SILYMARIN FOR PREVENTION OF ANTI-TUBERCULOSIS DRUG-INDUCED LIVER INJURY: A META-ANALYSIS

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Abstract

Background

There currently is no drug is proven to significantly prevent Anti-tuberculosis drug-induced liver injury. Silymarin, a traditional herbal drug, has been used as a hepatoprotectant, and has been shown to prevent AT-DILI in animals. Recent treatment trials in humans, have conflicting results

Objectives

The objective of this study is to evaluate the hepatoprotective effect of Silymarin in preventing drug-induced liver injury in adult patients being treated with anti-tuberculosis drugs.

Search methods

A search of Medline, Cochrane Library, Science Direct, Biomed Central and EMBASE was performed for literature about Silymarin and AT-DILI from inception to November 2016. Key words used were ("Silymarin" OR "Silybin" OR "Milk Thistle) AND ("DILI" OR "Hepatotoxicity" OR "Liver injury") AND ("Tuberculosis" OR "TB" OR "Anti-Kochs").

Included were randomized controlled trials comparing Silymarin to placebo in preventing the development of AT-DILI in adults diagnosed with Tuberculosis. Excluded studies were: children, no serum aminotransferase and bilirubin levels, multi- interventions, reviews, case reports, and expert opinions. Statistical analysis was done using RevMan 5.3 software.

Results

The search revealed 1695 abstracts, and 3 papers were included, comprising of 494 patients, of which 244 and 250 patients took Silymarin and placebo, respectively. The difference between Silymarin and placebo groups in incidence of AT-DILI in adult patients with tuberculosis was not significant (RR=1.04; 95%CI=0.34-3.23; p=0.03). Results show significant heterogeneity, and attributed to differences in AT-DILI definitions, frequency and duration of monitoring, and small sample sizes.

Conclusion

While Silymarin has an acceptable safety profile, available evidence from limited studies suggest that it exhibits no significant hepatoprotective effect against anti-Tuberculosis drug-related liver injury.

Key words

Silymarin, Drug induced liver injury, Tuberculosis

Background

Tuberculosis remains to be a major cause of morbidity and mortality in both developed and developing countries. The recommended treatment regimen consists an intensive phase of 2 months of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) followed by a maintenance phase of 4 months of INH and RIF.[1]

Hepatotoxicity is one of the more common adverse reactions of Anti-tuberculosis treatment. Pyrazinamide is said to be the most hepatotoxic, followed by Isoniazid, and Rifampicin.[2] A meta-analysis of studies involving different anti-tuberculosis treatment regimens estimates the incidence of liver toxcity at 2.6% with INH and RIF combined, 1.6% with INH alone, and 1.1% with RIF alone.[3] Anti-tuberculosis drug-induced liver injury (AT-DILI) ranges from asymptomatic elevation of liver enzymes to fulminant hepatic failure and results in increased morbidity and mortality, treatment interruption and withdrawal, and emergence of drug-resistant organisms.[4]

The exact mechanism of AT-DILI is still unknown, but it is postulated that DILI from INH and PZA may share similar mechanisms by inducing oxidative stress and increased oxygen free radical generation.[5] Several studies and reviews have reported that some drugs and herbal medicine, such as N-acetylcysteine or garlic, can help prevent AT-DILI, but such studies are small and most reports are anecdotal.[6]

Silymarin, a traditional herbal drug extracted from *Silybum marinums* seeds, has been used as a supplemental treatment for hepatoprotection. Active components found in silymarin extract are silybines, isosilybines, silychristine and silydianine, considered as active flavonoids.[7] It has been shown to be safe in animal models, and no signficant adverse reactions are reported in humans.[7,8]

Several studies have demonstrated a hepatoprotective effect of silymarin in prevention of AT-DILI in animal models. In these studies, silymarin administration significantly reduced the number of animals with pathological liver changes, as well as decreased liver enzyme elevations.[8] In-vitro and in-vivo studies have attempted to elucidate its mechanism of action, and it is postulated that this herbal drug acts as a free-radical scavenger and CYT P450 enzyme inhibitor.[9]

Silymarin is routinely prescribed as a hepatoprotectant in some countries. A large cohort study of 4304 patients in China showed that 63% of patients took hepatoprotectors during anti-TB treatment, with Silymarin as one of the most commonly prescribed hepatoprotectant.[6] Only small prospective observational studies and case reports suggest its efficacy, and recent clinical trials show conflicting results. To our knowledge, no meta-analysis has been done evaluating this drug's hepatoprotective effect in this particular subset of patients.

