

## Research Paper

# Effects of aqueous leaf extract of *Cola nitida* on Parasitaemia, *In vitro* antioxidant and biochemical parameters in *Plasmodium berghei* infected Mice

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Evaluation of the antimalarial activity of aqueous leaf extract of *Cola nitida* was carried out on *Plasmodium berghei* infected mice. Six groups of eight mice each were used. Groups D, E and F were treated with 100, 200 and 300 mg/Kg body weight of extract respectively. Group C was treated with 20 mg/Kg body weight chloroquine. Group A served as normal control while B was untreated control. Treatment commenced 3 days after inoculation for 3 days. Tail blood was collected on days 4, 7, and 10 after inoculation to check parasitaemia. Seven days after inoculation, five mice from each group were sacrificed and blood collected for analyses. Phytochemical screening revealed alkaloids, saponins, flavonoids, tannins, phlobatannins, and phenolics. The extract exhibited

antioxidant activity *in vitro* using DPPH radical scavenging and ferric reducing antioxidant power assays. Treatment significantly reduced ( $p < 0.05$ ) parasitaemia. Infection caused a significant decrease ( $p < 0.05$ ) in packed cell volume, haemoglobin, red blood cells and platelets and significant increase ( $p < 0.05$ ) in MCV, MCH, MCHC, white blood cells, neutrophils and the activities of all the enzymes studied compared to control. Treatment with the extract restored the altered parameters towards normal. *Cola nitida* leaves possess good antimalarial and antioxidant potentials that can be exploited.

**Key words:** *Cola nitida*, *Plasmodium berghei*, antimalarial, liver function indices, antioxidant

## INTRODUCTION

Malaria is one of the most serious vector-borne diseases transmitted by the bite of infected *Anopheles* mosquitoes which carry the *Plasmodium* parasites (Zahari *et al.*, 2016). Five species of *Plasmodium* including *Plasmodium knowlesi* which is originally a simian parasite can infect humans (Caraballo, 2014). *Plasmodium falciparum* causes the most severe form of the disease and results in the most deaths (WHO, 2015). Malaria is a major endemic disease with high mortality rate in many tropical and subtropical countries. Up to three million deaths due to malaria and close to five billion episodes of clinical illness requiring antimalarial therapy occur throughout the world each year. Of these, Africa accounts for more than 90% (Bremner *et al.*,

2004). The burden of this disease has been on the rise partly due to the increasing resistance of *Plasmodium falciparum* against the available antimalarial drugs (Caraballo, 2014).

In 2015, 95 countries and territories had ongoing malaria transmission. Sub-Saharan Africa carries a disproportionately high share of the global malaria burden and the region was home to 88% of malaria cases and 90% of malaria deaths in 2015 (WHO, 2016). Malaria is transmitted throughout Nigeria, with 97% of the population at risk; it accounts for about 60% of outpatient visits and 30% of hospitalizations in Nigeria (MOP, 2016). A significant number of malaria patients will progress to severe malaria with organ dysfunction, which

is more common among young children and travelers or other adults with only sporadic exposure to infected mosquitoes (Ackerman *et al.*, 2009; Wilson and Taskforce, 2011). Malaria parasite hemoglobin metabolism in red blood cells induces the generation of reactive oxygen species (ROS) which plays an important role in the development of systematic complication in hosts (Nethengwe *et al.*, 2012). This can have several consequences leading to changes in haematological parameters like malarial anaemia, decreased deformability of red blood cells (RBCs) under shear stress etc. Decreased deformability of RBCs is associated with mortality (Dondorp *et al.*, 1997; Calvo-Cano *et al.*, 2016) and can impede the transit of RBCs through capillaries thus impairing oxygen delivery and resulting in hypoxia (Charunwatthana *et al.*, 2009; Pivkin *et al.*, 2016). A wide variety of plants and compounds with antioxidative properties have been used to reduce ROS formation and damage from oxidative stress that can occur in malaria; this improves outcome of malaria management (Zahari *et al.*, 2016). Liver involvement in malaria infection is a common significant cause of morbidity and mortality; the clinical presentation of jaundice often reflects the degree of liver damage and is sometimes proportional to the parasite load (Viriyavejakul *et al.*, 2014).

