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IN VIVO ANTI-MALARIAL POTENTIALS OF SOME PLANTS EXTRACTS ON *ICR*-MICE, *Mus musculus*

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ABSTRACT
Five medicinal plants, Acacia nilotica (Fabaceae), Citrus aurantifolia (Rutaceae), Mangifera indica (Anacardiaceae) Carica papaya (Caricaceae), and Psidium guajava (Myrtaceae) used for the treatment of malaria/ fever by the Hausa people of Kano-Nigeria were selected based on their traditional claims. These were extracted using ethanol. The in vitro antiplasmodial activities of these extracts against laboratory adapted chloroquine susceptible strain of Plasmodium falciparum (3D7) were earlier reported (Dabo et al. 2013). This study was conducted to evaluate the in vivo antiplasmodial activity of four of these extracts using icr mice in the 4-day suppressive test against P. berghei. The extracts evaluated were: Acacia nilotica (Fabaceae) stem extract coded as ANSF1; Citrus aurantifolia (Rutaceae) leaf extract coded as CALF1; Mangifera indica (Anacardiaceae) leaf extract coded as MILF1 and combination of C. aurantifolia, Carica papaya (Caricaceae), M. indica and Psidium guajava (Myrtaceae) leaves extract coded as CBLF1. CALF1, ANSF1 and CBLF1 (combination) exhibited appreciable degree of suppression of 31.06%, 25.16 and %16.35% respectively. MILF1 did not exhibit any suppression against the P. berghei parasite in vivo at all. With the exception of haemoglobin content (P<0.0363), haematological analysis of the various blood parameters of mice in the control and treated mice indicated no statistical difference (P>0.05). Thus it can be concluded that, the apparent absence of changes in haematological profile alongside activities exhibited by CALF1 (Rutaceae), CBLF1 (a combination) and ANSF1 (Fabaceae),) suggest the bio-availability and antiplasmodial activities of the active substances in the plants evaluated.
Keywords: Antimalaria; In vivo, Plants Extracts; Mus musculus; Plasmodium berghei.

INTRODUCTION

Malaria still remains a serious disease with high mortality and morbidity especially in children and pregnant women with the Sub-Saharan Africa bearing the highest burden of the disease (WHO 2010). Due to the absence of an approved vaccine for use in the control of malaria, chemotherapy remains an effective intervention against the scourge of the disease. The spate of drug resistance by the etiologic parasites particularly *P. falciparum* among others to several drugs particularly chloroquine had necessitated the scientific evaluation of many traditional medicinal plants for alternative antimalarial drugs (Oyedeji *et al.*, 2005) particularly from plants as earlier antimalarial drugs were derived from compounds obtained from herbal plants used in treating fevers including malaria. Many plants are used in the traditional system as antimalarials especially in Africa (World Health Organisation, WHO / Tropical Disease Research TDR news, 2007 and Hostettmann *et al.*, 2000). It is for this reason that some plants used in the traditional treatment of malaria or fever by the Hausa people of Kano, Nigeria were selected and evaluated for efficacy against the Plasmodium parasite *in vivo*. The *in vitro* antiplasmodial activities of extracts from these plants against laboratory adapted chloroquine susceptible strain of *Plasmodium falciparum (*3D7) were earlier reported (Dabo *et al.* 2013*)*. This study was conducted to evaluate the *in vivo* antiplasmodial activity of these extracts against *P. berghei*.

MATERIALS AND METHODS

Collection and Handling of Plant Materials

Five plants used in the treatment of malaria or fever (Table 1) were collected on the basis of personal communication with the folks between 21/11/2008 to 01/01/2009. Taxonomic identification was confirmed by Professor B. S. Aliyu of the Department of Plant Biology, Bayero University Kano, Nigeria. Voucher specimens were deposited in the herbarium of the Bayero University, Kano.