Objectives

The objective of this study is to evaluate the hepatoprotective effect of Silymarin in preventing drug-induced liver injury in adult patients being treated with anti-tuberculosis drugs. Primary outcome is the incidence of anti-tuberculosis drug-induced liver injury. Secondary outcome is the incidence of adverse events such as anorexia, vomiting, abdominal pain, nonspecific elevation of liver enzymes, pruritus, eczema and diarrhea as reported.

Methods

Search methods for identification of studies

A literature search was performed using Medline (via Pubmed), the Cochrane Library, Science Direct, Biomed Central and EMBASE. The following subject heading terms were used (for Medline and the Cochrane Library) and text (for other databases) in the search, and MeSH terms were used when available for Medline: ("Silymarin" OR "Silybin" OR "Milk Thistle) AND ("DILI" OR "Hepatotoxicity" OR "Liver injury") AND ("Tuberculosis" OR "TB" OR "Anti-Kochs").

Search limits were placed to reduce the number of results and excluded review articles, books and reference work. Only reviews and published Cochrane reviews were searched in the Cochrane Library. Databases were searched from inception to November 2016.

Manuscripts were reviewed to identify and exclude duplicate reports of studies. Abstracts from all citations were reviewed by a single reviewer to identify potential sources of data. Only publications written in English were included in this meta-analysis. Animal experiments, case reports/series, expert opinions, editorials and review articles were excluded. The literature search is presented in Figure 1.

Selection of studies

Full articles were obtained for all selected abstracts and were further evaluated by three researchers for inclusion. Discrepancies were resolved in a consensus meeting, or, if agreement could not be reached, was referred to a third party investigator.

The inclusion criteria were: use of silymarin as hepatoprotectant in adult patients taking anti-TB drugs, no evidence of hepatotoxicity prior to anti-TB treatment, and reported AST, ALT, TB levels during treatment monitoring.

The exclusion criteria were: children, study with no serum aminotransferase and bilirubin levels, multi- interventions, presence of several diseases, duplicate publications, reviews, case reports, and expert opinions.

Data extraction and management

Demographic data (i.e. age gender, race), study characteristics (i.e. study design, inclusion and exclusion criteria), route of treatment (i.e. drug preparation, dose and duration of treatment), and AT-DILI definitions were further extracted from the selected articles by 3 reviewers. Compilation of extracted data was done by a single researcher and disagreements were resolved by referencing the primary source.

Assessment of risk of bias in included studies

Each eligible study was independently evaluated using the Cochrane Collaboration's tool for assessing risk of bias. Each study was assessed for the presence of the following: random sequence generation, allocation concealment, blinding of participants, personnel, and outcome assessors, presence of incomplete outcome data, selective reporting, and other bias.

Each criterion will be rated as ''low risk'' of bias (e.g., allocation concealment was done), ''high risk'' of bias (e.g., participants or outcome assessors were not blinded) or ''unclear risk'' of bias (e.g., methods used for randomization were not described in the manuscript). Disagreements were resolved by consensus.

A meta-analysis was performed if there were at least two studies found with similar objective, study design and population and outcome measurement.

Measures of treatment effect

The extracted data were analyzed using RevMan (Version 5.3; Cochrane Collaboration, Denmark) software. Binary outcomes were compared by risk ratio (RR), and then presented in forest plots. The 95% confidence interval (CI) was used with a p value of less than 0.05 considered to have a statistically significant difference.

In cases of missing data, the authors of the study in question were contacted for clarification.

Statistically significant heterogeneity of the included studies was assessed by use of the Chi^2-based Q statistic and the I^2 statistic for the extent of heterogeneity. There was significant heterogeneity if p is less than 0.1 and I^2 was more than 50%. The fixed-effect model was used in pooling data with no evidence of heterogeneity, while the random effects model was used if significant heterogeneity was present. Sensitivity analyses were performed by excluding studies with unclear quality. A funnel plot was used to detect possible publication bias or other study effects.