There is a wide use of plants among traditional healers for variety of human and animal diseases. In developing countries like Nigeria majority of people rely on herbal medicines for treatment due to cultural practices, high cost of orthodox drugs, incidence of drugs resistance and side effects (Arora and Kaur, 1999; Mathivanan *et al.*, 2014); one of such plants is *Cola nitida*. *Cola nitida* belongs to the family *Sterculiaceae*, having about 125 species of trees native to the tropical rainforest. *Cola nitida* is the second most important indigenous cash crop in Nigeria. *Cola nitida* has been used in folk medicine as an aphrodisiac and an appetite suppressant. It is also used to treat morning sickness, migraine headache, and indigestion (Esimone *et al.*, 2007; Uwabunkeonye *et al.*, 2015). We have previously reported the antimalarial activity of *Cola acuminata* aqueous leaf extract (Zailani *et al.*, 2016) which is used locally in the management of malaria in some localities in Nigeria. In line with this; the present study aims to determine if *Cola nitida* which belongs to the same family as *Cola acuminata* and has been reported by Okeke *et al.* (2015) to have only slight differences in chemical characteristics which suggests a close affinity of the two species also has potentials in malaria management.

## MATERIALS AND METHODS

### Chemicals and reagents

Chloroquine diphosphate salt was obtained from Sigma

Chemical Company, St. Louis, Mo, USA, Giemsa stain was obtained from Anosantec Laboratories, UK and immersion oil was obtained from Panzonar Laboratory Supplies, Canada, Assay kits for enzymes (ALT, AST and ALP) and liver function indices (Albumin, Total and direct Bilirubin and total protein) were obtained from Randox Laboratories Ltd, UK. All other reagents used were of analytical grade.

### Plant material and Extract preparation

Fresh leaves of *Cola nitida* were collected from a farmland in Emii, Owerri North Local Government of Imo State in the month of February, 2016; and were authenticated at the Department of Plant Sciences, Modibbo Adama University of Technology, Yola. The leaves of the plant were dried in the shade at room temperature and ground to powder using an electric blender (Arrow Mill S35, China). Two hundred gram of the leaf powder was macerated in a 1500mL of distilled water in dark bottles for 48 hours, filtered using Whatman filter paper No 1 and concentrated using water bath at 40°C. The dried extract was weighed and reconstituted with distilled water into desired doses.

### Phytochemical analysis

Phytochemical screening was carried out according to the methods described by Harbone (1973).

### Determination of *in vitro* antioxidant capacity

The scavenging capacity of 1,1 diphenyl-2-picryl-hydrazyl (DPPH) radical on the aqueous leaf extract of *Cola nitida* was determined using the method of Sunand Wang, (2010). While the ferric reducing antioxidant power assay (FRAP) of the extract was determined as described by Oyaizu, (1986).

### Experimental animals and malaria parasite

Swiss albino mice were obtained from the animal breeding unit of the Department of Biochemistry, University of Jos, Plateau State. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum*. The chloroquine sensitive strain of *Plasmodium berghei* (NK-65) used for the study was obtained from Department of Biochemistry, Federal University of Technology, Minna, Niger State and were maintained by weekly passage in to naïve mice.

### Animal inoculation and extract administration

Evaluation of the antimalarial potentials of the aqueous

extract of *Cola nitida* leaf was carried out as described by Nogueira and Virgilio, (2010). Forty eight (48) Swiss albino mice were divided into six groups (A-F) of eight (8) mice each. Groups B-F were inoculated with the rodent malaria parasite *Plasmodium berghei* from the same donormouse. Before inoculation, 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 through the intraperitoneal route on day 0 with 0.2 mL of infected blood containing about  $1 \times 10^7$  *Plasmodium berghei* parasitized red blood cells.

