Research Article

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Preparation, Characterization and *In-Vivo* Antiplasmodial Activity of Magnesium Oxide Nanoparticles on *Plasmodium Berghei* Infected Mice

Hadiza AM[1*] Taura DW[1] and Bashir Muhammad[2]

[1]*Department of Biological Sciences, Nigeria Police Academy, Kano, Nigeria.*

[2]*Department of Microbiology, Bayero University Kano, Nigeria.*

[*]Correspondence:
Hadiza AM, Department of Biological Sciences, Nigeria Police Academy, Kano, Nigeria, Tel: 08037039899 E-mail: haudiza@yahoo.com.

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ABSTRACT

Magnesium oxide (MgO) nanoparticles were synthesized and assayed for antiplasmodial activity in vivo. MgO nanoparticles were synthesized using sol-gel process and characterized using SEM, FTIR AND UV-VIS spectral study to confirm the formation and size of the nanoparticles. 20% of LD50 value was used to formulate a graded dose of 300, 200 and 100mg/kg. Then 30 mice were group into 5 containing six mice each and were inoculated with 0.2ml of ANKA strain of plasmodium berghei intraperitoneally and were left for the next 7 days before treatment with the graded doses based on their body weight.20/120mg/kg standard dose of Artemether lumefantrine was use as positive control while negative control were given no treatment at all. Data were analyses using mean percentage parasite clearance rate, analysis of variance (ANOVA) and Tukey honestly significant difference test. MgO nanoparticles showed a remarkable clearance rate of 98.8% just after 24hours of administration and at the end of the four day curative model all the parasites were cleared from the blood. There was statistically significant difference between groups as determine by one- way ANOVA F (3,16) =24.30,p=3.36E-06. This clearly showed that MgO nanoparticles are superior in the clearance of the ANKA strain of plasmodium berghei in infected mice.

Keywords

Plasmodium berghei, Magnesium Oxide, Nanoparticles, Synthesized, Resistance, Malaria.

Introduction

Malaria is the most important and most prevalent human infectious parasitic disease and still is the main reason of death in sub-Saharan Africa. There were 214 million new cases of malaria worldwide in 2015 with 88% of the cases occurring in Africa and an estimate of 438,000 malaria death worldwide with 90% occurring in Africa. Two African countries account for nearly 35% of the global total estimated malaria deaths: democratic republic of Congo and Nigeria [1]. People living in the poorest countries around the globe are at a higher risk of getting the disease mainly because of poor standard of living. About 1/5 of all childhood death between the ages of 1-6 are as a result of one or a combination of the human malaria parasite. According to Ranu Surolia et al. [2], malaria is caused by protozoan parasite of the genus *plasmodium*. Five species are common to human infection which includes *P.falciparum, P.knowlesi, P.ovale, P.malariea* and *P.vivix*. This parasite is transmitted to humans by the bite of the infected female anopheles mosquito. In the human body, the parasite reproduce in the liver before invading red blood cells where they multiply again before busting out and infecting more red blood cells this is called the erythrocytic phase. This causes high fever and sometimes in complicated cases damages some of the vital organs in the body.

Experimental research on *plasmodium falciparum* has improved seriously in the last decade, not only as a result of the cutting edge technology of recombinant DNA, but also as a result of the demonstration by Trager W, et al. [3] that the organism could be cultured in vitro. This made the organism much more accessible experimentally. Even though there have been some modification in the method, their method is still the most widely accepted method of culturing.

Drug resistance and resistance to insecticide Neto Z, [4] have caused a major drawback in the war against the parasite and the

insect as a whole. This may be in the form of either failure to clear the parasite from the blood or longer period of treatment as a result of relapse of the malarial symptoms. In the case of the insecticide, failure to kill the adult mosquito parasite in the environment leads to resistance.

The reduced efficacy of current antimalarial drugs is the major reason for worldwide resurgence of malaria and this has been happening for decades. This may also be as a result of the antimalarial drugs failing to reach the target *plasmodium* in the liver or red blood cells.

Development of either new antimalarial drugs or new ways to carry the antimalarial agents to specific target cells is required for better treatment of the parasite in cases of infection. This may be very expensive and time consuming but the end result may be worth the try because the spread of antimalarial resistance around the world can occur very fast and more effort must be put into the war against it [5].

