



The Effect of *Psidiumguajava* Linn Extract Toward Immunoglobulin G (IgG) As Anti Dengue

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ABSTRACT

In dengue infection, there is an immune system disorder which is characterized by decreased levels of immunoglobulin G (IgG). This study was conducted to determine the effect of *Psidiumguajava* Linn leaf extract against IgG in adult DF and DHF. The therapy group was categorized into treatment group (*Psidiumguajava* Linn. Leaf extract capsule) and control group (placebo capsule) dose of 3x2 capsules for 3-4 days. Examination of leukocytes by taking the patient's venous blood, for IgG blood samples used plasma. Independent *t*-test was used for data analysis. Provision of *Psidiumguajava* Linn leaf extract did not show a significant difference in increasing the number of leukocytes ($p = 0.99$) and IgG levels ($p = 0.72$) between the two therapy groups. *Psidiumguajava* Linn leaf extract did not significantly increase the IgG of adult DF and DHF patients. Further research is needed to evaluate the effect of *Psidiumguajava* Linn leaf extract against IgG as adjuvant therapy for DF and DHF patients.

KEYWORDS: Dengue Fever, Dengue Hemorrhagic Fever, leukocyte, Immunoglobulin G, *Psidiumguajava* Linn.

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I. INTRODUCTION

DHF is included in the ten biggest diseases in hospital inpatients in Indonesia with a total of 30,232 cases in men and 28,883 cases in women¹. The number of DHF cases in Wonosobo District in 2015 was 34 cases with Incidence Rate (IR) of 4.4 per 100,000 population². In DHF, the immune system decreases in protecting the body from antigens which are often characterized by leukopenia. One type of leukocytes that plays a role in the specific immune system is B lymphocytes. The immunopathogenesis mechanism of dengue virus infection involves a humoral response in the form of antibodies such as IgG by B lymphocytes that play a role in viral neutralization, complement mediated cytolysis and antibody mediated cytotoxicity. *Psidiumguajava* Linn leaf extract has immunostimulatory activity³.

Based on several research tests, *Psidiumguajava* Linn leaf extract contains flavonoids which have immunomodulatory effects by inducing tyrosine phosphorylation JAK2, TYK2, STAT3, STAT4 and producing Th1 derived from interferon- γ (IFN- γ) and decreasing Th2 derived from interleukin-4 (IL-4) by mononuclear peripheral blood cells (PBMC) which are characterized by an increase in total and differential leukocytes and antibodies⁴.

II. LITERATURE REVIEW

DF and DHF are acute febrile diseases caused by viral infections of flaviviridae species, namely dengue (DEN)-1, DEN-2, DEN-3 and DEN-4 which are transmitted through the bite of mosquitoes of the *Aedes* genus, especially *Aedes aegypti* and *Aedes albopictus* as vectors. DF and DHF are caused by the same virus, but the pathophysiological mechanisms are different and lead to clinical differences. The main difference is that there is a typical shock in DHF caused by plasma leakage which is thought to be due to an immunological process, in DF this does not happen⁵. Dengue virus infects dendritic cells via co-receptor dendritic cells (DC-SIGN), whereas monocytes are infected via an unknown receptor and in secondary infection, via an antibody-

dependent increase in crystallizable fragment (FcR) II antibody. B cells and endothelial cells can also be targets of dengue virus infection. Infected dendritic cells and monocytes produce cytokines and chemokines that attract cells from the adaptive immune system, but also have mechanisms to downregulate these immune responses such as interleukin (IL)-10). Infected dendritic cells and monocytes can then produce antigens to natural killer cells, naive or memory B cells, helper and cytotoxic T cells, which are then activated, producing anti-dengue E antibodies, non-structural proteins (NS1) B cells, cytokines and cytokines (natural killer and T cells). Pre-existing heterologous anti-NS1 antibodies can form immune complexes with NS1, either in the circulation or on the surface of infected cells. Infected hepatocytes release chemokines that attract T cells, which can lead to immune-mediated cellular injury and elevated liver enzymes. The result of this immune activation leads to changes in endothelial cell permeability and plasma leakage ⁶.

