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Anti-Plasmodial Effect of the Ethanolic Leaf Extract of Drum Stick Tree (*Cassia Sieberiana*) on Albino Mice Experimentally Infected With *Plasmodium Bergheiberghei*

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ABSTRACT

Anti-plasmodial effect of the ethanolic leaf extract of Drum stick tree (*Cassia sieberiana)* on albino mice experimentally infected with *Plasmodium bergheiberghei* were evaluated. The fresh leaves were harvested and transported to the laboratory aseptically. The leaves were defoliated, washed and dried at room temperature then macerated. The active ingredients were extracted with absolute ethanol. Twenty-four albino mice used for this experiment were divided into six groups of 4 per group based on body weight. The groups were denoted A_1, A_2, A_3, A_4, A_5 and A_6. All groups of A_4 to A_6 were infected with *P. b.berghei* and treated with the, A_1 normal control. A_2 parasitized untreated group, and A_3 treated with 10mg/kg weight of combisunate. Three days post inoculation with parasite; the experimental groups were treated with 200, 500 and 1000mg/kg per body weight of the ethanolic leaf extract respectively for five days. The mice were euthanized 6th day post inoculation. The result showed that the phytochemicals of the leaf present were alkaloids (5.39 ±0.21), anthraquinones (2.09±0.32), cyanogenic glycoside (0.24±0.52), flavonoids (8.02±0.82), saponins (0.34±0.2), steroids (0.34±0.26), tannin (3.24±0.12), and terpernoids (2.37±0.45). The chemo curative effect of the leaf extract showed 96.29%, 97.85% and 98.82% at concentration level of 250, 500 and 1000 mg/kg body weight respectively. Percentage inhibition of the extract were not significantly different at $p>0.05$ when compared with 98.85% inhibition rate exhibited by the standard drug. The haematological indices showed a mean value dose dependent increase in levels of Packed cell Volume, Haemoglobin, Red Blood cell and Neutrophill when compared to the positive control and a decrease in White Blood Cell when compared to the positive control. The plant extracts proved potent in clearing malaria parasite in the blood stream of the mice. This means that *C. sieberiana*can be included in pharmacology.

Keywords: ***Cassia sieberiana,*** **antiplasmodial effect,ethanolic extract,** ***Plasmodium bergheiberghei,*** **haematological indices**

1.0 INTRODUCTION

Malaria has not only been concerned to be the world main overwhelming human parasitic infection, however it has also been associated as one of the most important public health concerns globally (Foca *et al.,* 2012*)*. The female Anopheles mosquito is the vector transporting the causative agent which is a protozoa parasite belonging to the genus *Plasmodium* (Greenwood *et al.,* 2008*;* Singh, 2011*)*. The infection is a principal cause of mortality and morbidity amongst children below five years and pregnant women in prevalent regions (Greenwood *et al.,* 2008; Abdulrazak*et al.,* 2015; WHO, 2017). Malaria is one of the most hazardous parasitic diseases in developing countries particularly in Sub-Saharan Africa where it remains the main cause of morbidity and mortality among children below age 5 and pregnant women (WHO, 2008). The World Health Organization pinned the mortality rate of malaria in Africa to be 781,000 populace per year (WHO, 2010) and regardless of the declarations of the Africa government in the 1990s and harmonizing effort promised in the content of the Roll Back malaria declaration in Abuja in 2000, malaria remains the most important health dispute (WHO, 2005; Alaba and Alaba, 2005).

Regardless of the intrinsic difficulties in measuring changes in malaria in Africa, numerous aspects are not in distrust. It is obvious that an enormous international attempt has yielded spectacular increase in the coverage of malaria control intercession and that these intervention are successful at reducing malaria transmission, case incidence and fatality (WMR, 2012). In Nigeria it is anticipated that 300,000 deaths take place every year, 60% of outpatient visits and 30% hospitalization are accredited to malaria (FMOH, 2009). Furthermore, at least 50% of the populace has at least one occurrence of malaria per annum resultant in elevated efficiency losses whereas children that are aged less than five years have 2-4 attacks yearly (FMOH, 2009).

