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Original Article

Toll Like Receptor 4 Expression Profile in Mice Infected Mycobacterium Tuberculosis Given with Miana Leaves Extract (*Coleus scutellarioides* (L.) Benth) (Tuberculosis Preventive and Curative Mechanisms)

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ABSTRACT

Introduction: Tuberculosis is an infectious disease of the respiratory tract caused by Mycobacterium tuberculosis. During Mycobacterium tuberculosis infection, pathogens modulate TLR-4 receptor flow signaling, suggesting possible involvement of TLR-4 in the regulation of the host immune response. This study aims to determine the effect of miana leaf extract on the expression of toll like receptor 4 (TLR-4) in tuberculosis mice at the preventive and curative stages. **Methods**: Mice were divided into 3 groups with 7x replication. Providing 14 days of preventive treatment, 14 days of incubation and 14 days of curative treatment. Group 1 and group 3 were given Miana Leaves Extract (EDM) and placebo at all three stages. Group 2 was given EDM at the preventive and incubation stages, then they were given anti-tuberculosis drugs (OAT). **Results**: The results proved that EDM given as a preventive did not increase the expression of TLR-4 protein in healthy mice; Changes in expression of TLR-4 protein in M.tb-infected mice before and after curative EDM increased by 17%, after administration of placebo increased 97% and decreased 12% after OAT curative administration; Changes in expression of TLR-4 protein in M.tb-infected mice before preventive administration and after EDM curative administration increased by 20%, after administration of placebo increased 102% and decreased by 10% after the curative administration of OAT. **Conclusions**: EDM has potential as antituberculosis with TLR-4 regulatory mechanism.
Key words: Miana Leaf, TLR-4, Antituberculosis.

INTRODUCTION

Tuberculosis is a respiratory tract infection caused by Mycobacterium tuberculosis (M.tb). M. tb bacteria through the inhalation process infects the host and causes the host to be infected with tuberculosis and has caused millions of deaths in various countries including Indonesia.[1-5] In addition to using anti-tuberculosis drugs, the tuberculosis healing process can also be complementary alternative medicine that involves the immune mechanism.[6]

Infection control of various pathogens such as M. tb depends on introducing the pathogen to lipopolysaccharides and activating innate and adaptive immune responses.[7-14] Toll-like receptor (TLR) shows an important role in binding of several pathogens. Mycobacterial antigens recognize different TLRs that result in activation of innate / nonspecific immunity cells. The results of in vitro and in vivo investigations, show that TLR activation depends on the innate immune response,[15] whereas the induction of adaptive immunity to mycobacteria does not change against TLR.[16] Likewise, the miR-708-5p mechanism regulates mycobacterial vitality and inflammatory response in human macrophages by targeting TLR-4.[17]

During Mycobacterium tuberculosis infection, pathogens modulate TLR-4 receptor flow signaling, which suggests the possible involvement of TLR-4 in the regulation of host immune responses. Mycobacterium indicus pranii (MIP) has immunomodulatory properties that induce a proinflammatory response *via* induction of TLR-4 mediated signaling, thus it was concluded that TLR4 could represent a new therapeutic target for activation of the innate immune response during tuberculosis infection.[18]

Macrophage activity against Mycobacterium tuberculosis has been shown to involve Toll-like receptor (TLR) activation and production of ROS. Lipopolysaccharide (LPS) is able to limit the negative effects of M.tb and increase bactericidal activity including phagocytosis, production of ROS, and destruction of M.tb. However, all these mechanisms are disrupted when TLR4 is inhibited.[19]

The process of preventing the spread or killing of M. tb bacteria in infected hosts occurs by the T-lymphocyte response mechanism to proliferate and stimulates CD4 T-cells especially the helper 1 T-cell subset to produce IFN-γ, TNF-α and several other cytokines such as IL. Furthermore, TNF-α will activate macrophages to form granulomas and carry out the process of killing bacteria.[20] Leaf extract of Miana (EDM) (*Coleus scutellarioides* (L.) Benth.) Has been shown to have potential as a preventive and complementary curative for tuberculosis by increasing the proliferation of T lymphocytes, CD4, IFN-γ, TNF-α thus reducing the number of M. tb colonies in the lungs.[21]

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Various attempts have been made by researchers to find solutions to eradicate tuberculosis, especially M.tb which has been resistant to standard drugs. Many studies have been conducted intensively to find new anti-tuberculosis drugs that can overcome M.tb drug resistance, tuberculosis infection in HIV patients and the risk of drug side effects due to the use of interacting tuberculosis drugs. One example of a drug that has been found is Delamanid with a mechanism of inhibiting mycobacterial cell wall synthesis and has been approved for use by the European Medicine Agency (EMA) for the treatment of MDR-TB. Preclinical and clinical studies have shown that delamanid is very potential as an antituberculosis with a small risk of drug interactions with better tolerability.[22] These findings provide an idea to observe the effectiveness of herbal medicines that have been used empirically as antituberculosis by scientifically proven.

