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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 ± 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 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 = gammaglutamine 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-

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ciated with non-alcoholic steatohepatitis, which is characterized by inflammation and eventually fibrosis[1]. 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 prognosis[2]. 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, too[3].

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 modalities[4]. No proven treatment for patients with NAFLD is currently available[5]. Various treatment strategies such as thiazolidinediones, metformin, lipid-lowering agents and antioxidants have been studied[4]. 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 hematoxilin-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 ≥ 5[11].

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 = e0.953 × ln(triglycerides) + 0.139 × BMI + 0.718 × ln(GGT) + 0.053xwaist circunference – 15.745/1 + e0.953 × ln(triglycerides) + 0.139 × BMI + 0.718 × ln(GGT) + 0.053 × waist circunference – 15.745 × 100

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 × age (years) + 0.094 × BMI (kg/m^2) +1.13 × diabetes yes 1, no 0) + 0.99 × ratio AST/ALT -0.013 × platelets (× 10^9/L) – 0.66 × 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-chol), high density lipoprotein cholesterol (HDL-chol) were measured at baseline time and after 3 months of treatment. Anthropometric parameters as weight, body mass index (BMI) computed as 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 values[15].

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-chol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL-chol 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 handeled 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 ± 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)	*p*
Steatosis (leve-moderada/severa)	6/12	5/13	NS
NAS score (< 5/≥ 5)	11/7	10/8	NS
Fibrosis (F0/F ≥ 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 en 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 diet[18]. 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 activity[19].

Marchesini et al[20] have treated these patients with metformin: this drug reduced transaminase concentrations, insulin resistance and liver volume.

In another study[21], acarbose attenuated NAFLD progression in an experimental model of NAFLD in rats. Two drugs used in obese patients, orlistat and sibutramine[22-24] 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 report[25] 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 disease[29]. Mild to moderate elevation of serum aminotransferase

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

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

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

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

	Group I (n = 18)		Goup II (n = 18)	
	Basal	After	Basal	After
Glucose mg/dl	93.1 ± 13	93.8 ± 12	129.9 ± 46	114.6 ± 47*
TG mg/dL	189.6 ± 84	185 ± 85	180.8 ± 68	170.9 ± 63
AST (IU/L)	35.6 ± 16	34.6 ± 16	41.6 ± 20	36 ± 11.8
ALT (IU/L)	56.4 ± 27	52.7 ± 26	70.8 ± 41	54.7 ± 18*
ALT/AST	1.5 ± 0.4	1.5 ± 0.3	1.7 ± 0.5	1.5 ± 0.4*
GGT (IU/L)	81.5 ± 68	46.2 ± 27*	80.5 ± 46	50.3 ± 27*
HOMA-IR	3.4 ± 2.2	3.4 ± 2.2	5.4 ± 4.1	4.9 ± 4*
FLI	86.2 ± 19	76.9 ± 20*	85.2 ± 18	77.5 ± 23*
LAP	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.5	4.2 ± 0.6
NAFLD-FS	-1.6 ± 1.8	-2.1 ± 1.5*	-1 ± 1.9	-1.5 ± 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 al[30]. 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 NAFLD[31]. On the contrary, the AST/ALT ratio trends to increase with the development of cirrhosis, thus losing its diagnostic accuracy[32]. 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 studies[33-34] 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 al[35] 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 al[36] documented lower aminotransferases levels after two types of hypocaloric diets. The AGA guidelines[17] recommend a lost of 3 to 5% of body weight to reduce steatosis (Recommendation 1A). Hajmadi A et al[37] demonstrated that metformin, pioglitazona 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 authors[11-13] 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 scan foresee hepatic steatosis relatively well and can be used in clinical practice even before imaging studies or liver biopsy[38].

Conclusions

We have demonstrated that treatment whit 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 lost 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 ca 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 program's. 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.