Herbal or traditional medicine has been a most significant feature of the socio-cultural inheritance in Africa for hundreds of years even before the advent of conventional medicines. It was previously assumed to be ancient and erroneously challenged by foreign religious dating back throughout the colonial rule in Africa and subsequently by the conventional or orthodox medical practitioners (Okigbo*et al.,* 2006). plant derived medicines have been a component of traditional health concern in nearly all parts of the world for thousands of years and there is escalating concentration in them as sources in the management of disease (Mohana*et al.,* 2008). Majority of people in developing countries rely on herbalist for their medical care. The management and control of sickness by the use of accessible medicinal plants in a region will go on to participate in important roles in medicinal health care accomplishment in developing countries of the world (Akharaiyi et *al.,* 2010).

*Cassia sieberiana*is known as Drumstick tree, it's a savannah perennial legume tree, the plant sort from 10-

20 meters in height and has a yellow shining flowers. The plant is accustomed for several medicinal reasons. The bark ranges from a dark grey to black. The plant leaves are organized in leaflets that holds up to 7 -10 pairs of antipodes leaves and the leaves of are arranged spirally and possess short hairs. They have a cylindrical pod fruit which is up to 90cm, which contains rusty seeds which then changes to dark brown (Donkor *et al*., 2014). *C. sieberiana* belongs to the fabaceae family (leguminosae) which is a major group of angiosperm known as pea, legume or bean family. The genus *Cassia* is distributed from Eastern part of Gambia, Senegal, and Nigeria to Democratic Republic of Congo and Uganda (Ajayi*et a*l., 2015). Hence, this study aims to evaluate *C. sieberiana*anti-malarial activity in mice infected with *P.b.berghei.*

2.0 MATERIALS AND METHOD

2.1. Collection and identification of Reference Materials

The fresh leaves of the plants were obtained were obtained from Ignatius Ajuru University of Education which has a coordinate of latitude 4.80.45”N and longitude of 6.93.24”E. The plant were deposited in Rivers State University Botanical garden and identified by a plant taxonomist.

2.2. Preparation of Ethanolic Extracts of *C. sieberiana*

The ethanolic crude extract was carried out adopting the method of (Blight and Dyer, 1958) with slight modification. fresh leaf of *C. sieberiana*was washed in clean water and macerated using porcelain and electric blender. The leaves was weighed before grinder and weighed after been grinder. 100% ethanol solvent was used to extract the blended paste and allowed to stand overnight after which, the slurry was filtered using Whitman filter paper. The filtrate was subsequently concentrated in a rotary evaporator (45°C) and the residue was dried under reduced pressure to determine the dry weight of the leaves residue. The weight of dry residues was recorded and the percentage extraction yield was calculated. The extract was stored in air tight well labeled sample bottles in a refrigerator..

2.3 Phytochemical Analysis of the plant Extracts

Phytochemical analysis of the leaf extract of *C.sieberiana was* determined using the method of (Sofarawa, 1993).

2.4 Acute toxicity studies (LD_{50})

The median lethal dose (LD_{50}) of the extract of *C. sieberiana*that can kill 50% of the animals in a population was determined orally using the method described by (Alaribe et *al*., 2011). The mice were divided into four groups(B, C,D and F) of four mice each weighing between 18 g and 20 g. The mice were subjected to 24 hours starvation (with only water) before administration of the extracts. The extracts was dissolved in 20% Tween-80 and administered in different doses of body weight (b.wt) orally. The last group that was served as the control received only 20% Tween-80. Physical

observations such as their degree of restiveness, aggressiveness and calmness were observed. The mice were then observed for toxicity and fatalities within 72 hours. The LD_{50} was calculated using the modified formula of (Enegide*et al*., 2013). $LD_{50} = \sqrt{ab}$

Where:

a= least tolerable dose;

b = maximum tolerable dose.

2.5 Acquisition of *Plasmodium bergheiberghei* and mice

The Mice already parasitized with *Plasmodium berghei berghei* (NK65) was purchased from the animal house of the Faculty of Basic Medical Science, University of Port Harcourt, Rivers State, Nigeria. A total of twenty-four (24) adult healthy mice of both sexes weighing between 16 to 28g were used. They were purchased from Fluidic Medical Sciences animal farm at Aluu, University of Port Harcourt environment. They were housed in a specially designed wooden/wire gauze animal cage and left to acclimatize for two (3) days before use and was placed on standard feed (Vital feed growers) and given access to water. The mouse was handled in accordance with the United States of America National Research Council, Guidelines for the care and use of laboratory Animals (2003).

