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Original Research Article

Potential Antiplasmodial Activity of Artemether and Miconazole Combination against *Plasmodium berghei* in Preclinical Murine Malaria Model

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ABSTRACT

Following recent reports of resistance to currently approved artemisinin-based combination therapy (ACT), there is an urgent need for alternative ACT for effective treatment of malaria. Meanwhile, the antimalarial potential of artemether/miconazole combination has not been explored. In this work, we investigated artemether/miconazole combination as a new combo-therapy for malaria caused by chloroquine-sensitive and multidrug-resistant *Plasmodium berghei* (Pb) in murine models. The antimalarial activity of each drug and their combination [artemether (8mg/kg)/miconazole (2mg/kg)] was investigated using standard protocols for uncomplicated malaria (UM) and severe malaria (SM) in mice infected with chloroquine-sensitive Pb (CPb) and Pb ANKA (PbA), respectively. Hematological parameters (WBC, RBC, PCV and haemoglobin) and lethality of infected mice were assessed. Results revealed that the combination administered peorally (p.o.) gave greater antimalarial activity ($p<0.0001$) than monotherapies of pure artemether, miconazole and marketed chloroquine (p.o) although the effect was less than that of therapeutic dosage of marketed ACT (artemether-lumefantrine) (4mg/24mg/kg) against CPb. Furthermore, intraperitoneally (i.p.) administered artemether/miconazole combo-therapy gave greater antimalarial activity than artemether and miconazole monotherapies ($p<0.0001$) and the effect was comparable with commercial i.m. artemether against PbA. Moreover, the combo-therapies had similar effects to conventional antimalarials in preventing Pb-induced alterations in hematological parameters of the malariogenic mice. Results indicate miconazole as a promising repurposable drug with therapeutic potential against drug-sensitive and resistant parasites. Therefore, artemether/miconazole combination could serve as a plausible ACT option for UM and as an alternative to artemisinin derivatives in SM. An on-going research would seek to enhance the observed effects via nanotechnology-based drug delivery systems.

***Keywords*:** Malaria, Antimalarials, *Plasmodium berghei*, Miconazole, Drug Repurposing, Antimalarial activity.

Introduction

Over 200 million people worldwide are affected by malaria annually, and pregnant women, especially those in the first and second trimesters, are especially vulnerable because of their compromised immune systems.[1] Every year, between 75,000 and 200,000 baby fatalities linked to malaria are reported.[2] In contrast to the 218 million anticipated cases in 2015, 229 million malaria cases worldwide resulting in 409,000 fatalities, according to the World Health Organization's (WHO) annual report for 2019. [3] Africa is the continent that is most affected by malaria infection worldwide; among the 87 countries that have recorded instances, 94% of cases and Sub-Saharan Africa is where malaria deaths occur. [4]

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Five species of Plasmodium are responsible for this vector-borne parasite infection: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Sporozoites found in saliva migrate through the blood to the liver after a blood meal, when asexual reproduction takes place. *P. vivax* hypnozoites that are dormant and produce repeated malaria episodes are seen in the liver.[2] Merozoites, produced by asexual reproduction in hepatocytes, are then discharged into the bloodstream (erythrocytic stage).[5] Blood-borne merozoites and plasmodium parasites both kill red blood cells (RBCs). The main cause of severe malaria, which can be fatal and cause neurocognitive impairment with different clinical presentations in adults and children, is *P. falciparum*. [4] In contrast to cerebral malaria acidosis, which increases mortality risk in children both singularly and collectively, in adults the risk of death is dependent on the malfunction of important organs and increases thrice with acidosis and renal failure.[6] Approximately one-third of people who survive cerebral malaria experience chronic neurological and cognitive impairments.[7] Adults with multiple organ systems dysfunction and a high mortality rate are prevalent. [6] *P. falciparum* causes cerebral malaria coma, which is caused by sequestered parasite blockage of the brain's microvasculature.[6] Sequestered parasites also stick to the blood vessel's endothelium lining. Anemia and parasite blockage exacerbate hypovolemia, which lowers oxygen delivery to tissues and causes lactic acidosis and anaerobic metabolism. [8] Splenomegaly, which varies in degree, is also prevalent with severe malaria. [9]

In order to control malaria, antimalarial medications are important. Antimalarial monotherapy, however, is no longer effective due to the development and dissemination of parasite multidrug resistance. The plasmodium biomass per asexual cycle and the transmissibility of malaria are both decreased by artemisinin derivatives, which are potent antimalarials.[10] The cornerstone of current methods and initial courses of medication for simple *Plasmodium falciparum* malaria in the majority of malaria-endemic countries is artemisinin-based combination therapy (ACTs). [11] Infections resistant to artemisinin were lessened by longer-acting medications such as lumefantrine and amodiaquine. [12, 13] In Africa, an additional treatment option for simple malaria is artesunate-amodiaquine (AS-AQ). [14, 15] Later, dihydroartemisinin-piperaquine (DHAP) was additionally introduced. [16,17]

Furthermore, a significant global issue to the control of malaria is the current rise in ACT resistance.[18] There is ongoing proof of resistance even after chloroquine was stopped and artemether-lumefantrine was introduced.[19] There is documented amodiaquine resistance while treating simple falciparum malaria. [20–24] Treatment failure was caused by an accelerated reduced response to artemisinin derivatives in South Vietnam due to piperaquine resistance.[25–28] Moreover, dihydroartemisinin resistance was observed in regions of prior exposure, while piperaquine resistance is predominant in Ghana and northwest Thailand, respectively. [29–32] It has been determined that Pfmdr1 mutations linked to resistance to artemisinin-based combination treatment (ACT) are widely distributed throughout Tanzania.[33] There were biological indicators linked to lumefantrine resistance in Angola.[34] Pfmdr1 mutations and haplotypes linked to partner drug resistance were not significantly affected by retreatment with artemether-lumefantrine (AL) or artesunate-amodiaquine (ASAQ).[35] An urgent deployment of alternate drug combinations together with heightened vigilance are needed given the increasing rate of resistance.

