Investigation of herb-drug interaction between *MAMA powder* herbal antimalarial remedy and amodiaquine, chloroquine, artesunate in murine malaria

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ABSTRACT

Background: Antimalarial herbal remedies are traditionally presented as infusions, decoctions and extracts. There is limited information on antimalarial herbal powder presentations. The outcomes of possible co-administration of *MAMA POWDER* (MP), a herbal powder listed for malaria treatment, with orthodox antimalarial drugs, had not been explored.

Objectives: This study investigated MP as a reduced particle-sized powder, and its effects on amodiaquine (AQ), chloroquine (CQ), artesunate (AS) in mice.

Methods: *MAMA POWDER* comprises the powdered stem bark of *Alstonia boonei* De Wild (Apocynaceae) and seed of *Picralima nitida* H Durand (Apocynaceae). The barks were milled, sieved (particle size, 180 μm), mixed and suspended in propylene glycol (50 %). This suspension was assessed on chloroquine-sensitive and chloroquine-resistant *Plasmodium berghei* strains in varying oral doses (0.39-100 mg/kg), using the chemosuppressive and curative antimalarial test models. Co-administration of MP with the orthodox drugs was similarly investigated as follows: (MP 1.56+AQ 10; MP 6.25+AQ 10), (MP 1.56+CQ 10; MP 6.25+CQ 10), (MP 1.56+AS 4; MP 6.25+AS 4) mg/kg.

Results: MP suspension (6.25 mg/kg) exhibited considerable antimalarial chemosuppression (82 %) and it enhanced the activity of AQ (MP 6.25+AQ 10 mg/kg) in chloroquine-sensitive malaria infection (91 %). Significant antagonistic interactions were observed with the combinations of MP (1.56 mg/kg, 69%) and CQ (55.2 %) respectively.

Conclusion: MP exhibited significant antimalarial activity. The potentiation of AQ and antagonism with CQ, suggested herb-drug interactions, which called for further investigation.

Keywords: Antagonism, *Plasmodium berghei,* Powder dosage form, *Alstonia boonei, Picralima nitida*

Étude de l'interaction plante-médicament entre le remède antipaludique à base de plantes en *poudre MAMA* et l'amodiaquine, la chloroquine et l'artésunate dans le cas du paludisme murin

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RÉSUMÉ

Contexte: Les remèdes antipaludiques à base de plantes se présentent traditionnellement sous forme d'infusions, de décoctions et d'extraits. Les informations sur les présentations de poudres antipaludiques à base de plantes sont limitées. Les effets d'une éventuelle co-administration de *MAMA POWDER* (MP), une poudre à base de plantes indiquée pour le traitement du paludisme, avec médicaments antipaludiques orthodoxes n'ont pas été étudiés.

Objectifs: Par conséquent, cette étude a examiné le MP sous forme de poudre de taille de particules réduite et ses effets sur l'amodiaquine (AQ), la chloroquine (CQ) et l'artésunate (AS) chez la souris.

Méthodes: *MAMA POWDER* comprend l'écorce de tige en poudre d'Alstonia boonei De Wild (Apocynacées) et la graine de *Picralima nitida* H Durand (Apocynacées). Les écorces ont été broyées, tamisées (granulométrie : 180 µm), mélangées et mises en suspension dans du propylène glycol (50%). Cette suspension a été évaluée sur des souches de *Plasmodium berghei s*ensibles et résistantes à la chloroquine à différentes doses orales (0,39-100mg/kg), en utilisant les modèles de test antipaludique chimiosuppresseur et curatif. La co-administration de MP avec les médicaments orthodoxes a également été étudiée de la manière suivante : (MP 1,56 + AQ 10 ; MP 6,25 + AQ 10), (MP 1,56 + CQ 10 ; MP 6,25 + CQ 10), (MP 1,56 + AS 4 ; MP 6,25 + AS 4) mg/kg.

Résultats: La suspension de MP (6,25 mg/kg) a montré une chimio-suppression antipaludique considérable (82%) et a amélioré l'activité de l'AQ (MP 6,25 + AQ 10 mg/kg) dans l'infection paludique sensible à la chloroquine (91%). Des interactions antagonistes significatives ont été observées avec les combinaisons de MP (1,56 mg/kg, 69 %) et de CQ (55,2 %) respectivement.

Conclusion: Le MP a montré une activité antipaludique significative. La potentialisation de l'AQ et l'antagonisme avec la CQ ont suggéré des interactions entre les plantes et les médicaments, ce qui nécessite des recherches plus approfondies.

