Jundishapur Journal of Natural Pharmaceutical Products 2009; 4(1): 8-14

Jundishapur Journal of Natural Pharmaceutical Products

COMPARISON OF THERAPEUTIC EFFECTS OF SILYMARIN AND VITAMIN E IN NONALCOHOLIC FATTY LIVER DISEASE: RESULTS OF AN OPEN-LABELE, PROSPECTIVE, RANDOMIZED STUDY

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Received:*11 March 2009* ***Accepted:*** *28 December 2009*

Abstract

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic condition characterized by significant lipid deposition, despite a proposed role of oxidative stress in its pathogenesis antioxidant treatments have not been investigated sufficiently. During a five-year period a cross-sectional study was conducted among patients with NAFLD referred to the Ahvaz JundiShapour University Hospitals (AJSUH) and Hepatitis Clinic from 2003 to 2006. The study included 142 NAFLD patients attending our center for management of liver disease. Viral hepatitis and other hepatic diseases and patients who had intake of ethanol or drugs known to produce fatty liver disease within the previous 6 months were excluded. Patients were randomized to two groups to receive vitamin E 400 IU per day (71 patients) or Silymarin treatment, *Silybum marianum* extract containing silymarin 70 mg three times daily (71 patients). Treatment was continued for 12 weeks and cases were evaluated every 4 weeks in the out-patient clinic. 142 subjects (60.95% male, 39.05% mean age 45.2.5±10.3), who met the inclusion and exclusion criteria, were included in the analysis. At the end of the 12-week treatment period there was a significant decrease in the serum AST and ALT levels in both treatment groups. The mean serum baseline ALT level was 85 $\pm$ 10 IU/mL and AST level was 51.9 $\pm$ 10 IU/mL in study groups.

The mean aspartate transaminase (AST) levels changed to normal 56.30% (40 of 71 cases) in the vitamin E group and 74.6% (53 of 71 patients) in the *S. marianum* group (P=0.01).

The decrease in AST level in the *S. marianum* group as compared to the vitamin E group was significant (P<0.007). No side effects were reported in our cases.

S. marianum and vitamin E treatment appears to be significantly effective in biochemical improvement and decreasing the ALT and AST levels in patients with NAFLD.

Keywords

Non-alcoholic fatty liver disease, Milk thistle, Vitamin E, Silymarin

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathologic condition characterized by significant lipid deposition, usually greater than 5% of the liver weight deposited as triglyceride, inthe patient's liver parenchyma, without history of excessive alcohol consumption(1) . NAFLD is recognized today asthe most prevalent liver disease in the Western population, with estimated prevalence rates of 20–30% (1).

NAFLD seems to have become an important medical entity and this importance is mainly resulted from its

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potential to progress to cirrhosis and liver failure and its common occurrence in general population (2).

Because the pathogenesis of NAFLD remains unknown, the management of this condition is empirical. NAFLD may resolve with weight loss, although the benefits of weight loss have been inconsistent (3).

Since a considerable number of patients with NAFLD are not obese, or do not benefit from weight loss, use of medication that can directly reduce the severity of liver damage independent of weight loss is a reasonable alternative.

Despite a proposed role of oxidant stress in the pathogenesis of NAFLD, antioxidant approaches have not been investigated sufficiently in NAFLD therapy (4-5).

Milk thistle (Silybum marianum) as an antioxidant and a regulator of immune functions by modulating cytokine production was used in classical Greece to treat liver and gallbladder diseases to protect the liver against toxins (6-8). Vitamin E recently has been investigated for use as a cytoprotectant, and as an antioxidative scavenger of free radicals in treatment of liver damage from Amanita phalloides poisoning, viral infections and cirrhosis (9).

The aim of the present study was to compare the efficacy and safety of oral silymarin and Vitamin E, administered for three months in subjects with NAFLD.

