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In vivo Interaction between Extracts of *Khaya grandifoliola* (Welw) CDC (Meliaceae) and Artemisinin in a Murine Malarial Model

J. M. Agbedahunsi[1*], I. F. Umeevuruo[1], T. O. Elufioye[2] and A. O. Adepiti[3]

[1]Drug Research and Production Unit, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.
[3]Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author JMA designed the study, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Author IFU carried out the laboratory work under supervision of author TOE. Author AOA performed the statistical analysis, wrote the final draft and managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate the antimalarial activity of the aqueous (KGA) and n-hexane (KGN) extracts of the stem bark of *Khaya grandifoliola* (Meliaceae) in combination with artemisinin (ART) in mice.
Study Design: Preclinical antimalarial assessment in mice subjects.
Place and Duration of Study: Biological Laboratory, Drug Research and Production Unit, Obafemi Awolowo University, Ile-Ife between November 2006 and September 2007.
Methodology: In the first phase, 65 mice inoculated intraperitoneally with *Plasmodium berghei* were divided into groups of five mice each. Oral administration of KGA and KGN (50-400 mg/kg each) and artemisinin (0.2-1.6 mg/kg) was done for four days starting on the day of inoculation in the 4-day antimalarial test model. Giemsa-stained thin blood smear, from the tail of each mouse, was microscopically assessed for the parasitized and total number of red blood cells of ten fields. The combinations of the sub - effective doses of each of KGA and KGN (100 mg/kg) with ART (0.2 mg/kg) were similarly evaluated and assessed.

**Corresponding author: Email: jagbedah@oauife.edu.ng, foluagbedahunsi@yahoo.com;*

Results: The median effective doses (ED_{50}) were 0.29, 140 and 160 mg/kg for Artemisinin, KGA and KGN, respectively. The combinations (KGA: ART and KGN: ART) resulted in improved parasitemia suppression which were statistically significant (P = .05) when compared with the percentage parasitaemia obtained for individual agents of the combinations.
Conclusion: Extracts of *K. grandifoliola* potentiated the antimalarial activity of Artemisinin. The results showed beneficial interaction with potentials in antimalarial combination therapy.

Keywords: Khaya grandifoliola; artemisinin; malaria; combination therapy.

1. INTRODUCTION

The use of herbs and plant products in the treatment of febrile illness has grown over many generations in malaria-endemic areas. Medicinal plants are readily available, affordable and culturally acceptable to many people. It has been estimated that about 80 % of the world's population resort to the use of traditional medicine for their health needs [1]. Medicinal plants are the sources of the two most important drugs currently available to treat severe falciparum malaria. These are Quinine from *Cinchona succirubra* (Rubiaceae) and Artemisinin from *Artemisia annua* (Asteraceae). Artemisinin is a fast-acting drug with a high clearance of *Plasmodium* from infected erythrocytes. The short biological half-life precludes a long duration of action. Therefore, its use as monotherapy is discouraged [2]. In order to prevent the development of *Plasmodium* resistance and improve the efficacy of the treatment, artemisinin is currently administered in combination with a longer-acting antimalarial blood schizonticide in an Artemisinin-based combination therapy (ACT). These have been adopted as first-line drugs for the treatment of uncomplicated malaria in many sub-Saharan African countries [3]. The decoction of the stem bark of *Khaya grandifoliola* (Welw) CDC is commonly used in West Africa especially Nigeria, to treat malaria and other febrile illnesses. The aqueous extract and purified organic fractions of the stem bark have been reported to have antimalarial activity in *Plasmodium berghei*-infected mice [4,5]. Some studies have shown that combinations of plant-based compounds and extracts with conventional antimalarial drugs have promising antimalarial activities *in vitro* and *in vivo* [6,7,8,9,10] The authors reported that some local populations use medicinal plants in combination with conventional drugs in order to enhance their activities [9], It is therefore imperative to evaluate such combinations for possible interaction. This study reports the *in vivo* antimalarial activity of *Khaya grandifoliola* in combination with ART in *Plasmodium berghei*-infected mice.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation

The stem bark of *K. grandifoliola* was collected at Igasi village near Ikare Akoko, Ondo State, Nigeria in January 2005 and authenticated by Dr. C. Illoh of Botany Department, Obafemi Awolowo University, Ile Ife, Nigeria. A herbarium specimen with voucher specimen number IFE 15320 was deposited at the University herbarium located in the Botany department. The bark was chopped into pieces, oven - dried for 48 hours at 60ºC and powdered using a grinding machine (Christy Norris, UK). The powder was kept in a well closed, amber-coloured bottle until when needed.

