Clinical Trial of Propoelix (Extract of Propoelix) On Dengue Hemorrhagic Fever Patiens

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Abstract

Propolis, a substance obtained from bee activities, is known for years to contain antioxidant effect, anti-inflammatory, antiviral, antimitogenic, anticarcinogen and immunomodulatory effect, therefore it is assumed that propolis can be used in supportive therapy for DHF. There is no research on the effect of propolis administration as supportive therapy for DHF patients.

The objective of this research is to examine the effectiveness of propoelix, an extract of propolis as adjuvant therapy to improve laboratory parameters, clinical states and decreasing length of stay of Dengue Hemorrhagic Fever (DHF) patients.

This research is a clinical trial with randomized control trial design. Materials of the study are propoelix 100 mg capsules toward two groups of study i.e. Placebo and Propoelix 100 mg Group. The number of subjects who met criteria of inclusion and exclusion was 106. Carried out in the Functional Medical Staff of Internal Medicine at Persahabatan Hospital, Jakarta from December 2009 up to March 2010. 4 days serial examination of routine hematological, and clinical observations are conducted. Bivariat statistical analysis with unpaired t-Test was used to examine differences between variables or other tests that are appropriate with this study.

The results, both of the groups showed clinically improvement but statistically there were significant differences of platelet changes from baseline after second day (p=0,013), third day (p=0,000) between control group and experimental group. In experimental group, there were significant differences on observations of all research variables. There was significance difference of length of stay between control group and experimental group (p=0,002). The day of treatment of experimental group was shorter.

The conclusion is Propoelix, an extract of Propolis as adjuvant therapy is effective in improving laboratory parameters, clinical states and decreasing length of stay of Dengue Hemorrhagic Fever patients.

Keywords : Clinical Trial, Propolis, Propoelix, Dengue Hemorrhagic Fever

Abstrak

Propolis, suatu senyawa yang diperoleh dari aktivitas lebah telah lama diketahui mempunyai efek antioksidan, antiinflamasi, antiviral, antimitogenik, antikarsinogen, dan efek imunomodulator, sehingga diduga dapat berperan dalam terapi suportif Demam Berdarah Dengue (DBD). Belum ada penelitian yang membuktikan keefektifan propolis sebagai terapi tambahan pada pasien DBD.

Penelitian ini bertujuan mengetahui efektivitas propoelix yang merupakan ekstrak dari propolis sebagai terapi tambahan untuk memperbaiki parameter laboratorium, kondisi klinis, dan menurunkan lama perawatan pasien demam berdarah dengue (DBD).

Penelitian ini merupakan uji klinis dengan desain *randomized control trial* . Sebagai materi penelitian adalah propoelix kapsul 100 mg terhadap dua kelompok penelitian, yaitu kelompok plasebo dan propoelix 100 mg. Jumlah subjek penelitian 106 pasien yang memenuhi kriteria inklusi dan eksklusi. Penelitian dilakukan di SMF Penyakit Dalam RS Persahabatan, Jakarta, selama empat bulan, dari Desember 2009 sampai Maret 2010. Pemeriksaan serial hematologi rutin dan pengamatan klinis dilakukan selama 4 hari. Analisis statistik bivariat dengan uji t tidak berpasangan dilakukan untuk menguji perbedaan antarvariabel dan uji lain yang sesuai untuk penelitian ini.

Hasilnya, kedua kelompok menunjukkan perbaikan secara klinis, tetapi secara statistik didapatkan perbedaan bermakna perubahan trombosit dari *baseline* setelah hari kedua (p=0,013) dan hari ketiga (p=0,000) antara kelompok kontrol dengan kelompok eksperimen. Pada kelompok eksperimen terdapat perubahan yang bermakna pada semua pengamatan variabel penelitian. Terdapat perbedaan bermakna lama perawatan antara kelompok kontrol dan kelompok eksperimen (p=0,002). Kelompok eksperimen lebih singkat hari perawatannya.

Kesimpulannya, terapi tambahan propoelix yang merupakan ekstrak dari propolis efektif memperbaiki parameter laboratorium, kondisi klinis, dan menurunkan lama perawatan pasien demam berdarah dengue.

