In vivo antimalarial activity of propranolol

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In vivo anti-malarial activity of propranolol against experimental *Plasmodium berghei* ANKA infection in mice

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Abstract:

Background: Malaria is a mosquito-borne infectious disease caused by *Plasmodium* spp, which is widespread in tropical and subtropical regions of the world. The objective of this study is to evaluate *in vivo* antimalarial activity of propranolol against experimental *Plasmodium berghei* ANKA (PbA) infection in a mouse model.
Methods: A total of 36 mice weighing between 15 to 18g were randomly divided into six groups of six mice each. Mice in the first group (SAL) were non-infected with *P. berghei* but received normal saline (control), second group (PbA) were mice infected without treatment (control), third group (PRL) were non-infected mice treated with propranolol at the dose of 7.5 mg/kg/bid, fourth group (PbA+PRL) were mice infected and treated with same dose of propranolol, fifth group (QUN) were non-infected mice treated with quinine at a dose of 20 mg/kg stat, then 10 mg/kg bid, and sixth group (PbA+QUN) were infected mice treated with quinine. Parasitaemia, physiological conditions (cognitive function, temperature) and lethality of infected mice were monitored over 7-day period to assess the antimalarial activity of propranolol and quinine. The Y-maze paradigm was used to assess cognitive impairment induced by PbA infection. The effects of propranolol on malaria indices and cognitive impairment were compared with that of quinine and the control using T-test statistical method.
Results: Mortality of mice at day 7 in the infected group without treatment (PbA) was 100% (6/6) while mortality was 50% (3/6) in infected group treated with propranolol (PbA+PRL) and 33.3% (2/6) in infected group treated with quinine (PbA+QUN) (OR=2.000, p=1.000). No mortality was recorded in any of the three groups of uninfected mice. Propranolol reduced parasitaemia to a trough level of 1.40±0.07 three days after treatment, comparable to trough level of 1.39±0.0633 by quinine but did not reverse PbA-induced hypothermia, which quinine did.
Conclusion: Propranolol demonstrated *in vivo* antimalarial activity against experimental PbA infection in mice comparable to that of quinine.

Keywords: malaria, propranolol, quinine, *Plasmodium,* cerebral malaria

Activité antipaludique *in vivo* du propranolol contre l'infection expérimentale par *Plasmodium berghei* ANKA chez la souris

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Abstrait:

Contexte: Le paludisme est une maladie infectieuse transmise par les moustiques causée par *Plasmodium* spp, qui est répandue dans les régions tropicales et subtropicales du monde. L'objectif de cette étude est d'évaluer l'activité

antipaludique *in vivo* du propranolol contre une infection expérimentale à *Plasmodium berghei* ANKA (PbA) dans un modèle murin.
Méthodes: Un total de 36 souris pesant entre 15 et 18 g ont été réparties au hasard en six groupes de six souris chacun. Les souris du premier groupe (SAL) n'étaient pas infectées par *P. berghei* mais ont reçu une solution saline normale (contrôle), le deuxième groupe (PbA) était des souris infectées sans traitement (contrôle), le troisième groupe (PRL) était des souris non infectées traitées par propranolol à la dose de 7,5mg/kg/bid, le quatrième groupe (PbA+PRL) étaient des souris infectées et traitées avec la même dose de propranolol, le cinquième groupe (QUN) étaient des souris non infectées traitées avec de la quinine à une dose de 20mg/kg stat, puis 10mg/kg bid et le sixième groupe (PbA+QUN) étaient des souris infectées traitées avec de la quinine. La parasitémie, les conditions physiologiques (fonction cognitive, température) et la létalité des souris infectées ont été surveillées sur une période de 7 jours pour évaluer l'activité antipaludique du propranolol et de la quinine. Le paradigme du labyrinthe en Y a été utilisé pour évaluer les troubles cognitifs induits par l'infection au PbA. Les effets du propranolol sur les indices du paludisme et les troubles cognitifs ont été comparés à ceux de la quinine et du témoin à l'aide de la méthode statistique du test T.
Résultats: La mortalité des souris au jour 7 dans le groupe infecté sans traitement (PbA) était de 100% (6/6) tandis que la mortalité était de 50% (3/6) dans le groupe infecté traité avec du propranolol (PbA+PRL) et 33,3% (2/6) dans le groupe infecté traité par la quinine (PbA+QUN) (OR=2.000, *p*=1.000). Aucune mortalité n'a été enregistrée dans aucun des trois groupes de souris non infectées. Le propranolol a réduit la parasitémie à un niveau minimum de 1,40±0,07 trois jours après le traitement, comparable au niveau minimum de 1,39±0,0633 de la quinine, mais n'a pas inversé l'hypothermie induite par le PbA, ce que la quinine a fait.
Conclusion: le propranolol a démontré une activité antipaludique *in vivo* contre l'infection expérimentale au PbA chez la souris comparable à celle de la quinine.