2.2 Extraction

Stem bark powder (1.5 kg) was extracted with n-hexane using soxhlet extractor for 72 h. The extract was concentrated *in vacuo* at 40ºC to obtain 0.85% yield of the n-hexane extract (KGN).The aqueous extract was obtained by infusing 500 g of the stem bark powder with 1 L of boiled water, agitated for 12 h and thereafter filtered. The filtrate was at 50ºC *in vacuo* to obtain 7.5 % yield of KGA.

2.3 Animals

Swiss albino mice of both sexes, weighing 18 – 22 g, were obtained from The Animal House, Department of Pharmacology, Obafemi Awolowo University, Ile-Ife. Animals were housed under a 12 h light/dark cycle with free access to commercial food pellets (Premier Feed Mills Co Ltd., Ibadan, Nigeria) and water. They were acclimatized for at least 10 days before use. The mice were used in accordance with the "Guide for the care and use of laboratory" [11].

2.4 Parasites Strain

Plasmodium berghei NK 65 strain was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, Ibadan. The parasites were maintained in continuous blood passage in mice. A standard inoculum of 10^7 parasitized erythrocytes was prepared by dilution of blood, using normal saline, harvested from donor mouse (> 40 % parasitemia) and administered intraperitoneally (0.2 ml) to each experimental mouse. A single donor was used to infect test animals for each experiment.

2.5 Chemosuppression Assay

The evaluation of KGA, KGN and Artemisinin (ART) was determined using the 4–day antimalarial chemosuppressive method [12]. KGA, KGN and ART were dissolved in Tween 80 and made to give 5 % v/v solution. Thirteen groups of five mice each were inoculated intraperitoneally on day 0 with *Plasmodium berghei* NK 65 (CQ - sensitive). Four hours after inoculation, two sets of four groups of five mice each were orally dosed with 50, 100, 200 and 400 mg/kg of KGA and KGN respectively while another four groups of five mice each received ART at 0.2, 0.4, 0.8 and 1.6 mg/kg. The negative control-treated group received 5 % Tween 80. All the animals were treated for three more days. The combination experiments involved the use of six groups of five animals each similarly treated as above. They were orally dosed with 100 mg/kg each of KGA and KGN extracts; 0.2 mg/kg ART, and combination of 0.2 mg/kg ART + 100 mg/kg KGA and 0.2 mg/kg ART + 100 mg/kg KGN. The negative control group was treated as in the above experiment.

2.5.1 Parasite enumeration

On day 4, peripheral blood smears were prepared by using blood obtained from the tail veins of infected experimental mice. The thin films were fixed in methanol and stained with Giemsa stain. Blood smears were examined at x100 magnification by oil immersion light microscopy (Celestron). The parasitized and total numbers of red blood cells (RBC) in ten fields of view were counted. Parasitemia (%) was calculated by dividing the number of parasitized RBC by the total number of RBC. Average chemosuppression (%) was calculated as $100 \times [(A - B)/A]$, where A is the average parasitemia of the negative control group and B is the average parasitemia of the test group.

2.6 Data Analysis

Statistical analysis was performed using ANOVA followed by Dunnett post-hoc test [13].

3. RESULTS AND DISCUSSION

3.1 Chemosuppressive Activities of KGA, KGA and ART

The activities of the KGN and KGA extracts and their combinations were assessed in terms of average percentage parasitaemia or percentage chemosuppression with respect to untreated (negative) controls. The KGN and KGA extracts gave dose–dependent chemosuppressive activities as shown by the findings in this study. Table 1 shows the percentage parasitaemia for KGN and KGA extracts treated mice, on day 4 post-infection. At 50,100, 200 and 400 mg/kg, KGA and KGN extracts gave suppression of 22.0 %, 45.7 %, 57.8%, 69.6 % and 22.0 %, 37.8 % 61.7 %, 68.3 %, respectively. Compared to the negative control group, chemosuppression of the extracts at all the doses were statistically significant (*P* =0.01), whereas when the two extracts were compared dose for dose, their activities were not significantly different (P =.05) This shows a similar profile as can be observed in Fig. 1. Artemisinin (ART), the positive control drug, used at 0.2, 0.4, 0.8 and 1.6 mg/kg gave chemosuppression of 44.3 %, 56.0 %, 72.2 % and 78.2 %, respectively (Table 2).

