RESEARCH ARTICLE

The effect of *Carica papaya* L. leaves extract capsules on platelets count and hematocrit level in dengue fever patient

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Abstract: Indonesia is one of the countries with the highest cases of dengue fever in South East Asia. The number of patient and distribution area increases with increasing mobility and population density. *Carica papaya* L. (CP) belongs to Caricaceae family is a widely cultivated plant in Indonesia and has many health benefits. The leaves were believed to increase platelet count and dengue fever patient benefit, but there were very limited published reports on scientific evident. The objective of this study was to determine the effects of *C. papaya* leaves extract capsules (CPC) to dengue fever patient. The design of this study was randomized clinical trial with a sample size of 80 subjects. These subjects were randomized into two groups of 40, including the control and intervention group (received two CPC three times daily). The results showed that CPC had significant increased the platelet count ($p<0.05$), maintained stability of hematocrit in the normal level, shorten hospitalization ($p<0.05$) in dengue fever patients, and accelerates the increased in platelet count compared with control group.

Keywords: *Carica papaya*; clinical trial; dengue fever; hematocrit, platelet.

Introduction

Dengue infection is a viral infection spread by mosquitoes with the fastest spreading in the world. It occurs especially in tropical and subtropical countries. Epidemic dengue is a major public health problem in Indonesia, Myanmar, Sri Lanka, Thailand, and Timor Leste which are in tropical mansoon and equatorial zone where *Aedes aegypti* is widespread in both urban and rural areas, where multiple virus serotypes are circulating (WHO 2009). Indonesia has the highest incidence rate of dengue haemorrhagic fever in South East Asia (WHO 2004). The incidence rate has raised thirty times according to the raising of geographical expansion towards new countries and urban to rural change, high mobilization of the citizens, climate changes, and other epidemiologic factors. (WHO 2008)

Till now there is no approved vaccine or drug against this dengue virus, therefore there is an urgent need of development of alternative solutions for dengue. Several plants species have been reported with anti-dengue activity. Recently, the use of alternative medicine and the consumption of plant materials have increased in many countries in the world, mostly because plant-derived drugs and herbal formulation are commonly considered to be less toxic and side effects than synthetic ones.

Carica papaya L. (CP) otherwise known as the papaya pear were found in most tropical and subtropical countries of the world. It is a small tree, the single stem growing from 5 to 10 m tall. The leaves are large, 50 – 70 cm diameter, deeply palmate lobed with 7 lobes. The papaya plants is now cultivated commercially as a fruit crop in many countries of the world. In many parts of Indonesia, the fruits of papaya are much sought after by human as valuable foodstuff and have anti-hypertension activity (Eno et al. 2000). Many scientific investigations have been conducted to evaluate the biological activities of various parts of CP, including fruits, shoots, leaves, rind, seeds, roots or latex. The leaves of

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papaya have been shown to contain many active components that can increase the total antioxidant activity in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic-glucosides and glucosinolates (Otsuki et al. 2010).

The alkaloids, flavonoids, saponins, tannin, and glycosides are related with anti-inflammatory activity. CP leaves extract also found to have anti-bacterial effect (Romasi et al. 2011), anti tumor, and immunomodulator activities (Otsuki et al. 2010). The leaf of CP is categorized as non toxic because its $LD_{50} > 15$ g per kg body weight (Kardono et al. 2003). The leaves also contain cardiac glycosides, anthraquinones, carpaine, pseudocarpaine, phenolic compounds (Owoyele et al. 2008), (Zunjar et al. 2011).