Results

The systematic search produced 1695 possibly relevant abstracts. Only 16 studies meeting the inclusion criteria were selected and their full texts obtained for further review. After reviewing the full texts, 3 papers (Luangchosiri 2015, Marjani 2016, Zhang 2016) were identified and included in our analysis.[10,11,12] Eleven studies were excluded for a lack of a full-text in the English language precluding adequate assessment and 2 studies were prospective cohorts. The study selection process is presented in Figure 1.

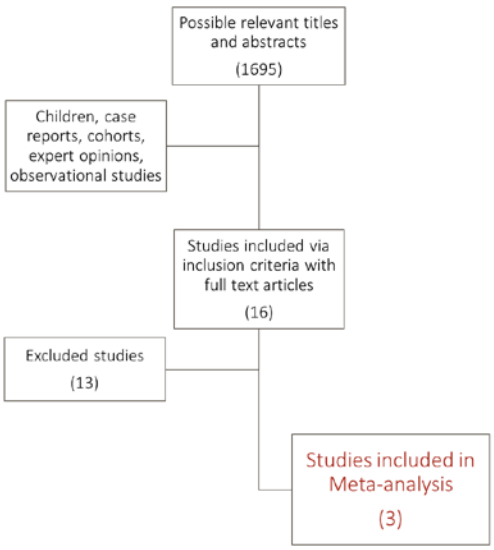

Figure 1. Study selection process.

All three studies were randomized controlled trials, were conducted in Asia (1 in China, 1 in Iran, 1 in Thailand), and compared Silymarin versus a placebo. Table 1 shows the characteristics of the studies included in the meta-analysis, including the outcomes measured, their definition of AT-DILI, Silymarin capsule preparation, dosing and frequency, and duration of study. The assessment of risk of bias in the individual studies is summarized in Figure 2.

Table 1. Characteristics of the included studies.

Study	Design	(n)	Intervention	Outcome	AT-DILI definition
Luangchosiri 2015	RCT	55	1 Silymarin 140mg tablet three times a day (420mg/day) for the whole duration of treatment	AST/ALT/TB at week 2/week 4 *Primary outcome:* Maximum ALT level at week4 *Secondary outcome:* AT-DILI, antioxidative enzymes at w4, adverse events	AT-DILI: presence of any of the following criteria: 1) having at least one of the following events: elevation of serum ALT level more than 2 times above the upper limit of normal, a rise in serum total bilirubin level to more than 1.5mg/dL, or any increase in ALT level above baseline levels combined with anorexia, nausea, vomiting or jaundice, 2) no other explainable causes of elevation of liver enzymes, and 3) normalization of liver enzymes after withdrawal of anti-TB drugs (based on ATS official statement: hepatotoxicity of antituberculosis therapy 2006)
Marjani 2016	RCT	70	1 Silymarin (Livergol®) 140mg tablet three	AST/ALT/TB at week 2 *Primary outcome*: AT-DILI *Secondary outcome:* adverse events	AT-DILI: 1) increasing of AST or ALT to three times more than upper limit of normal (40 IU/L), concomitant with symptoms of hepatic toxicity consisting

			times a day (420mg/day) for 2 weeks		nausea, vomiting, anorexia, weakness and abdominal pain 2) rise in AST or ALT more than five times or total serum bilirubin more than 2 mg/dl (based on ATS official statement: hepatotoxicity of antituberculosis therapy 2006)
Zhang 2016	RCT	369	1 Silymarin 200mg tablet two times a day (400mg/day) for the whole duration of treatment	AST/ALT/TB at week 8 , 6months Primary outcomes: probable and possible AT-DILI, the peak AST/ALT, maximum ALP/GGT value at 8 weeks Secondary outcomes: adverse drug reactions	Probable ATLI (Hy's Law): serum ALT or AST >3x ULN and a serum TBiL > 2x ULN in asymptomatic patients or in those with obvious hepatitis symptoms such as anorexia, nausea or vomiting, or abdominal pain Possible ATLI-A: abnormal level of ALT, AST, or TBiL Possible ATLI-B: serum level of ALT or AST > 2x ULN and the serum level of TBiL > 2x ULN.

