Efficacy of Oral Supplementation with Silymarin and S-Adenosyl-L-Methionine in Patients with Non Alcoholic Fatty Liver Disease – A Pilot Study

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Abstract

**Background and Aim:** Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide, affecting up to one-third of the population in industrialized countries. Silymarin and S-Adenosyl-L-Methionine (SAMe) have therapeutic potential for treatment of liver disease. The aim of the present pilot study was to evaluate the possible effect of a supplementation with Silymarin and SAMe in NAFLD patients.

**Methods:** Fifteen NAFLD patients (mean age: 46.3 ± 15 years) confirmed by ultra-sonographic findings were subjected to a 12-month oral supplementation with Silymarin and SAMe. Anthropometric measurements, biochemical parameters, liver function and hepatic fat content were assessed at baseline, at 6 months and at the end of treatment.

**Results:** After a 12-months’ oral supplementation a significant improvement (p<0.05) in several biochemical parameters such as total cholesterol (mean reduction: -8.67 mg/dL), insulin (-1.34 U/L) and HOMA-IR (-1.35) was observed. Similarly, serum levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP) significantly decreased by 13% (-3.87 U/L and -10.4 U/L respectively), with a small but significant amelioration in the risk profile. Finally, the ultrasonography examination revealed an improvement in liver echo-texture and a regression of the degree of hepatic steatosis, associated with a 22% increase of the Doppler Perfusion Index (DPI), which corresponds to better liver hemodynamic.

**Conclusion:** Our findings suggest that a prolonged Silymarin and SAMe supplementation could be used as an adjunctive therapy to improve metabolic risk profile and hepatic steatosis in patients with NAFLD. Further research is needed to confirm these preliminary results.

Keywords: Silymarin; S-adenosyl-L-methionine; NAFLD

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the industrialized countries [1]. Its prevalence increased from less than 10% in the 1980s, through 10% to 20% in the 1990s, to current rates of 15% to 30% or higher [1]. NAFLD represents the outcome of genetically-determined interactions between a changing environment and a susceptible host. Environmental factors include excess of energy intake, particularly in the form of cheap, highly processed simple carbohydrates and saturated fats, and reduced levels of physical fitness resulting from a sedentary lifestyle [2]. NAFLD embraces a pathological spectrum of liver disease, from cases of steatosis with virtually no evidence of hepatocellular injury or liver inflammation, often referred to as simple steatosis through steatohepatitis (NASH), to cases with cirrhosis [3]. Individuals with only hepatic steatosis (simple steatosis) infrequently show signs of any histologic progression, and are not at significant long-term risk of liver-related death [4], but those with advanced hepatic fibrosis are likely to experience liver-related complications (ascites, variceal bleeding, and/or hepatocellular carcinoma) [5]. Furthermore, hepatic necroinflammatory activity in patients with NAFLD is able to increase the risk of future cardiovascular disease [6], and confers a higher risk of unfavorable liver-related outcomes by promoting development of hepatic fibrosis. The lipid molecules that accumulate, together with triglycerides, in some NAFLD livers can participate directly in pathogenesis of the necroinflammatory element of NASH [7]. From the scientific literature it is known that high intracellular levels of Silymarin and S-Adenosyl-L-Methionine (SAMe) act in: reducing the uptake of exogenous toxic substances, increasing membrane fluidity, detoxifying the hepatic cell, preventing the depletion of glutathione and thereby increasing its availability, having anti-inflammatory activity and in promoting the regeneration of liver cells [8,9]. The aim of the present intervention study was to evaluate the possible effect of an oral supplementation of Silymarin and SAMe in NAFLD patients.

Material and Methods

**Study population**

Fifteen patients with a diagnosis of NAFLD referred to the Unit of Clinical Nutrition of the University Hospital of Careggi, Florence, Italy,
and characterized by ultrasonography features indicative of NAFLD were eligible for enrolment. Written informed consent was obtained from all patients. The study protocol followed the ethical guidelines of the Declaration of Helsinki and was approved by our departmental Ethics Committee.

