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Some phytochemicals extracted from *Cola acuminata* leaf have antimalarial activity and improve derangements in haematological indices of *Plasmodium berghei*-infected mice

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Abstract

Malaria, a disease caused by *Plasmodium* species has been linked to high morbidity and mortality especially in sub-saharan Africa due to the resistance developed by the parasite against all the existing drugs, thus, necessitating the search for novel antimalarials. *Cola acuminata* leaf has been reported to possess antimalarial activity and also contain pharmacologically active phytochemicals including alkaloids, flavonoids, tannins and phenolics. The effects of these phytochemicals in mice infected with *Plasmodium berghei* was evaluated in this study. For each phytochemical, 7 groups (A-G) of eight mice each were used. Groups A and B served as normal and untreated controls respectively. Group C was treated with 20mg/Kg body weight of chloroquine and served as the treated control. Groups D, E and F were administered 12.5, 25 and 50 mg/kg body weight of the different phytochemicals while group F was treated with 50mg/Kg body weight of the phytochemicals only without parasite inoculation thus serving as extract control. Treatment commenced 72hrs after inoculation and was done once orally for four a consecutive day after which parasitaemia was evaluated. All the phytochemicals were found to exhibit antimalarial activity in a dose dependent manner. The mean survival time of all the experimental groups were also prolonged in a dose dependent manner compared to that of untreated control. Similarly, all the phytochemicals improved the altered haematological indices towards normal. These phytochemicals of *Cola acuminata* exhibited significant antimalarial activities thus can be further studied in the search for novel antimalarial drugs.

Keywords: Antimalarial, phytochemical, *in vivo*, *Cola acuminata*, haematological indices.

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1. Introduction

Malaria, a disease caused by *Plasmodium* species has been linked to high morbidity and mortality has been declared an urgent public health challenge [1]. The morbidity and mortality of the disease is high particularly amongst children and women [2]. This disease is most prevalent in the sub-Saharan countries [3]. In 2017, Nigeria was ranked among the five countries that were responsible for almost half of the global malaria cases [4]. In Africa alone, 10,000 women and 200,000 infants die due to malaria [5].

Haematological changes are some of the most common complications in malaria and play a major role in malaria pathogenesis, morbidity and mortality. The changes affect major cell types that include red blood cells, leucocytes and thrombocytes [6]. Different strategies have been adopted by different nations and international communities on how to prevent, control and treat malaria. One of these strategies is chemotherapy which is the oldest method of managing and treating ailments. Quinine, chloroquine, mefloquine, and artemisinin among other drugs have been used in the treatment of malaria. However, the parasites have evolved to resist most of the current treatment regimens [1]. Most of these plant derived regiments which are often underexploited

serve as sources of medication to counter the evolutionary resistance developed by these parasites [7]. One of these plants used in the traditional management of malaria is *Cola* species [8,9]. This work therefore aims to evaluate the efficacy of some of these phytochemicals found in *Cola acuminata* leaves in a bid to discover novel anti-malarial agents.

2. Materials and Methods

2.1 Collection and identification of plant material

Fresh leaf samples of *Cola acuminate* was collected from Gembu, Sardauna Local Government of Taraba State, Nigeria. The leaves were identified and authenticated at the Department of Plant Sciences, Modibbo Adama University Technology Yola, Adamawa State.

2.2 Experimental Animals

One hundred and sixty eight (168) Swiss albino mice (about 6 to 8 weeks old) were obtained from the animal breeding unit of the University of Jos, Plateau State. The average body weight of mice (22.02±1.37g) was measured using a Shimadzu (UX4200H) top pan animal balance to the nearest 0.1g. The animals were then housed in well ventilated plastic cages, and acclimatized (in the Biochemistry laboratory) for 7 days prior to the commencement of the experiment with free access to rat pellets and tap water *ad libitum*.

2.3 Parasite Strain

Chloroquine sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University Zaria, Kaduna State Nigeria. The parasites were maintained weekly by serial passage of blood from the donor-infected mice to healthy uninfected mice via intraperitoneal (IP) injection [10].

2.4 Chemicals and reagents

Chloroquine diphosphate salt, immersion oil, and Giemsa strain were obtained from Sigma Chemical Company St. Louis, Mo, USA. Assay kits for enzymes and liver function indices were obtained from Randox Laboratory Ltd, UK.

