

Full Length Research Paper

# Comparative *in vitro* assessment of the antiplasmodial activity of quinine – zinc complex and quinine sulphate

O. O. Ogunlana<sup>1\*</sup>, O. E. Ogunlana<sup>2</sup> and O.G. Ademowo<sup>3</sup>

<sup>1</sup>Department of Biological Science, College of Science and Technology, Covenant University, Kilometer 10 Idi- Iroko Road, Canaan land, Ota, Ogun State, Nigeria.

<sup>2</sup>Department of Biochemistry and Biotechnology, Crawford University, Igbesa, Ogun state, Nigeria.

<sup>3</sup>Institute of Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, Oyo state, Nigeria.

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**Incessant malaria endemicity in the tropics and subtropical regions and the recent work done on the synthesis of metal drug complexes of antimalarial drugs and the evaluation of their antimalarial activities *in vitro*, has led to the development of this study. Quinine-Zinc complex (QZ) was synthesized using a modification of Singla and Wadhwa method. Melting point determination, TLC analysis, infra red, ultra violet, atomic absorption spectroscopy and mole ratio determination were all carried out on the complex synthesized. Direct measurement of the antimalarial activity of the potential new drug (QZ) against parasite growth *in vitro* was used to comparatively ascertain the antimalarial activity of QZ relative to Quinine sulphate (QS). Measurement of antimalarial activity was carried out based on the inhibition of parasite growth with respect to inhibition of schizont formation in freshly collected infected blood samples from patients. The results showed that QZ complex was formed and its antimalarial activity was three times that of QS alone. This study suggests that the quinine-zinc complex could have a better therapeutic activity than quinine.**

**Key words:** Quinine-zinc complex, quinine sulphate, *Plasmodium falciparum*.

## INTRODUCTION

Today, about 300 – 500 million malaria cases each year cause 1.5 – 2.7 million deaths. By this analysis, malaria accounts for more than 90% of deaths in children below 5 years of age in Africa (Good, 2001; Sachs and Malaney, 2002). The challenge of malaria especially to sub Saharan African nations continues to widen without easily defined limits as drug resistance to most antimalarial drugs, insecticide resistance in mosquitoes, and other climatic and social-cultural factors complicate malaria research (Krettli et al., 2001). The development of resistance to antimalarial drugs by malaria parasites is the most disturbing factor. Today, malaria parasite has been confirmed to show notable resistance to inexpensive drugs like chloroquine, quinine, sulphadoxine/ pyrimethamine and a number of other drugs in this category. Newer drugs however cost 7 - 60 times as much as these

(Olliaro et al., 1996). The challenge of drug resistance is leading malaria researchers in the direction of antimalarial medicinal plant research. Since many drugs e.g. quinine and artemisinin were isolated from plants, investigation of compounds within traditional plants is beginning to get attention (Phillipson, 1991). However, a number of these compounds within traditional plants may not have been thoroughly studied for toxicity and interaction with other xenobiotics.

Zinc in the therapeutic dosage form is not known to cause any immediate side effects other than occasional stomach upset usually when taken on an empty stomach (Sandstead, 1995). Instead, long term usage of zinc supplement has been shown to reduce the chances of getting sick (Marshall, 1998). Zinc supplement during pregnancy has been found to improve the birth weight and head size of new born (Goldenberg et al., 1995). Evidence has also shown that when zinc is taken at fairly high doses (100–10 mg/day), it will reduce the symptoms of acne to an extent comparable to that of tetracycline (Cunliffe et al., 1979), prevent the development of sickle

\*Corresponding author. E-mail: [kellybee2001@yahoo.com](mailto:kellybee2001@yahoo.com), [ogunlanaoo@gmail.com](mailto:ogunlanaoo@gmail.com). Tel: 234-802-342-8233

cell crisis in sickle cell anemia patients (Gupta and Chanbey, 1995) and speed up the healing of stomach ulcers (Frommer, 1975).

Due to the anti-ulcer and anti-inflammatory properties of zinc, aspirin-zinc complex has been synthesized in ratio 2:1 of aspirin and zinc respectively (Singla and Wadhwa, 1994). This has been shown to increase the anti-inflammatory effect of aspirin, enhance the gastrointestinal absorption of aspirin and present aspirin in a better tolerated dosage form (Singla and Wadhwa, 1994).