Miana leaf extract has been shown to be an immunomodulator, like Mycobacterium indicus pranii (MIP) which also has immunomodulatory properties that support the elimination of M.tb in macrophages through the TLR-4 receptor. In addition, EDM has also been proven as a hepatoprotector against anti-tuberculosis drugs in the treatment of tuberculosis.[23] So that research on the function of EDM as a preventive and curative in the treatment of tuberculosis based on the TLR-4 mechanism needs to be done.

MATERIALS AND METHODS

Preparation of test materials

Miana leaf test material was obtained from Tana Toraja Regency, South Sulawesi Province, Indonesia. The fresh leaves are sorted, chopped and then dried in an oven at 40-45^0 C. The dried simplicia was extracted by maceration method and solvent evaporation using an evaporator. The thick extract of miana leaves obtained was used to identify alkaloids, saponins, flavonoids, tannins and polyphenols based on phytochemical screening and *in vivo* testing on mice (*Mus musculus*). The treatment dose of miana leaf extract was 714mg/kg body weight of mice.[21] The comparison test material used anti-tuberculosis drugs (OAT) with the composition of rifampin 150 mg, ethambutol 275 mg, isoniazid 75 mg and pyrazinamide 400 mg. The dose of OAT treatment was calculated based on the conversion of the human dose to mice, namely 78mg/kg body weight of mice.

Identification of active compounds in miana leaves based on phytochemical screening

Phytochemical screening test using a color reaction with the appropriate reagent. **Alkaloid Test**. Extract ± 0.5 g + 2 mL ethanol 70% shaken + 5 mL HCl 2N, heated then added 3 drops of Mayer reagent to form a precipitate. **Saponin Test**. Extract ± 0.5 g + 2 mL 70% ethanol, shaken + 10 mL distilled water and shaken, then allowed to stand for 20 minutes. Then the solution causes foam. Tannin Test. Extract ± 0.5 g + 2 mL ethanol 70% shaken + FeCl3 1% 3 drops, a blue-black color occurs if the tannin compound is wrong and green-black color if the tannin compound is catechins. **Flavonoid Test**. Extract ± 0.5 g + 2 mL ethanol 70% shaken + 0.5 g magnesium powder and 3 drops of concentrated HCL. Formation of orange to red color indicates the presence of flavones, red to bright red indicates flavanols, bright red to purplish red indicates flavanones. **Polyphenol Test**. Ethanol extract + 10mL water and heated for 10 minutes, cooled and filtered. 3 drops of filtrate + FeCL3 will form a purple to blue solution. Tannin Test. Extract 1 ml + 3 drops of FeCl3 a green-blue black precipitate is formed. **Glycoside Test.** Extract 1 ml + 10 ml H2SO4 50% then heated for 15 minutes. Next + Fehling's solution and heated until a brick red precipitate is formed. **Steroid Test**. Extract 1 ml + 10 ml concentrated H2SO4 will form a reddish solution.

Analysis of the total content of polyphenols and flavonoids

Testing the amount of polyphenol and flavonoid content is calculated based on the results of the absorption spectrophotometer. Total polyphenols based on gallic acid standards and total flavonoids were calculated based on the comparison of quercetin.

TLR-4 expression test after preventive and curative treatment

This research was conducted with the recommendation of ethical research approval from the Makassar Health Polytechnic of the Ministry of Health Number: 00159 / KEPK-PTKMKS / III / 2020. The study used 21 mice (Mus musculus) which were divided into 3 groups, namely Group K 1 (EDM), The K2 group (OAT, namely a combination of rifampin, ethambutol, isoniazid and pyrazinamide) and the K3 group (Placebo). The treatment of each group is as follows:

Group K 1: oral EDM (miana leaf extract) treatment once a day for 14 days (day 1-14) with a preventive dose of 357 mg / kg BW of mice. Then on the 14th day the mice were infected with M.tb suspension intraperitonium (equivalent to 10,000 colonies). Furthermore, the incubation period of 14 days (days 15–28) mice were still given EDM treatment with a preventive dose of 357 mg / kg of mice orally once a day.

