

In Vivo Antiplasmodial Activities and Acute Toxicity Assessment of Two Plant Cocktail Extracts Commonly Used Among Southwestern Nigerians.

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IN VIVO ANTIPLASMODIAL ACTIVITIES AND ACUTE TOXICITY ASSESSMENT OF TWO PLANT COCKTAIL EXTRACTS COMMONLY USED AMONG SOUTHWESTERN NIGERIANS.

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Abstract

1 Discovering and developing the desired antimalarials continue to be a necessity especially due to treatment failures,
2 drug resistance, limited availability and affordability of pharmaceutical antimalarials, costs and logistical problems
3 especially in poor malarious countries. This study investigated the efficacies of two plant cocktails; CtA and CtB,
4 selected based on their traditional usage. Activities of the cocktail extracts, chloroquine and pyrimethamine against
5 *Plasmodium berghei berghei* were evaluated using the suppressive, curative and prophylactic test models, after oral
6 and intraperitoneal acute toxicity determination of the plant cocktails in accordance with Lorke method. Data was
7 analyzed using SPSS software version 23.0 with level of significance set at $P < 0.05$. The median lethal dose was
8 determined to be higher than 5000 mg/kg body weight orally for both CtA and CtB; and 316.23 mg/kg body weight
9 intraperitoneally for CtA. Each cocktail exhibited high dose dependent *Plasmodium berghei berghei* inhibition which
10 was 96.95%, 99.13% in the CtA800 mg/kg, CtB800 mg/kg doses in the curative groups respectively, 96.46%, 78.62%
11 for CtA800mg/kg, CtB800mg/kg doses in the suppressive groups respectively, and 65.05%, 88.80% for
12 CtA800mg/kg, CtB800mg/kg doses in the prophylactic groups respectively. Throughout the observation periods, the
13 standard drugs, chloroquine phosphate and pyrimethamine maintained higher inhibitions up to 100%. These findings
14 demonstrate that CtA and CtB possess good antimalarial abilities and calls for their development and standardization
15 as effective and readily available antimalarial options. The acute toxicity results obtained underscore the importance
16 of obtaining information on toxicities of medicinal plant remedies before their administration in both humans and
17 animals.

Key Words: Antimalarial efficacy, medicinal plants, cocktail remedies, acute toxicity

1.0 INTRODUCTION

Malaria remains a major health problem and continues to impact enormously on man's health and economy (WHO-WMR 2016). Effective malaria control and eradication depend largely on high-quality case management, vector control and surveillance (WHO 2006; 2012; 2014). Treatment with efficacious antimalarial drugs is crucial at all stages including the early control or "attack" phase to driving down transmission and the later stages of maintaining interruption of transmission, preventing reintroduction of malaria, and eliminating the last residual foci of infection (Bhatt *et al.*, 2015; WHO 2007; 2014; 2016). Substantial work is under way on new medicines to counter the resistance of vectors, to safely target hypnozoites (radical cure), to clear gametocytes and to prevent reinfection (Wells *et al.*, 2015; Burrows *et al.*, 2013; 2017).

Plants may well prove to be the source of new antimalarial drugs in view of the success with the two important chemotherapeutic agents, quinine and artemisinin (the mainstay of malaria treatment), both of which are derived from plants (Ojurongbe *et al.*, 2015). However, the use of Quinine and Artemisinin as antimalarials has been limited by incidences of *Plasmodium* strains developing resistance against them (WHO-WMR 2011; Ménard *et al.*, 2013; Ashley *et al.*, 2014). Medicinal plants traditionally used to treat malaria therefore continues to be increasingly investigated for ideal antimalarial drugs discovery and development.

There is a growing consensus that drug combinations are essential to the optimal control of malaria, since they offer improved efficacy through synergistic activities (Fidock *et al.*, 2004). Artemisinin-based Combination Therapies (ACTs) – particularly combinations of artemisinin or its semi-synthetic derivatives and a long-lasting drug – are recommended by WHO for treating *P. falciparum* infections (WHO 2001; 2015; Faurant, 2011; Dawaki *et al.*, 2016). Consequently, drug combination therapy, including the use of polyherbal products, has become the popular method of managing malaria morbidity (Arrey *et al.*, 2014). In recent times, several concoctions of two or more whole plant species or their parts that work in synergy are prepared and administered orally (Adjanohoun *et al.*, 1996; Adebayo

and Krettli 2011; Ajayi *et al.*, 2017). Despite the increasing level of dependence on this method of management, only a few pharmacological investigations have been carried out on the ever-increasing number of indigenous polyherbal preparations used to treat malaria fever (Table 1) (Nwabuisi 2002; Ofori-Attah *et al.*, 2012; Martey *et al.*, 2013; Arrey *et al.*, 2014; Idowu *et al.*, 2015.; Adepiti *et al.*, 2016; Okpe *et al.*, 2016; Ibukunoluwa 2017; Orabueze *et al.*, 2018; Omagha *et al.*, 2020). Also, toxicity assessment of these polyherbal preparations are rarely investigated, making it difficult to be generally accepted as safe to public health.

