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Co-administration effects and toxicity profile of ethyl acetate fraction of *Stemonocoleus micranthus* Harms and Artesunate in murine malaria model

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ABSTRACT

The increasing reports of multi-drug resistant malaria has necessitated the continuous search for new drug candidates, enhancing the efficacy and life span of existing agents. The study aims at evaluating antimalarial potentials and safety use of ethyl acetate fraction of *Stemonocoleus micranthus* Harms (SM-EF) in co-administration with antimalarial artesunate. Ethyl acetate fraction from 80% methanolic leaf extract was investigated for antimalarial activity in single and combination with different doses of artesunate (2.5, 5 and 10 mg kg^{-1}) against *Plasmodium berghei* in mice. The parameters measured were curative effect against established infection, mean survival time (MST), weight loss, rectal temperature changes and packed cell volume (PCV). Histopathological study was done on concerned internal organs. The daily monitored curative effect of SM-EF used singly and in combination with artesunate showed a daily, dose-dependent and progressively reduction of malaria parasite. Ethyl acetate fraction of *Stemonocoleus micranthus* Harms used alone showed a poor onset of activity but long acting effect. On day 9, percentage inhibition effect was 86.78, 85.42, 92.98, 100, 91.19 and 100% for SM-EF, SM-EF-artesunate 2.3, 5 and 10 mg kg-1, artesunate 10 mg kg^{-1} and chloroquine 10 mg kg^{-1} respectively. Highest MST was chloroquine (21 days) and SM-EF-artesunate 10 mg kg^{-1} (19.5 days). Safety study of the harvested organs showed no untoward effect. The combination of SM-EF with artesunate 10 mg kg^{-1}, showed an enhanced antimalarial activity. This study supports the basis for the suggestion of SM-EF as a prospective drug candidate in antimalarial drug combinations regimen.

1. Introduction

Literature search revealed a global renewed interest in herbal medicine or plant based medicines. The factors behind this re-insurgence of interest has been advocated to several reasons which include inadequate availability and distribution of health-care facilities and socioeconomic status of the citizens, high cost and unavailability of conventional drugs, increasing resistance to available drugs (Odugbemi et al., 2007). Medicinal plants have been in use for generations as source of basic primary healthcare system for the prevention, control and management of various health challenges. These plants contain a high diversity of biological active compounds that have been a major source of conventional drugs and drug templates. The development of phytoconstituents to drug use level requires a thorough evaluation of their efficacy and safety. There is a general false perception that medicinal plants being from nature are without safety issues, and have no adverse effect. This is misleading as experience has shown pure isolated compounds, semi purified extracts and crude extracts have been associated with cytotoxicity, hepatoxicity, neurotoxicity, nephrotoxicity, genotoxicity, tetragenecity (Canter and Ernst, 2004; Rietjens et al., 2005; Ekor, 2013).

The burden of malaria is a global health challenge. However, the sub-Saharan region of Africa is associated with a high mortality and morbidity risk value. It is estimated that about 93% of all global cases for the year 2018 occurred in this region and Nigeria recorded about 29% of this burden (WHO, 2019). The most vulnerable population to malaria are the pregnant women and children of under five years (Hartman et al., 2010; Murray et al., 2012; Mzena et al., 2018). In the past the incidence of malaria infection in Nigeria was predictable due to the intensity of the infection being dependent on seasonal parameters (Abeku, 2007). The incidence of the infection is at its peak during the rainy season. This pattern will likely change due to climatic changes which has affected the duration and time of the rainfall season and increase in the breeding period of the vector.

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The progress and eradication of malaria infection is dependent on interaction between three key factors, the host (human), the vector (mosquito) and causative agent (plasmodium). The degree of gametocyte load, which is the infective stage of parasitaemia in the environment has a direct effect on the level of infection in the society/environment (Zhou et al., 2012). This can be determined by the population density of patients that present high gametocyte load but are asymptomatic. They act as parasite reservoir and point of transmission. Malaria infection in children is a source of infection transmission due to high load of gametocyte parasitaemia in the blood produced (Zhou et al., 2012). The longevity and biting habit of the vector plays an important role in the spread of the infection. The parasite has been reported to develop resistance to most available drugs due to the ease of their ability to mutate thus leading to treatment challenges, the parasite living longer in the blood stream and may increase the volume of infection.

An effective intervention with antimalarial medicinal agent(s) which possess early onset of activity and long acting effect is desirable for quick and sustainable cure of the infection. The fast action potentiates the quick clearing of the parasite from the host system while the long effect ensures total eradication of the residual or reservoir gametocytes. The World Health Organization (WHO) recommended artemisinin combination therapy (ACTs) consists of 2 different drugs with different mechanism of activity. This means that artemisinin which has early onset of activity is given in combination with another effective drug with long acting effects. The drug combination augments efficacy and reduces the ease of developing resistance by the parasite (Bosman and Mendis, 2007; Noedl et al., 2008; WHO, 2010). There are reports of already observed parasite resistance strains to the ACTs and how long it may take to get to the level of impacting on clinical out-come is still unknown and of great concern (Ariey et al., 2014; Leang et al., 2015; Amaratunga et al., 2016; Tacoli et al., 2016). Thus, there is an urgency for the search of alternative drug candidates as well as identification of new and effective drug combinations. The combination could be herbal medicines as multi-component agents in combination with conventional drugs or plant derived compound(s). Interactions of such herbal combination could result in synergistic, additive or inhibition in desired effect. The combinations could also result in undesirable effect(s) (Lin, 2014). Concomitant use of medicinal plants and standard antimalarial drugs have been reported as a common practice by some malaria endemic areas of Africa, with little or known documented outcome (Waako et al., 2005; Vigneron et al., 2005; Chenniappan and Kadarkarai, 2010).

A lot of medicinal plants are used traditionally in Nigeria in the treatment of malaria fever due to its enriched biodiversity. Some of these plants have had their antiplasmodial potential evaluated and validated as crude drug, semi-purified fractions or isolated compounds. The efficacy of *Stemonocoleus micranthus* Harms (Leguminosae- Caesalpinioideae) (SM) used traditionally in the Southeast of Nigeria as antimalarial plant has been evaluated and reported by this research team (Orabueze et al., 2020). The antimalarial potential of the hydro-methanol crude extract and fractions of SM were reported to be effective and dose-dependent. Ethyl acetate fraction of SM (SM-EF) was recorded as the most effective fraction, based on its exhibited activities on both early and established malaria infection assay procedures.