Redox and antioxidant defense mechanisms exert strict control over oxidative stress. Involved in cell signaling pathways that protect cell functions during oxidative stress and related disease progression are redox systems, which include thioredoxin and glutathione linked enzyme.[36] Treatment approaches to control redox-activated cellular responses have been developed based on the roles concerning reactive oxygen species (ROS) and redox systems in many disorders. Oxygen and iron, two pro-oxidant species that are abundant in the Plasmodium habitat, produce reactive oxygen species (ROS).[37] In addition to harming biological macromolecules, ROS alter signal transduction pathways. [36] The redox metabolism of *P. falciparum* at the erythrocytic stage is targeted by the following therapeutic approaches: oxidant drugs that target essential parasite components and heme by-products; redox cycler drugs that target the parasitized red blood cell; and selective targeting and inhibition of a redox Plasmodial protein or enzyme".[38] Free radicals are produced via heme-dependent reduction of artemether. Parasite proteins are alkylated by free oxygen radicals produced when the artemisinin endoperoxide bridge is broken by heme.[39, 40] Proteins found in food vacuoles, particularly those involved in the redox process, are targeted by the alkylated protein.[41] Underlying oxidative stress leads to a redox imbalance and eventually the death of the parasite when the redox metabolism is disrupted.[42] Drugs that can so block the Plasmodia redox process can increase the effectiveness of artemether.

The process of developing new drugs is expensive, time-consuming, difficult, and rarely successful. Repurposing already approved medications for new indications is necessary due to the recent fall in the number of new drugs licensed for clinical usage. The necessity to repurpose currently available medications for antimalarial indications is highlighted by the mismatch between the development of antimalarial resistance and therapies. A number of medications have been modified for use in the treatment of malaria. There is a known relationship between the fungi's ability to withstand azoles and their antioxidant capability.[43]

A number of writers made suggestions on miconazole's possible use in the treatment of malaria based on the drug's now-understood mechanism. The azole-induced decrease in fungal antioxidant capacity is utilized in plasmodial therapy.[44] ROS accumulation was enhanced by the artemisinin-miconazole incubation.[45] The significant synergistic antimalarial impact is associated with the suppression of the erythrocyte-plasmodium complex antioxidant enzymes by miconazole.[46] Thus, the two medications caused an increase in oxidative stress. Furthermore, because *Plasmodium falciparum* relies on glycolysis for energy production, the enzyme Pfldf is a possible molecular target for antimalarial drugs. Miconazole interacts with this enzyme.[47] Moreover, miconazole reduces decreased glutathione-dependent heme polymerization and degradation by forming stable heme-azole complexes with nitrogenous ligands derived from imidazole moieties, leading to increased heme-induced hemolysis [48,49]. When combined with artemether-lumefantrine (AL), ketotifen dramatically lowered the percentage of parasitemia.[50] The bacterial proteins found in *P. falciparum* are targeted by azithromycin, auranofin, loperamide hydrochloride, amlodipine besylate, cyclosporin A, esomeprazole magnesium, and omeprazole.[51] Saquinavir, ritonavir, and indinavir are a few antiretroviral protease inhibitors (PIs) that may have antimalarial properties.[52] Miconazole boosted endogenous ROS generation in artemisinin annua culture after 24 hours. [53, 54]

Lastly, a significant amount of malaria-related deaths are ascribed to the pathogenic effects on hosts brought on by the spread of parasites and the toxicity of antimalarial drugs, or a combination of the two.[9]. Drugs with less dangerous pathophysiological consequences are therefore required. In addition, there has been a lot of support recently for the sensible combination of antibiotics and antimalarial medications due to the resistance concern. By doing this, you can kill parasites in a synergistic or additive way, preventing the establishment of treatment resistance.[55] Therefore, using mice infected with *Plasmodium berghei*, this study evaluated whether the antimalarial properties of artemether may be improved when coupled with miconazole. Currently, no *in vivo* study has been done to illustrate the synergistic properties of the combination of artemether and miconazole, which are structurally different entities. [56,57] This research aims to examine the novel artemether/miconazole combination against ANKA and sensitive *Plasmodium berghei* strains *in vivo*. It was also intended to determine how the mixture affected the lethality of the mice and hematological parameters.

Materials and Methods

Materials

Pure artemether sample (May and Baker PLC, Lagos, Nigeria), pure miconazole nitrate sample USP (Gutic Biosciences Limited, India), Capryol® 90 (Gattefosse, St-Priest, France), Solutol® HS 15 (BASF, Ludwigshafen, Germany) and distilled water (Lion Water, UNN, Nigeria) were utilized exactly as received from their makers, without additional purification. The remaining substances, reagents, and solvents were all of analytical grade and were acquired from commercial sources.

Ethical approval

The Research Ethics Committee of our university approved this inquiry, which was conducted in accordance with the Ethical Guidelines of Animal Care and Use (approval no. FPSRE/UNN/20/00064).

