PENGARUH PEMBERIAN MINYAK *PANDANUS CONOIDEUS* TERHADAP DERAJAT PARASITEMIA MENCIT *SWISS* YANG DIBERIKAN SEBELUM DIINFEKSI *PLASMODIUM BERGHEI ANKA*

PROPOSAL KARYA TULIS ILMIAH

Diajukan untuk memenuhi tugas dan
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HALAMAN PENGESAHAN

ARTIKEL KARYA TULIS ILMIAH

Pengaruh Pemberian Minyak *Pandanus conoideus* (Buah Merah) Terhadap Derajat Parasitemia Mencit *Swiss* Yang Diberikan Sebelum Diinfeksi *Plasmodium berghei ANKA*

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Pengaruh Pemberian Minyak *Pandanus conoideus* Terhadap Derajat Parasitemia Mencit *Swiss* Yang Diberikan Sebelum Diinfeksi *Plasmodium berghei* ANKA

Goretty Lusya Angelita[1], Edi Dharmana[2]

ABSTRAK

Latar Belakang: Minyak *Pandanus conoideus* (buah merah) merupakan tanaman berkhasiat yang berasal dari Papua, dengan kandungan zat-zat aktif yang dapat meningkatkan proliferasi sel-sel pembunuh alami dalam tubuh. Menurut pengamatan penemu buah merah terhadap masyarakat Papua yang mengkonsumsi buah merah dalam kurun waktu tertentu, ternyata didapati masyarakat tersebut lebih kuat dan lebih tahan terhadap penyakit. Sementara itu kita ketahui sampai saat ini Papua masih menjadi salah satu daerah endemis berat malaria. Karena itulah peneliti mencoba melihat bagaimana pengaruh pemberian minyak *Pandanus conoideus* yang diberikan sebelum diinfeksi *Plasmodium berghei* ANKA, terhadap derajat parasitemia mencit *Swiss*.

Metode: Penelitian eksperimental dengan rancangan *post test-only control group* ini menggunakan 14 ekor mencit *Swiss* betina, 25-30 gram, umur 8-12 minggu, dibagi acak menjadi dua kelompok. Kedua kelompok diberi pakan standar selama satu minggu. Pada hari ke-8, kelompok I (kontrol) diinfeksi *Plasmodium berghei* ANKA. Kelompok II (perlakuan) diberi minyak *P. conoideus* selama lima hari (hari ke-8 sampai hari ke-12), pada hari ke-13 diinfeksi *P. berghei* ANKA, kemudian pada hari ke-14 sampai hari ke-18 diberi kembali minyak *P. conoideus*. Pengambilan sampel kelompok kontrol untuk pemeriksaan derajat parasitemia dilakukan pada hari ke-9 sampai hari ke-13, sedangkan untuk kelompok perlakuan dilakukan pada hari ke-14 sampai hari ke-18.

Jumlah parasitemia diamati dari preparat darah tipis dari ekor, kemudian dihitung jumlah eritrosit yang terinfeksi parasit diantara 1000 eritrosit yang dihitung. Analisis data dilakukan dengan uji *Saphiro-Wilk* dilanjutkan dengan uji *t-test independent*.

Hasil: Rata-rata derajat parasitemia kelompok I sebesar 31,74 ± 11,03 dan kelompok II sebesar 13,36± 5,17. Derajat parasitemia kelompok II lebih rendah dari kelompok I. Berdasarkan uji *t-test independent* perbedaan bermakna terdapat pada hari pertama, ketiga, dan semua hari pemeriksaan, namun tidak signifikan pada hari kelima.

Kesimpulan: Pemberian minyak *Pandanus conoideus* sebelum infeksi *Plasmodium berghei ANKA* menyebabkan penurunan derajat parasitemia pada mencit *Swiss*.

Kata kunci: minyak *Pandanus conoideus*, derajat parasitemia, *Plasmodium berghei* ANKA.

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The Effect of Pandanus conoideus Lam Pretreatment on The Parasitemia Degree of Plasmodium berghei-infected Mice

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ABSTRACT :
Background: Pandanus conoideus Lam. (buah merah) is a well-known advantageous herbal from Papua, contains active substances that can increase Natural Killer proliferation in the human body. According to the observation of the P. conoideus' inventor on the native of Papua who consumed P. conoideus in the certain period of time, they became stronger and able to survive from disease. As we know, Papua is one of severe endemic area of malaria. For this reason, the researcher tries to evaluate the effect of P. conoideus Lam pretreatment on parasitemia degree of Plasmodium berghei-infected Swiss mice.

