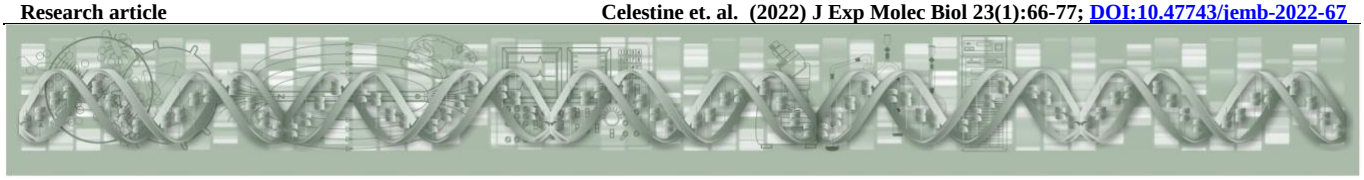

AQUEOUS AND ETHANOLIC LEAF EXTRACTS LEAF EXTRACTS OF *Lophira Lanceolata* PROPERTIES EFFECT IN MICE INFECTED WITH *Plasmodium Berghei*

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ABSTRACT

Background: This study was to determine the effects of *Lophira lanceolata* leaf extracts on haematological, biochemical, and histopathological parameters of the mice in the transmission of *Plasmodium* parasites using mice infected with *P.berghei* as a model. A total of 196 experimental mice divided into 15 groups in a randomized block design and replicated four times with four mice in each group were used for this study. Thirty six (36) mice were used for acute toxicity test, 50 each for haematological, biochemical, and histopathological analyses. Experimental mice were inoculated intraperitoneally with standard inoculum of 1 x 10^7 *P.berghei* parasitized red blood cells on first day (DO).

Results: Data analysis was done using student's t-test and one way analysis of variance with multiple comparison tests. Aqueous leaf extract exerted significant ($p < 0.05$) non-dose dependent reduction in parasitaemia level with chemosuppression of 48.79%, 24.26%, 29.38% and 80.32% at 10, 100, 200 mg/kg body weight and 5mg/kg body weight chloroquine treated groups respectively. Dose level of 10, 100, 200 mg/ kg body weight of both aqueous and ethanolic leaf extract produced significant increase in aspartate amino transferase, alanine amino transferase, total bilirubin, cholesterol, triglycerides and urea profiles of infected treated mice compared with the control. At 200mg /kg body weight of aqueous extract there was a significant decrease ($p < 0.05$) in weight of liver and kidney organs in the infected treated mice compared with the control. The results of histopathological examinations of the liver organs of the mice were characterized by tissue stromal proliferation, enlarged hepatocytes and disorganized sinusoids and lamina of hepatic cells. The results of histopathological examination of kidneys showed no histopathological changes.The aqueous and ethanolic leaf extracts of *Lophira lanceolata* plant showed significant antiplasmodium activities comparable to chloroquine drugs.

Conclusions: The leaf extracts of *Lophira lanceolata* plant had gametocytocidal action on *P. berghei*. The results of the haematological, biochemical and histopathological examinations indicated that caution should be exercised while taking the plant extract as an antimalarial agent.

Keywords: Leaf Extracts, *Lophira lanceolata*, Haematological, Biochemical, histopathological, Antimalarial

Introduction

Lophira lanceolata (Common Name: Dwarf Red Ironwood) is a tree commonly found in the savanna region. It often grows gregariously on fallow land at the edge of forests. It is widely distributed in African countries such as Uganda, Cameroun, Central Africa Republic, Benin, Gambia, Mali, Ghana, Guinea-Bissau, Sierra - Leone, Senegal, Sudan, Togo, Cote d'Ivoire and Nigeria (Igoli *et al*., 2005). The plant is 8 to 10m tall and usually straight or twisted, with alternate leaves clustered at the end of its branches. The branches are short, straight and bright with oblong-lanceolate blade. The bark surface is corkey grey; the young leaves are red and its fruits develop between February and March (Eromosele, 1993). This plant is used in traditional medicine to treat several illnesses. The infusion of the fresh leaves when administered orally is very useful against headaches, fever, dysentery, diarrhea, cough, abdominal pains and cardiovascular diseases Kouakou *et al*., 2013). It also has a wound healing effect on the skin (Kouakou *et al*., 2013; Igoli *et al*., 2005).

The word malaria originated from Medieval Italian term *mala aria*—"bad air"; the disease was formerly called *ague* or *marsh fever* due to its association with swamps and marshland (Reiter, 1999). The term first appeared in the English literature about 1829 (Strong and Richard, 1944). Malaria was once common in most of Europe and North America (Lindemann, 1999) where it is no longer endemic (Gratz, 2006) though imported cases do occur (Webb, 2009).

Scientific studies on malaria made their first significant advance in 1880, when Charles Louis Alphonse Laveran, a French army doctor working in the military hospital of Constantine in Algeria , observed parasites inside the red blood cells of infected people for the first time. He therefore proposed that malaria is caused by this organism, the first time a protist was identified as causing disease. For this and later discoveries, he was awarded the 1907 Nobel Prize for Physiology or Medicine. A year later, Carlos Finlay, a Cuban doctor treating people with yellow fever in Havana, provided strong evidence that mosquitoes were transmitting disease to and from humans. This work followed earlier suggestions by Josiah C. Nott (Chernin, 1983) and work by Sir Patrick Manson, the "father of tropical medicine", on the transmission of filariasis (Chernin,1977).

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Although the parasite responsible for *P. falciparum* malaria has been in existence for 50,000–100,000 years, the population size of the parasite did not increase until about 10,000 years ago, concurrently with advances in agriculture (Harper and Armaelagos, 2011) and the development of human settlements. Close relatives of the human malaria parasites remain common in chimpanzees. Some evidence suggests that the *P. falciparum* malaria may have originated in gorillas (Webb, 2009).
References to the unique periodic fevers of malaria are found throughout recorded history (Cox, 2002). Hippocrates described periodic fevers, labelling them tertian, quartan, subtertian and quotidian (Strong and Richard, 1944). The Roman Columella associated the disease with insects from swamps (Strong and Richard, 1944). Malaria may have contributed to the decline of the Roman Empire, and was so pervasive in Rome that it was known as the "Roman fever"(Sallares, 2002). Several regions in ancient Rome were considered at risk for the disease because of the favourable conditions present for malaria vectors. This included areas such as southern Italy, the island of Sardinia, the Pontine Marshes, the lower regions of coastal Etruria and the city of Rome along the Tiber River. The presence of stagnant water in these places was preferred by mosquitoes for breeding grounds. Irrigated gardens, swamp-like grounds, runoff from agriculture, and drainage problems from road construction led to the increase of standing water (Hays *et al*., 2004).
The first effective treatment for malaria came from the bark of cinchona tree, which contains quinine. This tree grows on the slopes of the Andes, mainly in Peru. The indigenous people of Peru made a tincture of cinchona to control fever (Achan *et al*., 2011). The present study was undertaken to ascertain the Aqueous and ethanolic leaf extracts of *lophira lanceolata* properties effect in mice infected with *plasmodium berghei*

Materials and Methods

Collection and Authentication of plant

The fresh leaves of *Lophira lanceolata* were collected in July 2017 at Obinze, Owerri West Local Government Area, Imo State, Nigeria. The plant materials were transported in polythene bags to the Research Laboratory of Department of Biology, Federal University of Technology Owerri, Imo State for identification. The specimen was identified and authenticated by Dr. C. M. Duru, a plant taxonomist in the Department of Biology, Federal University of Technology Owerri, Imo State, Nigeria. Voucher specimen number FUTO2546 was left in the Departmental herbarium.

