CHEMICAL SUBSTANCE OF MORINGA LEAF (MORINGA OLEIFERA) AS AN IMMUNOMODULATOR (EXPRESSION OF IFN-Γ AND IL-12) IN MYCOBACTERIUM TUBERCULOSIS INFECTION

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Abstract

Tuberculosis is a bacterial infection disease Mycobacterium tuberculosis which is usually transmitted through inhalation of droplets containing bacteria. Moringa oleifera is an alternative to overcome the problem of healing TB patients. M. oleifera leaf extract caused significant immunostimulatory effects on the cell-mediated and humoral immune system in Wistar albino rats. The design of this study was randomized posttest only design with a control group, which was conducted on 24 male mice. In the second week, the first group were infected with *Mycobacterium tuberculosis* and the second group were not infected. In the third week, the first group was divided into two groups, 6 mice were given Moringa leaf extract and 6 mice were not given. The second group was also divided into two groups, 6 mice were given Moringa leaf extract (KPE) and 6 mice were not given. At the tenth week, each group of mice was measured for IFN-γ, IL-12. Data were analyzed using Mann-Whitney U test (for IFN-γ) and independent sample t-test (for IL-12). The p-value for IFN-γ and IL-12 were less than 0.05. This study concluded that administration of moringa leaf extract acts as an immunomodulator in increasing cellular immunity (IFN-γ and IL-12).

Keywords: Mycobacterium tuberculosis; Moringa oleifera; immunomodulator

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1. Introduction

Tuberculosis (TB) is a bacterial infection disease *Mycobacterium tuberculosis* which is usually transmitted through inhalation of droplets containing bacteria. The World Health Organization (WHO) estimates that around 10 million people were diagnosed as new sufferers of TB in 2017 [1]. TB remains the leading cause of death worldwide among other infectious diseases. Indonesia is in third place for TB morbidity, while East Java is in second place for Indonesia [2]. TB and the Human Immunodeficiency Virus (HIV) can place a large burden on the health care system and are a major challenge for diagnostic and therapeutic programmes. Infection with HIV is the most common cause of *Mycobacterium tuberculosis*, which can accelerate the risk of reactivation of latent TB by up to 20 times. The emergence of strains that are resistant to anti-tuberculosis drugs indicates the need to find new drugs from local materials [3].

Moringa leaf (*Moringa oleifera*) is a plant that is rich in nutrients as a source of protein, fatty acids, minerals and vitamins for animal and human feed formulations, which can be used to improve health and nutrition [4]. Diet and nutrition are important factors in the promotion and maintenance of health. *Moringa oleifera*, known as the "Magic Tree", is one of the healthiest and most nutritious foods found in nature. Countless studies have described the benefits of Moringa leaves, pods, seeds and flowers. The potential health impact of phococlexes from African food crops in the context of cross-kingdom and endogenous microRNA regulation on improving the health and overall economic well-being of the continent is estimated to be enormous [5].

The body's defense against TB germs is played by cell mediated immunity (CMI = cellular immunity), namely T lymphocytes and macrophages. In CD4 T cells there is cell polarization based on the cytokine profile it produces, namely Th1 and Th2 cell groups. Th1 cells produce interleukin-12 (IL-12) and IFN-γ which have a protective role because they strengthen macrophages to kill and digest bacteria that have been phagocytized. Continuous exposure to mycobacterial antigens will increase memory immunity, causing delayed type hypersensitivity which is destructive to lung tissue [6].

Immunomodulatory therapy in the treatment of TB is divided into three groups according to their function and role. The first is the group that increases the Th1 immune response, the second is the group that suppresses the Th2 immune response and the third is the group that suppresses cytokines [7]. Methanol and ethanol from *Moringa oleifera* exhibit well-known therapeutic activity. This review explores and focuses on the phytochemical composition and various pharmacological activities such as immunomodulatory, antidiabetic, antiulcer, anti-inflammatory, analgesic, antiepileptic, cardioprotective, lipid-lowering, antihypertensive and antimicrobial [3].

Evaluation of the antimycobacterial efficacy of 10 ethnomedicinal plants against one clinical isolate of *Mycobacterium tuberculosis* and TB strain H37RV, the bacteria did not become resistant to phytochemicals, because of their complex structure. The goal of pure phytochemical isolation against MDR TB strains is rational and achievable, especially for A. vasica, and *Moringa oleifera* plants [8]. The incidence of TB patients with MDR is increasing, so Moringa oleifera is an alternative to overcome the problem of healing TB patients. M. oleifera leaf extract caused significant immunostimulatory effects on the cell-mediated and humoral immune system in Wistar albino rats [9].

