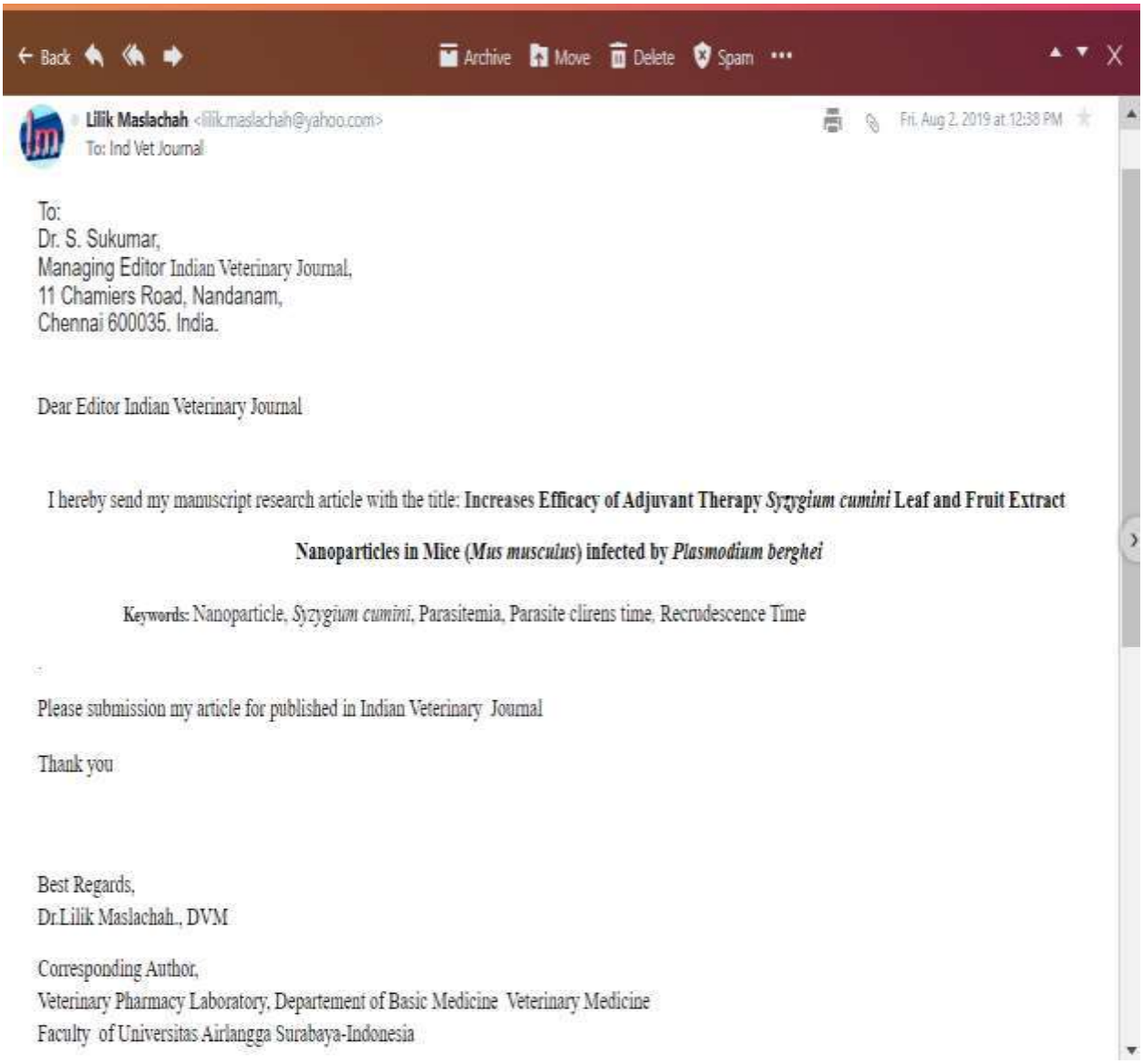

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Keywords: Nanoparticle, *Syzygium cumini*, Parasitemia, Parasite clirens time, Recrudescence Time

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Thank you

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Increases Efficacy of Adjuvant Therapy *Syzygium cumini* Leaf and Fruit Extract Nanoparticles in Mice (*Mus musculus*) infected by *Plasmodium berghei*

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Abstract

The aim is proving the efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles in mice infected with *Plasmodium berghei*. Forty-five mice were randomly divided into 9 groups consisting of 5 mice. K0: not infected and given chitosan-NaTPP polymer. K^+: infected and given chitosan-NaTPP polymer. K1: infected and given chloroquine. K2: infected and given *Syzygium cumini* leaf extract. K3: infected and given with *Syzygium cumini* fruit extract, P1: infected and given with *Syzygium cumini* leaf extract nanoparticles, P2: infected and given with *Syzygiumm cumini* leaf extract and chloroquine. P3: infected and given with nanoparticles of *Syzygium cumini* fruit extract. P4: infected and given with nanoparticles *Syzygium cumini* fruit extract and chloroquine. Treatment is given starting 24 hours post infection for 4 days. Observed on the 5^{th} day until 12^{th} day post infection. Hb levels, splenic and liver index, Mean Survival Time were analyzed by ANOVA, % parasitemia, % growth inhibition, Parasite Clirens Time (PCT) and Recrudescence Time (RT) were analyzed by linear regression. The results of % parasitemia, growth inhibition, Parasite Clirens Time, and Recrudescence Time of parasites that given with *Syzygium cumini* fruit nanoparticles with chloroquine were better than *Syzygium cumini* leaf nanoparticles with chloroquine, whereas in Survival time, Hb content, splenic index and liver index were the same. Efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles increases when used as an adjuvant therapy with chloroquin in mice infected with *Plasmodium berghei*.
Keywords: Nanoparticle, *Syzygium cumini*, Parasitemia, Parasite clirens time, Recrudescence Time

Introduction

The development of treatment, prevention and control of malaria is one of the substantial problems in the world, because until right now this disease still depends on the use of antimalarial drugs. Currently there has been a decrease in efficacy of antimalarial drugs and many parasites have been resistant to some antimalarial drugs, there is also no effective vaccine to control malaria infection because of the complex life cycle of the *Plasmodium* parasite (Good et al., 2018).

Malaria infection can cause systemic complications that are very serious, such as hematological abnormalities, splenomegaly, liver dysfunction, and cerebral malaria. Hematologic abnormalities during malaria infection are caused by high parasitemia in patients (Ngole et al., 2010). Malaria infection also causes the metabolic interactions that accompany clinical manifestations, namely decreased nutrition, micronutrients, and antioxidant status (Iribhogbe at al., 2013), and an increase in antigen will stimulate the activity of the immune system resulting in

the formation of reactive oxygen species (ROS) by inflammatory cells that can cause stress oxidative (Percario et al., 2013).

Imbalance of ROS formation in malaria infection, if it is not balanced with cytoprotective enzymes and antioxidants in the body can cause oxidative damage (George et al., 2012). Oxidative damage caused increases tissue and organ damage, increases cerebral malaria or severe malaria that can cause death or decrease survival time (Percario et al., 2013).

A decrease in the efficacy and spread of multidrug resistant malaria parasites is urgently needed for the development of new antimalarial drugs. The disadvantages of the original natural ingredients are the low stability, solubility and absorption, which reduces bioavailability and efficacy, and also has a workplace that is not specific to the target organ. The development of new drugs from nature with the delivery system of active compounds with nanoparticle technology will be able to increase, stability, solubility and absorption of the drugs (Mathur et al., 2013)

One of the many medicinal plants found in Indonesia is *Syzygium cumini*. *Syzygium cumini* has strong radical and antioxidant scavenging activity (Zhang et al., 2009). *Syzygium cumini* leaves as a therapeutic adjuvant have inhibitory effects on *plasmodium* better than the bark (Maslachah et al., 2018). Formulation of *Syzygium cumini L* leaf and fruit extract nanoparticles improve the efficacy as adjuvant therapy in *Plasmodium berghei* roden malaria as an in vivo model of malaria in mice animals (Craig et al., 2012).

Materials and Methods

This research has been approved by the animal ethics commission at the Faculty of Veterinary Medicine Universitas Airlangga with no certificate 1.KE.071.04.2008. The leaves and fruit of *Syzygium cumini* are aerated until dry. A total of 500 grams soaked with 4 L PA ethanol stirred and allowed for 3 x 24 hours and then filtered. The result of the filtrate is evaporated using Rotary Evaporator on 40-50^{o}C with low pressure (George et al., 2012). The ratio of making nanoparticles of *Syzygium cumini* leaf and fruit extract is 1:1:6. 10 ml of 5% extract was mixed with 10 ml sodium tripolyphosphate (0.1% NaTPP), added 60 ml of 0.2% chitosan solution. The mixture was sonication for 60 minutes in frequency 20 kHz until the suspension is formed. Then dried with freeze drying (Nguyen et al., 2017).

Forty-five mice were randomly divided into 9 groups consisting of 5 mice. The groups are K0: the mice is not infected and given chitosan-NaTPP polymer. K^{+}: infected and given chitosan-

NaTPP polymer. K1: infected and given chloroquine 25 mg/kgbb. K2: infected and given *Syzygium cumini* leaf extract 600mg/kgbb. K3: infected and given with *Syzygium cumini* fruit extract 600 mg/kgbb, P1: infected and given with *Syzygium cumini* leaf extract nanoparticles 600 mg/kgbb , P2: infected and given with *Syzygiumm cumini* leaf extract 600mg/kgbb and chloroquine 25 mg /kgbb . P3: infected and given with nanoparticles of *Syzygium cumini* fruit extract 600mg/kgbb. P4: infected and given with nanoparticles *Syzygium cumini* fruit extract 600mg/kgbb and chloroquine 25 mg/kgbb . Treatment is given starting 24 hours post infection for 4 days. Every day, a thin blood smear was taken from the tail and stained with giemsa 20% until the 12^{th} day post infection in order to see the percentage of parasitemia, growth inhibition, Parasite Clirens Time (PCT), Recrudescence Time (RT), and Mean Survival Time. On the 12^{th} day of infection, blood was taken from the heart to examine hemoglobin (Hb) levels and also splenic and liver organs were taken by surgery in order to examine the splenic index and liver index.

The percentage of parasitemia and growth inhibition was calculated by the formula from Garcia, 2008; Ljungstrom et al., 2004. Examination of *Parasite Clirens Time* and *Recrudescence Time* (RT) *Plasmodium berghei* were done by examinate the parasite growth since 1^{st} day (D1) until 12^{th} day (D12) post infection. The examination was done until there is no more parasite that can grow back (*Recrudescence Time* (RT) (Henrich et al., 2014). Deaths from control and treatment mice were observed daily from day 1 to 12 days post infection, to calculate Mean Survival Time that calculated by the formula from Tadesse et al, 2017. On the 12^{th} day post infection, the mice were anesthetized with ketamine (sigma), then thoracotomy was carried out, blood was taken from the heart (1 ml) in order to examine the hematological with SYSMEX XT 4000i automated blood analyzer. The value of the splenic index and liver index were calculated using the equations of the weight of the mice organs divided with the body weight of mice (Dkhil 2009).

Hb levels, splenic index and Mean Survival Time were processed using Analysis of Variants (ANOVA) followed by Duncan Multiple Range Test with a level of 5%. % parasitemia,% inhibition of growth, Parasite Clirens Time (PCT) and Recrudescence Time (RT) were analyzed by linear regression analysis.

Results and Discussion

The scanning results of electron microscope showed that there is different size between extracts and nanoparticles of *Syzygium cumini* leaf and fruit. Figure 1. In the nanoparticles extract of *Syzygium cumini* leaf and fruit using chitosan polymer and NaTPP with particle size of 10-1000 nm as an excellent carrier for active and passive drug control and carrier (S, Bathia. 2016).

The results percentage of parasitemia, inhibition of *Plasmodium berghei* growth and survival time as seen in Table 1 and Figure 2. Adjuvant therapy with the combination of *Syzygium cumini* leaf and fruit extracts with the main antimalarial chloroquine drug (P2 and P4) is more effective. Malaria immunopatogenesis is very complex so that single pathway targets are not sufficient to reduce mortality so that adjuvant therapy is able to reach complex target pathways with one intervention (Varo et al., 2018). Adjuvant therapy can increase the average survival time and reduce parasite growth and increase the inhibition of parasite growth. Compounds such as alkaloids, terpenoids, flavonoids and antraquinones from leaves, stems and fruit of *Syzygium cumini* have antioxidant and anti-inflammatory activity (Haroon, 2015). Syzygium cumini leaf extract as adjuvant therapy with chloroquine was able to inhibit the growth of *Plasmodium berghei* parasites infected in mice compared to *Syzygium cumini* stem bark extract (Maslachah et al., 2018). The higher ability of nanoparticles adjuvant of *Syzygium cumini* leaf and fruit extracts with chloroquine in reducing the percentage of parasitemia and increasing the inhibition of parasite growth is also due to short life time of nanoparticle extracts and long antimalarial chloroquine 150 hours or 6 days so that this combination can kill and inhibit Plasmodium more complete (Widyawaruyanti et al., 2017).