Table 1. Effect of the aqueous and n-hexane extracts of *Khaya grandifoliola* on *Plasmodium berghei*-infected mice using the 4 – day test

Dose (mg/kg)	KGA		KGN	
	Parasitaemia (%)*	Chemosuppression (%)*	Parasitaemia (%)*	Chemosuppression (%)*
0	11.5 ±1.1	--	11.5 ±1.1	--
50	9.0 ± 0.9 [a]	22.0 ± 4.4	9.0 ± 0.9 [a]	22.0 ±7.4
100	6.3 ± 1.9 [a]	45.7 ± 6.3	7.2 ± 1.1 [a]	37.8 ± 9.2
200	4.9 ± 0.5 [a]	57.8 ± 4.5	4.4 ± 0.4 [a]	61.7± 3.7
400	3.5 ± 0.1 [a]	69.6 ± 1.3	3.7 ± 0.4 [a]	68.3 ±3.1

* *Values presented as Mean ± Standard deviation of the mean of five animals per group*
[a] *Significantly different (P=0.05) compared with the negative control; Dose 0 = Negative control*

Table 2. Effect of Artemisinin on *Plasmodium berghei*-infected mice using the 4 – day test

Dose (mg/kg)	Mean parasitaemia (%)*	Chemosuppression (%)
0	11.5 ±1.1	--
0.2	6.4 ± 0.6 [a]	44.3 ±4.93
0.4	5.1 ± 2.2 [a]	56.0 ± 9.1
0.8	3.5 ± 0.5 [a]	72.2 ± 2.5
1.6	2.7± 0.6 [a]	78.2 ± 4.9
Negative control	11.5 ±1.1	0.0

** Values presented as Mean ± Standard error of the mean of five animals per group.*
[a] significantly different (P= .05) compared with the negative control.
Dose 0=Negative control

3.2 Chemosuppressive Activity of KGA: ART and KGN: ART Combinations

In the combination studies, drug activity was also assessed in terms of parasitaemia reduction / chemosuppression of sub optimal doses of the extracts and ART. Suboptimal doses were taken as doses less than the median effective doses (ED_{50}). Based on the plots of values in Table 1, values for the ED_{50} were calculated for KGA and KGN as 140 and 160.0 mg/ kg respectively while in Table 2, 0.29 mg/kg was calculated as the ED_{50} for ART. Thus, 100 mg/kg each of KGA and KGN, as well as 0.20 mg/kg of ART were taken as the suboptimal doses. Fig. 1 shows mean percentage parasitaemia of the suboptimal doses for each of KGA, KGN and ART and their respective combinations. Compared with the negative control group, the combinations of KGA with ART (KGA: ART) and KGN with ART (KGN: ART) were significantly different (P <0.001). However, at 100 mg/kg, KGA and KGN gave 48.39 % and 45.16 % chemosuppression respectively (Fig. 2) which were not significantly different (P=.05) from each other. In addition, when compared with 0.20 mg/kg of ART, the extracts did not show any significant difference (P=.05). The KGA: ART and KGN: ART combinations had percentage chemosuppression of 78.29 % and 68.24 % respectively (Fig. 2) which were significantly different (P=.05) from their individual agents. However, when compared with each other, the combinations were not statistically significant (P< 0.001).

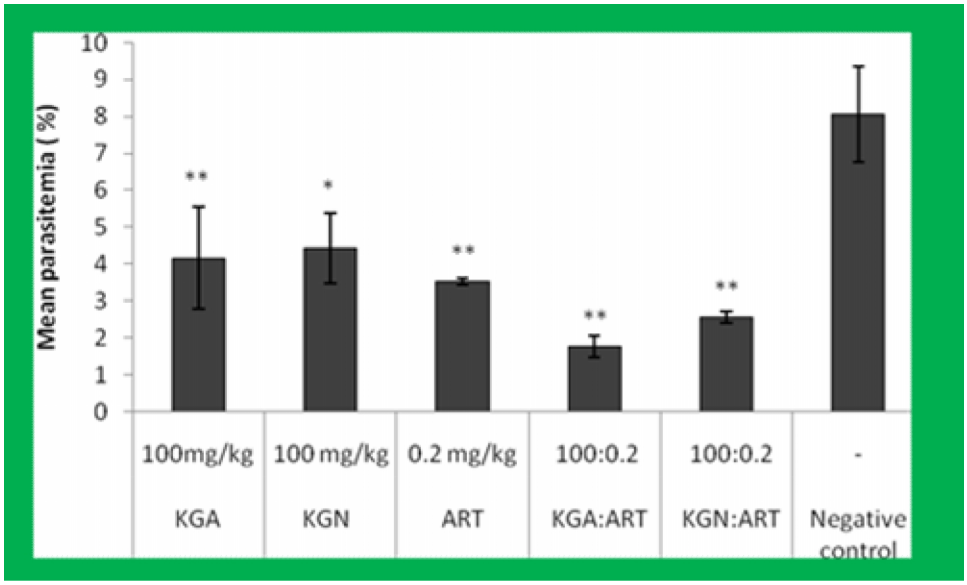

Fig. 1. Mean percentage parasitaemia due to aqueous (KGA) and n-hexane (KGN) extracts of *Khaya grandifoliola* alone and in combination with artemisinin on *Plasmodium berghei*-infected mice using the 4-day test

