ORIGINAL RESEARCH

Evaluation of the Potency and Safety of Model Clinic Polyherbal Anti-Malaria Tea

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ABSTRACT

Background: The Model Clinic Antimalaria Tea (AMT) is a product of the Herbal Clinic unit, Department of Pharmacognosy, University of Lagos. Its ingredients are the dried and powdered leaves of *Mangifera indica, Psidium guajava, Carica papaya* and *Cymbopogon citratus.*
Objective: The objective of this study is to evaluate the potency and safety of AMT.
Materials and methods: AMT was assessed for antimalarial activity against chloroquine-sensitive *Plasmodium bergheii* NK65 – infected mice using the suppressive and the curative test procedures at its half, full and double the prescribed dose (3.45, 6.90, and 13.80 mg kg^{-1} respectively). Chloroquine 5 mg kg^{-1} (CQ) was used as positive control while water was the normal control. The test parameters were parasitaemia, weight, PCV and temperature. AMT safety was assessed by acute toxicity test and microbial limit (using the pour plate method) and heavy metals limit test (using spectrophotometry).
Results: All the treated groups, except water, showed curative and suppressive antimalarial activity in the following order: CQ > AMT 13.80 mg kg^{-1} > AMT 6.90 mg kg^{-1} > 3.45 mg kg^{-1} which is dose-dependent. Oral administration of AMT at a dose of 4,000 mg kg^{-1} produced no noticeable deleterious effect. The microbial load and heavy metals content were within passable official limits. Arsenic and Mercury were not detected while Copper, Lead and Zinc occurred in 0.576 ppm, 0.108 ppm and 0.673 ppm respectively.
Conclusion: AMT has been shown to exhibit potent and dose-dependent anti-malarial activity against *P. bergheii* and it is also safe for consumption.

Keywords: Polyherbal; antimalarial; *Plasmodium berghei;* suppressive; curative; microbial load

INTRODUCTION

Herbal medicines have remained popular in the post-civilization healthcare system of various people globally despite the predominance of orthodox medicines, with over 80 % of the population of developing countries depending on herbal medicine for basic healthcare[1-2]. Dramatic increase in use reported during the last decade has led to growing concerns about efficacy and safety[3].
Malaria is a life-threatening infection with a high rate of mortality and morbidity. It is caused by Plasmodium parasites that are transmitted to people during blood meal by

infected vector. The causative vectors are female Anopheles mosquitoes. The World Health Organization reported that in 2019, Nigeria accounted for about 25% of all malaria cases in Africa while Africa accounts for 94% of global malaria[4]. Surveillance carried out in Nigeria on drug efficacy showed that chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) are no longer viable therapeutic options for the effective treatment of human malaria[2]. These two antimalarial drugs were commonly used in the past because of their efficacy and affordability in Nigeria. The efficacy of the WHO approved ACTs (Artemisinin Combination Therapy) has been documented as gradually being challenged by clinical failures[5]. Thus, the emergency of multi-drug resistant malaria parasites and insecticide-resistant vectors pose serious treatment limitations, control of the infection and a possible increase in death rate. And the survival of children under the ages of 5 year and pregnant women are highly threatened. These are the populations that are most endangered due to their compromised immunity status. This necessitates the discovery and development of novel antimalarial drugs urgently.

In most countries in Africa, the first line of treatment of fever and suspected malaria infection in children is treated at home with herbal medicine[6]. Almost every home in such African countries has procured or prepared herbal medicine for use. The gradual increase in the use of herbal medicine has been linked to many factors which include cultural beliefs, multidrug resistance, and the need to try something different and affordability[5,7].

Medicinal plants have been the direct or indirect sources of many conventional drugs on the shelves in pharmacies. Drugs are either developed from the natural product as a whole, semi-purified fraction or as a single isolated compound. Isolated pure compounds are either used as isolated and purified as it is or serve as "lead template" in the development of derivatives/analogues with more desirable properties concerning efficacy and safety. Chloroquine and artemisinin are good examples of antimalarial drugs derived from medicinal plants. Artemisinin is an isolate of *Artemisia annua*, while chloroquine is a synthetic analogue of quinine. Quinine is a compound isolated from Cinchona plant and has antiplasmodial activity[5,8]. Neither *Cinchona* nor *Artemisia annua*, from which these potent drugs (quinine and artemisinin) were isolated, are indigenous to Sub-Saharan Africa. It seems logical then to encourage studies on plants from these regions, especially since the major proportion of malaria-attributable deaths occurs in sub-Saharan African regions.

Antimalaria tea (AMT) is one of the herbal products under development by the Model Herbal Clinic, Department of Pharmacognosy, Faculty of Pharmacy University of Lagos.

