Does *Silybum Marianum* Play a Role in the Treatment of Chronic Hepatitis C?

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Objectives. **The recent boom in patient education on chronic hepatitis C has resulted in a worldwide increase in the diagnosis of this condition. Available treatment is expensive and associated with significant side effects; therefore, many patients seek for alternative medicine. *Silybum marianum* is a natural herb known to mankind for over 2,000 years that has been used as a liver-protecting agent due to its antioxidant properties. The objective of this study is to evaluate the safety profile and the effects of this herb, using a commercially available extract; in the liver chemistry and viral load of hepatitis C in chronically infected patients.**

Methods. **Patients aged 21-65 years old with a diagnosis of chronic hepatitis C who were not using antiviral therapy were asked to participate. Patients were randomized to treatment with S. *marianum* 160 mg orally three times a week for four weeks or to no-treatment (control). Blood tests for viral load and liver enzymes (ALT and AST) were done at randomization and at the end of treatment. Paired-t test was used to measure differences between baseline and week 4 values for ALT, AST and viral load. The percent change for ALT, AST and viral load of both groups was analyzed using the Mann Whitney statistical test.**

Results. **34 patients were enrolled. Men and women were equally distributed. Mean age was 50 years old. Mean baseline measurements of AST, ALT and viral load in the treatment group were 85 ± 12.41 IU/ml, 120 ± 20.57 IU/ml and 8.77± 4.12 copies x 10^6/ml while for the no-treatment group were 71 ± 9.46 IU/ml, 97 ± 15.35 IU/ml and 1.8 ± 0.62 copies x 10^6 /ml respectively. For treated subjects the mean values of AST, ALT and viral load demonstrated a decrease from baseline values, but this difference was not statistically significant. For control patients the values of ALT (p= .049), AST (p = .005) and viral load (p = .005) showed a statistically significant increase at week 4. Week 4 measurement changes from baseline values were calculated for each participant. The percent change for ALT (p = .014), AST (p = .002) and viral Ioad (p = .326) were compared between the treated and control group demonstrating a statistically significance difference for ALT and AST, but not for viral load. No side effects were reported using the herb extract.**

Conclusion. Sylibum marianum **is a well-tolerated plant extract associated with a decrease in liver chemistries but with no apparent effect on viral load when given for 4 weeks. These results suggest that S. *marianum* may have a protective effect in the inflammatory response to HCV, but no role as an antiviral agent. Further investigations may consider using this plant extract for a longer period of time or as adjuvant to the standard therapy of chronic hepatitis C.**

Key words: Complementary therapies, Silymarin, Hepatitis C

Hepatitis C, an RNA virus of the Flaviviridae family, is one of the leading causes of liver disease worldwide. At least 4 million people in the United States have been infected with this virus of which 2.7 are estimated to have chronic infection (1,2). There are 6 HCV genotypes and more than 50 subtypes. The extensive genetic heterogeneity of this virus explains the difficulties in the development of a vaccine as well as the low response to therapy (3). In Puerto Rico, as well as in the United States, infection with HCV genotype-1 prevails. A recent study from the Gastroenterology Association of Puerto Rico demonstrated that 82% of the chronically HCV infected patients visiting GI practices are genotype-1 (4). Since 1997, when the first Consensus Statement on the Management of chronic hepatitis C was published, therapeutic advances have occurred, specifically the introduction of pegylated interferons in combination with ribavirin. This combination has resulted in better results

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than monotherapy or the standard combination therapy of alfa- interferon three times a week and ribavirin (5).

Factors that have been consistently associated with successful therapy include: genotypes other than 1, less fibrosis or inflammation on liver histology, lower body mass index and lower viral loads. Sustained virological response for genotypes 2 and 3 have increased to around 80% while for genotype-1 are around 46% with new combination therapy (3).

Although the number of patients that fail to respond to therapy has decreased significantly from earlier treatment approaches, there is still a significant group of patients that are not cured. Studies are now geared to provide those patients with new treatment approaches or longer duration of treatment to achieve sustained virological response or to prevent the progression of liver disease. Failure to respond to optimal therapy presents a significant medical problem. Patients that have failed to all treatment approaches tend to seek non-conventional therapies.