References

1) Petta S, Muratore C, Craxi A. Non-alcoholic fatty liver disease pathogenesis: The present and the future. Dig Liver Dis 2009; 41: 615-625.
2) Lewis JR, Mohanty SR. Non-alcoholic fatty liver disease: a review and update. Dig Dis Sci 2010; 55: 560-578.
3) De luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Conde R, Bellido D. Influence of insulin resistance and adipocytokines on elevated serum alanine aminotransferase in obese patients. Arch Med Res 2008; 39: 110-114.
4) Socha P, Horvath A, Vajro P, Dziechciarz P, Dhawan A, Szajewska H. Pharmacological interventions for nonalcoholic fatty liver disease in adults and in children: a systematic review. J Pediatr Gastroenterol Nutr 2009; 48: 587-596.
5) Langmead L, Rampton DS. Review article: herbal treatment in gastrointestinal and liver disease. Aliment Pharmacol Ther 2001; 15: 1239-1252.
6) Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverts fatty liver disease in obese, leptin deficient mice. Nat Med 2000; 6: 998-1003.
7) Cadranel JF, Jouannaud V, Loison S. Improving insulin resisteance: certain progress in the management of patients with non-alcoholic steatohepatitis…but the story continues. Gastroenterology 2004; 28: 265-267.
8) Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, Rakela J, McGill DB. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study. Hepatology 1996; 23: 1464-1467.
9) Shaker E, Mahmoud H, Mnaa S. Silymarin. The antioxidant component and Silybum marianum extracts prevent liver damage. Food Chem Toxicol 2010; 48: 803-806.
10) Kazemifar AM, Hajiaghamohammadi AA, Samimi R. Hepatoprotective property of oral silymarin is comparable to n-acetyl cysteine in acetaminophen poisoning. Gastroenterol Res 2012; 5: 190-194.
11) Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005; 41: 1313-1321.
12) Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006; 6: 33.
13) Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC Gastroenterol 2010; 10: 98.
14) Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007; 45: 846-854.
15) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.
16) Mataix J, Mañas M. Tablas de composición de alimentos españoles. Ed: University of Granada, 2003.
17) Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K. 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.
18) de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Conde R. Effect of two different hypocaloric diets in transaminases and insulin resistance in nonalcoholic fatty liver disease and obese patients. Nutr Hosp 2010; 25: 730-735.
19) Zhao J, Agarwal R. Tissue distribution of silibinin, the major active constituent of silymarin, in mice and its association with enhancement of phase II enzymes: implications in cancer chemoprevention. Carcinogenesis 1999; 20: 2101-2108.
20) Marchesini G, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. Lancet 2001; 15: 893-894.
21) Lieber CS, Leo MA, Mak KM, Xu Y, Cao Q. Acarbose attenuates experimental non-alcoholic steatohepatitis. Biochem Biophys Res Commun 2004; 12: 699-703.
22) Harrison SA, Fincke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. Aliment Pharmacol Ther 2004; 20: 623-628.
23) Zelbersagi S, Kessler A, Brazowsky E, Webb M, Lurie Y, Santo M, Leshno M, Blendis I, Halpern Z, Oren R. A double blind randomized placebo controlled trial of orlistat for the treatment of nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2006; 4: 639-644.
24) Sabuncu T, Nazligul Y, Karaoglanoglu M, Ucar E, Kilic FB. The effects of sibutramine and orlistat on the ultrasonographic findings, insulin resistance and liver enzyme levels in obese patients with non-alcoholic steatohepatitis. Rom J Gastroenterol 2003; 12: 189-192.

25) Aller R, de Luis DA, Izaola O, Conde R, González Sagrado M, Primo D, de la Fuente B, González JM. Effect of a probiotic on liver aminotransferases in Non-alcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci 2011; 15: 1090-1095.

26) de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Conde R, Bellido D. Influence of insulin resistance and adipocytokines on elevated serum alanine aminotransferase in obese patients. Arch Med Res 2008; 39: 110-114.

27) Sanyal AJ. AGA technical review on nonalcoholic fatty liver disease. Gastroenterology 2002; 123:1705-1725.

28) Brea A, Mosquera D, Martín E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. Arterioscler Thromb Vasc Biol 2005; 25:1045-1050.

29) Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 1999; 107: 450-455.

30) Cacciapuoti F, Scognamiglio A, Palumbo R, Forte R, Cacciapuoti F. Silymarin in non alcoholic fatty liver disease World J Hepatol 2013; 27: 109-113.

31) Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980; 55: 434-438.

32) Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999; 30: 1356-1362.

33) Jacobs BP, Dennehy C, Ramirez G, Sapp J, Lawrence VA. Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. Am J Med 2002; 113: 506-515.

34) Kugelmas M, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003; 38: 413-419.

35) Stranges S, Trevisan M, Dorn JM, Dmochowski J, Donahue RP. Body fat distribution, liver enzymes, and risk of hypertension: evidence from the Western New York Study. Hypertension 2005; 46: 1186-1193.

36) de Luis DA, Aller R, Izaola O, González M, Conde R. Effect of two different hypocaloric diets in transaminases and insulin resistance in Non-alcoholic fatty liver disease and obese patients. Nutr Hosp 2010; 25: 730-735.

37) Hajiaghamohammadi A, Ziaee A, Oveisis S, Masroor H. Effects of metformin, pioglitazone, and silymarin treatment on non-alcoholic Fatty liver disease: a randomized controlled pilot study. Hepat Mon 2012; 12: e6099.

38) Cumberledge J, Angulo P. Noninvasive prediction of hepatic steatosis. Am J Gastroenterolgy 2014; 109: 1415-146.