Treatment was withheld for 72 h to allow for establishment of infection and it was commenced when parasitaemia was confirmed in the inoculated animals by screening their tail blood for malaria parasites. A drop of tail blood each from the animals was smeared on glass slides and allowed to dry; the slides were then fixed in methanol; stained with Giemsa and observed using X 100 objective lens. Treatment was given orally once daily for three consecutive days. Groups D, E and F were treated with 100, 200 and 300 mg/Kg body weight of the aqueous leaf extract of *Cola nitida* respectively. Group C was treated with 20 mg/Kg body weight of chloroquine. Groups A and B served as normal and untreated controls respectively. On days 4, 7 and 10 after inoculation, blood was collected from the tail of each mouse and thin films were made on a microscope slide and examined microscopically to determine the parasitaemia and calculate percentage chemosuppression; also on day 7, five mice from each group were sacrificed using a cotton wool soaked in diethyl ether for anaesthesia and blood collected through cardiac puncture for the evaluation of liver function and haematological indices. Percentage parasitaemia and percentage chemosuppression were calculated by the formula below as described by Manser *et al.* (2013).

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

Where RBC= Red Blood Cells and PRBC= Parasitized Red Blood Cells

$$\text{Chemo-suppression A} = \frac{C - B \times 100}{C}$$

Where B = parasitaemia in study group and C = parasitaemia in control.

### Test for hematological parameters

Blood was collected by cardiac puncture from all experimental groups and was placed in 1 ml vacutainer EDTA anticoagulated tubes for the analysis of the following heamatological parameters: Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red

Blood Cell (RBC), White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), neutrophils (NEU), lymphocytes (LYM) and platelet count. These were determined using the automated haematological analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan.

### Test for liver function indices

Bilirubin was determined by the method of Walter and Gerard, (1970). Serum total protein was determined by the method of Nishi *et al.* (1985) and serum albumin was determined by the method of Doumas *et al.* (1971).

### Enzyme assays

The activity of alkaline phosphatase in serum was determined as described by Wright *et al.* (1972) while that of alanine and aspartate amino transferase were assayed by the method Huang *et al.* (2006).

### Statistical analysis

The group means  $\pm$  SEM for each parameter was calculated and significant differences were determined by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% confidence level using SPSS-PC programme package (Version 22.0 SPSS Inc. Chicago).

## RESULTS

### Phytochemical Tests

The aqueous leaf extract of *Cola nitida* was found to contain alkaloids, saponins, flavonoids, tannins, phlobatannins, and phenolics.

### In vitro antioxidant activities

The extract exhibited antioxidant activities *in vitro* but the capabilities differed for the two indicators: DPPH had a lower IC<sub>50</sub> compared to FRAP but ascorbic acid, which was the standard, had the lowest IC<sub>50</sub> (Table 1).

**Table 1.** DPPH and FRAP scavenging activity (IC<sub>50</sub>) of *Cola nitida* aqueous leaf extract.

Parameter	DPPH ( $\mu\text{g}/\mu\text{L}$ )	FRAP ( $\mu\text{g}/\mu\text{L}$ )
<i>Cola nitida</i>	30.38	36.79
Ascorbic acid	2.8	2.8

**Table 2.** Effects of *Cola nitida* aqueous leaf extract on parasitaemia in *Plasmodium berghei* (NK-65) infected mice.

Parameter	Parasitaemia (%) (% Chemosuppression)		
	Day 4	Day 7	Day 10
Infected untreated	18.50	26.91	100
Chloroquine 20mg/kg b.w.	3.90(78.92)	5.38(80.00)	3.40(96.6)
100mg/kg b.w.	11.00(40.54)	8.78(67.37)	12.20(87.80)
200mg/kg b.w.	7.05(61.89)	8.20(69.52)	11.30(88.70)
300mg/kg b.w.	6.23(66.32)	5.50(79.56)	9.60(90.40)

Values are means of 4 replicates; b.w = body weight. The figures in brackets show percentage chemo suppression on each day.

