American Journal of Microbiological Research, 2015, Vol. 3, No. 1, 8-13
Available online at http://pubs.sciepub.com/ajmr/3/1/2
© Science and Education Publishing
DOI:10.12691/ajmr-3-1-2

The Effect of the Petroleum Ether Extracts from Mangosteen Pericarp (*Garcinia mangostana* L.) on Interferon-gammaand, Interleukin-12 Activities in AlbinoWistar Rats (*Rattus norvegicus*) Infected with *Mycobacterium tuberculosis*

Martha Kaihena[1], Syamsu[2], Yadi Yasir[3], Mochammad Hatta[4,*]

[1]Biology Study Programm, Faculty of Mathematic and Sciences, Pattimura University, Ambon, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine, Hasanudin University, Makassar, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Mulawarman University, Samarinda, Indonesia
[4]Molecular Biology and Immunology Laboratory for Infectious Diseases, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
*Corresponding author: hattaram@indosat.net.id

Received December 22, 2014; Revised December 30, 2014; Accepted January 07, 2015

Abstract **Background.** *Garcinia mangostana* L(GML) pericarp extract is known to contain active substances called Xanthones (α, β and γ Mangosteens) which is the biggest derivative and has strong antioxidant effects. This substance also has antiinflammatory, antilipid, anticancer, antibacteria and antituberculosis effects. However, its mechanism is still unclear. *Mycobacterium tuberculosis* (*M. tuberculosis*) invasion into the lungs through the respiratory tract can cause severe infection. The body has an immune system which controls infection by eliminating germs to ease the burden of infection. Interferon gamma (IFN- γ) and Interleukin 12 (IL-12) acts as positive feedback in stimulating macrophages to kill *M. tuberculosis*. During this process, oxidative compounds (*ROI,RNI,NO*) that plays an important role in the phagolysosome fusion process are produced. Not only does the consumption of GML pericarp extract as an antioxidant becomes the immunomodulator to enhance the immune's activities, it also functions as an antioxidant that can neutralize the oxidative compounds produced by the immune system. The aim of this study was to determine the effect of EPEBh GML on IFN-γ and IL-12 secretion activities in mice infected with *M. tuberculosis*. **Materials and method**. This study used 30 Wistar rats, 150-200g of weight and 8-10 weeks old. Rats were randomly divided into 6 groups each consisting of 5 rats, including the negative control (without infection and without EPEKBh GML intervention) and a positive control (rats were infected with *M.tb* H37Rv at a dose of 10^6cfuas much as 0.2 ml through the trachea for 6 weeks). Once infected, the rats were then intervened with EPEKBhGML 30, 60, 120 dose and 180 mg/kg bodyweigh/day for 1 month. Afterward the rats were necropsied and dissected for the blood to be taken directly from the heart. Levels of IFN-γ and IL-12 were analyzed using the *ELISA* method. Data were then analyzed using One-Way ANOVAtest followed by Post-Hoc test (LSD), a significant P <0.05, to assess the comparison between groups. **Results**. Results show that EPEKBh GML significantly affect the rise of IFN-γ levels, with a P value 0,000<0,05 and IL-12, with a P value of 0,045 <0,05. **Conclusion**. EPEKBh GML was effective in increasing the activity of IFN-γ and IL-12 and the most effective dose to increase IFN-γ and IL-12activity was 120 mg/kgbody weight/day. Also, increasing the dose to a higher dosage had no effect on IFN-γ and IL-12 activity, in fact it tended to decline.This results need to further study to understand what the reason.

Keywords: *extract, Garcinia mangostanal., IFN- γ, IL-12, Mycobacterium tuberculosis*

Cite This Article: Martha Kaihena, Syamsu, Yadi Yasir, and Mochammad Hatta, "The Effect of the Petroleum Ether Extracts from Mangosteen Pericarp (*Garcinia mangostana* L.) on Interferon-gammaand, Interleukin-12 Activities in AlbinoWistar Rats (*Rattus norvegicus*) Infected with *Mycobacterium tuberculosis*." *American Journal of Microbiological Research*, vol. 3, no. 1 (2015): 8-13. doi: 10.12691/ajmr-3-1-2.

1. Introduction

Mycobacterium tuberculosis is an acid-resistant bacteria that caused the death of nearly 2 million deaths and with more than 8 million new cases each year [1]. The World Health Organization (WHO) estimated that the bacteria has attacked a third of the world population [2].

