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Antimalarial properties of SAABMAL®: an ethnomedicinal polyherbal formulation for the treatment of uncomplicated malaria infection in the tropics

I.C. Obidike, B. Amodu** & M.O. Emeje*

*Departments of Pharmacology & Toxicology & *Pharmaceutical Technology & Raw Material Development, National Institute for Pharmaceutical Research & Development & **Halamin Herbal Center, Abuja, Nigeria*

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***Background & objectives*: Malaria is a serious problem in the countries of the developing world. As the malaria parasite has become resistant to most of the antimalaria drugs available currently, there is a need to search for newer drugs. This study reports the pharmaceutical quality and *in vivo* antimalarial activities of a polyherbal formulation (SAABMAL®) used as malarial remedy in Nigeria.**

***Methods*: The antiplasmodial activity of SAABMAL® was determined by using the 4-day suppressive test in *Plasmodium berghei*-infected mice. The formulation was tried on three different experimental animal models for *in vivo* antimalarial activities, which are prophylactic, suppressive and curative in mice. Chloroquine and pyrimethamine were used as standard drugs for comparison.**

***Results*: The suppressive study showed that, SAABMAL® (200 and 400 mg/kg/bw) significantly ($P<0.01$) produced a suppression (29.39 - 100%) of parasitaemia in a dose-dependent manner, while the curative study showed that SAABMAL® at 400 mg significantly ($P<0.01$) reduced (95.80%) parasitaemia compared with controls. The mean survival time of SAABMAL®-treated groups (100 and 200 mg/kg) was higher than that of the chloroquine-treated group. Histopathologically, no changes were found in the spleen of both untreated and treated groups. SAABMAL® capsules were of good mechanical properties with low weight variation and high degree of content mass uniformity.**

***Interpretation & conclusions*: The results obtained in this study showed the efficacy of SAABMAL®, a herbal antimalarial formulation against chloroquine sensitive malaria and its potential use in the treatment of uncomplicated malaria infection. Further studies need to be done in humans to test its efficacy and safety for its potential use as an antimalarial drug.**

Key words Antimalarial - efficacy - polyherbal - quality assessment

Malaria is a problem in every region of the developing world and contributes significantly to mortality, poverty and underdevelopment in endemic regions[1]. The problem is greatest in Africa, where over 80 per cent of malaria cases and death occur[1]. The disease affects all ages and economic groups with a devastating impact on pregnant women and children less than five years of age[2], and complications associated with the disease, such as anaemia and neurological sequelae are more severe in this group, owing to frequent attacks and their slow immunological response to infection[3].

Today, one of the biggest challenges in controlling malaria is combating drug resistance. Increasingly, one of the parasites that cause malaria (*Plasmodium falciparum*) is becoming resistant to chloroquine, the most widely used malaria treatment since the 1940s. The most common and affordable replacement for chloroquine in Africa, sulphadoxine-pyrimethamine, is also rapidly losing effectiveness against this parasite, while the current alternative is unaffordable by majority of Africans[4,5]. Continued use of ineffective pharmaceuticals not only contributes to the spread of drug resistance but also causes a disturbing increase in malaria-related morbidity and mortality. Complementary and alternative (herbal) preparations could prevent a substantial percentage of the deaths each year from malaria. Halting the spread of drug-resistant malaria needs to be a global priority, and resources must be focused on those areas of the world where the burden from the disease is greatest. One of such ways is to return to naturaceuticals, variously tagged; herbal medicines, natural products, complementary and alternative medicine (CAM) or even traditional medicines. Eisenberg *et al*[6] in an earlier study reported that in developed countries the number of visits to the alternative medicine practitioners was growing rapidly with the number of visits in US was estimated to be 629 million in 1997; it was believed to have exceeded the number of visits to all primary care physicians. Herbal therapy has been used extensively in Nigeria. Although more than 80 per cent of the people in both the underdeveloped and the developed countries depend on herbal medicines for their medical needs[7], the major problem with herbal medicines in such countries still remains their poor and sometimes unhealthy presentation. A major aspect of the standardization process includes the assessment of the efficacy and safety, as well as development of suitable dosage forms and stability for these herbal medicines[8]. One such polyherbal formulation used for various ethnomedicinal purposes in Nigeria including the treatment malaria is SAABMAL®. The constituents of SAABMAL® include; *Allium sativum, Cymbopogon citratus, Vernonia amygdalina, Saccharum officinarum, Amaranthus caudatus, Aloe barbadensis* and *Sesamum indicum.* Ethnomedicinally, four capsules of SAABMAL® (500 mg extracts/capsule) are taken as start dose, two capsules taken 8 h later, then daily for two additional days for the treatment of malaria, waist pain, arthritis, rheumatism, tuberculosis and as an adjunct in HIV/AIDS. The medicinal uses of the constituents of SAABMAL® are well documented in literature[9-22]. But like most traditional medicines in Africa, little or no scientific information is available on this polyherbal multicomponent preparation. This study was, therefore, carried out to evaluate and provide information on the efficacy and dosage form (herein referred to as pharmacological and pharmacotechnical) standardization of SAABMAL®.

