Potency Syzygium cumini Las Adjuvant Therapy On Mice Model Malaria

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Potency *Syzygium cumini L*as Adjuvant Therapy On Mice Model Malaria

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Abstract

The objective of the study was to prove the potential extract of Syzygium cumini L leaf and stem bark as an adjuvant therapy in mice as a malaria model. Antimalarial effects were assessed by the percentage of parasitemia, growth inhibition, 50% dose level (ED50), Parasite Clearance Time (PCT), Recrudescence Time (RT) of *Plasmodium berghei*. Male albino Swiss strain of mice were infected with $1x10^5$*P.berghei* parasite in 0.2 ml intraperitoneally. Treatment with chloroquine 25 mg/kgbb, chloroquine combination 25 mg/kgbb with leaf and stem bark extract of *Syzygium cumini L* dose 600 mg / kgbb for 4 days and 24 hours after infection, then its activity as antimalarial and adjuvant therapy were seen. The results showed that the extract of *Syzygium cumini L* leaf combined with chloroquine could higher inhibit the growth of parasites than the chloroquine alone. The conclusion is Syzygium cumini L leaf extract combined with chloroquine can give effect, faster theClearance Time Parasite (PCT), and longer Recrudescence Time (RT) than the other treatment.

Keywords:*Syzygium cumini L,Plasmodium berghei,*ParasiteClearanceTime, Recrudescence Time, 50 % dose level

1. Introduction

Malaria is one of infectious diseases caused by Plasmodium which is occurring especially in tropical countries. Every year, approximately 300-500 million people in the world are infected with malaria, and 1-3 million people die. The greatest deaths occur in Africa and the Sahara and 90% of deaths occur in children under 5 years old.This disease is still be a public health problem in 107 countries because malaria is still the fifth cause of death due to infectious diseases in the world (1).

During malaria infection, antigens will stimulate immune system activity that results the formation of Reactive oxygen species (ROS) by inflamantory cells. In trophozoite malaria infections, *Plasmodium* infects erythrocytes and producing H_2O_2 radical and OH^- which is twice larger than normal erythrocytes, excessive H_2O_2 can lead to breakdown of heme and release of free Fe ion which will form OH^- radicals again by Fenton reaction. All of these factors that cause increasing of lipid peroxide which be able to cause oxidative stress (2,3).

The potential effects of damage from normal oxidative stress can be eliminated by the antioxidants present in the body, but in malaria infections there is a decrease levels of antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase and other antioxidants such as albumin, glutathione and ascorbate. When the imbalance of ROS formation in malaria infections were not compansated with cytoprotective enzymes and antioxidants in the body, those can cause oxidative damage (4).Oxidative damage will be increasingthe tissue and organ damage (liver, kidney, lung and brain), increasing cerebral or severe malaria that be able to cause death or decreasing survival time (5).

The presence of multidrug resistant of malaria and decreased efficacy of antimalarial drugs make it essential for new therapies developmentwhich is adjuvant therapy (additional therapy that may affect on the pathophysiology of the cause of malaria). Adjuvant therapy that has been used in severe malaria is modulator of the immune system, antioxidants, anticoagulants and agents that have antiseizure activities (6).

One of the medicinal plants that can be found in Indonesia is *Syzygium cumini*. In India, this plant is used by brewed with hot water traditionally. This study used leaf and stem bark extract of *Syzygium cumini*. This plant has a high antioxidant activity because the content of anthocyanin, ellagitannin, ellegic acid, phenolic, flavonoids, and vitamins (7,8).The results of research showed that *Syzygium cumini* has radical *scavenging* activity and strong antioxidants (8).The use of medicinal plants as antimalarials drugs has been widely used but *Syzygium cumini* which has high potency of strong antioxidant as antimalarial drug had never been reported, so this study would like to know the potential of antioxidant *Syzygium cumini L* as adjuvant therapy in mice as malaria model. This study used *Plasmodium berghei* rodent malaria as an in vivo model by infecting that to the mice (9).