RCT: Randomized control trial; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma glutamyl transferase; AT-DILI: Anti-tuberculosis drug-induced liver injury; TBiL: Total bilirubin; ULN: upper limit of normal

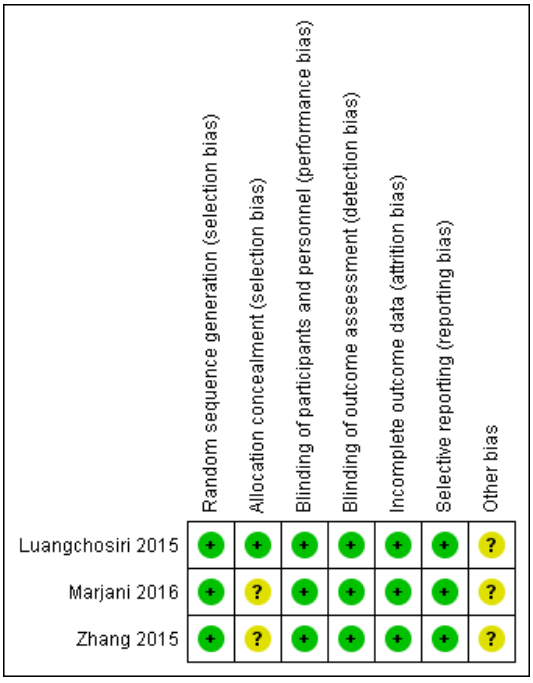

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

The total number of patients included in our meta-analysis was 494, of which 244 and 250 patients took Silymarin and placebo, respectively. The summary of results for 494 patients from the three studies examining the hepatoprotective effect of Silymarin

was a relative risk of 1.04 (95%CI=0.34-3.23) (Figure 3), suggesting that Silymarin has no significant hepatoprotective effect aganst AT-DILI compared to placebo. The Cochran Q test for heterogeneity of treatment effect was significant (p = 0.03). Visual inspection of the corresponding funnel plot revealed evidence of publication bias (Figure 4). Sensitivity analysis was done to reduce heterogeneity and the outlier study (Luangchosiri 2016) was removed. Figure 5 shows the pooled result of the two studies which shows with no evidence of heterogeneity based on Cochran's Q, favoring that of placebo (RR=1.72; 95%CI=1.15-2.57; P=0.81).

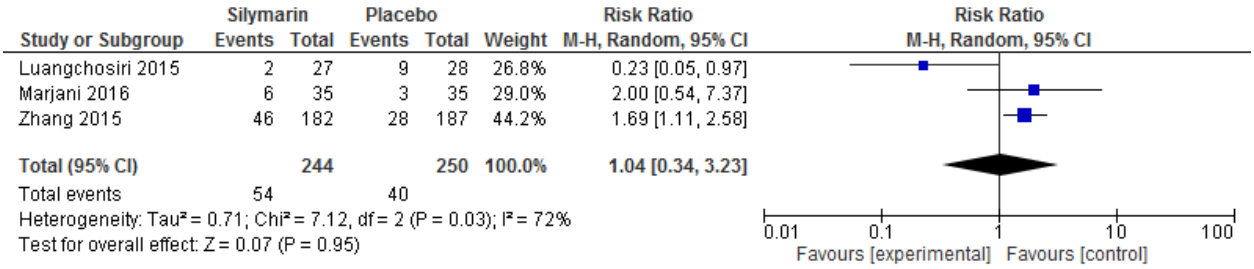

Figure 3. Forest plot of comparison of Silymarin vs. placebo on the occurrence of Anti-tuberculosis drug related liver injury (AT-DILI).

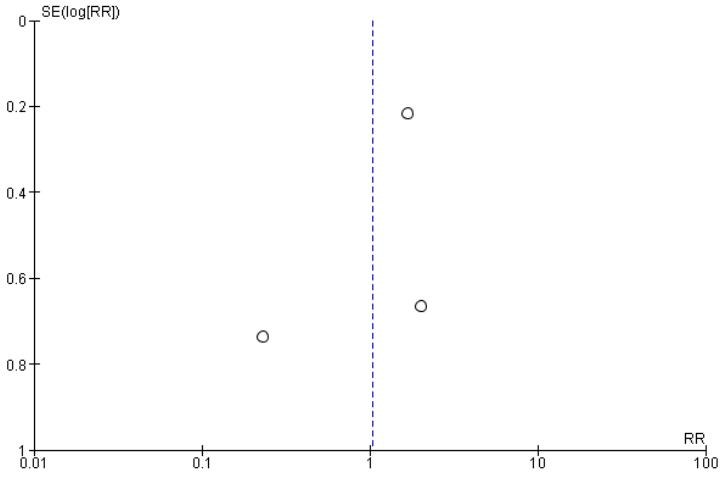

Figure 4. Funnel plot of comparison: Occurrence of Anti-tuberculosis drug related liver injury (AT-DILI).