Method: This was an experimental study using post test-only control group design with 14 female mice, 25-30 grams, 8-12 weeks, randomized into 2 groups. Both groups were given standard dietary for a week. At days 8th, group I (K) was infected by Plasmodium berghei ANKA. Group II (P) was given Pandanus conoideus oil for five days (days 8th until days 12th), At days 13th, it was infected by P. berghei ANKA, then continued giving P. conoideus oil from days 14th until days 18th. Some peripheral-blood samples of group I were taken from mice's tail for making preparat at days 9th until days 13th, and for group II, at days 14th until days 18th. Parasitemia degree was examined by counting the number of parasitized erythrocytes per 1000 cells and analyzed using Saphiro-Wilk and independent t-test.

Results: The mean of parasitemia degree of group I is 31,74 ± 11,03 and group II is 13,36± 5,17. Parasitemia degree of group II is lower than group I. There was a significant difference of parasitemia degree between group I and group II, analyzed by independent t-test at says 1st, 3rd, and all days of experiment, but not significant at days 5th of experiment.

Conclusions: The administration of Pandanus conoideus Lam, before Plasmodium berghei ANKA infection, may reduce the parasitemia degree of Swiss mice.

Key words: Pandanus conoideus Lam., parasitemia degree, Plasmodium berghei ANKA.

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INTRODUCTION

Malaria is a world-wide distributed disease between 60° north longitudes and 40° south longitudes, found throughout a hundred tropical and subtropical countries. There are 2,3 billion people who are at risk to this disease or it can be said approximately 41 % people of the world. [1] People have done so many efforts against this disease, but from WHO's data, there are still no significant reductions. Malaria is of overwhelming importance in the developing world today, with an estimated 300 million cases and more than 1 million deaths each year. [2,3] Whereas, until June 2003, there were fifteen outbreak regions of this disease in Indonesia.[4]

The etiology of malaria is *Plasmodium sp.*, which transmitted from an individual to another individual by the bite of the female anopheline mosquito. This *Plasmodium sp.* exist in a variety of forms and have a complex life cycle that enables them to survive in different cellular environments in the human host (asexual phase) and the mosquito vector (sexual phase). The erythrocytic phase of *Plasmodium* asexual development begins when the merozoites from the liver penetrate erythrocytes. Once inside the erythrocyte, the parasite transforms into the ring form which then enlarges to become a trophozoite. These latter two forms can be identified with Giemsa stain on blood smear, the primary means of confirming the diagnosis of malaria.[1,3] This parasitemia degree, that shows the number of parasitized erythrocytes, also can determine the severity of this disease. Erythrocytic phase also become the therapeutic target to overcome the clinical symptoms of malaria and also to prevent the relapse and the complications indeed. It can be said so because in this stadium or phase, the acute syndrome, that is fever, certainly appears and it usually becomes the main complaint of patients. [3,5]

Antimalarial drugs have been increasingly used along with the spread of the endemic areas. Chloroquine, a synthetic antimalarial drug, is also a blood-schizonticidal drug, used mostly in Indonesia; however the using by the people there become less controllable because of the low knowledge about the rational using of drugs.[1] As the impact, the chloroquine resistance has been ongoing.

Natural or herbal substances have been widely used as medicines by people nowadays, because of the progression of those resistance cases.[6]

Papua, the biggest province in Indonesia and also one of the severe endemic area of malaria, has a

well-known advantageous herbal, buah merah (*Pandanus conoideus*). For most people of Papua, *P. conoideus* oil has sufficient substances of nutrients, and also has many advantages to the various type of dangerous disease e.g. cancer, [7, 8] even the native people there become more resistant to the endemic disease, that is malaria, after consuming *P. conoideus* in a period of time.[9] *P. conoideus* oil has active substances that can stimulate the immune system by increasing the proliferation of Natural Killer cells and also the activation of T-helper and Lymphocyte.