The prevalence of tuberculosis in Indonesia is very high, as it is ranked ninth with an incidence of 185 cases per 100,000 people and ranks third in the Southeast Asian

Region with the number of cases reaching 82,799 new cases in 2012 which were reported from government and private hospitals in Indonesia. This is an increase from the previous year which was 71,454 cases [1]. *M. tuberculosis* is a pathogenic bacteria that has the ability to live and multiply in macrophages, after passing the mechanical barriers in the respiratory tract and then going into the alveoli [3]. The intracellular *M. tuberculosis* will stimulate macrophages to produce interleukin-12 (*IL-2*) that plays a role in the formation of T-helper-1 cells (Th1) which then secretes interferon gamma (IFN-γ) to activate macrophages so as to destroy the germs that have been phagocytized [4]. Thus, macrophages can serve as a key element in the process of *M.tuberculosis* limination ift here were germs that were not killed, so that the bacteria could survive and cause active tuberculosis infection [5,6].

Currently, tuberculosis treatment has become increasingly complicated because of the presence of antibiotic-resistant bacterias. The cause of the resistance is due to inadequate treatment, or the discontinuation of drug consumption, improper regimen and patient disobedience [7,8,9]. Patients with tuberculosis (TB) must use a combination of some common medications such as isoniazid, rifampicin, ethambutol and pyrazinamide [7,10,11]. Yet, there is still resistance to the combination of drugs known as MDR-TB (*Multi Drugs Resistance Tuberculosis*). MDR-TB is defined as TB cases that are resistant to at least isoniazid and rifampicin [12]. Currently, there is an increase of MDR-TB cases in several countries [1]. Therefore, it is necessary to find alternative new drugs that can help reduce the number of MDR-TB cases.

Experts suspect the presence of immune system disorders in patients with tuberculosis [13]. As a result, some researchers are using immunomodulators as an adjunct therapy for tuberculosis [14]. The rationale is, because of the fact that from one third of the human population who are exposed to *M. tuberculosis* only one-tenth becomes ill. This indicates that the immune system works quite effective against TB. Based on this evidence, the research on the role of cytokines in the body's defense mechanisms have been targeted in finding new strategies of treatment of tuberculosis.

GML is known to contain a variety of secondary metabolites with medicinal properties such as xanthone and its derivatives. It is estimated that there are about 190 types of active substances of the xanthone class in the world, with about 50 types contained by GML [15,16,17]. Those compounds are found in the pericarp, seed, bark, leaves, roots and flesh of the mangosteen. Besides xanthone, there are anthocyanin, folic acid and tannins, as well as various nutrients with antioxidant capacity of 84,6 to 86,3%, or 0.8 times higher than ascorbic acid thus it is called superantioxidant [18]. The mangosteen pericarp has a wide range of benefits such as antioxidant and anti-inflammatory. Chomnawang *et al.* stated that the xanthone compound has potential as an anti-inflammatory in the reduction of tumor Necrosis Factor Alpha (TNF-α) production [19]. Supiyanti *et al.* proved that the GML pericarp has anthocyanins which is a potential compound that has antioxidant activity [20]. Suksamran found that mangosteen can act as an in vitro anti-tuberculosis [21]. GML pericarp extract contains high levels of antioxidants and flavonoids that can be useful as immunomodulator in increasing TB patients' immune through increasing IFN-γand IL-12 cytokines which plays a key role for macrophage activation in phagocytosis of intracellular microbes such as *Mycobacterium tuberculosis.*

2. Materials and Method

This study is an experimental laboratory research that used a completely randomized design. After treatment was conducted on the rat blood sample it was then measured using the Enzyme Linked Immunosorbent Assay (ELISA) analysis technique. ELISA results were analyzed using the Shapiro-Wilktest [22] to determine normality in data distribution. If the data distribution is normal represented by P> 0,05, it is followed by the Analysis of Variance (ANOVA) and comparisons between groups using Post-Hoc analysis (LSD) with SPSS 16 software program.

2.1. Preparation of Animals

The samples in this study were male albino Wistar rats weighing 150-200 grams and aged 8-10 weeks at the beginning of the research. The numbers of samples used were 30 rats and an addition of 8 rats asbackup to anticipate the death risk factor and 2 rats to test infection by acid-fast bacili (AFB+). Rats were then placed randomly into 6 groups with 5 repetitions, and placed in a cage in the form of a plastic box. Rats were fed standard feed in the form of pellets anddrinking water was given *ad libitum*. Rat cage was placed in a special room with a non-moist environment, adequate ventilation, no direct sunlight and standard lighting at night.