Material & Methods

This study was conducted in the department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. Chloroquine (Sigma Aldrich, UK) and pyrimethamine (SKG Pharma Ltd., Nigeria) were procured. SAABMAL® capsules (Halamin, Nigeria) were specially prepared for this study in our laboratory at NIPRD, Abuja, using *Allium sativum, Cymbopogon citratus, Vernonia amygdalina, Saccharum officinarum, Amaranthus caudatus, Aloe barbadensis* and *Sesamum indicum* were collected from Ben Amodu farm, Abejikolo, Kogi State, Nigeria. Five hundred capsules were produced for analysis.The capsules were stored in air-tight cellophane bags inside a refrigerator at 4°C and protected from light until time of drug administration when it was opened, reconstituted in water and appropriate concentrations required were administered orally directly to the experimental animals.

Swiss albino mice (18-25 g) of either sex were acclimatized to laboratory conditions in the animal facility centre of the NIPRD, Abuja. The mice were housed in plastic cages in a well ventilated room (25 ± 5°C), fed with standard rodent feed and allowed free access to drinking water. Chloroquine-sensitive *Plasmodium berghei berghei* (NK 65 strain) was obtained from the National Institute for Medical Research (NIMR), Lagos, Nigeria. Parasite viability was maintained by continuous re-infection in mice, via intraperitoneal injection. All experiments were carried

out after ethical clearance was obtained from the ethics committee of the institute.

Parasite inoculation: One week after parasite infection of a naive mouse, a Leishman-stained thin blood film was prepared from its tail vein blood on the surface of a glass slide for the assessment of parasitaemia. The red blood cell count was determined with a haemocytometer (Thermo Fisher Scientific, Germany). Using the parasitaemia and red blood cell count of the donor mouse, the blood sample was diluted with normal saline such that 0.2 ml contained approximately 10^5 parasitized red blood cells. Each experimental mouse was injected intraperitoneally with 0.2 ml of the diluted infected blood.

Activity in early infection (4-day test): The method of Peters *et al*[23] was adopted. Thirty mice were randomized into five groups of six mice each and inoculated with the parasite on day 0. Four hours later, the mice were treated as follows: Group 1, distilled water, 0.3 ml/kg body weight (bw); Group 2, SAABMAL®, 100 mg/kg bw; Group 3, SAABMAL®, 200 mg/kg bw; Group 4, SAABMAL®, 400 mg/kg bw; and Group 5, chloroquine, 5 mg/kg bw.

Treatment was repeated at the same time on days 1, 2 and 3. On day 4, the level of parasitaemia in each mouse was determined. Thin films of tail vein blood were prepared and stained with Leishman's stain. The films were examined microscopically and parasitaemia was expressed as the average count per high power field (HPF).

