Assessment of Antiplasmodial Activity of *Terminalia Catappa* Leaf Extracts on *Plasmodium berghei* Infected Mice.

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Abstract: Antimalarial drug resistance is one of the factors that have contributed to malaria treatment failure especially in Asia and Sub-Sahara Africa where malaria is endemic. Globally, renewed interest in medicinal plants has focused on the herbal cures among indigenous populations. Of such medicinal plants is *Terminalia catappa*. The medicinal use of *T. catappa* in malaria treatment was investigated *in vivo* in this study. The fresh leaves of *T. catappa* were air dried for a period of four weeks and ground into powdery form. Two hundred and fifty grams of the powdered leaf was submerged in 1.3 litre of ethanol and hot water respectively for 72 hours to extract the bio-active ingredients. The antiplasmodial activity of the ethanol and water extracts were studied using *Plasmodium berghei* infected Swiss albino mice at 100 and 200mg/kg/day dosage. The 5 day curative test assay revealed that the administered dosages (100 and 200mg/kg/day) of *Terminalia catappa* ethanol extract caused chemo suppression of 32.88% and 39.48% respectively on day three and chemo suppression of 54.35% and 56.75% on day five. Similar dosages of hot water leaf extract caused chemo suppression of 26.63 and 30.45% respectively on day three and chemo suppression of 30.45% and 37.60% on day five. These values were statistically significant ($P<0.05$) when compared with the positive control which recorded 39.88% and 57.63% for day 3 and 5 respectively. Our finding showed that *Terminalia catappa* leaf contained active antiplasmodial compounds and therefore, might be useful in the formation of novel antimalarial drug.

Keywords: Chemo-suppression, antiplasmodial activity, malaria treatment,*Terminalia catappa*.

1.0 Introduction

Malaria is a major disease in tropical climate with high mortality rate. Despite recent advances in the development of a wide range of anti-malarials, the disease is emerging as the greatest threat to the people of tropical developing countries. It is a hemato-protozoan parasitic infection transmitted by certain species of *Anopheles* mosquitoes (Newman *et al*., 2008). Four species of the parasite, *Plasmodium*, commonly infect humans, but one, *Plasmodium falciparum*, accounts for the majority of instances of morbidity and mortality(Krettli*et al.*, 2001). Infection is often at peak during the rainy season and higher rates of bedridden workers affect the agricultural productivity of families, communities, and nations (Newman *et al*., 2008). This made the disease to be associated with poverty and regarded as a major hindrance to economic development (Joy and Feng, 2003). There has been a resurgence of interest in malaria in recent years due to the aforementioned burden it imposes on poor countries in the tropics. The control of this disease has traditionally relied on two arms: control of the *Anopheles* mosquito vector through removal of breeding sites, use of insecticides, and prevention of man- vector contact and effective case management (Newman *et al*., 2008).

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Case management has relied largely on antimalarial drugs. Chloroquine, which is inexpensive and widely available, was reported to be ineffective in the management of this disease due to misuse of the drug and resistance of the targeted etiological agent. The emergence of resistance, particularly in *P. falciparum*, to chloroquine has been a major contributor to the global resurgence of malaria in the last three decades (Marsh, 1998). According to Korenromp, *et al.* (2003), resistance is the most likely explanation for a doubling of malaria-attributable child mortality in eastern and southern Africa. In addition to this, the unavailability of newer anti-malarial drugs to the most vulnerable population coupled with high cost of available drugs prompts the need for a search on alternative, cheap and accessible remedies.

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization (Levetin and McMahon, 2003).The success of artmesinin isolated from *Artemisia annuus*and its derivatives for the treatment of malaria has focused attention on plants as sources of anti-malarial drugs (Tan *et al*., 1998). The use of herbal remedies for malaria treatment is endemic in many African countries. A cross section of both rural and urban dwellers, literate and illiterate in many African countries rely massively on herbal preparations for the treatment of fevers suspected to be malaria despite the existence of orthodox antimalarial drugs (Levetin and McMahon, 2003). In spite of the wide use of herbal medicines for the treatment of malaria, only a

small number of plant species have been studied and evaluated scientifically for possible antimalarial activities.