In addition to the nutritional value of its fruit, the leaves of CP possess medicinal properties and are widely used in traditional medicines as "Jamu" in Indonesia. Previous studies on papaya have shown that seed extract of CP possess pharmacological activities, including anthelmintic, contraceptive. The antifertility effects of CP seeds in rats and rabbits have been investigated (Lohiya et al. 1994), (Lohiya et al. 2000). The contraceptive of CP leaves had been contributed by increased oxidative stress either due to increased free radical release or the decreased antioxidant defense system as well as an associated derangement of protein content of the testes (Kusemiju et al. 2012). A hot-water extract of the leaves is taken orally as an antipyretic, and treatment of anemia, appetite stimulation. In other countries the leaves extract of CP had been effectively used for treatment of dengue fever disease (DFD) (Sathasivam et al. 2009). Although one of its indications has been historically as antipyretic there are very limited published reports on the efficacy of this herb to patients with DFD.

In Malaysia CP leaves as a suspension in coconut oil has been used to treat dengue fever patients. Preclinical study has been done to evaluate the effect of CP leaves suspension to platelet count in mice. The five mice given 15 mg/kg body weight of CP powder showed platelet count increase compared to the control group that was given saline solution and coconut oil (Sathasivam et al. 2009). Case report showed a 45 years old man with dengue fever has increased in platelet, leucocyte, and neutrophil count after given 25 ml water extract of CP twice daily for five consecutive days, compared with before treatment (Ahmad et al. 2011). In Srilanka has been done a study of CP for dengue fever patient which showed increased in platelet and leucocyte count of 12 patients in 24 hours after administration of 5 ml CP leaves extract twice daily (Hettige 2008).

The clinical usage of CP leaves in dengue fever patients should be evaluated in large comparative trial. In this study we observed the efficacy of CP leaves extract capsules in adults with dengue fever, included hospitalization period.

Materials and methods

Materials

Each 550 mg of CPC containing 70% ethanol extract of CP leaves and was registered for sale in Indonesia. The study was performed during November 2011 to April 2012.

Clinical Study

a. Design

The study was approved by Ethics Committee of Research and Development of Indonesian Ministry of Health. An open randomized controlled clinical trial design was conducted at the inpatient of the internal medicine ward hospital at Bekasi, West Java, Indonesia.

b. Methods

Each subject received 24 capsules to be taken twice daily. Eligible clinical trial subjects would receive study material and be monitored everyday to evaluate the outcome. Blood samples were obtained twice daily and the platelet count and hematocrit level were determined in the clinical hospital laboratory, during in the hospitalization period.

c. Subjects

The study protocol was approved by the Ethics Committee of Research and Develop-

ment, Indonesian Ministry of Health. The informed consent was explained to the patients and they agreed to participate by signing a written consent form. The eighty subjects were selected for the study using the following criteria. Inclusion criteria were patients with a diagnosis of DFD : high continuous fever (2-7 days), (thrombocytopenia under 150.000/µL, haemoconcentration (a rise in hematocrit by ≥20%), men and women aged ranged from 15 – 55 years old, to sign the *informed consent,* not used either supplement, drug/herbal that interfere the platelet count. Exclusion criteria were subjects with hematology disorders included Idiopathic Thrombocytopenia Purpura (ITP), leukemia, hemophillia; DFD patients with severe bleeding manifestation, and decreased of consciousness. All subjects received the standard therapy for DF patients, and the intervention group received additional 2 CPC three times daily while the control group received standard treatment. The blood of the subjects was taken for platelet count and hematocrit level every morning and afternoon during the study.

d. Statistical analysis

The data were presented in terms of mean and SD, analyzed by descriptive and analytical (dependent and independent t test) statistics, and all analysis was made using the SPSS statistical software package and P value of less than 0.05 was considered, statistically significant.

Results and discussion

The trial population consisted of adult patients with dengue fever who were hospitalized in internal medicine ward, between November 2011 and April 2012. Before the study, 464 patients were screened, and according to inclusion criteria 80 patients were recognized as eligible and randomized into two groups, each 40 patients. The eligible study subjects were patients, male or female, ages 15 - 55 years old, with clinical criteria as high continuous fever with acute onset (2 – 7 days). To support the diagnoses, in this study the thrombocytopenia (decrease in platelet count   150.000 / µL) and hemoconcentration (increase in hematocrit by

≥20%) were used as indicator of the subjects. All group treated with the standard therapy, one as control group and the intervention group received the CPC also. During the treatment period, no patient was excluded from this study. Before administration the CPC, the subject blood test had taken for estimation of biochemical parameters like platelets count (PLT) and hematocrit.