This work is designed with the intention of investigating the possible antiparasitic activity of a complex of two well-studied, readily available compounds, Quinine and Zinc, as a viable and affordable antimalarial option.

## MATERIALS AND METHODS

This research was carried out in three stages namely:

- Extraction and analysis of Quinine sulphate (QS)
- Synthesis and analysis of Quinine-zinc complex (QZ)
- Comparative determination of antiparasitic activity of Quinine sulphate and Quinine-zinc complex

The first two stages were carried out in the laboratory of the Pharmaceutical Chemistry department, University of Ibadan, Oyo state, Nigeria, while the third stage of this work was carried out at the Institute of Medical Research and Technology (IMRAT), College of Medicine, University of Ibadan, Oyo state, Nigeria.

### Extraction of Quinine sulphate

Quinine sulphate was extracted from its tablet form (Quinine sulphate, Cox Pharmaceuticals, AH Cox and Co Ltd., England) according to the method specified in British Pharmacopoeia (British Pharmacopoeia, 1998). Identity and purity assessment tests were carried out using melting point determination and thin layer chromatography (TLC) for both the extracted QS and the standard Quinine sulphate powder (BDH Chemicals, Poole, UK).

### Synthesis and analysis of QZ

A modification of Singla and Wadhwa method was employed (Singla and Wadhwa, 1994). QZ was formed in 60% v/v aqueous ethanolic extract. 7.83 g Quinine sulphate (0.1 moles) was added to 700 ml ethanol. The solution was stirred until dissolution was complete. The solution was then added slowly with constant stirring to a solution of  $ZnSO_4 \cdot 7H_2O$  (1.4375 g, 0.005 moles) in 467 ml water. Turbidity appeared which (between 10 – 15°C) led to formation of white crystals. The crystals were filtered, washed with minimum quantity of cold distilled water, dried under vacuum to constant weight to give QZ.

Infra red spectra of both QS and QZ in the range of 600 – 4000  $cm^{-1}$  were taken with BUCK M500 spectrometer (Buck Scientific, East Norwalk) in nujol. 0.01 g QZ and QS were dissolved separately in 5 ml (0.01 M) HCl. Ultra violet spectroscopy was also carried out on the two compounds using PERKINS ELMER  $\lambda 3b$  pp uv/ visible spectrophotometer (Perkin Elmer Life and Analytical Sciences Inc., USA) with recorder. Zinc content was determined with atomic absorption spectroscopy using AANALYST 200 (PerkinElmer Life and Analytical Sciences Inc., USA). Atomic absorption spectrometry was used in the determination of mole ratio of Quinine and Zinc in QZ.

### Comparative determination of antiparasitic activity of QS and QZ *in vitro*

WHO (1990) standard micro *in vitro* susceptibility (MARK II) technique was used to determine the *in vitro* sensitivity of *P. falciparum* to the QZ and QS drugs. The test kits were obtained from IMRAT. 0.0134 g of QS was dissolved in 100 ml distilled water (stock solution 1). 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  $\mu$ l of samples containing 4, 8, 16, 32, 64, 128, 160, 256, 320, 620, 1280, 2560 pmols of QS and used to medicate wells in the columns labeled 3 and 9 of B - H. 0.0128 g of QZ was dissolved in 100 ml water and similar procedure as that used for the preparation of different dilutions of QS was followed to obtain same concentrations of QZ as that of QS in pmols and used to medicate wells in columns labeled 5 and 11 of the micro plate. 150  $\mu$ l 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.