2.6 Inoculation of Mice with Malaria parasite

The parasitized mice with *p. b. berghei* (NK65) were anaesthetized in a glass jar containing cotton wool soaked in chloroform after five days of observing clinical symptoms of malaria. Blood was collected from the anaesthetized mice by cardiac puncture suing sterilize springs and needles. The blood was diluted in normal saline in the ration of 1:10, which is 1ml of blood in 10ml of normal saline the parasitized erythrocyte in volume of 0.2ml was used to infect each of the experimental mice intraperitoneally 6 days before treatment.

Experimental design

At the commencement of the experiment, 24 albino mice weighing between 16-28g were divided into six (6) groups of four (4) mice each in a group. The groups were labeled as A_4, A_5 and A_6. They were grouped according to the doses of plant extract. Three control groups were introduced which include the normal control group ' A_I ' which was fed with food and water only no inoculation of parasite or administration was given to this group. The next control group 'A_2' was the untreated group where the mice were inoculated with the malaria parasite but were not treated in the experiment. And finally, the Combisunate treated control group 'A_3' which was inoculated with the parasite and treated with Combisunate.

The reconstituted extracts of the leaves of the plant were administered to the mice six (6) days after inoculation with *P. b. berghei*. The extracts were administered orally with the help of feeding cannula. Group A_4 to A_6 was treated for five (5) days with 250, 500 and 1000 mg/kg dose of leaf extracts of *C. sieberiana*/kg b.wt orally daily respectively; three control groups were used. Control group A_3 was inoculated with the parasite and treated with 10 mg of

Combisunate/kg (Artemether + Lumefantrin at 80/ 480 mg) b.wt orally daily. Group A_2 was infected with the parasite but was not treated with any extract. Group A_1 was not infected with the parasite and was not treated with any extract but only fed on food and water.

2.7 Determination of Parasitaemia

Six days after inoculation of parasite, blood was collected from the tail of each mouse in the various groups before administration of extracts. This was used to make thin and thick blood smears to determine the baseline parasitaemia. Percentage of parasitaemia was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Percentage parasitaemia and average parasitaemia were calculated according to the following formula as adopted by (Ablishek*et al*., 2010).

$$\text{PP} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} * 100$$

Where :

PP = Percentage parasitaemia,

PRBC = parasitized red blood cells,

RBC = red blood cells

2.8 Determination of Percentage Average Suppression

The percentage chemo suppression was determined by using the method of Ebiloma*etal*., (2012). It was calculated by subtracting the average percentage parasitaemia in the least group from average percentage parasitaemia in control group NCO_2 (Infected untreated group). The value obtained was expressed as a percentage of the average parasitaemia in the control group NCO_2.

$$\text{APP} = \frac{\text{APPC} - \text{APPT}}{\text{APPC}} * 100$$

Where:

APP= average percentage parasitaemia,

APPC= average percentage parasitaemia in control group,

APPT= average percentage parasitaemia in test group.

2.9 Euthanization and Blood Collection for analysis

On the fifth day, at the end of administration, each mouse was withdrawn from the cage and euthanized. The mouse was stunned by cervical dislocation. The thoracic region was opened up to reveal the heart and blood was collected by cardiac puncture. The blood was collected in a well labeled sample bottles (EDTA) and was used to make thin and thick blood films for parasite count and determination of parasitaemia and for hematological assays of the mice.

2.10 Hematological Parameters of the Mice

An EDTA bottle was used to collect blood samples and was used to investigate the hematological indices immediately such as Red blood cell count (RBC), Haemoglobin concentration (Hb), Packed cell volume (PCV) and

(WBC) White blood cell Differentia.

2.11 Statistical Analysis

Data obtained are expressed as Mean $\pm$ Standard Error of Mean ($\pm SEM$). The Statistical significance was

determined using ANOVA with the SPSS (Statistical Package for Social Sciences) Version 20.0. A P-value less than 0.05 were simply statistical significance.

3.0 RESULTS

3.1 Phytochemical Screening of the EthanolicExtract of*C.sieberiana*

The qualitative phytochemical screening of the ethanolic extract of *C.sieberiana*leaves conducted indicated the presence of alkaloids, Anthraquinones, cyanogenic glycosides, Flavonoids, Saponins, steroids, tannins and Terpernoids (Table 1).