This research has been done to the mice, and based on the previous experiment, the best mice for malaria research is Swiss mice.[11] *Plasmodium berghei* ANKA is used for inducing the disease, which have been molecularly proven having similarities with *Plasmodium falciparum*.[12,13]

MATERIALS AND METHODS

The field of this study is Parasitology, and has been done for a month at Parasitology Laboratory Medical Faculty of Diponegoro, where all mice was raised and treated, and also where the parasitemia degree was examined.

The population of this study is *Swiss* mice with the criteria: eight-to twelve-week-old female mice, with the 25-30 grams average weight, and no anatomy abnormalities. There were 14 *Swiss* mice, randomized into two groups, control and treated group; each has 7 mice in it.

The *Federer* formula was used to determine the amount of mice in this experiment, that is: (t-1) (r-1) < 15, where t = the amount of group, r = the amount of sample.

Both groups were given standard dietary for a week. Infection at both groups was initiated by intraperitoneal injection of 10^4 parasitized red blood cells (pRBCs). In the mean time, the treated group was administrated by *P. conoideus* oil 0,05 cc/day/mouse orally for five days before the infection and continued the administration for five days too after the infection.

Blood samples were daily taken from the peripheral blood of tail's infected mice at days 9th until days 13th in control group and at days 14th until days 18th in treated group. The parasitemia degree, examined from thin smears, was counted based on the formula: parasitized erythrocytes per 1000 cells times 100%.

Independent variable of this study is the effect of *P. conoideus* with nominal scale and the dependent variable is parasitemia degree of *Swiss* mice with numeric scale.

This was an experimental study, with primary data, using the *post test only control group* design. *Saphiro-Wilk* was used for normality test of all data distribution, and since the data distribution was normal, independent t-test was used then for hypothetical analysis. All data was processed using SPSS 13.0. A *p* value of < 0, 05 was considered significant and a *p* value of < 0, 01 was considered highly significant.
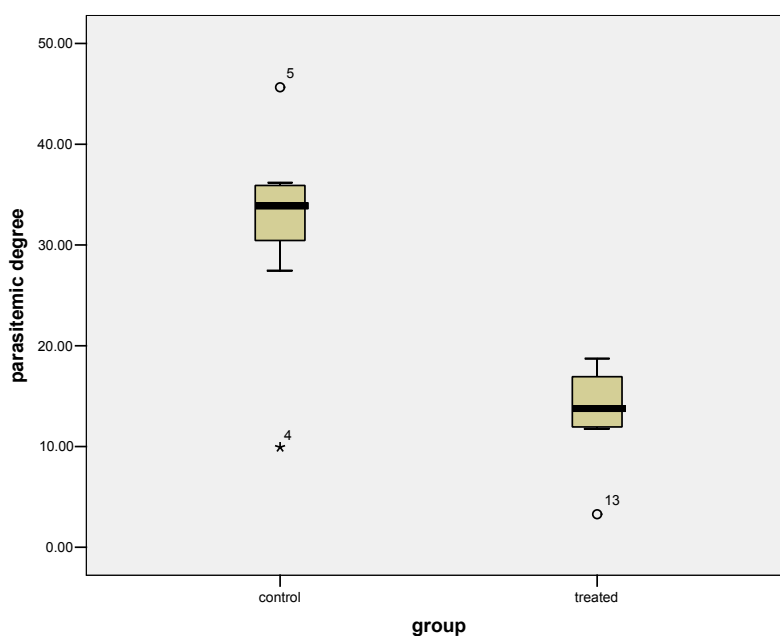
RESULTS

