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Effect of Crude Ethanolic Extract of Mangosteen Pericarp (*Garcinia mangostana Linn.)* on IFN-γ and IL12 Level in Mice Infected by *Salmonella Typhimurium*

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Abstract: *Garcinia mangostana* L pericarp extract is known has active substances called Xanthones which has strong antioxidant effects. This substance also has anti-inflammatory and immunomodulation effects. However, the intercellular infection of *Salmonella* stimulates macrophage to produce interleukin IL12 for enhancement of IFN-γ secretion. lFN γ, which plays role for activates macrophage, acts as positive feedback in stimulating macrophages to kill *S.* Typhimurium. Macrophages play role in the phagolysosome fusion process are produced. This study aims to determine the effect of Crude Ethanolic Mangosteen Extract (CEME) on IFN-γ and IL-12 secretion in mice infected with *S.* Typhimurium. This study used 25 mice Balb/c. Mice divided into 5 groups consist of 5 mice in each group, including positive control (mice were infected with *S.* Typhimurium), T0 (control with 40 mg/ml of crude extract mangosteen pericarp without *S.* Typhimurium), T1, T2 and T3 (treatment with 20 mg/ml, 40 mg/ml and 60 mg/ml of crude extract mangosteen pericarp). Then, the mice were necropsied and dissected to take the blood directly from the heart. Levels of IFN-γ and IL-12 were analyzed using ELISA. Data were analyzed by One-Way ANOVA followed by Post-Hoc test (LSD) to assess the comparison between groups. Results showed that CEME increase serum IFN-γ and IL12 without infection. CEME increase serum IL12 in T1, T2and T3 with a p-value 0,000. Other results showed decreases serum IFN-γ in T2 and T3 with a p-value 0,000. CEME improve immune response through the increases serum IL-12 and decreases serum IFN-γ in mice infected with *S.* Typhimurium.
Keywords : Extract, *Garcinia mangostana*, IFN- γ, IL-12, *S.* Typhimurium.

Introduction

Mangosteen (*Garcinia mangostana*) is a tropical fruit has been used as a traditional indigenous medicine across Southeast Asia, especially the pericarp (rind/peel/hull) with widespread biological activities, including anti-inflammatory[1,2], anti-oxidant[3], anti-proliferative[4,5], immunostimulatory[6]. Ethanol extract from mangosteen pericarp is the highest content of total polyphenols and the strongest antioxidant activity, as well as the relatively higher antibacterial activity[7].

While, the fruit pericarps are most nature abundant sources of xanthenes. These natural chemical substances possessing of numerous bioactive properties that help to maintain intestinal health, neutralize free radicals, help and support immunomodulation systems and treatment of skin infections[8].

Salmonella is a very successful enteric pathogen because it has developed strategies to cope with most of the immune defenses employed by the host during the different phases of the disease[9]. *S.* Typhimurium results in systemic infection and a disease similar mechanism as *S. typhi*[10]. During the early, innate host response against Salmonella, high levels of gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-a) are produced, Lipoproteins and peptidoglycans on the surface of the bacteria ligate receptors on macrophages and dendritic cells include the Toll Like receptors (TLRS). Particularly, TLRs and mannose receptor and their ligation stimulates nitric oxide production with the cells, which is toxic to bacteria. Signaling by the TLRs stimulates the release IL12, which in turn drives NK cells to produce IFN-γ in the early phase of immune response. IL12 also stimulates TH1 cells (antigen specific CD4) to release IFN- γ[11]. Consequently, production of macrophage activating cytokines, particularly IFN-γ and IL12 are major hallmark in the host response against all intracellular bacteria[12].

In this study ethanol mangosteen pericarp extract may contains high levels of flavonoids which have antioxidants that can be useful to improve immune response through increasing IFN- γ and IL12 cytokines which plays a key role for macrophage activation in phagocytosis of intracellular microbes such as *Salmonella* infection.