Percentage suppression of parasitaemia was calculated using the following equation[24]:

$$\text{Mean parasitaemia}_{\text{control}} - \text{mean parasitaemia}_{\text{treated}} \times 100 / \text{mean parasitaemia}_{\text{control}}$$

Activity in established infection: The curative activity of the crude extract and in established infection was evaluated using the method of Ryley and Peters[25]. Thirty mice were inoculated intraperitoneally with dilute infected blood. On day three, the mice were randomized into five groups of six mice each such that mean parasitaemia level of the groups was almost similar. The treatment was carried out as follows: Groups 1 and 2 served as controls and received distilled water and chloroquine (5 mg/kg bw), respectively, while groups 3, 4 and 5 were treated with 100, 200 and 400 mg SAABMAL® /kg bw, respectively. Treatment was continued once daily on days 4 to 6. On day 7, blood films were made and the level of parasitaemia assessed. The mice were subsequently monitored for mortality and mean survival time of each group was recorded.

Prophylactic activity: The prophylactic effect of the extract against infection was assessed using the method of Peters[26]. Thirty mice were randomized into five groups (n = 6) and treated once daily for three consecutive days. Control groups received distilled water (5 ml/kg b.w) and pyrimethamine (1.2 mg/kg b.w), while the other groups were treated with 100, 200 and 400 mg SAABMAL®/kg bw, respectively. On the fourth day, the mice were inoculated with *P. berghei berghei*. After 72 h, a thin blood film of each mouse was made and parasitaemia was assessed. The mice were then euthanized by chloroform inhalation and the liver, kidneys and spleen were examined macroscopically. After inspection, the organs were immediately transferred to a plastic containers containing 10 per cent v/v formal saline tissue processing and histopathological examination. Tissue slices were embedded in paraffin and sections stained with hematoxylin and eosin. Light microscopic examination of multiple tissue sections from each organ in all groups was performed.

Evaluation of capsules properties:

Weight and content uniformity - Twenty capsules selected randomly from each of the three batches supplied were weighed individually and collectively on an electronic balance (Metler P 167). The contents of each capsule were simultaneously determined. The mean weights and coefficient of variations were then calculated.

Disintegration test - The disintegration time of the capsules was determined as specified in the British Pharmacopoeia[27] using an Erweka 6 – station disintegration tester (Erweka, Dreiech, Germany). Three media (distilled water, simulated gastric and intestinal fluids) simulating three *p*H conditions (7.0, 1.2 and 7.5, respectively) of the gastrointestinal tract were used. Distilled water was used as the disintegration medium. The results shown are the average of three replicate determinations.

Statistical analysis: Data were analysed by one-tailed t test and one way ANOVA followed by Dunnet's post hoc test.

Results

Suppressive antiplasmodial activity: SAABMAL® showed suppressive antiplasmodial activity at the

doses employed. The activity of SAABMAL®was dose-dependent and ranged from 29.39 to 100 per cent. At doses of 200 and 400 mg/kg, SAABMAL® significantly (P<0.01) suppressed parasitaemia relative to the untreated control group. A dose of 400 mg SAABMAL® /kg bw showed remarkable activity (100 % suppression), similar to the activity of the reference drug, chloroquine (Table I).

Curative antiplasmodial activity: SAABMAL® reduced the levels of parasitaemia in a dose-dependent manner by 56.20-95.89 per cent. These effects were significant (P<0.01, P<0.05) with all doses of SAABMAL® compared with control and chloroquine showing similar activity. Survival time was also increased in a non-dose dependent fashion, relative to the untreated control group. The mean survival time of SAABMAL® -treated groups (100 and 200 mg/kg) was noticeably higher than that of the chloroquine-treated group (Table I).

Prophylactic antiplasmodial activity: The prophylactic treatment with 100, 200 and 400 mg SAABMAL® / kg bw reduced parasitaemia dose-dependently by 4.67, 53.39 (P<0.05) and 99.75 per cent (P<0.01), respectively. The activity of 400 mg SAABMAL® /kg bw was comparable to that of pyrimethamine.

