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Comparative efficacy of five brands of Arthemeter Combination Therapy (ACTS) purchased in Ekiti State, Nigeria

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Abstract

Malaria is one of the most severe public health problems and a leading cause of morbidity and mortality where children and pregnant women are the most affected groups. This may be as a result of resistant of malaria parasites which are mostly traceable to drug adulteration. In this study, *Plasmodium berghei* NK65, a rodent malaria parasite was used as a model organism to simulate human malaria parasite. The efficacy of five brands of arthemeter combination therapies (ACTs) purchased from pharmacy stores in Ekiti state were evaluated. The drugs were coded Art-Ar, Art-Ph, Art-Ki, Art-Na, and Art-Gy respectively. Twenty-one mice were constituted into seven different groups of three mice. Each mouse was inoculated inter-peritoneally with *Plasmodium berghei* NK65 and left for 7days for the parasite to manifest. The prepared drug solution was administered on five of the seven groups orally for 10 days, while the remaining groups were used as the positive and the negative control groups. One of the control groups was given chloroquine (positive) and the other normal saline (negative). The antimalarial efficacy of the drugs (ACTs) was determined by examining their influence on the increase or decrease in the number of parasites (parasitemia level). Giemsa stained thin blood smears from the tails were used to assess parasitemia level and the parasites clearance rate. Results show that the average parasite clearance rates were 14.1, 40.6, 25.3, 44.9 and 27.9 for Arthemeter coded as Art-Ar, Art-Ph, Art-Ki, Art- Na and Art-Gy respectively. All the brands significantly cleared all the parasites within 7-9days of the treatment, which shows that adulterated Arthemeter is not presently in the community studied. Therefore, arthemeter combination therapy is recommended as a drug of choice for the treatment of malaria fever in Ekiti State.

Keywords: Arthemeter Combination Therapies (ACTs); Malaria; Mice; *Plasmodium beighei*

1. Introduction

Plasmodium berghei is a model organism for the study of human malaria due to its ability to infect rodents, relative ease of genetic engineering and its usefulness in research programs for the development and screening of anti-malarial drugs [1]. *P. berghei* is transmitted by *Anopheles* mosquitoes, like all malaria parasites of mammals and the symptoms are comparable to symptoms of cerebral malaria in patients infected with the human malaria parasite *Plasmodium falciparum* [2].

Malaria is one of the most severe public health problems and a leading causes of morbidity and mortality in many developing countries, with children and pregnant women the most affected groups [3]. This may be as a result of resistant of malaria parasites to antimalarial drugs which are mostly caused by drug adulteration. According to WHO [4], an estimated 219 million cases of malaria occurred worldwide in 2017, in same year, there were an estimated 435,000 deaths. Globally, it is estimated that 3.2 billion people are at risk of contracting malaria annually [5]. In Nigeria, malaria is transmitted throughout the year, with more than 194 million people predisposed to contracting malaria infection [6]. There is a vaccine currently available for malaria as of 2021. The vaccine (RTS, S vaccine) with the brand

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name mosquirix provides a high level of protection for children living in areas with moderate- to-high malaria transmission, but not all areas were covered. Hence, antimalarial drugs must be taken continuously to reduce the risk of infection in such situations where vaccines are yet to be administered [4]. Malaria infections are treated through the use of antimalarial drugs such as quinine, artesunate, artemisinin, halofantrine and a host of others. But malaria parasites have evolved to be resistant to many of these drugs [3], this may be as result of drug adulteration.

There had been cases of drug adulteration in some part of the world most especially in Nigeria [7]. A review conducted by Buckley [8], showed that about 35 % of the antimalarial medicines in Southeast Asia were substandard, and 36 % can be classified as falsified. Similar patterns were also found in sub-Saharan Africa, where about 35 % of antimalarial drugs were substandard and 20 % were falsified; 8 of the 12 major antimalarial drugs used in the world have been found to be falsified. Examples of these faked drugs include products labeled as mefloquine, but containing sulphadoxine-pyrimethamine and no mefloquine, and a product labeled as artesunate, but containing 6 % chloroquine and no artesunate [8]. Wogu *et al*. [9] also found out that the NAFDAC MAS (Mobile Authentication Service) scheme has not reduced or eliminated the production, distribution and consumption of fake or counterfeit drugs in the South-east Nigeria, this is due to the inappropriate media awareness campaign.

