

Review Paper

Ascorbic Acid Supplementation for Adjunctive Treatment of Pulmonary Tuberculosis: Review of Laboratory Research and Clinical Trials in Indonesia

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ABSTRACT

Background: Tuberculosis (TB) is a highly lethal global disease caused by *Mycobacterium tuberculosis* (Mtb), which has affected approximately a quarter of the world's population. Ascorbic acid is acknowledged for its strong antioxidant properties, its ability to modulate the immune system, and its effectiveness against Mtb infections. Researchers plan to conduct a comprehensive literature review to examine the potential of ascorbic acid as an adjunct therapy in the management of TB. **Method:** literature search based on specific keywords following the PICO (Patient, Intervention, Comparison, Outcome) research question was carried out on Pubmed, Scopus and Google Scholar databases, as well as Garuda for clinical research in Indonesia. The flow of the literature search followed PRISMA and risk of bias analysis using the Rob2 tool. **Discussion:** Laboratory studies have shown that ascorbic acid, whether given by itself or in conjunction with standard anti-TB medications, can decrease the quantity of Mtb colony-forming units (CFU). Similar outcomes were witnessed in experiments conducted with mice. The administration of ascorbic acid to mice infected with Mtb also led to a reduction in lung tissue damage. Clinical trials carried out in Indonesia demonstrated that the addition of ascorbic acid resulted in a greater sputum conversion rate in comparison to patients who did not receive this supplementation. **Conclusion:** Ascorbic acid exhibits several clinical attributes that prove beneficial in the management of TB.

ABSTRAK

Latar Belakang: Tuberkulosis (TB) merupakan penyakit global yang sangat mematikan yang disebabkan oleh *Mycobacterium tuberculosis* (Mtb), yang telah menyerang sekitar seperempat populasi dunia. Asam askorbat dikenal karena sifat antioksidannya yang kuat, kemampuannya memodulasi sistem kekebalan tubuh, dan efektivitasnya melawan infeksi Mtb. Para peneliti berencana untuk melakukan tinjauan literatur yang komprehensif untuk mengkaji potensi asam askorbat sebagai terapi tambahan dalam pengelolaan TB. **Metode:** pencarian literatur berdasarkan kata kunci spesifik berikut pertanyaan penelitian PICO (Patient, Intervention, Comparison, Outcome) yang dilakukan pada database Pubmed, Scopus dan Google Scholar, serta Garuda untuk penelitian klinis di Indonesia. Alur pencarian literatur mengikuti PRISMA dan analisis risiko bias menggunakan alat Rob2. **Diskusi:** Penelitian laboratorium menunjukkan bahwa asam askorbat, baik diberikan sendiri atau bersamaan dengan obat anti-TB standar, dapat menurunkan jumlah unit pembentuk koloni (CFU) Mtb. Hasil serupa juga diamati dalam percobaan yang dilakukan dengan tikus. Pemberian asam askorbat pada tikus yang terinfeksi Mtb juga menyebabkan penurunan kerusakan jaringan paru-paru. Uji klinis yang dilakukan di Indonesia menunjukkan bahwa penambahan asam askorbat menghasilkan tingkat konversi dahak yang lebih besar dibandingkan pasien yang tidak mendapat suplementasi tersebut. **Kesimpulan:** Asam askorbat menunjukkan beberapa sifat klinis yang terbukti bermanfaat dalam pengelolaan TB.

INTRODUCTION

Tuberculosis (TB) is one of the deadliest diseases in the world with a mortality rate of 1.7 million deaths worldwide.^{1,2} According to World Health Organization (WHO), *Mycobacterium tuberculosis* (Mtb) has infected about one fourth of the world's population. Mtb infects the lungs and spreads between persons relatively easily.³ In Indonesia, TB case occurrence has reached 1,020,000 infected people, thus ranking Indonesia second for the most cases of TB in the world after India^{1,4,5} although there are several regions in Indonesia that are successful in TB management programs.⁶

Currently, drug-susceptible TB disease is treated quite effectively with the use of first-line anti-TB drugs, such as Isoniazid (INH) and rifampicin (RIF) for 6 months.⁷ However, this treatment presents several major problems, both in terms of treatment duration and the emergence of drug-resistant strains. The extensive duration of TB treatment regimen, often decrease patient compliance which may lead to the development of multidrug-resistant Mtb (MDR-TB).^{3,7} MDR-TB is a mutant strain of Mtb that developed resistance to first-line TB drugs, thus requiring second-line drug treatment and up to 2 years of treatment. In some cases, drug resistance in TB occurs more significantly, resulting in the development of extensively drug-resistant TB (XDR-TB).⁸ Based on these problems, TB treatment needs to be more time-efficient, thereby increasing patient obedience and consequently preventing the emergence of drug resistance,^{3,9} while the treatment must still be effective against MDR-TB and XDR-TB.

