

## RED YEAST RICE (*Monascus Purpureus*) EXTRACT INCREASES INTERLEUKIN-2 LEVEL IN DENGUE INFECTION PATIENTS

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### ABSTRAK

Trombositopenia merupakan salah satu manifestasi klinis utama dan komponen penting dalam infeksi Dengue. Penelitian praklinis telah menunjukkan bahwa ekstrak beras ragi merah (*Monascus purpureus*) dapat meningkatkan jumlah platelet. Namun mekanisme tersebut belum dipahami. Dikemukakan bahwa mekanisme tersebut disebabkan efek anti-inflamasi dan peningkatan thrombopoiesis. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh ekstrak *Monascus purpureus* pada peningkatan thrombopoiesis. Kami menguji efeknya terhadap kadar IL-3 sebagai salah satu faktor pertumbuhan hematopoietik pada pasien dengan infeksi Dengue. Percobaan acak terkendali ini membandingkan pasien DHF yang menerima *Monascus purpureus* ekstrak dengan pasien Dengue pada kelompok kontrol. Hitung darah lengkap dan kadar IL-3 dievaluasi pada saat pendaftaran dan setelah pemberian ekstrak *Monascus purpureus*. Di antara 15 pasien yang menerima *Monascus purpureus* dan 15 pasien dalam kelompok kontrol, ada peningkatan kadar IL- $0,59 \pm 8,72$  pg/mL di pada kelompok *Monascus purpureus*, dan  $0,25 \pm 6,67$  pg/mL pada kelompok kontrol. Kelompok *Monascus purpureus* memiliki peningkatan kadar IL-3 lebih tinggi tetapi secara statistik tidak signifikan ( $p = 0,91$ ). Kesimpulannya, ekstrak *Monascus purpureus* meningkatkan kadar IL-3 pada pasien dengan infeksi dengue dibandingkan dengan kelompok kontrol, walaupun secara statistik tidak signifikan. (FMI 2012;48:58-66)

**Kata kunci:** infeksi dengue, IL-3, *Monascus purpureus*

### ABSTRACT

Thrombocytopenia is one of the main clinical manifestations and key components in Dengue infection. Preclinical research has shown that the extract of red yeast rice (*Monascus purpureus*) can increase platelet count. However the mechanism has not been understood. It has been suggested that the mechanisms are due to anti-inflammatory effect and increase of thrombopoiesis. The aim of this study was to investigate the effect of *Monascus purpureus* extract on increasing thrombopoiesis, we examined its effect on IL-3 level as one of the hematopoietic growth factor in patients with Dengue infection. This randomized controlled trial compare Dengue patients who received *Monascus purpureus* extract with Dengue patients in the control group. Complete blood count and IL-3 level were evaluated at enrollment and after administration of *Monascus purpureus* extract. Among 15 patients who received *Monascus purpureus* and 15 patients in control group, there are increase of IL-3 level  $0,59 \pm 8,72$  pg/mL in *Monascus purpureus* group, and  $0,25 \pm 6,67$  pg/mL in control group. *Monascus purpureus* group had higher increase of IL-3 level but statistically not significant ( $p = 0,91$ ). In conclusion, *Monascus purpureus* extract increased IL-3 level in patients with Dengue infection compared to control group which was statistically not significant. (FMI 2012;48:58-66)

**Keywords:** Dengue infection, IL-3, *Monascus purpureus*

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### INTRODUCTION

Indonesia is one of the endemic countries, with incidence between 6 to 15 per 100,000 population (1989 to 1995), and rose sharply when the extraordinary events of up to 35 per 100,000 population in 1998 (Suhendro et al 2006). Thrombocytopenia is one of the major manifestations in dengue infection. Studies by Makroo et al (2007) found that in the case of dengue infection was confirmed by serological tests that the prevalence of thrombocytopenia (platelet count less than 100,000/mm<sup>3</sup>) was 84.88% while the patient was hospitalized. Thrombocytopenia in dengue infection has

been associated with bleeding manifestations and severity of dengue. The study by Díaz-Quijano et al (2006) showed that severe thrombocytopenia associated with bleeding manifestations and signs of plasma leakage. Severe thrombocytopenia was also observed in 74% of cases of death due to dengue (Navarrete-Espinosa et al 2005).