Guava leaves contain various components that are efficacious to overcome DHF, such as flavonoid compounds containing quercetin. Research conducted by Zandi et al. (2011) to determine differences in four types of bioflavonoids: quercetin, naringin, hesperetin and daidzein against dengue virus RNA replication in C6/36 cells of *Aedes albopictus* mosquitoes and vero cells (kidneys of green African macaques) which were injected five hours before DENV-2 virus inoculation using qRT-PCR showed that quercetin was found to be most effective against DENV-2 in vero cells (67%), daidzein (25%) by inhibiting viral RNA polymerase activity and binding to viral nucleic acid or protein capsid, whereas hesperetin and naringin did not differ significantly in inhibiting DENV-2 RNA levels.

Quercetin is categorized as a flavonol, one of six subclasses of flavonoid compounds. In vivo studies on carrageenan-induced and high-fat Zucker rats at Northeast Agricultural University Japan showed that quercetin can inhibit TNF- and nitric oxide production, and reduce nitric oxide synthase (NOS) expression (Rivera et al., 2008). In in vitro studies, quercetin can increase immunity by inducing phosphorylation of tyrosine JAK2, TYK2, STAT3, STAT4 and producing Th1 derived from interferon- γ (IFN- γ) and reducing Th2 derived from interleukin-4 (IL-4) by cells peripheral blood mononuclear cells (PBMCs). Quercetin inhibits TNF- by activating the extracellular signaling pathway (ERK), c-Jun NH2-terminal kinase (JNK) and nuclear factor- κ B (NF- κ B). In addition, quercetin increases peroxisome proliferator-activated receptor γ (PPAR γ) which can activate NF-B antagonists ⁷.

Guava leaf extract also contains polyphenolic compounds, which are antioxidants whose strength is 100 times more effective than vitamin C and 25 times higher than vitamin E. One of the polyphenol components is epigallocatechingallate (EGCG) which plays a role in stimulating the production of interleukin-1 alpha (IL-1 α), interleukin-1 beta (IL-1 β), tumor necrosis factor alpha (TNF-). EGCG can also help the phagocytosis process, increase lymphocyte resistance, lymphocyte proliferation, IL-12 macrophage secretion, increase IFN and produce antibodies such as immunoglobulin G (IgG) ³.

A pre-clinical study at the University of Lahore Pakistan in 2016 on 36 rats divided into a control group and a treatment group using intraperitoneal guava leaf extract. The treatment group showed a significant increase in the total and differential leukocyte count, red blood cell count, platelet count and hemoglobin level compared to the control group ($p < 0.001$). This shows that guava leaf extract has an immunostimulant effect. The immunostimulant effect on humoral immunity is evidenced by a decrease in the number of rat deaths and an increase in the antibody produced ⁸.

Another pre-clinical study at Ranchi University in 2013 on 18 albino rats divided into a control group and a treatment group using oral guava leaf extract, the results showed that the treatment group significantly ($p < 0.05$) increased the number of leukocytes compared to the control group, so it can be recommended in the treatment of immune disorders ⁹.

III. METHOD

This study used 37 patients at PKU Muhammadiyah Wonosobo Hospital during March to August 2018 which met the inclusion criteria, namely diagnosed DF and DHF and adult age ≥ 18 years. Patients with hematological, cardiac and lung abnormalities were excluded in this study. The *sample size* calculation uses the open epi calculator with a 5% confidence interval. The total patients were divided into two groups randomly, namely the treatment group (*Psidiumguajava* Linn Leaf extract capsules) and controls (placebo capsules containing starch) dose 3x2 for 3-4 days. Dosage determination is based on previous study in adult DF and DHF patients.

Measurement of the number of leukocytes with a *flowcytometer* was obtained from routine blood tests (venous blood) every 24 hours as long as the patient was admitted to the hospital. IgG measurement used the ELISA method after the patient has received either treatment or control therapy. Blood samples were then stored at -20°C at GadjahMada University Clinical Pathology Laboratory. *Independent t-test* was used for data analysis. This study has been reviewed and obtained permission from the Ahmad Dahlan University ethics committee.

IV. RESULTS AND DISCUSSION

Patient Characteristics

Based on patient characteristics data in table 1, all variables between groups have a p value > 0.05. This shows the similarity of the characteristics of patients involved in this study.

