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Effetto di un nuovo complesso farmacologico (sillibina vitamina E+fosfolipidi) su alcuni indicatori di sindrome metabolica e di fibrosi epatica in pazienti con epatopatia steatosica

Studio pilota

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Aims. This open preliminary pilot study was aimed to evaluate the effect of a new pharmaceutical complex (sylibin+vitamin E+phospholipids - RealSIL-IBI-Lorenzini Pharmaceutical, Italy) on some parameters of metabolic syndrome and of liver fibrosis in patients with non alcoholic fatty liver disease (NAFLD) with or without the contemporaneous presence of hepatitis C (HCV)-related chronic hepatitis.
Methods. **Eighty five patients were consecutively enrolled in the study and divided in 2 groups; the first group was represented by 59 patients affected by NAFLD, negative for other known causes of chronic liver damage (M/F= 39/20; median age and range: 44 years, 22-76, group A). The second group was represented by 26 patients (M/F=19/7; median age and range 51 years, 20-75, group B) with HCV-related chronic hepatitis associated to NAFLD. Adverse events and drop-outs were absent in all group and compliance at the study was absolute.**
Results. **This open preliminary study shows that the new compound sylibin+vitamin E+ phospholipids is active, in vivo, and produces some therapeutic effects in patients with different forms of chronic liver damage. In particular, it improves insulin resistance and plasma levels of markers of liver fibrosis in patients in whom these parameters are particularly altered.**
Conclusions. **Our data have a role of suggestion to further evaluate, through a controlled trial, a possible therapeutic use of this new compound in the management of patients with NAFLD.**

Key words: **Sylibin - Non alcoholic fatty liver diseases - Markers - Liver fibrosis.**

Flavonoids are a class of natural components with antioxidant and antifibrotic actions.[1] From these, sylimarin and sylibin (the main component of sylimarin), are able to reduce plasma levels of aminotransferase and gamma-glutamyl-transpeptidase in experimental animals and men.[2, 3] The conjugation of sylibin with vitamin E and phopsholipids, increases both the absorption and the bioavailability of sylibin, that results in an enhanced its pharmacogical action.[4] Recently, it has been documented that this new compounds (RA) is able to reduce body weight and liver damage in rats.[5]

Non alcoholic fatty liver diseases (NAFLD) is a pathological condition extremely com-

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mon, and of emerging and increasing relevance.[6, 7] This type of chronic liver damage may occur as a "primarily" condition generally associated to obesity and metabolic syndrome (insulin resistance or diabetes mellitus, dyslipidemia, arterial hypertension, visceral obesity), but it also may complicate the liver damage due to other causes, such as that due to chronic hepatitis C (HCV) infection. In this last group of patients the contemporaneous presence of NAFLD negatively affects the progression to fibrosis and the response to antiviral treatment.[8-10] The histological pictures of NAFLD are various and they range from steatosis until cirrhosis and hepatocellular carcinoma.[11] The diagnosis of the various types of NAFLD is based on liver histology, but a recent literature discussion is open about the validation of ultrasonographic scores of bright liver to quantify liver steatosis and of plasma markers to evaluate liver fibrosis.[12, 13]

This open preliminary pilot study was aimed to evaluate the effect of a new pharmaceutical complex (REALSIL-IBI-integrator of Vitamin E with Cardo Mariano. Silybin complexed with phospholipids, RA) on some parameters of metabolic syndrome and of liver fibrosis in patients with NAFLD with or without the contemporaneous presence of chronic hepatitis by HCV.

Materials and methods

Patients

The study protocol was in keeping with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by Ethics Committee of our Department; 85 patients were consecutively enrolled in the study and divided in 2 groups; the first group was represented by 59 patients affected by NAFLD, negative for other known causes of chronic liver damage (M/F=39/20; median age and range: 44 years, 22-76, group A). The second group was represented by 26 patients (M/F= 19/7; median age and range 51 years, 20-75) with HCV-related chronic hepatitis associated to NAFLD, all non responders to previous treatments with interferon and ribavirin, stopped from almost one year (group B). The diagnosis of NAFLD in both groups was performed on the basis of the presence of bright liver at ultrasonographic examination, graded from 0 to 3 according to Joseph *et al*.[14] None of these patients were drug users, and none was previously exposed to environmental toxins, such as pesticides or other xenobiotics. All enrolled patients reported a constant daily alcohol intake of <20 g throughout their life, considered not exclusion criterion according to literature data.[15, 16] Other associated diseases, including autoimmune, genetic or other possible causes of chronic liver damage were excluded on the basis of the results of a global screening in all patients, prior to enrolment into the study.

