# Effect of Mangosteen (*Garcinia mangostana)* PEEL Extract towards CD4+, CD8+ T LYMPHOCYTES, CD38 Expression, NK Cells, IL-2 and IFNγ in Hiv Patients with Antiretroviral Therapy

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## ABSTRACT

**Introduction:** HIV/AIDS still being an emerging & epidemic disease in Indonesia. Humans infected with HIV have shown to be under chronic oxidative stress. Experimental studies have shown that obtained xanthones from mangosteen have remarkable biological activities as an antioxidant. This study aims to analyze the effects of Mangosteen Peel Extract (MPE) toward CD4+ T cells, CD8+ T cells, NK cells, CD8+CD38 expression, levels of IL-2 and IFN-γ, in HIV patients with antiretroviral therapy.
**Method:** This experimental study was designed using double-blind, randomized control group which randomized by the permuted table. Subjects were HIV-positive patients receiving antiretroviral therapy more than six months. Patients were divided into 2 groups; treatment group (n=20) and placebo group (n=20). The treatment group had been given MPE 2400 mg/day for 30 days the same as the placebo group. The variables were measured before and after treatment using FacsCalibur Becton-Dickinson flowcytometry.
Results: There was significant increase in the number of CD4+T cells (p=0.001). There was significant decrease in CD38 expression (p=0.001). There were no significant changes in CD8+T cells (p=0.601), NK cells (p=0.911), IL-2 (p=0.260) and IFN-γ (p=0.588).
**Conclusion:** Mangosteen peel extract increases the number of CD4+ T cells and decreases the level of CD38 expression, whereas the effect of CD8+ T cells, NK cells, IL-2 and IFN-γ in HIV patients with antiretroviral therapy were not significant.

***Keywords***: Mangosteen, CD4+, CD8+, CD38, NK cells, IL-2, IFNγ, HIV, Antiretroviral

The AIDS epidemic in Indonesia is one of the fastest growing in Asia. Indonesia will have almost twice the number of people living with HIV and AIDS in 2014 as compared to 2008, rising from an estimated 227,700 to 501,400.(1)(2)(3)

The hallmark of HIV infection is the progressive loss or depletion of CD4+ T lymphocytes.(4)(5)(6)(7)(8)(9) The changes in the CD4+ T lymphocytes counts are important indicators of the response to antiretroviral treatment.(10)(11)(12)(13)(14) Also, the analysis of CD38 expression on lymphocytes has become an important tool for monitoring patients during HIV-1 infection.(15)(16)(17) The role of CD38 expression in CD8+ T cells as a prognostic marker of virological failure. CD8 cells are also known to produce many cytokines(18)(19) including IFN-γ, TNF-α, and IL-2, in meticulous broadly related aspects of infection. Levels of IL-2 in HIV infection may be an indicator of the improved immune system in HIV infection.(20) The levels of IL-2 has an essential influence on the proliferation of CD4.(21) IL-2 have a major role to help the development of CD8+ T cytotoxic cells, CD4+ T cells, B cells, and increase the killing power of macrophages and regulate T cells multiplication.(22)(23) IFN-γ also produced by NK cells, it can stimulate dendritic cells which are non-specific and specific immune cell coordinator.(24) IFN-γ function prevents

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viral replication in infected cells and the surrounding cells that induce anti-viral environment. IFN-γ produced by NK cells, also has positive feedback on its own of NK cells, NK cells which can become more active in its function.(25)

Humans infected with HIV have been shown to be under chronic oxidative stress. Oxidant production could enhance HIV replication via activation of NFκB and indirectly through activation genes that further promotes oxidative stress and hence HIV replication.(24)(25)(26) Interestingly, HAART proved to have deleterious effects as a result of mitochondrial dysfunction, increase in oxidative status and it plays an important role in the occurrence of oxidative stress.(27) Antioxidant may have a role in the treatment of HIV infection.(28)

