IN VIVO ANTIPLASMODIAL ACTIVITY AND SHORT TERM SAFETY ASSESSMENT OF METHANOL LEAF EXTRACT OF *FICUS ASPERIFOLIA* (MORACEAE)

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Abstract: Malaria is a global health problem that is still a major cause of morbidity and mortality in the tropical and sub-tropical areas of the world. *Ficus asperifolia* (Moraceae) has been ethno medicinally used as analgesic, anti-tumor, diuretic, abortificient, and antimalarial. The aim of this study was to evaluate the antiplasmodial activity and short term safety profile of methanol leaf extract of *Ficus asperifolia* (FaMLE). Phytochemical screening was conducted as described by Trease & Evans and oral median lethal dose (LD_{50}) of the extract was estimated using the OECD guidelines. Subchronic toxicity was evaluated by administering1000, 500, and 250 mg/kg graded doses of FaMLE daily for 28 days p.o. This is followed by biochemical and hematological analysis. The antiplasmodial activity was evaluated in mice infected with chloroquine-sensitive *plasmodium berghei-berghei* using suppressive, prophylactic, and curative experimental models. Oral LD_{50} of the extract was estimated to be >5000 mg/kg. The FaMLE at all doses produced a significant ($p<0.05$) suppressive, prophylactic, and curative antiplasmodial activity. The extract also significantly prolonged the survival time of the treated mice compared to the DW group. In liver function test FaMLE did not show any significant changes in functional indices compared DW group except AST at the dose of 250 mg/kg. No significant changes were observed in both kidney function tests and hematological analysis. However, FaMLE produced slight histopathological changes among various organs examined. In conclusion FaMLE possesses suppressive, prophylactic, and curative antiplasmodial activity and may be safe during short term administration.

Keywords: Antiplasmodial, Chloroquine, Ethnomedicinal, *Ficus asperifolia*, *Plasmodium berghei-berghei*,

Introduction

Malaria is caused by single-celled microorganisms of the Plasmodium group. Clinically, *Plasmodium falciparum* is the most fatal and the major cause of malaria incidences. Also, it is responsible for high prevalence of mortality. Other species such as *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* generally cause a milder form of malaria. However, *Plasmodium knowlesi* specie rarely causes disease in humans (WHO, 2020). Malaria is mostly spread by an infected female Anopheles mosquito. This mosquito bites and introduces the parasites into the person's blood through its saliva which then travels to the liver where they mature and reproduce (WHO, 2020).

The populations that are at highest risk of malaria infection include pregnant women, children under 5 years, infants, people with HIV/ AIDS and travellers. Symptoms of severe malaria include fatigue, convulsions, impaired consciousness, difficulty breathing, bloody urine and jaundice (WHO, 2023). Recent data suggested that, about 50% of the world populations are at risk of malaria. The prevalence of malaria infection is estimated as 247 million cases globally with 619,000 mortality reported in the year 2021. Notably, 80% of death caused by malaria affected children under 5 years (WHO, 2023). Sub-Saharan Africa suffers the major global burden of malaria and its prevalence in Nigeria is about 31% (WHO, 2022).

Ficus asperifolia has different forms; it can be an average size tree, scrambling shrub, or epiphyte (Ojo and Akintayo, 2014; Adjanohoun *et al.* 1996). Common names of the plant are *Sandpaper tree* (English), *Kawusa* (Hausa), *Ipin* (Yoruba) and *Anmerenwa* (Igbo) (Burkill, 1997). Ethno-medicinal uses include menstrual pain, inflammation, diabetes mellitus, hypertension, dysentery, liver problems, urinary and respiratory tract infections (Watcho *et al*. 2009; Burkill, 1997).

Medicinal plants have been acknowledged as primary source of therapeutic interventions since the medival periods (Faustino *et al*. 2010; Abdullahi *et al*. 2023). The plant *Ficus asperifolia* possesses hypoglycemic properties (Omoniwa and Luka, 2012) and antibacterial effect (Lawal *et al*. 2016; Nwanko and Ukaegbu-obi, 2014). Also, it was reported to have uterotonic activities (Pierre *et al*. 2009; Watcho *et al*. 2011). Another study carried out revealed that *Ficus asperifolia* has antioxidant property (Ojo *et al*. 2016; Ojo and Akintayo, 2014). Other scientific reports have revealed that the plant possesses gastro protective (Raji *et al*. 2011). *Ficus asperifolia* has also lipid lowering property (Omoniwa *et al*. 2013). Recent study reported that methanol leaf extract of *Ficus asperifolia* possesses anti-inflammatory and analgesic activities (Abdullahi *et al.* 2020).

Materials and Methods

Experimental Animals

Wistar rats and mice of both sexes weighing 100-150g and 20-25g respectively were obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were allowed free access to standard feed and water ad libitum. They were kept in clean cages filled with saw dust, which was replaced every three days. The study was conducted according to ethical guidelines on laboratory animal use and care policy, which is in compliance with Ahmadu Bello University Research Policy (Revised 2010).

Drugs and Chemicals

Giemsa solution, Immersion Oil, Pyrimethamine, Chloroquine (Fluka, Germany), Agappe diagnostic kit (Switzerland), Randox diagnostic kit (UK), Artesunate (Cusnat, China), Pyrimethamine (SKG, Nigeria), Methanol (JHD Sci-Tech. Co. Ltd, China).etc.

Equipment

Thermostat Oven (DHG-9101, USA), What man's Filter Paper No. 1, Microscope, EDTA bottles, Whatman No. 1 filter paper (1mm mesh size), Eppendorf micro pipettes, Centrifuge (Hawksley-15006, England), Micro-hematocrit reader (Hawksley-15006, England), Biobase auto hematological analyzer (BK 6300, UK).

Preparation of plant material

The fresh leaves of *Ficus asperifolia* Miq were collected from Toro Local Government Area, Bauchi State. The plant was identified and authenticated by the Department of Biological Sciences, Bayero University, Kano. A voucher specimen number BUKHAN 0106 was given and documented.

Extraction

Powdered plant material 2kg was macerated with 7L of 70%v/v methanol at room temperature for 7 days with occasional agitation. At the end of the extraction, the methanol extract of *Ficus asperifolia* (FaMLE) was filtered using Whatman's filter paper (1mm mesh size) and then concentrated on water bath at 45ºC.

Phytochemical Screening

The chemical composition of FaMLE was determined using phytochemical screening (Abubakar and Haque, 2020).

Acute toxicity study in mice (LD_{50})

LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD, 2001) guidelines. In this method, a dose of 2000 mg/kg was administered to one mouse and observed for 48 hours. There was no death and the test proceeded to the second phase. A dose of 5000 mg/kg was administered to another mouse and observed for 24 hours. There was no death recorded and the observation continues daily for 14 days. Changes monitored include skin, eyes, mucous membranes, somatic activity and behavior pattern. Animals were also observed for tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted.

