



Antiplasmodial Potential of Combination Therapy of Methanolic Bark Extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* and Its Effect on Haematological Parameters on Mice Infected with *Plasmodium berghei*

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: The resistance of *Plasmodium* species to drugs has necessitated the search for more potent drugs. *Anogeissus leiocarpus* and *Terminalia avicennioides* have been considered traditionally for the treatment of malaria.

Aim: This study assessed the efficacy of combination therapy of methanolic bark extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* on malaria parasite and its effect on haematological and lipid profile on mice infected with *Plasmodium berghei*.

Methodology: Thirty-six mice were distributed into six groups. The first group was not infected with the parasite (normal control). The second group was infected but not treated (negative control). The

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third group was infected and treated with 5.0 mg/kg of Artemether-Lumefantrine (positive control), while the third, fourth and fifth groups were infected and treated with 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight of combined methanolic bark extracts of *T. avicennioides* and *A. Leiocarpus* respectively. Treatments were administered for four days. Blood was taken daily from the tail of mice for the assessment of parasitaemia. The animals were sacrificed on the fifth day. Whole blood and serum were collected into EDTA and plain bottles respectively. The blood in the EDTA was used to determine haematological parameters, while lipid profile was determined using serum.

Results: The results showed that the parasite clearance was highest in the group treated with 400 mg/kg and lowest in the group treated with 100 mg/kg. White blood cell, lymphocyte, haemoglobin, red blood cell and platelets levels were significantly higher ($P<0.05$) in normal control than in other groups. White blood cell count was lowest in the group treated with 400 mg/kg when compared with other treated groups. Haemoglobin was significantly reduced in the groups treated with 100 and 400mg/kg when compared with the group treated with 200 mg/kg. Platelet count was significantly higher ($P<0.05$) in the group treated with 400 mg/kg than in all other treated groups. Mean HDL and triglyceride levels were significantly reduced in the group treated with 400 mg/kg than in all other treated groups.

Conclusion: Antiplasmodial potential of combination therapy of the bark extracts of *A. leiocarpus* and *T. avicennioides* was highest at 400 mg/kg but that concentration had serious adverse effect on the lipid profile, body weight and haematological parameters on mice.

Keywords: Malaria; combination therapy; antiplasmodial; *A. leiocarpus*; *T. avicennioides*; blood count; lipid profile.

1. INTRODUCTION

The episode of parasitic diseases is becoming alarming despite all the concerted effort of different organs of government and private organisation to curb it. Among the tropical parasitic diseases, malaria infection is one of the great concern to World Health Organization (WHO), because it is responsible for the highest number of morbidity and mortality especially in children and pregnant women [1,2]. Recently, the mortality has been greatly reduced as a result of tremendous activity of WHO [3], but malaria is still prevalent among the rural dwellers where there is no special documentation of mortality rate. Among many factors that have been attributed to the prevalence of malaria infection in the tropics, drug resistance and poverty have been considered to be the major factors. Most people living in malaria endemic areas have resulted into the use of traditional herbal medicine for the treatment of malaria infection because of those factors. Many cultures have replaced orthodox medicine with the traditional herbal medicine and presently traditional herbal medicine has become a comprehensive system of health care delivery in some community [4]. The last two decades had witnessed a globally renewed interest in the use of traditional and Complementary and Alternative Medicine [5,6]. Some of the best-known Traditional medicine systems include traditional Indian (Ayurveda) medicine, traditional Chinese medicine (TCM), and traditional Arabic (Unani) medicine [7].

In Nigeria the use of traditional herbal medicine is becoming common than before. Among the medicinal plants used for the treatment of malaria infection are *Terminalia avicennioides* and *Anogeissus leiocarpus* [8]. The antihelmintic and antimicrobial activities of the extracts of these two plants have been studied [9,10]. The antiplasmodial activities of leaf and bark extracts of each of these plants have been also reported [11,8], but there is no record about the antiplasmodial activities of combined bark extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus*. It is therefore imperative to study the combination therapy of bark extracts of these plants against malaria infection, especially now that WHO is clamouring for combination therapy as a strategy to prevent or delay resistance of parasites to antimalarial drugs [12,13]. This work therefore study the antiplasmodial activities of bark extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* and its effect on the haematological parameters and lipid profile on mice infected with *Plasmodium berghei*.