Methods

During a five-year period a cross-sectional study was conducted among patients with NAFLD referred to Ahvaz JundiShapour University Hospitals (AJSUH) and Hepatitis Clinic from 2003 to 2006. This is an open-label, prospective, randomized study. The study included 142 consecutive NAFLD patients attending our center for management of liver disease. Patients were excluded if they had an intake of ethanol (more than 20 g/day) or when they ingested drugs known to produce fatty liver disease, such as steroids, estrogens, amiodarone, tamoxifen, or other chemotherapeutic agents within the previous 6 months. Viral hepatitis B and C were excluded by HBsAg and anti HCV tests. Other hepatic diseases including autoimmune hepatitis, Wilson's disease, hemochromatosis and alpha-1 antitrypsin deficiency were also ruled out. Patients with severe co morbid medical conditions (such as severe cardiac, pulmonary, renal or psychological problems) or those not consenting to participate in the study were also excluded. The patients were followed up with a weight-reducing diet at least three months by checking alanine aminotransferase (ALT) levels. In this screening period, liver biopsies were performed in order to rule out other causes of liver diseases and to prove the histologic diagnosis of NAFLD in six cases. The major indication was persistent elevation of ALT and AST after an attempt to control the metabolic conditions and after 6 or more months of follow-up which showed fatty liver.

At the end of this period, the patients having ALT levels at least 1.2 times the upper limit of normal, despite a three-month weight reducing diet, were included in the study.

Ultrasonographic evaluations of the liver were performed at the entry of the study and at the end of the treatment. In the ultrasonographic examination, fatty liver was diagnosed according to the modified criteria. (10). The four parameters used in this criteria are brightness of the liver, attenuation of echogenicity, blurred vessels, and the contrast ratio of the liver-to-kidney on ultrasonography (US) (General Electric LOGIQ 400 CL).

Patients consecutively were randomized to two groups to receive vitamin E 400 IU per day (71 patients) or Silymarin treatment, *Silybum marianum* extract containing silymarin 70 mg three times daily (71 patients).

Treatment was continued for 12 weeks and cases were evaluated every 4 weeks in the out-patient clinic. Serum levels of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), fasting plasma glucose (FPG),serum triglycerides, and cholesterol values were monitored at each visit during treatment.

Statistical analysis

Sample size calculations were based on the comparison of paired data. With a sample size of 71 subjects, this study had an 95% power to detect a difference in ALT levels equivalent to one standard deviation with a two-sided *P* -value of 0.05. Statistical analysis was performed using a repeat measures analysis of variance and Pearson's correlation, with baseline and population comparisons made using student *t*-tests and chi-squared tests for equal proportion where appropriate. For all comparisons, a two sided *P* -value of 0.05 was considered to be statistically significant. All calculations were made using SAS, version 8.2 (SAS Institute, Cary, NC, USA).

Results

142 subjects (no=87 male (60.95%) no=55female,(39.05%) mean age (45.2.5±10.3), who met the inclusion and exclusion criteria, were included in the analysis. No patient had clinical evidence of cirrhosis or decompensated liver disease. There was no patient with a BMI greater than 40 in our study.

In the whole treatment groups there were 11 patients with diabetes mellitus who were on diabetic diet without medication (7.7%) and 79 patients with hyperlipidemia (55.6%). There was no significant difference in age, gender, BMI, and frequency of diabetes mellitus and hyperlipidemia between the two treatment groups. Table 1 shows the demographic data of the studied cases.

At the end of the 12-week treatment period there was a significant decrease in the serum AST and ALT levels in both treatment groups. The mean serum baseline ALT level was 85µ 10 IU/mL and AST level was 51.9µ 10 IU/mL in study groups.

The percentage of patients with alanine transaminase (ALT) normalization was 45% (32 of 71 patients) in the vitamin E-treated group and 41% (29 of 71 patients) in the *S. marianum* -treated group, but the difference between them was not significant (*P*=0.79).