Enantia chlorantha (African yellow wood), *Cymbopogon citratus* (Lemon grass), *Carica papaya* (Pawpaw), *Mangifera indica* (mango), *Curcuma longa* (Turmeric), *Alstonia boonei* (Pattern wood) are some of the predominantly used antimalarial medicinal plants in most endemic countries including Nigeria. Despite their popular application in combination antimalarial ethnomedicine, there is no scientific evidence to justify the acclaimed antimalarial efficacy when combined. Therefore, the present study evaluated *in vivo* antimalarial efficacy and acute toxicity of two plant cocktail extracts in mice following ethno botanical survey of traditional medicine practitioners (manuscript A). The scientific justification of the plant cocktails being investigated may be a springboard for new phytotherapies that could be affordable to treat malaria in Nigeria and in other parts of Sub-Saharan Africa, the most malaria endemic areas in the world.

Table 1: Previous studies validating plant cocktails in malaria treatment

Plant species	Antiplasmodial activities	Safety studies	References
<i>Cajanus cajan</i> + <i>Euphorbia lateriflora</i> + <i>Mangifera indica</i> + <i>Cassa alata</i> + <i>Cymbopogon giganteus</i> + <i>Nauclea latifolia</i> + and <i>Uvaria chamae</i>	Significant antiplasmodial activity	No significant side effects	Nwabuisi 2002.
<i>A. indica</i> + <i>C. papaya</i> + <i>M. indica</i>	Significant antiplasmodial activity	Not done	Ofori-Attah <i>et al.</i> 2012
<i>Azadirachta indica</i> + <i>Nauclea latifolia</i> + <i>Morinda lucida</i>	Significant antiplasmodial activity	No adverse effects.	Martey <i>et al.</i> , 2013
<i>Nauclea latifolia</i> + <i>Artocarpus altilis</i> + <i>Murraya koenigii</i> + <i>Enantia chlorantha</i>	Significant antiplasmodial activity	Not done	Adebajo <i>et al.</i> , 2014
<i>Mangifera indica</i> + <i>Psidium guajava</i> + <i>Carica papaya</i> + <i>Cymbopogon citratus</i> + <i>Citrus sinensis</i> + <i>Ocimum gratissimum</i>	Significant antiplasmodial activity	No adverse acute toxicity	Arrey <i>et al.</i> , 2014
<i>Ficus exasperata</i> + <i>Anthocleista vogelii</i>	Significant antiplasmodial activity	Increased size of liver, spleen	Okpok <i>et al.</i> , 2014
<i>P. nitida</i> + <i>A. boonei</i> + <i>G. Latifolia.</i>	Significant antiplasmodial activity	Elevated ALT,AST and creatinine.	Idowu <i>et al.</i> , 2015.
<i>Mangifera indica</i> + <i>Alstonia boonei</i> + <i>Morinda lucida</i> + <i>Azadirachta indica</i>	Significant antiplasmodial activity	Not done	Adepiti <i>et al.</i> , 2014; 2016
<i>Vernonia amygdalina</i> + <i>Carica papaya</i>	Significant antiplasmodial activity	Increased RBC and PCV. Histology indicated hepatic cell damage	Okpe <i>et al.</i> , 2016
<i>Anthocleista djalensis</i> A. Chev. + <i>Azadirachta indica</i> A. Juss + <i>Cajanus cajan</i> (L.) Huth. + <i>Crescentia cujete</i> L. + <i>Lawsonia inermis</i> L. + <i>Lophira alata</i> Banks ex C.F. Gaertn. + <i>Myrianthus pruessii</i> Engl. + <i>Nauclea latifolia</i> Sm. + <i>Olex subscorpioidea</i> Oliv. + <i>Terminalia glaucescens</i>	Significant antiplasmodial activity	Histological studies revealed some pathology.	Ibukunoluwa 2017
<i>Fadogia cienkowskii</i> + <i>Lophira lanceolata</i> + <i>Vernonia conferta</i> + <i>Protea madiensis</i>	Significant antiplasmodial activity	No adverse effects	Orabueze <i>et al.</i> , 2018

2.0 MATERIALS AND METHODS

2.1 Preparation of plant cocktail extracts and reference drugs: Based on information collected from herbal practitioners in south west Nigeria (manuscript A), powdered hot water extracts of *A. boonei* (stem bark), *C. papaya*

(fruits), *C. citratus* (leaves), *C. longa* (roots), *M. indica* (stem bark) and *E. chlorantha* (Stem bark) were combined in ratios to prepare two popularly used polyherbal remedies from indigenous plants. Cocktail treatment A (CtA): 4:2:1 (*E. chlorantha*:*C. citratus*:*C. longa*). Cocktail treatment B (CtB): 4:2:1:1 (*E. chlorantha*:*A. boonei*:*C. papaya*:*M. indica*). The two cocktail treatments were obtained by separately dissolving 10g of each components (CtA = 5.70g + 2.87g + 1.43g; and CtB = 5.00g + 2.33g + 1.27g + 1.27g) in 200ml distilled water equivalent to 50mg/ml concentration. The resulting combinations were separately heated over a water bath for 30minutes and left to cool, labelled and refrigerated at 4°C in air-tight bottles. Doses administered for CtA and CtB, (200mg/kg, 400mg/kg and 800mg/kg) were appropriately chosen for antimalarial evaluation based on acute toxicity results established by this study. Chloroquine phosphate (CQ) and Pyrimethamine (PY) manufactured in Ikeja, Lagos Nigeria by Vitabiotics Limited, and SKG-Pharma Limited respectively are the standard drugs used as positive controls. The doses required for each of the standard drugs, 25 mg/kg and 5 mg/kg respectively (Iwalokun 2008; Alli *et al.*, 2011) were given according to weight of each animal. They were each prepared by diluting: 250mg tablet strength of CQ in 25mls of distilled water (10mg/ml), and 25mg tablet strength of PY in 5mls of distilled water (5mg/ml). A measure of 10ml/kg distilled water (DW) was given as negative control.