Demographic Characteristics

Descriptive statistics were used to describe basic demographic characteristics. Distribution of sex of the subjects in the two groups did not show significant difference, men were slightly more than women, most subjects infected are in the age of 15-34 years old (Table 1).

Table 1: Demographic Parameters of Dengue Fever patients (n=80)

Variables	Subjects (%) n=80
Sex :	
Male	45 (56.25)
Female	35 (43.75)
Age (years):	
15 - 24	14 (35.0)
25 - 34	15 (37.5)
35 - 44	7 (17.5)
45 – 55	4 (10)

The distribution of subject according to sex shown that male is more than female, similar to the WHO data of dengue incidence in Asian countries (Anker M 2011). It might be related with the higher exposure of male subjects to the dengue vector in the working place or during travelling periods. National data shown that male is slightly more than female but no significant difference, it means that male and female have the same risk to have dengue infection. This study used 15 - 55 years old subject since national epidemiological data in 2009 shown the dengue patient proportion has changed from 5 – 14 years old patients to the group of more than 15 years old (Ministry of Health 2010). Subject under 15 years old and above 55 years old is not included regarding the risk of incompliance of patient to take the medicine in pediatric and ger-

iatric. The result is similar to the national data that dengue patients from 5 - 14 and more than 45 years old decreased, while 15 – 44 years old increased (Ministry of Health 2010). It might be related to the transportation development that increased the geographical expansion of dengue virus (WHO 2009).

Changes in platelet count and hematocrit level in all subjects of intervention and control group are shown in Figure 1 and Figure 2. Platelet count in intervention group raised faster and higher than in the control group (Figure 1 and Table 2). Hematocrit changes in intervention and control group are not significantly different (Figure 2).
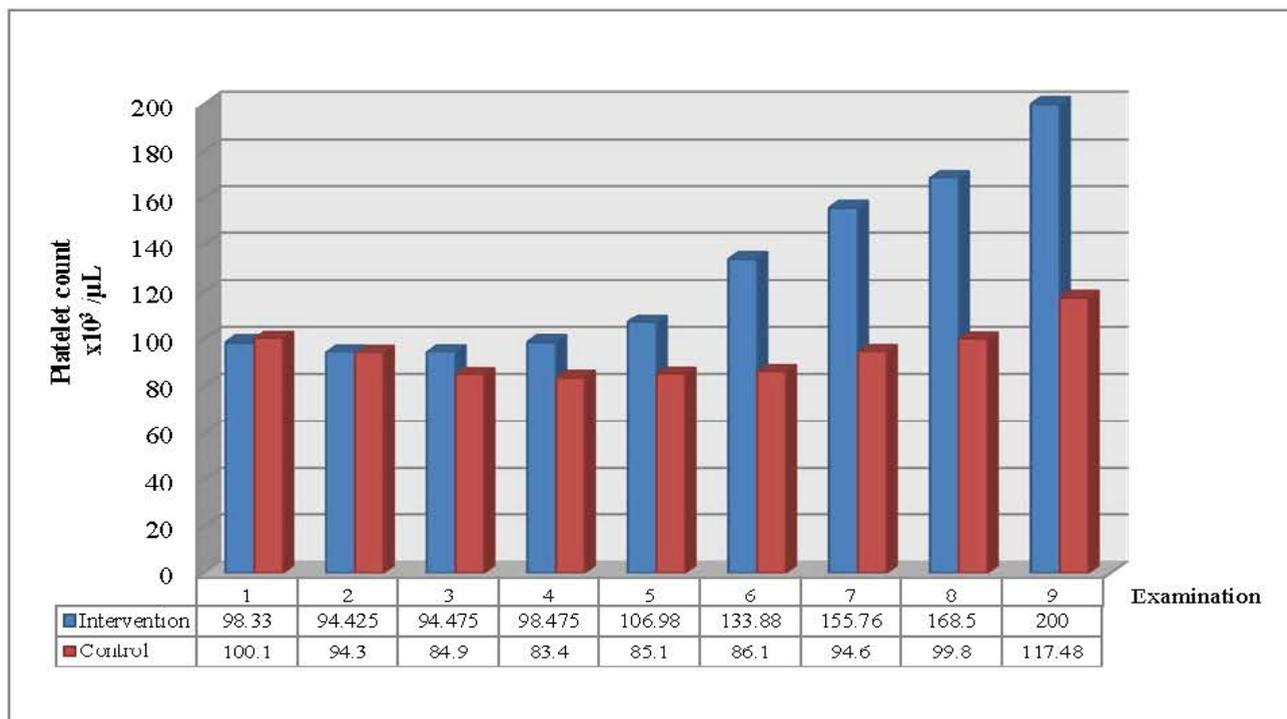