Methods

All patients were asked to follow a balanced diet performed on the basis of body weight for each patient during all the study (12 months). The two groups were subdivided in patients treated (N. 53) and no treated (N. 32). The treatment consisted in an association of sylibin+vitamin E+phospholipids (RealSIL - IBI - Lorenzini Pharmaceutical, Italy, RA) at high dose (47 mg×4/die) for 6 months, followed by other 6 months of follow-up without treatment.

Parameters evaluated

At 0, 6 and 12 months we evaluated the following parameters:

1) compliance to treatment (diet and/or drug), adverse events, body mass index (BMI) (weight kg/height^2 in metres).

2) Liver ultrasonograpy (US) and plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transpeptidase (gGT), blood glucose and insulin by commercial kits. We also calculated insulin-resistance by HOMA test model.[17-19]

3) Plasma levels of markers of liver fibrosis: transforming growth factor β (TGF-β), hyaluronic acid (HA), metalloproteinase 2 (MMP-2) by ELISA test (Elisa human quantikine RεD systems).

TABLE I.—*BMI (kg/m²) values (M±SD) and percentage (%) of patients with altered BMI in A and in B group in basal condition, at 6 and 12 months.*

	Treated			No treated		
	Basal	6 months	12 months	Basal	6 months	12 months
Group A						
BMI (M±SD)	26.9±2.4	25.5±2.2	27±1.2	26.7±2.3	26.9±2	27±1.1
%↑	83.3	75	78	85	83	80
Group B						
BMI (M±SD)	26.9±2.1	25.5±1.5	27.9±2	26.5±2	27±1.8	27.2±1.6
%↑	66.6	44*	49*	49	60	80

*) $p<0.01$ *vs* basal values.

TABLE II.—*Plasma values (IU/L) of ALT and γGT in A and B group in basal condition, at 6 and 12 months (M±SD).*

	Treated			No treated		
	Basal	6 months	12 months	Basal	6 months	12 months
Group A						
ALT (nv <40 IU/L)	79±51	40±15*	59±5*	54.2±20	55.2±24	45.2±34
γGT (nv <50 IU/L)	75±112	59±100*	60±33*	64±20	64±24	64±35
Group B						
ALT (nv <40 IU/L)	69±28	45±18*	62±16	47.8±30	50.3±32	60.3±33
γGT (nv <50 IU/L)	118±70	56±20*	83±24	115±20	64±24	64±35

*) $p<0.01$ *vs* basal values.

Statistical analysis

Results were expressed as median and range, and as number (percentage) of patients with a condition. SPSS program 11.0 was used for the statistical analyses. The analysis of variance (ANOVA) and Wilcoxon test were used to evaluate the differences between data and groups. Differences in frequency were calculated by χ^2. Correlations between results were evaluated by Pearson bivariate correlation test. A value of $p \leq 0.05$ was considered significant.[20]

Results

Adverse events and drop-outs were absent in all group and compliance at the study was absolute.

BMI was increased (>25 kg/m²) in approximately 70% of cases; with a clear prevalence of obese individuals more in females than males, even if no significant difference was revealed in relationship to sex. In terms of mean, the treatment with RA not induced any significant variation of BMI in all groups; however, it significantly reduced BMI values in a number of patients from HCV positive group greater than in others, with a persistence of the reached effect (Table I).

NAFLD group (group A) showed a significant and persistent reduction of ultrasonographic score of liver steatosis that ranged from 2-3 in basal conditions to 1-2 at 6 and 12 months ($P<0.01$). This effect was absent in the other groups.

Plasma levels of liver enzymes ameliorated in treated and not in control groups, with a persistence of the effects only in NAFLD patients (Table II).