Table 1: Phytochemical Composition of Ethanolic Extract of *C. sieberiana*

Phytochemicals	***C. sieberiana* l eaf**
Alkaloids	**+++**
Anthraquinones	**++**
Cyanogenic glycoside	**+**
Flavonoids	**+++**
Saponins	**+**
Steroids	**+**
Tannins	**++**
Terpernoids	**++**

Keys: ++: moderately present; +: present in trace

3.2 Evaluation of Toxicity Studies (LD_{50})

The experimental animals were observed for 72 hours for morbidity and mortality. It was observed that at 2000 mg/kg b.wt, there were no noticeable physical signs. However after 48 hours of extract administration few physical signs were noticed such as aggressiveness, restiveness and calmness at (3000 and 5000 mg/kg body weight) (Table 4.2).

Table 2: Acute toxicity (LD_{50}) of *C. sieberiana*after 72 hours

Group	**No. of mice**	**Dosage (mg/kg b.wt)**	**% Mortality**
1	4	1000	0
2	4	2000	0
3	**4**	3000	0
4	4	5000	0
5	4	-	-

3.3 Effect of Ethanolic Extract of C. *sieberiana* on Malaria parasite.

The results showed a significant difference ($p<0.05$) in percentage parasitaemia inhibition for the treated groups relative to the untreated groups. Whereas, the control group treated with standard drug Combisunate (Artemether + Lumefantrine) 10mg/kg dose showed a percentage inhibition of 98.85%, the 1000mg/kg dose of the combined leaves had the highest percentage inhibition of 99.75% followed by 99.55% of 500 mg/kg of the combined leaf extract respectively. The leaf of *C. sieberiana* at 1000 mg/kg and 500 mg/kg showed inhibition of 98.82% and 97.85% respectively. The plant showed a dose dependent inhibitory behavior (Table 3.3)

Table 3: Effect of Ethanolic Extract of C. *sieberiana* on Malaria parasite.

Group	Extract	Dosage (mg/kg)	Average parasitaemia level before administration	Average % inhibition after administration
A_1	Food +Distilled water	_	0.00 ± 0.00^{bc}	100%
A_2	Food +Distilled water	_	11.35 ± 1.16^{a}	0.00%
A_3	Combisunate	10	13.49 ± 1.36^{a}	98.85%
A_4	*C.sieberiana*	250	10.42 ± 0.76^{ac}	96.29%
A_5	*C. sieberiana*	500	13.83 ± 1.53^{a}	97.85%
A_6	*C. sieberiana*	1000	12.39 ± 2.17^{a}	98.82%

3.4: Effect of Ethanolic Extracts on Hematological parameters of the Mice.

The result of the hematological parameters of the mice evaluated indicated a reduction in packed cell volume (PCV) of the untreated group. The PCV of the normal group 49.75±1.79 were significantly (P<0.05) different from the untreated group 19.00±1.08. The treated groups and positive control group were significantly (P<0.05) different when compared to the untreated control group. A lowered Packed cell volume (PCV) in the malaria infected patients may reflects anemia which is often mainly due to mechanical destruction of parasitized red cells as well as spleenic clearance of parasitized and defected erythrocytes. Also it is most probably that relative neutropenicleukocytopenia develops subsequently in malaria infected mice. (Table 3.5).

Table 4: Effect of Ethanolic Extracts on the Hematological parameters of the Mice.