2. MATERIALS AND METHODS

Adult Swiss albino mice used for this study were obtained from the Institute for Advanced Medical Research and Training, College of Medicine, University Teaching Hospital, University of Ibadan, Ibadan, Nigeria. The animals were kept in well aerated wired cages, and they were kept for two weeks to acclimatize with the new

immediate environment before they were infected with the malaria parasite.

2.1 Parasite Acquisition

The parasite used (*Plasmodium berghei* NK 65) was a donation from Professor O.G. Ademowo, Institute for Advanced Medical Research and Training, College of Medicine, University Teaching Hospital, University of Ibadan, Ibadan, Nigeria. The parasites were maintained in the animals by serial passage of blood collection from a parent donor to a naïve recipient.

2.2 Plant Materials

The barks of *Anogeissus leiocarpus* (locally called *Ayin* in Yoruba language) and *T. avicennioides* (locally called *Udi* in Yoruba language) were collected in Akungba-Akoko, Ondo State, Nigeria, and was identified by Dr. A.O. Obembe, Plant science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo state, Nigeria. The Herbarium specimen with voucher number UIH22318 *Anogeissus leiocarpus* and UIH22319 *T. avicennioides* were deposited at the Herbarium unit of the University of Ibadan, Ibadan, Nigeria.

2.3 Plant Extraction

Anogeissus leiocarpus and *Terminalia avicennioides* barks were harvested and air dried under shade and was later ground into powder. 300g of both barks powder was soaked into 1000ml of methanol for 72 hours. The extracts were filtered and evaporated to dryness with a rotary evaporator. 1.17 g of each methanolic bark extract was diluted into 65ml of distilled water to make the solution for treatment.

2.4 In-vivo Antimalarial Assay

Thirty six Swiss albino mice weighing from 18 – 21 g were distributed into six groups with six animals in each group. The first group (normal control) was not infected with *Plasmodium berghei*. All the infected mice were infected intraperitoneally with an aliquot of 0.2 ml of standard inoculum (1×10^7 *Plasmodium berghei* strain NK 65 parasitized erythrocytes). Among the infected groups, the second group was infected with the *Plasmodium berghei* but not treated (negative control). The third group was infected with the *P. berghei* and treated with

5 mg/kg body weight of Artemether-Lumefantrine (positive control), the fourth group was infected with the *Plasmodium berghei* and treated with 100 mg/kg body weight of combination of bark extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus*, the fifth group was also infected with the *P. berghei* and treated with 200 mg/ kg body weight of combination of bark extracts of *T. avicennioides* and *A. leiocarpus* and the sixth group was also infected with *P. berghei* and treated with 400 mg/kg body weight of combined bark extracts of *T. avicennioides* and *A. leiocarpus*. The treatments were done orally with the intubator once daily for four consecutive days. Blood was taken from the tail vein of the mice daily during the period of treatment to assess daily parasitaemia count.

2.5 Determination of Weight Gain

The initial weights of mice were taken before the animals were infected with the parasite. The weight was taken daily before the treatment and the final weight was taken on the last day before the animals were sacrificed. Weight gained was calculated by deducting initial weight from the final weight.

2.6 Parasitaemia Count

Blood was collected from the tail vein of the infected mice and both thick and thin smear were prepared on a microscope slide. The thin film was fixed with 30% methanol. Staining was done with Giemsa stain and the thick film on slide was screened under light microscope with X100 magnification and parastaemia count was done. On the fifth day of treatment, mice were anaesthetized using chloroform. Blood was collected into EDTA and plain bottles by cardiac puncture. The blood in EDTA bottle was used to determine haematological parameters and the final parasitaemia count, while serum obtained from the blood in the plain bottle was used to determine biochemical parameters.

2.7 Biochemical Assay

Serum LDL-cholesterol level was calculated using Friedewald [14] formula: $LDL = (TC - HDL) - (TG/5.0)$. Serum total triglycerides concentration was measured by the Tietz [15] method, as described in the manual of the Randox Total triglycerides kit. Serum total cholesterol level was measured by the Trinder [16] method, as

described in the manual of the Randox Total cholesterol kit. Serum HDL-cholesterol concentration was measured by the NIHCDS [17] method, as described in the manual of the Randox HDL-cholesterol kit.

2.8 Determination of Haematological Parameters

Blood sample collected into EDTA bottle was used to determine haematological parameters by the automated machine; Diatron Abacus 380 haematocrit machine and the procedure was carried out according to the manual of the automated machine.

