ANTIPLASMODIAL ACTIVITY OF NANOPARTICLES ENHANCED CHLOROQUINE ON *Plasmodium berghei* In-Vivo

*Hadiza, A.M.*, ¹ Taura, D.W.² and Mohammed, B.²

¹ Department of Biological Sciences, Nigeria Police Academy, Kano, Nigeria
² Department of Microbiology, Bayero University Kano, Nigeria  
*Corresponding author: haudiza@yahoo.com/ 08037039899

**ABSTRACT**

The search for new antimalarial agents is at the forefront for the global fight against antimalarial drug resistance as malaria treatments take longer than the three days plan. These changes pose a threat to the progress made thus far and search for newer and more rapid antimalarial agents is needed to maintain that success. The potentials of Magnesium Oxide Nanoparticles (MgO NPs) enhanced Chloroquine were assessed on *Plasmodium berghei* in vivo. Magnesium Oxide Nanoparticles were synthesized using sol-gel method and characterized using TEM (transmission electron microscope) and EDS (energy dispersive x-ray spectroscopy), SEM, UV-VIS and FTIR. Concentration of 10 mg/ml of MgO NPs were prepared and combined with graded doses of chloroquine (25mg, 12.5mg and 6.25mg per kg) and assayed on *Plasmodium berghei*. Mice for this research were cared for at the Pharmacology Department Aminu Kano Teaching Hospital (AKTH) and weighed before the start of the experiment. *Plasmodium berghei* ANKA (Pba) infection was successfully inoculated in all the mice from a donor. The mice were grouped into five A, B, C, D and E consisting of 6 mice each. Group A- no treatment, Group B-50mg of chloroquine Group C were treated with 10mg/ml MgO NPs and 25mg chloroquine and Group D were treated with 10mg/ml MgO NPs and 12.5mg chloroquine and group E- 10mg/ml MgO NPs and 6.25mg of chloroquine once a day for 4 days. TEM shows that the particles are all under 100nm cylindrical and spherical in shape. EDS shows that the sample contains magnesium and oxygen in the ratio of 2:1. In-vivo antiplasmodial activity shows that all the groups that were given NPs combined with chloroquine had better parasite clearance rate \( (F_{3, 20}) = 47.39, p<0.00 \) at 0.05 level of significance. By all counts chloroquine enhanced with MgO NPs is considered to be more effective. The T-HSD shows that there is statistically significant difference between groups. It is hoped this study will inform malaria agencies of the potentials of this promising agent in the treatment of *Plasmodium* infection and further studies be carried out on the said compound.

**Key words**: magnesium oxide nanoparticles, chloroquine, ANKA, *Plasmodium berghei*, geimsa stain

**INTRODUCTION**

Malaria still remains a burden in sub-Saharan Africa even though the disease last between some selected months when it is endemic. The major drug for treatment as listed on the WHO protocol is any artemisinin-based combination therapy and this depends on the region and what the Plasmodium parasite has been known to be sensitive to (W.H.O., 2015). In Nigeria, all the combinations are being recommended by doctors depending on what is available in stock, but recently treatments have turned to IV for 3 days followed by ACTs for another 3 days. This development is telling us that the Plasmodium is gradually developing some form of resistance to ACTs and something has to be done promptly to prevent a disaster from occurring. (Ashley, et al, 2014)

Malaria statistics as reported by Felix Brambaifa in February of 2018 of the natural health news tagged under malaria statistics stated that malaria kills about 300,000 children per annum and is the cause of about 4000 maternal death too. This statistics is baffling considering the facts that antimalarial drugs are administered free of charge across major hospitals in the country. And the roll back malaria program distributes insecticides treated mosquito nets to both rural and urban areas of the country. However, this continual resistance of *Plasmodium* to antimalarial drugs is causing a major limitation to the global prospect to “Roll Back Malaria”.