Subchronic (28 days daily administration)

This test was conducted according to the method described by OECD, guidelines 407 (OECD, 2008) with slight modification. Twenty Wistar rats (10 males and 10 females) weighing (180-200g) were selected and divided into four groups of 10 rats each. Group I, II, and III received 1000, 500 and 250 mg/kg of FaMLE respectively. Group IV the last group received distilled water 1 ml/kg to serve as negative control. The administration of FaMLE was done every 24 hour for 28 days (OECD, 2008). At the 29th day, the rats were anaesthetized and sacrificed. The blood samples were collected for haematological analysis using EDTA bottles (1 mg/ml of blood). The blood samples for biochemical analysis were collected in plain bottles and centrifuged at 1500 rpm for 15 minutes. Subsequently, the rats were dissected and the liver, heart, kidney and spleen were removed for histological studies (OECD, 2008).

Antimalarial Studies

Inoculation of mice

A *Plasmodium berghei berghei* infected mouse (parasitemia of 34 %) was used as a parasite donor and blood sample was collected retro-orbitally into an EDTA containing bottle. The inoculum was prepared by determining the percentage parasitemia and erythrocyte count of the donor mouse and further diluting the blood with isotonic saline (Okokon and Nwafor, 2009).

Parasitemia determination

Thin blood smears were applied on microscope slides, fixed with absolute methanol for 10 minutes and stained with 10% Geimsa stain for 30 minutes. The number of parasitized red blood cells was counted using light microscope with an oil immersion eye piece of 100x magnification power (Laychiluh, 2011).

Parasitemia and suppression were determined as follows:

$$\% \text{ parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

$$\% \text{ Suppression} = \frac{Po - Pt}{Po} \times 100$$

Where Po is the average parasitemia of the control group and Pt is the average parasitemia of the test group.

Antiplasmodial activity against early infection (Suppressive test)

Adult Swiss albino mice weighing 22 g to 24g were infected by intraperitoneal *(i.p)* injection with standard inoculums of *P. berghei* with 1×10^7 infected erythrocytes (Peters, 1967). The mice were randomly divided into 6 groups of 6 mice each. Group I was given 1000 mg/kg, group II 500mg/kg, group III 250 mg/kg orally for 4 consecutive days. Group IV treated daily with 5 mg chloroquine kg-1 (positive control-I) and Group V received 2 mg kg artesunate (positive control-II). Group VI received distilled water 10 ml kg-1 (Negative control). On day 5, the blood was collected from the tail of each mouse and smear made (Khan *et al*. 2015). The blood films was fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 min and *parasitaemia* determined microscopically (Peter *et al*. 1975).

The average % suppression was calculated as follows:

Average % suppression = A-B/A X 100

Where A = Average percentage *parasitaemia* in negative control group, and B = Average percentage *parasitaemia* in test group

Determination of packed cell volume

The packed cell volume (PCV) of each mouse was measured before infection and on day 4 after infection. Blood was collected from the retro orbital sinus and centrifuged at 12,000 rpm for 5 minutes. The PCV measurement was taken using micro haematocrit reader using the modified Win Trobe's Method (Munzer *et al*. 1980).

PCV volume is calculated as follows

PCV = Volume of erythrocytes in a given volume of blood X 100 / Total blood volume

Prophylactic (repository) test

The prophylactic activity of the extract was assessed using the method described by Peters (1967). Adult mice were randomly divided into 5 groups of 6 mice each. Group I

received 10 ml/kg of distilled water (negative control), Group II, III and IV received 250, 500 and 1000 mg/kg FaMLE respectively. Group V received 1.2 mg/kg of pyrimethamine orally (Positive control). Treatment was continue for five consecutive days (D_0–D_4). On the sixth day (D_5), the mice were inoculated with *Plasmodium berghei berghei*. After 72 hours later, the blood was collected by tail bleeding and parasitemia was determined.

Antiplasmodial activity against established infection (Rane or Curative test)

Evaluation of the schizontocidal activity of the extract against established infection was carried out as described by Ryley and Peters (1970). Adult mice were inoculated with *Plasmodium berghei berghei* on the first day (D_0). 72 hours later (D_3), the mice were divided randomly into 6 groups of 6 mice each. Group I received 10 ml/kg of distilled water (negative control), Group II, III and IV received 250, 500 and 1000 mg/kg of the leaf extract respectively. Group V received 5 mg/kg of chloroquine (positive control-I) and Group VI received 2 mg/kg of artesunate (positive control-II) for five consecutive days (D_3–D_7) orally. The blood sample was collected from each mouse by tail bleeding on day three (post parasite inoculation). On the day seven (post treatment), the parasitemia was determined microscopically

Determination of mean survival time

Mortality was monitored daily and the number of days from the time of *inoculation* of the *parasit*e to time of death was recorded (Mengistie *et al*. 2012).

The mean survival time (MST) for each group was calculated as follows:

MST= Sum of survival time (days) of all mice in a group /Total number of mice in that group

$$MST = \frac{\text{Sum of survival time of all the mice in a group}}{\text{Total number of mice in the group}}$$

Data analysis

Results were expressed as Mean ± Standard Error of Mean (S.E.M) and were analyzed using one way analysis of variance (ANOVA) followed by Dunnett ' s post hoc test. Values of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

Phytochemical constituents of the methanol leaf extract of Ficus asperifolia

The results indicated that FaMLE contains alkaloids, flavonoids, tannins, saponins, steroids, cardiac glycosides, and terpenoids (Table 1).

Table1: Phytochemical Constituents of Methanol Leaf Extract of *Ficus asperifolia*

Chemical constituents	FaMLE
Alkaloids	+
Anthraquinone	-
Steroids	+
Terpenoids	+
Cardiac glycosides	+
Saponins	+
Tannins	+
Flavonoids	+

+ =present, - = absent, FaMLE = *Ficus asperifolia* methanol leaf extract.

Median Lethal Dose (LD_{50}) Values

The oral median lethal dose (LD_{50}) of FaMLE in mice and rats was above 5000 mg/kg (Table 2).

Table 2: Median Lethal Dose (LD_{50}) of FaMLE

Extract	Animal	Route	(mg/kg)
FaMLE	Mice	Oral	>5000
FaMLE	Rats	Oral	>5000

FaMLE = *Ficus asperifolia* Methanol leaf extract

In vivo antiplasmodial Suppressive test

There was a statistically significant ($p<0.05$) reduction in the mean percentage parasitaemia values of the FaMLE treated groups compared to the distilled D/W group. The FaMLE, at the doses of 1000, 500, and 250 mg/kg produced percentage suppression of 81.5, 80.4 and 67.1% respectively in dose dependent. The standard drug chloroquine 5mg/kg and artesunate 2 mg/kg produced a percentage suppression of 82.0 and 84.3 respectively (Table 3). The result of PCV determination after 4 days suppression test showed no statistically significant difference compared to D/W group (Table 4).