The inclusion criteria were as follows: age >18 years, lack of excessive alcohol consumption (<20 g/d), ultrasound examination demonstrating fatty liver, negative diagnostic tests for viral hepatitis and absence of other causes of liver disease. Exclusion criteria included a history of alcohol intake >20 g/d, use of drugs known to be associated with liver steatosis, undernutrition, chronic viral hepatitis and chronic liver disease of other causes (autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangiitis, hereditary haemochromatosis, Wilson's disease, α1-anti-trypsin deficiency, coeliac disease). Pregnant and breastfeeding women were also excluded.

Study design

All patients were subjected to a 12-months' oral supplementation with 1 tablet per day of Duoliver (Biocure Srl, Milan), a food supplement containing Silymarin (140 mg) and SAMe (200 mg) without dietary guidelines or other lifestyle changes. The products were packed in labelled boxes. Compliance was verified by counting the empty boxes on return. A medical examination with measurement of body mass index (BMI), blood tests and liver ultrasonography was performed by the same expert operator using a high-quality ultrasound device equipped with a 5 MHz Convex array transducer (Voluson 530 DMT; Kretz Technik AG, Zipf, Austria). The primary inclusion criteria were as follows: age >18 years, lack of excessive alcohol consumption (<20 g/d), ultrasound examination allowing a semi-quantitative measurement of fatty liver storage on the basis of the Doppler Flow Index (DFI) (i.e. the ratio between hepatic artery blood flow and total liver blood flow).

Laboratory parameters

Venous blood samples anticoagulated with 0.129 M sodium citrate (ratio 9:1) were collected from the antecubital vein into evacuated plastic tubes (Vacutainer; BD, Milan, Italy), after overnight fasting. Whole venous blood was also collected in tubes without anticoagulant. Citrated and serum samples were centrifuged at 2,000× g for 10 min at 4°C, and supernatants were stored in aliquots at −80°C until assays. Lipid variables, blood glucose, insulin, and hepatic function tests were assessed by conventional methods, as well as the HOMA-IR.

Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software for Macintosh (Version 20.0). Results were expressed as means ± standard deviation. The Chi-square test was used to identify statistically significant differences between categorical variables. The Wilcoxon test for related data was used to evaluate differences between two time-points. A general linear model for repeated measurements, with adjustments for age, sex and BMI change, was used to compare differences between baseline and the end of the intervention period. A P value ≤ 0.05 was considered significant for all the analyses.

Results

Between January, 2015 and May, 2015 a total of 15 patients (11 females, 4 males; mean age: 46.3 ± 15 years) with NAFLD were included in the protocol. Participants received a Silymarin plus SAMe supplement for 12 months. None of them withdrew from the study during the research period and no adverse events were observed. At baseline, the average BMI was 30.9 ± 8.9 kg/m², with 7 (47%) patients categorized as obese or extremely obese and 4 (27%) patients classified as overweight. The assessment of liver steatosis showed a moderate/severe steatosis in 6 (40%) patients.

After 6 and 12 months of food supplementation with Silymarin and SAMe, BMI did not change significantly with respect to baseline (p=0.65). In order to analyse the changes in the biochemical variables after the intervention, a general linear model adjusted for age, sex and BMI change was performed. As reported in Table 1, supplementation was found to result in a significant reduction by 4% (mean reduction: -8.67 mg/dL; p=0.01) for total cholesterol levels with respect to baseline. Also insulin levels and HOMA-IR decreased significantly by 9% and 15%, respectively (-1.34 U/L; p<0.01 and -1.35; p=0.03) with an amelioration of the metabolic status of patients. Non-significant differences were observed for LDL-cholesterol, HDL-cholesterol and triglycerides.

