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Research Article

Faster Sputum Conversion with Addition *Phyllanthus niruri* Extracts to Standard Anti-Tuberculosis Therapy: A Double-Blind Randomized Controlled Trial

Zulkifli Amin[1,2]*, Ceva Wicaksono Pitoyo[1,2], Cleopas Martin Rumende[1,2], Jamal Nasir[1,2], Feni[2] and Sari Purnama Hidayat[2]

[1]Division of Respiratory and Critical Illnes, Internal Medicine Department, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia

[2]Faculty Medicine University of Indonesia, Indonesia

***Corresponding Author**: Zulkifli Amin, Division of Respiratory and Critical Illnes, Internal Medicine Department, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.

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Abstract

Background: Sputum conversion of active pulmonary TB occurs in 48% and 58% patients after four and eight weeks of standard anti-tuberculosis treatment, respectively. Without an effective immune system, standard anti-tuberculosis therapy are not able to eradicate mycobacterium completely, thus using adjunctive immunomodulator *Phyllanthus niruri* extracts is hypothesized to hasten sputum conversion.

Method: A double-blind randomization of 67 newly diagnosed tuberculosis patients into treatment group (RHZE and PNE thrice daily) or control group (RHZE and placebo) was conducted.

Result: Bivariate statistical analysis revealed no significant difference in sputum conversion, radiologic score, and cytokine levels between two groups. Logistic regression analysis showed that adjuvant PNE and higher IL-6 levels were correlated with faster sputum conversion (RR 0.32 and 0.14, respectively). No correlation was found between sputum conversion and reduction of pulmonary lesion area.

Discussion: Faster sputum conversion, as reported in the treatment group, benefits at the community level despite the insignificant statistical difference. Further, faster conversion may assist in controlling the spread of tuberculosis as most lost to follow-up TB cases were happened during the intensive anti-tuberculosis phase. Additionally, sputum conversion mostly occurred within the first week of treatment, thus evaluation of the role of cytokines in the second month was not representative of its function in eliminating *mycobacterium tuberculosis*. As severe lung damage is correlated with oxygen free radicals, PNE prescription as an antioxidant may be rationalized.

Conclusion: PNE hastens sputum conversion in pulmonary tuberculosis patients which may lower tuberculosis burden by decreasing spread of the disease.

Keywords: *Sputum Conversion; Pulmonary Tuberculosis; Phyllanthus niruri; Herbal Medicine; Phytopharmaca; Loss to Follow Up*

Introduction

Tuberculosis is one of the most threatening disease in the world. Implementation of DOTS strategy in 1995 has greatly impacted in reaching the Millenium Development Goals of controlling TB. A successful decreased in the TB cases rate and TB mortality rate have been reported. Additionally, there was an impressive growth in successful TB treatment cases, from 1.0 million in 1995 to 4.2 million in 2005 and 5.4 million in 2013, reaching a percentage of around 86% globally [1]. However, tuberculosis keeps spreading and infecting people, with around 9,6 million new cases each year [1].

Tuberculosis bacilli spreads from active pulmonary tuberculosis patients, especially those with positive sputum smears, which indicates a higher number of living Mycobacterium. However, proper anti-tuberculosis treatment, especially in the first two months (the intensive phase), most patients may undergo negative sputum conversion. Unfortunately, negative sputum conversion only occurs in 48% and 58% of the population after four weeks and eight weeks of treatment, disregarding their compliance status [2]. In studies with higher patient compliance, sputum conversion was around 40% after 4 weeks and 70 - 85% after 8 weeks of anti-tuberculosis treatment [2,3].

Antituberculosis drugs are not able completely eradicate bacilli tubercles without an effective immune system [4]. The lipoarabinomannan (LAM) in the mycobacterium wall may have role in reducing the number of CD4+ by disrupting the blastogenesis process and inhibiting IFN-ϒ activity, thus patients undergo secondary immunodepression, making the elimination of Mycobacterium bacilli more difficult [4].