2. Materials and methods

2.1. Experimental animals

This study used male albino Swiss strain of mice as an animal trial which is the weight is approximately 20g - 30g and the age is 2,5 months. The mices were got from Pusat Veterinaria Farma (Pusvetma) Surabaya. Every mice were kept in a cage. Food and drink were given *ad libitum*. This study has got approval with certificate No. 722-KE from Animal Care and Use Committee onVeterinary Medicine Airlangga University Surabaya Indonesia.

2.2. Inoculation of parasites in experimental animals

The parasite that is used to infect mice is *Plasmodium berghei*ANKA strain. Mice is infected with red blood cells containing $1x10^5$*P.berghei* parasites in 0.2 ml intraperitoneally. In order to find out the infection has occurred, microscopic examination of erythrocytes of mice is daily taken by a thin blood smear which is taken from the vein of the tail and stained with Giemsa 20% (10).

2.3 Drugs and plant materials

Chloroquine that is used from Sigma Chemical Co. Leaf and stem bark of*Syzygium cumini*is got from Kediri Jawa Timur Indonesia, and identified on plant laboratory Kebun Raya Purwodadi Pasuruan. Chloroquine dose that is used is 25 mg/Kgbb and given everyday for 4 days (11).*Syzygium cumini*dose is 600mg/kgbb (12).

2.3. Preparation of leaf and stem bark extract of *Syzygium cumini*

The leaves and stem bark of *Syzygium cumini* are dried until dried, after that, it was smoothed into smooth simplicia. Extracted with methaonl. Maseration for 3x24 hours. Filtrate was evaporated using a Rotary Evaporator at a temperature of 40-50^{0}C with low pressure. The result of extraction is saved on temperature of -8 ^{0}C until ready to use (4).

2.4.Reseacrh design

Thirty mice were randomly divided into 6 groups of treatment and each group consists of 5 mices. Each groups are explained below.
K1: Control group that is infected with 0,2 ml *P. berghei* $1x10^5$ intaperitoneally and did not get any treatment.K2: Control group that is infected with 0,2 ml *P. berghei* $1x10^5$ intaperitoneally and given chloroquine which is the dose is 25 mg/kgbb. P1 : Treatment group that is infected with 0,2 ml *P. berghei* $1x10^5$ intaperitoneally and given leaf extract of *Syzygium cumini* which is the dose is 600 mg/kg bb.P2 : Treatment group that is infected with 0,2 ml *P. berghei* $1x10^5$ intaperitoneally and given chloroquine which is the dose is 25 mg/kgbb and leaf extract of *Syzygium cumini* which is the dose is 600 mg/kgbb. P3 : Treatment group that is infected with 0,2 ml *P. berghei* $1x10^5$ intaperitoneally and given stem bark extract of *Syzygium cumini* which is the dose is 600 mg/kgbb.P4 : Treatment group that is infected with 0,2 ml *P. berghei* $1x10^5$ intaperitoneally and given chloroquine which is the dose is 25 mg/kgbb and stem bark extract of *Syzygium cumini* which is the dose is 600 mg/kgbb. Therapy is given for 4 days, 24 hours post infection. Everyday, thin blood smear was taken from the tail and stained with giemsa 20% until 21 days post infection in order to see the percentage of parasitemia, growth inhibition, 50% effective dose level, Parasite Clearance Time (PCT), Recrudescence Time (RT).

2.5. Calculation of parasitemia and growth inhibition

The calculation of parasitemia percentage and growth inhibition of *Plasmodium berghei*on the mice is done on the first day post infection by calculate the sum of erythrocytes that infected in every 1000 cells of erythrocytes under the light microscope with 1000x magnification. Percentage of parasitemia and growth inhibition is calculated by using formulas from (13,14).

2.6. Measurement of 50 % effective dose level

Measurement 50 % effective dose levelin every treatment after 24 hours post infection is using formula(A-B)/A)x100. where A is the mean of parasitemia in control group and B is parasitemia in treatment group.$ED_{50}$is determined by using linear regression program(15).