### Cultivation procedure

The methods of Trager and Jensen, (1976) and Jensen and Trager, (1977) were employed for cultivation of *Plasmodium* culture. Positive blood sample collected from malaria patients in bijou bottle containing ACD (acid citrate dextrose) was suspended and washed three times with incomplete RPMI (Rosewell Park Memorial Institute) 1640 medium (Gibco BRL, Scotland) by centrifugation at 1500 g for 5 min at room temperature. The supernatant and buffy coats were carefully removed aseptically under the lamina flow hood at the end of each wash. In 35 mm sterile plastic Petri dishes, 0.5 ml of the washed packed parasitized red blood cells and 4.5ml of complete medium were mixed gently to start the culture. All the wells with appropriate dilutions of sample drugs in them were dosed with 50  $\mu$ L of the blood/medium mixture in ratio 1:9 respectively using the 50  $\mu$ L Eppendorf pipette and disposable sterile tips. The blood/medium mixture was gently shaken from time to time to ensure that the blood was kept in suspension. The microlitre plates were covered and labeled appropriately and placed in a glass incubator into which a lit candle was put. The desiccator's lid was removed. The culture plates were maintained at 37°C in a water-jacketed incubator for a period of 24 - 30 h.

### Pre-harvest analysis of cultures

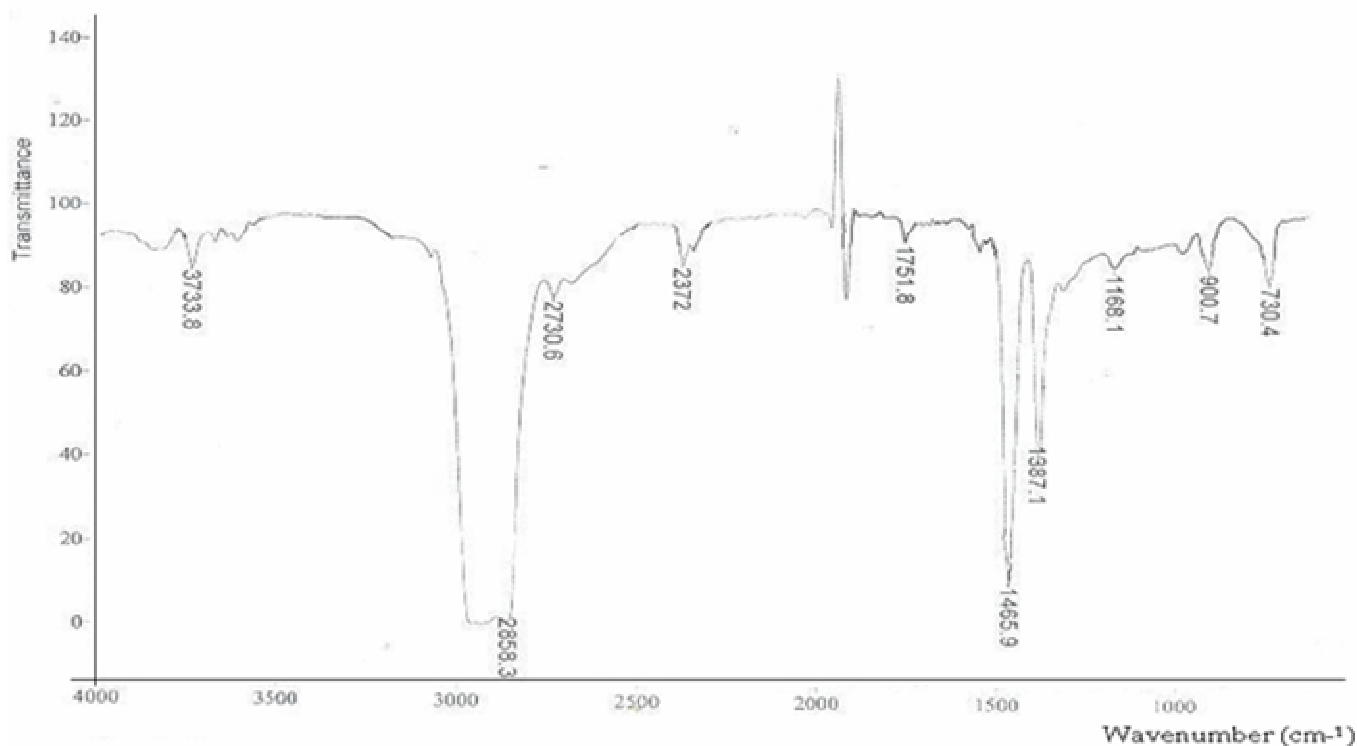
Smear made 24 h after the start of the culture was stained and examined. Harvesting was delayed because majority of the parasites had not matured into schizonts. Cultures were further incubated for another 2 - 6 h until control wells revealed mature schizonts. Harvesting was then carried out.