Kata kunci: uji klinis, propolis, propoelix, demam berdarah dengue

(Rochsismandoko dkk., Medika 2013, Tahun ke XXXIX, No. 2, p. 103—111)

Introduction

Since 1994, Dengue Hemorrhagic Fever (DHF) has spread to every province in Indonesia. Nowadays, DHF is endemic in a number of big cities. Since 1975 the disease has spread in the rural areas. Considering the absence of medicine and vaccine for DHF until presently, efforts to eradicate the disease is emphasized on intensive vector control and supportive therapy. A number of researches on natural substances in Indonesia have been carried out as supportive therapy, but the results are not satisfactory. There is no intervention efforts made on the reactions that occurred in the DHF patients' bodies and the contributing factors, therefore, new efforts for an effective therapy or management to obstruct pathogenesis and progressivity of DHF are required (11).

Propolis, a substance obtained from bee activities, is known for years to contain antioxidant effect, anti-inflammatory, antiviral, anti-mitogenic, anti-carcinogen and immunomodulatory effect, therefore it is assumed that propolis can be used in supportive therapy for DHF (2,11).

Up to the present time, in Indonesia no research has been published on the effect of propolis administration as supportive therapy for DHF. This research is expected to contribute knowledge and better treatment of dengue fever patients by utilizing existing potential sources in Indonesia.

The objective of the study is to examine if propolis extract can be used as adjuvant therapy on DHF patients. The other objective is to prove if there is a significant difference on the increase in the number of platelets, leukocytes, and hematocrits between DHF patients administered by propolis extract compared to those who only receive standard therapy for DHF.

This study is expected to help DHF patients in reducing clinical symptoms and decreasing length of stay for DHF patients at the Hospital. Also to help establish SOP of therapy toward management of DHF patients at Persahabatan Hospital.

Dengue fever and dengue hemorrhagic fever (DHF) are caused by dengue virus of the genus flavivirus, family flaviviridae. Flavivirus is a virus with 30 nm diameter, consisting of ribonucleic acid, single stranded with the total molecule weight of 4x106. There are 4 serotypes, namely DEN-1, 2, 3, and 4, all of which may cause dengue fever and DHF. The four serotypes are found in Indonesia, with DEN-3 as the most commonly found. Dengue fever shows mild clinical manifestation, while DHF may cause severe symptoms and Dengue Shock Syndrome (DSS). A number of hematologic disorders accompany DHF so that they can be used to support diagnosis and the parameter of clinical improvement. The pathogenesis of DHF is still debatable; there is strong evidence that immunopathologic mechanism plays an important role. Dengue virus infection activates macrophage which causes phagositosis on virus-antibody complex. On DHF there is a decrease in complementary level, the more severe the symptoms are, the higher the decrease of complementary level such as C proactivator. Radioactively, it was proved that the decrease is not caused by lower production or extravasations (6,10).

Propolis or bee glue, propolis balsam, propolis resin, propolis wax, hive dross, is the product of honey bees (Apis melifera) which collected from tree buds or sap and chewed, then mixed with certain enzyme from their body, and stored in the pollen basket located in their hind legs. The propolis are used to seal and sterilize the beehive. It is well known as a medication for centuries. The name propolis originated from Greek (pro = before, polis = city). In constructing their hive, in the beginning honey bees will build a wall with the entrance which is antimicrobial to prevent antigens which may pollute their hive. The natural disinfectant contained in propolis is very effective as a bactericidal agent.

The chemical composition of propolis is still not fully revealed. It is a resin-like material which normally is green or brown in color, with poplar buds, honey, wax, or vanilla taste, but it may also taste bitter. The chemical composition, color, and taste of propolis depend on the geographic location. It is hard and brittle at low temperature, and very sticky when it is warm. Kaczmarek, et al (1983) found B-Amylase in propolis, Bankova, et al (1982,

1983 & 1988) found that the main composition of propolis are polyphenols, flavones, flavonones, phenolic acid and ester. Polyakof, et al (1998) found fatty acid in propolis. Hegazi and Abd el Hady (1997) analyzed Egyptian propolis with gas chromatography-mass spectrometry and found phenolic acid esters (72.7%); phenolic acids (1.1%); aliphatic acids (2.4%); dihydrochalcones (6.5%); Chalcones (1.7%); flavonones (1.9%); flavones (4.6%) and tetrahydrofuran derivatives (0.7 %). Nikolaev (1978) found minerals such as Mg, Ca, I, K, Na, Cu, Zn, Mn, Fe, and vitamin B1, B2, B6, C, E, fatty acid (1,3,4,5,7).

The general composition of propolis varies, depending on the type of vegetations found by the bees; some of the identified compositions are (1,3,8,9): 50-55% resin & balsam, 30% bee wax, 10-15% essential oil, and 5% bee pollen.