**Table 3.** Effects of aqueous leaf extract of *Cola nitida* on PCV, Hb and red blood cells of mice infected with *Plasmodium berghei* (NK-65).

Treatment	PCV (%)	Hb (g/L)	RBC ( $\times 10^{12}/L$ )
Normal Control	35.68 $\pm$ 2.75 <sup>a</sup>	10.32 $\pm$ 0.72 <sup>a</sup>	7.36 $\pm$ 0.46 <sup>a</sup>
Untreated Control	13.67 $\pm$ 1.31 <sup>b</sup>	4.76 $\pm$ 0.42 <sup>b</sup>	2.20 $\pm$ 0.16 <sup>b</sup>
Chloroquine (20 mg/Kg b.w.t)	39.48 $\pm$ 3.61 <sup>a</sup>	11.72 $\pm$ 1.09 <sup>a</sup>	7.43 $\pm$ 0.77 <sup>a</sup>
100 mg/Kg b.w.t	14.88 $\pm$ 1.52 <sup>b</sup>	4.76 $\pm$ 0.40 <sup>b</sup>	2.54 $\pm$ 0.31 <sup>b</sup>
200 mg/Kg b.w.t	21.36 $\pm$ 4.73 <sup>c</sup>	6.66 $\pm$ 1.13 <sup>c</sup>	3.80 $\pm$ 0.82 <sup>bc</sup>
300 mg/Kg b.w.t	23.60 $\pm$ 1.70 <sup>c</sup>	7.32 $\pm$ 0.52 <sup>c</sup>	4.19 $\pm$ 0.20 <sup>c</sup>

Values are means of 5 replicates  $\pm$ SEM. Means in the same column with different superscripts are significantly different ( $p < 0.05$ ). PCV=Packed cell volume, Hb=Haemoglobin, RBC=Red blood cells.

### Antimalarial test

Extracts that cause less than 30% chemosuppression in *in vivo* antimalarial studies are considered inactive. Those that cause between 30-50% chemosuppression are considered partially active. Only those that cause above 50% chemosuppression are considered active. Going by this, all of the doses were active on day 4 except the lowest dose which only showed partial activity. On days 7 and 10 post inoculation all the doses were active; the group treated with the highest dose (300 mg/kg b.w) of extract compared favorably with the group treated with chloroquine (Table 2).

### Red blood cell indices

Infection with *Plasmodium berghei* significantly reduced ( $p < 0.05$ ) the packed cell volume, haemoglobin and red blood cell concentration compared to normal control (Table 3). Although treatment with the extract increased these parameters in a dose dependent manner, the increase was not significant ( $p > 0.05$ ) compared to normal control. Infection also caused a significant increase ( $p < 0.05$ ) in MCV, MCH, MCHC. This was improved by treatment (Table 4).

### White blood cell indices

Infection of experimental animals with *Plasmodium*

*berghei* caused a significant increase ( $p < 0.05$ ) in the WBC count and some of its differentials (Neutrophils and Lymphocytes) in the mice.

These were ameliorated by treatment especially in the chloroquine group where the reduction was significant ( $p < 0.05$ ) (Table 5).

There was as a significant decrease ( $p < 0.05$ ) in the platelet count of the mice as a result of infection; treatment with the extract improved platelet count towards normal in a dose dependent manner; the group treated with chloroquine compared favourably with the normal control (Table 5).

### Liver function indices

Infection with *Plasmodium berghei* (NK-65) caused a significant increase ( $p < 0.05$ ) in all the liver function indices except serum albumin compared to control (Table 6). These changes were ameliorated by treatment, especially in the group treated with chloroquine.