2.2. Treatment of Rats

Rats were then placed randomly into 6 groups with 5 repitition. Groups of rats were differentiated based on the treatment that is: the negative control group K (-) was a group of rats that were not based ont he treatment that is: the negative control group K (-) was a group of rats that were not given any treatment other than standard food and drink. Positive control group K (+) was a group of rats infected with *M. tuberculosis* for 6 weeks without GML extract therapy. Treatment group (P1, P2, P3 and P4) were groups of rats infected by *M. tuberculosis* for 6 weeks until it is infected then treated with GML petroleum ether extracts with concentrations of 30, 60, 120 and 180 mg/kg body weight/day. At the end of the treatment, the rats were released from any treatment. The rats were then anesthetized with ketamine-xylazine 75-100 mg/kg body weight by intraperitoneal administration then euthanized based on the Institutional Animal Careand Use Committee (IACUC) using the cervical dislocation method [23]. Blood samplewas taken directly from the heart as much as ±3 ml and put into an EDTA tube. Examination of IFN-γ and IL-12 were performed by using ELISA Kit.

2.3. Examination of *IFN-γ* and *IL-12* Cytokines usingELISA.

The 3 ml rat blood sample was inserted into an EDTA tube, then was shook back and forth. Next, the tube was centrifuged at 5000 rpm for 30 minutes. The centrifuged blood was separated into erythrocytes, buffy coat layer and blood plasma. Plasma was separated and stored in a

microtube. Standard solution was prepared by dissolving lyophilize each for IFN-γ and IL-12. Standard and assay diluent was then vortexed. Standard solution was then examined in duplo, while other wells were filled with samples that have been combined with assay diluent in advance. Each well was then givenrabbit anti-rat IFN-γ and IL-12 polyclonal antibodies. The plate was then covered with sealer (Acetate Plate Sealer) to prevent evaporation and incubated at room temperature for 3 hours. After incubation, the plate's sealer was opened and washed with wash buffer. Alkalinephosphatase conjugated goat anti-rabbit was added to each well and then resealed. The plate was incubated for 45 minutes at room temperature. Sealer is then opened and the liquid discarded. The plate was washed with wash buffer. Dye reagent was added and incubated at room temperature for 6 minutes and after that stop solution was added. The result was read in an ELISA Reader, to obtain the levels of IFN-γ and IL-12.

2.4. Extraction of GML

Pieces of GML pericarp was dried usingan oven at a temperature below 50°C, ground and sieved to produce a fine powder, the powder was then weighed each 1,000 grams to be extracted. The extraction process used the soxhlation method. Each extraction process used 60 grams of powdered pericarp and was extracted using petroleum ether solvent resulting in 300 ml of Petroliumetet every 24 hours at 59°C. The result of the extraction process was collected and evaporated to dryness using a rotary evaporator.

3. Results

3.1. *IFN-γ* Expression on Wistar Rats Infected with *Mycobacterium tuberculosis*

Based on the measurement of IFN-γ levels in Wistar rats blood intervened by GML pericarp extract using the ELISA Kit,the following group means were obtained: P3 with 199,80 ± 17,610; P4 with 161,02 ± 32,061; P2 with 135,88 ± 35,551; P1 with 110,30 ± 52,295; followedby K(+) with 84,52 ± 24,424 and K(-) with 17,32 ± 8,511. Results of the ANOVA test that was followed by an LSD test can be seen in Table 1.

Table 1. Results of One-Way ANOVA Test on *IFN-γ* and *IL-12* Secretion

No.	Variable	p	Description
1	*IFN-γ*	0,000	There is a significant difference between each group
2	*IL-12*	0,045	There is a significant difference between each group

Table 2. Results of Multiple Comparison-Post Hoc (LSD) Test of IFN-γ Levels in Rats with TB