Propolis contains substances required to build a strong immune system and to activate thymus gland. It can be concluded from various researches: propolis contains every Vitamin except vitamin K; it contains every Mineral required by our body except Sulfur; propolis contains 16 essential amino acid chains required for cell regeneration; it contains bioflavonoid, an antioxidant which functions as cell supplement. Based on a research, bioflavonoid in a drop of propolis equals to bioflavonoid produced from 500 oranges.

Propolis has been used since 300 BC for cosmetic. Then it is used as an anti-inflammatory,, antibacterial, antiviral, antifungal, local anesthesia, antiulcer, immunostimulant, hipotensive, and cytostatic agent. Propolis is also known to have effect of antimicrobial, antifungal, antiviral, antioxidant, nutrient absorption, and immunity.

A number of researches have been carried out to evaluate the benefit of propolis administration as anti-inflammatory. The effect of ethanol extract of propolis (EEP) on chronic inflammation is proved on a research using rats with arthritis. In that research, arthritis index could be decreased by administering EEP 50-100 mg/kg body weight/day. The analgesic effect of propolis equals to prednisolone (2.5 mg/kg/day, P.O.) and acetylsalicylic acid (100 mg/kg/day). It is also proved that propolis contains acute and chronic anti-inflammatory effect (Park and Kahng, 1999), especially due to the activity of caffeic acid phenethyl ester (CAPE) in propolis (4,9).

Propolis with various benefits can increase the activation of macrophages (Sforcin, 2007) where propolis may stimulate the production of cytokines such as IL-1β and TNF-α in mice (Moriyasu et al., 1994). One content of propolis extract is CAPE (Caffeic Acid Phenethyl Ester). It has anti-inflammatory activity by inhibiting the release of arachidonic acid from cell membranes, suppress the activity of the enzyme COX-1 and COX-2. (Borelli, 2002). CAPE (1.5 and 10 µM) also has the effect of inhibiting transcription from NF-kB factor and NFAT of T cell in the inflammatory process (4,9).

An unique Propolis extract (Propoelix) was tested by BrunswickLaboratories, USA on 13 January 2012 was found to contain an Oxygen Radical Absorbance Capacity (ORAC), a measurement of Antioxidant Activity to be 21,921 compared to orange which has an ORAC value of 24.

Treatment of dengue fever today was limited to supportive therapy, and therefore propolis is expected to be one option in supportive treatment for patients with dengue fever diagnosis. Properties of propolis that can suppress inflammation and enhance the activity of macrophages are considered theoretically capable of improving immunity and capillary permeability.

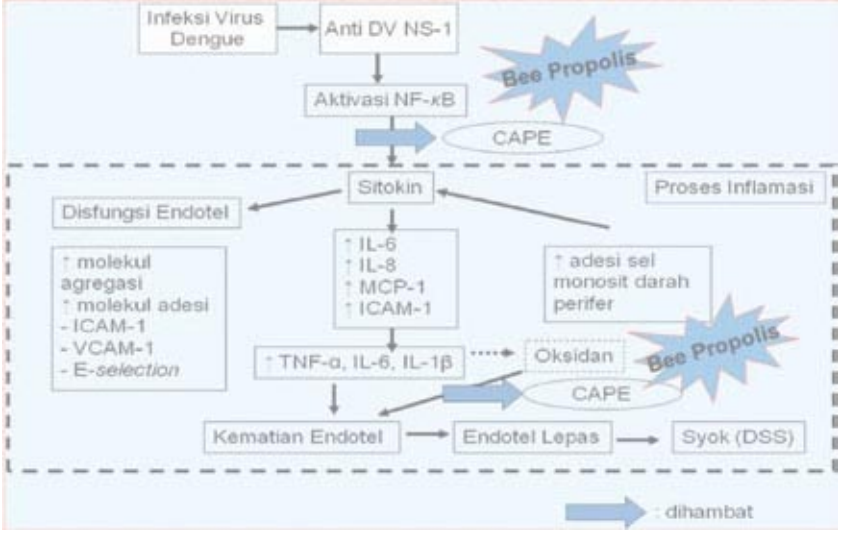
Figure 1 illustrates the schematic relationship of inflammatory process on DHF and the role of CAPE in obstructing the process (4,9).

Material and Method

This research is a clinical trial with design of randomized control trial that was carried out by the Functional Medical Staff of Internal Medicine at Persahabatan Hospital,

Skema 1:

The mechanism of action of propolis in the pathophysiology of DSS



Jakarta for four months from December 2009 up to March 2010. Materials of the study are HDI Propoelix 100 mg capsules.