Hyperinsulinemia was present, in basal conditions, in 65% of NAFLD and 46% of HCV positive patients globally considered. This percentage was significantly reduced in NAFLD treated (from 65% to 47% at 6 months

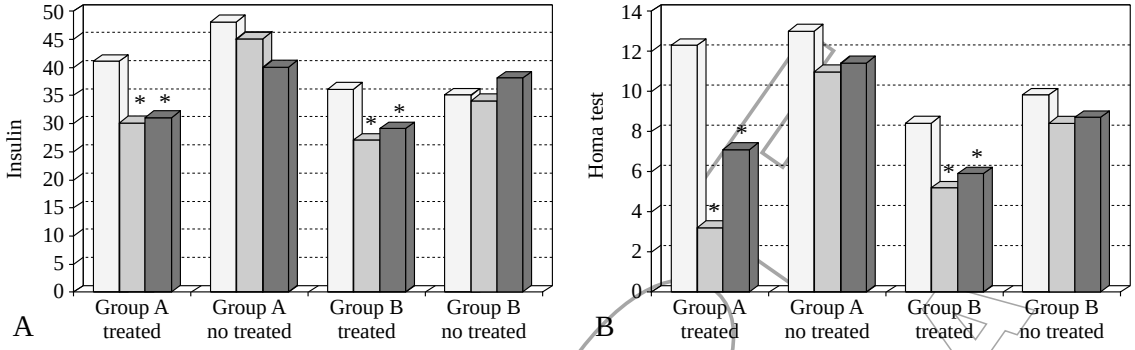

Figure 1.—Plasma values (μU/ml) of insulin and HOMA values in A and B group in basal condition, at 6 and 12 months, before and after treatment (M±SD) (*p<0.01).

TABLE III.—*Percent of patients that ameliorate ultrasonographic steatosis, ALT, γGT, and insulin plasma levels in relationship to BMI.*

	Treated		No treated	
	BMI improved	BMI not improved	BMI improved	BMI not improved
US steatosis	71	14	20	15
ALT	86	43	22	20
γGT	85	100	75	23
Insulinemia	71	67	17	24

and 51% at 12 months, p<0.05). In terms of mean values, insulinemia was significantly and persistently improved by treatment both in NAFLD and HCV groups. Similar trends was registered for HOMA values. These data are summarized in Figure 1 (part A and B).

To further evidenciate a possible effect of the treatment with RA, we evaluate the relationship occurring among BMI, ultrasonographic score of liver steatosis, hyperinsulinemia and plasma levels of ALT and γGT in treated and no treated groups at the various times of observations. As reported in Table III, by dividing patients in those with BMI improved and not, data indicate that the treatment affected the parameters considered, independently, almost in part, by the modifications of BMI.

Plasma indexes of liver fibrosis have a different rate between NAFLD and HCV groups. In fact, a more marked increase of these parameters was registered in HCV positive patients in respect to those of NAFLD; the treatment with RA significantly reduced all these parameters, but the significant persistence of the effect also after the follow-up period was registered only in HCV group. Table IV summarizes the plasma values of markers of fibrosis in all groups.

BMI, insulinemia, plasma levels of TGFβ and degree of steatosis (P<0.01) resulted significantly related to γGT. A significant correlation was also documented among indices of fibrosis (P<0.001).

Discussion and conclusions

This open preliminary study shows that the new compound sylibin+vitamin E+phospholipids is active, *in vivo*, and produces some therapeutic effects in patients with different forms of chronic liver damage. In particular, it improves insulin resistance and plasma levels of markers of liver fibrosis in patients in whom these parameters are particularly altered. Finally, as documented in animals,[5] it affects BMI values also in men. These effects, associated to a good compliance and to an absence of adverse events, suggest that RA should be evaluated in controlled trials.

TABLE I.—*BMI (kg/m²) values (M±SD) and percentage (%) of patients with altered BMI in A and in B group in basal condition, at 6 and 12 months.*

	Treated			No treated		
	Basal	6 months	12 months	Basal	6 months	12 months
Group A						
HA	383±627	176±184*	331.1±293.2	422±504	517±283	498±145
MMP-2	151±132	141±84*	158.2±165.5	160±183	173±102	183±97
TGF-β1	45.3±17.7	32.9±24.3*	42.9±22	41.2±22	43.3±27.3	44.7±31
Group B						
HA	1295±259	625±122*	586.7±496.2*	1180±703	1372±806	1332±452
MMP-2	292±201	137±53*	196.6±94.2	280±300	311±278	299±123
TGF-β1	54.1±21.7	27±12.2*	21.2±17.4	53.3±18.3	45.2±28.5	49.6±30

*) $p<0.05$ *vs* basal values.