Tropical almond (*Terminalia catappa*) which is also known locally in Nigeria as 'Ebelebo' (Benin) is a large, spreading tree distributed throughout the tropics in coastal environments(Joy and Feng, 2003). In Nigeria, it is one of the numerous medicinal plants used by both rural and urban dwellers. Due to its chemical richness, the leaves and the bark are used in different herbal medicines for various purposes (Joy and Feng, 2003).

This study aimed at investigating the chemical components of the plant extracts of *Terminalia catappa* obtained from different solvent, the antiplasmodial activity on *Plasmodium berghei* and the effect of the different extracts on hematological parameters of Wistar rats inoculated with the extracts.

2.0 Methodology

2.1 Sample Collection

Leaves of *Terminalia catappa* were collected from the campus of The Federal University of Technology, Akure (FUTA), Nigeria. The leaves were identified at the Department of Crop, Soil and Pest, FUTA, Nigeria.

2.2 Extraction of the Leaves of Test Plant

The fresh leaves of *Terminalia catappa* were air dried for a period of one month. The dried leaves were ground into powder with an industrial blender. A weight of 250g of the leaf powder was submerged in 1.3Litre of ethanol, methanol and hot water respectively for 72hrs, stirring every four hours for homogenicity and was drained with the aid of a muslin cloth. The filtrate was collected into a beaker with appropriate labeling, and allowed to evaporate using rotary evaporator to yield the extract concentrate. The stock solution was prepared by dissolving 10g and 20g of the powdered extract in 100 ml (ratio 1:10) of Dimethylsulphodioxide, to give a stock concentration of 0.10g/ml and 0.20g/ml respectively for the ethanol extract and the same ratio was used for water extract using sterile distilled water respectively. The concentration was filtered using Whatman filter paper, and filtered using membrane filter (0.002 mm pore size) according to Owolabi, (2010).

2.3 Phytochemical Screening of *Terminalia catappa* Extracts

The ethanolic, and hot water leaf extracts of *Terminalia catappa* were screened for the presence of phytochemicals according to standard methods (Harbone, 1984). The phytochemical screening was carried out at the Department of Biochemistry, The Federal University of Technology, Akure.

2.4 Experimental Animals

Twenty apparently healthy albino mice weighing between 18-25g were obtained from Animal House at Obafemi Awolowo University, Ile-Ife, Nigeria, and were housed in a screened cage and acclimatized for a period of 14 days. They were fed with growers mash and water before infecting them with *Plasmodium berghei* for the experiment.

2.4.1 Sourcing of Malaria Parasites

Artesunate sensitive *Plasmodium berghei* strain maintained in mice were obtained from Animal House at Obafemi Awolowo University, Ile-Ife, Nigeria and transported to the Microbiology laboratory at FUTA, Nigeria.

2.4.2 Inoculum Preparation

A stock of parasitized erythrocytes was obtained from infected mice, with a minimum peripheral parasitemia of 37% by cardiac puncture in heparin-coated tube. Each mouse was inoculated on day 1; intraperitoneally, with 0.2ml of infected blood containing *Plasmodium berghei* parasitized red blood cell having parasite load of $1x10^3$parasitaemia. This was prepared by determining both the percentage parasitemia and the erythrocyte count of the donor and diluting with normal saline in proportion indicated by both determinations (Cheesbrough, 2014).

$$\%Parasitemia = \frac{\text{Total Number of Parasitized RBCs counted}}{\text{Total Number of RBCs counted}} \times 100$$

2.5 Evaluation of Malaria Parasite

The parasite load of the infected mice was evaluated after 72 hours, by preparing thick blood film, from blood collected by transection of the distal end of their tail, with a pair of scissors after cleaning with methylated spirit cotton swab. Three drops of blood were allowed to drop on a slide and with the smooth angle edge of another clean slide used as a spreader; thick blood smears were prepared. Each smear was air-dried, and stained with Leishman for 10 minutes. The slides were rinsed carefully and thoroughly under running tap water and left, to stand in an upright position to dry, and then examined under the objective lens x100 of Olympus microscope oil immersion. Each slide was observed at three different fields and the parasitized red blood cells (RBCs) were recorded (Cheesbrough, 2014).