2.5 Preparation of plant sample

Whole fresh leaves of *Cola nitida* were washed with water and dried in the shade at room temperature and ground to powder using an electric blender (Mazeda Mill, MT 4100, Japan). It was then packed in a sealed plastic bottle until extraction.

2.6 Extraction of alkaloids

Alkaloids were extracted gravimetrically according to the method described by Harborne [11].

2.7 Extraction of flavonoids

Flavonoids were extracted according to the method described by Yahaya [12].

2.8 Extraction of phenolics

Phenolics were extracted according to the method described by Velioglu *et al* [13].

2.9 Parasite inoculation

The mice were inoculated from the same donor mouse. The percentage parasitaemia and the red blood cell count of the donor mouse was first determined using a haemocytometer and appropriate dilutions of the infected blood with isotonic saline were made. Each mouse was inoculated intraperitoneally on day 0 with 0.2 ml of infected blood containing about 1 x 10^7 *Plasmodium berghei* parasitized red blood cells. They were then monitored for 72 hours after which infection was confirmed by observing tail blood microscopically prior to commencement of treatment.

2.10 Extract and chloroquine administration

Experimental groups to receive extract or treatment (chloroquine) started receiving treatment 72 hours after infection. All treatment administration was done orally using intra-gastric tube once daily for 4 days.

2.11 Experimental design

For each phytochemical, the mice were divided into seven groups (A-G) of eight mice per group. Groups A was not inoculated and not treated and served as normal; group B was inoculated but untreated and served as untreated control. Group C was treated with 20mg/Kg body weight of chloroquine (treated control). Groups D, E and F were inoculated with *Plasmodium berghei* (pb) and administered 12.5, 25 and 50 mg/kg body weight of the different phytochemicals respectively while group F was treated with 50mg/Kg body weight of the phytochemicals only without parasite inoculation thus serving as extract control. A week after treatment, five (5) mice from each group were sacrificed and blood collected to evaluate the effect of treatment on haematological parameters which is usually greatly affected by malaria infection. The remaining three (3) mice in each group were monitored for changes in parasitaemia for up to twenty eight (28) days and mean survival times for up to thirty (30) days.

2.12 Parasitaemia count

Parasitaemia count was carried out on days 4, 6, 8, 10, 14, 21, 26 and 28 after commencement of treatment. A drop of tail blood from each of the animals was smeared on glass slides and allowed to dry; the slide was then fixed in methanol; stained with Giemsa and observed using X100 objective lens. The number of parasitized red blood cells seen per film was counted and recorded using the formula:

$$\% \text{ Parasitaemia} = \frac{\textbf{Total number of PRBC X 100}}{\textbf{Total number of RBC}}$$

Where **RBC**= Red Blood Cells;
PRBC= Parasitized Red Blood Cells

The percentage parasitaemia suppression of the extracts compared with that of the untreated control and parasitaemia suppression was calculated using the following formula below as described by Dikasso *et al* [14].

$$\% \text{ chemosuppression} = \frac{\text{Mean parasitemia of untreated control} - \text{mean parasitemia of treated group}}{\text{Mean parasitemia of untreated control}} X\ 100$$

2.13 Determination of mean survival time (MST)

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse. The mean survival time (MST) was calculated as follows:

$$\text{MST} = \frac{\text{sum of survival days of all mice in a group}}{\text{Total number of mice in that group}} \text{ (days).}$$

2.14 Haematological analysis

A week after treatment, five (5) animals from each sub-group of the four phytochemical groups were sacrificed under diethyl ether anaesthesia, and the blood was collected by cardiac puncture into clean EDTA containers for hematological analysis. Determination of white blood cell (WBC) and its differential count, red blood cell count (RBC), platelet (thrombocyte) counts**,** packed cell volume (PCV), hemoglobin concentration (HGB), the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and the mean corpuscular hemoglobin concentration (MCHC) indices were done using an automated electronic blood analyser "Abacus 380".

2.15 Statistical analysis

The group data was expressed as mean ± standard error of mean (SEM) and the significant differences were determined using Statistical Package for Social Sciences (SPSS V. 25).

