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Full Length Research Paper

In-vitro Comparison of Antiplasmodial Efficacy of Chloroquine Formulations from Local and International Manufacturers Available in Kano

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The world Health Organization's committee for the review of medicines encourages a continuous post-marketing surveillance for quality of essential drugs among which chloroquine is one. In compliance with that, samples of oral chloroquine brands belonging to twenty manufacturers both foreign and Nigerian, ten in each case that were on sale in Kano market between January and August, 2004 were investigated for antimalarial quality. *Plasmodium falciparum* infected erythrocytes were used for the assay of the samples at 24, 48 and 72 h of incubation at 37°C. Overall, antiplasmodial activity of 90% of the Nigerian brands were found to be in conformity with the WHO acceptable limits revelations, against 50% of their foreign counterparts. Nigerian products had the highest frequency of passes in the test. It can therefore be inferred that Nigerian pharmaceutical industries are complying with the current mandate of ensuring good manufacturing practice, a crusade by NAFDAC.

Keywords: Antiplasmodial activity, Nigerian, foreign, chloroquine brands, quality.

INTRODUCTION

Malaria (Plasmodiasis) continues to be a growing health problem of global dimensions. Clinical cases reached 300 to 500 million annually most of which are in sub-Saharan Africa (Hanne et al., 2002). In addition to a yearly mortality rate of 1.1 – 5 million and mostly among child-ren (< 5 years), malaria puts a heavy burden on the developing world by exhausting the resources of their health system and by associated loss of economic activity (Patrick, 2001). Factors, such as the species of malaria parasites that occur in a given area, for example *P. falciparum* in west and sub-Saharan Africa, susceptibi-lity to commonly used antimalarials, the distribution and efficiency of mosquito vectors, climate, environmental sanitation and drainage are what make the disease to vary widely in epidemiology and clinical manifestations in different parts of the world. These are what continue to necessitate a daily malaria control and prevention efforts (Bloland, 2001).

James et al. (1996) reported that inspite of available promising preventive measures, such as house and bed netting, use of mosquito repellants and environmental hygiene, chemoprophylaxis (using currently available drugs) is to date the easiest and most readily available strategy in the world wide fight against the scourge of malaria in endemic areas. Most of these drugs were developed on the basis of their action against asexual erythrocytic forms of the parasites namely, *P. falciparum, P. ovale,* and *P. malariae.*

Bakare et al. (2004) reported that there are different brands of antimalarials including chloroquine, quinine, mefloquine and halfanthrin. Other useful but slower acting antimalarial drugs includes pyrimethamine, sulfo-namide and tetracyclines. These are usually used in combination with other antimalarial compounds.

The increasing demand for antimalarial drugs has resulted into an increasing number of pharmaceutical manufacturers both local and foreign that produce such drugs to cater for the lucrative market that ensured. However, against this background, the drug users and

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prescribers expressed fear on the quality in some brands that circulates. The issue of faking and counterfeiting is now a much talked about matter especially with such popular drugs like chloroquine. A portion of the public are at a view that the local brands of the drugs are incriminated for appearing with poor quality in terms of antimalarial activity. However, another segment of the population suspect that the foreign brands have poor quality (Mustapha and Mukhtar, 2006). This is inspite of the well known effort of Nigeria's National Agency For Drug Administration and Control (NAFDAC) in its crusade to ensure the supplies of safe and efficacious drugs. There could have been a greater opportunity for connivance with importers and the manufacturers towards increasing profit at the expense of quality (Mustapha and Mukhtar, 2006).

The present work was therefore planned with the aim of assessing the antiplasmodial activity of both the foreign and local brands of oral chloroquine formulations that were sold in Nigeria via Kano drug outlets. This is with a view to comparing the degree of compliance of the industries to NAFDAC (2002) acceptability specifications and good manufacturing practice. This can resolve speculations and can encourage healthy competition among the manufacturers when considering economic point of view.

MATERIALS AND METHODS

Sampling sites and sample selection criteria

The Kano main market for drugs – Abubakar Rimi market pharmacy and patent medicine shops within the metropolitan Kano served as the sampling points for any available tablet, capsule and/or caplet of chloroquine. Samples of ten Nigerian and ten foreign brands of the drugs were purchased anonymously as any other purchaser in duplicate from shops in ten different sites. The samples from 10 local industries were labelled A – J while the foreign ones K – T. The British Pharmacopoeia reagent standard (BPCRS) chloroquine (used as control) was kindly supplied by the Drug Manufacturing Unit of Kano State Drug Management Agency.

