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The Antiplasmodial Activity of an Herbal Antimalarial, AM 207 in *Plasmodium berghei*-infected Balb/c Mice: Absence of Organ Specific Toxicity

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The antiplasmodial activity in *P. berghei*-infected Balb/c mice and safety in rats of the herbal antimalarial, AM 207 were investigated. The results of the 4-day parasite suppression test showed a high antiplasmodial activity of AM 207 (40 and 200 mg kg^{-1}) comparable to that of Nibima (80 mg kg^{-1}) and chloroquine evidenced by the similar reductions in parasitemia (6.5-6.7%) and degrees of chemo-suppression (48.3-50.6%). In the 6-day suppression test, results showed significant differences in chemo-suppression ($p<0.05$) between chloroquine (5 mg kg^{-1}) and AM 207 at 20 and 40 mg kg^{-1} (35.9-52.8%) on one hand and Nibima at 40 and 200 mg kg^{-1} (42.6-59.2%) on the other. However, AM 207 (200 mg kg^{-1}) and Nibima (80 mg kg^{-1}) showed the highest chemo-suppressions (65.9-71.4%), which were comparable to that of chloroquine (86.6%). The effects of treatments on animal survival time showed that it was increased from 10 days for negative control to a maximum of 23 days for animals receiving AM 207 (20-200 mg kg^{-1}) and 17 days for animals which received Nibima (40-200 mg kg^{-1}). However, no deaths were recorded for chloroquine over the 23 day period. There were no adverse effects of AM 207 on selected rat organs/tissues as evidenced by absence of any significant untoward changes in haematological, urine and serum biochemical parameters as well as organ/body weights. These results indicate that AM 207 at 200 mg kg^{-1} exhibited antiplasmodial activity comparable to chloroquine and Nibima (80 mg kg^{-1}) and there is no apparent organ specific toxicity associated with it on sub-chronic administration to rats.

Key words: *Azadirachta indica*, *Nauclea latifolia*, *Morinda lucida*, *Cryptolepis sanguinolenta*, chemo-suppression

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INTRODUCTION

Malaria is a potentially deadly tropical disease characterized by cyclical bouts of fever with muscle stiffness, shaking and sweating. It is caused by protozoan parasites of genus plasmodium and is transmitted by the female mosquito of genus Anopheles when it feeds on blood for its developing eggs (Perrin *et al*., 1982).

According to The Roll Back Malaria Campaign of WHO, 90% of the more than one million deaths worldwide caused by malaria every year take place in Africa and malaria constitutes 10% of the continents burden (Yamey, 2000; Roll Back Malaria, 2004). In Ghana, malaria is responsible for 40% of daily out-patients consultantations at hospitals and clinics and over 23% deaths in children under 5 years (Ofori-Adjei and Arhinful, 1996).

Malaria is treated with chloroquine, quinine and other derivatives of artemisinine and other herbal antimalarials by suppressing the activity of the parasites or kill them. The World Health Organization (WHO) has recommended the use of artesunate/amodiaquine combination therapy as first line treatment for malaria. Most of these synthetic drugs are expensive, have a lot of side effects and have developed resistance to the parasites because of long term usage. It therefore calls for the development of alternatives and cheaper medicines such as herbal antimalarials for the treatment of malaria.

In Ghana many herbal medicines for the treatment of malaria are available in the market. These include: Nibima, Phytolaria, malaherb, malacure, herbaquin to name but a few. However, all of these products are *Cryptolepis sanguinolenta* based (Grellier *et al*., 1996; Ansah *et al*., 2005). This plant is becoming extinct and therefore, the cost of production is becoming more and more expensive.

There is therefore, the need to develop new herbal medicine for malaria from plant sources other than *C. Sanguinolenta.* Hence the development of the aqueous extract AM 207 which is made from three medicinal plants; leaves of *Azadirachta indica*, leaves and roots of *Morinda lucida* and roots of *Nauclea latifolia*, known to have anti-malarial activity individually (Akanmori *et al*., 1994; Benoit-Vical *et al*., 1998; Zirihi *et al*., 2005).

Azadirachta indica A Juss. belongs to the family Meliaceae, It is used traditionally to treat fever, chikenpox, HIV/ AIDS, birth control, chagas disease, eczema, ringworm, cancer, septic sores, infected burns and malaria. The active chemical compounds present in the plant include, nimbin, nimbinin and nimbidin. Gedunnin, quercetin and irodin A compounds are also present in Neem leaves (Iwu, 1993).