Thrombocytopenia in dengue infection has the potential to cause significant bleeding can even be life threatening. Until now there has been a specific treatment against dengue infection so that its management is supportive and symptomatic. In case of

severe thrombocytopenia, causing life-threatening bleeding, platelet transfusion may be considered (Soewandojo 2002, Nasronudin 2005). But in addition to platelet transfusion is only temporary, it requires no small cost, are also at risk for transfusion reactions (Spiess 2010).

Some traditional medicines have been developed in the community as an effort to improve clinical outcomes of patients with dengue infection, such as the extract of red rice (red yeast rice) or red yeast rice is rice fermented with *Monascus purpureus*. Research in the Laboratory of Genetics Biosystematics and Microorganisms, Research Center for Biology LIPI Bogor show that in the mice that were given rice *Monascus purpureus* obtained a significant increase in platelet count. In addition, during the study the mice remained active and not observed any significant change in condition (Tisnadjaja 2006). Another study by Muharni et al (2011) showed that in patients with DHF who received guava leaf extract and *Monascus purpureus* it was found that platelets increase significantly compared with the control group (Muharni et al, 2011).

Increased platelets in the extract of *Monascus purpureus* rice could be expected to increase through thrombopoiesis and supported by anti-inflammatory effects of metabolites, Monacolin K, Ankaflavin and Monascin. Monacolin K which have similar structure with Lovastatin has statin-like effects could be expected to affect the activity of macrophages (Lin et al, 2011). Increased thrombopoiesis process requires stem cell factor, including IL-3 produced by activated monocytes or macrophages (Miyajima et al 1992). To prove whether there was an increase in provision of *Monascus purpureus* thrombopoiesis can go through the process of hematopoiesis in the form of changes in markers of hematopoietic growth factor levels, so this study was undertaken to determine the effect of extract of *Monascus purpureus* rice on levels one of which is a hematopoietic growth factor interleukin-3 in patients with dengue infection. The installation Inpatient Medical space Tropical Diseases-Infection Hospital Dr. Soetomo.

## MATERIALS AND METHODS

The research is a single-blind randomized controlled trials or single-blind randomized controlled trial. This research was conducted in the Tropical Room Installation Inpatient-Hospital Infections Dr. Soetomo during the period from July 2011 to December 2011. The population in this study was inpatients in-room Tropical Infectious Diseases Hospital Dr. Soetomo, the diagnosis of dengue infection is established based on the

diagnostic criteria according to the WHO (1997), which consists of clinical and laboratory criteria. Samples were taken from patients who met the inclusion criteria and did not meet the exclusion criteria by using simple random sampling.

Criteria for inclusion in this study were: 1) Patients of dengue infection based on the diagnostic criteria according to the WHO (1997); 2) Have a positive result on examination of IgM and/or Ig G anti-Dengue were performed after the fifth day of fever; 3) more than 13 years old and less than 65 years; 4) fever on admission of less than 5 days; 5) Willing to be involved in clinical trials with signed informed consent. For patients younger than 21 years old and unmarried; 6) informed consent was signed by the parents of the patient. Exclusion criteria were performed in patients who met the inclusion criteria, but have the following circumstances: 1) Patients who experience complications of dengue infection or serious complications for example sepsis, shock, DIC, moderate or severe bleeding (DHF Grade III or IV), loss of consciousness; 2) Receiving a transfusion of whole blood, packed red cells, platelets, fresh frozen plasma during treatment. Samples were excluded from the analysis if the research results obtained during the study the following circumstances: 1) Return the forced; 2) Withdrawing from research participation; 3) The data is not complete; 4) There was an allergy or serious side effects of the extract of red yeast rice (*Monascus purpureus*). Large total sample in this study 30 samples, which consisted of 15 patients as a group without the extract of *Monascus purpureus* (control) group and 15 patients who received the extract of *Monascus purpureus* (treatment). Both groups, both the control group and the treatment group, treated with standard therapy based on the same study protocol.

