Original Article

The Effectiveness of Silymarin in the Prevention of Anti-tuberculosis Drug-induced Hepatotoxicity: A Randomized Controlled Clinical Trial

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Abstract

Background: Several animal studies have shown the protective effect of silymarin (the extract of *Silybum marianum* seeds) against anti-tuberculosis drug-induced hepatotoxicity (ATDH). However, the knowledge of ATDH of silymarin in humans is scarce. In this study, we aimed to clinically evaluate it. **Methods:** During this randomized controlled clinical trial, 36 new cases of tuberculosis (TB) were enrolled to receive either silymarin 150 mg twice daily for two weeks along with a standard anti-TB therapeutic regimen (experimental group; n = 16) or standard anti-TB therapeutic regimen alone (control group; n = 21). Liver function tests (serum AST, ALT, ALP, and total bilirubin) at the end of weeks 1 and 2 as well as the rate of ATDH during the study were determined and compared between the groups. **Results:** No significant differences between the experimental and control groups were observed at the end of the first week regarding liver function tests; However, at the end of the second week, the mean serum levels of AST (P = 0.03) and ALP (P = 0.04) were significantly lower in the experimental group. ALT (P = 0.016) and ALP (P = 0.027) levels in the experimental group significantly decreased during the study, while the changes in the control group were not significant. Two patients in the control group (9.5%) developed ATDH, while no one in the experimental group manifested this adverse effect. **Conclusions:** Our study suggests that silymarin use has the potential for the reduction of anti-TB drug-induced hepatotoxicity.

Keywords: *Anti-tuberculosis drug, chemical and drug-induced liver injury, silymarin*

Introduction

Worldwide, tuberculosis (TB) infection caused by *Mycobacterium Tuberculosis*, remains one of the top 10 deadly diseases having a significant socioeconomic burden in the world, especially in developing countries.[1-3] Despite successful protocols for TB prevention and treatment, roughly 1.3 million people died from active TB in 2017 despite the fact that it is.[4]

TB is a preventable and curable disease.[5] Combination therapy with rifampin (Rif), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) is the classic treatment of TB.[6] However, TB treatment has serious problems such as disease relapse and the emergence of resistance to drugs.[7] Adverse effects of anti-tuberculosis drugs (ATDs) can affect patient adherence and the effectiveness of treatment and lead to severe morbidity and even mortality.[8] It is well known that drug-induced liver injury is major toxicity of anti-TB treatment.[7] Among the patients receiving the combination treatment, anti-TB drug-induced hepatotoxicity (ATDH) is most prevalent within the first three months of treatment,[9] which occurs in 4.8% to as high as 36% of patients.[7]

Previous studies have shown that ATDH is mediated by oxidative stress, and therefore, several antioxidant agents have been proposed to prevent it.[10] Several studies have been conducted in animal models to show the beneficial hepatoprotective effect of herbal drugs.[9,11-14] Silymarin, a standardized extract of the milk thistle (the plant *Silybum marianum*) seeds, has been used as a hepatoprotective supplement for over 2000 years.[15] It is a complex mixture of flavonolignan isomers consisting of silybin, isosilybin, silydianin, silychristin, and others[16,17] as well as other polyphenolic compounds.[18] Its components have interesting traits like anti-cancer, neuroprotective, and antioxidant properties.[17,19] Animal studies have shown the hepatoprotective activity of silymarin against some toxins and drugs, especially

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against the hepatotoxicity of anti-TB drugs.[10,14,20] It has been proposed that hepatoprotective actions of silymarin may result from the scavenging of free radicals and inhibition of lipid peroxidation, leading to faster recovery after injury.[21,22]

Despite the results of animal studies showing the protective effect of silymarin against ATDH, randomized clinical trials (RCT) addressing this issue are limited with inconclusive results.[8,23] Therefore, the present RCT was designed to evaluate the preventive effect of silymarin against the development of ATDH in new TB cases on a standard anti-TB drug regimen.

Methods

This study was a randomized double-blind controlled clinical trial performed at Al-Zahra hospital affiliated to Isfahan university of medical sciences, Isfahan, Iran, from April 2017 to August 2018. The university ethical committee approved the study protocol. Also, it was recorded in the Iranian Registry of Clinical Trials with the code IRCT20150721023282N9.