The ingredients are the dried and ground leaves of:

Carica papaya L. (Caricaceae); Common name is Pawpaw, also known as Ibepe in Yoruba. One of the chemical constituents is papain. In a study carried out on *C. papaya* leaf extracts, the plant extracts showed moderate to good antiparasitic effects. The extracts exhibited promising inhibitory activity against the CQ sensitive and CQ resistant strains and also against *P. falciparum*[9].

Cymbopogon citratus Stapf. (Poaceae); Common name is Lemon grass, also known as Koriko oba in Yoruba. Citral is one of its chemical constituents. In a previously reported study, the *C. citratus* plant exhibited prolonged antimalarial activity against both *P. chabaudi* AS and *P. berghei* ANKA. As a prophylactic treatment, the plant exhibited higher antimalarial activity than chloroquine. In addition, the combination of the *C. citratus* plant and chloroquine displayed higher activity than chloroquine alone against *P. berghei* ANKA patent infection[10].

In another study, designed to evaluate the antiplasmodial activity of aqueous leaf and root extracts of Cymbopogon citratus

against *Plasmodium berghei* in mice, the extracts exhibited significant antiplasmodial activity in all the experimental doses used. The suppressive effect of the extracts followed a dose-dependent pattern[11]. The aqueous leaf extract of *Cymbopogon citratus* has been reported to exhibit good antimalarial property and was recommended for possible use in prophylactic and chemotherapeutic antimalarial care[12].

Mangifera indica L. (Anacardiaceae): Common name is Mango, also known as mangoro in Yoruba. One of its commonly known phytoconstituents is mangiferin. In an ethnographic study carried out in Oyo state Southwestern Nigeria, a total of 514 respondents were interviewed on their choice of anti-febrile herbal remedy. Of the 112 herbs mentioned, *Mangifera indica* was one of the four most frequently mentioned (75.0%)[13]. The plant has been reported to exhibit antipyretic activity[14]. Its schizontocidal effect during early infection and repository activity has been also demonstrated.

Psidium guajava L. (Myrtaceae): Common name is Guava, contains quercetin and guajanoic acid. The antimalarial activity of the various morphological parts of the plant has been demonstrated and documented[15]. Rajendran and colleagues[16] reported the more efficacy of aqueous fresh leaf extract of *P. guajava* compared to its unripe fruit extract against the NK65- chloroquine-resistant strain of *Plasmodium berghei*.

The preparation of this polyherbal product involves drying to constant weight and homogeneously mixing at a fixed ratio known by the manufacturer. Each tea bag contains 2 g of the mixed dried herbal blend. They are packed in a number of 10 tea bags in a pack. The prescribed dosage is one bag to be taken in the morning and one at night for 5 days.

This study aimed to evaluate the efficacy and safety of the AMT. The outcome of this investigation is expected to provide information concerning the antimalarial efficacy and the safety potential of a newly developed polyherbal product for malarial fever. This will be a contribution towards creation of more treatment alternative and safe antimalarial options.

MATERIALS AND METHODS

Materials

Chloroquine (May & Baker), AMT (Anti Malaria Tea), Swiss albino mice, frosted slides, cannula, Geimsa stain, suction tubes, weighing balance. Nutrient agar, disposable petri dishes, biological safety cabinet, autoclave, chilling incubator for warm and cold temperature, Anderman colony counter, Aqua regia

AMT extraction

Twenty tea bags were weighed, and the average weight was derived. The teabags were infused in 200 ml of just boiled distilled water, the process was repeated twice to ensure adequate extraction, and freeze-dried to obtain a brownish powdered extract.

Animals

Swiss albino mice (18 - 25 g) of the male sex were used for the study. They were bred and kept in the Laboratory Animals Center, College of Medicine, University of Lagos, Nigeria. The animals were maintained on standard rodent feed and water ad libitum under 12 h light and dark cycle at room temperature. The animals were allowed to acclimatize for 7 days preceding the experiments. All experimental protocols were in compliance with internationally accepted principles for laboratory animal use and care. Ethical approval was obtained from the Health Research Ethics Committee of the College of Medicine of the University of Lagos (CMUL/HREC/05/17/129).

Malaria Parasite

The rodent parasite *Plasmodium berghei* chloroquine-sensitive strain NK-65 was obtained from the National Institute of Medical Research (NIMR), Yaba, Lagos and identified by Dr Aina of National Institute of Medical Research (NIMR) Yaba

Lagos Nigeria. The *Plasmodium* was maintained in University Animal House Laboratory by serial passage of the parasite into uninfected mice every 7 days. A standard inoculum of 1 X 10^7 of parasitized erythrocytes from the donor mice (0.2 ml) was used to infect the experimental animals intra-peritoneally.