3. Results

Table 1 shows the results of the effects of some phytochemicals extracted from *Cola acuminata* leaf and chloroquine on percentage parasitaemia and chemosuppressionin *Plasmodium berghei* (NK-65) infected Mice. Percentage parasitaemia increased continuously with time (days 4, 6, 8, 10, 14, 21 and 28) in the untreated control group. Percentage parasitaemia decreased in a dose dependent manner with time, with the treated control having the lowest percentage parasitaemia followed by group treated with phenolics at the highest dose on day 28. On days 8, 10 and 28, all the extract concentrations as well as the treatment groups were active (chemosurpression> 50%). But on day 4 and 6, only the treated and highest doses of the flavonoids and alkaloids were active.

Table 2 shows the results of the effects of some phytochemicals extracted from *Cola acuminata* leaf and chloroquine on mean survival time of mice infected with *Plasmodium berghei* (NK-65). The results revealed that treatment of the infected mice with all the phytochemical extracts increased the mean survival time of the infected mice in a dose dependent manner.

However, chloroquine, phenolics and flavonoids exhibited the highest mean survival time of 30 days and alkaloids; 29 days at the highest dose.

Table 3 shows results of the effects of some phytochemicals extracted from *Cola acuminata* leaf on Packed Cell Volume, Haemoglobin and Red Blood Cells of *Plasmodium berghei* (NK-65) infected Mice. Infection of the mice with the parasite decreased the level of these haematological parameters significantly ($P<0.05$). However, treatment with all the phytochemical extracts significantly ($P<0.05$) improved the haematological parameters towards normal in a dose dependent manner with the highest dose of all the phytochemicals having no significant difference ($P>0.05$) with the normal.

Table 4 shows the results of the effects of some phytochemicals extracted from *Cola acuminata* leaf on Mean Corpuscular Volume, Mean Corpuscular Heamoglobin and Mean Corpuscular Haemoglobin Concentration of *Plasmodium berghei* (NK-65) infected Mice. Infection of the mice with the parasite was found to increase these haematological parameters significantly ($P<0.05$). All the phytochemicals were found to significantly ($P<0.05$) restore thesehaematological parameters towards normal in a dose dependent fashion with doses of 50mg/kg b.w.t of all the phytochemicals having no significant difference with the normal.

Table 5 shows results of the effects of some phytochemicals extracted from *Cola acuminata* leaf on White Blood Cell count, lymphocytes and Platelets of *Plasmodium berghei* (NK-65) infected Mice. Infection of the mice with the parasite significantly ($P<0.05$) increased white blood cell and lymphocytes count, and significantly ($P<0.05$) decreased platelets. Treatment of the infected mice with all the phytochemical extracts significantly ($P<0.05$) improved these haematological parameters towards normal with the dose of 50mg/kg b.w.t of all the phytochemicals having no significant difference from the normal.

Table 1: Effects of some phytochemicals extracted from *Cola auminata* leaf on parasitaemia and chemosuppression in *Plasmodium berghei* (NK-65) infected Mice

