

Administration Of High Dose Of Promethazine And Artemether-Lumefantrine Does Not Reduce Parasitaemia And Body Weight In Mice Infected With *Plasmodium Berghei Berghei*

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Abstract

Introduction: Malaria is Africa's second leading cause of death from infectious diseases after HIV/AIDS. It is a major public health problem in Nigeria.

Objective: This study evaluated the effects of co-administration of promethazine (an antiemetic) and Artemether-Lumefantrine (antimalarial) on mean parasitaemia and body weight in mice infected with *Plasmodium berghei*.

Method: The mice were grouped into six groups of six mice each and inoculated with *Plasmodium berghei*. Parasitaemia was noticeable 72 hours post-inoculation, after which artemether-lumefantrine was co-administered orally and for five days. The dose administered was Promethazine 25mg/kg and Artemether-Lumefantrine 10/60mg/kg across all the groups.

Results: Promethazine did not show any significant effect on mean parasitaemia. The average percentage inhibition obtained with Promethazine 25mg/kg (23.32%) was lower than that of the reference standard drug, Artemether-Lumefantrine 10/60mg/kg (85.56%), which was significantly different at level 0.05 ($P \leq 0.05$). The combination of promethazine 25mg/kg and Artemether-Lumefantrine (10/60mg/kg) gave a higher percentage inhibition (69.20%) than promethazine (50mg/kg) and Artemether-Lumefantrine (10/60mg/kg), (41.93%), while Artemether-Lumefantrine (85.56%) alone gave a higher inhibition than the drugs co-administered. Administration of A/L reduced parasitaemia by 85.56%, A/L-promethazine (25mg) afforded a 69.20% inhibition, while A/L-promethazine (50mg) caused a 41.93% inhibition.

Conclusion: The study showed that co-administration of Promethazine and Artemether-lumefantrine possesses no advantage over Artemether-Lumefantrine alone.

Keywords: Artemether-lumefantrine, promethazine, co-administration, Parasitaemia, Inhibition.

Introduction

Malaria is a life-threatening disease caused by protozoan parasites that are transmitted to people via the bites of infected mosquitoes. The disease is caused by *Plasmodium* transmitted to humans from infected female *Anopheles* mosquitoes ^[1]. Malaria is an acute febrile illness in which symptoms appear seven days or more (usually 10–15 days) after the infective mosquito bite in a non-immune individual. The first symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognise in malaria. If not treated within 24 hours, *Plasmodium falciparum* malaria can progress to severe illness often leading to death. Children with severe malaria frequently develop one or more symptoms: severe anaemia, respiratory distress with metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent. Malaria detection depends mainly on microscopy and rapid diagnostic tests ^[19]. However, it suffers poor sensitivity in scenarios of low-level parasitemia of below 10 parasites/ μ l ^[11]. Polymerase chain reaction (PCR) based assays have been developed to detect and identify malaria parasites, which have proven to be more specific and sensitive than conventional microscopy. It has detected as few as one parasite/ μ L of blood and is also more expensive ^[13].

Today, the treatment of choice in most malaria cases is Artemisinin-based Combination Therapy (ACT). The key to artemisinin's activity is believed to lie within the presence of its peroxide

bridge^[11]. Artemisinin derivatives, such as artesunate, artemether, and dihydroartemisinin, are short-acting antimalarial agents that kill parasites more rapidly than previous-generation antimalarials. They can achieve 10,000-fold reductions in parasite burden per asexual cycle. As a result, fever clearance time is reduced to 32 hours compared to days. While highly effective, the concern is that parasites resistant to artemisinin will develop and spread if used alone, making the disease potentially untreatable. Therefore, in 2006, the World Health Organisation (WHO) produced new treatment guidelines stipulating that the treatment of choice for uncomplicated *P. falciparum* malaria should be fixed-dose ACT (fACTs), where artemisinin is protected against the emergence of resistance by the presence of a second medicine^[26].

Over the last 5 years, more than 300 million patients have been treated with ACTs, a tremendous achievement. However, with such large treatment numbers and the fact that 28 countries still allow the use of artemisinin monotherapy (against the advice of the WHO), resistance is almost bound to occur. Resistance to the partner drug has always been a hazard. However, it is manageable now that we have a range of fACTs launched or submitted to stringent regulatory authorities. Artemisinin resistance, however, is a much bigger issue since there are fewer alternatives. The first signs of emerging resistance have been reported recently in Southeast Asia^[3]. Promethazine is an antihistamine that acts by competing with histamine for H-1 receptor sites on effector cells. This H-1 antagonist is also used as adjunct therapy in the treatment of malaria in English-speaking West African countries. The drug is given as an antiemetic and also with chloroquine to prevent or alleviate chloroquine-associated pruritus^[8]. It is also given with Artemeter- Lumefantrine commonly, a dose of 5.0–10 mg is given simultaneously, or just prior to administration of chloroquine in children with falciparum malaria in Nigeria; in adults, daily doses of 25 mg are well tolerated.