GROUP	PCV (%)	HGB	RBC (X 10^3)	WBC (X 10^3dL)	NEU (%)	LYM (%)	MON (%)
A_1	49.75 ±1.79^{bc}	15.02 ±0.26^{c}	4.85± 0.12^{bc}	3.35± 0.19^{bc}	73.25 ±1.31^{b}	23.00 ±0.40^{c}	1.00 ±0.40^{b}
A_2	19.00 ±1.08^{a}	5.82± 0.49^{ac}	2.12± 0.14^{ac}	12.10 ±0.72^{ac}	60.75 ±2.56^{a}	31.75 ±1.70^{c}	2.50 ±0.28^{ac}
A_3	43.**50** ±1.55^{b}	13.80 ±0.23^{b}	4.42± 0.14^{ab}	5.20± 0.09^{ab}	72.75 ±1.03^{b}	21.25 ±0.48^{b}	0.50 ±0.28^{b}
A_4	27.67 ±1.33^{bc}	10.53 ±0.17^{bc}	3.53± 0.08^{c}	7.07± 0.15^{c}	70.67 ±0.67^{b}	25.67 ±1.20^{bc}	1.00 ±0.57^{b}
A_5	35.33 ±1.45^{bc}	12.03 ±0.34^{bc}	4.07± 0.13^{c}	6.47± 0.17^{c}	73.00 ±1.15^{b}	20.00 ±0.57^{b}	0.33 ±0.33^{b}
A_6	39.67 ±1.45^{bc}	14.03 ±0.12^{bc}	4.47± 0.13^{ab}	5.80± 0.12^{c}	79.00 ±1.73^{bc}	19.67 ±1.45^{bc}	0.33 ±0.33^{b}

Data are expressed as Mean ± SEM. Values found in column with superscript letter a, are significantly different ($p<0.05$) when compared to the normal control. Values with superscript b, are significantly different ($p<0.05$) relative to the untreated control. While values with the superscript c, are significantly different ($p<0.05$) compared to the Combisunate treated control.

4.0 DISCUSSION

Numerous indigenous plants have been found with astonishing anti-parasitic attribute (Fajimi and Taiwo, 2005). Plant derived-compounds had played crucial role over the years in drug discovery and development for the treatment of several diseases. The separation of new bioactive compounds from medicinal plants based on their traditional therapeutic use appears to be very easy. (Newman, 2008). Therefore, several phytochemicals such as Flavonoids, Saponins, tannins, steroids, Terpernoids alkaloids etc are the keys to drug formation.

The phytochemicals identified in this work corroborate with the findings of Awomukwu*et al.*, (2015), "on comparative chemical constituents of some *Cassia* species and other Pharmacognistic importance" *which* in their study revealed tannins, Saponins, Flavonoids, alkaloids, and terpernoids to be present in the leaves of *C. sieberiana* and these biologically active compounds are said to be secondary metabolite which are used from the foundation of drug prescription which has been recommended for anti-microbial, anti-oxidant, anti-hormonal, anti-protozoan and anti-plasmodic activities.

Igboh*et al.*, (2015), in their findings on the Chemical profile of *C. odorata* leaf revealed cyanogenic glycoside, alkaloids, Flavonoids, Saponins to be present, which can serve as a potential source to protein supplement and malaria cure. Also Okpok*et al.*, (2014), in their findings indicated tannins, Saponins, Flavonoids, alkaloids steroids, terpernoids to be present in the combined effect of the leaf of *Ficus exasperate* and stem bark of *Anthoclesistavogelii* on parasite clearance.

Furthermore, Dada and Oloruntola, (2016), in their studies indicated the ethanolic leaf extract of Marigold tree *(Tithoniadivesifolia)* against*P.b. bergheit*o have the following phytochemicals such as Saponins, tannins, alkaloids, cyanogenic glycosides and Flavonoids which can also be used in the treatment of other diseases. Lavanya*et al.*, (2018), also revealed the secondary metabolite of *C. sieberiana* to be tannins, Saponins, alkaloids, anthraquinones and Flavonoids which are used in the cure and treatment of other ailments such as fungal infection, bacterial and skin diseases like scabies, eczema

The LD_{50} (median lethal dose) was estimated at 3162 mg which did not cause any death when administered orally at concentration up to 5000 mg/kg b.wt. This means that the plant extract could be used within the ranges of 5000 mg/kg b.wt without causing any hepatic damage or being cytotoxic. Similar study of Enechi*et al.*, (2019) recorded no lethality of Artar root *(Fagarazanthoxyloides)* even at the highest dose of 5000 mg/kg b.wt administered no behavioral changes

occurred within the 24hrs acute toxicity test. Study of Akuodor *et al.,* (2014) "on the Antimalarial potency of the methanolic leaf extract of Jiga plant (*Maeruacrassifolia*)" also collaborates with the study of which at dose level of 5000 mg/kg b.wt showed no behavioral signs of toxicity such as dizziness, salivation, reduced physical activity and death.