Criteria of Inclusion: above 15 years of age, dengue Hemorrhagic Fever Patient, the fever lasts for 72 hours or less, able to eat and drink (compos mentis), platelets below 100,000/µL, willing to participate in the study. Criteria of Exclusion: suffer from serious diseases or other chronic diseases, The DHF patient is mentally ill, allergic to propolis, pregnant and breastfeeding woman, receives blood transfusion / platelet concentrates, taking medications other than symptomatic drugs (analgesic, decongestant, etc.)

Clinical trial in this study is to determine the effectiveness of propoelix, a propolis extract as adjuvant therapy to handling DHF in addition to applicable standard therapy in the hospital. The effectiveness is assessed from the differences on the changes in clinical, laboratory parameters and length of stay between the two groups of study. In other words the difference in changes between the two groups caused by the influence of propoelix.

Independent variable: provision of additional therapeutic propoelix. Dependent variable: laboratory, temperature and length of hospitalization. Laboratory parameters included hemoglobin, hematocrit, leucocytes and platelets. Clinical condition is a measurement of the patient's body temperature. Length of stay is the number of day patients undergoing treatment in hospital.

Trial was started by studying the status for every subject. After that, patients who met the criteria were randomly categorized into two groups: group I as control, received placebo; group II received propoelix capsules (100 mg). Patients were followed for 4 days, conducted serial routine examination of hematology, serology for DHF and clinical observations. Length of study is four months, with number of subjects for each group is 30-50. Data and statistical calculations are processed by unpaired t test or Mann Whitney test to compare treatment effects on various clinical and laboratory parameters with numerical data. Repeat Anova test or Friedman test to compare changes in parameters of each group every day, followed by post hoc analysis. Chi Square test for comparing categorical data of the two groups. A limit of significance is based on the value of p <0.05

Result

There were 114 patients, who met criteria of inclusion and exclusion. 13 other patients were excluded from the study for consuming non-standard medicines/herbal medicines without the doctor's consent of the 114. 5 were excluded for co-morbid infection, and 3 other patients were unwilling to continue the study. The basic data of the two groups is elaborated in table 1.

Comparison of demographic characteristics showed there was no significant difference between two groups before medication was administered on proportion characteristic of gender by using chi square test (p=0,205). By unpaired t test or Mann Whitney test on numeric variable there was no significant difference between two groups before medication was administered, including characteristic of age (p=0,817), height (p=0,199), systolic blood pressure (p=0,628), diastolic blood pressure (p=671). There was significant difference on weight (p=0,024).

From Table 1 illustrates the demographic characteristics between the placebo

Tabel 1: Basic Characteristics of the Three Medication Groups

No.	Variabel	Plasebo n=46		Propoelix 100 mg n=47		P
		Mean	SD	Mean	SD	
1.	Jenis Kelamin L/P	31/15		25/22		0,205
2.	Umur (tahun) ·	23,78	8,27	25,15	8,14	0,817
3.	Berat Badan (Kg)	53,09	7,61	56,68	8,20	0,024
4.	Tinggi Badan (cm)	161,35	5,49	159,98	4,69	0,199
5.	TD Sistolik (mmHg) ·	111,30	8,06	110,21	6,75	0,628
6.	TD Diastolik (mmHg) ·	72,17	6,64	72,77	5,40	0,671

Uji Chi Kuadrat. Uji t tidak berpasangan, · Uji Mann Whitney

Tabel 2: Comparison of clinical variable in 4 days monitoring on Placebo/Control

No	Variabel	Hari 1		Hari 2		Hari 3		Hari 4		p
		Rerata	SD	Rerata	SD	Rerata	SD	Rerata	SD	
1.	Hemoglobin	14,31	1,57	13,91	1,51	14,00	1,46	13,91	1,30	0,069
2.	Hematokrit	41,61	4,13	40,50	3,58	40,72	3,83	40,30	3,64	0,150
3.	Leukosit	4559,56	2658,92	4776,52	2614,82	5488,48	2337,98	5771,09	1868,64	0,000
4.	Trombosit	73573,91	33266,11	69521,74	32620,20	77108,70	46471,61	81304,35	59094,98	0,505
5.	Suhu (^{O}C)	37,43	0,92	37,24	1,05	36,91	0,80	36,78	0,77	0,000

Uji Repeat Anova, Uji Friedman, p<0,05

group (n = 46) and propoelix group (n = 47) are equal or from demographic variable there was no significant difference. Randomization process dividing the two groups by demographic worked out well.