Nauclea latifolia Smit. belongs to the family Rubiaceae and has traditionally been used for the treatment of malaria (Iwu, 1993). Studies have shown that the aqueous extracts of *Nauclea latifolia* inhibited chloroquine-resistant *P. falciparum* mainly at the end of the erythrocytic cycle (Benoit-Vical *et al*., 1998). The plant contains several indole-quinolizidine alkaloids and glycoalkaloids. The major ones include angustine, angustoline, nauclefine and nauclectine (Iwu, 1993).

Morinda lucida Benth also belongs to the family Rubiaceae and a decoction from the stem back and/or the leaves has been used for the treatment of severe jaundice, hypertension, cerebral complications dysentery, but the leaves for malaria and as a general febrifuge and analgesic (Iwu, 1993; Awe and Makinde, 1998). The plant contains tannins, methylanthraquinones and heterosides. Studies have showed that *Morinda lucida* leaves extract appears to have schizontocidal and repository effects in mice infected with *P. berghei* (Akanmori *et al*., 1994).

This study was therefore, aimed at evaluating the safety and efficacy of AM 207 in male Sparague-Dawley rats and balb/c mice infected with *Plasmodium berghei*, respectively.

MATERIALS AND METHODS

Reagents and chemicals: Test kits; Aspartate Aminotransferase (AST), Alamine aminotransferase (ALT), Gamma-Glutamyltranspeptidase (GGT), total bilirubin, albumin, creatinine, urea were purchased from Cypress Diagnostics (Belgium). Urine test strips (Accu-Tell$^{(R)}$) AccuBioTech Co. Ltd (Beijing, China), Giemsa stain, methanol and sodium chloride, were obtained from British Drug House Ltd (Poole, England). Chloroquine phosphate was obtained from Troge Medical GMBH (Hamburg Germany).

Plant raw materials and herbal standard: *Azadirachta indica* leaves, *Nauclea latifolia* leaves and the leaves and roots of *Morinda lucida* were obtained from the Plant Production Department (PDD), CSRPM (Mampong-Akuapem, Ghana) and authenticated by Dr. Yaw Ameyaw, a botanist of the department. Nibima (aqueous herbal medicine standard) was obtained from the Production department, CSRPM.

Animals: Male Sprague Dawley rats (170-250 g) and seven-week old female Balb/c mice (30 g) were obtained from the Animal unit of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapem, in the Eastern Region of Ghana. The animals were fed on powdered feed obtained from Ghana Agro Food Company

(GAFCO), Tema, Ghana. They were allowed free access to sterile distilled water. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the US guidelines (NIH, 1985).

Preparation of herbal extract: To 150 g each of chopped *Azadirachta indica* leaves, *Nauclea latifolia* leaves and the leaves and roots of *Morinda lucida* was added 10x the equivalent volume of distilled water. The mixture was boiled for 45 min, sieved through a wire mesh and allowed to cool. The extract was freeze dried and stored in a cool dry place. This was reconstituted in sterilized distilled water before use.

Malaria parasites: *Plasmodium berghei* NK65 strain from the University of Copenhagen, Denmark and maintained for more than 20 years in liquid nitrogen with occasional passage in Balb/c mice in the Department of Immunology, Noguchi Memorial Institute of Medical Research (NMIMR), University of Ghana, Legon, Accra, Ghana, was used for the experiment.

Inoculum preparation: A stock of parasitized erythrocytes was obtained from infected Balb/c mice, with a minimum peripheral parasitemia of 20%, by cardiac puncture in heparin-coated tube. The cell concentration of the stock was determined and diluted with physiological saline such that 0.2 mL of final inoculum contained 10^6 parasitized RBCs.

Treatment of animals: In acute toxicity studies, single oral dose (2 mL/rat) of AM 207 was administered at 5000 mg kg^{-1} to six rats each with an oral gavage needle. Mortality and general behavior of the animals were observed over a 48 h period. Surviving animals were observed for a further period of 12 days for toxic symptoms such as the arrangement of hairs, condition of eyes, movement and breathing of animals.