Results were analyzed using descriptive analysis and inferential analysis. Inferential analysis includes qualitative test 2-independent samples (Chi-square or Fisher's Exact test) to analyze the homogeneity of the basic data, t-test two sample pairs (paired t-test) to analyze the levels of interleukin-3 before and after treatment in each group treatment (*Monascus purpureus* extract and control), and two independent samples t test or Mann-Whitney to compare changes in levels of interleukin-3 between treatment groups (*Monascus purpureus* extract) with a control group.

## RESULTS

The results showed that patients with more males than females with a ratio of 3.28. The mean age of patients hospitalized dengue infection was  $27.03 \pm 11.45$  years,

with the youngest 14 and the oldest 53 years. In the group of *Monascus purpureus* extract the mean age of the patients of dengue infection are older than the group without *Monascus purpureus* respectively  $30.53 \pm 11.63$  years and  $23.53 \pm 10.49$  years.

Days of fever Dengue infection when the patient entered the hospital averages almost no difference between the two groups:  $4.87 + 0.35$  ke- day for the group without *Monascus purpureus* and ke-  $4.60 + 0.63$  for the group with the extract of *Monascus purpureus*. The mean axillary temperature without *Monascus purpureus* group does not differ much from the group with *Monascus purpureus* respectively  $37.74 \pm 0.18^\circ\text{C}$  and  $37.73 \pm 0.19^\circ\text{C}$ .

Results of initial laboratory tests on admission to hospital showed hemoglobin level was slightly higher in the group without *Monascus purpureus* is  $14.62 \pm 1.45$  g/dL, compared to  $14.10 \pm 1.63$  g/dL in the group with *Monascus purpureus*. Leukocyte levels in the absence of *Monascus purpureus*  $4530.67 \pm 2023.71/\text{mm}^3$ , slightly higher than the group with *Monascus purpureus* is  $4426.67 \pm 2428.84/\text{mm}^3$ . Acquired thrombocytopenia in both groups, with  $69.853 \pm 20203.07$  platelets/ $\text{mm}^3$  in the absence of *Monascus purpureus* and  $57393.33 \pm 24485.75/\text{mm}^3$  in the group with *Monascus purpureus*.

The mean hematocrit patients of dengue infection on admission  $43.16 \pm 4.58\%$ , with the group without *Monascus purpureus*  $43.23 \pm 4.58\%$  and *Monascus purpureus* group with  $43.1 \pm 4.74\%$ . During the course of the disease during hospitalization had a mean decrease in hematocrit be  $40.68 + 4.11\%$  with a change of  $1.79 \pm 5.82\%$  for the group without *Monascus purpureus* and  $3.18 \pm 4.44\%$  for the group with *Monascus purpureus*.

Levels of AST and ALT liver function patients of dengue infection appears to be higher in the group with *Monascus purpureus* respectively  $107.07 \pm 78.68$  U/L and  $69.47 \pm 39.31$  U/L compared to the group without *Monascus purpureus*  $96.87 \pm 55.94$  U/L and  $67.93 \pm 44.83$  U/L. Plasma albumin levels in the absence of *Monascus purpureus* higher at  $3.95 \pm 0.29$  g/dL compared to  $3.8 \pm 0.36$  g/dL for the group with *Monascus purpureus*. Examination of serological tests IgM and IgG anti-dengue performed to refine the diagnosis of dengue infection. The percentage of positive results of serological investigations of anti-dengue IgM or IgG anti-dengue necessary to sharpen the diagnosis of dengue infection in the absence of *Monascus purpureus* respectively 100% and 53.33%, while the group with *Monascus purpureus* 100% and 73, 33%.