Group K 2: oral EDM treatment (miana leaf extract) once a day for 14 days (day 1-14) with a preventive dose of 357 mg / kg BW of mice. Then on the 14th day the mice were infected with M.tb suspension intraperitonium (equivalent to 10,000 colonies). Furthermore, the incubation period of 14 days (days 15–28) mice were still given EDM treatment with a preventive dose of 357 mg / kg of mice orally once a day. Furthermore, 14 days (29-42 days) mice were given further treatment of anti-tuberculosis (OAT) drugs equivalent to Rifampisisn dose of 78 mg / kg orally once a day.

Group K 3: oral placebo (Na CMC) treatment once a day for 14 days (day 1-14). Then on the 14th day the mice were infected with M.tb suspension intraperitonium (equivalent to 10,000 colonies). Furthermore, the incubation period of 14 days (days 15–28) mice were still given a placebo (Na CMC) orally once a day. Furthermore, 14 days (29-42 days) mice were given further treatment with placebo (Na CMC) orally once a day.

The first test blood sample was taken on day zero before the mice received treatment. The second test blood sample was taken on the 14th day (after being given preventive treatment) and the third test blood sample was taken on the 43rd day (after being given curative treatment). TLR4 testing using the ELISA method with Mouse Toll Like Receptor 4 (TLR4) ELISA Kit Cat. No: MBS026966. The data were analyzed by SPSS ANOVA and Least Significant Different method.

RESULTS AND DISCUSSION

Miana leaf extract yield and screening results

The simplicia of miana leaves from Tana Toraja Regency was macerated using 96% ethanol solvent and the yields listed in table 1 were obtained.

The results of phytochemical screening of the extract showed that the content of chemical compounds: alkaloids, glycosides, steroids, saponins, phenols, tannins, flavonoids. Polyphenol content 20.35mg / g EDM (gallic acid standard) and flavonoid content 7.35mg / g EDM (quercetin standard).

Test results of blood samples from mice

Blood collection of mice was carried out in 3 stages, namely

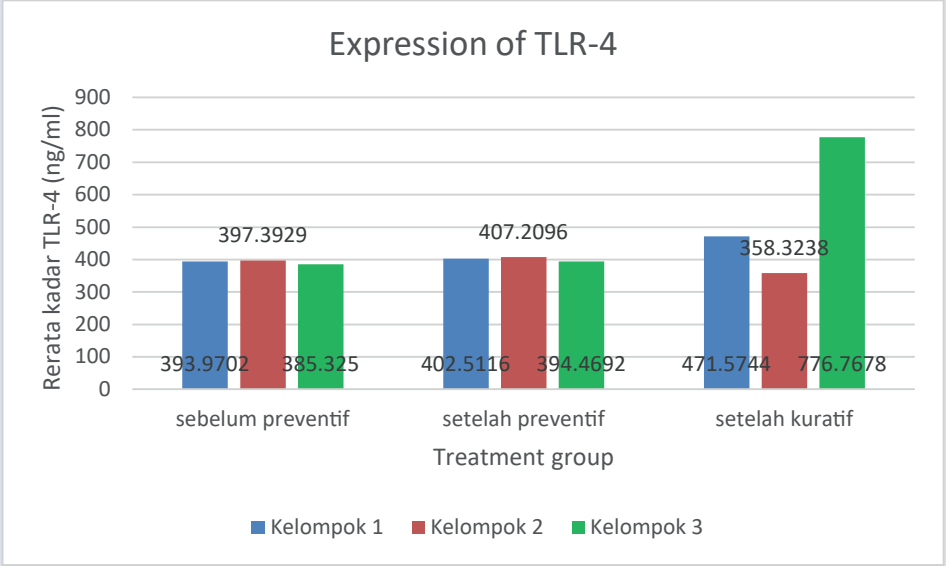

Figure 1: Expression of TLR-4 at each treatment stage.

Table 1: Rendement of simplicia and miana leaf extract.

