lipoprotein cholesterol; HDL-Chol = high density lipoprotein cholesterol; BMI = body mass index; NS = non significant.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly recognized health problem. Increased fat accumulation in the liver is observed in 20-30% of the population in the Western World, and in approximately 10% of this cohort it is asso-

ciated with non-alcoholic steatohepatitis, which is characterized by inflammation and eventually fibrosis¹. Disease presentation of NAFLD ranges from asymptomatic disease to cirrhosis with the complication of liver failure and hepatocellular carcinoma. NAFLD is suspected on the basis of various clinical aspects (an elevated alanine aminotransferase concentration, presence of obesity and diabetes) that alone are not sufficient to establish diagnosis or prognosis². Obesity is considered the most important risk factor. In different series, abdominal fat was correlated with degree of steatosis in liver biopsy and insulin resistance has been associated with fat liver and NAFLD, too³.

The major diagnostic procedure is liver biopsy, which allows assessment of liver injury. In most cases, NAFLD is associated with insulin resistance which is, therefore, the target of most current NAFLD treatment modalities⁴. No proven treatment for patients with NAFLD is currently available⁵. Various treatment strategies such as thiazolidinediones, metformin, lipid-lowering agents and antioxidants have been studied⁴. So far, no single intervention has convincingly improved liver histology. It is recommended that patients at high risk of developing advanced liver disease, who are not part of controlled studies, should receive nutritional counselling and take physical exercise to achieve moderate weight loss and improve insulin sensitivity. Weight reducing (phentermine, pioglitazone), cytoprotective, antioxidant (ursodesoxycholic acid, vitamin E, n-acetylcysteine), and others drugs have been tested for the treatment of NAFLD; but no generally accepted conclusions has been reached (6-8)

Experience of using silymarin alone or in combination with other agents in patients with NAFLD is limited in medical literature (9-10). The present study was conducted to evaluate the efficacy of silymarin in the treatment of NAFLD.

Patients and Methods

Patients

A sample of 36 patients was enrolled. Exclusion criteria were hepatitis B, C, cytomegalovirus, Epstein Barr infections, non organ-specific autoantibodies, alcohol consumption, diabetes mellitus, impaired glucose tolerance, medication (blood-pressure lowering medication and statins) and hereditary defects (iron and copper storage diseases and alpha 1-antitrypsin deficiency).

The study was approved by the institutional Ethics Committee and all patients signed an informed consent.

Liver Biopsy

The diagnosis of NAFLD was confirmed by percutaneous liver biopsy performed in all subjects with a 1.6 mm Menghini-type biopsy needle. Liver samples were routinely processed, sectioned, and stained with hematoxylin-eosin and Manson's trichome. A semi quantitative scoring system for NAFLD had been applied. Defined as the unweighted sum of scores for: Steatosis (< 5% = 0, 5 to 33% = 1, > 33 to 66% = 2, > 66% = 3). Lobular inflammation (no foci = 0, < 2 foci per 200 × field = 1, 2 to 4 foci per 200 × field = 2, > 4 foci pr 200 × field = 3). Ballooning (none = 0, few balloon cells=1, many cells/prominent ballooning = 2) Fibrosis was not include in the NAS score. The maximum score was 8. Definitive non alcoholic steatohepatitis (NASH) was defined as a NAS score ≥ 5¹¹.

Procedure

All patients were randomized (table of numbers) to one of the following interventions: group I, treated with 2 tablets of silymarin per day (*Silybum marianum* Gaerth, e.s. 540.3 mg) plus vitamin E (36 mg) (Eurosil 85®, MEDAS SL) plus a lifestyle modification program and group II: only with a lifestyle modification program (hypocaloric diet (1520 kcal, 52% of carbohydrates, 25% of lipids and 23% of proteins) and exercise. The exercise program consisted of aerobic exercise at least 4 times per week (60 minutes each) and group II treated only with lifestyle modification program for 3 months.

Validated index of NAFLD, were determined in all patients before and after therapeutic intervention.