According to WHO, Malaria is the single most important cause of ill health, death and economic loss in Sub-Saharan Africa^[26]. The management of malaria is complicated because the parasites that cause malaria are resistant to most of the safest and cheapest first-line treatments developed so far. There is an urgent need for the discovery of new antimalarial combinations. Malaria vaccines such as FMPI/ASO2 that target falciparum malaria protein1 (FMP1) show promising effects in malaria prevention^[3]. Pfspsz, R21, pre-erythrocytic stage and full-length circumsporozoite protein malaria vaccine are being improved in efficacy to protect against the infection^[3]. However, attempts are ongoing to develop a perfect vaccine for malaria prevention. Nausea and vomiting are some of the major symptoms of malaria. For it to be well managed, in treating the malaria infection with antimalarials, artemether-lumefantrine, it is a common practice clinically to prescribe promethazine for nausea and vomiting. This is very common, particularly in children above 2 years, as fluid loss in children is very dangerous.

Also, with the resistance to artemether-lumefantrine, it is essential to know what added advantage promethazine has on artemether-lumefantrine aside from its antiemetic properties. The potentiating effect of promethazine on amodiaquine has been established.^[17]

Materials and Methods

Male and female albino mice weighing 18 – 22 grams, obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for this study. The mice were kept (males and females separately) in cages floored with wood shavings and fed with standard rodent feed and clean water *ad libitum*. The mice were handled following the National Institute of Health (NIH) Guide for the care and use of laboratory animals^[15].

Plasmodium berghei

Mice infected with *Plasmodium berghei* were obtained from the Department of Microbiology, National Medical Research Institute Yaba, Lagos. Fresh mice were inoculated every 2 weeks to preserve the plasmodium parasite. The re-infected mice were kept in the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

Drugs and Chemicals

The drugs and materials used are promethazine (Sanofi Aventis Pharmaceuticals) and artemether-lumefantrine (Novartis Pharma AG, Basel, Switzerland). Formalin (2.5%) (BDH Pooled, U.K) Normal saline solution (0.9%), (Dana Pharmaceuticals Ltd, Nigeria). Drug administration was done orally with the aid of an oro-gastric tube.

Experimental protocol

Blood from a donor mouse infected with *Plasmodium berghei* ANKA strain with rising parasitaemia (about 20-30% infected erythrocyte) was used for this study. Blood was collected using a needle and syringe from the saphenous vein, transferred into a sterile bottle and mixed with normal saline so that 0.2 ml contained approximately 1×10^7 infected red blood cells [20].

Thirty-six (36) male and female albino mice weighing 18 – 22 g were randomly divided into six groups of six mice each. In Group 1, which served as the negative control given 0.2 ml of normal saline daily, mice were not infected with *P. berghei*. Groups 2-6 were inoculated intraperitoneally with 1×10^7 red blood cells (0.2ml) infected with the chloroquine-resistant *P. berghei* ANKA strain on day 0 (Hilou A. et al., 2006), and parasitaemia was observed 72 hours post-inoculation. Animals in Groups I and II received normal saline throughout the experiment. , Mice in Group III received 10/60 mg/kg of artemether-lumefantrine for five days, those in Group IV were treated with promethazine 25 mg/kg, while those in Group V were administered artemether-lumefantrine 10/60mg/kg and of promethazine 25 mg/kg. Group VI mice received artemether-lumefantrine 10/60 mg/kg and promethazine 50mg/kg. The parasiticidal activity was assessed on days 3, 5, 7 and 8 post-infection via thin blood smears prepared from blood collected from the tip of the tail of each mouse. The smears were fixed in absolute ethanol, stained with 10% Geimsa stain and examined microscopically. The parasitaemia was determined by counting infected erythrocytes in hundred fields, it was then divided by the total erythrocytes in the hundred fields and multiplied by one hundred. parasitemia = $\frac{\text{infected erythrocytes in hundred fields}}{\text{total erythrocytes in hundred fields}} \times 100$ Mortality was monitored daily until the mice were sacrificed

on day 8. Inhibition of parasite growth in the drug-treated group was calculated with parasite growth in the infected (untreated) control group using the formula below:

$$\text{Average \% inhibition of parasitemia} = \frac{\text{average \% parasitemia in control} - \text{average \% parasitemia in test}}{\text{average \% parasitemia in control}} \times 100$$

All animals were sacrificed by anaesthetic chloroform 24 hours after the last treatment on day 7. Blood was then collected by cardiac puncture into heparin bottles. Plasma was separated after centrifugation at 4,500 g at room temperature for 25 minutes.