Silybum marianum (milk thistle) is a natural herb, very commonly used by patients with liver maladies. The plant is native to Europe and grows wild in the United States and South America. It can also be found in the Caribbean and the Mediterranean sea. Mankind has known its medical benefits for over 2000 years. History of Medicine records indicates that in the first century Romans used the plant as a liver protecting medicine. In the 1960 German scientists first isolated the active ingredient and identified its antioxidant properties (6). The active ingredient of this ancient herb, sylimarin, is found in the ripe seeds of the plant. Sylimarin is composed of three isomer flavonoligans: silybin, silychristin, and silydianin (7). Sylibin makes up 50% of silymarin and is regarded as the most biologically active constituent. Most standardized milk thistle extracts contain 70% to 80% of silymarin (6). Numerous studies indicate that silymarin, has protective effects on the liver without associated toxicity. It is also easy to obtain and inexpensive. In vitro assays suggest that milk thistle acts as an antioxidant reducing free radical production and lipid peroxidation in the setting of hepatotoxicity (8). As an antifibrotic agent, it reduces markers for collagen accumulation in the liver measured by serum procollagen type III formation (9). As a toxin blockade agent, it may bind to the hepatocyte cell membrane receptor site, inhibiting binding of toxins to these sites (10). In animals, milk thistle reduces liver injury caused by acetaminophen, carbon tetrachloride, radiation, iron overload, phenylhydrazine, alcohol, cold ischemia, and *Amanita phalloides* (8,11). In human clinical trials, milk thistle has been used to treat alcoholic liver disease, acute and chronic viral hepatitis, and toxin-induced liver injuries. Despite inconsistencies in study outcomes, the use of milk thistle remains prevalent throughout Europe and in the United States (3).

The purpose of this study is to evaluate the safety profile and the effect of silymarin in the liver profile and viral load of patients with chronic HCV infection.

Methods

Patients with an age range of 21 to 65 years of age and an established diagnosis of chronic hepatitis C at the San

Table 1. Silymarin Treated Group Percent Difference from Baseline Values

Subject	Baseline AST	Week 4 AST	% change	Baseline ALT	Week 4 ALT	% change Viral load	Baseline Viral load	Week 4	% change
1	32	26	19	70	46	34	60.25	52.67	13
2	50	39	22	55	48	12	41.54	36.1	13
3	181	185	**-2**	235	305	**-29**	2.8	3.13	**-12**
4	49	37	24	57	52	9	0.54	2.11	**-290**
5	127	172	**-35**	293	301	**-3**	0.39	0.8	**-105**
6	72	78	**-7**	44	59	**-34**	0.5	0.58	**-16**
7	39	39	0	72	68	5	10	6.89	31
8	72	58	19	83	78	6	18.54	12.39	33
9	72	54	25	116	115	1	0.89	0.48	45
10	198	240	**-21**	295	311	**-5**	0.58	0.6	7
11	166	117	29	177	97	45	0.61	14.42	226
12	83	89	**-7**	179	154	14	0.51	0.86	**-68**
13	47	44	6	84	78	7	10.28	8.2	20
14	76	61	20	35	25	29	0.83	0.55	34
15	60	71	**-18**	106	122	**-15**	0.39	0.62	**-59**
16	47	45	4	48	48	0	0.05	0.08	**-58**
17	78	49	37	98	72	27	0.55	4.73	**-760**

Juan VA Medical Center from December 2001 to March 2002 were invited to participate. Eligible patients were those who had serological evidence of hepatitis C infection, compensated liver disease and had not received antiviral therapy for at least 6 months before entering the study. Patients with ascites, coagulopathy, encephalopathy and/or cancer were excluded. The Institutional Review Board of the San Juan VA Medical Center approved this research protocol. Those who agreed to participate signed an informed consent. The patients were randomized into two groups: the treatment group who received Silybum marianum 160 mg administered orally three times a day during 28 days and the no-treatment group (control group) who did not receive medication. A commercially available milk thistle preparation (GNC™) was used; medication bottles were from the same batch to prevent dose variability. Medication was dispensed by the hospital pharmacy. Bottles were collected at the end of the experimental period to assess for compliance. Patients were oriented to abstain from using alcohol and/or medications that may cause alteration in liver enzymes during their participation.