Table 2: Concentrations for treatments administered.

Treatments		Treatments and doses administered		
Standard drugs	Chloroquine (CQ)	CQ25 mg/kg		
	Pyrimethamine (PY)	PY5 mg/kg		
Cocktail extracts	Cocktail treatment A (CtA)	CtA200 mg/kg	CtA400 mg/kg	CtA800 mg/kg
	Cocktail treatment B (CtB)	CtB200 mg/kg	CtB400 mg/kg	CtB800 mg/kg
Distilled water (DW)		DW10 mg/kg		

2.2 Plasmodium parasite species and animals: Chloroquine-sensitive *Plasmodium berghei berghei* parasites was obtained from Institute for Advanced Medical Research and Training, (IMRAT), University of Ibadan, Ibadan, Nigeria, by intraperitoneal inoculation of uninfected mice with 0.2 ml of the diluted blood from previously infected mice maintained by successive intra-peritoneal inoculation of parasite-free mice every four days. The donor mice were then transported to the University of Lagos animal house where they were kept under standard laboratory conditions with constant access to food and water until the desired level of parasitemia was achieved. Infected blood from the donor mouse was obtained by cardiac puncture. Infected red cells/ μ l was calculated using the relative value method, count of infected red cells X 5000000/total red cells counted (infected + non-infected). This was done to determine the required standard inoculum of 1×10^6 using thin blood films of donor mice. Five millilitres normal saline, a quantity determined by the level of parasitaemia of the infected donor mice was used to dilute 2ml of the donor blood.

A total of 120 adult male mice of about 7 - 8 weeks old, weighing between 16 - 20g were obtained from the Animal House, University of Lagos, where the animal exposures during this study was conducted. Before being subjected to experiment they were left for two weeks to acclimatize to laboratory conditions, and had constant access to feed on a standard rodent's diet consisting of crude protein, fat, calcium, available phosphate, vitamins, crude fiber and tap water. They were kept in plastic cages with metal covers for free passage of air, and at room temperature of about 27°C.

2.3 Acute toxicity (LD₅₀) test of the plant cocktails: A lethal dose for fifty percent of the mice (LD₅₀) test was performed on the two cocktail treatments, CtA and CtB in accordance with (Lorke 1983) method. This test was done to observe the mice for signs of toxicity including clogging together, weakness, anorexia, micturition, respiratory distress, coma, and mortality for the first 24 h and thereafter daily for 14 days. The tests were replicated in two groups, group 1 treated orally and group 2 treated intraperitoneally. A total of 48 mice was used for this test. In the phase 1 of the method, 9 animals divided into three groups of 3 animals was orally administered different doses (10 mg/kg, 100 mg/kg and 1000 mg/kg) of the test substances CtA and CtB, and observed for 24 hours. Another 9 animals divided into three groups of 3 animals each was intraperitoneally administered different doses (10 mg/kg, 100 mg/kg and 1000 mg/kg) of the test substances CtA and CtB, and observed for 24 hours. In the phase 2 of the method, further specific doses were administered to calculate an LD₅₀. 3 animals divided into three groups of 1 animal each were orally administered different doses (1600 mg/kg, 2900 mg/kg and 5000 mg/kg) of CtA. This was replicated for CtB. Another 3 animals divided into three groups of 1 animal each were intraperitoneally administered different doses (1600 mg/kg, 2900 mg/kg and 5000 mg/kg) of CtA. This was replicated for CtB. The LD₅₀ was calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose, i.e., the geometric mean of the consecutive doses for

which 0 and 100% survival rates were recorded in the second phase, using the formula: $LD_{50} = \sqrt{(D0 \times D100)}$; D0 = highest dose that gave no mortality, D100 = lowest dose that produced 100 % mortality.

2.4 Antimalarial activities of CtA and CtB: The antimalarial efficacy of the cocktail extracts CtA and CtB in rodent malaria parasites was evaluated using the suppressive, curative and prophylactic test models. The body weight of each mouse for all the tests was taken before and after exposure. 0.2ml of the prepared *P. berghei berghei* parasitized erythrocytes suspension in normal saline was injected intraperitoneally into each mouse to be used for the tests using one (1) ml syringe and needle. The drugs and plant cocktails were orally administered with the aid of oral cannula. Thick and thin smears fixed in 100 % methanol stained with 10 % Giemsa's stain at pH 7.2 for 15 min were prepared from the tail blood of each mouse. Prepared blood films were air dried at room temperature and examined microscopically under oil immersion (X100 magnification). The parasitaemia was determined by counting the number of parasitized erythrocytes in 2000 cells in random fields of the microscope. The percentage parasitaemia was determined using the formular: $\text{Number of parasitized RBCs} / \text{Total number of RBCs (infected + Non-infected)} \times 100\%$ (Fidock *et al.*, 2004). The mean parasite count for each group were determined and the percentage chemo inhibition for each dose was calculated as $[(A-B)/A]$, where A is the average percentage of parasitaemia in the negative control and B is the average percentage of parasitaemia in the test groups (Kalra *et al.*, 2006).