### Cellular enzymes

Infection with *Plasmodium berghei* (NK-65) caused a significant increase ( $p < 0.05$ ) in the activities of all the enzymes studied compared to control. Treatment with the aqueous leaf extract of *Cola nitida* was able to restore the altered enzyme activities towards normal in a dose dependent manner (Table 7).

**Table 4.** Effects of administration of *Cola nitida* aqueous leaf extract on MCV, MCH and MCHC of mice infected with *Plasmodium berghei*.

TREATMENT	MCV (fL)	MCH (pg)	MCHC (g/L)
Normal	49.07±2.23 <sup>a</sup>	14.62±0.53 <sup>a</sup>	29.98±0.42 <sup>a</sup>
Infected untreated	63.40±3.97 <sup>b</sup>	23.60±0.70 <sup>b</sup>	38.39±1.35 <sup>b</sup>
Chloroquine 20mg/kg b.w.t	53.20±1.20 <sup>a</sup>	15.86±0.26 <sup>a</sup>	29.64±0.20 <sup>a</sup>
100mg/kg b.w.t	55.00±3.08 <sup>a</sup>	19.06±1.30 <sup>c</sup>	34.62±1.19 <sup>b</sup>
200mg/kg b.w.t	55.60±1.44 <sup>a</sup>	18.28±0.94 <sup>c</sup>	32.94±2.17 <sup>ab</sup>
300mg/kg b.w.t	56.20±2.22 <sup>a</sup>	17.42±0.61 <sup>c</sup>	31.08±0.62 <sup>ab</sup>

Values are means of 5 replicates ±SEM. Means in the same column with different superscripts are significantly different (p<0.05). MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration.

**Table 5.** Effects of administration of *Cola nitida* aqueous leaf extract on white blood cell indices and platelet count of mice infected with *Plasmodium berghei*.

TREATMENT	WBC(x10 <sup>9</sup> /L)	NEUT (%)	LYM (%)	PLT(x10 <sup>9</sup> /L)
Normal	13.20±1.14 <sup>a</sup>	11.42±0.70 <sup>a</sup>	75.54±1.63 <sup>a</sup>	471.60±51.14 <sup>a</sup>
Infected untreated	46.21±3.58 <sup>b</sup>	17.71±1.16 <sup>b</sup>	60.78±2.74 <sup>b</sup>	222.20±23.60 <sup>b</sup>
Chloroquine 20mg/kg	13.53±1.60 <sup>a</sup>	12.26±1.27 <sup>a</sup>	72.42±0.70 <sup>a</sup>	468.80±74.22 <sup>a</sup>
100mg/kg b.w	38.10±6.51 <sup>c</sup>	14.88±2.72 <sup>a</sup>	61.90±2.93 <sup>b</sup>	244.80±40.22 <sup>b</sup>
200mg/kg b.w	26.11±3.17 <sup>d</sup>	12.56±1.68 <sup>a</sup>	71.10±4.70 <sup>a</sup>	367.80±84.32 <sup>c</sup>
300mg/kg b.w	25.95±3.68 <sup>d</sup>	12.34±5.42 <sup>a</sup>	72.90±4.94 <sup>a</sup>	344.60±25.77 <sup>c</sup>

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, NEUT=Neutrophils, LYM=Lymphocytes, PLT=Platelet count.

**Table 6.** Effects of administration of aqueous leaf extract of *Cola nitida* on liver function indices in *Plasmodium berghei* (NK-65) infected mice.