Variable	Comparison between Groups		p-value	Description
IFN-γ	K (-)	K (+)	0,003	Significant Difference
		P1	0,000	Significant Difference
		P2	0,000	Significant Difference
		P3	0,000	Significant Difference
		P4	0,000	Significant Difference
	K (+)	P1	0,219	No Significant Difference
		P2	0,017	Significant Difference
		P3	0,000	Significant Difference
		P4	0,001	Significant Difference
	P1	P2	0,213	No Significant Difference
		P3	0,000	Significant Difference
		P4	0,018	Significant Difference
	P2	P3	0,004	Significant Difference
		P4	0,221	No Significant Difference
	P3	P4	0,064	Significant Difference

Table 3. Results of Multiple Comparison-Post Hoc (LSD) Test of *IL-12* Levels in Rats with TB

Variable	Comparison between Groups		p-value	Description
IFN-γ	K (+)		0,504	No Significant Difference
	K (-)	P1	0,652	No Significant Difference
		P2	0,068	No Significant Difference
		P3	0,052	No Significant Difference
		P4	0,074	No Significant Difference
	K (+)	P1	0,448	No Significant Difference
		P2	0,016	Significant Difference
		P3	0,012	Significant Difference
		P4	0,018	Significant Difference
	P1	P2	0,156	No Significant Difference
		P3	0,126	No Significant Difference
		P4	0,171	No Significant Difference
	P2	P3	0,897	No Significant Difference
		P4	0,996	No Significant Difference
	P3	P4	0,863	No Significant Difference

Based on the ANOVA test results, the table shows that there are significant differences in interferon gamma levelsbetween each group with a p-value = 0.000 (<0.05). It is the same as the Interleukin-12 levels in which the p-value = 0.045 <(0.05) which means that the administration of petroleum ether extracts of GML pericarp (EPEKBh) affects the increase of IFN-γ and IL-12.

Treatment using petroleum ether extracts of GML pericarp (EPEKBh) in Table 3 can be compared with the negative control group mean: K(-) with the positive control group K (+) showed a significant difference where p = 0,003 <0,05. Between the negative control group K(-) and all other concentration treatment groups which are P1, P2, P3 and P4 showed a significant difference with a P value of <0,05. There were also significant differences between the positive control K (+) and P1, P2, P3 and P4 with each p-value of (0,017; 0,017; 0,000; 0,001) <0,05 respectively. Between treatment groups, P1 also differed significantly with P3 and P4 with a p-value of (0,000; 0,018) <0,05 respectively. While between the treatment group P1 and P2, with a p-value of 0,213> 0,0;P2 and P4 with a p-value of 0,221> 0,05; P3 and P4 was not significantly different with a p-value of 0,064> 0,05.

Figure 1, Shows that intervention using petroleum ether extracts of GML pericarp (EPEKBh) onrats infected with *M. tuberculosis* resulted in the mean levels of IFN-γ in the negative control group K (-) and the positive control group K (+) showing a significant difference in which the p-value= 0,003 <0,05. Between the negative control group K (-) and all other concentration treatment groups which were PA1, PA2, PA3 and PA4, there was a significant difference with a p-value of <0,05. There were also significant differences between the positive control K (+) and PA1, PA2, PA3 and PA4 with each p-value of (0,017; 0,017; 0,000; 0,001) <,05 respectively. The P1 treatment group also differed significantly with P3 and P4 with a p-value of (0,000; 0,018) <0,05 respectively. While between treatment groups PA1 andPA2, with a p-value of 0,213> 0,0; PA2 and PA4 with a p-value of 0,221> 0,05; and between PA3 and PA4 was not significantly different with a p-value of 0,064> 0,05.

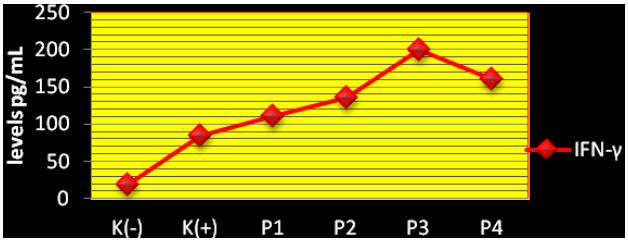

Figure 1. Graph of *IFN-γ* Levels in the Group of Rats Infected with TB Receiving EPEKBh GML Intervention

3.2. *IL-1* Expression on Wistar Rats Infected with *Mycobacterium tuberculosis*

Based on the One-Way ANOVA test resultsof mean *IL-12* levels in Wistar rats blood which received intervention using Petroleum ether extracts of GML pericarp (EPEKBh), the following group means were obtained:P3 with 35,11 ± 17,492, P4 with 33,42 ± 8,921, P2 33,84 ± 28,68, P1 with 19,75 ± 10,913 followed by K (-) with 15,3 ± 7,119 and K(+) with 8,78 ± 5,086.