Study Design

The study adopted randomized complete block design.

Preparation and administration of leaf extracts

Fresh leaves of the plant were sorted to remove any dead matter and other unwanted particles. The plant leaves were washed in tap water and air-dried for two (2) weeks (Odeghe *et al*., 2012). The crude extracts were prepared by cold maceration technique according to 'O' Neil *et al.* (1985).

Ethanol extraction

One hundred grams (100g) of the air-dried powdered leaf were mixed with ethanol 95% (500 ml) using a rotary shaker at 200 rpm for 24 hours at room temperature (25 $\pm$ 3^0C). The filtrates were pooled and filtered two times on cotton wool and once on Whatman (No 1) filter paper. The ethanol was evaporated at 50^0C using a rotary evaporator (Odeghe *et al*., 2012). The prepared extract was weighed, labeled and stored at 4^0C in air tight bottles until ready for use.

Aqueous extraction

One hundred grams (100g) of leaf powder was dissolved in 500ml of distilled water. The solvents were evaporated using a rotary evaporator at 100 rpm. The prepared extract was weighed, labeled and stored at 4^0C in air tight bottles until ready for use. The yields of the extract obtained were calculated by the formula as shown below:

$$\text{Extraction yield} = \frac{\text{Weight of concentrated extract}}{\text{Weight of plant dried powder}} \times \frac{100}{1}$$

The weighed extracts were administered orally to animals using a metal orpharyngeal cannula.

Experimental design and Treatment

The animals were divided in a completely randomized design (CRD) into fifteen groups: I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, XIII, XIV and XV of four mice in each. Animals in groups I, VI, VII,VIII, XII,XIII and XIV were not inoculated with parasite while those in groups II, III, IV, V,IX,X, XI, and XV were intraperitoneally inoculated with 0.2ml of infected blood containing $1x10^7$ *P. berghei* parasitized red blood cells.
The mice were treated thus:
Group I: (uninfected mice): received 10ml of distilled water (positive control).
Group II: (infected mice): received 10ml of distilled water (negative control).
Group III: (infected mice): received the aqueous extract of 10mg/kg body weight.
Group IV: (infected mice): received the aqueous extract of 100mg/kg body weight
Group V: (infected mice): received the aqueous extract of 200mg/kg body weight
Group VI: (unnfected mice): received the aqueous extract of 10mg/kg body weight.
Group VII: (uninfected mice): received the aqueous extract of 100mg/kg body weight.
Group VIII: (uninfected mice): received the aqueous extract of 200mg/kg body weight.
Group IX: (infected mice): received the ethanolic extract of 10mg/kg body weight.
Group X: (infected mice): received the ethanolic extract of 100mg/kg body weight
Group XI: (infected mice): received the ethanolic extract of 200mg/kg body weight.
Group XII: (uninfected mice): received the ethanolic extract of 10mg/kg body weight.

Group XIII: (uninfected mice): received the ethanolic extract of 100mg/kg body weight.
Group XIV: (uninfected mice): received the ethanolic extract of 200mg/kg body weight.
Group XV: (infected mice): received chloroquine solution of 10mg/kg body weight.
Thin films from tail blood samples obtained on Day 7, Day 14, Day 21 and Day 28 were examined microscopically to monitor gametocytaemia level. On 29th Day, thin film from tail blood sample of each mouse was prepared. The percentage gametocytaemia was obtained by counting the number of red blood cells (RBC) having gametocytes in every 500 erythrocytes in random fields of the microscope
Percentage gametocytaemia was calculated using the formula below:

$$\% \text{ gametocytaemia} = \frac{\text{No of RBC infected with gametocytes}}{\text{Total No of RBC counted}} \times \frac{100}{1}$$

Body weight measurement
Body weight of all groups of mice was taken using a sensitive digital weighing balance before the commencement of the first oral administration of the plant extracts. These were considered to be the initial body weight. The body weights of all groups were also taken on the last day of oral administration and these were considered to be the final body weight. The mean body weight per group was calculated using the formula shown below:

$$\text{Mean body weight} = \frac{\text{Total weight of the mice in the group}}{\text{Total number of mice in that group}}$$

Blood collection for haematological and biochemical investigation
At the end of the experiment, all the experimental mice were fasted overnignt and their blood samples were collected by cardiac puncture into specimen containers using 5ml sterile needles and 5ml syringes. The specimen containers for haematology tests contained ethylene- diamine-tetra-acetic acid (EDTA) to prevent adhesion proteins (coagulation factors) in cell-cell and cell-matrix interactions while the specimen containers for biochemistry tests did not have anticoagulants (EDTA) to avoid difficulty during centrifugation. Also, a drop of blood sample from each mouse was placed on a clean and labeled microscope slide for immediate processing of blood films for the differential white blood count.
Determination of Haematological Parameters:
Haematological parameters were done using automated haematological analyzer
The hematological components including Total White Blood Cells Counts (WBC), Haemoglobin (HB), Haematocrit (HCT), Red Blood Cells (RBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), platelet count (PLT), Mean Platelet Volume (MPV), prolactin Count (PCT) and Platelet Distribution Width (PDW) were determined using an autohaematology analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan employing the methods described by Dacie and Lewis (2002) and Bashir *et al*., (2015). The differential white blood cell counts (WBC) including neutrophils, lymphocytes, monocytes eosinophils, and basophils were carried out using stained thin blood film examined under the microscope. Slides were viewed using a compound microscope with oil immersion (x100 magnification). Each smear was examined in a systematic pattern, scanning from side to side within the "feathered edge" and counting cells consecutively. The different white blood cells were identified based on their morphologic features such as the size and structure of their nucleus and the colour and texture of their cytoplasm.
Determination of Biochemical Parameters
The blood samples collected from the experimental mice were kept at 4ºC for 4 hours to let it clot. The clotted blood samples were centrifuged (using Humax 4k bench top Centrifuge with a capacity of 12x15ml; Germany, Max-Planck-ring 21D-65205 Wiesbaden) at 5000 rpm maximum speed for 10 minutes to obtain the serum. The serum samples were kept in -22ºC refrigerator until ready to be used for biochemical analysis. Then, biochemical parameters were measured using different biochemical kits as follows:
Determination of Aspartate Aminotransferase (AST)
Aspartate Aminotransferase (AST) was assayed colorimetrically using clinipak kit.
Aspartate aminotransminase catalyses the reaction between a ketoglutarate and L-Aspertate, giving glutamate and oxaloacetate. Oxaloacetate in presence of malate dehydrogenase reacts with NADH giving malate and NAD. There is decreased in absorbance at 340nm as NADH is converted to NAD. The rate of decrease in absorbance is measured and is proportional to AST in the sample.
Histopathological Examinations
On the 30th day, one mouse from each replicate treatment group and control were sacrificed after anesthetizing with tricaine methanesulfonate (MS 222) to minimize stress. Animals from each group were sacrificed at the end of 30 treatment days, after the body weight of mice were taken one by one using a digital electronic balance. Animals under diethyl ether anesthesia were laid on a clean paper towel and had all four extremities pinned to thin corkboard. A vertical midline incision with scissors cut from the neck to pubis opened the peritoneum. Then, 3-4mm wide strips of tissue samples were randomly taken from the right lobe of the liver and coronal section of right kidneys were cut lengthwise with a scalpel through the renal pelvis after each of these organs was weighed. These tissue samples were taken from each organ and transferred by a blunt forceps to a test tube containing 10% buffered formalin that completely immerses the tissues for the purpose of fixation.
Histological Tissue Processing Procedure