2.7.*Parasite*Clearance *Time*(PCT)*andRecrudescence Time* (RT) *Plasmodium berghei*

Parasite Clearance TimeandRecrudescence Time(RT)*Plasmodium berghei*is done by examination of parasite growth from day 1 (D1) post infection to day 20 (D20) post infection and showed by the absence of parasite on the thin blood smear that taken from vein of the tail and stained by Giemsa 20% for 20 minutes and that examination is using light microscope with 1000x magnification. That examination is done everyday until the parasites that can grow back is found (16,17).

2.8. Data Analysis

The result of observation of % parasitemia, % growth inhibiton, is processed by *Analysis of Varian (* ANOVA), then continued with *Duncan Multiple Range Test* with level 5% in order to know the differences of treatment that given. 50 % *effective dose levelParasiteClearance Time*(PCT)and*Recrudescence Time* (RT) is processed by linear regression using *SPSS System* 17.0

3. Results

3.1. Result of *Plasmodium berghei* parasitemia percentage in control group and treatment group that given leaf and steam bark extract of *Syzygium cumini*

Result of *Plasmodium berghei* parasitemia percentagein control group and treatment group that given leaf and stem extract of *Syzygium cumini* which is using analysis of varians (Anova) showed that there is difference on α 0.05 ($p<0.05$). Then, the test is continued with the difference test between the treatment groups, the results showed that the control group that infected without treatment (K1) with the control group that infected and given chloroquine (K2), treatment group that infected and given leaf and steam bark extract of *Syzygium cumini*(P1,P2,P3,P4) showed that there is a difference on α 0.05 ($p<0.05$), whereas between group (K2) and group (P1, P2, P3, P4) did not show any difference, also between treatment group (P1, P2, P3, P4) did not show difference at α 0.05 ($p> 0.05$) (Figure**1**).

3.2. growth inhibition percentage of *plasmodium berghei* on control group and treatment group that given leaf and steam bark extract of *syzygium cumini*

The result of growth inhibition of *Plasmodium berghei* on control group and treatment group that given leaf and steam bark extract of *Syzygium cumini* showed that there is a difference on α 0.05 ($p<0.05$). Then, the test is continued with the difference test between the treatment groups, the results showed that the control group that infected without treatment (K1) with the control group that infected and given chloroquine (K2), treatment group that infected and given leaf and steam bark extract of *Syzygium cumini*(P1,P2,P3,P4) showed that there is a difference on α 0.05 ($p<0.05$), whereas between group (K2) and group (P1, P2, P3, P4) did not show any difference, also between treatment group (P1, P2, P3, P4) did not show difference at α 0.05 ($p> 0.05$) (Figure2).

3.3.Parasite*Clearance Time* (PCT) and*Recrudescence Time* (RT) of *Plasmodium berghei* on the control group and treatment group that given leaf and steam bark extract of

Syzygium cumini

Control group that infected without treatment (K1) % parasitemia after 6th day post infection is 1.43%, whereas treatment that given for 4 days on the control group that infected and given chloroquine (K2), leaf extract (P1), combination leaft extract and chloroquine (P2), stem bark extract of *Syzygium cumini*(P3) and combination theraphy of stem bark extract and chloroquine (P4) *Parasit Clearance Time* (PCT) on 6th day post infection % parasitemia are K2: 1.01%, P1: 1.33%, P2: 0.94 %, P3:1.21%and P4: 1.09 %. That result showed that K2, P1, P2, P3, P4 % parasitemia is lower than the control group K1. Provision of adjuvant therapy with combination of leaf extract and chloroquine showed the lowest % of parasitemia when compared with other treatment groups (K2, P1, P3 dan P4). *Recrudescence Time* (RT) *Plasmodium berghei* is calculated after parasitemia reach 2.5 % after receive drugs for 4 days. The result of *Recrudescence Time* (RT) on K1, parasitemia reach 2.5 % after 6th days with regression model Y = -2.002 + 0.717X. On K2, after 11th days with regression model Y= -0.233+0.209X. On P1, after 10th days with regression model Y= -0,063+0,217X. On P2, after 11th days with regression model Y= -0.485+0.247X. On P3after 10th days with regression model Y=-0.325+0.262X**.** On P4 after 10th days with regression model Y= -.434+0261X. The administration of chloroquine and leaf extract of *Syzygium cumini*showed a longer *Recrudescence Time* (RT) compared to the control group K1(Figure 3).