Source of miana leaf	Weight (grams)				Percentage
	wet leaves	Extract yield	simplicia	extract	Extract yield
Tana Toraja Regency	4.000 grams	621 grams	500 grams	78.08 grams	15,62%

Stage 1 (blood sample 1) day zero before the mice were given preventive treatment.

Stage 2 (blood sample 2) on the 14th day after the mice received preventive treatment before the mice were given Mycobacterium tuberculosis infection.

Stage 3 (blood sample 3) on the 42nd day after the mice experienced the incubation phase of infection and were given curative treatment.

DISCUSSION

Research has been carried out using test animals Balb C strain, as one of the suitable mammals for use in preclinical testing. Mus musculus (mice) are called house mice and belong to the group of mice. The use of rat test animals, especially transgenic mice, is often used for preclinical testing, especially the body's immune mechanism because there is a similarity between the human immune system and mice, namely having strong natural immunity.[24] In addition to selecting the right experimental animal, the number of uses of tested animals also plays a very important role in determining the quality of research data. This study used replication of 7 mice tested animals according to the results of the calculation of the number of replications for a sample of 3 groups. The calculation of the number of samples plays a role in the research design including studies using test animals. If replication using small numbers of animals tends to produce non-significant data. However, if the number of replications is too large, it will be problematic with research ethics due to waste of test animal resources.[25]

One of the factors that is a critical point in this study is the process of taking blood from test animals. Since blood sampling is carried out for up to 3 periods during the treatment period, the animal must be made comfortable or avoid stress. The stress level of the tested animals can cause changes in the physiology and immunology of the tested animals so that it has an impact on the measured test parameter values of each test animal. Collecting blood samples from experimental animals should not stress the animals, which in turn has an impact on the results of the study. In this case, a permit from the Institute's Animal Ethics Committee is required to be submitted and approved before conducting treatment.[26] This study also obtained approval from the institutional ethics commission before starting treatment.

The selection of miana leaf test material is a part of the miana plant obtained from Tana Toraja Regency. The basis for selecting the source of the test material is based on the content of active substances and antioxidant potential, the potential for previously proven immunomodulators.[21] The results of the screening test in this study proved that the secondary metabolites in EDM were steroids, glycosides, saponins, phenols, flavonoids, alkaloids and tannins. Secondary metabolites in EDM function as antibacterials with various mechanisms such as immunostimulants, cytokine immunodepressants, inhibiting cell DNA synthesis, agglutinating proteins, damaging cell wall membranes.[23,27-29] The use of OAT as antituberculosis takes 3 months, 6 months and even 9 months. The length of the treatment period allows side effects that cause treatment to be interrupted and cure is not achieved. Overcoming this requires herbal medicine that can support safe long-term treatment of tuberculosis. Previous research stated that EDM has the potential to be a hepatoprotector against liver function and organ damage in tuberculosis treatment using rifampin.[23] Furthermore, also concluded the EDM toxicity with an LD50 value of ethanol extract of 9757.14 mg / kg body weight of mice.[30]

This study discusses the effect of giving miana leaf extract (EDM) on the prevention and cure of tuberculosis with the toll like receptor 4 (TLR-4) protein expression mechanism. The choice of TLR-4 protein expression mechanism is because TLR-4 is related to the bacteriocidal mechanism of Mycobacterium tuberculosis by macrophages. Macrophage activity against Mycobacterium tuberculosis has been shown to involve Toll-like receptor (TLR) activation and production of ROS. Previous research has shown that lipopolysaccharide, through TLR4, can activate macrophages, increase the production of ROS which are bacteriocidal, and facilitate anti-infective immune responses.[19]

The effect of EDM on the expression of TLR-4 was observed in 3 stages, namely preventive, curative and preventive curative. This study used a controlled placebo test and anti-tuberculosis drugs (OAT). The OAT used is a combination of 4 types of drugs that are used as standard drugs in the treatment of tuberculosis. OAT contains (rifampin, ethambutol, Isoniazide and pyrazinamide). The preventive phase was observed when the mice were still healthy before and after being given EDM maintenance. The preventive objective was to determine the natural effect of EDM on TLR-4 expression, so that the differences in TLR-4 expression when mice were sick and healthy could be compared. The curative stage was observed before and after the mice were infected with Mycobacterium tuberculosis and were given EDM, OAT and placebo treatment. This stage was used to observe the effect of treatment on recovery, in this case the removal of tuberculosis bacteria from the body of sick mice based on TLR-4 expression and animal comparisons that were given placebo. The preventive curative stage was observed before being given EDM as maintenance and after the mice were given EDM as a treatment. The aim of this stage is to prove the potential of EDM as an herb to prevent tuberculosis infection and as a complement to tuberculosis treatment.