The average percentage suppression of parasitemia was calculated of control as shown below.

$$\textbf{Average of suppression} = \frac{\text{average \% parasitaemia in control groups} - \text{average of parasitaemia in treated groups} \times 100}{\text{Average \% parasitaemia in control group.}}$$

2.6 Treatment of Experimental Animals

Thirty two (32) mice of both sexes were selected and put into four (4) groups of eight mice (four males and four females) per group. Each mouse was inoculated intraperitonaelly with the parasite. Group 1 (Gp1) animals received 0.2ml/mouse of distilled water (negative control), group 2 (Gp2) animals received 5mg/kg Artemether (Art.) positive drug control, group 3 (Gp 3) animals received ethanolic leaf extract of *T. catappa* (TCE) and group 4 (Gp4) animals received water leaf extract of *T. catappa* (TCW). Within each group, the animals were further divided into two sub-groups of four (2 male and 2 female). The sub-groups within the groups were given different treatment dosages (100 and 200mg/kg/day) respectively for five days. The drugs were administered orally. Blood counting was done for the third and fifth day after drug administration (Odeghe *et al*., 2012). The parasitemia was determined to find the qualitatively presence and degree of activity at the screening doses.

2.7 Determination of the haematological parameters of the experimental animals

The haematological parameters such as the erythrocyte sedimentation rate, total red blood cells, packed cell volume, white blood cell and its differentials were carried out according to Odeghe *et al*., (2012) at the Department of Animal Production and Health, Federal University of Technology, Akure.

2.8 Data Analysis

The data were analyzed using student's t-test. The results were expressed as mean ± SEM and differences between means were considered significant when $p< 0.05$ (Kirkwood *et al*., 2003).

3.0 Results

3.1 Phytochemical Screening

The phytochemical screening result showed the distribution of the presence of secondary metabolites in the extracts. The ethanol, and hot water extractsshowed the presence of saponins, tannin, flavonoids,terpenoid, steroid and alkaloid while phlobatannin and anthraquninous were absent. The screening also indicated positive test to Cardiac Glycosis test. The extracts were positive to Salkowski's test and Legals' test, while ethanol extract tested positive to Keller-Killiani's test and hot water extract tested negative (Table 1).

Table 1: Phytochemical constituents of the *Terminalia catappa* Extracts

	Ethanol	Water
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Terpenoid	+	+
Steroid	+	+
Alkaloid	+	–
Phlobatannin	–	–
Anthraquninous	–	–
Cardiac Glycosis		
Salkowski's test	+	+
Legals' test	+	+
Keller killiani's test	+	–
Lieberman's test	+	+

(+) indicates present and (–) indicates absent

3.2 Body Weight Measurement

The initial body weights of the mice were observed before infecting them with malaria parasite which were recorded as mean weight per group indicated that Gp 1 had 22.05g, Gp 2 had 21.20g, Gp 3 had 20.08g, and Gp 4 had 20.16g. The body weights of the mice were observed to reduce after they were infected with malaria parasite and the following results were observed; Gp 1 had 18.10g, Gp 2 had 17.12g, Gp 3 had 17.01g, and Gp 4 had 18.25g. Also, during the course of treatment, it was observed that there was still decrease in the weight of the Gp 1 mice treated with distilled water (negative control). Their mean weights reduced to 16.36g and slight increase was observed in the body weights of the mice in the remaining groups; Gp2 treated with artemether had mean weight of 18.92g, Gp 3 treated with TCE had 17.75g, and Gp 4 treated with TCW had 18.85g (Table 2).

Table2: Mean Body Weight of the Mice.

Each value expressed as Mean±S.E; Values followed by the same superscript letter in a row are not significantly different at P≤0.05

Table2: Mean Body Weight of the Mice.

Groups	Drug/extract	Weight before infection (g)	Weight during infection (g)	Weight after treatment (g)
Gp1	(distil water)	22.05± 2.21[c]	18.10±1.82[b]	16.36±2.21[a]
Gp2	(Artemether)	21.20±1.83[c]	17.21±1.92[a]	18.92±2.61[b]
Gp3	(TCE)	20.08±2.50[c]	17.01±2.51[a]	17.75±0.42[b]
Gp4	(TCW)	20.16±2.01[c]	18.25±1.13[a]	18.85±0.58[b]

Each value expressed as Mean±S.E; Values followed by the same superscript letter in a row are not significantly different at P≤0.05

Key: TCE- Ethanolic extracts; TCW-Hot water extracts.

