Medical Scope Journal 2024;6(2):243-249
DOI: https://doi.org/10.35790/msj.v6i2.51870
URL Homepage: https://ejournal.unsrat.ac.id/index.php/msj

Exploring the Therapeutic Benefits of Silymarin Herbal Extract as a Supplement to Pulmonary Tuberculosis Treatment: A Comprehensive Review from Laboratory to Clinical Trials

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Received: October 17, 2023; Accepted: February 29, 2024; Published online: March 3, 2024

Abstract: Indonesia is ranked second in the number of tuberculosis (TB) cases with an incidence rate of 300 per 1000 population. A combination of antibiotics with a minimum six-month-administration regimen is an effective first line of TB treatment. Silymarin (Sm) is a plant extract which is known to have hepatoprotective and anti-microbial effects. This literature review aimed to discuss the potential of Sm in pulmonary TB treatment, starting from laboratory studies to clinical trials in humans. Studies on the use of Sm in tuberculosis literatures were obtained from a rapid systematic search in Pubmed and ScienceDirect databases. Supporting articles were searched based on specific keywords with inclusion criteria. The *in vitro* test showed immunomodulatory and bactericidal capacities of Sm against *Mycobacterium tuberculosis*. The *in vivo* test of Sm administration showed that Sm was able to increase the percentage of macrophage cells expressing the cytokines NF-κB and IFN-β. Sm had a bactericidal effect at levels >50 μM. The hepatoprotective character of Sm could prevent the increase in liver enzymes in mice receiving anti-TB drugs. Clinical trials showed that administration of Sm could prevent anti-tuberculosis drug hepatoxicity. In conclusion, silymarin has the potential to be an adjuvant therapy for the treatment of anti-TB drug-sensitive or resistant tuberculosis, as well as protection against the hepatotoxic properties of anti-TB drugs.
Keywords: tuberculosis; silymarin; supplementation therapy; antituberculosis therapy

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) infection, is still a global health problem and one of the biggest causes of death worldwide. This disease caused the deaths of ±1.64 million people in 2016.[1,2] In 2017, there were 10 million incident cases and 1.57 million deaths. The highest incidence of cases is in Asia, and in Indonesia the incidence of TB cases is recorded at 300 per 100,000 population.[3]

Tuberculosis treatment in Indonesia follows the World Health Organization (WHO) guidelines which consist of two phases, namely, the initial phase (bactericidal) and the continuous phase. Treatment of patients who have been diagnosed with pulmonary TB for the first time uses a combination of four types of antibiotics which are consumed for six months.[4] In cases of drug withdrawal or secondary TB patients, second-line anti-TB drugs such as clofazimine and kanamycin are given to patients with a therapy period of 18-24 months.[5] Problems arise from the length of therapy given, so that patient compliance in carrying out treatment is a key factor in TB treatment.[6] To increase patient compliance, WHO recommends having medication supervisors/ directly observed treatment, short-course (DOTS, also known as TB-DOTS), but research shows that DOTs are not a solution to patient on treatment with low compliance.[7] Until now, the problem of patient compliance and the hepatotoxic side effects of anti-TB drug are still a separate focus in TB treatment.[8]

As herbal extraction technology advances, research related to plant extracts is developing rapidly. Silymarin (Sm) is an extract of the herbal plant *Silybum marianum* which has been used to treat liver disease. Research shows that silymarin has anti-bacterial properties against Mtb and works synergistically with anti-TB drug, allowing for shorter treatment.[9,10] Looking at its characteristics, researchers conducted a rapid literature review regarding the effects of Sm in TB treatment.

METHODS

This literature review was compiled based on the latest research sources that focused on the development of pulmonary tuberculosis therapy. Literature search was carried out on Pubmed, Cochrane, ScienceDirect and Google Scholar by three independent researchers (MID, MTK, IRD). The literature search flow followed PRISMA guidelines with minor modifications (Figure 1).[11]