OAT is a standard drug in the treatment of tuberculosis which contains several types of drugs with an antibacterial function (bacteriostatic and bacteriocide). The study compared the function of OAT with herbal medicine, namely EDM to determine the potential of EDM as a preventive against tuberculosis infection and complementary in the treatment of tuberculosis. The function of EDM as an antibacterial tuberculosis has been previously studied which states that EDM is effective as an antibacterial for M.tb. The function of EDM as complementary in the treatment of tuberculosis produces immunomodulatory potentials that increase the proliferation of T lymphocytes, T cells, IFN-γ, TNF-α.[21] Furthermore also proven the potential of EDM as a complementary treatment for tuberculosis by increasing IL-6 mRNA expression and decreasing IL-10 mRNA expression.[27] The potential of these cytokines can support the function of macrophages to swallow and eliminate M.tb in the lungs and blood, thus providing healing for the host. The function of cytokines that play a role in eliminating bacteria is related to the function of TLR-4 as a receptor on macrophages to ingest M.tb.

Expression of TLR-4 at the preventive stage

The preventive observation was carried out by taking two blood samples, namely before and after giving EDM and placebo as test materials. The EDM dose as maintenance was 357 mg / kg BW in mice. This dose is converted from previous research where EDM can function as an immunomodulator in the prevention of tuberculosis.[21] Placebo was given as a control comparison of EDM effects to determine differences in the results of TLR-4 expression. TLR-4 expression was observed before and after preventive treatment to determine the effect of EDM administration in a healthy state to prevent tuberculosis infection. Previous studies have stated that the results of *in vivo* and *in vitro* investigations show that TLR activation depends on the innate immune response.[15,16]

The results of the observations in Table 2 indicate that all mice express TLR-4 naturally. Table 3 shows the changes in TLR-4 expression at the preventive stage where the administration of EDM and placebo increased the number of TLR-4 in healthy conditions. This shows that the immune function involving TLR-4 can be activated when cells are influenced from outside the body, for example in this case EDM. However, under normal conditions or no Mycobacterium tuberculosis infection, the increase in TLR-4 should not be as excessive as in EDM administration in this study. When the response to TLR-4 expression is excessive it allows an over-response of immunity that is not needed under normal circumstances because it can activate cytokines excessively when not needed. In this study, the provision of EDM which is immunomodulatory is able to regulate the immune response as needed by showing differences in TLR-4 expression as a preventive which is not significantly different before and after treatment. Thus the TLR-4-mediated immune response such as cytokines, NO, TNF α and others does not occur when it is not needed. Hosts who live together with tuberculosis sufferers need to have a good immune defense to prevent tuberculosis and other infections from the environment. EDM has the potential as an immunomodulator to prevent tuberculosis infection by increasing the proliferation of T lymphocytes, T cells, IFN-γ, TNF-α.[21] Where it is known that these cytokines are needed as a preventive and can be activated *via* the TLR-4 receptor on M.tb-sensitized macrophages or other pathogens. This is consistent with the conclusions of Das *et al*. (2016)[18] who stated that increasing TLR-4 can activate cytokines, NFkB, NO and P38 MAP when M.tb.

Expression of TLR-4 at the curative stage

The observations at the curative stage showed that the TLR-4 values were different before and after the curative treatment. The condition of group 3 mice (the group given placebo) changed from healthy to sick due to M.tb infection given. Illness was evidenced by the increased expression of TLR-4 which significantly changed from the start and end of infection. This is because M.tb which infects the host (mice) triggers host immunity activity in macrophages to inhibit growth, eliminating the presence of M.tb and killing M.tb in host cells. Host immunity to fight M.tb infection involves macrophages to recognize, ingest and eliminate M.tb and stimulate macrophage activation involving TLR-4 as pathogen binding receptors. The results of this study are in line with research which states that the survival of M.tb will increase miR-21-5p expression and expression of miR-708-5p and attenuates the secretion of inflammatory cytokines such as IL-6 and TNF-α in macrophages involving TLR-4 as a target pathogen in macrophages.[17,31]