Mots-clés: Antagonisme, *Plasmodium berghei,* Forme posologique en poudre, *Alstonia boonei, Picralima nitida*

INTRODUCTION

Malaria, caused in humans majorly by *Plasmodium falciparum*, is a disease of global public health concern that occurs mainly in tropical and subtropical regions of the world. According to the World Health Organization (WHO), 263 million malaria cases and 0.6 million mortalities occurred in 2023.[1] Significant increases from 2022 cases were seen largely due to disruptions with the COVID-19 infection. The African region still bears the highest burden of malaria cases with 233 million cases in 2022, accounting for about 94 % of global cases with Nigeria as one of the four countries responsible for nearly half of all malaria cases globally.[1] Ethnomedical use of herbal remedies in the treatment of malaria is a common practice in sub-Saharan Africa.[2] Modern herbal formulations of various plant materials have evolved through research and development efforts. The antimalarial and toxicity properties of the combination of the powdered stem bark of *Alstonia boonei* De Wild (Apocynaceae) and the powdered seed of *Picralima nitida* (Stapf) T.Durand & H.Durand (Apocynaceae), were reported.[3,4] This combination was patented and registered as an antimalarial agent by the Faculty of Pharmacy, Obafemi Awolowo University, Nigeria. Although MP exists as a powder, previous studies have been on the extracts of the plants.[4,5] The present study aimed at evaluating the fine powder dosage form of the herbal product with a view to simulating the current mode of use in humans. In traditional herbal medicine, powder dosage formulations are culturally acceptable and cost-effective compared to other formulations. They also have been shown to have rapid bioavailability relative to compacts and capsule dosage forms.[6] The smaller particle size of powdered dosage forms has been shown to result in rapid absorption from the gastrointestinal tract compared to tablets. This, in turn, leads to reduced local irritation of the gastrointestinal tract which may be caused by the local concentration of a drug as encountered when taking an equivalent tablet.[7] Powder dosage forms are generally considered to be stable compared to other dosage forms, however, their stability is largely determined by the type and nature of the plant material, the moisture content, and the type of packaging used in storing the powder.[8]

In malaria therapy, the practice of concurrent administration of orthodox and herbal medicines is widespread due to the belief that such combinations have increased efficacy, short duration of treatment, and reduced side effects.[6] A beneficial combination of an herbal antimalarial, *MAMA* Decoction and amodiaquine with total parasite clearance in CQ-sensitive *Plasmodium berghei*-infected mice was reported.[9] Due to the dearth of knowledge about possible interactions between MP and orthodox drugs, it was necessary to investigate the potential effects of MP on the activities of some orthodox antimalarial drugs and therefore provide information on the possible co-administration of these drugs.

METHODS

Plant collection and authentication

The fresh stem bark of *Alstonia boonei* De Wild. (Apocynaceae) was collected in the rainy season (March 2019) at the Obafemi Awolowo University (OAU), Ile Ife (7.5183 ^{o}N, 4.5228 ^{o}E) while the seed of *Picralima nitida* (Stapf) T. A Durand & H. Durand (Apocynaceae) was purchased at Oja-Oba market, Osogbo, (7.767 N, 4.567^{o}E) Nigeria. The two plants were identified by Mr. Ifeoluwa Isaac Ogunlowo, the plant curator of the Department of Pharmacognosy, OAU. Voucher specimens were deposited in the Ife Herbarium as well as in the herbarium of the Faculty of Pharmacy, OAU, where voucher numbers IFE 16534 and FPI 2225 were given, respectively.

Preparation of *MAMA POWDER*

The *A. boonei* stem bark and *P. nitida* seed were oven-dried at 50 ^{o}C, individually pulverized, and sieved to give powders with particle size of 180 µm. Fifty grams (50 g) of *A. boonei* powder was thoroughly mixed with two parts (100 g) of *P. nitida* powder to obtain MP which was subsequently suspended in propylene glycol (50 %) before drug administration. The combination ratio of the *A. boonei*: *P. nitida* (1:2) was based on the published data by Ajayi *et al*., 2015.[3]

Parasites and experimental animals

Chloroquine-sensitive *Plasmodium berghei* NK 65 and chloroquine-resistant *P. berghei* ANKA parasites were obtained from the Institute for Advanced Medical Research and Training, University College Hospital, Ibadan, Nigeria. The animals were sourced from the Department of Pharmacology, Faculty of Pharmacy, OAU. The study followed the "Principles of laboratory animal care",[10] and the method reviewed by the Postgraduate Committee of the Faculty.

The donor mice were monitored until the *Plasmodium* infections were 30 % and 15 %, respectively. Blood was withdrawn by cardiac puncture into heparinized sterile bottles and diluted with normal saline to contain 1x107 parasitized red blood cells in 0.2 mL suspension of

inoculum. Experimental mice were inoculated intraperitoneally with diluted blood (0.2 mL).[11]

Evaluation of acute and repeated-dose toxicity studies

The toxicity tests were carried out according to the Organisation for Economic Co-operation and Development (OECD) method.[12] Five healthy female albino mice were given MP (1000 mg/kg) for 7 days and they were observed for morbidity and mortality, first for 24 h and thereafter for 14 days.