Fixation: The organs were preserved in 10% neutral buffered formalin for 24 hrs. Cut-Up: The organs were examined grossly and pieces of tissue cut out/selected and placed in tissue cassette for histological processing. **Dehydration**: Tissues were placed in 4(four) changes of increasing grades/concentrations of Isopropyl –Alcohol (IPA) i.e. 70%, 80%, 90%, 100% for 1hr each. **Clearing/Dealcoholization**: Tissues were placed in 2 (two) changes of Xylene for 30mins each that was to remove IPA. **Impregnation/Infiltration**: Tissues were transferred into 2 (two) changes of wax bath containing molten paraffin wax, so that the Xylene could diffuse out into the surrounding melted wax and the wax in turn replacing it**. Embedding**: Tissues were embedded in molten paraffin wax using stainless steel embedding mould and then placed on ice block to solidify. **Sectioning**: Tissue blocks were cut into thin sections of 5 micrometer using Leica RM 212 Rt Rotary Microtome. **Floatation**: Cut sections were floated on a thermostatically regulated water bath of 45^0C to spread out and then picked with a clean grease free slide. **Flattening**: Tissue sections on slides were placed on a thermostatically regulated hot plate of 65^0C to allow the sections stick firmly on the slide.

Staining:

The sections of tissue were stained using haematoxylin and Eosin (H & E) method and Giemsa stain.

Data Analysis

The data was analysed using the Statistical Package for Social Sciences (SPSS) version 17. T-test and and one way analysis of variance (ANOVA) with multiple comparison tests were used to compare parameters.

RESULTS

The doses of 10, 100 and 200 mg/kg of ethanolic leaf extract in the infected mice produced a significant decrease in total WBC, neutrophil, lymphocyte, monocyte, eosinophils and basophil counts compared with the mice in the control group (**Tables 1 and 2**). Specifically, the dose level of 10mg/kg body weight of ethanolic extract caused a significant decrease in neutrophil profiles of infected and uninfected mice compared to control. However, there was a significant increase ($P < 0.05$) in lymphocyte counts at dose level of 10mg/kg body weight of ethanolic extract of infected and uninfected mice compared with the control. There were significant differences ($P< 0.05$) between the infected and uninfected mice in levels of lymphocyte, monocyte, eosinophil and basophil counts at the dose levels of 100mg/kg body weight and 200mg/kg body weight of the ethanolic extract.

Table 1: Effects of aqueous leaf extracts of *L. lanceolata* on total white blood cell counts (WBC) and differential counts

Treatments (mg / kg)	WBC (X109/l)	Neu (%)	Lymph (%)	Mon (%)	Eos (%)	Bas (%)
Distilled water	14.82^{d}	28.62^{a}	66.10^{c}	2.25^{c}	2.50^{a}	0.68^{b}
10						
Infected, untreated	18.33^{a}	22.50^{d}	71.40^{a}	2.75^{b}	2.50^{a}	0.48^{bc}
0						
Infected, aqueous extract treated						
10	15.87^{c}	26.75^{b}	68.25^{b}	2.75^{b}	2.00^{b}	0.25^{c}
100	15.23^{c}	27.50^{b}	67.15^{c}	2.75^{b}	2.00^{b}	0.60^{b}
200	16.40^{b}	23.98^{d}	69.80^{a}	3.50^{a}	2.25^{ab}	0.48^{bc}
Uninfected, aqueous extract treated						
10	15.03^{c}	24.85^{c}	70.22^{a}	2.50^{bc}	2.00^{b}	0.45^{c}
100	14.25^{b}	28.03^{b}	67.15^{c}	2.75^{b}	1.75^{b}	0.85^{a}
200	15.40^{c}	25.33^{bc}	69.80^{ab}	2.75^{b}	1.75^{b}	0.58^{b}
Infected, chloroquine treated	15.50^{c}	28.75^{a}	65.65^{c}	2.75^{b}	2.25^{ab}	0.50^{b}
5						
SEM	1.01	1.24	1.15	0.56	0.43	0.39

Values are mean $\pm$ S. D n = 4. *Mean values having different superscripts along the same column are significantly different (P<0.05).* Legend: **WBC**, white blood cell; **Neut**, Neutropils; **Lymph**, Lymphocytes; **Mon**, Monocytes; **Eos**, Eosinophils; **Bas**, Basophils

The dose levels of 10mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight of ethanolic leaf extract in the infected mice produced a significant decrease in total WBC, neutrophil, lymphocyte, monocyte, eosinophils and basophil counts compared with the mice in the control group (**Table 2**). Specifically, the dose level of 10mg/kg body weight of ethanolic extract caused a significant decrease in neutrophil profiles of infected and uninfected mice compared to control. However, there was a significant increase ($P < 0.05$) in lymphocyte counts at dose level of 10mg/kg body weight of ethanolic extract of infected and uninfected mice compared with the control. There were significant differences ($P< 0.05$) between the infected and uninfected mice in levels of lymphocyte, monocyte, eosinophil and basophil counts at the dose levels of 100mg/kg body weight and 200mg/kg body weight of the ethanolic extract.