Weight of the animals

The animals were weighed three (3) days after infection with *P.berghei*, on alternate days, 3,5,7 and the last day of the experiment before the animals were sacrificed. The mice were sacrificed on the fifth day of drug treatment and seven (7) days post-infection, and the weights were recorded.

Statistical Analysis

All quantitative data were expressed as the mean \pm standard error of mean (SEM). Statistical analysis was carried out using ANOVA and Duncan's post hoc test. Statistical differences in means between

treatment and control were assessed at a 95% level of significance, i.e. P -values less than 0.05 ($P \leq 0.05$) were considered significant.

Results

1.1 Effects of treatment on established *Plasmodium berghei berghei*

The mean level of parasitaemia did not differ significantly on day 3 and day 5 (Fig 1) for the different treatment combinations. A significant difference ($p < 0.05$) was observed for the mean level of parasitaemia on day 7 and day 8.

Compared to the negative (or positive control) control group, A/L significantly ($p < 0.05$) reduced average parasitaemia, promethazine did produce a decrease in parasitaemia levels, while A/L and promethazine (25 and 50 mg/kg) caused a decrease in mean parasitaemia count. Details are presented in Fig 1

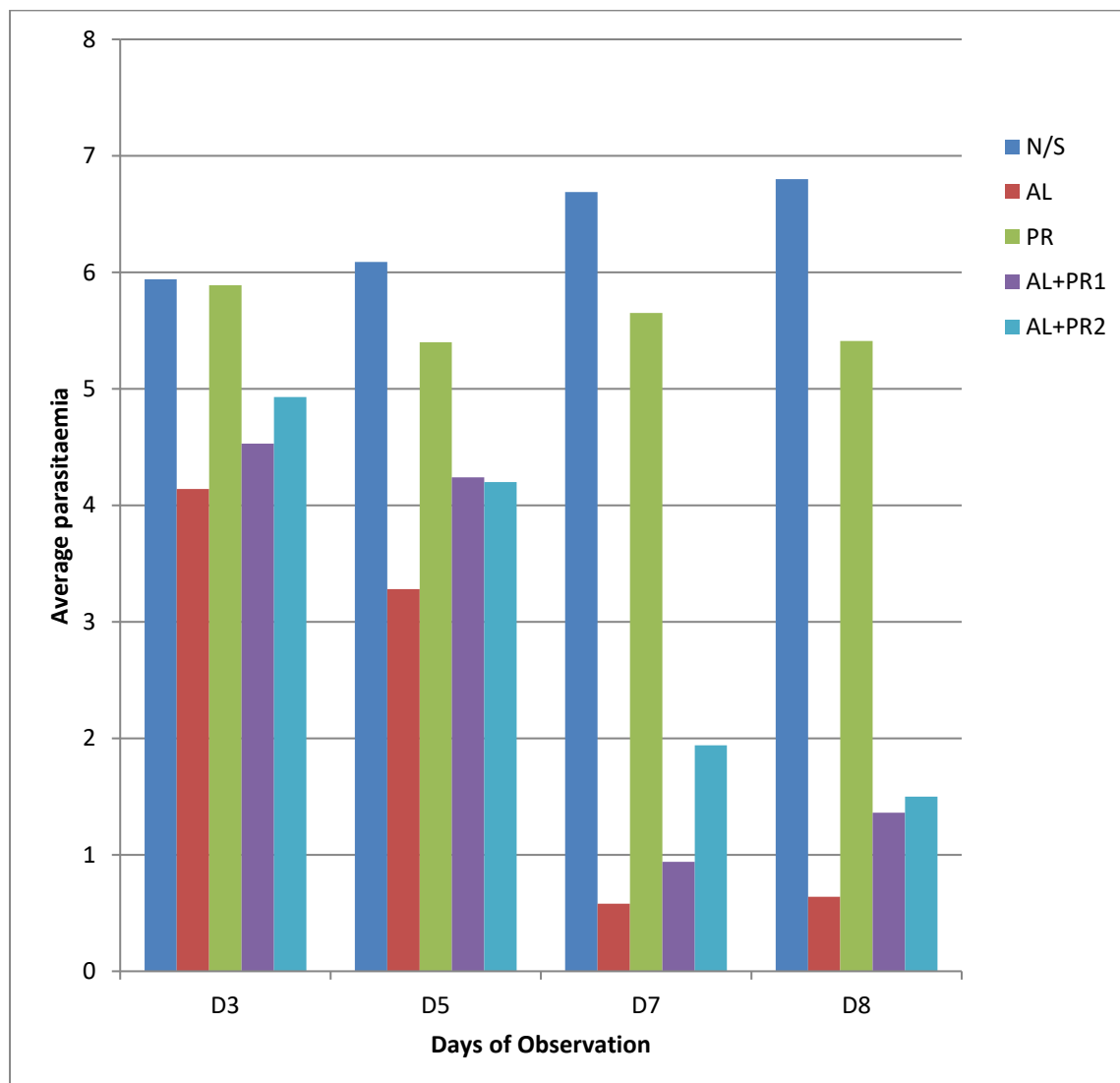


Fig 1: Effects of drug administration on average parasitaemia in mice

$p \leq 0.05$; $n=5$

N/S- Normal saline AL-Artemether-lumefantrine (10-60 mg/kg)

PR-Promethazine (25 mg/kg)

AL+PR1-Artemether-lumefantrine plus Promethazine (25 mg/kg)

AL+PR2-Artemether-lumefantrine plus Promethazine (50 mg/kg)

D3- DAY 3 D5-DAY 5 D7-DAY 7 D8- DAY 8

The average percentage of inhibition and parasitaemia for the 8th day is presented in Table 1 below.