Treatment using petroleum ether extracts of GML pericarp (EPEKBh) showed that the average between the negative control group K (-) and the positive control group K (+) did not differ significantly in which the p-value = 0,268> 0,05; the same is true between the positive control group K (+) and the PA1 treatment group in which the p-value = 0,268> 0,05.The P1 with P2, P3 and P4 treatment groups with a p-value of 0,158; 0,126; 0,171> 0,05 respectively, and between P2 with P3 and P4 with a p-value of 0,897; 0,966> 0,05 respectively, as well as P3 with P4treatment group did not differ significantly with a P-value of 0,89> 0,05. While between the positive control group K (+) and the PA3 and PA4 treatment groups with a p-value of 0,523 and 0,055> 0.05 respectively, P1 and P2 treatment groups with a p-value of 0,185> 0,05; as well as between the PA2 with the P3 and P4 treatment groups with a p-value of 0,093 and 0,124> 0,05 respectively, meaningthere was no significant difference. The same thing wasevident in the P3 and P4 groups with a p-value of 0,863> 0,05. The data is presented in Figure 2 below.

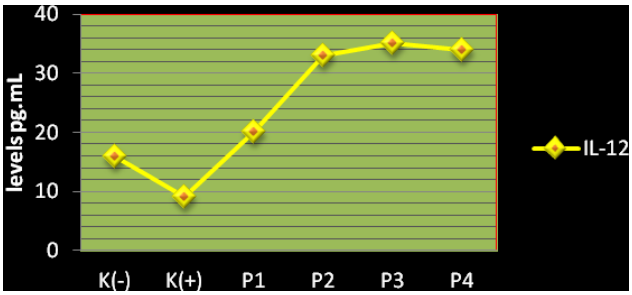

Figure 2. Graph of *IL-12* Activity in the Group of Rats Infected with TB ReceivingEPEKBh GML Intervention

4. Discussion

Results of Post-Hoc LSD analysis on the secretion of IFN-γ showed that there were significant differences in treatment groups which was between K (-) and K (+), in contrast also with the P1, P2, P3 and P4 groups, K (+) was different from the P2, P3, and P4 groups, while P1 was different fromthe P2 and P3 groups, P2 was different from P3, and P3 differed from the P4 groupin which the p-value = (0,000) p <(0,05). The groups that did not differ significantly were K (+) with P2, P3 and P4.

Results of Post-Hoc LSD analysis on the secretion of *IL-12* showed that the average group that differed significantly was found in: K (+) to the P2, P3 and P4 groups with a p-value = (0,048) p<(0,05). Meanwhile, between the K (-) and K (+) group, K (-) and P1, P2, P3 and P4 groups, as well as the P1 and P2, P3 and P4 groups, P2 and P3, and P3 and P4 did not differ significantly. Significant differences between the K (-) and K (+) group in the secretion of IFN-γ indicated that the entry of foreign antigens in the form of *M.tuberculosis* with the size of 10^6 CFU into the rat's body through the alveolar had stimulated the T lymphocytes, in this case Th1, to secrete IFN-γ so that the levels increased in the blood. The results of this study showed that the petroleum ether extracts of GML pericarp affectedthe increased secretion of IFN-γ and that a dose of 120/mg/kg body weight /day was the best dose because it resulted in the p-value of 0,000 <0,05. In P4 treatment with a higher dose of 180/mg/kg body

weight/day the results showed that it tended to reduce IFN-γ secretion. While for the IL-12, a significant differenceoccurredbased on P2, P3and P4 treatmentsin which the p-value was 0,045 <0,05. Between P2, P3 and P4 were not significantly different, but the same trend was also observablein the P3 treatment that showed ahigher increase in IL-12 levels, but tended to decrease at higher concentrations.

Increasing the dose of medication should in turn increase the response proportional to the dose increased [24]. However, the reality in this study was that with the increase in concentration, the increase in response ultimately decreased. This is because the dosage where it cannot increase the response anymore had been reached[24]. This often occurs in natural medicines, particularly in the use of extracts, because the compound components that it contains is not a single compound but rather it consists of various chemical compounds, in which these components work together to cause an effect. If the extract dose is increased, the number of chemical compounds contained was increase, resulting in adverse interactions that lead to a decrease in effect [25].