Table 1: Plant Names, Voucher Number, Family and Parts Collected

Plant Name	Herbarium Voucher #	Common name	Family	Part used
Acacia nilotica	*287/05*	*Bagaruwa*	Fabaceae	Stem bark
Citrus aurantifolia	*20/07*	*Lemon-tsami*	Rutaceae	Leaves
Mangifera indica	*55/10*	*Mangwaro*	Anacardiaceae	Leaves
Carica papaya	*70/12*	*Gwanda*	Caricaceae	Leaves
Psidium guajava	*66/01*	*Goba*	Myrtaceae	Leaves

Fresh plant materials were collected and shade dried to minimize deterioration of active components. Each dried plant material was then ground into powder using mortar and pestle. Two hundred and fifty grams (250g) portions were then weighed out and extracted im accordance with Fatope *et al*. (1993) by percolating in absolute ethanol (Rjedel-de Haèn®) at 1:5 w/v (plant part: solvent) for two weeks and filtered using Whatman® No.1 filter paper. A portion of the extract was transferred into pre-weighed specimen bottle and designated F1. The four plant leaves used as combination (*M. Indica, C. Papaya, P. guajava* and *C, aurantifolia*) were prepared by weighing equal proportions of the respective plants (12.5g each). Four extracts which exhibited appreciable inhibitory activities *in vitro* against the laboratory reared strain of chloroquine susceptible *P. falciparum* (Dabo *et. al., 2013)* were thus evaluated for *in vivo* activity against a rodent malaria parasite *P. berghei*. These comprised of *A. nilotica* stem extract (designated as ANSF1); *C. aurantifolia* leaf extract (designated as CALF1); *M. indica* leaf extract (designated as MILF1) and combination of *C. aurantifolia, C. papaya, M. indica* and *P. guajava* leaves extract (designated as CBLF1).

Evaluation of Antiplasmodial Activity on Early Infection

The Knight and Peters (1980) 4-day suppressive test for evaluating parasitaemia in early infection was employed (Chandel and Bagai, 2010; Matur *et al*., 2009). Animal model for the *in vivo* study were *icr* mice obtained from the Animal Experimentation Department, Noguchi Memorial Institute of Medical Research, NMIMR University of Ghana, Legon. These were acclimatized in the laboratory for 5 days prior to the commencement of the experiment with food and water provided *ad libitum. P. berghei,* a rodent malaria parasite obtained from the Immunology Department, NMIMR was used for the evaluation. The parasites were stabilised by continuous re-infection in recipient mice by passaging with 200µL of normal saline diluted parasites through intraperitoneal route (David *et al.*, 2004; Peter and Anatoli, 1998) using a 26G needle and 1mL Terumo® syringe. Parasitaemia was allowed to build- up for three days, after which blood smears were made, by taking blood obtained from tail snips of the mouse, to assess the level of parasitaemia before another passage was done.

Once 30-40 % parasitaemia was attained in the donor mouse, it was anaethesised under chloroform. Following the technique of Hoff, (2000), 0.5ml of blood was drawn by cardiac puncture from it and transferred into a screw capped sterile plastic tube and then topped up to14 ml with normal saline. This served as the inoculums for the *in vivo* assay. Thirty six (36) experimental *icr* mice were then injected intra-peritoneally (ip) with 0.2ml of the suspension (Innocent *et al*., 2009; Jigam *et al*., 2009) and randomly assigned into 5 treatment groups of six mice per group. Twenty four (24) hours after inoculation and infection confirmed, the extracts were administered via oral gavage at dose levels of 100 mg/kg body weight daily for 4 days using 1 ml syringe fitted with a trachea needle (Table 2 below). Artesunate was however administered to the fifth group at the dose of 1.6 mg/kg body weight for 4 days. A sixth and seventh groups of mice with neither plant extract no artesunate served as the control II group. The control II group was given normal saline. A set of mice which had neither infection nor treatment were also maintained along the experimental sets. These were designated control I group.

Table 2: Doses and Concentrations Administered to Experimental and Control *icr* Mice

GROUP	NUMBER OF MICE	TREATMENT	DOSE
1	6	Artesunate	1.6 mg/kg
2	6	ANSF1	100mg/kg
3	6	CALF1	100mg/kg
4	6	CBLF1	100mg/kg
5	6	MILF1	100mg/kg
6	6	Control 1**	
7	6	Normal Saline*	-0.1ml

Lagend: ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae), *M. indica* and *Psidium guajava* (Myrtaceae) leaves extract * Mice that had been infected but not treated; ** Mice that had not been infected no treated.

Further evaluation of the most potent plant extract CALF1 at the same concentration as mentioned above was carried out alongside artesunate and di-methyl-sulfoxide (DMSO) following the protocol explained above on 3 groups of the *icr* mice. This was aimed at confirming the earlier observation and to ascertain the noninterference of the DMSO diluent used for the extract on the *P. berghei* and hence the result of the experiment in general.