In contrast to the placebo group that was not given post-infection treatment, in groups 1 and 2 after which mice were infected with M.tb, they were given treatment after the incubation period. The treatment given was EDM for group 1 and OAT (a combination of rifampin, ethambutol, pyrazinamide and isoniazid) for group 2. The results showed that the expression of TLR-4 before infection and after treatment was significantly different. Likewise, Table 4 shows the significant differences in changes in TLR-4 expression between the groups treated with placebo. The number of TLR-4 expressions in the group given placebo was very high compared to the group given treatment. Placebo TLR-4 expression was 9.7 times that of the OAT group and 5.5 times that of the EDM group. This means that EDM treatment provides a healing effect of tuberculosis infection with a potential of 57% of the potency of OAT. This potential is very promising because the OAT used is a combination of 4 types of drugs (rifampin, ethambutol, INH and pyrazinamide), while EDM is a single extract. Cure of infection in this study was related to TLR-4 expression because TLR-4 is a receptor on macrophages for the recognition and destruction of the pathogen M.tb. This is in line with the research who concluded that TLR-4 could represent a new therapeutic target for activating innate immune responses during tuberculosis infection.[14,18]

When a pathogen infects the host, the body activates macrophages at the TLR-4 receptor to modulate other immune responses such as IL-6 (interleukin 6), IL-1β (interleukin 1β), IFN-γ (interferon gamma) and TNF-α (tumor necrosis factor alpha).[32] This study showed that levels of TLR-4 expression in the placebo group increased by 97%, whereas those in the OAT-treated group increased by 12% and EDM increased by 19%. These data show the potential of EDM as an antibacterial which is very promising, considering that one dose of EDM gives an effect close to the potency of OAT which is a combination of 4 doses of anti-tuberculosis drugs. Analysis of these data is possible when EDM is given as a complement to OAT in the treatment of tuberculosis so that it can

Table 2: Expression of TLR-4 protein at stages 1,2 and 3.

Treatment group	n	TLR-4 expression level (ng / ml)					
		Blood sample 1		Blood sample 2		Blood sample 3	
		Mean	SD	Mean	SD	Mean	SD
K1	7	393,9702	32,9676	402,5116	41,9432	471,5744	58,2291
K2	7	397,3929	54,7211	407,2096	61,8159	358,3238	46,2681
K3	7	385,325	63,6832	394,4692	52,3806	776,7678	40,3707

K1: group 1 (mice were given EDM as a preventive and curative)
K2: group 2 (mice were given EDM as a preventive and OAT as a curative)
K3: group 3 (mice were given Na CMC as a placebo for preventive and curative)

Table 3: Changes in expression of TLR-4 before and after preventive.

Treatment group	n	Changes in levels of TLR-4 (ng / ml)				
		Mean	SD	Median	Min	Maks
K1a -K1b	7	8,546	25,991	17,71	-23,726	40,818
K2a -K2b	7	9,874	19,349	19,92	-14,152	33,899
K3a -K3b	7	9,142	39,326	9,142	-39,687	57,971

a is a blood sample 1
b is a blood sample 2
K1a -K1b: blood sample 1 and blood sample 2 from group 1
K2a -K2b: blood sample 1 and blood sample 2 from group 2
K3a -K3b: blood sample 1 and blood sample 2 from group 3

Table 4: The LSD test results of changes in TLR-4 expression before and after curative.

Treatment group	n	Changes in levels of TLR-4 (ng / ml)				
		Mean	SD	Median	Min	Maks
K1b -K1c	7	69,06	30,245	79,66	31.5058	106,614
K2b -K2c	7	-48,89	28,6512	40,85	-84,4653	-13,3147
K3b -K3c	7	382,3	54,6368	378,4	314,459	450,14

b is a blood sample 2
c is a blood sample 3
K1b -K1c: blood sample 2 and blood sample 3 from group 1
K2b -K2c: blood sample 2 and blood sample 3 from group 2
K3b -K3c: blood sample 2 and blood sample 3 from group 3

Table 5: The LSD test results of changes in TLR-4 expression before prevention and after curative.