Table 2 illustrates the use of repeat Anova test or Friedman test to compare variables during the observation. At least there are two different levels of leukocyte and temperature in the control group (placebo) on measurement day 1, 2, 3 and 4, then performed post hoc test to determine which of two different measurements follow: For level of leucocytes there were differences between day 1 and day 3 (p = 0,002), day 1 to day 4 (p = 0,001), day 2 with day 3 (p = 0,005) and day 2 and day 4 (p = 0,004). For the temperature of the patient there was a difference between day 1 and day 3 (p = 0,000), day 1 to day 4 (p = 0,000), day 2 with day 3 (p = 0,004) and day 2 and day 4 (p = 0,000).

For level of leucocytes there was a difference between day 1 and day 3 (p = 0,003); day 1 to day 4 (p = 0,016).

For level of Platelet there was a difference between day 1 and day 3 (p = 0,000); day 1 to day 4 (p = 0,000); day 2 with day 3 (p = 0,000); day 2 with day 4 (p = 0,000).

For the temperature of the patient there was a difference between day 1 and day 2 (p = 0,000); day 1 to day 3 (p = 0,000); day 1 to day 4 (p = 0,000); day 2 with day 3 (= 0,000); day 2 by day 4 (p = 0,000); and day 3 with day 4 (p = 0,002).

All laboratory and clinical variables that were measured during four days in the propoelix group showed statistically significant changes in all observed variables and in leucocytes and temperature variables, compared to the control group.

Graph 1 illustrates the observation of

Tabel 3:
Comparison of clinical and laboratory variables in 4 days observation toward Experiment / Propoelix Group

No	Variabel	Hari 1		Hari 2		Hari 3		Hari 4		p
		Rerata	SD	Rerata	SD	Rerata	SD	Rerata	SD	
1.	Hemoglobin	14,24	1,22	13,95	1,42	13,57	1,43	13,41	1,49	0,000
2.	Hematokrit	41,57	2,65	40,05	3,65	39,74	3,45	38,64	3,61	0,000
3.	Leukosit	4787,02	2656,86	5368,72	2119,20	5760,64	1673,52	5565,74	1403,86	0,001
4.	Trombosit	69531,91	64363,05	58553,19	27978,88	73893,62	32834,39	96808,51	40878,21	0,000
5.	Suhu (^{O}C)	37,22	0,67	36,88	0,43	36,38	0,38	36,44	0,39	0,000

Uji Repeat Anova, Uji Friedman, p<0,05

While the observations for levels of hemoglobin (p = 0,069), hematocrit (p = 0,150) and platelets (p = 0,505) over four days showed no statistically significant differences in the placebo group.

Table 3 shows there are at least two statistically significant differences in levels of hemoglobin, hematocrit, leucocytes, platelets and Temperature in the experimental group (100 mg propoelix) measured on day 1, 2, 3 and 4, then performed post hoc test to determine which measurement differs as follows:

For Hb level there was a difference between day 1 and day 2 (p = 0,003); day 1 to day 3 (p = 0,000); day 1 to day 4 (p = 0,000); and day 2 to day 4 (p = 0,000).

Ht level there was a difference between day 1 and day 2 (p = 0,011); days 1 to 3 (p = 0,008); day 1 to day 4 (p = 0,000); and day 2 to day 4 (p = 0,000).

Tabel 4: The differences of clinical variables of both study groups on the first day of

No.	Variabel	Plasebo		Propoelix 100 mg		P
		Mean	SD	Mean	SD	
1.	Hemoglobin	14,31	1,57	14,24	1,22	0,481
2.	Hematokrit	41,61	4,13	41,57	2,65	0,871
3.	Leukosit	4559,56	2658,92	4787,02	2656.86	0,776
4.	Trombosit	73573,91	33266,11	69531,91	64363,05	0,705
5.	Suhu (^{O}C)	37,43	0,92	37,22	0,67	0,211

Uji t tidak berpasangan; Uji Mann Whitney; p < 0,05

Tabel 5: The differences of clinical variables of both study groups on the second day of treatment

No.	Variabel	Plasebo		Propoelix 100 mg		P
		Mean	SD	Mean	SD	
1.	Hemoglobin	13,91	1,51	13,95	1,42	0,817
2.	Hematokrit	40,50	3,58	40,05	3,65	0,822
3.	Leukosit	4776,52	2614,82	5368,72	2119,20	0,097
4.	Trombosit	69521,74	32620,20	58553,19	27978,88	0,073
5.	Suhu (^{O}C)	37,24	1,05	36,88	0,43	0,036