Determination of Parasitaemia

Parasitaemia in experimental mice in all groups was monitored by Giemsa-stained thin blood films (WHO (1991) obtained from tail snips of the mice in all the groups (Ene, *et al.* 2009; Lamb and Langhorne 2008) starting from day 1, (24hours) after inoculation of parasites until the sixth day. At least five hundred uninfected RBCs were counted against the infected to determine percent parasitaemia. Percentage suppression of parasitaemia was then calculated using values from control animals using the relation:

[(a – b/a) x100],

Where a = average % Parasitaemia in control groups,

b= average % parasitaemia in extract or artesunate.

Parasite density was expressed as number of parasite per 200 white blood cells x 8000 (Greenwood and Armstrong 1991).

Measurement of Body Weight

Body weights of mice were measured on D0 (the 1st day of the commencement of the experiment) and D7 (the 7th day of the commencement of the experiment) as described by Dikasso *et al*, (2006), using TOLEDO® Metler Balance (Japan), to observe whether the test prevented the weight loss that is common with increasing parasitaemia in infected mice.

Daily Measurement of (Body) Rectal Temperature

Body temperature, of the mice was measured rectally daily for six days starting from the day prior to parasite inoculation. This was achieved by inserting the round tip of a digital thermometer into the anus of the mouse until a beeping sound was heard to signal the reading was confirmed.

Haematogram of Study Mice

This was achieved using terminal blood collected from fully anesthetized mice taken out via a cardiac puncture into ethylenediamine tetra-acetic acid, (EDTA) tubes as described by Hoff (2000). This was then gently and thoroughly mixed to prevent clotting and blood cells disintegration. Haematogram of control and treated mice were achieved using Sysmex KX - 2IN automated haematology analyzer (Baars, 2010).

RESULTS

***In vivo* Antiplasmodial Activities of Selected Extracts**

Body Temperature Recordings

Control I group (mice that had not been infected nor treated with any substance) had the highest mean body temperature of 34.95±0.48°C (Figure 1). CALF1 treatment group had the lowest mean body temperature of 33.39±0.31°C. There was a mean drop in temperature in all the infected mice ranging from 0.15°C to 1.56°C in the treated group. Statistical analysis of the data also confirmed there was significant difference ($P < 0.05$; $P=0.0118$) in the mean body temperatures of the experimental mice. Temperatures recorded for the control I group, the control II and artesunate treated groups were similar but different with that of mice in the four extract treated groups (Figure.1).

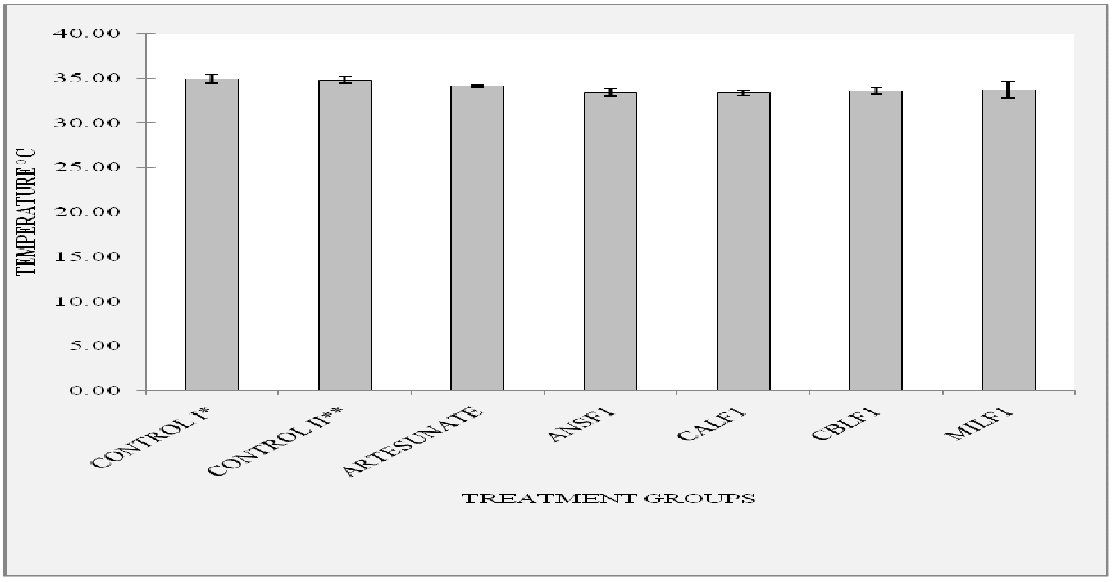

Figure 1: Mean Body Temperature in Experimental and Control Mice Infected with *P. berghei*