3.4 Examination of 50 % dose level (ED_{50}) *Plasmodium berghei* on the control group and treatment group that given Leaf and Steam Bark Extract of *Syzygium cumini*

The result of linear regression test showed that 50% dose level (ED_{50}) *Plasmodium berghei* in control group K2 was on the 9th days post infection with regression equation is Y =29.975+ 2.243X, P1 group on the 13th days post infection with regression equation is Y = 10.881+3.194X, P2 group on the 9th days post infection with regression equation is Y = 36.344+1.661X, P3 group on the 13th days post infection with regression equation is Y =20.921+2.275X andP3 group on the 13th days post infection with regression equation is Y = 27.239+2.055X.(Figure 4)

4.Discussion

4.1. Parasitemia percentage and growth inhibition

There is significant difference between decreasing percentage of parasitemia and increasing growth of ihibition on the control group that infected *Plasmodium falciparum* without treatment (K1) with control group that is infected *Plasmodium falciparum* and given chloroquine (K2) showed that chloroquine as antimalarial drug can inhibit parasite growth. Chloroquine as antimalarial drug is shizontosid blood, that antimalaria only active on the erythrocytes stadium. Malaria does not active on hypnozoite parasites, preerythrocytes in the liver, and gametocytes. Chloroquine works exclusively on the intraerythrocytes cycle when active parasite degrade hemoglobin in *digestive vacuole*. Chloroquine works by inhibit the change of free heme so that it does not become hemozoin, heme is toxic to membrane. Free heme can lyse membrane and cause peroxidative damage on bilayer lipid and protein with reative oxygen intermediete. Accumulation of heme cause the death of parasite (18).

The significant difference between parasitemia percentage and growth inhibition on the control group that is infected *Plasmodium berghei* without treatment (K1) and control group that is infected *Plasmodium berghei*and given leaf extract of *Syzygium cumini* (P1), leaf extract of *Syzygium cumini*combine with chloroquine (P2) also with treatment group that is infected with *Plasmodium berghei* which is given stem bark extract of *Syzygium cumini* (P3) and combination of stem bark extract of *Syzygium cumini*and chloroquine (P4) showed that leaf and stem bark extract of *Syzygium cumini* can inhibit the growth of *Plasmodium berhgei*. The decreasing growth and increasing inhibition are caused by active ingredients of

Syzygium cumini leaf and stem bark extracts containing flavonoids, polyphenols, acetyl oleanolic acid, tannins. gallic acid, ellagic acid, quercetin, isoquercetin, kaemferol, myricetin, flavonol, glycosides, saponins, triterpenoids (S.Ramya *et al*, 2012). Active metabolites that is found in leaves and stem bark of *Syzygium cumini* have potency as an antioxidants which is shown from the research results (7,8). Results of research conducted by showed that leaves, stems and fruit of *Syzygium cumini* have antioxidant and antiinflammatory activity(19). Activities as antioxidants are thought to be due to the presence of flavonoids and polyphenols in the plant (20).