Several papers in the last years have been focused their attention on NAFLD and on its treatment,[21-23] because this condition is frequently associated to overweight, that, as known, is of increasing relevance in all industrialized countries.[24] The presence of non alcoholic steatohepatitis (NASH) in NAFLD is estimated about 20% and cirrhosis in 15-30% of individuals with NASH within a 10-year period of follow-up.[21] NAFLD and NASH may also complicate the liver damage due to other causes, such as that due to chronic HCV infection. In these patients, the contemporaneous presence of NAFLD negatively affects the progression of fibrosis and the response to antiviral treatment.[8-10] The pathogenic steps involved in the induction and progression of this chronic liver damage are very complex and they include as substrate the metabolic syndrome or associated metabolic alterations (particularly over weight, insulin resistance, diabetes, hyperlipemia), as mediators a large series of peptides, hormones, cytokines (particular TNF-α, adiponectin, leptin, etc.) and, finally, as cellular messengers, reactive oxygen and nitrosative species that are capable of induce lipid peroxidation of cellular membranes.[25, 26] An ideal treatment of NAFLD should improve the liver damage and or its progression, both directly or trough the modulation of its pathophysiological events. Therefore drugs should ameliorate body weight, insulin resistance and other metabolic associated alterations, reduce the linkage between adipose tissue and liver by acting as antinflammatory and/or immonomodulatory agents, modulate the progression of liver steatosis to inflammation and fibrosis by blocking the oxidative and nitrosative stress.

In patients with NAFLD, weight loss was accompanied by a significant improvement of all parameters of metabolic syndrome as well as of liver function tests.[27] However, in an our previous experience,[28] the improvement of liver tests with weight loss was obtained in about 40% of overweight patients with NAFLD. Two main problems are related to weight management. The first is that the adherence to an hypocaloric diet is generally discontinuous; secondly a very rapid weight loss may cause a worsening of steatohepatitis.[29] Data of this study show that, despite patients were continuously controlled on the basis of the design of the study, the adherence to diet was obtained in about 50% of cases, as documented by the modifications of BMI values. Similar data were also registered by using lowering weight agents, such as orlistat.[30] Drugs used for insulin resistence, such as thiazolidinediones and metformin, even if had various positive effects on insulin resistence and liver histology, globally failed to respond to the initial promising their efficacy in patients with NAFLD.[31, 32]

Similar not conclusive effects were also documented for some cytoprotective agents such as ursodeoxicholic acid, vitamin E alone, N-acetyl-L-cysteine or dietary supplementation with lecithin and other antioxi-

dants.[33, 34] At the moment many authors focused their attention on the possibility to reverse liver fibrosis or by reducing the activation of hepatic stellate cells or by promoting their apoptosis or degradation of formed collagen.[35, 36] The main activators of hepatic stellate cells are the reactive oxygen species and the lipid peroxidation products. Therefore also antioxidants [37] may be considered antifibrotic agents. Silybin is the main component of the flavonoid silymarin. It acts as a radical scavenger, stimulates hepatocite RNA synthesis and suppresses the proliferation of hepatic stellate cells and the collagen deposition *in vitro*.[38] In rats whit induced fibrosis silybin reduces collagen accumulation, as well as lipid peroxidation.[5]

We do not explain, on the basis of our results, the key through RA improves BMI. It is possible that this effect may be mediated by the action on insulin resistance. Infact it is suggested that insulin resistance following injury may be a consequence of receptor defect of the target cells and silybin may be used with benefit.[39] The effects registered on plasma markers of liver fibrosis should be related to a direct effect of sylibin on the activation of hepatic stellate cells [40] or it may be mediated by its antioxidant action, because, as know, free radicals are the main mediators of fibrogenesis.[41] Finally, RA also improves some markers of liver damage, such as liver enzymes, even if differently in NAFLD and HCV groups, and this effect in only in part due to the variations of metabolic patterns.

In conclusion, our data have a role of suggestion to further evaluate, through a controlled trial, a possible therapeutic use of this new compound in the management of patients with NAFLD.

Riassunto

Effetto di un nuovo complesso farmacologico (sibillina+vitamina E+fosfolipidi) su alcuni indicatori di sindrome metabolica e di fibrosi epatica in pazienti con epatopatia steatosica: studio pilota

Obiettivi. Questo studio pilota è stato effettuato per valutare un nuovo complesso farmacologico (silibina+vitamina E+fosfolipidi - RealSIL-IBI-Lorenzini Pharmaceutical, Italia) su alcuni parametri di sindrome metabolica e di fibrosi epatica in pazienti con steatosi epatica non alcolica (NAFLD) con o senza la contemporanea presenza di epatite cronica epatite C (HCV)-correlata.