Suppressive test

The four-day suppressive test described by Peters[17] was employed for antimalarial activity evaluation of the crude extract of AMT against *P. berghei*. The weight, temperature and PCV of the mice were taken before the animals were infected intraperitoneally with blood containing 1×10^7 parasitized red blood cells contained in 0.2 ml inoculum on the first day (D_0). They were then randomly divided into 5 groups of 6 mice each. Two hours post-infection, 3 test groups were administered orally with the AMT extract at the three different dose levels (3.45, 6.90, 13.80 mg kg^{-1}), corresponding to the half, full and double the currently prescribed human dosing. One test group received the reference drug (chloroquine 5 mg kg^{-1}), while another group received the vehicle, water (normal control, 0.2 mL). Drug administration was repeated 24, 48, and 72 hours post-infection (3 consecutive days, D_1 to D_3). On D_4 (day 5 post-treatment), tail blood smears were prepared, stained with 10% Giemsa in phosphate buffer, pH 7.2 for 15 min and examined under a microscope at 100x to determine the level of parasitaemia. The weight, temperature and PCV were also taken on D_4.

Curative test

Evaluation of the curative potential of the AMT against established infection was carried out as described by Ryley and Peters[18] and Iyiola *et al.*[19]. The weight, temperature, PCV and parasite load of the mice were taken before the animals were infected intraperitoneally with a standard inoculum of 1×10^7 *Plasmodium berghei* NK – 65 infected erythrocytes on the first day (D_0) and left for 72 hours before commencement of treatment (D_3). The mice were divided into 5 groups of 6 mice each. Three test groups were administered with the AMT orally at three dose levels of (3.45, 6.90, 13.8 mg kg^{-1}), corresponding to the half, full and double the human dosing daily for 5 days (D_3 – D_7). The positive control group received chloroquine 5 mg kg^{-1} daily while the normal control group received water. Blood smears were collected and examined microscopically on D_3 to establish parasitaemia levels. Then parasitaemia level in each mouse was monitored daily for the remaining four dosing days (D_4 –D_7) and D_8 (24 h after last drug administration), using Giemsa stained thin smear film. The weight, temperature and PCV of the mice were also taken daily, from D_3 – D_8. On D_{15}, that is 7 days after the last drug administration, the weight, temperature, PCV and parasite load of the mice were measured to test the drug for sustained action.

Thereafter, the animals were observed till day 30 (D_0 – D_{29}) post-inoculation of parasite for death. Any death that occurred during this period was noted and used to determine the survival rate and mean survival time during this period.

The survival rate is the number of deaths that occurred in each group between D_0 and D_{29}. Mean survival time (MST) for each group was determined by calculating the average survival time (days) of mice from the date of infection over a period of 30 days (D_0 – D_{29}).

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

Acute Toxicity Test

A modified method as described by Lorke[20] was used to test for the acute toxicity of the AMT. The mice were fasted for 24 h prior to drug administration. They were divided into five groups of eight mice each. The first four groups received single doses of 500, 1000, 2000 and 4000 mg kg^{-1} of the freeze-dried extract each while the untreated group (negative group) was given the water only.

The animals were given food and water 4-hour post drug administration and observed closely for signs of distress or death. The observation was continued for up to 7 days.

Microbial Limit Test

Media Preparation

Media were weighed according to the manufacturers' inscriptions. They were heated in the water bath to melt and then autoclaved at 121°C for 15 minutes.

Diluent Preparation

Tween 20 (4%) was prepared according to the United States Pharmacopoeia, 2007 edition. Forty millilitres of the tween 20 was diluted to 1000 mL with distilled water. Ninety-millilitre portion was put in each sample bottle and then autoclaved at 121°C for 15 minutes.

Sampling

There were two working dilutions, one - in - ten (1 in 10) and one- in- hundred (1 in 100) dilutions. A portion of the sample (10 g) was weighed aseptically and transferred into the 1 in 10 bottles. It was shaken and then 10 mL portion was transferred into the 1 in 100 bottles. The 1mL of each of the dilutions was aseptically transferred to appropriately labelled petri dishes. They were overlaid with 19 mL of sterile agar, allowed to set and incubated. Plates for total plate count and other bacterial counts were inverted and incubated at 37°C and observed daily for 72 hours. Plates for total yeast and mould counts were incubated at 25°C and observed daily for one week at lid-up position. Open control plates were set up to validate the biological safety cabinet and all the assay media yielded no growth. The environment was therefore good for the assay.

Heavy Metals Limit Test

Two grams of the freeze-dried tea was dissolved in sufficient water and digested with Aqua Regia over heat in a fume cupboard for about 2 hours. The resulting clear solution was filtered. The filtrate was diluted to 100 mL, collected in a sample bottle and taken to CTX – ION Analytics Ltd. Ikeja Lagos for detection and assay of arsenal, copper, lead, mercury and zinc metals. The Microplasma Atomic Emission Spectrophotometer MP-AES (Agilent Technologies USA) with a detection limit of 2.5×10^{-5} mgL^{-1} was used.