Histopathology: Table II summarizes histopathological findings in the liver, spleen and kidney of groups administered SAABMAL® prophylactically prior to infection with the parasite. Compared to the control group which showed moderate amounts of parasitized red blood cells (RBCs) in the liver, livers of SAABMAL® -treated groups were devoid of parasitized RBCs, although occassional parasitized cells were seen in the kidney of 100 mg SAABMAL® /kg group. Occasional vascular dilatation and pigment granules were observed in the liver of 100 mg SAABMAL® / kg group but these changes were absent in 200 and 400 mg/kg SAABMAL® -treated groups. No pathological changes were found in the spleen of both untreated and treated groups.

Pharmacotechnical properties of the capsules: The pharmacotechnical properties of the capsules were also evaluated (data not shown); there was low weight variation and high degree of content uniformity. Disintegration test results showed that all capsules disintegrated in less than 15 min, thus complying with Pharmacopoeial requirement.

Discussion

In vivo screening for antimalarial activity is usually done with rodent malaria parasites, especially *Plasmodium berghei* which has been used extensively in antimalarial drug discovery and development[28]. These models are useful screening tools in the prediction of treatment outcome of human infection. Consequently, SAABMAL® was screened for antimalarial activity using three models; prophylactic, suppressive and curative. SAABMAL® suppressed parasitaemia in a dose-dependent manner and at 400 mg/kg b.w completely suppressed parasitaemia, implying that this natural product could be a potent prophylactic antimalarial agent. The significant anti-plasmodial activity of SAABMAL® in established infection placed this product at an advantageous position in terms of malaria treatment in developing countries. The reduction in parasitaemia and the comparative prolongation of mean survival times were indicative of the curative potential of SAABMAL®. Although there has been no previous study on the pharmacological activities of SAABMAL®, many of the medicinal plants that constitute SAABMAL® have been individually reported to exhibit diverse pharmacological actions[9-22,29-32].

Herbal medicines have the advantage of an impressive and long history of safety and efficacy profile, the major problem however, has been lack of adequate documentation. This study has provided scientific evidence on the efficacy and dosage form assessment of SAABMAL® against experimentally induced malaria infection in mice. It is hoped that randomized, controlled clinical trials will be conducted to evaluate the efficacy, tolerability, and safety of SAABMAL® in the human subject.

The physical appearance, weight variation, content uniformity as well as the disintegration times of the sampled capsules were found to be satisfactory as they met official compendia specifications[27]. All the capsules evaluated showed low weight variation and high degree of content uniformity indicating that the method of formulation used was acceptable for preparing good quality SAABMAL® capsules.

The efficacy of a drug mixture can be dependent on the rate at which the tablet or capsule disintegrates in the patient's gastrointestinal tract. Although rapidly disintegrating tablets or capsules do not necessarily ensure fast bioavailability, but it is still an important parameter as it is a pointer to the probable performance

Table I. Effect of SAABMAL® on early infection (4-day suppression test), established infection and prophylactic antiplasmodial activity of SAABMAL®

		Early infection		Established infection			Prophylactic activity	
Treatment	Dose (mg/kg)	Parasitaemia (%) (mean ± SEM)	% Suppression of parasitaemia as compared to control	Parasitaemia (%) (Day 6) (mean ± SEM)	% Suppression of parasitaemia as compared to control	Mean survival time (days)	Parasitaemia (%) (mean ± SEM)	% Suppression of parasitaemia as compared to control
Distilled Water	-	11.33 ± 1.71	-	12.17 ± 2.09	-	13.83 ± 2.56	11.80 ± 2.20	-
SAABMAL®	100	8.00 ± 1.03	29.39	5.33 ± 1.45*	56.20	20.40 ± 1.89	11.25 ± 2.50	4.67
	200	1.45 ± 0.57**	87.20	2.82 ± 0.99**	76.83	19.40 ± 1.83	5.50 ± 0.29*	53.39
	400	0.00 ± 0.00**	100	0.50 ± 0.12**	95.89	15.50 ± 1.29	0.03 ± 0.03**	99.75
Chloroquine	5	0.00 ± 0.00**	100	0.00 ± 0.00**	100	18.80 ± 4.47		
Pyrimethamine	1.2						0.00 ± 0.00**	100