TREATMENT	TB(mg/dL)	CB(mg/dL)	TP(mg/mL)	ALB(g/dL)
Normal	0.48±0.16 <sup>a</sup>	0.30±0.6 <sup>a</sup>	0.36±0.80 <sup>a</sup>	26.50±1.16 <sup>a</sup>
Infected untreated	2.40±0.90 <sup>b</sup>	0.90±0.25 <sup>b</sup>	0.70±0.02 <sup>b</sup>	29.01±0.54 <sup>a</sup>
Chloroquine 20mg/kg b.w	0.73±0.31 <sup>a</sup>	0.36±0.07 <sup>a</sup>	0.42±0.53 <sup>a</sup>	27.39±1.17 <sup>a</sup>
100mg/kg b.w	2.04±0.60 <sup>b</sup>	0.71±0.22 <sup>ab</sup>	0.65±0.12 <sup>ab</sup>	27.71±0.45 <sup>a</sup>
200mg/kg b.w	1.63±0.30 <sup>b</sup>	0.50±0.20 <sup>ab</sup>	0.64±0.03 <sup>ab</sup>	27.69±1.52 <sup>a</sup>
300mg/kg b.w	1.10±1.09 <sup>b</sup>	0.40±0.10 <sup>a</sup>	0.62±0.5 <sup>ab</sup>	27.58±0.84 <sup>a</sup>

Values are means of 5 replicates ±SEM. Means in the same column with different superscripts are significantly different (p<0.05). TB=Total bilirubin, CB=Conjugated bilirubin, TP=Total protein, ALB=Albumin.

## DISCUSSION

Phytochemical analysis of the aqueous leaf extract of *Cola nitida* revealed the presence of alkaloids, saponins, flavonoids, tannins, phlobatannins, and phenolics. This agrees with the findings of Okeke *et al.* (2015). The observed antimalarial activity of the aqueous leaf extract of *Cola nitida* may be attributed to its phytochemical components such as alkaloids, tannins, flavonoids and phenolics which have been identified in this study and which have been previously shown to have antimalarial activity (Liu *et al.*, 1992; Soh *et al.*, 2008; Oliveira *et al.*, 2009; Eleazu *et al.*, 2012; Ntie-Kang *et al.*, 2014; Pivatto *et al.*, 2014; Zailani *et al.*, 2016). Oxidative stress occurs

when there's an imbalance between the generation of reactive oxygen species (ROS) and endogenous antioxidant systems that mop them up (Chanda and Dave, 2009; Abubakar *et al.*, 2016). Since ROS play a big role in the pathophysiology of malaria, a scavenger of these species may prevent free radical-mediated tissue damage associated with malaria (Chanda and Dave, 2009; Balogun *et al.*, 2014). Phenolic compounds which are a class of antioxidant agents can act as reducing agents, hydrogen donors and singlet oxygen quenchers and thus have been used to reverse, retard, or delay the onset of some diseases including malaria (Farombi and

**Table 7.** Effects of administration of aqueous leaf extract of *Cola nitida* on the activities of Aspartate amino transferase (AST), Alanine amino transferase (ALT), and Alkaline phosphatase (ALP) in *Plasmodium berghei* (NK-65) infected mice.

TREATMENT	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
Normal	36.90±4.60 <sup>a</sup>	12.44±2.10 <sup>a</sup>	43.50±9.21 <sup>a</sup>
Infected untreated	73.70±22.80 <sup>b</sup>	46.63±18.12 <sup>b</sup>	57.52±10.10 <sup>b</sup>
Chloroquine 20mg/kg b.w	41.62±1.10 <sup>a</sup>	17.04±5.30 <sup>a</sup>	47.20±5.10 <sup>a</sup>
100mg/kg b.w	58.90±12.50 <sup>c</sup>	29.03±3.91 <sup>c</sup>	55.30±10.92 <sup>b</sup>
200mg/kg b.w	52.00±8.70 <sup>c</sup>	19.50±3.10 <sup>a</sup>	49.60±15.63 <sup>a</sup>
300mg/kg b.w	51.45±5.52 <sup>c</sup>	18.05±5.50 <sup>a</sup>	48.80±17.51 <sup>a</sup>

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

Owoeye, 2011). Tannins have been reported to have antioxidant effects in addition to antibacterial, antiviral and antifungal activities (Cowan, 1999; Wafa and Sofiane, 2016).

