AMERICAN JOURNAL OF PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY, 2020
VOL 10, NO. 1, PAGE 1–6
10.5455/ajpbp.20190822021151

ORIGINAL RESEARCH

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Artesunate (ART) antagonizes antiplasmodial activity of *Vernonia amygdalina* methanol leaf extract in murine model of malaria

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ABSTRACT

Context: Malaria is a life-threatening disease in sub-Saharan Africa. *Vernonia amygdalina* (VAM), a popular Nigeria vegetable, is commonly consumed by the patients on antimalarial therapy, as a means of curbing resistance posed by *Plasmodium* species to conventional antimalarial drugs.

Objective: This study was designed to evaluate the effect of artemisinin combination-based therapy, Artesunate (ART)/Amodiaquine (A/A) on the antimalarial efficacy of VAM leaf extract.

Materials and Methods: *Plasmodium berghi* infected mice were randomized into six groups as follows: Group 1 (untreated control), group 2 (ART/VAM, 35.14 mg/kg/125 mg/kg), group 3 (ART, 35.14 mg/kg), group 4 (ART/A, 35.14 mg/kg/105 mg/kg), group 5 (VAM, 125 mg/kg), and group 6 (ART/VAM, 2.86 mg/kg/125 mg/kg). Administration lasted for three consecutive days and antiplasmodial activity was assessed using Rane's curative test.

Results: Parasitemia clearance of 98.8% and 52.83% were recorded for VAM (125 mg/kg) and ART (35.14 mg/kg), respectively. However, lower parasitemia clearance of 97.05% and 80.49% were produced by the combinations of VAM/ART (125 mg/kg/2.86 mg/kg) and VAM/ART (125 mg/kg/35.14 mg/kg), respectively, when compared to that of VAM, 125 mg/kg. A/A, (35.14 mg/kg/105 mg/kg) produced a parasite clearance of 89.69%.

Conclusion: ART produced dose dependent antagonism on the antimalarial efficacy of VAM. Combination of higher dose of ART and VAM should be discouraged, while the combination of low dose of ART and VAM could be encouraged for malaria patients.

ARTICLE HISTORY

Received August 22, 2019
Accepted November 30, 2019
Published January 10, 2020

KEYWORDS

Antagonism; *Vernonia amygdalina*; ACT; *Plasmodium berghei*; Artesunate; interaction

Introduction

Malaria, a common disease in tropical countries, especially sub-Saharan Africa, is an endemic infection caused by *Plasmodium falciparum* (*P. falciparum*). An estimate by the World Health Organization (WHO) in 2016 revealed that about 429,000 deaths occurred out of 212 million malaria cases reported, with majority from Africa [1,2]. Pregnant women and children below 5 years suffer mostly from malaria [3].

Plasmodium falciparum is known to be resistant to monotherapeutic agents, such as 4-minoquinolines, quinine, chloroquine, anti-folates, atovaquone, and Artesunate (ART). Consequently, WHO recommended other combination therapies, especially the artemisinin-based combination therapy (ACT) [4]. Unfortunately, the parasite has been demonstrated to resist the combination therapy [4].

These limitations posed by conventional antimalarial agents have inspired scientific assessment of herbal remedies in an attempt to discover an alternative safe, affordable, effective, and readily available antimalarial therapy [5,6]. Also, combination of conventional antimalarial drugs and herbal remedies is now being recognized as probable means of eliminating further resistance posed by

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P. falciparum to the WHO approved ACT. This practice had aroused scientific interest on the therapeutic efficacy and safety, as such combination could result in increase or decrease in the therapeutic efficacy or toxicity [1].

Vernonia amygdalina (VAM) (Asteraceae) commonly known as bitter leaf is a perennial shrub growing up to 2–5 cm high with a rough bark and thick black straits. Its leaves are elliptic with about 6 mm long [7]. It is a medicinal plant used for malaria and other diseases in various countries in sub-Saharan Africa, including Nigeria [8]. It is called "Ewuro," "Onugbu," and "Chusar doki or fatefate", in Yoruba, Igbo, and Hausa Nigeria populace, respectively. It is used in the preparation of vegetable soup due to numerous health benefits embedded in it [9].