Table 1: The effect of Promethazine and Artemether-Lumefantrine co-administration on established *Plasmodium berghei berghei* malaria infection in mice

| Treatment | Dose (mg/kg) | Average Parasitaemia | Average percentage inhibition |
|-----------------|---------------|----------------------|-------------------------------|
| Normal Saline-A | 0.2 ml/kg | 0.00 | 0.00 |
| Normal Saline-B | 0.2 ml/kg | 6.80±2.821 | 0.00 |
| A-L | 10/60 | 0.64±0.304* | 85.56±6.898 |
| Promethazine | 25 | 5.41±2.821 | 23.32±94.363a |
| A-L + Pro | 10/60 + 25 | 1.36±1.229* | 69.20±27.873 |
| A-L + Pro | 10/60 + 50 | 1.50±3.7427* | 41.93±68.078 |

n =6, * $p \leq 0.05$ (versus infected control)

a = $p \leq 0.05$ (versus A-L)

(ANOVA, post-hoc=Duncan)

A-L = Artemether-lumefantrine, Pro = Promethazine

A= Normal Control (uninfected), B= Infected Control

Values presented as SEM±

The effect of drug co-administration of promethazine and Artemether-lumefantrine on the body weight of the malaria-infected mice

No significant difference was produced in the body weight for the drug treatment combinations across the groups and days.

Table 2: Mean effects of drug treatment on body weight of malaria-infected mice

| Treatment combinations | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
|---|-------|-------|-------|-------|-------|-------|
| Uninfected + NS | 19.6 | 20.2 | 20.7 | 21.1 | 21.2 | 20.5 |
| Infected + NS | 19.4 | 20.1 | 20.0 | 19.2 | 18.8 | 18.9 |
| Infected + AL (10/60 mg/kg) | 19.0 | 20.5 | 20.6 | 20.8 | 21.1 | 21.2 |
| Infected + Pro (25 mg/kg) | 19.4 | 19.4 | 19.1 | 18.9 | 18.8 | 18.8 |
| Infected + A-L(10/60 mg/kg)+Pro (25mg/kg) | 19.6 | 20.0 | 20.6 | 20.8 | 21.2 | 21.4 |
| Infected + A-L (10/60 mg/kg) + Pro (50 mg/kg) | 19.7 | 20.5 | 20.5 | 20.8 | 20.5 | 20.2 |