Animals and parasites

In this investigation, 8 to 10 weeks old seemingly healthy Swiss albino mice of both sexes weighing between 16 and 22 g, were produced locally. The animals were acquired from the University of Nigeria, Nsukka's Faculty of Veterinary Medicine, and were first kept in a room in the animal house. The cages made of plastic and metal held the animals, and they were cleaned and had new bedding on a regular basis. The animals were kept at ambient temperature and humidity, on a typical commercial animal feed diet, with access to unlimited amounts of drinking water, and a 12-hour light/dark cycle.

A chloroquine-sensitive strain of *Plasmodium berghei* (CPb) and a resistant strain, *Plasmodium berghei* ANKA (PbA), were the rodent malaria parasites used. The parasites were employed as a model to imitate *Plasmodium falciparum*, which causes severe/cerebral malaria (SM) and uncomplicated malaria (UM) in humans. [58,59] They were obtained from the Institute of Medical Research and Training, University College Hospital (UCH), Ibadan.

Preparation of Pb NK-65 and Pb ANKA Inoculums

By serially presaging blood drawn from a donor mouse and diluting it with normal saline, a standard inoculum of parasitized erythrocytes was created. [58] In short, through the retro-bulbar plexus of the median canthus of its eye, a supply of parasitized erythrocytes was collected from infected mice, requiring a minimum peripheral parasitemia of 20%. A tube coated with EDTA was used to collect the blood. The amount of parasitized red blood cells relative to the total number of red blood cells was used to calculate the percentage of parasitaemia in each case. Determined by dilution with normal saline, the stock's cell content resulted in 0.2 ml of the final inoculums containing parasistized red blood cells, which are the standard inoculums for infecting a single mouse.

Preparation of extemporaneous injections and oral solutions

In this study, reported procedures were followed with slight modifications. [60,61] Injections containing artemether, miconazole nitrate (MN) or dual drug were successfully applied by intraperitoneal injections of each solubilized drug or rational combination of artemether and MN (4:1) in dimethylsulphoxide (DMSO) [60] at dosages stated under the experimental protocol in the subsequent section. Similarly, oral extemporaneous preparations containing the dosages of artemether, MN or combination of artemether and MN (4:1) dissolved in a homogenous mixture containing Solutol® HS 15 and Capryol® 90 at 1:3 (w/w) [61] in distilled water, were administered perorally as stated under the experimental protocol in the subsequent section.

Experimental protocols

As indicated in Table 1, seventy-two (72) mice were split up into twelve groups, each consisting of six mice.

The first six groups (A-F) were infected with chloroquine-sensitive strain of *Plasmodium berghei* (CPb), the next five groups (G-K) were infected with *Plasmodium berghei* ANKA (PbA) while the last group (L) was not infected and serves as non-infected control, NC. Thereafter, after five and seven days of the inoculation of the mice with CPb and PbA, respectively, the percentage parasitaemia were determined and, after the establishment of malaria, treatment was started on the same day (day 1) on the malariogenic mice and was repeated till day 3 and day 5 for CPb-infected and PbA-infected mice, respectively. Details of the experimental treatments are shown in Table 1. Giemsa-stained tail blood smears obtained after treatment were used to measure parasitemia. Blood samples were taken from the mice's tails, and after fixing the blood with methanol and staining it with 10% Giemsa, thin blood films were created. The mice infected with CPb and PbA were given slides containing the parasites. Each case's stained blood smear slide was placed under a binocular microscope and coated with an immersion oil drop.[59] Red blood cells (RBCs) with and without parasites were analyzed microscopically (Leica DM300 LED Binocular microscope, New York, USA) (x1000 magnification) in each slide field. The total number of RBCs was calculated by counting the number of parasitized RBCs in each field. Mean parasitemia (%) and percentage reduction in parasitemia were obtained using equations 1 and 2. [62]

$$Mean\ parasitemia\ (\%) = \frac{Number\ of\ infected\ red\ blood\ cells\ (RBCs)}{Total\ number\ of\ RBC\ count} x\ 100 \quad (1)$$

$$Percentage\ reduction\ in\ parasitemia\ (\%) = \frac{Parasitemia\ of\ negative\ control\ (\%) - Parasitemia\ of\ treated\ group\ (\%)}{Parasitemia\ of\ negative\ control\ (\%)} x\ 100 \quad (2)$$

Determination of hematological parameters

For both the Pb NK-65-infected and Pb ANKA-infected mice and normal control (uninfected and untreated), blood samples were gathered from every mouse's tail (post-treatment for the treatment groups) and evaluated with respect to red blood cells (RBCs), white blood cells (WBCs), packed cell volume (PCV) and hemoglobin (Hb) using an auto analyzer, consistent with an established procedure. [62]

Table 1: Treatments administered to the mice

Group	Sample code	Treatment	Dosing	Route
A	Art-NK-65	Art monotherapy	8 mg/kg x 3 days	PO
B	MN-NK-65	MN monotherapy	10 mg/kg x 3 days	PO
C	Art-MN-NK-65	Art and MN 4:1 combo therapy	8 mg/2 mg/kg x 3 days	PO
D	AL-NK-65	Marketed ACT (Art and LMF combo therapy)	4 mg/24mg/kg x 3 days	PO
E	CQ-NK-65	Marketed CQ monotherapy	10 mg/kg on day 1, then 5 mg/kg on day 2 and 3	PO
F	DW-NK-65	Distilled water	2 ml/kg x 3 days	PO
G	Art-ANKA	Art monotherapy	8 mg/kg x 5 days	IP
H	MN-ANKA	MN monotherapy	10 mg/kg x 5 days	IP
I	Art-MN-ANKA	Art and MN 4:1 combo therapy	8 mg/2 mg/kg x 5 days	IP
J	Mktd Art-ANKA	Marketed Art injection	8 mg/kg x 5 days	IM
K	NS-ANKA	Normal saline	2 ml/kg x 5 days	IP
L	NC	Normal control (uninfected and untreated) but received distilled water	2 ml/kg x 5 days	PO

Key: Art: artemether, MN: miconazole nitrate, AL: artemether plus lumefantrine marketed dosage regimen, LMF: lumefantrine, ACT: artemisinin-based combination therapy, CQ: chloroquine, NC: normal control, PO: peroral, IP: intraperitoneal, IM: intramuscular, Groups A to F were inoculated with chloroquine sensitive strain of *P. berghei* (NK 65) while Groups G to K were inoculated with *P. berghei* ANKA.