Parasite Clirens in the K^+ 4.158, K1 0.465, K2 2.363, K3. 2.745, P1 1.863, P2 0.303, P3 1.843 and P4 0.290. Recrudescence Time (RT) *Plasmodium berghei* calculated after parasitemia reached 5% after 4 days of treatment showed that in the K^+ 6.36 days, K1 11.34 days, K2 7.23 days, P1 7.17 days, P2 10.26 days, P3 7.27 days and P4. 13.32 days (Figure 3) The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) is better if seen from the ability to increasing the Parasite Clirens Time with % parasitemia at least 0.290% after 4 days of treatment, and prolonging the parasitic Recrudescence Time to 13.32 days. This suggests that therapeutic adjuvants of *Syzygium cumini* fruit extract nanoparticles have a better antimalarial effect. Adjuvant therapy using a combination of primary antimalarial drugs with other drugs aimed to improve the efficacy and reduce the complications because of malaria (Varo et al., 2018). Adjuvant antimalarial drug combination therapy with immunomudulation glucocorticosteroid

betamethasone hemisuccinat encapsulted in liposomes that given after neurological symptoms can improve survival time and improve clinical symptoms (Guo et al., 2014). along with curcumin can improve survival time and reduce parasitemia (Dende et al., 2015).

The co-administration of neuroprotexion such as lithium chloride, nimodipine can improve survival and reduce nerve dysfunction and reduce inflammation (Dai et al., 2012; Martins et al., 2013). Combination with improving endothelial function like recombinant human Ang 1 (Higgins et al., 2016), artovastatin (Wilson et al., 2013), and vitamin D (Dwivedi etal., 2016) can improve survival, clinical symptoms, decrease cerebrovascular leak and inflammation. There are three processes that play a role in the clearance of malaria parasites from the peripheral blood circulation namely the host defense mechanism, the effects of antimalarial drugs and sequestration. Immune host response plays an important role in the severity of malaria infection, and immunomudulatory administration in adjuvant therapy can improve clinical outcomes.

Measurement of hemoglobin (Hb), splenic index, and liver index can be seen in Table 2 and Figure 4. The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) had the same ability as *Syzygium cumini* leaf nanoparticles as adjuvant therapy with chloroquine (P2) in mice infected with *Plasmodium berghei* compared to single extracts and nanoparticles of *Syzygium cumini* leaf and fruit extracts (K2,P1,P3) when viewed from the ability to increase hemoglobin levels, splenic index and heart index. In malaria infection there is an increase in TNFα levels which can indicate the severity of the disease because it can cause erythropoesis suppression, reduction of erythropoetin production and increase erythropagocytosis with anemia clinical manifestations. Malaria infection is also caused by parasitic or non-parasitic erythrocytes which undergo hemolysis due to increased osmotic fragility as well as osmotic autohemolysis from increasing parasitic or non-parasitic erytrocytes and also shortened erythrocyte life time (Irawati, L, 2014). Administration of adjuvant therapy *Syzygium cumini* leaf and fruit extract nanoparticles was able to increase hemoglobin levels in mice infected with *Plasmodium berghei* because of inhibition on the percentage of parasitemia and inhibition of *Plasmodium* growth. This shows a correlation between an increase in the percentage of parasitemia, a decrease in growth inhibition, a level of cytokines with hemoglobin levels (anemia). In malaria, there is an increase in levels of cytokines IL-5, IL-13 and TNF (Megnekou et al., 2013).

The administration of adjuvant therapy *Syzygium cumini* leaf and fruit extract nanoparticles was able to reduce the splenic index of mice infected with Plasmodium. In splenic

organ, malaria infection plays an important role in the control and clearance of intraerythrocytic stage of malaria infection. Malaria infection can cause splenic enlargement to be more than 3 times normal and clearance function also increases (Kotlyar et al., 2014). Splenic can recognize erythrocytes that have deformability due to infection, and eliminated by splenic by increasing the function of splenic clearence and splenomegaly (White, 2017). There is a decrease in parasitemia and an increase in growth barriers by adjuvant therapy of *Syzygium cumini* leaf and fruit extract given to mice infected with *Plasmodium* so that the index of splenic mice infected with *Plasmodium* can decrease.

The stadium form of sporozoites malaria parasites infects liver cells causing the liver to undergo congestion, sinusoid blockade and cellular inflammation. The clinical manifestations are jaoundice, hepatomegaly and increased liver enzymes. This enzyme increase in malaria patients correlates with an increase in parasite density (Al-Salahy et al., 2016). The ability of adjuvant therapy of nanoparticles leaves and fruit extracts of *Syzygium cumini* parasitemia and increase the inhibition of plasmodium parasite growth so that the liver index of mice infected with *Plasmodium* can decrease.

Summary

Syzygium cumini leaf and fruit nanoparticles have increased efficacy when used as an adjuvant therapy with antimalarial chloroquine drugs in mice infected with *Plasmodium berghei*. Efficacy Adjuvant therapy of *Syzygium cumini* fruit nanoparticles with chloroquine is better than *Syzygium cumini* leaves in reducing % parasitemia, increasing growth barriers, increasing Parasite Clirens Time, and extending Recrudescence Time parasites, while the ability to Survival time, increasing Hb levels, splenic index and liver index have the same efficacy.

References

Al-Salahy, M., Shnawa, B., Abed, G., Mandour, A. and Al-El, A. 2016. Parasitaemia and its relation to hematological parameter and liver fuction among patients malaria in Abs Hajjah North westyemen. Interdiciplinary perspective in Infectious Diseases. http//dx.; doi.org/10.1155/2016/ID.595439

Craig., Alister, G., Georges, E., Grau., Chris Janse., James W., Kazura., Milner., John W.. Barnwell., Gareth Turner., Jean Langhorne. 2012. The Role of Animal Models for Research on Severe Malaria. PLoS Pathogens., 8 (2)

Dai, M., Freeman, B., Shikani, H.J., Bruno, F.P., Collado, J.E., Macias, R. 2012. Altered regulation of signaling with murine cerebral malaria,effects on longterm neuro-cognitive function, restoration with lithium treatment. Plos One., 7: e44117.

Dende, C., Meena, J., Nagarajan, P., Panda, A.K., Rangarajan, P.N., Padmanaban, G. 2015. Simultaneously targeting inflammatory response and parasite sequestration in brain to treat experimental cerebral malaria. Sci Rep., 5:12671.
Dkhil, M.A.E. 2009. Apoptotic changes induced in mice splenic tissue due to malaria infection.J Microbiol Immune Infect., 42:13-18.
Dwivedi, H., Singh, S.K., Chauhan, B.S., Gunjan, S., Tripathi, R. 2016. Potential cerebral malaria therapy. Intramuscular arteether and vitamin D administration. Parasitology., 143:1557-68.
Garcia, C.R., M.F. de Azevedo, G., Wunderlich, A., Budu., J.A.Young and L. 2008. Bannister. Plasmodium in the Post Genomic Era: New Insight Into Moleculer Cell Biology of Malaria Parasites. Int.Rev. Cell Mol Biol., 266: 8.
George, B.O., Okpoghono, J., Osioma, E., Aina, O.O. 2012. Changes in oxidative indices in Plasmodium berghei infected mice treated with aqueous extract of *Aframomum sceptrum*. Frontiers in Science., 2(1): 6-9.
Good, M.F., Doolan, D.L. 2018. Malaria Vaccine Design : Immunological Consideration. Immunity., 33(4): 555-566.
Guo, J., H. Judith., Grinberg, W., Mitchele, A.J., Barenhol, Y. and Golenser, J. 2014. Reduction of experimental cerebral malaria and its related pro inflamatory responses by the novel liposom based B methasone nano drug. Biomed Research International. http//dxdoi.org/10.1155/2014/292471.
Haroon, R. 2015. Comparative analysis of antioxidant profile of bark, leaves and seeds of *Syzygium cumini* (Indian blackberry). International Journal of Research (Indian blackberry). International Journal of Research.Granthaalayah., 3(5): 11-22.
Henrich, P.P., O. Brien, C., Saen, F.E., Cremers., Kyle, D.E., Fidock, D.A. 2014. Evidence for pyronaridine as highly effective partner drug for treatment of artemisinin resistant malaria in a rodent model. Antimicrob Agents Chemother., 58(1): 183-95. Higgins, S.J., Purcell, L.A., Silver, K.L., Tran, V., Crowley, V. 2016. Dysregulation of angiopoietin 1 plays amechanistic role in the pathogenesis of cerebral malaria. Sci Transl Med., 8:128.
Irawati, L. 2014. Relation level of TNF alfa with haemoglobin and parasitemia on malaria falciparum infection. http://jurnal.fk.unand.ac.id
Iribhogbe, O.I., Agbaje, E.O., Oreagba, I.A., Aina, O.O., and Ota, A.D. 2013. Oxidative stress and micronutrient therapy in malaria : An in vivo study in Plasmodium berghei infected mice. Pakistan Journal of Biological Sciences., 16(4):160-167.
Kotlyar, S., Nteziyaremye, J., Olupot-olupot, P., Akech, S.O., Moore, C.L., Maitland, K. 2014. Spleen volume and clinical diseases manifestations of severe Plasmodium falciparum malaria in African children. Trans R Soc.Trop Med Hyg., 108:283-9.
Ljungstrom, I., Perlmann, H., Schlictherle, M., Scherf, A., Wahlgren, M. 2004. Methods in Malaria Research Ed 4., 1-240.
Martins, Y.C., Clemmer, L., Orjuela Sanche, P., Anini, GM., Ong, P.K., Frangos, J.A. 2013. Slow and continuous delivery of a low dose of nimodipine improves survival and electrocardiogram parameters in rescue therapy of mice with experimental cerebral malaria. Malar Journal, 12:138.

Mathur, M. and Govind, V. 2013. Role of nanoparticles for production of smart normal drug. Overview Indian Journal of natural Products and Resources., 4(4):329-338.
Maslachah, L. and Sugihartuti, R. 2018. Potency *Syzygium cumini L* as adjuvant therapy on mice model malaria. Iraqi Journal of Veterinary Sciences., 31(1): 73-80. Megnekou, R., Staalsoe, T.,

Hviid, L. 2013. Cytokine response to pregnancy associated recrudescence of Plasmodium berghei infection in mice with pre exixting immunity to malaria., 12:387
Ngole, S.I.U., N.A Theresa., Moses, S., Thomas, N et al. 2010. Haematological changes and recovery associated with treated and untreated *Plasmodium falciparum* infection in children in the Mount Cameroon Region. Clinical Medicine and Research., 2(9) : 143-151.
Nguyen, T.V., Nguyen, T.T.H., Wang, S.L., Khanvo, T.P., Nguyen, A.D. 2017. Preparation of chitosan nanoparticle by TPP ionic gelation combined with spray drying and the antibacterial activity of chitosan nanoparticles and a chitosan nanoparticle amoxicillin complex. Res Chem Intermed., 43:3527-3537
Percario, S., Moreira, D.R., Gomes, B.A.Q., Ferreira, M.E.S., Goncalves, A.C.M., Laurindo, P.S.O.C., Vilhena, T.C., Dolabela, M.F. and Green, M.D. 2013. Oxidative stress in malaria. *Int. .J. Mol. Sci.*, 13: 16346-16372.doi :10.3390/ijms/31216346.
Queiroz, N.L., Riteau, N., Eastman, R.T., Bock, K.W., Orandle, M.S., Moore, I.N., Sher, A., Long, C.A., Jankovic, D. and Su, X.Z. (2017). Mechanism of splenic cell death and host mortality in Plasmodium yoelii malaria model. Scientific Repots., 7:10438.
S, Bathia. 2016. Natural Polymer Drug Delivery System. Springer International Publishing Switeland., doi.10.1007/978-3-319-41129-3-2.
Tadesse, S.A., Wubneh, Z.B. 2017. Antimalarial activity of Syzygium guineense during early and established Plasmodium infection in rodent models. Biomed Central.Complementary and Alternative Medicine., doi 10.1186/s12906-016—1538-6
Varo, R., Crowley, V.M., Sitoe, A., Madrid, L., Serghides., Kain, K.C. and Bassat, Q. 2018. Adjuctive therapy for severe malaria: a review and critical appraisal. Malaria Journal., 17: 47
White, J.N. 2017. Malaria parasite clearance. Malaria Journal., 16:88
Widyawaruyanti, A., Tumewu, L., Ilmi, H., Setyawan, D., Widiastuti, E., Dachilyati, L., Tantular, I.S. and Hafid, A.F. 2017. Antimalarial activity and survival time of *Adrographis paniculata* fraction (AS202-1) on *Plasmodium berghei* infected mice. Research Journal of Pharmaceutical, Biological and Chemical Sciences., 8(1S).49-54. Wilson, N.O., Solomon, W., Anderson, L., Patricson, J., Pitts, S., Bond, V. 2013. Pharmacologic inhibition of CXCL10 in combination with antimalarial therapy eliminates mortality associated with murine model of cerebral malaria. Plos One., 8.e60898.
Zhang, L.L., Lin, Y.M. 2009. Antioksidant tannins from *Syzygium cumini* fruit. *African Journal of Biotechno.logy.*, 8(10): 2301-2309.

Table 1 Mean of parasitemia, inhibition growth of *Plasmodium berghei* and survival time on control and treatment groups.