Earlier animals studies have revealed that VAM to possess a significant antimalarial properties [10–12]. For instance, *Plasmodium* strains of *falciparum, vivax, ovale*, and *malariae,* which are resistant to conventional antimalarial drugs were found to be susceptible to aqueous leaves extract of VAM at higher concentration [13,14]. Its aqueous extract was found to enhance the antimalarial efficacy of chloroquine [11]. Also, its anti-plasmodial activity has been validated in clinical trials [15].

Consumption of VAM vegetable soup while on antimalarial medications, especially ART is a common practice by some of the South Eastern Nigeria populace. To our knowledge, there is paucity of studies to ascertain the effect of concurrent use of VAM and ART to validate their herb-drug synergism/antagonism.

Materials and Methods

Animals

A total of 24 albino mice of both sexes were used in the study. Following procurement from the Faculty of Veterinary Sciences, University of Nigeria Nsukka, they were acclimatized for 7 days at the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu. Animals were fed with grower feed and water *ad libtum* for the entire duration of the study. Good hygiene was maintained by regular cleaning of the cages and replacement of their beddings. They were handled in compliance with the National Institute of Health Guidelines for the care and use of laboratory animals (Pub No. 85-23, revised 1985).

Drugs and chemicals

ART/Amodiaquine was sourced from Right-health pharmacy Awka, methanol (JHD, Guangdong Guanghua Schi Tech, Ltd., China), animal feed (Vital feed Ltd., Jos, Nigeria).

Preparation of extract

VAM leaves were harvested in the morning hours, by 6 am. The sample was authenticated by the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu. The leaf sample was crisply air-dried and milled. Thereafter, the pulverized leaf was macerated for 48 hours using methanol as a solvent. The filtrate recovered was evaporated to dryness using water bath at 45°C.

Experimental protocol

A standard inoculation of 1×10^7 *Plasmodium berghei* infected red blood cell was prepared by diluting the infected blood of donor mice with normal saline, such that 0.2 ml of the diluted blood contained approximately 1×10^7 infected erythrocytes. Each animal was intraperitoneally injection with 0.2 ml of the standard inoculum which produced a steadily rising infection in the animal.

After infection, mice were grouped into six groups of four mice per group. Group 1 served as negative control and received distilled water only, 10 ml/kg. Group 2 received ART/VAM, 35.14 mg/kg/125 mg/kg. Group 3 received ART (35.14 mg/kg). Group 4 received ART/A (35.14 mg/kg/105 mg/kg). Group 5 received VAM (125 mg/kg). Group 6 received ART/VAM (2.86 mg/kg/125 mg/kg). The doses were determined from adult clinical human dose.

At 72 hours post infection, when the parasitemia level had risen >4%, animals were treated accordingly. Average weights of the animals were taken in each group and were used as the actual weigh for determining doses. Treatments were given for three consecutive days [6] Parasitemia level was examined the following day after the third day of treatment.

Parasitemia monitoring

Parasitemia was determined by collecting the blood samples from the tail of mice for thin blood films fixed on slides with methanol for 15 seconds. The fixed samples were stained with 10% Giemsa for 25 minutes. Thereafter, the slides were washed with

phosphate buffer of pH 7.2 and allowed to dry and were examined with microscope using ×100 magnification in oil immersion. The number of parasitized erythrocytes out of 200 erythrocytes in random field of the microscope was determined. The percentage parasitemia was determined as follows:

% Parasitemia = (total no. of parasitized RBC (red blood cell)/total no. of RBC) ×100 [6]. Parasitemia clearance was calculated as follows: (Pre-treatment – Post-treatment)/Pre-treatment × 100.

Statistical analysis

All result obtained were expressed as mean ± standard error of mean (SEM) (n = 4) and were statistically analyzed by parried sample t-test, using Statistical Package for Social Sciences (SPSS-20). $p < 0.05$ considered to be statistically significant.

