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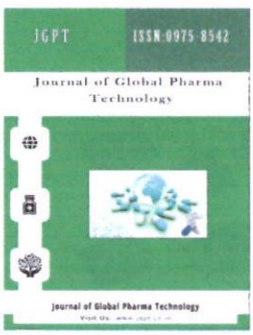

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RESEARCH ARTICLE

The Effect of Ethanolic Extract of Mangosteen Peel As Adjuvant Therapy to TNF-A And IL-10 in Wistar Rats Infected with *M.tuberculosis H37rv*

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Abstract

Objective: This study was an experimental study of wistar rats infected with *M. tuberculosis H37 Rv* and then given isoniazid INH as an anti-tuberculosis drug and ethanolic extract of mangosteen peel as adjuvant therapy. Method: Rats infected with *M tuberculosis* H37rv 10^6 Mc Farland, as 100µl intratracheal. Rats were randomly divided into six groups control (K1), rats infected with M tuberculosis H37rv only (K2), and rats infected with *M tuberculosis* H37rv and then treated with INH 100,200, 300 and 400 mg/kg body weight/ day (P1, P2, P3 and P4, respectivelyAfter administration of 4 weeks of therapy, the rats were sacrificed and the right lung tissue was collected to measured the levels of TNF α and IL 10 by using ELISA. Results The mean level of TNF α was found in the lowest P2 group compared to the treatment group (P1, P3 and P4) and the control was 24.16pg/ml. The –level of IL 10 was found in the lowest P2 group compared to the treatment group (P1, P3 and P4) and the control was 232.75pg/ml. Ethanolic extract of mangosteen peel has a significant effect on decreasing levels of TNF α with a value of P = 0.000 <0.005 and IL 10 with a value of p = 0.001 <0.005. Conclusion Ethanolic extract of mangosteen peel is effective in reducing levels of TNF α and IL10 in adjuvant tuberculosis therapy, especially at doses of 200mg/kgbw/day, in smaller or larger doses there is an increased possibility of interaction between INH and the active substances in the extract. Therefore further studies are needed to find the cause.

Keywords: *M.tuberculosis, Mangosten, TNFa, IL10.*

Introduction

Tuberculosis is an infectious disease caused by a bacterium called *Mycobacterium tuberculosis* which can enter the host body (human) through the airway. Indonesia occupies the world position, the increase in cases in Indonesia is partly due to long treatment times, the presence of HIV infection, and antibiotic-resistant cases.

This is a problem for obtaining supportive therapy that can reduce the number of tuberculosis sufferers [1]. *Mycobacterium tuberculosis* is an intracellular bacterium that has cell walls that contain lots of lipids and have the ability to avoid phagocytosis from macrophages. There are two kinds of defense immune response of the body against tuberculosis infection, namely the cellular immune response (T cells and activated macrophages) along with a number of cytokines and defenses in humoral (anti-body-mediated). The cellular immune response plays a role in defense of the body against tuberculosis infection [2].

M. tuberculosis is inhaled so that it enters the lungs, and then is swallowed by macrophages. Macrophages have 3

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functions,: such as ; producing prothelytic enzymes and metabolites that have a microbactericidal effect; producing cytokines in response to *M. tuberculosis* IL (Interleukin) -1, IL-6, IL-8, IL-10, Tumor Necrosis Factor Alpha (TNF-α), Transforming Growth Factor Beta (TGF-ß); to process and present anti genes against T lymphocytes [3]. Mangosteen *Garcinia mangostana L*(GML) contains various secondary metabolites with medicinal properties such as xanthones and their derivatives. About 190 types of active substances found in the xanthones class in the world, with about 50 species contained by GML [4,5].

The active substance is found among others in the peel of the mangosteen. In addition to xanthones, other active substances anthocyanins and tannins, as well as various nutrients with an antioxidant capacity of 84.6 to 86.3%, or 0.8 times higher than ascorbic acid, so-called super antioxidants [4,5]. Mangosteen peel has various benefits such as antioxidants and anti-inflammation [6] Chomnawang *et al*. shown that xanthone compounds have the potential as anti-inflammatory in reducing the production of *tumor Necrosis Factor Alpha* (TNF-α). Supiyanti *et al*. prove that GML pericarp has anthocyanin which is a potential compound that has antioxidant activity[7].Suksamran report that mangosteen can act as an anti-tuberculosis in vitro which has antioxidant activity [8].