Table 2: Effects of ethanolic leaf extracts of *L. lanceolata* on total white blood cell counts (WBCs) and differential WBC in mice

Treatments (mg / kg)	WBC ($X10^9$/l)	Neu (%)	Lymph (%)	Mon (%)	Eos (%)		Bas (%)
Distilled water							
10	14.82^{c}	28.65^{a}	66.10^{b}	2.25^{c}	2.50^{c}	0.68^{b}	
Infected, untreated							
0	18.33^{a}	22.50^{d}	71.40^{a}	2.75^{bc}	2.50^{a}	0.48^{c}	
Infected, ethanolic extract treated							
	9.63^{d}	25.25^{b}	68.48^{a}	2.50^{c}	2.00^{b}	0.65^{c}	
10	15.09^{b}	24.45^{b}	68.75^{a}	3.50^{b}	2.50^{a}	0.80^{a}	
100	16.12^{b}	20.75^{c}	72.45^{a}	4.00^{a}	2.25^{ab}	0.80^{a}	
200							
Uninfected, ethanolic extract treated							
	15.85^{b}	25.32^{b}	68.60^{a}	3.25^{b}	2.50^{a}	0.33^{d}	
10	15.62^{b}	28.60^{a}	67.18^{ab}	2.25^{c}	1.50^{c}	0.48^{c}	
100	15.38^{b}	27.88^{a}	66.77^{b}	3.00^{b}	2.00^{b}	0.35^{d}	
200							
Infected, chloroquine treated							
5							
	15.50^{b}	28.75^{a}	65.65^{b}	2.75^{bc}	2.25^{ab}	0.50^{c}	
SEM	1.94	1.41	1.20	0.66	0.50	0.34	

Values are mean ± S. D n = 4. Mean values having different superscripts along the same column are significantly different (P<0.05). Legend: **WBC**, white blood cell; **Neut, Neutropils**; **Lymph**, Lymphocytes; **Mon**, Monocytes; **Eos**, Eosinophils; **Bas**, Basophils

Effect of aqueous and ethanolic leaf extract of *L. lanceolata* on erythrocytes, platelets and the related parameter profiles in mice

The aqueous leaf extract of *L. lanceolata* induced changes in the erythrocytes, platelets, and their related parameter profiles in mice as shown in **Table 3**. The dose levels of 10mg/kg body weight of aqueous extract in the infected mice had a significant decrease (p <0.05) in red blood cell counts (RBC) but had no significant difference (p>0.05) on haemoglobin (HGB), haematocrit (HCT), mean platelet volume (MPV), and plateletcrit (PCT) profiles compared with the control. Furthermore, there was a significant increase (p<0.05) in levels of mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular haemoglobin (MCH) profiles at the dose level of 200mg/kg body weight in the infected mice compared with the control. The uninfected mice at the dose level of 100mg/kg body weight of aqueous extract caused a significant increase (p <0.05) in levels of MCV, MCHC and MCH profiles compared with the control.

Table 3: Effect of aqueous leaf extract of *L. Lanceolata* on erythrocytes, platelets and the related parameter profiles in mice

Treatments (mg / kg)	RBC ($X10^{12}$/l)	HGB (gldl)	HCT	MCV (fl)	MCHC (gldl)	MCH (Pg)	PLT (10^{9}/l)	MPV (fl)	PDW	PCT (ml/l)
Distilled water										
10	4.81^{a}	12.75^{a}	0.370^{a}	78.95^{b}	31.90^{b}	28.75^{b}	364^{c}	8.95^{a}	15.05^{a}	1.82^{b}
Infected, untreated										
0	4.11^{b}	10.38^{a}	0.429^{a}	84.68^{a}	33.23^{a}	29.60^{a}	343^{a}	8.95^{a}	15.05^{a}	1.82^{b}
Infected, aqueous extract treated										
10	4.22^{ab}	12.65^{a}	0.388^{a}	85.45^{a}	33.15^{a}	31.25^{a}	362^{a}	8.08^{b}	15.08^{a}	1.62^{b}
100	4.68^{a}	12.68^{a}	0.437^{a}	84.65^{a}	32.23^{a}	31.62^{a}	345^{a}	6.62^{c}	15.08^{a}	1.86^{a}
200	4.59^{a}	11.17^{a}	0.389^{a}	86.30^{a}	34.25^{a}	30.0^{a}	358^{a}	7. 35^{bc}	13. 30^{a}	2.01^{a}
Uninfected, aqueous extract treated										
10	4.32^{a}	11.63^{a}	0.395^{a}	76.85^{b}	29.17^{c}	27.85^{b}	374^{a}	7.43^{bc}	15.38^{a}	2.05^{b}
100	4.31^{a}	10.62^{a}	0.422^{a}	89.25^{a}	31.75^{a}	31.62^{a}	364^{a}	8.10^{b}	15.05^{a}	2.12^{a}
200	4.88^{a}	10.63^{a}	0.360^{a}	88.75^{a}	32.20^{b}	30.10^{b}	355^{a}	7. 20^{c}	15. 55^{a}	1.93^{a}
Infected, chloroquine treated										
5	5.22^{a}	11.63^{a}	0.384	85.8^{a}	32.50^{a}	30.50^{a}	360^{a}	8.73^{a}	15.25^{a}	1.33^{a}

SEM	0.42	0.77	0.131	1.76	1.00	0.97	2.78	0.76	0.75	0.35

Values are mean ± S. D. n = 4 Mean values having different superscripts along the same column are significantly different (P<0.05). Legend: **HGB**, Haemoglobin; **HCT**, hematocrit; **RBC**, Red Blood Cells **MCV**, Mean Corpuscular Volume; **MCH**, Mean Corpuscular Haemoglobin; **MCHC**, Mean Corpuscular Haemoglobin Concentration; **PLT**, platelet count; **MPV**, Mean Platelet Volume; **PCT** ,Plateletcrit Count ;**PDW**, Platelet Distribution Width

The ethanolic leaf extract of *L. lanceolata* equally induced changes in the erythrocytes, platelets and their related parameter profiles in mice (**Table 4**). The dose levels of 10mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight of ethanolic extract caused non significant decrease (p>0.05) in RBC counts of both infected and uninfected mice compared with the control. The infected untreated mice produced a significant decrease (P<0.05) in HGB, MCV, and PLT but had no significant effect (p>0.05) on platelet distribution weight (PDW), HCT, MCHC, MPV and PCT compared with the control. The infected mice treated with chloroquine at the dose level 5mg/kg body weight caused no significant increase (p > 0.05) in levels of MCH, PLT, MPV, PDW and RBC profiles compared with the negative control. However, there was a significant decrease (p<0.05) in RBC in infected mice treated at the dose levels of 10mg/kg body weight and 100mg/kg body weight compared with the controls.