Groups	% Parasitemia	% Growth inhibition	% Survival time
K0	-	-	$100.000^{d} \pm 0.000$
K^{+}	$7.132^{c} \pm 0.776$	$0.000^{a} \pm 0.000$	$90.000^{bcd} \pm 9.122$
K1	$2.087^{a} \pm 0..527.$	$88.815^{c} \pm 3.624$	$100.000^{d} \pm 0.000$
K2	$4.806^{b} \pm 0.376$	$43.175^{ab} \pm 1.747$	$80.950^{b} \pm 17.577$
K3	$7.724^{c} \pm 0.057$	$28.547^{ab} \pm 1.122$	$61.150^{a} \pm 34.294$
P1	$5.817^{b} \pm 1.775$	$55.201^{bc} \pm 2.082$	$85.416^{bc} \pm 12.873$
P2	$2.190^{a} \pm 0.493$	$92.724^{c} \pm 1.501$	$98.817^{d} \pm 4.099$
P3	$5.521^{b} \pm 0.181$	$55.683^{bc} \pm 2.503$	$90.317^{bcd} \pm 8.547$
P4	$1.498^{a} \pm 0.558$	$93.025^{c} \pm 0.520$	$97.233^{cd} \pm 6.462$

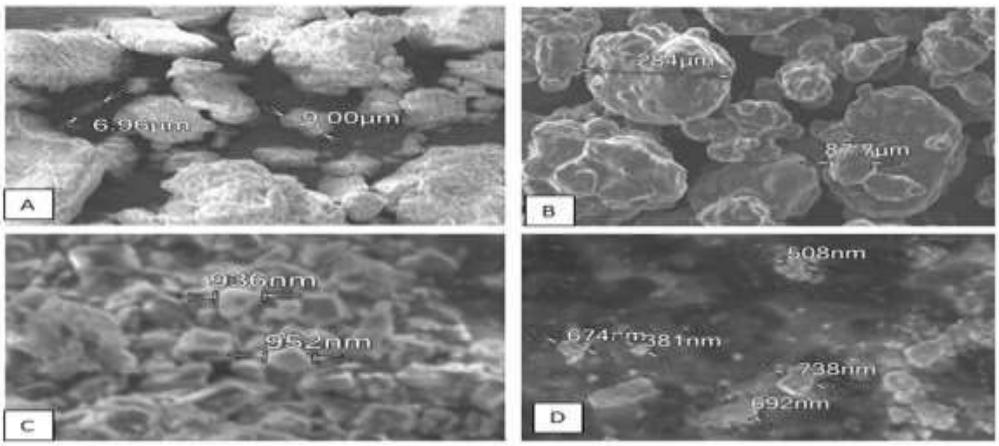
Note: different superscript on the same column showed the significant difference with significant level 0.05 %.

Table 2 Hb level, splenic index and liver index on control and treatment group

Groups	Hb	Splenic index	Liver index
K0	$14.750^{d} \pm 0.957$	$0.4600^{a} \pm 0.050$	$3.5850^{a} \pm 0.523$
K^{+}	$3.925^{ab} \pm 1.027$	$2.8700^{c} \pm 0.341$	$9.550^{d} \pm 0.744$
K1	$14.400^{d} \pm 0.141$	$1.2600^{ab} \pm 0.327$	$4.4100^{a} \pm 2.022$
K2	$6.6500^{b} \pm 2.533$	$1.3473^{ab} \pm 0.834$	$6.1800^{b} \pm 0.579$
K3	$3.1000^{a} \pm 0.081$	$1.1525^{ab} \pm 0.127$	$7.0950^{bc} \pm 1.544$
P1	$6.1500^{ab} \pm 3.307$	$1.6910^{b} \pm 0.368$	$6.5125^{b} \pm 0.476$
P2	$12.400^{cd} \pm 1.630$	$1.2950^{ab} \pm 0.193$	$6.3300^{b} \pm 0.498$
P3	$4.9750^{ab} \pm 3.094$	$1.8175^{b} \pm 0.440$	$8.4525^{cd} \pm 1.665$
P4	$11.2500^{c} \pm 2.409$	$1.0425^{ab} \pm 0.316$	$6.1850^{b} \pm 0.920$

Note: different superscript on the same column showed the significant difference with significant level 0.05 %.

Figure 1.



A: *Syzygium cumini* leaf extract, B: *Syzygium cumini* fruit extract, C: *Syzygium cumini* leaf extract nanoparticles, D: *Syzygium cumini* fruit extract nanoparticles

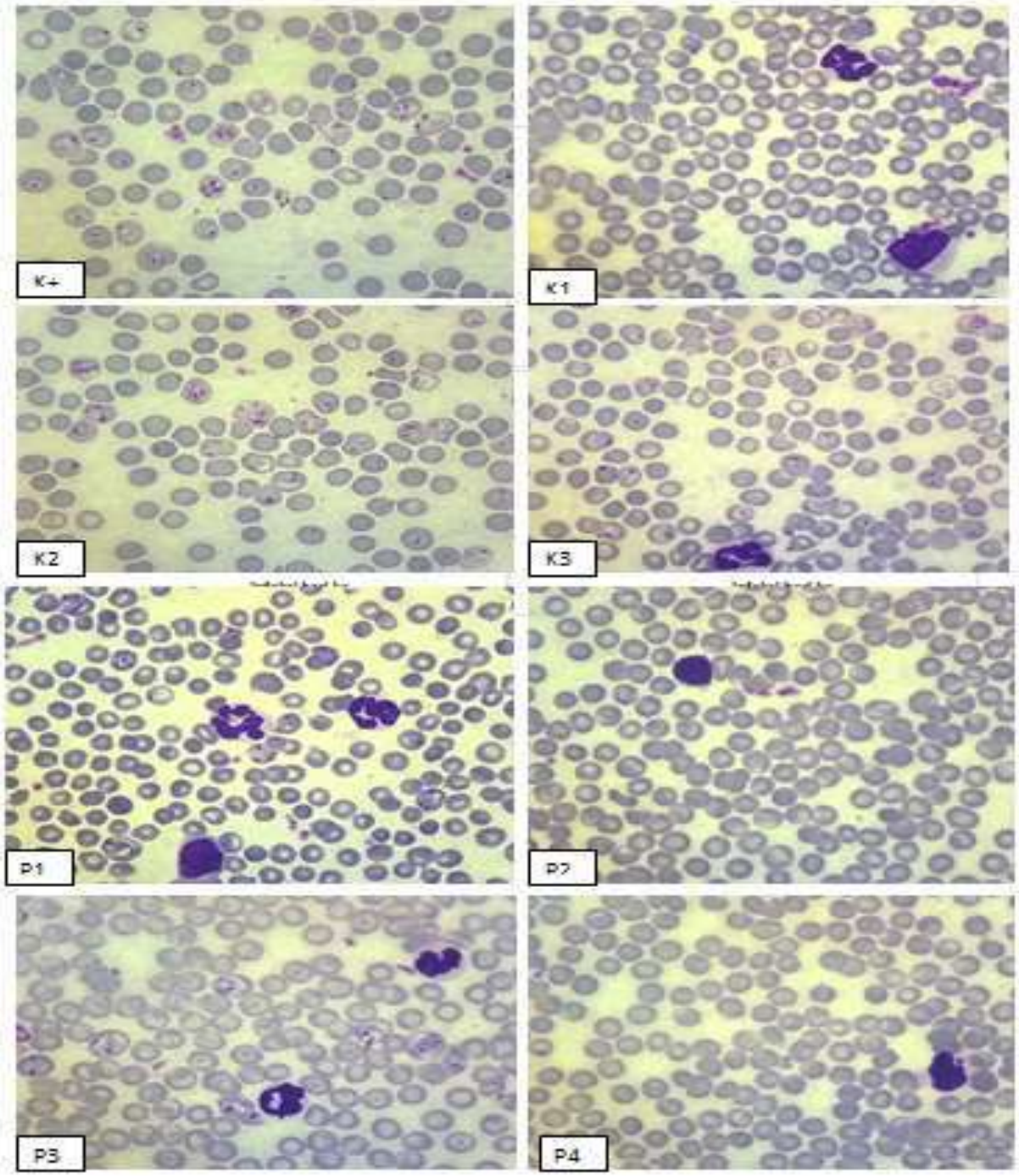


Figure 2. Thin blood smear on 5th post infection after given treatment for 4 days on the control and treatment groups. The microscope magnification is 100x and the stained is using HE

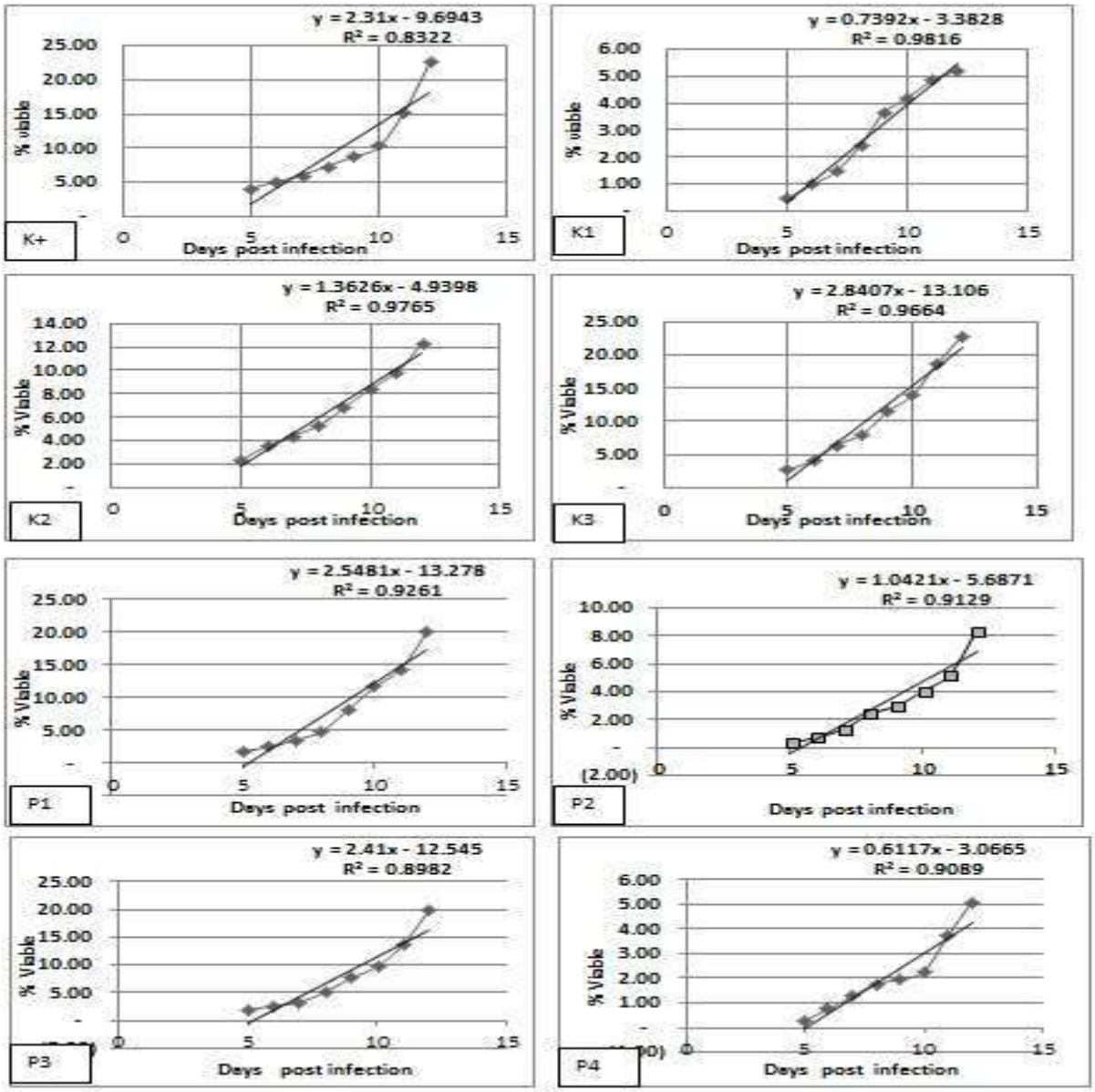


Figure 3. *Parasite Clirens Time* dan *Recrudescence Time* (RT) *Plasmodium berghei* on control and treatment groups.