For the sub-chronic toxicity studies, four groups of six rats each were kept in four separate metal cages. Group 1 was kept as control and received sterilized distilled water for six weeks. Groups 2, 3 and 4 were treated with 40, 400 and 800 mg kg^{-1} daily in their drinking water, representing the normal dose, 10x and 20x the normal dose, respectively of freeze dried AM 207 for six weeks. The volume of the extract taken by test animals were not different from that of controls over the period of study. The animals in each group were weighed on day zero (baseline) and weekly thereafter After the end of the six-week treatment period, three rats from each group were euthanized by cervical dislocation and the heart, lungs, liver, kidney and spleen were excised and weight. The spleen was discarded whereas the remaining organs were preserved in 10% formalin.

In the *in vivo* antiplasmodial assay, forty eight mice were selected and divided into eight groups of six mice per group. Each mouse in each group set were inoculated intraperitonaelly with 10^6 parasitized RBCs (0.2 mL) on day zero. Group 1 received distilled water (negative control), group 2 received 5 mg kg^{-1} chloroquine (positive drug control), groups 3, 4 and 5 received 40, 80 and 200 mg kg^{-1} respectively of Nibima (positive herbal control) and groups 6, 7 and 8 received 20, 40 and 200 mg kg^{-1}, respectively of AM 207. Chloroquine, Nibima and AM 207 were administered to the animals orally, 2-3 h after the mice were inoculated with the parasites, for six days.

Monitoring of parasitemia and antimalarial activity: On the fifth and seventh days after drug administration, thin blood smears were prepared using blood from the tail vein of each mouse. Each smear was air-dried, fixed in methanol, air–dried and again stained with Giemsa for 10 min and examined under the microscope. The slides were observed under oil immersion. Each slide was observed at three different fields and the parasitized RBCs and total number of RBCs for each field was also recorded:

$$\text{Percentage parasitemia} = \frac{\text{Total number of parasitized RBCs counted}}{\text{Total number of RBCscounted}} \times 100$$

$$\text{Activity (\%Chemo}-\text{suppression)} = 100 - \left[\frac{\text{Parasitemia of test}}{\text{Parasitemia of control}}\right] \times 100$$

Urinalysis: Urine samples of rats in each treatment group produced as a result of involuntary discharges by the groups were collected on clean ceramic tiles at day zero (baseline) and termination of treatment. Analysis of urine for glucose, bilirubin, ketones, specific gravity, pH, proteins, urobilinogen, nitrate, blood and leukocytes was done using urine reagent strips (Accu-Tell$^{(R)}$) AccuBioTech Co. Ltd (Beijing, China).

Blood sampling: Blood samples (3 mL) of rats in each treatment group were obtained by tail bleeding (at baseline and week 6) into Eppendorf tubes without anticoagulant, centrifuged at 4000x g for 5 min after coagulation (Denley BS 400, England) and serum stored at -4°C for biochemical analysis. Other blood samples (0.5 mL) were collected into separate tubes already coated with trisodium citrate (Westergreen E.S.R, UK) for haematological analysis within 24 h.

Haematological and serum biochemical analyses: In haematological analysis, Red Blood Cells (RBC), White Blood Cells (WBC), Haemocrit (HCT), Haemoglobin (HGB) and Platelet (PLT) counts and other haematological parameters like Mean Cell Volume (MCV), Means Corpuscular Haemoglobin Concentration (MCHC), Mean Platelet Volume (MPV), Red cell Distribution Width (RDW), Means Corpuscular Haemoglobin (MCH), per cent lymphocytes and lymphocytes number (LYM#) of citrate-treated blood of control and test animals were measured using Sysmex KX 21 (Europe) in accordance with established protocol. The equipment is computerized to automatically determine and display the above profile for each blood sample. In serum biochemistry analysis, ALT, AST, GGT, albumin, total bilirubin, creatinine and urea in serum samples of control and test animals were determined using protocols from Cypress diagnostic kits (Belgium) with a semi-auto blood chemistry analyser, photometer 4040 (Robert Riele G and Cole-2000, Germany).

Phytochemical screening of AM 207 and nibima extracts: The aqueous herbal extracts AM 207 and Nibima were screened for the presence or absence of groups of phytochemicals such as saponins, reducing sugars, phenolics, cyanogenic glycosides, polyamides, phytosterols, triterpenes, anthracenosides, flavonoids and alkaloids (Sofowora, 1982; Harborne, 1983).

Statistical analysis: One-way Analysis of Variance (ANOVA), independent sample t-Test and log rank test of the Kaplan-Meier method was used to determine the toxicity and activity between control groups and test groups. The 5% level of probability was used as criterion of significance in all instances. All statistical tests were performed with SPSS statistical software version 11.0 and graph pad prism version 4.0.