Table 4: Effect of ethanolc leaf extract of *L. lanceolata* on erythrocytes, platelets and the related parameter profiles in mice

Treatments (mg / kg)	RBC ($X10^{12}$/L)	HGB (gldL)	HCT	MCV (fl)	MCHC (gldl)	MCH (Pg)	PLT (10^{9}/l)	MPV (fl)	PDW	PCT (ml/l)
Distilled water										
10	4.81^{a}	12.75^{a}	0.370^{a}	78.95^{b}	31.90^{a}	28.75^{a}	364^{a}	8.95^{a}	15.05^{a}	1.82^{a}
Infected, untreated 0	4.11^{b}	10.38^{c}	0.429^{a}	84.68^{a}	33.23^{a}	29.60^{a}	343^{b}	8.95^{a}	15.05^{a}	1.82^{b}
Infected, aqueous extract treated										
10	4.24^{ab}	12.82^{b}	0.388^{a}	76.50^{b}	13.15^{a}	29.75^{a}	3.30^{b}	7.23^{b}	13.85^{b}	2.11^{a}
100	3.94^{b}	11.38^{b}	0.400^{a}	83.65^{b}	33.50^{a}	31.20^{a}	376^{a}	7.58^{a}	16.15^{a}	2.10^{a}
200	4.47^{a}	12.78^{b}	0.430^{a}	84.57^{a}	32.10^{a}	30.52^{a}	366^{a}	8. 95^{a}	14.00^{ab}	2.18^{a}
Uninfected, aqueous extract treated										
10	4.52^{a}	10.90^{c}	0.399^{a}	88.32^{a}	33.50^{a}	31.57^{a}	389^{a}	7.20^{b}	15.15^{a}	2.03^{a}
100	4.36^{a}	11.17^{bc}	0.425^{a}	83.38^{b}	30.55^{a}	30.90^{a}	397^{a}	7.38^{b}	16.17^{a}	1.86^{b}
200	4.63^{a}	10.55^{c}	0.367^{a}	84.22^{a}	31.40^{a}	29.30^{a}	355^{a}	8.05^{ab}	15. 25^{a}	1.97^{b}
Infected, chloroquine treated										
5	5.22^{a}	11.63^{b}	0.384^{a}	85.8^{a}	32.50^{a}	30.50^{a}	360^{a}	8.73^{a}	15.70^{a}	1.33^{c}
SEM	0.44	1.22	0.031	4.69	1.48	1.23	27.0	0.86	1.09	0.43

Values are mean ± S. D n = 4.Mean values having different superscripts along the same column are significantly different (P<0.05).

Legend: **HGB**,Haemoglobin; **HCT**, hematocrit ;**RBC**, Red Blood Cells **MCV**,Mean Corpuscular Volume ;**MCH**,Mean Corpuscular Haemoglobin;**MCHC**,Mean Corpuscular Haemoglobin Concentration ;**PLT**, platelet count ;**MPV**,Mean Platelet Volume; **PCT** ,Prolactin Count :**PDW**, Platelet Distribution Width.

Effect of aqueous extracts on biochemical parameters of mice

The aqueous extract of *L. lanceolata* brought about some changes in biochemical parameters of the mice. There were statistically significant increases (P<0.05) in alanine aminotransferase (ALT), total bilirubin (T. Bil), direct bilirubin (D. Bil), cholesterol, triglyceride, and urea profiles in infected untreated mice compared with the control (**Table 5**). The dose level of 10mg/kg body weight and 100mg/kg body weight of aqueous extract caused statistically significant increases (p <0.05) in AST, ALT, T. Bil, cholesterol, triglyceride, and urea profiles in infected treated mice compared with the control. However, there was no significant increase (p >0.05) in direct bilirubin at dose level of 10mg/kg body weight of the aqueous extract in infected treated mice compared with the control. The dose level of 200mg/kg body weight of the aqueous extract caused a significant increase (p < 0.05) in AST, ALT, T. Bil, and triglyceride profiles in uninfected treated mice at the dose levels of 10mg/kg body weight and 100mg/kg body weight of aqueous extract compared with the control.

Table 5: Effect of aqueous extract on biochemical parameters

Treatments (mg / kg)	AST (U/L)	ALT (U/L)	T.BIL (mg/dL)	D.BIL (Mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Distilled water								
10 Infected, untreated	6.87^{e} ± 0.33	17.14^{c} ± 52	0.05^{c} ± 0.02	0.02^{c} ± 0.01	6.86^{d} ± 0.33	10.26^{d} ± 1.40	12.19^{e} ± 1.44	0.59^{b} ± 0.09

0	32.28^{a} ± 1.71	37.11^{a}± 1.76	1.19^{a} ± 0.14	0.28^{a} ± 0.07	86.48^{a} ± 2.16	66.91^{a} ± 2.02	57.14^{a} ±1.94	0.91^{a} ± 0.12
Infected, aqueous extract treated								
10	17.46^{c} ± 1.52	22.02 ^{b} ± 1.59	0.65 ^{b}±0.10	0.05^{c}±0.02	42.78^{b}±4.82	40.92^{b}±1.80	33.33^{b}±1.72	0.64^{b}±0.10
100	13.09^{d}±1.45	25.31^{b}±1.63	0.77^{a}±0.11	0.11^{b}±0.04	42.78^{b}±1.82	39.83^{b}±1.79	24.95^{c}±1.62	0.67^{b}±1.10
200	20.08^{b}±1.56	28.69^{b}±1.67	0.81^{a}±0.11	0.15^{b}±0.05	44.09^{b}±1.83	40.78^{b}±1.80	22.90^{c}±1.60	0.68^{b}±0.10
Uninfected, aqueous extract treated								
10	12.97^{d}±1.45	11.40^{d}±1.42	0.25^{b}±0.02	0.02^{c}±0.02	28.28^{c}±1.66	27.39^{c}±1.65	24.33^{c}±1.61	0.63^{b}±0.09
100	11.78^{d}±1.43	11.92^{d}±1.43	0.50^{a}±0.09	0.03^{c}±0.02	24.44^{c}±1.62	21.40^{c}±1.38	18.72^{d}±1.54	0.65^{b}±0.10
200	17.87^{c}±1.33	14.65^{c}±1.48	0.49^{a}±0.09	0.04^{c}±0.03	22.79^{c}±1.60	21.07^{c}±1.57	21.99 ^{b}±1.59	0.73^{b}±0.11
Infected, chloroquine treated								
5	9.63^{d}±0.39	10.71^{d}±1.40	0.53^{a}±0.09	0.02^{b}±0.01	27.58^{c}±1.66	24.48^{c}±1.62	28.62 ^{b}±1.67	0.46^{c}±0.08

Mean values having different superscripts along the same column aresignificantly different (P<0.05).
Legend: **ALT**, alanine aminotransferase; **AST** , aspartate aminotransferase; **T**.**Bil** , total bilirubin; **D. Bil** , direct bilirubin

