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Full Length Research Paper

Antiplasmodial activity of quinine-zinc complex and chloroquine: A comparative *in vitro* assessment

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The evolving and complicating drug resistance exhibited by strains of *Plasmodium falciparum* to existing antimalarials like chloroquine and quinine (which are relatively more affordable than recent drugs) and advances in metal-drug complex research instigated this work. The antiplasmodial activity of the Quinine-Zinc complex (QZ) synthesized by a modification of the method of Singla and Wadhwa was assessed relative to that of Chloroquine diphosphate (C) *in vitro*. Measurement of antiplasmodial activity was carried out based on the inhibition of parasite growth measured by the inhibition of schizont formation in freshly collected infected blood samples from malaria patients. A comparative analysis of the antiplasmodial activity of QZ against C showed that its antiplasmodial activity was significantly better than that of C ($p < 0.05$). The result of this study suggests that the QZ could have a better therapeutic activity against malaria than C.

Key words: Quinine-Zinc complex, Chloroquine diphosphate, *Plasmodium falciparum*, antiplasmodial activity.

INTRODUCTION

Malaria today accounts for more than 90% of deaths, has a record of about 300 to 500 million infections yearly and kills about 1.5 to 2.7 million people yearly (Goods, 2001; Sachs and Malaney, 2002). Malaria is one of the most common infectious diseases and an enormous public health problem. The disease is caused by protozoan parasite of the genus *Plasmodium*. Five species of the plasmodium parasite can infect humans; the most serious forms of the disease are caused by *Plasmodium falciparum*. Malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* causes milder disease that is not generally fatal in humans. A fifth species *Plasmodium knowlesi*, causes malaria in macaques and can also infect humans. This group of human- pathogenic *Plasmodium* species is usually referred to as malaria parasites.

The efficiency of the malaria parasite in developing resistance to antimalarials including even recently discovered drugs is the most disturbing issue related to malaria research. Today, malaria parasite has been confirmed to show notable resistance to inexpensive drugs like chloroquine, quinine, sulphadoxine/ pyrimethamine and a number of other inexpensive drugs, leaving us with newer drugs which cost 7 to 60 times more (Olliaro et al., 1996).

The resistance of malaria parasite to almost all antimalarials has led scientific researchers into the exploitation of plant materials for the treatment of malaria. Since many drugs for example, quinine and artemisinin were isolated from plants, investigation of active components of plants has gained prominence (Phillipson, 1991). Many of these bioactive phytochemicals may not have been thoroughly studied for toxicity and interaction with other xenobiotics.

Zinc is becoming a topic of interest to scientific researchers in our times. Within therapeutic dosage, it is not known to cause any immediate side effects (Sandstead, 1995; Fosmere, 1990). On the contrary, usage

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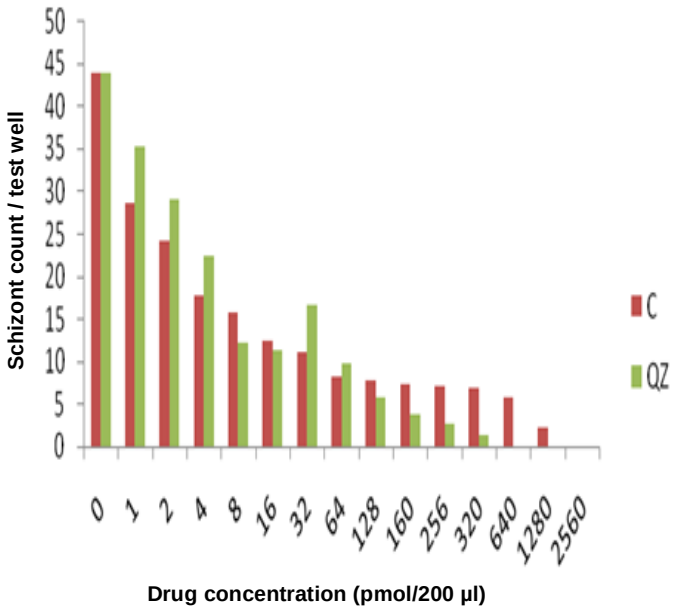

Figure 1. Bar Chart of Schizont count against drug concentration.

of zinc over a long time has been shown to be beneficial in pregnancy (Goldenberg et al., 1995), dermatological issues like acne (Cunliffe et al., 1979), sickle cell anaemia (Gupta and Chanbey, 1995), ulcers (Frommer, 1995) and in sickness (Marshall, 1998, Muller et al., 2001).