In order to combat the menace of malaria, arthemeter combination therapy was recommended by the Centre for disease control and prevention (CDC) in the treatment of chloroquine resistant and multidrug resistant malaria [10]. Arthemeter is a phenanthrene methanol antimalarial which is schizonticidal with high degree of activity against the asexual erythrocytic stage of malaria parasites. It also has different modes of action and act at different points in the parasite life cycle [11]. Presently not less than ten brands of Arthemeter is circulated and marketed in Nigeria. Despite the use of this drug, cases of malaria fever are increasing at alarming rate. It is not clear whether the arthemeter in circulation is also adulterated, since there is no information about it. Therefore, this study investigates the therapeutic efficacy of five brands of arthermether combination therapies purchased in Ekiti State, Nigeria.

2. Material and methods

2.1. Experimental Animals

Swiss albino mice weighing between 12g and 17g were used in this study. These albino mice were brought from the animal house of the Nigeria Institute for Advanced Medical Research and Training, UCH, Ibadan, Oyo State (IAMRAT). The twenty-one mice were grouped into seven different groups of three mice, kept in cages, fed with standard mouse cubes and maintained at the same environmental condition throughout the period of the investigation.

2.2. Malaria Parasite *(Plasmodium berghei)*

Plasmodium berghei (NK65) strain was obtained from malaria unit of Nigeria Institute for Advanced Research and Training, UCH, Ibadan, Oyo State (IAMRAT). The parasites were maintained by serial passage of blood collected from an infected donor animal to an uninfected mouse using the method of Aina *et al*. [11]. Each mouse was inoculated with $1x10^6$ parasitized red blood cells suspension blood in phosphate buffer saline. The newly infected animals were left for 7days for the parasite to manifest.

2.3. Drug Preparation and Treatment of Infection

Five Arthemeter drugs bought from different geographical locations in Ekiti State (Iyin, Otun, Ido, Ijero and Ilawe Ekiti) were used for this study, they were coded Art-Ar, Art-Ph, Art-Ki, Art- Na and Art-Gy respectively. The method of Okafor *et al*. [13] was employed for the drug preparation and administration. The weights of the mice were measured in order to determine the quantity of the drug to be administered, each Arthemeter injection was dissolved in 10ml of distilled water (which is equivalent to 50 mg in 1 ml of water), 0.01 ml of the prepared drug solution was administered into the mice following 24 mg/kg body weight standard of oral Arthemeter administration for 10 days with the exception of the control groups. Chloroquine and 0.5ml of 0.9% normal saline (placebo) was administered to the positive control group and the negative control group respectively for the same period of time.

2.4. Determination of Parasitemia

Thin blood film was made by smearing a drop of blood from the tail snip of each mouse on a microscopic slide from day one of the treatment according the method used by David *et al*. [14]. Each smear was dried at room temperature, fixed with methanol, stained with Giemsa stain and allowed to dry for 30 minutes. They were flooded with water, dried and viewed under the oil immersion objective lens of light microscopy. The parasitemia level was determined by counting the number of parasite against the number of red blood cells using the following relation

$$P\% = \frac{E_n}{E_T} \times 100$$

Where P% = Percentage parasitemia, E_n = number of infected erythrocytes counted and E_T = total erythrocytes (infected and non - infected).

2.5. Responses of Infected Mice to Arthemeter

The responses of the mice to the drugs were determined by counting the number of parasites in a thin blood film made every 24 hours for a period of 10 days according to the method of Ologunde *et al*. [15]. Percentage Clearance rate was calculated using the following relation.

$$C\% = \frac{E_c - E_t}{E_c} \times 100$$

Where C% = percentage clearance rate, E_c = counted infected erythrocytes (control) and E_t = counted infected erythrocytes (test).

3. Results and discussion

The results presented in Figures 1 – 7 show mice responses to treatment in the seven groups respectively. All the animals in the negative control group died after 10days while those in the experiment survived. Paired test was used for statistical significance. There was no significant difference in the average parasite clearance time (PCT) between the Arthemeter drugs.

In Figure 1 to 5, the parasitemia level increases from day 0 to day 3, the drug administered (arthemeter) had not started to manifest on the malaria parasites, but begin to decrease till the end of the treatment period. Also, the parasites clearance rate was very low from day 0 to day 2 and later begin to increase, this time the drug had started to show it effects on the malaria parasites and was effective and had killed nearly all the parasites. The drugs significantly cleared the parasites in less than 10 days in all the mice treated when compared with the negative control. This shows that there is no adulterated arthemether in Ekiti State, this result corroborates the work of Udeme and Omotayo [3], that observed efficacious in the five brands of artemisinin combination therapies used on swiss albino mice infected with *Plasmodium berghei*