Effect of ethanolic extracts on biochemical parameters of mice

The oral administration of ethanolic leaf extract of *L. lanceolata* caused changes in the biochemical parameters of the mice (**Table 6**). Specifically, there were significant increases (p < 0.05) in ALT, T. Bil, D. Bil, cholesterol, triglyceride and urea profiles of the infected untreated mice compared with the control. There was no significant increase (p >0.05) in aspartale aminotransferase (AST) and creatinine in the infected untreated mice compared with the control. The dose level of 10mg/kg body weight of ethanolic extract caused statistically significant increases (p< 0.05) in AST, T. Bil, cholesterol, triglyceride and urea profiles of infected treated mice compared with the control. However, there was no significant increase (P >0.05) in ALT, D. Bil, and creatinine profiles of infected mice at dose level of 10mg/kg body weight of the ethanolic extract compared with the control. The dose level of 200mg/kg body weight of the ethanolic extract caused significant increases (p<0.05) in AST, ALT, T. Bil, cholesterol, triglyceride and urea profiles of the infected treated mice compared with the control. However, there was no significant increase (p>0.05) in creatinine profile of the infected mice treated with 200mg/kg body weight of the ethanolic extract compared with the control. The dose levels of 10mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight of ethanolic extract caused significant increases (p<0.05) in AST, ALT, and T. Bil profiles of uninfected treated mice compared with the control.

Table 6: Effect of ethanolic extract on biochemical parameters of mice

Treatments (mg / kg)	AST (U/L)	ALT (U/L)	T.BIL (mg/dL)	D.BIL (Mg/dL)	Chlorestorol (mg/dL)	Triglyceride (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Distilled water								
10	6.87^{a}±0.33	17.14^{c}± 52	0.05^{c}±0.02	0.02^{c}±0.01	6.86^{d}±0.33	10.26^{d}±1.40	12.19^{d}±1.44	0.59^{a}±0.09
Infected untreated 0	32.28^{a}±1.71	37.11^{a}±1.76	1.19^{a}±0.14	0.28^{a}±0.07	86.48^{a}±2.16	66.91^{a}±2.02	57.14^{a}±1.94	0.91^{a}±0.12
Infected ethanolic extract treated								
10	25.75^{b}±1.63	23.20^{c}±1.61	0.69^{a}±0.10	0.07^{c}±0.03	[41.26^{b}±1.80	42.96^{b}±1.82	21.61^{c}±1.58	0.75^{a}±0.11
100	24.88^{b}±1.62	24.83^{bc}±1.62	0.67^{a}±0.10	0.13^{b}±0.05	44.97^{b}±1.84	42.09^{b}±1.84	27.55^{c}±1.66	0.64^{a}±0.10
200	26.15^{b}±1.50	30.83^{b}±1.69	0.89^{a}±0.12	0.14^{b}±0.05	44.43^{b}±1.83	35.08^{c}±1.74	39.53^{b}±1.79	0.65^{a}±0.10
uninfected ethanolic extract treated								
10	10.47^{c}±1.40	12.13^{d}±1.44	0.37^{b}±0.08	0.03^{c}±0.02	23.75^{c}±1.61	12.95^{e}±1.45	22.89^{c}±1.60	0.58^{b}±0.09
100	11.78^{c}±1.43	11.97^{d}±1.43	0.38^{b}±0.08	0.03^{c}±0.02	24.53^{c}±1.62	21.40^{d}±1.58	20.30^{c}±1.56	0.68^{a}±0.10
200	13.09^{c}±1.45	16.72^{c}±1.51	0.52^{a}±0.09	0.03^{c}±0.02	25.97^{c}±1.64	23.55^{d}±1.61	21.65^{c}±1.58	0.68^{a}±0.10

Infected treated	chloroquine 5	9.63^{c}±0.39	10.71^{d}±1.40	0.53^{a}±0.09	0.02^{c}±0.01	27.58^{c}±1.66	24.48^{d}±1.62	28.62^{c}±1.67	0.46^{a}±0.08

Mean values having different superscripts along the same column are significantly different (P<0.05).
Legend: **ALT** , alanine aminotransferase; **AST**, aspartate aminotransferase ;
T.**Bil** , total bilirubin; **D. Bil**, direct bilirubin

Histopathological effects of aqueous and ethanolic leaf extract of *Lophira lanceolata* on mice organs (liver and kidney)

The microscopic examination of kidney sections of all the mice showed no histopathologic alteration. The microscopic architecture of sections of the kidney in both infected treated and uninfected treated groups had a similar appearance to that of the control groups in which normal renal corpuscles and tubular structures were maintained.

The microscopic examination of liver sections showed some histopathologic alterations in the architecture of structural units in both infected treated and uninfected treated groups. Specifically, liver section of infected mice administered with 100mg/kg aqueous extract showed liver tissue with slightly enlarged central vein and increased cellularity. Also, liver section of infected untreated mice showed liver with tissue stromal proliferation and enlarged central viein; the hepatocytes appeared enlarged with some undergoing ballooming degeneration; the sinusoids and lamina of hepatic cells were disorganized**.**

The microscopic examination of liver section from control mice showed the normal architecture of the structural units of the liver, the hepatic lobules formed by cords of hepatocytes separated by hepatic sinusoids.

Histopathological effects of aqueous and ethanolic leaf extract of *lophira lanceolata* on mice organs (liver and kidney)