Fatty Liver Index (FLI)¹²

$$FLI = e^{0.953} \times \ln(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \ln(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745$$

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Liver Accumulation Product (LAP)¹³

In Men: waist circumference cm -65 × triglycerides (mmol/L)

In Woman: waist circumference cm -58 × triglycerides (mmol/L)

NAFLD-Fibrosis score (FS)¹⁴

NAFLD fibrosis score = $-1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{diabetes yes 1, no 0} + 0.99 \times \text{ratio AST/ALT} - 0.013 \times \text{platelets (} \times 10^9/\text{L)} - 0.66 \times \text{albumin (g/dl)}$.

Subject food intakes were recorded at baseline and after dietary advice after 3 months with 3 days written food record. Basal glucose, liver enzyme levels (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamine transpeptidase (GGT)), insulin resistance measured by homeostasis model (HOMA-IR), triglycerides, total cholesterol, low density lipoprotein cholesterol (LDL-cho), high density lipoprotein cholesterol (HDL-cho) were measured at baseline time and after 3 months of treatment. Anthropometric parameters as weight, body mass index (BMI) computed as $\text{body weight/height}^2$, waist circumference and dietary intakes were controlled at basal time and after three months of treatment.

Assays

Plasma glucose levels were determined using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was measured by enzymatic colorimetric (Insulin, WAKO Pure-Chemical Industries, Osaka, Japan) and the homeostasis model assessment for insulin sensitivity (HOMA-IR) was calculated using these values¹⁵.

AST, ALT activity, bilirubin and GGT levels were determined by enzymatic colorimetric assay Hitachi 917 (Roche Diagnostics, Geneve, Switzerland). Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), while HDL-cho was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL-cho was calculated using Friedewald formula.

Dietary Intake

Patients received prospective serial assessment of nutritional intake with 3 days written food

records. All enrolled subjects received instructions to record their daily dietary intake for three days including a weekend day. Dietary data was handled via a personal computer (Dietsource, Novartis, Geneve, Switzerland), incorporating use of food scales and models to enhance portion size accuracy. National composition food tables were used as reference (16). Physical activity remained unchanged during the follow up period.

Statistical Analysis

Sample size was calculated to detect differences over 5 UI/L on transaminases levels with 90% power and 5% significance. The results were expressed as means \pm standard deviation. The distribution of variables was analyzed with Kolmogorov-Smirnov test. Quantitative variables with normal distribution were analyzed with a two-tailed, paired Student's-*t* test. Nonparametric variables were analyzed with the Wilcoxon test. Qualitative variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. A *p*-value under 0.05 was considered statistically significant.

Results

Thirty-six patients were included in the protocol. We first assessed distribution of variables before using parametric analyses and results showed that all of them were normal. None of patients withdrew from the study during the research period. There were no differences in gender and age distributions of patients between both groups. Average age was 47.4 ± 11.2 years. Sex distribution was 22 males and 14 females. In the group I, 11 patients (61%) had a NAS-score ≥ 5 and 10 (55.5%) in the group II (non significant) (NS) (Table I).

There were no adverse events during the treatment period and study compliance was absolute. All patients were asked to follow a well balanced individual diet and exercise during the time of the study.

Table I. Histological findings in enrolled subjects in both groups (group I: silymarin + vit E + diet and group II: diet).

| | Group I (n = 18) | Goup II (n = 18) | <i>p</i> |
|----------------------------------|------------------|------------------|----------|
| Steatosis (leve-moderada/severa) | 6/12 | 5/13 | NS |
| NAS score (< 5/ \geq 5) | 11/7 | 10/8 | NS |
| Fibrosis (F0/F \geq 1) | 13/5 | 13/5 | NS |

Patients treated with Silymarin-vitamin E plus diet (group I) showed a significant improvement in anthropometric parameters such as waist circumference and body weight (Table II).

Patients in both groups showed a decrease in GGt levels after treatment (group I: 81.5 ± 68 IU/L vs. 46.2 ± 27 IU/L; $p < 0.05$ and group II 80.5 ± 46 IU/L vs. 50.3 ± 27 IU/L; $p < 0.05$). Only in group II we observed also, a significant decrease in AST and ALT levels. In both groups, we observed a significant decrease in FLI and NAFLD-FS index (Table III).