Results

From the result as shown in Table 1, VAM (125 mg/kg) alone and ART (35.14 mg/kg) alone recorded parasite clearances of 98.9% and 52.83%, respectively. A combination of VAM/ART (125 mg/kg/35.14 mg/kg) gave a lower parasite clearance of 80.49%. A repeat of the combination at lower dose VAM/ART (125 mg/kg/2.86 mg/kg) recorded 97.05%. ART/Amodiaquine (35.14 mg/kg/105 mg/kg) produced a parasites clearance of 89.69%.

Discussion

This present study had demonstrated the potentials of ART to antagonize the antimalarial efficacy of VAM *in vivo* model of *P. berghei* infected mice.

Emerging resistance posed by artemisinin monotherapy, even the modern ACT has aroused scientific attentions into their combination with medicinal plants. Some benefits attributed to these combinations include decreased cytotoxicity, delay, or prevention of the development of drug resistance as well as increase in potency and efficacy [16,17].

ART, a pro-drug of dihydroartemisinine, is the most commonly used of all the artemisinin derivatives, as approved by the WHO in curbing malaria resistance to other conventional antimalarial drugs. ART has been postulated to act by DNA damage mediated by reactive oxygen species (hydroxyl radicals, superoxide anions, and carbon-centered free radicals) followed by alkylation of *P. falciparum* protein [18]. It exhibits rapid absorption and elimination after oral and intramuscular administration [3]. Esterase-mediated hydrolysis and CYP2A6 enzyme followed by uridine diphosphate glucuronosyltransferases into the active metabolite dihydroartemisinin has been known to be the chief metabolic process of ART [19].

From this study, VAM (group 5) elicited the highest parasitemia clearance of 98.9%, which substantiates its antimalarial potency than conventional antimalarial agents. This is supported by the study of Odeh and Usman [13] where VAM elicited better antimalarial activity more than the conventional antimalarial agents against various plasmodium stains. Although its mechanism (s) of antiplasmodial activity is yet to be established, VAM had been established by various authors as a promising antimalarial remedy against various stains of plasmodium [14] due to its phytoconstituents, such as sesquiterpene lactones, especially the highly oxygenated derivatives vernolide isolated from its leaves which exhibited significant antimalarial activity against *P. falciparum* blood stages *in vitro* [8]. According to Ijeh and Ejike [7], flavonoids, saponins, alkaloids and sesquiterpene, and steroidal constituents in VAM mediated its antiplasmodial activity. Metabolism of VAM extract using Western blot and real time polymerase chain reaction analyses in MCF-7 cells revealed a dose and time-dependent induction of phase 1 (CYP3A4) and

Table 1. Effects of co-administration of ART and VAM on parasite clearance of *P. berghei* in murine models.

Treatment	Pre-treatment	Post-treatment	Clearance (%)
	Percentage parasitemia		
Group 1: Negative control, distilled water	13.06 ± 0.00	12.30 ± 0.02*	05.82
Group 2: ART/VAM (35.14 mg/kg/125 mg/kg)	15.38 ± 2.11	3.00 ± 1.39**	80.49
Group 3: ART (35.14 mg/kg)	15.50 ± 1.44	7.30 ± 2.83*	52.83
Group 4: ART/Amodiaquine (35.14 mg/kg/105 mg/kg)	17.56 ± 1.08	1.81 ± 1.10**	89.69
Group 5: VAM (125 mg/kg)	22.75 ± 2.08	0.25 ± 0.10**	98.90
Group 6: ART/VAM (2.86 mg/kg/125 mg/kg)	16.94 ± 2.29	0.50 ± 0.29**	97.05

Values are presented as mean ± SEM, n = 4. *$p < 0.05$, **$p < 0.01$: Significantly different from baseline.

phase 2 (microsomal epoxide hydrolase) enzyme gene expression [20].

Combination of medicinal plants with conventional drugs could cause interaction with drug transporters thereby causing increase or decrease in pharmacological activity [21,22]. Other studies have also shown that concomitant administration of synthetic antimalarials with or medicinal plants produced adverse effects [23], since medicinal plants also undergo pharmacokinetic processes thereby affecting the outcome of synthetic drugs in the body [24].