Ascorbic acid has been recognized for its potent antioxidant, immune-regulating, and anti-infection properties since the 1930s.¹⁰ Scientific investigations have unveiled its ability to act as an antimicrobial agent,

reducing the susceptibility to infections, especially when present in high concentrations.¹¹ In laboratory settings, ascorbic acid concentration of 0.31 mg/mL has been shown to effectively impede the growth of *Pseudomonas aeruginosa*.¹² Furthermore, ascorbic acid exhibits both preventative and therapeutic benefits in cases of active tuberculosis (TB).¹³ Researchers intend to compile a literature review discussing the role of ascorbic acid as additional therapy in the treatment of TB.

MATERIALS AND METHOD

Literature search regarding ascorbic acid supplementation in the treatment of tuberculosis was carried out on the Pubmed, Scopus and Google Scholar databases. Specifically, a search for clinical trial literature in Indonesia was carried out on the Garuda database. The specific keywords used follow the PICO (Patient, Intervention, Comparison, Outcome) research question guidelines. The literature search was carried out by 3 authors (MID, GK, MFF) independently. We included articles published in the last 10 years and journals in English or Indonesian. The flow of the literature search followed PRISMA guidelines (Figure 1).¹⁴ For clinical trial articles, we do not limit patient characteristics (children or adults) to be able to obtain a complete picture of the potential of ascorbic acid in TB. Risk of bias analysis using the Rob2 tool was carried out by 2 authors (MID, MT) independently.¹⁵ Differences in risk assessment between authors were resolved by discussion.

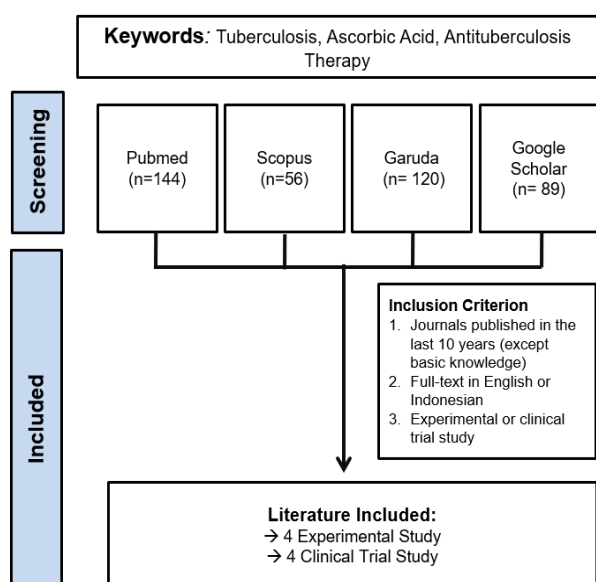


Figure 1. Literature Search Flow¹⁴

RESULT

We identified a total of four in vitro^{16,17} and in vivo^{18,19} research literature related to ascorbic acid for treating Mtb infections. Study characteristics are shown in table 1. Additionally, there were four clinical trials conducted in Indonesia²⁰⁻²³ that examined the effects of ascorbic acid supplementation in the treatment of pulmonary TB. Information about these clinical trials can be found in Table 2, and the assessment of potential bias is available in the supplementary file.

