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Original Research Article

ANTIPLASMODIAL POTENTIAL OF COMBINATION THERAPY OF METHANOLIC BARK EXTRACTS OF *Terminalia. avicennioides* and *Anogeisus leiocarpus* AND ITS EFFECT ON HAEMATOLOGICAL PARAMETERS IN MICE INFECTED WITH *Plasmodium berghei*

Background: The resistance of *Plasmodium* species to drugs has necessitated the search for more 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 in 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 third group was infected and treated with 5.0mg/kg of combisunate (positive control), while the third, fourth and fifth groups were infected and treated with 100mg/kg, 200mg/kg, and 400mg/kg body weight of combined methanolic bark extracts of *T. avicennioides* and *A. leiocarpus*. 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 determine using serum.

Results: The results showed that the parasite clearance was highest in the group treated with 400mg/kg and lowest in the group treated with 100mg/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 counts was lowest in the group treated with 400mg/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 200mg. Platelets was significantly higher ($P<0.05$) in the group treated with 400mg/kg than in all other treated groups. Mean HDL and triglyceride levels were significantly reduced in the group treated with 400mg/kg than in all other treated groups..

Conclusion: Antiplasmodial activities of combination therapy of the bark extracts of *A.leiocarpus* and *T.avicennioides* was highest at 400mg/kg but that concentration had serious adverse effect on the system of the organism.

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1. INTRODUCTION

The episode of parasitic diseases has 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 WHO, because it is responsible for the highest number of morbidity and mortality especially in children and pregnant women [1]. Recently, the mortality has been greatly reduced as a result of tremendous activity of WHO [2], but malaria still prevalent among the rural dweller 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. Majority of people living in malaria endemic areas have resulted into use of traditional medicine because of those factors. Many cultures have replaced orthodox medicine with the traditional medicine and presently traditional medicine has become comprehensive system of health care delivery in some community [3]. The last two decades have witnessed globally renewed interest in the use of traditional and Complementary and Alternative Medicine [4, 5]. Some of the best-known Traditional medicine systems include traditional Indian (Ayurveda) medicine, traditional Chinese medicine (TCM), and traditional Arabic (Unani) medicine [6].

In Nigeria the use of traditional medicine is becoming common than before. Among medicinal plants use for the treatment of malaria infection are *Terminalia avicennioides* and *Anogeissus leiocarpus* [7]. The antihelmintic and antimicrobial activities of the extracts of these two plants have been studied [8, 9].The antiplasmodial activities of leaf and bark extracts of each of these plants have been also reported [10, 7], but there is no yet record about the antiplasmodial activities of combined bark extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus*. It becomes imperative to study the combination therapy 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 [11, 12]. The work therefore study the antiplasmodial activities of bark extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* and its effect on the haematological parameters in 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 Advance 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 patent donor to a naïve recipient.

2.2 Plant Materials

The barks of *Anogeissus leiocarpus* (locally called *ayin* in Yoruba language) amd *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

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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 extract was filtered and evaporated to dryness with a rotary evaporator. 1.17g 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 - 21g 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 0.2ml of standard inoculum (1x107 *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 5mg/kg body weight of combisunate (positive control), the fourth group was infected with the *Plasmodium berghei* and treated with 100mg/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 200mg/ 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 400mg/kg body weight of combined bark extracts of *T. avicennioides* and *A. leiocarpus*. The treatments were done once daily with the intubator 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 on each day 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 slide was screened under light microscope with X100 magnification and parastemia count was done. On the fifth day of treatment, mice were dazed using chloroform. Blood was collected into EDTA and plain bottles. The blood in EDTA bottle was used to determine haematological parameters and the final parasitamia count, while serum obtained from the blood in the plain bottle was used to determine biochemical parameters.

2.7 Biochemical Assay

2.7.1 Total triglycerides assay

Serum total triglycerides concentration was measured by the Tietz [13] method, as described in the manual of the Randox Total triglycerides kit.

Total cholesterol assay

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Serum total cholesterol level was measured by the Trinder [14] method, as described in the manual of the Randox Total cholesterol kit.

2.71 HDL-cholesterol assay

Serum HDL-cholesterol concentration was measured by the NIHCDS [15] method, as described in the manual of the Randox HDL-cholesterol kit.

2.72 LDL-cholesterol assay

Serum LDL-cholesterol level was calculated using Friedewald formula: LDL= (TC – HDL) – (TG/5.0).

2.73 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 manure of the automated machine.

2.8 Statistical Analysis

The differences among the groups were analysed by the one-way analysis of variance and the significant test was done using Microsoft excel 2007 and SPSS 17.0 software and descriptive and inferential statistics for this analysis. The results were expressed as Mean± Standard Error (SE), where the ANOVA level of significance was considered as $P<0.05$.

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3. RESULTS

Table 1 shows 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 rates of decreasing were higher in the groups treated with 200mg/kg and 400mg/kg than in other groups. The parasite clearance rate was highest in the group treated with 400mg/kg as shown in table 2.