The treatment with the plant extracts showed a lowering of parasitaemia density in all extract treated group. The mice showed that there was decrease in malaria parasite when the extracts were administered in various dosages within the b.wt. This is an indication that the ethanolic leaf extracts of *C. sieberiana*had a great effect on *Plasmodium* parasites. Physical signs of illness such as piloerection (erection of hairs on the skin), lethargy (lack of energy) diarrhea, reduced locomotor activity often seen in malaria infected mice were absent in treated groups of malaria mice, and they appeared healthier after five days post treatment. This study collaborates with that of Awoibi*et al.,* (2019) using the ethanolic extract of three different plant in malaria suppression revealed that there were no sign of behavioral changes after five day post treatment of the mice. The fifth day curative effects of the extract on malaria parasites showed theparasitaemia level to be very high ranging from the control groups to the treated group. The curative activity of the plant showed a dose independent decrease especially at 1000 mg/kg b.wt which almost abolished the parasite on day five of the treatment which revealed to be more potent than doses of 250 and 500 mg/kg b.wt. This conforms to the study of Okon*et al.,* (2014) which showed high level of suppression of parasite in all groups after five days of inoculation which also was consistent with the findings of Fidock*et al*., (2004) who also observed high parasitaemia in *P. berghei* infected mice after five days of inoculation. This is also consistent with previous study of Okolie and Awoibi, (2018) on anti-plasmodial activities of ethanolic extract of Mango (*Magnifera indica*) leaf and the seed of Bitter kola (*Garcinia kola*) on albino mice which also revealed high curative activity at dose level of 1000 mg/kg. The high potency of the extract could result from the presence of certain phytochemicals such as Flavonoids, Saponins, alkaloids which were both present in both extracts and their synergetic action might have elevated the suppressive activity of 99.23%, 99.55% and 99.75% reduction of parasitaemia at dose level of 250, 500 and 1000 mg/kg b.wt respectively which also conforms to previous report of Okon*et al.,* (2014) where the combined extract of Sandpaper fig (*Ficus exasperate*)leaf and stem bark of Cabbage tree (*Anthocleistavogelli*) on albino mice showed dose dependent level of parasite at dose level of 200, 400 to be 79.5% and 91.7% which may be as a result of the phytochemicals present in both extract such as Flavonoids, steroids and Saponins. In our studies, maximum of 98.82% suppression for *C. sieberiana*, respectively. Similar report of Okolie and Awoibi*et al.,* (2018) of 99.26% and 98.19% chemo suppression of *p.berghei* in mice using the leaf of mango and the seed of bitter kola at dose level of 1000mg/kg body weight

respectively. In the present day study, Combisunate was used as a positive control, was observed to significantly decrease the parasitaemia in the infected mice with 98.85% which also conforms to the study of Okolie and Awoibi, (2019) which reported 99.45% parasitaemia suppression on the infected mice using Combisunate as the standard drug. Similar study also of Awoibi*et al.,* (2019) also reported the extract of *Psidiumguajava*to be more potent in suppressing the parasite with 99.12% at dose level of 800 mg/kg b.wt which is higher than the standard drug (Combisunate) of 98.48% that was used to inhibit the parasite. This study of Awoibi*et al.,* 2019 contradicts that of Okon*et al.,* (2014) which reported 100% inhibition of the standard drug to the extract which as higher.