2. Methods

This study was a true experimental study with a randomized posttest only design with a control group, which was conducted on 24 male mice aged 8-10 weeks, which were randomly allocated to two groups. In the second week, the first group (12 mice) were infected with Mycobacterium tuberculosis and the second group (12 mice) were not infected with Mycobacterium tuberculosis. In the third week, the first group was divided into two groups, namely 6 mice were given Moringa leaf extract (PPE) and 6 mice were not given Moringa leaf extract (KP). The second group was also divided into two groups, 6 mice were given Moringa leaf extract (KPE) and 6 mice were not given Moringa leaf extract (K). At the tenth week, each group of mice was measured for IFN-y, IL-12.

For the data that had been collected, a normality test was carried out, namely the distribution distribution test, followed by the Kolmogorov-Smirnov normality test, which shows that the IFN in all groups was not normally distributed so that it was followed by a difference test using the Mann-Whitney U test; while IL-12 was normally distributed so that it was continued with the difference test using the independent sample t-test.

3. Results

Table 1 shows the results of ELISA examination of serum IFN-y levels in the treatment group infected with *Mycobacterium tuberculosis* and given Moringa leaf extract (PPE), the group infected with *Mycobacterium tuberculosis* (KP), the control group given Moringa leaf extract (KPE) and the control group receiving not given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (K).

Table 1: Results of examination of IFN-y levels (pg/ml)

No	Treatment group with Moringa leaf extract (PPE) (p/g/ml)	Treatment group with Mycobacterium tuberculosis (KP) infection (pg/ml)	Control group with Moringa leaf extract (KPE) (pg/ml)	Control group (K) (pg/ml)
1	1.174	0.754	0.866	0.824
2	1.098	0.77	0.979	0.870
3	1.118	0.705	0.934	0.987
4	1.054	0.731	0.965	0.911
5	1.021	0.794	0.965	0.898
6	1.047	0.717	0.924	0.946

Table 2 shows the results of ELISA examination of serum IL-12 levels in the treatment group infected with *Mycobacterium tuberculosis* and given Moringa leaf extract (PPE), the group infected with *Mycobacterium tuberculosis* (KP), the control group given Moringa leaf extract (KPE) and the control group receiving not given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (K).

Table 2: Results of examination of IL-12 levels (pg/ml)

No	Treatment group with Moringa leaf extract (PPE) (p/g/ml)	Treatment group with Mycobacterium tuberculosis (KP) infection (pg/ml)	Control group with Moringa leaf extract (KPE) (pg/ml)	Control group (K) (pg/ml)
1	1.174	0.754	0.866	0.824
2	1.098	0.77	0.979	0.870
3	1.118	0.705	0.934	0.987
4	1.054	0.731	0.965	0.911
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6	1.047	0.717	0.924	0.946

From the results of the ELISA examination of the levels of IFN-y, IL-12 in the control group treated with Moringa leaf extract (KPE) and the control group (K) statistical tests were carried out to determine differences in immune responses both given Moringa leaf extract and those not given Moringa leaf extract.

Table 3: Differences in IFN-y and IL-12 levels in mice between the control group and the KPE group

	KPE				K				p
	Min	Max	Mean	SD	Min	Max	Mean	SD	
IFN-y	0.90	1.00	0.94	049	0.61	0.68	0.64	0.02	0.002
IL-12	0.87	0.98	0.93	0.41	0.82	0.99	0.90	0.56	0.395

The mean for IFN-y levels in mice that were given Moringa leaf extract and not infected with Mycobacterium tuberculosis (KPE) was 0.94. While the mean for IFN-y levels in mice that were not given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (K) was 0.64; with p value of difference test result = 0.002. The mean for IL-12 levels in mice that were given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (KPE) was 0.93. While the mean for IL-12 in mice that were not given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (K) was 0.90, with a p value of difference test results = 0.395.

Table 4: Differences in IFN-y and IL-12 levels in mice between the PK group and the K group

	KPE				K				p
	Min	Max	Mean	SD	Min	Max	Mean	SD	
IFN-y	0.62	0.76	0.71	0.04	0.61	0.68	0.64	0.02	0.026
IL-12	0.71	0.79	0.74	0.33	0.82	0.99	0.90	0.56	0.000

The mean for IFN-y levels in mice that were not given Moringa leaf extract and infected with *Mycobacterium tuberculosis* (PK) were 0.71; while the mean for IFN-y levels in mice that were not given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (K) was 0.64; with p value of difference test result = 0.026. The mean for IL-12 in mice that were not given Moringa leaf extract but infected with *Mycobacterium tuberculosis* (PK) was 0.74; while the mean for Il-

12 in mice that were not given Moringa leaf extract and not infected with *Mycobacterium tuberculosis* (K) was 0.90; with the p value of the difference test result = 0.000.

