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IN-VITRO EVALUATION OF POTENCY OF BRANDS OF CO-ARTEMISININ AGAINST *Plasmodium falciparum* STRAINS IN KANO, NIGERIA

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ABSTRACT
The short term in-vitro growth of plasmodium falciparum in asexual erythrocyte stage and the in-vitro activities of fourteen different standard artemisinin combination antimalarial drugs bought in duplicates were assessed and compared using the Roswell park memorial institute media (RPMI 1640) medium supplemented with 10% rabbit serum. The test shows that about 80% 0f the drugs were active in-vitro against P. falciparum. No statistical significant difference was observed in terms of antiplasmodial activities between the open market brands and the brands bought from the pharmacy within the prescribed shelf life of the drugs.

INTRODUCTION

Malaria is a disease of global health importance. It is one of the world most problematic disease endemic to many tropical regions in which Africa to be precise Nigeria is one of them. Its social and economic burden is a major obstacle to human development in many of the world poorest countries. In heavily infected countries, malaria alone accounts for as 40% of public health expenditure, 30% - 50% hospital admissions and up to 60% of outpatient visits (WHO, 2007). It has an annual incidence of approximately 250 million episodes and is the cause of 3 million deaths, most of them infants, young children and pregnant women (WHO, 2008).

Malaria is caused by a parasite in the genus *plasmodium* and five different species affecting humans have been identified so far. At the present, there is no known successful researched protective vaccine for the disease but there are available treatment options worldwide.

The center for disease control (CDC, 2008) has stated that drug resistance has rapidly become an issue in malaria control and eradication especially in *Plasmodium falciparum* malaria.

The control and eventual complete elimination of the disease depends on some set of factors that though small but seems complicated (Tjitra *et al.*, 2008). These include control of the anopheline mosquito vector by using insecticide –impregnated bed nets and indoor residual spraying (okumo, et al,2011) but their efficacy will be next to useless without coincident effort directed towards the parasite it selves in cases of infection. As a result of this, an effective and appropriate use of antimalarial drugs remains the best option in the control of *falciparum* malaria

Drugs have two key roles in malaria control. First immediate and effective treatment of malaria prevents progression to severe cases of the disease and limits the development of gametophytes, thus blocking the transmission to mosquito. Gosling et al (2001). Second drug can be used to prevent malaria in endemic populations, including various strategies of chemoprophylaxis, intermittent prevention therapy, and mass drug administration (Greenwood (2010).

Antimalarial drugs acts principally to eliminate the erythrocytic stages of malaria parasites that are responsible for human illness and artemisinin based combination therapy ACTs are recommended by (WHO) as the best treatment option so far and the combination include artemether/lumefantrine, artesunate/amodiaquine, artesunate/mefloquine, dihydroartemisinin/piperaquine, artesunate/pyronaridine and artesunate/ sulphadoxine pyrimethamine these are mostly available in the market (WHO, 2006).

Due to the unusual high incidence of drug resistance in Thailand and Cambodia, and some high clearance and treatment failure rate that were being clinically reported with some ACTs use, a conclusion was made that preliminary evidence had been gathered that artemisinin resistance had emerged along the thai-Cambodia border (Penh, 2007). Recently another study was published in the New England Journal of Medicine that revealed out of 60 people receiving artesunate treatment two were classified as having artemisinin resistant infection Noedl (2008). In Africa because of the low research carried out in the area it is yet uncertain whether co-artemisinin resistance has emerged hence this is what prompts the current investigation.

It is therefore the aim of this study to carry out a comparative study on the potency of various brands of co-artemisinin bought from the open market and pharmacy against strains of *Plasmodium falciparum* in Kano. This is with a view to examining the impact of storage conditions of the drugs on its activity against *P.falciparum* strains in Kano Metropolis

MATERIALS AND METHODS

Sample collection

The sabon Gari Market a central open drug centre was surveyed for the various types of co-artemisinin brands. These brands were purchased and each of the brand combination that was purchased from the market was also purchased from the pharmacies in Kano city. Shops in the market were searched visually and via oral interview for the different combinations of co-artemisinin they have and asking them to present sample for visual analysis.

Sampling of co-artemisinin

Samples of fourteen brands of artemisinin combination were obtained between the periods September 2010 to march 2011. Shops were selected at random and all the five different brands specified by W.H.O were purchased and analyzed. The samples were coded A – N and the duplicated samples from the pharmacy were numbered 1 – 14.