---

27
Chloroquine and sulfadoxine/pyrimethamine are now inactive which has increased malaria-related deaths, economic and social cost of managing the disease. Some are advocating the development of resistance drugs or deploying an artemisinin-based combination therapy. The short come of this combination through effective is the cost and the uncertainty about their impact in real life victims (Caroline and Holly, 2004.) Chloroquine, which is one of the cheapest and most affordable drugs in the 90’s, has since been abolished due to the development of resistance in most of the Plasmodium parasite’s species. In cases where it is used, it is in combination with another strong active drug. Though still present in the Nigerian marketplace, many still look to it as an option when down with malaria. (Matins, et al, 2009) Since chloroquine-resistant was discovered in Southeast Asia and South America, it has posed a big threat to controlling malaria in areas that are plagued by malaria-like Cambodia and Thailand and even sub-Saharan Africa. Even though chloroquine is a cheaper and faster drug for curing malaria in the 90’s, its resistance spread across the eastern parts of Africa and then throughout Africa and the progressive deterioration of the potency of the drug has made the need for better drugs that can combat the resistant strain. Pfcr polymorphisms are molecular markers that have been identified with chloroquine resistance. Polymorphisms in pfmdr1 are also associated with resistance to quinine, artemisinin, and other quinine derivatives. This information is important in developing a drug that is potent to Plasmodium. It is obvious that malaria is a huge health problem in major parts of Asia and South American; however, the burden of malaria affects sub-Saharan Africa more than anywhere else where 90% of deaths are malaria-related. The main reason is the presence of the vector Anopheles gambiae that feeds on humans and lives long making it an effective medium in transmitting malaria from one person to another. The EIR which is the rate of individuals bitten by an infectious mosquito hardly ever passes 5 years in Asia and South America but a record of over 1000 has been registered in sub-Saharan Africa as reported by Moles et al (2015). In fact in West Africa, it is not unusual to collect more than one species of mosquito in a particular room with the Anopheles Gambiae complex which is 1-5% infectious. The makes the task of preventing the transmission of malaria in Africa difficult. There is a notable difference in the prevalence of malaria in Africa even between to community in the same locality. Fortunately, new techniques are now been employed to track and trace the spread of malaria and contain the complex epidemiological situation such as satellite imagery. This allows for an accurate location of areas where the infection is predominant and areas where the resistant strain is plenty. (Porter-Kelly et al., 2010) Malaria is most dangerous during pregnancy as the body immunity lowers or rather does more work and an infection of malaria may lead to the death of the mother, abortion, stillbirth or deformity in babies that get to term. Even with such technology on the ground to track the Plasmodium, accessing the full impact of malaria is still difficult. This is due to deaths at home caused by malaria and illnesses with symptoms related to malaria. Even with the limitations, efforts are been put in place to tackle the global epidemic and the early detection of the malaria parasite as it is now believed that there is a death by malaria every 30 seconds through an intervention in malaria endemic area such as the provision of malaria vaccine might be able to save more lives.

Chloroquine, as mentioned above, is the most affordable drug to cure malaria and has been used since it was created in 1934 by Hans Andersag. It is an excellent drug for the treatment and cure of malaria-like P. vivax, P. Ovale, and P. malariae (Cann, 1961). It is not used in preventing the parasite P. falciparum because it started to develop resistance to the drug. The reason for this resistance is due to the mass administration of the drug to contain the emergence and spread of the malaria epidemic. The CDC guard against using chloroquine alone to prevent malaria except in combination with other antimalarial drugs (Snow et al., 1999) The rise of malaria in Africa has hit over 500 million people and still raising. This epidemic has triggered a response from international societies and donor organization such as The Gates Foundation and the National Institute for Health and The Welcome Trust. Another organization is the Roll Back Malaria initiative that has the profile of malaria control for the past years. Their achievement includes access to malaria medications and treatments, protection and prevention of malaria in pregnant women and appropriate measures to reduce continental and global mortality. However, the world is skeptical but they have proven to keep to their word and are fighting to curb the spread of malaria.

As technology disrupts every sector of our world, the health and research sectors are not left out. Malaria remains one of the most greatest threats to man that still claims lives regardless of the number of drugs or combination that are been employed.
Multiple drugs resistance and continuous test drug is a problem that needed to be overcome. This is where nano-based drugs are being developed to hit the targeted cells infected by malaria and minimize the development of resistance of malaria drugs. So, how exactly can nano-based drug delivery system help in treating malaria across the globe?

Nanoparticles technology will help to promote the drug or vaccine against cellular degradation and improve the sensitivity of the malaria drug and limit resistance when administered. The importance effect of nano carriers anti malaria drugs is the ability for the drug to remain longer in the blood stream combating and interacting with the infected red blood cells and the parasitic membrane thereby eliminating malaria from the body. Although the technology is still new and under testing where it has been developed, nanoparticles technology will help combat malaria better and provide a better treatment for malaria case in sub-Sahara Africa. (Molina et al, 2012)

In this research, Chloroquine was used as a base drug for the treatment of Plasmodium berghei infected mice and was enhanced with magnesium oxide nanoparticles to compare the efficacy of the drug.