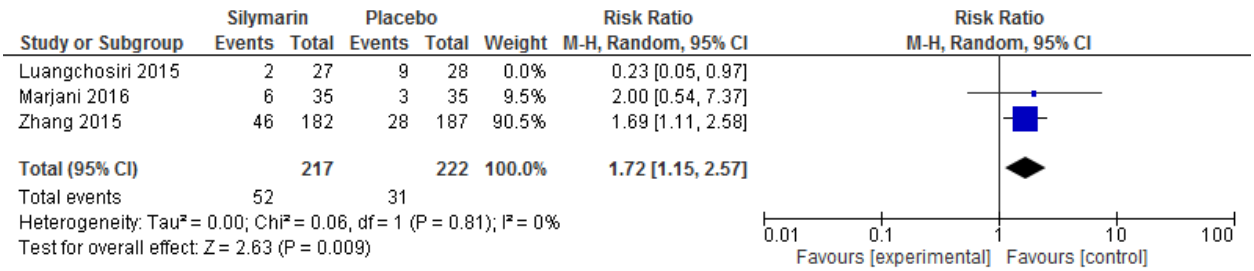

Figure 5. Sensitivity Analysis: Silymarin vs. placebo on the occurrence of Anti-tuberculosis drug related liver injury (AT-DILI).

FIgure 6 shows the pooled effect of the difference in occurrence of reported adverse events, which was also not statistically significant, with an equal occurrence between the two groups. (RR=1.07; 95%CI=0.84-1.37; P=0.02). This suggests that Silymarin was generally well tolerated, with an incidence of adverse events not significantly different from that of placebo.

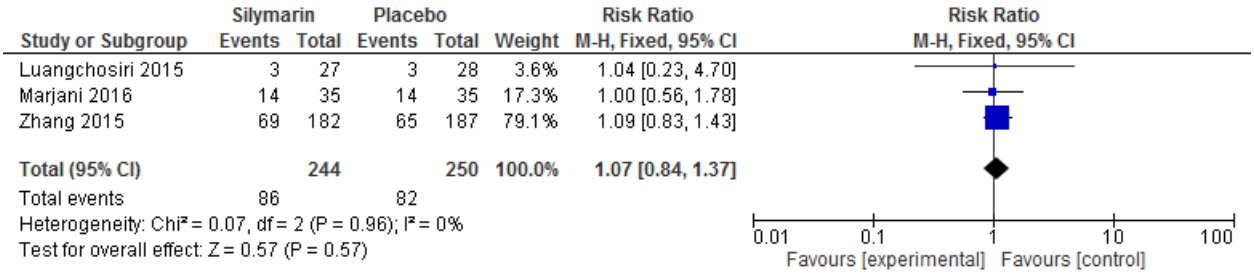

Figure 6. Forest plot of comparison of Silymarin vs. placebo on the occurrence of adverse events during anti-TB treatment period

Discussion

Drug induced liver injury remains the most common adverse drug reaction among patients receiving Anti-TB treatment, with reported incidence rates ranging from 2.6-28% in various cohorts.[13] The occurrence of AT-DILI can lead to delays in treatment, significant morbidity and mortality, with a fatality rate of up to 6-12% with continued drug use. Among the most widely accepted

risk factors are advanced age (above 60 years), female sex, low body mass index, malnutrition, those with existing chronic or active liver disease, as well as Hepatitis B or C coinfection.[2]

A diagnosis of AT-DILI is made when it satisfies all three of the following: 1) AST or ALT elevated more than 5 times the upper limit of normal regardless of symptoms, AST or ALT levels more than 3 times the upper limit of normal with symptoms suggestive of liver injury (ie jaundice, ascites, encephalopathy, coagulopathy, unexplained anorexia or fatigue), or elevation of total bilirubin levels more than twice the upper limit of normal, 2) known intake of anti-TB medications and 3) exclusion of other liver diseases.[2]