Pharmaceutical analysis on the drugs

Examination of pharmacopeia depends on the drugs. The drug generic name/ indication, country of origin, NAFDAC number, Manufacturing and Expiring date, batch number, nature of pack, combinations, stated weight in mg, color of tablets, quality of pack, availability of the Product were all physically assessed and recorded accordingly.

Sourcing of Malaria Parasites

Infected blood sample with malaria parasite were obtained from Lamco Medical Laboratory and the Bayero University campus clinic. The blood samples were transported to the Laboratory in an ice cooler at temperature of 4^0C in EDTA coated disposable plastic sample bottle.

Identification of Plasmodium in Blood Sample

Preparation of the thin smear was carried out using the sample in the laboratory to check for percentage parasite in the sample before the experiment begins. This was examined using a light microscope under 40x and 100x objectives. The percentage of parasitized cell was obtained using the formulate of Catelli and Carosi (1997)

% parasite= number of parasitized red blood cell/total no of red blood cell x100

Preparation of culture medium

With a sterile disposable 5ml syringe, 2ml venous blood was withdrawn from the main jugular vein of a rabbit. The sample was placed in a sterile test tube and defibrinated as demonstrated by Decie and Levies (1968). The defibrinated blood sample was centrifuged at 1500rpm and the supernatant layer was collected in a sterilized test tube. The serum was collected and 10% was supplemented with 18ml of the salts of Roswell park memorial institute culture medium (RPMI 1640 media) , (Kirsten, et al,2008) after autoclaving. And this was sterilized by filtering with membrane filter

Preparation of the ACT concentration for the test.

All the artemisinin combination drugs were prepared in dimethylsulphuroxide (DMSO). 0.1 g of the grounded powder of each drug was dissolved in 1ml of DMSO to prepare a standard constant concentration of each of the drugs. The calculation of the equivalence of artemisinin present in each of the drug was calculated based on the amount of active compound present in each 100mg of the test drug .(Sharon et al,2010)

Drug sensitivity Testing

The blood samples were centrifuged at 1500rpm for 7 minutes and the plasma decanted followed by tenfold volume of complete RPMI 1640, without supplementing with rabbit serum. After re-suspending the cells, the cell medium mixture was centrifuged at 1500rpm for 7 minutes and the supernatant medium decanted and washing again. From the final cell concentrate, a thin blood film was prepared for determination of pre-incubation parasite density.

The cell concentrate was then diluted with 19parts RPMI -1640 medium in 10% rabbit serum (Richards and Maples 1979).

The cell-medium mixture (CMM) was added in 0.5ml aliquots to the vial bottles which were predisposed with the 0.1ml of the scheduled drugs after re-suspending the cell layer. These were closed and placed in a candle jar with burning candle upon extinction of the candle, the closed jar was then placed in an incubator and gas phase held at 37.5^0C (Trager and Jensen(1976)). Incubation lasted for 72houes, with a renewal of the gas phase after every 24hours by lighting the candle again. The growth inhibition of the drugs upon the parasites was monitored by preparing thin films of the cell sediments wells with for subsequent staining and reading after incubation. The rate of growth inhibition was evaluated as percentage at 24, 48 and 72 hours bases.

Percentage parasite cleared was calculated as number of parasite remaining after each incubation is been divided by the initial numbers before incubation multiply by one hundred.

RESULTS AND DISCUSSION

Storage conditions of the drugs were up to standard. With all pharmacological information intact. All were tablets in sachets form.

Table 1- Pharmacological features of the co-artemisinin

S/N	DRUG NAME	NATURE OF PACK	COMBINATION	STATED WEIGHT
1	COARINATE	3 WHITE TABS	Artesunate sulfamethoxy-pyrazine-pyrimetiamine	100/250/12.5mg
2	FARENEX	3PINK AND WHITE TAB	Artesunate sulfadoxine pyrimrthamine	200/500/25mg
3	AMALAPLUS	3WHITE AND PEACH TABS	Artesunate sulfadoxine pyrimrthamine	200/500/25mg
4	MALACT	12YELOW AND 3 CREAM TAB	Artesunate amodiaquine hydrochloride	50/153.1mg
5	DART	3YELLOW AND 3CREAM TABS	Artesunate amodiaquine	200/199mg
6	SYNARTEM	12 CREAM AND YELLOW	Arthemether lumefantrine	40/240mg
7	LATESEN	12YELLOW TABS	Arthemether lumefantrine	40/240mg
8	ACTIMAX	12YELLOW TABS	Arthemether lumefantrine	40/240mg
9	ARTELUM	12YELLOW TABS	Arthemether lumefantrine	40/240mg
10	WAIPAACT	12YELLOW TABS	Dihydroartemisinin piperaquine phosphate	30/225mg
11	PALAXIN	6 BLUE TABS	Dihydroartemisinin piperaquine phosphate	40/320mg
12	ARTECXIN	8 GREEN OBLONG TABS	Dihydroartemisinin piperaquine phosphate trimethoprim	32/90/90mg
13	SYNAMEF	3 WHITE AND 3 OBLONG	Artesunate mefloquine	200/250mg
14	THYDOP	3 WHITE AND 3 OBLONG	Artesunate mefloquine	200/275mg