Metodi. Ottantacinque pazienti sono stati consecutivamente arruolati e divisi in 2 gruppi. Nel primo gruppo sono stati arruolati 59 pazienti affetti da NAFLD, non affetti da altre cause conosciute di danno epatico cronico (rapport M/F=39/20; mediana ed estremi: 44 anni, 22-76, gruppo A). Nel secondo gruppo sono stati arruolati 26 pazienti (rapporto M/F= 19/7; mediana ed estremi 51 anni, 20-75, gruppo B) con epatite cronica HCV-correlata associata a NAFLD. Non si sono avuti effetti collaterali in entrambi i gruppi e l'aderenza allo studio è stata assoluta.

Risultati. Questo studio preliminare mostra che il nuovo composto silibina+vitamina E+fosfolipidi è attivo, *in vivo*, e produce alcuni effetti terapeutici nei pazienti con diverse forme di danno epatico cronico. In particolare, esso migliora il grado di insulino resistenza ed i livelli plasmatici dei marcatori di fibrosi epatica nei pazienti nei quali questi parametri sono alterati.

Conclusioni: I nostri dati suggeriscono ulteriori valutazioni, attraverso un trial controllato, sul possibile uso terapeutico di questo nuovo composto nella gestione del paziente con NAFLD.

Parole chiave: Silibina - Steatosi epatica non alcolica - Fibrosi epatica.

References

1. Kravchenko LV, Morozov SV, Tutel'yan VA. Effects of flavonoids on the resistance of microsomes to lipid peroxidation *in vitro* and ex vivo. J Hepatol 2003;39: 333-40.
2. Valenzuela A, Lagos C, Schmidt K, Videla L. Silymarin protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat. Biochem Pharmacol 1985;34:2209-12.
3. Wellington K, Jarvis B. Silymarin: a review of its clinical properties in the management of hepatic disorders. Bio Drugs 2001;15:465-89.
4. Morazzoni P, Magistretti MJ, Giachetti C, Zanolo G. Comparative bioavailability of Silipide, a new flavanolignan complex, in rats. Eur J Drug Metab Pharmacokinet 1992;17:39-44.
5. Di Sario A, Bendie E, Taffetani S. Realsil, a new sylibinphospatidylcoline complex, reduces dimethylnitrosamine-induced hepatic fibrosis in the rat. J Hepatol 2003;38: Suppl:76.
6. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37:1202-19.
7. Loguercio C, De Simone T, D'Auria MV, de Sio I, Federico A, Tuccillo C *et al.* Italian AISF Clinical Group. Non-alcoholic fatty liver disease: a multicentre clinical study by the Italian Association for the Study of the Liver. Dig Liver Dis 2004;36:398-405.
8. Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G, Day CP *et al.* Steatosis and hepatitis C virus: mechanism and significant for hepatic and extrahepatic disease. Gastroenterology 2004;126:586-97.