2.9 Statistical Analysis

The differences among groups were analyzed by the one-way analysis of variance (ANOVA). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 15.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. The results were expressed as mean ± standard error mean (SEM). The level of significance was estimated at $P < .05$.

3. RESULTS

Table 1 showed that there was a sharp increase in the parasitaemia count on day 2 in the negative control and this was reduced in day 3. The parasitaemia was later significantly increased in day 4 and 5 when compared with day 3. Among the treated groups, the parasitaemia was decreasing from day 2 to day 5

when compared with day 1. The rate of reduction was higher in the groups treated with 200 mg/kg and 400 mg/kg than in other groups. The parasite clearance rate was highest in the group treated with 400 mg/kg.

Fig. 1 showed that the body weight gain was higher in the normal control and groups treated with 100 mg/kg, and 200 mg/kg when compared with the positive and negative control. Although the body weight gain was higher in the group treated with 400 mg/kg when compared with the negative control, but the difference was not significant. The body weight gain was highest in the group treated with 100 mg/kg while it was least in the negative control.

The WBC count was significantly higher ($p < 0.05$) in the normal control than in all other groups. The level of WBC was significantly lower in the group treated with 400 mg/kg than in the negative and positive control (Table 2). Among the treated groups, WBC and RBC levels were highest in the group treated with 100 mg/kg than in the other treated groups. Haemoglobin was significantly higher in the normal control than in the group treated with 100 mg/kg and 400 mg/kg. Red blood cell was significantly reduced in the group treated with 400 mg/kg than in the normal control. Platelets was significantly higher ($p < 0.05$) in the group treated with 400 mg/kg than in all other groups except normal control, while it was lowest in the group treated with 100 mg/kg. Lymphocyte level was significantly reduced in the group treated with 100 mg/kg than in the negative control. All the test groups had lower lymphocyte levels when compared with normal, negative and positive controls.

Table 1. Parasitaemia counts in mice infected with *P. berghei* and treated with combined methanolic bark extracts of *T. avicennioides* and *A. leiocarpus*

Day	Negative control	Positive control	MP+100 mg/kg	MP+200 mg/kg	MP+400 mg/kg
1	980±52.0 ^a	1401.5±34.0 ^a	1261.5±20.0 ^a	1219.8±32.0 ^a	1491.5±27.0 ^a
2	1136.80±44.0 ^b	1121.2 ±20.0 ^b	1059.66±35.0 ^b	975.84±33.0 ^b	1163.37±26.0 ^b
3	1097.60 ±23.5 ^b	910.98±23.0 ^{bc}	946.13±28.0 ^c	646.49±18.0 ^c	894.90±18.0 ^c
4	1274.00±22.0 ^{bc}	574.62±22.0 ^{bd}	782.13±32.0 ^d	317.15±20.0 ^d	343.05±27.0 ^d
5	1568.00±32.0 ^{bd}	252.27±21.0 ^{be}	252.3±43.0 ^e	146.38±16.0 ^e	00 ^e

*The data showed mean parasitaemia counts in the negative control, positive control and the groups treated with combined methanolic bark extracts of *A. leiocarpus* and *T. avicennioides*.

**Mean parasitaemia count in day 1 was considered as the initial count and this was compared with the mean parasitaemia count in subsequent days.

***MP represents Malaria parasite

Table 2. Effect of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioides* on haematological parameters in mice infected with *P. berghei*