Table 5. Differences in IFN-y and IL-12 levels in PK and PPE group mice

	PPE				PK				Sig
	Min	Max	Rerata	SD	Min	Max	Rerata	SD	
IFN-y	0.98	1.25	1.13	0.09	0.9	1.00	0.71	0.45	0.009
IL-12	1.02	1.17	1.08	0.05	0.87	0.98	0.74	0.41	0.000

The mean for IFN-y levels in mice given Moringa leaf extract and infected with *Mycobacterium tuberculosis* (PPE) was 1.13; while the mean for IFN-y not given Moringa leaf extract and infected with *Mycobacterium tuberculosis* (PK) was 0.71; with p value of difference test result = 0.009. The mean for IL-12 levels in mice given Moringa leaf extract and infected with *Mycobacterium tuberculosis* (PPE) was 1.08; while the mean for IL-12 levels that were not given Moringa leaf extract and infected with *Mycobacterium tuberculosis* (KPE) was 0.74; with p value of difference test result = 0.000.

4. Discussion

The results of this study showed that serum levels of IFN-γ were lower in the control group than in the group infected with *Mycobacterium tuberculosis*, and there was a statistically significant difference. This is consistent with a previous study by Ribeiro-Rodrigues et al. [10] on 15 new pulmonary TB patients during June to December 2000 in Brazil and compared them with 7 healthy controls. Interferon levels were checked from sputum and serum using the ELISA kit. The results they got were: the control group had low serum interferon levels, while pulmonary TB patients had higher serum interferon gamma levels, and there was a relationship between increased serum IFN-γ levels and the degree of sputum smear positivity. This is probably because serum levels of IFN- increase when there is a reaction to *Mycobacterium tuberculosis* infection so that IFN- levels increase to activate macrophages that kill Mycobacterium tuberculosis.

The results of this study showed that IL-12 levels were higher in the control group than the group infected with *Mycobacterium tuberculosis*, and there was a relationship. This is in accordance with the results of Linawati's study [11], which showed that the secretion of IL-12 in macrophage cultures of healthy individuals at risk of pulmonary tuberculosis after 24 hours of infection with *Mycobacterium tuberculosis* (3.156 ng/ml) was higher than in patients with pulmonary tuberculosis (1,593 ng/ml) and IL-12 secretion in macrophage culture of healthy individuals was more at risk of pulmonary tuberculosis after 48 hours of infection with *Mycobacterium tuberculosis* (3.446 ng/ml) than patients with pulmonary tuberculosis (1.8 ng/ml). This indicates that the greater bacterial load will suppress the production of IL-12. The decrease in IL-12 production causes low IFN-γ levels, the biological effect of IL-12 is to stimulate the production of IFN-γ by NK cells and T cells and IL-12 also increases the cytolytic function of NK cells, due to this cytolytic role. To fight *Mycobacterium tuberculosis*, IL-12 levels are reduced in the group infected with *Mycobacterium tuberculosis*.

The results of this study showed an increase in IFN-γ levels in mice that were given Moringa leaf extract and were infected with *Mycobacterium tuberculosis* (PPE) compared to mice that were not given Moringa leaf extract and were infected with *Mycobacterium tuberculosis* (KP), and statistically there is a relationship. In previous studies it was said that there were 2 pathways for the production of IFN-γ that had been discovered using CD4+ T cells, namely: (i) T cell receptor (TCR) mediated antigen-dependent pathway, cyclosporine sensitive, and (ii) cytokine-induced, cyclosporine-insensitive pathways. Experimentally, IFN-γ production can also be induced using stimuli that mimic activation via TCR, which include the use of mitogens (such as concanavalin A or phytohemagglutinin), cross-linking antibodies, or pharmacological agents (such as a combination of phorbol myristate acetate and calcium ionophore). In non-activated (resting) T cells, the IFN-γ gene is not expressed so that the protein cannot be detected. However, after activation of T cells, IFN-γ can be detected within 6-8 hours, its levels will reach a maximum level at 12-24 hours, and then will decrease back down to baseline values [12].

Thus, the effect of Moringa leaves is as a stimulus (imonomodulator) that can enhance the cellular immune response to increase IFN-γ production by using T cells so as to limit the intracellular growth of Mycobacterium tuberculosis.