Weight Determination
The various weights of the mice were determined using an electronic veterinary/animal weighing balance (SRV930, USA), before inoculation and after establishment of parasitemia, and after treatment to evaluate the impact of the parasitemia and the treatments on the weights of the animals. The average body weights of the groups were computed and averaged, in line with our earlier report. [58]

Assessment of CPb and PbA induced lethality in mice
The lethality of CPb and PbA infections were assessed by determining the quantity of sick mice that perished in the absence of treatment control and those who receive treatment with various samples.

Statistical analysis
The data was analyzed on GraphPad prism 10.3, using ordinary one-way or two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Values were expressed as mean ± SEM (standard error of means) of n = 6. A *P* value less than 0.05, 0.01, 0.001 and 0.001 was considered statistically significant and was flagged with one star (*), two stars (**), three stars (***), and four stars (****), respectively.

Results and Discussion

Most of the currently approved front line antimalarial drugs are rendered ineffective due to the spread of multidrug-resistant *Plasmodium* parasites, thus necessitating the use of approaches such as drug repurposing to introduce novel compounds as potential antimalarials. [63] In this study, we attempted to realign an antimycotic drug, MN and evaluated it's *in vivo* ability to combat drug-sensitive and drug-resistant types of Pb in murine malaria model. Although researchers have reported additive and/or synergistic antimalarial activity of rational combinations of artemisinin derivatives and imidazole antifungals against *Plasmodium falciparum* and *Plasmodium yoelii nigeriensis*, the antimalarial potential of the combinations against *Plasmodium berghei* (Pb) has not been explored. MN exhibited potent *in vivo* anti-malarial effects against sensitive (CPb) and -resistant (PbA) strains. In combination-treated mice, significant reduction in parasitemia was observed compared to monotherapies. Meanwhile, artemether is predominantly metabolized by CYP 3A4 enzyme. [64] Recently, our group reported enhanced antimalarial activity of nanosized miconazole nitrate in murine malaria model. [65] Besides, imidazole antifungals are cytochrome P_{450} inhibitors and have been reported to potentiate the antimalarial action of artemisinin derivatives. [66] In addition, Solutol® HS 15 employed as surfactant in formulating extemporaneous oral medication inhibits CYP 3A4. [67,68]
Besides, it has been established that drugs which can block the Plasmodia redox process can also increase the effectiveness of artemether. Miconazole can cause a decrease in fungal antioxidant capacity which is utilized in plasmodial therapy.[44] In fact, ROS accumulation was enhanced by the artemisinin-miconazole incubation [45] and the significant synergistic antimalarial impact was associated with the suppression of the erythrocyte-plasmodium complex antioxidant enzymes by miconazole.[46] Thus, the two medications (artemether and miconazole) caused an increase in oxidative stress. Furthermore, miconazole molecularly interacts with an enzyme, Pfldf I, which is a possible molecular target for antimalarial drugs.[47] In terms of pharmacokinetic profiles, miconazole has a long half-life of about 24 hours, while artemether has a short half-life (2 to 4 hours); therefore, the combination of the two drugs is germane due to the fact that artemether would provide fast onset of action to reduce the high parasite load whereas the long-acting miconazole would take care of the residual parasites.

Antimalarial efficacy of artemether and MN against chloroquine sensitive P. berghei infection in mice
Figure 1 shows the mean parasitemia levels in the experimentally chloroquine-sensitive *Plasmodium berghei*-infected groups of mice with or without per-oral treatment whereas percentage reduction in parasitemia of mice infected with NK-65 strain of *Plasmodium berghei* after three days of per-oral treatment is depicted in Figure 2. The group treated with artemether alone saw a mean reduction in parasitemia from 33.4 to 28 after inoculation; the group treated with MN alone saw a mean reduction in parasitemia from 47 to 41 after inoculation; the group treated with artemether/MN combination saw a mean reduction in parasitemia from 45 to 33 after inoculation; the group treated with Coartem® (a commercial artemisinin-based combination product containing artemether and lumefantrine) saw a mean reduction in parasitemia from 32.8 to 20.2 post-inoculation, and the group treated with marketed chloroquine phosphate saw a mean reduction in parasitemia from 38.6 to 32.4 post-inoculation. On the other hand, the untreated group's mean parasitemia count increased (i.e. DW-NK-65 that served as negative control) from 26 post-inoculation to 38 post-treatment. Moreover, while the percentage decrease in parasitemia of commercial products (Coartem® with chloroquine phosphate) were 37.7 and 15.76%, respectively, post-treatment, the percentage decrease in parasitemia attained at study's conclusion (i.e., post-treatment) by Art, MN and Art/MN combination was respectively 14.88, 12.90 and 26.70%. In this research, the reduction in parasitemia caused by artemether/miconazole combo therapy in CPb-infected mice was significantly greater than artemether monotherapy (****p<0.0001), miconazole monotherapy (****p<0.0001) as well as marketed chloroquine (****p<0.0001) via peroral administration (Figure 2). In addition, from the results presented in Figures 1 and 2, it was obvious that the treatments were successful in lowering the level of parasitemia, in contrast to the untreated group's (negative control's) increased parasitemia. These preclinical findings implicate miconazole as a possible repurposed medication with medicinal antimalarial activity, consistent with our recent report[65] and as a potential partner medication to artemether for possible treatment of uncomplicated malaria.