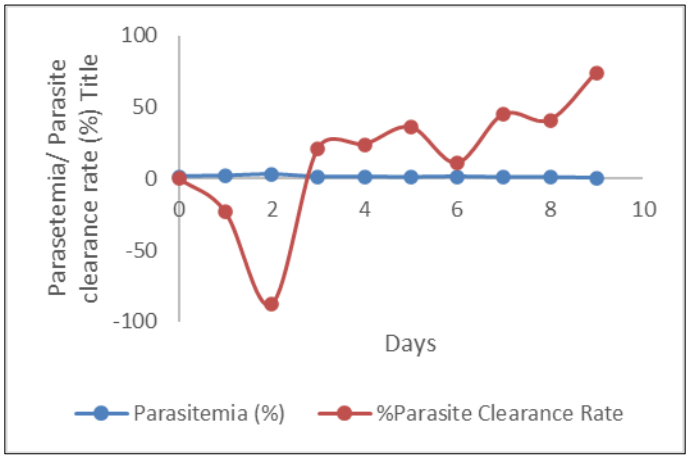

Figure 1 Percentage parasitemia and parasite clearance rate (Art – Ar) in group 1

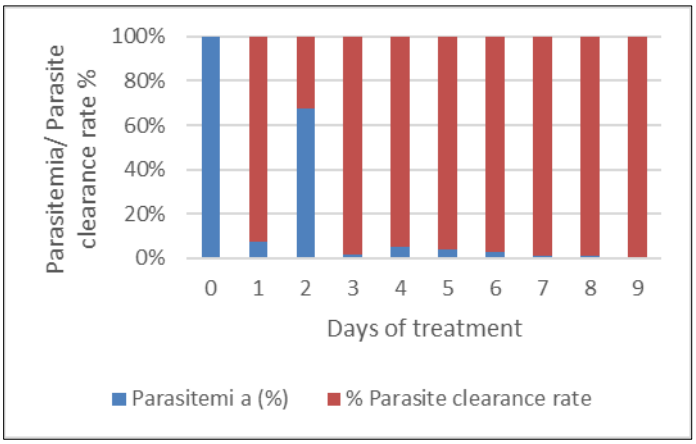

Figure 2 Shows percentage parasitemia and parasite clearance rate (Art – Ph)

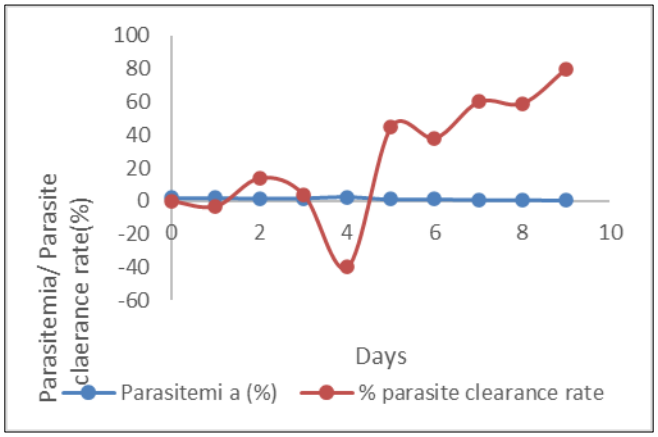

Figure 3 Parasitemia level and parasite clearance rate (Art – Ki)

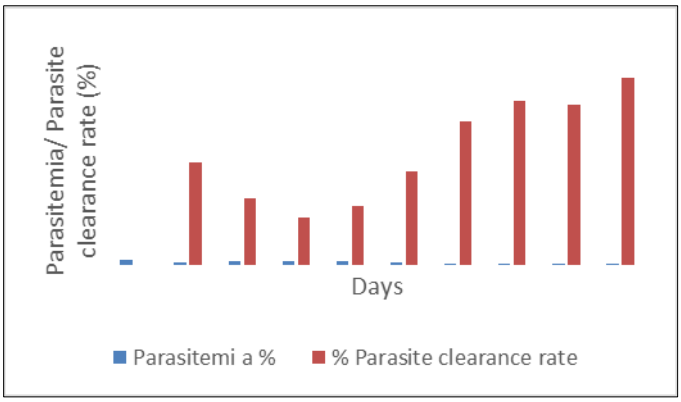

Figure 4 Percentage parasitemia and parasite clearance rate (Art – Na)