In this research, nanoparticles are going be evaluated for their antiplasmodial activities on mice inoculated with the ANKA strain of *Plasmodium berghei*. Nanoparticles are particles between 1 and 100 nano meter. These particles behave as a whole unit with respect to its transport and properties. Nanoparticles are considered a discovery of science, and possess a variety of biological activities and considered a rich source of novel antiplasmodial agent and these potential resources are scarcely explored [6], and they actually have a long history. Nanoparticles were used by artisans in the ninth century in Mesopotamia for generating a glittering effect on the surface of pots.

Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structure. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus the properties of materials changes as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk material larger than one micron, the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material [7].

The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to large surface area of the material which dominates the contribution made by the small bulk of the material. The high surface area to volume ratio provides a tremendous force for diffusion. This also makes the particles activity better. The primary aim of this study is to investigate the antiplasmodial activity of nanoparticles on *Plasmodium berghei* infected mice.

Materials and Methods

Materials

Materials and reagents and standard drug preparation for this experiment will be purchased from sigma Aldrich through Ceman Scientific Limited Kano, Nigeria.

Synthesis and Characterization of Magnesium Oxide Nanoparticles

Magnesium oxide nanoparticles were synthesized according to the procedure of [8] using magnesium nitrate ($MgNO_3.6H_2O$) as a source material with sodium hydroxide, 8g of magnesium nitrate and 2.4g of polyethylene glycol was dissolved in 200 ml of de-ionize 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. White precipitate of magnesium hydroxide appeared in beaker after few minutes. The stirring was continued for 30 minutes. The pH of the solutions was increased to 11.5, as measured by the PH meter. The precipitate was filtered and washed with methanol five times to remove ionic impurities and then centrifuged for 5 minutes at 1500 rpm/min and dried at room temperature for a week. The dried white powder samples were annealed in an oven for twenty four hours at 200°C. The morphological investigation was carried out by the scanning electron microscopy [9], (SEM, Inspect S50) at the National Geological Survey Agency, Kaduna. Samples composition of the synthesized magnesium oxide nanoparticles were analyzed by the Fourier transform infrared (FTIR) spectroscopy at the Department of Biochemistry, Bayero University Kano, in the range of 650-4000 nm and infrared spectroscope was carried out at a range between 200 and 800 nm.

Experimental Animals and Methodology for Intraperitoneally Ld50 Procedure

Acute toxicity study was conducted in accordance with Locke's method [10]. The study was conducted in two phases using a total of thirteen mice. In the first phase, nine mice were divided into 3 groups of 3 mice each. Groups 1, 2 and 3 animals were given 10, 100 and 1000 mg/kg body weight of the MgO nanoparticles, respectively, to possibly establish the range of doses producing any toxic effect. Each mouse was given a single dose and after 24 hours results were observed and recorded. Based on the mortality recorded in the first phase a second phase was initiated and a total of four mice was used and they were given doses of (200, 400, 800 and 1600mg/kg) of the nanoparticles (one mice per dose) to further determine the correct LD50 value. The nanoparticles was dissolved in normal saline solution and given via intraperitoneally route [11]. All animals were observed frequently on the day of treatment and surviving animals were monitored after 24 hours for acute toxicity mortalities. And the geometric mean calculated to give the LD 50 dose.

Preparation of Stock Concentration of Nanoparticles

Fresh stock solution of nanoparticles was prepared based on the value of LD 50 calculated from the Locke's method. The LD 50 was approximated to 1131.37mg/kg. To do this, 0.30g of the powdered MgO nanoparticles with 0.01g of acacia powder was dissolved in 10ml of normal saline to prepare a 30mg/kg of the MgO nanoparticles. From which graded doses of 300,200 and 100mg/kg were prepared. This was given to the experimental mice through the course of the experiment. The preparation was stored

in a refrigerator at 40°C until required.

Preparation of Stock Concentration of Artemether Lumefantrine Standard Drug 20mg/120mg (Coatem)

Artemether lumefantrine branded tablets was obtained from lamco pharmaceutical store in Kano state and taken to the laboratory for the experiment. One tablet was grounded in a mortar and dissolved in 10 ml of normal saline. This is to prepare a stock concentration of 2:12mg/ml of artemether lumefantrine, and this was given to the positive control group though out the curative model.