LEGEND * mice that had not been infected nor treated with any substance; ** mice that had been infected but not treated; ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract

Body Weight Analysis

Changes in body weights recorded in control and treated icr mice infected with *P. berghei* for the various treatment groups indicated that only mice in the control Group I recorded a positive gain (1.5g or 6%) (Figure 2). Those in the control II group had -5.08 or 17% loss. CBLF1 group had the highest mean final weight loss of -8.19g [27% loss]). This was closely followed by MILF1 with -6.81g [24 % loss]. ANSF1 and CALF1 had -3.95g [13%loss] and -3.13 [11%loss] respectively. The group treated with artesunate recorded the least mean weight loss -2.5g (8%).

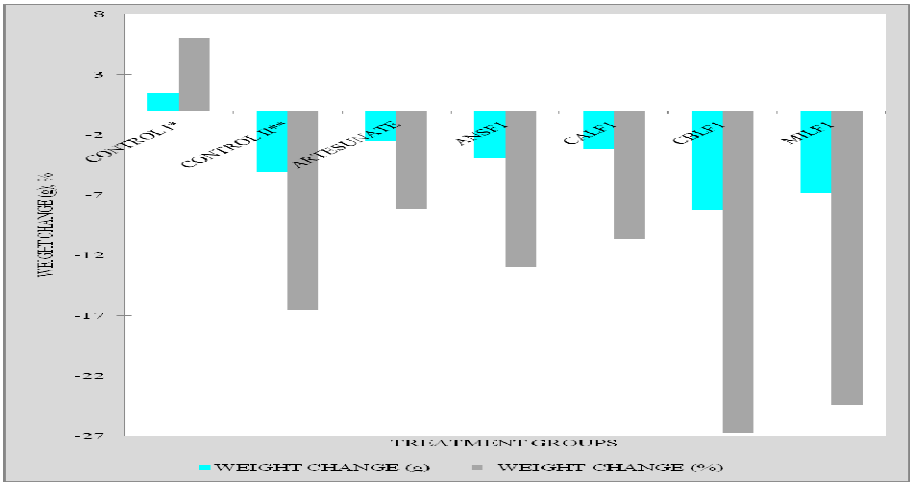

Figure 2: Illustrates Changes in Body weights in Control and Treated icr mice Infected with *P. berghei*
LEGEND * mice that had not been infected nor treated with any substance; ** mice that had been infected but not treated; ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract

Daily Parasitaemia Levels during Treatment

Parasitaemia levels in control and treated *icr* mice on Day 0 (zero), recorded no statistically significant difference in the parasitaemia among the groups (Table 3). Generally, parasitaemia increased in all the groups. Mean % parasitaemia for the 3rd day of the assay were significantly different statistically (P<0.05; 0.0015) as evident from the statistical comparison of the mean % parasitaemia. Mean % parasitaemia values for artesunate (4.32±0.96$^{\mathbf{a}}$) and CALF1 (15.50±6.64$^{\mathbf{a}}$) were lowest compared to the rest. Parasitaemia build up on the third day was highest in the MILF1 treatment group [46.04±8.77$^{\mathbf{d}}$].

Table 3: Daily Parasitaemia (%) in Control and Treated *icr* Mice Infected with *P. berghei* over a 4 – day Treatment Period.

TREATMENT	n	DOSE(mg)	MEAN DAILY PARASITAEMIA			
			DAY 0	DAY 1	DAY 2	DAY 3
CONTROL II**	6	100	2.15±0.32^{a}	3.46±0.64	17.44±2.04	17.24±7.24^{ab}
ARTESUNATE	6	1.6	2.37±0.59^{a}	2.00±0.47	3.82±1.75	4.32±0.96^{a}
ANSF1	6	100	1.52±0.24^{a}	4.346±2.00	56.36±13.25	41.76±17.07^{cd}
CALF1	6	100	2.38±0.24^{a}	4.53±1.70	11.84±3.85	15.50±6.64^{a}
CBLF1	6	100	2.62±1.01^{a}	8.12±2.42	14.27±3.70	24.938±6.62^{bcd}
MILF1	6	100	1.80±0.26^{a}	12.42±1.23	29.91±7.14	46.04±8.77^{d}
LSD			4.74			25.18