Materials and method

The study required 15 days to accomplish on 25 male mice were randomly divided into 5 groups each consisting of 5 mice, including the positive control group, T0, T1, T2 and T3. The experimental animals of group C1 was infected with dose of 108cfu bacteria *S. typhimurium* as control positive, group T0 was administered with 40 mg/ml (CEMP) for 14 days, group T1 was administered with 20 mg/ml (CEMP) for 7 days and 7 days later for *S. typhimurium* infection, group T2 was administered with 40 mg/ml (CEMP) for 7 days and 7 days later for *S.* Typhimurium infection, group T3 was administered with 60 mg/ml crude extract mangosteen pericarp for 7 days and 7 days later for *S.* Typhimurium infection . Afterward the mice were necropsied and dissected for the blood to be taken. Ethical clearance approved by the studies Faculty of Medicine Brawijaya University NO. 360/EC – S2 / 05 /2015 at date 09 JUN 2015.

Extract Garcinia mangostanal Preparation

Pieces of G. Mangosteen pericarp was dried, ground and sieved to produce a fine powder, the powder was then weighed each 100 grams to be extracted. The extraction process used the macerated method. Each extraction process used 100 grams of powdered pericarp and was extracted using ethanol 96%. The extract was left to settle overnight (Pharmacology Laboratory, Brawijaya University). (Every mouse received 0.5cc/mice of concentration CEMP)

Inoculum Preparation

Four days (96 hours) after the last immunization (mangosteen extract), mice were infected with 300 μL *Salmonella* Typhimurium bacteria (concentration 2x108 cells / mL) two times with 2-days interval orally. Four days (96 hours) after the last infection, mice were sacrificed and taken the whole the blood[13].

Statistical Analysis

Each data was subjected to statistical analysis. We analyzed our data using One-Way ANOVA test followed by Post-Hoc test (LSD), a significant $p < 0.05$, to assess the comparison between groups. We considered < 0.05 as significant.

Results

Based on the Figure above showed that the difference in dose of CEMP influenced IL-12 levels. By looking where the IL-12 levels in Mice was affected by *S.*Typhimurium, the treatment of CEMP which was started at a dose of 20 mg/mL (T1), dose of 40 mg/mL (T2) and dose of 60 mg/mL (T3), compared with IL-12 levels in the positive control group became higher after giving CEMP.Then, IL-12 levels increased when given higher doses of CEMP. Thus, based on the descriptive assessment according to the mean of IL-12 levels, it can be said that the administration of treatment in the form of CEMP at a dose of 20 mg/mL (T1), 40 mg/mL (T2),

and 60 mg/mL (T3) showed different influences, where higher dose of provided Mangosteen extract further increased the IL-12 levels and showed CEMP at a dose60 mg/mL.