Haematological parameters	Normal control	Negative control	Positive control	100 mg/kg Test 1	200 mg/kg Test 2	400 mg/kg Test 3
WBC×10 ⁹ /L	22.10±14.04 ^b	11.43±0.36 ^a	9.66±3.03 ^a	8.04±3.00 ^a	7.97±0.40 ^{ac}	7.66±0.99 ^{ac}
LYM×10 ⁹ /L	16.48±12.38 ^b	8.68±0.97 ^{ac}	6.64±3.47 ^a	4.80±1.26 ^{ac}	5.55±2.80 ^{ac}	4.53±0.80 ^{ac}
MID×10 ⁹ /L	2.00±0.93 ^c	1.06±0.50 ^{ab}	1.19±0.40 ^{ab}	1.55±0.72 ^{bc}	0.92±0.23 ^a	0.72±0.25 ^a
GRA×10 ⁹ /L	3.68±2.19 ^b	1.67±0.76 ^a	1.97±0.66 ^a	1.96±1.12 ^a	1.37±0.61 ^a	2.05±1.16 ^a
PLT×10 ⁹ /L	693.00±66.41 ^a	500.00±70.0 ^b	465.66±91.4 ^{bc}	505.00±14.85 ^{bc}	515.33±15.0 ^{bc}	544.00±00.79 ^b
LYM %	70.26±16.65 ^{ab}	76.10±10.20 ^b	72.33±16.90 ^{ab}	59.14±10.40 ^a	67.17±12.97 ^{ab}	62.20±16.52 ^{ab}
MID %	9.20±1.54 ^a	9.17±4.11 ^a	13.16±4.67 ^b	18.79±3.72 ^c	12.00±2.10 ^{ab}	12.90±4.33 ^b
GRA %	20.26±17.29 ^a	14.43±6.20 ^a	21.83±11.31 ^a	21.83±7.67 ^a	20.56±11.51 ^a	24.63±12.22 ^a
HCT %	43.08±2.97 ^b	43.42±1.20 ^b	43.24±0.97 ^b	32.29±9.51 ^a	40.87±4.51 ^b	33.89±10.59 ^a
PCT %	0.37±0.07 ^{ab}	0.36±0.46 ^a	0.32±0.62 ^a	0.37±0.08 ^{ab}	0.51±0.04 ^c	0.42±0.03 ^b
RDWC %	17.90±0.53 ^a	18.33±0.15 ^a	18.40±1.57 ^a	17.36±0.10 ^a	18.20±1.16 ^a	19.06±2.00 ^a
PDWC %	35.89±2.19 ^a	35.78±0.71 ^a	36.00±1.53 ^a	36.54±0.39 ^a	35.16±0.65 ^a	38.36±1.78 ^b
HGB g/dl	15.03±1.13 ^b	10.76±0.22 ^b	13.76±0.27 ^b	12.56±2.57 ^a	12.00±1.42 ^b	10.79±1.20 ^a
MCHC g/dl	32.61±0.36 ^{ab}	31.76±1.03 ^a	32.00±1.16 ^a	33.33±2.11 ^b	31.97±0.23 ^a	32.01±0.52 ^a
RBC×10 ¹² /L	9.73±0.11 ^b	9.04±0.35 ^a	8.92±0.25 ^a	9.07±0.08 ^a	8.18±0.19 ^a	6.91±2.37 ^a
MCV (fl)	48.33±2.78 ^a	46.66±1.32 ^a	47.33±2.91 ^a	46.66±1.58 ^a	49.00±2.44 ^a	48.66±2.91 ^a
MPV (%)	7.36±0.90 ^a	6.97±0.15 ^a	7.16±0.15 ^a	7.46±0.27 ^a	7.46±0.23 ^a	7.97±0.74 ^a
MCH (Pg)	16.09±0.91 ^a	15.13±0.48 ^a	15.43±0.21 ^a	15.87±0.62 ^a	15.92±0.62 ^a	15.86±1.04 ^a
LYSE ml	0.99±0.00 ^a	0.99±0.00 ^a	0.99±0.00 ^a	0.99±0.00 ^a	0.99±0.00 ^a	0.99±0.00 ^a

*Positive control was infected with *P. berghei* and treated with 5 mg/kg (kilogram/body weight) of combisunat

*Test groups were infected with *P. berghei* and treated with combined methanolic bark extracts of *A. leiocarpus* and *T. avicennioides*
Means with different superscripts are significantly different at $P < 0.05$