Examination	1	2	3	4	5	6	7	8	9
Intervention	98.33	94.425	94.475	98.475	106.98	133.88	155.76	168.5	200
Control	100.1	94.3	84.9	83.4	85.1	86.1	94.6	99.8	117.48

Figure 1: Change of platelet count of all subjects.

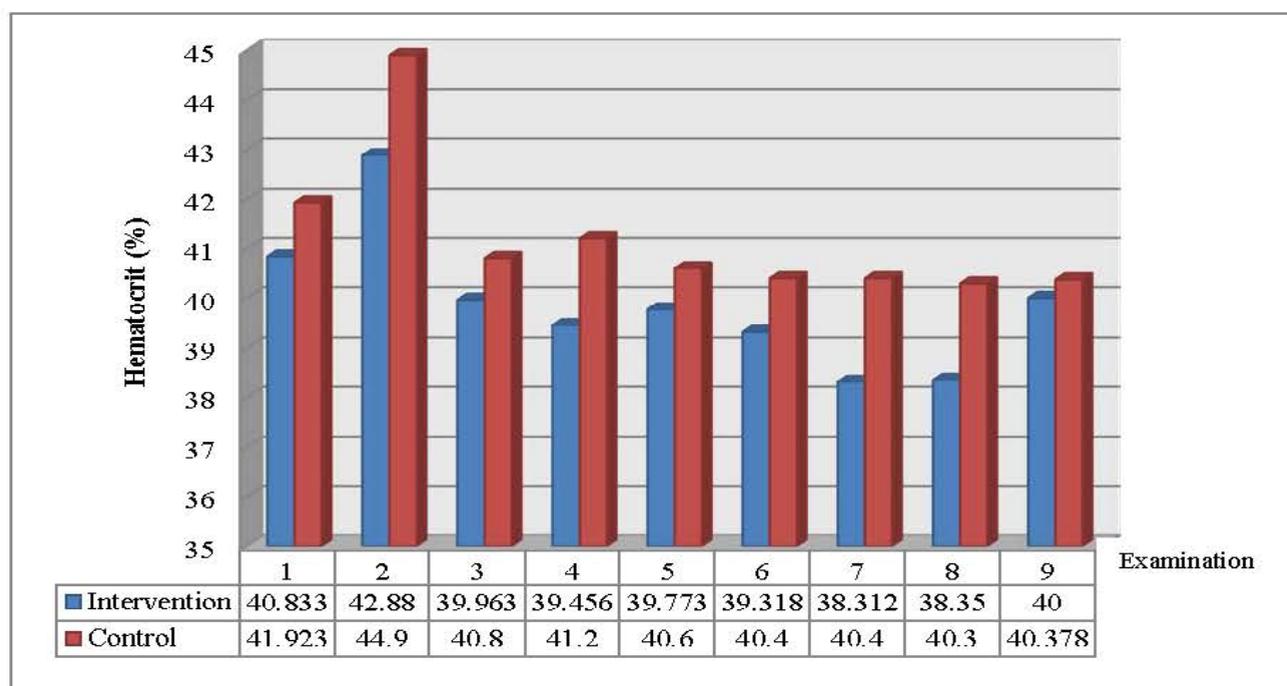| Examination	1	2	3	4	5	6	7	8	9
Intervention	40.833	42.88	39.963	39.456	39.773	39.318	38.312	38.35	40
Control	41.923	44.9	40.8	41.2	40.6	40.4	40.4	40.3	40.378