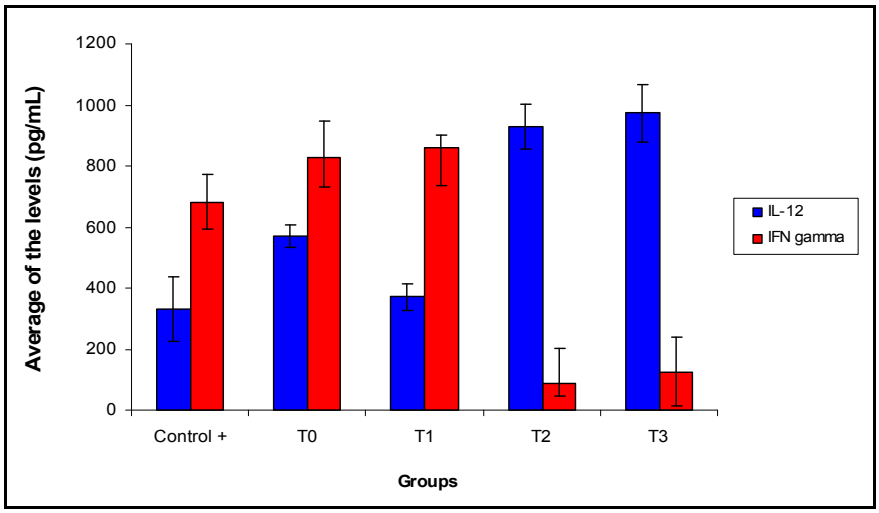

Figure 1. Graph of IFN- γ and IL-12 Levels at each dose of Mangostine extract

As for the overall differences in IFN- γ Levels in each group above can see in the graphic .Shows that the difference on IFN- γ levels. Starting to look where the IFN- γ levels in positive control (+) group effected by *S.*Typhimurium is higher, but in group of Mangostine extract was given started at a dose of 20 mg/mL (T1), dose of 40 mg/mL (T2) and dose of 60 mg/mL (T3), compared with IFN- γ levels in the positive control group. show that the effect of Mangostine extract is starting to look where the concentration of IFN-γ Levels in mice induced (20 mg/mL Mangostine + *S. typhimurium*) was higher,and then the treatment groups dose of 40 mg/mL (T2) and dose of 60 mg/mL (T3) becomes lower compared with IFN gamma Levels in the positive control group. Then the IFN- γ Levels decreased when given higher doses. Thus, based on the assessment descriptively according to the mean IFN- γ Levels is, it can be said that the administration of treatment in the form of Mangostine extract at a dose of 20 mg, 40 mg and 60 mg showed different influences, where the higher dose of Mangostine extract provided will further lowering the IFN gamma Levels.

Discussions

This study showed that the ethanol extract of Garcinia mangosteen pericarp affected the increased levels of IL-12 in groups T0, T2 and T3. Giving Mangostine extract at a dose of 60 mg/mL could increase the IL-12 levels higher than the provision of Mangostine extract at a lower dose of 40 mg/mL. Thus, the dose of 60 mg/mL was more effective to increase IL-12 levels than the dose of 40 mg/mL. However, the provision of Mangostine extract at a dose of 40 mg/mL could increase the average IL-12 levels better than a dose of 20 mg/mL. Hence, a dose of 40 mg/mL was more effective to increase IL-12 levels than a dose of 20 mg/mL. The average of IL-12 levels in granting Mangostine extract at a dose of 20 mg/mL was more effective to increase IL-12 levels rather than the positive control group. Mangosteen pericarp extracts contain 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, supposed to act as an immunomodulator in modulating the increase of IFN-γ and IL-12 secretion. IFN-γ secretion by macrophages also stimulates the formation of free radicals to destroy components of M. tuberculosis bacteria which are the DNA and the bacteria's cell walls that play a role in combating the *M. tuberculosis* bacteria[14] in other studies showed that Mangosteen Pericarp extract can induce an increasing value of TLR5 and CD14 expression by mice PBMCs, with optimal dose 40mg/ml. Since the level of cytokines plays an important role during the protective response in a biological system, it is interesting to investigate the effect of Mangosteen on cytokines[15]. IL-12 has multiple biological activities, and it is a key factor that drives Th1 responses and IFN-γ production. Early application or production of IL-12 during infection may activate Macrophages and augment a host's cell-mediated immunity while shaping the ultimate antigen-specific immune responses. As a result, IL-12 may play a key role in protection against bacterial and viral infections, and IL-12 immunotherapy could be important in the treatment of diseases where a Th1 response is desirable. Meanwhile, cytokines including IL-12 is short in vivo half-life, and in the development of advanced drug delivery systems[16, 17]. There were

studies reporting that the activity of petroleum ether extracts of mangosteen pericarp at a dose of 120 mg/kg body weight/day has a significant effect in increasing the secretion of IFN-γ exceeding the increase at the time rats were infected by M. tuberculosis. Also, the activity of Petroleum Ether Extracts of mangosteen pericarp is significantly influential in increasing the secretion of IL-12[18]. In this present study showed that (CEME) improve immune response through the increases serum IL-12 production in mice infected with *S.* Typhimurium.