Of the three hepatotoxic drugs in the standard Anti-TB regimen, Pyrazinamide is said to be the most hepatotoxic, followed by Isoniazid, then Rifampicin.[13] In vitro and in vivo results in an animal study by Eminzade et al in 2008 suggested several underlying mechanisms for AT-DILI: 1) activation of CYTP450 2E1 by the Anti-TB drugs Rifampicin and Pyrazinamide, 2) generation of drug metabolites as well as oxygen free radicals leading to imbalance of oxidant/antioxidant defense at the cellular level, and 3) eventual peroxidation of cell membrane lipids that leads to the loss of hepatic cell membrane integrity and ultimately loss of liver cell function.[8]

Silymarin has been used as a hepatoprotectant supplement for over 2000 years, and is still used routinely in some countries such as China. It has been shown to be safe in animal models, and no significant adverse reactions are reported in human studies. This was also demonstrated in our meta-analysis, which showed no significant difference in the incidence of adverse reactions from placebo.[6]

It is suggested that Silymarin inhibits several isoforms of CYTP450 enzymes and potentiates the antioxidant capacity of the liver, acts as a free-radical scavenger, and inhibits the production of inflammatory cytokines.[8] Luangchosiri 2015, one of the included studies, also measured and compared the antioxidant enzyme levels of patients taking Silymarin vs that of placebo, and demonstrated higher levels of one of the enzymes (superoxide dismutase, SOD) in the treatment group. This may serve as a more specific mechanism at which Silymarin demonstrates its hepatoprotective effect.[10]

The results of our meta-analysis shows that while Silymarin is generally well tolerated, it is no better than placebo in preventing anti-tuberculosis drug-induced liver injury in patients receiving the standard anti-TB regimen. In fact, more cases of AT-DILI were noted in the group taking Silymarin compared to placebo, the difference however was not significant.

The analysis yielded results with significant heterogeneity. This may be due to several differences among the studies' design and methods. While all three based their AT-DILI definition to that of the 2006 ATS Consensus statement, each had their own set of cut-off in diagnosing AT-DILI. Only one of the three studies (Luangchosiri, 2015) demonstrated a significant hepatoprotective effect of Silymarin, as opposed to the other two studies. This study had the lowest threshold of all the studies in terms of the aminotransferase cut- off (at only >2x ULN, vs >3x ULN in the other two studies). This implies that the study of Luangchosiri may have overestimated the prevalence of AT-DILI.

The choice of placebo may have also affected the pooled result. Marjani 2016 and Luangchosri 2015 both used similar placebo tablets for the control group, while Zhang 2016 used vitamin C tablets, which in itself has antioxidant properties. There have been small studies (both in vivo and in vitro) done to demonstrate vitamin C's potential hepatoprotective effects - and while this is still yet to be proven in larger trials, it may have contributed to the large placebo effect in this particular study.[14]

The Silymarin was given at the same dose and preparation (at 400-420mg/day) in all included studies, but at different durations (2 weeks vs whole duration of the treatment regimen). While there exists no published recommendations regarding the length at which this drug is to be given, there is not enough evidence to show that the Silymarin given at a longer duration exhibits better hepatoprotective effect. The frequency of monitoring and length of follow-up also differed among the three (2 weeks vs 4 weeks vs 8 weeks). Most cases of AT-DILI occur during the first month of treatment,[15] the short follow-up time of the two studies (Luangchosiri 2015 and Marjani 2016) may have missed some cases of late-onset AT-DILI.

This meta-analysis has several limitations. Only publications in the English language were included, which may have left out potentially larger studies in regions where Silymarin use is prevalent and more extensively studied, such as China. All three studies had small sample sizes, and excluded those patients who are at high risk of developing AT-DILI, ie, those with active liver disease, HCV/HBV/HIV co-infection, those taking concomitant hepatotoxic drugs, as well as those with abnormal baseline liver function tests.

The three studies also dwelled on only one aspect of AT-DILI, which was largely based on serum AST, ALT and bilirubin levels. Signs and symptoms pertaining to AT-DILI were not extensively investigated, which may have missed some AT-DILI cases in the study population.

The results of this meta-analysis clearly show a lack of large controlled trials to evaluate Silymarin's efficacy as a hepatoprotectant.

Conclusions

Based on the results of this meta-analysis, it appears that while Silymarin is generally well tolerated, it is no better than placebo in the prevention of drug induced liver injury among patients taking Anti-tuberculosis drugs. However, further research is required to determine its efficacy in larger, randomized, comparative clinical trials.

