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Research Article

THE ANTIMALARIA EFFECT OF DIFFERENT DOSAGE REGMEN OF ARTEMISININ-NAPHTHOQUINE ON *PLASMODIUM BERGHEI* INFECTED MICE

Onasanya Seun Sunday [1], Ademowo Olusegun George[2]

1. *Department of Pharmaceutical Technology, Moshood Abiola Polytechnic, PMB 2210 Abeokuta, Ogun State, Nigeria.*
2. *Institute of Medical Research And Training, University College Hospital, Ibadan, Oyo State, Nigeria*

ABSTRACT

In view of the recent development where the widespread use of antimalarial agents has contributed to selection pressure and the emergence of *P. falciparum* resistance to almost all existing monotherapy antimalarial drugs , Artemisinin combination based therapy (ACT) has been adopted by most endemic countries for the treatment of malaria. Artemisinin-naphthoquine (ANQ) is a relatively new ACT with a dearth of information on its activity and toxicity We therefore design this study to compare the efficacy of single dose regimen of artemisinin-naphthoquine combination ARCO® with 3 days divided doses of artemisinin-naphthoquinein *P. berghei* infected mice, as well as the efficacy of the known ACT like Artemether-lumefantrine (A-L), Dihydroartemisinin-piperaquine (DPQ), and established monotherapies like artemether, (ART) and chloroquine.(CQ)

BALB/c mice (n = 105) were infected with standard inoculums (1x 10^7) of *Plasmodium berghei* intraperitoneally. Albino mice were divided into seven groups of fifteen mice each. Group 1 (control) were infected but not treated, Group 2 were infected and treated with Chloroquine (10mg/kg) for three days. Group 3 were infected and treated with artesunate (ART) 4mg/kg for three days. Group 4 were infected and treated with Artemether-lumefantrine (A-L) 56 mg/kg, stat then after 8 hours then twice daily for the next two days. Group 5 were infected and treated Dihydroartemisinin-piperaquine (18mg/kg for two days and 12mg/kg for the third day). Group 6 were infected and treated with Artemisinin-naphthoquine (ANQ), 23mg/kg once daily for just one day. Group 7 were infected and treated with Artemisinin-naphthoquine combination (ANQ), 7.6mg/kg once daily for three days. All drugs were administered orally with a canula starting from the day the parasitemia was established and were subsequently monitoredfor clearance of parasitemia. Result showed that the mice infected and treated with CQ alone and ANQ once daily for three days (3CD) had a significant decrease ($p<0.05$) in percentage parasitaemia on days 2, when compared with day 0.while a significant decrease ($p<0.05$) was observed in days 3, 4 with A-L, ARTCOP,treated groups. However, in ART treated group a significant decrease was observed on days 2, and 3 followed by a resurgence and most of the animal died. Parasite infected untreated group had their parasitaemia increased with the days of infection. ANQ 3 CD had the highest mean survival time (MST) followed by CQ while ART had the least MST. PCV was rapidly restored to baseline after treatment with ARTCOP group followed by ANQ 3CD. AND CQ.

All these suggested that, taking ANQ for three consecutive days at 7.3mg/kg gave a significant difference in parasiteamia clearance in mice on day 2 compared to ANQ single dose, restored PCV faster than ANQ single dose , confers a higher mean survival rate compared to ANQ taken for a single day.

Key-words: Artemisinin-naphthoquine, *Plasmodium berghei*, antimalarial,

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INTRODUCTION

Malaria is a major public health problem in the world. It continues to afflict the poor nations and the poor most. Freedom from malaria is the basic right of humankind, yet malaria is among the top 10 killer diseases in the world. Annual estimates vary between 300 to 500 million clinical episodes of malaria and 1.5 to 2.7 million deaths worldwide, 90% of which occur in tropical Sahara. Outside Africa, some two-thirds of the remaining cases occur in just three countries; Brazil, India and Sri Lanka. However, malaria is exists in some 100 countries [1].In 1992, Global Malaria Control Strategy was introduced to control malaria with four basic elements e.g., early diagnosis, prompt treatment, selective and sustainable vector control and early detection of forecasting of epidemics. Though the revised malaria control strategy has been implemented in all countries of the region but however, the desired results could not be achieved. However, in 1998, Maldives has been granted Malaria free status as no indigenous cases were reported from Maldives since 1984. But in contrast, in DPR Korea, which has malaria free status, reported the malaria outbreak in its southern states bordering to South Korea in 1998.