Table 1. General Characteristics of Patients Dengue Infection

Variables	Without <i>Monascus purpureus</i> (n=15)	<i>Monascus purpureus</i> (n=15)	p	Note
Age (years)	$23.53 \pm 10.49$	$30.53 \pm 11.63$	0.095	NS
Sex:				
Male (n) / (%)	12 (40)	11 (36.67)		
Female (n) / (%)	3 (10)	4 (13.33)		
Total	15 (50)	15 (50)	1.000	NS
Day of fever	$4.87 + 0.35$	$4.6 + 0.63$	0.071	NS
Axillar temperature ( $^\circ\text{C}$ )	$37.74 \pm 0.18$	$37.727 \pm 0.19$	0.831	NS
Hb (g/dL)	$14.62 \pm 1.45$	$14.10 \pm 1.63$	0.371	NS
Leukocyte (/mm $^3$ )	$4.530.67 \pm 2023.71$	$4.426.67 \pm 2428.84$	0.90	NS
Thrombocyte (/mm $^3$ )	$69.853 \pm 20203.07$	$57.393.33 \pm 24.485.75$	0.140	NS
Hct (%)	$43.23 \pm 4.58$	$43.1 \pm 4.74$	0.941	NS
$\Delta$ _Hct (%)	$1.79 \pm 5.82$	$3.18 \pm 4.44$	0.467	NS
SGOT (U/L)	$96.87 \pm 55.94$	$107.07 \pm 78.68$	0.686	NS
SGPT (U/L)	$67.93 \pm 44.83$	$69.47 \pm 39.31$	0.921	NS
Plasma Albumin (g/dL)	$3.95 \pm 0.29$	$3.8 \pm 0.36$	0.226	NS
<u>Anti dengue serologic test:</u>				
IgG + (n) / (%)	8 (26.67)	11 (36.67)		
IgG - (n) / (%)	7 (23.33)	4 (13.33)		
Total	15 (50)	15 (50)	0.256	NS
IgM + (n) / (%)	15 (50)	15 (50)		
IgM - (n) / (%)	0 (0)	0 (0)		
Total	15 (50)	15 (50)	1.000	NS

Characteristics of the study sample in each group showed no significant difference between the groups without *Monascus purpureus* and *Monascus purpureus* group for the variables examined on admission to hospital as a hot day, the temperature of the axilla, the levels of hemoglobin, leukocytes, platelets, hematocrit, SGOT , ALT, albumin, plasma, and likewise for other variables such as age, changes in hematocrit, anti-Dengue IgG and IgM anti-dengue (Table 1). Percentage of first-degree subjects of dengue patients is as much as 50% and DHF grade 2 by 50%. In the group without *Monascus purpureus* proportion of subjects with DHF degree 1 and degree 2 respectively by 60% and 40%, while for the group with *Monascus purpureus* respectively 40% and 60%. There is no significant difference between the control group and the treatment group (Table 2).

Patients of dengue infection in this study mostly came during the 5th day of fever by 18 patients (60%), while the fever that comes on day 3 and day 4, respectively 2

patients (6.67%) and 10 patients (33.33%). By using the Chi-square test found no significant difference in the distribution of patients by days of fever in *Monascus purpureus* extract group and the group without *Monascus purpureus* ( $p = 0.733$ ) (Table 3). Average value of the initial amount of IL-3 in the absence of *Monascus purpureus* by  $22.07 \pm 4.4$  pg/mL, while in the group of *Monascus purpureus* at  $24.227 \pm 6.315$  pg/mL, which by using two sample t-test is free there is no difference between the two group with  $p = 0.288$ , as well as when analyzed based on each day fever group (Table 4). The mean levels of IL-3 in the control group before therapy without *Monascus purpureus* was  $22.07 \pm 4.40$  pg/mL, whereas levels of IL-3 after therapy without *Monascus purpureus* was  $22.33 \pm 5.39$  pg/mL. Changes in levels of IL-3 before and after therapy without *Monascus purpureus*  $0.25 \pm 6.67$  pg/mL. Found no statistically significant difference between the levels of IL-3 before and after therapy without *Monascus purpureus* ( $p = 0.885$ ).

Table 2. The degree of severity of dengue infection

DHF Grades	Without <i>Monascus</i> <i>purpureus</i> (n=15)	With <i>Monascus</i> <i>Purpureus</i> (n=15)	Total (n=30)	p	Note
DHF grade 1 (n) / (%)	9 (30)	6 (20)	15 (50)		
DHF grade 2 (n) / (%)	6 (20)	9 (30)	15 (50)		
Total	15 (50)	15 (50)	30 (100)	0.281	NS

Table 3. Occurrence day of hospitalization of DHF patients without *Monascus purpureus* and with *Monascus purpureus*