A possible interaction of ethyl acetate fraction of SM with standard drugs, chloroquine and artemisinin is not known. The present study is a continuation of a previous study and is aimed at evaluating anti-malarial interaction (*in vivo*) and effect on organs of concern when SM-EF is combined with an antimalarial conventional drug (artesunate). The outcome of the study is expected to provide information on the antimalaria combination activity, safety, and possible alternative treatment of malaria therapy targeted to delay resistance development.

2. Materials and methods

2.1. Reagents and chemicals

All reagents and solvents used for the study were of analytical grades and were obtained from reputable bio-chemical outlets.

2.2. Plant material collection and identification

The stem bark of SM was collected in February 2016 at Eha-Alumona village, Nsukka, Enugu in the South-Eastern part of Nigeria and authenticated by Mr. Ozioko Fred, of International center for Medicine and Drug Development (INTERCEDD). Voucher number was assigned to the plant, INTERCEDD/877 and deposited as herbarium specimen. The stem bark was washed and dried on laboratory table and then powdered. Grinding mill (Hamburg 76 West Germany) was used for the grinding of the sample. The coarse powder was immediately subjected to extraction process.

2.3. Preparation and extraction of plant materials

The methods for extraction and fractionation of SM stem bark have been previously described by Orabueze et al., 2020. Briefly, the dried and powdered stem bark of SM (1 kg) was exhaustively extracted with 80% methanol using Soxhlet apparatus (2000 mL) for 70 h. The filtrate obtained was concentrated under vacuum using a Buchi Vacuum Module V-801 Easy Vac rotary evaporator at 45 rpm and 40 °C to obtain the crude extract. The yield was 156.36 g, crude extract. The crude extract (40 g) was partitioned successively with different solvents (3 × 300 ml) to obtain n-hexane (FH), chloroform (FC), ethyl acetate (SM-EF) and aqueous (FA) fractions. The fractions were evaporated to dryness using rotary evaporator and later water bath at 40 °C. The ethyl acetate fraction (SM-EF) was 46.65% (Orabueze et al. (2020) and stored in a refrigerator at 4 °C in an air tight plastic container until used.

2.4. Collection of animal and parasite

The animals used in this study were male albino mice, with average weight of 24 – 28 g. They were procured from the Laboratory Animal center, College of Medicine, University of Lagos and kept in the University to acclimatized for 7 days. The animals were housed in standard clean plastic cages with soft wood shaving as bedding. They had free access to water and commercial animal food. Their housing was maintained under 12-h light-dark cycles. They were fasted overnight (12 h) from food only prior to dosing but has access to water. The use and handling of animals was in agreement with the international accepted principles for laboratory animal use and care. Ethical protocol was approved and issued by the Health Research Ethics committee of College of Medicine of the University of Lagos

2.5. Isolation of parasite

A mice infected with trophozoite stage of *Plasmodium berghei NK-65* (chloroquine (CQ)-sensitive strain) was procured from the National Institute of Medical Research (NIMR), Lagos and was maintained by serial passage of the parasite from donor mice to uninfected mice. Blood was drawn using heparized syringe from the donor mice and diluted in physiological saline solution (0.9% NaCl). A standard inoculum of 1×10^7 of parasitized erythrocytes from the donor mice parasite suspension (0.2 mL) was used to infect the experimental animals intra-peritoneally.

2.6. Acute toxicity study

The median lethal dose (LD_{50}) of the fraction SM-EF was determined, to know the dose that would kill 50% of the experimental animal population. A modified method as described by Lorke (1983) was used to test for toxicity of the extract (Lorke, 1983). The mice were fasted for 12 h (all night) prior to drug administration. The mice were divided into 3 groups of 5 mice namely, SM-EF drug groups and no-treatment group. The first 2 groups received a single doses of 1000 and 2000 mg kg^{-1} of SM-EF respectively while the no-treated group (negative group) received reconstitution solvent, 5% DMSO. The animals were allowed to live normally while observation took place. Signs of distress or death were observed throughout. This observation continued for 7 days.

The LD_{50} will be determined using Lorke's equation

$$LD_{50} = \sqrt{ab}$$

Where a = Least tolerable dose
b = Maximum tolerable dose

2.7. Antimalarial studies

2.7.1. Evaluation of curative antimalarial potential of sm-ef (Rane's test

This method aimed at investigating the curative potential of the plant extract fraction (SM-EF) in established infection as described by Ryley and Peters (1970) and modified by Iyiola et al. (2011). The fixed dose for the SM-EF has been pre-determined in a previous study Orabueze et al. (2020). The animals selected (35) for the assay were injected intraperitoneally with standard inoculum of 1×10^7 *Plasmodium berghei* NK - 65 infected erythrocytes on the first day (D_0). The mice were left for 72 h (3 days) to allow enough time for the infection to be established before commencement of any intervention. After Giemsa-stained thin blood smear confirmation of parasitaemia, the 35 mice were grouped into 7 groups of 5 mice per Group. Test animal were grouped into:

Group 1 (GP1): treated with 40 mg kg^{-1} of SM-EF
Group 2 (GP2): treated with SM-EF 40 mg kg^{-1} in combination with artesunate 2.5 mg kg^{-1}
Group 3 (GP3): treated with SM-EF 40 mg kg^{-1} in combination with artesunate 5 mg kg^{-1}
Group 4 (GP4): treated with SM-EF 40 mg kg^{-1} in combination with artesunate 10 mg kg^{-1}
Group 5 (GP5): treated with 0.2 mL 5% DMSO (negative control group)
Group 6 (GP6): treated with 10 mg kg^{-1} of artesunate (positive control group I)
Group 7 (GP7): treated with 10 mg kg^{-1} of Chloroquine (positive control group II)

All groups were treated orally, once daily for 5 days (D_3-D_7). Blood smears were done daily through the dosing days (D_3-D_7) and D_{14} and D_{21} to monitor the rate of parasite clearance. The plasma cell volume (PCV) was checked during dosing D_0, D_3 - D_7 and post dosing days of D_{14} and D_{21}.