Material and Methods

Preparation of Experimental Animals

The animals used were male wistar rats aged 8-10 weeks with a weight of 150 to 200 grams. The number of rats was 24 divided into 6 groups Male wistar rats weighing 150 to 200g were used. They were obtained from Pusvetma Surabaya. The rat were keep under standard conditions of temperature of 25-27◦C, relative humidity (55±5%), and 12h/12h light/dark cycle. Rats were given normal drinking water ad libitum during the experimental periods. All experiments were conducted according to the principles of Guide for the Care and Use of Laboratory Animals in Indonesia and were approved by the Ethical Committee of Wijaya Kusuma University, Surabaya, Indonesia number 10198/SLE/FK/UWKS/2017

The Preparation of Mangosteen Peel Extract

The extraction of mangosteen peel was performed according to Shahidah, 2008.Briefly,; the powder **of** mangosteen peel (250 g) was defatted with petroleum ether and extracted with 1 liter of 80% ethanol at room temperature by using Soxhlet apparatus for 48 hour. The resultant extract was filtered and concentrated in a rotary evaporator under reduced pressure to obtain a paste, which was stored at-20°C until used.

Experimental Infection Procedure

Rats were anesthetized with Ketamine and Xylazine and then incised in the coli region until the trachea appeared. Trachea was added to 100 µl of Isolate M. tuberculosis H37Rv 106 Mcfarland. Rats were randomly divided into six groups [control (K1), rats infected with M tuberculosis H37rv 106 Mc Farland only (K2), and rats infected with M tuberculosis H37rv 106 Mc Farland and then treated with INH 100,200, 300 and 400 mg/kg body weight/ day (P1, P2, P3 and P4, respectively)]. The administration of drugs or extracts were carried out for 4 weeks then the rats were switched off and their right lung organs were taken to be taken as samples for TNF α and IL 10 examination.

TNF-α Assay According to Skerry et al, 2012 [9]

TNF-α concentrations were measured by sandwich ELISA in a 96 well plate pre-coated with capture antibodies (Legend MAXTM TNF-α ELISA Kit). The plates were washed with Wash Buffer [PBS-Tween 20 (0.05% v/v)] and blocked using 300 µl of 10% (w/v) milk powder for 2 hours at room temperature before the addition of recombinant standards and samples and a 2 hour incubation at room temperature. Following incubation, plates were washed and detection antibody added for 1 hour at room temperature. Following this, streptavidin-HRP was added followed by 3,3′,5,5′ - tetramethylbenzidine (TMB) substrate and color allowed to develop in the dark. Plates were then read on a spectrophotometer at an optical density of 450 nm.

IL 10 Assay According to Skerry, [9]

IL-10 concentrations were measured by sandwich ELISA in a 96 well plate pre-coated with capture antibodies (RayBioR Rat IL10

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ELISA Kit). The plates were washed with Wash Buffer [PBS-Tween 20 (0.05% v/v)] and blocked using 300 µl of 10% (w/v) milk powder for 2 hours at room temperature before the addition of recombinant standards and samples and a 2 hour incubation at room temperature. Following incubation, plates were washed and detection antibody added for 1 hour at room temperature. Following this, streptavidin-HRP was added followed by 3,3′,5,5′ - tetramethylbenzidine (TMB) substrate and color allowed to develop in the dark. Plates were then read on a spectrophotometer at an optical density of 450 nm.

Statistical Analysis

Data were expressed as mean ± standard error of mean and statistically evaluated using one-way analysis of variance, followed by Tukey's multiple comparison tests using SPSS software version 20. $P<0.05$ was considered to be significant

Results

The Exract ethanolic of mangosten peel able to decrease TNFα and IL10. The lung tissue was homogenized in PBS and followed by measuring of TNF α levels: K1 was 201.51 pg /ml ± 28.12; K2 was 75.41 pg / ml ± 2.58; P1 was 316.87 pg / ml ± 1.82; P2 (4) was 24.16 pg /ml ± 1.83; P3 was 32.98 pg / ml ± 18.68; P4 was 40.29 pg / ml ± 25.66.

The results of examination of IL 10 levels from the right lung tissue: K1 was 390.25 pg / ml ± 50.74; K2 was 221 pg / ml ± 5.83; P1 was 594.42 pg / ml ± 53.6; P2 was 232.75 pg / ml ± 60.53; P3 was 434.0 pg / ml ± 165.4; P4 was 606.08 pg / ml ± 206.16.