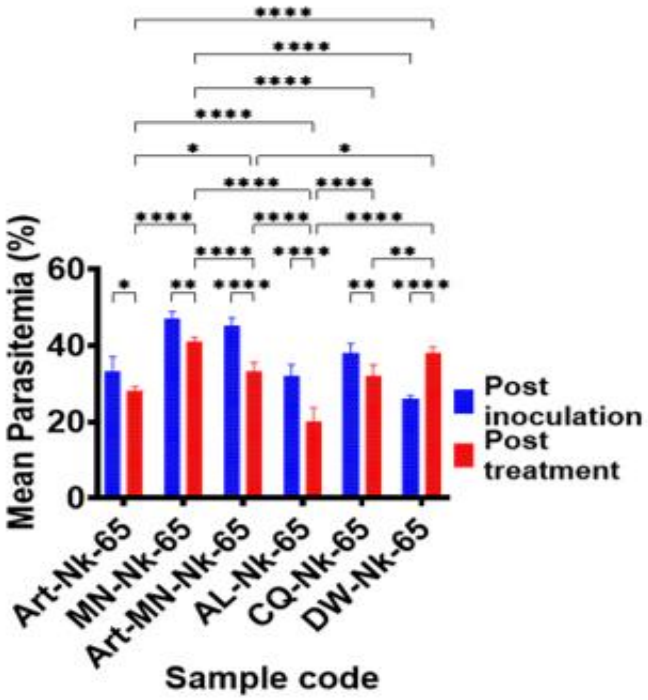

Figure 1: Mean parasitemia levels obtained post inoculation and post treatment with the samples (p.o.) in mice infected with NK-65 strain of *Plasmodium berghei*. Data were expressed as mean ± SEM (standard error of mean) n = 6, differences were considered significant for *p<0.05, **p<0.01 and ****p<0.0001.
Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, AL: artemether plus lumefantrine marketed oral dosage regimen, DW: distilled water and CQ: conventional chloroquine phosphate oral dosage regimen.

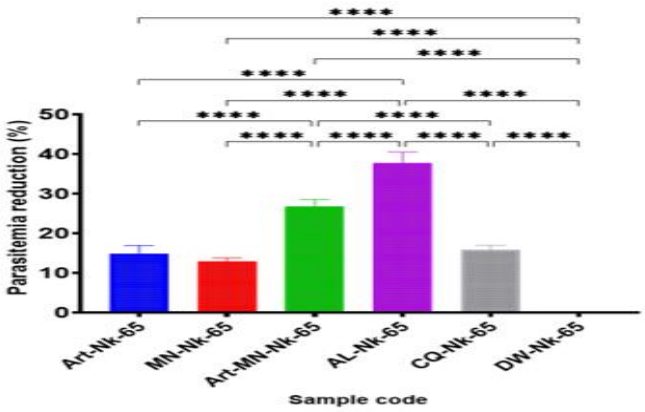

Figure 2: Percentage reduction in parasitemia of mice infected with NK-65 strain of *Plasmodium berghei* after three days of treatment with the samples (p.o). Data were expressed as mean ± SEM (standard error of mean) n = 6, differences were considered significant for ****p<0.0001.
Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, AL: artemether plus lumefantrine marketed oral dosage regimen, DW: distilled water and CQ: conventional chloroquine phosphate oral dosage regimen.

Antimalarial efficacy of artemether and MN against multidrug-resistant P. berghei ANKA infection in mice

Figure 3 shows the mean parasitemia levels in the experimentally *Plasmodium berghei* ANKA-infected groups of mice with or without intraperitoneal or intramuscular treatment whereas percentage reduction in parasitemia in mice carrying a *Plasmodium berghei* ANKA infection following five days of intraperitoneal or intramuscular treatment is depicted in Figure 4. Antimalarial efficacy in PbA-infected mice revealed that the average number of parasites in the group receiving artemether alone reduced from 48 post-inoculation to 44 after treatment; the group's average parasitemia count after treatment with MN alone decreased from 38 post-inoculation to 35 after treatment; the group's average parasitemia count after treatment with artemether/MN combination lowered from 43 post-inoculation to 35 after treatment; wherein the group that was given marketed artemether injection lessened from 41 post-inoculation to 33 after treatment. On the other hand, the untreated group's (i.e. NS-ANKA that served as vehicle or negative control's) mean parasitemia count increased from 32 post-inoculation to 40 after treatment. Besides, while commercial artemether injection gave a percentage decrease in parasitemia of 19.17%, posttreatment, the percentage of parasitemia that was reduced at study completion (i.e., day 5 post-treatment) by Art, MN and Art/MN combination was respectively 8.35, 7.91 and 18.88%. Statistically, the parasitemia reduction caused by artemether/miconazole combo therapy in PbA-infected mice was significantly greater than artemether monotherapy (****p<0.0001) and miconazole monotherapy (****p<0.0001) via parenteral administration (Figure 4). From the results presented in Figures 3 and 4, it was obvious that the treatments were able to lower the level of parasitemia, in contrast to what the untreated group (negative control) showed: rise in parasite infections. Importantly, Art/MN combination reduced the increasing parasitemia in PbA-infected mice, which further confirms its antimalarial property, and this was comparable to the antimalarial activity of artemether injection, a medicine commonly used conventionally for the treatment of severe and cerebral malaria. [69-71]