Figure 2: Change of hematocrit level of all subjects.

Table 2: Onset of increase in platelet count of all subjects.

Group	Examination							
	2nd	3rd	4th	5th	6th	7th	8th	9th
Control n (%)	*	3 (7.5)	3 (7.5)	9 (22.5)	3 (7.5)	8 (20.0)	1 (2.5)	13 (32,5)
Intervention n (%)	1 (2)	10 (25)	8 (20)	6 (15)	15 (38)	**	**	**

*= At the 2nd examination no subject have increased in platelet count
**= At the 7, 8 and 9th examination the subjects have been permitted by the physician to discharge from the hospital.
It is shown that the platelet count in control group mostly increased in the 9th examination (5th day), while in the intervention group is in the 6th examination (3rd day).

Table 3: Platelet count and hematocrit changes of all subjects.

Parameters	Control		Intervention	
	1st	9th	1st	6th
Platelet count (x 10^3/µL)	100.10 ± 28.981	117.48 ± 24.550	98.33 ± 38.721	133.88 ± 33.956
Hematocrit (%)	41.922 ± 3.8122	40.378 ± 4.1075	40.680 ± 4.9684	39.318 ± 4.7649

Statistical analysis with dependent t test showed the significant differences of platelet count ($p<0.05$) and hematocrit level ($p<0.05$) from baseline level to 9th examination (5th day) during observation periods in the control group. And also significant differences of platelet count ($p<0.05$) and hematocrit level ($p<0.05$) from baseline level and 6th examination (3rd day) during observation periods in the intervention group (Table 3).

Statistical analysis with independent t test showed no significant differences of platelet count ($p>0.05$) and hematocrit ($p>0.05$) baseline level between control and intervention group. There is significant difference of platelet count ($p<0.05$) but no significant difference of hematocrit ($p>0.05$) between the 9th examination during observation periods in the control group and 6th examination during observation periods in the intervention group (Table 3).

Table 4: Hospitalization period of the patients.

Hospitalization (days)	Group	
	Control (n=40)	Intervention (n=40)
3	---	23 (57%)
4	---	15 (38%)
5	29 (72%)	2 (5%)
6	7 (18%)	---
7	4 (10)	---

The hospitalization period in control group is 5.38 ± 0.67 days, while in intervention group is 3.48 ± 0.60 days (Table 4). Statistical analysis

with independent t test showed significant difference in hospitalization period between control and intervention group ($p<0.05$). It is shown that the subjects in the intervention group that received CPC can reach faster and higher increase in platelet count compared to the control group. This result are similar to those reported by Sathasivam et al. (2009) that CP leaves extract can increase platelet count in mice, and also in dengue fever patient as reported by Ahmad et al. (2011) and Hettige S. (2008). But this study used more subjects and all the subjects are hospitalized and this study used CPC that has been standardized and more comfortable for the patient. CPC also can maintain the hematocrit level of the subjects within the normal level.

Tai (1999) reported the hospitalization period of dengue fever is 4.2 ± 1.5 days. In this study the hospitalization period of subjects in control group are similar to the previous study as reported by Tai (1999), but it is shorter in the intervention group. It means that as CPC can increase the platelet count faster, so the subject who received CPC will be discharged more quickly, which also means that it could minimize the hospitalization cost. There are no side effects found during the observation in this study.