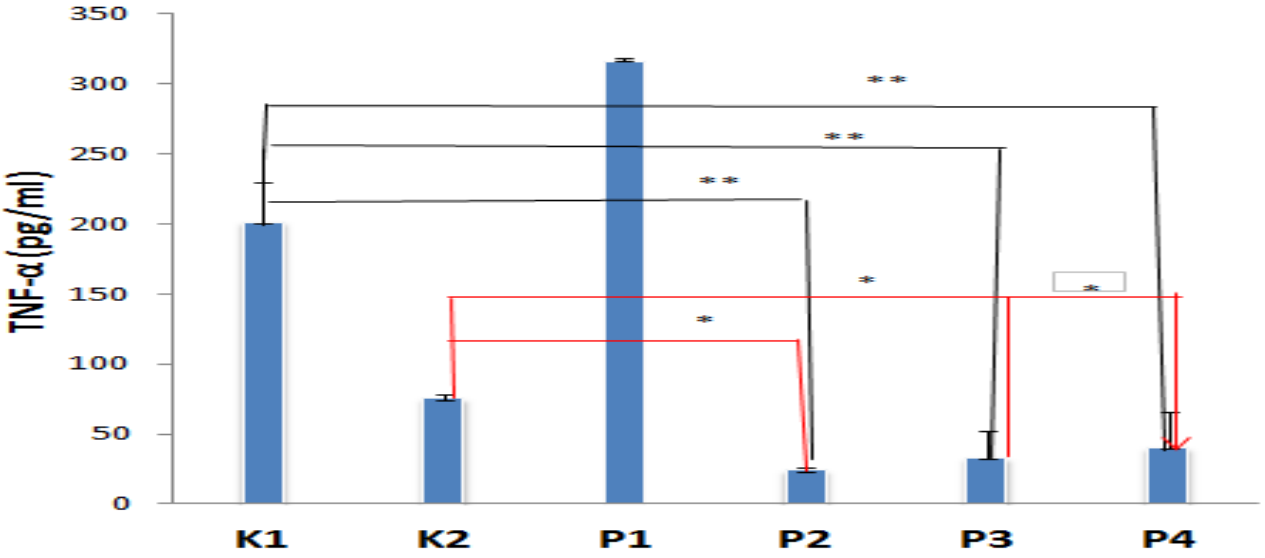

Figure 1: Level of TNF α K1 infected rat group; K2 infected given INH. P1 group infected given INH and mangosteen peel ethanolic extract dose of 100 mg/kgbw/day. P2 group given INH and ethanolic extract of mangosteen peel 200mg/kgbw/day. P3 groups given INH and ethanolic extract of mangosteen peel were 300 mg/kgbw/day. P4 groups given INH and ethanolic extract of mangosteen peel were 400 mg/kgbw/day. *P=0.001, **P=0.005