Effects of artemether and MN on hematological parameters of mice infected with NK-65 P. berghei or P. berghei ANKA

The results of the hematological determinations are shown in Tables 2 and 3. Based on the findings of the comprehensive hematological investigations conducted on the animals (Tables 2 and 3), it was possible to infer that differences in the hematological parameters resulted from the diverse impacts of distinct treatments given to the various animal groups. There was a notable rise in WBCs with a noteworthy decline in HB, PCV and RBCs in the mice infected with both Pb NK-65 and Pb ANKA (negative controls) when compared to normal control (uninfected and untreated) mice (Table 2 and 3). However, treatment with MN and Art and combination of MN and Art as well as with Artemether Inj., CQ and AL significantly decreased WBCs and significantly increased HB, PCV and RBCs, when compared to the negative control groups (DW for Pb NK-65 and NS for Pb ANKA) (Tables 2 and 3). The variations in the hematological parameters of the negative control groups indicate that the unfavorable consequences that followed must have had a factor in the demise of several animals in these specific groupings, which corresponds with our previous reports. [72,73]

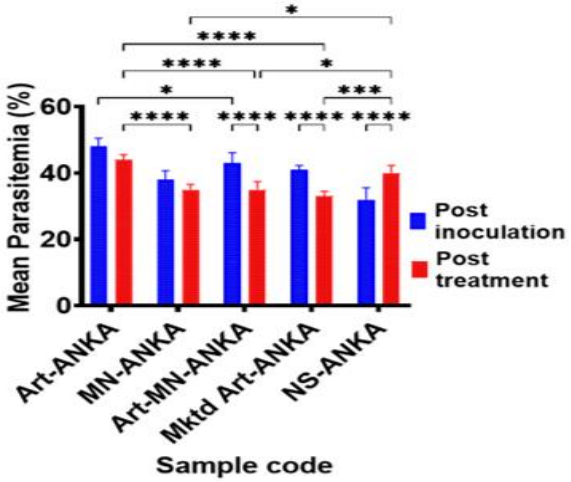

Figure 3: Mean parasitemia levels obtained post inoculation and post treatment with the sample (i.p. and i.m.) in mice infected with *Plasmodium berghei* ANKA. Data were expressed as mean ± SEM (standard error of mean) n = 6, differences were considered significant for *p<0.05, ***p<0.001 and ****p<0.0001.
Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, Mktd Art: marketed artemether injection dosage regimen and NS: normal saline.

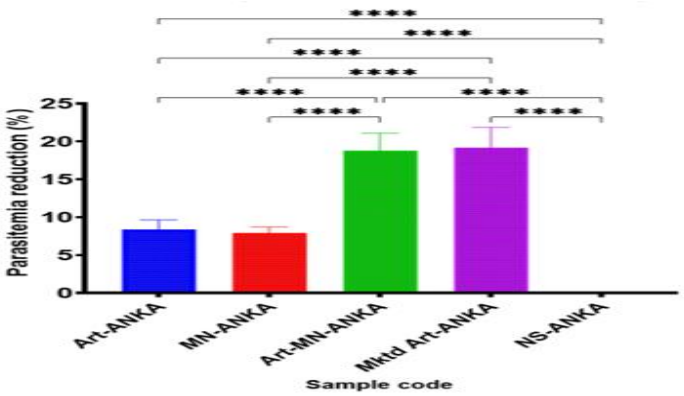

Figure 4: Percentage reduction in parasitemia of mice infected with *Plasmodium berghei* ANKA after five days of treatment with the samples (i.p. and i.m.). Data were expressed as mean ± SEM (standard error of mean) n = 6, differences were considered significant for ****p<0.0001.
Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, Mktd Art: marketed artemether injection dosage regimen and NS: normal saline.

Mice infected with *Plasmodium berghei* are susceptible to anemia because of erythrocyte destruction brought on by either the parasite's growth or the reticuloendothelial cells in the spleen's generation of phagocytes as a result of aberrant erythrocytes. [62,74] In the present investigation, mice treated with *P. berghei* showed clear signs of anemia, as evidenced by decreased levels of PCV, Hb, and RBCs and elevated levels of WBCs. Nevertheless, Pb–induced anemia was vividly reduced in mice treated with Art and MN monotherapies, Art/MN combo therapy, CQ, AL and Art injection which showed a substantial reduction in Pb-induced anemia, which manifested as increased PCV, Hb, and RBCs with decreased WBCs levels. Interestingly, it could be clearly seen from Tables 2 and 3 that for both CPb-infected mice and PbA-infected mice, the anti-anemic activity of Art/MN combo therapy was best when compared to Art and MN monotherapies (i.e. individual doses of Art and MN). However, marketed artemisinin-based combination therapy (AL) and marketed artemether injection gave the overall best anti-anemic activity when compared to other treatments among the CPb-infected mice and PbA-infected mice, respectively, which confirms the established antimalarial property of AL and Art injection, especially against uncomplicated malaria[75-77] and severe or cerebral malaria[69-71], respectively.