Table 1. Characteristics of Laboratory Studies being Included

No	Author	Year	Design Study	Mtb Strain Being Tested	Outcome
1	Vilcheze E, et al	2013	In Vitro Study	Drug-sensitive and drug-resistant strains	Ascorbic acid demonstrated potent bactericidal activity against Mtb, with a sterilizing effect observed at 4 mM concentration within three weeks. This effect was specific to Mtb, as other Gram-positive and Gram-negative bacteria exhibited significantly higher minimum inhibitory concentrations (MICs) for ascorbic acid.
2	Sikri K, et al	2018	In Vitro Study	Drug-sensitive and drug-resistant strains	Mtb that have adapted to ascorbic acid exposure exhibit typical signs of dormancy, such as ceasing growth, transitioning to a viable but non-culturable state (VBNC), losing their acid-fastness, becoming shorter in length, accumulating triglycerides (TAG) to alleviate reductive stress, developing a defensive response to oxidative stress, and demonstrating resilience to conventional tuberculosis drugs.
3	Vilcheze E, et al	2018	In Vitro and In Vivo Study	Drug-sensitive strain	Combination of ascorbic acid with the primary TB drugs isoniazid and rifampin resulted in a quicker reduction of the bacterial load in the lungs of the infected mice compared to using isoniazid and rifampin together. In live animal experiments, researcher demonstrated that injecting ascorbic acid intraperitoneally into mice could elevate the level of ascorbic acid in their serum to concentrations similar to those necessary for in vitro effectiveness.
4	Song F, et al	2022	In Vitro and In Vivo Study	Drug-sensitive strain	Study revealed that ascorbic acid significantly lessened the harm inflicted on cells by Mtb infection. Furthermore, ascorbic acid reduced the expression of proteins associated with Mtb-induced apoptosis (including Cleaved-caspase-9, Cleaved-caspase-3, Bcl-2, and Cyt-c) and inflammatory factors (such as IL-1β, IL-6, NLRP3, TNF-α, IL-8, and NF-κB) in THP-1 cells, resulting in a reduction in apoptosis.

Table 2. Characteristics of Clinical Studies being Included

No	Author	Year	Subjects	Intervention	Comparison	Outcome
1	Zakiyyah et al.	2014	30 TB patients aged 1-14 years	Ascorbic acid according to Recommended Dietary Allowance (RDA) in 2 weeks	Placebo	Total serum antioxidant levels by time (mmol/L)
2	Susanto et al.	2018	62 adult TB patients	Standard tuberculosis treatment + Ascorbic acid Ester type 500 mg/day in 8 weeks	Standard tuberculosis treatment only	Sputum culture conversion rate
3	Jefri et al.	2020	80 adult TB patients	Anti Tuberculosis Treatment + ascorbic acid 500 mg/day in 2 months	Anti Tuberculosis Treatment + Placebo	Improvements in CXR image
4	Safitri et al.	2022	80 adult TB patients	Standard tuberculosis treatment + Ascorbic acid Ester type 500 mg/day in 8 weeks	Anti Tuberculosis Treatment + Placebo	Conversion of AFB sputum

DISCUSSION

Ascorbic acid is a vital nutrient to some mammals and possesses some highly potent prooxidant and antioxidant properties depending on its interaction with the environment.¹⁰ Although the inclusion of ascorbic acid to the human diet in an attempt to improve treatment of various diseases such as viral and bacterial infections, cancer, and cardiovascular diseases have yielded some promising results, its impact on the treatment of TB has not yet been conclusively described.¹⁰

In Vitro Study

The in vitro test of administering ascorbic acid to Mtb showed that at sufficiently high doses ascorbic acid induces in vitro sterilization of Mtb cultures. The required minimum inhibitory concentration (MIC) of ascorbic acid against Mtb was 1 mM. Five cultures of Mtb was treated with different concentration of VC (single dose of 0,1 mM, 1 mM, 2 mM, 4 mM and a daily dose of 1 mM). After 4 weeks of colony-forming units (CFU) plating a dose-response relationship was

observed. The first bactericidal activity was observed at 2 mM treatment, while 1 mM treatment produced a brief bacteriostatic period followed by continuation of growth.¹⁷ This is evidenced by the fact that daily dose treatment of 1 mM for the first 4 days achieves the same, if not better, result compared to 4 mM treatment.¹⁷ MIC of ascorbic acid was also compared to other mycobacteria and Gram-positive and Gram-negative bacteria. It is found that the MIC of ascorbic acid on other *Mycobacteria* is 8 times higher than Mtb, while Gram-positive and Gram-negative bacteria is 16 to 32 times higher.¹⁷ This result could be evidence that the treatment of ascorbic acid will not disrupt the normal flora of the human body.