In recent years, it was reported that there had been a resurgence of interest in malaria as the immensity of the burden it imposed on poor countries in the tropics had become apparent, and as effects at control had been unsuccessful after the failure of the global eradication campaign in the 1960s [2]. This is largely due to the increasing resistance of mosquitoes to the insecticides and of malaria parasites to the drugs. *P.Falciparum* is now highly resistant to Chloroquine in most malaria-affected area. Resistance to sulphadoxine-pyrimethamine (SP) is also widespread and has developed much more rapidly. However, despite the established resistance and ineffectiveness of these drugs, they are still being used in most malaria affected countries, which unfortunately have less than $10 per capital to spend on all aspect of health. However, Intermittent preventive treatment with sulphadoxine-pyrimethamine (IPT-SP) is currently the recommended regimen for prevention of malaria in pregnancy in endemic areas [3].*Plasmodium vivax*malaria is the most geographically widespread and the second prevalent cause of malaria globally. Among 2.6 billion people are at risk of malaria infection, from 130 to 435 million [4]. 90% of these infections occur outside Africa [5]. Since then, cases of resistance have been reported from places such as Indonesia, island of Nias, in Papua and Irian[6]. To counter the threat of resistance of *P. falciparum* to monotherapies, and to improve treatment outcome, WHO recommends that artemisinin-based combination therapies be used for the treatment of uncomplicated *P. falciparum* malaria. Although the evidence base confirming the benefits of artemisinin-based combinations has grown substantially in recent years, there is still substantial geographic variability in the efficacy of available ACT options, underlining the importance of countries regularly monitoring the efficacy of the ACTs in use to ensure that the appropriate ACT option(s) is being deployed. Artemisinin derivatives have short half-life but the component combination drug with a longer duration of action, makes up for this by providing sustained activity to eliminate remaining parasite [7]. Among the combination treatments recommended by WHO for the treatment of uncomplicated falciparum malaria are artemether plus lumefantrine, artesunate plus amodiaquine , artesunate plus mefloquine artesunate plus sulfadoxine-

pyrimethamine, dihydroartemisinin plus piperaquine [8].

All artemisinin-based combination therapies (ACTs), recommended by the World Health Organization, are 3-day regimen. A considerable level of non-compliance on ACTs has been reported from some countries this led to the formulation of a new generation ACT containing artemisinin plus naphthoquine. An oral single dose of eight tablets (400 mg of naphthoquine+1000 mg artemisinin) [9]. He use of anti-malarial drug combinations with artemisinin or with one of its derivatives is now widely recommended to overcome drug resistance in falciparum as well as vivax malaria. The fixed oral dose artemisinin-naphthoquine combination (ARCO™) manufactured by the Kunming Pharmaceuticals in China and marketed by Nigerian-German chemicals Plc in Nigeria is a newer artemisinin-based combination (ACT) therapy. However, Francis Hombhanje reported in 2009 that the drug was undergoing clinical assessment to assess the safety, efficacy and tolerability of the combination in areas of multi-drug resistance to generate preliminary baseline data in adult population of Papua New Guinea [10].

However, the World health organisation had stipulated a three days therapy for malaria treatment with artemisinin derivatives, few studies have separately describe the safety, efficacy and tolerability of Artemisinin plus naphthoquine combination in adult of Papua new Guinea with few therapeutical failure seen in few patients [10] whileEmilanaTjitra et al [11]carried out a study on the efficacy and safety of artemisinin - naphthoquine versus dihydroartemisinin-piperaquine in adult patients with uncomplicated malaria in Indonesia.

Most recent Published studies by Battykt et al [12] looked at the pharmacokinetic study of Artemisinin plus Napthoquine therapy for uncomplicated paediatric malaria in 2012.

No studies on the impact or effect of dose regmen of Artemisinin plus Napthoquine on malaria treatment has ever been published. Furthermore there has never been any published studies comparing the efficacy and effect of the different dosage regmen of Artemisinin plus napthoquine with Artemether-lumenfantrine, Dihydroartemisinin-piperaquine , Chloroquine, artemether on malaria treatment in human, mice or rat infected with Plasmodium berghei.

MATERIALS AND METHODOLOGY

Experimental Animals

One hundred and five BALB/c mice were used and each animal weighed between 20-25 grams. The animals were obtained from the animal house of Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria. The animals were housed in well-aerated cages, fed with standard mouse cubes and supplied with clean drinking water ad libitum.