From this study, combination of ART and VAM (35.14 mg/kg/125 mg/kg) (group 2) as well as ART/VAM, 2.86 mg/kg/125 mg/kg (group 6) produced lower clearance of 80.48% and 97.05% as against 98.9% produced by VAM alone. This suggests a dose dependent antagonizing effect of ART on the antimalarial efficacy of VAM. Thus, it would not be appropriate to combine ART and VAM in malaria therapy. The interaction of drug metabolizing enzymes of ART with VAM aforementioned could be responsible for the observed antagonism. We suggest that reactive oxygen species generated from the action of ART [18] may interfere with antioxidants, especially flavonoids which could play a significant anti-plasmodial role in VAM [25,26], thereby causing a reduced efficacy of VAM. We also hypothesis that the physicochemical nature of ART could interfere with the proper absorption of VAM thereby reducing its bioavailability or increase in its half-life.

Contrary to this finding, a bioavailability study on Dihydroartemisinin and VAM single dose in normal Wistar rats revealed a reduction in area under curve (AUC). The authors suggested that the extract components, like Zinc, could bind the drug and prevent its transport from the intestine into the body systemic circulation [27]. Similarly, a reduction in bioavailability, increase in elimination process, and clearance of dihydroartemisinin (the most potent metabolite of ART) was observed following the co-administration with *Chrysolina sanguinolenta* and ART [28]. Also, a reduction in effectiveness of ART was observed following its concurrent administration with aqueous extract of *C. sanguinolenta* [29]. In a related study, the leaf extract of VAM significantly altered the pharmacokinetic process of chloroquine via reduced C max, AUC and elimination half-life [30]. On the other hand, the extract of *Ageratum conyzoides* potentiated the antimalarial activity of chloroquine and ART, probably due to prolongation of elimination half-life [17]. Dose variability could account for differences observed in this study and other authors cited above.

From this study, a parisitemia clearance of 52.83% was produced by ART at a dose of 35.14 mg/kg (group 3) as against the value of 89.6% found when it was combined with Amodiaquine (group 4). This suggests that Amodiaquine enhances the parasite clearance of ART. This is corroborated by the study of Ajuik et al. [31] who reported that the combination of ART and Amodiaquine, a potential combination in Africa for malaria enhanced the treatment efficacy in Gabon, Kenya, and Sénégal.

Conclusion

From this study, ART exhibited a dose-dependent antagonism on the antimalarial efficacy of VAM. Thus, malaria patients should avoid the consumption of VAM soup while still on ART therapy. Also, pharmacokinetic and safety evaluation of this combination in murine model is recommended.

References

[1] Onyeji CO, Igbinoba SI, Olayiwola G, Adehin A. Insight into clinically effective herbal antimalarial products: effects on drug metabolizing enzymes and p-glycoprotein. Afri J Pharm Pharmaco 2017; 12(48):591–613; doi:10.5897/AJPP2017.4870

[2] Luiz CSP, Livia MF, Flavia FDS, Nubia B. Current antimalarial therapies and advances in the development of semi-synthetic artemisinin derivatives. An Acad Bras Ciênc 2018; 90(1 Suppl. 2):1251–71; doi:10.1590/0001-3765201820170830

[3] Adegbolagun OM, Emikpe BO, Woranola IO, Ogunremi Y. Synergistic effect of aqueous extract of Telfaria occidentalison the biological activities of ART in *Plasmodium berghei* infected mice. Afr Health Sci 2013; 13(4):970–6; doi:10.4314/ahs.v13i4.16

[4] Adepiti AO, Elujoba AA, Bolaji OO. Evaluation of herbal antimalarial MAMA decoction-amodiaquine combination in murine malaria model. *Pharm* Biol 2016; 54(10):2298–303; doi:10.3109/13880209.2016.1155626