3.3 Haematological Parameters of the experimental mice.

The haematological parameters of the mice in each group were observed and recorded in average mean per group. The parameters were recorded before the mice were infected with malaria parasite.The packed cell volume (PCV) from Gp1-Gp4 were 33.50, 38.75, 35.75, and 34.50 (%) respectively, the white blood cell are 141.50, 156.75, 156.50, and 159.50(x50/cubic mm) respectively and the differential counts were also recorded (Table 3). After 72 hours of infection with malaria parasite, there was an observable decrease in the PCV of the mice across the groups; 30.75, 33.75, 30.50, and 30.50 (%) and the WBC increased across the groups from Gp 1-Gp 4; 146.33, 159.33, 157.00, and 160.00 (x50/cubic mm) respectively. Other differentials were also recorded (Table 4). The after treatment haematological parameters of the mice in each group recorded revealed that the PCV of the Gp 1 mice treated with distil water reduced to 29.25%. Also the WBC reduced slightly to 144.00 x50/cubic mm. The PCV and the WBC of other groups showed slight increase; Gp 2 mice treated with artemether has 35.50% and 160.50 x50/cubic mm, Gp 3 mice treated with TCE has 32.50% and 159.00 x50/cubic mm, while Gp 4 treated with TCW has 31.25% and 162.25 x50/cubic mm respectively (Table 5).

Parameters	Distilled Water Gp1	Artemether Gp2	Ethanolic extract Gp3	Hot water extract Gp 4
Erythrocyte Sedimentation rate (mm/hr)	1.75 ±0.48[b]	1.00±0.00[a]	2.00±0.41[b]	2.00±0.41[b]
Packed Cell Volme (%)	33.50±0.95[a]	38.75 ± 0.48[d]	35.75±0.94[c]	34.50±1.44[b]
Red Blood Cells (x10^3 cubic mm)	469.25±44.42[d]	455.25±35.81[c]	371.25±14.10[a]	389.25±18.60[b]
White Blood Cells (x50/cubic mm)	141.50±9.56[a]	156.75±2.21[b]	156.50±2.90[b]	159.50±2.78[c]
Haemoglobin(g/100ml)	11.18±0.31[a]	12.93±0.14[c]	11.90±0.30[b]	11.50±0.49[b]
Lymphocyte (%)	62.00±1.08[a]	64.00±1.47[c]	63.00±1.08[b]	63.25±0.85[b]
Neutrophils (%)	28.00 ± 1.23[b]	25.50±1.19[a]	29.00±0.91[c]	27.50±0.64[b]
Monocyte (%)	6.25 ± 0.48[b]	6.00±1.22[b]	5.25±0.63[a]	6.25±0.48[b]
Eosinophil(%)	2.75 ± 0.48[b]	3.75±0.25[c]	2.00±0.41[a]	2.50±0.29[b]
Basophil (%)	1.00 ±0.41[d]	0.750±0.25[c]	0.50±0.27[b]	0.250±0.25[a]

Table 3: Haematological Parameters for Full Blood Count before infection of Mice.
. Each value expressed as Mean±S.E; Values followed by the same superscript letter in a row are not significantly different at P≤0.05

Table 4: Haematological Parameters for Full Blood Count 72 hours after infection of Mice.
Each value expressed as Mean±S.E; Values followed by the same superscript letter in a row are not significantly different at P≤0.05

Parameters	Distilled Water Gp1	Artemether Gp2	Ethanolic extract Gp3	Hot water extract Gp4
Erythrocyte Sedimentation rate (mm/hr)	1.50 ± 0.29^{a}	1.50 ± 0.29^{a}	1.50 ± 0.29^{a}	2.00 ± 0.41^{b}
Packed Cell Volme (%)	30.75 ± 1.11^{a}	33.75 ± 0.85^{b}	30.50 ± 0.65^{a}	30.50 ± 1.04^{a}
Red Blood Cells (x10^{3} cubic mm)	446.25 ± 35.02^{d}	433.50 ± 38.24^{c}	356.25± 11.79^{a}	376.25 ± 17.60^{b}
White Blood Cells (x50/cubic mm)	146.33 ±11.80^{a}	159.33± 2.73^{c}	157.00 ± 2.35^{b}	160.00 ± 4.04^{d}
Haemoglobin (g/100ml)	10.25 ± 0.36^{a}	11.25 ± 0.28^{b}	10.18 ± 0.21^{a}	10.15 ± 0.35^{a}
Lymphocyte (%)	63.50 ± 1.94^{c}	62.50 ± 1.32^{b}	61.75 ± 1.18^{a}	61.50 ± 0.65^{a}
Neutrophils (%)	26.00 ± 1.87^{a}	29.00 ± 0.41^{d}	28.00 ± 1.47^{c}	27.25 ± 1.18^{b}
Monocyte (%)	7.25 ± 0.25^{c}	4.50 ± 0.87^{a}	6.50 ± 0.50^{b}	7.50 ± 0.50^{d}
Eosinophil(%)	2.50 ± 0.29^{a}	2.75 ± 0.48^{a}	2.75 ± 0.48^{a}	2.75 ± 0.48^{a}
Basophil (%)	0.67 ± 0.67^{a}	1.00 ± 0.41^{c}	1.25 ± 0.25^{c}	0.75 ± 0.25^{b}