Assessment of chemosuppressive activity

The chemosuppressive activities were carried out using the early malaria infection test model described by Peters *et al*. 2002[13] on *P. berghei* NK65-infected mice randomly divided into five mice per group. First, treatment of the experimental group was initiated two hours post-inoculation by oral administration of MP at 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, and 25 mg/kg for four days. Mice in the positive control groups were given CQ, AQ and AS at 10, 10, and 4 mg/kg (200 µL), respectively, for four days while mice in the negative control group were given propylene glycol (50%, 200 µL).

Secondly, the herb-antimalarial drug combinations were evaluated as follows - MP combined with AQ: (MP 1.56+AQ 10 mg/kg; MP 6.25+AQ 10 mg/kg), with CQ (MP 1.56+CQ 10 mg/kg; MP 6.25+CQ 10 mg/kg) and with AS (MP 1.56+AS 4 mg/kg; MP 6.25+AS 4 mg/kg).

Furthermore, the herb-antimalarial drug combinations were evaluated on mice infected with the chloroquine-resistant *P. berghei* ANKA as follow: Eight (8) groups of infected mice were orally treated with MP (6.25 mg/kg) as well as combinations of MP (6.25 mg/kg) with each of AQ (MP 6.25+AQ 10 mg/kg), CQ (MP 6.25+CQ 10 mg/kg) and AS (MP 6.25+AS 4 mg/kg). Mice in the positive control groups were given CQ, AQ and AS at 10, 10, and 4 mg/kg, respectively.

Assessment of curative activity

The curative antimalarial activities were carried out using the established infection test model described by Ryley and Peters.[14] Fifty-five CQ-sensitive *P. berghei*-infected mice were divided into eleven (11) groups. Treatment started 72 h post-inoculation by oral administration of MP (0.39, 0.78, 1.56, 3.12, 6.25, and 12.5 mg/kg)[3] for five days. Also, combinations of MP and AQ (MP 1.56+AQ 10 mg/kg; MP 6.25+AQ 10 mg/kg), MP and CQ (MP 1.56+CQ 10 mg/kg; MP 6.25+CQ 10 mg/kg) MP and AS (MP 1.56+AS 4 mg/kg; MP 6.25+AS 4 mg/kg) were similarly evaluated. Mice in the positive control group were given CQ, AQ, and AS at 10, 10, and 4mg/kg, respectively, for five days while those in the negative control group received propylene glycol (50 %).

Furthermore, in chloroquine-resistant *P. berghei* ANKA strain, MP (25, 50, and 100 mg/kg) and its combination with AQ (MP12.5+AQ10 mg/kg) were evaluated as earlier described.

Blood smear preparation

Twenty-four hours after the drug administration, a thin film blood smear was prepared from each mouse by snipping the tip of the tail of each mouse and dropping its blood on a clear slide, fixed with drops of methanol, and stained with giemsa (10 %). Parasitaemia assessments of the giemsa-stained slides were carried out by light microscope (x 100). The number of parasitised red blood cells (RBC) was counted against the total number of RBCs present in 10 different fields of view and the percentage parasitaemia calculated.

Survival time and percentage survival determination:

The mice were observed for 28-days post-treatment and mortality, which occurred during the period, was recorded. The survival time was determined as the percentage of the ratio of the number of mice that survived during the period to the total number of mice in the group.

Data analysis

The average parasitaemia was obtained by calculating the percentage of the ratio of parasitised RBCs (^{Np}) to the total number (^{Nt}) of RBCs per view of the count:

Average parasitaemia = $^{Np}/_{Nt}$ X 100. The average percentage chemosuppression and parasite clearance were calculated as 100 x $\frac{(A-B)}{A}$, where A and B represent the average parasitaemia of the negative control and test groups, respectively.

Data was presented as mean±standard error of the mean. The statistical analysis - ANOVA and Student's-Newman Keuls test- was performed with Microsoft Excel (2010) and Graphpad Instat (2003). A P value of < 0.05 was considered statistically significant.

RESULTS

Toxicity tests

The suspension of MP (1000 mg/kg) was well-tolerated by the mice. No visible sign of morbidity nor mortality was observed within the period of the experiment.

Chemosuppressive antimalarial activities

The chemosuppressive antimalarial activity of MP on chloroquine-sensitive *P. berghei*-infected mice is presented in Table 1. The activity (81.81 %, 6.25 mg/kg) of MP was comparable (p>0.05) to the activities of AQ (85.24 %, 10 mg/kg), CQ (83.24 %, 10 mg/kg) and AS (82.13 %, 4 mg/kg). A similar observation was made with the survival time of the mice treated with MP at the same dose.

In the herb-antimalarial drug combination study, there were no significant differences in the activities of MP (1.56 mg/kg) when combined with AQ and AS, respectively. However, significant bi-directional reductions in the activities of CQ (83.2 to 54.76 %) and MP (68.9 to 55.76 %) were observed when the two drugs were combined (Fig. 1).