2.4.1 Rane Curative Test: was conducted according to Ryley and Peters (1970) in a similar method adopted by Iwalokun (2008); Alli *et al.*, (2011). Seventy-two hours after infection with chloroquine sensitive *P. berghei*, 40 infected mice were divided into 8 groups of 5 animals each. 3 groups were orally administered different doses (200mg/kg, 400mg/kg and 800mg/kg respectively) of CtA. The same treatment was replicated for CtB using another 3 groups of the animals. The 7th group was administered chloroquine phosphate (25 mg/kg) as positive control, while the 8th group received 10 mg/kg distilled water as negative control. Each mouse was treated orally once daily with the dose given according to body weight of animal for 5 consecutive days (D4-D8) post inoculation during which the parasitaemia level were monitored daily.

2.4.2 The 4-day Suppressive Test: Peters (1965) 4-day suppressive test in mice was conducted in a similar method reported by Mesia *et al.*, (2005); Iwalokun (2008); Alli *et al.*, (2011). 40 mice divided into 8 groups of 5 animals each were inoculated intraperitoneally on the first day (Do) with *Plasmodium berghei berghei* parasitized red blood cells. The mice were then treated immediately after day 0 to day 3. 3 groups were orally administered different doses (200mg/kg, 400mg/kg and 800mg/kg respectively) of CtA according to body weight of animal. The same treatment was replicated for CtB using another 3 groups of the animals. The 7th group was administered chloroquine phosphate (25 mg/kg) as positive control, while the 8th group received 10 mg/kg distilled water as negative control. All treatments were given according to body weight of animal. Parasitaemia levels were monitored daily from day 4 to day 7.

2.4.3 Repository (Prophylactic) Test: Adopted the method of Peters (1965) and as similarly followed in Alli *et al.*, (2011). A total of 40 mice were divided into 8 groups of 5 animals. 3 groups were orally administered different doses (200mg/kg, 400mg/kg and 800mg/kg respectively) of CtA. The same treatment was replicated for CtB using another 3 groups of the animals. The seventh group was given 5 mg/kg of pyrimethamine, while the last group (negative control) received 10 mg/kg distilled water for four consecutive days. On the fourth day (D4), the mice were inoculated with *P. berghei berghei*. Seventy two hours later (D7), smears were made from the mice (D7-D11) to assess parasitaemia levels.

2.5 Ethical considerations

The Ethics Committee at the Nigerian Institute of Medical Research Institutional Review Board (NIMR IRB) reviewed and granted approval (assigned number IRB/17/036) for this study.

2.6 Data analysis: Data from anti-plasmodial curative, suppressive and prophylactic assays were entered and analyzed using Microsoft Excel version 2016 and Statistical Package for Social Sciences (SPSS) version 23.0. The differences between means among negative and positive controls as well as treatment groups were compared for significance using one way analysis of variance (ANOVA), followed by Dunnett's multiple post hoc test. Differences were considered significant to negative control when Probability value ($P < 0.05$). Results obtained were presented using bar charts.

3.0 RESULTS

3.1 Acute toxicity of CtA and CtB in mice: Acute oral toxicity assessment of both CtA and CtB showed dose-dependent reduced activity and clogging together within the first six hours at the phase 2 treatments. All the mice survived both phases within the 24h and 2-weeks observation periods. The oral median lethal dose of both CtA and CtB was calculated to be greater than 5000 mg/kg in mice (Table 3a). In the intraperitoneal acute toxicity assessment, there were remarkable dose-dependent reduced activity and clogging together in the phase 1 treatments with 1000

mg/kg dose of both cocktails, and at all doses in phase 2 with CtB. Within 24h, 100% mortality was recorded for CtB and 33% for CtA, though mortality for CtA reached 100% within 7days observation period. Within 24h, 100% mortality was recorded for CtA at all doses in phase 2. No further exposure was done for the group receiving CtA following a 100% mortality observed at 1000mg/kg in phase 1. Acute intraperitoneal toxicity assessment was calculated to be 316.23 mg/kg in mice for CtA. (Table 3b).

Table 3a: Acute toxicity (LD₅₀) results from oral administration

Cocktails	Route of Administration	No of mice	Dose (mg/kg)	Weakness/Reduced activity	Clogging Together	Day 0 Mortality	% Mortality	LD ₅₀ (mg/kg)	Day 7 Mortality	Day 14 Mortality
LORKE'S PHASE 1										
CtA	Orally	3	10	No	No	0	0		0	0
		3	100	No	No	0	0		0	0
		3	1000	No	No	0	0		0	0
CtB	Orally	3	10	No	No	0	0		0	0
		3	100	No	No	0	0		0	0
		3	1000	No	No	0	0		0	0
LORKE'S PHASE 2										
CtA	Orally	1	1600	Yes	Yes	0	0	CtA >5000	0	0
		1	2900	Yes	Yes	0	0		0	0
		1	5000	Yes	Yes	0	0	CtB >5000	0	0
CtB	Orally	1	1600	No	Yes	0	0		0	0
		1	2900	Yes	Yes	0	0		0	0
		1	5000	Yes	Yes	0	0		0	0