This reseacrh showed that there is no significant difference between parasitemia percentage and growth inhibition on the control group that is infected *Plasmodium falciparum* and given chloroquine (K2) with control group that is infected *Plasmodium berghei*and given leaf extract of *Syzygium cumini* (P1), leaf extract of *Syzygium cumini*combine with chloroquine (P2) also with treatment group that is infected with *Plasmodium berghei* which is given stem bark extract of *Syzygium cumini* (P3) and combination of stem bark extract of *Syzygium cumini*and chloroquine (P4) showed that leaf and stem bark extract of *Syzygium cumini* have the same potential as chloroquine in inhibiting parasite growth which is indicated by the decrease of % parasitemia with different working mechanisms. Chloroquine works by inhibiting the formation of hemozoin in digestive vacuoles while the active metabolite of leaf and stem bark extract of *Syzygium cumini* acts as an antioxidant. In trophozoite malaria infection, Plasmodium infects erythrocytes and produces twice larger H_2O_2 and OH^- than normal erythrocytes, excessive H_2O_2 can be also cause heme breakdown and release of free Fe ion which will form again the OH^- radical through Fenton's reaction. All of these factors lead to an increase of lipid peroxide that causes oxidative stress in malaria infections (3). The results of research showed that there is total decreasing antioxidant status in malaria patients (21), showed the same result which is there is an imbalance between oxidants and antioxidants in pregnant women with malaria(22).

There is no significant difference between control group that is infected *Plasmodium berghei*and given leaf extract of *Syzygium cumini* (P1), leaf extract of *Syzygium cumini*combine with chloroquine (P2) also with treatment group that is infected with *Plasmodium berghei* which is given stem bark extract of *Syzygium cumini* (P3) and combination of stem bark extract of *Syzygium cumini*and chloroquine (P4) showed that *Syzygium cumini* leaf and stem bark extracts containing flavonoids, polyphenols, acetyl oleanolic acid, tannins. gallic acid, ellagic acid, quercetin, isoquercetin, kaemferol, myricetin, flavonol, glycosides, saponins, triterpenoids which have potency as antioxidant (7, 23, 24).

4,2 Parasit *Clearance Time* (PCT) and *Recrudescence Time* (RT) *Plasmodium berghei*

Provision of adjuvant therapy combination with leaf extract and chloroquine showed the lowest % of parasitemia when compared with other treatment groups (K2, P1, P3 and P4). The administration of chloroquine and *Syzygium cumini* leaf and stem bark extracts also showed a longer Recrudescence Time (RT) when compared to the K1 control group. These results suggest that adjuvant therapy be able to maximize the effectiveness of the drug and accelerate the healing. Adjuvant therapy in malaria is intended to modify the pathophysiological processes caused by malaria so that the symptoms can be relieved, reduce the possibility of complications and death. Antioxidant is one of the drug which is used as adjuvant therapy (6).

Several studies have shown that adjuvant therapy with MMP inhibitors may improve survival of mice with cerebral malaria, other studies have also shown that adjuvant therapy with dexametasone may decrease the inflammation in the lung of mouse malaria

models, also addition of quercetin flavonoids, kinase inhibitors are able to suppress hemozoin induction in order to regulate MMP9, TNF alpha and IL1 beta (25,26)

Some medicinal plant extracts or natural products that have potency as antioxidant can interact by modulating cellular signaling pathways so as to accelerate the shizontosid and antiparasitic activity by altering the balance of redox reactions on the host. Increasing of total antioxidant capacity and decreasing lipid peroxidation may decrease parasitemia and increase survival of mice which is infected with Plasmodium berghei (20).

50 % dose level (ED_{50}) *Plasmodium berghei*

The results of this study showed that the use of combination *Syzygium cumini* leaf extract with chloroquin require a shorter time in parasitic growth than the P1, P3 and P4 groups. This suggests that the effectiveness of active metabolites from *Syzygium cumini* leaf extract is better when used together with chloroquine as adjuvant therapy. These results are consistent with the Hiben et al study, 2016 using chloroquin with *S.singueana* leaf extracts that able to enhance the antimalarial effect and provide benefits when used as adjuvant therapy to support chloroquine in malaria treatment. These results were proved by all mices that suvive though infected with *P.berghei* and then treated with combination of chloroquin with *S.singueana* leaf extract (6).Conclusionsof the researchprovision of *Syzygium cumini L* leaf extract combined with chloroquine can increase Clearance Time Parasite (PCT) more rapidly and longer Recrudescence Time (RT)than other treatments.