Legend: Values are means± standard error of the means; n = initial number of animals in a group.** mice that had been infected but not treated; ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract
All values were compared with each others on days 0, and 3 at P = 0.05; Values with different superscripts vertically differ statistically (P < 0.05)

Daily Parasitaemia Levels after Treatment

Generally, parasitaemia increased in all the groups after the four day treatment period (Table 4). Percent (%) parasitaemia on the 6th day were significantly different with one another with respect to the treatments. It was lowest in the artesunate treated group (15.98±2.34$^{\mathbf{a}}$), followed by the CALF1 treated group (34.44±7.6$^{\mathbf{ab}}$).

Table 4: Daily Parasitaemia (%) Levels in Control and Treated *icr* Mice Infected with *P. berghei* after the Three Days Treatment.

TREATMENT	N	DAY 4	DAY 5	DAY 6
CONTROL II**	6	28.26±6.42	43.74±9.57	49.96±10de
ARTESUNATE	6	2.61±0.73	7.72±1.34	15.98±2.34^{a}
ANSF1	6	24.95±13.69	32.215±16.33	41.79±19.5^{dc}
CALF1	6	22.57±8.55	33.09±6.26	34.44±7.6^{ab}
CBLF1	6	36.86±10.10	47.81±14.03	37.39±12.8^{bc}
MILF1	6	54.06±2.66	74.52±3.77	66.67±0.00^{e}
LSD			22.2	

Legend: Values are means± standard error of the means; n = initial number of animals in a group. ** Mice that had been infected but not treated; ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract; Values were compared with each others on day 6 at P = 0.05; Values with different superscripts vertically differ statistically (P < 0.05)

Daily (%) Parasitaemia Suppression

Daily (%) suppression of parasitaemia levels (Figure 3) in artesunate treated group yielded a suppression of 42% on day 1. This increased to 91% on day 4 of the treatment days. Percentage suppression however gradually dropped to 68.01% by the sixth day. CALF1 produced a suppression of 32.11% on day 2 and the suppression fluctuated and finally dropped to 31.06% by the 6th day. ANSF1 however produced an initial suppression of 11.71% on day 4, which increased to 26.35 and then dropped to 16.35% on the 6th day. CBLF1 exhibited 18.18% suppression on day 2 and increased to 25.16% by the 6th day. MILF1 however exhibited no parasite suppression throughout the period of the experiment.

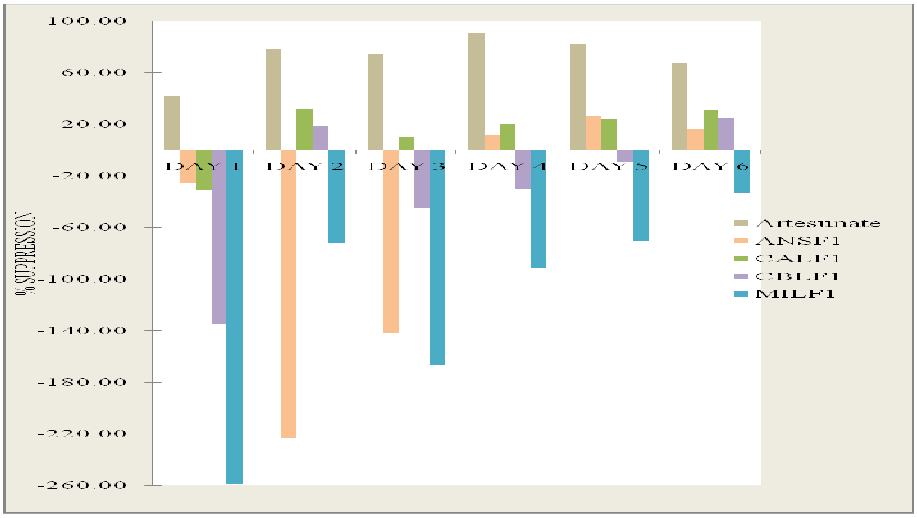

Figure 3: Parasitaemia Suppression (%) in Artesunate and Plant Extracts Treated *icr* Mice Infected with *P. berghei.*