Experimental studies have shown that obtained xanthones from mangosteen have remarkable biological activities as antioxidant, antitumor, antiallergic, anti-inflammatory, antibacterial, antiviral activities, antiplasmodial, cytotoxic and potential cancer chemoprotective activities.(26) Moreover, some of the xanthones from mangosteen have been found to influence specific enzyme activities, such as aromatherapy, inhibitor kB kinase, quinone reductase, sphingomyelinase, topoisomerase and several protein kinases.(26) Several studies showed that xanthones from Garcinia mangostana act as an active constituent against HIV-1 protease.(29)(30)(31) A randomized controlled trial study in healthy adults showed that intake of a xanthone-rich mangosteen product elevated of the frequency of peripheral Th cells frequency 2.6% (SD 5.7%).(32)

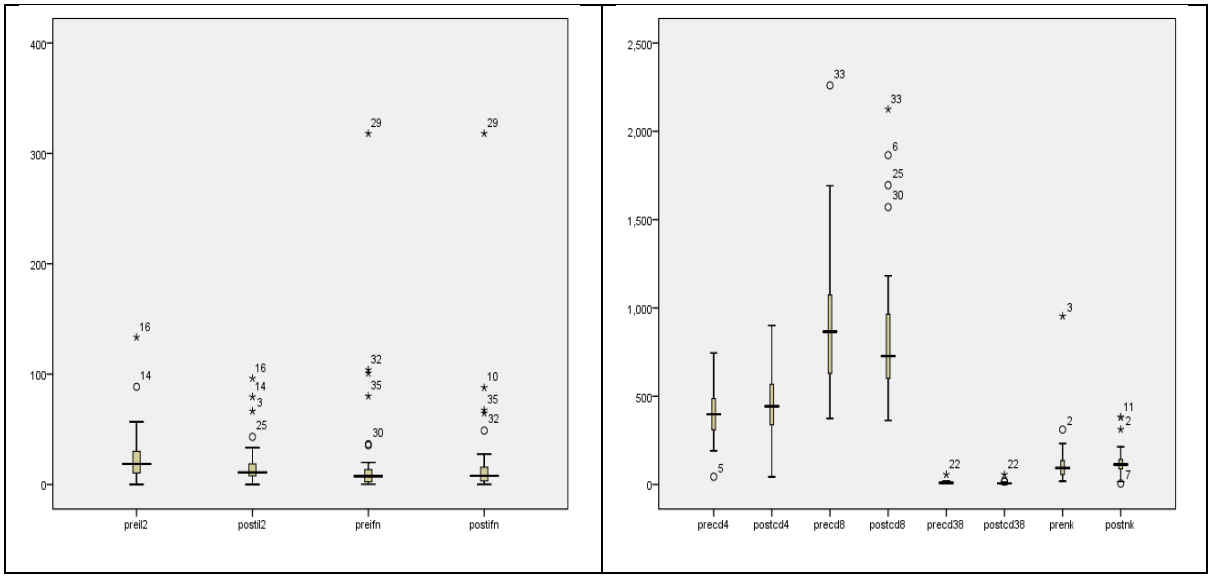
Although it has been many research related to Garcinia mangostana performed, research on the effects of mangosteen (*Garcinia mangostana)* peel extract towards $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, NK cells, IL-2, IFN-γ and CD38 expression in HIV patients has not been investigating. Thus not known whether mangosteen *(Garcinia mangostana)* peel extract affects the $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, NK cells, IL-2, IFN-γ and CD38 expression in HIV patients with HAART.

## MATERIALS AND METHODS

This experimental study was designed using double-blind, randomized control group which randomized to the permuted table. Subjects were HIV-positive patients receiving antiretroviral therapy more than six months. Patients were divided into 2 groups; treatment group (n=20) and placebo group (n=20). A treatment group had been given MPE 2400 mg/day for 30 days the same as the placebo group. The number of $CD4^+$ cells, $CD8^+$ T cells, NK cells, $CD8^+CD38$ expression, the level of IL-2 and IFN-γ were measured before and after treatment using FacsCalibur Becton-Dickinson flowcytometry.

## RESULTS AND DISCUSSION

Analyses of immune cells before and after treatment revealed significant increase in CD4+ T cells, but reduced another immune cells and cytokine shown in the Graphic 1. Boxplot pre-post test treatment group.