[5] Ogundapo, SS, Ezeanyika LUS, Uzoegwu PN, Soniran OT, Parker EJ, Olugbue, VU, et al. Potentiantion of ART by antiplasmodial fraction of *Moringa oleifera* leaves in drug resistance *Plasmodium berghei* infection. Nig J Pharm Sci 2019; 16(1):81–92; doi:10.31183/imcp.2019.00022

[6] Ihekwereme CP, Melidem CO, Maduka IC, Okoyeh JN. *In vivo* antiplasmodial and toxicological effects of extracts of fruit pulp of *Chrysophyllum albidium* G. Don (Sapotaceae). Trop J Nat Prod Res 2018; 2(3):126–31; doi.org/10.26538/tjnpr/v2i3.5

[7] Ijeh II, Ejike CE. Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. J Med Plants Res 2011; 5(7):1051–61. Available via http://www.academicjournals.org/JMPR (Accessed 14 April 2019).

[8] Abay SM, Lucantoni L, Dahiya N, Dori G, Dembo EG, Esposito FE, et al. *Plasmodium* transmission blocking activities of *Vernonia amygdalina* extracts and isolated compounds. Malar J 2015; 14:288; doi:10.1186/s12936-015-0812-2

[9] Clement E, Erharuyi O, Vincent I, Joy A, Christopher A, Anthony AU, et al. Significance of bitter leaf (*Vernonia amagdalina*) in tropical diseases and beyond: a review. Malar Chemoth Cont 2014; 3:120; doi:10.4172/2090-2778.1000120

[10] Abosi A, Raseroka H. *In vivo* antimalarial activity of vernonia amygdalina. Br J Biomed Sci 2013; 60(20):89–91; *doi:*10.1080/09674845.2003.11783680

[11] Iwalokun BA. Enhanced anti-malarial by *Vernonia amygdalin l*eaf extract in mice infected with *plasmodium berghei*. Afr. Health Sci 2008; 8(1):25–35. Available via https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2408548/ (Accessed 16 April 2019).

[12] Masaba SC. The antimalarial activity of *Vernonia amygdalina* Del (Compositae). Trans Royal Society Trop Med Hyg 2000; 94:694–5; *doi:*10.1016/S0035-9203(00)90236-0

[13] Odeh AP, Usman AA. Antimalarial activity and phytochemical analysis of aqueous leaf extract of *Vernonia amygdalina*. J Zool Biosci Res 2014; 3:28–31. Available via http://www.journalzbr.com/issues.html (Accessed 14 April 2019).

[14] Bihonegn T, Fentahun S. Review on malaria and antimalarial activity of *Vernonia Amygdalina* in Ethiopia: a review article. JHMN 2019; 60:1–15; doi:10.7176/JHMN

[15] Challand S, Willcox M. A clinical trial of the traditional medicine *Vernonia amygdalina* in the treatment of uncomplicated malaria. J Altern Complement Med 2009; 15:1231–7; doi:10.1089/acm.2009.0098

[16] Dondrop AM, Nosten F, Poravuth Y, Das D, Phyo AP, Tarning J, et al. Artemsinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 2009; 361:455–67; doi:10.1056/NEJMoa0808859

[17] Ukwe CV, Ekwunife O, Epueke EA, Ubaka CM. Antimalarial activity of *Ageratum conyzoides* in combination with chloroquine and ART. Asian Pac J Trop Med 2010; 412–420; *doi*:10.1016/S1995-7645(11)60005-9

[18] Gopalakrishnan AM, Kumar N. Antimalarial action of ART involves DNA damage mediated by reactive oxygen species. Antimicrob Agents Chemother 2015; 59(1):317–25; doi:10.1128/AAC.03663-14

[19] Ilett KF, Ethell BT, Maggs JL, Davis TM, Batty KT, Burchell B, et al. Glucuronidation of dihydroartemisinin *in vivo* and by human liver microsomes and expressed UDP-glucuronosyltransferases. Drug Metab Dispos 2002; 30(9):1005–12. Available via https://www.ncbi.nlm.nih.gov/pubmed/12167566 (Accessed 7 March 2019).