With regard to the liver function tests, at the end of the study we observed a significant reduction by 13% in the levels of both ALT (-3.87 U/L; p=0.01) and ALP (-10.4 U/L; p=0.04), with non-significant changes in the other parameters (Table 2). The echo-Doppler examination revealed a 22% increase in the hepatic DFI, moving from a mean of 0.18 ± 0.07 at baseline to 0.22 ± 0.05 at the end of the treatment (normal value range: 0.20-0.30), although this was not
significant (p=0.08). However, ultrasonography indicated a regression of steatosis in a consistent proportion of patients: after a 12-months’ oral supplementation with Silymarin and SAMe, 5 patient out of 6 with moderate/severe steatosis regressed to mild steatosis (Figure 1).

![Diagram showing steatosis grades]

**Figure 1:** Modification of steatosis grade in enrolled subjects after a 12-months' oral supplementation with Silymarin plus SAMe.

<table>
<thead>
<tr>
<th>Variable</th>
<th>baseline (T0)</th>
<th>6-months (T1)</th>
<th>12-months (T2)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, mg/dL</td>
<td>211.3 ± 31.5</td>
<td>204.0 ± 27.4</td>
<td>202.6 ± 30.6*</td>
<td>0.014</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>143.9 ± 26.8</td>
<td>144.1 ± 24.1</td>
<td>140.7 ± 22.2</td>
<td>0.366</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dL</td>
<td>45.9 ± 9.51</td>
<td>47.0 ± 8.28</td>
<td>48.3 ± 8.84</td>
<td>0.246</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>120.1 ± 53.6</td>
<td>116.3 ± 45.1</td>
<td>119.3 ± 44.5</td>
<td>0.93</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>93.1 ± 10.9</td>
<td>90.8 ± 7.42</td>
<td>91.1 ± 6.80</td>
<td>0.352</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>16.1 ± 5.33</td>
<td>15.5 ± 4.9</td>
<td>14.7 ± 5.04*</td>
<td>0.006</td>
</tr>
<tr>
<td>HOMA-Index</td>
<td>3.67 ± 1.75</td>
<td>3.39 ± 1.29</td>
<td>3.11 ± 1.15*</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Data are reported as mean and standard deviation, HOMA=Homeostatic Model Assessment, *p<0.05 for Wilcoxon signed-rank test (T0 vs T1 and T0 vs T2), †General linear model for repeated measurements adjusted for age, sex and BMI change.

**Table 1:** Modification of biochemical parameters in enrolled subjects after 6- and 12-months’ oral supplementation with Silymarin plus SAMe.
ALT and ALP was also observed biochemical parameters such as total cholesterol, insulin, HOMA-IR, regression of the degree of hepatic steatosis, associated with a examination revealed an improvement in liver echo-texture and a Indeed, remarkable increase of DPI, which corresponds to better liver supplementation on the metabolic risk the most difficult the milk thistle plant (Silybum marianum) is a modern drug that arose from traditional medicine practices. This study is one of the few clinical trials that assessed the potential role of Silymarin and SAMe in the treatment of NAFLD. Supplementation with Silymarin alone has been investigated in two Iranian studies [12,13], exhibiting large effects in liver function tests in enrolled subjects after 6- and 12-months’ oral supplementation with Silymarin plus SAMe. Discussion

The current study reported a beneficial effect of oral supplementation on the metabolic risk profile in patients with NAFLD. Indeed, after a 12-month oral supplementation, the ultrasonography examination revealed an improvement in liver echo-texture and a regression of the degree of hepatic steatosis, associated with a remarkable increase of DPI, which corresponds to better liver hemodynamic. Moreover, a significant improvement in several biochemical parameters such as total cholesterol, insulin, HOMA-IR, ALT and ALP was also observed after the intervention period.