Table 3b: Acute toxicity (LD₅₀) results from intraperitoneal administration

Cocktails	Route of Administration	No of mice	Dose (mg/kg)	Weakness/Reduced activity	Clogging Together	Day 0 Mortality	% Mortality	LD ₅₀ (mg/kg)	Day 7 Mortality	Day 14 Mortality
LORKE'S PHASE 1										
CtA	Intraperitoneally	3	10	No	No	0	0		0	0
		3	100	No	No	0	0		0	0
		3	1000	Yes	No	1	33%		2	-
CtB	Intraperitoneally	3	10	No	No	0	0		0	0
		3	100	No	No	0	0		0	0
		3	1000	Yes	No	3	100		-	
LORKE'S PHASE 2										
CtA	Intraperitoneally	1	1600	Yes	Yes	1	100	CtA =	-	
		1	2900	Yes	Yes	1	100	316.23		
		1	5000	Yes	Yes	1	100			
CtB	Intraperitoneally	1	1600	ND					-	
		1	2900	ND						
		1	5000	ND						

3.2 *In vivo* antimalarial efficacies of CtA and CtB

Curative ability on established infection: Curative effects in all the treated groups increased from day 4 to day 8. Parasite inhibition was 100% with CQ25 mg/kg from day 2 till day 5 of observation. In the extract treated groups, inhibition was observed to be 88.50%, 94.77% and 96.95% in the CtA200 mg/kg, CtA400 mg/kg and CtA800 mg/kg doses respectively; and 74.82%, 91.81% and 99.13% in the CtB200 mg/kg, CtB400 mg/kg and CtB800 mg/kg doses respectively.

Suppressive ability on early infection: Throughout the four days of observation, parasitaemia was 100% inhibited in the CQ25 mg/kg group. In the groups treated with the plant cocktails, inhibition increased at all doses, and by day 6, reached 63.82%, 89.54% and 96.46% at the CtA200 mg/kg, CtA400 mg/kg and CtA800mg/kg doses respectively; and 33.84%, 51.92% and 78.62% for CtB200 mg/kg, CtB400 mg/kg and CtB800mg/kg doses respectively.

Prophylactic ability on residual infection: Result shows inhibition with PY5 mg/kg to be 100% throughout the 5 days of observation. With the cocktail extract-treated groups, at the beginning of observation (D7), parasite inhibition was 42.34%, 50.88% and 65.05% for the CtA200 mg/kg, CtA400 mg/kg and CtA800mg/kg doses respectively; and 65.05%, 60.57% and 88.80% for CtB200 mg/kg, CtB400 mg/kg and CtB800mg/kg doses respectively. However, a decreased parasite inhibition was observed as the observation proceeded, and by D11, parasite inhibition was 26.12%, 55.14% and 57.09% for the CtA200 mg/kg, CtA400 mg/kg and CtA800mg/kg doses respectively; and 8.53%, 25.59% and 47.42% for the CtB200 mg/kg, CtB400 mg/kg and CtB800mg/kg doses respectively.

Figures 1 (a-c) below shows the percentage inhibition of *P. berghei berghei* in mice.