Uji t tidak berpasangan; Uji Mann Whitney; p < 0,05

Tabel 6: The differences of clinical variables of both study groups on the third day of treatment

No.	Variabel	Plasebo		Propoelix 100 mg		P
		Mean	SD	Mean	SD	
1.	Hemoglobin	14,00	1,46	13,57	1,43	0,147
2.	Hematokrit	40,72	3,83	39,74	3,45	0,204
3.	Leukosit	5488,48	2337,98	5760,64	1673,52	0,289
4.	Trombosit	77108,70	46471,61	73893,62	32834,39	0,914
5.	Suhu (^{O}C)	36,91	0,80	36,38	0,38	0,018

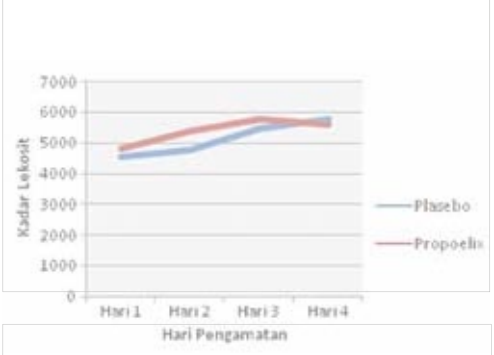
Uji t tidak berpasangan; Uji Mann Whitney; p < 0,05

Tabel 7: The differences of clinical variables of both study groups on the fourth day of treatment

No.	Variabel	Plasebo		Propoelix 100 mg		p
		Mean	SD	Mean	SD	
1.	Hemoglobin	13,91	1,30	13,41	1,49	0,127
2.	Hematokrit	40,30	3,64	39,64	3,61	0,463
3.	Leukosit	5771,09	1868,64	5565,74	1403,86	0,727
4.	Trombosit	81304,35	59094,98	96808,51	40878,21	0,030
5.	Suhu (^{O}C)	36,78	0,77	36,44	0,39	0,009

Uji t tidak berpasangan; Uji Mann Whitney; p < 0,05

Grafik 1: Comparison of Measurement on levels of leucocytes during the four days of observation in both study groups



Grafik 2: Comparison of Measurement on platelet levels during the four days of observation in both study groups

Grafik 3: Comparison of Body Temperature Measurement during four days of observation in both study groups

leukocyte levels in both groups beginning with levels of leucocytes lower than normal (5000-10000) or easy leukopenia. It began to increase on the second day and approached normal levels. No significant differences between the two groups at each measurement. Level of leucocytes in experimental group likely to remain after the leucocytes were within normal limits.

Graph 2 illustrates Comparison of platelet levels on the first day of both groups showed mean scores were equal. Until the measurement of day 2 and 3, there was no significant differences. While the observation of the next day showed there was no significant difference in the measurement of day 2, 3 and 4. But the change of platelet levels from second baseline of the two groups differed on measurement of the fourth day, in which the platelet levels in group of propoelix increased faster than the placebo group.

Graph 3 shows the comparison of body temperature on the first day of two groups. It showed no significant difference in mean score. The observation showed there were significant differences in measurements on day 2, 3 and 4. On the fourth day, the body temperature still showed a tendency to go down. Propoelix group showed a faster decrease in temperature.

Table 4 shows no clinical and laboratories difference in terms of levels of hemoglobin, hematocrit, leukocytes, temperature on the first day of treatment in the control group and the group that received propoelix capsules. This showed the initial conditions of two groups were equal from the variables to be measured.

Table 5 shows no difference in levels of hemoglobin, hematocrit, leukocytes and platelets on the second day in both study groups. There was a significant difference in body temperature on the second day between the two groups. Experimental group was lower than placebo on measurement of the second day (p=0,036). Where this indicates the mean score of body temperature measurement on the second day, propoelix group had a faster decrease than the placebo group.

Table 6 shows no differences in levels of hemoglobin, hematocrit, leukocytes and platelets on the third day in both study groups. There was a significant difference in body temperature on the third day between the two groups. Mean score of temperature for experimental group was lower than the placebo group. Where this indicates the mean score of body temperature measurement on the third day (p = 0,018) propoelix group experienced a

Variabel	Plasebo				Propoelix 100 mg				p
	Mean	SD	Min	Maks	Mean	SD	Min	Maks	
Hari Perawatan	5,48	1,26	4	9	4,79	0,78	4	6	0,002

Uji t tidak berpasangan; Uji Mann Whitney; p < 0,05

Tabel 8:
The differences of length of stay of both study groups

faster decrease than the placebo group.