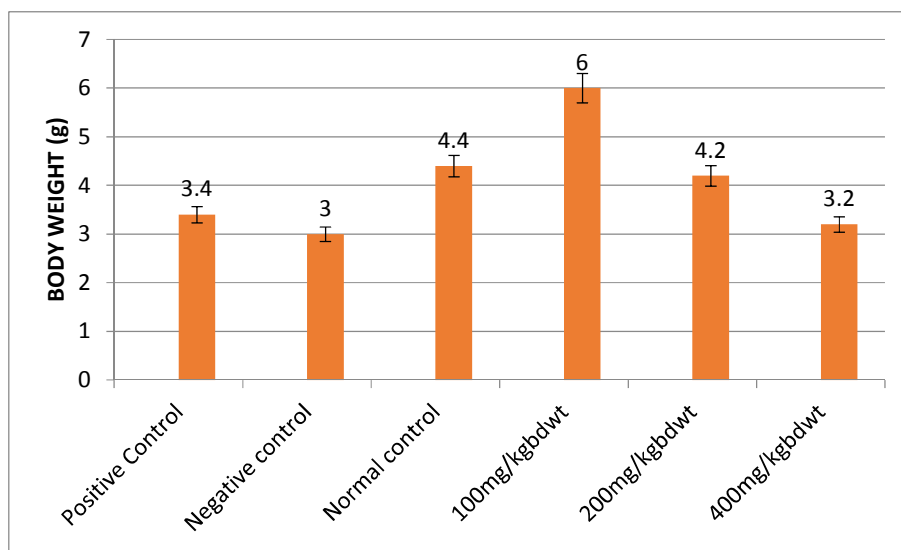


Fig. 1. Effect of combined methanolic bark extracts of *A. leiocarpus* and *T. avicennioides* on the body weight of mice infected with *Plasmodium berghei*
**bars represents standard error means*

Table 3. Effect of treatment with combination bark extracts of *A. leiocarpus* and *T. avicennioides* and malaria parasite on some lipid profile in mice

Treatment	HDL	Triglyceride	LDL	Cholesterol
Normal Control	1.34±0.14 ^a	1.47±0.13 ^a	0.95±0.02 ^a	2.97±0.14 ^a
Positive Control	0.83±0.01 ^b	1.50±0.80 ^a	1.11±0.00 ^b	2.58±0.00 ^b
Negative Control	0.79±0.04 ^b	1.23±0.07 ^a	1.22±0.00 ^c	2.61±0.00 ^c
100mg/kg	0.80±0.00 ^c	0.68±0.30 ^b	1.43±0.19 ^c	2.59±0.13 ^c
200mg/kg	0.81±0.78 ^b	0.92±0.58 ^b	1.21±0.02 ^d	2.41±0.09 ^c
400mg/kg	0.34±0.15 ^d	0.55±0.24 ^b	0.69±0.31 ^a	1.29±0.50 ^d

**Positive control was infected with P. berghei and treated with 5 mg/kg (kilogram/body weight) of Artemether-Lumefantrine.*

***Test groups were infected with P. berghei and treated with combined methanolic bark extracts of A. leiocarpus and T. avicennioides.*

****Means with different superscripts are significantly different at P<0.05*

Table 3 showed that the mean HDL level was significantly higher ($p<0.000$) in the normal control than in all other groups. Mean HDL was significantly reduced in the group treated with 400 mg/kg than in all other groups. The HDL level was significantly higher ($p<0.05$) in the positive control than in the groups treated with 100 mg/kg and 400 mg/kg. The triglyceride level was significantly higher ($p<0.001$) in the normal and positive controls than in all the treated groups and negative control. Among all the groups studied, the level of triglyceride was lowest in the group treated with 400 mg/kg. Cholesterol level was significantly increased ($p<0.05$) in the normal control than in all other treated groups. LDL level was significantly higher ($p<0.05$) in the group treated with 100 mg/kg than in all other treated groups.

4. DISCUSSION

The resistance of malaria parasite to some conventional antimalarial drugs and high cost of these drugs make it unaffordable to the people living in malaria endemic areas. This therefore necessitated the use of medicinal plants for the treatment of malaria infection. This study considered the potency of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioides* in the treatment of malaria infection over the combination therapy of Artemether-Lumefantrine which is one of the conventional drugs against malaria infection in Nigeria.

The result of this study showed that the parasite clearance of combination therapy of methanolic

back extract of *A. leiocarpus* and *T. avicennioids* at 100 mg/kg body weight was similar with the group treated with Artemether-Lumefantrine. This showed that the potency of the combination therapy of *A. leiocarpus* and *T. avicennioids* at this dosage was similar to that of Artemether-Lumefantrine. The parasite clearance in the groups treated with 200 mg/kg and 400 mg/kg was higher when compared with the positive control, especially in the group treated with 400 mg/kg of combination therapy of *A. leiocarpus* and *T. avicennioids*. This showed that parasite clearance of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioids* was dose related. This agreed with our previous studies where the treatment was done with the bark extracts of *A. leiocarpus* and *T. avicennioids* separately [11,8].