Parasite and standard inoculation

Plasmodium berghei, donated by Malaria Reference Reagent Resource Center to a drug research unit laboratory was obtained from the animal house of Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria. Parasites were maintained through serial blood passage in mice wherein the mice previously infected with *p. berghei* and with high parasitemia level serve as the donor. Blood samples was taken from the donor and diluted with phosphate-buffered saline such that 0.2ml injected intraperitoneally into the experimental animals contained 1×10^7infected erythrocytes.

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Study of the course of infection and antimalarial activity

The course of infection following intraperitonial inoculation in mice was studied in each experimental mouse that received 1×10^7 parasitized red blood cells in 0.2ml inoculums. Thin blood films were prepared from the tail vein of infected mice, fixed with methanol and stained with 10% Giemsa stain for 30 min and then rinsed with tap water. Parasitemia was monitored daily using ×100 objective of a light microscope. The numbers of parasitized cells (infected red blood cells) was expressed against 1000 infected and uninfected red blood cells and were evaluated. Treatments commenced when about 10% parasitemia were established in infected mice.

$$\% \text{ Parasitemia} = \frac{\text{Infected red blood cells}}{\text{Total red cell}} \times 100$$

Distribution of mice

Mice weighing 20- 25g were distributed and the experimental animals used were divided into groups based on their body weight. They were divided into eight groups, each group comprising of fifteen animals. The mice were randomly assigned to a given treatment group

Group 1: Animals in this group were infected with Plasmodium berghei and were not treated with any drug. Animals in this group served as the control.

Group 2 (Chloroquine only): Animals in this group were infected with Plasmodium berghei and starting from day 4 post infection, they were treated with chloroquine for 3 days. The dose of chloroquine that was administered to these animals is 10mg/kg.

Group 3 (Artesunate only): Animals in this group were infected with Plasmodium berghei and starting from day 4 post infection, they were treated with Artesunate for 3 days. The dose of Artesunate that was administered to these animals is 4mg/kg, once daily for three days.

Group 4 (Artemether-lumefantrine):Animals in this group were infected with Plasmodium berghei and starting from day 4 post infection, they were treated with Artemether-lumefantreine for 3 days. The dose of Artemether-lumefantrine that was administered to these animals is 56mg/kg, stat then after 8 hours then twice daily for the next two days.

Group 5 (Dihydroartemisinin-piperaquine): Animals in this group were infected with Plasmodium berghei and starting from day 4 post infection, they were treated with Dihydroartemisinin- piperaquine for 3 days. The dose of dihydroartemisinin-piperaquine that was administered to these animals is mg/kg once daily for four days, and the dose for Chloroquine is 18mg/kg once daily for two days and 12mg/kg for the third day.

Group 6 (Artemisinin-naphthoquine): Animals in this group were infected with Plasmodium berghei and starting from day 4 post infection, they were treated with Artemisinin- naphthoquine for just one day. The dose of Artemisinin-naphthoquine that was administered to these animals is 23gmg/kg once daily for just one day.

Group 7 (Artemisinin-naphthoquine): Animals in this group were infected with Plasmodium berghei and starting from day 4 post infection, they were treated with Artemisinin-naphthoquine daily for 3 days. The dose of Artemisinin-naphthoquine that was administered to these animals is 23mg/kg once daily for three days.

Evaluation of Blood Antimalarial Activity on Established Infections

In vivo antimalarial activity against Plasmodium berghei infection in mice was done according to Rane's test as described by Elufioye and Agbedahunsi[13] TheRane's test relies on the ability of standard inoculums of P. Berghei to kill the

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recipient mouse within six days of inoculation. Extension of survival beyond 12 days is regarded as activity. Adult Swiss albino mice weighing 20- 25g were inoculated by intraperitoneal injection with 1× 107infected erythrocytes on the first day of the experiment(day 0). The mice were not treated until the parasitemia was established. On day 4, after the experimental animals were infected, treatments started. Each day, beginning on the day the treatment commenced until the end of the experiment, the packed cell volume (PCV) and animals weight were determined daily, blood films were made from the tail of the infected mouse, fixed with methanol and stained with 10% Giemsa stain. Examined microscopically to assess the level of parasitemia. Percentage parasitemia was evaluated as follows

Total number of PRBC× 100
Total number of RBC
PRBC = Parasitized red blood cells
RBC = Red blood cells.

After the last day of drug administration, the animals were observed weekly for 3 weeks, through which PCV, weight and percentage parasitemia were determined. The number of deaths was recorded and the mean survival time (MST) for the various groups was recorded.

Determination of packed cell volume

Packed cell volume is a measure of the proportion of red blood cells to whole blood. Small volume of blood was collected from the tip of the animal tail (tail tip amputation) into a heparinized capillary tube. The capillary tube was sealed with plasticin and spun for 5 minutes using haematocrit centrifuge to separate the blood into plasma and packed cells. The percentage of the packed cells was calculated using a hematocrit reader.