Table 2: Effect of the samples on hematological/biochemical parameters of mice infected with or without *Plasmodium berghei* NK-65

Sample code	Treatment	RBC ($x10^{12}$/L)	WBC (Cells/µL)	PCV (%)	Hb (g/dL)
NC	Normal control (uninfected and untreated)	5.51±0.29	7,250±13.96	56.7±3.71	15.8±0.16
Art-NK-65	Art monotherapy	3.92±0.12 [c]	6,180±47.92 [c]	36.8±4.49 [c]	9.82±0.37 [c]
MN-NK-65	MN monotherapy	3.78±0.05 [b]	6,970±54.30 [b]	30.7±3.56 [b]	9.28±0.64 [b]
Art-MN-NK-65	Art and MN 4:1 combo therapy	4.03±0.16 [c]	5,360±67.18 [c]	45.1±5.07 [c]	11.3±0.18 [c]
AL-NK-65	Marketed ACT (Art and LMF combo therapy)	5.14±0.17 [d]	4,280±35.67 [d]	53.6±3.54 [d]	13.6±0.35 [d]
CQ-NK-65	Marketed CQ monotherapy	4.16±0.09 [c]	6,590±28.71 [c]	38.4±3.17 [c]	11.7±0.92 [c]
DW-NK-65	Distilled water	2.87±0.07 [a]	12,640±98.53 [a]	24.8±4.94 [a]	7.65±0.81 [a]

Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, AL: artemether plus lumefantrine marketed oral dosage regimen, DW: distilled water and CQ: conventional chloroquine phosphate oral dosage regimen. Data as mean SEM (Standard Error of Mean) n = 6, [a]p<0.001 when compared to normal control (NC), [b]p<0.01, [c]p<0.05, [d]p<0.001 when compared to distilled water (negative control).

Table 3: Effect of the samples on hematological/biochemical parameters of mice infected with or without *Plasmodium berghei* ANKA

Sample code	Treatment	RBC ($x10^{12}$/L)	WBC (Cells/µL)	PCV (%)	Hb (g/dL)
NC	Normal control	5.51±0.29	7,250±13.96	56.7±3.71	15.8±0.16
Art-ANKA	Art monotherapy	3.84±0.17	6,290±46.78	35.3±2.50	9.14±0.45
MN-ANKA	MN monotherapy	3.47±0.09 [b]	6,990±72.50 [b]	29.5±1.98 [b]	8.73±0.27 [b]
Art-MN-ANKA	Art and MN 4:1 combo therapy	3.98±0.11 [c]	5,490±69.97 [c]	44.6±3.69 [c]	10.8±0.32 [c]
Mktd Art-ANKA	Marketed Art injection [d]	4.72±0.24 [d]	4,370±32.64 [d]	50.8±3.15 [d]	14.61±0.15 [d]
NS-ANKA	Normal saline	2.65±0.08 [a]	13,700±89.35 [a]	21.3±3.76 [a]	6.91±0.54 [a]

Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, Mktd Art: marketed artemether injection dosage regimen and NS: normal saline. Data as mean SEM (Standard Error of Mean) n = 6, [a]p<0.001 when compared to normal control (NC), [b]p<0.01, [c]p<0.05, [d]p<0.001 when compared to normal saline (negative control).

Effects of the drugs on weights of infected mice

Figure 5a shows the weights of the mice infected with NK-65 strain of *Plasmodium berghei* before and after three days of treatment with the samples while Figure 5b shows the weights of *Plasmodium berghei* ANKA-infected mice before and five days following the treatment with the samples. As depicted in the figures, the mice's weight increased as a result of an increase in the size of their liver, spleen, and maybe other blood-forming tissues. [72] These minor weight changes suggest that the medication is working to control the mice's malaria, which is becoming worse. It could be seen from Figures 5a and 5b that, in line with our previous research, the weight of the animals increased in all groups overall, although at a slower rate in the treatment groups than in the negative control group.[73]

Effects of the drugs on lethality of infected mice

Table 4 presents the mortality of PbA and CPb infections in the animals. For CPb-infected animals (groups A-F), mortality among those infected and untreated mice (i.e. DW-NK-65) was 66.67% (4/6) while mortality was 33.33% (2/6) in the afflicted cohorts administered with Art and MN monotherapies (i.e. Art-NK-65 and MN-NK-65) and 16.67% (1/6) within the afflicted cohorts administered with Art and MN combo therapy (i.e. Art-MN-NK-65), marketed artemether/lumefantrine combo therapy (i.e. AL-NK-65) and marketed chloroquine monotherapy (i.e. CQ-NK-65).

For PbA-infected animals (groups G-K), mortality among those infected and untreated mice (i.e. NS-ANKA) was 66.67% (4/6) while mortality was 33.33% (2/6) in the afflicted cohort administered with artemether monotherapy (i.e. Art-ANKA) as well as afflicted cohort administered with artemether and MN combo therapy (i.e. Art-MN-ANKA, 50% (3/6) in the afflicted cohorts administered with MN monotherapy (i.e. MN-ANKA) and 16.67% in the afflicted cohort given commercial artemether injectable monotherapy (i.e. Mktd ANKA). The mice in the untreated and uninfected group that functioned as the normal control (NC) did not die.