In this study, the number of male patients (56.76%) was more than that of women (43.24%) (p > 0.05). Wangsa and Lestari Research (2014) at RSUP Sanglah and Valentino (2012) at RSUP Dr. Kariadi Semarang in dengue infection patients stated that > 50% of patients were male, with the reason that the active *Aedes aegypti* mosquito bites during the day with two activity peaks, namely at 08.00-12.00 and 15-17-17.00 where at those hours men more frequent activities outside the home^{10,11}.

Table 1. Characteristics of DF and DHF patients

Patients Characteristics	Capsules of <i>Psidiumguajava</i> Linn leaf extract. (n=18)	Non Capsules of <i>Psidiumguajava</i> Linn leaf extract. (n=19)	pvalue
Male	10 (47.60%)	11 (52.40%)	1.00
Age (year)	42.22±14.84	37.53±14.48	0.29
Suhu (°C)	37.18±0.73	37.63±0.96	0.13
Initial leukocyte level (x10 ³ /μL)	5.03±2.36	4.37±2.44	0.42
Initial Platelet Level(x10 ³ /μL)	109±4.28	94.79±3.77	0.31
Fever Day	4.00±0.91	3.68±0.82	0.27
Complaint			
Fever	18 (100%)	19 (100%)	0.47
Nauseous vomit	10 (55.55%)	8 (42.11%)	
Headache	10 (55.55%)	8 (42.11%)	
Heartburn	4 (22.22%)	3 (15.79%)	
Joint pain	0 (0%)	4 (21.05%)	
Weakness	5 (27.78%)	6 (31.58%)	
Bleeding (nosebleeds, gums, hematuria, melena)	0 (0%)	1 (5.26%)	
Diarrhea	1 (5.55%)	2 (10.52%)	
Cough	1 (5.55%)	2 (10.52%)	
Tingling	0 (0%)	1 (5.26%)	
Myalgia	1 (5.55%)	3 (15.79%)	
BAK difficulty	0 (0%)	1 (5.26%)	
Out of breath	1 (5.55%)	0 (0%)	

In this study, the mean age of the treatment group was 42 years, and the control group was 38 years with a value of p > 0.05. The study conducted by Wangsa and Lestari (2014) stated that the age of majority of DF and DHF patients is ≥15 years old, where adults are more likely to engage in activities outside the house at the peak of the activity of *Aedes aegypti* mosquitoes¹⁰.

The mean number of leukocytes in the treatment group was 5.03±2.36, while the control group was 4.37±2.44 (p > 0.05). In dengue infection, the number of leukocyte is usually normal or decreases with neutrophil cell domination¹². Valentino's study (2012) also showed that 51.8% of DF and DHF patients had initial leukocytes ≥5,000 / mm³¹¹.

The mean initial platelet level in the treatment group was 109±4.28, and the control group was 94.79±3.77 with p value > 0.05, which means that there were no significant differences between therapy groups.

The mean temperature and fever day of the treatment group were 37° C and 4.00±0.91, while the control group was 38° C and 3.68±0.82 (p > 0.05). One typical symptom of DF and DHF is a fever between 2-7 days. These symptoms appear as the body's response to dengue virus infection and as a defensive mechanism of the body against antigens¹³. Study conducted by Ho et al. (2013) at Cheng Kung Hospital showed that many dengue patients began hospitalization on day 4 of fever. Nurjannah's study (2010) at Faisal Islamic Hospital, Makassar Hajj and LabuangBaji Hospital also showed that dengue patients were hospitalized on 4-7 days (60%) due to insufficient knowledge of early recognition of early symptoms of DF and DHF and alone treatment habits at home so that it is too late to visit a hospital^{14,15}.

Complaints experienced by the treatment group with the control group in this study have p > 0.05. Patient complaints in this study are in accordance with the clinical manifestations of DF and DHF patients listed

in WHO (2011) which include fever 2-7 days, nausea vomiting (57.9%), heartburn or stomach pain (50%), headache (44.6%), cough (21.5%), limp (21.5%), diarrhea (6.4%)¹⁶. Cohort study by Phuong et al. (2004) at Dong Nai Pediatric Center (DNPC) Vietnam in DF patients and DHF children showed that >60% of patients experienced almost the same as this study¹⁷.