[20] Howard CB, Stevens J, Izevbigie EB, Walker A, McDaniel O. Time and dose-dependent modulation of phase 1 and phase 2 gene expression in response to treatment of MCF-7 cells with a natural anti-cancer agent. Cell Mol Biol 2003; 49(7):1057–65. Available via https://www.semanticscholar.org/paper/Time-and-dose-dependent-modulation-of-phase-1-and-2-Howard-Stevens/543c2814d43bd57138f9a73cc57c0ed5653eca6a (Accessed 7 March 2019).

[21] Guengerich, FP. Role of cytochrome P450 enzymes in drug-drug interactions. Adv Pharmacol 1997; 43:7–35.

[22] Fugh-Berman A. Herb-drug interactions. Lancet 2000; 355:134–8.

[23] Igbinoba SI, Onyeji CO, Akanmu MA, Soyinka JO, Pullela SS, Cook JM, et al. Effect of dehusked Garcinia kola seed on the overall pharmacokinetics of quinine in healthy Nigerian volunteers. J Clin Pharmacol 2015; 55(3):348–54; *doi*:10.1002/jcph.414

[24] Izzo AA. Interactions between herbs and conventional drugs: overview of the clinical data. Med Princ Pract 2012; 21(5):404–8; doi:10.1159/000334488

[25] Bansal T, Jaggi M, Khar RK, Talegaonkar S. Emerging significance of flavonoids as P-glycoprotein inhibitors in cancer chemotherapy. J Pharm Pharm Sci 2009; 12(1):46–8. Available via https://www.ncbi.nlm.nih.gov/pubmed/19470292 (Accessed 14 April 2019).

[26] Oga EF, Sekine S, Horie T. Ex vivo and in vivo investigations of the effects of extracts of Vernonia amygdalina, Carica papaya and Tapinanthus sessilifolius on digoxin transport and pharmacokinetics: assessing the significance on rat intestinal P-glycoprotein efflux. Drug Metab Pharmacokinet 2013; 28(4):314–20. Available via https://www.ncbi.nlm.nih.gov/pubmed/23291634 (Accessed 14 April 2019).

[27] Eseyin AO, Igboasoiyi CA, Igbo C, Igboasoiyi A, Ekarika J, Dooka B. Effects of the leaf extract of Venonia amygdalina on the pharmacokinetics of dihydroartemisinin in rat. Pharmacologia 2012; 3(12):713–8. Available via https://scialert.net/abstract/?doi=pharmacologia.2012.713.718

[28] Sakyiamah MM, Ocloo A, Adjimani JP, Sittie AA. Effect of aqueous extract of *Cryptolepis sanguinolenta* on the pharmacokinetics of chloroquine. Int J Drug Res Technol 2012; 2(4S):378–87. Available via http://ijpsdr.org/index.php/ijpsdr/article/view/475 (Accessed 14 April 2019).

[29] Ocloo A, Okpattah WE, Quasie O, Sakyiamah MM, Okine LKN. Concurrent administration of aqueous extract of Cryptolepis sanguinolenta reduces the

effectiveness of ART against *Plasmodium berghei* in Rats. J Appl Pharm Sci 2014; 4(03):024–028; *doi*:10.7324/JAPS.2014.40306

[30] Igboasoiyia C, Eseyin OA, Odoma, NF. The effect of ethanolic extract of *Vernonia amygdalina* leaves on some Pharmacokinetic parameters of Chloroquine in rats. Res J Pharmacol 2008; 2(2):24–27. Available via http://medwelljournals.com/abstract/?doi=rjpharm.2008.24.27 (Accessed 12 April 2019).

[31] Ajuike M, Agnamey P, Babiker A, Borrmann S, Brasseur P, Cisse M, et al. Amodiaquine-ART versus amodiaquine for uncomplicated *Plasmodium falciparum* malaria in African children: a randomised, multicentre trial. Lancet 2002; 359:1365–72. Available via https://www.ncbi.nlm.nih.gov/pubmed/11978332 (Accessed 14 April 2019).