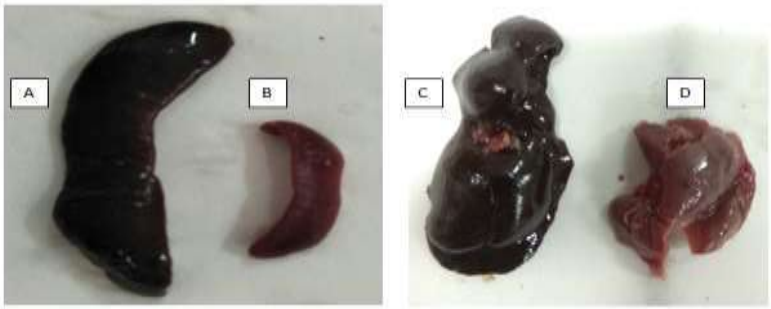


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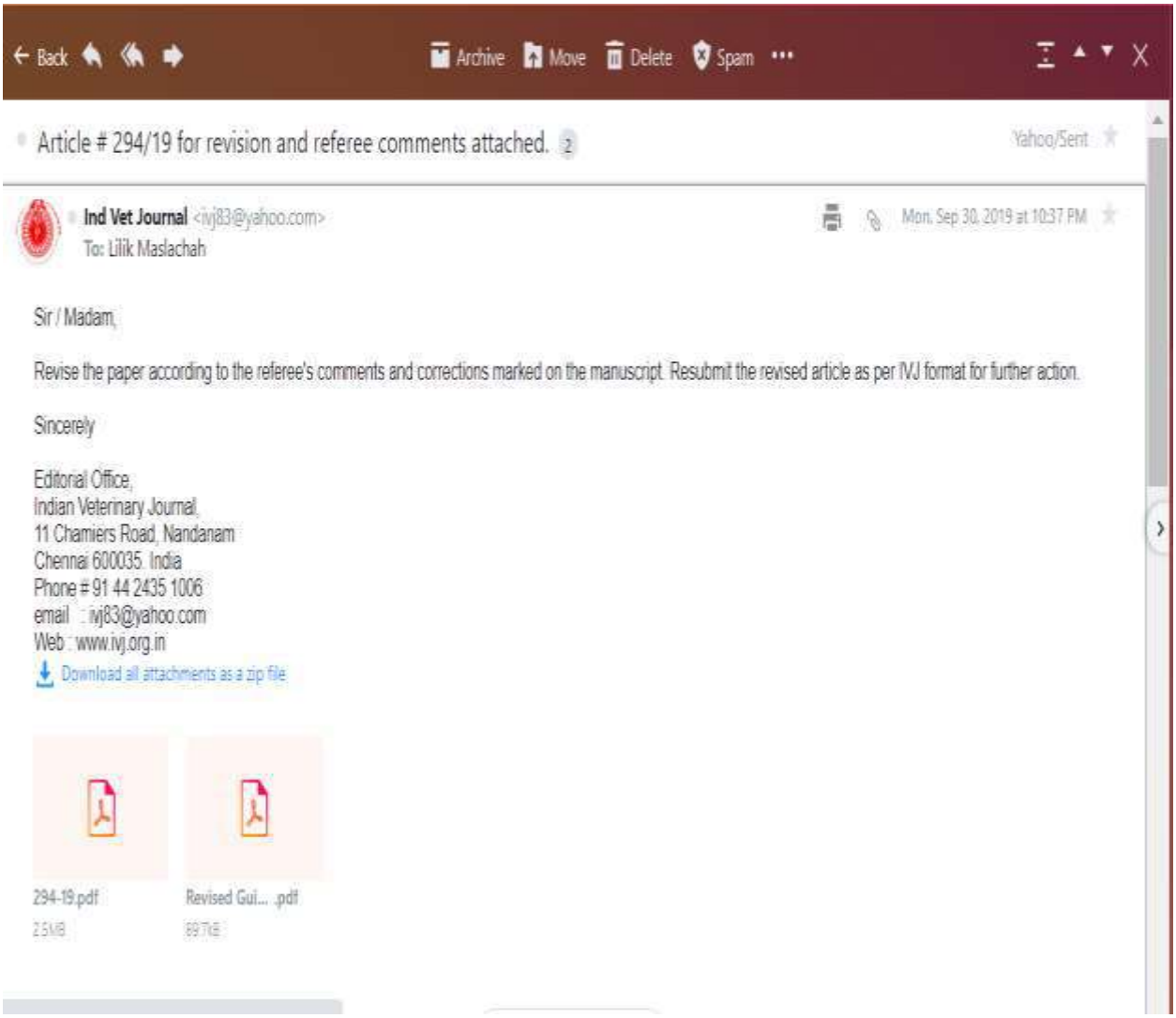
Figure 4. A dan C. splenic and liver organ on K^+ group, B and D: splenic and liver organ on K0 group. Blackish splenic enlargement in the group infected with *Plasmodium berghei* (A) was compared with the group which is not infected with *Plasmodium berghei* (B). The liver is enlarged with a blackish color (C) compared to the group not infected with Plasmodium berghei (figure D).



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Increases Efficacy of Adjuvant Therapy *Syzygium cumini* Leaf and Fruit Extract Nanoparticles in Mice (*Mus musculus*) infected by *Plasmodium berghei*

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Abstract

The aim is proving the efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles in mice infected with *Plasmodium berghei*. Forty-five mice were randomly divided into 9 groups consisting of 5 mice. K0: not infected and given chitosan-NaTPP polymer. K+: infected and given chitosan-NaTPP polymer. K1: infected and given chloroquine. K2: infected and given *Syzygium cumini* leaf extract. K3: infected and given with *Syzygium cumini* fruit extract, P1: infected and given with *Syzygium cumini* leaf extract nanoparticles, P2: infected and given with *Syzygiumm cumini* leaf extract and chloroquine. P3: infected and given with nanoparticles of *Syzygium cumini* fruit extract. P4: infected and given with nanoparticles *Syzygium cumini* fruit extract and chloroquine. Treatment is given starting 24 hours post infection for 4 days. Observed on the 5th day until 12th day post infection. Hb levels, splenic and liver index, Mean Survival Time were analyzed by ANOVA. % parasitemia, % growth inhibition, Parasite Clirens Time (PCT) and Recrudescence Time (RT) were analyzed by linear regression. The results of % parasitemia, growth inhibition, Parasite Clirens Time, and Recrudescence Time of parasites that given with *Syzygium cumini* fruit nanoparticles with chloroquine were better than *Syzygium cumini* leaf nanoparticles with chloroquine, whereas in Survival time, Hb content, splenic index and liver index were the same. Efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles increases when used as an adjuvant therapy with chloroquin in mice infected with *Plasmodium berghei*.

Keywords: Nanoparticle, *Syzygium cumini*, Parasitemia, Parasite clirens time, Recrudescence Time

Introduction

The development of treatment, prevention and control of malaria is one of the substantial problems in the world, because until right now this disease still depends on the use of antimalarial drugs. Currently there has been a decrease in efficacy of antimalarial drugs

and many parasites have been resistant to some antimalarial drugs, there is also no effective vaccine to control malaria infection because of the complex life cycle of the *Plasmodium* parasite (Good et al., 2018).

Malaria infection can cause systemic complications that are very serious, such as hematological abnormalities, splenomegaly, liver dysfunction, and cerebral malaria. Hematologic abnormalities during malaria infection are caused by high parasitemia in patients (Ngole et al., 2010). Malaria infection also causes the metabolic interactions that accompany clinical manifestations, namely decreased nutrition, micronutrients, and antioxidant status (Iribhogbe et al., 2013), and an increase in antigen will stimulate the activity of the immune system resulting in the formation of reactive oxygen species (ROS) by inflammatory cells that can cause stress oxidative (Percario et al., 2013).

Imbalance of ROS formation in malaria infection, if it is not balanced with cytoprotective enzymes and antioxidants in the body can cause oxidative damage (George et al., 2012). Oxidative damage caused increases tissue and organ damage, increases cerebral malaria or severe malaria that can cause death or decrease survival time (Percario et al., 2013).

A decrease in the efficacy and spread of multidrug resistant malaria parasites is urgently needed for the development of new antimalarial drugs. The disadvantages of the original natural ingredients are the low stability, solubility and absorption, which reduces bioavailability and efficacy, and also has a workplace that is not specific to the target organ. The development of new drugs from nature with the delivery system of active compounds with nanoparticle technology will be able to increase, stability, solubility and absorption of the drugs (Mathur et al., 2013)

One of the many medicinal plants found in Indonesia is *Syzygium cumini*. *Syzygium cumini* has strong radical and antioxidant scavenging activity (Zhang et al., 2009). *Syzygium cumini* leaves as a therapeutic adjuvant have inhibitory effects on *plasmodium* better than the bark (Maslachah et al., 2018). Formulation of *Syzygium cumini L* leaf and fruit extract nanoparticles improve the efficacy as adjuvant therapy in *Plasmodium berghei* roden malaria as an in vivo model of malaria in mice animals (Craig et al., 2012).

Materials and Methods

This research has been approved by the animal ethics commission at the Faculty of Veterinary Medicine Universitas Airlangga with no certificate 1.KE.071.04.2008. The leaves and fruit of *Syzygium cumini* are aerated until dry. A total of 500 grams soaked with 4 L PA

ethanol stirred and allowed for 3 x 24 hours and then filtered. The result of the filtrate is evaporated using Rotary Evaporator on 40-50°C with low pressure (George et al., 2012). The ratio of making nanoparticles of *Syzygium cumini* leaf and fruit extract is 1:1:6. 10 ml of 5% extract was mixed with 10 ml sodium tripolyphosphate (0.1% NaTPP), added 60 ml of 0.2% chitosan solution. The mixture was sonication for 60 minutes in frequency 20 kHz until the suspension is formed. Then dried with freeze drying (Nguyen et al., 2017).

Forty-five mice were randomly divided into 9 groups consisting of 5 mice. The groups are K0: the mice is not infected and given chitosan-NaTPP polymer, K+: infected and given chitosan-NaTPP polymer, K1: infected and given chloroquine 25 mg/kgbb. K2: infected and given *Syzygium cumini* leaf extract 600mg/kgbb. K3: infected and given with *Syzygium cumini* fruit extract 600 mg/kgbb, P1: infected and given with *Syzygium cumini* leaf extract nanoparticles 600 mg/kgbb , P2: infected and given with *Syzygiumm cumini* leaf extract 600mg/kgbb and chloroquine 25 mg /kgbb . P3: infected and given with nanoparticles of *Syzygium cumini* fruit extract 600mg/kgbb. P4: infected and given with nanoparticles *Syzygium cumini* fruit extract 600mg/kgbb and chloroquine 25 mg/kgbb . Treatment is given starting 24 hours post infection for 4 days. Every day, a thin blood smear was taken from the tail and stained with giemsa 20% until the 12th day post infection in order to see the percentage of parasitemia, growth inhibition, Parasite Clirens Time (PCT), Recrudescence Time (RT), and Mean Survival Time. On the 12th day of infection, blood was taken from the heart to examine hemoglobin (Hb) levels and also splenic and liver organs were taken by surgery in order to examine the splenic index and liver index.

The percentage of parasitemia and growth inhibition was calculated by the formula from Garcia, 2008; Ljungstrom et al., 2004. Examination of *Parasite Clirens Time* and *Recrudescence Time* (RT) *Plasmodium berghei* were done by examinate the parasite growth since 1st day (D1) until 12th day (D12) post infection. The examination was done until there is no more parasite that can grow back (*Recrudescence Time* (RT) (Henrich et al., 2014). Deaths from control and treatment mice were observed daily from day 1 to 12 days post infection, to calculate Mean Survival Time that calculated by the formula from Tadesse et al, 2017. On the 12th day post infection, the mice were anesthetized with ketamine (sigma), then thoracotomy was carried out, blood was taken from the heart (1 ml) in order to examine the hematological with SYSMEX XT 4000i automated blood analyzer. The value of the splenic index and liver index were calculated using the equations of the weight of the mice organs divided with the body weight of mice (Dkhil 2009).

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Hb levels, splenic index and Mean Survival Time were processed using Analysis of Variants (ANOVA) followed by Duncan Multiple Range Test with a level of 5%. % parasitemia,% inhibition of growth, Parasite Clirens Time (PCT) and Recrudescence Time (RT) were analyzed by linear regression analysis.

Results and Discussion

The scanning results of electron microscope showed that there is different size between extracts and nanoparticles of *Syzygium cumini* leaf and fruit. Figure 1. In the nanoparticles extract of *Syzygium cumini* leaf and fruit using chitosan polymer and NaTPP with particle size of 10-1000 nm as an excellent carrier for active and passive drug control and carrier (S, Bathia. 2016).

The results percentage of parasitemia, inhibition of *Plasmodium berghei* growth and survival time as seen in Table 1 and Figure 2. Adjuvant therapy with the combination of *Syzygium cumini* leaf and fruit extracts with the main antimalarial chloroquine drug (P2 and P4) is more effective. Malaria immunopatogenesis is very complex so that single pathway targets are not sufficient to reduce mortality so that adjuvant therapy is able to reach complex target pathways with one intervention (Varo et al., 2018). Adjuvant therapy can increase the average survival time and reduce parasite growth and increase the inhibition of parasite growth. Compounds such as alkaloids, terpenoids, flavonoids and antraquinones from leaves, stems and fruit of *Syzygium cumini* have antioxidant and anti-inflammatory activity (Haroon, 2015). Syzygium cumini leaf extract as adjuvant therapy with chloroquine was able to inhibit the growth of *Plasmodium berghei* parasites infected in mice compared to *Syzygium cumini* stem bark extract (Maslachah et al., 2018). The higher ability of nanoparticles adjuvant of *Syzygium cumini* leaf and fruit extracts with chloroquine in reducing the percentage of parasitemia and increasing the inhibition of parasite growth is also due to short life time of nanoparticle extracts and long antimalarial chloroquine 150 hours or 6 days so that this combination can kill and inhibit Plasmodium more complete (Widyawaruyanti et al., 2017).

Parasite Clirens in the K^+ 4.158, K1 0.465, K2 2.363, K3. 2.745, P1 1.863, P2 0.303, P3 1.843 and P4 0.290. Recrudescence Time (RT) *Plasmodium berghei* calculated after parasitemia reached 5% after 4 days of treatment showed that in the K^+ 6.36 days, K1 11.34 days, K2 7.23 days, P1 7.17 days, P2 10.26 days, P3 7.27 days and P4. 13.32 days (Figure 3) The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) is better if seen from the ability to increasing the Parasite Clirens Time with

% parasitemia at least 0.290% after 4 days of treatment, and prolonging the parasitic Recrudescence Time to 13.32 days. This suggests that therapeutic adjuvants of *Syzygium cumini* fruit extract nanoparticles have a better antimalarial effect. Adjuvant therapy using a combination of primary antimalarial drugs with other drugs aimed to improve the efficacy and reduce the complications because of malaria (Varo et al., 2018). Adjuvant antimalarial drug combination therapy with immunomudulation glucocorticosteroid betamethasone hemisuccinat encapsulted in liposomes that given after neurological symptoms can improve survival time and improve clinical symptoms (Guo et al., 2014). along with curcumin can improve survival time and reduce parasitemia (Dende et al., 2015).