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

The WBC 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 400mg/kg than in the negative and positive control (Table 3). Among the treated groups, WBC and RBC levels were highest in the group treated with 100mg/kg than in the other treated groups. Haemoglobin was significantly higher in the normal control than in the group treated with 100mg/kg and 400mg/kg. Red blood cell was significantly reduced in the group treated with 400mg/kg than in the normal control. Platelets was significantly higher ($P<0.05$) in the group treated with 400mg/kg than in all other groups except normal control, while it was lowest in the group treated with 100mg/kg. Lymphocyte level was significantly reduced in the group treated with 100mg/kg than in the negative control. All the test groups had lower lymphocyte levels when compared with normal, negative and positive controls.

Table 4 shows 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 400mg/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 100mg/kg and 400mg/kg while it was only marginally higher than in the group treated with 200mg/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 400mg/kg. Cholesterol level was significantly increased ($P<0.05$) in the normal control than in all other treated groups. LDL level was significantly lower ($P< 0.05$) in the group treated with 100mg/kg than all other treated groups.

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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+100mg/kg	MP+200mg/kg	MP+400mg/kg
1	980±52.0	1401.5±34.0	1261.5±20.0	1219.8±32.0	1491.5±27.0
2	1136.80±44.0	1121.2 ±20.0	1059.66±35.0	975.84±33.0	1163.37±26.0
3	1097.60 ±23.5	910.98±23.0	946.13±28.0	646.49±18.0	894.90±18.0
4	1274.00±22.0	574.62±22.0	782.13±32.0	317.15±20.0	343.05±27.0
5	1568±32.0	252.27±21.0	252.3±43.0	146.38±16.0	00

*The data shows mean parasitaemia counts in the negative control and the groups treated with combined methanolic bark extracts of *A.leiocarpus* and *T. avicenniodes*.

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

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Table 2: Parasite clearance rate 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+100mg/kg (%)	MP+200mg/kg (%)	MP+400mg/kg (%)
1	100	100	100	100	100
2	116	80	84	80	78
3	122	65	75	53	60
4	130	41	62	26	23
5	160	18	20	12	00

*The data shows mean percentage parasitaemia levels in negative control and groups treated with combined methanolic bark extracts of *A.leiocarpus* and *T. avicenniodes*.

**Mean percentage for day 1 were considered as 100%, while mean percentages of other days were compared with the mean percentage of day 1.

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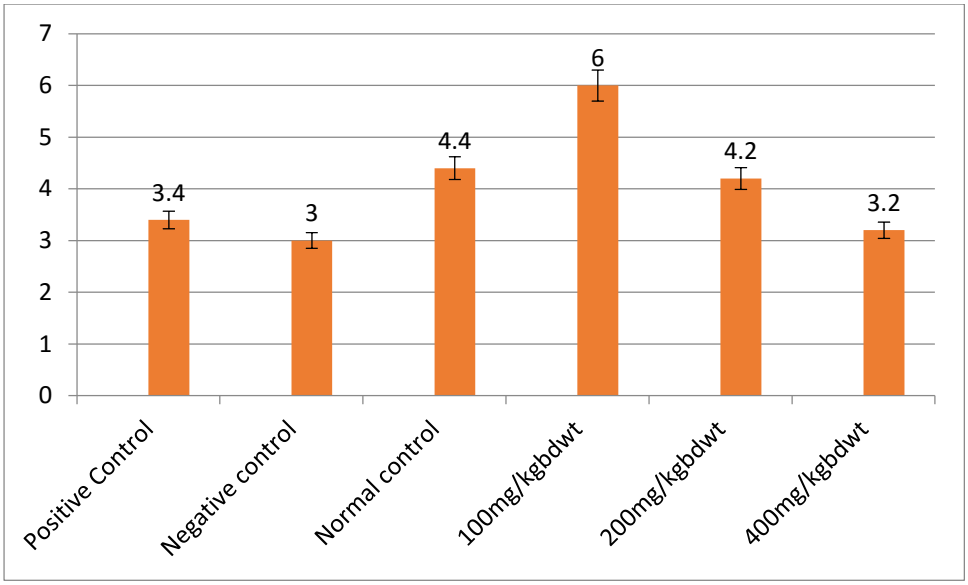

FIGURE 1: EFFECT OF COMBINED METHANOLIC BARK EXTRACTS OF *A. leiocarpus AND T. avicennioides* ON THE BODY WEIGHT OF MICE INFECTED WITH *Plasmodium berghei*

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Table 3. Effect of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T. avicenniodes* on haematological parameters in mice infected with *P.berghei*

Haematological Parameters	Normal Control	Negative Control	Positive Control	100mg/kg Test 1	200mg/kg Test 2	400mg/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^{ab}	18.33±0.15^{ab}	18.40±1.57^{ab}	17.36±0.10^{a}	18.20±1.16^{ab}	19.06±2.00^{b}
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±0.23a	32.01±0.52^{a}
RBC×10^{12}/L	9.73±0.11^{b}	9.04±0.35^{b}	8.92±0.25^{b}	9.07±0.08^{b}	8.18±0.19^{ab}	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 fl	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 5mg/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*