### Harvesting and preparation of post-culture blood slides

After incubation, the contents of the test wells were harvested by the removal of the supernatants with Pasteur pipettes. The red blood cells deposited on the flat bottom of each well were mixed and transferred carefully to a clean microscope slide, to form a series of thick films. The slides were air-dried for four days and then stained for 20 min with giemsa stain (10%v/v in water, pH 7.2).

### Analysis of the post-culture blood slides

WHO standard counting procedure for quinine-chloroquine post culture thick films (WHO, 1990) was employed. In each thick film, the number of schizonts was expressed as parasites with 3 or more



**Figure 1.** Infra-red spectrum of quinine-zinc complex.

nuclei per 200 asexual parasites, that is, total number of schizonts and trophozoites per 200 ring forms after incubation. The count was then expressed as a percentage of the control.

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 (paired two-sample for means) was used for analysis of data for statistical significance ( $p < 0.05$ ).

## RESULTS

### Identity and purity assessment test

Quinine sulphate was extracted from Quinine sulphate tablets with percentage yield of 98.33%. The extracted QS and reference QS changed color at 190°C and melted with decomposition at 210°C. Single spot was observed for the extracted QS and reference QS on the chromatoplate with the same average retention factor ( $R_f$ ) of 0.87 in methanol and ammonia and 0.26 in chloroform and methanol. Percentage yield of QZ obtained from complexing was 23.57% (1.6061 g). The melting point of the complex was 111°C, melting point of Quinine sulphate was 209°C, while the mixture of QS and QZ had a melting point of 162°C. Thin layer chromatography analysis of QS and QZ gave single spot for each of QS and QZ, and  $R_f$  values 0.45 and 0.20 for QS and QZ respectively.

The major IR peaks for QS in nujol were 730, 1077, 1156, 1235, 1381, 1457, 1624, 2962 while the IR peaks for the synthesized QZ in nujol were 730, 901, 1168, 1387, 1466, 2858, 3733.8 as shown in Figures 1 and 2. The uv spectra of both QS and QZ showed two maxima 203 and 246 nm as represented in Figure 3. There was therefore no difference in the maxima readings of the uv spectra of the two substances.

### Schizont inhibition

From the graph of percentage schizont inhibition versus drug concentration Figure 5 as obtained from data from Figure 4, the minimum inhibitory concentration (MIC) of QS was 2560 pmol/200  $\mu$ l while that of QZ was 640 pmol/200  $\mu$ l, indicating that the amount of QZ required for effective schizonticidal action was three times less than that of QS. Drug concentration corresponding to 50% schizont inhibition ( $IC_{50}$ ) for QZ and QS are 3.98 and 14.13 pmol/200  $\mu$ l of drugs respectively. Schizont inhibition of QZ was significant over schizont inhibition of QS ( $p < 0.05$ ).

## DISCUSSION

The difference in the melting point of the complex (111°C) and that of the mixture of QS and QZ (162°C) indicated that the substances mixed were different, and supported the claim that QZ was synthesized. Comparative TLC analysis of QS and QZ showed that  $R_f$  for QS was 0.45