Parasitaemia (%) **(% chemosuppression)**

Phytochemicals	Treatment	Day 4	Day 6	Day 8	Day 10	Day 14	Day 21	Day 28
Alkaloids	Untreated Control	16.80	30.28	53.08	90.30	95.00	98.00	100
	Treated control 20mg/kg b.w.tchloroquine	4.48 (73.33)	6.30 (79.19)	7.32 (86.21)	5.21 (94.23)	4.80 (94.95)	3.10 (96.84)	2.19 (97.81)
	12.5 mg/kgb.w.t	12.20 (27.38)	11.54 (61.89)	14.09 (73.46)	14.00 (84.50)	22.30 (76.53)	25.11 (74.38)	28.05 (71.95)
	25mg/kg b.w.t	8.40 (50.00)	14.20 (53.10)	3.45 (74.66)	10.20 (88.70)	11.32 (88.42)	10.51 (89.28)	12.10 (87.90)
	50mg/kg b.w.t	6.20 (63.10)	7.28 (75.96)	6.67 (87.43)	8.55 (90.53)	6.23 (93.44)	5.12 (94.78)	4.50 (95.5)
Flavonoids	Untreated Control	15.10	22.25	38.75	87.60	100	100	100
	Treated control 20mg/kg b.w.t chloroquine	5.15 (65.89)	3.25 (85.39)	1.00 (97.42)	0.00 (100)	0.00 (100)	0.00 (100)	0.00 (100)
	12.5mg/kg b.w.t	10.30 (31.79)	10.10 (54.61)	9.97 (74.27)	9.50 (89.15)	8.80 (91.20)	7.60 (92.40)	5.85 (94.15)
	25mg/kg b.w.t	7.80 (48.34)	6.90 (68.98)	5.75 (85.16)	4.98 (94.32)	3.90 (96.20)	2.50 (97.50)	2.00 (98.00)
	50mg/kg b.w.t	6.05 (59.93)	5.50 (75.28)	4.85 (87.48)	3.80 (95.66)	2.50 (97.50)	1.67 (98.35)	0.90 (99.10)
Phenolics	Untreated Control	17.15	20.32	**37.52**	65.25	**100**	**100**	**100**
	Treated control 20mg/kg b.w.t chloroquine	12.33 (28.10)	11.45 (43.65)	9.56 (74.52)	7.47 (88.55)	0.10 (99.85)	0.00 (100)	0.00 (100)
	12.5mg/kg b.w.t	14.76 (13.94)	13.85 (31.84)	12.34 (67.11)	11.75 (81.99)	10.52 (89.48)	10.05 (84.60)	9.55 (90.45)
	25mg/kg b.w.t	**13.56 (20.93)**	12.35 (39.22)	**11.93 (68.20)**	**10.82 (83.42)**	**9.62 (90.38)**	**8.55 (91.45)**	**5.32 (94.68)**
	50mg/kg b.w.t	**12.75 (25.66)**	**11.54 (43.21)**	**10.25 (72.68)**	**8.75 (86.59)**	**5.43 (94.59)**	**4.52 (95.54)**	**0.25 (99.75)**

Values are means of 5 replicates. Figures in brackets are the percentage chemosuppression on each day.

Table 2: Effects of some phytochemicals extracted from *Cola acuminata* leaf on mean survival times (MST) of *Plasmodium berghei* (NK-65) infected Mice

Phytochemicals	Treatment	Mean Survival Time (days)
Alkaloids	Untreated control	10.00
	Treated control 20mg/kg b.w.t chloroquine	28.00
	12.5mg/kg b.w.t	23.00
	25mg/kg b.w.t	22.25
	50mg/kg b.w.t	29.00
Flavonoids	Untreated control	10.00
	Treated control 20mg/kg b.w.t chloroquine	30.00
	12.5mg/kg b.w.t	24.00
	25mg/kg b.w.t	24.67
	50mg/kg b.w.t	30.00
Phenolics	Untreated control	10.00
	Treated control 20mg/kg b.w.t chloroquine	30.00
	12.5mg/kg b.w.t	20.00
	25mg/kg b.w.t	25.00
	50mg/kg b.w.t	30.00

Table 3: Effects of some phytochemicals extracted from *Cola acuminata* leaf on PCV, HB and RBC of *Plasmodium berghei* (NK-65) infected Mice