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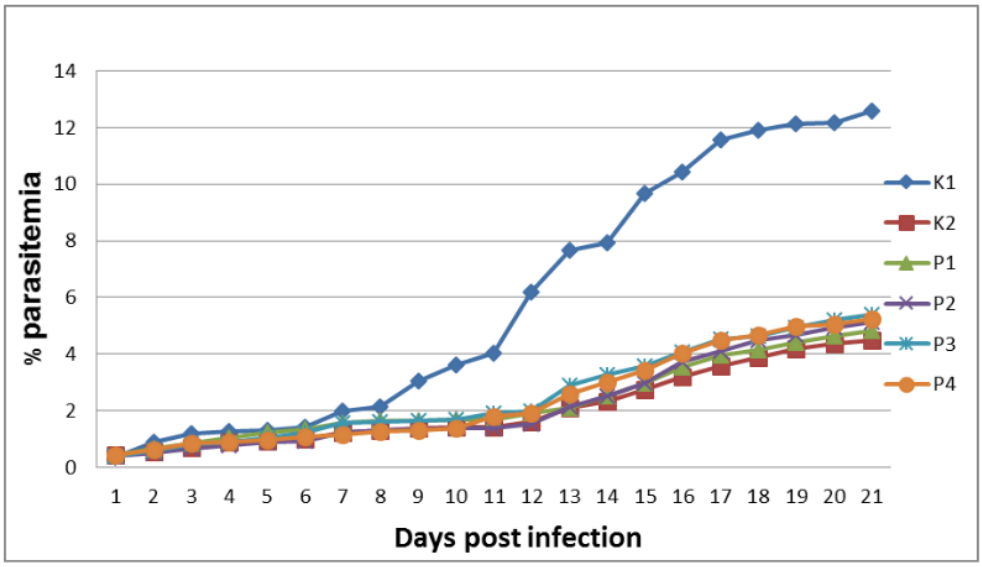

Figure.1 Graph of Percentage of *Plasmodium berghei* Parasitemia on Control Group and Treatment Group that given Leaf and stem bark Extract of*Syzygium cumini*. K1: control group infected untreated. K2: control group infected treated chloroquine. P1 : infected treated leaf extract. P2 : infected treated chloroquineand leaf extract. P3 : infected treated stem bark extract P4 : infected treated chloroquineand stem bark extract