RESULTS

Chemo-suppression of Parasitemia in a 4-day Test: Table 1 shows the blood antiplasmodial activity of varying oral doses of AM 207 compared to chloroquine (positive drug control), Nibima (positive herbal control) and distilled water (negative control) during an early P. berghei infection (4-day test) in mice. These results showed a high antimalarial activity of AM 207 (40 and 200 mg kg^{-1}) comparable to that of Nibima (80 mg kg^{-1}) and chloroquine as evidenced by the similar reductions in parasitemia (6.5-6.7%) and degrees of chemo-suppression (48.3-50.6%). The activities expressed by Nibima and AM 207 were, however, not dose-dependent.

Table 1: The Effect of AM 207 on Parasitemia in *P. berghei*-infected Mice in a 4-day suppression test

Treatment	Dose (mg kg^{-1})	Parasitemia (%)	Chemo-suppression (%)
Control	0	13.3±4.14	0
Chloroquine	5	6.7±1.19	49.1±8.9
AM 207	20	10.1±1.60	37.7±14.4
	40	6.8±1.90	48.3±14.5
	200	6.8±1.21	48.4±9.06
Nibima	40	12.3±1.21	13.6±10.0
	80	6.6±0.4	50.6±2.9
	200	9.8±1.60	33.6±11.5

Results are means±SEM of n = 6

Table 2: The effect of AM 207 on Parasitemia in *P. berghei*-infected Mice in a 6-day Suppression Test

Treatment	Dose (mg kg^{-1})	Parasitemia (%)	Chemo-suppression (%)
Control	0	51.9±6.8	0
Chloroquine	5	6.9±1.2	86.6±4.7
AM 207	20	25.6±5.2	50.7±10.1*
	40	37.4±4.7	33.8±9.1*
	200	14.9±3.9	71.4±7.5
Nibima	40	43.6±7.5	27.4±12.9*
	80	17.7±7.4	65.9±14.2
	200	29.1±3.4	44.0±6.4*

Results are means±SEM of n = 6, *Value significantly different from positive drug control (chloroquine); $p<0.05$

Chemo-suppression of Parasitemia in a 6-day Test: Table 2 shows the antiplasmodial activity of varying oral doses of AM 207 compared to chloroquine, Nibima and negative control during an early *P. berghei* infection (6-day test) in mice. The results showed significant differences in chemo-suppression ($p<0.05$) between chloroquine and AM 207 at 20 and 40 mg kg^{-1} (35.9-52.8%) on one hand and Nibima at 40 and 200 mg kg^{-1} (42.6-59.2%). However, AM 207 (200 mg kg^{-1}) and Nibima (80 mg kg^{-1}) showed the highest chemo-suppressions (65.9-71.4%), which were comparable to that of chloroquine (86.6%). The activities expressed by Nibima and AM 207, once again, were not dose-dependent.

Survival times of *P. berghei*-infected Balb/c Mice: Figure 1 show the percentage survival time of the *P. berghei*-infected Balb/c mice treated with chloroquine, Nibima and AM 207 compared to negative control. This graph shows that the last animal in the negative control died on day 10, the last animals which received AM 207 at 20, 40 and 200 mg kg^{-1} died on days 21, 21 and 23, respectively. The last animals which received Nibima at 40, 80 and 200 mg kg^{-1} died on days 10, 15 and 17, respectively. However, no deaths were recorded for chloroquine over the 23 day period.

Acute toxicity studies: The effect of a single oral dose of AM 207 (5000 mg kg^{-1}) administered to six animals showed no deaths within 48 h and no physical signs of

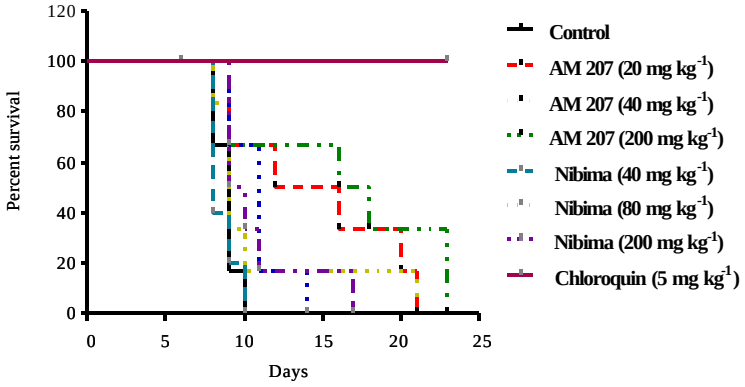

Fig. 1: Percentage survival of *P. berghei*-infected Balb/c mice at different times of treatment with chloroquine (positive drug control), Nibima (positive herbal control), distilled water (negative control) and AM 207