Effect of mangosteen Extract pericarps on Levels of IFN- γ

IFN-γ plays a key role in enhancing immune responses, in particular by modulating macrophage activation, which enhances the killing of intracellular bacteria[19]. An increase in IFN-γ which also increases the activity of macrophages in secreting ROI and NO during infection proved that the bacterial infection can lead to an immune response in the body Furthermore, macrophages produce IL- 12, which then stimulates NK cells to produce IFN-γ which in turn activates other macrophages[20]. Giving ethanol extract of Garcinia mangosteen pericarp affected the increasing secretion of IFN-γ and that a dose of 20 mg/mL Mangostine was the best dose because it resulted in the p-value of 0,000 <0,05. In T3 treatment with a higher dose of 60 mg/mL Mangostine, the results showed that it tended to reduce IFN-γ secretion. While for the IL-12, a significant difference occurred based on T1, T2 and T3 treatments in which the p-value was 0,000 <0,05. Between T2, and T3 they were not significantly different, but the same trend was also observable in the T3 treatment which showed higher 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[21]. However, this study confirmed that with the increase in concentration, the response ultimately decreased. This often occurs in natural medicines, particularly in the use of extracts, because the components within the compound is not a single compound but rather they consist of various chemical compounds, in which these components work together to cause an effect. If the extract dose is increased, the number of contained chemical compounds will increase, resulting in adverse interactions that lead to a decrease in effect[22]. In this present study showed that (CEMP) decreases serum IFN-γ at higher dose which that back to several reasons could be led to the couse here we suggest that the (CEMP) affected or intervention directly on Th1 cells to depress production of IFN-γ at higher dose that may IFN-γ expression is regulated by various chemical compounds, and these components work together to cause an effect. If the extract dose is increased, the number of contained chemical compounds will increase, resulting in adverse interactions that lead to a decrease in effect. Or probably regulation of IFN-γ not similar with IL-12 at different concentrations of (CEMP). That could be conclude the lower dose of (CEMP) affected better then higher dose. There were studies reporting that the (CEMP) could be significantly reduce TNF-α production generated from peripheral blood mononuclear cells by stimulating with P.acen[25].

Other studies showed that (CEMP) increasing number of cells expressed CD14 and also could altered the innate immune response and increase its activity and works optimal at 40mg/ml without any vaccination. That can be useful as immunomodulation [26]. In this study showed giving CEMP only increased the IL-12 and IFN-γ level at 40mg/ml without any infection in (T0 group) could improve immune response as immunoistimlut. While there studies showed that (CEMP) had the highest content of flavonoids and anthocyanin respectively and also exhibited higher antioxidant and antibacterial activities [7].

Based on the above mechanisms it is essential to maintain and conclude the intracellular infection of Salmonella which will stimulate macrophages to produce IL-12 playing a role in the formation of Th1 which then secretes IFN-γ that acts as positive feedback in activating macrophages to destroy the phagocytized germs. The Mangosteen ethanolic pericarp extract improve immune response through increases the secretion of IL-12 and decreases secretion of IFN-γ at higher dose with *S.*Typhimurium infection in mice. The probably mechanisms of CEMP towards Th1. May be there is paralysis effect due to direct effect to suppress the Th1. May be caused by small samples (only 5) so the SD is large.

Conclusion

The effected of Crude Ethanolic Mangosteen Extract pericarp at a dose of 60 mg/ml has a significant effect in increases the serum of IL-12 secretion and the activity of CEME pericarp were significantly influential in decreases the secretion of IFN-γ in mice were infected by *S. typhimurium* and CEME pericarp might be as increasing the serum of IL-12 and IFN-γ secretion by negative control group (T0 group) to the dose of CEME at 40mg/ml without any infection could altered the innate immune response.

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