Experimental Animals and Methodology for Oral Drug Administration

For the curative model, 40 white male and female albino mice (Wister stock) and 2 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. Animals' weights ranged from 18g to 26g just before the commencement of the experiment. Experimental design for curative model was used. The animals were inoculated with the parasite by removing blood from an ocular puncture and about 10 drops of blood was collected in an EDTA container and 2ml of normal saline was added and mixed well. 0.2ml of this blood containing $1x10^7$ of *Plasmodium berghei* parasitized red cells was injected into each mouse intraperitoneally. This involves the use of 30 mice of 6 each in a group and five groups were made. Mice were allow rest for 3 before treatment begins days this is to enable the parasite time to multiply to the 20-30% parasitamia mark. On the 7th day, thin blood film were made from each mouse taken from the tail vein and stained using 3% geimsa for 45minutes this is to know the final percentage of parasite in each mice after the start of treatment. Group one were given no treatment this is the negative control group, group 2 were given 20mg/kg coatem alone based on the weight of the mice drugs were administered, group 3 was given 300mg/kg nanoparticles, group 4 were given 200mg/kg nanoparticles, group 5 were given 100mg/kg nanoparticles. Thin blood was made from the tail of the mice after a day and at the end of a four days treatment and stained with 3% geimsa and read using a light model OPTIKA B150 microscope x100 oil emersion objective.

Microscopy

Thick and thin blood films were prepared from each blood sample and stained with 3% geimsa stain for 45 minutes [12] and were examined under the microscope with the assistance of an expert microscopes'. Three micro liter of the culture was placed on a slide and another slide was used to smear it to get a thin film the slides were dried at room temperature and fixed by immersing in absolute methanol for 2 seconds [6] and allow drying. The slide were then stained and viewed with x 100 oil immersion objective.

Percentage Parasitamia Estimation from Microscopic View

An area of stained thin blood film where the erythrocytes are evenly distributed was observed, approximately 150-200 erythrocytes were counted without moving the slide and both infected and uninfected erythrocytes were counted. If more than one parasite is present in an erythrocyte it is still one erythrocyte that was infected. All this was done for the nanoparticles concentrations and ACTs to determine the inhibition concentration (IC 50) that is the concentration of drug that cleared 50% of the parasite. Percentage inhibition was plotted against concentration.

Percent infected RBCs = (number infected RBCs ÷ total number of RBCs counted) ×100.

Statistical analysis

One way ANOVA followed by Tukey HSD were used to carry out the analysis of data.

Results and Discussions

Scanning Electron Microscope Image of the Synthesized Magnesium Oxide Nanoparticles

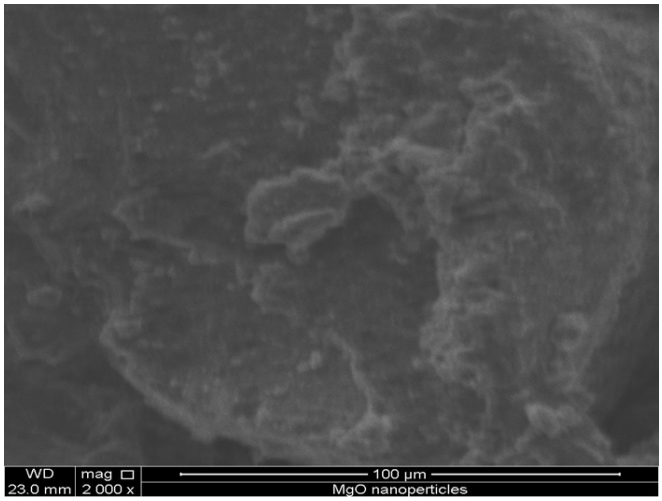

Figure 1: Shows the MgO NPs were composed of sheets and plates which are closely stacked.

FTIR Analysis of magnesium oxide nanoparticles synthesised using sol-gel process

Here also FTIR spectra shows bands at 3434 cm^{-1}, 3692 cm^{-1}, 2113 cm^{-1}, 1648 cm^{-1} and 1354 cm^{-1}. 3434 cm^{-1} and 3692 cm^{-1} which indicates the presence of (O-H) which indicates strong broad bonded and non bonded hydroxyl group and water (socrates, 2001), and 2113 cm^{-1} and 1648 cm^{-1} indicate a strong presencee of alkyne (C≡C) and alkene (C=C). The last absorption band 1354 cm^{-1} indicate the presence of alkane (C-H) [13].