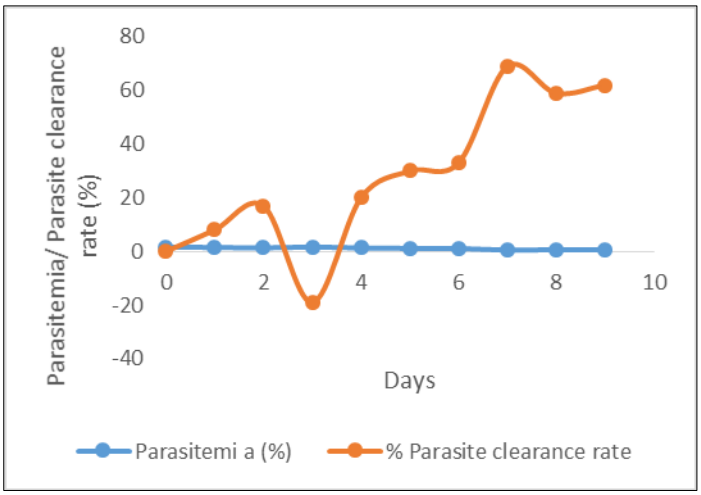

Figure 5 Parasitemia and parasite clearance rate of (Art – Gy)

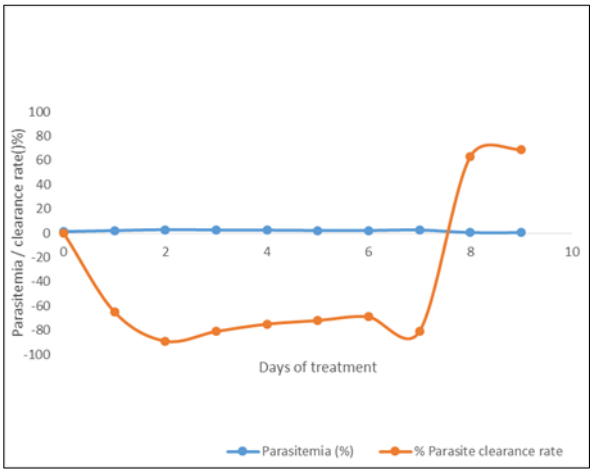

Figure 6 Parasitemia level and the clearance rate in the negative control group

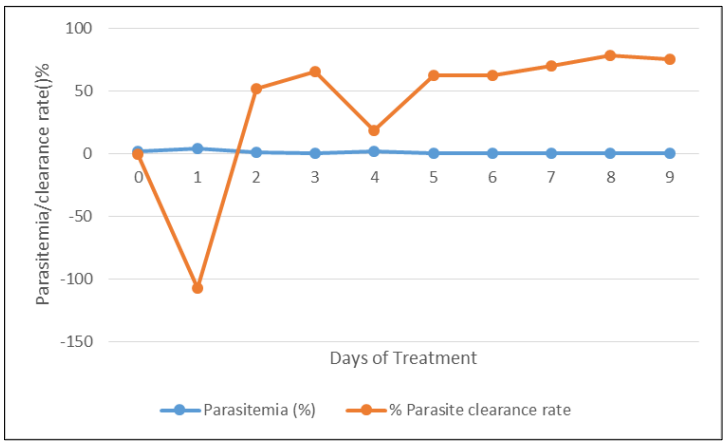

Figure 7 Parasitemia level and the clearance rate in the positive control group

Figure 6 shows the parasitemia level and parasite clearance rate in the negative control group, the parasitemia level increases until day 7 and decreases again from day 8 and 9, this may be due to sudden development of immunity in the mice at day 8 and 9. The parasite clearance rate was very low compare to the other treatment groups.

In the positive control, the parasitemia level decreases and the clearance rate was high, this is an indication that the drug (chloroquine) used was effective on the malaria parasites. The present level of chloroquine (CQ) administered to mice seems to significantly cleared the parasite**,** this results suggest that there is a threshold level of parasitemia in bloodstream of mice. but the use of chloroquine as the first line of treatment for malaria in Nigeria and other parts of the world had been stopped (Federal Ministry of Health, Nigeria).

4. Conclusion

The result shows that Arthemeter drugs brought from different locations showed an effective clearance of the *Plasmodium beighei* injected into the mice without any significant difference. This is an indication that there was no adulterated drug among the arthemeter drugs purchased in Ekiti State. As a result of this, the government should adopt Arthemeter antimalarial drug as the first line of treatment of malaria in Nigeria and other parts of the Africa. In addition, Government should empower the Drug Enforcement Agency in the country in search for adulterated drugs.

Compliance with ethical standards

Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

Statement of ethical approval

Ethical approval for the study was obtained from the Research Committee of the Federal Polytechnic, Ado-Ekiti, Ekiti State, Nigeria.

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