Table 7 illustrates there was no difference in levels of hemoglobin, hematocrit and leukocytes on the fourth day in both study groups. There was a significant difference in level of platelets and body temperature on the fourth day between the two groups. Level of platelets in experimental group were increased more compared to control group (p=0,030). Temperature on fourth day remained lower compared to placebo (p=0,009).

Table 8 shows there were significant differences in length of stay between placebo group and propoelix group (p=0,002). From the minimum and maximum number of days of treatment if also showed that placebo group had longer days of treatment. Clinically demonstrated propoelix effectively reduce hospital length of stay. In other words, with the addition of propoelix as an adjuvant therapy, it can shorten hospital length of stay.

In graph 4 illustrates there was a difference but statistically not significant. There were differences in Hb levels from day 1 (baseline) and on the changes in day 1, 2 and 3 between the control and experimental group. This means that Hb levels of both treatment groups are equal and the possibility is that Hb level is not affected by propoelix.

In graph 5, it illustrates there was no difference in changes in hematocrit levels from day 1 (baseline) and on the changes in day 1 and 3 between the control and experimental group. However, the changes in hematocrit statistically showed significant difference on the changes in day 2 where propoelix group had higher decrease of hematocrit.

Graph 6 illustrates there was no statistically significant difference in changes in leucocytes levels from day 1 (baseline) and on the changes in day 1, 2 and 3 between the control and experimental group.

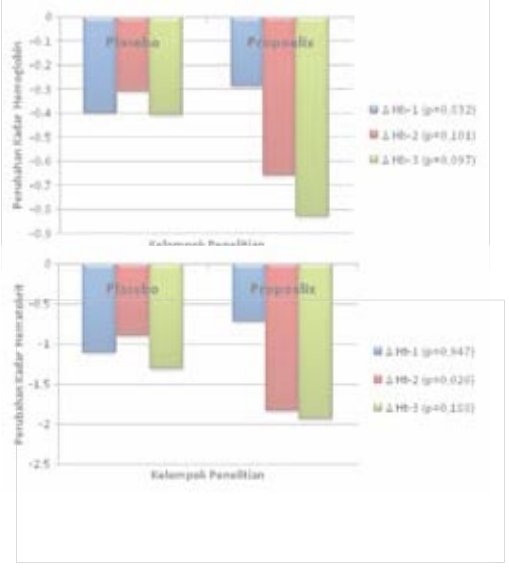
Graph 7 illustrates there was no significant difference in changes in platelets levels from the first day (baseline) on the changes in day 1. While there was a significant difference changes in platelets levels on day 2 (p=0,013) and day 3 (p=0,000) between the placebo and propoelix group. Propoelix group showed more rapid changes in platelets levels increment start from day 3 and 4 (measurement of platelet changes on day 2 and 3).

Graph 8 illustrates there were differences in decrease in temperature. Propoelix group is better than placebo, but statistically there was no significant difference in temperature changes from baseline on day 1, 2 and 3 between the two study groups p <0.05.

Discussion

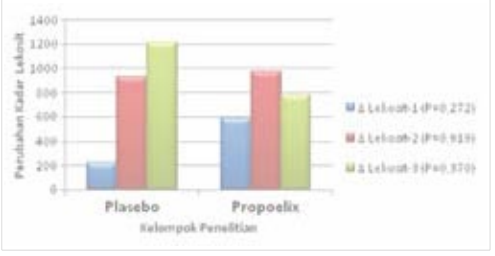
Hemoglobin of both groups showed no significant difference from the first day until the fourth day observation. This means there was no difference in both placebo and experimental groups. This is likely that Hb level is not directly affected by propoelix.