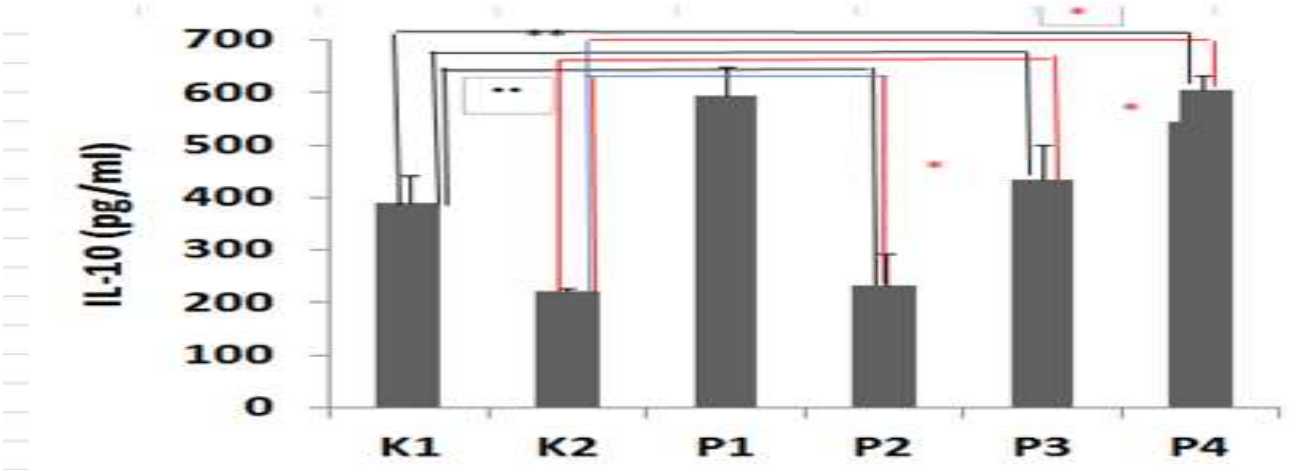

Figure 2: Level of IL 10 K1 infected rat group; K2 infected then given INH. P1 group infected given INH and mangosteen peel ethanolic extract dose of 100 mg/kgbw/day. P2 group given INH and ethanolic extract of mangosteen peel 200mg/kgbw/day. P3 groups given INH and ethanolic extract of mangosteen peel were 300

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mg/kgbw/day. P4 groups given INH and ethanolic extract of mangosteen peel were 400 mg/kgbw/day. *P=0.001, **P=0.005

Based on the ANOVA test results, Figure 1 and 2 showed that there were significant differences in TNF α levels between each group with p-value = 0,000 (<0.05). Likewise, IL 10 levels indicated that there were differences between groups where p-value = 0.001 (<0.005). This results indicated that the addition of mangosteen peel ethanolic extract able to decrease the levels of TNF α and IL 10.

The effect of adjuvant ethanolic extract on mangosteen peel in mice infected with *M.tuberculosis H37Rv* on TNF α levels in figure 2 could be compared between groups (K1) and groups (K2) showing a significant difference where p-value = 0,000 (<0.05) In group (K1) with all groups (P1); (P2); (P3); (P4) showing a significant difference with p-value = 0,000 (<0.005), 0.005. In group (K2) with group (P1); (P2) and (P3) there were significant differences with p-value <0.005. Whereas in group (K2) with group (P4) showed no significant difference with p-value = 0.10 (> 0.005).

Results of Interleukin 10 Examination in Rats Infected with *M.tuberculosis H37Rv*

The effect of adjuvant ethanolic extract on mangosteen peel in rats infected with *M.tuberculosis H37Rv* on TNF α levels (figure 2) could be compared between groups K1 with groups K2, P1 and P4 indicating there were significant differences where p-value <0.005, whereas when compared between groups K1 with groups P2 and P3 there were no significant differences. In group K2 with groups P1, P3 and P4 there were significant differences with p value <0,005, whereas with group P2 there were no significant differences.

Discussion

At the time of *M.tuberculosis* infection, an increase in activation of NF-kB was found increase in various types of genes that mediate immune responses include TNF α and interleukin 10. TNF α is a proinflammatory cytokine which will occur after *M. tuberculosis* enters the host body through the airway. In the study of Yuni *et al* 2017, reported that the levels of TNF α in the microglia of mice infected with *M.tuberculosis H37Rv* are increased [10,11]. In this study, it was found that rats were infected with *M. Tuberculosis* showed an inflammatory process in lung of rats. Our result proved that lung tissue of rats were infected with *M. Tuberculosis* indicated an increasing of both TNF α and IL10 levels [10, 11].The increasing of TNF α and IL 10 levels (P1 group) were probably due to the amount of active substances in the ethanolic extract of mangosteen peels affecting the work of INH and this possibility need further research.

The increase in the ajuvant dose then found a decrease in the levels of TNF α and IL 10 due to the effects of the ethanolic extract of mangosteen peel which can cause NFkB activity, especially in the P2 group, which is a dose of 200mg/kgbw/day [12, 13,14].

Increasing the dosage 300 and 400mg/kgbw/day of the ethanolic extract of mangosteen peel will cause the active substances contained in the extract to increase so that the immune response actually increases the levels of TNF α and IL 10. This result supported with Susanto's study, which examined the effect of ethanolic extract on mangosteen peels in rats given INH [15, 16].

Conclusion

Ethanolic extract of mangosteen peel can reduce levels of TNF α and IL 10 at doses of 200mg/kgbw/day of mangosteen peel ethanolic extract could be used as new adjuvant therapy in tuberculosis.

Abbreviation

GML: *Garcinia mangostana L*

TNFα: tumour necrosis factor alpha

IL10: interleukin10

NFkB: nuclear faktor kappa beta

TGFß: transforming growth factor ß

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