Mean ± S.E.M = Mean values ± Standard error of means of five animals.
*Test doses significant from negative control,(Dose 0) * P=0 .01, ** P<0.001.*

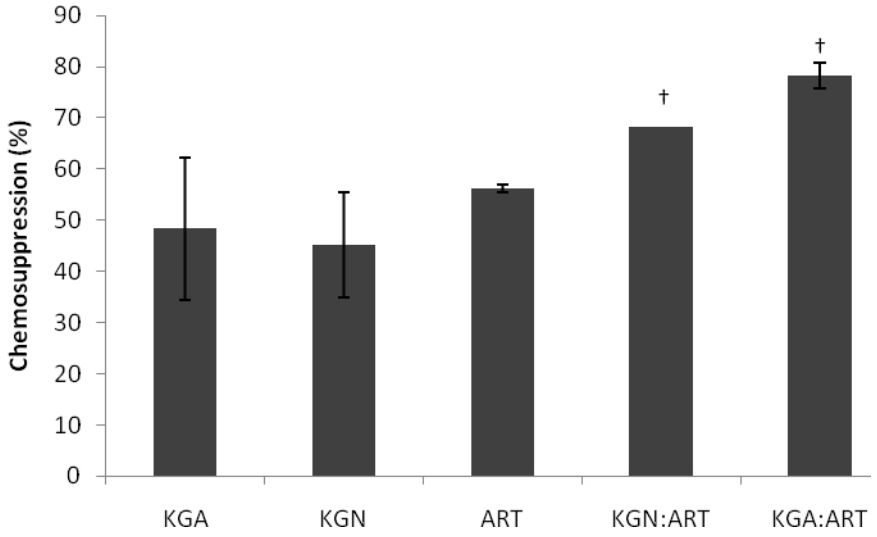

Fig. 2. Mean percentage chemosuppression due to aqueous (KGA) and *n* hexane (KGN) extracts of *Khaya grandifoliola* alone and in combination with artemisinin on *Plasmodium berghei*-infected mice using the 4-day test

Mean ± SD = Mean values ± Standard deviation of means of five animals
Test doses significance when compared with each other and in combinations † P <0.001

4. DISCUSSION

This study demonstrates a model for the pharmacodynamic evaluation of herb-drug antimalarial combinations using murine malaria chemosuppression test model. The results in this study showed remarkable suppression on day 4 post-inoculation. The antimalarial activity of an extract is considered good if more than 50 % reduction in parasitaemia was obtained at 200 mg/kg body weight [14]. Limonoids have been implicated as the antimalarial constituents of *Khaya grandifoliola* and *Entandophragma angolense* [15,16] both of the Meliaceae family. Grandifolin, a novel bicycle (3,3,1) nonane derivative, was isolated from the stem bark [17]. This compound was classified as a mexicanolide class of limonoid [18]. Artemisinin, isolated from *Artemisia annua* L., is an endoperoxide antimalarial drug with a high parasite-density clearance. The highest dose of artemisinin used in this study (1.6 mg/kg) was chosen based on *in vivo* antimalarial ED_{50} values obtained with *P. berghei* infected mice [12]. Sub-therapeutic doses of both extracts and ART were chosen to take advantage of the combination formula and also to demonstrate possible interactions. The results showed a potentiation in the chemosuppressive effects of KGA, KGN and ART suggestive of synergistic interactions. This type of pharmacodynamic interaction arises when the observed activity is greater than that of the individual agents. Thus, doses of the drugs can be reduced, side effects minimised without altering the overall drug activity [19]. Similar synergistic activity was obtained for *K. grandifoliola* with halofantrine while *K. grandifoliola* with chloroquine exhibited additive interaction at doses less than their respective ED_{50} values [6]. In addition, some combinations of natural products have been shown to potentiate the antimalarial activity of artemisinin. Curcumin (from *Curcuma longa*, Zingiberaceae) was reported to exhibit additive antimalarial effect with artemisinin *in vitro* [10]. Also, the extract of *Eurycoma longifolia* enhanced the antimalarial activity of artemisinin

in *Plasmodium yoelii*-infected mice [20]. This study gives information on the possibility of coadministration of herbal extracts with conventional drugs in a combination therapy in order to improve efficacy.

4. CONCLUSION

The combination of the n-hexane and aqueous extracts of *Khaya grandifoliola* with artemisinin suggested synergistic interactions. The investigation of the mechanism of action and evaluation of the combination using chloroquine-resistant *Plasmodium berghei* parasites to determine the effectiveness of the combination in CQ-resistant malaria, are in view in our laboratory.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication, revised 1996) were followed; All experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee of the Obafemi Awolowo University, ile Ife, Nigeria, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared no competing interests exist.

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