In the chloroquine-resistant malaria infection, the activities of MP (6.25 mg/kg) in combination with AQ, AS and CQ were not significantly different (p>0.05) from their activities as single drug agents (Fig. 2).

Table 1: Effects of *MAMA POWDER* (0.39-25 mg/kg) on parasitaemia and survival time of chloroquine-sensitive *Plasmodium berghei* NK65-infected mice using the early malaria infection test model

Dose (mg/kg)	Parasitaemia (%±SEM)*	Chemosuppression (%±SEM)*	Survival time (Day±SEM)*
MP 0.39	6.94±0.43^{a}	33.26±6.28^{a}	9.20±2.78^{a}
MP 0.78	5.50±0.27^{a}	39.62±4.29^{a}	9.60±2.57^{a}
MP 1.56	2.83±0.48^{b}	68.89±4.62^{b}	10.60±6.00^{a}
MP 3.12	3.07±0.77^{b}	66.24±8.55^{b}	14.40±4.80^{b}
MP 6.25	1.55±0.55^{d}	81.81±6.34^{d}	13.40±4.17^{b}
MP 12.5	2.68±2.47^{b}	70.53±7.59^{c}	14.00±3.30^{b}
MP 25.0	3.05±0.62^{b}	72.45±6.81^{c}	20.40±1.79^{b}
AQ (10)	1.34±0.67^{d}	85.24±7.39^{d}	16.40±1.79^{b}
AS (4)	1.23±0.18^{d}	82.13±2.70^{d}	19.00±2.00^{b}
CQ (10)	1.46±0.70^{d}	83.24±7.39^{d}	19.40±2.00^{b}
NC (0)	9.11±1.68^{e}	00.00±0.00	10.85±5.85^{a}

Keys: MP=*MAMA POWDER*, AQ=Amodiaquine, AS=Artesunate, CQ=Chloroquine, NC=Negative control; *Values with the same superscript letters were not significantly different (p>0.05) from each other while values with different superscript letters were significantly different from each other (p<0.05).