Analysis of the combined effect of ascorbic acid and first line anti-TB drugs showed inconsistent results.^{16,17} A study by Vilcheze et al, showed that 4 mM treatment of ascorbic acid achieved almost the same effects as first-line drug isoniazid (INH) on the first week. What differs however is that afterwards the Mtb cultures treated with INH started to

develop INH-resistant mutants while the ascorbic acid treated culture continuously produced less CFU until sterilization occurs. When combined, ascorbic acid and INH acts synergistically that resulted in relatively faster decrease in the amount of CFU on the first week compared to the other treatments.¹⁷

A study by Sikri et al showed the emergence of Mtb resistance to first-line anti-TB drugs after being treated by ascorbic acid. Mtb that had been exposed to ascorbic acid showed a notably increased ability to survive when anti-TB drugs (rifampicin, isoniazid, ethambutol, streptomycin) were introduced. Approximately 15% to 50% of the bacteria demonstrated resistance to anti-TB drugs, even when the antibiotics were administered at concentrations as high as 32 to 128 times their MIC₉₀. In contrast, the untreated bacteria were vulnerable to the drugs, with <10% survival at the MIC₉₀ and <1% survival at twice the MIC.¹⁶ Although it confers resistance to rifampicin, isoniazid, streptomycin and ethambutol, ascorbic acid increases the susceptibility of Mtb to pyrazinamide. Further analysis by Sikri et al showed the synergistic effect of ascorbic acid with a combination of anti-TB drugs (rifampicin+isoniazid+pyrazinamide+ethambutol).¹⁶

Data regarding the effect of ascorbic acid administration on drug-resistant Mtb is very limited with a study by Vilcheze et al conducting a study on the XDR-TB strain. INH and RIF-resistant strains of Mtb, drug-susceptible strain and XDR-TB strain was treated with 4 mM ascorbic acid. It is found that 4 mM treatment of VC has no significant effect to XDR-TB. This could be caused by the higher MIC required to sterilize XDR-TB culture which was not explored further nor given alternative solutions.¹⁷

Bactericidal activity is not due to the acidification of the media as the addition of the highest ascorbic acid concentration used in the experiment (4mM) did not significantly alter the pH of the cultures.¹⁷ However, because the statement was not elaborated further nor it is supported with the pH data of the cultures, for the purpose of coherence researchers assume that the pH is valued between 5.8 and 6.7 which is the optimal pH for the growth of Mtb in liquid medium²⁴ and the decrease is not enough to induce the sterilization of the Mtb cultures on its own.

In Vivo Study

Activity observed in vitro does, to some extent, correspond to activity in vivo.¹⁸ Considering that the 4 mM serum concentration of ascorbic acid is difficult to achieve in mice, they compromised to use the sub-inhibitory concentration of 1 mM which was injected intraperitoneally to achieve the closest concentration required for any sterilizing or bacteriostatic effect that can be observed in vitro.^{17,18} The result is that when given alone, sub-inhibitory dose ascorbic acid did not have any effect on the Mtb infected mice as reflected by the relatively similar CFU with the untreated mice. However, when given in combination with first line drug INH or rifampicin, the bacterial burden in the lungs is reduced much faster compared to each drug and ascorbic acid treatment independently. This synergistic response is also observed in the in vitro experiments which suggests that ascorbic acid can potentially be used to increase the efficacy of first line drug INH or rifampicin.¹⁸

Song et al conducted a study that examined the impact of giving ascorbic acid to mice infected with *Mycobacterium tuberculosis* (Mtb). They assessed the histopathological changes in the lung alveoli tissue of these mice, comparing those that received ascorbic acid orally and those that did

not. The results indicated that, in the group that received ascorbic acid in addition to Mtb infection, there was a noteworthy reduction in the extent of damage to the alveolar walls and a decrease in the presence of inflammatory cells infiltrating the tissue, in comparison to the group infected with Mtb alone.¹⁹