Table 2. Differences in mean patients' leukocytes between groups based on fever days

Fever days	Capsules of <i>Psidiumguajava</i> Linn leaf extract. (n=18)	Non Capsules of <i>Psidiumguajava</i> Linn leaf extract. (n=19)	pvalue
3 mean±SD n (%)	4.77±2.75 4 (33.33)	4.20±2.03 8 (66.67)	0.69
4 mean±SD n (%)	3.99±1.92 12 (48)	3.81±2.32 13 (52)	0.82
5 mean±SD n (%)	3.93±2.07 18 (50)	3.89±2.19 18 (50)	0.97
6 mean±SD n (%)	4.24±2.01 15 (46.87)	4.21±2.36 17 (53.13)	0.97
7 mean±SD n (%)	5.24±2.37 13 (44.83)	5.00±2.79 16 (55.17)	0.81
8 mean±SD n (%)	5.78±3.24 8 (50)	6.08±3.37 8 (50)	0.86

After processing the data with an independent t-test, it was found that there were no significant differences in the mean leukocytes between groups based on fever days ($p > 0.05$) (Table 2).

Independent t-test showed that there were no significant differences ($p > 0.05$) on the mean number of leukocytes between treatment and control groups (Table 3).

Table 3. Differences in mean patients' leukocytes between groups

Groups	N	Mean	Std. Deviation	p value
Standard therapy+extract guava leaf capsule	18	4,67	1,88	0,99
Standard therapy (control)	19	4,66	1,88	

Based on the independent t-test showed that there were no significant differences in IgG levels between treatment and control groups.

Table 4. Differences in patient IgG levels between groups

Groups	N	Mean	Std. Deviation	pvalue
Standard therapy+extract guava leaf capsule	18	272,82	33,57	0,72
Standard therapy (control)	19	279,14	67,86	

One of the causes of leukopenia in dengue virus infection is bone marrow suppression as a result of direct viral suppression or indirect mechanism through the production of proinflammatory cytokines that suppress bone marrow¹⁸. At the end of the fever phase (3rd day) leukopenia will usually occur significantly. In this study, not all subjects experienced leukopenia, this is the same as the results obtained in the retrospective study of Masihor et al. (2013), in which the number of leukopenia patients was 26.8% and those who did not have leukopenia as much as 73.2% with mean leukocytes 8.65 ± 0.51 ¹⁹.

The study conducted by Bandiola and Corpuz (2018) on 24 *Sprague-Dawley* rats induced hydroxyurea to cause leukopenia and thrombocytopenia, were then given leaves extract of *Syzygiumcumini* Linn from the family Myrtaceae (one family with *Psidiumguajava* Linn.) containing flavonoids at a dose of 400 mg / kgBB and 800 mg / kgBB showed that the leaf extract of *Syzygiumcumini* Linn can increase leukocytes at a dose of 400 mg / kgBB compared to the control group but did not differ statistically significantly ($p=0.054$) and with a dose of 800 mg/kgBB compared to the control group significantly ($p=0.021$). These results are consistent with this study that *Psidiumguajava* Linn leaf extract. no effect on the number of leukocytes²⁰.

Immunoglobulin is a type of globulin protein that acts as an antibody or has the same chemical structure as antibodies. In adolescents and adults, total immunoglobulin levels (IgG, IgM, IgA) >600 mg/dL with a normal antibody response, excluding humoral deficiency. The category is sufficient if the total immunoglobulin level is 400-600 mg/dL or 200-400 mg/dL IgG level, and less if the total immunoglobulin level is less than 400 mg/dL or serum IgG levels <200 mg/dL²¹.

This study showed that the leaf extract of *Psidiumguajava* Linn did not significant effect on IgG levels ($p > 0.05$). These results are consistent with the study conducted by Zhan et al. (2017) in Holstein dairy cattle to determine the effect of flavonoids contained in *Medicago sativa* on the immune system and blood cell count, divided into four groups: control, 20, 60 and 100 mg/kgBW showed that flavonoids did not affect the concentration of immunoglobulin such as IgA ($p = 0.57$), IgM ($p = 0.04$), IgG ($p = 0.29$) and leukocytes ($p = 0.83$) between groups²². Another study conducted by Yano et al. (2006) in male rats aged 8 weeks about the influence of flavonoids for two weeks on the C57BL/6N immune system as measured by the ELISA method showed that there were no significant differences ($p > 0.05$) between flavonoid groups (treatment) $2,1 \pm 0.1$ with the group not given flavonoids (control) $4,3 \pm 1,2$ for IgG²³.