The co-administration of neuroprotexion such as lithium chloride, nimodipine can improve survival and reduce nerve dysfunction and reduce inflammation (Dai et al., 2012; Martins et al., 2013). Combination with improving endothelial function like recombinant human Ang 1 (Higgins et al., 2016), artovastatin (Wilson et al., 2013). and vitamin D (Dwivedi et al., 2016) can improve survival, clinical symptoms, decrease cerebrovascular leak and inflammation. There are three processes that play a role in the clearance of malaria parasites from the peripheral blood circulation namely the host defense mechanism, the effects of antimalarial drugs and sequestration. Immune host response plays an important role in the severity of malaria infection, and immunomudulatory administration in adjuvant therapy can improve clinical outcomes.

Measurement of hemoglobin (Hb), splenic index, and liver index can be seen in Table 2 and Figure 4. The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) had the same ability as *Syzygium cumini* leaf nanoparticles as adjuvant therapy with chloroquine (P2) in mice infected with *Plasmodium berghei* compared to single extracts and nanoparticles of *Syzygium cumini* leaf and fruit extracts (K2,P1,P3) when viewed from the ability to increase hemoglobin levels, splenic index and heart index. In malaria infection there is an increase in TNFα levels which can indicate the severity of the disease because it can cause erythropoesis suppression, reduction of erythropoetin production and increase erythropagocytosis with anemia clinical manifestations. Malaria infection is also caused by parasitic or non-parasitic erythrocytes which undergo hemolysis due to increased osmotic fragility as well as osmotic autohemolysis from increasing parasitic or non-parasitic erytrocytes and also shortened erythrocyte life time (Irawati, L., 2014). Administration of adjuvant therapy *Syzygium cumini* leaf and fruit extract nanoparticles was able to increase hemoglobin levels in mice infected with *Plasmodium berghei* because of inhibition on the percentage of parasitemia and inhibition of *Plasmodium* growth. This shows a correlation

between an increase in the percentage of parasitemia, a decrease in growth inhibition, a level of cytokines with hemoglobin levels (anemia). In malaria, there is an increase in levels of cytokines IL-5, IL-13 and TNF (Megnekou et al., 2013).

The administration of adjuvant therapy *Syzygium cumini* leaf and fruit extract nanoparticles was able to reduce the splenic index of mice infected with Plasmodium. In splenic organ, malaria infection plays an important role in the control and clearance of intraerythrocytic stage of malaria infection. Malaria infection can cause splenic enlargement to be more than 3 times normal and clearance function also increases (Kotlyar et al., 2014). Splenic can recognize erythrocytes that have deformability due to infection, and eliminated by splenic by increasing the function of splenic clearence and splenomegaly (White, 2017). There is a decrease in parasitemia and an increase in growth barriers by adjuvant therapy of *Syzygium cumini* leaf and fruit extract given to mice infected with *Plasmodium* so that the index of splenic mice infected with *Plasmodium* can decrease.

The stadium form of sporozoites malaria parasites infects liver cells causing the liver to undergo congestion, sinusoid blockade and cellular inflammation. The clinical manifestations are jaoundice, hepatomegaly and increased liver enzymes. This enzyme increase in malaria patients correlates with an increase in parasite density (Al-Salahy et al., 2016). The ability of adjuvant therapy of nanoparticles leaves and fruit extracts of *Syzygium cumini* parasitemia and increase the inhibition of plasmodium parasite growth so that the liver index of mice infected with *Plasmodium* can decrease.

Summary

Syzygium cumini leaf and fruit nanoparticles have increased efficacy when used as an adjuvant therapy with antimalarial chloroquine drugs in mice infected with *Plasmodium berghei*. Efficacy Adjuvant therapy of *Syzygium cumini* fruit nanoparticles with chloroquine is better than *Syzygium cumini* leaves in reducing % parasitemia, increasing growth barriers, increasing Parasite Clirens Time, and extending Recrudescence Time parasites, while the ability to Survival time, increasing Hb levels, splenic index and liver index have the same efficacy.

References

Al-Salahy, M., Shnawa, B., Abed, G., Mandour, A. and Al-El, A. (2016) Parasitaemia and its relation to hematological parameter and liver fuction among patients malaria in Abs Hajjah North westyemen. Interdiciplinary perspective in Infectious Diseases. http//dx.: doi.org/10.1155/2016/ID.595439

Craig., Alister, G., Georges, E., Grau., Chris Janse., James W., Kazura., Milner., John W.. Barnwell., Gareth Turner., Jean Langhorne. 2012. The Role of Animal Models for Research on Severe Malaria. PLoS Pathogens., 8 (2) page ?

Dai, M., Freeman, B., Shikani, H.J., Bruno, F.P., Collado, J.E., Macias, R. 2012. Altered regulation of signaling with murine cerebral malaria,effects on longterm neuro-cognitive function, restoration with lithium treatment. Plos One., 7: e44117.

Dende, C., Meena, J., Nagarajan, P., Panda, A.K., Rangarajan, P.N., Padmanaban, G.(2015.) Simultaneously targeting inflammatory response and parasite sequestration in brain to treat experimental cerebral malaria. Sci Rep., 5:12671.

Dkhil, M.A.E. (2009.) Apoptotic changes induced in mice splenic tissue due to malaria infection.J Microbiol Immune Infect., 42:13-18.

Dwivedi, H., Singh, S.K., Chauhan, B.S., Gunjan, S., Tripathi, R. 2016. Potential cerebral malaria therapy. Intramuscular arteether and vitamin D administration. Parasitology., 143:1557-68.

Garcia, C.R., M.F. de Azevedo, G., Wunderlich, A., Budu., J.A.Young and L.(2008.) Bannister. Plasmodium in the Post Genomic Era: New Insight Into Moleculer Cell Biology of Malaria Parasites. Int.Rev. Cell Mol Biol., 266: 8.

George, B.O., Okpoghono, J., Osioma, E., Aina, O.O.(2012.)Changes in oxidative indices in Plasmodium berghei infected mice treated with aqueous extract of *Aframomum sceptrum*. Frontiers in Science., 2(1): 6-9.

Good, M.F., Doolan, D.L. 2018. Malaria Vaccine Design : Immunological Consideration. Immunity., 33(4): 555-566.

Guo, J., H. Judith., Grinberg, W., Mitchele, A.J., Barenhol, Y. and Golenser, J.(2014.) Reduction of experimental cerebral malaria and its related pro inflamatory responses by the novel liposom based B methasone nano drug. Biomed Research International. http//dxdoi.org/10.1155/2014/292471.

Haroon, R. (2015.) Comparative analysis of antioxidant profile of bark, leaves and seeds of *Syzygium cumini* (Indian blackberry). International Journal of Research (Indian blackberry). International Journal of Research.Granthaalayah., 3(5): 11-22.

Henrich, P.P., O. Brien, C., Saen, F.E., Cremers., Kyle, D.E., Fidock, D.A. 2014. Evidence for pyronaridine as highly effective partner drug for treatment of artemisinin resistant malaria in a rodent model. Antimicrob Agents Chemother., 58(1): 183-95. Higgins, S.J., Purcell, L.A., Silver, K.L., Tran, V., Crowley, V. 2016. Dysregulation of angiopoietin 1 plays amechanistic role in the pathogenesis of cerebral malaria. Sci Transl Med., 8:128.

Irawati, L. 2014. Relation level of TNF alfa with haemoglobin and parasitemia on malaria falciparum infection. http://jurnal.fk.unand.ac.id

Iribhogbe, O.I., Agbaje, E.O., Oreagba, I.A., Aina, O.O., and Ota, A.D. 2013. Oxidative stress and micronutrient therapy in malaria : An in vivo study in Plasmodium berghei infected mice. Pakistan Journal of Biological Sciences., 16(4):160-167.

Kotlyar, S., Nteziyaremye, J., Olupot-olupot, P., Akech, S.O., Moore, C.L., Maitland, K. 2014. Spleen volume and clinical diseases manifestations of severe Plasmodium falciparum malaria in African children. Trans R Soc.Trop Med Hyg., 108:283-9.

Ljungstrom, I., Perlmann, H., Schlictherle, M., Scherf, A., Wahlgren, M. 2004. Methods in Malaria Research Ed 4., 1-240.

Martins, Y.C., Clemmer, L., Orjuela Sanche, P., Anini, GM., Ong, P.K., Frangos, J.A.(2013.) Slow and continuous delivery of a low dose of nimodipine improves survival and electrocardiogram parameters in rescue therapy of mice with experimental cerebral malaria. Malar Journal, 12:138.

Mathur, M. and Govind, V. (2013) Role of nanoparticles for production of smart normal drug. Overview Indian Journal of natural Products and Resources., 4(4):329-338.

Maslachah, L. and Sugihartuti, R. (2018) Potency *Syzygium cumini L* as adjuvant therapy on mice model malaria. Iraqi Journal of Veterinary Sciences., 31(1): 73-80 Megnekou, R., Staalsoe, T., Hviid, L. 2013. Cytokine response to pregnancy associated recrudescence of Plasmodium berghei infection in mice with pre exixting immunity to malaria., 12:387

Ngole, S.I.U., N.A Theresa., Moses, S., Thomas, N et al. 2010. Haematological changes and recovery associated with treated and untreated *Plasmodium falciparum* infection in children in the Mount Cameroon Region. Clinical Medicine and Research., 2(9) : 143-151.

Nguyen, T.V., Nguyen, T.T.H., Wang, S.L., Khanvo, T.P., Nguyen, A.D. (2017) Preparation of chitosan nanoparticle by TPP ionic gelation combined with spray drying and the antibacterial activity of chitosan nanoparticles and a chitosan nanoparticle amoxicillin complex. Res Chem Intermed., 43:3527-3537

Percario, S., Moreira, D.R., Gomes, B.A.Q., Ferreira, M.E.S., Goncalves, A.C.M., Laurindo, P.S.O.C., Vilhena, T.C., Dolabela, M.F. and Green, M.D. 2013. Oxidative stress in malaria. *Int. J. Mol. Sci.,* 13: 16346-16372.doi :10.3390/ijms/31216346.

Queiroz, N.L., Riteau, N., Eastman, R.T., Bock, K.W., Orandle, M.S., Moore, I.N., Sher, A., Long, C.A., Jankovic, D. and Su, X.Z. (2017). Mechanism of splenic cell death and host mortality in Plasmodium yoelii malaria model. Scientific Repots., 7:10438.

S. Bathia. 2016. Natural Polymer Drug Delivery System. Springer International Publishing Switeland., doi.10.1007/978-3-319-41129-3-2.

Tadesse, S.A., Wubneh, Z.B. 2017. Antimalarial activity of Syzygium guineense during early and established Plasmodium infection in rodent models. Biomed Central.Complementary and Alternative Medicine., doi 10.1186/s12906-016—1538-6

Varo, R., Crowley, V.M., Sitoe, A., Madrid, L., Serghides., Kain, K.C. and Bassat, Q.(2018.) Adjuctive therapy for severe malaria: a review and critical appraisal. Malaria Journal., 17: 47

White, J.N. 2017. Malaria parasite clearance. Malaria Journal., 16:88

Widyawaruyanti, A., Tumewu, L., Ilmi, H., Setyawan, D., Widiastuti, E., Dachilyati, L., Tantular, I.S. and Hafid, A.F. (2017) Antimalarial activity and survival time of *Adrographis paniculata* fraction (AS202-1) on *Plasmodium berghei* infected mice. Research Journal of Pharmaceutical, Biological and Chemical Sciences., 8(1S).49-54. Wilson, N.O., Solomon, W., Anderson, L., Patricson, J., Pitts, S., Bond, V. 2013. Pharmacologic inhibition of CXCL10 in combination with antimalarial therapy eliminates mortality associated with murine model of cerebral malaria. Plos One., 8.e60898.

Zhang, L.L., Lin, Y.M. (2009.)Antioksidant tannins from *Syzygium cumini* fruit. *African Journal of Biotechno.logy.*, 8(10): 2301-2309.

Table 1 Mean of parasitemia, inhibition growth of *Plasmodium berghei* and survival time on control and treatment groups.

Groups	% Parasitemia	% Growth inhibition	% Survival time
K0	-	-	$100.000^{d} \pm 0.000$
K^{+}	$7.132^{c} \pm 0.776$	$0.000^{a} \pm 0.000$	$90.000^{bcd} \pm 9.122$
K1	$2.087^{a} \pm 0..527.$	$88.815^{c} \pm 3.624$	$100.000^{d} \pm 0.000$
K2	$4.806^{b} \pm 0.376$	$43.175^{ab} \pm 1.747$	$80.950^{b} \pm 17.577$
K3	$7.724^{c} \pm 0.057$	$28.547^{ab} \pm 1.122$	$61.150^{a} \pm 34.294$
P1	$5.817^{b} \pm 1.775$	$55.201^{bc} \pm 2.082$	$85.416^{bc} \pm 12.873$
P2	$2.190^{a} \pm 0.493$	$92.724^{c} \pm 1.501$	$98.817^{d} \pm 4.099$
P3	$5.521^{b} \pm 0.181$	$55.683^{bc} \pm 2.503$	$90.317^{bcd} \pm 8.547$
P4	$1.498^{a} \pm 0.558$	$93.025^{c} \pm 0.520$	$97.233^{cd} \pm 6.462$

Note: different superscript on the same column showed the significant difference with significant level 0.05 %.