Assessment of pharmaceutical information on the labels of the samples

As specified by the BP (1993) label and details of presentation such as NAFDAC registration number, manufacturing and expiry dates, batch number, stated amounted of active ingredients, indications, address of manufacturers as well as nature of packaging were noted.

Preparation of *P. falciparum* culture medium

Venous blood (2 ml) from the main vein of white healthy rabbits pinnae was withdrawn using a disposable 5m syringe (BD, 205WG). This was defibrinated by allowing it to settle for at least one hour (Dacie and Lewis, 1968). The defibranated blood was centrifuged at 1500 rpm using Spectra Merlin Centrifuge for 10 min and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500 rpm for five minutes, and the supernatant layer was added to the first test tube. The sediments were discarded and the serum collected was supplemented with the salts of RPM1 1640 medium (KCl 5.37 mM, NaCl 10.27 mM, $MgSO_4$ 0.4 mM, Na_2HPO_4 17.73 mM, Ca $(NO_3)_2$ 0.42 mM, $NaHCO_3$ 2.5 mM and glucose 11.0 mM (BDH, Ltd, UK), as demonstrated by Devo et al. (1985). The medium was sterilized by 40 g/ml gentamicin sulphate (Trager, 1982).

Sourcing of chloroquine sensitive *P. falciparum*

P. falciparum positive human blood samples were obtained with kind permission from Lamco, Yantawa medical laboratories and Murtala Muhammad Specialist Hospital, Kano Haematology Department.

Infected erythrocytes were concentrated after a stepwise centri-fugation at 2500 rpm for 15 and 10 min. Geimsa's staining techni-que was employed to search thoroughly for the parasite infected erythrocytes under x100 oil immersion objective of a microscope (CETI, Belgium). An average parasitaemia of 5% per microscopic field using five fields were determined with appropriate dilution of the erythrocytes.

A 0.5 ml of 0.5 mmol/L solution of chloroquine BPCRS and 0.2 ml of the culture medium were added into a tube containing 0.1 ml of 5% parasitaemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to chloroquine BPCRS was determined microsco-pically after incubation for 24, 48 and 72 h at 37^{O}C. The incubation was undertaken in glass bell jar containing a lighted candle to ensure the supply of required quantity of CO_2 (about 5%), O_2 gas (2%) and about 93% nitrogen gas as demonstrated by Hanne et al. (2004).

Assay for the anti-plasmodial activity of the chloroquine samples

The same procedure as used in the determination of BPCRS chloroquine sensitive *P. falciparum* described above was equally followed in this section. The control tubes contain the parasite but without drug samples (negative control) and parasite with BPCRS chloroquine (positive control).

Determination of the activity of the samples

At the end of the incubation periods (24, 48, 72 h) a drop of a thoroughly mixed aliqout of the culture was smeared on a microscopic glass slide and stained by Giemsa's staining technique. The mean number of erythrocytes appearing as blue discoid cells containing life ring of the parasite (that appeared red pink) were estimated and the average percentage elimination by the samples was determined in compliance with that of the BPCRS control tube. The activity of the test samples was calculated thus;

Percentage activity of sample = $(N_S/N_{BPCRS}) \times 100$

Where; N_S = number of parasite eliminated by the sample, N_{BPCRS} = number of parasites eliminated by the control (BPCRS chloroquine).

RESULTS AND DISCUSSION

The detail pharmaceutical information about the samples of the brands of chloroquine from both foreign and local sources is shown in Table 1. Ninety percent (90%) of the Nigerian brands and 60% of the foreign ones registered with NAFDAC.

Table 1. Pharmacological information of the Nigerian and foreign brands of the oral chloroquine formulations sampled in Kano, Nigeria.