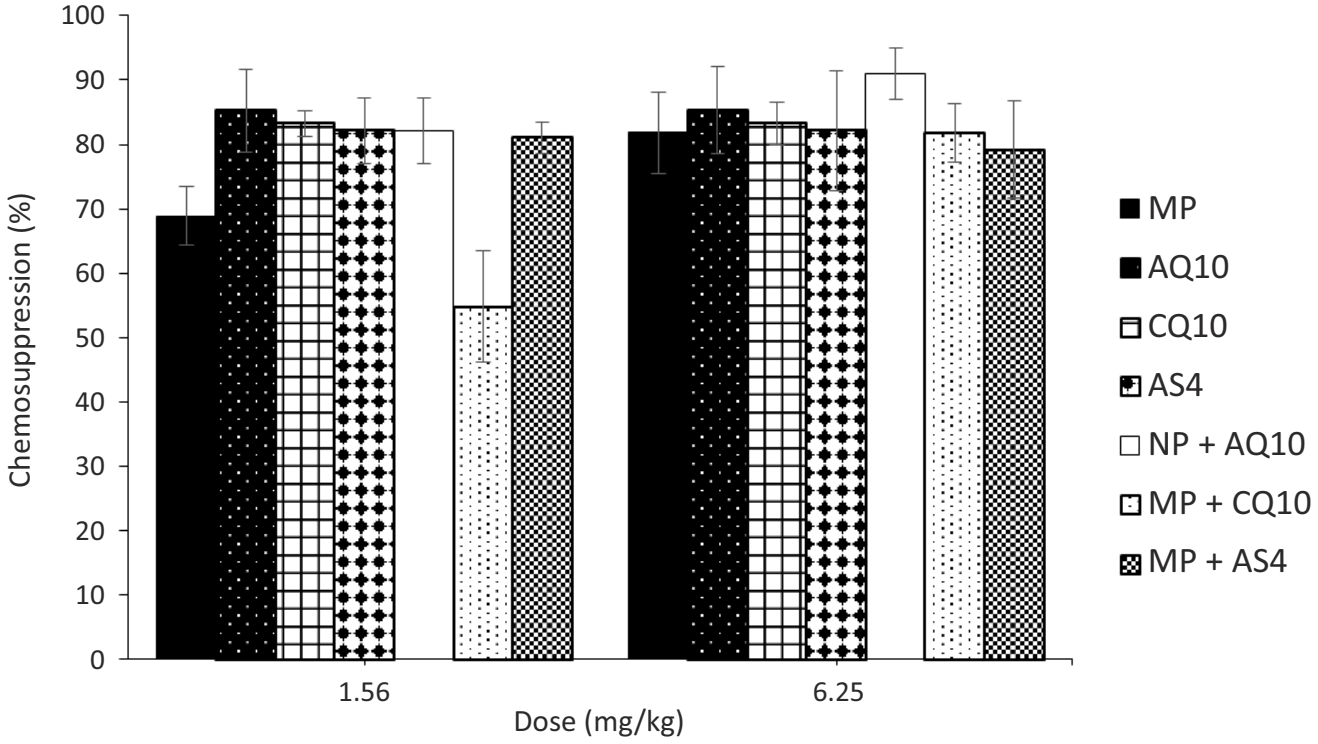

Fig. 1: Chemosuppressive activities of *MAMA Powder* (1.56 and 6.25 mg/kg) combined with amodiaquine, chloroquine, artesunate in chloroquine-sensitive *Plasmodium berghei*-infected mice.

Data expressed as mean±SEM, MP-*MAMA Powder*; AQ-amodiaquine; CQ-chloroquine; AS- artesunate.

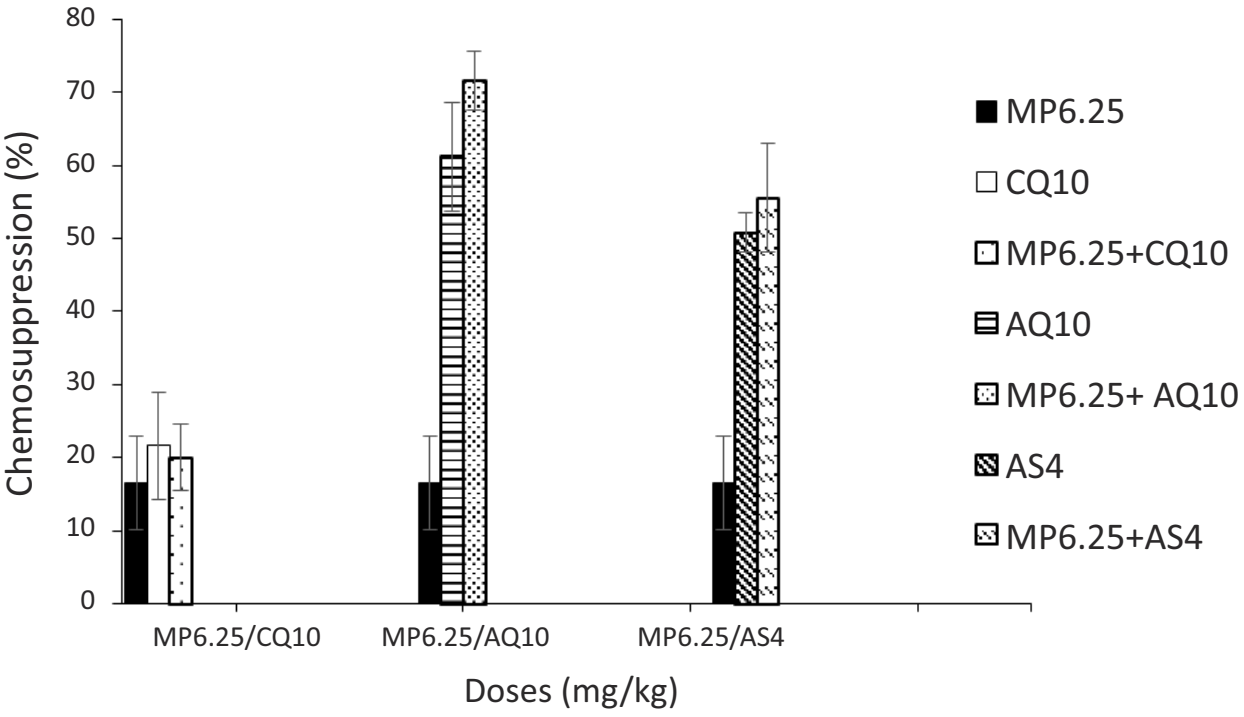

Fig. 2: Chemosuppressive effects of *MAMA Powder* combined with selected orthodox antimalarials on parasitaemia of CQ-resistant *Plasmodium berghei* ANKA-infected mice

Data expressed as mean ±SEM, MP-*MAMA Powder*; AQ-amodiaquine; CQ-chloroquine; AS- artesunate.

Curative antimalarial activities

In the chloroquine-sensitive malaria infection, the highest parasite clearance was observed with MP at 6.25 mg/kg on the 5th day (78.06 %), and this was not significantly different ($p>0.05$) from the activities of AQ (80.63 %) and CQ (83.61 %) (Fig. 3). The herb-antimalarial drug combination of MP at a higher dose of 6.25 mg/ kg with AQ 10mg/kg exhibited the highest curative activity (71.3 %) (Fig. 4).