Statistical Analysis

The results were expressed in terms of mean± standard deviation (SD). Parameters in the groups were compared by one-way (ANOVA) using the computer software Statistical Package for Social sciences (SPSS) Version 15. All data was analyzed at a 95% confidence interval and values were considered statistically significant at $p<0.05$.

RESULTS

EFFECTS OF TREATMENTS ON PERCENTAGE PARASITEMIA

Figure 2 showed that the parasite- infected untreated group had their parasitemia increased with days of infection. The parasite-infected treated groups had slight initial increase in parasitemia level followed by a gradual decline post treatment. At baseline (day 0) all treatment groups were not significantly different ($P>0.05$) compared to untreated control group. (group 1). At day 1, all treatment groups were significantly different compared to untreated control group ($p<0.05$). Thus at day 2 the Chloroquine treated group (group 2), the parasite –infected AL group (group 4) and artemisinin-naphthoquine 3 daily dosing (group 7) had virtually a complete clearance of parasitaemia with significant difference between artemisinin-naphthoquine 3 daily dosing (group 7) and ANQ 1 day treatment group (group 6). At day 9 the parasite –infected CQ group, ARCO 3 c. d group, AL, ARTCOP had a complete parasitaemia clearance however, arco group 1 had a sharp increase in parasitemia level at day 16 which might probably be due to recrudescence.

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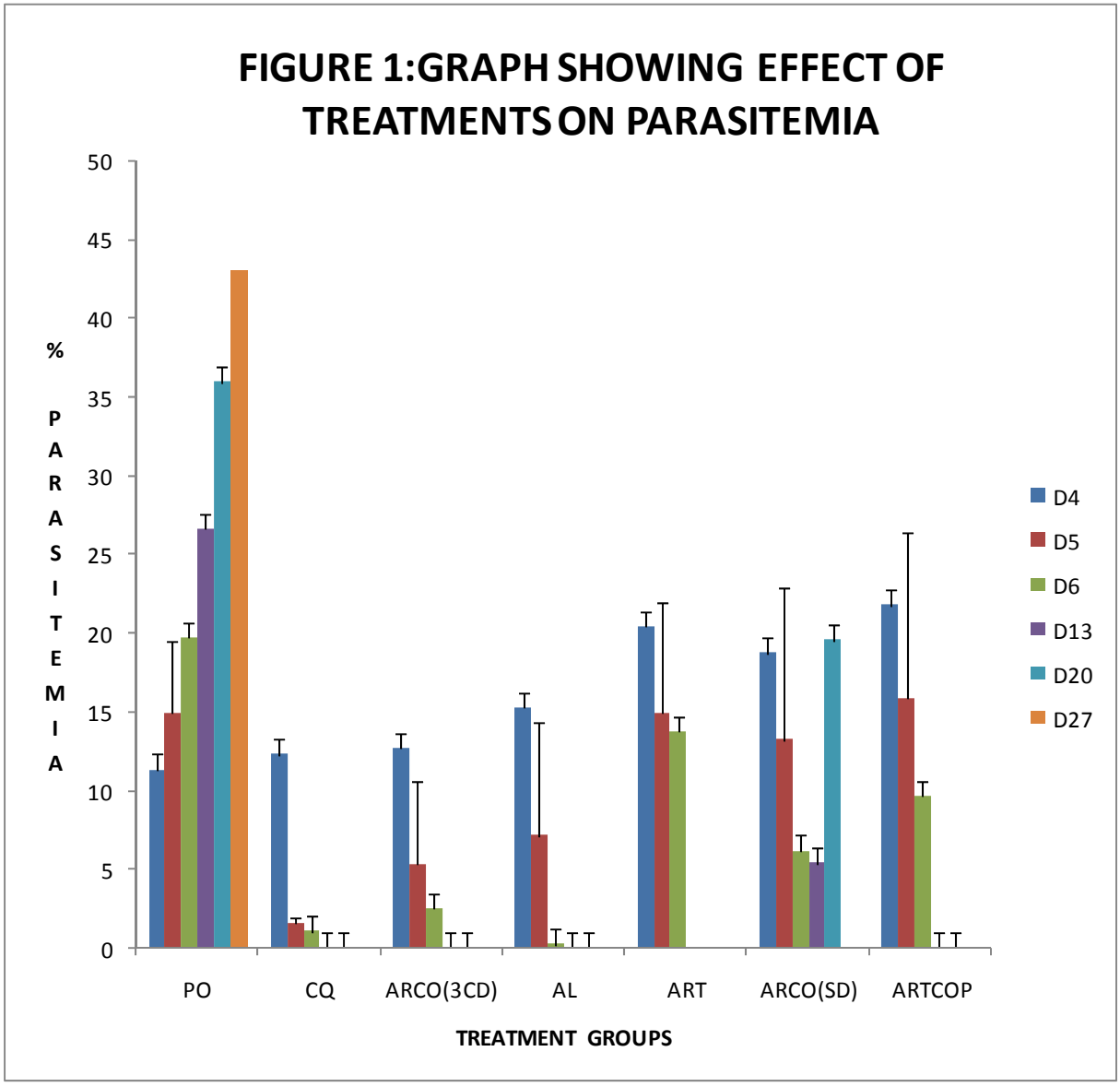
Figure 1: Graph Showing Effect of treatments on Parasitemia