Table 3: Suppressive Effect of the FaMLE in Mice Infected with *Plasmodium berghei*

Treatment (mg/kg)		Average % Parasitemia (D4)	% Suppression
D/W	10ml	20.60±1.42	_
FaMLE	1000	3.81±0.12*	81.5
FaMLE	500	4.03±0.10*	80.4
FaMLE	250	6.77±0.23*	67.1
CQ	5	3.77±0.78*	82.0
ART	2	3.24±0.36*	84.3

Values presented as Mean ±SEM, and percentage, n=6, * significantly different from negative control at $p<0.05$ using one-way ANOVA and Dunnettes Post Hoc tests. FaMLE = Methanol leaf extract of *Ficus asperifolia*, DW = Distilled water, D4 indicates day 4, ART = Artesunate, CQ = Chloroquine Phosphate.

Table 4: PCV Values in Mice Infected with *Plasmodium berghei* and Treated with Methanol Leaf Extract of *Ficus asperifolia*

Dose (mg/kg)	Average PCV (%)		% Change
	D0	D4	
D/W 10ml	60.11±1.08	57.33±1.08	- 4.6
FaMLE 1000	57.67±0.72	59.17±1.72	+2.6
FaMLE 500	61.50±0.89	58.67±2.89	- 4.6
FaMLE 250	65.33±0.67	59.50±1.67	- 8.9
CQ 5	58.50±0.24	55.67±2.24	- 4.8
ART 2	53.30±0.06	51.20±2.06	- 3.9

Values presented as Mean ±SEM, and percentage, n=6, No significant difference from control at $p<0.05$ using one-way ANOVA and Dunnettes Post Hoc tests. FaMLE = Methanol leaf extract of *Ficus asperifolia*, D4 indicates day 4, ART = Artesunate, CQ = Chloroquine Phosphate.

Prophylactic test

There was a statistically significant ($p<0.05$) reduction in the mean percentage parasitaemia at all doses of FaMLE treated groups compared to D/W group. The extract at 1000, 500 and 250 mg/kg produced percentage activity of 93.3, 81.9 and 99.5% in a dose independent manner respectively. Also, a standard drug pyrimethamine at 1.2 mg/kg produced activity of 81.6 (Table 5).

Table 5: Prophylactic Effect of the FaMLE in Mice Infected with *Plasmodium berghei*

Treatment (mg/kg)	Percentage Parasitemia (D7)	% Prophylaxis
D/W 10ml	16.45±1.92	_
Pyrimethamine 1.2	3.03±0.42*	81.6
FaMLE 1000	1.10±0.23*	93.3
FaMLE 500	2.99±0.93*	81.9
FaMLE 250	0.09±0.08*	99.5

Values presented as Mean ±SEM, and percentage, n=6, * significantly different from negative control at $p<0.05$ using one-way ANOVA and Dunnettes Post Hoc tests. FaMLE = Methanol leaf extract of *Ficus asperifolia*, D7 indicates day 7.

Curative test

There was a statistically significant ($p<0.05$) reduction in the mean percentage parasitaemia in the FaMLE treated groups compared to the D/W group. The FaMLE extract at 1000, 500 and 250 mg/kg produced percentage parasite clearance of 87.1, 78.6 and 80.0% in a dose independent manner respectively. The standard drug chloroquine 5mg/kg and artesunate 2 mg/kg produced a parasite clearance of 87.7 and 90.2 respectively (Table 6). Also, the FaMLE at 1000 mg/kg, 500 mg/kg and 250 mg/kg significantly extended the mean survival of the animals to 26, 25 and 27 days respectively. Lastly, the standard drugs Artesunate 2mg/kg and Chloroquine 5mg/kg produced mean survival days of 27 each (Table 6).

Table 6: Curative Effect of the FaMLE in Mice Infected with *Plasmodium berghei*

Treatment (mg/kg)	Average % Parasitemia		% Clearance	Mean Survival (Days)
	Pre (D4)	Post (D7)		
D/W 10ml	18.00±2.65	23.17±1.79	_	11.83±3.61
ART 2	19.00±2.41	2.27±0.56*	90.2	27.83±1.37*
CQ 5	19.38±1.70	2.86±0.15*	87.7	27.67±0.33*
FaMLE 1000	18.78±2.65	2.99±0.21*	87.1	26.67±0.54*
FaMLE 500	17.17±2.70	4.95±0.54*	78.6	25.33±1.10*
FaMLE 250	18.88±2.21	2.78±0.94*	88.0	27.33±1.02*

Values presented as Mean ±SEM, and percentage, n=6, * significantly different from negative control at $p<0.05$ using one-way ANOVA and Dunnettes Post Hoc tests. FaMLE = Methanol leaf extract of *Ficus asperifolia*, D7 indicates day 7, D/W = Distilled water, ART = Artesunate, CQ = Chloroquine Phosphate, % = Percentage.

Sub-chronic toxicity

Haematological parameters

Results of haematological parameters determination after 28 days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FaMLE did not show any statistically significant difference from that of the D/W group (Figure 1).

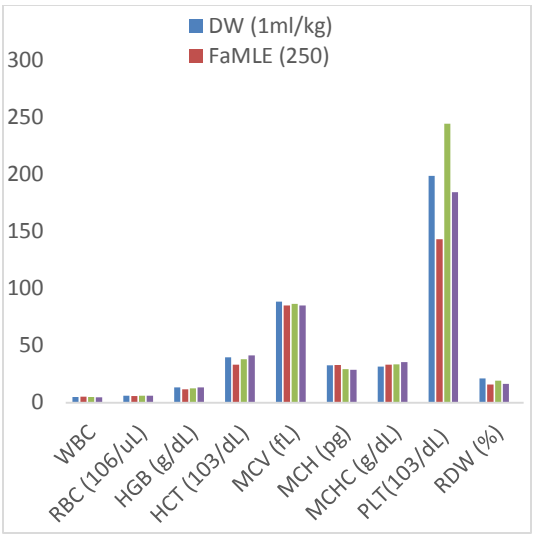

Figure 1: Effect of 28 days Oral Administration of FaMLE Extract on Hematological indices in Rats Key: White blood cell (WBC), Red blood cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red distribution width (RDW), Platelet (PLT), Distilled Water (DW), Methanol Leaf Extract of *Ficus asperifolia* (FaMLE), n= 5.

Kidney function test

Results of kidney function test after 28days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FaMLE did not show any statistically significant difference from that of the D/W group (Figure 2).