Fever day	Types of treatment		Total	Nilai p*
	Without <i>Monascus</i> <i>purpureus</i>	<i>Monascus</i> <i>purpureus</i>		
3	1 (3.33%)	1 (3.33%)	2 (6.67%)	
4	4 (13.33%)	6 (20%)	10 (33.33%)	
5	10 (33.33%)	8 (26.67%)	18 (60%)	
Total	15 (50%)	15 (50%)	30 (100%)	0.733

Table 4. The mean levels of IL-3 (pg/mL) before treatment in group with and without *Monascus purpureus*

Fever day	Without <i>Monascus</i> <i>purpureus</i> (Mean $\pm$ SD)	<i>Monascus</i> <i>purpureus</i> (rerata $\pm$ SD)	p value*
3	18.5	22.9	
4	$22.35 \pm 4.39$	$24.033 \pm 3.73$	0.532
5	$22.32 \pm 4.71$	$24.54 \pm 8.33$	0.485
Total	$22.07 \pm 4.40$	$24.23 \pm 6.32$	0.288

Table 5. Analysis of IL-3 levels before and after treatment in groups with and without *Monascus purpureus*

Types of treatment	Fever day	Total IL-3 in examination (pg/mL)		
		Before Treatment	After Treatment	p
Without <i>Monascus purpureus</i>	3	Mean	18.50	-
		SD		
	4	Mean	22.35	
		SD	4.39	0.231
	5	Mean	22.32	
		SD	4.71	0.609
<i>Monascus purpureus</i>	Total	Mean	22.07	
		SD	4.39	0.885
<i>Monascus purpureus</i>	3	Mean	22.90	-
		SD		
	4	Mean	24.03	
		SD	3.73	0.302
<i>Monascus purpureus</i>	5	Mean	24.54	
		SD	8.33	0.554
	Total	Mean	24.23	
		SD	6.32	0.798
			7.20	

Table 6. Comparison of changes in levels of IL-3 between groups with and without *Monascus purpureus*

Fever day	IL-3 level (pg/mL)		P
	Without <i>Monascus purpureus</i>	<i>Monascus</i> <i>purpureus</i>	
3	2.40	- 3.30	-
4	-2.85 + 3.79	-1.40 + 2.98	0.517
5	1.28 + 7.64	2.56 + 11.65	0.782
Total	0.25 ± 6.67	0.59 ± 8.72	0.907

In the treatment group who received therapy *Monascus purpureus*, levels of IL-3 before therapy was  $24.23 \pm 6.32$  pg/mL, whereas levels of IL-3 after administration of *Monascus purpureus* therapy was  $24.82 \pm 7.20$  pg/mL. Changes in levels of IL-3 before and after administration of *Monascus purpureus* therapy of  $0.59 \pm 8.72$  pg/mL. Found no statistically significant difference between the levels of IL-3 before and after administration of *Monascus purpureus* therapy ( $p = 0.798$ ).

In both groups the group that received both therapy and groups *Monascus purpureus* there is no change in levels of IL-3 after therapy *Monascus purpureus* or without each of  $0.59 \pm 8.72$  pg/mL ( $24.07 \pm 6.43$  pg/mL be  $7.14 \pm 24.9$  pg/mL) in the group receiving therapy *Monascus purpureus* and  $0.25 \pm 6.67$  pg/mL (from  $22.07 \pm 4.40$  pg/mL be  $22.33 \pm 5.39$  pg/mL) in the absence of *Monascus purpureus*. The changes increased levels of IL-3 in the group receiving therapy outweigh *Monascus purpureus* group without *Monascus purpureus*, but found no statistically significant difference ( $p = 0.907$ ).

as shown in Table 6. In the group with *Monascus purpureus* extract treatment of dyspepsia occurred in 5 patients (33.33%), dizziness complaints are not obtained, headache in 3 patients (20%), and found no symptoms of serious side effects. While the group without the extract of *Monascus purpureus* dyspepsia seen in 4 patients (26.67%), and headache in 2 patients (13.33%).

Clinical manifestations of dengue is usually preceded by a sudden onset of fever with an increase in body temperature can reach  $39^\circ\text{C} - 40^\circ\text{C}$  and lasted for about 5-6 days. In this study, the mean days of fever when he came to the hospital on the day of  $4.63 \pm 0.61$  and fastest day 3 and no later than the 5th day. This result is slightly longer than the results of the study by Carlos et al (2005) which shows the average days of fever on admission  $3.50 \pm 1.40$  days. In this study, the mean temperature at presentation  $37.73 \pm 0.24^\circ\text{C}$ , where the lowest temperature of  $37.5^\circ\text{C}$  and  $38.5^\circ\text{C}$  the highest. The mean temperature in this study were similar to the average in secondary dengue infection on a study conducted by Thomas et al (2008) is  $37.80^\circ\text{C}$ .