The effect of treatment with combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioids* on the weight gain by the mice infected with *P. berghei* was also considered. The result showed a significant increase in the weight gain of the infected mice treated with 100 mg/kg when compared with other groups. This significant increase in the weight gain of the mice treated with 100 mg/kg showed that the combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioids* at this dosage may boost the body weight of the animal. The significant reduction in the weight gain by the mice treated with 400 mg/kg when compared with the normal and positive controls showed that treatment at this dosage may have adverse effect on the bodyweight of the organism.

The abnormalities that occur in haematological parameters during malaria infection have been reported by some studies [18,19,20]. In this study the white blood cell count was significantly higher in the normal control as compared with all the infected groups. This study confirmed the previous report that white blood cell counts are generally low during malaria infection when compared with normal individual [21]. The reduction in WBC counts may be due to localization of leucocytes away from the peripheral circulation and to the spleen and other marginal pool instead of total depletion from the system [21]. There was reduction in the WBC count in all the groups treated with combination therapy of *A. leiocarpus* and *T. avicennioids* extracts when compared with the positive control group. The group treated with 400 mg/kg had lowest WBC count in this study. The total clearance of the parasite in this group could be

responsible for the reduction in the WBC counts when compared with other groups. The significant reduction in lymphocytes in all the infected groups as seen in this study when compared with the normal control could be as a result of redistribution of lymphocyte with sequestration in the spleen. This has also been reported by [18]. The reduction in lymphocyte count reported in the group treated with 400 mg/kg combination therapy of *A. leiocarpus* and *T. avicennioids* crude extract may be due to the adverse effect of high dosage of the extracts.

Anaemia is one of the most common complications in malaria infection. Different aetiology of anaemia during malaria infection has been reported by different authors [18,22]. The significant increase in Hb level in the normal control group in this study when compared with other infected groups showed that malaria parasite could be responsible for the destruction of the Hb. Though the parasitaemia was lowest in the group treated with 400 mg/kg (Table 1), but the Hb and RBC levels were significantly reduced in this group than in other treated groups (Table 3). This showed that apart from the effect of parasite on the Hb level and RBCs count, treatment with combination therapy of *A. leiocarpus* and *T. avicennioids* extracts at high dosage may also contribute to the destruction of Hb and RBC. There was no significant difference in MCV, MPV, and MCH in all the treated groups when compared with normal and negative control groups.

The significant increase in platelet in the normal control than in the infected groups indicated that there was thrombocytopenia in those that were infected with malaria parasite. This concurred with the report of Coelho, et al. [23] which showed that thrombocytopenia is a common phenomenon in malaria patient when compared with non-infected person. The significant increase in the mean platelet count of the group treated with 400 mg/kg, and the significant reduction in the group treated with 100 mg/kg when compared with other treated groups is a reflection of the parasitaemia count in each of the group (Table 1). This showed that the level of parasitaemia count in individuals could affect the platelet count in the body.

It has been reported that the acute phase response to malaria infection is associated with changes in lipid metabolism including a moderate increase in serum triglyceride and VLDL with a decrease in HDL and LDL levels [11,24]. The

significant increase in HDL level in the normal control when compared with all other infected groups in this study showed that malaria infection may have impact on the HDL level. Though the parasitaemia count was significantly lower in the group treated with 400 mg/kg body weight of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioids* but HDL, triglyceride, LDL and Cholesterol levels were significantly reduced in this group when compared with other treated groups. This showed that treatment with combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioids* at higher dosage could be dangerous and may be responsible for arteriosclerosis. The HDL level was highest in the group treated with 200 mg/kg body weight of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicennioids*. This agreed with the previous study [25].

5. CONCLUSION

Though the antiplasmodial potential of combination therapy with methanolic bark extracts of *A. leiocarpus* and *T. avicennioids* was higher at 400 mg/kg body weight in this study, but treatment at this dosage had serious adverse effect on haematological parameters, lipid profile and body weight. Hence it cannot be considered as the best dosage. The potency of the treatment with combination therapy of bark extracts at 100 mg/kg was similar with the positive control and the adverse effect at this dosage was mild when compared with other groups treated with combination therapy of bark extracts of *A. leiocarpus* and *T. avicennioids*. Thus, it is possible to conclude that the best dosage of combination treatment with bark extracts of *A. leiocarpus* and *T. avicennioids* is at 100 mg/kg.

CONSENT

It is not applicable to this study.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 8523, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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