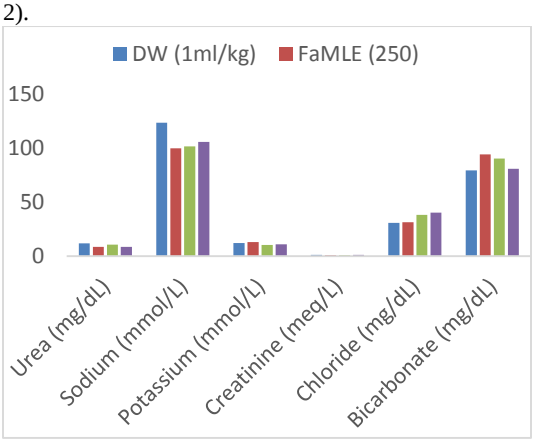

Figure 2: Effect of 28 days Oral Administration of FaMLE Extract on Kidney function Test in Rats. Key: Methanol Leaf Extract of *Ficus asperifolia* (FaMLE), n= 5, Distilled Water (DW).

Liver function test

Results of liver function test after 28 days daily graded doses (1000 mg/kg, 500 mg/kg and 250 mg/kg) administration of FaMLE did not show any statistically significant difference from that of the distilled D/W group except AST where a significant decrease was observed at the dose of 250 mg/kg (Figure 3).

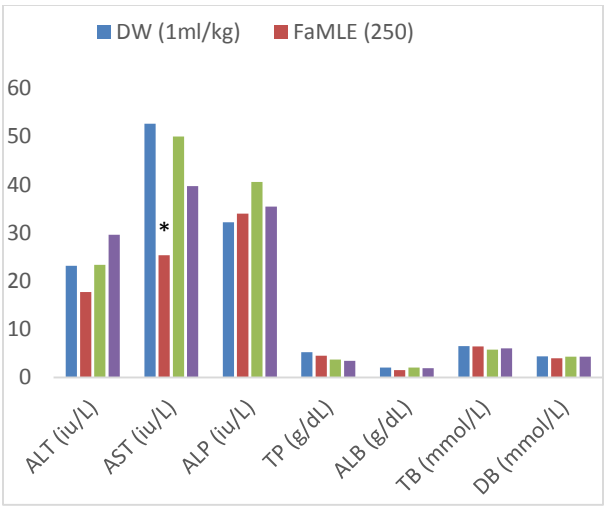

Figure 3: Effect of 28 days Oral Administration of FaMLE Extract on Liver function Test in Rats. Key: Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Methanol Leaf Extract of *Ficus asperifolia* (FaMLE), * significantly different from control at $p<0.05$ analysed using one-way ANOVA followed by Dunnettes post hoc test, n=5.

Histopathological examination after the sub chronic toxicity studies

Relative body organ weight

Body organs harvested include Brain, liver, heart, lungs, kidney and spleen. The result revealed that FaMLE at 250, 500 and 1000 mg/kg doses did not produced statistically significant change in relative organs weight compared to D/W group (Table 7).

Table 7: Effect of the FaMLE on Body and Organ Weight of Rats

Parameters / Treatments (mg/kg)	Control	FaMLE (250)	FaMLE (500)	FaMLE (1000)
Brain (g)	1.50±0.06	1.46±0.01	1.48±0.02	1.51±0.04
Heart (g)	0.57±0.18	0.58±0.04	0.53±0.17	0.56±0.04
Kidneys (g)	0.74±0.13	0.70±0.04	0.71±0.30	0.71±0.08
Liver (g)	3.55±0.16	3.43±0.28	3.21`±0.25	3.71±023
Lungs (g)	1.21±0.11	1.18±0.19	0.99±0.18	1.01±0.11
Spleen (g)	0.71±0.03	0.78±0.04	0.70±0.30	0.69±0.08

Data presented as Mean ± SEM, analysed using one-way ANOVA followed by Dunnettes post hoc test: Control = distilled water (10 mL/kg), n = 6, FaMLE = *Ficus asperifolia* methanol leaf extract

Liver

The group of rats that received distilled water showed normal hepatocytes (Plate I). It was also observed that the FaMLE treated rats showed normal hepatocytes after 28 days of daily oral administration (Plate II) respectively.

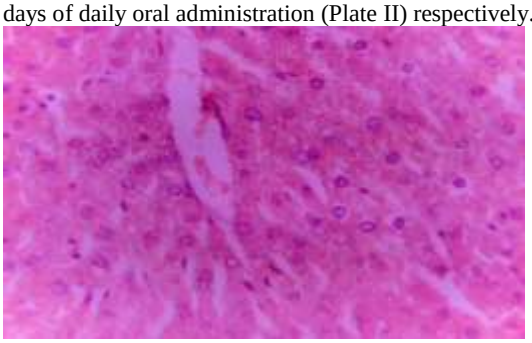

Plate I: Photomicrograph of Liver Section of Distilled Water 28 days Oral daily treated rats showing normal Hepatocytes. Hematoxylin & Eosin (H&E) stain (× 400).

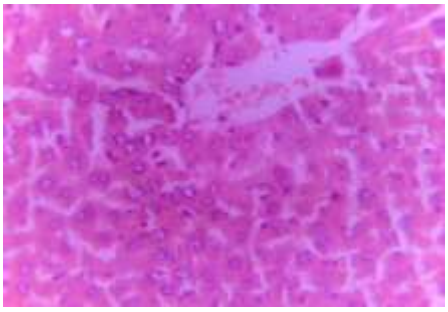

Plate II: Photomicrograph of Liver Section of 250mg/kg FaMLE 28 days Oral daily treated Rats showing normal Features. Hematoxylin & Eosin (H&E) stain (× 400).

Heart

The group of rats that received distilled water showed normal features of heart section (Plate III). However slight myocardial necrosis was observed in the heart features of the FaMLE treated rats after 28 days of daily oral administration (Plate IV).

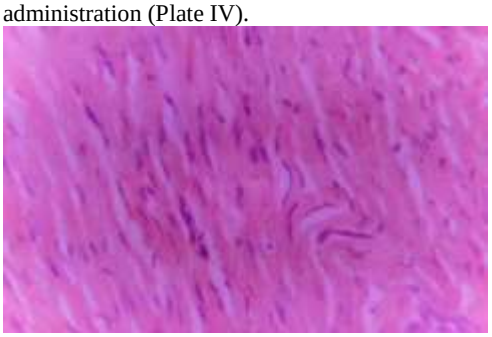

Plate III: Photomicrograph of Heart Section of 28 days Distilled Water Oral daily treated Rats showing normal Myocardium. H&E stain (× 400).