Table 7. Dyspepsia in patients with dengue Infection

Dyspepsia	Without <i>Monascus purpureus</i> (n=15)	With <i>Monascus purpureus</i> (n=15)	Total (n=30)	p	Note
Yes (n) / (%)	4 (13.33)	5 (16.67)	9 (30)		
No (n) / (%)	11 (36.67)	10 (33.33)	21 (70)		
Total	15 (50)	15 (50)	30 (100)	0.690	NS

Table 8. Head pain in patients with dengue infection

Head Pain	Without <i>Monascus purpureus</i> (n=15)	With <i>Monascus purpureus</i> (n=15)	Total (n=30)	p	Ket
Yes (n) / (%)	2 (6.67)	3 (10)	5 (16.67)		
No (n) / (%)	13 (43.33)	12 (40)	21 (83.33)		
Total	15 (50)	15 (50)	30 (100)	0.624	NS

Dengue virus infection can be found leukopenia caused by the destruction of mature leukocytes and exacerbated by damage to the bone marrow (Kohli et al 2008). The study by Lee et al (2005) showed that leukopenia occurred in 54.8% of patients with DHF. In this study based on the results of laboratory tests of blood by the time the patient entered the hospital obtained leukopenia  $4000/\text{mm}^3$  occurred in 46.67% of patients, slightly below the results of the study by Lee et al (2007) and obtained a mean leukocyte  $4478.67 + 2197.23/\text{mm}^3$ , slightly higher than the average of leukocytes in the study by Itoda et al (2006 ) is  $3062/\text{mm}^3$ .

In this study, the mean hematocrit value on admission was  $43.16 + 4.58\%$  with a maximum value of 50.5%. This result is higher than the average hematocrit was found in the study by Gomber et al (2001) in patients with DHF, which is  $38.34 + 6.02\%$ . All patients have experienced thrombocytopenia on admission with a mean of platelets on admission was  $63\ 623 + 22948.53/\text{mm}^3$ , with a minimum value of  $20,800/\text{mm}^3$  and a maximum of  $97,000/\text{mm}^3$ , slightly above the average of platelets obtained in the study by Kittigul et al (2007), ie  $60\ 722 + 48\ 350/\text{mm}^3$ .

Transaminase levels obtained in the study of Nguyen et al (1997) who obtained the results of the mean levels of SGOT 128.4 U/L (24-537) and the mean ALT level of 54.2 U/L (4.1 to 279) in which the increase occurred at 60 -90% of DHF patients, and in most cases are mild to moderate increase in transaminases, but a fraction, 7-10%, an increase of up to 10x the upper limit of normal values???. In addition it is also not found a statistically significant difference between the levels of SGOT and SGPT with primary and secondary infection. In this study, the mean increase in AST and ALT levels that row is  $101.97 + 67.28 \text{ U/L}$  and  $68.7 + 41.44 \text{ U/L}$ , where

an increase occurred in 76.67% of patients, similar to the study by Itoda et al (2006) is 78%.

Hypoalbumin associated plasma leakage in dengue infection can be an indicator of disease exacerbation, where albuminemia  $> 4\text{g/dL}$  was associated with a lower risk of dengue fever, it is because of the high levels of integrity albuminemia vascular endothelium, while albumin  $< 4\text{ g/dL}$  may be an early indicator of changes in vascular permeability (Gubler 1998). Villar-Centeno et al (2008) in their research on the biochemical changes as a marker of dengue indicates that the relationship between the development of DHF with early changes of serum albumin. Of the 169 patients with DD average albumin level of  $4.11\text{ g/dL}$  compared to 30 patients with DHF albumin levels on average  $3.95\text{ g/dL}$  showed a significant difference between them ( $p = 0.049$ ). In this study, the average albumin level of  $3.88 + 0.33\text{ g/dL}$ , which is 0.07% below the average albumin dengue patients in the study conducted by Villar-Centeno et al (2008) is  $3.95\text{ g/dL}$  can indicate the presence of disorders ranging vascular permeability.