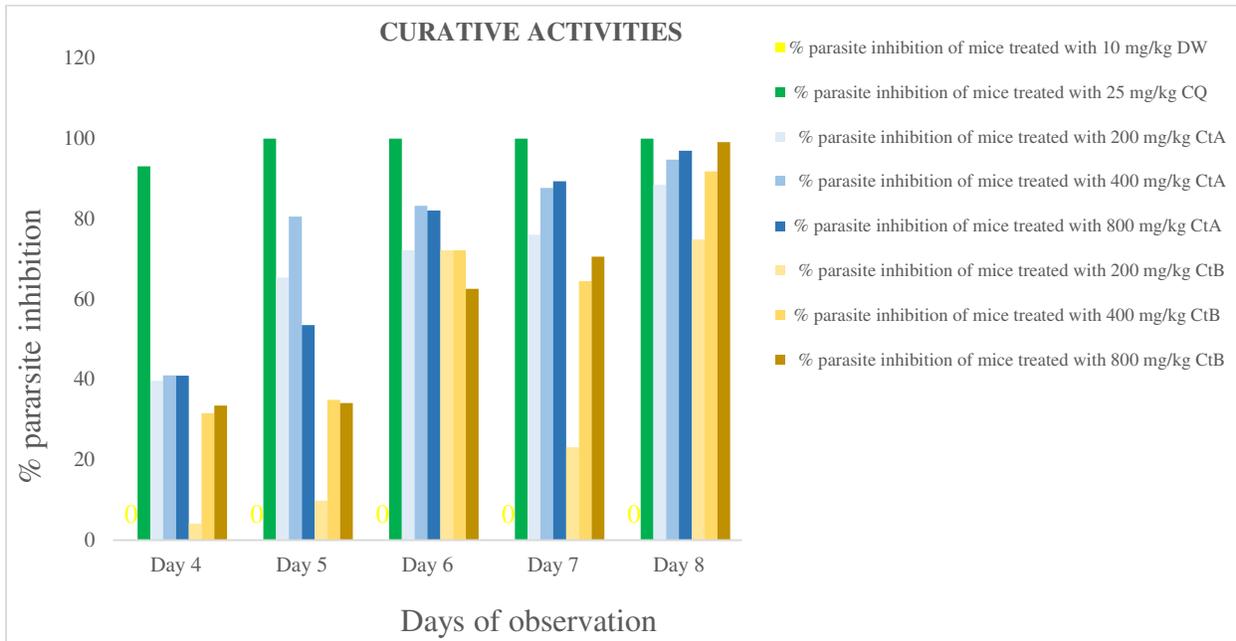


Figure 1a: Curative ability of treatments on established infection

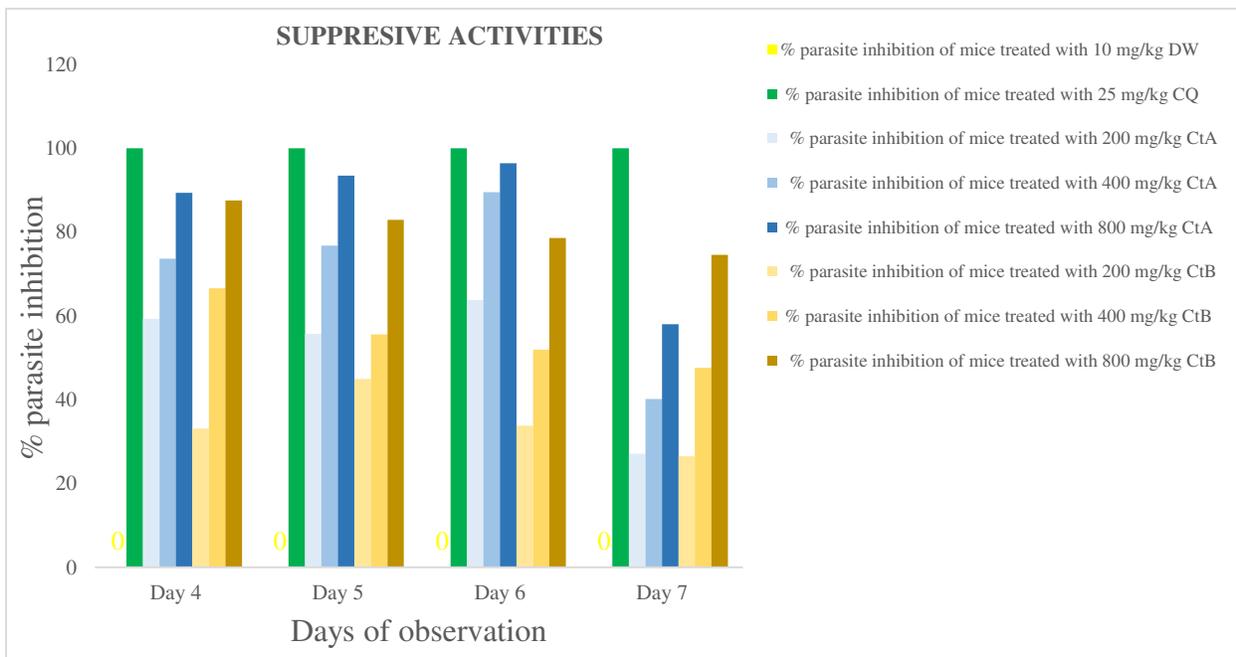


Figure 1b: Suppressive ability of treatments on early malaria infection

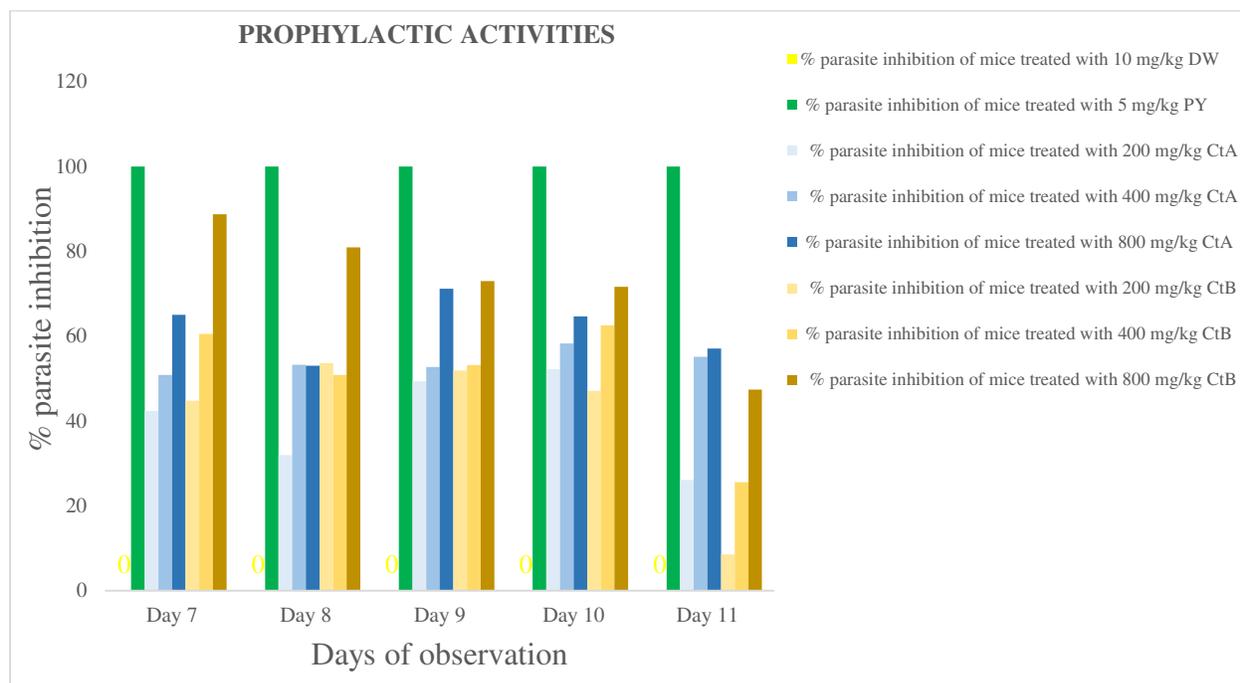


Figure 1c: Prophylactic ability of treatments on residual infection

4.0 DISCUSSION

Acute toxicity was evaluated to observe for toxicity signs and mortality associated with administration of CtA and CtB in mice. The oral and intraperitoneal routes' toxicity effects in this study varied with dosage concentrations. Lethal oral dose of CtA and CtB was evaluated to be less toxic than the intraperitoneal administration, emphasizing a need to establish any toxicity before choosing the routes of administration. This findings suggests the safe use of the plants by the locals who reported oral route as the most popular mode of administration of these cocktail recipes in malaria treatment (manuscript A). The toxic effects reported of these cocktail recipes in mice is expected to serve as baseline for further studies, for comparison in mammalian anatomy and physiology (Ibukunoluwa 2017; Jutamaad *et al.*, 1998) and encourage advocacies for their regulated oral use as complementary therapies. The results of acute toxicity generally serve in choosing appropriate test doses of CtA and CtB for antimalarial evaluation in investigation.

Earlier studies have reported the traditional antimalarial uses and the monotherapeutic activities of *C. papaya*, *C. longa*, *A. boonei*, *M. indica*, *E. chlorantha* and *C. citratus* (Agbaje and Onabanjo 1991; Awe *et al.*, 1998; Bhat and Surolia 2001; Udeh *et al.*, 2001; Aiyeloja and Bello 2006; Idowu *et al.*, 2010). This study reports the antiplasmodial activity of two polyherbal recipes from the above mentioned plants: CtA comprising *E. chlorantha* + *C. citratus* + *C. longa*, and CtB comprising *E. chlorantha* + *A. boonei* + *C. papaya* + *M. indica*. Similar investigation on the antiplasmodial activities of some other polyherbal recipes in malaria treatments have reported noteworthy efficacies (Nwabuisi 2002; Ofori-Attah *et al.*, 2012; Martey *et al.*, 2013; Adebajo *et al.*, 2014; Arrey *et al.*, 2014; Idowu *et al.*, 2015; Adepiti *et al.*, 2016; Okpe *et al.*, 2016, Ibukunoluwa 2017; Orabueze *et al.*, 2018).

In all three models (curative, suppressive and prophylactic), results showed that CtA and CtB significantly inhibited parasitaemia, with inhibition levels up to 90% in the 400 and 800 mg/kg doses of each cocktail extracts in the curative and suppressive models. The findings confirms antiplasmodial potency of CtA and CtB, provides scientific basis for their usage as antimalarial remedies and proves they are potential sources that should be explored for new antimalarial drugs. Chloroquine, an antimalarial drug still widely used in Nigeria because it is accessible and affordable, and despite WHO recommendation for Artemisinin Combination Therapies ACTs, (WHO 2001; Oladipo *et al.