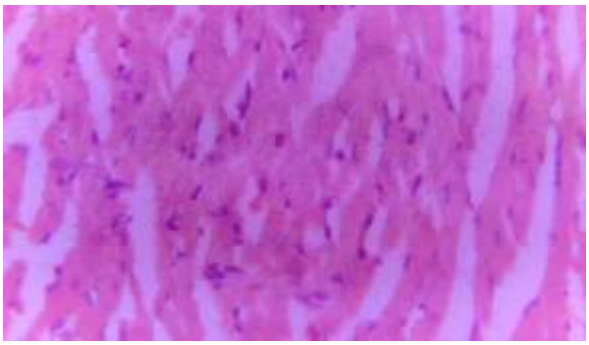

Plate IV: Photomicrograph of Heart Section of 250 mg/kg FaMLE 28 days Oral daily treated Rats showing features of Slight Myocardial Necrosis. H&E stain (× 400).

Kidney

The group of rats that received distilled water showed normal features of kidney section (Plate V). However slight tubular adhesions were observed in the kidney features of the FaMLE treated rats after 28 days daily oral administration (Plate VI).

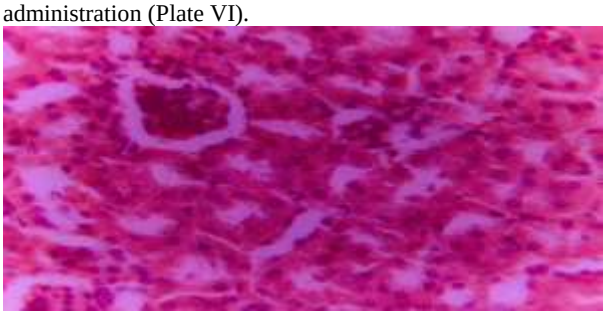

Plate V: Photomicrograph of Kidney Section of Distilled Water 28 days Oral daily Treated Rats showing normal features. H&E stain (× 400).

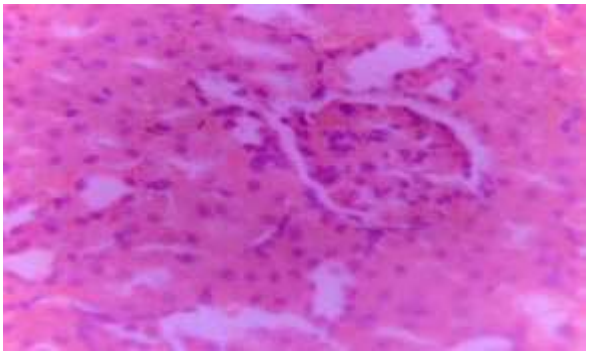

Plate VI: Photomicrograph of Kidney Section of 250 mg/kg FaMLE 28 days Oral daily treated Rats showing features of Slight Tubular Adhesion. H&E stain (× 400).

Spleen

The group of rats that received distilled water showed normal features of spleen section (Plate VII). However slight hyperplasia of inflammatory cells was observed in the spleen features of the FaMLE treated rats after 28 days of daily oral administration (Plate VIII).

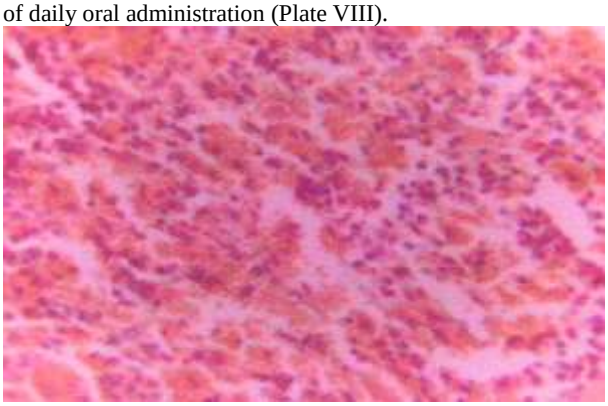

Plate VII: Photomicrograph of Spleen Section of Distilled Water 28 days Oral daily treated Rats showing normal features. H&E stain (× 400).

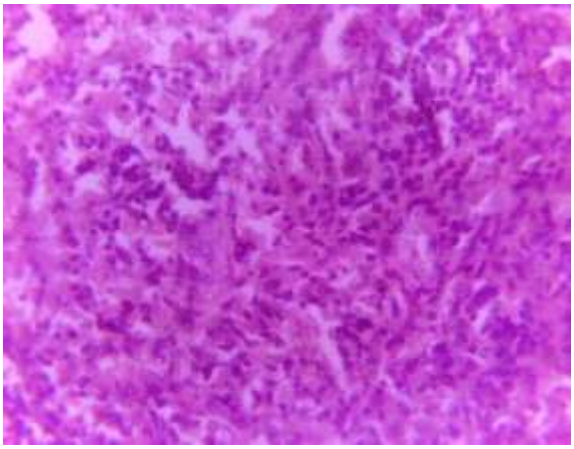

Plate VIII: Photomicrograph of Spleen Section of 250mg/kg FaMLE 28 days Oral daily treated Rats showing features of Slight Hyperplasia of Inflammatory cells. H&E stain (× 400).

Discussion

Medicinal plants, especially in developing countries, have been widely used in the management of various diseases and disorders (Zhang, 2004). Researches on medicinal plants have led to the discovery of novel lead compounds for potential development of drugs (Adebayo *et al*. 2015; Seo *et al*. 2018). During this study, preliminary phytochemical screening of the methanol leaf of *Ficus asperifolia* (FaLME) revealed the presence of saponins, flavonoids tannins, alkaloids, terpenoids, steroids and cardiac glycosides which might be responsible for the observed antiplasmodial activity. Similar metabolites were reported in previous studies (Omaniwa and Luka, 2012; Abdullahi *et al*. 2020).

Medium lethal dose serves as a useful indicator of margin of safety of a medicinal plants or drugs under study. However, it should not be regarded as a comprehensive evaluation of toxicity profile. In this test, the oral LD_{50} value of the FaMLE was found to be above 5000 mg/kg. This is indicating relative safety of this medicinal plant and the outcome agrees with work reported earlier (Abdullahi *et al*. 2020).

Sub-chronic toxicity studies involve assessment of hematological indices which can be used to determine the safety of medicinal plants on blood parameters (Yakubu *et al*. 2007). On one hand, toxicity of a medicinal plant may causes anemia which may be manifested as decrease in red blood cells (RBCs), pack cell volume (PCV) and hemoglobin concentration (Shittu *et al.* 2013). On the other hand, an increase in WBC, neutrophils or lymphocyte is an indication of phagocytosis which signifies presence of infection. Additionally, an increase in WBC has been implicated in inflammation, coronary artery disease, diabetes and leukemia (Wolford *et al.* 1986). In this study, FaMLE did not cause any change in blood parameters indicating wide margin of relative safety.