N =6, ANOVA, Duncan posthoc test

NS=Normal Saline

Discussion

World Health Organization (WHO) recommends the use of artemisinin-based combination therapies (ACTs) as the first-line treatment of uncomplicated *Plasmodium falciparum* malaria [26]. ACTs therapy is currently regarded as more effective relative to non-artemisinin regimens like chloroquine and also yields rapid symptomatic improvement, parasite clearance and a reduction in gametocyte carriage, which could help to reduce malaria transmission [17]. Consequent to the use of Artemether-Lumefantrine and promethazine as a treatment for malaria, promethazine mainly used for nausea and vomiting, this study sought to elucidate the effects of the combination on parasitaemia in recipients. In this study, promethazine produced no significant effect on the mean parasitaemia during drug treatment compared to the infected control group. This is consistent with observations from earlier study [17]. Artemether-Lumefantrine produced a significant effect ($p < 0.05$) on the mean parasitaemia, consistent with the study [6] and the highest average percentage inhibition among all the treatment groups. This confirms the antimalarial potentials of Artemether-lumefantrine.

The combination of Artemether-Lumefantrine with promethazine (25mg/kg) produced a higher value in the average mean parasitaemia inhibition compared to the combination of Artemether-lumefantrine and promethazine at dose (50mg/kg) when the two combinations were compared to the infected control. This may be explained by the metabolism and protein binding properties of the drugs Artemether-Lumefantrine and Promethazine, which are metabolised by the enzyme Cytochrome P450 system. Artemether-Lumefantrine is metabolised by CYP3A4 and to a lesser extent by CYP2A6 amongst other substrates, while promethazine is predominately by the enzyme CYP2A6, [9]. Lumefantrine inhibits CYP2D6; therefore, there is a potential pharmacokinetic interaction of Artemether-lumefantrine with promethazine [16]. The co-administration may increase the plasma concentrations of promethazine, which has no significant effect on the average percentage parasitaemia inhibition and quick metabolism of artemether-lumefantrine in the system.

Artemether-Lumefantrine has a higher plasma protein binding than promethazine [12]; therefore, in combination, Artemether-Lumefantrine will be bound while promethazine will be free, giving a lower average percentage inhibition. The Promethazine 25mg/kg dose inhibits parasitaemia more than the 50mg/kg dose. It gives a lower inhibition but higher tendencies of adverse effects (dizziness, anaemia, fatigue) as it is more in the blood in its free state [6]. In the analysis of the body weight for the different treatment combinations, no significant difference was observed even though there was a gradual increase in the weight of the mice treated with Artemether-Lumefantrine, indicating effects of the treatment (antimalarial properties) compared to the other treatments.

Promethazine did not show any significant effect on mean parasitaemia. The average percentage inhibition obtained with Promethazine 25mg/kg (23.32%) was lower than that of the reference standard drug, Artemether-Lumefantrine 10/60mg/kg (85.56%), which was significantly different at level 0.05 ($P < 0.05$). The combination of promethazine 25mg/kg and Artemether-Lumefantrine (10/60mg/kg) gave a higher percentage inhibition (69.20%) than promethazine (50mg/kg) and Artemether-Lumefantrine (10/60mg/kg), (41.93%), both of which were lower than the reference drug Artemether-Lumefantrine (85.56%).

This study established that co-administration of Artemether-Lumefantrine and Promethazine possesses no adverse effect on parasitaemia level over Artemether-Lumefantrine.

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References

1. Centre for Disease Control 2020, [cdc.gov/malaria](https://www.cdc.gov/malaria) 2020
2. Duffy P.E, Patrick G.J., 2020. Malaria vaccines since 2000: progress, priorities, products. *NPJ Vaccines*.;5:48. <https://doi.org/10.1038/s41541-020-0196-3>.
3. Edagah, I. A. ,Atting, I. A. , Bassey, R. B. , Bassey, E. I. , & Ukpe, S. J. Erythropoietic and Hepatoprotective Potential of Ethanolic Extract of *Nauclea latifolia* in Mice Infected with *Plasmodium berghei berghei*. *Am J Med Sci*, 2014, 2(1), 7-12.