9. Ramesh S, Sanyal AJ. Hepatitis C and nonalcoholic fatty liver disease. Semin Liver Dis 2004;24:399-413.
10. Younossi ZM, McCullough AJ, Ong JP, Barnes DS, Post A, Tavill A *et al*. Obesity and non-alcoholic fatty liver disease in chronic hepatitis C. J Clin Gastroenterol 2004;38:705-9.
11. Angulo P. Nonalcohollc fatty liver disease. N Engl J Med 2002;346:1221-31.
12. Joy D, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? Eur J Gastroenterol Hepatol 2003;15:539-43.
13. Rossi E, Adams L, Prins A, Bulsara M, de Boer B, Garas G *et al*. Validation of the fibro test biochemical markers score in assessing liver fibrosis in hepatitis C patients. Clin Chem 2003;49:450-4.
14. Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchimal liver disease. Clin Radiol 1991;9:91-4.
15. Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001;121:710-23.
16. Fletcher LM, Kwoh-Gain I, Powell LW, Halliday JW. Markers of chronic alcohol ingestion in patients with nonalcoholic steatohepatitis: an aid to diagnosis. Hepatology 1991;13:455-9.
17. Melchionda N, Forlani G, Marchesini G, Baraldi L, Natale S. WHO and ADA criteria for the diagnosis of diabetes mellitus in relation to body mass index. Insulin sensitivity and secretion in resulting subcategories of glucose tolerance. Int J Obes Relat Metab Disord 2002;26:90-6.
18. American Diabetes Association. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. Diab Care 1998;21:310-4.
19. Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity. Am J Gastroenterol 2001;96:2957-61.
20. Armitage P, Berry G. Statistical methods in medical research. Rd ed. Oxford: Blackwell Science Ltd; 1994.
21. Ramesh S, Sanyal AJ. Evaluation and management of non-alcoholic steatohepatitis. J Hepatol 2005;42:S2-S12.
22. Clark JM, Brancatf FL, Diehl AM. Nonalcoholic fatty liver disease. Gastrcenterology 2002;122:149-57.
23. Cua IH, George J. Non-alcoholic fatty liver disease. Hosp Med 2005;66:106-11.
24. Hu KQ, Kyulo NL, Esrailian E, Thompson K, Chase R, Hillebrand DJ *et al*. Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States. J Hepatol 2004;40:147-54.
25. Loguercio C, Federico A. Oxidative stress in viral and alcoholic hepatitis. Free Rad Biol Med 2003;34:1-10.
26. Mehta K, Van Thiel DH, Shah N, Mobarhan S. Nonalcoholic fatty liver disease: pathogenesis and the role of antioxidants. Nutr Rev 2002;60:289-93.
27. Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic Fatty Liver Disease: Improvement in Liver Histological Analysis With Weight Loss. Hepatology 2004;39:1647-54.
28. Loguercio C, De Girolamo V, de Sio I, Tuccillo C, Ascione A, Baldi F *et al*. Non alcoholic fatty liver disease in an area of southern Italy: main clinical, histological and pathophysiological aspects. J Hepatol 2001;35:568-74.
29. Luyckx FH, Desaive C, Thiry A, Dewe W, Scheen AJ, Gielen JE *et al*. Liver abnormalities in several obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Relat Metab Disord 1998;22:222-6.
30. Harrison SA, Finke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. Aliment Pharmacol Ther 2004:20:623-8.
31. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin revers fatty liver disease in obese, leptin-deficient mice. Nat Med 2000;6:998-1003.
32. Cadranel JF, Jouannaud V, Loison S. Improving insulin resistance: certain progress in the management of patients with non-alcoholic steatohepatitis...but the story continues. Gastroenterol Clin Biol 2004;28:265-7.
33. Laurin J, Lindor KD, Crippin JS, Gossard A, GoresGJ, Ludwing J. Ursodeoxycolic acid or clofibrate in the treatment of non alcohol induced steatohepatitis; a pilot study. Hepatology 1996;23:1464-7.
34. Lazaridis KN, Gores GJ, Lindor KD. Ursodeoxycolic acid "mechanism of action and clinical use in hepatobilary disorders". J Hepatol 2001;35:134-46.
35. Bataller R, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. Semin Liver Dis 2001;21:437-51.
36. Di Sario A, Bendia E, Macarri G, Candelaresi C, Taffetani S, Marzioni M *et al*. The anti-fibrotic effect of pirfenidone in rat liver fibrosis is mediated by downregulation of procollagen α1(I), TIMP-1 and MMP-2. Dig Liver Dis 2004;36:744-51.
37. Parola M, Muraca R, Dianzani I. Vitamin E dietary supplementation inhibits TGF-β1 gene expression in the rat liver. FEBS Lett 1992:308:267-70.
38. Bindoli A, Cavallini L, Siliprandi N. lnhibitory action of sily-marin of lipid peroxide formation in rat liver mitochondria and microsomes. Biochem Pharmacol 1977; 26:2405-9.
39. Tang ZJ. Alterations in insulin receptor in injured rats and the effects of dexamethasone and silybin. Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi 1991;7:214-7.
40. Pietrangelo A, Montosi G, Garuti C, Contri M, Giovannini F, Ceccarelli D *et al*. Iron-induced oxidant stress in nonparenchymal liver cells: mitochondrial derangement and fibrosis in acutely iron-dosed gerbils and its prevention by silybin. J Bioenerg Biomembr 2002;34:67-79.
41. Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 2004;34:9-19.