Statistical analysis

All experiment results were evaluated by analysis of variance (ANOVA), followed by Tukey's (post-hoc test) which was used to determine the statistical significance of means and $p < 0.05$ was regarded as statistically significant.

RESULTS

Chemo-suppressive effect in mice

Using parasitaemia, weight, temperature and PCV as indices, all the extracts and CQ showed suppressive effect in the mice in various measures

Chemo-suppressive effect on parasitaemia in mice

Apart from water which expectedly gave no suppressive activity, all the extracts suppressed the parasites in a dose-dependent pattern with AMT 13.8 mg kg^{-1} giving the highest suppression of 89.19% among the extract treated groups (Table 1). CQ gave a total suppression (100%) after the 4-day test. However, the difference in activity between AMT 6.90 and 13.80 mg kg^{-1} was not significant at $p < 0.05$ (Table 1).

Table 1: Chemo-suppressive effect of AMT and control agents on parasitaemia in mice

Mice Group	Treatment	Dose (mg kg^{-1})	Parasitaemia (%)	Suppression (%)
1	Water	0.2 ml	10.73 ± 0.69	0
2	AMT	3.45	3.24 ± 0.11	69.80*
3	AMT	6.90	1.90 ± 0.09	82.29*
4	AMT	13.80	1.16 ± 0.07	89.19*
5	Chloroquine	5.00	0	100*

Values are expressed as mean ± SEM, n = 6; *significant at $p < 0.05$ compared to negative control

Effect of AMT and control agents on weight and temperature in mice during chemo-suppressive study

The effect of the extract on the weight and temperature of the mice in the suppressive model did not follow any specific pattern and cannot be said to be dose dependent. Slight temperature increase was recorded for CQ treated group (Table 2).

Table 2: Chemo-suppressive effect of AMT and Control agents on weight and temperature in mice

Mice Group	Treatment Dose (mg kg^{-1})	W_0 (G)	W_4 (G)	% Change W	T_0 (°C)	T_4 (°C)	% Change T (°C)
1	Water 0.2 mL	16.17 ± 0.54	15.83 ± 0.60	-5.44	38.22 ± 0.12	38.18 ± 0.24	-0.10
2	AMT 3.45	15.00 ± 0.00	13.67 ± 0.67	-8.86	38.72 ± 0.29	37.87 ± 0.16	-2.20
3	AMT 6.90	15.83 ± 0.48	13.33 ± 0.33	-15.79	38.90 ± 0.26	37.57 ± 0.80	-3.42
4	AMT 13.80	16.83 ± 0.48	16.50 ± 0.56	-1.96	38.45 ± 0.17	38.12 ± 0.30	-0.86
5	CQ 5.00	16.33 ± 0.33	16.00 ± 0.58	-2.02	38.22 ± 0.16	38.28 ± 0.28	+0.16

Values are expressed as mean ± SEM; n = 6; significant at $p < 0.05$; W_0 = weight on DAY $_0$;W_4 = weight on DAY_4;T_0 = Temperature at DAY_0; T_4 = Temperature at DAY_4;CQ = Chloroquine

Effect on PCV in mice during Chemo-suppressive study

A decrease in PCV was observed for all the agents employed but water gave the highest percentage decrease (28.61%) while CQ gave the least decrease (6.63%) (Figure 1). Other figures obtained were AMT 3.45 mg kg-1 (26.85%), AMT 6.90 mg kg-1 (12.57%) and AMT 13.80 mg kg-1 (12.50%) (Figure 1).

Curative effect of AMT and Control agents on % parasitaemia in mice

All the AMT extract treated groups effected reduction in the mice parasite load, attaining a statistical significance by D_8, with AMT 13.80 mg kg^{-1} attaining the highest % CURE of 83.24% (Table 3). CQ attained a statistically significant cure of 89.36% by D_5 and continued with 100% cure from D_6 through to D_{15}. However, for the AMT extracts, by D_{15}, the curative effect on parasitaemia had waned to insignificant levels. Water did not cause any decrease in parasite load throughout the duration of treatment. The difference in activity between AMT 6.90 and 13.80 mg kg^{-1} curative effect was not significant at $p < 0.05$ (Table 3)