Kidney and liver play important roles in drug metabolism within the body and are necessary organs for the survival of animals (Olorunnisola *et al.* 2012). Damage to the kidney tubules may cause non reabsorption of electrolytes, retention of urea and cretenine in the blood. Henceforth, measurement of these parameters may be used to assess kidney damage (Afolabi *et al*. 2014). During this study, there was no significant change in the above parameters following 28 days FaMLE daily administration. This suggested that the plant extract is relatively safer in kidney during short term use.

In liver function test, increase in AST, ALT, ALP, bilirubin and total protein imply liver damage, but in clinical practice the most important parameter is ALP (Kachmar and Moss, 1976; Dhariyal *et al*. 2016). Increased activities of serum AST, ALT and ALT are indicative of cellular leakage and loss of functional integrity of liver cell (Sabiu *et al*. 2015). ALT is more elevated than AST in various necro-inflammatory conditions of the liver (Willianson *et al*. 1996). In this study, FaMLE significantly lowers the AST values. This suggested that the plant maybe having hepato protective effect. Related findings were reported earlier (Opotu *et al*. (2017).

Histological examination of the rodents' internal organs following repeated doses of extract provides some evidence of organ toxicity, similar to those caused by known hepatotoxins and nephrotoxins (Hussain *et al*. 2018; Mukherjee and Ahmad, 2018). Histological abnormalities including fatty cells changes, hydropic degeneration, fibrosis, and vascular abnormalities are usually seen in liver damage (Khleifat *et al*. 2002; Nigatu *et al*. 2017). In this study, 28 days daily administration of FaMLE produced only slight histopathological changes in body internal organs. This further suggested that the plant has margin of safety on short term use. A number of researchers have reported comparable outcome (Raji *et al*. 2011; Opotu *et al*. 2017).

In vivo screening provides a comprehensive approach for evaluating the effectiveness of drugs within a biological system. However, *in vivo* studies are influenced by various host factors, such as drug disposition and inherent anti-parasitic activities, which may affect the outcomes. *In vivo*

screening of antimalarial drugs is generally carried out using *Plasmodium berghei* because it is the most suitable species for animal model of malaria infection. It has good face, construct and predictive validity (Fidock *et al*. 2004; Asanga *et al*. 2017).

The result of the prophylactic study revealed that FaMLE at all doses significantly reduced the parasite density. The activity reported FaMLE was superior to the Pyrimithamine used as positive control. This finding is consistent with the report published in another study Nkafamiya *et al.* (2010) where the nutritious property of the plant is suggested to boast immunity. Prophylactic activity of FaMLE is an indication of its non-selectivity in its mechanism of action. This is because drugs use in prophylactic of malaria work either by the disruption of the parasite at the early liver stage or by suppressing the emergence of asexual blood stages or largely by preventing the relapse induced by hypnozoites (Hill *et al*. 2006).

During curative activity testing, FaMLE at all doses significantly reduced the parasite densities. Malaria is known to triggers the release of pain and inflammatory mediators, generates free radicals, and activates phospholipase activity. As such, a curative property of FaMLE is believed to occur via the inhibition of the release of inflammatory mediators and or through the direct cytotoxic effect on the parasites (Boampong *et al*. 2013). In addition, FaMLE significantly increased the mean survival time (MST) of the animals experimented signifying its efficacy as an antimalarial drug. This finding is in line with earlier study conducted (Udobre *et al*. 2013). The FaMLE contains abundant of secondary metabolites which are believed to be responsible for its antimalarial activity. Notably, cardiac glycosides have been implicated in prophylactic antiplasmodial activity of a medicinal plant. This was believed to occur through their ability to impede developments of gametocytes (Yoo *et al*. 2008). Also, alkaloids have been implicated in blocking protein synthesis in *Plasmodium falciparum* (Nergiz, 1993).

Previous experiment conducted *in vivo* revealed that FaMLE possess chemo suppressive activity. Its antimalarial activity was categorized based on the dose and level of parasite suppression. *Plasmodium berghei* suppression ≥ 50% at 100 mg/kg (very good), suppression ≥ 50% at 250 mg/kg (good), and suppression ≥ 50% at 500 mg/kg body weight/day (moderate) (Deharo *et al.* 2001; Tajbakhsh *et al*. 2021). Based on this classification the result obtained from this study indicated that FaMLE has good parasite suppression ability and can be used as a candidate for development of antimalarial drug.

Conclusion

The data obtained from this experiment indicated that the methanol leaf extract of *Ficus asperifolia* possesses antiplasmodial activity. This was seen based on the plant's ability to produced significant suppressive, prophylactic, and curative antiplasmodial property. *Ficus asperifolia* has higher margin of safety based on the result of acute toxicity studies. On subchronic administration, the plant did not produce any changes on hematological or biochemical parameters signifying safety and kidney and liver. There were only slight histopathological changes observed among the internal organs studied. This further suggested that the plant is safe based on the result of short term administration. It can also be suggested that the methanol leaf extract of *Ficus asperifolia* be fractionated and the bioactive compound (s) responsible for the observed antiplasmodial activity isolated. The safety testing should be extended to chronic administration up to 9 months daily administration.

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Conflict of Interest

The Authors have declared that there is no any conflict of interest with regards to this work or its publication.

References

Abdullahi, I. D., Umar H. Y., Suleiman U, B., Abdullahi R. A., Maitama A. Y., Abdullahi R. A and Yaro, A. H. (2023). Quantitative Determination of Phytochemical Constituents of Fractions Obtained from *Ficus asperifolia* Leaves Miq (Moraceae) and the Characterization of Compounds Identified in the Residual Aqueous Fraction; *FUDMA Journal of Sciences* Vol. 7 No.2, 330-343.

Abdullahi, I. D., Yaro, A. H and Nazifi, A. B. (2020). Anti-nociceptive and Anti-inflammatory Activities of Solvent Fractions of Methanol Leaf Extract of *Ficus asperifolia* miq. (Moraceae) in Murine Models; *Nigerian Journal of Pharmaceutical Sciences* Vol. 19 No.2, 25-37.

Adebayo S. A., Dzoyem J. P., Shai L. J and Eloff J. N. (2015). The Anti-inflammatory and Antioxidant Activity of 25 Plant Species used Traditionally to Treat Pain in Southern African. *BMC Complimentary Alternative Medicine*; 15:1-10.

Adjanohoun, J. C., Aboubaker, N., Dramane, K., Ebot, M. E., Ekpere, J.A. and Enow, O. E.G. (1996). Contribution to Ethnobotanical and Floristic Studies in Cameroon. Lagos, Nigeria: OUA/STRC; *Traditional Medicine and Pharmacopoeia*. 301–325.

Afolabi B. A., Obafemi T. O., Akinola T. T., Adeyemi A.S and Afolabi O. B. (2014). Effect of Ethanolic Extract of Alstonia boonei Leaves on Serum Electrolyte Levels in Wistar Albino Rats. *Phol-Pharmacology Online*; 3: 85-90.