PO – UNTREATED GROUP **ARCO – ARTEMISININ-NAPHTHOQUINE**
CQ –CHLOROQUINE **AL- ARTEMETHER – LUMEFANTRINE**
ART – ARTESUNATE **ART- DIHYDROARTEMISININ-PIPERAQUINE**
SD- SINGLE DOSE **3CD- 3 CONSECUTIVE DOSES.**

Table I: Mean Survival times of the mice.

Table I shows the mean survival time of parasite-infected treated groups with the parasite-infected untreated group with the artemisinin-naphthoquine 3 days daily dosing having the highest survival rate during the observation.

GROUPS	Mean Survival time (DAYS)
Untreated Control	9
CQ treated- infected	NO DEATH
ART treated – infected	10
AL treated- infected	17
ARTCOP treated- infected	14
ARCO treated (single dose)- infected	11
ARCO treated (3 consecutive doses)-infected	NO DEATH

CQ -Chloroquine ART- Artesunate AL- Artemether-lumefantrine

ARTCOP- Dihydroartemisinin-piperaquine ARCO- Artemisinin-naphthoquine

Effects of treatments on Packed cell volume.

Figure II shows that the PCV of parasite-infected untreated animals decreased progressively while parasite-infected CQ-treated, ART-treated, AL-treated, ARCO (single dose)- treated and ARCO (3 daily dosing)- treated group

At day 0, there was no significant difference between Arco (single dose), arco (3 days daily doses), AL, ARTCOP ($p<0.05$) when compared with infected untreated group. At day 2, all the treatments groups had significantly ($p<0.05$) higher than PCV compared to parasite-infected untreated control.

However, starting from day 4, the second day of treatment, the PCV increases significantly in all the groups when compared with the untreated group, with Artcop- treated group having a sharp increase in PCV but this was not statistically significant ($p<0.05$) when compared with other treated groups. Fig 3 shows that the control and parasite-untreated group had a gradual decrease in the body weight while CQ-treated, ARCO (S.D) –treated group, ARCO (3C.D) – treated group, ARTCOP – treated group, ART – treated group and AL-treated group all had a slight decrease in body weight followed by an increase in body weight.