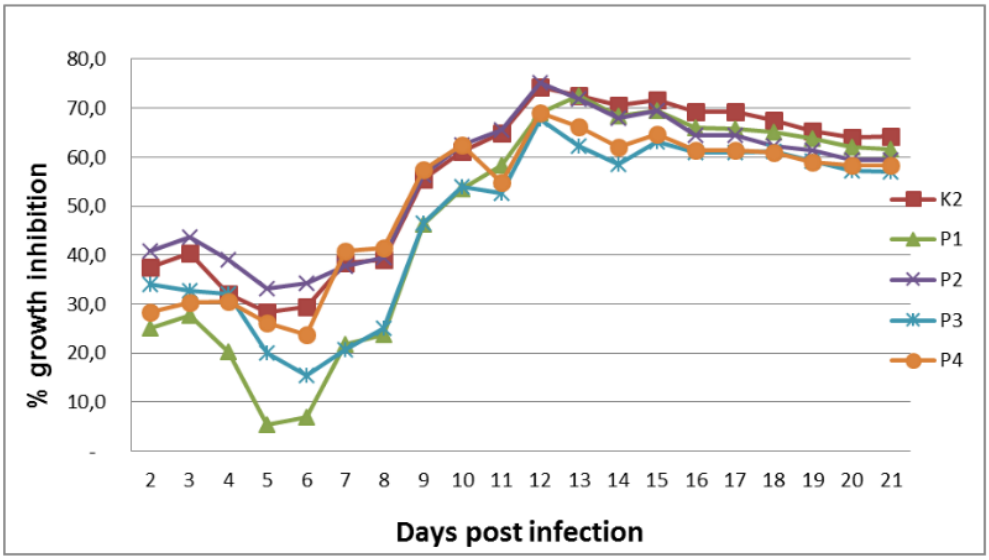

Figure 2. Graph of Percentage of Growth Inhibition of*Plasmodium berghei* on Control Group and Treatment Group that given Leaf and stem bark Extract of*Syzygium cumini*K1: control group infected untreated. K2: control group infected treated chloroquine. P1 : infected treated leaf extract. P2 : infected treated chloroquineand leaf extract. P3 : infected treated stem bark extract P4 : infected treated chloroquineand stem bark extract