Mots-clés: paludisme, propranolol, quinine, *Plasmodium*, paludisme cérébral

Introduction:

Malaria is a parasitic disease caused by protozoan parasites of the genus *Plasmodium*, which is transmitted by female anopheles' mosquitoes. Only five plasmodia species develop in humans, these are *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium vivax, Plasmodium knowlesi* and *Plasmodium malariae*. Of these, only *Plasmodium vivax* and *Plasmodium ovale* have persistent liver forms that may lead to relapses after the initial blood infection. However, *P. falciparum* malaria is the main species of public health importance as a result of its lethality and virulence (1).

Cerebral malaria is the most severe neurologic complication of infection caused by *P. falciparum* (2) and collectively involves the clinical manifestations of malaria that induce changes in mental status and coma. It presents as acute widespread infection of the brain accompanied by fever (3). Even though this type of malaria is most common in children living in sub-Saharan Africa, it should be considered in any patient with impaired consciousness that has recently travelled to a malaria endemic area. Cerebral malaria is a major cause of acute non-traumatic encephalopathy in tropical countries with high mortality, and over the past two decades, the extent of persistent neuro- cognitive deficits after recovery has become very apparent (4).

Cerebral malaria is fatal within days if left untreated, immediate treatment is therefore crucial (5). Because natural immunity to malaria is not fully understood and thus cannot yet be artificially imitated by drugs, control and prevention strategies are significant, two of which include anti-malarial chemotherapy and adjunctive measures. Chemotherapy for cerebral malaria now primarily involves the use of artesunate (6), and if not available, artemether or quinine can be given as alternatives (7). However, in most health facilities, quinine still finds great use especially because it is still safe in the first trimester of pregnancy. Adjunctive measures for cerebral malaria include the use of anti-pyretics such as paracetamol and anti-convulsants such as the benzodiazepines.

The mature human erythrocyte is a terminally differentiated cell that lacks sub-cellular organelles such as nucleus or secretory structures, de-novo protein or lipid biosynthesis, and does not endocytose its plasma membrane (8). Parasite entry into erythrocytes is a complex, dynamic process, but it has been shown that Gs peptide signaling is involved and β-adrenergic blockers inhibit the signaling process *in vitro* (9). It has been documented that propranolol, an antagonist of G protein-coupled β-adrenergic receptors, dampens Gs activity in erythrocytes (10). This prevents the invasion and growth of the parasite in the erythrocyte and hence easy clearance by the immune system.

Meanwhile, cerebral malaria has been attributed to be a major cause of cognitive impairment in sub-Saharan Africa (11). This impairment has been postulated to be due to engorgement of brain microvasculature as a result of parasites movement into the brain (12). *Plasmodium berghei* ANKA (PbA) infection mimics human cerebral malaria and this strain of plasmodium is widely used in the study of

diffuse encephalopathy arising from cerebral malaria (13,14).

The objectives of this study are to assess *in vivo* anti-malarial activity of propranolol by monitoring end stage symptoms (hypothermia, high parasitaemia and lethality) and to eva- luate the effects of propranolol on cognitive impairment, in a mouse model experimental PbA infection.

Materials and method:

Experimental animal study

Locally bred apparently healthy albino mice of both sexes, 6 to 8 weeks old, with weights ranging between 15 g to 18 g were used. The animals were obtained from and initially housed in a room in the animal house of the Department of Pharmacology, Faculty of Pharmacy of the Obafemi Awolowo University, Ile-Ife. The animals were housed in plastic and metal cages that were regularly cleaned and beddings changed. The animals were main- tained on regular commercial animal feed diet and drinking water provided *ad libitum*.

Experimental drugs

The experimental drugs used were Quinine hydrochloride (Laborate Pharmaceuti- cals, India) and Propranolol (BDH chemicals), which were administered intraperitoneally.