Phyllanthus niruri is a medicinal plant grown in many tropical countries, known in Indonesia as green meniran. Its extract has been approved by the national government as phytopharmaca for modulation of the immune system since 2006. It plays a role in increasing macrophage activity and T lymphocyte proliferation. In addition, it increases the level of proinflammatory cytokines, such as TNF-α, and suppresses IL-10, which then stimulates monocyte function, macrophage phagocytosis and chemotactic activity, and natural killer cell cytotoxicity [5,6]. Adding this plant extract to standard tuberculosis treatment is expected to improve the immunological status and treatment outcome. Therefore, this study was conducted to investigate the effectivity of adjunctive *Phyllanthus niruri* extract (PNE) with the standard anti-tuberculosis drugs as compared to the standard anti-tuberculosis alone in enhancing negative sputum conversion.

Material and Methods

Study Population

All the newly diagnosed tuberculosis cases at the Pulmonology outpatient clinic at Cipto Mangunkusumo Hospital from February 2001 until April 2003 were screened for enrollment in the trial. The illness history, physical examination, and radiological evaluation of the patients were reviewed to identify eligibility. The inclusion criteria were: new tuberculosis patients who have never received anti-tuberculosis drugs, aged 15-55 years old, confirmed by three sputum specimens positive for acid-fast bacilli (AFB) by direct microscopy and culture, clinical and radiological signs consistent with pulmonary TB, and have agreed to be included in this study. The exclusion criteria were: drug resistance at baseline and during follow up, extra pulmonary TB, pregnancy, lactation, use of steroids or other supplements that modulate immune status during the previous months, any comorbidities (AIDS, renal failure, heart failure, malignancy, etc), and severe malnutrition.

Study Design

Patients who fulfilled the inclusion criteria and not the exclusion criteria were randomized and then given a treatment code number: standard tuberculosis regimen with *Phyllanthus niruri* extract or standard tuberculosis regimen with placebo. The study was held for the two months of the intensive phase of standard anti-tuberculosis treatment. The investigator, health staff and patients were unaware of the treatment code until the study was completed. Standard TB treatment used in the trial was based on the WHO dosage recommendations as shown in Table 1.

Trial groups will get addition PNE thrice daily which packed in capsules for two months study. PNE were prepared by PT Dexa Medica Indonesia, with every capsules contained 50 mg of PNE. Controlled groups got placebo capsules comprised lactose which made indistinguishable in appearance with PNE capsules.

Every sample was given logbook to record timed when medication was taken. At least one family member or neighbour were asked to help in monitoring patients compliance during study. Patients were also asked to come to clinic every week to take TB drugs and supplements or placebo, and health staff checked the compliance at their home each day during study. Patients who didn't take their medication

regularly, even missing one day were dropped from the study. The primary end points of this study were the rate and time to negative sputum conversion. Secondary end points were radiological changes, reversed body wasting syndrome (Body weight and BMI), cytokines profiles.

Body weight	Dossage
33 - 50 kg	Rifampicin 450 mg Isoniazid 300 mg Pyrazinamide 1500 mg Ethambutol 750mg daily
> 50 kg	Rifampicin 600 mg Isoniazid 600 mg Pyrazinamide 2000 mg Ethambutol 1500mg daily

Table 1: *WHO Standard Antituberculosis Dossage Recommendation.*

Clinical Examination

Eligible patients were thoroughly examined at diagnosis by investigator. Clinical assessment were performed at the start of study, including Karnofsky scale, body temperature, and the presence of Bacillus Calmette Guerin (BCG) Scar. Health status was rated using karnofsky scale which score range from 0 (dead) to 100 (normal). Besides body mass index was also calculated on the start and the end of study. Body weights was assessed using an electronic platform model weighing scale (770 alpha; SECA, Hamburg, Germany) to nearest 0.1 kg. Height was recorded to nearest 0.1 cm using a microtoise.