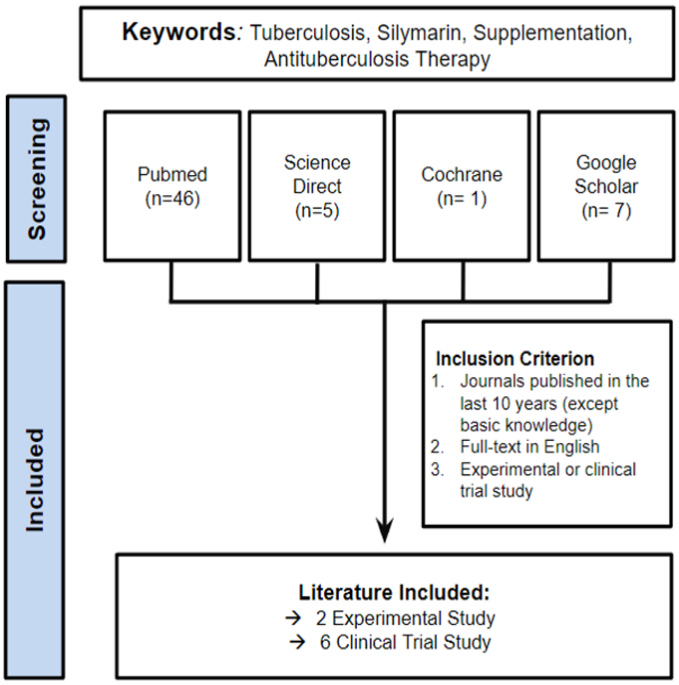

Figure 1. Literature search flow of silymarin review

The inclusion criteria used for the literature search were design studies of experimental or clinical trial studies and full text in English. This review did not include journals that compared combinations of Sm with other compounds or treatments for mycobacteria other than tuberculosis (MOTT). The search terms employed included tuberculosis, silymarin, supplementation, anti-tuberculosis therapy, and synonymous terms for each of these keywords. After the articles were collected, comprehensive analysis and synthesis of the study results were carried out. The risk of bias assessment of the study was carried out using the OHAT Risk of Bias Rating Tool for Human and Animal Studies which was carried out by two authors independently (GCP, MZB) and if there were differences in the assessment it would be discussed with the main researcher (MID). Data collection is done by taking data from the source, reading, synthesizing, and extracting the required data.

RESULTS

From the literature search, eight articles discussed Sm in TB therapy. Of the total eight literatures, two literatures were experimental trials[12,13] and the other six were clinical trials.[14–19] The literatures were written within the time frame of 2008-2023. All literatures, except one (Rodrıguez-Flores et al, 2019),[13] discussed the hepatoprotective effects of Sm against anti-tuberculosis drugs. Table 1 showed the characteristics of all literatures in this study. The results of the risk of bias analysis for each study were presented in Figure 2.

DISCUSSION

For more than 2000 years, the seed extract of the *Silybum marianum* plant, known as silymarin (Sm), has been used as a medicine to help maintain liver health. Sm is a complex combination of flavonolignan isomers which includes silybin, isosilybin, silydianin, silychristin, and other polyphenolic compounds. Its components have interesting characteristics, such as anticancer, neuroprotective and antioxidant properties.[9]

In *in vitro* studies, analysis of the immunomodulating capacity of Sm was carried out by *in vitro* assays. Using flow cytometry, the capacity of Sm to promote the production of proinflammatory cytokines was evaluated. NF-κB expression was increased after receiving 50 µM Sm.

Additionally, treatment of Sm at doses of 50 and 100 µM induced the production of IL-12 and IFN-γ. After the injection of 50-100 µM Sm, the expression of TNF-α increased (not considerably).[13,20]

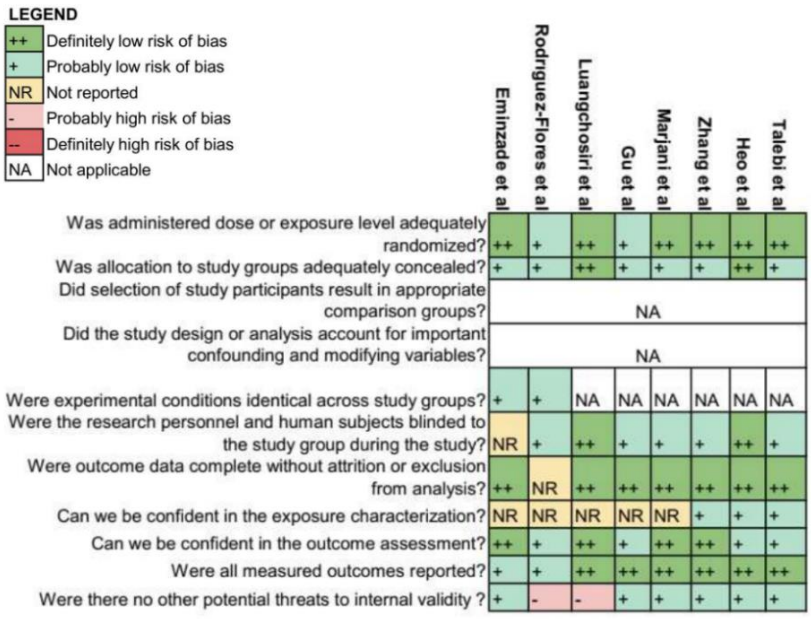

Figure 2. Risk of bias assessment of included studies

After determining its immunomodulatory capacity, a study was conducted to assess the *in vitro* bactericidal activity of streptomycin (Sm) against both susceptible and drug-resistant strains of *Mycobacterium tuberculosis* (Mtb). The total number of both Mtb strains increased in a dose-dependent manner after Sm treatment. Administration of Sm at a dose of >50 μM resulted in a significant reduction in the number of both strains observed from the absorbance. Calculation of the number of colony-forming units (CFU) supported these results.[13]