WHO (2012) classified the degree of thrombocytopenia in two categories: mild thrombocytopenia ($>50,000$ cells/ μ L) and severe thrombocytopenia or *severe thrombocytopenia* ($<50,000$ cells/ μ L)²⁴. Based on Figures 1 and 2 show that patients who have mild or severe thrombocytopenia in day 2 fever until day 11 fever between the two groups during treatment showed no significant difference ($p > 0.05$). In *dengue* infection, leukopenia is usually followed by thrombocytopenia. Leukopenia can predict the critical period of plasma leakage which will cause thrombocytopenia. Thrombocytopenia has an important role in the pathogenesis of *dengue* infection. Thrombocytopenia in *dengue* infection occurs through a mechanism of bone marrow suppression, platelet destruction and shortening of platelet life¹⁹.

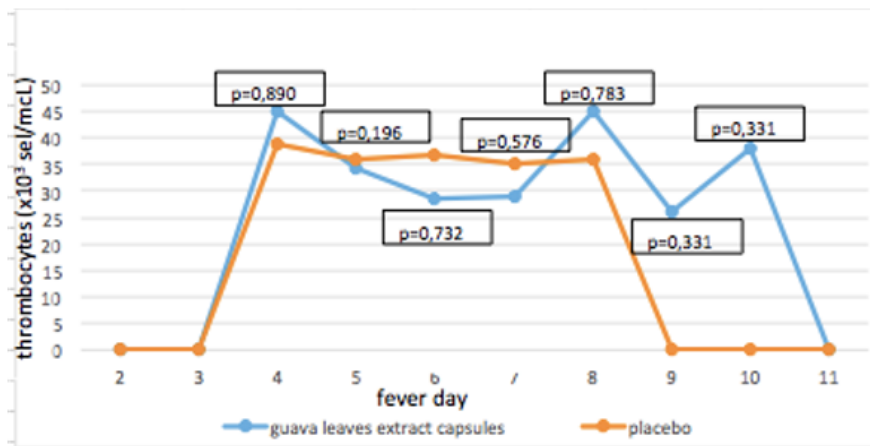


Figure 1. Effect of *Psidiumguajava* Linn leaf extract capsules in severe thrombocytopenia

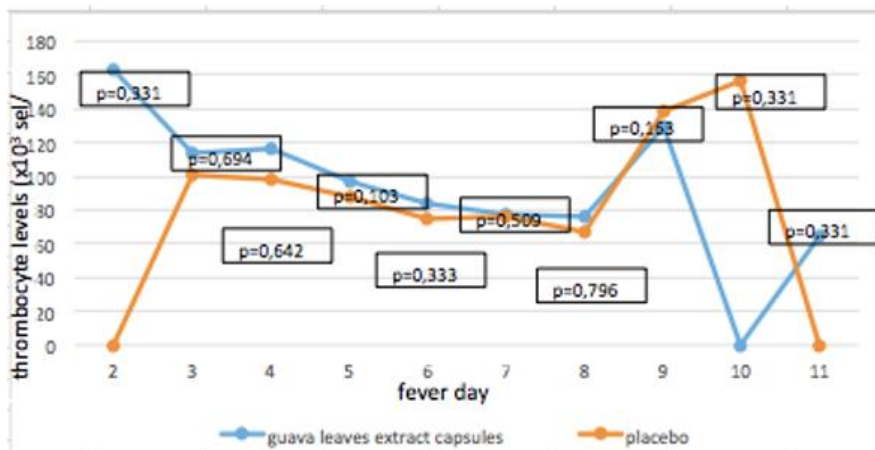


Figure 2. Effect of *Psidiumguajava* Linn leaf extract capsules in mild thrombocytopenia

V. CONCLUSION

Provision of *Psidiumguajava* Linn leaf extract for adult patients DF and DHF did not give a significant difference ($p > 0.05$). This is in accordance with WHO (1997) that adjuvant therapy such as *Psidiumguajava* Linn leaf extract did not need to be given to patients with dengue infection.

Ethical Clearance: The ethical clearance was taken from research committee of Ahmad Dahlan University, Yogyakarta, Indonesia.

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Conflict of Interest: The author confirm that there are no conflicts of interest to disclose.

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