Table 3: Curative effect of AMT and control agents on parasitaemia in mice

Treatment (mgkg^{-1})		D_0	D_3	D_4	D_5	D_6	D_7	D_8	D_{15}
Water	% PARA	9.96 ± 0.05	12.55 ± 0.16	15.23 ± 0.20	17.98 ± 0.38	20.58 ± 0.30	21.48 ± 0.26	32.54 ± 0.32
	% CURE		0	0	0	0	0	0
AMT 3.45	% PARA	9.88 ± 0.13	11.29 ± 0.20	9.80 ± 0.19	8.98 ± 0.13	7.09 ± 0.12	4.66 ± 0.21	19.21 ± 1.03
	% CURE		10.04	35.65	50.05	65.55	78.31*	40.96
AMT 6.90	% PARA	9.88 ± 0.18	11.27 ± 0.23	9.69 ± 0.09	7.76 ± 0.17	5.82 ± 0.28	3.60 ± 0.12	15.74 ± 0.30
	% CURE		10.20	36.38	56.84	71.72	83.24*	51.63
AMT 13.80	% PARA	9.87 ± 0.12	10.74 ± 0.24	8.85 ± 0.24	6.72 ± 0.33	4.74 ± 0.26	2.92 ± 0.09	12.95 ± 0.13
	% CURE		14.42	41.89	62.63	76.97	86.41*	60.20
CQ 5	% PARA	10.22 ± 0.32	5.08 ± 0.17	1.62 ± 0.08	0	0	0	0
	% CURE		59.52	89.36*	100	100	100*	100

Values are expressed as mean ± SEM, n = 6; *significant at $p < 0.05$ compared to negative control on D_8, % PARA = % Parasitaemia

Table 4: Curative effect of AMT and Control agents on weight and temperature in mice

Mice Gp	Treatment Dose (mg kg^{-1})	W_0 (g)	W_3 (g)	W_8 (g)	% Change W_0-W_8	W_{15} (g)	T_0 (ºC)	T_8 (ºC)	% Change T_0 – T_8T (ºC)
1	Water 0.2mL	22.33 ± 1.38	23.67 ± 1.43	22.60 ± 1.81	+1.21	18.50 ± 2.33	37.67 ± 0.14	37.66 ± 0.37	-0.03
2	AMT 3.45	17.17 ± 0.40	17.83 ± 0.65	16.50 ± 0.65	-3.90	13.00 ± 1.00	38.70 ± 0.16	37.63 ± 0.43	-2.76
3	AMT 6.90	20.17 ± 0.95	21.83 ± 1.25	20.75 ± 1.25	+2.88	15.00 ± 3.00	38.02 ± 0.20	37.48 ± 0.50	-1.42
4	AMT 13.80	24.17 ± 1.11	25.50 ± 1.18	24.20 ± 1.66	+0.12	21.33 ± 0.33	38.65 ± 0.12	36.50 ± 0.67	-5.56
5	CQ 5.00	19.00 ± 1.71	21.17 ± 1.96	20.50 ± 1.82	+7.89	24.60 ± 1.33	38.23 ± 0.20	37.40 ± 0.36	-2.17

Values are expressed as mean ± SEM, n = 6; significant at $p < 0.05$

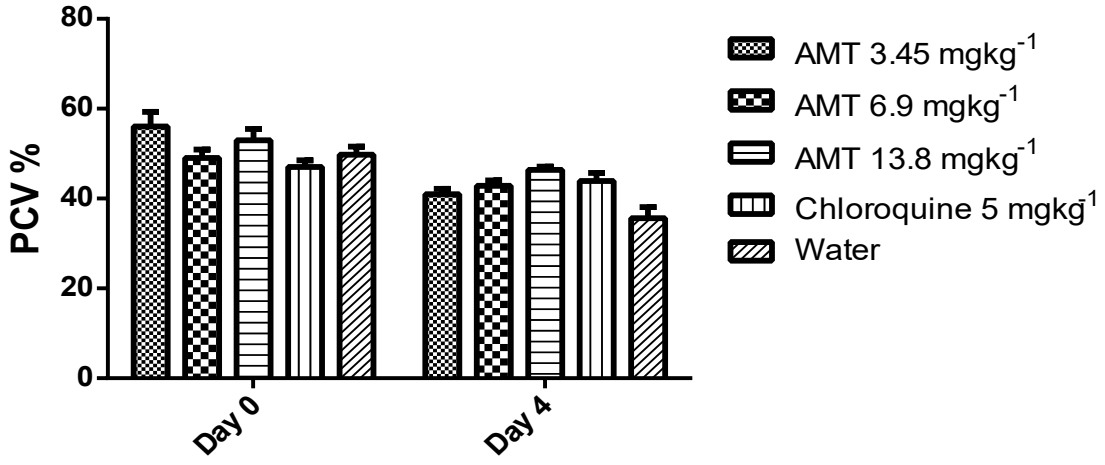

Figure 1: Graph showing Chemo-suppressive effect of AMT and Control agents on PCV in mice (Values are expressed as mean + SEM, n = 6; significant at $p < 0.05$)

Effect on Weight and temperature in Mice during curative study

The effect on weight did not follow any particular pattern. CQ gave the highest weight increase of 7.89%, and water 1.21% increase (Table 4). All the test agents, including water caused the body temperature of the mice to drop, though not in any dose-dependent manner (Table 4).