Preparation of PbA parasite inoculum

The rodent malaria parasite used was a quinine sensitive strain, *Plasmodium berghei* ANKA (PbA). The parasite was obtained from the Institute of Medical Research and Training, University College Hospital (UCH), Ibadan, and is used as a model to mimic *Plasmodium falci- parum* that causes cerebral malaria in human (13,14). A standard inoculum of 10^6 parasitized erythrocytes was prepared by dilution of blood harvested from a donor mouse with normal saline by serial persaging.

Experimental study protocol

The animals (total of 36 mice) were allowed to acclimatize to their new environment before randomly dividing them into six groups of six mice each. In each group, the mice were weighed and marked for identification. The first group (SAL) comprised non-infected (control) mice which received normal saline, the second group (PbA) comprised infected (control) mice without treatment, the third group (PRL) com- prised non-infected mice treated with propra- nolol at the dose of 7.5 mg/kg/bid (10), the fourth group (PbA+PRL) comprised infected mice treated with propranolol, the fifth group (QUN) comprised non-infected mice treated

with quinine at a dose of 20 mg/kg stat, then 10 mg/kg bid (15), and the sixth group (PbA+QUN) comprised infected mice treated with the same dose of quinine.

The experimental study was conducted over a period of 7 days while cognitive functions of the mice were assessed on day 8. Mice were infected on day 0 of the study by intraperitoneal administration of the standard inoculum of the parasitized erythrocytes. Propranolol and quinine were administered starting from day 5 to day 7, as cerebral malaria is known to develop on day 5 of PbA infection in mice (14,16). The non-infected mice were also treated with propanol and quinine to assess their effects on cognitive functions of the mice in the absence of parasitaemia.

Assessment of PbA induced lethality in mice and effects of propranolol and quinine

The lethality of PbA infection was dete- rmined by the number of infected mice that died in the untreated (PbA) and those treated with propranolol (PbA+PRL) and quinine (PbA +QUN).

Assessment of PbA induced hypothermia and effects of propranolol and quinine

Hypothermia, which is infection induced observation in mice seen as an end stage symptom (16), was determined by measuring rectal temperature of mice with an electronic thermometer daily for each group during the period of the study

Assessment of PbA induced cognitive impair- ment and effects of propranolol & quinine

The Y-maze, a measure of short-term memory (17), was used to assess cognitive function in the mice. Each animal was dropped in one of the arms of the maze. The movement of the mouse into other arms was then observed and recorded. The assessment was done for six (6) minutes each. The Y-maze experiment was done for each group before infection and after experimental treatment.

Assessment of PbA parasitaemia and effects of propranolol and quinine

Parasitaemia was obtained by pre- paring slides from thin blood smears made from the tail of the mice, fixing with methanol and staining with Giemsa. Slides for the parasites were prepared for the three groups of mice infected with PbA. The stained slide of the blood smear was mounted on a binocular microscope and a drop of immersion oil applied to the slide (15). The 100x objective lens (with total magnification of 1000 x) of the micro-

scope was used to examine a slide field for parasitized and non-parasitized red blood cells. In each field, the number of parasitized red blood cells was counted, and the total number of red blood cells was determined. The slides were prepared daily for each group during the period of the study.

Statistical analysis
The percentage parasitaemia in each field was calculated as; % Parasitaemia = number of parasitized cells/total number of cells (x100). For each slide, 7 fields were counted and the average of the 7 fields was calculated as the average % Parasitaemia (total % parasitaemia/7). The T-test was used to compare percentage parasitaemia between the infected groups while Fisher Exact was used to compare differences in mortality rate

between groups of treated mice. A *p* value less than 0.05 was considered significant.

Results:

Effects of propranolol and quinine on lethality of infected mice
Table 1 shows the lethality of PbA infection on the mice in treated and untreated groups. Mortality of mice at day 7 in the infected group without treatment (PbA) was 100% (6/6) while mortality was 50% (3/6) in the infected group treated with propranolol (PbA+PRL) and 33.3% (2/6) in the infected group treated with quinine (PbA+QUN). There was no mortality of mice in uninfected group that received saline (SAL), propranolol (PRL) and quinine (QUN) throughout study period.