Fig 1. *Box-plot* graphic of parasitemia degree at all days

The mean of parasitemia degree of control group was 31, 74 ± 11, 03 and treated group was 13, 36 ± 5, 17. Parasitemia degree of treated group was lower than control group. There was a significant difference of parasitemia degree between both groups, analyzed by independent t-test with p =0,002. (p< 0,05).
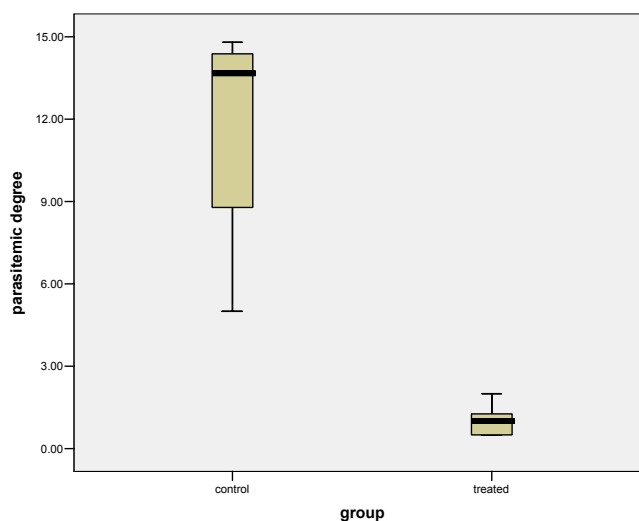
Fig 2. *Box-plot* graphic of parasitemia degree at days 1st

The mean of parasitemia degree of control group was 11, 40 ± 3, 99 and treated group was 1, 00 ± 0, 58. Parasitemia degree of treated group was lower than control group. There was a significant difference of parasitemia degree between both groups, analyzed by independent t-test with p < 0,001.

Fig 3. *Box-plot* graphic of parasitemia degree at days 3rd

The mean of parasitemia degree of control group was 41, 29 ± 19, 51 and treated group was2, 39 ± 1, 36. Parasitemia degree of treated group was lower than control group. There was a significant difference of parasitemia degree between both groups, analyzed by independent t-test with p < 0,001.
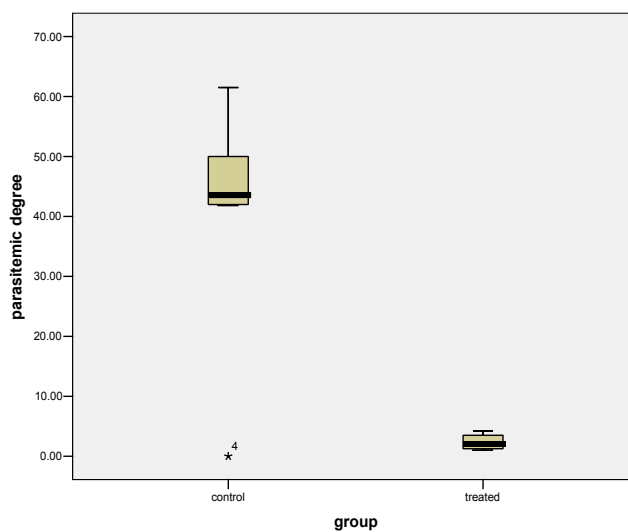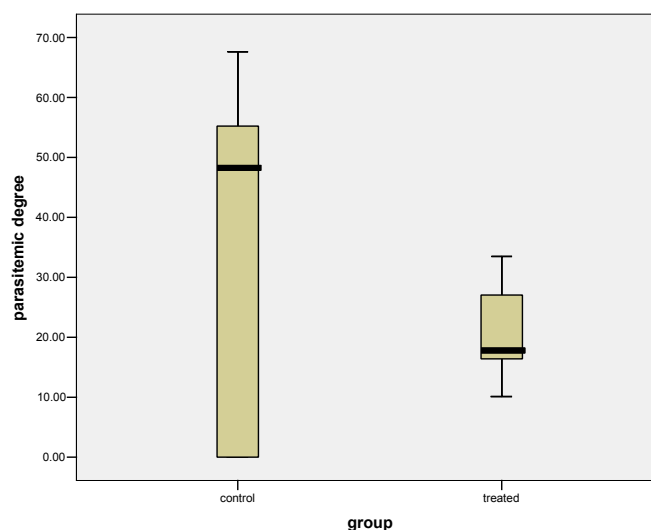
Fig 4. *Box-plot* graphic of parasitemia degree at days 5th

The mean of parasitemia degree of control group was 32, 33 ± 30, 84 and treated group was 21, 18 ± 8, 19. Parasitemia degree of treated group was lower than control group. But the difference of parasitemia degree between both groups was not significant, analyzed by independent t-test with p = 0,386 (p > 0, 05)

DISCUSSION

Plasmodium is an intracellular parasite, so cellular immune response plays an important role to defend this disease. Those cellular defenses, such as T lymphocytes, macrophages, polymorphonuclear leukocytes, and the spleen, involve many cytokines both as direct effectors and as triggered factors. [2,3]