Sputum Examination

Three specimen of patients sputum were examined by direct microscopy after Kinyoun-Gabbet staining and cultured in Kudoh medium. Sputum smears were graded according to International scale based on the number of acid-fast bacilli (AFB) visible on microscope. We collected three sputum specimen every weeks for AFB direct microscopy assessment until 8 weeks when mostly sputum conversion was reached [4].

Chest X ray examination

Chest X ray were performed at the time when diagnosis performed and after finished intensive phase in both groups. Chest X-ray then assessed by pulmonologist calculating based on American tuberculosis association criteria for TB radiology lession.

Infiltrate	Score
No Infiltrate	0
< 5 cm	1
> 5 cm in 1 lung field	2
More than 1 lung field	3

Table 2: *American Tuberculosis Criteria for Pulmonary TB Lesion [4].*

Laboratory Examination

Assessment of blood glucose, hepatic function index (SGOT, SGPT, total bilirubin, total protein, albumin, and globulin), renal function index (Ureum, Creatinine) were done at the start of study. It's were used to determine population baseline and exclude patients whose

detected have any exclusion criteria. Besides we also assess cytokines profile, which is IFN-Ɣ, TNF-α, and IL-6 at the start and the end of second month.

Statistical Analysis

Analysis were performed using SPSS 20. One sample kolmogorov-smirnov test was used to investigate normality distribution of sample. Then independent t-test was used to compare numeric data with normal distributed parameters between groups, while unnormal distributed parameters were tested using Mann-Whitney U test. For nominal data, we used a chi-square test. Pearson test was also applied to determine the correlation between variables. The sputum conversion rate of both groups was compared by Wilcoxon rank sum test. Logistic regression analysis was fitted to investigate significant covariates as factors in sputum conversion among: age, gender, history of previous vaccine or infection by presence of BCG scar, BMI, sputum AFB grade, and mean lession area. Statistical significance was considered if p value < 0.05.

Ethics

This study had already been approved by independent Ethics Committee of University of Indonesia, prior to trial initiation. The trial was performed in accordance with the Good Clinical Practice (GCP) and Declaration of Helsinki.

Results

This study involved 67 newly diagnosed pulmonary TB patients whose randomized blindly into two groups, 34 patients were received PNE capsules, 33 patients were received placebo capsules. All patients were also treated with standard first category of anti-tuberculosis regimens. No patients were dropped out during two months of study.

Baseline Characteristic

All samples had mean age 31.52 ± 11.24 years old, most of them were male with evenly distributed into two groups, on average they were underweight with mean BMI 16.77 in treatment group and 17.49 in control group,7 less than 50% had history of BCG vaccination which shown by BCG scar, and no hepatic or renal abnormality were record in the start of trial. Demographics as well as symptoms in both group were comparable prior to treatment. None of both groups were resistant to anti-tuberculosis drugs regimen. Between trials and controlled groups, there was no significant difference (p > 0.05) in all categories of baseline characteristics which showed in Table 3.

Categories	EPN Group	Placebo Group
Age	30.91 ± 10.95	32.15 ± 11.54
Sex, N (%)		
Male	20 (58,8)	22 (66.7)
Female	14 (41.2)	11 (33.3)
Weight (kg)	44.57 ± 5.54	42.66 ± 6.99
BMI (kg/m2)	16.77 ± 2.63	17.49 ± 2.29
BCG Scar, N (%)	14 (41.2)	14 (42.4)
Karnofsky score	88.9 ± 0.70	89.1 ± 0.69
Sputum smear	34 (100%)	33 (100%)
1+	18 (52.9)	18 (54.5)
2+	10 (29.4)	8 (24.2)
3+	6 (17.6)	7 (21.2)
Radiology score (mean)	2.26	2.30
1 (N)	5	5