The average daily parasitaemia observed was calculated using the formula below;

$$\text{Average \% parasitaemia} = \frac{\text{Number of parasitized ErythrocytesX100}}{\text{Total number of Erythrocytes}}$$

$$\%\text{Inhibition} = \frac{\text{Mean \% parasitaemia in control} - \text{mean parasitaemia in treated group X100}}{\text{Mean \% parasitaemia in control group}}$$

On day 14 post malaria infection, an animal from each group was sacrificed and some organs: kidney, liver and spleens were harvested for histopathological analysis.

2.7.2. Determination of body weight and rectal temperature

The body weight and rectal temperature of each individual mouse in all groups were recorded before initiation of any treatment regimen on day 0, 3 (D_3) D_7 and after treatment on D_{14}, D_{21}. Temperature change was monitored by measurement of rectal temperature using digital thermometer and the weight using LA 164 analytical balance (B. Bran Scientific and Industries Company, England). The body weight and rectal temperature changes for the extract-treated groups were compared with the control groups.

2.7.3. Determination of packed cell volume (PCV)

Blood sample was collected from the eye socket of each mouse in heparinized microhematocrit capillary tubes. The PCV of each mouse was measured after infection with *Plasmodium berghei*. The tubes were sealed with plasticin after each sample collection and centrifuged at 3000 rpm for 10 min. The volume of the erythrocytes and total blood were measured, and used for the determination of the PCV.

$$\text{PCV} = \frac{\text{Volume of erythrocytes in a given blood volume x100}}{\text{Total volume of blood in capillary tube}}$$

2.7.4. Mean survival time and survival rate

Death records were kept and used to determine the survival rate per group and then in calculation of the Mean survival time (MST) according to the method of Alli et al. (2011). Survival rate is the number of death that occurred in each group between D_0 and D_{21}. Mean survival time (MST) for each group from date of infection over a period of 22 days ($D_0 - D_{21}$) was calculated using the formula:

$$\textbf{MST} = \frac{\text{Sum of survival time of all mice in each group(days)}}{\text{Total number of mice in that group}}$$

2.7.5. Toxicological assessment of co-administration in the Rane's test: histological analysis

The histological analysis of the kidneys, liver spleen excised from all the animals were done in the histopathology laboratory of the Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, Nigeria.

The organ samples collected were trimmed, processed, embedded fixed in 10% buffered formalin for 10 days before embedding in paraffin wax. The organs were prepared for viewing under the microscope by sectioning and staining with hematoxylin and eosin (H.E). The view under the microscope was done in oil immersion with magnification of x100. Any pathological changes were examined and compared with the sections of harvested organs from healthy and non-infected mice. Photomicrographs were taken for analysis and documentation.

2.7.5.1. Statistical analysis. Values were expressed as Mean ± Standard error of mean (SEM). Graphpad version 5.00 was used to analyze the data. All experiments results were evaluated by analysis of variance (ANOVA), followed by a post test (Tukey Kramer multiple comparison test) to compare result between treatment and control groups. And $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Acute toxicity study on mice

The acute toxicity study of orally administered SM-EF 2000 mg kg^{-1} caused no visible or physical distress or behavioural changes or death within 7 days in treated group. Thus, no toxicity (LD_{50}) concern or mortality was recorded. The dose of the semi-purified fraction SM-EF used

for the study was considered to be relatively within safe dose range of the herbal drug.

3.1.1. Effect on established malaria infection (Rane's test)

Decrease in the percentage parasitaemia was inversely proportional to percentage of inhibition (degree of cure, reduction of the volume of parasite) by a drug. Effect of the SM-EF at fixed dose of 40 mg kg^{-1} in combination with varying doses of artesunate (2.5, 5 and 10 mg kg^{-1}) on established *P. berghei* infected mice exhibited a dose-dependent and significant ($p<0.05$) schizonticidal effect (Table 1). This indicates that the concomitant use of SM-EF-artesunate had pharmacological effect against established malaria infection. The group, GP4 had the highest percentage of inhibition on D_7 which is not significantly different ($p<0.05$) from that of GP3 on the same day. On D_9, no parasite was detectable in GP4, 100% inhibition (100% cure) was established while GP3 represented 92.98% inhibition. The GP4 maintained the 100% inhibition (100% cure) while GP3 had 100% inhibition on D_{14} and started presenting parasitaemia again on D_{21}. The regrowth observed with GP3 could be recrudescence of undetectable reservoir or re-infection. The GP2 showed daily and gradual increase in percentage of inhibition until D_9 (85.42%) but the drug started losing its effectiveness as seen on D_{14} with an increased value of percentage of parasitaemia, 11.74 ± 0.26.

There was a significant difference ($p<0.05$) between GP1 and GP2 in terms of onset of action. The drug (artesunate) combination dose of GP2 seems to delay the time of onset of activity. This onset of activity effect was noticed to be dose-depended in relation to artesunate dose (mg kg^{-1}).

The 2 positive controls GP6 and GP7 (artesunate and chloroquine, respectively) treated groups, showed different percentages of parasite reduction (% inhibition) compared to negative control (GP5). The group that received chloroquine only, GP7 showed early and fast parasite reduction. By D_6 there was a 100% inhibition (100% cure) and no parasite was detected until the end of the study on D_{21}. The group that was treated with artesunate only at 10 mg kg^{-1} show fast onset of activity (33.47% percentage inhibition on D_4) but did not present a total parasite reduction and by D_7 was showing recrudescence, and the percentage parasitaemia started increasing (Table 1).

3.1.2. Body weight and rectal temperature change

The effect of the fraction SM-EF used individually and in combination with artesunate on weight following treatment, showed a continuous or gradual loss in body weight in the animals in all the groups (Table 2). The rate of weight loss between D_0 and D_4 across the groups, was reduced on introduction of the intervention but general weight loss continued. The positive control group (GP7) showed the same pattern of controlled weight loss. However, weight loss was fairly similar between groups treated with CQ and those that received the extract fractions.

Rectal temperature analysis showed daily and progressive reduction in rectal temperature across the groups compared to the negative control group (Table 3). The negative control group (GP5) maintained a constant temperature through the treatment duration D_3 to D_7 and the animals died before D_{21}. On D_{14} significantly reduction in rectal temperature was observed in group 1.