**Graphic 1. Boxplot pre-post test treatment group**

***Changes in CD4⁺ T cells***

The statistical analysis showed that the P value 0.000 (P<0.05) in each group. There is a significant increase in pretest and posttest group. Means treatment group 401.9±152.2 and placebo group 492.8±196.2 Mean value between pre-test and post value difference is larger, so the result means that there is a significant difference of Mean.

***Changes in IL2***

IL-2 level decreased both two groups. But the decreased in the treatment group was not significant (p=0.26). Delta decreased in the treatment group was 5.94 and 8.72 in the placebo group. It shows delta placebo group was 1.4 times higher than the treatment group.

***Changes in CD8⁺ T cells***

The frequency of peripheral blood CD8⁺ T cells decreased in both groups. But the decreased was not significant in extract group (p=0.601) neither on placebo group (p=0.135). Delta decline of the number of CD8⁺ T cells from extract group is 51.65 and 59 in placebo group which is delta decline of placebo group 1.14 times higher than the Delta of treatment group. There is an increase in Th/Tc ratio between baseline and post-treatment. In the extract group Th/Tc ratio increase from 0.44 to 0.51, and 0.45 to 0.54 in the placebo group.

***Changes in CD38 expression***

CD38 expression decreased both two groups. The decreased was significant in extract group (p=0.001) either in the placebo group (p=0.001). Delta decline of CD38 expression from extract group is 3.23 which is 1.035 times higher than the Delta of placebo group whose delta is 3.12.