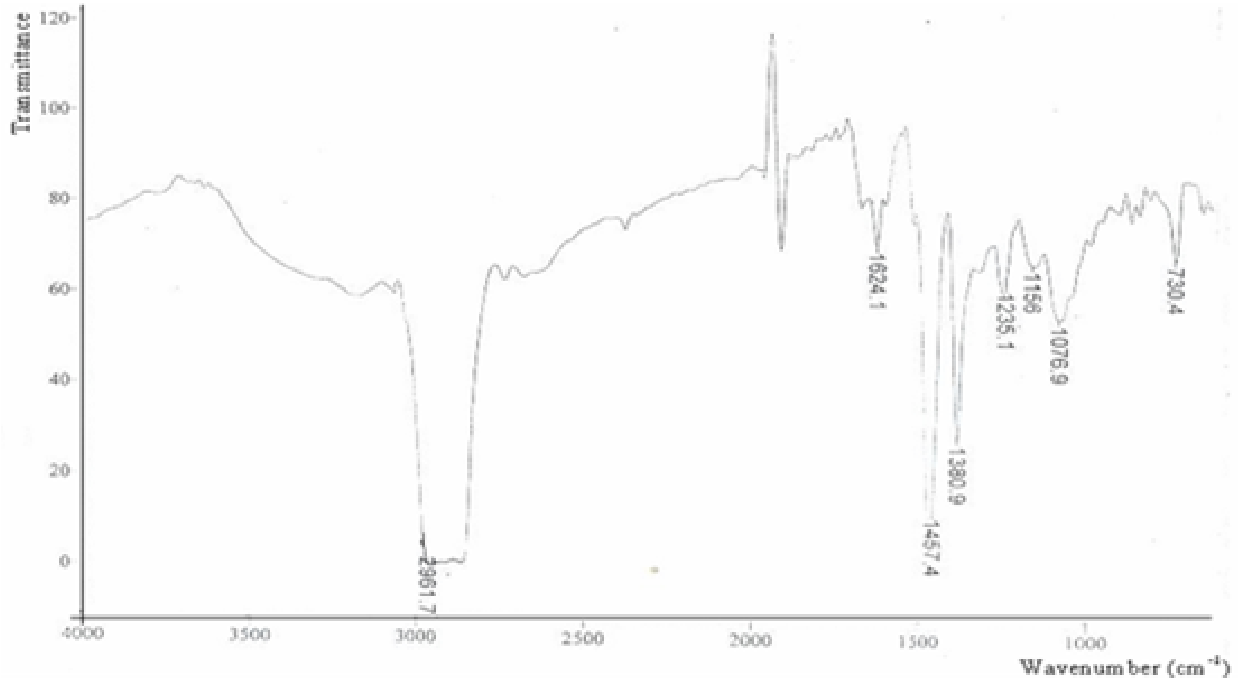


Figure 2. Infra-red spectrum of quinine sulphate.

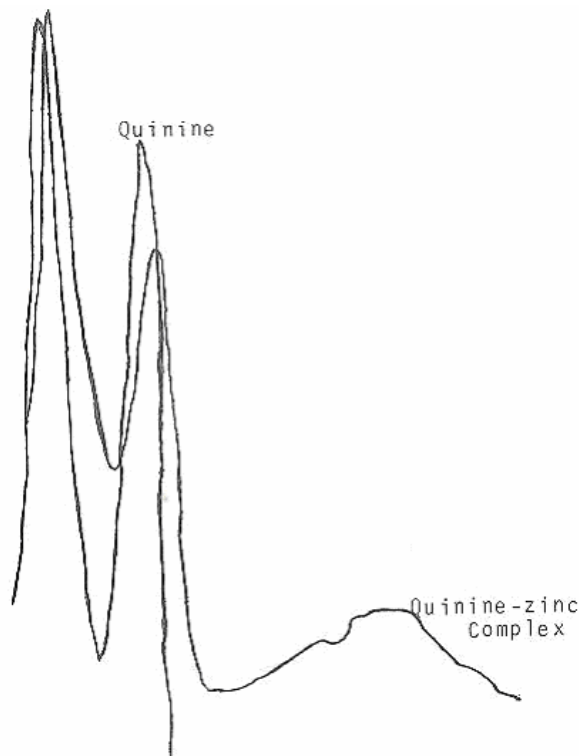


Figure 3. UV spectra of quinine sulphate and quinine-zinc complex.

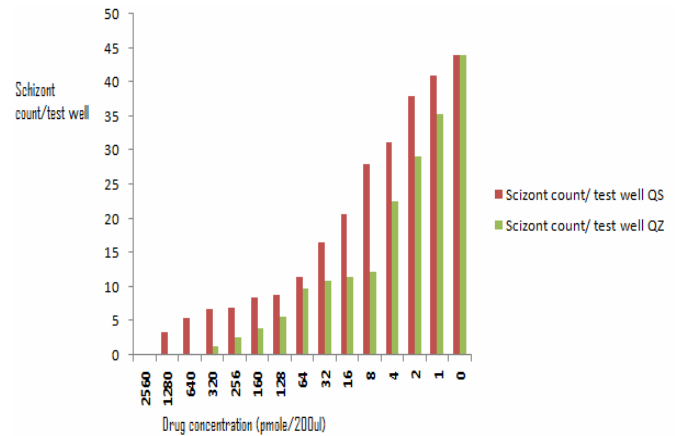


Figure 4. Schizont count per 200 parasite test well following treatment with of quinine sulphate and quinine-zinc complex.