MATERIALS AND METHODS

Synthesis and Characterization of Magnesium Oxide Nanoparticles

Magnesium oxide nanoparticles were synthesized using magnesium nitrate (MgNO3.6H2O) as a source material with sodium hydroxide according to the procedure of Rizwan et al. (2007) eight grams ( 8g) of magnesium nitrate and 2.4g of polyethylene glycol was dissolved in 200 ml of de-ionized water. 1M (4g in 100ml distilled water) sodium hydroxide solution was added drop wise to the prepared magnesium nitrate solution while stirring it continuously on a magnetic stirrer at 60°C. A white precipitate of magnesium hydroxide appeared in a beaker after a few minutes. The stirring was continued for 30 minutes. The pH of the solutions was increased to 11.5 by adding the sodium hydroxide solution drop wise and monitored and measured by the PH meter. The precipitate was filtered and washed with methanol once to remove ionic impurities and then centrifuged for 5 minutes at 1500 rpm/mint and the samples were annealed in an oven for twenty-four hours at 200 oC. Samples were characterized using TEM (transmission electron microscope) and EDS (energy dispersive x-ray spectroscopy) to determine size and composition.

Preparation of Stock Concentration of Nanoparticles

A fresh stock solution of nanoparticles was prepared based on the value of IC50 calculated from the in-vitro anti plasmodial test. 100mg/kg that is 0.010g of the powdered MgO nanoparticles with 0.01g of acacia powder was dissolved in 10ml of normal saline to prepare a 100mg/kg of the MgO nanoparticles according to the procedure of Ojurongbe et al. (2015). This was given to the experimental mice through the course of the experiment. The preparation was stored in a refrigerator at about 6°C throughout the treatments.

Preparation of Stock Concentration of Chloroquine

The stock solution of 5mg/ml of chloroquine was prepared in a bottle and was diluted serially to produce graded doses that were used in this experiment. Doses of 2.5mg/ml, 1.25 mg/ml and 0.625mg/ml were prepared and administered together with the 10mg/ml on MgO NPs to each mouse.

Experimental Animals and Methodology for Oral Drug Administration

For the curative model, 36 white male and female albino mice (Wister stock) and 6 infected donor mice were obtained from the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. The animals were fed on diet specially prepared from chick Grower, cassava flour, and maize bran and were given water throughout the study period. The experimental animals were divided into 5 groups (A, B, C, D, and E) and numbered 1-6 respectively. Group A mice were given no treatment, Group B mice were given 100mg/kg of the MgO nanoparticles according to the procedure of Ojurongbe et al. (2015). This was given to the experimental mice orally. Animals’ weights ranged from 18g to 26g just before the commencement of the experiment. The animals were inoculated with the parasite by removing blood from an ocular puncture and about 10 drops of blood containing trophozoites and ring stage of the parasites was collected in an EDTA container and 2ml of normal saline was added and mixed well. 0.2ml of this blood containing 1x10⁷ of Plasmodium berghei parasitized red cells was injected into each mouse intraperitoneally. Mice were allowed rest for 3 days this is to enable the parasite time to multiply to the between 20-30% parasitemia mark. On the 3rd day, thin blood film was made from each mouse taken from the tail vein and stained using 3% Gelmsa for 45minutes this is to know the initial percentage of the parasite in each mice before the start of treatment.
Thin blood films were made from the tail of the mice after every 24 hours stained and read using a light microscope Optika-B 150 x 100 oil emersion objectives (Yufan, et al, 2014)

Statistical Analysis
The results was analyzed using a one way analysis of variance (ANOVA) and the Tukey Honestly Significant Difference test (T-HSD)

RESULTS AND DISCUSSION
Figures 1 and 2 shows the transmission electron microscopic image of the MgO NPs, Clearly showing the spherical and some elongated cylindrical shapes of the MgO NPs

Figure 1:- TEM at 50nm
Figure 2:- TEM at 20nm

Figure 3:- Shows the Energy Dispersive X-Ray Spectroscope (EDS) Profile of MgO NPs
The profile below clearly shows that the synthesized substance contains magnesium and oxygen in the ratio of 2:1.