Each value expressed as Mean±S.E; Values followed by the same superscript letter in a row are not significantly different at P≤0.05

Table 5: Haematological Parameters for Full Blood Count after treatment of Mice.

Parameters	Distilled Water Gp1	Artemether Gp2	Ethanolic extract Gp3	Hot water extract Gp4
Erythrocyte Sedimentation rate (mm/hr)	1.75 ± 0.28^{b}	1.250 ± 0.25^{a}	2.00 ± 0.41^{c}	2.25 ± 0.25^{c}
Packed Cell Volme (%)	29.25±0.30^{a}	35.50 ± 0.65^{d}	32.50 ± 0.87^{c}	31.25 ± 1.49^{b}
Red Blood Cells (x10^{3} cubic mm)	452.50 ± 36.63^{d}	437.75 ± 37.88^{c}	360.50 ± 13.09^{a}	379.25 ± 17.99^{b}
White Blood Cells (x50/cubic mm)	144.00 ± 8.90^{a}	160.50 ± 2.60^{c}	159.00 ± 2.55^{b}	162.25 ± 2.81^{d}
Haemoglobin (g/100ml)	9.78 ± 0.35^{a}	11.83 ± 0.21^{c}	10.83 ± 0.28^{b}	10.53 ± 0.49^{b}
Lymphocyte (%)	65.25 ± 1.70^{a}	68.50 ± 0.65^{c}	68.00 ± 0.41^{b}	68.25 ± 0.85^{b}
Neutrophils (%)	24.00 ± 1.08^{b}	20.75 ± 1.11^{a}	21.00 ± 0.71^{a}	20.75 ± 0.85^{a}
Monocyte (%)	8.00 ± 0.913^{a}	8.00 ± 0.41^{a}	7.75 ± 0.48^{a}	8.50 ± 0.29^{b}
Eosinophil(%)	1.75 ± 0.48^{a}	2.25 ± 0.48^{b}	2.25 ± 0.48^{b}	2.00 ± 0.20^{a}
Basophil (%)	1.00 ± 0.41^{b}	0.50 ± 0.29^{a}	1.00 ± 0.41^{b}	0.50 ± 0.29^{a}

Each value expressed as Mean±S.E; Values followed by the same superscript letter in a row are not significantly different at P≤0.05

3.4 Antiplasmodial Activity of the leaf Extracts of *Terminalia catappa*

The percentage pre-parasitemia counts for each group were recorded after 72hours of infection. From Gp 1-Gp 4 the percentage pre-parasitemia were; 24.68, 31.78, 30.83, 32.10 (%) respectively. Treatment commenced after 72hours of infection, and the average parasitemia counts and average suppressions were recorded for the third day and fifth day as follows Gp 1 treated with distil water (negative control) had parasitemia count of 50.38% and 57.60% with no suppression for both days, Gp 2 treated with artemether (positive control) has parasitemia count of 20.30% and suppression of 39.88% for third day and 9.98% and 57.63% respectively for the fifth day. The extract treated groups were treated in dosages of 100mg/kg and 200mg/kg, Gp 3 treated with ethanolic extract (TCE) has parasitemia count and suppression for the third and fifth day of 21.23% and 32.88%, 13.26% and 54.35% respectively. With 100mg/kg and for 200mg/kg treatments 19.93% parasitemia and 39.48%, 11.85% parasitemia 56.75%were recorded for the third and fifth supressions respectively. Group 4 treated with hot water extract (TCW) had; 30.75% and 26.63% third day, 25.15% and 30.45% fifth day for 100mg/kg and for 200mg/kg we have 23.83% and 30.45% third day, 17.57% and 37.60% on the fifth day respectively (table 6).