Legend**:** ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract

Parasite Densities in CALF1, Artesunate and DMSO Treated *icr* Mice

Mean daily parasite densities in CALF1 extract treated icr mice in relation to artesunate and Dimethyl sulfoxide, DMSO treated set further confirm the potency of CALF1 extract. The parasite densities on Day 1 were not statistically different from one another in all the treatment groups (P > 0.05; P=0.7367). However, on Day 4, the parasite densities became significantly different among the treatments groups (P< 0.05; P=0.0075). The group treated with DMSO recorded the highest parasite density which increased steadily from Day 3 (15,836 parasite/ml) to Day 7 at about 96 000 parasite/ml (Figure 4). The parasite densities in both artesunate and CALF1 treated groups for both day 4 and 7 were not statistically different.

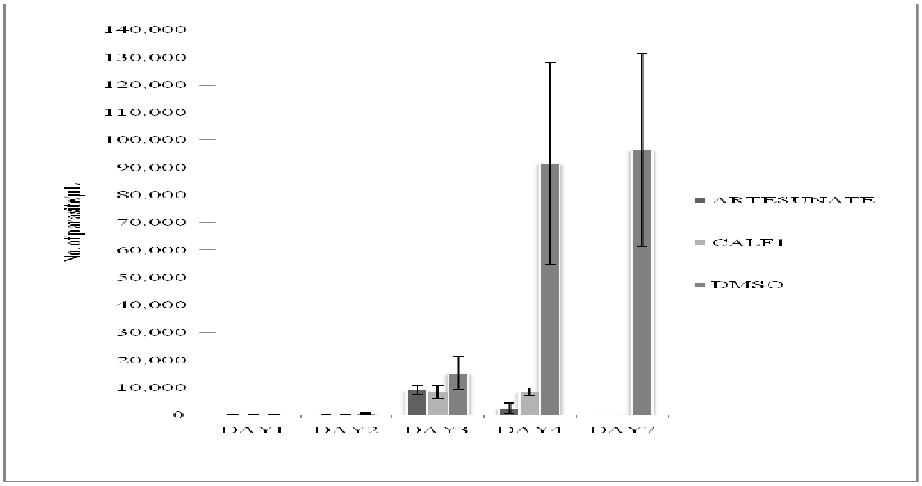

Figure 4: Mean Daily Parasite Densities in Artesunate, CALF1 and DMSO Treated icr - Mice Infected with *P. Berghei*
Legend: ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract

Mice Survival in Control and Treated Groups
All mice (100%) in the control group I survived the period of the experiment (Figure 5). Similarly, all (100%) the parasite inoculated mice in the artesunate treated group survived during the experimental period. Percent survival of parasite infected mice was 67%. Fifty percent (50 %) of the mice in the CALF1 and CBLF1 group survived the experimental period, while only 1 survived to the seventh day in the MILF1 extract treated group. Twenty five or 60% of the 42 mice used survived the experimental period.