To rule out that these changes were due to the effect of diet, patients achieving a weight loss equal to or greater than 5% in group I (which is the percentage weight recommended by AGA 2012 guidelines)¹⁷ were excluded ($n=8$). We analyze this group of patients without loss of body weight and we also observed a significant decrease in GGT levels (89 ± 78 IU/L vs. 48 ± 32 IU/L; $p < 0.01$), FLI (84.3 ± 18 vs. 76.6 ± 21 ; $p < 0.05$) and NAFLD-FS (-1.5 ± 1.3 vs. -2 ± 1.4 ; $p < 0.05$) without changes in the other parameters.

In group II, patients who failed to lose 5% of body weight showed no change in the biochemical parameters.

Discussion

The aim of our study was to evaluate the efficacy of anti-oxidant (vitamin E) treatment with silymarin and hypocaloric diet in comparison with hypocaloric diet, in patients affected by NAFLD.

Treatment of steatohepatitis is difficult. Based on some data, a 4-5% reduction in body weight could be recommended as an initial therapeutic target in patients with NAFLD to improve liver function. ALT and AST levels improve with low fat diet¹⁸. It has been suggested that in the future a therapeutic approach to chronic liver disease

could consist of a number of complementary approaches considering the multitude of pathogenic mechanisms. In our study we observed that patients who underwent life style changes with a weight lost at least 5% showed an improvement in AST, ALT and specially GGt levels.

This study also shows that treatment with silymarin plus vitamin E and lifestyle changes achieved significantly decrease GGt levels even when they were not able to decrease body weight.

It is interesting to note that GGt values decreased significantly ($p < 0.01$) and in the silymarin treated patients, which confirms that silymarin is able to restore normal liver membrane permeability by reducing enzyme dispersion in the extracellular medium. Silymarin is a natural flavonoid that has been conjugated to vitamin E and phospholipids to improve its bioavailability, and antioxidant and antifibrotic activity¹⁹.

Marchesini et al²⁰ have treated these patients with metformin: this drug reduced transaminase concentrations, insulin resistance and liver volume.

In another study²¹, acarbose attenuated NAFLD progression in an experimental model of NAFLD in rats. Two drugs used in obese patients, orlistat and sibutramine²²⁻²⁴ have shown that drug-induced weight losses result in reduction of insulin resistance and improvements in biochemical markers of NAFLD. Other potential treatment is probiotics. In other report²⁵ we have documented, with a randomized clinical design that *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, improved liver aminotransferase levels in patients with NAFLD.

In our case load, NAFLD is associated with insulin resistance (HOMA-IR)²⁶.

Previous studies^{27,28} demonstrated that insulin resistance almost universally induces NAFLD. It is known that this condition may precede the development of cardiovascular disease²⁹. Mild to moderate elevation of serum aminotransferase

Table II. Changes in anthropometric parameters (mean \pm SD) (group I: silymarin + vit E + diet and group II: diet).

| | Group I (n = 18) | | Group II (n = 18) | |
|--------------------------|------------------|------------------|-------------------|------------------|
| | Basal | After | Basal | After |
| BMI (kg/m ²) | 36.8 \pm 7.9 | 31.7 \pm 5.8* | 35 \pm 7.4 | 32.1 \pm 7.1 |
| Weight (kg) | 91.6 \pm 14 | 89.5 \pm 13* | 92.2 \pm 13 | 87.4 \pm 13.9* |
| WC (cm) | 102.6 \pm 10.2 | 101.6 \pm 9.7* | 103.7 \pm 13 | 100.6 \pm 13* |

BMI: body mass index, WC: waist circumference * $p < 0.05$.

Table III. Biochemical and NAFLD-index in enrolled subjects (mean \pm SD) (group I: silymarin + vit E+ hypocaloric diet), group II (only hypocaloric diet).