Flavonoids also have an established antioxidant activity (Harbonne and Williams, 2000; Farombi and Owoeye, 2011). The presence of flavonoids in the extract indicates their potentiality in serving as antioxidants. Antioxidant action of flavonoids is important in malaria infection because flavonoids can traverse the blood brain barrier as does *Plasmodium* species making it very useful in management of some complications of malaria like cerebral malaria (Youdim *et al.*, 2003; Ferri *et al.*, 2015). Therefore, these antioxidant species in the extract in addition to direct parasite clearance may play an important role in scavenging the ROS produced during malaria infection and thus ameliorating some of the complications of malaria (Balogun *et al.*, 2014).

Changes in haematological parameters is one of the most common complications in malaria and plays a major role in malaria pathogenesis. These changes involve the major cell types such as red blood cells, leucocytes and thrombocytes (Maina *et al.*, 2010; Bakhubaira, 2013; van Wolfswinke *et al.*, 2013; Warimwe *et al.*, 2013). Anaemia which is characterized by a decrease in the levels of circulating RBC, Hband PCV is a common problem in malaria (Chang and Stevenson, 2004; Pathak and Ghosh, 2016) and it was also observed in this study. The amelioration of these deficits after the administration of the extract suggests that the extract may be able to stimulate the production of red blood cells by stimulating erythropoietin which is the humoral regulator of red blood cell production in the kidney (Balogun *et al.*, 2009).

The white blood cells which fight infection by phagocytosis of foreign organisms; produce, transport and distribute antibodies in immune responses were significantly increased in this study. This may imply a reduction in the ability of the mice to resist the infection (Yakubu *et al.*, 2007; Kotepui *et al.*, 2015). There was also a decrease in platelets levels in the infected groups which is also common in malaria and may result from increased sequestration of platelets in the spleen (Ogboi

*et al.*, 2011). The observed significant increase in platelet count by the infected extract-treated compared to the infected untreated, suggests a stimulatory effect of the extract on platelet production. The liver is an important organ involved during the hepatic stage of the malaria parasite's life cycle thus interfering with the liver and its functions. The invasion of liver cells by malaria parasites can cause organ congestion, sinusoidal blockage and cellular inflammation (Petit and Wamola, 1994; Jarike *et al.*, 2002; Akbari *et al.*, 2016). When these happen, enzymes in the liver like aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase leak out through altered membranes into the circulation leading to the observed increase in enzyme activities (Burtis and Ashwood, 2001; Ignatius *et al.*, 2006). Ignatius *et al.*, (2006) also reported that the increase in enzyme activities could be attributed to the destruction of the liver parenchyma by the malaria parasites leading to the leakage of the liver enzymes into circulation. Elevated bilirubin levels as seen in this study are also common in malaria due to increased lysis of red blood cells (Kayode *et al.*, 2011). The decrease towards normal in bilirubin levels after treatment maybe as a result of the observed decrease in parasitaemia which may have led to less lysis of red blood cells. The results obtained in this study show that aqueous leaf extract of *Cola nitida* was able to reduce parasitaemia and it contains some phytochemical constituents that possess antioxidant activity which may also help to ameliorate some complications of malaria infection.

## Conclusion

This study has demonstrated the antimalarial and antioxidant activity of the aqueous leaf extract of *Cola nitida* which was found to be dose dependent. Some antioxidant phytochemicals which have been shown to be beneficial in the management of malaria were detected in the leaf extract. Derangements in liver function indices and haematological parameters usually associated with malaria infection were also improved after treatment

probably by these phytochemicals. The results obtained in this study suggest that the aqueous leaf extract of *Cola nitida* possesses significant pharmacological potentials which can be exploited in the management of malaria.

## AUTHOR'S DECLARATION

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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