There was a decrease in hematocrit levels starting from the second day of treatment in the group received propoelix which leads to the normal level. In both groups there were differences in changes on the 2nd day of measurement, where in propoelix group there was significant decreases compared to control group. Concluded that the group that received propoelix decreased hemoconcentration more rapid than control group. Increase in level of hematocrit is a



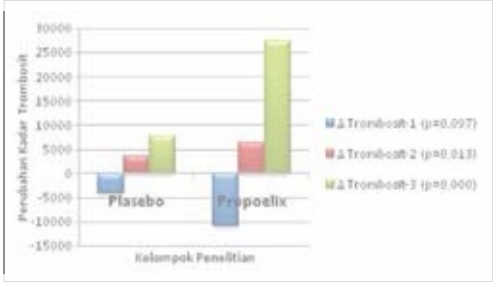
Grafik 4:
Comparison of (Δ) hemoglobin levels changes on day 1, 2 and 3 from baseline in the Placebo and Propoelix group

Grafik 5:
Difference of (Δ) hematocrit levels changes on day 1, 2 and 3 from baseline in the Placebo and Propoelix group

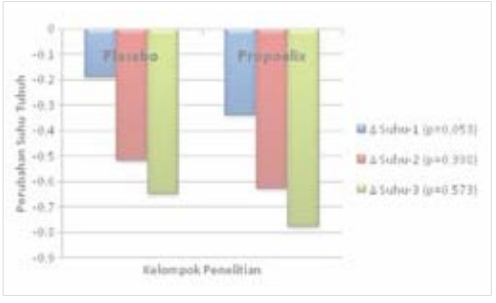


Grafik 6:
Difference of (Δ) Leukocyte levels changes on day 1, 2 and 3 from baseline in the Placebo and Propoelix group

Grafik 7:
Difference of (Δ) Platelet levels changes on day 1, 2 and 3 from baseline in the Placebo and Propoelix group



Grafik 8:
Perbedaan perubahan (Δ) suhu (^{O}C) pada hari ke-1, 2, dan 3 dari *baseline* pada kelompok plasebo dan propoelix



manifestation of hemoconcentration caused by plasma leakage into the extra vascular space through the damaged capillaries.

In DHF patients usually leukopenia (reduction in the number of leucocytes) occurs. Normal leucocytes is between 5-10 thousands. In this study there were improvement in the conditions of leukopenia that occurred in experimental group, it was increasing and maintained after reaching normal level, though changes in leucocytes in both study groups showed no statistically significant difference.

There was no significantly increase in platelets level in the control group until the fourth day of treatment. In the group that received propoelix, significant increase of platelets began on the third day of treatment and continued until the fourth day. There was a significant difference on fourth day of observation, where level of platelets in propoelix group experienced better increase than control group. Significant difference in changes in platelets already occurred on day 2 and 3. Changes in platelets in experimental group increase more significantly than placebo group.

One indication of DHF patient treatment is platelet level less than 100 thousand / µL (normal level between 150-400 thousand platelets / µL). After third and fourth day of measurement, levels of platelets in propoelix group still showed a significant tendency to increase compared with placebo.

The decrease in body temperature occurred more rapidly in the group that received propoelix, which was evident since the second day of treatment and continued until close to normal on the fourth day. Until the fourth day the temperature was still trending downwards. Much longer observation is required to see when the temperature becomes stable.

There was a significant difference in the length of stay between the two groups that received propoelix compared to the control group, where the experimental group has shorter days of treatment in hospital. It means the administration of propoelix significantly shorten the hospital length of stay.

During the study there were no unwanted side effects in everybody in the two groups. No patients experienced clinical deterioration leading to shock or Dengue Shock Syndrome (DSS).

It can be concluded that administration of propoelix as an adjuvant therapy or support in dengue cases significantly improve clinical conditions that are showed by decrease in hematocrit (prevention of hemoconcentration), improvement of leukopeni condition, increase the number of platelets, more rapid decrease of body temperature, and reduce hospital length of stay.

Propolis as one of the few natural healers, in many studies that has been proven to have anti-inflammatory effects, anti-bacterial, anti fungal, immunomodulatory activity, anti-cancer. In this study, propoelix, a propolis extract has been proven to improve all the parameters of clinical, laboratory and hospital length of stay.

Limitation of the Study

Observation of the study was made only during four days. Mean score of platelet measurement showed there was still a tendency to increase higher than 100,000. Likewise, the observation in body temperature, there was a decrease and lasted until the fourth day but still has not reached a stable condition.

Conclusion

Propoelix, a propolis extract is effective as an adjuvant therapy in patients with Dengue Hemorrhagic Fever, as it can improve the clinical conditions (lowering the temperature faster), improve laboratory parameters (accelerating the increase of platelets, decrease hematocrit) and shorten hospital length of stay.

Suggestion

To conduct further study on the effectiveness of propoelix, as an adjuvant therapy with a longer study time for DHF and also for other diseases. Require much longer

observation to get an overview of clinical and laboratory until the condition is stable and normal. Require observation with wider subject and population in other places as a comparison so that the results can be generalized.

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