The same is seen in the research conducted by Susanto, *et al*., [26] using 40% ethanol extract on the activity of Aspartate aminotransaminase (AST) and Alanine aminotransferase (ALT) in male Sprague-Dawley albino rats (*Rattus norvegicus*) induced by isoniazid, using doses of 20,40, 80 and 200 mg/100g body weight. Study results showed that the increase in dosage up to 100 mg/100g body weight did not change, in factthe most effective dose was 80mg/100g body weight. Other studies on the effect of ethanol extractsof GML pericarpon the decrease in blood glucose levels of mice using doses of 50,100, and 200 mg/kg body weight showed that the response to a decrease in blood glucose levels of laboratory animals was more effective at doses of 100 mg/kg body weight compared to the higher dose of 200 mg/kg body weight [27]. Mangosteen pericarp extracts contains various active compounds that have great potential as an alternative to various treatments. The dominant active compound contained in the extract is xanthone which consists of various derivatives including α-mangostin and γ-mangostin, as well as flavonoids that have the potential as super antioxidants, thought to act as an immunomodulator in modulating the increase of IFN-γ and IL-12 secretion. IFN-γ secretion by macrophages also stimulate the formation of free radicals to destroy components of *M. tuberculosis* bacteria which are the DNA and the cell walls of bacteria that play a role in combating the *M. tuberculosis* bacteria [28], in addition IFN-γ increases the activity of macrophages to destroy the germs that havebeen phagocytized and stimulate macrophages to produce a variety of substances namely Reactive Oxygen Intermediates (ROI) and nitrogen oxide (NO) which serves to inhibit the growth of bacteria [29,30].

An increase in IFN-γ which also increases the activity of macrophages in secreting ROI and NO during *M. tuberculosis* infection proved that the bacterial infection can lead to an immune response in the body. Infection of *M. tuberculosis* in rats resulted in the development of both non-specific and specific immune receptors. Non-specific immune response begins with phagocytosis by NK cells and macrophages. Furthermore, macrophages produce IL-12, which then stimulates NK cells to produce IFN-γ which in turn activates other macrophages [31]. While in the specific immune system, Cell-Mediated Immunity (CMI) macrophages will be activated by T cells so that it can serve as effector cells which are more effective in eliminating *M. tuberculosis* [32]. IFN-γ is also said to be a major activator of macrophages to stimulate phagocytosis, oxydative burst and Reactive Nitrogen Intermediates (RNI) for intracellular killing [29,30]. However respiratory burst also cause an increase in Reactive Oxygen Species (ROS) and Nitrogen Reactive Intermediate (RNI). Increased ROS in the lungs can result in tissue damage and lung inflammation. This condition further contributes to immune deficiency [33]. Increased ROS and RNI lead to a greater use of endogenous antioxidants such as glutathione to neutralize ROS. But if there is an increase in ROS in the lungs while the detoxification capacity of endogenous antioxidants was stable or decreasing then oxidants and antioxidants imbalance occurs causing oxidative stress in TB patients [34,35]. It is known that glutathione apart from having antibacterial properties, this endogenous antioxidant alsoplay an important role in innate immunity against TB infection [36]. Glutathione affetcsthe in vitro growth of H37RV. This shows that decreased levels of (glutathione) intracellular GSH in the continuity of living cells change the function of T cells, the activity of NF-kB, the sensitivity of TNFα and cell death. Venketaraman *et al.*, reported that reduced glutathione levels in individuals with pulmonary tuberculosis, is associated with an increase in the growth of *M. tuberculosis* and proinflammatory cytokines [36]. This means that the decrease in GSH correlated with the increase of *M. tuberculosis* growth in macrophages [37]. Based on the above mechanisms it is necessary to maintain a balance between endogenous antioxidants against ROS and RNI, one way requires exogenous antioxidants which in this study is Petroleum Ether Extracts of GML pericarp to enhance macrophage functions through the activity of the IFN-γ and IL-12 cytokines. Results of this study showed that GML pericarp extract was able to modulate and activate phagocytosis as an early signal to produce the IL-12 cytokines and then trigger the secretion of IFN-γ which is a positive feedback effect for the phagocytosis mechanism and destruction of *M. tuberculosis.*

5. Conclusions.

The activity of the Petroleum Ether Extracts of GML pericarp at a dose of 120 mg/kg body weight/day has a significant effect in increasing the secretion of interferon gamma (IFN-γ) exceeding the increase at the time the rats were infected by *M. tuberculosis.* And the activity of Petroleum Ether Extracts of GML pericarpwas significantly influential in increasing the secretion of IL-12. Also, petroleum ether extracts of GML pericarpat a higher dose that was 180 mg/kg body weight/day caused IFN-γ and IL-12 secretion tended to decline.