| | Group I (n = 18) | | Group II (n = 18) | |
|---------------|------------------|-----------------|-------------------|-----------------|
| | Basal | After | Basal | After |
| Glucose mg/dl | 93.1 \pm 13 | 93.8 \pm 12 | 129.9 \pm 46 | 114.6 \pm 47* |
| TG mg/dL | 189.6 \pm 84 | 185 \pm 85 | 180.8 \pm 68 | 170.9 \pm 63 |
| AST (IU/L) | 35.6 \pm 16 | 34.6 \pm 16 | 41.6 \pm 20 | 36 \pm 11.8 |
| ALT (IU/L) | 56.4 \pm 27 | 52.7 \pm 26 | 70.8 \pm 41 | 54.7 \pm 18* |
| ALT/AST | 1.5 \pm 0.4 | 1.5 \pm 0.3 | 1.7 \pm 0.5 | 1.5 \pm 0.4* |
| GGT (IU/L) | 81.5 \pm 68 | 46.2 \pm 27* | 80.5 \pm 46 | 50.3 \pm 27* |
| HOMA-IR | 3.4 \pm 2.2 | 3.4 \pm 2.2 | 5.4 \pm 4.1 | 4.9 \pm 4* |
| FLI | 86.2 \pm 19 | 76.9 \pm 20* | 85.2 \pm 18 | 77.5 \pm 23* |
| LAP | 4.3 \pm 0.1 | 4.3 \pm 0.1 | 4.2 \pm 0.5 | 4.2 \pm 0.6 |
| NAFLD-FS | -1.6 \pm 1.8 | -2.1 \pm 1.5* | -1 \pm 1.9 | -1.5 \pm 2.1* |

TG: triglycerides; AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. GGT: Gamma glutamyl transpeptidase; HOMA-IR: Homeostatic model assessment-insulin resistance; FLI: fatty liver index. LAP: liver accumulation product. NAFLD-FS: non alcoholic fatty liver disease-fibrosis score; * $p < 0.05$.

(ALT and AST) found in our subjects at baseline represents the most common abnormality found in patients with NAFLD also documented by F Cacciapuoti et al³⁰. In their study serum levels significantly reduced after diet and silymarin treatment. Unlike subjects with alcohol-induced steatohepatitis (who typically manifest disproportionate increases in the AST level), patients with NAFLD usually have an AST/ALT ratio < 1 because the ALT level is higher than AST in NAFLD³¹. On the contrary, the AST/ALT ratio trends to increase with the development of cirrhosis, thus losing its diagnostic accuracy³². The reduction both of AST and ALT after silymarin treatment seems due to the antioxidant effect of *Silybum marianum* that is also able to protect the liver against toxins. Previous studies³³⁻³⁴ also demonstrated that silymarin acts as a cytoprotectant, anticarcinogenic and supportive agent for liver damage from *Amanita phalloides* poisoning. In our study, silymarin was demonstrated to reduce high GGt serum levels, probably due to stabilization of the hepatocyte membrane structure, thereby preventing toxins from entering the cells. Stranges et al³⁵ have demonstrated that mean serum levels of GGt were above the normal limits in selected subjects with NAFLD. This is due to obesity, hyperinsulinemia, oxidative inflammation and changes in hepatocyte membrane permeability.

Weight loss is one of the first line recommendations to individuals with NAFLD. DA de Luis et al³⁶ documented lower aminotransferases levels after two types of hypocaloric diets. The AGA guidelines¹⁷ recommend a loss of 3 to 5% of body

weight to reduce steatosis (Recommendation 1A). Hajmadi A et al³⁷ demonstrated that metformin, pioglitazone and silymarin treatment decreases AST, ALT and GGT levels but in the silymarin group it was more than of the other group.

In our study there is absence of histological confirmation of improvement after treatment. Several authors¹¹⁻¹³ have validated noninvasive test for the diagnosis of NAFLD and its severity. Although further studies should focus on developing a references standard score more accurate than ultrasonography or less invasive than liver biopsy, in the meantime, the FLI, LAP and NAFLD-FS score can foresee hepatic steatosis relatively well and can be used in clinical practice even before imaging studies or liver biopsy³⁸.

Conclusions

We have demonstrated that treatment with silymarin associated with vitamin E and a hypocaloric diet ameliorate function hepatic test, and non-invasive NAFLD test, and this change is also present in patients without loss of 5 % of weight. Life style modification continues to be a valid treatment for these patients. In patients who fail to lose weight with diet, silymarin can be a valid alternative therapeutic option particularly when other drugs are not indicated or have failed or as a complementary treatment associated with other therapeutic programs. However these results should be confirmed by prospective randomized clinical trials in the future.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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