$P^{*}<0.05$ $^{**}<0.01$ compared with distilled water (control). N=5 for control, N=6 for the treatments

Table II. Effect of prophylactic treatment with SAABMAL® on liver, spleen and kidney of infected mice

Organ	Distilled water	SAABMAL® 100 mg/kg
Liver		
Vascular dilatation	++	+
Parasitized RBC	++	-
Pigment granules	++	+
Spleen		
Vascular dilatation	-	-
Parasitized RBC	-	-
Pigment granules	-	-
Kidney		
Vascular dilatation	+	-
Parasitized RBC	+	+
Pigment granules	-	-

-, absent; +, occasional; ++, moderate
Effect of SAABMAL® at 200 and 400 mg/kg as also pyrimethamine (1.2 mg/kg) was absent in all organs

of the preparation following administration. The ability to interact strongly with water is also known to be essential for disintegration. In the present study all the capsules disintegrated in less than 15 min irrespective of the *p*H of the investigating media, implying that *p*H may not have detrimental effect on the disintegration of SAABMAL® capsules.

In conclusion, our results confirmed the efficacy of SAABMAL® as an herbal antimalarial formulation with acceptable capsule qualities. However, there is a need for development of an appropriate analytical technique for monitoring drug release from the formulation. This will assist in developing appropriate stability parameters and bioavailability/bioequivalence studies during clinical trials.

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***Conflicts of interest*:** None.

References

1. Sachs J, Malaney P. The economic and social burden of malaria. *Nature* 2002; *415* : 680-5.
2. Management Sciences for Health (MSH). Management strategies for improving health services. *Manager* 2003; *12* : 1-7.
3. Winzeler EA. Malaria research in the post-genomic era. *Nature* 2008; *455* : 751-6.
4. Watsierah CA, Ouma C. Access to artemisinin-based combination therapy (ACT) and quinine in malaria holoendemic regions of western Kenya. *Mal J* 2014; *13* : 290.
5. Coleman PG, Morel C, Shillcutt S, Goodman C, Mills AJ. A threshold analysis of the cost-effectiveness of artemisinin-based combination therapies in sub-Saharan Africa. *Am J Trop Med Hyg* 2004; *71* : 196-204.
6. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rompay M, *et al*. Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA* 1998; *280* : 1569-75.
7. World Health Organization (2008). Traditional Medicine. Fact Sheet no. 134. Geneva:WHO. Available from: *http://www.who.int/mediacentre/factsheets/fs134/en*, accessed on January 12, 2015.
8. Emeje M, Izuka A, Isimi CY, Ofoefule S, Kunle O. Preparation and standardization of a herbal agent for the therapeutic management of asthma. *Pharm Dev Technol* 2011; *16* : 170-8.
9. Ojo OO, Anibijuwon II, Ojo OO. Studies on extracts of three medicinal plants of south-western Nigeria: *Hoslundia opposita, Lantana camara* and *Cymbopogon citratus*. *Adv Nat Appl Sci* 2010; *4* : 93-8.
10. Devi RC, Sim SM, Ismail R. Spasmolytic effect of citral and extracts of *Cymbopogon citrates* on isolated rabbit ileum. *J Smooth Muscle Res* 2011; *47* : 143-56.
11. Cheel J, Theoduloz C, Rodr´ıguez J, Schmeda-Hirschmann G. Free radical scavengers and antioxidants from lemon grass (*Cymbopogon citratus* (DC.) Stapf.). *J Agric Food Chem* 2005; *53* : 2511-7.
12. Akah PA, Alemji JA, Salawu OA, Okoye TC, Offiah NV. Effects of *Vernonia amygdalina* on biochemical and hematological parameters in diabetic rats. *Asian J Med Sci* 2009; *1* : 108-13.
13. Michael UA, David BU, Theophine CO, Philip FU, Ogochukwu AM, Benson VA. Antidiabetic effect of combined aqueous leaf extract of *Vernonia amygdalina* and metformin in rats. *J Basic Clin Pharm* 2010; *1* : 197-202.
14. Nwanjo HU, Nwokoro EA. Antidiabetic and biochemical effects of aqueous extract of *Vernonia amygdalina* leaf in normoglycaemic and diabetic rats. *J Innov Life Sci* 2004; *7* : 6-10.
15. Nwanjo HU. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. *Niger J Physiol Sci* 2005; *20* : 39-42.