2 (N)	15	13
3 (N)	14	15
Laboratory baseline		
Blood Glucose	86.13 ± 3.91	93.29 ± 8.21
SGOT	39.63 ± 2.24	44.78 ± 4.18
SGPT	28.30 ± 3.38	33.84 ± 3.68
Bilirubin (total)	0.707 ± 0.8	0.64 ± 0.6
Ureum	19.67 ± 1.49	20.5 ± 1.2
Creatinine	1.11 ± 0.345	0.71 ± 2.8
Protein	7.69 ± 0.158	7.73 ± 0.147
Albumin	3.59 ± 0.125	3.38 ± 9.34
Globulin	4.1 ± 0.139	4.39 ± 0.14

Table 3*: Sample Demographics.*

Sputum conversion

Negative sputum conversion were happened in both group as shown in table 4. Total 30 of 34 patients in treatment group going through negative sputum conversion, while 28 of 33 patients in controlled group going through negative sputum conversion, which is not significantly difference by statistical analysis. Greater difference between two groups were shown in the first week, when negative sputum conversion in treatment group were 52,9% and controlled group only 36% with also not significantly difference statistically, but has great clinical implication.

Week	EPN N, (%)		Placebo N, (%)		P value
1	18	(52,9%)	12	(36,4%)	0.172
2	21	(61,8%)	18	(54,5%)	0.549
3	23	(67,6%)	23	(69,7%)	0.856
4	23	(67,6%)	23	(69,7%)	0.856
5	26	(76,5%)	25	(75,8%)	0.318
6	28	(82,4%)	25	(75,8%)	0.507
7	28	(82,4%)	25	(75,8%)	0.507
8	30	(88,2%)	28	(84,8%)	0.684

Table 4: *Negative Sputum Conversion.*

Pulmonary Lesion

Radiologic resolution was seen in both groups in the second month of treatment. Additionally, chest x-ray was examined in the sixth month of treatment on patients who were still compliant to treatment, with a total of 31 samples from the treatment group and 29 samples from the control group. However, no significant difference between mean radiologic score in PNE and placebo groups were found. The radiologic evaluation scores are shown in Figure 1.

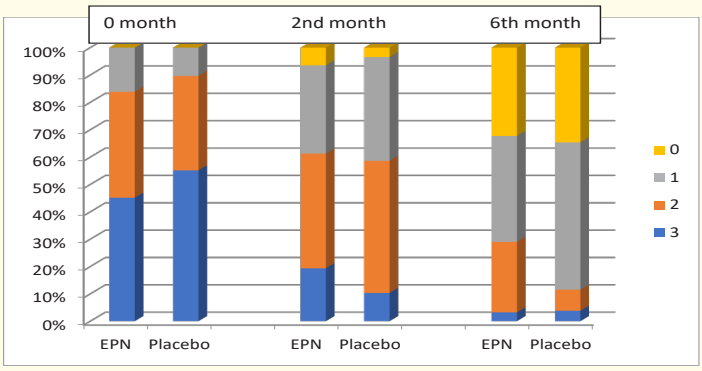

Figure 1: *Radiologic evaluation score.*

In addition, the relation between sputum conversion and this radiologic score were examined, and no relation was found between the two categories after the first two months of the study.

Cytokine Concentration

The concentration of IFN-Ɣ significantly increased in both groups following two months of treatment, but there was no statistically significant difference between the two groups. in addition, decreased TNF-α concentration was found in both groups, as seen in Table 5. However, the reduction in IL-6 concentration was greater in the placebo group. There was a correlation between the reduction in mean lesion score and the concentration of IL-6 in the PNE group.

	0 months		2 months	
Cytokines	EPN	Placebo	EPN	Placebo
TNF-α	17.31 ± 6.25	46.08 ± 21.69	4.34 ± 2.49	6.59 ±3.96
IFN-Ɣ	2.76 ± 0.70	2.32 ± 0.65	5.38 ± 1.90	5.03 ± 2.11
IL-6	27.57 ± 10.89	33.36 ± 9.82	16.19 ± 5.52	17.17 ± 7.80

Table 5: *Cytokines Profile.*

Clinical Assessment

Both, the PNE and placebo groups, showed improvement in body weight and increase in BMI value. However, no statistically significant difference was found between the two groups. The BMI in the PNE group was smaller from the start of the treatment. However, both groups also had subjects in the underweight BMI classification.