Brands code	Country of Origin	NAFDAC No.	Man. Date	Exp. Date	Batch No.	Nature of pack	Stated weight of chloroquine (mg)	Colour of tablets, caplets, or capsules	Quality of pack	Availability of the Product
A	Nigeria	04 – 0564	11/03	10/06	262	Sachet of tabs dark brown	250 mg (Eq. 150 mg base)	White tabs	Very good	Readily
B	Nigeria	04 -2601	07/02	07/07	22076	Sachet of 6 caplets	500 mg (Eq. 300 mg base)	White caps	Very good	Readily
C	Nigeria	04 -3652	05/03	04/06	32041	1000 pack	250 mg (Eq. 150 mg)	White tabs	Good	Not Readily
D	Nigeria	04 -2214	06/03	05/07	30221100	Sachet of 50 capsules	500 mg (Eq. 300 mg)	Yellow/white caps	Very good	Readily
E	Nigeria	04 -0946	05/03	05/08	410	Sachet of 5 capsules	400 mg (Eq. 300 mg)	White powder	Very good	Readily
F	Nigeria	04 -2855	07/02	06/05	07053	1000 pack	250 mg (Eq. 150 mg)	Light/green caps with white powder	Good	Readily
G	Nigeria	-	-	04/06	001	1000 pack	250 mg (Eq. 150 mg base)	White tabs	Good	Readily
H	Nigeria	04 -2378	05/03	03/07	0283	1000 pack	250 mg (Eq. 1500 mg)	White tabs	Good	Readily
I	Nigeria	04 –0333	04/04	07/06	16122004	Sachet of 10 capsules	250 mg (Eq. 150 mg)	White tabs	Very good	Readily
J	Nigeria	04 -2912	07/03	09/06	5733	Sachet of 6 capsules	500 mg (Eq. 300 mg)	White tabs	Very good	Readily
K	India	04 -785	09/03	01/06	3008	1000 pack	250 mg (Eq. 150 mg)	Yellow/green caps White powder	Good	Not Readily
L	India	04 – 933	02/03	01/06	3302	1000 pack	250 mg (Eq. 150 mg)	white tabs	Good	Not Readily
M	India	-	09/02	08/05	G189	Sachet of 10 tabs	250 mg (Eq. 150 mg)	White tabs	Very Good	Not Readily
N	India	-	02/03	01/06	3001	Sachet of 10 tabs	250 mg (Eq. 150 mg base)	White tabs	Very Good	Not Readily
O	China	04 -1688	02/03	02/06	030307	1000 pack	250 mg (Eq. 150 mg)	White caps	Good	Not Readily
P	China	04 -3332	03/03	03/06	030307	Sachet of 10 tabs	250 mg (Eq. 150 mg)	White caps	Good	Readily
Q	China	-	08/02	08/05	20020801	1000 pack	250 mg (Eq. 150 mg base)	White tabs	Good	Readily
R	Germany	-	03/02	02/06	540	1000 pack	250 mg (Eq. 150 mg)	White tabs	Very good	Not Readily
S	India	04 – 3005	03/03	02/06	009	Sachet of 10 tabs	250 mg (Eq. 150 mg)	White caps	Very good	Readily
T	England	04 - 2102	07/02	07/05	20020701	Sachet of 10 tabs	250 mg (Eq. 150 mg base)	White tabs	Very good	Readily

The manufacturing dates of up to 70% of local varieties were between May and November, 2005 and 50% of them were due to expire between April and October, 2006. 60% of the foreign ones were manufactured bet-ween February and March, 2003 and all expired by February to August, 2005. Furthermore, both local and foreign chloroquine brands had standard batch numbers. Fifty percent (50%) of the foreign brands were imported from India, thirty percent (30%) from China, and the remaining twenty percent from Germany and England. Nigerian products were available in 80% of the shops visited compared to 40% of their foreign counterparts. The manner of presentation was variable and is indepen-dent of the country of origin. Some appeared in a sachet of 10 tablets or in a jar of 100 tablets. Some (15%) are however formulated in caplet and another 15% in capsular form but all with their stated amount of the active ingredients between 150 mg and 300 mg chloro-quine base in an easily divisible form (excepting the powdered capsule) to suite the Kg body weight regimen during administration.

The antimalarial activities of the samples showed that all the Nigerian brands had 100% elimination power at the end of 72 h of incubation except sample D which recorded 80% (Table 2). Comparatively, 50% of the fore-ign brands had 100% elimination power. These samples included K, L, M, N and O while the remaining (R, S, T, P, and Q) had eliminated between 27.8 – 77.8% of the *P. falciparum* at the end of incubation period (Table 3).