Study population

Participants were eligible for enrolment if they were new cases of TB and aged ≥18 years old who received a standard 4-drug anti-TB regimen (i.e. isoniazid 5 mg/kg + rifampin 10 mg/kg + ethambutol 15 mg/kg + pyrazinamide 25 mg/kg) for active pulmonary/extrapulmonary TB infection. Patients who used any first-line drugs within the last 8 weeks, any silymarin supplement within the last 4 weeks, and/or concomitant hepatotoxic medications/substances (e.g., sodium valproate, methotrexate, any sulfonamide, regular-dose acetaminophen, and alcohol), systemic corticosteroids, antioxidants (e.g. vitamins E and C), patients with acute or chronic liver and/or renal disease (including viral hepatitis diseases), HIV-positive patients, and pregnant/lactating women were excluded from the enrollment. Furthermore, the participants were excluded from the study if they discontinued ATDs for any reason other than hepatotoxicity. Figure 1 shows the flowchart of patients' enrollment in the study. As shown, a total of 53 new cases of TB met the inclusion criteria and gave consent to participate, of whom, however, a total of 37 patients completed the study including 16 and 21 patients in experimental and control groups, respectively.

Study protocol

After written informed consent was obtained, patients were randomly allocated to experimental and control groups to receive either silymarin150 mg twice daily (as 150-mg capsules containing dried extract of Milk thistle, Nutri Century, Canada) started concurrently with the standard anti-TB regimen and continued for the first two weeks of therapy or only standard regimen, respectively. Simple randomization was used for patients' allocation to the groups. For this, an online random number generator was used (available at https://www.random.org/sequences) so that even and odd numbers were considered for drug and control groups, respectively. Before the intervention, baseline information on the demographic and clinical characteristics of participants including age, sex, nationality (Iranian vs. non-Iranian), any chronic disease comorbidity, and current smoking status was obtained. Liver function tests (LFTs) including serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin were assayed at the beginning of the intervention (time 0) and weekly for two weeks. For this, at each time point, 5 mL of forearm venous blood was obtained and centrifuged (Kubota, Japan) at 2000 rpm for 10 minutes. The obtained serum was stored at −80°C (Jaltajhiz, Iran) until assay. At the end of sampling, the serum levels of the markers mentioned earlier were detected using specific spectrophotometric kits (Pars Azmoon, Iran).

During the study period, patients were monitored for signs and symptoms of liver toxicity (i.e. nausea, vomiting, right upper quadrant pain, anorexia, jaundice, and change in urine/stool color). ATDH was defined as either 1) serum AST and/or ALT elevation more than three times (ULN, 40 U/L) with hepatitis symptoms or 2) serum AST and/or ALT elevation more than five times the ULN regardless of the presence or absence of symptoms.[24]

Outcome measures

The primary outcome measures were the differences in the mean of serum levels of AST, ALT, ALP, and bilirubin between the groups to compare the magnitude of changes in these parameters from baseline to the end of the intervention. The secondary outcome measure was the difference in the frequency of ATDH in both groups at the end of the trial.

Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0). To evaluate the normality of quantitative variables distribution, Kolmogorov–Smirnov test was applied. Due to the normal distribution of all data, an independent samples *t* test was used to compare the LFT parameters between the groups at each time point. Repeated-measures ANOVA was used to assess within-group changes. Qualitative variables (gender and nationality) were compared by the Chi-square test. Differences with a *P* value of less than 0.05 were considered statistically significant.

Results

As Table 1 shows, all recruited patients in the two groups were matched regarding demographic and baseline clinical characteristics.