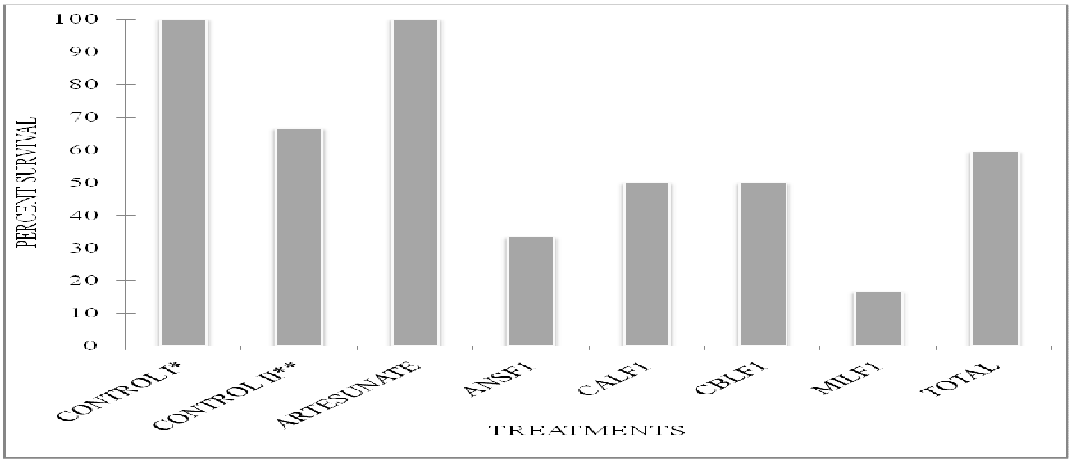

Figure 5: Survival *of icr* mice in control and Experimental Groups Infected with *P. berghei*
LEGEND ANSF1 = *Acacia nilotica* (Fabaceae) stem extract; CALF1 = *Citrus aurantifolia* (Rutaceae) leaf extract; MILF1 = Mangifera *indica* (Anacardiaceae) leaf extract; CBLF1 = Combination of *C. aurantifolia, Carica papaya* (Caricaceae)*, M. indica* and *Psidium guajava* (Myrtaceae) leaves extract * mice that had not been infected nor treated with any substance; ** mice that had been infected but not treated

Haematological Analysis
Analysis of haematological parameters (cardiac blood) in the experimental mice *i* are presented in Table 5 below. The highest haematocrit (%) was measured in the mice in control group I (55%) and the lowest was found in the mice treated with the MILF1 (15%). Haemoglobin was highest in mice in the control I group (13.92±0.4 g/dL) and the lowest was again found among the group treated with MILF1 (3.7±0.0 g/dL). The group of mice treated with CALF1 recorded the highest number of lymphocyte of 9, 733/µL of blood with the lowest number being recorded by the control group I (2,485/µL). Mixed granulocytes (basophils and eosinophils) and neutrophil granulocytes were only detected in the control I group and the DMSO treated mice. Mean platelets counts were highest in mice in the control group I (1,123,500/µl), closely followed by mice in the MILF1 treated group (1,181,000±0.0/µl). The least amount of platelets /µ was observed in mice in the control group II (295,750/µl).

Highest mean RBC counts of 8,226,666/µl and 7,268,000/µl were found among mice in the control group I and the DMSO treated groups respectively; while the least count of 2,314,444/µl was found among the mice treated with MILF1. The highest mean WBC count of 24,650/µl was found among the ANSF1 treatment group and the lowest was found among the control group I (5,066/µl). All the haematological parameters were not statistically different except haemoglobin which had a p-value of p=0.0363.

Table 5: Haematogram in the Control and Treated *icr* Mice Infected with *P. berghei*

PARAMETER	TREATMENT							
	CONTROL I* (n=6)	CONTROL II** (n = 4)	DMSO (n=6)	ARTESUNATE(n= 6)	ANSF1 (n= 2)	CALF1 (n= 3)	CBLF1 (n= 3)	MILF1 (n=1)
HCT%	55.03±1.5	42.03±3.8	39.4±4	29.65±4.2	42.±27.5	39.33±12	22.87±2.8	15.00±0
HGBg/dL	13.92±0.4a	10.25±1.07ab	12±1	7.18±1c.	10.30±6.5ab	9.67±2.8ba	5.7±0.8c	3.7±0.0c
LYM#/uL	5067±537	5567±625	3633.3±1194	6133.±1241	8404.±8396	9733.±1338	2807.±2793	7400±0.0
LYM%	78±1.73	66±6.5	35±13	84±11	50±6	54±4.5	44±7	68±0.0
MCHCg/dL	25.±0.18	24.3±0.53	31±1	24.±.02	25.±1	25±0.5	25±0.9	25±0.0
MCHpg	17±0.26	17±0.3	18±1	16.72±0.41	17.4±1.7	17±0.72	17±0.53	16±0.0
MCVfL	67±0.83	69.4±1.9	59±1	69.3±2.1	69±4.2	67±2.15	68±1.73	65±0.0
MPVfL	6.±0.11	8.4±.37	7.4±0.42	9.6±0.6	10±1.2	9.±0.15	6.8±0	9.7±0.0
MXD#/uL	300±45	0±0	1500±469.04	0±0	0±0	0±0	0±0	0±0
MXD%	6.±1.53	0±0	17±5.3	0±0	0±0	0±0	0±0	0±0
NEUT#/uL	800±84	0±0	4975±1716	0±0	0±0	0±0	0±0	0±0
NEUT%	16±0.73	0±0	53.03±17.3	0±0	0±0	0±0	0±0	0±0
P_LCR%	4±0.35	22.3±2.4	12.03±2.2	28±3.4	26.±8.65	25±0.42	12.1±0	28.5±0.0
PDWfL	7±0.15	15.4±1.5	9.4±0.99	16±1.15	15±2.65	15.±1.18	9.2±0	16.2±0.0
PLT/uL	1123500±103305	295750±101334	53100±14004	357500±96138.7	806500±321500	692333.±229008	707667±154985	1181000±0.0
RBC/uL	8226666±256874	6095000±625333	7268000±641058	43766679±685228	6380000±439000	5973333±190830	3413333±515337	2314444±0
RDW_CV%	12.±0.45	16.1±1.9	12.03±1	16±1.7	15±1.15	16.3±2.14	20.2±2.1	21.2±0.0
RDW_SDfL	32.4±0.8	38.4±6	28±2	40.±3.5	30±0	40±5.22	44.6±2	0±0.0
WBC/uL	5066±537	9333±2151.	8933.3±1969	12100±3160	24650±5650	18033±1225	7400±3600	10900±0