To date, lifestyle modifications, healthy diet and physical activity are the most effective treatment for NAFLD, but for many people they are difficult to achieve and maintain [10]. Therefore, over the past years, several therapeutic proposals such as weight reducing, lipid-lowering, and insulin-sensitizing drugs have been proposed. These agents should act to reduce the link between adipose tissue and liver function by acting as anti-inflammatory and immunomodulatory agents. However, a validated and unique approach has not yet been obtained [10]. On the other hand, the use of complementary medicines, such as natural antioxidants, may have therapeutic potential. In fact, oxidative stress is thought to play a central role in the aetiology of NAFLD, as fatty acid oxidation produces reactive oxygen species, which cause direct cellular damage and activate pro-inflammatory cytokines. (1) Derived from the milk thistle plant (Silybum marianum), Silymarin is an example of a modern drug that arose from traditional medicine practices. The hepatic protection of this flavonoid complex has been attributed to its antioxidant, anti-inflammatory and antifibrotic properties [11].

Although results are promising, the number of participants represents a limitation. Further and larger studies are needed before drawing any firm conclusions. In addition, up to now, there is no evidence on the ideal quantity and duration of supplementation with Silymarin plus SAMe for NAFLD. We cannot exclude that a higher

<table>
<thead>
<tr>
<th>Variable</th>
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<th>6-months (T1)</th>
<th>12-months (T2)</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST, U/L</td>
<td>21.9 ± 7.30</td>
<td>21.3 ± 7.07</td>
<td>21.2 ± 6.71</td>
<td>0.443</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>30.0 ± 15.4</td>
<td>28.6 ± 13.3</td>
<td>26.1 ± 13.5†</td>
<td>0.013</td>
</tr>
<tr>
<td>γ-GT, U/L</td>
<td>44.4 ± 39.9</td>
<td>45.7 ± 60.1</td>
<td>44.6 ± 59.1</td>
<td>0.974</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>80.7 ± 34.3</td>
<td>77.5 ± 31.6</td>
<td>70.3 ± 30.7†</td>
<td>0.039</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>0.75 ± 0.23</td>
<td>0.70 ± 0.20</td>
<td>0.81 ± 0.16</td>
<td>0.235</td>
</tr>
<tr>
<td>Direct bilirubin, mg/dL</td>
<td>0.18 ± 0.08</td>
<td>0.21 ± 0.10</td>
<td>0.23 ± 0.09*</td>
<td>0.097</td>
</tr>
<tr>
<td>Indirect bilirubin, mg/dL</td>
<td>0.57 ± 0.22</td>
<td>0.49 ± 0.22</td>
<td>0.57 ± 0.16</td>
<td>0.875</td>
</tr>
</tbody>
</table>

Data are reported as mean and standard deviation.
†p<0.05 for Wilcoxon signed-rank test (T0 vs T1 and T0 vs T2).
General linear model for repeated measurements adjusted for age, sex and BMI change.

Table 2: Modification of hepatic function tests in enrolled subjects after 6- and 12-months’ oral supplementation with Silymarin plus SAMe.
dosage and/or a prolonged intake could reach even better outcomes. A current problem of clinical trials conducted on NAFLD patients is how to measure the outcome [23]. In particular, routine laboratory tests are helpful but not sufficient to assay the effectiveness of treatment, while needle liver biopsy, that provides the most conclusive evaluation both for diagnosis and follow-up, cannot be carried out in all patients because of invasiveness and possible complications. In the present study, in order to improve the accuracy of the evaluation, besides the liver function tests we performed ultrasonography to assess the hepatic fat content, and duplex doppler ultrasound, which provides a quantitative, non-invasive evaluation of NAFLD by measuring hepatic artery and portal vein blood flow.

In conclusion, our findings suggest that a prolonged Silymarin plus SAMe oral supplementation might have positive effects in patients with NAFLD, improving hepatic steatosis and insulin resistance, and reducing cholesterol and liver enzymes level. In patients who fail to change their lifestyle, Silymarin and SAMe could be a valid alternative therapeutic option, where appropriate as a complementary treatment associated with other therapeutic programs. Further research is needed to confirm these preliminary results.

Acknowledgments

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References