16. Okolie UV, Okeke CE, Oli JM, Ehiemere IO. Hypoglycemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. *Afr J Biotechnol* 2008; *7* : 4581-5.
17. Emeje M, Boyi S, Obidike I, Isimi C, Kunle O, Ofoefule S. Natural antidiabetic compound for the therapeutic management of diabetes mellitus and its drug delivery system. *J Diet* (Suppl) 2011; *8* : 266-79.
18. Londhe VP, Gavasane AT, Nipate SS, Bandawane DD, Chaudhari PD. Role of garlic (*Allium sativum)* in various diseases: An overview. *J Pharm Res Opin* 2011; *1* : 129- 34.
19. Singh VK, Singh DK. Pharmacological effects of garlic (*Allium sativum* L.). *Annu Rev Biomed Sci* 2008; *10* : 6-26.
20. Repo-Carrasco-Valencia R, Hellstrøm JK, Pihlava JM, Mattila PH. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*). *Food Chem* 2010; *120* : 128-33.
21. Pallavi R, Elakkiya S, Tennety SR, Suganya Devi P. Anthocyanin analysis and its anticancer property from sugarcane (*Saccharum officinarum* L) peel. *Int J Res Pharm Chem* 2012; *2* : 338-45.
22. Ferro VA, Bradbury F, Cameron P, Shakir E, Rahman SR, Stimson WH. *In vitro* susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. *Antimicrob Agents Chemother* 2003; *47* : 1137-9.
23. Peters W, Robinson BL, Tovey G, Rossier JC, Jefford CW. The chemotherapy of rodent malaria L. The activities of some synthetic 1, 2, 4 - trioxanes against chloroquine-sensitive and chloroquine-resistant parasites. Part 3: Observations on 'Fenozan-50F' a difluorinated 3,3'-spirocyclopentane 1,2,4-trioxane. *Ann Trop Med Parasitol* 1993; *87* : 111-23.
24. Warhurst DC, Williams JE. ACP Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. *J Clin Pathol* 1996; *49* : 533-8.
25. Ryley JF, Peters W. The antimalarial activity of some quinoline esters. *Ann Trop Med Parasitol* 1970; *64* : 209-22.
26. Peters W. Drug resistance in *Plasmodium berghei*. Vincke and Lips, 1948; 3. Multiple drug resistance. *Exp Parasitol* 1965; *17* : 97-102.
27. *British Pharmacopoeia 2004*: London: Her Majesty's stationery Office; 2004.
28. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. *Nat Rev Drug Discov* 2004; *3* : 509-20.
29. Coppi A, Cabinian M, Mirelman D, Sinnis P. Antimalarial activity of allicin a biologically active compound from garlic cloves. *Antimicrob Agents Chemother* 2006; *50* : 1731-7.
30. Tchoumbougnang F, Zollo PH, Dagne E, Mekonnen Y. *In vivo* antimalarial activity of essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* on mice infected with *Plasmodium berghei*. *Planta Med* 2005; *71* : 20-3.
31. Masaba SC. The antimalarial activity of *Vernonia amygdalina* Del (Compositae). *Trans R Soc Trop Med Hyg* 2000; *94* : 694-5.
32. Chenniappan K, Kadarkara M. *In vitro* antimalarial activity of traditionally used Western Ghats plants from India and their interactions with chloroquine against chloroquine-resistant *Plasmodium falciparum*. *Parasitol Res* 2010; *107* : 1351-64.

Reprint requests: Dr M.O. Emeje, Department of Pharmaceutical Technology & Raw Materials Development, National Institute for Pharmaceutical Research & Development, Abuja, Nigeria
e-mail: memeje2011@hotmail.com