Effect on PCV in mice during curative study

The PCV dropped in all the mice in the following order: Water > AMT 3.45 mg kg^{-1} > AMT 6.90 mg kg^{-1} > AMT 13.80 mg kg^{-1} > CQ. CQ however caused an increase in PCV as from D_3 of treatment (Figure 2)

Curative effects in mice

Using parasitaemia, weight, temperature and PCV as indices, all the extract treated groups showed curative effect in the mice to varying degrees.

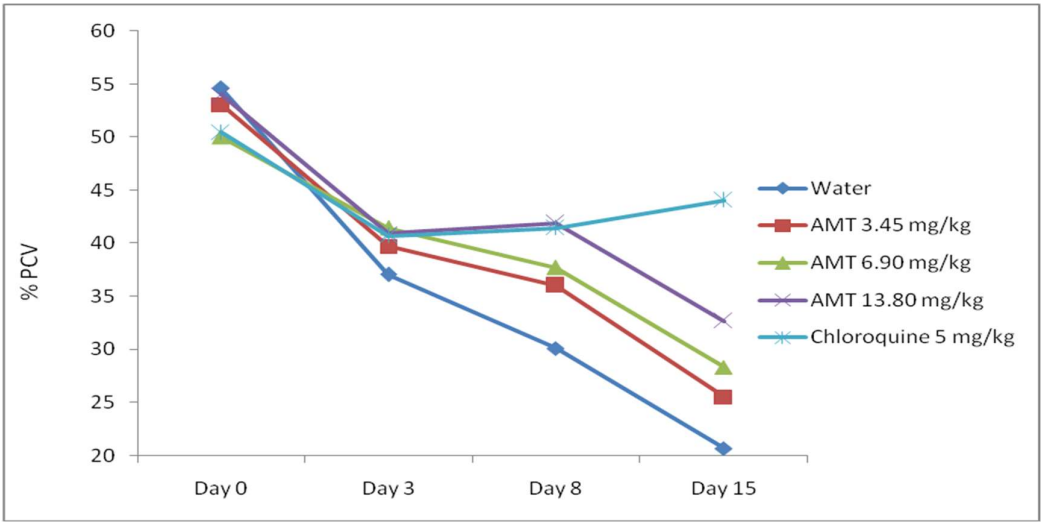

Figure 2: Graph showing effect of AMT and Control agents on PCV in mice during curative study (Values are expressed as mean ± SEM, n = 6; significant at $p < 0.05$)

Mean Survival Time

With the CQ group outstripping the others with an MST of 30 days, the MST followed this order: CQ > AMT 13.8 $mgkg^{-1}$ > 6.9 $mgkg^{-1}$ > 3.45 $mgkg^{-1}$ > Water in the curative model (Table 5). The MST for the suppressive model didn't show any dose-related pattern. The study animals that received the AMT treatment (the three dose levels) lived longer than the negative control group, but they were not fully protected. While the CQ treatment group lived out the 30 days of observation (Table 5).

Table 5: Mean Survival Time for suppressive and curative models

Treatment	Suppressive MST (days)	Curative MST (days)
Water	17.50	14.33
AMT 3.45 $mgkg^{-1}$	24.25*	22.17*
AMT 6.90 $mgkg^{-1}$	19.17*	23.00*
AMT 13.80$mgkg^{-1}$	26.67*	26.50*
Chloroquine 5 $mgkg^{-1}$	23.33*	30.00*

*Significant at $p < 0.05$ compared to negative control

Acute Toxicity Test

No death or abnormality was recorded, even at the highest dose of 4,000 mg kg^{-1} body weight.

Microbial Limit Test

Going by the British Pharmacopoeia criteria, the microbial limit for tea products was not exceeded (Table 6). Also, none of the following objectionable organisms was detected after culturing: *Enterobacteria* and other Gram-negative bacteria, *Pseudomonas aeruginosa, Salmonella spp, Shigella spp, Staphylococcus aureus, Vibrio cholerae* (Table 7).