Table 2 Hb level, splenic index and liver index on control and treatment group

Groups	Hb	Splenic index	Liver index
K0	$14.750^{d} \pm 0.957$	$0.4600^{a} \pm 0.050$	$3.5850^{a} \pm 0.523$
K^{+}	$3.925^{ab} \pm 1.027$	$2.8700^{c} \pm 0.341$	$9.550^{d} \pm 0.744$
K1	$14.400^{d} \pm 0.141$	$1.2600^{ab} \pm 0.327$	$4.4100^{a} \pm 2.022$
K2	$6.6500^{b} \pm 2.533$	$1.3473^{ab} \pm 0.834$	$6.1800^{b} \pm 0.579$
K3	$3.1000^{a} \pm 0.081$	$1.1525^{ab} \pm 0.127$	$7.0950^{bc} \pm 1.544$
P1	$6.1500^{ab} \pm 3.307$	$1.6910^{b} \pm 0.368$	$6.5125^{b} \pm 0.476$
P2	$12.400^{cd} \pm 1.630$	$1.2950^{ab} \pm 0.193$	$6.3300^{b} \pm 0.498$
P3	$4.9750^{ab} \pm 3.094$	$1.8175^{b} \pm 0.440$	$8.4525^{cd} \pm 1.665$
P4	$11.2500^{c} \pm 2.409$	$1.0425^{ab} \pm 0.316$	$6.1850^{b} \pm 0.920$

Note: different superscript on the same column showed the significant difference with significant level 0.05 %.

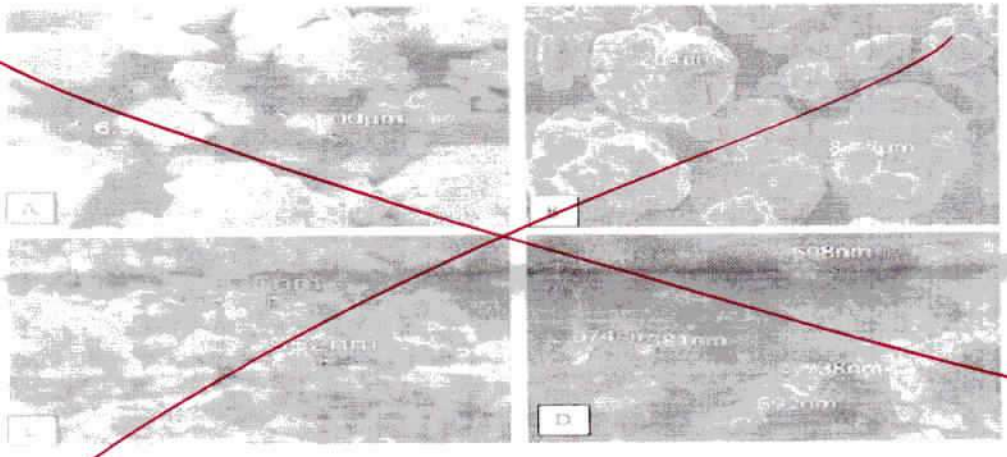

Figure 1. A: *Syzygium cumini* leaf extract, B: *Syzygium cumini* fruit extract, C: *Syzygium cumini* leaf extract nanoparticles, D: *Syzygium cumini* fruit extract nanoparticles

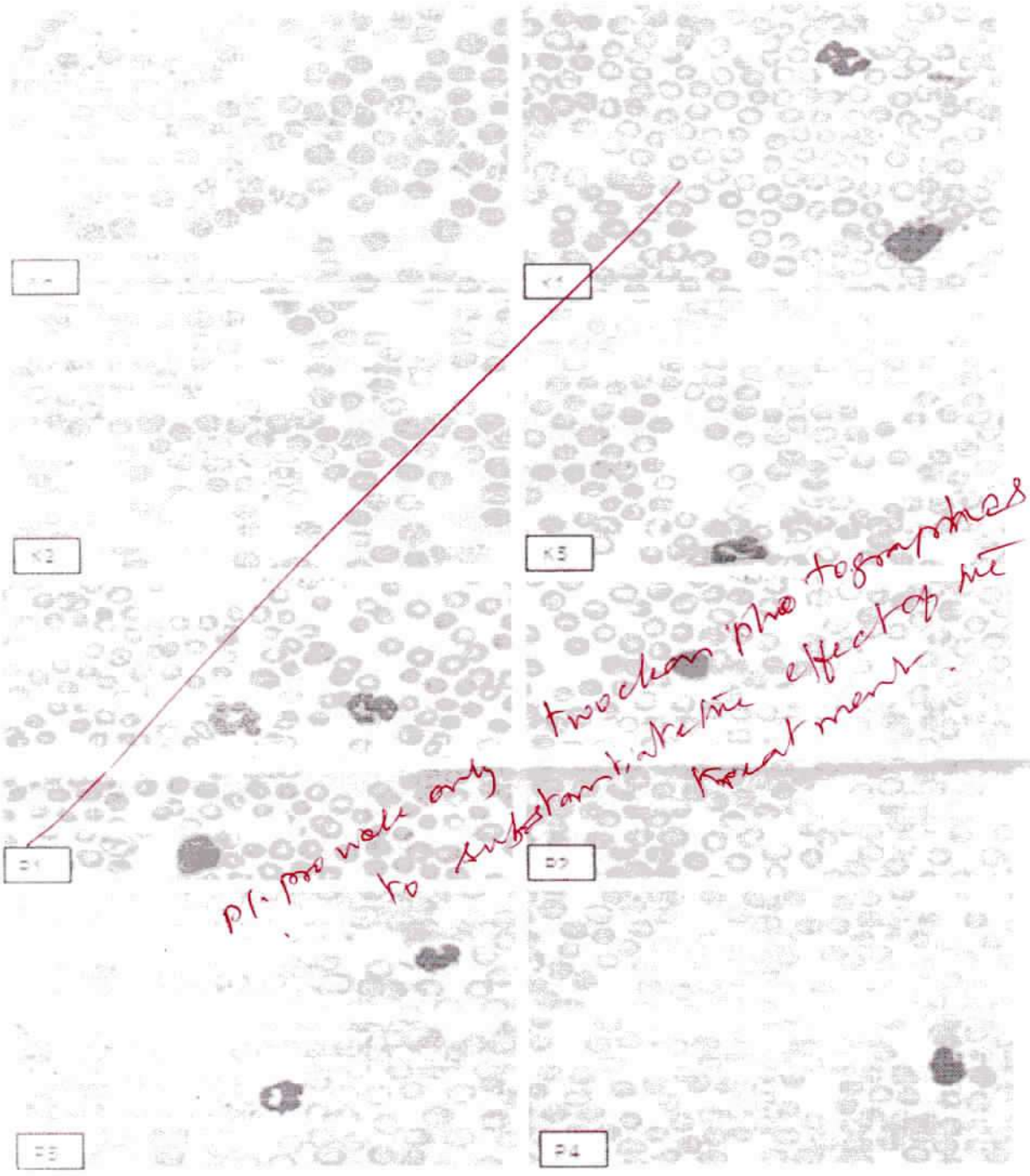

Figure 2. Thin blood smear on 5th post infection after given treatment for 4 days on the control and treatment groups. ~~The microscope magnification is 100x and the stained is using HE~~ Stain used X 100

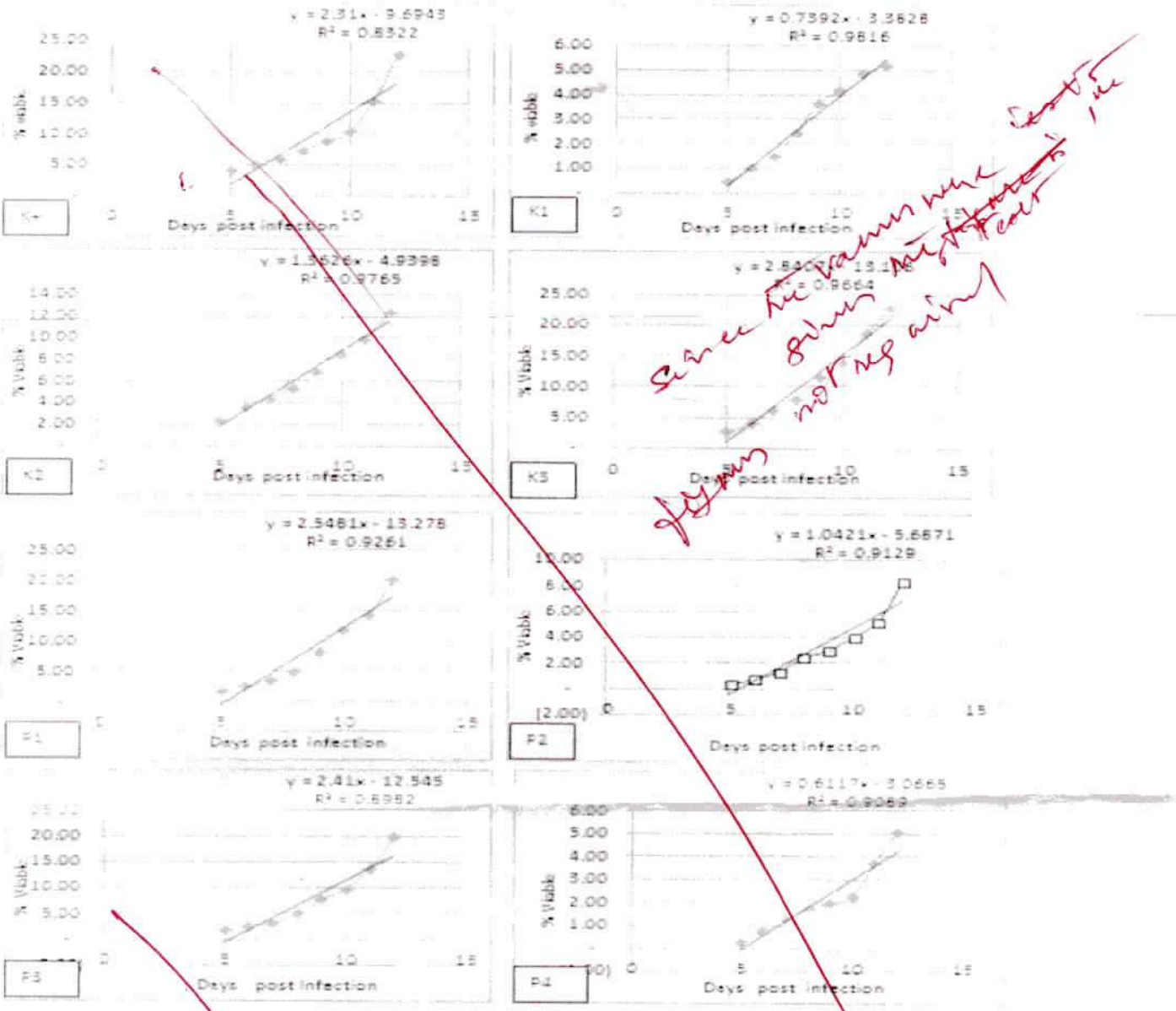

Figure 3. *Parasite Clirens Time* dan *Recrudescence Time* (RT) *Plasmodium berghei* on control and treatment groups.

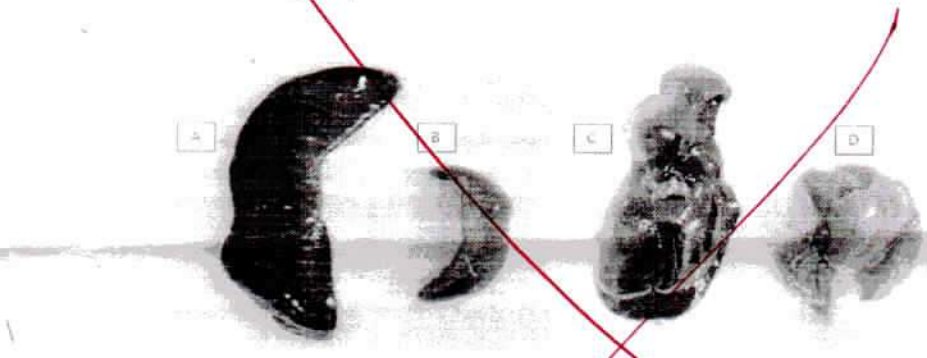

Figure 4. A dan C. splenic and liver organ on K^+ group, B and D: splenic and liver organ on K0 group. Blackish splenic enlargement in the group infected with *Plasmodium berghei* (A) was compared with the group which is not infected with *Plasmodium berghei* (B). The liver is enlarged with a blackish color (C) compared to the group not infected with Plasmodium berghei (figure D).