The mean aspartate transaminase (AST) levels changed to normal 56.30% (40 of 71 cases) in the vitamin E group and 74.6% (53 of 71 patients) in the *S. marianum* group (*P*=0.01).

The decrease in AST level in the *S. marianum* group as compared to the vitamin E group was significant (P<0.007). The mean alanine transaminase (ALT) levels changed from 78.2 to 58.8 in the vitamin E group and from 77.3 to 56.4 in the *S. marianum* group, respectively. The difference was not significant between two groups Table 2. Patient compliance with medication was good in both groups and all cases completed the study. No side effects were reported in our cases.

Table 1. Demographic data of the cases

variable	vitamin E group	*S. marianum* group
age	38.3±8	37.9±10
sex	F/M 87/55	F/M 86/56
Mean age	45.2.5±10.3	45.2.5±10.3
Hyperlipidemia n (%)	79 (55.6%)	79 (55.6%)

Diabetes mellitus n (%)	5(3.5%)	6(4.2%)

Table 2. Laboratory values before and after therapy

variable	baseline ALT level(IU/mL)	ALT normalization n(%)	baseline AST level(IU/mL)	AST normalization n(%)
vitamin E group	85µ 10 IU/mL	32/71(45%) (*P*=0.79)	51.9µ 10	40/71(56.30%) (*P*=0.01)
S. marianum group	85µ 10 IU/mL	29 / 71(41%) (*P*=0.79)	51.9µ 10	53 /71(74.6%) (P<0.007)

Discussion

NAFLD is now considered to be the hepatic manifestation of the metabolic syndrome which includes central obesity, hypertension, hypertriglyceridemia, low levels of high-density lipoprotein (HDL) and hyperglycemia (11). The strong association of NAFLD with insulin resistance and the metabolic syndrome has been documented in a growing body of literature (12). The prevalence of NAFLD may be increasing because of changing of dietary habits, less physical activity, and increasing incidence of obesity.

In most patients oxidative stress is thought to be the cause of lipid accumulation in the hepatocytes and hepatic inflammation and fibrosis (13).

There is no strong evidence supporting any effective therapeutic agents for reducing inflammation and fibrosis or preventing the progression of NAFLD. Nonetheless, weight reduction and the drugs that overcome oxidative stress may be appropriate to slow the disease process.

Silybum marianum and vitamin E were used as an anti-oxidant to protect the liver against toxin in several studies. They have been investigated for use as a cytoprotectant, an anticarcinogen, and a supportive treatment for liver damage from Amanita phalloides poisoning (14-15).

The active component of the silymarin complex is silybin and the main mechanism of its action is not completely understood, but a variety of mechanisms have been proposed. Silymarin is reported to have antioxidant properties, by increasing superoxide dismutase activity in erythrocytes and lymphocytes (16).

It is also reported to stabilize hepatocyte membrane structure, thereby preventing toxins from entering the cell through enterohepatic recirculation, and to promote liver regeneration by stimulating nucleolar polymerase A and increasing ribosomal protein synthesis (17).

Silybin selectively inhibits leukotriene formation by Kupffer cells and is a mild chelator of iron. It also prevents glutathione depletion in human hepatocyte protecting cells from damage *in vitro* (18-19).

Previous studies that have suggested a therapeutic role for *S. marianum* in NAFLD have either been uncontrolled and/or conducted in a heterogeneous group of patients with fatty liver.

There are not sufficient studies about the treatment of NAFLD in our area. In most of the publications about the treatment of NAFLD, focus is on improvement of associated conditions like obesity, diabetes mellitus and hyperlipidemia (20-21).

Recently, in Tehran, Merat et al. (21), in a double-blind randomized controlled study, Probucol which is a lipid-lowering agent with strong antioxidant properties, was compared with placebo.

In this study liver tests normalized or were significantly improved after six months of treatment with Probucol.