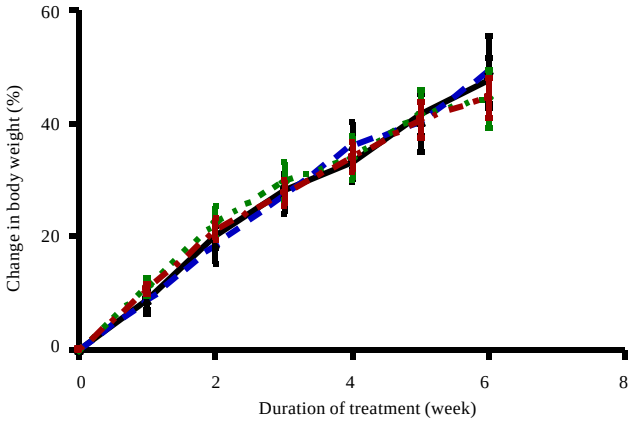

Fig. 2: Percentage changes in mean body weight of rats with duration of AM 207 treatments. Each point represents mean±SEM of n = 6

toxicity as evidenced by normal breathing and movement. There were also no signs of bulging eyes and pilo-erection. Observations of animals over the next 12 days showed no adverse effects of treatment.

Body and organ weight changes: Figure 2 shows a graphical representation of the percentage change in body weight following treatment of rats with AM 207. There were significant increases ($p<0.05$) in body weight with time over the experimental period for all treatment groups but no significant differences ($p>0.05$) between the control and test groups. Table 3 shows the effect of AM 207 on mean organ wet weights expressed as percentage of body weight at termination of treatment. There were no significant differences in mean organ weights between control and test animals.

Table 3: Mean wet organ/body weight at termination of treatment with AM 207

	Organ /body weight (%)			
		AM 207		
Organs/Tissue	Control	40 mg kg^{-1}	400 mg kg^{-1}	800 mg kg^{-1}
Heart	0.34±0.02	0.33±0.03	0.37±0.03	0.25±0.11
Lungs	0.60±0.02	0.53±0.02	0.60±0.03	0.59±0.03
Liver	3.55±0.27	2.92±0.22	3.61 ±0.13	3.25±0.30
Kidney	0.54±0.03	0.63±0.06	0.68±0.03	0.63±0.08
Spleen	0.26±0.02	0.23±0.02	0.24±0.02	0.29±0.01

Results are means±SEM of n = 6

Urinalysis data: Dipstick urinalysis data of control and test animals at termination of treatment with AM 207 is shown in Table 4. There were no noticeable differences in urine blood, bilirubin, urobilinogen, proteins, ketones, glucose, nitrite, leucocytes, pH and specific gravity of control and test animals.

Table 4: Effect of AM 207 on some rat urine parameters at termination of treatment

		Treatment groups		
		AM 207		
Parameters	Control	40 mg kg^{-1}	400 mg kg^{-1}	800 mg kg^{-1}
Urobilinogen (mg dL^{-1})	-	-	-	-
Glucose (mg dL^{-1})	-	-	-	-
Bilirubin (mg dL^{-1})	-	-	-	-
Ketones (mg dL^{-1}	-	-	-	-
S.G. (g mL^{-1})	1.026	1.023	1.023	1.021
Blood (RBC µL^{-1})	-	-	-	-
PH	6.5	6.7	6.8	6.8
Protein (g L^{-1})	+	+	+	+
Nitrite	-	-	-	-
Leukocytes (WBC µL^{-1})	-	-	-	-

Figures represent means of 6 determinations, –: Absent, +: Positive

Table 5: Effect of AM 207 on rat haematological parameters after termination of treatment