*, 2015) was used as the reference drug in curative and suppressive models (Iwalokun 2008; Alli *et al.*, 2011), and Pyrimethamine was the standard antimalarial drug used in the prophylactic model (Alli *et al.*, 2011). These standard drugs inhibited parasitaemia to undetectable levels and the findings agrees with results from other studies validating medicinal plants in malaria treatments (Alli *et al.*, 2011; Ogbole *et al.*, 2014). The total clearance of parasitaemia by chloroquine compares very closely to the 99.13% curative ability demonstrated in this study at the 800mg/kg of CtB.

Phytochemical analysis plays a vital role in the observation of the efficacies of the plant materials in therapeutic preparations. The antiplasmodial activity observed in these plant cocktails could be attributed to the presence of some of the antimalarial proven phytochemicals including saponin, alkaloid, flavonoid, phenol and lactones (Kirby *et al.*, 1989; Philipson and Wright 1991; Christensen and Kharazmi 2001; Onifade and Maganda 2015; Ibukunoluwa 2017; Omagha *et al.*, 2020) present in each of the plants used in the combinations for CtA and CtB. These phytoconstituents affect the condition and function of body organs, clear up residual symptoms of the disease. They help increase the body's resistance to disease or facilitate the adaptation of the organism to certain conditions (Forantisek 2001). However, the potential toxicity of plant products is understudied. A few scientific evidences available from toxicological studies have reported some phytochemicals to be potentially toxic thus affecting their safe use (Bode and Dong 2015; Mensah *et al.*, 2019).

Multidrug strategy in therapeutic applications is expected to increase efficacy of two or more anti-infective agents (Bennet *et al.*, 2015), improve clinical cure, shorten the duration of therapy so as to minimize the risk of recrudescence, and provide a way in which resistance can be delayed (WHO 2001). Our results showed that the action of the combination therapies in this study significantly differed from the plant materials acting individually in previous studies (Agbaje and Onabanjo 1991; Longdet and Adoga 2017; Awe *et al.*, 1998; Onifade and Maganda 2015). The improved efficacy demonstrated by these findings and extent of use by locals who rely on them to combat high burdens of malaria morbidity and mortality calls for further studies to understand activities and actual behaviours of these combined plant materials in malaria treatments.