In the study, it was reported that it is the antioxidant effects of probucol regardless of its lipid-lowering effect was the responsible mechanism for the normalization of ALT levels.

In another study, treatment of NASH patients with vitamin E resulted in significant improvement in hepatic steatosis and ALP, ALT, and GGT levels (22).

In another study, with higher dosages of vitamin E (1000 IU/day) showed that the therapy improved fibrosis, but its effect was not different from placebo in terms of ALT levels (23).

Open-label study was performed to compare the therapeutic benefits of vitamin E plus vitamin C combination treatment compared with UDCA in patients with NAFLD (24)

In the study they noted that vitamin E plus vitamin C combination treatment is as effective as UDCA in normalization of ALT in NAFLD patients.

In another study, with higher dosages of vitamin E (1000 IU/day) showed that the therapy improved the fibrosis, but its effect was not different from placebo in terms of ALT levels (25).

However, in the study by Ugelmas et al (26), vitamin E was detected to have no effect on cytokine profile and levels of hyaluronic acid, one of the fibrosis markers.

Recently, a placebo- controlled double-blind study in Iran reported the results of treatment with vitamin E in 63 cases and compared with placebo led to a significant improvement in liver tests and histologic changes (26).

Although promising results have been obtained with silymarin in patients with alcoholic liver disease, the results of clinical trials have produced conflicting results.

There are no studies with silymarin in patients with NAFLD but according to the data of one study they found that silymarin treatment in patients affected by cirrhosis and diabetes was associated with a reduction of insulin resistance and a significant decrease in fasting insulin levels, suggesting an improvement of the activity of endogenous and exogenous insulin, this substance could be interesting for the treatment of NAFLD(27).

In a case study, silymarin was well tolerated in HCV-infected persons, but did not significantly affect serum HCV RNA, alanine transaminase levels, quality of life or psychological well-being in subjects with this condition (28).

Finally, in a recent study on 85 consecutive cases of NAFLD treated with 4 pieces/day of the complex silymarin - vitamin E-phospholipids for six months followed by another six months of follow up, they found an improvement in treated individuals including insulinaemia,liver enzyme levels, degree of steatosis (p,0.01)(29).

In two groups of NAFLD patients, we investigated the effect of vitamin E and silymarin on biochemical tests. We found normalization of ALT in 45% of cases in vitamin E group and 41% in silymarin group, the difference between these two groups was not significant (P=0.79).

an the other hand, AST normalization was 56.30% and 74.6% in the vitamin E group and silymarin group, respectively (P=0.01).

The decrease in AST level in the silymarin group as compared to the vitamin E group was significant (P<0.007).

Another finding of the study is that treatment with *S. marianum and* vitamin E in subjects with NAFLD is safe and well-tolerated. Patient compliance with medication was good in both groups and all cases completed the study. We could not reach a conclusion about the histologic change because we did not biopsy the patients after the treatment period. But transaminase levels are generally accepted as the reflection of liver injury, and decrease in transaminase levels generally accompanies histologic healing.

In conclusion, in terms of biochemical improvement, vitamin E and silymarin treatment is effective in the treatment of NAFLD, particularly when other drugs have failed or as a complementary treatment associated with other therapeutic modalities.
Treatment with vitamin E and silymarin costs less than any other treatment and there are negligible side effects. In the future, our data must be confirmed in larger scale studies with pre- and post-treatment biopsies.

Abbreviations
ALP, alkaline phosphatase; ALT, alanine transaminase;
AST, aspartate aminotransferase; CAM, Complementary
and Alternative Medicine; GGT, c-glutamyltransferase;
NAFLD,Nonalcoholic fatty liver disease

Acknowledgements
We thank the medical and nursing staff of Imam Hospital for their help in the conduct of this study. The authors sincerely thank the participants of the study. The authors also thank Prof. Manucheher Makvandi who performed the statistical analyses of data for this project and AJUMS medical students for their excellent assistance.

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