Table 6: Percentage Pre-Parasitemia Count and the Antiplasmodial Activity of the Extracts
The mean values with the same letter within the same column are not significantly different from each other at p<0.05

			Dose/Extract	Third Day		Fifth Day	
Groups	**Drug/extract**	**Pre-parasitemia count**	**(mg/kg/day)**	**Average parasitemia %**	**Average suppression %**	**Average parasitemia %**	**Average suppression %**
Gp1	**Distilled water (negative control)**	$24.68^{a} \pm 1.89$	0.2ml	$50.38^{b} \pm 2.81$	0.00	$57.60^{c} \pm 3.28$	-
Gp2	**Artemether (positive control)**	$31.78^{b} \pm 0.48$	5	$20.30^{c} \pm 0.85$	39.88±2.55	$9.98^{b} \pm 1.60$	57.63±2.93
Gp3	**TCE** (ethanolic	$30.83^{b} \pm 0.50$	100	$21.23^{c} \pm 0.32$	32.88±2.84	$13.26^{b} \pm 0.12$	54.35±3.24
	extracts)	30.83 ± 0.50	200	19.93±0.31	39.48±2.88	11.85±0.34	56.75±3.08
Gp4	**TCW (Hot water**	$32.10^{c} \pm 1.89$	100	$30.75^{a} \pm 0.14$	26.63±2.79	$25.15^{a} \pm 0.66$	30.45±3.45
	extract)	32.10 ± 1.89	200	23.83±0.14	30.45±2.69	17.57±0.41	37.60±3.01

4.0 Discussion

The antimalarial activity of *Terminalia catappa* leaf extract recorded in the present study might be due to the presence of the observed phyto-constituents. This is because the presence of glycosides, alkaloids, saponins, tannins, flavonoids and carbohydrates (Nwanjo and Alumanah, 2005) has been implicated in anti-plasmodial activities and as such, anti-plasmodial activity observed in this study might be due to either the presence of one of these compounds or synergistic action of all the compounds (Okwu, 2004). Some plants are known to exert antiplasmodial activity either by causing red blood cell oxidation or by inhibiting protein synthesis (Kirby *et al.*, 1989) depending on their phytochemical constituents. Thus, the active involvement of these phytochemicals in the plant extracts antiplasmodial activity cannot be overemphasized.

The body weights of the mice were observed to be reducing in measurement (g), when they were infected with the malaria parasite. Also, some other symptoms were observed in the mice such as sluggishness and loss of fur. All this symptoms could be attributed to loss of appetite and stimulation of the immune response that is associated with malaria illness in humans. However, the body weights showed slight increase after treatment with the extracts (TCE, and TCW) and artemether respectively which could be as a result of the efficacy of the drug in alleviating the disease. On the other hand, Gp1 animals treated with distil water (negative control) has a drastic reduction in weight to 16.36g because water does not have any effect on the parasitemia level of the mice in the group.

In this study, the extract and artemether produced a significant positive ($p < 0.05$) difference on the haematological (PCV or haematocrit), haemoglobin (Hb), white blood cells (WBC), platelets, lymphocyte, neutrophils and monocyte when compared with the negative control. The slight increase in haematological values demonstrated an improvement in disease progression (Chang and Stevenson, 2004). Literature has shown that ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters (Odeghe *et al.*, 2012). This decrease may also be due to multiple causes, of which repeated haemolysis of infected red cells is the most important. The haemolysis may be due to non-immune destruction of parasitized red cells in case of high parasitemia or immune mediated destruction of parasitized as well as non-parasitized red cells because the changes in the red cell antigen structure brought about by the parasitic invasion stimulate the production of antibodies against the red cell (Cheesbrough, 2014). This triggers immune-mediated red cell lysis. This may account for further decrease in Hb. These decreases however were considerably reversed in the infected extract-treated and infected artemether -treated groups on day 5 post-infection. This suggests that the extract may have some stimulatory effect on the production of red blood cells (erythropoiesis). This might have contributed to the increase in Hb and PCV observed in the infected extract-treated group on day 5 post infection.

Anaemia is a fairly common problem encountered in malaria. The observed anaemia in *P. berghei* infected mice may be due to RBC destruction caused either by parasite multiplication or by spleen reticuloendotelial cell action (Odeghe *et al.*, 2012).