References

[1] WHO: Global Tuberculosis Report 2013. WHO Press 2013: http://www.who.int/tb/publications/global_report/en/.

[2] Zumla A, Raviglione M, Hafner R, von Reyn CF. Tuberculosis. N Engl J Med. 2013, 368:745-55.

[3] Smith I. Mycobacterium tuberculosis Pathogenesis and Molecular Determinants of Virulence. Clinical Microbiology Reviews 2003, 16(3): 463-496.

[4] Subagyo A., T.Y Aditama., Sutoyo D.K. dan L.G Partakusuma. Pemeriksaan *Interferon-gamma* Dalam Darahuntuk DeteksiInfeksi Tuberkulosis, JTI 3(2). 6-15.

[5] Schluger NW,Ro WN. The Host Immune To Tuberculosis. AmJRespir; Crite Care Med 1998, Vol157. pp 79-691.

[6] Gouzy A, Larrouy-Maumus G, Bottai D, Levillain F, Dumas A, et al. Mycobacterium tuberculosis Exploits Asparagine to Assimilate Nitrogen and Resist Acid Stress during Infection. PLoSPathog. 2014, 10(2): e1003928.

[7] Lange C, Abubakar I, Alffenaar J-WC, et al. Management of patients with multidrug-resistant/extensively drug-resistant tuberculosis in Europe: a TBNET consensus statement. *The European Respiratory Journal* 2014; 44(1): 23-63.

[8] Gaude GS, Hattiholli J, Kumar P. Risk factors and drug-resistance patterns among pulmonary tuberculosis patients in northern Karnataka region, India. *Nigerian Medical Journal: Journal of the Nigeria Medical Association* 2014, 55(4):327-332.

[9] Mendez AP., RaviglioneMC.,Laszlo A., Binkin N., Rieder HL., Bustreo F., Cohn DL., Weezenbeek CSBI., Kim SJ., Chaulet P., Nun P., Global surveillance for antituberculosis-drug resistance. 1994-1997. NEJM 1998, 338: 1641-9.

[10] Indonesia Health Department,Guideline for Tuberculosis management. 8th Edition, Jakarta. Indonesia 2002.

[11] Muchtar,A. Farmakologi Obat Antituberkulosis (OAT) Sekunder.JTI 2006, 3(2): 24-25.

[12] WHO: Drug-Resistant Tb Surveillance & Response Supplement Global Tuberculosis Report 2014. WHO Press 2014: http://who.int/tb/publications/global_report/gtbr14_supplement_web_v3.pdf?ua=1.

[13] vanCrevel R, Ottenhoff THM, van der Meer JWM. *Innate immunity to Mycobacterium tuberculosis. Clin. Microbiol. Rev. 2002, 15:* 294-309.

[14] Arora A, Nadkarni B, Dev G, Chattopadhya D, Jain AK, Tuli SM, Kumar S.The use of immunomodulators as an adjunct to antituberculous chemotherapy in non-responsive patients with osteo-articular tuberculosis. J Bone Joint Surg Br. 2006 Feb, 88(2): 264-9.

[15] Jing J. Wang a,b, Qing H. Shi a,c, Wei Zhang a,b, Barbara J.S. Sanderson. Anti-skin cancer properties of phenolic-rich extract from the pericarpofmangosteen (Garciniamangostana Linn.). Food and Chemical Toxicology. 2012, 50:3004-3013.

[16] Wang JJ, Zhang W, Sanderson BJS. Altered mRNA Expression Related to the Apoptotic Effect of Three Xanthones on Human Melanoma SK-MEL-28 Cell Line. *BioMed Research International* 2013, 2013:715603.

[17] Jing J. Wang, Barbara J.S. Sanderson, Wei Zhang. Cytotoxic effect of xanthones from pericarp of the tropical fruit mangosteen (Garciniamangostana Linn.) on human melanoma cells.Food and Chemical Toxicology, Volume 49, Issue 9, September 2011, Pages 2385-2391

[18] Marcin Barański, Dominika Średnicka-Tober, Nikolaos Volakakis,, et al. Higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops: a systematic literature review and meta-analyses. *Br J Nutr*. 2014; 112(5): 794-811.