References

1 Payam Nahid, Susan E. Dorman, Narges Alipanah, Pennan M. Barry, Jan L. Brozek, Adithya Cattamanchi, Lelia H. Chaisson, Richard E. Chaisson, Charles L. Daley, Malgosia Grzemska, Julie M. Higashi, Christine S. Ho, Philip C. Hopewell, Salmaan A. Keshavjee, Christian Lienhardt, Richard Menzies, Cynthia Merrifield, Masahiro Narita, Rick O'Brien, Charles A. Peloquin, Ann Raftery, Jussi Saukkonen, H. Simon Schaaf, Giovanni Sotgiu, Jeffrey R. Starke, Giovanni Battista Migliori, and Andrew Vernon. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. *Clinical Infectious Diseases.* 2016 63: e147-e195.

2 Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, Peloquin CA, Gordin FM, Nunes D, Strader DB, Bernardo J, Venkataramanan R, Sterling TR, on behalf of the ATS Hepatotoxicity of Antituberculosis Therapy subcommittee. An official ATS Statement: Hepatotoxicity of Antituberculosis therapy. *Am J Respir Crit Care Med.* 2006; 174:935-952.

3 Steele M, Burk RF, DesPrez RM. Toxic hepatitis with isoniazid and rifampin. A meta-analysis. *Chest* 1991, 99:465-471.

4 Aziz MA, Wright A. The World Health Organization/International Union Against Tuberculosis and Lung Disease Global Project on Surveillance for Anti-Tuberculosis Drug Resistance: a model for other infectious diseases. *Clin Infect Dis*. 2005;41 Suppl 4:S258–62.

5 Attri S, Rana SV, Vaiphie K, Katyal R, Sodhi CP, Kanwar S, et al. Protective effect of N-acetylcysteine in isoniazid induced hepatic injury in growing rats. *Indian J Exp Biol*. 2001;39(5):436–40.

6 Wu S, Xia Y, Lv X, Tang S, Yang Z, Zhang Y, Wang X, Hu D, Liu F, Yuan Y, Tu D, Sun F, Zhou L, Zhan S. Preventive use of hepatoprotectors yields limited efficacy on the liver toxicity of anti-tuberculosis agents in a large cohort of Chinese patients. *J Gastroenterol Hepatol.* 2015;30:540-545.

7 Au AY, Hasenwinkel JM, Frondoza CG. Hepatoprotective effects of Sadenosylmethionine and silybin on canine hepatocytes in vitro. J Anim Physiol Anim Nutr (Berl). 2013;97(2):331–41.

8 Emizande S, Uras F, Izzetin FV. Silymarin protects liver against toxic effects of anti-tuberculosis drugs in experimental animals. *Nutrition Metabol.* 2008; 5:18.

9 Adhvaryu MR, Reddy N, Parabia MH. Effects of four indian medicinal herbs on izoniasid-and pyrazinamide-induced hepatic injury and immunosuppression in guinea pigs. Word J Gastroenterol 2007, 13:3199-3205.

10 Luangchosiri C, Thakkinstian A, Chitphuk S, Stitchantrakul W, Petraksa S, Sobhonslidsuk A. A double-blinded randomized controlled trial of silymarin for the prevention of antituberculosis drug-induced liver injury. *BMC Complementary and Alternative Medicine.* 2015; 15:334.

11 Marjani M, Baghaei P, Dizaji MK, Bayani PG, Fahimi F, Tabarsi P, Velayati AA. Evaluation of hepatoprotective effect of silymarin among under treatment tuberculosis patients: A randomized clinical trial. *Iran J Pharmacol Research.* 2016;15(1):247-252.

12 Zhang S, Pan H, Peng X, Lu H, Fan H, Zheng X, Xu G, Wang M, Wang J. Preventive use of a hepatoprotectant against anti-tuberculosis drug-induced liver injury: A randomized controlled trial. *J Gastroenterol Hepatol.* 2016; 31:409-416.

13 Marra F et al. Adverse drug reactions associated with first-line anti-tuberculosis drug regimens. *Int J Tuberc Lung Dis* 2007; 11: 868-75

14 Su M, Chen H, Wei C, Chen N, Wu W. Potential protection of vitamin C against liver-lesioned mice. *Int Immunopharmacol*. 2014; 22:492-7.

15 Tostman A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R. Antituberculosis drug induced hepatotoxicity: concise up-to-date review*. J. Gastroenterol. Hepatol*. 2008; 23: 192-202.