The observed increase in WBC in the infected animal groups on day 3 post-infection may have resulted from stimulation of the immune system of the animals to fight the malaria parasites. White blood cells function mainly to fight infection, defend the body by phagocytosis against invasion by foreign organisms,

and to produce, transport and distribute antibodies in the immune response.

Lymphocytes are the main effector cells of the immune system (Mcknight *et al*., 1999). The increase in the lymphocytes of the infected mice in this study may be the immune system response to the parasites that may have invaded the system. Although Artemether showed superior antiplasmodial activity but *Terminalia catappa* extract still stand a chance in the traditional malarial treatment. The result did not show any special activity regarding the sex of the rats.

Summarily, the antiplasmodial activity of the extracts was found to be statistically significant compared with the negative control. However, this study showed that the ethanolic extract of *Terminalia catappa* has significant anti-malarial effect on *P.berghei* strains. It can therefore be said that if the ethanol extract of *Terminalia catappa* is subjected to purification of its active constituents, it might have more anti-malaria effect than artemether. Findings in this study therefore suggest that the leaf extracts of *Terminalia catappa* are potential therapy in the search for novel antimalarial drugs. Further work on the bioactive constituents is on-going.

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References

Chang, K. H., and Stevenson, M. M. (2004). Malarial anaemia: mechanisms and implications of insufficient erythropoiesis during blood-stage malaria, *International Journal of. Parasitology*, **34**: 1501-1516.

Cheesbrough, M. (2014): District Laboratory Practice in Tropical Countries. Low price 2nd edition update, Cambridge University Press, United Kingdom. Pp588.

Harbone, J. B. (1984). *Phytochemical methods. A guide to modern technique of plant analysis* (2rded.). London: Chapman and Hall.

Joy, D., and Feng, X. M. (2003). Early Origin and Recent Expansion of *Plasmodium falciparum*. *Science,***300** (5617): 31821.

Kirby, G. C., O'Neil, M. J., Philipson, J.D., and Warhurst, D. C. (1989). *In vitro* studies on the mode of Chloroquine-resistant Plasmodium falciparium. *BiochemPharmacol*, **38**: 4367-4374.

Kirkwood, B. R., and Sternc, J. A. C. (2003). *Essential Medical statistics*. (2nd ed). MA: Blackwell Science Ltd., U.S.A.

Korenromp, E. L, Williams, B. G., Gouws, E., Dye, C., and Snow, R. W. (2003). Measurement of trends in chSildhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy. *Lancet Infect. Dis.***3**:349–358.

Krettli, A. U., Andrade-Neto, V. F., Brandão, M. G. L., and Ferrari, W. M. S. (2001). The Search for New Antimalarial Drugs from Plants Used to Treat Fever and Malaria or Plants Ramdomly Selected. *Rio de Janeiro*, **96**(8): 1033-1042.

Levetin, E. and McMahon, k. (2003). *Plants and Society* (3rd edition). Iowa: McGraw-Hill.

Marsh, K. (1998). Malaria disaster in Africa. *Lancet*, **352**:924–925

Mcknight, D. C., Mills, R. G., Bray, J. J. and Crag, P. A. (1999). Human Physiology, (4th ed.) Churchill Livingstone, p 290-294.

Newman, J . L., Mahretu, A., Shillington, K., Stock R (2008). *Africa*. Microsoft Student 2008 [DVD]. Redmond, WA: Microsoft Corporation.

Nwanjo, H. U., and Alumanah, E. O. (2005). Effect of aqueous extract of G.latifolium leaf on some indices of liver functions in rats. *Global J.Med.Sci*.,**4**(1): 29-32.

Odeghe, O. B., Uwakwe, A. A. and Monago, C. C. (2012). Some Biochemical and Haematological Studies on the Methanolic Extract of *Anthocleista Grandiflora* Stem Bark. *International Journal of Applied Science and Technology*, **2**: 5.

Okwu, D. E. (2004). Phytochemical and vitamins content of indigenous species of South Eastern Nigeria. *Journal of Suitable Agriculture Environment.* **6** (1):30-31.

Owolabi, C. (2010). Modern methods in organic chemistry. Longman University Press, Ibadan, Nigeria. Pp276.

Tan, R. X., Zheng, W. F., and Tang H. Q. (1998). Biologically active substances from the genus *Artemisia*. *Planta Med*, **64**: 295–302.