Conclusions

Malaria control continues to rely upon antimalarial plant remedies increasingly used as combination therapies. Findings in this study demonstrates that plant-based cocktail treatments CtA and CtB possess good antimalarial abilities and safer in oral administrations compared to intraperitoneal administrations. Following the efficacies established in this study, further investigations concerning their safety is currently ongoing to determine the potential toxicity-related adverse reactions while using CtA and CtB in malaria treatment.

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Figures

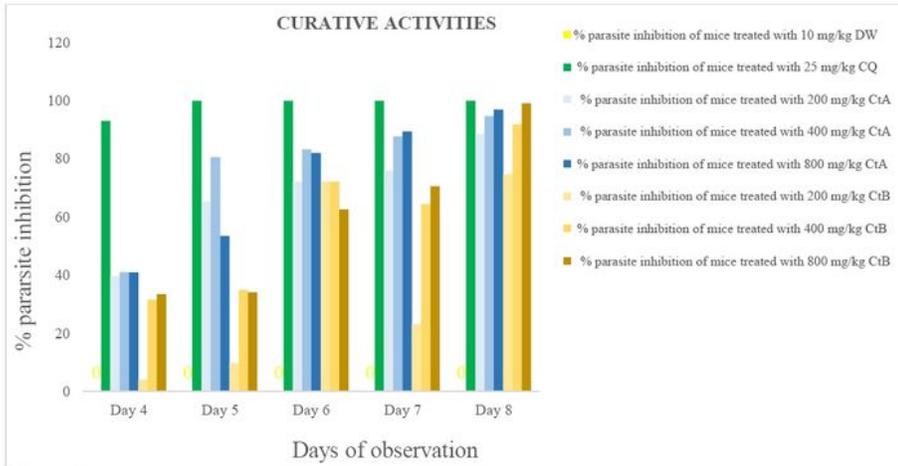


Figure 1a:

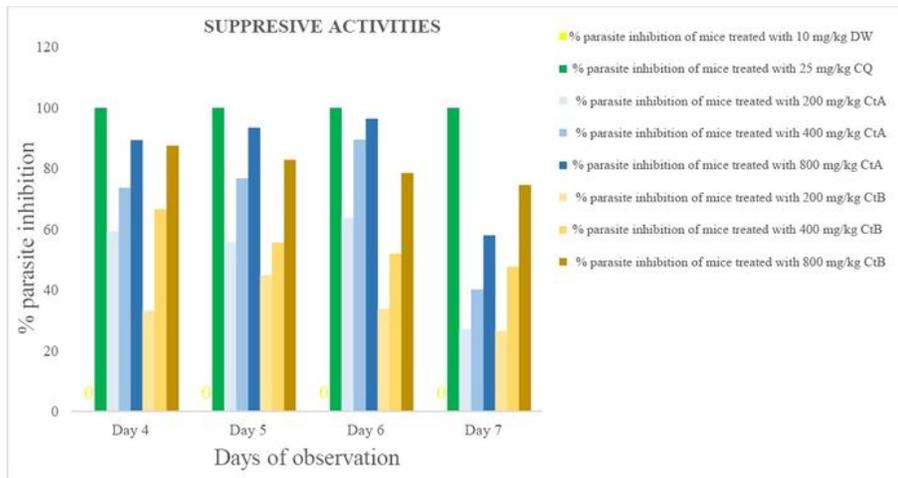


Figure 1b:

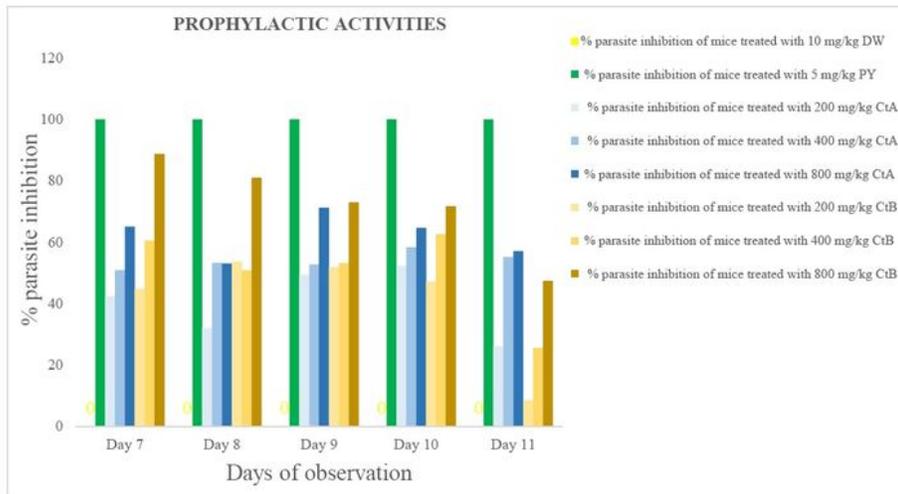


Figure 1c:

Figure 1

1a: Curative ability of treatments on established infection 1b: Suppressive ability of treatments on early malaria infection 1c: Prophylactic ability of treatments on residual infection