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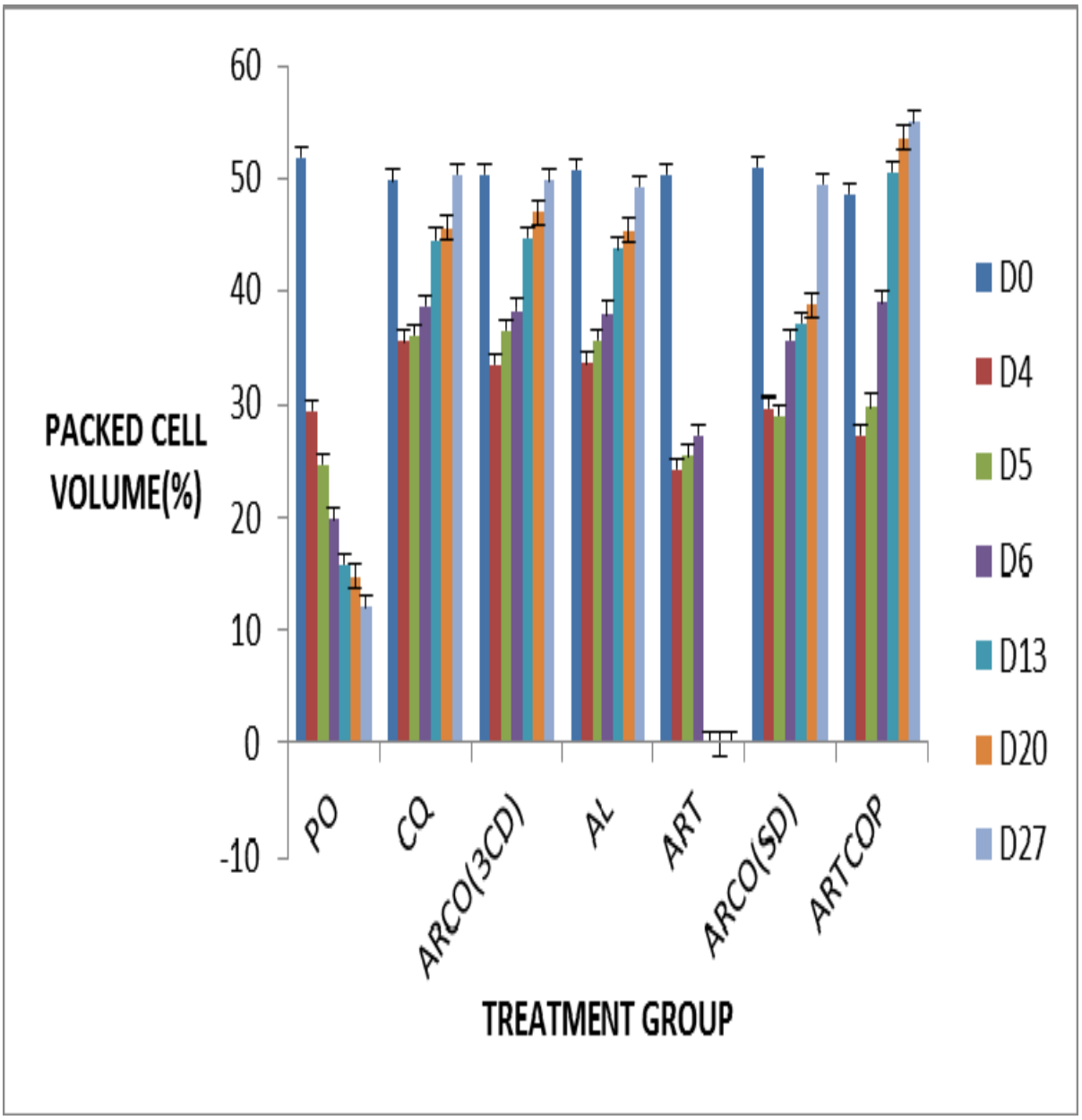
This implies that ARCO treated groups both 3daily doses and single dose prevented loss of body weight in infected mice with higher increase in parasitemia being brought about by ARCO (3 daily dose

Figure 2 Graph Showing the Effect of Treatment on Packed Cell Volume



PO - Untreated; CQ – Chloroquine; ARCO- Artemisinin-naphthoquine; ART - Artesunate; AL – Artemether-lumefantrine ARTCOP – Dlhydroartemisinin-Piperaquine