Table 1: Number of surviving animals in the uninfected and infected (with and without treatment) groups of mice

Day	No of surviving mice in groups of mice					
	PbA	SAL	PbA+PRL	PRL	PbA+QUN	QUN
Day 3	6	6	6	6	6	6
Day 4	6	6	6	6	6	6
Day 5	3	6	5	6	5	6
Day 6	1	6	4	6	4	6
Day 7	0	6	3	6	4	6

PBA = *Plasmodium berghei* ANKA; SAL = Normal Saline; PRL = Propranolol; QUN = Quinine; PBA+PRL = *Plasmodium berghei* ANKA and Propranolol; PBA+QUN = *Plasmodium berghei* ANKA and Quinine.

Table 2: The average percentage parasitaemia in the three experimentally infected groups of mice with and without treatment

DAY	% Parasitaemia PbA	% Parasitaemia PbA+PRL	% Parasitaemia PbA+QUN
Day 3	6.53±0.3971	6.67±0.1512	6.80±0.1219
Day 4	7.91±0.0734	8.23±0.0618	7.90±0.1247
Day 5	9.37±0.1770	4.53±0.2072**	4.12±0.0872**
Day 6	11.78±0.0000	2.78±0.2148**	3.06±0.1777**
Day 7	[+]0	1.40±0.0651**	1.39±0.0633**

*Significantly different from control at *p* < 0.05; **Significantly different from control at *p* <0.01
PBA – *Plasmodium berghei* ANKA, PBA+PRL – *Plasmodium berghei* ANKA + Propranolol, PBA+QUN - *Plasmodium berghei* ANKA + Quinine
The average parasitaemia was calculated for the surviving mice on day 5 upward, the parasitaemia for mouse in PbA group on day 6 was the parasite count of the only one surviving mouse in the group (Table 1). + = no surviving mouse on day 7

Parasitaemia in infected mice

Table 2 shows the average percentage parasitaemia in the three experimentally infected groups of mice with and without treatment. Infected mice without treatment (PbA) showed increasing parasitaemia, with mortality (50%) occurring on day 5 (average parasitaemia of 9.37±0.1770) and 100% mortality on day 7. Infected mice treated with propranolol (PbA+PRL) on day 5 showed a statistically significant decrease in average parasitaemia of mice on day 5 (4.53±0.2072) from that of day 4 (8.23±0.0618) ($p<0.05$), with mortality of 16.7% (1/6) and the lowest parasitaemia was recorded on day 7 (1.40±0.0651), with mortality of 50% (3/6).

Infected mice treated with quinine (PbA+QUN) also showed statistically significant decrease in average parasitaemia on day 5 (4.12±0.0872) from average parasitaemia on day 4 (7.90±0.1247), with mortality of 16.7% (1/6), and the lowest parasitaemia was recorded on day 7 (1.39±0.0633), with mortality of 33.3% (2/6).

Rectal temperature in infected and non-infected mice

Mice infected with PbA showed reduction in rectal temperature as the infection progressed compared to the uninfected groups (SAL, PRL and QUN) in which the temperature did not show any significant variation throughout the study period ($p>0.05$) (Table 3). Infected mice treated with propranolol showed an increase in rectal temperature from the day of treatment as the reduction in rectal temperature caused by PbA infection from day 3 was reversed by day 5 propranolol treatment on days 6 and 7 of the study.

Similarly, infected mice treated with quinine showed an increase in rectal temperature from the day of treatment as the reduction in rectal temperature caused by PbA infection from day 3 was reversed by quinine treatment on days 5, 6 and 7 of the study.

Table 3: Mean of rectal temperature of uninfected and infected (with and without treatment) groups of mice

Day	Mean temperature in groups of mice					
	SAL (℃)	PBA (℃)	PRL (℃)	PBA+PRL(℃)	QUN (℃)	PBA+QUN (℃)
Day 0	36.51±0.1428	36.73±0.0799	36.80±0.2039	37.17±0.2348	36.73±0.0732	36.50±0.3678
Day 1	36.69±0.1378	36.75±0.1675	36.99±0.2651	36.79±0.4344	36.90±0.1188	36.29±0.3475
Day 2	36.90±0.1152	36.94±0.0221	36.55±0.1886	35.26±0.2263**	36.58±0.1755	35.42±0.0641**
Day 3	36.70±0.2563	35.23±0.1030**	36.98±0.1575	35.05±0.1258**	36.59±0.1868	34.93±0.0771**
Day 4	36.73±0.1145	34.43±0.0844**	36.56±0.1121	33.95±0.1367**	36.59±0.1670	33.40±0.0949**
Day 5	36.54±0.1485	32.40±0.2000**	36.72±0.0760	34.48±0.1190**	36.40±0.0949	34.49±0.0886
Day 6	36.70±0.1057	32.00±0.0000**	36.57±0.1301	35.12±0.2252	36.69±0.9610	35.32±0.2278
Day 7	36.57±0.1605	+0	36.62±1.0955	36.00±0.1528	36.69±0.1177	36.08±0.1652