Akah, P.A, Orisakwe, O.E., Gamanieel, K.S. and Shittu, A. (1998). Evaluation of Nigerian Traditional Medicines: 11. Effects of some Nigerian Folk Remedies on Peptic Ulcer. *Journal of Ethnopharmacolology*. 62 (2): 123-7.

Akanbi O. M., Elekofehinti O., Olatokunbo A., Adejuyigbe A and Jegede A. (2018). Anti-malarial Activity of Total Saponins from *Terminalia avicennioides* and Its Effect on Liver and Haematological of Infected Mice. *Drug Designing*; 7: 2. DOI: 10.4172/2169-0138.1000161.

Arbonnier, M. (2004). Trees, Shrubs and Lianas of West African Dry Zones. 1st Edn., CIRAD, Margraf Publishers GMBH MNHN,USA., Pp:574.

Arthur, S.J. and John, B. (1978). A colour Atlas of Histopathological Staining Techniques. Wolf MED.Pub. Ltd. London, pp 14-20.

Asanga Edet E., Eseyin Olorunfemi, Ebong Patrick, Igile Godwin,Thomas Paul S and Ebong A. (2017). Antiplasmodial Activity of Ethanol Extract and Fractions of *Nauclea Latifolia* Smith (Rubiacea) Roots. *World J Pharm Sci* ; 5(5): 106-118.

Burkill, H.M. (1997). The Useful Plants of West Tropical Africa. Vol. 4 2nd edition. Royal Botanic Gardens, Kew, Richmond, Survey TW 8 3AE Pp 293.

Caliskan, O. and Polat A. A. (2008). Fruit Characteristics of Fig Cultivars And Genotypes Grown In Turkey. *Science Horticulture*, 115: 360-367.

Caraballo H, King K (May 2014). "Emergency Department Management of Mosquito-borne illness: Malaria, Dengue, and West Nile virus". *Emergency Medicine Practice*. 16 (5): 1–23, quiz 23–4.

Deharo, E., Bourdy, G., Quenevo, C., Munoz, V., Ruiz, G., & Sauvain, M. (2001). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. *Journal of Ethnopharmacology*, *77*(1), 91-98.

Dhariyal KK, Farooq U, Singh S, Shariq M, Kaur N, Bharti AK. (2016). Malaria positive cases with reference to liver function test among patients attending in teerthankermahaveer medical college and research centre, Moradabad, Uttar Pradesh. *India International Journal of Science;* 3(12):62–66

Hill D. R et al. (2006). "Primaquine: Report from CDC Expert meeting on Malaria Chemophylaxis I". *The American Journal of Tropical Medicine and Hygiene*; 75 (3): 402 – 15.

Husain H, Latief U, Ahmad R. (2018). Pomegranate action in Curbing the Incidence of Liver Injury triggered by Diethylnitrosamine by Declining Oxidative Stress via Nrf2 and NFkB regulation. *Science Rep*; 8(1):8606. https://doi.org/10.1038/ s41598-018-26611-1.

Kachmar, J. F. and Moss, D. N. (1976). Enzymes (Transferase). In: Fundamentals of Chemistry (Nobert, W. Tietz Ed.) W. B. Saunders company, Philadelphia, pp.672-682.

Kalra BS, Chawla S, Gupta P, Valecha N. (2008). Screening of Antimalarial Drugs. *Indian journal of Pharmacol*ogy; 38:5-12.

Khleifat K, Shakhanbeh J, Tarawneh K. (2002). The chronic effects of Teucrium polium on some blood parameters and histopathology of liver and kidney in the rat. Turkish Journal of Biology; 26:65–71.

Lawal, O.A., Adebayo, M.A., Sikiru, A.A. and Ogunwande, I.A. (2016). Chemical Composition and Antimicrobial Activity of Essential Oils of *Ficus asperifolia* Miq. and *Ficus capensis* Thunb From Nigeria. *Journal of Essential Oil Bearing Plants*; 19: 1693- 1700.

Laychiluh, Bantie (2011). In vivo Antimalarial Activity of the Leaf Extract of *Croton macrostachys* (Euphorbiaceae) against *Plasmodium berghei* in mice. *Addis Ababa University*. 20-34.

Mengiste, B., Makonnen, E. and Urga, K (2012). Invivo Antimalarial Activity of *Dodonaea angustifolia* Seed Extracts against *Plasmodium berghei* in Mice Model. *Momona Ethiopian Journal of Science*; 4(1): 47-63.

Mukherjee D, Ahmad R. (2018). Resveratrol attenuates Nitrosodiethylamine-induced Liver injury in Anti-inflammatory manner via Regulating Cyclooxygenase-2. *Journal of Food Biochemistry*; 42: (5), e12594.

Nadjm B and Behrens RH (2012). "Malaria: An Update for Physicians". In fectious Disease Clinics of North America. 26 (2): 243–59.

Nergiz C and Otles S. (1993). Chemical Composition of Nigella sativa I. Seeds. *Food Chem*; 48: 259 – 61.

Nkafamiya I.I., Osemeahon, S.A., Modibbo, U.U. and Aminu, A. (2010). Nutritional Status of Non-conventional Leafy Vegetables, *Ficus asperifolia* and *Ficus sycomorus. African Journal of Food Science;* 4(3): 104-108.

Nwanko, I.U. and Ukaegbu-Obi, K.M. (2014). Preliminary Phytochemical Screening and Antibacterial Activity of two Nigerian Medicinal Plants (*Ficus asperifolia* and *Terminalis catappa*). *Journal of Medicinal Plant and Herbal Therapy Research*, 2: 1-5.

Odiba, P., Yusuf, D., Ali, E., Yusuf, M. and John, E. (2012). Effect of Aqueous Extract of *Ficus exasperata* Leaf on the body Weight and Haematological Parameters of wistar rats. *Journal of Applied Science Environment*. 3: 80-831.

Ojo, A.O. and Akintayo, C.O. (2014). Assessment of Antioxidant Activity of *Ficus asperifolia* Miq Aqueous Extract –*Invitro* Studies. *TheJjournal of Phytopharmacology*, 3(1):16-21.

Ojo, O.A., Ojo, A.B., Ajiboye, B., Fadaka, A., Imiere, O.D., Adeyonu, O. and Olayide, I. (2016). Protective Influence of Ficus asperifolia Miq. Leaf Extract on Carbon Tetrachloride (CCl4)-Induced Testicular Toxicity in Rat's Testes. *Journal Applied Pharmaceutical Science*; 6: 37-41.

Olurunnisola O. S., Bradley G and Afolayan A. J. (2012). "Acute and Sub-chronic Toxicity Studies of Methanolic Extract of Tulbaghiaviolacea rhizomes in Wistar rats," *African journal of Biotechnology*; 11: 14934-14940.