Loban and Polazok (1989) reported that all qualitative chloroquine samples should be able to produce a pharmacologic effect of clearing 100% of the sensitive malarial parasites in the first three days of the therapy. For this reason, 10% of Nigerian and 50% of foreign chloroquine oral formulations were said to have failed the test. The brand R which was labeled as coming from an industry in Germany had no NAFDAC registration number and has grossly failed by showing only 27.8% activity. This is followed by brands S, P and T from India, China and England with 75.5 – 76.0 and 76.5% quality index respectively. In an effort to assess the quality of some antimalarials other than chloroquine, Mustapha and Mukhtar (2006) did not find any drug that failed. Perhaps adulteration was low in these drugs since they were not as popular or marketable as chloroquine brands. Possibly the shortfall in the activity of such brands encountered in the present study was due to a presence of inadequate concentrations of the active ingredients either due to photoreaction with light on transit, chloroquine being highly sensitive to light or because of a deliberate act of counterfeiting (NAFDAC, 2002) which appeared easier abroad than within Nigeria inspite of all stringent measures being taken before importation.

Table 2. Average percentage activity of the Nigerian chloroquine brands as compared with that of BPCRS against *P. falciparum* at different incubation periods.

Brands codes	Average % elimination of parasites during incubation period (Hrs)		
	24	48	72
A	56	89	100
B	60	92	100
C	60.5	91	100
D	45	63.3	80
E	56.3	88.8	100
F	57.8	90	100
G	59.8	90.5	100
H	58.5	91.3	100
I	60.5	92	100
J	54.3	86.5	100
BPCRS	60	95	100

BPCRS = British pharmacopoeia reagent substance (chloroquine phosphate).

Table 3. Average percentage activity of the foreign chloroquine brands as compared with that of BPCRS against *P. falciparum* at different incubation periods.

Brands codes	Average % elimination of parasites during incubation period (h)		
	24	48	72
K	58.5	89.3	100
L	55	90	100
M	58.3	91	100
N	58.3	91	100
O	54.3	87.5	100
P	42.5	59.8	76.5
Q	44.5	59.5	77.8
R	17.5	22.5	27.8
S	43	66.3	75.5
T	44.5	61.5	76
BPCRS	60	95	100

BPCRS = British pharmacopoeia reagent substance (chloroquine phosphate).

Conclusion

There were many brands of both local and foreign chloroquine oral formulations on sale at Kano within the study period although locally manufactured brands were most available and qualitative. This shows that the Nigerian pharmaceutical industries had vigorously imbibed the active campaign of the nation for good manufacturing practice advocated by NAFDAC. It can be recommended that clinicians and patients could patronize the chloroquine oral formulations produced in Nigeria.

REFERENCES

Bakare OMT, Garba M, Balat I, Yakasai IA (2004). Chemical quality control of some brands of chloroquine. Proceedings of the 31st annual

conference of the West African Society for Pharmacology. Bayero University, Kano 11 – 16th Oct. 2004.

Bloland PB (2001). Malaria treatment policy in the face of drug resistance. Trop. Med. Parasitol. 93(1):5–23.

BP (1993). British Pharmacopoeia. Quality indices of chemotherapeutic agents. Her Majesty Stationery Office, London. Vol. 2.

Dacie JV, Lewis SM (1968). eds. Practical Haematology. 4th ed. Blackwell Publishers, USA. pp. 65 – 66.

Devo AA, Greary TG, Davis NL, Jensen JB (1985). Nutritional requirements of *P. falciparum* in culture exogenously supplied with dialyzable components necessary for continous growth. J. Protozool. 32: 59 – 64.

Hanne IZ, Dan S, Jette C, Lars H, Henry H, Jerzy WJ (2002). *In-vitro P. falciparum* sensitivity assay. Antimicrobial agents and chemotherapy, 42(6): 1441–1446.

James W, Leslie T, Webster J (1996). Drug used in chemotherapy of protozoal infection In: Goodman A, Gilman E, hardman JG, Limbird LE (1996). The Pharmacological basis of therapeutics. 9th ed. Mc Graw Hill Ltd, pp. 965 – 980.

Loban KM, Polazok ES (1989). eds. *Malaria.* 2nd ed. Mir Publishers Moscow. pp. 17 – 248.

Mustapha Y, Mukhtar MD (2006). *In-vitro* assay for antimalarial quality of antimalarial drugs dispensed in Kano. Nig. J. Microbiol. 20(1):729 – 736.

NAFDAC (2002). National Agency for Food, Drugs Administration and Control fight against fake and substandard drugs in Nigeria. NAFDAC Quarterly Bulletin, June, 2002. p. 4.

Patrick J (2001). The role of rapid diagnosis tests in malaria. Africa Health, 23(6):28.

Trager W (1982). Cultivation of malaria parasites. British Med. Bull. 38(2):129 – 13.