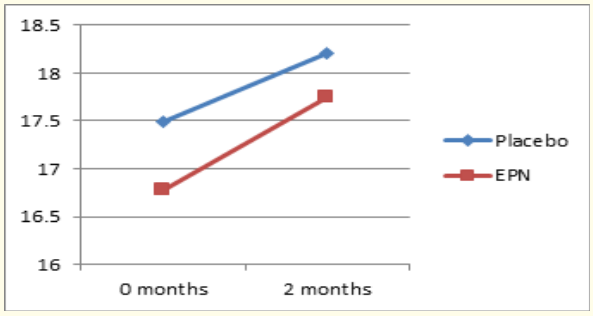

Figure 2: *Body Mass Index.*

There was no difference in the adverse events that occurred in both groups, indicating that six months of Phyllanthi extract supplementation is safe.

Discussion

Phyllanthus niruri is a well-known medicinal plant that has been used widely in Africa, Asia, and South America. It is known in many languages; as Stonebreaker (English), Chanca Piedra (Spanish), and Quebra Pedra (Portuguese), and meniran hijau (Bahasa). The extracts from this plant has been proven to have therapeutic effects, evident from *in-vitro, in-vivo*, and animals studies for antioxidant activity, immune modulating activity, antimicrobial activity, lipid lowering activity, antihyperglycemic effects, analgesic effects, and as having hepatoprotective properties [8,9].

In this study, we found that the treatment group had faster sputum conversion than the control group. After one week of treatment, more than half of the patients (52.9%) in the treatment group had undergone conversion, compared to 36.4% in the control group had ($p = 0.172$). Nevertheless, this difference has a great clinical implication because the common, old, but unsolved problem of loss to follow up cases of tuberculosis patients still remains. Therefore, faster sputum conversion will provide benefit at the community level by controlling the spread of tuberculosis from positive-sputum TB patients. Evident from the Tupasi TE., *et al*. study, more than 70% of patients are loss to follow-up during the intensive phase of anti-tuberculosis treatment in the Philippines [10]. In our setting, there were loss to follow up patients after a week or two weeks of anti-tuberculosis treatment, which is the time when patients experience improvement in their clinical symptoms. If they were loss to follow-up, at least, after achieving negative sputum conversion, they would be less contagious to others in the community.

Radiological resolution results were also not different between the treatment and control groups after 2 and 6 months of treatment. However, we found no correlation between the rate of bacilli elimination, as shown by sputum conversion, and the reduction of mean lesion area after two and six months of treatment. On the other hand, severe lung damage or lung lesion is correlated with oxygen free radicals, and tuberculosis augments the level of free radicals as macrophages eliminate the mycobacteria [11]. Therefore, use of an antioxidizing agent may show benefit in this situation. *In-vitro* studies show that PNE aqueous extracts significantly scavenges reactive oxygen species (ROS) such as DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis3-ethylbenzothiazoline-6-sulfonic acid) free radicals, and reduces ferric ion free radical activity, which directly protects the organs and indirectly provides a better immune response [8].

Using PNE supplementation to standard anti-tuberculosis treatment may also benefit immunodepressed patients, whether it is primary condition in the patient or it has occurred as a result of TB disease. In some studies, *Phyllanthus niruri* showed both specific and non-specific, cellular and humoral immunomodulator activity. PNE is able to activate immune responses such as the proliferation of PBMC by more than four-fold (450.5%) as compared to controls; proliferation of B and T lymphocytes, increased expression of surface activation marker (CD69), enhanced production of IL-4 and IFN-Ɣ, as well as the activation of macrophages and the maturation of dendritic cells [8,9,13].