Meanwhile, the lethality of CPb and PbA illnesses in mice that are untreated and afflicted confirms these parasites to be virulent. [58] These parasites (Pb NK-65 and Pb ANKA) are laboratory models that are frequently employed to imitate *Plasmodium falciparum* infections that cause uncomplicated malarial illness and severe or cerebral malaria, respectively, in humans. [58,59]

Lethality can be used as a measure of antimalarial activity of a drug. [59] In this study, MN reduced the mortality in CPb-infected mice. Mortality rate can be employed to gauge a medication's effectiveness against malaria. [59] In this investigation, MN decreased the death rate of CPb-infected and PbA-infected mice by 50 and 25%, respectively, implying that MN is more effective against UM than SM in Pb murine malaria model. Comparatively, artemether decreased mortality in mice infected with PbA and CPb by 50%, confirming the drug's known antimalarial properties, particularly against cerebral or severe malaria in contrast to MN (a repurposed antimalarial agent). [65, 69-71] Interestingly, the reduction in mortality of PbA-infected mice achieved with artemether/MN combo therapy was equal to that of artemether; this further confirms that the antimalarial activity of artemether/MN was similar to the antiplasmodial activity of artemether. However, the marketed artemether injection achieved the best mortality reduction of PbA-infected mice (75%), thus adjudging it as better than the combo therapy against PbA. Nonetheless, the reduction in mortality of CPb-infected mice achieved with artemether/MN combo therapy was equal to that of marketed chloroquine and artemether/lumefantrine combo therapy. This not only confirms the established antimalarial activity of chloroquine and artemether/lumefantrine, especially against uncomplicated malaria[58,72-73,75-78] but also implies that the use of artemether/MN combo therapy in UM should be initiated.

Table 4: Survivability of uninfected and infected groups of mice (with and without treatment)

Group	Sample code	Treatment	Number of surviving mice post inoculation (before treatment)	Number of surviving mice post treatment	Mortality (%)
A	Art-NK-65	Art monotherapy	6/6	4/6	33.33
B	MN-NK-65	MN monotherapy	6/6	4/6	33.33
C	Art-MN-NK-65	Art and MN 4:1 combo therapy	6/6	5/6	16.67
D	AL-NK-65	Marketed ACT	6/6	5/6	16.67
E	CQ-NK-65	Marketed CQ monotherapy	6/6	5/6	16.67
F	DW-NK-65	Distilled water	6/6	2/6	66.67
G	Art-ANKA	Art monotherapy	6/6	4/6	33.33
H	MN-ANKA	MN monotherapy	6/6	3/6	50.00
I	Art-MN-ANKA	Art and MN 4:1 combo therapy	6/6	4/6	33.33
J	Mktd Art-ANKA	Marketed Art injection	6/6	5/6	16.67
K	NS-ANKA	Normal saline	6/6	2/6	66.67
L	NC	Normal control (uninfected and untreated)	6/6	6/6	0.00

Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, AL: artemether plus lumefantrine marketed oral dosage regimen, DW: distilled water, CQ: conventional chloroquine phosphate oral dosage regimen, Mktd Art: marketed artemether injection dosage regimen and NS: normal saline. Groups A to F were inoculated with chloroquine sensitive strain of *Plasmodium berghei* (NK 65) while Groups G to K were inoculated with *Plasmodium berghei* ANKA.

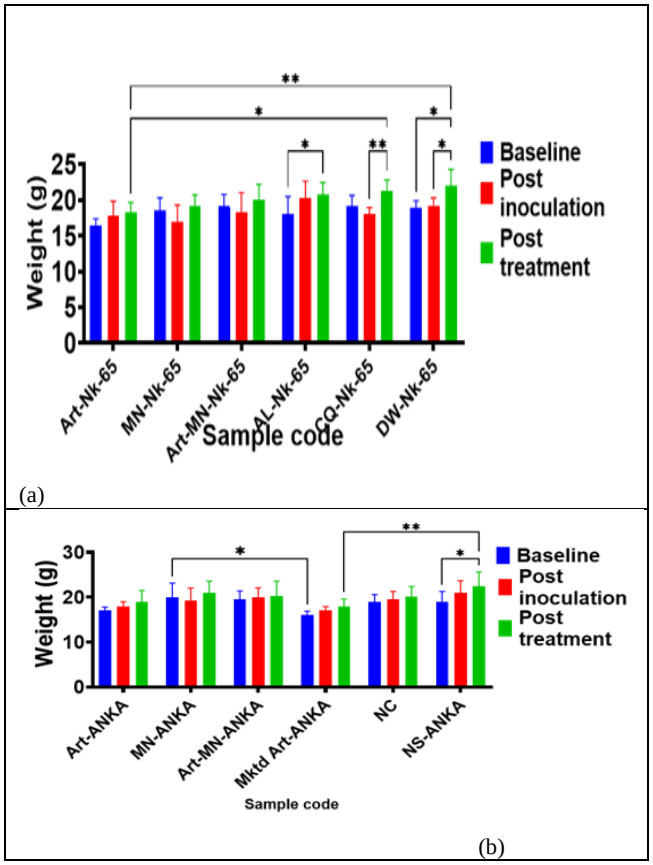

Figure 5: Weights of the mice infected with (a) NK-65 strain of *Plasmodium berghei* before and after three days of treatment with the samples (p.o) (b) *Plasmodium berghei* ANKA before and after five days of treatment with the samples (i.p. and i.m.). Data were expressed as mean ± SEM (standard error of mean) n = 6, differences were considered significant for *p<0.05 and **p<0.01.
Key: Art: pure artemether sample, MN: pure miconazole nitrate sample, AL: artemether plus lumefantrine marketed oral dosage regimen, DW: distilled water, CQ: conventional chloroquine phosphate oral dosage regimen, Mktd Art: marketed artemether injection dosage regimen and NS: normal saline.

Conclusion

This study has shown that miconazole both by itself and when combined with artemether demonstrated its activity *in vivo* in drug-sensitive and resistant murine malaria model using CPb and PbA. Overall, the findings suggest further research into repositioning miconazole as possible antimalarial component and so suggests a novel approach to support the development of antimalarial drugs via combo-therapy with first-line antimalarial drugs (artemisinin and its derivatives).

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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