Figure 3:- EDS Shows pure MgO NPs
TABLE 1: Mean Percentage Parasitamia at Day Three after Inoculation

The mean percentage parasitaemia was calculated by counting infected red blood cells against the total number of cells per field for three microscopic fields multiply a hundred.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Group C1 %</th>
<th>Group C2 %</th>
<th>Group C3 %</th>
<th>Group C4 %</th>
<th>Group C5 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.0</td>
<td>14.4</td>
<td>17.0</td>
<td>21.0</td>
<td>16.4</td>
</tr>
<tr>
<td>2</td>
<td>30.6</td>
<td>15.0</td>
<td>20.0</td>
<td>21.0</td>
<td>15.0</td>
</tr>
<tr>
<td>3</td>
<td>20.0</td>
<td>18.9</td>
<td>20.0</td>
<td>15.2</td>
<td>20.8</td>
</tr>
<tr>
<td>4</td>
<td>21.7</td>
<td>20.0</td>
<td>16.0</td>
<td>21.6</td>
<td>17.0</td>
</tr>
<tr>
<td>5</td>
<td>17.6</td>
<td>22.6</td>
<td>15.7</td>
<td>17.6</td>
<td>18.1</td>
</tr>
<tr>
<td>6</td>
<td>30.0</td>
<td>18.1</td>
<td>20.0</td>
<td>20.0</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Total mean 22.7 18.2 17.3 19.4 16.7

Table 2: Mean Percentage Parasitamia at Day Seven after Treatment with Chloroquine and Nanoparticles

<table>
<thead>
<tr>
<th>S/N</th>
<th>Group C1 NO TREATMENT</th>
<th>Group C2 5MG/ML CHLOROQUINE</th>
<th>Group C2 2.5MG/ML CQ +10MG/ML NPs</th>
<th>Group C3 1.25MG/ML CQ +10MG/ML NPs</th>
<th>Group C4 0.625MG/ML CQ +10MG/ML NPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.1</td>
<td>14.0</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>40.0</td>
<td>21.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>38.8</td>
<td>16.0</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>34.0</td>
<td>28.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>32.0</td>
<td>34.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>37.1</td>
<td>19.1</td>
<td>0</td>
<td>0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

% 34.6 22.5 0 0.06 0.13

The no treatment group (C1) had almost doubled the percentage of parasite at the end of the seven days experiment. The group that was treated with only Chloroquine (C2) also had an increase in the parasitamia level of 4.3% and with 77.5% clearance rate in vivo at the end of the experiment and the three separate groups that was treated with a combination of different dose of chloroquine with MgO nanoparticles is had 99.9% clearance rate.

Result was statistically significant with p-value < 0.00 at 0.05, level of significance. This result tally with the findings of Elende and Nnenna (2017) that reported in vivo parasitamia levels to be high after treatment with chloroquine but when he added Aluminum Magnesium Silicate Nanoparticles to the CQ, a significant clearance rate was obtained. This result does not tally with the work of Rajnish, et al (2014) that reported 99% clearance rate with chloroquine in vivo.

Table 3: Summary of the result of the research showing initial parasitamia after inoculation, parasitaemia after treatment and percentage inhibition.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>INITIAL PARASITAEMIA</th>
<th>PARASITAMIA AFTER TREATMENT</th>
<th>% PERCENTAGE INHIBITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>22.7</td>
<td>34.6</td>
<td>0.00</td>
</tr>
<tr>
<td>C2</td>
<td>18.2</td>
<td>22.0</td>
<td>77.5</td>
</tr>
<tr>
<td>C3</td>
<td>17.3</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>C4</td>
<td>19.4</td>
<td>0.06</td>
<td>99.94</td>
</tr>
<tr>
<td>C5</td>
<td>16.7</td>
<td>0.13</td>
<td>99.87</td>
</tr>
</tbody>
</table>

From table 3, it can clearly be seen that the chloroquine doses that were combined with magnesium oxide nanoparticles had the best percentage inhibition.

CONCLUSION

This result clearly shows that magnesium oxide nanoparticles can be synthesized using sol-gel method in the laboratory as shown by the images from the Transmission Electron Microscope (TEM) and the Energy Dispersive X-Ray Spectroscope (EDS). The activities of the MgO NPs also were remarkable with the in-vivo mean percentage clearance rate of above 99 % at the conclusion of all the experiment.

This Research presentation is part of a series of research publications and some of the results had already been published online and can be found under the titles “preparation, characterization and in vivo antiplasmodial activities on magnesium oxide nano particles on Plasmodium berghei infected mice” and “in vivo antimalarial activity of nanoparticles enhanced artemether lumefantrine (coatem) on Plasmodium berghei infected mice.”
REFERENCES


Felix Brambaifa (2018). The natural health news tagged under malaria statistics