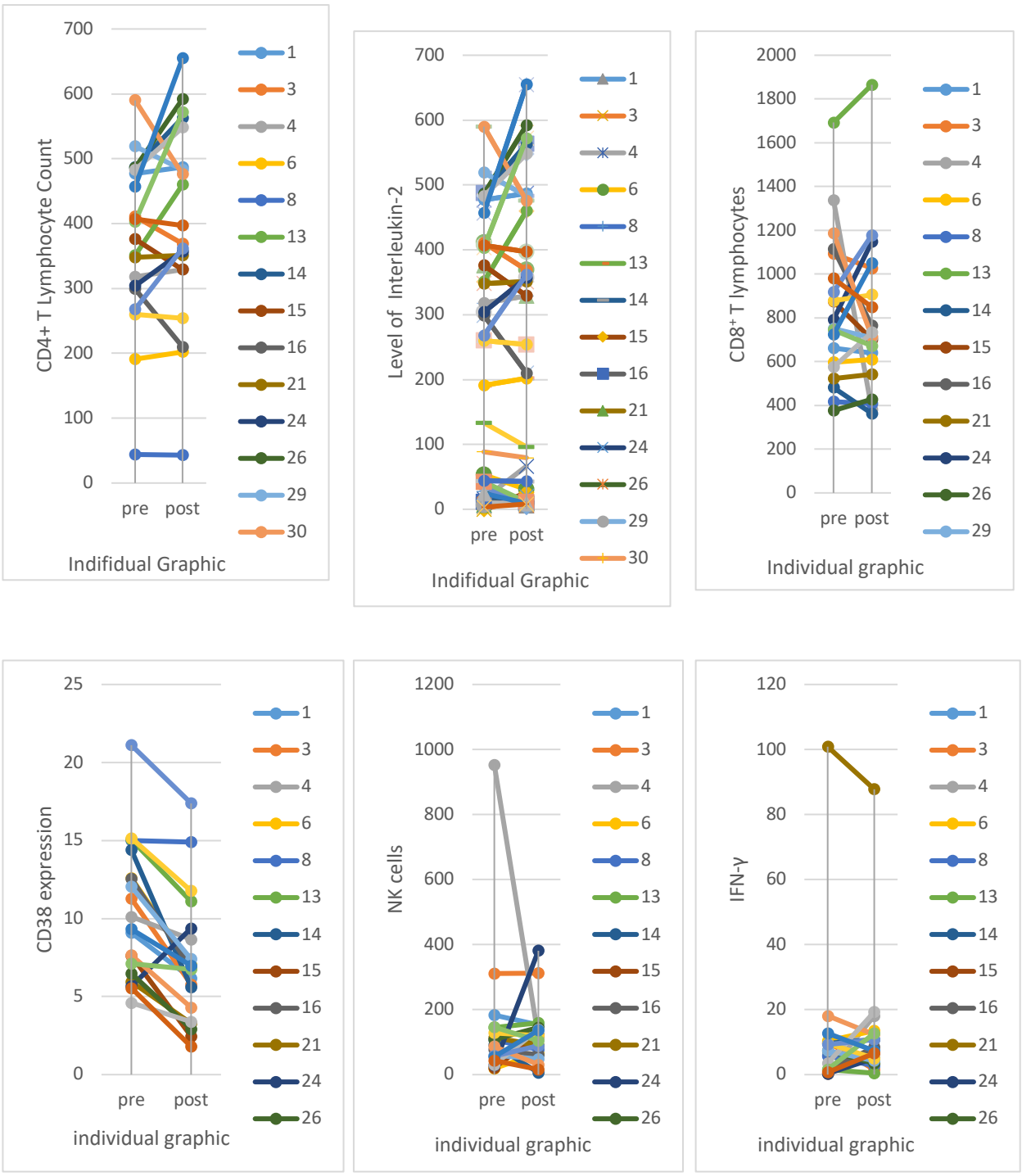
***Changes in NK Cell***

NK cells in the treatment group decreased but not significant (p = 0.911). The number of NK cells in the placebo group increased, but the increase was not significant (p= 0.121).

***Changes in IFN-γ Level***

There is no significant increase in IFN-γ levels in the treatment group (p = 0.588), and there is no significant decreased of the placebo group (p=0.444).

The other than that, the authors report the changes of each indicator in individual graphic




**Graphic 2. Individual graphic treatment group**

The authors report here that after the treatment of mangosteen peel extract, CD4+ T cells have shown a significant increase in the treatment group, resulting in decreased levels of IL-2. Otherwise, $CD8^+$ T cells decreased in line with a decrease of CD38 expression. In the other side, there were no significant changes in IFN-γ caused the decreased of NK cells.

The increasing IFN-γ and CD4+ Lymphocytes is closely related to the antioxidant content contained in Mangosteen Peel Extract/MPE. In this study, HIV patients had high levels of ROS, along with high viral levels and the side effect of consumption the drugs can increase the level of ROS. Antioxidants in MPE can inhibit the high levels of ROS (Reactive oxygen species)

in HIV patients. The mangosteen peel extract contains xanthones compounds which have a high antioxidant function that can be used to protect and reduce cell damage mainly caused by free radicals. These results indicate a role of MPE as an antioxidant which also suppresses apoptosis that would maintain the number of CD4+ T lymphocytes.

Decreased levels of IL-2 could be due to the role of cytokines that influenced easily soluble factor or a local effect on a particular cellular environment. It would affect T cell stimulation and the effect of fluctuations of the immune activation. In this study, increase the number of $CD4^+$ T cells not accompanied by elevated levels of IL-2 resulting in the balance of the immune system in HIV patients. The descent of IL-2 cytokines, which decrease the number of NK cell can happen because of several factors. It could be due to exhausted factors on the condition of HIV patients to increase so that the number of $CD4^+$ T cells not accompanied by elevated levels of IL-2 resulting in the balance of the immune system in HIV patients. Increase in IFN-γ levels was due to a decrease in CD8 cells due to the mechanism of HIV.[38] The work of IFN-γ above indicates that the results of this study are not enough to seek changes in IFN-γ levels, but still require research looking for the effectiveness of mangosteen peel extract on changes in function and work of IFN-γ. The function have done by working with NK cells; this cooperation occurs after NK cells identify microbes and perform its cytolytic function, then NK cells produce IFN-γ. The impairments of NK cell are associated with expansion of an "anergic" NK cell. In HIV infection, CD4 T cells induce IFN-γ and IL-2 in an attempt to suppress viral infections.