		Treatment groups		
		AM 207		
Parameters	Control	40 mg kg^{-1}	400 mg kg^{-1}	800 mg kg^{-1}
WBC (x10^3 µL^{-1})	10.70±1.15	11.54±0.64	12.82±1.52	13.75±0.71
MPV (f L)	6.48±1.62	6.08±0.15	6.06±0.18	6.00±0.14
RBC (x10^6 µL^{-1})	6.80±0.17	7.29±0.17	7.03±0.24	7.09±0.16
Hb (g dL^{-1})	12.28±0.14	13.68±0.23	13.12±0.38	13.53±0.21
HCT (%)	42.89±0.94	46.06±0.85	43.76±1.00	46.05±1.21
MCV (f L)	63.07±0.66	62.93±0.82	61.7±0.97	64.98±1.49
MCH (pg)	18.86±0.47	18.97±0.24	18.68±0.21	19.10±0.50
MCHC (g d L^{-1})	29.92.±0.74	30.11±0.51	29.58±0.81	29.40±0.48
PLT (x10^3 µL^{-1})	844.8±149.0	865.75 ±55.67	768.60±125.81	904.00±128.50
LYM (%)	78.98±5.44	81.93±2.87	90.52±1.93	84723±2.67
LYM (#)	9.18±1.14	9.78±0.68	11.80±0.64	11.65±0.65
RDW (f L)	48.57±1.61	45.42±2.47	47.88±5.30	47.18±2.45

Results are means±S.E.M. of n = 6

Table 6: Rat serum biochemical data on termination of treatment with AM 207

		Treatment groups		
		AM 207		
Parameter	Control	40 mg kg^{-1}	400 mg kg^{-1}	800 mg kg^{-1}
AST (U L^{-1})	141±11.3	133±9.47	117±8.71	106±9.67
ALT (U L^{-1})	84.0±8.83	78.2±4.12	75.8±8.24	109±12.3
Total bilirubin (µmol L^{-1})	2.72±0.77	2.66±0.12	2.76±0.90	2.67±0.84
GGT (U L^{-1})	4.45±0.43	4.55±0.43	4.67±0.88	5.33±0.67
Albumin (g L^{-1})	47.2±2.92	56.2±3.94	72.7±2.26*	78.5±3.53*
Creatinine (µmol L^{-1})	68.6±3.97	64.9±7.97	62.7±5.17	65.1±15.0
Urea (mmol L^{-1})	7.50±0.09	6.90±0.81	6.73±0.36	7.00±0.29

Results are means±SEM of n = 6, *Value significantly different from control; $p<0.05$

Haematological and serum biochemical data: Table 5 and 6 show the effects of AM 207 on some rat haematological and serum biochemical parameters, respectively compared to control at termination of treatment. No significant differences ($p>0.05$) were observed between test and control animals for all the haematological parameters measured over the treatment period. Also, no significant differences ($p>0.05$) were observed between the test and control animals in all the serum biochemical parameters determined with the exception of albumin level, which increased with increasing dosage but not in a dose dependent fashion.

Phytochemical composition of extracts: The results of the screening of the groups of phytochemicals present in the aqueous herbal extracts, AM 207 and Nibima are shown in Table 7. The AM 207 extract showed the presence of phenolics, anthracenosides, phytosterols and flavonoids

Table 7: Phytochemical screening of aqueous extracts of AM 207 and nibima

	Characterization of test results	
Groups of phytochemicals	AM 207	Nibima
Saponins	-	-
Reducing sugars	-	+
Phenolics	+	-
Cyanogenic glycoside	-	-
Polyamides/ polyuronides	-	+
Triterpenes	-	-
Phytosterols	+	-
Anthracenosides	+	-
Flavonoids	+	-
Alkaloids	-	+

+: Positive, -: Negative

whilst the Nibima extract contained reducing sugars, polyuronides and alkaloids.

DISCUSSION

Malaria is a major disease that affects millions of children and adults in tropical and sub-tropical regions of the world with serious debilitating effects. The astronomical cost of treatment has become a major cause of concern for most governments in developing countries, warranting the need to find cheaper and effective herbal alternatives. The herbal extract AM 207 is one of such alternatives formulated by the CSRPM, Manipong, Akuapem, in the Eastern Region of Ghana, for the treatment of malaria. The antiplasmodial activity of this product has been assessed in a *P. berghei*-infected mouse model and male Sprague-Dawley rats, respectively in this study.

From the results of the *in vivo* antiplasmodial activity studies, there were no significant differences ($p>0.05$) in % parasitemia and degree of chemo-suppression between chloroquine and the varying doses of herbal medicines (Nibima and AM, 207) used in the 4-day parasitemia suppression test (Table 1), indicating that the chemo-suppression of parasitemia in the *P. berghei*-infected mice by AM 207 were comparable to those of chloroquine and Nibima.