4. Edet, E.E., Atangwho, I.J., Akparabiatu, M.I., Uboh, F.E. and David-Oku, E.. Effect of *Gongromenalatifolium* Leaf Extract on Some Liver Enzymes and Protein Levels in Diabetic and non-Diabetic Rats. *J Pharm Biomed Sci*, 2013, 1(5): 104-107
5. Ezike A.C, Ukekwe I.F, Akah P.A, Okoli C.O. Assessment of delayed toxicity of Artemether-lumenfantrine combinations in rats. *Int J Pharm*, 2013; 4 (2):168-176.
6. Ganong, W F., *Blood*, Review of medical physiology (21st edition.). New York: Lange Medical Books/McGraw-Hill. 2003, p. 518.
7. Gbotosho, G.O., Happi, C.T., Ganiyu, A. et al., 2009. The potential contribution of prescription practices to the emergence and spread of chloroquine resistance in south-west Nigeria: caution in the use of artemisinin combination therapy. *Malaria Journal* 8, 313 <https://doi.org/10.1186/1475-2875-8-313>
8. Guyton A.C, Hall J.E. *Blood*, Textbook of Medical Physiology (15 edition.). Philadelphia: Elsevier Saunders. 2020, p.411
9. Hilou A., Nacoulma, O.G and Guiguemde T.R, "In vivo antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice," *Journal of Ethnopharmacology*, vol. 103, no. 2, pp. 236–240, 2006.
10. Jourdan, J., Walz, A., Matile, H., Schmidt, A., Wu, J., Wang, X., Dong, Y., Vennerstrom, J. L., Schmidt, R. S., Wittlin, S., & Mäser, P. (2019). Stochastic Protein Alkylation by Antimalarial Peroxides. *ACS infectious diseases*, 5(12), 2067–2075. <https://doi.org/10.1021/acsinfecdis.9b00264>
11. Law V, Knox C, Djoumbou Y, Jewison T, Guo A.C., Liu Y., Adamjee S, Dame Z.T., Han B., Zhou Y., Wishart D.S., *DrugBank 4.0: shedding new light on drug metabolism*. *Nucleic Acids Res*, 2014, 1:42
12. Leski, T.A., Taitt, C.R., Swaray, A.G. et al., 2020. Use of real-time multiplex PCR, malaria rapid diagnostic test and microscopy to investigate the prevalence of Plasmodium species among febrile hospital patients in Sierra Leone. *Malaria Journal* 19, 84, <https://doi.org/10.1186/s12936-020-03163-2>
13. Nakamura, K., Yokoi, T., Inoue, K., Shimada, N., Ohashi, N., Kume, T., Kamataki, T., CYP2D6 is the principal cytochrome P450 responsible for the metabolism of the histamine 111 antagonists in human liver microsomes. *Pharmacogenet and Genom*, 1996, 2(16)
14. NIH-National Institute of Health, Revised Guide for the care and use of laboratory animals, NIH Guide, 2019, volume 30, number 38,
15. Novartis, Novartis Drug Regulatory Affairs: Coartem®/Riamet® Basic Prescribing Information.Coartem (artemether/lumefantrine) Tablets. Novartis Pharmaceuticals Corporation. 2014 Accessed April 30, 2015 from <http://www.pharma.us.novartis.com/product/pi/pdf/coartem.pdf>
16. Olalubi, O.A., Ogunlana, O.E., Fagbemi, B.O. "In-Vivo Evaluation of the Antiplasmodial Effect of Amodiaquine and Amodiaquine-Promethazine Combination in Plasmodium berghei Infected Mice", *J Health Res*, 2011, 4(2):83-88
17. Parnpe, V., Vasant, K., and Chuchard, P., "Liver Changes in Severe Plasmodium falciparum. *Malar J*, 2014,13:106.
18. Peipei, L., Zhenjun, Z., Ying, W., Nested PCR Detection of Malaria Directly Using Blood Filter Paper Samples from Epidemiological Surveys. *Malar J*. 2014; 13:175-178.
19. Phillipson J.D (1991). Assays for antimalarial and amoebicidal activities in *methods in plant biochemistry*. 6: 135-149.
20. Rxlist, <http://www.rxlist.com/promethazine> 2015.
21. Sellers, R.S., Morton, D., Micheal, B., Roome, N, Johnson, J.K. and Yano, B.L. Society of Toxicologic Pathology Position Paper: Organ Weight Recommendations for Toxicological Studies. *Toxicol Pathol*, 2007,33:751-755.
22. Singh, B., Bobogane, A., Cox Singh, J., et al., A Genus and Species-specific Nested Polymerase Chain Reaction Malaria Detection Assay for Epidemiologic Studies *Am J Trop Med*.1999; 60(4): 687-692.
23. Weiss, J.B DNA Probes and PCR for Diagnosis of Parasitic Infections in *Clinical Microbiology Reviews*, 1995, 8(1):113-130.
24. WHO, World Health Organization. 'World Malaria Report 2020', 2020. Geneva, Switzerland.