Exhausted factor mechanisms began in early infection (primary) where an increase in $CD4^+$ T cells and $CD8^+$ T cells, followed by the rate of infection cannot stop, and the production of IL-2 levels was high. This situation makes $CD4^+$T cells, and $CD8^+$T cells do not respond, characterized by the inability to produce cytokines, perforin, and granzyme (partial exhaustion I). Furthermore, the value chronic immune activation has been reasoned to be a significant contributor to disease progression in HIV-1 infected patients which has prompted the use of the expression of cell surface activation markers such as CD38 to monitor disease progression. Results from several such studies have documented a correlation between plasma viral load and the increased expression of CD38 as strong predictor of disease progression, and it may because of the response to ARV despite to mangosteen peel extract.

Variations in individual results can be due to differences in allele and Polymorphism in every individual which caused by the variation of the expression of DNA base composition and chromosome differences. Polymorphism in enzymes can increase the toxic effects of the drug. The involvement of the gene and the protein will affect the body's response to an adjuvant and drug products. Thus, the need for pharmacogenomics approach to that may explain individual variations in the response of each of the drugs given; this response is closely related to the genetic differences of each. Another factor that affects the immune response is different for every individual who is affected by immunogenetics factors, one of which is an HLA (Human Leukocyte Antigen) system on each that will be an expression of different characteristics. HLA's role associated with the number of CD4+ T lymphocytes. HLA associated with MHC class II or the so-called APC cells for antigen presentation to T CD4+ lymphocytes. MHC (Major histocompatibility complex) controls the immune response and antigen expression.

Bass(33) in his research stated that the correlation absence between the number of CD4+ T lymphocytes and the levels of IL-2 might be due to response disturbance of the mitogens proliferation which correlates with decreased expression of the IL-2 receptor and increased expression of HLA-DR. CD4+ T lymphocytes. Kawamura(34) added function decline in the role of Dendritic cells (DC) as immunopathogenesis in HIV disease that will affect the APC response in CD4+ T lymphocytes, which will stimulate CD4 cell proliferation and production of IL-2 production which is mediated by the binding of gp120 in HIV patients.The decrease result in this study, because the bioavailability of mangosteen xanthones is limited as it is for many phytochemicals, the gastrointestinal (GI) tract is exposed to high concentrations of these compounds and their metabolites.It may lead to improvement in patients defecation.

On the previous study about mangosteen, the study population was very homogeneous regarding nutritional status and other

lifestyle factors. They are representative of ordinary, generally healthy adults.It may take a different result dealing with this study, whereas study population was an HIV patient wherein HIV patients, there was progressive dysregulation of the immune response to progressive disease. Although they were accepted antiretroviral therapy, ART only partially corrects these deficits.

Another factor that affects the immune response is different for every individual who is affected by immunogenetics factors, one of which is an HLA (Human Leukocyte Antigen) system on each that will be an expression of different characteristics. HLA's role associated with the number of CD4+ T lymphocytes. HLA associated with MHC class II or the so-called APC cells for antigen presentation to T CD4+ lymphocytes. MHC (Major Histocompatibility Complex) controls the immune response and antigen expression. The literature presents equilibrium between oxidants and antioxidants is crucial to the body; it would be important to look into the products consumed, that have a protective effect on the organism and encourage other population to consume it for its beneficial effect against certain diseases such as the metabolic syndrome.

Correlation towards the negative in the extract group may be due to the of the increase mechanism in the number of CD4 + T lymphocytes. It can express CD38, and HLA-DR showed the activation of immune chronic affecting the deregulation of cytokines, resulted in increased production pro-inflammatory cytokines such as IL-1, TNF, IL-6 as well as a decrease in TH1, such as IL2 and IFN. Decreased IL-2 production will affect the endogenous IL-2 receptor. Unbalanced lymphocytes T CD4 + and CD8 number can damage certain antigens. (17)

However, this study did not analyze the viral load; further research needed with viral load as a gold standard. Other than that the results will be seen to variance with the patient in the early stages (primary), before the patient given anti-retroviral and herbal research done ± six months, so it can be seen how effective the concoction in the immune system and process deregulation immune balance. This study also report that MPE can be the adjuvant therapy for HIV patients, while consumption the anti-retroviral therapy.

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