Phytochemicals	Treatment	PCV (%)	HB (g/dL)	RBC ($X10^{12}$/L)
Alkaloids	Normal control	47.38±1.61[a]	12.78±0.69[a]	8.46±0.37[a]
	Untreated control	30.03±0.70[b]	10.16±0.59[b]	5.99±0.18[b]
	Treated control (20mg/kg b.w.t chloroquine)	40.10±0.59[a]	11.80±0.96[a]	7.60±1.54[a]
	12.5mg/kg b.w.t of extract + *P.b*	31.90±0.97[b]	10.85±0.72[b]	7.19±0.47[a]
	25mg/kg b.w.t of extract + *P.b*	36.94±1.02[b]	10.60±1.24[b]	7.61±0.48[a]
	50mg/kg b.w.t of extract + *P.b*	38.45±3.92[b]	11.08±0.51[a]	7.59±0.72[a]
	50mg/kg b.w.t of extract without *P.b*	43.92±2.25[a]	12.30±0.68[a]	7.78±0.66[a]
Flavonoids	Normal control	35.50±0.56[a]	12.30±0.69[a]	8.79±0.32[a]
	Untreated control	24.48±2.31[b]	8.95±1.19[b]	5.45±1.35[b]
	Treated control (20mg/kg b.w.t chloroquine)	34.80±0.58[a]	12.21±0.61[a]	8.33±0.46[a]
	12.5mg/kg b.w.t of extract + *P.b*	26.10±2.00[b]	9.15±0.59[b]	6.19±0.47[b]
	25mg/kg b.w.t of extract + *P.b*	**32.89±0.97**[a]	11.65±0.34[a]	8.20±0.66[a]
	50mg/kg b.w.t of extract + *P.b*	**34.07±0.65**[a]	12.15±0.72[a]	**8.30**±0.25[a]
	50mg/kg b.w.t of extract without MP	**35.80±0.69**	**12.63±0.77**[a]	**8.64±0.34**[a]
Phenolics	Normal control	34.80±0.97[a]	12.30±0.69[a]	8.79±0.32[a]
	Untreated control	28.48±2.51[d]	8.95±1.10[d]	5.48±1.35[d]
	Treated control (20mg/kg b.w.t chloroquine)	32.97±0.83[a]	11.99±0.96[a]	8.45±0.55[a]
	12.5mg/kg b.w.t of extract + *P.b*	29.51±1.99[c]	9.08±0.56[c]	5.75±0.73[c]
	25mg/kg b.w.t of extract + *P.b*	30.99±1.23[b]	10.84±0.32[b]	7.21±0.66[b]
	50mg/kg b.w.t of extract + *P.b*	31.77±0.89[a]	11.73±0.61[a]	8.23±0.46[a]
	50mg/kg b.w.t of extract without *P.b*	35.89±1.20[a]	13.37±0.70[a]	9.59±0.52[a]

Values are means of 5 replicates± SEM. Means in the same column with different superscripts are significantly different (P<0.05).

Key: PCV: packed cell volume, HB: haemoglobin, RBC: red blood cells, *P.b: Plasmodium berghei.*

Table 4: Effects of some phytochemicals extracted from *Cola acuminata* leaf on MCV, MCH and MCHC of *Plasmodium berghei* (NK-65) infected Mice

Phytochemicals	Treatment	MCV(fl)	MCH(pg)	MCHC(g/l)
Alkaloids	Normal control	61.25±3.64[a]	17.13±0.85[a]	33.13±1.70[a]
	Untreated control	38.75±1.11[b]	12.40±2.11[b]	23.58±2.45[b]
	Treated control 20mg/kg b.w.t chloroquine	50.50±2.75[a]	15.88±1.13[a]	30.71±2.67[a]
	12.5mg/kg b.w.t of extract + *P.b*	43.50±1.76[b]	13.15±0.75[b]	26.10±1.87[b]
	25mg/kg b.w.t of extract + *P.b*	45.00±7.60[b]	13.80±1.31[b]	29.08±2.39[a]
	50mg/kg b.w.t of extract + *P.b*	47.25±2.43[b]	13.28±0.69[b]	28.78±0.96[a]
	50mg/kg b.w.t of extract without *P.b*	57.00±4.71[a]	15.69±1.19[a]	31.43±1.81[a]
Flavonoids	Normal control	30.28±2.86[a]	13.15±0.75[a]	29.10±2.39[a]
	Untreated control	47.25±2.43[b]	18.69±1.19[b]	42.75±3.14[b]
	Treated control (20mg/kg b.w.t chloroquine)	30.40±2.25[a]	13.20±1.62[a]	30.00±1.60[a]
	12.5mg/kg b.w.t of extract + *P.b*	41.75±1.11[b]	16.83±0.52[b]	39.28±2.86[b]
	25mg/kg b.w.t of extract + *P.b*	33.28±1.38[a]	13.70±0.52[a]	31.12±2.69[a]
	50mg/kg b.w.t of extract + *P.b*	33.12±1.69[a]	13.27±0.68[a]	30.28±2.86[a]
	50mg/kg b.w.t of extract without *P.b*	30.71±2.66[a]	13.28±0.50[a]	30.05±2.66[a]
Phenolics	Normal control	30.28±2.86[a]	13.15±0.75[a]	29.99±1.70[a]
	Untreated control	47.25±2.45[d]	15.69±1.19[d]	39.05±1.87[d]
	Treated control (20mg/kg b.w.t chloroquine)	33.12±1.69[a]	13.45±0.52[a]	30.74±2.66[a]
	12.5mg/kg b.w.t of extract + *P.b*	40.78±2.45[c]	14.35±0.99[c]	38.55±1.03[c]
	25mg/kg b.w.t of extract + *P.b*	37.23±1.82[b]	13.85±0.53[b]	34.23±1.24[b]
	50mg/kg b.w.t of extract + *P.b*	33.89±1.52[a]	13.89±0.97[a]	31.60±1.43[a]
	50mg/kg b.w.t of extract without *P.b*	31.15±1.25[a]	14.53±0.52[a]	30.80±1.80[a]

Values are means of 5 relplicates ± SEM. Means in the same column with different superscripts are significantly different.