The antiplasmodial activity of QZ *in vitro* has been investigated and reported (Ogunlana et al., 2009). This work was designed to compare the antiplasmodial activity of a complex of two well-studied, readily available substances, quinine and zinc (Quinine-zinc complex) with that of Chloroquine diphosphate *in vitro*.

MATERIALS AND METHODS

Quinine-zinc complex had been synthesized and its synthesis has been authenticated by melting point determination, thin-layer chromatography (TLC) analysis, infra red, ultra violet, atomic absorption spectroscopy in our previous work (Ogunlana et al., 2009). The mole ratio of quinine to zinc in the Quinine-zinc complex was also determined in our previous work (Ogunlana et al., 2009).

This work was carried out in the laboratory of the Pharmaceutical Chemistry department, University of Ibadan, Oyo state, Nigeria, and the Institute of Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Oyo state, Nigeria.

Culture procedure

Cultivation of *P. falciparum* from positive blood samples collected from malaria parasites infected individuals, pre-harvest analysis of cultures, harvesting and analysis of post culture slides were carried out using the methods of Trager and Jenson (1976) and Jenson and Trager (1977) as reported in our previous work (Ogunlana et al., 2009).

Comparative determination of antimalarial activity of QZ and chloroquine

In vitro test

World health organization (WHO) standard micro *in vitro* susceptibility (MARK II) technique (WHO, 1990) was used to determine the *in vitro* sensitivity of *P. falciparum* to the QZ and C drugs. The test kits were obtained in IMRAT. 0.0111 g of C was dissolved in 500 ml distilled water (Stock solution1). 1 ml of Stock solution 1 was drawn with pipette into 10 ml falcon tube and made up to 10 ml with complete medium (Stock solution 2). Series of dilution of stock solution 2 were used to obtain 150 µl of samples containing 1, 2, 4, 8, 16, 32, 64, 128, 160, 256, 320, 620, 1280, 2560 pmols of C and used to medicate wells in the columns labeled 1 and 7 of B-H and B_1-H_1 containing 50 µl of culture medium. 0.0128 g of QZ was dissolved in 100 ml water and similar procedure as that used for the preparation of different dilutions of C was followed to obtain same concentrations of QZ as that of C in pmols and used to medicate wells in columns labeled 5 and 11 of the micro plate. 200 µl of complete culture medium was used to medicate wells in row labeled A in the columns as control. The experiment was carried out in duplicates.

Schizont inhibition and maturation were assessed in each well and the data obtained from the wells were processed to determine comparative sensitivity or resistance of *P. falciparum* to the drugs.

Statistical analysis

Students T-test was used for analysis of data for statistical significance at 95% significance level ($p < 0.05$).

RESULTS

From the graph of percentage schizont inhibition versus drug concentration shown in Figure 3, the minimum inhibitory concentration (MIC) of C was 2560 pmol/200 µl while that of QZ was 640 pmol/200 µl, indicating that the amount of QZ required for effective schizonticidal action was three times less than that of C. Drug concentration corresponding to 50% schizont inhibition (IC_{50}) for QZ and C were 3.98 and 3.35 pmol/200 µl of drugs, respectively. Schizonticidal activity of QZ was significantly higher than that of C ($p < 0.05$). It is important to note that from Figures 1, 2 and 3, at concentrations lower than 128 pmol/200 µl, C exhibited better efficiency at inhibiting schizont growth. However, at drug concentrations of 128 pmol/200 µl, QZ showed better efficiency at inhibiting schizont growth or maturation. Hence schizont inhibition was better in the C-medicated wells than the QZ-medicated wells at concentrations less than 128 pmol/200 µl but schizont maturation was better inhibited in the QZ- medicated wells than the C- medicated wells for drug concentrations greater than 128 pmol/200 µl.