Examination of serological tests Ig M and Ig G anti-Dengue was done after fever on the 5th of each patient to confirm the diagnosis of dengue. Of the 30 samples of the study showed that the results of serological tests positive IgG anti-dengue was found in 19 patients (63.33%), the 8 patients in the group without *Monascus purpureus* and 11 patients in the *Monascus purpureus*. There were no patients with serological tests of anti-dengue IgM negative. IgM antibody is an immunoglobulin isotype that appears first. Anti-dengue IgM was detected in 50% of patients are at day 3 to 5 fever, increased to 80% of patients on day 5 and 99% of patients on day 10. Peak levels of IgM anti-dengue occurred in the second week after the onset of the disease and usually drop to undetectable levels in 2-3

months later. While Ig G anti-Dengue generally detected at low titers at the end of the first week of fever, increased slowly, and levels remain detectable after a few months, and possibly for life. In secondary dengue virus infection, antibody titers increased rapidly. The dominant isotype immunoglobulin ie Ig G anti-Dengue detected at high levels that last up to 10 months or even a lifetime. In the early convalescent stage, the levels of Ig M anti-dengue are lower than the time of primary infection, even in some cases undetectable (WHO 2009).

The study by Nguyen et al (1997) found dengue cases due to secondary infection of 39 cases and 5 cases due to primary infection. Similarly, Krishnamurti et al (2001) found 13% of cases are caused by primary infection and 87% of cases due to secondary infection. In this study, the percentage incidence of dengue due to secondary infection (63.33%) more than the primary (36.67%), according to the study by Nguyen et al (1997) and Krishnamurti et al (2001).

The results show the percentage of first-degree subjects dengue patients by 50% and DHF grade 2 by 50%. In the group without *Monascus purpureus* proportion of subjects with DHF degree 1 and degree 2 respectively by 60% and 40%, while for the group of *Monascus purpureus* respectively 40% and 60%. There is no significant difference between the groups without *Monascus purpureus* and *Monascus purpureus* group ( $p = 0.281$ ), so it can be said that the severity of disease between the two groups was not significant.

In this study the majority of patients with dengue infection fever came upon the 5th day total of 18 patients (60%), while the fever that comes on day 3 and day 4, respectively 2 patients (6.67%) and 10 patients (33.33%). By using the Chi-square test found no significant difference in the distribution of patients by days of fever in *Monascus purpureus* extract group and the group without *Monascus purpureus* ( $p = 0.733$ ), so it can be said that the profile of fever between the two groups was not significant.

In addition to the degree of fever and dengue fever, to assess samples homogeneity, this research analyzed the differences between the groups with *Monascus purpureus* and *Monascus purpureus* group without prior treatment is given, which includes age, gender, time of start of treatment, the temperature, the mean number of leukocytes, the mean platelet count, hematocrit, albumin, and transaminase levels before the start of treatment. Statistical analysis on all the above variables did not show any difference between the *Monascus purpureus* extract group and the group without *Monascus purpureus*. Thus it can be said fairly

homogeneous sample of this study. So when it is found that the difference between the two groups occurred after treatment, then this difference is not expected due to variable factors mentioned above.

The mean number of IL-3 before the start of treatment was found in the control group amounted to  $22.07 \pm 4.4$  pg/mL, while in the group of *Monascus purpureus* by  $24.23 \pm 6.32$  pg/mL, which by using two sample t-test free there is no difference between the two groups with  $p = 0.288$ , as well as analysis based on the group's fourth day of fever ( $p = 0.557$ ) and fifth ( $p = 0.193$ ). This suggests that the levels of IL-3 early in the two groups did not differ significantly.