In vivo study research is aimed at assessing the immunomodulatory and bactericidal capabilities of Sm in the natural environment in living organisms. *In vivo* Sm studies were carried out using Balb/c mice injected with antibiotic sensitive Mtb strains. Mice were then given Sm with or without antibiotics for two months. Compared to the group of mice that did not receive treatment, administration of Sm was able to reduce bacillary loads. In addition, giving Sm together with antibiotics showed a significant decrease compared to the group that was only given antibiotics. The decrease in bacillary loads was supported by morphometric results which showed a decrease in the area of the lungs experiencing post-infection pneumonia. Giving Sm and antibiotics showed a significant reduction in the area of pneumonia compared to giving antibiotics or Sm separately. Examination of the amount of mRNA showed that administration of Sm and antibiotics was able to increase the expression of pro-inflammatory cytokines such as Il-12 and IFN-γ. *In vivo* tests on MDR TB showed similar results.[13]

Hepatotoxicity is a major side effect of tuberculosis treatment, with manifestations varying from asymptomatic elevations in liver enzymes to acute liver failure. Liver impairment due to anti-TB drug can result in increased morbidity and mortality; interrupted/interrupted treatment; dose reduction; and bacterial resistance trigger. However, the exact mechanism of liver disorders that occurs is not yet known, but is thought to be through increased oxidative stress and free radicals which trigger membrane lipoperoxidation and cell death.[21] Silymarin maintains hepatocyte membrane integrity and prevents the entry of toxic substances, as well as stabilizing free radicals and reactive oxygen species (ROS) through its phenolic properties.[9] Experiments on animals show histopathological and biochemical improvements such as reduced levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).[12,22]

Clinical trial of five randomized clinical trial (RCT) studies involving a total of 585 patients given silymarin and 613 patients given placebo, also stated that Sm administration had a significant positive effect in preventing liver disorders due to tuberculosis treatment. This is characterized by a reduced risk of developing liver disorders and liver enzymes (AST, ALT, ALP).[14–16,18,19] The results of this review are confirmed by the latest clinical trial research in 2023 which shows a significant difference in the incidence of anti-tuberculosis drug hepatotoxicity (ATDH) between the intervention group and the control group.[17]

Silymarin is highly hydrophobic and non-ionizable, thus exhibiting low water solubility and bioavailability. The mechanism of Sm action is a complex process (Figure 3). After oral administration, Sm is rapidly absorbed in the stomach (with a Tmax of about 2-4 hours and a half-life of about 6-8 hours), but with low absorption efficiency.[23] Some organic solvents such as transcutol, ethanol, polysorbate, and glyceryl mono-oleate are used to increase the solubility of Sm.[24] The bioavailability of Sm in the gastrointestinal tract depends on various factors, such as the incoming concentration and the presence of additional substances with solubilizing properties (such as fat, protein, amino acids, cholesterol, or other flavonoids). Silymarin concentrations after oral administration using conventional preparations based on silymarin extract alone are considered quite low.[25]

Following oral intake, Sm enters the enterohepatic circulation, and its primary component, silybin, undergoes phase I and phase II biotransformation within liver cells. Research indicates that silybin interacts with multiple cytochrome enzymes (CYP).[23,26] In laboratory conditions, the enzyme CYP450 2C8 has been recognized as the catalyst for transforming silybin into its primary metabolite, O-demethylated silybin, along with secondary metabolites such as mono- and dihydroxy-silybin.