Table 2 showed that there was a continuing increase in the chemo-suppression of parasitemia with increasing period of treatment with the medicines. However, there was a significant difference ($p<0.05$) between chloroquine and AM 207 (20 and 40 mg kg^{-1}) on one hand and Nibima (40 and 200 mg kg^{-1}) on the other, indicating that these doses were not as effective as chloroquine. The level of parasitemia suppression by AM 207 (200 mg kg^{-1}) and Nibima (80 mg kg^{-1}) were 71.4 % and 65.9 %, respectively and were comparable to chloroquine which showed 86.6% suppression. The antimalarial effect of these herbal medicines was not dose-dependent since the degree of *P. berghei* suppression by the 20 mg kg^{-1} dose of AM 207 was higher than that of the 40 mg kg^{-1} dose and that of 80 mg kg^{-1} Nibima dose was higher than the 200 mg kg^{-1} dose.

It has been shown that *Cryptolepis sanguinolenta*, the plant raw material base of Nibima, contains mainly alkaloids (Dwuma-Badu *et al*., 1978; Tackie *et al*., 1993) some of which are known to be responsible for its antimalarial activity (Bierer *et al*., 1998). Qualitative phytochemical screening in this study led to the detection of alkaloids in Nibima but not in AM 207 (Table 7). The observation that AM 207 (200 mg kg^{-1}) exhibited a higher parasite chemo-suppressive activity than Nibima seems to suggest that the antimalarial activity of AM 207 could be attributed to phenolics, anthracenosides, phytosterols or flavonoids as detected in AM 207, other than alkaloids. It is known that some flavonoids and phenolics have antiplasmodial activity (Hilou *et al*., 2006; Builders *et al*., 2011).

It was observed that the animals which received Nibima (80 mg kg^{-1}) showed a higher parasite chemo-suppressive activity than Nibima (200 mg kg^{-1}) but died earlier (Fig. 1). This indicates that although the higher dose may extend the survival time of the mice, probably as a result of higher concentrations of certain chemical entities present, it had opposite effect on antiplasmodial activity. However, that of AM 207 (200 mg kg^{-1}) produced the highest antiplasmodial activity and the longest survival time of animals as compared to the lower doses.

Despite the extensive use of *Morinda lucida* leaves and roots, *Nauclea latifolia* roots and *Azadiractha indica* leaves, either singly or in combination, in the treatment of malaria (Iwu, 1993), there are no available scientific data on their safety. In this study the AM 207 was investigated in male Sprague-Dawley rats. Acute toxicity studies findings indicated that the oral LD_{50} of the aqueous extract was above 5000 mg kg^{-1} body weight of rat, and animals did not show any observable signs of toxicity over the 14-day period of study. This suggests that AM 207 may be tolerable as a result of accidental overdosage.

The absence of significant difference ($p>0.05$) between AM 207-treated rats and controls in haematological profile (Table 5) suggests that AM 207 may not affect the bone marrow or the blood cells (Guyton and Hall, 2005). The serum biochemical data (Table 6) showed that AM 207 over the period of administration did not cause any significant elevation of

AST, ALT, GGT and total bilirubin, which are used as indices of hepatocellular damage and/or cholestasis (Allan and James, 1995). Brett *et al.* (2006). However, albumin levels increased significantly with increasing dosage of the extract. This suggests that treatment with AM 207 may lead to increase in protein biosynthetic activity of the liver (Allan and James, 1995).

The kidney is a major excretory and osmoregulatory organ of mammals. This therefore, makes it a target for toxic chemicals by concentrating xenobiotics and their metabolites to high levels resulting in the immediate organ failure or delayed malfunctioning (Lash, 1994). The lack of effect on creatinine and urea levels (Table 6) in the blood indicates that AM 207 may not cause any failure or dysfunction of the kidney. This is corroborated by urinalysis data (Table 4) and organ/body weight of kidneys (Table 3).

CONCLUSION

In conclusion it may be said that AM 207 at 200 mg kg^{-1} exhibited antiplasmodial activity comparable to chloroquine (5 mg kg^{-1}) and Nibima (80 mg kg^{-1}). There is, however, no overt organ specific toxicity associated with AM 207 on sub-chronic administration. This appears to support the traditional use of the constituent plants of AM 207 in the treatment of malaria.

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