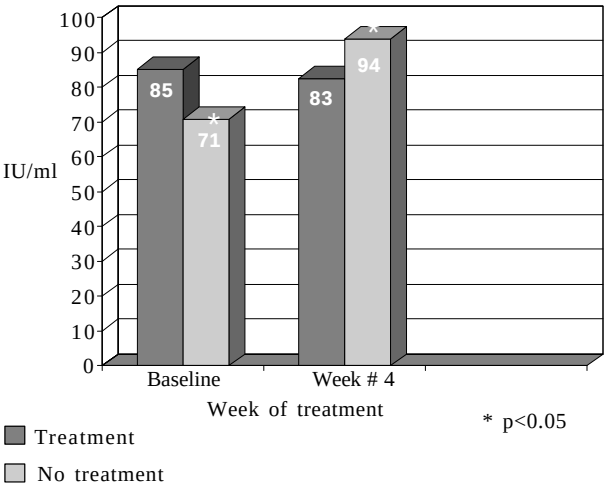
All the participating patients had a basal HCV viral load and liver enzymes (AST, ALT). These tests were repeated four weeks later. Viral load was measured using the Roche Amplicor method. Basic demographic data about the participants was collected.

The statistical analysis for the outcome measures employed the paired t-test to determine if there is a significance difference in the explored variables (AST, ALT, HCV viral load) from baseline to week 4. This analysis was performed separately for each group (treated and untreated). Differences with a p value less than 0.05 were considered statistically significant. Once the changes in AST, ALT and HCV viral load were calculated for each group, the percentage of change for AST, ALT and viral load were compared between the two groups using the Mann Whitney statistical test. Differences with a p value less than 0.05 were considered statistically significant.

Results

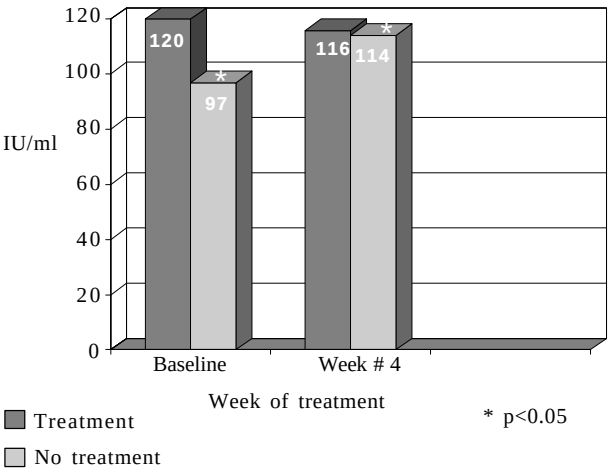
A total of thirty-four patients who met the inclusion criteria were enrolled in the study; seventeenin each group. A similar gender distribution was observed between both groups; treated (male (53%), female (47%)) vs. untreated: male (47%), female (53%)). Age range extended from twenty-one (21) to sixty-five (65) years with a similar mean age in both groups (treated: 49 years / untreated: 51 years). The mean baseline AST decreased from 85 ± 12.41 IU/ml to 83 ± 14.81 IU/ml at week 4 in the treated group. This decrease was not statistically significant (p>0.05) (Fig.1) .

Figure 1. Mean AST Level



The mean baseline AST increased from 71 ± 9.46 IU/ml to 94 ± 13.98 IU/ml at week 4 in the untreated group. This increase was statistically significant (p<0.05). The mean baseline ALT decreased from 120 ± 20.57 IU/ml to 116 ± 23.22 IU/ml at week 4 in the treated group. This decrease was not statistically significant (p>0.05). The mean baseline ALT increased from 97 ± 15.35 IU/ml to 114 ± 14.41 IU/ml at week 4 in the untreated group. This increase was statistically significant (p<0.05). (Fig. 2)

Figure 2. Mean ALT Level



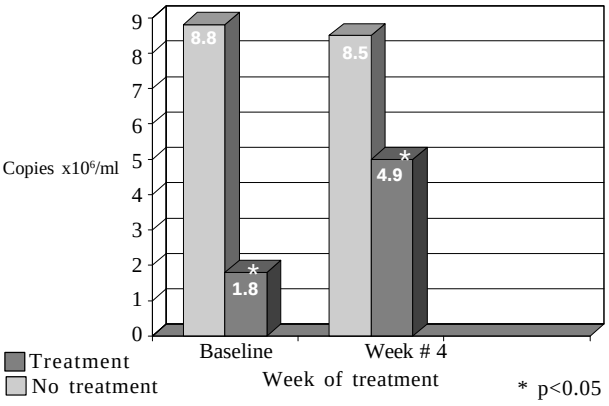
Despite randomization, a disproportion for the mean baseline HCV viral load level was noted between both groups (treated: 8.8 copies x 10^6/ml / untreated: 1.8 copies x 10^6/ml). The mean baseline HCV viral load decreased from 8.8 ± 4.11 copies x 10^6/ml to 8.5 ± 3.51 copies x 10^6/ml at week 4 in the treated group. This decrease was not