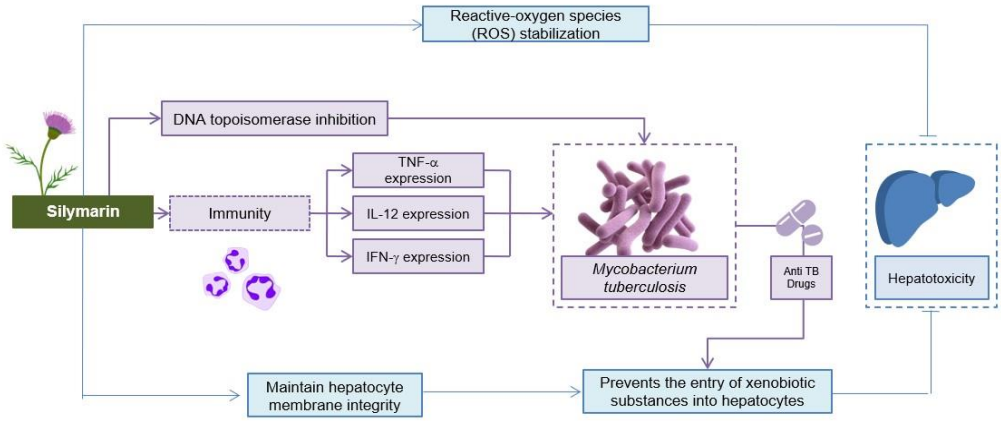

Figure 3. Proposed mechanism of action of silymarin as an adjuvant to anti-TB therapy in tuberculosis, maintain hepatocyte membrane integrity, and prevent hepatotoxicity

The half-life for the elimination of silymarin is approximately six hours, and roughly 3-8% of the orally administered portion is excreted unchanged through urine. The majority, about 80%, of silymarin is excreted in the form of its metabolites, specifically as glucuronide and sulfate conjugates via bile.[26]

CONCLUSION

Tuberculosis remains a global health problem with approximately 10 million people infected with *Mycobacterium tuberculosis*. Current antibiotic combination treatments, although effective in treating TB, still have a number of challenges. The first is patient compliance in carrying out therapy for at least six months, and the second is the hepatotoxic side effects of anti-TB drugs. Therefore, it is necessary to identify drug candidate compounds that can overcome this problem. Silymarin is an active metabolite from the *Silybum marianum* which has long been used for liver treatment. *In vitro* and *in vivo* studies showed that Sm displayed bactericidal effects against Mtb, as well as stimulating cytokine expression in macrophages. Clinical trials show the interesting characteristics of Sm which is able to prevent the occurrence of hepatotoxicity in TB patients on anti-TB treatment.

Conflict of Interest

The authors declare no conflict of interest in this study.

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Table 1. Studies of silymarin for TB included in the review

No	Authors	Year	Design Study	Number of subjects	Properties being studied	Outcome
1	Eminzade et al[12]	2008	In vivo study	N/A	Hepatoprotective properties	Treatment of rats with anti-TB drugs induced hepatotoxicity as evidenced by biochemical measurements: serum ALT, AST and ALP activities and the levels of total bilirubin were elevated, and the levels of albumin and total protein were decreased in drugs-treated (Sm) animal
2	Rodrıguez-Flores et al[13]	2019	In vitro and in vivo study	N/A	Immunomodulatory and microbisidal properties	In vitro study: Sm had microbicidal activity against drug-sensitive and MDR mycobacteria, induced the production of protective cytokines from infected macrophages, and improved the growth control of mycobacteria ($p<0.0001$). In vivo study: Sm induced significant expression of Th-1 cytokines such as IFN-γ and IL-12 as well as TNFα, which produced significant therapeutic activity when administered alone and apparently had a synergistic effect with chemotherapy
3	Luangchosiri et al[14]	2015	RCT	58	Hepatoprotective properties	There were 3.7 % and 32.1 % patients who developed anti TB-DILI in the silymarin and the placebo groups with risk reduction rate 0.28 (0.10-0.47)
4	Gu et al[18]	2015	RCT	568	Hepatoprotective properties	At 2, 4 and 8 weeks of treatment, the incidences of liver injury in experimental group and control group showed no difference in the incidence between the two groups. Numbers of patients diagnosed of DILI in experimental and control groups showed no difference
5	Marjani et al[15]	2016	RCT	72	Hepatoprotective properties	Six patients of silymarin group (17.1%) and three of placebo group (8.6%) experienced ATLI, not statistically significant.
6	Zhang et al[19]	2016	RCT	379	Hepatoprotective properties	The risk of developing probable ATLI was not significantly different between the two groups. During the follow-up period, 43.72% of cases in the experimental group and 35.83% of cases in the control group were determined to have possible ATLI
7	Heo et al[16]	2017	RCT	121	Hepatoprotective properties	The proportions of elevated serum liver enzymes more than 3 times of normal ranged at week 2, week 4, and week 8 did not show any significant difference between the silymarin and placebo groups
8	Talebi et al[17]	2023	RCT	36	Hepatoprotective properties	ALT and ALP levels in the experi-mental group significantly decreased during the study, while the changes in the control group were not significant

Notes: Sm = silymarin; MDR = multi-drug resistance; Th-1 = helper T cell-1; IFN = interferon; IL = interleukin; TNF = tumor necrosis factor; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; RCT = randomized clinical trial; ATLI = anti-tuberculosis drug-induced liver injury