Key: MCV= mean corpuscular volume, **MCH**= mean corpuscular haemoglobin, **MCHC**= mean corpuscular haemoglobin concentration, ***P.b:*** *Plasmodium berghei.*

Table 5: Effects of some phytochemicals extracted from *Cola acuminata* leaf on WBC, LYM and PLT of *Plasmodium berghei* (NK-65) infected Mice

Phytochemicals	Treatment	WBC($X10^9$L)	LYM (%)	PLT($X10^9$/L)
Alkaloids	Normal control	13.08±1.69[a]	10.59±1.57[a]	732.25±21.54[a]
	Untreated control	19.82±0.70[b]	72.07±4.95[c]	481.00±95.42[b]
	Treated control (20mg/kg b.w.t chloroquine)	15.10±0.20[a]	17.48±0.95[a]	598.25±6.29[a]
	12.5mg/kg b.w.t of extract + *P.b*	19.22±1.01[b]	65.44±3.25[b]	497.75±62.50[b]
	25mg/kg b.w.t of extract + *P.b*	19.03±0.38[b]	62.67±3.93[b]	503.75±13.81[a]
	50mg/kg b.w.t of extract + *P.b*	17.50±0.72[b]	58.01±5.19[b]	521.25±37.84[a]
	50mg/kg b.w.t of extract without *P.b*	14.41±0.72[a]	15.98±0.67[a]	722.75±13.60[a]
Flavonoids	Normal control	12.98±0.78[a]	74.44±1.62[a]	464.75±44.28[a]
	Untreated control	19.82±0.69[b]	81.68±2.72[b]	232.20±24.60[b]
	Treated control (20mg/kg b.w.t chloroquine)	13.20±1.62[a]	73.43±0.60[a]	461.70±50.14[a]
	12.5mg/kg b.w.t of extract + *P.b*	19.22±1.01[b]	80.90±3.93[b]	258.60±39.88[b]
	25mg/kg b.w.t of extract + *P.b*	13.60±1.63[a]	72.80±4.93[a]	440.73±28.77[a]
	50mg/kg b.w.t of extract + *P.b*	13.28±0.50[a]	73.10±0.80[a]	454.38±38.88[a]
	50mg/kg b.w.t of extract without *P.b*	12.93±0.96[a]	74.58±2.22[a]	514.50±39.88[c]
Phenolics	Normal control	12.79±0.78[a]	14.24±1.58[a]	722.75±13.59[a]
	Untreated control	19.82±1.69[d]	8.29±0.32[d]	514.50±39.88[d]
	Treated control (20mg/kg b.w.t chloroquine)	12.83±1.05[a]	13.89±0.98[a]	720.28±28.43[a]
	12.5mg/kg b.w.t of extract + *P.b*	18.03±0.99[c]	10.45±1.23[c]	560.75±34.39[c]
	25mg/kg b.w.t of extract + *P.b*	17.83±0.67[b]	10.59±1.57[b]	654.25±28.24[b]
	50mg/kg b.w.t of extract + *P.b*	13.12±1.68[a]	12.79±1.00[a]	719.10±31.22[a]
	50mg/kg b.w.t of extract without *P.b*	12.99±0.78[a]	14.92±0.52[a]	723.26±26.7[a]

Values are means of 5 replicates ±SEM. Means in the same column with different superscripts are significantly different ($p<0.05$).

WBC=White blood cells, **LYM**=Lymphocytes and **PLT**=Platelet count, *P.b: Plasmodium berghei.*

4. Discussion

Natural products have been proven as promising sources of effective drugs for the treatment of several diseases. For example, Quinine, chloroquine, mefloquine, and artemisinin among other drugs have been used in the treatment of malaria [1,15]. Some phytochemicals have been found to exhibit antimalarial activity individually or in synergy with other phytoconstituents to elicit their biological activities [16].