**Significantly different from control at $p <0.01$; *Significantly different from control at $p < 0.05$; (n=6, mean ± SEM); SAL – Normal Saline, PBA – *Plasmodium berghei* ANKA, PRL – Propranolol, PBA+PRL – *Plasmodium berghei* ANKA and Propranolol, QUN – Quinine, PBA+QUN - *Plasmodium berghei* ANKA and Quinine. Average rectal temperature was calculated for the surviving mice on day 5 upward, the rectal temperature for mouse in PbA group on day 6 was the rectal temperature of the only one surviving mouse in the group (Table 1). + = no surviving mouse on day 7

Table 4: Cognitive functions of uninfected and infected (treated and non-treated) groups of mice

Day	SAL	PbA	PRL	PbA+ PRL	QUN	PbA +QUN
Day 0	61.67±8.6923	74.39±4.6644	56.38±4.4795	73.68±8.3500	64.43±1.8524	74.39±5.8723
Day 8	74.90±5.242	+0	82.97±7.5013	47.62±9.9143*	67.03±5.2421	57.43±9.7463

*Significantly different from control at p < 0.05; (n = 6, mean ± SEM); SAL – Normal Saline, PBA – *Plasmodium berghei* ANKA; PRL – Propranolol, PBA+PRL – *Plasmodium berghei* ANKA and Propranolol, QUN – Quinine, PBA+QUN - *Plasmodium berghei* ANKA and Quinine. + = no surviving mouse on day 7

Effects of propranolol and quinine on cognitive functions of infected and uninfected mice

The cognitive functions of PbA infected untreated mice could not be assessed as 100% mortality had occurred on day 7 (Table 4). Expectedly, there was no significant change in the cognitive functions of the un-infected mice that received normal saline. Propranolol (PRL) and quinine (QUN) had no effect on the cognitive function of un-infected mice as there was no significant difference between the cognitive functions before and after treatment.

However, there was significant decrease ($p<0.05$) in the cognitive functions in infected mice treated with propranolol (PbA+ PRL), indicating that propranolol did not reverse the impaired cognitive function caused by PbA infection. In contrast, there was no significant difference ($p>0.05$) in the cognitive function of infected mice treated with quinine, indicating that quinine protected the mice from impaired cognitive function caused by PbA infection.

Discussion:

In this study, the lethality of *P. berghei* ANKA (PbA) infections in infected untreated mice from 50% on day 5, 83.3% on day 6 and 100% on day 7 confirms this parasite to be virulent. This parasite is a laboratory model that is often used to mimic *P. falciparum* infection which causes cerebral malaria in human (13,14). Lethality can be used as a measure of anti-malarial activity of a drug. In this study, propranolol reduced mortality in infected mice by 50% on day 7 implying that although propranolol is effective, its use for treatment of cerebral malaria should be initiated as soon as possible (5). In comparison, quinine reduced mortality of infected mice by 66.7% on day 7 which confirms the established antimalarial property of quinine, especially against cerebral malaria (7,15). Propranolol reduced the increasing parasitaemia in infected mice when treated was initiated on day 5 of the study, which further confirms its anti-malarial property, and this was comparable to the antimalarial property of quinine (15).

The use of rectal temperature as a parameter is borne out of the fact that one of the end stage manifestations of cerebral malaria is hypothermia (18). In the group infected with the parasite, there was a marked significant decrease in the rectal temperature which confirms the establishment of PbA infection and induced hypothermia from cerebral affectation. Our study demonstrated that propranolol reversed the hypothermia induced by PbA infection with recovery of some of the mice, and this action was comparable to that of quinine on the infected mice as previously established (15).