[19] Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Effect of *Garciniamangostana*on inflammation caused by Propionibacteriumacnes. Fitoterapia 2007, 78: 401-8.

[20] Supiyanty W, Wulansari ED, Kusmita L. Ujiaktivitasantioksidandanpenebtuankandunganantosianin total kulitbuahmanggis (*Garciniamangostana* L.) Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang. Majalah Obat Tradisional. 2010, 15 (2): 64-70.

[21] Suksamrarn S., Panseeta P., Kunchanawatta S., Distaporn I., Ruktasing S., Suksamrarn A. Ceanothane–and Lupane-Type Triterpenes with Antiplasmodial and Antimycobacterial Activities with Antiplasmodial and from Ziziphuscambodiana. Chem. Pharm. Bull. 2006, 54(4)535 – 537.

[22] Dahlan MS.Statistic for health and medicine (in Indonesia).SPSS : 5th edition. Jakarta:Salemba Medika, Indonesia 2012.

[23] The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition: https://www.avma.org/KB/Policies/Documents/euthanasia.pdf.

[24] Peper A. Aspects of the Relationship Between Drug Dose and Drug Effect. *Dose-Response* 2009, 7(2): 172-192.

[25] A. Gurib-Fakim. Review: Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 2006, 1-93.

[26] Susanto M., Zulfian, B. Kurniawan, A. Tjiptaningrum, Effects of *Garciniamangostana* L. against AST and ALT activity in *Rattusnovergicus* induced by Isoniazid (in Indonesia). *Medical Faculty Lampung University journal*. 2013. 23: 378-381.

[27] Pasaribu F, P.Sitorus S.Bahri, Effects of *Garcinia Mangostana* L.extraction in decreasing level glucosa in blood (in Indonesia). *Journal of Fakultas Farmasi USU*. 2012. 12: 142-149.

[28] Darwich L, Coma G, Peña R, et al. Secretion of interferon-γ by human macrophages demonstrated at the single-cell level after costimulation with interleukin (IL)-12 plus IL-18. *Immunology* 2009, 126(3): 386-393.

[29] Michel Denis. Interferon-gamma-treated murine macrophages inhibit growth of tubercle bacilli via the generation of reactive nitrogen intermediates. *Cellular Immunology, Volume 132, Issue 1, January 1991, Pages 150-157*.

[30] Christian Bogdan, Martin Röllinghoff, Andreas Diefenbach. Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Current Opinion in Immunology, Volume 12, Issue 1, 1 February 2000, Pages 64-76*.

[31] Kubota K. Innate IFN-γ production by subsets of natural killer cells, natural killer T cells and γδ T cells in response to dying bacterial-infected macrophages. Scand J Immunol 2010, 71: 199-20910.

[32] Janis E. Wigginton and Denise Kirschner. A Model to Predict Cell-Mediated Immune Regulatory Mechanisms During Human Infection with Mycobacterium tuberculosis. J Immunol. 2001, 166: 1951-1967.

[33] Emily Gwyer Findlay and Tracy Hussell, "Macrophage-Mediated Inflammation and Disease: A Focus on the Lung," Mediators of Inflammation 2012, Article ID 140937, 6 pages, 2012.

[34] Tostman A, Boeree MJ, Aarnouts RE, de Lange WC, Van der Ven AJAM, and Dekhuijen R.. Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. *Journal of Gastroenterology and Hepatology* 2007.

[35] Akiibinu MO., Ogunyemi EO, Arinola OG, AdenaikeAf, and Adegoke OD. Level of Oxidatif Metabolites Antioxidants and Neopterin in Nigerian Pulmonary Tuberculosis PatientsEur.J.Gen.Med. 2011, 5(4): 208-211.

[36] Venketaraman V, Dayaram YK, Talaue MT, dan Connell ND. 2005. Glutathione and Nitrosoglutathione in macrophage defense against M. tuberculosis. *Infect Immunity.*

[37] Guerra C, Johal K, Morris D, et al. Control of *Mycobacterium tuberculosis* growth by activated natural killer cells. *Clinical and Experimental Immunology* 2012, 168(1): 142-152.