In the chloroquine-resistant infection, MP (100 mg/kg) gave the highest activity (64.87 %) comparable to AQ (65.70 %) while the herb-drug combination of MP 12.5 + AQ 10 mg/kg exhibited parasite clearance of 70.85% (Fig. 5).

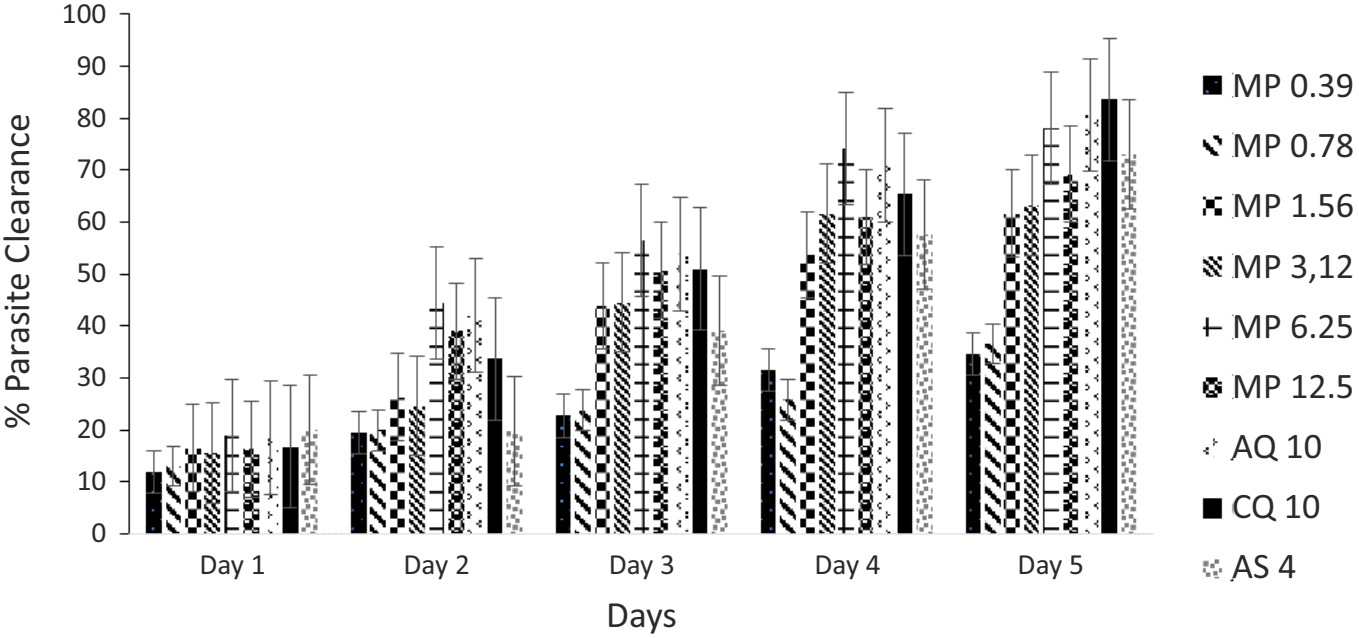

Fig. 3: Curative activity of *MAMA Powder* (0.39-12.5 mg/kg) on chloroquine-sensitive *Plasmodium berghei* NK65 infected mice

Data expressed as mean ±SEM, MP-*MAMA Powder*; AQ-amodiaquine; CQ-chloroquine; AS- artesunate