All the phytochemicals in this study demonstrated the ability to reduce parasitaemia in a dose dependent manner. This finding agrees with the work of Gebrehiwot *et al* [17], Ounjaijean *et al* [10] and Okello and Kang [18] that the phytochemicals used in this research exhibit antimalarial activity via diverse mechanisms. Some phenolics act through haem detoxification by inhibiting β-haematin formation [19,20]. Reported that some Alkaloids affects protein folding in the parasite through the inhibition of the parasite's chaperon function. Flavonoids work by inhibiting the influx of L-glutamine and myoinosol into the infected red blood cell which is specifically deleterious to the growth and live of the parasite [18,21]. The antioxidant activities of these compounds as reported by Builders *et al* [22]; Zailani *et al* [8] might have supported the exhibited antimalarial activity since antioxidative activity inhibits haem polymerization and unpolymerized haem is toxic to the parasite [23]. The remarkable parasite suppression exhibited by the compounds translated in to longer survival times.

Hematological changes that are thought to characterize malaria may be related to the biochemical alterations that occur during the asexual stage of the life cycle of the malaria parasite. Entry of the parasiteinto erythrocytes usually leads to a marked increase in secretion of inflammatory cytokines (TNFα, IL-1, IL-10, and IFNα), endothelial cell activation (due to overexpression of cell adhesion molecules; ICAM-1, VCAM-1), activation of the coagulation cascade (due to platelet consumption and endothelial damage), and sequestration of parasitized RBCs (due to overexpression of cell adhesion molecules, pfEMP, and iNOS) which mostly results in morphological and numerical changes in all the blood cell line in during malaria infection. However, these changes depend on the parasite specie, severity of illness and the immune status of the host [3]. In this study, infected mice were found to have reduced PCV, haemoglobin and RBC and

increase in RBC indices levels (Tables 3 and 4). This agrees with the report of Meraiyebu *et al* [24]; Francis *et al* [25]; Ounjaijean *et al* [10] which can be attributed to increase in haemolysis of parasitized red blood cells and increase in fragility of the normal cells. All the phytochemical treatment groups improved PCV, haemoglobin and RBC and its indices towards normal. At the highest dose, the results of the phytochemical treated groups were comparable to the standard control group. This can be due to the observed chemosuppressive effects of these phytochemicals.

Results of the extract control group shows that these phytochemicals are relatively safe at this dose since there was no significant difference ($P>0.05$) between this group and the normal group with regards to PCV, haemoglobin, RBC and its indices. The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. Lymphocyte is a type of white blood cell that fights against invading pathogens including *Plasmodium* species [3]. In this study, infection of mice was found to increase both WBC and lymphocytes substantially ($P<0.05$) when compared with the normal. This can be due to immune response by the host mice to the presence of the parasite. All the phytochemicals improved both WBC and LYM towards normal in dose dependent manner, thus suggesting that these phytochemicals possesses immunomodulatory activities.

Thrombocytopenia is a major complication of malaria [26]. This is characterized by low platelets in the blood probably due to activation of the coagulation cascade as a result of platelet consumption and endothelial damage [3]. Results from the untreated control group confirmed this reduction in the platelet count which was significantly improved by all the phytochemicals used in treatment in a dose dependent manner with the dose of 50mg/kg b.w.t of all the phytochemicals having no significant difference from the normal control. This may be due to the observed chemosuppressive effects of the phytochemicals which agrees with the findings of Ifeanyichukwu and Esan [28] that thrombocytopenia usually disappears with the treatment of malaria infection. The results of this study suggest that all these phytochemicals possess antimalarial activity in dose dependent manner thereby supporting the folkloric claim of the useof this plant and other plants containing these phytochemicals in the treatment or management of malaria [8-10,17].

5. Conclusion

All the phytochemicals of *Cola acuminata* leaf used in this study individually exhibited antimalarial activity. This can be due to the plasmocidal or immunomodulatory activities of the phytochemicals. All the phytochemicals were also able to restore altered haematological indices probably through parasite clearance activities elicited by the phytochemicals though effects were better at higher doses. As such, these phytochemicals are promising novel antimalarial agents.

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