Table 6: Microbial limit report of the samples: AMT interpretive table (British Pharmacopoeia criteria)

Microbial Parameter	AMT Parametric Colony Count	Official Limit (Bp, Appendix Xvi D)	Unit
Total Viable Aerobic Count	16000	NMT 10^7	CFU/G
Total Yeast and Mold Count	<10	NMT 10^5	CFU/G
Escherichia coli	Not detected	10^2	CFU/G

Table 7: Investigation of objectionable Organisms

OBJECTIONABLE ORGANISM	STATUS
Enterobacteria and other Gram-negative bacteria	Not detected
Pseudomonas aeruginosa	Not detected
Salmonella spp	Not detected
Shigella spp	Not detected
Staphylococcus aureus	Not detected
Vibrio cholera	Not detected

CFU/G: Colony Forming Unit PerGram; < 10: No growth seen

Heavy Metals Limit Test

Heavy metals Copper, Mercury and Zinc were all present within W.H.O permissible limits, except for lead which was detected at 0.108 ppm as against the 0.100 ppm limit, and arsenic, which was not detected at all (Table 8).

Table 8: Level of Heavy metals detected in AMT

S/N	Metal	Amount Present (ppm)	WHO Permissible Limit (ppm)
1	Arsenic	Not Detected	0.050
2	Copper	0.576	1.000
3	Lead	0.108	0.100
4	Mercury	Not Detected	0.500
5	Zinc	0.673	5.000

Chemo-suppressive effect in mice

Using parasitaemia, weight, temperature and PCV as indices, the extracts showed suppressive effect in the mice in the following order: CQ > AMT 13.8 $mgkg^{-1}$ > 6.9 $mgkg^{-1}$ > 3.45 $mgkg^{-1}$ > water.

Curative effect in mice

Using parasitaemia, weight, temperature and PCV as indices, all extracts showed suppressive effect in the mice in the following order: CQ > AMT 13.8 $mgkg^{-1}$ > 6.9 $mgkg^{-1}$ > 3.45 $mgkg^{-1}$ > water.

Mean Survival Time

With the CQ contingent outstripping the others with an MST of 30 days the MST followed this order: CQ > AMT 13.8 $mgkg^{-1}$ > 6.9 $mgkg^{-1}$ > 3.45 $mgkg^{-1}$ > water

Acute Toxicity Test

No death or abnormality was recorded, even at the highest dose of 4,000mg/kg body weight

DISCUSSION

The resistances of pathogens to orthodox medicine have been pointed out as a contributing factor in the recent increase in the use of herbal medicine. The ease of development of resistance by the malaria parasite to commonly available drugs is a good example. Animal models were used for the study due to the noted advantages it has over *in vitro* studies. *In vivo* models are challenged with some drawbacks like difference in biokinetic parameters or extrapolation of results to humans, however, they are more reliable than *in vitro* tests[21]. *In vivo* models take into account factors present in a living body such as pro-drug effect, bioavailability, and first–pass effect[22]. *Plasmodium berghei* ANKA has been a useful tool in the study and prediction of malaria-treatment outcomes[23]. Several conventional antimalarial agents such as chloroquine, halofantrine, mefloquine and more recently, artemisinin derivatives have been identified using the rodent malaria model[24]. The 4-day suppressive test, which primarily evaluates the antimalarial activity of candidate agents on early infections and Rane's test, which evaluates the curative capability of candidate extracts on established infections, are commonly used for antimalarial drug screening. In both methods, the determination of the percent inhibition of parasitaemia is the most reliable parameter[25]. In the evaluation of the suppressive potency of AMT, all the treatment groups suppressed the multiplication of the parasites at varying degrees indicating its ability to prevent malaria at an early stage of the infection.

Anaemia, bodyweight loss, and body temperature reduction are the general features of malaria-infected mice[26]. These parameters are measurable and can be indicators of the rate of efficacy for antimalarial agents. The PCV is an important indicator of cure, as malaria

parasites damage red blood cells and haemoglobin, leading to anaemia in severe episodes[27]. In this suppressive study, each treatment group had a significantly higher redeeming effect on PCV than the negative control, water, while there was no difference of statistical significance among the treatment options of 6.90 $mgkg^{-1}$, 13.80 $mgkg^{-1}$ and chloroquine 5 mg kg^{-1}. Temperature is considered a parameter as red blood cells have the role of regulating body temperature. Destruction of red blood cells by malaria parasites impairs this role. Unlike human malaria fever, however, mice experience a drop in rectal temperature during malaria. A drop in temperature in mice challenged with malaria parasite is noticed before death occurs[28]. In a study carried out to observe the pathogenesis of malaria in mice, the rectal temperature dropped progressively till the animals died without intervention[29]. However, going by the tables, the effect on temperature did not follow any particular pattern in both models, with chloroquine alone giving an increase in temperature in the suppressive test, but failing to repeat the feat in the curative. The four parameters employed in both models, only parasitaemia and PCV showed constant pattern (i.e. Chloroquine 5 mg kg^{-1} > AMT 13.80 mg kg^{-1} AMT 6.90 mg kg^{-1} > AMT 3.45 mg kg^{-1} > water in activity). A similar observation was made in the study of the antimalarial activity of *Croton macrostachyus* roots and fruit extracts, where the increase in mice weight was not consistent with the increase in dose[30] This does not come as a surprise as parasitaemia and PCV are primary parameters, malaria being a blood infection, while temperature and weight drop are consequences of the parasitic activity in the blood. This implies that the blood parameters may not readily translate to weight and temperature, especially in animals with high endurance to stress.

