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## AUTHOR INDEX

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

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**Abstract**

The aim was determine the efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles to mice infected with *Plasmodium berghei* at $1x10^5$ of *Plasmodium berghei.* Treatment was given 24 hours post infection for 4 days. Observed from the 5th day to 12th day post infection. The results of per cent parasitemia, growth inhibition, Parasite Clearance Time, and Recrudescence Time of parasites. *Syzygium cumini* fruit nanoparticles with chloroquine were better than *Syzygium cumini* leaf nanoparticles with chloroquine, whereas the Survival time, Hb content, splenic index and liver index were same. Efficacy of *Syzygium cumini* leaf and fruit extracts nanoparticles increased 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. Currently there has been a decrease in efficacy of antimalarial drugs and many parasites were resistant to antimalarial drugs. (Good and Doolan, 2018).

The disadvantages of the natural ingredients are low stability, solubility and absorption, which reduces bioavailability and efficacy. The development of new drugs with nanoparticle technology will be able to increase, stability, solubility and absorption of the drugs (Mathur and Govind, 2013)

*Syzygium cumini* has strong radical and antioxidant scavenging activity (Zhang and Lin, 2009). *Syzygium cumini* leaves as a therapeutic adjuvant have inhibitory effects on *plasmodium* better than their bark (Maslachah and Sugihartuti., 2018). The *Syzygium cumini L* leaf and fruit extract nanoparticles improve the efficacy as adjuvant therapy in *Plasmodium berghei* rodent malaria as an *in vivo* model in mice (Craig *et al.*, 2012).

## Materials and Methods

The leaves and fruit of *Syzygium cumini* were air dried, and 500 grams was 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), and 60 ml of 0.2% chitosan solution. The mixture was sonicated for 60 minutes in frequency 20 kHz until the suspension is formed and freeze dryed (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

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**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 ($p<0.05$).

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, blood smear were 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 Clearance 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 harvested after sacrifice to examine the splenic index and liver index.

On the 12th day post infection, the mice were anesthetized with ketamine (sigma), thoracotomy was carried, blood was taken from the heart (1 ml) 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 at 5%. Per cent level parasitemia, % inhibition of growth, Parasite Clearance Time (PCT) and Recrudescence Time (RT) were analyzed by linear regression analysis.

## Results and Discussion

The per cent parasitemia, inhibition of *Plasmodium berghei* growth and survival time are presented in Table I and Fig I. Adjuvant therapy with the combination of *Syzygium cumini* leaf and fruit extracts with the chloroquine (P2 and P4) was more effective. Malarial immunopatogenesis is very complex so that single pathway targets are not sufficient to reduce mortality,

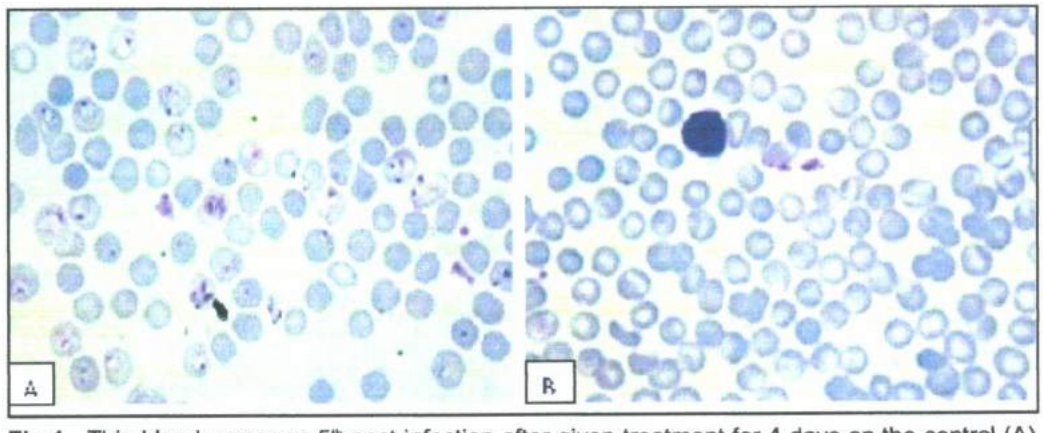


**Fig 1.** Thin blood smear on 5th post infection after given treatment for 4 days on the control (A) and combination treatment groups (B). (x100) Giensa stained.

**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).

the adjuvant therapy was able to reach complex target pathways with one intervention (Varo *et al.*, 2018). Adjuvant therapy can increase the average survival time and reduce parasitic growth and inhibit the growth of parasites. 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 an adjuvant with chloroquine was able to inhibit the growth of *Plasmodium berghei* in mice compared to *Syzygium cumini* stem bark extract (Maslachah and Sugihartuti, *loc cit*). The higher ability of *Syzygium cumini* leaf and fruit extracts with chloroquine in reducing the percentage of parasitemia, even though the activity of nanoparticles is short lined their combination with chloroquine can extend their antimalarial activity up to 150 hr or 6 days there by effectively eliminating the *Plasmodium* completely (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 by their 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 and other drugs can improve the efficacy and reduce the complications of malaria (Varo *et al.*, *loc cit*). Adjuvant antimalarial drug combination therapy with immunomudulation glucocorticosteroid betamethasone hemisuccinat encapsulted in liposomes given after neurological symptoms can improve survival time and improve clinical symptoms were reported by (Guo *et al.*, 2014) along with curcumin as adjument can improve survival time and reduce parasitemia (Dende *et al.*, 2015).