Table 2. Control Group: Percent Difference from Baseline Values

Subject	Baseline AST	Week 4 AST	% change	Baseline ALT	Week 4 ALT	% Change	Baseline Viral load	Week 4 Viral load	% Change
1	110	177	**-61**	266	220	17	0.34	.52	**-52**
2	41	47	**-15**	67	72	**-7**	1.6	8.7	**-400**
3	38	41	**-8**	74	77	**-4**	10.38	21.41	**-106**
4	35	52	**-49**	56	56	0	2.37	9.7	**-309**
5	65	74	**-14**	137	172	**-25**	0.741	1.89	**-153**
6	83	71	14	89	86	3	1.28	2.96	**-132**
7	57	51	10	56	54	3	3.59	10.35	**--188**
8	55	57	**-4**	94	96	**-2**	0.19	0.18	2
9	119	162	**-36**	142	153	**-7**	1.2	3.39	**-182**
10	37	37	0	53	52	2	0.32	0.69	**-118**
11	100	127	**-27**	124	129	**-4**	0.81	10.9	10
12	183	237	**-32**	218	258	**-18**	4.89	12.11	**-431**
13	69	91	**-32**	67	140	**-108**	0.745	0.282	62
14	62	150	**-142**	31	126	**-306**	0.182	0.2	**-9**
15	31	61	**-92**	45	79	**-75**	1.15	0.52	55
16	69	55	20	77	68	10	0.509	0.37	27
17	52	106	**-104**	60	99	**-65**	0.34	0.154	55
Mean	70.94	93.6	**-33.64%**	97.4	113.94	**-34.47**	1.8	4.96	**-109.9**

statistically significant ($p>0.05$). The mean baseline HCV viral load increased from 1.8 ± 0.62 copies x 10^6/ml to 4.9 ± 1.49 copies x 10^6/ml at week 4 in the untreated group. This increase was statistically significant ($p<0.05$) (Fig. 3).

Figure 3. Mean Viral Load Level



Due to the large variability of viral load and liver enzymes values within each group a percent change for AST, ALT and HCV viral load for each participant was calculated using the following formula:

$$\frac{\text{end of treatment value - baseline value X 100}}{\text{baseline value}} = \%\ \text{change}$$

This calculation provides a percent difference from the baseline values of each participant. The mean percent changes in AST, ALT and HCV viral load for the treated group were: AST: -6.76%, ALT: -6.06% and HCV viral load: +56%. For the control group the mean percent changes were: AST: +33.6%, ALT: +34.5% and HCV viral load: +109.9%. Positive percents represent worsening or increasing values after the intervention, while negative percents represent lower values or improvement from baseline values. When the percent changes of both groups were compared, a significant difference ($p<0.05$) was noted in the liver enzymes (AST, ALT) between the treated and the untreated group (Fig. 4), although a not significant

Figure 4. Liver Enzymes % of Change

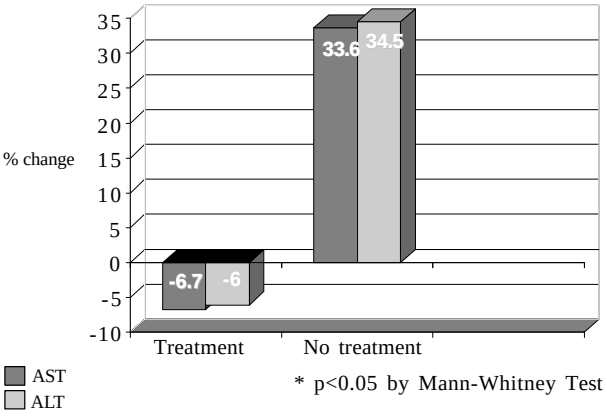
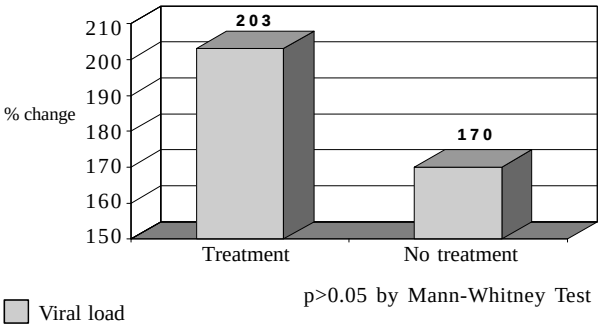


Figure 5. Viral Load % of Change



difference was observed for the HCV viral load ($p>0.05$). (Fig.5). There were no side effects reported by patients during their participation in the study.