All treated groups (positive controls and the SM-EF treated groups) significantly prevented reduction in PCV when treatment was initiated on D_3 as compared to the negative control group. Gradual increase in PCV was seen from D_3 to D_7. *Re*-evaluation of PCV on D_{14} showed that groups 1, 2, and 3 could no longer prevent reduction of PVC on withdrawal of the treatment. The treatment group 4 and positive control groups 6 and 7 were able to increase PCV from D_3 and maintained such increased value of PCV compared to the negative control until end of the study.

3.1.3. Mean survival time

Groups 1 and 2 lived longer but were not fully protected. Group 4 (GP4) experimental animals lived longer than GP1 and other groups except GP7, but were not fully protected though the drug combination showed 100% inhibition of parasite presence by D_9. An animal died on day 14 unexplainably.

Group 6 (ART 10 mg kg-1) did not present total reduction of parasite and the animals lived longer that GP1 but were not fully protected.

Group 7 (CQ 10 mg/kg-1) treated group displayed total parasite clearance and full mean survival time (21 days).

3.1.4. Histology report

The positive control groups treated with known standard drugs (GP 6 and 7) which received 10 mg kg^{-1} artesunate and chloroquine respectively, showed a vascular congestion in the histologic section of the liver. No significant alteration was seen in the groups that received SM-EF and its combinations (Supplementary figure 1).

Table 1
Curative antimalarial activity of Stem bark of SM-EF in single and in combination on mice infected with Chloroquine-sensitive *Plasmodium berghei* (NK-65).

		D3	D4	D5	D6	D7	D9	D14	D21
0.2 mL 5% DMS0	**% Parasitaemia**	10.11 ± 0.15	12.01 ± 0.34	15.21 ± 0.82	16.37 ± 0.28	18.69 ± 0.20	20.65 ± 0.16	–	–
GP 5	**% Inhibition**	0	0	0	0	0	0	0	0
GP1	**% Parasitaemia**	11.49 ± 0.18	9.92 ± 0.07	6.57 ± 1.67	6.50 ± 0.09	4.77 ± 0.05	2.73 ± 0.07	2.59 ± 0	–
	% Inhibition	−13.65	17.40	56.80	60.29	74.48	86.78	–	–
GP2	**% Parasitaemia**	11.08 ± 0.27	10.18 ± 0.48	7.79 ±	5.70 ± 0.14	2.96 ± 0.09	3.01 ± 0.41	11.74 ± 0.26	–
2.5	**% Inhibition**	−9.59	10.07	48.78	65.18	84.16	85.42	–	–
GP3	**% Parasitaemia**	11.29 ± 0.15	9.50 ± 0.10	6.53 ± 0.39	2.63 ± 0.37	1.53 ± 0.24	1.45 ± 0.02	0.00 ± 0.00	3.04 ± 0.00
5	**% Inhibition**	−11.67	20.9	57.07	83.93	91.81	92.98	–	–
GP4	**% Parasitaemia**	11.73 ± 0.22	8.41 ± 0.37	5.75 ± 0.75	3.58 ± 0.24	1.32 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	**% Inhibition**	−16.02	29.98	62.20	78.13	92.94	100	–	–
GP6 Art 10	**% Parasitaemia**	11.69 ± 0.19	7.99 ± 0.18	4.53 ± 0.11	1.15 ± 0.09	1.57 ± 0.00	1.82 ± 0.21	3.22 ± 0.19	–
	% Inhibition	−15.63	33.47	70.22	92.97	91.60	91.19	–	–
GP7	**% Parasitaemia**	11.35 ± 0.19	5.66 ± 0.25	1.32 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CQ 10		−12.27	52.87	91.32	100	100	100	–	–

Values for mean% parasitaemia are expressed as mean ± standard error of mean (PD ± SEM), $P < 0.05$, $n = 5$ mice per group.
Key.
GP1 = SM-EF only (40 mg kg^{-1}).
GP2= SM-EF + 2.5 mg kg^{-1} Artesunate.
GP3 = SM-EF + 5 mg kg^{-1} Artesunate.
GP4 = SM-EF +10 mg kg^{-1} Artesunate.
GP5 (0.2 mL 5% DMSO; -ve control).
GP6= 10 mg kg^{-1} of ART.
GP7= 10 mg kg^{-1} of Chloroquine CQ.
SM-EF: *Stemonocoleus micranthus* Ethyl-acetate fraction.

Table 2
Body weight change during curative test of stem bark of SM_EF in individual and combination therapy.

GROUPS	D0	D3	D5	D7	D14	D21
Gp5	25.70 ± 1.40	24.00 ± 0.40	25.00 ± 0.80	22.00 ± 0.70	–	–
GP1	26.50 ± 0.46	24.20 ± 1.12	24.50 ± 1.06	23.00 ± 1.30	23.50 ± 0.00*	_
GP2	27.20 ± 1.08	26.10 ± 1.12	26.50 ± 1.53	25.00 ± 1.43	22.60 ± 0.62*	–
GP3	25.50 ± 2.20	24.00 ± 0.50	24.00 ± 1.30	25.00 ± 0.72	23.00 ± 1.03*	22.25 ± 0.00*
Gp4	26.50 ± 01.42	25.00 ± 1.10	25.00 ± 1.40	24.00 ± 1.30	24.80 ± 1.49*	21 ± 0.88*
Gp6	24.00 ± 1.54	20.60 ± 0.97	22.00 ± 1.50	20.00 ± 2.90	22.50 ± 2.50*	–
Gp7	26.00 ± 1.70	23.60 ± 0.61	24.00 ± 1.040	22.80 ± 0.47	24.00 ± 0.40*	21.4 ± 0.93*

Values are expressed as mean ± SEM; $n = 5$.
Significant at $^{*}p<0.05$ on D_{14} and D_{21} in comparison with value on D_0 (pre-infection).