Haematological indices such as Packed Cell Volume (PCV), Haemoglobin (HGB), White blood cell (WBC), Neutrophils, Lymphocytes and Monocytes level are common biomarkers of malaria infection and frequently monitored as indicators of drug efficacy against *plasmodium* infection. Haemoglobin has the physiological function to transport oxygen to tissues of the animal for the oxidation of ingested food so as to release energy for the other body functions as well as transporting carbon (iv) oxide out of the body (Ugwuene, 2011; Isacc*et al.,* 2013). The study shows that the total haemoglobin (Hgb) was higher in cases of malaria treated group. The reduction of Hgb was as a result of the parasite infection and the extracts where able to increase the Hgb of mice especially the dose level of 1000 mg/kg which was more potent than the standard drug in increasing the Hgb almost too normal. This conforms to the study of Enechi*et al.,* (2019) which reported the extract of Artar root (*Fagarazanthoxyloides*) leaves on the haematology of infected mice with *P. berghei* where the treated mice with the extract was able to have an increased Hgb when compared to the untreated mice. PCV is used to access anaemia, erthrocytosis, haemodilution and haemaconcentraion. An increased PCV shows transportation capacity of RBC and a decreased PCV indicates anaemia (Ugwuene, 2011; Isacc*et al.,* 2013). The present study indicated that the mice had a decreased PCV when they were infected with *P. berghei* which showed traces of anaemia but when the extract was administered orally to the mice it was able to increase the PCV as a result of time frame in administration. Even at the lowest dose level of 250mg/kg the extract was still able to increase the PCV and a better potency of increased PCV showed in dose level of 1000 mg/kg body weight. This is in line with the study of Enechi*et al.,* (2019). It also agrees with the report of Omoya and Olaiya, (2014) on the efficacy of Neem (*Azadirachtaindica*) leaf on *P. berghei* infected mice which had e reduced PCV after infection and on administration of the extract the PCV increased which is one of the major factor in Neem (*Azadirachtaindica*)effectiveness against malaria infection (Saxena*et al.,* 2003). So those the study agrees with the report of Ukpanukpong*et al.,* (2018) on haematological effect of ethanolic leaf extract of Barbados nut (*Jatrophacurcas*)on *P. berghei* infected

mice, reported the increase of PCV in the treated groups with extract and a decrease in infected but untreated and in the standard drug which indicates anaemia but the extract was able to clear the anaemia. RBC showed an increased in the mice treated with the extract while there was a reduction in RBC in the infected and untreated mice. When compared to the untreated group there is high potency of the extract in increasing the RBC of the mice. The decrease in the untreated group shows severe malaria infection and anaemia which the extract was able to clear with its potency. The study conforms with the report of Enechi*et al.,* (2019) on the leaf extract of Artar root (*Fagarazanthoxyloides*) on albino mice infected with *P. berghei*, reported that there was a reduction of RBC in mice of infected untreated group but when compared to the treated mice with extract there was an increase in the RBC. It also conforms to report of (Adewale*et al.,* 2016; Omoya and Oaiya, 2014; Eluu*et al.,* 2019) which had similar result of increased RBC. The WBC of the mice increased in the infected untreated group when compared to the positive group and all the treated groups with the extracts. This WBC function mainly in body defense against foreign bodies and this is achieved through antibody production (Moisola*et al.,* 2013). Study of Johnson and Campbell, (2014) also had similar result in their study. An increased WBC has been associated to be linked to severe malaria (Modiano*et al.,* 2001) during acute malaria, WBC counts are observed to be low or normal (white *et al.,* 2001) this indicates that the extract was potent in normalizing the WBC status . The Neutrophils in the study showed reduction in the infected untreated group which indicates serve malaria infection compared to the treated group which is in agreement with the report of (Ekvall, 2003). Lymphocytes and monocytes level were seen to be high in the untreated group. The extract of the plants were potent enough to bring them to normal as a result of the time frame. The reduction of monocytes by the treated groups with the extract can be said to be as a result of the phytochemical like phenol, Saponins, Flavonoids and glycoside in the plant extract which have antioxidant effects (Omonkhua*et al.,* 2013).

5.0 Conclusion

The studyshowed that the plants possessed anti-malarial activity that were potent enough to combat malaria parasite in the blood stream of the mice. This effect might have been attributed to the presence of pharmacological active compounds like saponins, flavonoids, tannins, alkaloids, terpenoids, cyanogenic glycoside and anthraquinones in the extract which may have acted singly or in synergy with one another to show the anti-malarial activity observed in the study with percentage suppression of 98.20% for *C. sieberiana*extract respectively. The study revealed that both plants were at tolerable range and without any physical and behavioral changes with a recorded LD_{50} of 3162 mg/kg b.wt against the highest treatment dose of 1000mg/kg b.wt. The extract significantly restored altered hematological parameters such as increasing RBC, PCV, HGB,

LYM, NEU, when compared to the untreated group and lowering of the WBC and MON to almost normal. This study revealed that the plant could be listed as part of plant that has futuristic anti-malarial properties when fully exploited also in Pharmacognosy, health Services and likes.

6.0 Ethical Consideration

Application for the approval for the use of animals for the study was made to the ethical committee of the faculty of Natural and Applied Sciences, through the Department of Biology, Ignatius Ajuru University of Education and approval was granted by the committee.

REFERENCE

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