Omoniwa, B.P., Luka, C.D. and Soji-Omoniwa, O. (2013). Effect of Aqueous Leaf Extract of *Ficus asperifolia* on Cardiac Enzymes and Lipid Profile in Male Albino Rats. *Journal of Medical Sciences*; 13: 373-378.

Omoniwa, B.P. and Luka, C.D. (2012). Antidiabetic and Toxicity Evaluation of Aqueous Stem Extract of *Ficus asperifolia* in Normal and Alloxan-induced Diabetic Albino Rats. *Asian Journal Experimental Biological sciences*; 3:726-732.

Opotu, R.O., Uwakwe, A.A., Chuku, L.C and Olua, V. (2017). Hepatoprotective Potentials of Aqueous extracts of *Ficus asperifolia* MIQ leaves on CCl4induced liver damage in Wistar rats; *International Journal of Advanced Research in Biological Sciences*; 4(12): 27-39.

Organization for Economic Co-operation and Development (OECD). (2001). Guidelines for Testing Chemicals: Repeated Dose 28 Day Oral Toxicity in Rodents (No. 407), Section 4, *OECD Publishing*; Paris, France: pp1e8.

Organization for Economic Co-operation and Development (OECD). (2008). Guidelines for Testing Chemicals: Acute Oral Toxicity e Fixed Dose Prpcedure (No .420), Section 4, *OECD Publishing*, Paris, France: pp1e14.

Peters W (1967). Rational methods in the search for anti-malaria drugs. *Trans. R. Soc. Tropical Medicine Hygiene*; 61:400-410.

Peters W., Portus H and Robinson L. (1975). The four-day suppressive in vivo antimalarial test. Ann *Tropical Medical Parasitology*; 69:155–71.

Pierre, W., Esther. N., Pepin, A. N., Telesphore, B. N. and Albert, K. (2009). Evaluation of *In- vitro* Uterotonic Activities of Fruit Extracts of *Ficus asperifolia* in Rats. *Evidence-Based Complementary and Alternative Medicine*, 2(11): 7.

Raji, Y., Oyeyemi, W.A., Shittu, S.T. and Bolarinwa, A.F. (2011). Gastro-Protective Effect of Methanol Extract of *Ficus asperifolia* Bark on Indomethacin Induced Gastric Ulcer in Rats. *Nigerian Journal of Physiological Sciences*; 26: 43-48.

Ryley JF, Peters W (1970). The Anti-malaria Activity of some Quinolone esters. *AmericanTropical Medical Parasitology*; 84:209-222.

Sabiu S, Sunmonu TO, Ajani EO, Ajiboye TO (2015). Combined administration of silymarin and vitamin C stalls acetaminophenmediated hepatic oxidative insults in Wistar rats. Rev Bras Farmaco.

Seo, E., Efferth, T. and Panossian, A. (2018). Curcumin Down Regulates Expression of OpioidRelated Nociceptin Receptor Gene (OPRL1) in Isolated Neuroglia Cells. *Phytomedicine*; 50: 285-299.

Shittu OK, Olayemi IK, Omalu ICI, Adeniyi AK. (2013). Antiplasmodial properties of methanolic extract of Musca Domestica Maggot On P. berghei-infected mice. *IJBPAS*; 2(5): 1064–1070.

Tajbakhsh E., Tebit Emmanuel Kwenti, Parya Kheyri, Saeed Nezaratizade, David S. Lindsay and Faham Khamesipour. (2021). Antiplasmodial, Antimalarial Activities and Toxicity of African Medicinal Plants A Systematic Review: *Journal of Malaria*; 20:249. https://doi.org/10.1186/s12936-021-03866-0.

Trease, G.E. and Evans, W.C. (2000). Pharmacognosy 16th Ed. *Bailliere Tindall Ltd*., London, pp: 60-75.

Udobre A. S *et al.* (2013). Effect of Methanol Leaf Extract of *Nauclea latifolia* on Albino Mice Infected with *Plasmodium berghei berghei*. *African Journal of Pharmacology and Therapeutics*; 2 (3): 83 – 87.

Watcho, P., Ngadjui, E., Alango, N.P., Benoit, N.T. and Kamanyi, A. (2009). Reproductive Effects of *Ficus asperifolia* (Moraceae) in Female Rats. *African Health Sciences*; 9: 49-53.

Watcho, P., Ngadjui, E., Nkeng-Efouet, P.A., Nguelefack, T.B. and Kamanyi, A. (2011). Evaluation of In-Vitro Uterotonic Activities of Fruit Extracts of *Ficus asperifolia* in Rats. *Evidence Based Complimentary Alternative Medicine*; 783413: 1-7.

Weatherall DJ (2008). "Genetic variation and susceptibility to infection: The red cell and malaria". British Journal of Haematology. 141 (3): 276–86. doi:10.1111/j.1365-2141.2008.07085.x. PMID 18410566.

White NJ. (2008). Quighaosu (artemisin): The Price of Success. *Science*; 320: 330 – 4.

WHO (2020). World Malaria Report 2020. Switzerland: World Health Organization. ISBN 978-92-4-001579-1.

Willianson EM, Okpako DT, Evans FJ. (1996). Selection Preparation and Pharmacological Evaluation of Plant Material. John Wiley, London.

Wolford, S. T., Schroer, R. A., Gohs, F. X., Gallo, P. P., Brodeck, M., Falk, H. B and Ruhren, R. (1986). Reference Range Database for Serum Chemistry and Hematology Values in Laboratory Animals. *Journal of Toxicology and Environmental Health, Part A Current Issues;* 18(2): 161-188.

World Health Organization. (2019). *World malaria report 2019*. World Health Organization

World Health Organization. (2020). *World malaria report 2020*. World Health Organization

World Health Organization. (2022). *World malaria report 2022*. World Health Organization. world Malaria report 2023 - Google Scholar

World Health Organization. (2023). WHO malaria policy advisory group (MPAG) meeting report, 18–20 April 2023. World Health Organization.

World Health Organization. (2023). WHO guidelines for malaria, 14 March 2023 (No. WHO/UCN/GMP/2023.01). World Health Organization.

Yakubu MT, Akanji MA, Oladiji AT. (2007). Hematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogia agrestis stem. *Pharmacogny Mag* ; 3(9):34.

Yoo HJ et al. (2008). Anti-angiogenic Antinociceptive and Anti-inflammatory Activities of *Lonicera japonica* Extract. *Journal of Pharmacy and Pharmacology*; 60: 779 – 86.

Zhang Yawei, Qiaofen Cheng, Yao Yao, Xiuyun Guo, Rongrong Wang and Zengqi Peng. (2014). A Preliminary Study: Saltiness and Sodium Content of Aqueous Extracts from Plants and Marine animal shells; *European Food Research Technology;* 238:565–571 Doi: 10.1007/s00217-013-2136-1.