Discussion

In recent years, there has been a substantial increase in the use of so-called complementary and alternative therapies by patients with liver disease. Medical professionals have been open-minded to the possibility that some benefit may come from therapies currently regarded as alternative. *Silybum marianum* (milk thistle) is most widely ascribed to as a remedy for liver diseases. Although a beneficial effect of silymarin has not been clearly established, it is been used by a large number of patients due to its good safety record. There is limited data to address the effect of *Silybum marianum* in liver chemistries and viral load of patients with chronic HCV infection. This study provides some insight to this issue. Our results suggest that the group of subjects receiving *Silybum marianum* for a period of 28 days had a stabilization of their liver enzymes, whereas the group of subjects who did not receive therapy had worsening. The lack of increase in the liver enzymes levels in those patients taking the medication suggests that *Silybum marianum* may have a protective effect in the inflammatory response to hepatitis C virus (HCV). This effect probably results from its activity as antioxidant agent and in protecting the liver cell membrane preventing cell destruction. The effect on viral load was not significant which suggests that *Sylibum marianum* has no or less efficacy as an antiviral agent.

This small short-term pilot study suggests that chronic hepatitis C patients may benefit from the use of *Silybum marianum*, a natural herb that has a little or no side effects. Short-term use of *Sylibum marianum* is safe and appears to have protective effects in the liver. Further studies with a larger number of patients treated for a longer period of time should be considered to evaluate the persistent effect on liver enzymes and to exclude that these changes could be related to the fluctuations seen in patients with chronic hepatitis C.

Resumen

El virus de la hepatitis C es una de las causas principales de enfermedad hepática en el ámbito mundial. La cirrosis que se desarrolla a causa de la infección crónica con este virus es la razón principal de transplante de hígado en los Estados Unidos. Un número significativo de pacientes con hepatitis C crónica no responden a la terapia antiviral convencional, principalmente los de genotipo 1. Los pacientes que no responden a la terapia antiviral o que no toleran los efectos adversos de la misma, tienden a usar terapias o remedios no convencionales. El *Sylibum marianum* ("milk thistle") es una hierba natural que es comúnmente usada por pacientes con enfermedad hepática. Su ingrediente activo, silymarin, parece tener efectos protectivos en el hígado. El sylimarin actúa como antioxidante reduciendo la producción de radicales libres. También tiene efecto antifibrótico reduciendo los marcadores para la acumulación de colágeno en el hígado y actúa como agente bloqueador de toxinas enlazándose a la membrana celular del hepatocito. En este estudio se evalúo el efecto del silymarin en los niveles de enzimas hepáticas (AST, ALT) y en la carga viral de pacientes con hepatitis C crónica. Estos pacientes estaban compensados de su enfermedad hepática y no habían recibido terapia para la hepatitis C por lo menos desde seis meses antes de entrar al estudio. Un total de 34 pacientes llenaron los criterios de inclusión. Estos pacientes fueron aleatorizados en dos grupos: diecisiete pacientes recibieron *Sylibum marianum* (160 mg tres veces al día x 28 días) y los otros diecisiete pacientes no recibieron terapia. Los niveles de enzimas hepáticas y de carga viral fueron medidos antes de comenzar el estudio y a la cuarta semana. Los pacientes que recibieron *Sylibum marianum* revelaron una estabilización de sus enzimas hepáticas, mientras que los pacientes que no recibieron terapia mostraron un aumento significativo de éstas. Esto sugiere que el *Sylibum marianum* tiene un efecto protector en la respuesta inflamatoria al virus de hepatitis C. Probablemente, este efecto es resultado de su actividad como antioxidante y de protector de la membrana celular previniendo la destrucción celular. El efecto en la carga viral no fue significativo lo que sugiere que *Silybum marianum* tiene poca eficacia como agente antiviral.

Este estudio piloto sugiere que los pacientes de hepatitis C crónica se podrían beneficiar del uso de *Silybum marianum*. A corto plazo, su uso es seguro y parece tener efectos protectores en el hígado. Estos resultados estimulan el desarrollo de otros estudios con un mayor número de pacientes que sean tratados por un período de tiempo más largo y así para poder evaluar el efecto a largo plazo de sylimarin en el hígado.

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