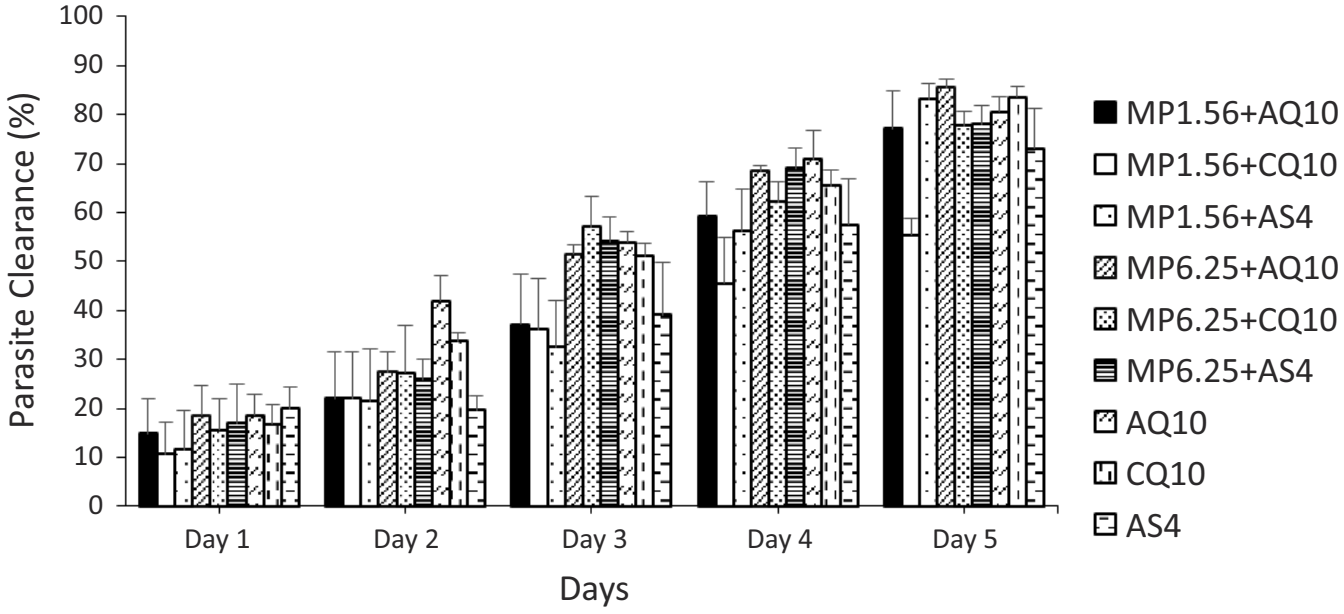

Fig. 4: Effects of amodiaquine, chloroquine and artesunate on the curative activity of *MAMA Powder* (1.56 and 6.25 mg/kg) in chloroquine-sensitive *P. berghei*-infected mice

Data expressed as mean±SEM, MP-*MAMA Powder*; AQ-amodiaquine; CQ-chloroquine; AS-artesunate.

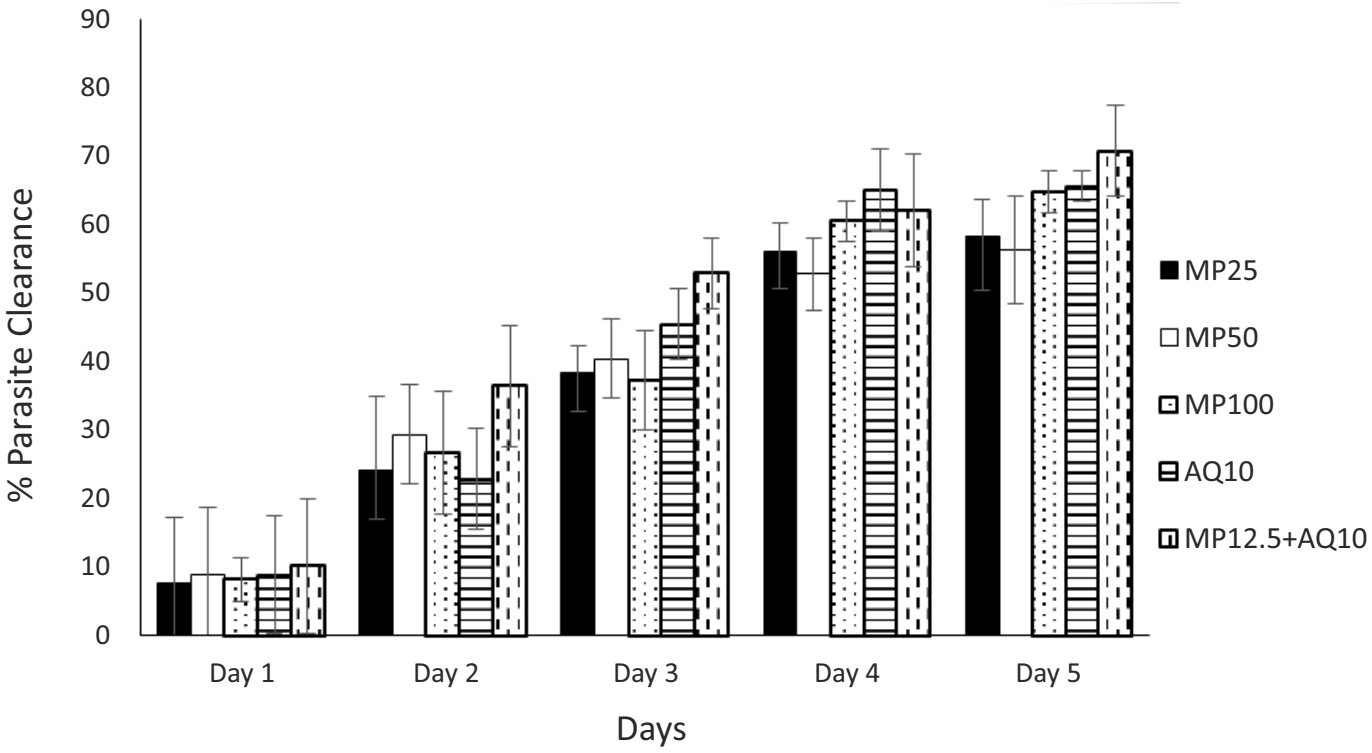

Fig. 5: Effects of *MAMA Powder* (25-100 mg/ kg) on chloroquine-resistant *Plasmodium berghei*-infected mice

Data expressed as mean±SEM, MP-*MAMA Powder*; AQ-amodiaquine.