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Thank you for the revision gives in my article manuscript No 294/19 with the title " Increase Efficacy of Adjuvant Therapy Syzygium cumini leaf and Fruit Extract Nanoparticles in Mice(Mus musculus) infected by Plasmodium berghei" I have corrected all revision in accordance with the advice and input provided by the editor. I hope there is good news. Thank you very much for your cooperation

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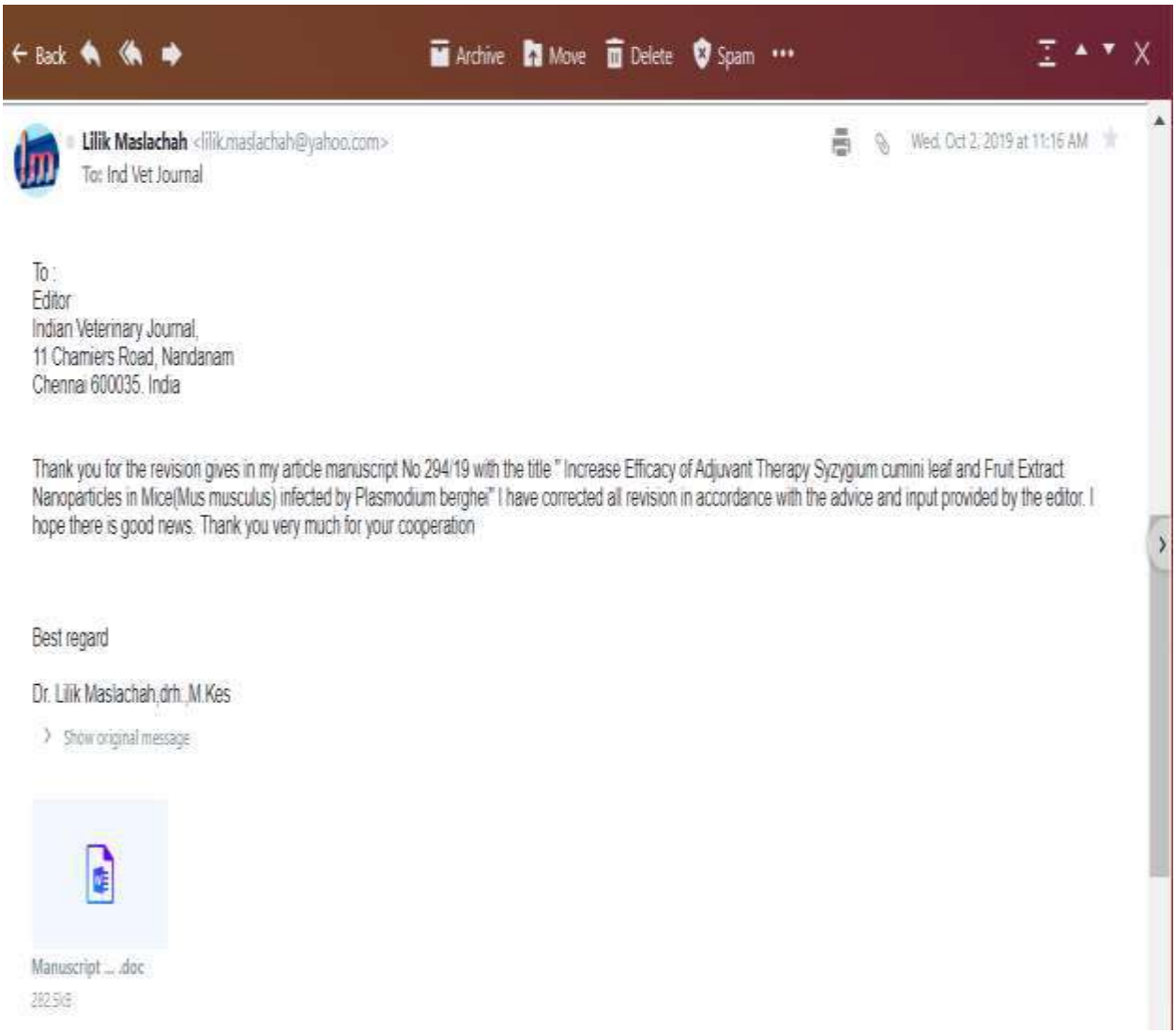

Increases Efficacy of Adjuvant Therapy *Syzygium cumini* Leaf and Fruit Extract Nanoparticles in Mice (*Mus musculus*) infected by *Plasmodium berghei*

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Abstract

The aim was proving the efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles in mice infected with *Plasmodium berghei*. Mice infected by $1x10^5$ of *Plasmodium berghei.* Treatment was given starting 24 hours post infection for 4 days. Observed on the 5^{th} day until 12^{th} day post infection. The results of % parasitemia, growth inhibition, Parasite Clearance Time, and Recrudescence Time of parasites that given with *Syzygium cumini* fruit nanoparticles with chloroquine were better than *Syzygium cumini* leaf nanoparticles with chloroquine, whereas in Survival time, Hb content, splenic index and liver index were the same. Efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles increases when used as an adjuvant therapy with chloroquin in mice infected by *Plasmodium berghei*.

Key words: Nanoparticle, *Syzygium cumini*, *Plasmodium berghei*, Therapy

The prevention and control of malaria is one of the substantial problems in the world, which is still depends on the use of antimalarial drugs. Currently there has been a decrease in efficacy of antimalarial drugs and many parasites have been resistant to some antimalarial drugs. (Good *et al*., 2018).

The decrease in the efficacy and spread of multidrug resistant malaria parasites warranty need for the development of new antimalarial drugs. The disadvantages of the original natural ingredients are the low stability, solubility and absorption, which reduces bioavailability and efficacy, and also has a workplace that is not specific to the target organ. The development of new drugs from nature with the delivery system of active compounds with nanoparticle technology will be able to increase, stability, solubility and absorption of the drugs (Mathur *et al*., 2013)

One of the many medicinal plants found in Indonesia is *Syzygium cumini*. *Syzygium cumini* has strong radical and antioxidant scavenging activity (Zhang *et al*., 2009). *Syzygium cumini* leaves as a therapeutic adjuvant have inhibitory effects on *plasmodium* better than the bark (Maslachah *et al*., 2018). Formulation of *Syzygium cumini L* leaf and fruit extract nanoparticles improve the efficacy as adjuvant therapy in *Plasmodium berghei* roden malaria as an *in vivo* model of malaria in mice animals (Craig *et al*., 2012).

Materials and Methods

The leaves and fruit of *Syzygium cumini* were air dried. A total of 500 grams soaked with 4 L PA ethanol stirred and allowed for 3 x 24 hours and then filtered. The result of the filtrate is evaporated using Rotary Evaporator on 40-50^{o}C with low pressure (George *et al*., 2012). The ratio of making nanoparticles of *Syzygium cumini* leaf and fruit extract is 1:1:6. 10 ml of 5% extract was

mixed with 10 ml sodium tripolyphosphate (0.1% NaTPP), added 60 ml of 0.2% chitosan solution. The mixture was sonicated for 60 minutes in frequency 20 kHz until the suspension is formed. Then dried with freeze drying (Nguyen *et al*., 2017).

Forty-five mice were randomly divided into 9 groups consisting of 5 each. The K^- groups mice were not infected and given chitosan-NaTPP polymer; K^+ groups: infected and given chitosan-NaTPP polymer; K1: infected and given chloroquine 25 mg/kgbb; K2: infected and given *Syzygium cumini* leaf extract 600mg/kgbb; K3: infected and given with *Syzygium cumini* fruit extract 600 mg/kgbb; P1: infected and given with *Syzygium cumini* leaf extract nanoparticles 600 mg/kgbb; P2: infected and given with *Syzygiumm cumini* leaf extract 600mg/kgbb and chloroquine 25 mg /kgbb; P3: infected and given with nanoparticles of *Syzygium cumini* fruit extract 600mg/kgbb; P4: infected and given with nanoparticles *Syzygium cumini* fruit extract 600mg/kgbb and chloroquine 25 mg/kgbb. Treatment was given starting 24 hours post infection for 4 days. Every day, a thin blood smear was taken from the tail and stained with giemsa 20% until the 12^{th} day post infection in order to see the percentage of parasitemia, growth inhibition, Parasite Clearance Time (PCT), Recrudescence Time (RT), and Mean Survival Time. On the 12^{th} day of infection, blood was taken from the heart to examine hemoglobin (Hb) levels and also splenic and liver organs were taken by surgery in order to examine the splenic index and liver index.

On the 12^{th} day post infection, the mice were anesthetized with ketamine (sigma), then thoracotomy was carried out, blood was taken from the heart (1 ml) in order to examine the hematological parameters with SYSMEX XT 4000i automated blood analyzer. The value of the splenic index and liver index were calculated using the equations of the weight of the mice organs divided with the body weight of mice (Dkhil, 2009).

Hb levels, splenic index and Mean Survival Time were processed using Analysis of Variants (ANOVA) followed by Duncan Multiple Range Test with a level of 5%. Percent parasitemia,% inhibition of growth, Parasite Clearance Time (PCT) and Recrudescence Time (RT) were analyzed by linear regression analysis.

Results and Discussion

The results percentage of parasitemia, inhibition of *Plasmodium berghei* growth and survival time as seen in Table I and Figure II. Adjuvant therapy with the combination of *Syzygium*

cumini leaf and fruit extracts with the main antimalarial chloroquine drug (P2 and P4) was more effective. Malaria immunopatogenesis is very complex so that single pathway targets are not sufficient to reduce mortality so that adjuvant therapy is able to reach complex target pathways with one intervention (Varo *et al*., 2018). Adjuvant therapy can increase the average survival time and reduce parasite growth and increase the inhibition of parasite growth. Compounds such as alkaloids, terpenoids, flavonoids and antraquinones from leaves, stems and fruit of *Syzygium cumini* have antioxidant and anti-inflammatory activity (Haroon, 2015). Syzygium cumini leaf extract as adjuvant therapy with chloroquine was able to inhibit the growth of *Plasmodium berghei* parasites infecting mice compared to *Syzygium cumini* stem bark extract (Maslachah *et al*., *loc cit*). The higher ability of nanoparticles adjuvant of *Syzygium cumini* leaf and fruit extracts with chloroquine in reducing the percentage of parasitemia and increasing the inhibition of parasite growth is also due to short life time of nanoparticle extracts and long antimalarial chloroquine 150 hours or 6 days so that this combination can kill and inhibit *Plasmodium* more complete (Widyawaruyanti *et al*., 2017).

Parasite Clearance Time (PCT) in the K^+ 4.158, K1 0.465, K2 2.363, K3. 2.745, P1 1.863, P2 0.303, P3 1.843 and P4 0.290 and Recrudescence Time (RT) *Plasmodium berghei* calculated after parasitemia reached 5% after 4 days of treatment showed that in the K^+ 6.36 days, K1 11.34 days, K2 7.23 days, P1 7.17 days, P2 10.26 days, P3 7.27 days and P4. 13.32 days.The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) is better if seen from the ability to increasing the Parasite Clearance Time with % parasitemia at least 0.290% after 4 days of treatment, and prolonging the parasitic Recrudescence Time to 13.32 days. This suggested that therapeutic adjuvants of *Syzygium cumini* fruit extract nanoparticles had a better antimalarial effect. Adjuvant therapy using a combination of primary antimalarial drugs with other drugs aimed to improve the efficacy and reduce the complications because of malaria (Varo *et al*., *loc cit*). Adjuvant antimalarial drug combination therapy with immunomudulation glucocorticosteroid betamethasone hemisuccinat encapsulted in liposomes that given after neurological symptoms can improve survival time and improve clinical symptoms (Guo *et al*., 2014). along with curcumin can improve survival time and reduce parasitemia (Dende *et al*., 2015).

Measurement of hemoglobin (Hb), splenic index, and liver index can be seen in Table 2. The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) had the same ability as *Syzygium cumini* leaf nanoparticles as adjuvant therapy with

chloroquine (P2) in mice infected with *Plasmodium berghei* compared to single extracts and nanoparticles of *Syzygium cumini* leaf and fruit extracts (K2,P1,P3) when viewed from the ability to increase hemoglobin levels, splenic index and heart index. The administration of adjuvant therapy *Syzygium cumini* leaf and fruit extract nanoparticles was able to reduce the splenic index of mice infected with *Plasmodium*. There is a decrease in parasitemia and an increase in growth barriers by adjuvant therapy of *Syzygium cumini* leaf and fruit extract given to mice infected with *Plasmodium* so that the index of splenic mice infected with *Plasmodium* can decrease. The ability of adjuvant therapy of nanoparticles leaves and fruit extracts of *Syzygium cumini* parasitemia and increase the inhibition of *Plasmodium* parasite growth so that the liver index of mice infected with *Plasmodium* can decrease.

Summary

Syzygium cumini leaf and fruit nanoparticles have increased efficacy when used as an adjuvant therapy with antimalarial chloroquine drugs. Efficacy of *Syzygium cumini* fruit nanoparticles with chloroquine is better than *Syzygium cumini* leaves in reducing % parasitemia, increasing growth barriers, increasing Parasite Clearance Time, and extending Recrudescence Time parasites.

References

Craig., Alister, G., Georges, E., Grau., Chris Janse., James W., Kazura., Milner., John W.. Barnwell., Gareth Turner., Jean Langhorne. (2012) The Role of Animal Models for Research on Severe Malaria. *PLoS Pathogens*. **8**(2):e1002401.

Dende, C., Meena, J., Nagarajan, P., Panda, A.K., Rangarajan, P.N., Padmanaban, G. (2015) Simultaneously targeting inflammatory response and parasite sequestration in brain to treat experimental cerebral malaria. *Sci Rep*. **5**:12671.

Dkhil, M.A.E. (2009) Apoptotic changes induced in mice splenic tissue due to malaria infection. *J. Microbiol Immune Infect*. **42**:13-18.