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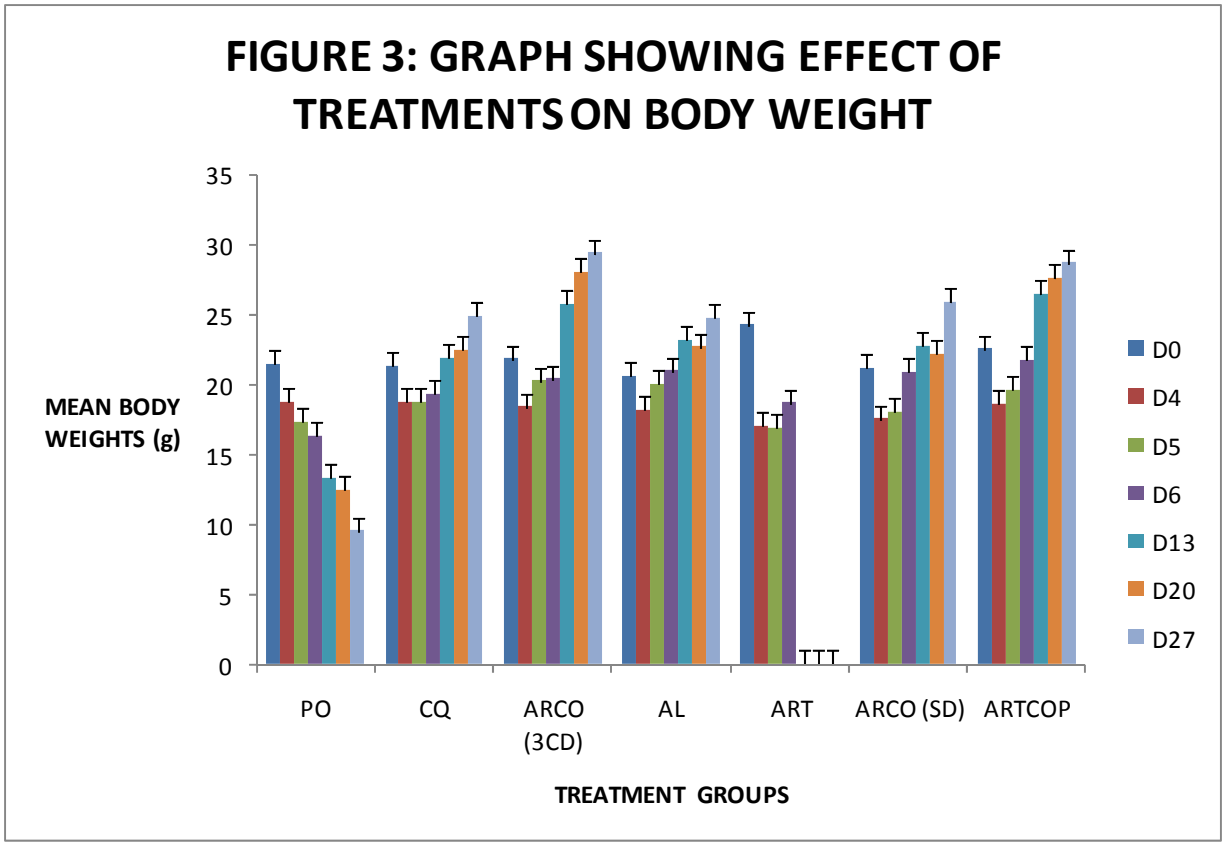
Figure 3: Effect of treatment on weight of *Plasmodium berghei* infected mice



PO- Untreated group; CQ- Chloroquine; ARCO (3CD) – Artemisinin-naphthoquine 3 consecutive days; ART – Artesunate; ARTCOP – Dihydroartemisinin- piperaquine.

DISCUSSION

The high prevalence of malaria in Africa, the ever increasing drug resistance which made the continent a target for new antimalarial drugs has also made it imperative to develop a cost effective antimalarial drug since antimalarial resistance has now become a serious global

Challenge. However, preserving the life span of antimalarial drugs is a key part of the strategy for roll back malaria. The WHO had adopted ACT as a policy standard for antimalarial in areas where Plasmodium falciparum is the predominant infecting specie and had stipulated at least three days regimen for these ACT [14].HoweverA considerable level of non-compliance on ACTs has been reported from some countries, also there is too much pressure on the available ACT ; some level of resistance has been reported to available ACT but not from this part of the world. This led to the formulation of a new generation ACT containing artemisinin plus naphthoquine. An oral single dose of eight tablets (400 mg of naphthoquine+1000 mg artemisinin) [9] Naphthoquine also belong to the group of 4 amino quinolones, several studies have been carried out to evaluate the safety, and efficacy of Artemisinin- naphthoquine combination. However in a study carried out Hombhanje [10] in 2009 reported that the

drug was undergoing clinical assessment to assess the safety, efficacy and tolerability of the combination in areas of multi-drug resistance to generate preliminary baseline data in adult population of Papua New Guinea [10]. EmilanaTjitra et al [11] carried out a study on the efficacy and safety of artemisinin - naphthoquine versusdihydroartemisinin-piperaquine in adult patients with uncomplicated malaria in Indonesia and found the drug to be effective but therapeutic failure in few patients.

However, due to the claim and the fact that malaria remains a major public problem in Africa, the present study focused on investigation of the antimalarial activity of the artemisinin-combination single dose therapy when compared to using the same combination for three days and with other ACTs like dihydroartemisinin-piperaquine, artemether-lumefantrine.in *Plasmodium berghei*-infected mice. We also reported the effect of the combination on packed cell volume, weight, and antioxidant properties.