As with all chemotherapeutic agents, the "all or none" effect is desirable in any malaria curative[31]. In this study curative model, all the agents reduced the parasite load progressively within duration of dosage, with the chloroquine group attaining a 0% mean parasitaemia by the 4th day of therapy. The chloroquine group showed rapid onset of action, reducing parasite load with the first dose, and attaining total clearance after 3-4 days of intervention. A decrease in parasite load with the extract agent (AMT) was observed after the first dose (that is, early onset of activity) but significantly low compared to the effect of chloroquine. Increasing the start dose (initial loading boost dose) in case of emergency may improve on this slow activity within the first 24 h of dosage, curtail damage and provide quick relief. And 24 hours post the final dose (D_8), the groups that received AMT still showed parasite infection. Although AMT 3.45 $mgkg^{-1}$ reduced parasitaemia significantly more than the negative control, it performed significantly less than the other drug treated groups, namely AMT 6.90 $mgkg^{-1}$, AMT 13.80 $mgkg^{-1}$ and Chloroquine 5 $mgkg^{-1}$. Thus, may not be considered as a dosing option. The difference in percent-cure between the AMT 6.90 $mgkg^{-1}$ and the 13.80 $mgkg^{-1}$ groups was not statistically significant.

Evaluation of the parameters at D_{15} was relevant to monitor relapse or recrudescence, as remnant parasites may multiply, some develop to the schizonts (expressive) stage, while beyond D_{15}, and some might still be left in the liver. According to Cogswell (1992)[32], *P. vivax* and *P. ovale* infections, patients who have recovered from the first episode of illness may suffer several additional attacks (relapses) after months or even years without symptoms. Relapses occur because *P. vivax* and *P. ovale* have dormant liver stage parasites known as hypnozoites that may reactivate. Treatment to reduce the chance of such relapse is advised following treatment of the first attack[32]. On D_{15}, it was observed that the chloroquine treatment sustained the 0% parasitaemia, while mean parasitaemia increased with AMT groups after conclusion of the 5 – day therapy,

suggesting replication of the remnant parasites (recrudescence).

Animals in the 6.90 and 13.80 80 mg kg^{-1} AMT groups were noted to be very physically active compared to other groups, including the chloroquine treated group. However, this parameter was not measurable. The rapid onset of activity is one of the desired characteristics of a possible antimalarial agent. The insignificant differences noticed between suppression and cure effects of 6.90 and 13.80 80 mg kg^{-1} AMT groups is an indicator that increasing the dose to improve on its parasite clearance effect may not be encouraged. However, increasing the dosage days from 5 to 7 days may result in 100% parasite clearance (effect 100% eradication) considering the rapid increase in percentage cure noticed after the second dose administration. And such high activity was still retained 24 h post (D_8) withdrawal of the drug. Thus, the inability of AMT at the currently prescribed dose of 6.90 mg kg^{-1}, and even at 13.80 mg kg^{-1} to effect plasmodia eradication after 5 days of use suggests the need to extend the duration of therapy. Indeed, most herbal products are dosed at 10 -14 days[33]. The multi-component nature of herbs however prevents the build-up of pathogen resistance in the long run.

The question of acute toxicity is, "If an unusually high dose is mistakenly or intentionally ingested, what are the consequences?" This question is essential as medications could get grabbed by children, dosage misunderstood by patients or even suicide attempted. With AMT, even at a dose of 4,000 mg kg^{-1} neither death nor deleterious effect was observed, thus indicating a broad therapeutic index, one of the indices of safety. Heavy metals are highly undesirable in oral medicines because they accumulate in the body as they are not biodegradable. The copper and zinc level (0.576 ppm and 0.673 ppm respectively) of AMT were found to be within W.H.O permissible limits (1.000 ppm and 5.000 ppm), while no mercury nor was arsenic detected. Lead, however, was detected in 0.108 ppm of AMT, slightly higher than the WHO permissible limit of 0.100 ppm. This deviation is insignificant and maybe due to sourcing environment.

CONCLUSION

Based on this study, the AMT currently prescribed dose of 6.90 $mgkg^{-1}$ daily (1 tea bag twice daily) for 5 days possesses suppressive and curative antimalarial activity. AMT is also safe for oral consumption. The study result suggests that increasing dosage of AMT to at least 7 - 10 days may ensure total eradication of plasmodia and prevent relapse. Further studies may however be carried out to determine the exact duration that is most effective.

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