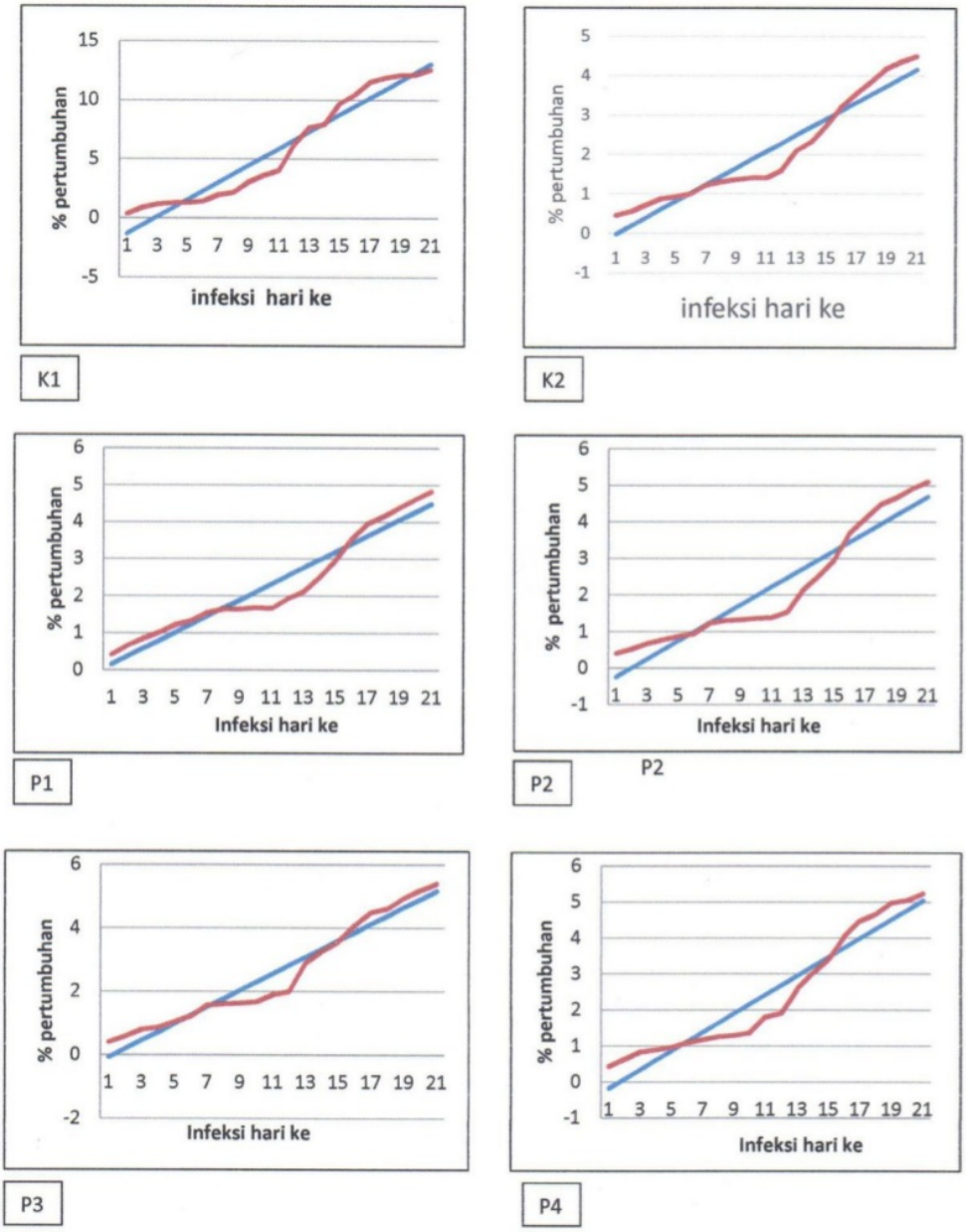

Figure 3. Graph of Parasite*Clearance Time* (PCT) and*Recrudescence Time* (RT) of *Plasmodium berghei* on the control group and treatment group that given leaf and steam bark extract of *Syzygium cumini*K1: control group infected untreated. K2: control group infected treated chloroquine. P1 : infected treated leaf extract. P2 : infected treated chloroquineand leaf extract. P3 : infected treated stem bark extract P4 : infected treated chloroquineand stem bark extract

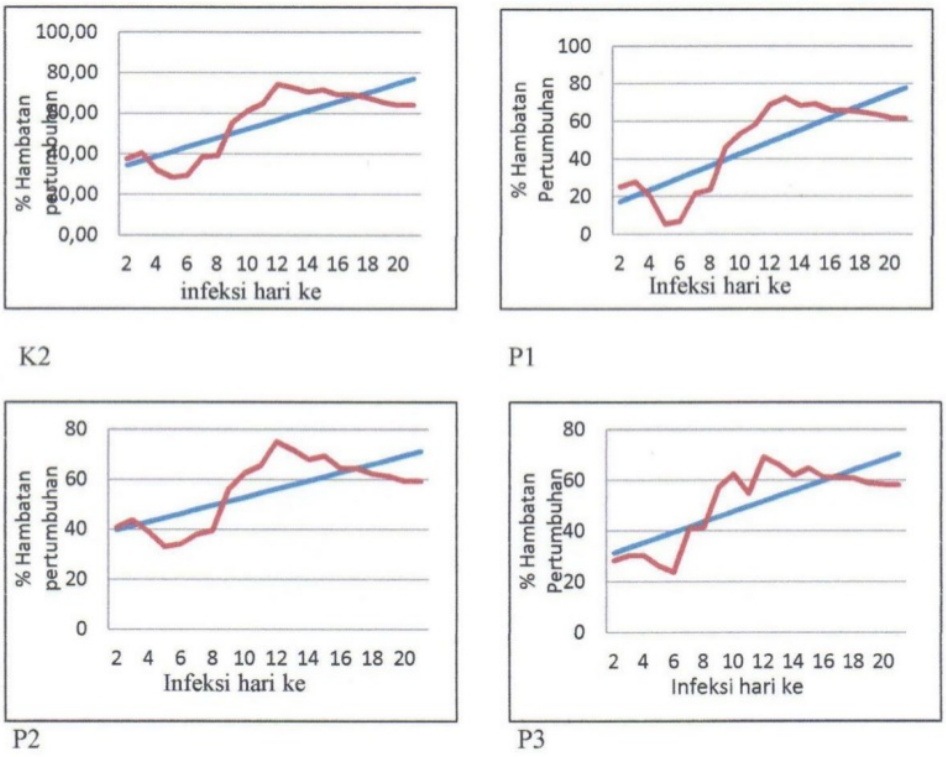

Figure 4 Graph of 50 % dose level (ED_{50}) linear regression of *Plasmodium berghei* on control group and treatment group that given Leaf and stem bark Extract of*Syzygium cumini*K2: control group infected treated chloroquine. P1 : infected treated leaf extract. P2 : infected treated chloroquineand leaf extract. P3 : infected treated stem bark extract P4 : infected treated chloroquineand stem bark extract

Potency Syzygium cumini Las Adjuvant Therapy On Mice Model Malaria

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