4. Discussion

Malaria is an infectious disease of global interest due to its effects on the socioeconomic life of the people and regions affected. The increasing number of recorded morbidity or mortality is likely due to the increasing rate of parasite resistance to available drugs and resistance of vectors to insecticides (Fidock et al., 2004; Willcox, 2011). The use of chloroquine for clinical management of uncomplicated P. falciparum malaria was replaced by WHO recommended Artemisinin-based combination therapy (ACT) for the same reason of resistance to available drugs. The reason behind the success of this recommendation was its ability to reduce the ease of the parasite developing resistance due to ACTs two drug-component nature. The advantage of this 2 component drug being that artemisinin is a fast action drug, and early onset of parasite's clearance increases the rate of patient recovery; however, it is easily metabolised. The second drug in the combination is normally slow in initiating bioactivity but has a long duration of effect in the blood system, thus aiding in clearing the remaining and reservoir malaria parasite load. In this present study SM-EF which has been reported as presenting long duration activity in a previous study, is assayed singly (as individual) and in various dose combinations with artemisinin and the results evaluated against standard drugs, such as artemisinin and chloroquine. The use of medicinal plant preparations together with conventional antimalarial drugs in traditional herbal healthcare facilities is a regular practice (Waako et al., 2005; Vigneron et al., 2005; Chenniappan and Kadarkarai, 2010; Rodríguez et al., 2018). The outcome of most of these concomitant administration of plant extracts and orthodox drugs have not been evaluated for efficacy and safety.

The researchers of this study employed an *in vivo* model to assess the treatment outcome of SM-EF in individual and combinations with artesunate on mice infected with P. *berghei* (Bantie et al., 2014). The curative (Rane) antimalarial assay evaluates the antimalarial activity of drug candidates on established infection. The parameters measured were percentage parasitaemia, percentage inhibition of parasitaemia, suppression of malaria oriented symptoms (anemia, weight loss and temperature control), and survival time of the infected mice.

The curative test of SM-EF only, showed a slow onset of parasite clearance at the tested dose level (40 mg kg^{-1}), which progressed daily till D_9 (Table 1). The slow onset but long acting parasite reduction suggests that this fraction (SM-EF) may not be suitable to be used in emergency or as a single drug but may be a good candidate for combination therapy with antimalarial drug like artemisinin to enhance its efficacy (Orabueze et al. (2020). When given concurrently or as a fixed dose combination with another antimalarial agent with fast but short duration of activity it may result in a more effective additivity-synergy product (Orabueze et al. (2020). Groups treated with SM-EF in combination with varying doses of artesunate (2.5, 5 and 10 mg kg^{-1}) showed a dose dependent activity in relation to onset of activity and efficacy (Table 1). The percentage parasitaemia clearance obtained 48 h post last drug administration (D_9) in the various dose levels of drug combination treated groups indicated possible prolonged (long acting) antimalarial bio-active. Group 4 that was treated with SM-EF - 10 mg kg^{-1} artesunate showed the most rapid onset of parasite clearance which progressed daily until all the parasites were relatively eliminated from the animal body. Long acting effects may suggest that the drug has a slow rate of metabolism or acts by inducing the body's immunological system to heal itself. Another study on this plant reported an immunomodulatory activity of *Stemonocoleus micranthus Harms* (Mbaoji et al., 2020).

The animals in groups 4 (SM-EF 40 mg kg^{-1} - Artesunate 10 mg kg^{-1}) and group 7 (CQ treated group) had high levels of protection. They showed full recovery of 100% inhibition (100% cure) and parasitaemia level of 0% at the end of the experiment. Lower dose treated groups exhibited prolonged days of survival, compared to the untreated group but were not fully protected. Death at lower doses instead of higher doses of the extract is a likely indication that death occurred possibly due to malaria infection and not toxicity. Group 3 showed zero parasitaemia level on D_{14} but re-growth of malaria parasite was observed on D_{21} blood smear. This could possibly be attributed to undetectable levels of the parasite on $D_{14,}$ of which the immunological activity of the body system or the long acting effect of SM-EF could not prevent parasite multiplication with the progression of time. The re-appearance of parasitaemia could also be due to re-infection, which is an indication of a compromised immune system

Table 3
Rectal temperature change during curative test of SM-EF.

GROUPS	D0	D3	D5	D7	D14	D21
Gp5	38.33 ± 0.30	37.95 ± 0.20	38.25 ± 0.14	38.63 ± 0.06	-	
GP 1	38.68 ± 0.14	36.86 ± 0.34	36.78 ± 0.43	37.15 ± 0.03	34.8 ± 0.00*	–
GP2	38.62 ± 0.17	37.90 ± 0.27	37.47 ± 0.06	37.7 ± 0.40	37.08 ± 1.03	–
GP3	38.85 ± 1.37	37.32 ± 0.78	37.80 ± 1.21	37.57 ± 5.25	37.75 ± 3.20	37.72 ± 0.00
Gp4	38.57 ± 0.61	37.04 ± 0.27	37.40 ± 0.24	37.60 ± 1.54	36.33 ± 1.40	37.54 ± 0.7
Gp6	38.35 ± 0.43	37.20 ± 0.62	38.00 ± 0.30	37.10 ± 0.10	36.20 ± 0.87	–
Gp7	38.23 ± 1.06	38.02 ± 0.51	38.48 ± 0.18	37.08 ± 0.16	37.44 ± 0.40	39.24 ± 1.20

Values are expressed as mean ± SEM; $n = 5$.
Significant at $^{*}p<0.05$ on D_{14} and D_{21} in comparison with value on D_0 (pre-infection).

so soon after an infection. *Re*-occurrence of infection after a prolonged survival could mean recrudescence of *P. berghei* after the apparent cure. Recrudescence which is re-emerging of symptoms of infection after an apparent cure indicates possible relapse and treatment failure or re-infection. Only groups 4 and 7 maintained 100% inhibition of parasite (cure), zero detection of parasite on D_{21} blood smear and high survival rate.

The outcome exhibited by GP4 is similar to the report of the antimalaria effect of combining *Gynostemma pentaphyllum* with artesunate, *Moringa oleifera* leaf extracts with artesunate, *Uvaria chamae* and amodiaquine and Cryptolepine (CPE) with artesunate (ART) combinations (Somsak et al., 2016; Forkuo et al., 2016; Adepiti and Iwalewa, 2016). The combination or co-administration of leaf or stem bark extract of *Chrysophyllum albidum* with chloroquine produced an activity that was lower than those of the individual extracts when used singly (Odediran et al., 2020). Thus the outcome of combination therapy may not always be favourable and may have adverse effects.