Ten of the brands bought from the market had 100% elimination of p. falciparum samples B, C, I and L did not clear the parasites completely. The initial parasite count was 14 infected cells per 200 red blood cells counted.

Table 2 Rate of inhibitory action of some co-artemisinin drugs on *plasmodium falciparum*

S/N	BRANDCODE	%NO OF PARASITES CLEARED AFTER		
		24HRS	48HRS	72HRS
1	COARINATE	4(29%)	6(43%)	14(100%)
2	FARENEX	4(29%)	6(43%)	12(86%)
3	AMALA PLUS	4%)	5(36%)	13(93%)
4	MALACT	3(21%)	7(50%)	14(100%)
5	DART	6(43%)	10(71%)	14(100%)
6	SYNATEM	2(14%)	7(50%)	14(100%)
7	LATESEN	6(43%)	12(86%)	14(100%)
8	ACTIMAX	1(7%)	8(57%)	14(100%)
9	ARTELUM	2(14%)	3(21%)	6(43%)
10	WAIPAACT	5(36%)	9(64%)	14(100%)
11	P.ALAXIN	6(43%)	10(71%)	14(100%)
12	ATECXIN	2(14%)	7(50%)	12(86%)
13	SYNAMEF	0(0%)	7(50%)	14(100%)
14	THYDOP	6(43%)	9(63%)	14(100%)
CONTROL	O	NON	NON	NON

Eleven of the brands bought from the pharmacy had 100% elimination of *P. falciparum* parasites. Samples B, I and L did not clear the parasite completely after 72% of incubation.

Table 3-Anti-plasmodia activity of the brands bought from the pharmacy

S/N	BRANDCODE	%NO OF PARASITES CLEARED AFTER		
		24HRS	48HRS	72HRS
1	COARINATE	5(36%)	7(50%)	14(100%)
2	FARENEX	3(21%)	6(43%)	13(93%)
3	AMALA PLUS	2(14%)	6(43%)	14(100%)
4	MALACT	3(21%)	9(64%)	14(100%)
5	DART	6(43%)	12(86%)	14(100%)
6	SYNATEM	4(29%)	7(50%)	14(100%)
7	LATESEN	6(43%)	13(93%)	14(100%)
8	ACTIMAX	2(14%)	6(43%)	14(100%)
9	ARTELUM	2(14%)	3(21%)	8(57%)
10	WAIPAACT	5(36%)	9(64%)	14(100%)
11	P.ALAXIN	4(29%)	8(57%)	14(100%)
12	ATECXIN	2(14%)	7(50%)	12(86%)
13	SYNAMEF	4(29%)	9(64%)	14(100%)
14	THYDOP	6(43%)	10(71%)	14(100%)
CONTROL	O	NON	NON	NON

CONCLUSION

Malaria is a major global health problem, and the emergence of multi-drug resistance to existing drugs is an increasing problem. Drug testing through in-vitro evaluation test is crucial for malaria research. It is important to determine if resistance is occurring early on in order to look for a workable measure to prevent it, which include formulation and alteration of drug regiments, education and raising awareness of the impact of this in the nearest feature . From the research work carried out; six different combinations were seen to have eliminated the parasites completely totaling up to 78.6% while 21% did not totally eliminate the parasites. The combination that eliminated the parasites 100% included artesunate amodiaquine (AA) and artemether lumefantrin (AL) which is consistent with previous clinical and in-vitro studies performed in the past (Aminu et al, 2012). Storage conditions did not affect the efficacy of the drugs irrespective of the source. There was no significant difference in the antiplasmodial activity. This finding also means that for drugs with a delayed effect a longer incorporation time is needed to determine the real antimalarial activity after a full cycle. The incubation time should therefore be increased past the 72hrs that was selected for this research. Thus artemisinin combination can still be effectively used to treat uncomplicated *falciparum* malaria in effectively in Kano metropolis.

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