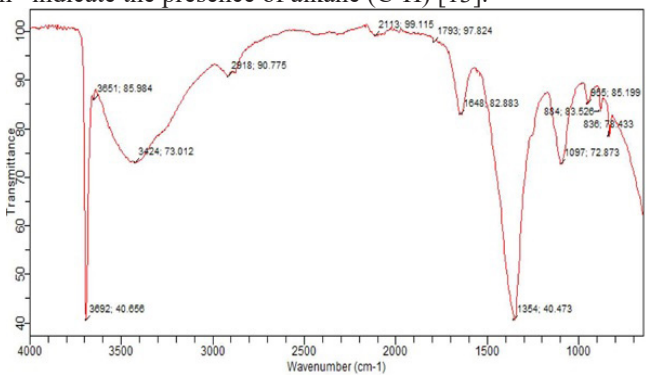

Figure 2: FTIR analysis of synthesized magnesium oxide nanoparticles.

UV-VIS Spectral Analysis

Absorption spectroscopy results found that absorbance decreases sharply with an increase in wavelength near the band edge (300 nm) indicating the formation of nanostructure samples and thereafter the value of absorption coefficient becomes more or less constant indicating the uniformity of size of synthesized Nanoparticles.

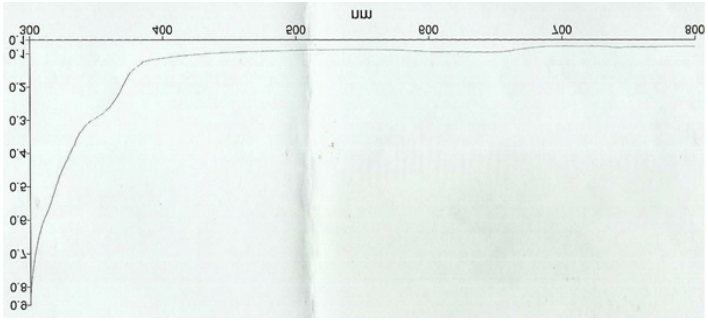

Figure 3: UV-VIS Scan of MgO Nanoparticles.

Absorption graphs of MgO nanoparticles calcinated at 200°C for 24 hours.

S/N	A	B	C	D	E
1	30.0	41.5	16.5	18.0	25.2
2	28.2	18.4	16.8	12.8	19.2
3	11.2	29.2	14.8	18.6	15.5
4	16.8	19.7	17.2	12.8	16.3
5	30.0	17.6	14.0	14.0	14.0
6	14.4	17.5	12.8	12.8	13.5
	21.8	24.2	15.35	14.8	17.3

Table 1: mean percentage parasitamia of the mice after inoculation with the parasite.

Table 1 shows the initial mean parasite load before the onset of treatment.

Mean Percentage Parasite clearance at Day Seven after Inoculation and Treatment

Percentage parasitamia and survival time were used to assess the therapeutic potency of the synthesized nanoparticles. Table 3 below shows the mean percentage parasitamia of the mice after treatment with different doses of magnesium oxide nanoparticles. Standard dose of coatem was given to B group while nanoparticles (NPs) were given to groups C, D and E and at the end of the experiment the result discovered that MgO nanoparticles were more effective in clearing the ANKA strain malaria parasite from the mice than coatem did. [14-16] also reported failure of artemether and lumefantrine to completely cure *P.berghei* malaria *in vivo* in mice with cure rate of 84%, 79%, 81.43% and 70% reported respectively. There was statistically significant difference between groups as determine by one- way ANOVA F (3,16) = 24.30, p=3.36E-06. This is the first time this research has ever been done and from all indication MgO nanoparticles is superior and better and more active on P. berghei infection in mice clearing 99.9% of the parasite.

S/N	A (no treatment)	B (20mg/kg coatem)	C (300mg MgO NPs)	D (200mg MgO NPs)	E (100mg MgO NPs)
1	27.6	98.8	100	99.6	99.6
2	76.8	97.5	100	100	100
3	21.2	98.0	100	99.6	100
4	38.4	99.2	100	100	100
5	43.2	98.4	100	100	100
6	38.0	98.8	100	100	99.6
	41.0	98.6	100	99.6	99.6

Table 2: Percentage Parasitamia Clearance after Treatment.

Table 2 shows the final mean percentage parasite load after treatment.

Conclusion

The research clearly indicates that other antimalarial agents are out there waiting to be uncovered. This research is a major breakthrough in the fight against malaria and antimalarial drug resistance by *Plasmodium* species. To the best of my knowledge this is the first time this type of research has been reported. There is no call for alarm as per the *Plasmodium* becoming resistance to known anti *plasmodium* drugs available, all we need is to find other agents that are also active and can reduce the burden of over dependent on just a single combination. That is what this research has discovered.

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