Changes in some body indices such as the body weight, PCV and temperature are indicators for evaluating the general health status (Sharma et al., 2009). Loss of body weight, anemia and body temperature increase are some expected or commonly seen clinical symptoms of malaria infection (Saganuwan et al., 2011). Such information can be used to fairly monitor the recovery progress of the infection.

All the experimental groups lost weight even after the commencement of therapy. However, the groups that received treatment intervention were able to control the rate of weight loss between D_4 and D_7 (Table 2). This was possible due to their effectiveness in initiating antiparasitic activity, effectiveness in reducing the parasite load, and the likelihood of reducing the discomfort of the illness. Furthermore, it is likely there was improvement in feeding of the animals. The inconsistent loss of weight that occurred post withdrawal of treatment, could be indicative of after-drug exposure (adverse effect) effects on the body's system or the body's immunological system suppressing the re-growth (replication) of parasite load. Thus, the use of this parameter to evaluate the efficacy of performance of a given drug can be ruled out

Daily but progressive reduction of rectal temperature was exhibited by the groups with significant maintenance of temperature (Table 3). A decrease in internal body temperature is an indicator that the metabolic rate of infected mice is compromised and usually is observed before death occurs (Mengiste et al., 2012; Fentahun et al., 2017). The value of rectal temperature can be used to predict the group that is under distress and threat. All the groups that received treatment intervention experienced a halt in temperature reduction during the treatment regime. Further but controlled reduction in temperature as seen in D_{14} can be explained to have occurred as a result of the inefficacy of the drugs at some dose level to clear all plasmodia parasites. The groups that achieved 100% inhibition (cure) of parasites recovered and temperature elevation was observed on D_{21}. Another factor that could alter temperature without total parasite clearance from the body's system can be attributed to antipyretic potentials of an administered candidate drug (Falade et al., 2018; Muregi, 2010).

Packed cell volume (PCV) was measured to evaluate the efficiency of SM-EF administered in single or in combination with artesunate in preventing haemolysis, due to malaria infection (Table 4). The change in value of PCV (Table 4) followed the pattern of the level of parasitaemia as observed in Table 1. Thus, treatment groups 2, 3, 4, 5, and 7 showed increase in the PCV of the animals when drug treatment started on D_3 to D_7. Post D_{14}, the groups that experienced recrudescence had their PCV decreased again. The treatment group with 100% inhibition of parasitaemia (cure) exhibited no lysing of the Red Blood Cell because of the relative absence of parasites in the RBC to induce lysis. This confirms the efficacy of SM-EF – Artesunate at 10 mg kg^{-1} in clearing *plasmodium berghei* in an established infection. Decrease in PCV is an indication of anemia, haemolysis of red blood cells and decrease in blood oxygen transportation (Briggs and Bain, 2017)

The internal organs, especially the ones involved with drug and substance metabolism are monitored and evaluated during preclinical safety studies of new drugs (Kramp et al., 1974). This involved functional studies in toxicology and histological studies of the appropriate organs. Liver, kidney and the spleen are organs that are involved with malaria parasite lifecycle, infection and metabolism of antimalaria drugs. Histological analysis of liver, kidney and the spleen, of some randomly selected animals per group, showed no significant morphological or pathological changes or alterations in the organs harvested from groups treated with fixed dose of SM-EF and various combinations of SM-EF-artesunate compared to the negative and positive controls. The absence of histological damage of the liver as seen in the groups treated with SM-EF only and SM-EF combinations could be due to the hepatoprotective potential of *Stemonocoleus micranthus* stem bark. Past investigations on *Stemonocoleus micranthus* stem bark demonstrated that the plant had antioxidant, nephroprotective and hepatoprotective potentials (Mbaoji et al., 2017; Orabueze et al., 2020). The antioxidant effect of the plant may explain its ability to protect the organs. The result of an acute toxicity test of SM-EF showed that the extract was not lethal at 2000 mg kg^{-1} when administered orally. Furthermore, no behavioural changes, toxidromes or death, was observed in similar studies by (Mbaoji et al., 2020).

The results of the mean survival time (MST) calculated showed that group 4 which was treated with 40 mg kg^{-1} SM-EF and 10 mg kg^{-1} artesunate, showed the highest MST of all the treated groups indicating that this dose combination is the most effective. Furthermore, other treated groups showed a slightly high mean survival time when compared to the negative control which had the lowest MST (Table 4). Most deaths occurred early in the study before the full effect of the drug intervention was able to attenuate the effect of the malaria parasite.

Table 4
Packed cell volume (PCV) of mouse selected randomly in each group.

GROUPS	D3	D5	D7	D14	D21	MST (days)
GR5	32.20 ± 0.30	29.50 ± 1.15	27.10 ± 0.50	–	–	8.3 ± 3.04
GR1	32.20 ± 0.32	35.95 ± 0.38	32.85 ± 0.42	27.80 ± 00.00	–	11 ± 5.20
GR2	32.20 ± 0.87	37.70 ± 0.76	38.10 ± 00.37	26.20 ± 00.70	–	9 ± 0.160
GR3	32.05 ± 0.35	39.30 ± 0.30	39.40 ± 00.51	28.00 ± 0.64	22.7 ± 00.00*	14 ± 1.20
GR4	32.20 ± 0.62	40.30 ± 0.43	40.50 ± 00.17	40.95 ± 1.01	42.40 ± 0.00*	19.50 ± 1.79
GR6	32.50 ± 0.41	39.00 ± 0.07	42.50 ± 00.97	40.80 ± 0.22	–	15.5 ± 1.02
GR7	32.00 ± 1.27	39.20 ± 0.82	42.90 ± 00.54	42.20 ± 0.35	42.25 ± 0.00*	21 ± 0.12

Data show the mean ± SEM $n = 5$,.
Significant at $^*p<0.05$ on D_{21} in comparison with value on D_3 (pre-drug administration).
MST: mean survival time.

5. Conclusion

This study investigated the potency and efficacy of the combination of a fixed dose of the ethyl-acetate extract fraction of the stem bark of *Stemonocoleus micranthus* (SM-EF) with low, medium and high doses of artesunate against *Plasmodium berghei* infected mice. Current malaria control programs advocate the use of ACTs while at the same time encouraging the search for new active compounds and efficacious drug combinations in other to be ahead of drug possible resistance to malaria infection. All SM-EF combinations with artesunate at different doses in the study showed significant reduction in the level of parasitaemia and longer survival time. The combination of 10 mg kg^{-1} artesunate and 40 mg kg^{-1} SM-EF gave the best antimalarial effect significantly comparable to chloroquine. Thus, the concomitant administration enhanced the antimalaria potency of the two drugs involved, compared to when used as individual drugs. Histopathological study of the harvested organs showed no signs of adverse morphology or toxicity.