LEGEND: Values are means± standard error of the means; n = # of animals in a group;
HCT= haematocrit; HGB= haemoglobin; LYM= lymphocyte; MCHC=mean corpuscular haemoglobin concentration; MCH=mean cell haemoglobin; MCV=mean cell volume; MPV=mean platelets volume; MXD=mixed (eosinophiles, basophiles &monophiles); NEUT=neutrophiles; P_LCR= platelets - large cell ratio; PDW= platelets distribution width; PLT= platelets; RBC=red blood cells; RDW=RBC distribution width and WBC=white blood cells.
* Mice that had not been infected nor treated with any substance; ** mice that had been infected but not treated

DISCUSSION

All the extracts evaluated did not prevent phenomenal drop in body temperature with increasing parasitaemia in infected mice (Figure 1) as earlier observed by Jutamaad *et al.*, (1998). The drop in temperature in the artesunate group was minimal and points to the efficiency of artesunate in suppressing the *P. berghei* parasitaemia *in vivo*. The assay of the artesunate and CALF1 indicated no significant difference between the treatments ((P < 0.05)). The activities exhibited by CALF1 (Rutaceae) ANSF1 (Fabaceae) and CBLF1 (a combination) in the i*n vivo* assay corroborates the *in vitro* activity reported earlier (Dabo *et al*., 2013). The result for CBLF1 suggests the synergistic effects of the constituent plants in CBLF1, (*M. indica, C. papaya, P. guajava and C. aurantifolia*).

Premature destruction of RBCs during the course of malaria infection (Claire *et al.* 2004; Rowe *et al.* 2009; Pasvol, 1986) is a very good index for anaemia (Dikasso, 2006). The value obtained for all the *P. berghei* infected groups were found to be appreciably below the normal. This is consistent with Dikasso (2006) assertion that as 'parasite count increased haematocrit decreased'. Reduction in the leukocyte count is attributed to hypersplenism or sequestration in the spleen (Claire *et al.* 2004). The results obtained in this work indicated that the relatively low WBC count (5066±537/µl) in the control group mice and higher (8933±1969/µl to 24650±5650/µl) in the other groups is consistent with Claire *et al.* (2004) and Patients Against Lymphoma, P.A.L. (2011), that increased leukocyte count indicates severe infection.

The absence of the neutrophil granulocytes and the mixed granulocytes (i.e. basophils, eosinophils and monocytes) as well as the observed elevation of lymphocytes is consistent with the occurrence of malaria induced changes in the differential white cell counts associated with human malaria due to *P. falciparum* (Taylor, 2008).

Thrombocytopenia which is an index of severe infection (Claire *et al.* 2004) including falciparum malaria (Maina *et al*., 2010) had been observed in all the experimental *icr* mice except MILF1 group.

It is noteworthy that the DMSO control group suffered the highest degree of thrombocytopenia. It may therefore be inferred that DMSO used may have some impact on this phenomenon. Weight loss was worse in the control group II mice (*icr* mice infected with *p. berghei* but not treated), compared to other groups. This may suggest bioactivity effects of these extracts in reducing the weight loss associated with malaria.

CONCLUSION

The activities exhibited by CALF1 (Rutaceae), CBLF1 (a combination) and ANSF1 (Fabaceae),) when evaluated against *P. berghei*, *in vivo* using *icr* mice as animal model establishes the bio-availability and antiplasmodial activities of the active substances in the extracts evaluated and is consistent with the observed *in vitro* reported earlier by Dabo, *et al*., (2013).

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