Clinical Trial in Indonesia

Several clinical trials in Indonesia were conducted to determine the impact of ascorbic acid supplementation on pulmonary TB patients in Indonesia with varying outcomes. In a study on pediatric TB patients aged 1-14 years, ascorbic acid supplementation according to the Recommended Dietary Allowance (RDA) for 2 weeks was found to increase total serum antioxidant levels significantly compared to placebo²². Higher serum antioxidants may provide benefits to TB patients undergoing treatment. In adult TB patients, ascorbic acid supplementation provides benefits that can be seen in sputum culture conversion. A study found that routine daily ascorbic acid supplementation was associated with significantly higher sputum culture conversion rates as early as 2 weeks from the start of administration and consistently significant up to 2 months. At the end of this 8 week study, there was 100% sputum conversion from positive to negative in the group receiving ascorbic acid supplementation while only 83.9% in the control group.²³ In another study, higher sputum conversion was also found in adult TB patients who received ascorbic acid supplementation for 8 weeks compared to placebo, with a greater difference of 100% conversion in the experimental group compared to 20% in the placebo group.²⁰

Apart from bacteriological indicators from sputum examination, ascorbic acid is also known to have benefits in improving radiological conditions in TB patients. Study found that adult TB patients who received

anti-tuberculosis therapy plus ascorbic acid supplementation for 2 months experienced more improvement in lesions on chest radiographs than TB patients who received anti-tuberculosis and placebo. 52.5% of the experimental group had no more lesions found on chest x-ray after being given ascorbic acid supplementation for 2 months compared to 37.5% in the control group.²¹ Although chest radiology alone is not significant in determining the recovery status of pulmonary TB patients, improvements in radiological images can indicate improvement²⁵ and regeneration of lung tissue which can also be related to lung function and the patient's clinical condition.

Proposed Mechanism of Action

Vilcheze et al in 2013 tried to explain the mechanism of action of ascorbic acid against TB through the Fenton reaction.¹⁷ The production of ROS increased DNA damage partly through oxidation of the guanine nucleotide pool that resulted in cell death. This effect was observed by comparing the level of ROS and DNA damage in Mtb treated with ascorbic acid and treated with vitamin E relative to the untreated which was measured by flow cytometry. The result was that ascorbic acid-treated Mtb culture had nearly 3 times increase in ROS levels and nearly 20% increase in percentage of double-stranded DNA breakage over 4 and 9 days respectively compared to other treatments.¹⁷

Ascorbic acid disrupts lipid biosynthesis. The other mechanism proposed is that ascorbic acid affects lipid which are often the most significant target of oxidative damage. The detection of accumulated free 2-hydroxylated long-chain fatty acids which was toxic to mycobacteria and not normally produced by Mtb could be evidence that the bactericidal activity of ascorbic acid does not solely owe to DNA damage. In addition, the general reduction of phospholipid content in ascorbic

acid-treated Mtb could jeopardize its mycobacterial cell wall integrity and consequently its survival.^{17,26} Proposed mechanism of action of ascorbic acid against Mtb infection shown in Figure 2.^{17,26,27} In addition to the two mechanisms mentioned earlier, it's worth noting that ascorbic acid has

recognized properties such as anti-inflammatory and antioxidant effects, as well as immunomodulatory properties, and it can influence autophagy.²⁷ However, its specific connection to tuberculosis remains uncertain and requires further investigation



Figure 2. Mechanism of Action of Ascorbic Acid against *Mycobacterium tuberculosis*^{17,26,27}

CONCLUSION

Tuberculosis remains a significant health challenge in Indonesia despite extensive awareness and treatment efforts. On the other hand, ascorbic acid, a micronutrient known for its anti-infective properties, has shown promise in both laboratory and animal studies. In vitro research indicates that ascorbic acid, whether administered alone or in combination with conventional anti-TB drugs, can reduce the number of Mtb CFU (colony-forming units). Similar results were observed in studies involving mice. When given to mice infected with Mtb, ascorbic acid administration also reduced lung tissue damage. Clinical trials in Indonesia showed supplementation with ascorbic acid resulted in a higher rate of sputum conversion compared to patients without supplementation. These findings provide a compelling rationale for considering ascorbic acid supplementation as part of TB

treatment, particularly in the context of Indonesia.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Supplementary File

Risk of bias of clinical trial included in the review

		Risk of bias							
		D1	D2	D3	D4	D5	D6	D7	Overall
Study	Susanto et al, 2018								
	Jefri et al, 2020								
	Zakkiyah et al, 2020								
	Safitri et al, 2021								

D1: Random sequence generation
 D2: Allocation concealment
 D3: Blinding of participants and personnel
 D4: Blinding of outcome assessment
 D5: Incomplete outcome data
 D6: Selective reporting
 D7: Other sources of bias

Judgement
 High
 Unclear
 Low