George, B.O., Okpoghono, J., Osioma, E., Aina, O.O. (2012) Changes in oxidative indices in Plasmodium berghei infected mice treated with aqueous extract of *Aframomum sceptrum*. *Frontiers in Science*. **2**(1): 6-9.

Good, M.F., Doolan, D.L. (2018) Malaria Vaccine Design : *Immunological Consideration. Immunity*. **33**(4): 555-566.

Guo, J., H. Judith., Grinberg, W., Mitchele, A.J., Barenhol, Y. and Golenser, J. (2014) Reduction of experimental cerebral malaria and its related pro inflamatory responses by the novel liposom based B methasone nano drug. *Biomed Research International*. *http//dxdoi.org/10.1155/2014/292471*.

Haroon, R. (2015) Comparative analysis of antioxidant profile of bark, leaves and seeds of *Syzygium cumini* (Indian blackberry). *International Journal of Research.Granthaalayah*. **3**(5): 11-

22.
Mathur, M. and Govind, V. (2013) Role of nanoparticles for production of smart normal drug. *Overview Indian Journal of natural Products and Resources*. **4**(4):329-338.
Maslachah, L. and Sugihartuti, R. (2018) Potency *Syzygium cumini L* as adjuvant therapy on mice model malaria. *Iraqi Journal of Veterinary Sciences*. **31**(1): 73-80.
Nguyen, T.V., Nguyen, T.T.H., Wang, S.L., Khanvo, T.P., Nguyen, A.D. (2017) Preparation of chitosan nanoparticle by TPP ionic gelation combined with spray drying and the antibacterial activity of chitosan nanoparticles and a chitosan nanoparticle amoxicillin complex. *Res Chem Intermed*., **43**:3527-3537
Varo, R., Crowley, V.M., Sitoe, A., Madrid, L., Serghides., Kain, K.C. and Bassat, Q. (2018) Adjuctive therapy for severe malaria: a review and critical appraisal. *Malaria Journal*. **17**: 47
Widyawaruyanti, A., Tumewu, L., Ilmi, H., Setyawan, D., Widiastuti, E., Dachilyati, L., Tantular, I.S. and Hafid, A.F. (2017) Antimalarial activity and survival time of *Adrographis paniculata* fraction (AS202-1) on *Plasmodium berghei* infected mice. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. **8**(1S).49-54.
Zhang, L.L., Lin, Y.M. (2009) Antioksidant tannins from *Syzygium cumini* fruit. *African Journal of Biotechnology*. **8**(10): 2301-2309.

Table I Mean of parasitemia, inhibition growth of *Plasmodium berghei* and survival time on control and treatment groups.

Groups	% Parasitemia	% Growth inhibition	% Survival time
K^-	-	-	$100.000^{d} \pm 0.000$
K^+	$7.132^{c} \pm 0.776$	$0.000^{a} \pm 0.000$	$90.000^{bcd} \pm 9.122$
K1	$2.087^{a} \pm 0..527.$	$88.815^{c} \pm 3.624$	$100.000^{d} \pm 0.000$
K2	$4.806^{b} \pm 0.376$	$43.175^{ab} \pm 1.747$	$80.950^{b} \pm 17.577$
K3	$7.724^{c} \pm 0.057$	$28.547^{ab} \pm 1.122$	$61.150^{a} \pm 34.294$
P1	$5.817^{b} \pm 1.775$	$55.201^{bc} \pm 2.082$	$85.416^{bc} \pm 12.873$
P2	$2.190^{a} \pm 0.493$	$92.724^{c} \pm 1.501$	$98.817^{d} \pm 4.099$
P3	$5.521^{b} \pm 0.181$	$55.683^{bc} \pm 2.503$	$90.317^{bcd} \pm 8.547$
P4	$1.498^{a} \pm 0.558$	$93.025^{c} \pm 0.520$	$97.233^{cd} \pm 6.462$

Note: different superscript on the same column showed the significant difference ($p<0,05$).

Table II Hb level, splenic index and liver index on control and treatment group

Groups	Hb	Splenic index	Liver index
K^-	14.750^{d}±0.957	0.4600^{a}± 0.050	3.5850^{a}± 0.523
K^+	3.925^{ab}±1.027	2.8700^{c}± 0.341	9.550^{d}± 0.744
K1	14.400^{d}± 0.141	1.2600^{ab}± 0.327	4.4100^{a}± 2.022
K2	6.6500^{b} ± 2.533	1.3473^{ab}± 0.834	6.1800^{b}± 0.579
K3	3.1000^{a}± 0.081	1.1525^{ab}± 0.127	7.0950^{bc}± 1.544
P1	6.1500^{ab}± 3.307	1.6910^{b}± 0.368	6.5125^{b}± 0.476
P2	12.400^{cd}± 1.630	1.2950^{ab}± 0.193	6.3300^{b}± 0.498
P3	4.9750^{ab}± 3.094	1.8175^{b}± 0.440	8.4525^{cd}± 1.665
P4	11.2500^{c}± 2.409	1.0425^{ab}± 0.316	6.1850^{b}± 0.920

Note: different superscript on the same column showed the significant difference ($P<0.05$).

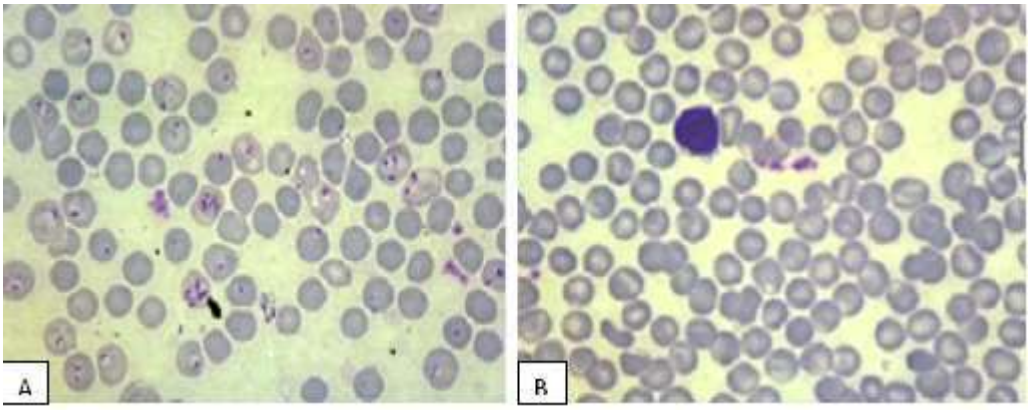

Figure 2. Thin blood smear on 5th post infection after given treatment for 4 days on the control (A) and combination treatment groups (B). Stain used x100.

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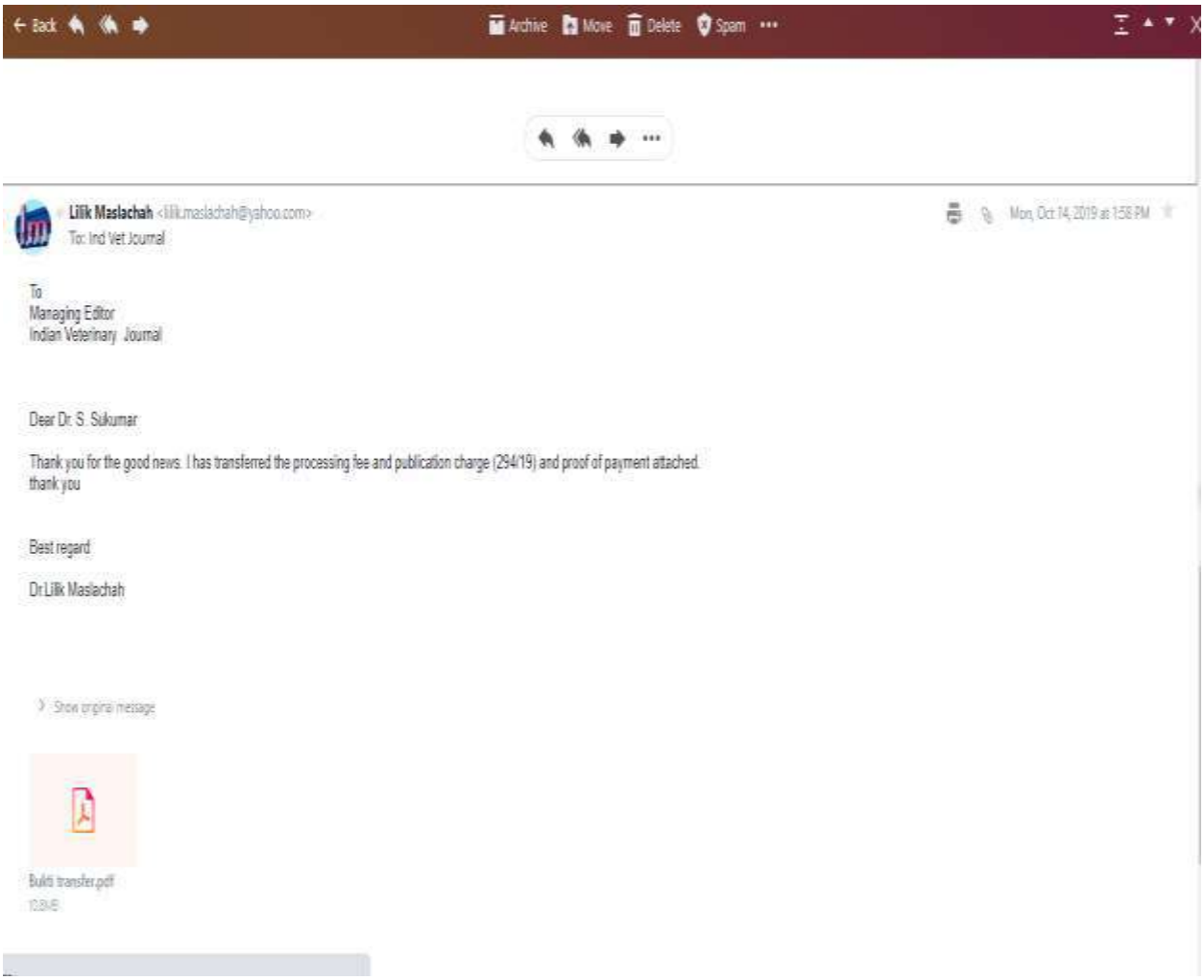
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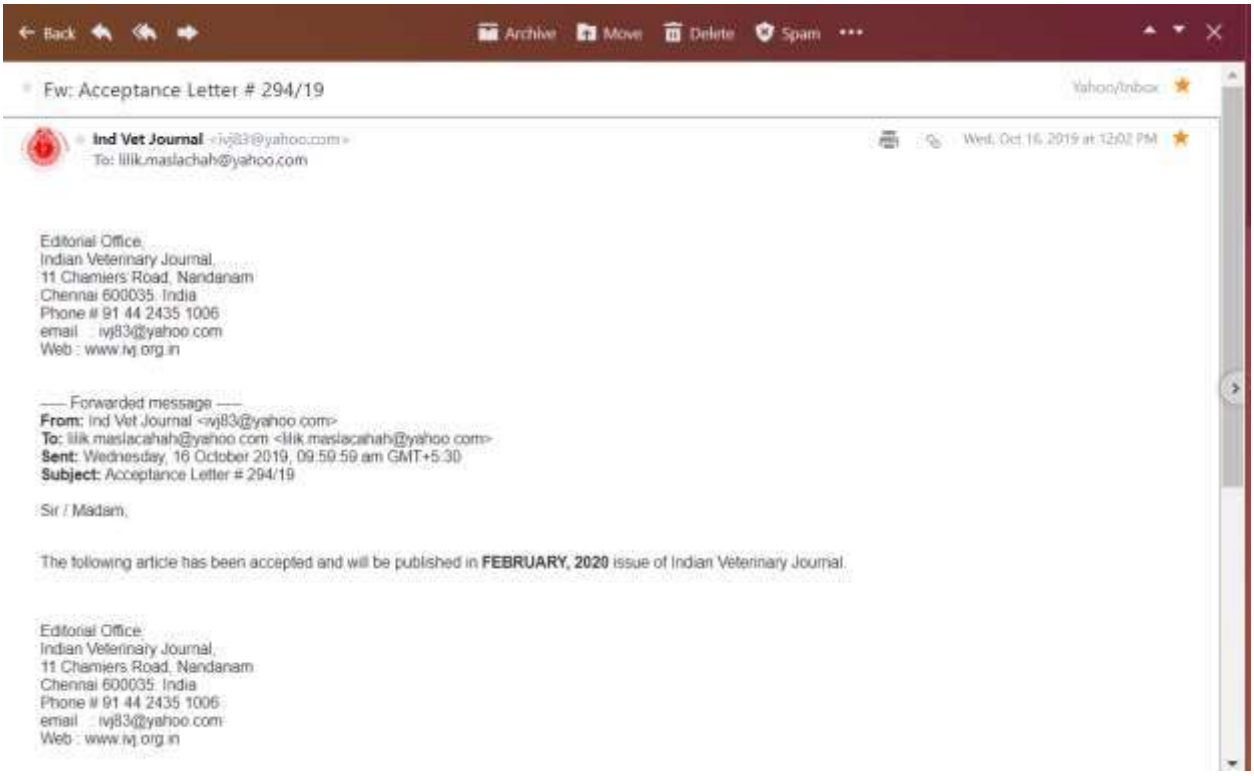

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