P. conoideus contains many active substances, such as betacaroten (provitamin A), tocopherol (vitamin E), many essential fatty acids (oleic acid, palmitoleic acid, linoleic acid, decanoic acid, linolenic acid), and also complete vitamins and essential minerals. Antioxidant in *P. conoideus* is found in the high contents of betacaroten and tocopherol. This antioxidant can stimulate the immune response and break the free radical chains, which can cause the genetic cell mutation. [14]

Lymphocyte and mononuclear cells in the human body contain high concentration of tocopherol, that's the reason why tocopherol is very important in the immunity function especially in the making of antibody.[10]

Consuming betacaroten 30-60 mg/day for two months can increase the proliferation of Natural Killer cells and also the activation of T helper and lymphocyte cells. The increasing proliferation of Natural Killer cells is important to defend cancer cells and to control free radical, which is dangerous to the human body. [10,14]

This high concentration of betacaroten in *P. conoideus* oil could also be the factors that increase the activation of T Lymphocytes, which could stimulate the activation of macrophage directly, and neutrophyl and B Lymphocytes indirectly. All of them played an important role in killing the parasites, and the result was the reduction of parasitemia degree.

In this study, those things could be the reason why the parasitemia degree of treated mice at days 1st, 3rd, and all days was significantly decrease. But because the *P. conoideus* oil has been given since five days before the infection, the role of that oil to stimulate immune system may be decrease at longer period of treatment. That could be the reason why the decreasing of parasitemia degree at days 5th was not significant.

There were confounding factors of the mice, that have been controlled, which also could have a role too in the process of this experiment, such as age, weight, sex, food of mice and also the infection history of mice.

CONCLUSION

Based on the experiment *P. conoideus* oil can reduce the parasitemia degree of P*lasmodium berghei* ANKA infected *Swiss* mice, which has been administrated before the infection, or it can be said that *P. Conoideus* has a parasiticidal -pretreatment effect, but there was no significant reduction of parasitemia degree on the 5th days of experiment.

SUGGESTIONS

1. Need further research to compare the results of preinfection and postinfection experiments.
2. Need further research to find out the specific substances in *P. conoideus* oil that can cause the reduction of parasitemia degree.
3. Need further research about *P. conoideus* oil in a stratified-dose.
4. Continue the research to find out another pretreatment effect of *P. conoideus*.

ACKNOWLEDGMENTS

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LAMPIRAN

Explore

Case Processing Summary

	F	100	7	0	.0%	7	100
	K	100	7	0	.0%	7	100

1

Descriptives

Tests of Normality

t-test

Group Statistics

Independent Samples Test

F	1.466	
Sig.	.249	
t	-3.992	-3.992
df	8.513	12
Sig.	.004	.002
M	-18.378	-18.378
S	4.60344	4.60344
Lower	-28.883	-28.408
Upper	-7.87347	-8.34857

t-test hari ke-1

Group Statistics

	N	Mean	Std. Deviation	Std. Error Mean
F	7	1,00	.57746	,218
K	7	11,	3,9892	1,5

Independent Samples Test

F	18,574	
Sig.	,001	
t	-6,824	-6,824
df	6,251	12
Sig.	,000	,000
M	-10,3957	-10,3957
S	1,52349	1,52349
Lower	-14,087:	-13,715
Upper	-6,70389	-7,07631

t-test hari ke-3

Group Statistics

	N	Mean	Std. Deviation	Std. Error Mean
F	7	2,39	1,3617:	,514
K	7	41,	19,50:	7,3

Independent Samples Test

F	F		3,629	
E	S		,081	
t-	t	-5,262	-5,262	
M	df	6,058	12	
	S	,000	,002	
	M	-38,8971	-38,8971	
	S	7,39162	7,39162	
9	L	-56,941	-55,002	
o	L	-20,852	-22,792	

t-test hari ke-5

Group Statistics

L	F	7	21,	8,1872	3,0
	K	7	32,	30,83	11

Independent Samples Test

F	F		45,576	
E	S		,000	
t-	t	-,925	-,925	
M	df	6,842	12	
	S	,386	,373	
	M	-11,1529	-11,1529	
	S	12,0586	12,0586	
9	L	-39,801	-37,426	
o	L	17,4954	15,1205	