Ethical approval

The research work was approved by the Health Ethics Research committee of College of Medicine of the University of Lagos, with protocol ID number CMUL/HREC/05/17/129

Declaration of Competing Interest

The authors declare that there is no conflict of interest concerning this research paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2021.12.005.

References

Abeku, T.A., 2007. Response to malaria epidemics in Africa. Emerg. Infect Dis. 13, 681–686.

Adepiti, A.O., Iwalewa, E.O., 2016. Evaluation of the combination of *Uvaria chamae* (P. Beauv.) and amodiaquine in murine malaria. J. Ethnopharmacol. 193, 30–35.

Alli, L.A., Adesokan, A.A., Salawu, O.A., Salawu, O.A., Akanji.and, M.A., Tijani, A.Y., 2011. Antiplasmodial activity of aqueous root extract of *Acacia nilotica*. Afr J Biochem Res 5 (7), 214–219.

Amaratunga, C., Lim, P., Suon, S., Sreng, S., Mao, S., Sopha, C., Sam, B., Dek, D., Try, V., Amato, R., Blessborn, D., Song, L., Tullo, G.S., Fay, M.P., Anderson, J.M., Tarning, J., Fairhurst, R.M., 2016. Dihydroartemisinin–piperaquine resistance in *Plasmodium falciparum* malaria in Cambodia: a multisite prospective cohort study. Lancet Infect Dis 16, 357–365.

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A.C., Khim, N., Kim, S., Duru, V., Bouchier, C., Ma, L., Lim, P., Leang, R., Duong, S., Sreng, S., Suon, S., Chuor, C.M., Bout, D.M., Ménard, S., Rogers, W.O., Genton, B., Fandeur, T., Miotto, O., Ringwald, P., Le Bras, J., Berry, A., Barale, J.C., Fairhurst, R.M., Benoit-Vical, F., Mercereau-Puijalon, O., Ménard, D.A., 2014. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature 505, 50–55.

Bantie, L., Assefa, S., Teklehaimanot, T., Engidawork, E., 2014. *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hochst. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement. Altern. Med. 14, 79.

Bosman, A., Mendis, K.N., 2007. A major transition in malaria treatment: the adoption and deployment of artemisinin-based combination therapies. N. Engl. J. Med. 77 (6), 193–197.

Briggs, C., Bain, B.J., 2017. Basic Haematological Techniques In: Dacie and Lewis practical Haematology, 12th ed Elsevier, New York (NY), pp. 18–49. 2017.

Canter, P.H., Ernst, E., 2004. Herbal supplement use by persons aged over 50 years in Britain: frequently used herbs, concomitant use of herbs, nutritional supplements and prescription drugs, rate of informing doctors and potential for negative interactions. Drugs Aging 21, 597–605.

Chenniappan, K., Kadarkarai, M., 2010. *In vitro* antimalarial activity of traditionally used Western Ghats plants from India and their interactions with chloroquine against chloroquine-resistant *Plasmodium falciparum*. Parasitol. Res. 107, 1351–1364.

Ekor, E., 2013. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 4, 177. https://doi.org/10.3389/fphar.2013.00177.

Falade, M.O., Komoni, F., Nwuba, R.I., 2018. Efficacy of *Lophira alata* leaf extract and its combination with artesunate in mice prior exposed to *Plasmodium berghei*. Drug Res. (Stuttg) 68 (4), 232–237. https://doi.org/10.1055/s-0043-120759.

Fentahun, S., Makonnen, E., Awas, T., Giday, M., 2017. *In vivo* antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice. BMC Complement. Altern. Med. 17, 13.

Fidock, D.A., Rosenthal, P.J., Croft, S.L., Nwaka, N.C., 2004. Antimalarial drug discovery: efficacy models for compounds screening. Nat. Rev. Drug Discov. 3 (6), 509–520.

Forkuo, A.D., Ansah, C., Boadu, K.M., Boampong, J.N., Ameyaw, E.O., Gyan, B.A., Arku, A.T., Ofori, M.F., 2016. Synergistic anti-malarial action of cryptolepine and artemisinins. Mal. J. 15 (89), 1–12.

Hartman, T.K., Rogerson, S.J., Fischer, P.R., 2010. The impact of maternal malaria on newborns. Ann. Trop. Paediatr. 30, 271–282.

Iyiola, O.A., Tijani, A.Y., Lateef, K.M., 2011. Antimalarial activity of ethanolic stem bark extract of *Alstonia boonei* in mice. Asian J. Biol. Sci. 4, 235–243.

Kramp, R.A., MacDowell, M., Gottschalk, C.H., Oliver, J.R., 1974. A study in microdissection and micropuncture of the structure and the function of kidneys and the nephrons of rats with chronic renal disease. Kidney Int. 5 (2), 147–176.

Leang, R., Taylor, W.R., Bouth, D.M., Song, L.J., Tarning, M.C., Char, M.C., Kim, S., Witkowski, B., Duru, V., Domwergue, A., Khim, N., Ringwald, P., Menard, D., 2015. Evidence of *Plasmodium falciparum* malaria multidrug resistance to artemisinin and piperaquine in Western Cambodia: dihydroartemisinin–piperaquine open-label multicenter clinical assessment. Antimicrob. Agents Chemother. 59, 4719–4726.

Lin, G., 2014. Beneficial herb–drug interaction in combinational therapy of herbal medicine with drug. J. Integr. Med. 6, 697.

Lorke, D., 1983. A new approach to practical acute toxicity testing. Arch. Toxicol. 54, 275–287.

Mbaoji, F.N., Ezike, A.C., Nworu, C.S., Onyeto, C.A., Nwabunike, I.A., Okoli, I.C., Akah, P.A., 2017. Antioxidant and hepatoprotective potentials of *Stemonocoleus micranthus* Harms (Fabaceae) stem bark extract. *Int*. J. Pharm. 8 (7), 47–51.