In the control group gained an increase in mean levels of IL-3 from  $22.07 \pm 4.40$  pg/mL  $\pm 5.39$  become  $22.33$  pg/mL, but this increase was not statistically significant ( $p = 0.885$ ). While in the treatment group who received therapy *Monascus purpureus* obtained a mean increase in levels of IL-3 from  $24.23 \pm 6.32$  pg/mL be  $7,20 \pm 24.81$  pg/mL. However, changes in levels of IL-3 was also not statistically significant ( $p = 0.798$ ). This shows that in both groups obtained a mean increase in levels of IL-3 were not significant in the whole group. To be able to find out more detail changes in levels of IL-3 that occurs we perform the analysis of IL-3 before and after the therapy group based on the fever.

When viewed based on each day fever group, in this study, decreased levels of IL-3 after four days ie from  $26.05 + 6.78$  pg/ml and  $24.03 \pm 3.73$  pg/mL be  $21.42 + 3.77$  pg/mL and  $22.63 + 3.30$  pg/mL in the control and treatment groups, respectively. Meanwhile, after the fifth day found increased levels of IL-3 from  $20.84 + 2.15$  pg/mL and  $24.54 + 8.33$  pg/mL be  $22.83 + 6.29$  pg/ml and  $27.10 \pm 9, 06$  pg/mL in the control and treatment groups, respectively. The fluctuating levels of IL-3 may explain the small changes in the combined analysis of the entire group day fever.

Intervention dengue virus in the bone marrow, especially in the first 3 days leading to suppression of platelet production. On examination of serial bone marrow biopsy in patients with DHF young adults, on the 4th day of fever obtained hypocellular picture, a decrease in the number of megakaryocytes, decreased erythropoiesis, and decreased granulopoiesis. Repeat biopsy on day 7 and 10 fever, showed improvement towards the cellular picture normocellular or hypercellular as a compensatory mechanism in all blood components occurs through stimulation by a variety of hematopoietic growth factors such as GM-CSF, Thrombopoietin, IL-3 and IL-6 (La Russa and Innis, 1995, Nasronudin 2007). This could account for the

fluctuations in the levels of IL-3 and a new rise in the levels obtained after the fifth day of fever.

IL-3 is produced mainly by T cells after cell activation by antigens and also produced by keratinocytes, NK cells, mast cells, endothelial cells and monocytes (Kaushansky et al 1992). Monocytes and T cells are the main cells involved in the pathogenesis of dengue hemorrhagic fever (Kurane 2007). The occurrence of elevated levels of IL-3 is likely to be influenced by the activity of activated macrophages or monocytes. But gains were obtained in this study were not statistically significant and was not in line with the significant increase in platelets obtained results, which show that in addition to IL-3, there are still other factors that play a role in the increase in platelets.

The results showed that both groups contained increased levels of IL-3 whereas in the group without treatment *Monascus purpureus* obtained change increase of  $0.25 \pm 6.67$  pg/mL ie from  $22.07 \pm 4.40$  pg/mL be  $22.33 \pm 5.39$  pg/mL, whereas in the group with *Monascus purpureus* obtained changes in levels of IL-3 by  $0.59 \pm 8.72$  pg/mL ie from  $24.07 \pm 6.43$  pg/mL be  $24.9 \pm 7$ , 14 pg/mL. The changes increased levels of IL-3 in the treatment group is greater than the control group, but found no statistically significant difference ( $p = 0.907$ ). Thus *Monascus purpureus* extract in patients with dengue infection can increase the levels of IL-3 but not statistically significant.

Based on existing literature, this study is the first to examine the effects of extract of *Monascus purpureus* on levels of IL-3 and in this study showed increased levels of IL-3 in the treatment group were higher than controls, but not statistically significant. The results of this study are expected to be additional literature for further study.

Results showed the treatment group occurred dyspepsia in 5 patients (33.33%), dizziness complaints are not obtained, headache in 3 patients (20%), and found no symptoms of serious side effects. While the treatment group dyspepsia seen in 4 patients (26.67%), and headache in 2 patients (13.33%). Because these symptoms are not only caused by the side effects of *Monascus purpureus* (Edrogul & Azirak, 2004) but also the clinical symptoms can be found in patients with DHF (Guglani & Kabra 2005), we conducted analyzes to compare it with the control group. The analysis showed no significant difference between the acquisitions of the two groups so that the symptoms that arise may still be a part of the manifestation of dengue disease.

## CONCLUSION

Extract *Monascus purpureus* increase levels of IL-3 in patients with dengue infection compared to the control group, although not statistically significant.

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