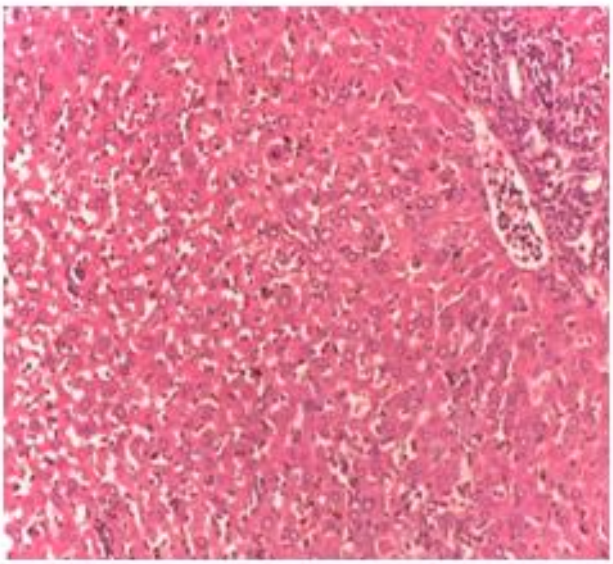

Plate 1
Liver: No visible lesion H & E
10mg/kg Aqueous Extract Treated infected

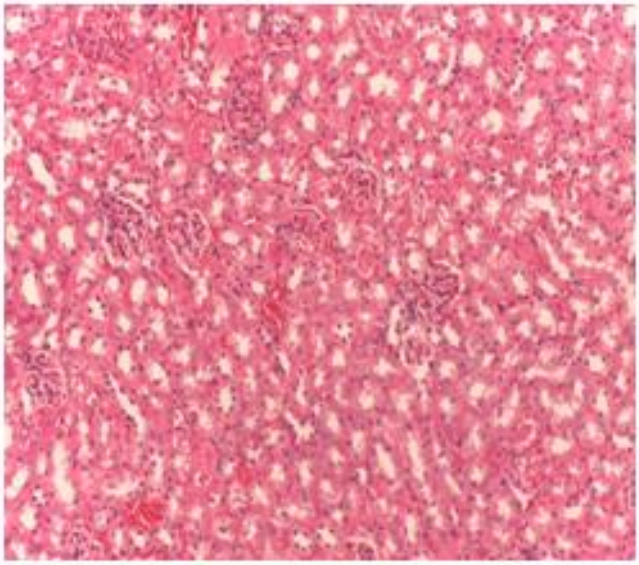

Plate 2
Liver: No visible lesion H & E X 200
10mg/kg Aqueous extract treated uninfected

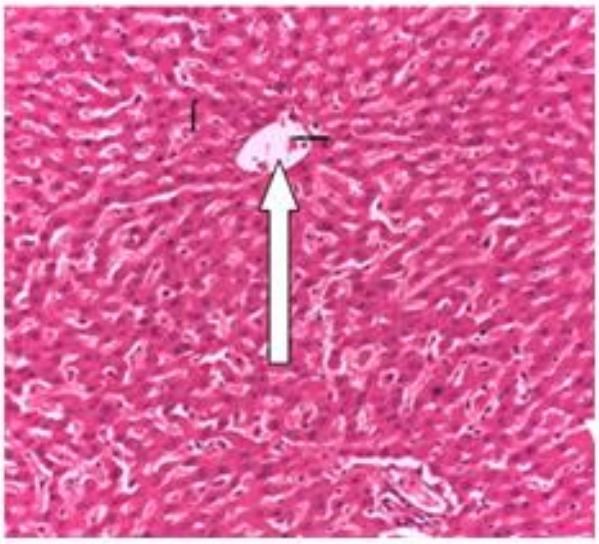

Plate 3
Liver: Section shows liver tissue with slightly enlarged central vein and increased cellularity.
100mg/kg Aqueous Extract Treated infected

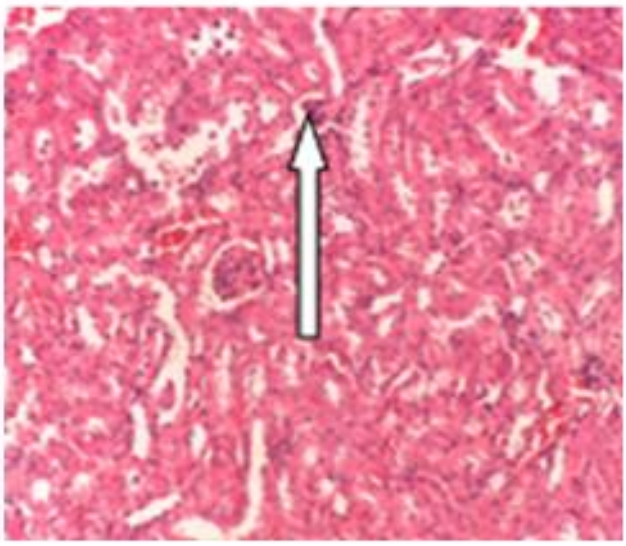

Plate 4
Liver: Section showing liver tissue with distorted stromal arrangement
100mg/kg Aqueous extract treated uninfected

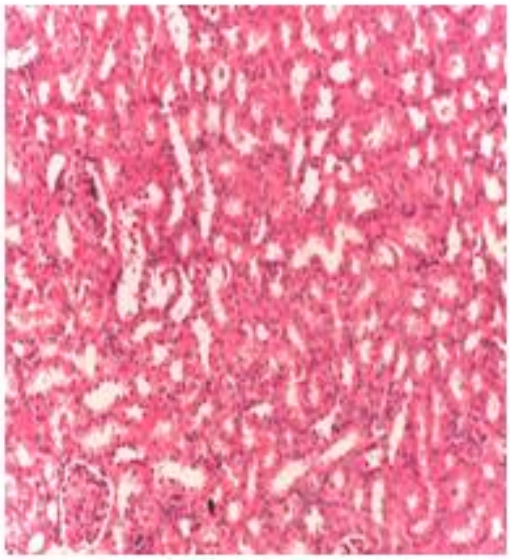

Plate 16
Kidney: No visible lesion H & E X 200
10mg/kg Aqueous extract treated infected

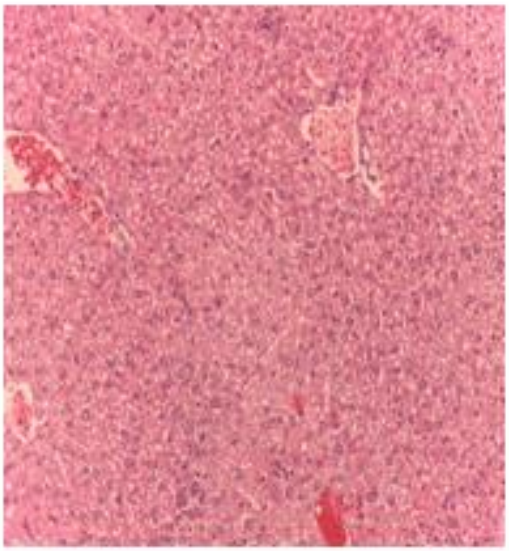

Plate 17
Kidney: No visible lesion H & E X 200
10mg/kg Aqueous extract treated uninfected

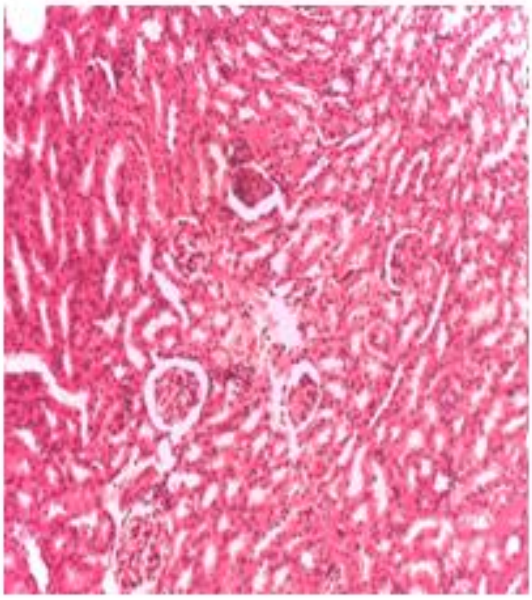

Plate 18
Kidney: No visible lesion H & E X 300
100mg/kg Aqueous extract treated infected