There are several strategies for using immunomodulators as adjunctive therapy in the treatment of tuberculosis: (1) Using Th-1 which triggers the production of Th-1 type cytokines such as IFN-γ, IL-12, IL-8, GM CSF and TNF-alpa. (2) Using Th-2 type cytokine inhibitors especially TGF-beta and IL-10. (3) Using non-cytokine imonomodulators [7].

The results of this study provide important information that the effect of giving Moringa leaf extract to mice infected with *Mycobacterium tuberculosis* can increase the intracellular immune response by increasing IFN-γ and IL-12. This finding is consistent with the measurement of increased IFN-γ levels in the group given Moringa leaf extract and infected with *Mycobacterium tuberculosis*. Also, the measurement of IL-12 levels increased in the group given Moringa leaf extract and infected with *Mycobacterium tuberculosis*. This is in accordance with previous research which stated that Moringa leaf extract (*Moringa oleifera*) has an effect as an immunomodulator, namely immunostimulam [13].

The results of this study provide information that an increase in IFN-γ and IL-12 in TB patients who receive Moringa leaf extract can be used as an adjuvant in the success of countermeasures to increase the recovery of TB patients. This finding provides information that administration of moringa leaf extract plays a role in increasing cellular immunity (IFN-γ. IL-12).

The results of this research further enrich the results of scientific studies in the health sector related to chemical substances, as well as previous findings with various focuses such as the impact of chemicals on workers' health [14,15], chemicals for organ restoration [16], and others.

5. Conclusion

This study concluded that administration of moringa leaf extract acts as an immunomodulator in increasing cellular immunity (IFN-γ and IL-12).

6. References

WHO. Tuberculosis Global Tuberculosis Report 2017. Geneva: WHO; 2017.

Kemenkes RI. Riset Kesehatan Dasar (RISKESDAS). Jakarta: Kemenkes RI; 2018.

Lakshmana Prabu, Sakthivel. Phytopharmacological potential of the natural gift Moringa oleifera Lam and its therapeutic application: An overview. Asian Pacific Journal of Tropical Medicine. 2019.

Busani M, Patrick A. Nutitional characterization of Moringa (Moringa Oleifera Lam) Leaves, South Africa. 2011.

Matic, Ivana. Investigation of medicinal plants traditionally used as dietary supplements: a review on Moringa oleifera. Journal of Public Health in Africa; 2018.

Siagian H. The Role of Vitamin D against to Lung Damage on Infected Mice by Mycobacterium tuberculosis. Chemistry and Materials Research. 2011;7:50-56.

Siagian H, Christyaningsih J. The effect of vitamin D on the lung tissue damage in mice infected with Mycobacterium tuberculosis. Research Journal of Pharmaceutical. Biological and Chemical Sciences. 2015;6:460-465.

Rath, Shakti. Study on Antimycobacterian properties of Selected Ethnomedicinal Plants: An alternative Therapeutic Choice Against Multidrug Resintant Mycobacterium tuberculosis. Indian Journal of Public Hralth Research & Development. 2018.

Nfambi, Joshua. Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats. Journal of Basic & Clinical Physiology & Pharmacology. 2015.

Ribeiro-Rodrigues R, Resende Co T, Rojas R, Toossi Z, Dietze R, Boom WH, Maciel E, Hirsch CS. A role for CD4+CD25+ T cells in regulation of the immune response during human tuberculosis. Clinical and Experimental Immunology. 2006;144(1):25–34.

Linawati NM, Sukrama DM, Mertaniasih M. The influence of Euphorbia milii Flower Extract in the activity of Th17 through IL-17 Secretion In Mycobacterium tuberculosis Infected Mice. International Journal of Science and Research. 2014;626-627.

Wahyuni N. Peran Interferon Gamma Pada Infeksi. Jurnal Kedokteran Syiah Kuala. 2017;17(2):126-132.

Dillasamola, Dwisari A, Fakhri Y, Skunda M, Skunda P. Immunomodulatory effect test from moringa leaf extract (Moringa oleifera l.) with carbon clearance method in male white mice. Asian Journal of Pharmaceutical and Clinical Research. Biomechy & Noverial. 2018;11.

Rajan AP, Dayalan MSD. Assessment of the work-related health status of iron industry workers. European Chemical Bulletin. 2015;4(6):303-305.

Azab A. Assessment of the work-related health status of iron industry workers. European Chemical Bulletin. 2017;6(10):456-469.

Subramanian EMG, Kumar KS, Vignesh R. Assessment of the work-related health status of iron industry workers. European Chemical Bulletin. 2022;11(1):12-16.