Rodent models have been validated through identification of several conventional antimalarials,such as Chloroquine, mefloquine and Artemisinin derivatives, though primate models seem to provide better prediction of efficacy in human. When Chloroquine is used in mice infected with *P.berghei,* it suppresses parasitemiato a non-detectable levels, which is in agreement with the effects of Chloroquine in this study. However, artemisinin-naphthoquine 3 consecutive suppresses and clear parasitemia to a non-detectable level on the second day of treatment and hence suggesting efficacy but with artemisinin-naphthoquine single dose, it suppresses parasitemia but a notable significant resurgence occurs, this might probably due to the fact that artemisinin has a half-life of between 4 to 6 hours, and since it's taken for a day, a very high pressure will be on the partner drug naphthoquine. However, resistance to naphthoquinemonotherapy by *Plasmodium berghei* K173 have been reported [15], this further explain why resurgence of parasitaemia occurs in a single dose of artemisinin-naphthoquine..

Furthermore, in untreated mice, the percentage parasitemiaincreased while packed cell volume (PCV) decreases markedly from day to day which is consistent with previous studies. The increases in PCV of the parasite –infected CQ-treated, AL-treated, ARTCOP- treated, ARCO- treated result in hemo-concentration and may be due to increased red blood count mass while the decrease observed in parasite- infected untreated group is due to hemolysis.

REFERENCES

1. WHO (2007): Guidelines for the treatment of malaria, http// www.who.int /malaria /docs./ treatment guidelines 2007.pdf.
2. Nicholas JW (2004) . Antimalarial drug resistance J. Clin invest. 113: 1094-1092.
3. FaladeCO,Bidemi Yusuf, Francis F Fadero, Olugbenga A. Mokuolu, Davidson H. Hamer, Lateef A. Salako. (2007). Intermittent preventive treatment with sulphadoxine-Pyrimethamine is effective in preventing maternal and placental malaria in ibadan, south western Nigeria. M.J 2007, 6:88.
4. Guerra CA, Snow RW, Hay SI, (2006): Mapping the global extent of malaria in 2005. *Trends Parasitol*2006, 22: 353-358
5. Mendisk, Sina BJ, Marchesini P, Carter R, (2001). The neglected burden of *Plasmodium vivax* malaria.*Am J. Trop. Med Hyg.* (2001) 64 (1-2) Suppl : 97-106
6. Baird JK, Basri H, Purnomo, Bangs M j, Subianto B, PatchenLc, Hoffman SL, (1991). Resistant to Chloroquine by Plasmodium falciparum, Plasmodium vivax in indonesiaAmj Trop. Med.Hyg 1991, 44:547-552
7. White NJ (1999)Antimalaria drug resistance and combination chemotherapy. Philos Trans R Soc.Lond. B. Biol. Sci. 354: 739-49

8. WHO (2010): Guidelines for the treatment of malaria, (online). Geneva: WHO 2010
9. Tun T, Tint HS, Lin K, Kyaw TT, Myint MK, Khaing W, Tun ZW,(2009) Efficacy of or Single dose therapy with artemisinin-naphthoquine phosphate in uncomplicated falciparum Malaria . Actra Trop.111 (3): 275-8
10. Hombhanje FW, Linge D, Saweri A, Kuanchi C, Jones R, Toraso S, Sapuri M, (2009):Artemisinin-naphthoquine Combination (ARCO) Therapy for Uncomplicated Falciparum Malaria in Adults of Papua New Guinea: A premilinary report on safety and efficacy. Malaria journal, 8,196
11. EmilanaTjitra, Armedy R, Hasugian, Hadjarsisiwantoro, Budiprasetyorim, RiyantiEkowatinmgshi, EndohA,Yusnito, TellyPurnama s.(2012).Efficacy and safety of artemisinin-naphthoquine versus dihydroartemisinin-piperaquine in adult patients with uncomplicated malaria. A multi centered study in Indonesia. Malar J. 2012 Jun 14;11:153
12. Batty KT, Tran QuangBinh, Kenneth F lleft, Timothy M.E Davis, Nguyen Canh Hung, Shane M Powell, Vu Duong Bich Phuong.(2001). Oral bioavailability of dihydroatemisinin in Vietnamese volunteers and in patients with *falciparum* malaria. Br J Clin Pharmacol. 51(6): 541–546.
13. Elufioye and Agbedahunsi (2004) Antimalaria activities of *Tithoniadiversifolia* (Asteraceae) and *Crossopteryxfebrifuga* (Rubiaceae) on mice in vivo. *J.Ethnopharmacology*. 93 (2-3); 167-71.
14. WHO (2003): Assessment and Monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. Geneva, World Health Organization, WHO/HTM/RBM/2003, 50.
15. Wang J, Huang L, Li J, Fan Q, Long Y, et al. (2010):Artemisinin Directly Targets Malarial Mitochondria through Its Specific Mitochondrial Activation. PLoS ONE 5(3): e9582. doi:10.1371/journal.pone.0009582