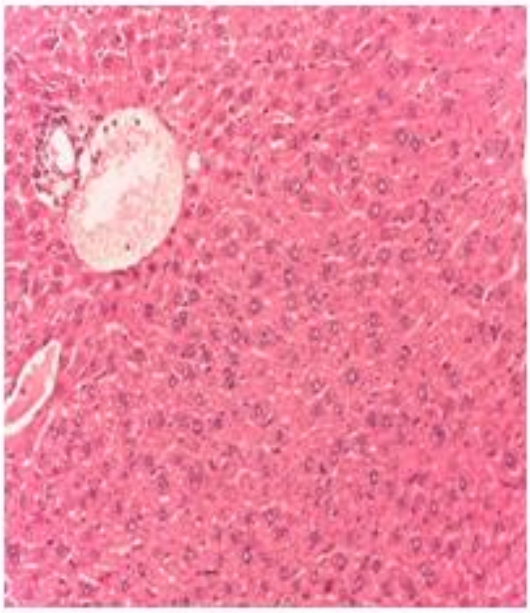

Plate 19
Kidney: No visible lesion H & E X 300
100mg/kg Aqueous extract treated infected

DISCUSSION

The ethanolic leaf extract of *L. lanceolata* equally induced changes in the erythrocytes, platelets and their related parameter profiles in mice. The dose levels of 10mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight of ethanolic extract caused non significant decrease ($p>0.05$) in RBC counts of both infected and uninfected mice compared with the control. Nonetheless, the dose levels of 10mg/kg body weight of aqueous extract in the infected mice had a significant decrease ($p<0.05$) in red blood cell counts (RBC) but had no significant difference ($p>0.05$) on haemoglobin (HGB), hematocrit (HCT), mean platelet volume (MPV), and plateletcrit (PCT) profiles compared with the control. The infected untreated mice produced a significant decrease ($P<0.05$) in HGB, MCV, and PLT but had no significant effect ($p>0.05$) on platelet distribution weight

(PDW), HCT, MCHC, MPV and PCT compared with the control. The infected mice treated with chloroquine at the dose level 5mg/kg body weight caused no significant increase ($p > 0.05$) in levels of MCH, PLT, MPV, PDW and RBC profiles compared with the negative control.

The red blood cells and factors relating to them are major indices for evaluating circulating erythrocytes and are very important in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cell as in mammals (Peters *et al*., 2011). The insignificant decreases in red blood cells, platelets and factors relating to them in treated mice could mean that the extracts of *Lophira lanceolata* did not have adverse effect on erythropoiesis and thrombopoiesis potency of the blood components. In other words, the insignificant effect of the extract on the red blood cells may be an indicator that the balance between the rate of production and destruction of the blood corpuscles was not altered. The result of the study corresponds with earlier findings by Lawal *et al*. (2015) who reported similar findings on *Apis mellifera*. Also, the result of this study is line with the result reported by Oussou *et al*. (2015) in which an ethyl acetate fraction of *Lophira lanceolata* leaves in albino rats produced non-significant effect on red blood cells and factors relating to them. However, the findings of this work on the effect of *Lophira lanceolata* leaf extract on mice infected with *P. berghei* contradict the results of the effect of methanol extract of *Telfaira occidentalis* on haematological parameters in wister rats as reported by Bashir *et al*. (2015). According to Bashir *et al*. (2015), there were significant increases in red blood cells, platelets and factors relating to them in the treated rats. The observed significant increases in red blood cells, platelets, and factors relating to them following the administration of *T. occidentalis* was an indication of erythropoiesis stimulation of the extract. The extract must have increased the rate of erythropoietin release in the kidney, which is the humoral regulator of red blood cell production (Mishira and Tandon, 2012).

The administration of the plant extract in both infected and uninfected mice produced a significant increase in levels of cholesterol and triglycerides.The increase in total cholesterol and triglyceride profiles may reflect an increase in anaerobic metabolism via intensified glycolytic processes and an enhanced protein catabolism leading to an increase in glucogenic amino acids. There was a significant increase in levels of urea profiles in both infected and uninfected mice treated with the leaf extracts.

The administration of aqueous and ethanolic leaf extract of *Lophira lanceolata* on mice caused significant decreases in the weight of kidney and liver compared with the control. However, there was no significant decrease in weights of lungs of mice examined compared with the control. This difference in the weight of organs may be as a result of the selective toxicity of the plant extract. Also, the significant decreases in the weight of mouse organs might be due to the anti-nutritional bioactive components (e.g tannins) present in the plant extract. The results of this research work on the effects of *Lophira lanceolata* leaf extracts on the liver weight is not in agreement with the findings of Ousson *et al*. (2015) in which there was absence of significant effect on the liver weight which was an indication that the *Lophira lanceolata* leaf extract did not adversely affect the liver organs. Nonetheless, the findings of this work on the effects of the leaf extract on lung weights of the mice were in conformity with the results of Ousson *et al*. (2015) on the effects of leaf extract on lung weights of the mice.

Conclusions

The aqueous and ethanolic leaf extract of *L. Lanceolata* produced increases in the erythrocytes, platelets, and their related parameter profiles in mice. The dose levels of 10 mg/kg body weight of aqueous extract in the infected mice had a significant decrease ($p<0.05$) in red blood cell counts (RBC) but had no significant difference ($p > 0.05$) on haemoglobin (HGB), hematocrit (HCT), mean platelet volume (MPV), and plateletcrit (PCT) profiles compared with the control.

The dose level of 200mg/kg body weight of the aqueous extract caused a significant increase ($p<0.05$) in AST, ALT, T. Bil, triglyceride and creatinine profiles in uninfected treated mice at the dose levels of 10mg/kg body weight and 100mg/kg body weight of aqueous extract compared with the control. The administration of aqueous and ethanolic leaf extract of *Lophira lanceolata* on mice caused significant decreases in the weight of kidney, liver and heart compared with the control. However, there was no significant decrease in weights of lungs of mice examined compared with the control.

The histopathological examination of the liver of mice treated with aqueous and ethanolic leaf extract of *Lophira lanceolata* revealed some histopathological changes. The signfcant changes observed in the liver were characterized by tissue stromal proliferation, enlarged central veins, enlarged hepatocytes, and the disorganized sinusoids and lamina of hepatic cells. However, the results of histopathological examination of kidneys showed no significant changes.

The findings of this research work revealed that both aqueous and ethanolic leaf extracts of *Lophira lanceolata* plant showed significant *antiplasmodium* activities comparable to chloroquine drugs. However, the results of the haematological, biochemical and histopathological examinations indicated that caution should be exercised while taking the plant extract as an antimalarial agent.

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