The increase in IFN-Ɣ was also seen in this study, though there was no significant difference between the control and treatment groups. Additionally, no correlation was found between positive AFB index and the concentration of IFN-Ɣ. However, in this study, TNF- α tend to decrease in both groups. In contrast, the Nworu CS., *et al. in-vitro* study reported an increase in TNF- α as macrophages were activated within as a result of PNE stimulation, thus allowing effective phagocytosis [13]. However, in the Nikolaeva clinical study, lower TNF-α showed a better clinical outcome, a finding which has yet to be explained [12]. Further, it is speculated that the lower cytokine levels were found in the second month of this study does not represent the role of cytokines in eliminating mycobacterium, as most patients in the treatment group had experienced sputum conversion during the first and second week of this study. Hence, assessing cytokine levels in the first and second weeks of treatment would represent a clearer picture of the role of cytokines in the elimination of *Mycobacterium tuberculosis*.

Frequently in active pulmonary TB, hyperglobulinemia occurs, and a high level of acute phase proteins are synthesized, thus elevating the IL-6 levels. In this study, the concentration of IL-6 decreased in both groups after the first 2 months, with lower concentrations reported in the PNE group. IL-6 is produced by macrophages when TNF-α increases and stimulates the production. Higher the level of TNF-α, more macrophages are stimulated and more IL-6 is produced, so phagocytosis would occur faster. With logistic regression analysis, we found that higher IL-6 levels is correlated with higher negative-sputum conversion (RR = 0.14).

It has been said that standard anti-tuberculosis drugs does not completely eliminate mycobacterium, in the absence of an effective immune system. Thus, using supplementary immunomodulators should give benefit. However, comparing this result with the bivariate analysis, no significant difference was found between the treatment and the control group. Therefore, we suggest that both groups have an effective immune response, because other factors that would affect the immune system, such as geriatric age, severe malnourishment, diabetes mellitus, HIV, etc., were excluded in this study. In an unpublished study on the role of PNE-stimulated specific immune responses in mice, also it was found that PNE may increase the levels of tetanus toxoid IgG significantly in the fasting-treatment group mice better than in the fasting control mice. However, there was no significant difference between the groups of mice receiving PNE supplementary but not fasting, than in the group of mice not fasting and not receiving PNE supplementary. Further, logistic regression analysis was performed to identify any confounding factors to the first week AFB sputum conversion in the patient demographic characteristics such as variant of age, sex, history of BCG vaccine, history of TB contact, supplementary PNE, BMI, AFB positive-index, lung lesion size, and cytokine levels. It was found that adjuvant PNE to standard anti-tuberculosis therapy has RR = 0.32, indicating that PNE has a likelihood to enhance AFB conversion three times faster than treatment with standard anti-tuberculosis drugs alone.

Side effects and reversed wasting syndrome were similar in both groups. There was complaint of itchiness, painful joints, and erythema, distributed relatively evenly between the two groups. Hence it may be deducted that PNE is safe as there were no additional side effects as compared to the placebo group, when it is given in adjunct to standard anti-tuberculosis therapy. However, this study also did not investigate any hepatoprotective activity of PNE against drugs induced hepatotoxicity, which has been shown to occur in some animals and *in-vivo* studies. The body weight of all subjects had an increasing pattern, however that the mean BMI of both groups were still underweight, thus necessitating further observations regarding this parameter.

Conclusion

The addition of adjunctive PNE to standard anti-tuberculosis drugs showed faster AFB sputum conversion, even at the first week of treatment. There was no significant statistical difference (p = 0.172), but the clinical implications are still notable. Earlier sputum conversion may lead to less spreading of the bacteria to the environment and fewer new infected cases, especially with regard to those patients who were loss to follow up. PNE supplementation is safe, even when consumed simultaneously with standard anti-tuberculosis drugs.

Conflicts of Interest

All authors have no potential conflict of interest relevant to this article.

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