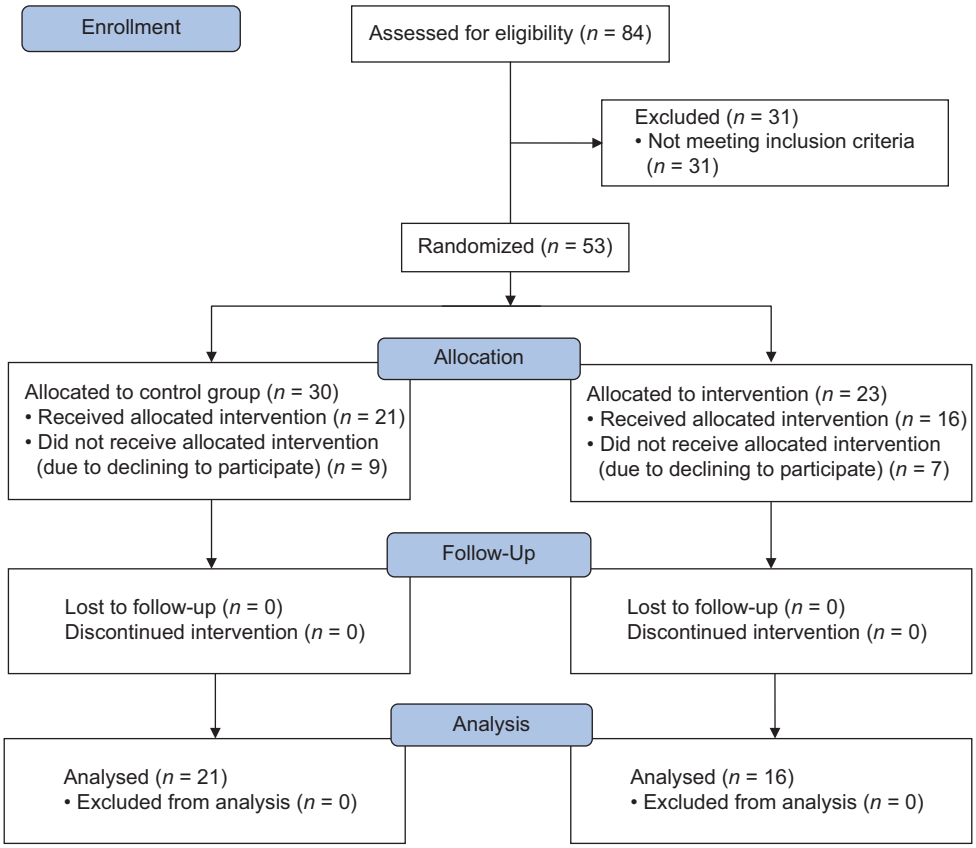

Figure 1: Diagram of the patients' enrollment in the study

Table 1: Comparison of demographic and baseline characteristics between the two groups

Parameter	Group		*P*
	Experimental (*n*=16)	Control (*n*=21)	
Age (years, mean±SD)	49.92±19.12	57.66±20.20	0.265
Sex (male/female)	10/6	11/10	0.728
Nationality			0.133
Iranian	16	17	
Non-Iranian	0	4	
Chronic disease	3 (18.75%)	6 (28.57%)	0.490
Smoking	5 (31.25%)	7 (33.33%)	0.893

The study results for laboratory parameters at evaluated times are presented in Table 2. As shown, at the end of week one, the mean serum levels of all parameters were not statistically different between the groups; however, at the end of the intervention (week two), the mean serum levels of AST (P = 0.033) and ALP (P = 0.037) were significantly lower in the experimental group compared to the control group.

According to repeated measures, ANOVA, ALT (P = 0.016), and ALP (P = 0.027) levels significantly decreased during the study, while the changes in the control group were not significant, albeit an increasing trend was observed in this group. Furthermore, the mean levels of AST increased significantly in the control group, whereas the values in the experimental group had decreasing trend with no significant change. No significant within-group change and between-group differences were observed for bilirubin. Comparing the trend of changes between the groups by repeated-measures ANOVA showed that silymarin significantly prevented the increase of AST (P = 0.036), ALT (P = 0.046), and ALP (P = 0.004) during the study period. Regarding the secondary outcome measure, two patients in the control group (9.5%) developed ATDH, while no one in the experimental group manifested this adverse effect. However, the difference was not statistically significant (P = 0.501).

Discussion

In the present study, the administration of silymarin 150 mg BID for a 14-day course was associated with a significant reduction in LFTs. However, it did not significantly reduce ATDH. The preventive effects of silymarin against the increase of hepatic markers in our study show the potential ability of this substance to protect the liver from ATDs-induced liver damage.