Mbaoji, F.N., Onwuka, A.M., Onu, S., Peter, I.E., Nweze, J.A., Okonta, L.E., 2020. Evaluation of methanol-dichloromethane extract of *Stemonocoleus micranthus* Harms (Fabaceae) stem bark for anti-inflammatory and immunomodulatory activities. Evid. Based Complement. Altern. Med.. https://doi.org/10.1155/2020/1738163.

Mengiste, B., Makonnen, E., Urga, K., 2012. *In-vivo* antimalarial activity of *Dodonaea angustifolia* seed extracts against *Plasmodium Berghei* in mice model. Momona Ethiopian J. Sci. 4, 47–63.

Muregi, F.W., 2010. Antimalarial drugs and their useful therapeutic lives: rational drug design lessons from pleiotropic action of quinolines and artemisinins. Curr. Drug Discov. Technol. 7, 280–316.

Murray, C.J.L., Rosenfeld, L.C., Lim, S.S., Andrews, K.G., Foreman, K.J., Haring, L.C., Fullman, N., Naghavi, M., Lozano, R., ALopez, D., 2012. Global malaria mortality between 1980 and 2010: a sysyematic analysis. Lancet 379 (9814), 413–431. https://doi.org/10.1016/S0140-6736(12)60034-8.

Mzena, T., Swai, H., Chacha, M., 2018. Antimalarial activity of *Cucumis metuliferus* and *Lippia kituiensis* against *Plasmodium berghei* infection in mice. Res. Rep. Trop. Med. 9, 81–88.

Noedl, H., S, Y., Schaecher, K., Smith, B.L., Socheat, D., Fukuda, M.M., 2008. Evidence of artemisinin-resistant malaria in Western Cambodia. N. Engl. J. Med. 359, 2619–2620.

Odediran, S.A., Awosode, K.E., Adegoke, T.A., Odebunmi, K.A., Oladunjoye, B.B., Obasanya, A.A., Omilani, I.M., Soyinka, K.T., Ugbo, J.U., Ogunleke, A.J., Adesida, S.A., Adenajo, A.C., 2020. Combinations of *Chrysophyllum albidum* and *Citrus aurantifolia* as antimalarial agents and their effects on orthodox antimalarial drugs in mice. Ann. Complement. Altern. Med. 2 (1), 1007.

Odugbemi, T.O., Akinsulire, O.R., Aibinu, I.E., Fabeku, P.O., 2007. Medicinal plants useful for malaria therapy in Okeigbo, Ondo state, South-west, Nigeria. Afr. J. Tradit. Complement. Altern. Med. 2, 191–198.

Orabueze, I.C., Ota, D.A., Coker, H.A., 2020. Antimalarial potentials of *Stemonocoleus micranthus* Harms (leguminoseae) stem bark in *Plasmodium berghei* infected mice. J. Trad. Complement. Med. 10 (1), 70–78. https://doi.org/10.1016/j.jtcme.2019.03.001.

Rietjens, I.M., Martena, M.J., Boersma, M.G., Spiegelenberg, W., Alink, G.M., 2005. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol. Nutr. Food Res. 49 (2005), 131–158.

Rodríguez, Y.V., Arias, M.H., García, J.O., Deharo, E., Garavito, G., 2018. Pharmacological activity of *Curarea toxicofera* in combination with classical antimalarial treatments. J. Ethnopharmacol. 222, 288–294. https://doi.org/10.1016/j.jep.2018.04.008.

Ryley, J.F., Peters, W., 1970. The antimalarial activity of some quinolone esters. Ann. Trop. Med. Parasitol. 84, 209–222.

Saganuwan, S.A., Onyeyili, P.A., Ameh, E.G., Etuk, E.U., 2011. *In vivo* antiplasmodial activity by aqueous extract of *Abrus precatorius* in mice. Rev. Latinoam. de Quimica. 32–44.

Sharma, V., Kalim, S., Srivastava, M.K., Nanda, S., Mishra, S., 2009. Oxidative stress and coxsackie virus infections as mediators of beta cell damage: a review. Sci. Res. Essay. 4, 42–58.

Somsak, V., Borkaew, P., Klubsri, C., Dondee, K., Bootprom, P., Saiphet, B., 2016. Antimalarial properties of aqueous crude extracts of *Gynostemma pentaphyllum* and *Moringa oleifera* leaves in combination with artesunate in *Plasmodium berghei*-infected mice. J. Trop. Med. 1–6. https://doi.org/10.1155/2016/8031392.

Tacoli, C., Gai, P.P., Bayingana, C., Sifft, K., Geus, D., Ndoli, J., Sendegeya, A., Gahutu, J.B., Mockenhaupt, F.P., 2016. Artemisinin resistance-associated K13 polymorphisms of *Plasmodium falciparum* in Southern Rwanda, 2010–2015. J. Trop. Med. Hyg. 95, 1090–1093.

Vigneron, M., Deparis, X., Deharo, E., Bourdy, G., 2005. Antimalarial remedies in French Guiana: a knowledge attitudes and practices study. J. Ethnopharmacol. 98, 351–360.

Waako, P.J., Smith, P., Folb, P.I., 2005. *In vitro* interactions of *Aspilia africana* (Pers.) C.D. Adams, a traditional antimalarial medicinal plant, with artemisinin against *Plasmodium falciparum*. J. Ethnopharmacol. 102, 262–268.
Willcox, M., 2011. Improved traditional phytomedicines in current use for the clinical treatment of malaria. Planta Med. 77, 662–671.
World Health Organization (WHO), 2010. World Malaria Report 2010. Roll Back Malaria Partnership, New York, p. 6.
World Health Organization (WHO) World malaria report 2019. Available from https://www.who.int/malaria/publications/world-malaria-report-2019/en/. Accessed January 25, 2020
Zhou, Z., Mitchell, R.M., Kariuki, S., odero, C., Otieno, P., Onyona, P., Were, V., Wiegand, R., Gimnig, J., Walker, E., Desai, M., Shi, P., Y, 2.0.1.6, 2012. Assessment of submicroscopic infections and gametocyte carriage of *Plasmodium falciparum* during peak malaria transmission season in a community-based cross-sectional survey in western Kenya. Malar. J. 15, 421. https://doi.org/10.1186/s12936-016-1482-4.