Treatment group	n	Changes in levels of TLR-4 (ng / ml)				
		Mean	SD	Median	Min	Maks
K1a -K1c	7	77,606	46,5376	66,39	19,821	135,39
K2a -K2c	7	-39,016	31,4815	-24,34	-78,105	-0,0735
K3a -K3c	7	391,442	67,5449	402,74	307,573	475,31

a is a blood sample 1
c is blood sample 3
K1a -K1c: blood sample 1 and blood sample 3 from group 1
K2a -K2c: blood sample 1 and blood sample 3 from group 2
K3a -K3c: blood sample 1 and blood sample 3 from group 3

Table 6: Percentage change in expression of TLR-4 at each treatment stage.

Groups	Percentage change in expression of TLR-4 at each treatment stage (%)		
	Preventive	Curative	Preventive Curative
Group 1	2	17	20
Group 2	2	-12	-10
Group 3	2	97	102

Group 1: a group of mice given EDM
Group 2: a group of mice given OAT
Group 3: the group of mice given a placebo
positive: increased TLR-4 expression
negative: decreased TLR-4 expression

reduce the dose of OAT. So that it can further reduce the side effects of treatment. In line with this study, it was stated that giving C4T4 agonists could increase immunity and reduce the amount of M.tb from the lungs of mice infected with M.tb. So that the combined use of C4T4 agonists and OAT (isoniazid or rifampin) can reduce the dosage of OAT by up to 10 times lower than using C4T4 alone to provide the same effect. The mechanism that occurs is that complementary C4T4 and OAT can reduce TLR-4 even though the dose of OAT is reduced by 10 times.[33] Thus, it can be stated that TLR-4 is a parameter of the condition of the host infected with tuberculosis which can be lowered by the combined administration of OAT and complementary EDM for the treatment of tuberculosis. Evidence that the combination of rifampin and OAT was able to kill M.tb colonies to zero compared to OAT alone, still found the colony count was 81.67 CFU / g and EDM alone was found to be 187.5 CFU / g at the end of treatment based on the results tuberculosis rat lung culture.[21] The number of colonies in the end is a parameter of the host's recovery from tuberculosis and one of the factors that play a role in the elimination of M.tb is TLR-4. The increase in TLR-4 is needed when the host is infected to activate immunity against infection but will decrease again with elimination of the pathogen in the host.

Expression of TLR-4 at the preventive curative stage

Treatment at the preventive curative stage analyzed differences in the expression of TLR-4 protein at the beginning of the preventive treatment and after treatment. In this stage the mice as hosts experienced a period of EDM administration in preventive doses before experiencing infection, then received curative dose EDM treatment after going through the infection incubation stage. The results of the observation show that the provision of EDM and OAT has an effect on the expression of TLR-4 because it is significantly different from giving placebo according to table 5. Data showed an increase in the expression of TLR-4 before preventive and after successive curative for EDM increased 20%, OAT decreased 10% and placebo increased 102%. This proves that the placebo group the host experienced active infection because of the very high expression of TLR-4. The increase in TLR-4 expression in the EDM and OAT groups was significantly different from the placebo group. LSD analysis showed that giving EDM was significantly different from giving OAT to TLR-4 expression. The results in table 1 show that giving OAT can return the state of the host infected with tuberculosis to normal as it was based on its TLR-4 expression which is not significantly different.

Analysis of the results of EDM treatment as a preventive during the incubation period has been shown to support the ability of host immunity to limit the spread of infection. In this case the expression of TLR-4 at the preventive stage before infection and during the incubation period after tuberculosis infection has been shown to have the potential to activate the immune system as an initial defense when infection occurs. Initial defense is important to restrain the rate of infection during the incubation period and support the elimination of bacteria during the curative period. The administration of preventive doses of EDM given during the incubation period was proven to activate macrophages to recognize, ingest and eliminate M.tb which involved TLR-4 as a receptor. TLR-4 which is sensitized by the pathogen M.tb can further promote the activation of proinflation cytokines such as IFN-γ, IL-6, IL-1β and TNF-α.[34] Thus the growth of M.tb during the incubation period is limited. These findings can be applied to healthy hosts who live together or are in an environment with active tuberculosis sufferers. The function of EDM as a preventive can activate the production of proinflammatory cytokines such as IFN-γ and TNF-α for the prevention of tuberculosis.[22]