Several mechanisms for the liver injury of anti-TB drugs have been proposed including oxidative stress,[23] suppression of antioxidant defense mechanisms accompanied by enhanced lipid peroxidation, stimulation of metabolic activation by CYP 2E1, and elevation of intracellular Ca2+ that is

Table 2: Comparison of the values of laboratory parameters at the evaluated time points between the two groups

Parameter	Time	Group		*P*[a]
		Experimental (*n*=16)	Control (*n*=21)	
AST (mg/dL)	0 (baseline)	29.71±12.34	26.90±11.58	0.502
	Week 1	29.10±14.36	39.95±29.50	0.166
	Week 2	22.70±15.53	86.70±123.70	0.033
	P[b]	0.256	0.026	0.036[c]
ALT (mg/dL)	0 (baseline)	31.79±17.83	23.65±12.48	0.127
	Week 1	39.07±21.30	29.90±28.28	0.313
	Week 2	25.32±11.11	46.95±46.00	0.056
	P[b]	0.016	0.812	0.046[c]
ALP (mg/dL)	0 (baseline)	323.71±205.48	295.14±136.14	0.630
	Week 1	293.84±146.90	329.21±107.71	0.438
	Week 2	236.14±81.76	323.22±140.68	0.037
	P[b]	0.027	0.812	0.004[c]
Total Bil (mg/dL)	0 (baseline)	0.31±0.18	0.47±0.35	0.14
	Week 1	0.26±0.07	0.51±0.33	0.121
	Week 2	0.40±0.56	0.46±0.42	0.517
	P[b]	0.665	0.856	0.544[c]

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Bil, bilirubin. [a]Between-group comparison at each study point (independent samples *t* test). [b]Within-group comparison during the study period (repeated-measures ANOVA). [c]Between-group comparison during the study period (repeated-measures ANOVA)

known to be involved in hepatocellular damage,[25] cell membrane damage as evidenced by increased serum levels of liver enzymes.[9] Silymarin has been shown to block or reverse these effects including enhancement of catalase and glutathione peroxidase (GPx) activity and glutathione content, suppression of lipid peroxidation, a reversal of CYP 2E1 activation, and prevention of Ca2+ accumulation.[9] Furthermore, silymarin prevents ATDs-induced membrane disintegration[9] and mitochondrial dysfunction[26] as evidenced by the inhibition of LFT elevation in our study. The anti-inflammatory, immunomodulatory, and antioxidant properties of silymarin including scavenging free radicals[10,17,27] may be responsible for these protective effects.

Although evidence for the hepatoprotective effects of silymarin against ATDH is present from several animal studies, they are limited clinical data with inconsistent conclusions.[8,16]

In an animal study conducted by Eminzade *et al*.,[10] simultaneous administration of silymarin with ATDs significantly decreased the biochemical and histological changes induced by the drugs. According to a recent 8-week trial performed by Heo *et al*.[8] on 121 TB patients, no significant preventive effect of silymarin against ATDH was observed. Similarly, in the study of Marjani *et al*.[23] on 70 new cases of TB patients, silymarin had no significant protective effect in this regard. Another study by Gu *et al*.[28] which used silibinin, the major active constituent of silymarin, showed similar results. The discrepancies in the study population, sample size, study duration, and drug preparation may be responsible for the observed differences between the results of these studies and ours.

Although no case of ATDH was detected in the experimental (silymarin) group versus two cases in the control group in our study, this difference did not reach statistical significance. This may be due to the small sample size and relatively short duration of the intervention (two weeks) as ATDH usually occurs in the first months of treatment.[29,30] Therefore, expanding the study duration may reveal more significant differences regarding ATDH incidence. However, considering the obtained results, our study provides initial evidence for the effectiveness of silymarin as it showed preventive effect against AST and ALP increase and a tendency toward ameliorating the rate of ATDH.

Our study limitations were mainly the short duration of intervention, small sample size, and lack of a placebo. Future investigations are necessary to confirm the benefits of silymarin in larger, longer, and multicenter studies.

In conclusion, the consumption of Milk thistle seed extract equivalent to 150 silymarin twice daily has the potential for the reduction of anti-TB drug-induced hepatotoxicity. However, more studies are needed to confirm this effect.

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Conflicts of interest

There are no conflicts of interest.

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