DISCUSSION

MAMA POWDER is an herbal antimalarial product comprising the combination of the powdered stem bark of *Alstonia boonei* and the seed of *Picralima nitida,* both Apocynaceae plants. It is produced in the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, as a scientifically validated herbal medicinal product.3 However, previous studies have been limited to the evaluation of the water and methanol extracts of the Powder. Therefore, this study explored the possibility of evaluating the whole powder in a fine-particle (180µm) presentation in murine malaria as well as investigating possible herb-drug interaction with orthodox antimalarials drugs.

The present investigation has shown relative safety on repeated administration of MP at the experimental dose (1000 mg/kg). This corroborated an earlier study on the aqueous extract of MP and reported neither morbidity nor mortality at single doses of 1000-5000 mg/kg and repeated doses of 12.5-50 mg/kg while histopathology of essential organs also revealed normal architecture.4

In this study, the suspension of MP exhibited significant chemosuppressive (82 %) and curative (78.1 %) antimalarial activities which further justified the use of MP as an antimalarial agent in chloroquine-sensitive infections. It supported a previous report that the aqueous extract had antimalarial activities (75 %), which was optimal at 12.5 mg/kg.[3] However, the optimal dose of the fine-powder formulation in this present study was lower (6.25 mg/kg) and, therefore, should be more therapeutically and economically beneficial. Expectedly, a much higher dose of MP (100 mg/kg) was needed to achieve significant parasite clearance in the chloroquine-resistant infection.

In traditional medicine, powder dosage forms, containing one or more medicinal plants, constitute a large percentage of herbal drug formulations.15 Historically, the use of powder dosage form had existed as far back as 220AD in Traditional Chinese Medicine (TCM). A higher bioavailability was observed with the ultrafine powder formulation of *Salvia miltiorrhiza*, used in TCM because of the absorption of more chemical compounds, for the treatment of coronary heart disease, when compared with its preparation as a decoction.16 Chemical compounds identified in the MP included the aglycone of loganic acid, akuammine, alstonine, picraline, and picratidine, some of which have been implicated in the

antimalarial activities of *P. nitida*.[5,17] Hence, it is possible that the activity of MP in this study may be as a result of the effect of one or more of these compounds. Consequently, some of these compounds can be proposed for standardising the pharmaceutical formulations of MP products.

The herb-drug interaction studies revealed a potentiation in the activities of amodiaquine when combined with the optimal dose of MP in the chloroquine-sensitive infection. Enhanced activities in herb-drug combination therapy were reported with *Ageratum conyzoides* and *chloroquine* as well as with artesunate when combined.[18] In addition, *Morinda lucida, Alstonia boonei* and *Curcuma longa* enhanced the antimalarial effects of sulphadoxine-pyrimethamine and quinine.[19] Although it was assumed that a combination of the drugs at their therapeutic doses may be more beneficial, however, the combinations at their suboptimal doses also elicited significantly high parasite clearance. The combination of drugs at their sub-therapeutic doses may reduce the possibility of side effects of either drug. Furthermore, in this study, it is interesting to note that antagonistic interactions were observed at the sub-therapeutic dose of MP (1.56 mg/kg) with CQ. The overall antimalarial effect (55 %) of the combination of MP (69 %, 1.56 mg/kg) and CQ (83 %, 10 mg/kg) was less than their individual effects, which could result in the reduction of therapeutic efficacy when both drugs are concurrently administered. Further investigations may be necessary to evaluate the observed antagonistic interaction, especially as such observation was also recorded with the same combinations in the curative (treatment) antimalarial model.

The antimalarial activity of chloroquine was significantly reduced when concurrently administered to patients using Catha edulis 20 as well as the activity of artesunic acid in combination with Carica papaya.21

The research and development effort into the development of MP is an ongoing process. Continuous assessment of herbal medicines should be encouraged. One of such is the investigation of Yoyo Bitters, a polyherbal preparation that showed noticeable antimalarial activity.22 This present study showed that MP at this particle size may be ideal for liquid and solid dosage pharmaceutical forms. Furthermore, it revealed the possibilities for newer formulation approaches to oral delivery of MP such as sustained- and extended-release formulations, microparticles, and nanoparticles. Consequently, with an array of bioactive chemical constituents and supported with future clinical trials, MP may serve as a veritable alternative to the currently used artemisinin-based combination therapy.

CONCLUSION

The study concluded that the fine powder of MP exhibited significant antimalarial activity at a relatively reduced dose. Also, MP potentiated the activity of amodiaquine in the chloroquine-sensitive malaria infection. Further investigations are needed to understand the mechanism of the observed antagonism effect between MP and chloroquine

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