The analysis of the results of curative preventive observations proves the potential of EDM as a preventive has supported the success of tuberculosis treatment using OAT. Differences in TLR-4 expression before preventive and after treatment showed significantly different levels between treatment with EDM or with OAT. It is known that OAT is a combined drug of 4 types of anti-tuberculosis with therapeutic doses of each drug, however, EDM provides a curative potential that is close to the curative potential of using OAT. The analysis for these data is based on the content of active substances or secondary metabolites such as flavonoids from EDM which support their antibacterial function. The flavonoid content of 7.35mg / g EDM and total polyphenols of 20.35mg / g EDM is a promising amount as an active component of the miana plant *(Coleus scutellarioides* (L). Benth.). Flavonoids as antioxidants can be immunostimulant and antibacterial in general, including M.tb. Various evidences have been gathered to prove the mechanism of flavonoids as anti-tuberculosis. All flavonoids from plants, especially the flavone and flavonone groups have been proven to be antimikobacterial, anti-M.tb or anti-tuberculosis.[29]

The findings in this study were the potential of EDM as a preventive and curative for tuberculosis based on TLR-4 expression. This is evident based on the expression of TLR-4 from the EDM group approaching the OAT group but very low compared to the placebo group, especially after curative. The role of TLR-4 in modulating the body's defenses against mycobacterial infections has provided new information about the pathogenesis of tuberculosis thus providing useful assumptions for developing potential therapeutic interventions for disease.[35]

CONCLUSION

EDM given as a preventive does not increase the expression of TLR-4 protein in healthy mice.

Changes in expression of TLR-4 protein in M.tb-infected mice before and after curative EDM increased by 17%, after giving placebo increased 97% and decreased 12% after OAT curative administration.

Changes in expression of TLR-4 protein in mice infected with M.tb before preventive administration and after EDM curative administration increased by 20%, after administration of placebo increased 102% and decreased by 10% after OAT curative administration.

SUGGESTION

It is recommended to carry out further research to determine the potential use of miana leaves as a complementary treatment for infectious diseases, especially tuberculosis with B cell antibody mechanisms.

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AUTHORS CONTRIBUTION

Sesilia Rante Pakadang as the lead author and other writers took part in the research at each stage. All authors contributed to each stage of the study.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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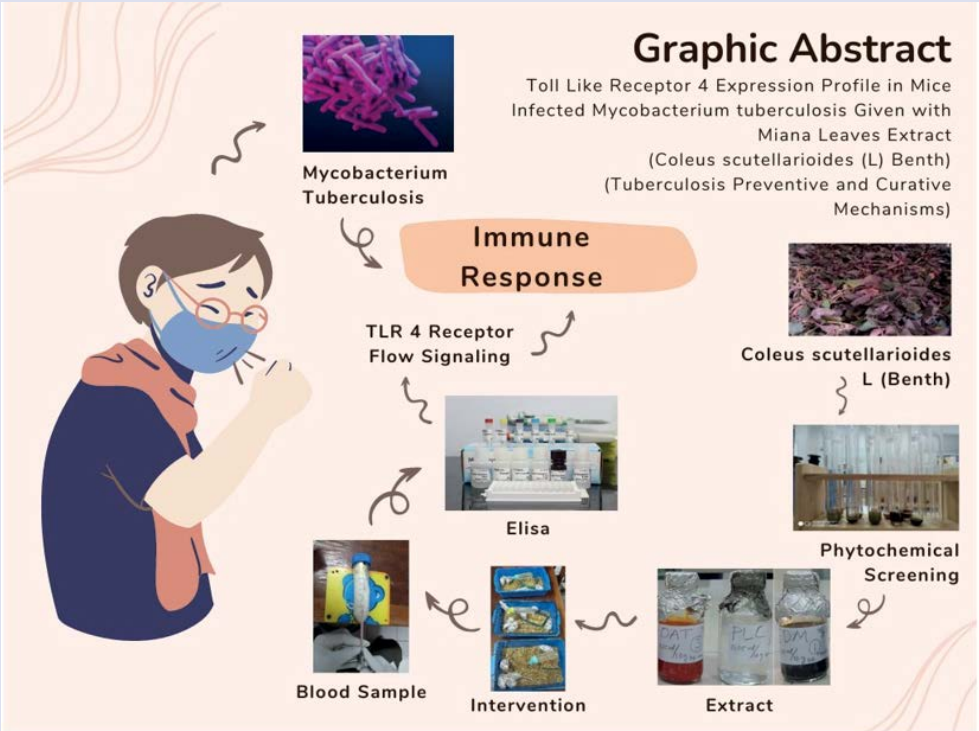
GRAPHICAL ABSTRACT



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