NOTE: *Means with different superscripts are significantly different at P=0.05*

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Table 4. Effect of treatment with combination bark extracts of *A. leiocarpus* and *T. avicennoindes* and malaria parasite on some lipid profile in mice

Treatment	HDL	Triglyceride	LDL	Cholesterol
Normal Control	1.34±0.14	1.47±0.13	0.95±0.02	2.97±0.1.4
Positive Control	0.83±0.01	1.50±0.80	1.11±0.00	2.58±0.00
Negative Control	0.79±0.04	1.23±0.07	1.22±0.00	2.61±0.00
100mg/kg	0.80±0.00	0.68±0.30	1.43±0.19	2.59±0.13
200mg/kg	0.81±0.78	0.92±0.58	1.21±0.02	2.41±0.09
400mg/kg	0.34±0.15	0.55±0.24	0.69±0.31	1.29±0.50

*Positive control was infected with *P.berghei* and treated with 5mg/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*.

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4. DISCUSSION

The resistance of malaria parasite to some of the conventional antimalarial drugs and inability of the people who live in malaria endemic areas to afford the potent antimalarial drugs have necessitated the use of medicinal plants for the treatment of malaria infection in the endemic areas. This study considered the potency of combination therapy methanolic bark extracts of *A. leiocarpus* and *T.avicennioides* in the treatment of malaria infection over the combination therapy of combisunate which is one of the conventional drugs against malaria infection. The result of this study shows that the parasite clearance of combination therapy of methanolic back extract of *A. leiocarpus* and *T.avicennioids* at 100mg/kg body weight was similar with the group treated with combisunate. This shows that the potency of the combination therapy of *A. leiocarpus* and *T.avicennioids* at this dosage was similar to that of combisunate. The parasite clearance in the groups treated with 200mg/kg and 400mg/kg was higher when compared with the positive control, especially in the group treated with 400mg/kg of combination therapy of *A. leiocarpus* and *T.avicennioids*. This shows that parasite clearance of combination therapy of methanolic bark extracts of *A. leiocarpus* and *T.avicennioids* was dose related. This agreed with our previous study where the treatment was done with the bark extracts of *A. leiocarpus* and *T.avicennioids* separately [10, 7].

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

The abnormalities that occur in haematological parameters during malaria infection have been reported by some studies [17, 18, 19]. 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 [20]. 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 [20]. There was reduction in the WBC count in all the groups treated with combination therapy of *A. leiocarpus* and *T. avicennioides* extracts when compared with the positive control group. The group treated with 400mg/kg had lowest WBC count in this study. The reduction in the WBC counts in the group treated with 400mg/kg could be as a result of total clearance of the parasite in this group as 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 [17]. The reduction in lymphocyte count reported in the group treated with 400mg/kg combination therapy of *A. leiocarpus* and *T. avicennioides* crude extract may be due to the adverse effect of high dosage of these 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 [17, 21]. The

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significant increase in Hb level showed in the normal control group in this study when compared with all the infected groups show that malaria parasite could be responsible for the destruction of the Hb. Though the parasitaemia was lowest in the group treated with 400mg/kg (Table 1), but the Hb and RBC levels were significantly reduced in this group than in other treated groups (Table 4). This shows that apart from the effect of parasite on the Hb level and RBCs count, treatment with combination therapy of *A. leiocarpus* and *T. avicennioides* extract at high dosage may also contribute to the destruction of Hb. 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 all the infected group indicate that there was thrombocytopenia in those that were infected with malaria parasite. This concurs with the report of Coelho *et al* [22] which shows 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 400mg/kg, and the significant reduction in the group treated with 100mg/kg when compared with other treated groups is a reflection of the parasitaemia count in each of these group (Table 1 and 2). This shows that the level of parasitaemia count in an individual 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 [10, 23]. The significant increase in HDL level in the normal control when compared with all other infected groups in this study shows that malaria infection may haves impact on the HDL level. Though the parasitaemia count was significantly lower in the group treated with 400mg/kg body weight of combination therapy of methanolic bark extract 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 shows that treatment with combination therapy of methanolic bark extracts of *A. leiocarpus* and *T.avicennioids* at higher dosage could be dangerous as it may cause arthesclerosis. The HDL level was highest in the group treated with 200mg/kg body weight of combination therapy of methanolic bark extract of *A. leiocarpus* and *T.avicennioids*. This concurs with the previous study [24].

5. CONCLUSION

In conclusion, antiplasmodial activities of combination therapy with methanolic bark extracts of *A. leiocarpus* and *T.avicennioids* was at its best at 400mg/kg body weight in this study, but this dosage was dangerous to the organism as a result of the adverse effect on some parameters studied.

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