Conclusion

In conclusion the results of our study, the CPC could be used as an additional or as a com-

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plementary drug in dengue fever patients, accelerates the increased in platelet count, and shorten the hospitalization period.

References

Ahmad, N., Faizal, H., Ayaz, M., Abbasi, B.H., Mohammad, I., Fazal, L. 2011. Dengue fever treatment with *Carica papaya* leaves extracts. *Asian Pacific Journal of Tropical Biomedicine,* 330-333.

Eno, A.E., Owo, O.I., Itam, E.H., Konya, R.S. 2000. Blood pressure depression by the fruit juice of *Carica papaya* (L.) in renal and DOCA-induced hypertension in the rat. *Phytotheraphy Research*, **(14)**: 235–239.

Hettige, S. 2008. Salutary Effect of *Carica papaya* leaf extract in dengue fever patients - a pilot study. *Sri Lankan Family Physician*, **(29)**: 17-19.

Kusemiju, T.O., Yama, O.E., Okanlawon, A.O. 2012. Effect of *Carica papaya* bark extract on oxidative stress parameters in testes of male albino mice. *International Journal of Applied Research of Natural Product*, **4(4)**: 1-6.

Kardono, L.B.S., Artanti, N., Dewiyanti, I.D., Basuki, T., Padmawinata, K. 2003. *Carica papaya* L., Selected Indonesian Medicinal Plants Monographs and Description. Vol 1. Grasindo, Jakarta, pp. 167-182.

Lohiya, N.K., Pathak, N., Mishra, P.K., Manivannan, B. 2000. Contraceptive evaluation and toxicological study of aqueous extract of the seeds of *Carica papaya* in male rabbits. *Journal of Ethnopharmacology*, **(70)**: 17-27.

Lohiya,N.K., Goyal R.B., Jayaprakash, D., Ansari, A.S., Sarma, S. 1994. Antifertility effects of aqueous extract of *Carica papaya* seeds in male rats. *PlantaMedica*, **(60)**: 400-404.

Ministry of Health. 2010. Dengue Hemorrhagic Fever in Indonesia 1968 – 2009. *Epidemiologic Bulletins*, **(2)**: 1-14.

Otsuki, N., Dang, N.H., Kumagai, E., Kondo, A., Iwata, S. Morimoto, C. 2010. Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects. *Journal of Ethnopharmacology*, **(127)**: 760–767.

Owoyele, B.N., Adebukola, O.M., Fumilayo, A.A., Soladeye, A.O. 2008.Antiinfammatory activities of ethanolic extract of *Carica papaya* leaves. *Inflammopharmacology,* **(16)**: 168-173.

Romasi, E.F., Karina, J., Parhusip, A.J.N. 2011. Antibacterial activity of papaya leaf extracts against pathogenic bacteria. *Makara Teknologi*, **15(2)**: 173-177.

Sathasivam, K., Ramanadhan, S., Mansor, S.M., Haris, M.R., Wernsdorfer, W.H. (2009). Thrombocyte counts in mice after the administration of papaya leaf suspension. *The Middle European Journal of Medicine,* **(121)**: 19-22.

Tai, D.Y.H., Chee, Y.C., Chan, K.W. 1999. The natural history of dengue illness based on a study of hospitalized patients in Singapore. *Singapore Medical Journal*, **(1)**: 40.

World Health Organization. 1999. Guidelines for treatment of dengue fever / dengue hemorrhagic fever in small hospitals. WHO, Geneva.

World Health Organization. 2008. Dengue hemorrhagic fever in Indonesia. Dengue report: Asia-Pacific dengue program manager meeting. WHO, Geneva.

World Health Organization. 2009. Dengue fever in Indonesia, dengue guideline. WHO Geneva.

Zunjar,V., Mammed, D., Trivedi, B.M., Daniel, M. 2011. Pharmacognostic, physicochemical, and phytochemical studies on *Carica papaya* Linn. leaves, *Pharmacognosy Journal*, **(3)**: 5-8.