DISCUSSION

From the result shown in Figure 1, the parasite was found

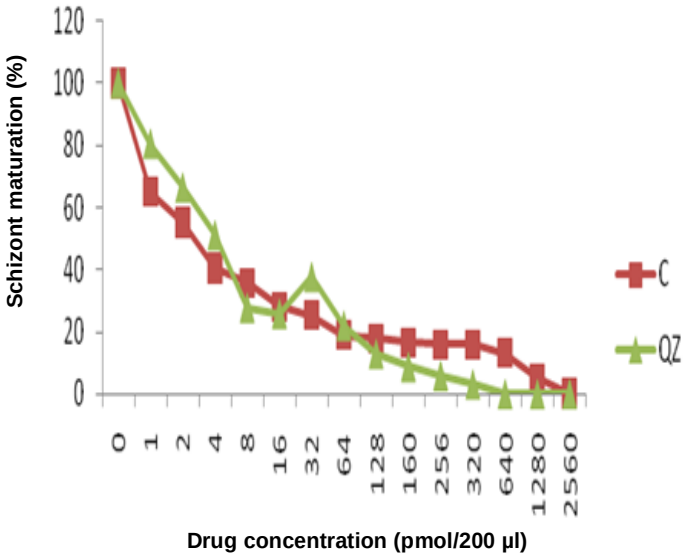

Figure 2. Graph of percentage Schizont maturation against drug concentration.

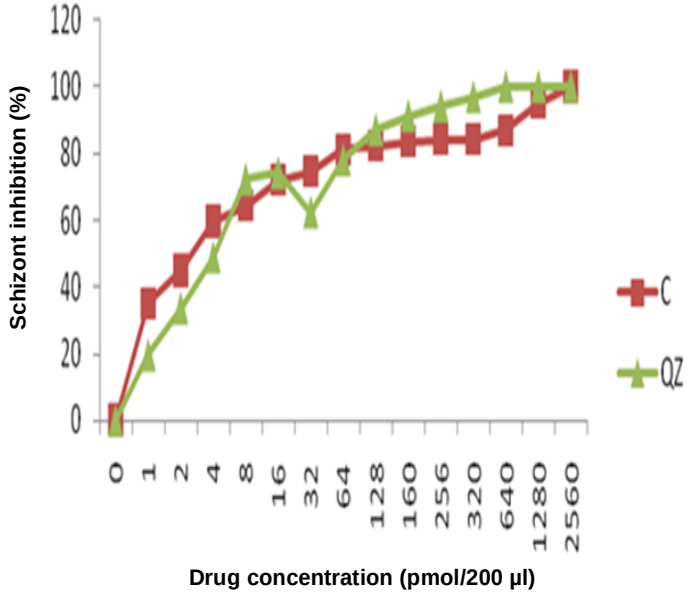

Figure 3. Graph of percentage Schizont inhibition against drug concentration.

to be resistant to chloroquine because there was schizont growth at 8 pmol/200 µl and above. Parasite growth at 8 pmol/200 µl and above is an indication of resistance (WHO, 1990). The parasite was however sensitive to both C and QZ with complete schizont inhibition at 2560 and 640 pmol/200 µl, respectively. The report of Obaleye et al. (2009) on the increased antimalarial activity of some quinolinemethanol complexes compared with chloroquine and parent ligands corroborated the current findings. The antimalarial activity of 7-chloro-4-(1, 4, 7, 10-tetraaza-cyclododec-1-yl)-quinoline-Zn^{2+} complex as well as other 7-chloro-4-aminoquinoline complexes between strong to moderate activities have also been reported (Khan et al., 2009).

Although the exact mechanism of action of QZ is unknown, the report of Chevion et al. (1995) supports the reasoning that the higher schizonticidal activity of QZ compared to that of C may not be unconnected with better permeability of parasite erythrocyte to QZ than that of C and the possibility of the exchange of zinc metals in QZ for ferric ions, thus, rendering the iron unavailable for vital parasite functions. It has also been demonstrated that metal complexes mediate antimalarial activity by inhibiting hemozoin formation through binding to a dimer of hematin (Dorn et al., 1998) and as a result, might lead to accumulation of heme thereby, preventing intraerythrocytic growth and proliferation of the malaria parasite.

Conclusion

From *in vitro* micro test determination, complex was confirmed to have 3- times antimalarial potency over Chloroquine diphosphate. However, before Quinine Zinc complex can be recommended as a better alternative to chloroquine and its various salts forms, its acute toxicity levels *in vivo* must be tested and established. Further research is also required to establish its therapeutic advantage with the use of well characterized susceptible and resistant strains of malaria parasite.

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