The Y-maze is generally acceptable as a model to assess cognitive function (17). Although cognitive function of the mice in the PbA infected group could not be assessed because there was 100% mortality of the mice on day 7, impaired cognitive function, which we believed was caused by the PbA infection in the mice as previously reported (11,12), was not reversed by propranolol treatment. This was demonstrated by significant reduction in the cognitive function of the infected mice inspite of the propranolol treatment. Propanol and quinine also did not show any effect on cognitive functions of uninfected animals which may imply their safety. However, quinine appears to protect the infected mice from impaired cognitive function as the cognitive function of the PbA infected mice was not significantly reduced upon treatment with quinine. This protective quinine phenomena has similarly been described for *P. falciparum* cerebral malaria in humans (11).

Conclusion:

This study demonstrated anti-malarial property of propranolol in an *in vivo* mice

experiment with comparable antimalarial property to that of quinine, a widely used anti-malarial drug.

References:

1. Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y., and Hay, S. I. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 2005; 434 (7030): 214–217.
2. Newton, C. Severe Falciparum Malaria in Children Current Understanding of Pathophysiology and Supportive Treatment. Pharmacol Ther. 1998; 79 (1): 1–53.
3. MacPherson, G. G., Warrell, M. J., White, N. J., and Looareesuwan, S. Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. Am J Pathol. 1985; 119 (3): 385.
4. Idro, R., Jenkins, N. E., and Newton, C. R. J. Pathogenesis, clinical features, and neurological outcome of cerebral malaria. Lancet Neurol. 2005; 4 (12): 827-840.
5. Reyburn, H., Mbatia, R., Drakeley, C., et al. Association of transmission intensity and age with clinical manifestations and case fatality of severe *Plasmodium falciparum* malaria. 2005; 293 (12): 1461-1470.
6. World Health Organization. A practical handbook. Management of severe malaria. Geneva: World Health Organization; 2012.
7. Stauffer, W., and Fischer, P. R. Diagnosis and Treatment of Malaria in Children. Clin Infect Dis 2003; 37 (10): 1340–1348.
8. Chasis, J. A., and Schrier, S. L. Membrane Deformability and the Capacity for Shape Change in the Erythrocyte. Blood. 1989; 74: 2562-2568.
9. Miller, L. H., McAuliffe, F. M., and Johnson, J. G. Invasion of erythrocytes by malaria merozoites. Prog Clin Biol Res. 1979; 30: 497–502.
10. Harrison, T., Samuel, B. U., Akompong, T., Hamm, H., Mohandas, N., Lomasney, J. W., and Haldar, K. Erythrocyte G protein-coupled receptor signaling in malarial infection. Science. 2003; 301 (5640): 1734-1736
11. Boivin, M. J., Bangirana, P., Byarugaba, J., Opoka, R. O., Idro, R., Jurek, A. M. and John, C. C. Cognitive impairment after cerebral malaria in children: A prospective study. Pediatrics 2007; 119 (2): e360-e366.
12. Holding, P. A., Stevenson, J., Peshu, N., and Marsh, K. Cognitive sequelae of severe malaria with impaired consciousness. Trans R Soc Trop Med Hyg. 1999; 93 (5): 529–534.
13. Peters, W., and Robinson, B. L. The chemotherapy of rodent malaria XXXV. Further studies on the retardation of drug resistance by the use of a triple combination of mefloquine, pyrimethamine and sulfadoxine in mice infected with *P. berghei* and "*P. berghei* NS." Ann Trop Med Parasitol. 1984; 78 (5): 459–466.
14. Clark, I. A., Ilschner, S., MacMicking, J. D., and Cowden, W. B. TNF and *Plasmodium berghei* ANKA-induced cerebral malaria. Immunol Lett. 1990; 25 (1–3): 195–198.
15. Pasvol, G., Newton, C. R., Winstanley, P. A., et al. Quinine treatment of severe falciparum malaria in African children: A randomized comparison of three regimens. Am J Trop Med Hyg. 1991; 45 (6): 702–713.
16. Adepiti, A. O., Elujoba, A. A., and Bolaji, O. O. *In vivo* antimalarial evaluation of MAMA decoction on *Plasmodium berghei* in mice. Parasitol Res. 2014; 113 (2):505–511.
17. Prieur, E., and Jadavji, N. Assessing Spatial Working Memory Using the Spontaneous Alternation Y-maze Test in Aged Male Mice. Bio-Protocol. 2019; 9 (3): 1-10.
18. McGugan, E. A. Hyperpyrexia in the emergency department. Emerg Med Australas. 2001; 13 (1): 116–120