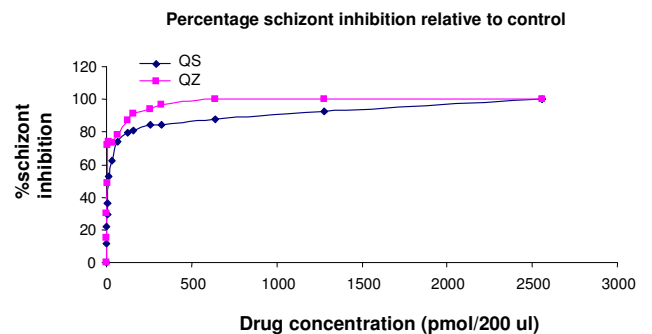


Figure 5. Percentage schizont inhibition by of quinine sulphate and quinine-zinc complex.

while that of QZ was 0.20. The melting point of the mixture of QS and QZ was 162°C. This melting point

value, that is, 162°C is between the melting points of QZ (209°C) and QS (111°C). This melting point value obtained for the mixture of QS and QZ supported the idea that the substances mixed could have been QZ and QS. The IR peaks for QS in nujol as shown in Figure 2 were 730 (aryl C-H deformation), 1077 (methoxy C-O stretching), 1156 (alcoholic O-H deformation), 1235 (methoxy C-O stretching), 1381 (aryl C-N stretching), 1457 (alkyl C-H deformation), 1624 (alkenes C=C stretching), 2962 (alkyl C-H stretching) while the IR peaks for QZ in nujol as presented in Figure 1 were 730 (aryl C-H deformation), 901 (alkene C-H deformation), 1168 (alcoholic O-H deformation), 1387 (aryl C-N stretching), 1466 (alkyl O-H deformation), 2858 (aryl C-H stretching), 3733.8 (alcoholic O-H stretching). The expressions in parenthesis describe the functional groups responsible for the IR peaks. An examination of the IR spectra of QS and QZ revealed a definite shift in the absorption of the alcoholic O-H group and an introduction of a new band for the complex at 3734 correlating with O-H stretching group. This shift occurred in the direction of longer wavelength for the group indicating that the O-H group of Quinine might be involved in the complexation with Zinc. Donation of electrons to metals by the ligand has been reported to produce lower excitation state, hence shifts to longer wavenumber (Mulliken, 1952). The ultraviolet spectra of QS and QZ complex in 0.1 M HCl showed two maximum absorptions at 230nm and 246nm indicating that there was no difference in the UV absorbance of QS and QZ. This is because Quinine has no chromophore for absorption in the UV region. Assessment of the zinc content of QZ prepared was done through Atomic absorption spectroscopy. The zinc content obtained was used to calculate the mole ratio of complexation. The mole ratio was calculated to be 2:1 (Quinine:Zinc) supporting the report of Singla and Wadhwa (1994). From the graph of percentage schizont inhibition versus drug concentration, the minimum inhibitory concentration (MIC) of QS was 2560 pmol/200 µl while that of QZ was 640 pmol/µl, indicating that the amount of QZ required for effective schizonticidal action was three times less than that of QS. Drug concentration corresponding to 50% schizont inhibition (IC<sub>50</sub>) for QZ and QS were 3.98 and 14.13 pmol/200 µl respectively. Using students T-test (paired sample for means), statistical analysis of the data obtained showed that the difference in schizonticidal activities of QZ and QS were significant at 95% confidence limit (p < 0.05).

## Conclusion

Quinine- Zinc complex (QZ) was synthesized in this experiment and its identity confirmed with UV, TLC, AAS and IR analysis. From *in vitro* micro test determination, complex was confirmed to have 3 times antimalarial potency over Quinine sulphate. However, before Quinine Zinc complex can be recommended as a better alternative to Quinine 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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