Measurement of hemoglobin (Hb), splenic index, and liver index is presented in Table II. The administration of *Syzygium cumini* fruit nanoparticles as adjuvant therapy with chloroquine (P4) had the same leaf nanoparticles effect within the combination of leaf and fruit extract with anti malarial drugs were more effective in reducing the splenic index and the liver index in the treatment groups.

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

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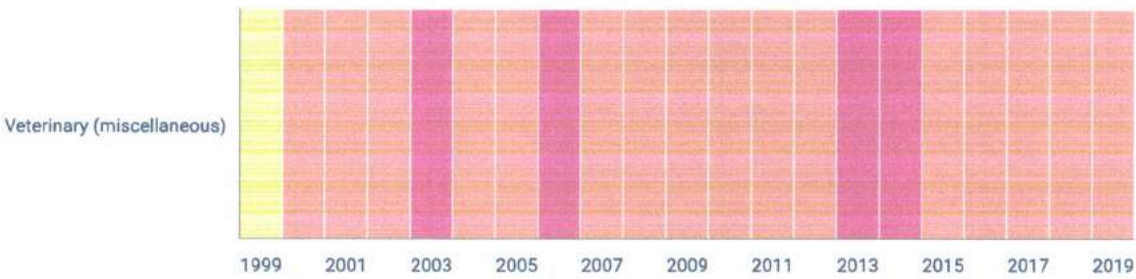


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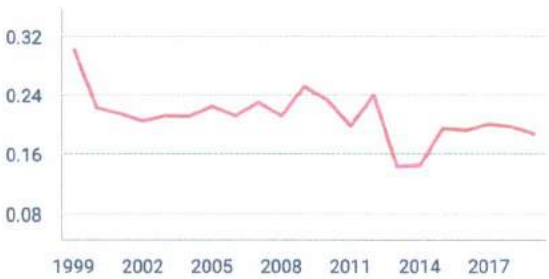


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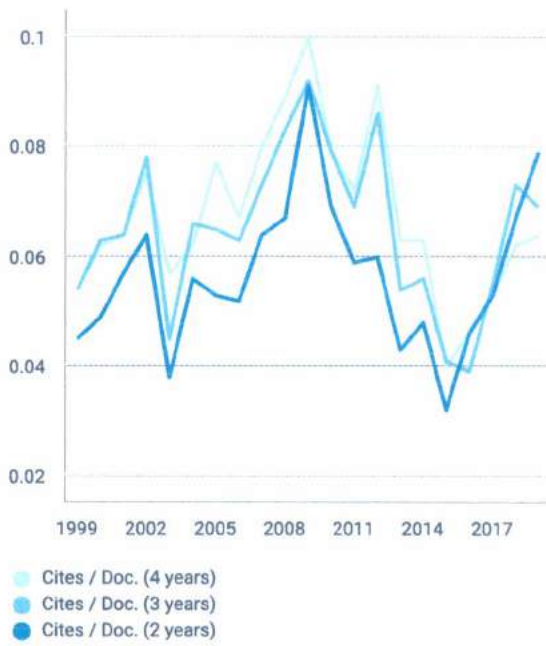


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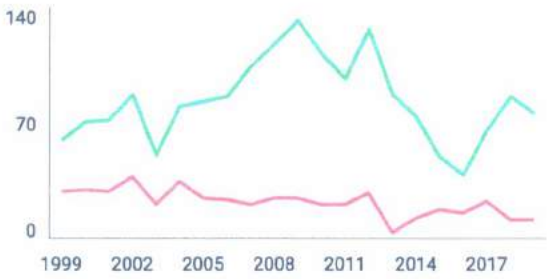


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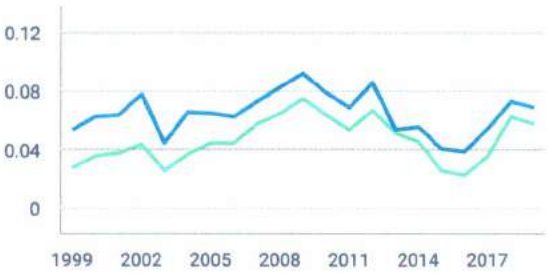


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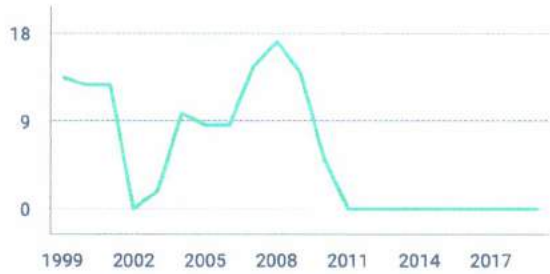


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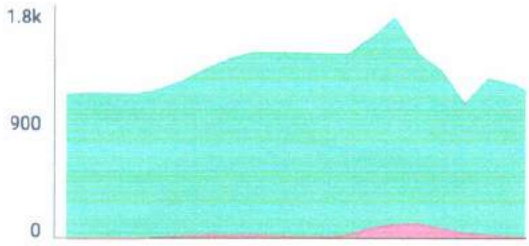


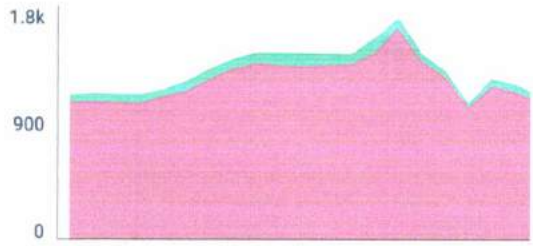



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