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Original Research Article

In-vivo and Histological Studies of Matrix-based Artemether-Lumefantrine Oral Tablets Derived from *Cissus populnea* Gum

John D.N. Ogbonna[1], Chinelo N. Aguiyi-Ikeanyi[2], Paul A. Akpa[1], Samuel N. Uzondu[1], Adaeze C. Echezona[1], Akachukwu M-T. Onwuka[3], Emmanuella T. Ogbonna[1], Emmanuel I. Onah[1], Ifebuche N. Ugwuagbo[1], Victoria O. Ezeala[1], Anthony A. Attama[1]

[1]*Department of Pharmaceutics, Drug Delivery and Nanomedicines Research Group, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Nigeria*
[2]*Department of Clinical Pharmacy and Pharmacy Management, Faculty of Pharmaceutical Sciences, University of Nigeria, 410001 Nsukka, Nigeria*
[3]*Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria*

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ABSTRACT

Malaria incidences has been a public health concern and it has increased in recent years because of resistance of the malaria parasite to available antimalarial drugs. This research explored the anti-plasmodial effect of the *Cissus populnea* matrixed in artemether-lumefantrine against *Plasmodium berghei* infected mice. The matrixed drug was evaluated for prophylactic, curative, and suppressive effect in *Plasmodium berghei* mice. The swiss Albino mice were grouped into 4 and were inoculated with *Plasmodium berghei* intraperitoneally except the naïve group. The matrixed drug significantly inhibited parasitemia in prophylactic, curative, and suppressive infections ($p<0.05$) (45.75%, 37.75, and 60.99%, respectively). However, a significant decrease in PCV, Hb, RBC and increased total and differential white blood cell count was observed in the mice that were treated with the matrixed tablet formulation for prophylaxis and suppression, but no significant hematological effect was observed in the groups that received the matrixed tablet for curative effect. The histopathological analysis of the matrixed drug on different organs showed no observable damage in any of the organs This study suggests that incorporation of *Cissus populnea* gum in artemether-lumefantrine based tablets possessed promising antimalarial potential with minimal histological and hematological effects.

Keywords: *Cissum populnea*, Artemether/lumefantrine, Malaria, *Plasmodium berghei*

Introduction

Malaria is a disease of public health concern affecting about 241 million people and resulting in death of about 627,000 people which increased to 227 million cases and 558,000 deaths in 2020.[1] The causative organism is a protozoa parasite from a *Plasmodium* specie. It is transmitted through the bite of a female anopheles mosqutio.[2] Children below age five and pregnant women are mostly affected in the endemic regions of the world.[3] In some endemic areas, it is the leading cause of death and morbidity.[4] In 2018, 95% of the global malaria burden was from Africa.[5]

There have been recent trends of resistance of malaria parasites to the available antimalaria agents resulting in increased malaria incidences.[6-8] The resistance due to plasmodial parasite to the available Artemisinin-Based Combination Therapy (ACTs) is primarily due to the Artemisinin component of the medicine which causes a resultant delay in the clearance of the parasite from the blood hence resulting to a resistance of the parasite to the partner drug.[9] The development of new antimalaria have been challenging because of insufficient information and understanding of the life cycle, biology and pathology of malaria parasite.[10]

*****Corresponding author. E mail: chinelo.aguiyi@unn.edu.ng
Tel: +2348167886250

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Most of the antimalarial agents currently in use have adverse effects, therefore cost-effective, non-toxic, low tendency to develop resistance and efficacious medicines for malaria treatment especially for infected pregnant women and children below 5 years needs to be developed.[11] Most citizens of tropical countries rely on traditional medical remedies for treatment because conventional medicines are sometimes not accessible and may not be available.[12] The use of safe and efficacious herbal medicines in the treatment of disease conditions have been approved in Nigeria and in other parts of the world.[13] Most regions across the globe already use herbal medicines to treat some minor ailments and disease conditions.[14] Most medicinal plants are used as medicines because they have active chemicals that have therapeutic benefits. A lot of indigenous plants had considerable antiplasmodial and anti-malaria activity.[15]

Experimental animal models are adopted in the research and development of new drugs and vaccines to understand the nature of the disease.[16] Animals are used as models of human diseases for ethical reasons. The mouse model is commonly used in research because of the similarities of the malaria parasite antigen in the mouse and human and the similarities in the defense mechanism of the mouse and human against the parasite.[17]

Plasmodium berghei causes severe infection which manifests as high level of parasitemia and anemia.[17] It is used as a model organism for investigation of human malaria because of its similarity in life cycle and clinical manifestation to the *Plasmodium* spp which causes malaria in humans. *Plasmodium berghei* NK 65 is suitable for this study because it is used to determine the effectiveness of a new herbal antimalarial agent due to its unique sensitivity to chloroquine making it a suitable parasite for this study.[18]

Cissus populnea is a medicinal plant that has lots of therapeutic uses and benefits.[19] It is commonly found in Sub-Sahara Africa.[20] It possessed anti- sickling potential,[21] antioxidant properties,[22] antimicrobial activities[23] and spermatogenic enhancer.[24] This study

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assayed the antimalarial activity of *Cissus populnea* matrixed in artemether-lumefantrine tablet against *Plasmodium berghei* infection in mice.

Materials and Methods

The materials were sourced from the locality and were used in their original form. They include sodium hydroxide (Merck, Germany), hydrochloric acid, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate, absolute ethanol, sodium chloride, acetone (BDH, England), Microcrystalline cellulose (Vivapur VR 102, JRS Pharma, Germany), lactose monohydrate (Acofarma, Spain), and magnesium stearate (Faci, Italy).
Cissus populnea root was sourced and collected in April 2016 from Uvuru Town in Uzo-Uwani LGA Enugu State, Nigeria. Artemether and lumefantrine were gift samples from Emzor Pharma (Lagos, Nigeria). Reference samples of artemether and lumefantrine were obtained from Sigma Aldrich (Taufkirchen, Germany). The reagents were all analar grade.

Extraction of Cissus gum
Fresh roots of Cissus populnea were washed in running water, the outer layers were removed, and then they were cut into smaller sizes. The plant's roots were then soaked in 10 times its weight of distilled water containing 1% sodium metabisulphite (an antioxidant). The slimy mucilage was collected after straining the root particles and the mucilage dispersion screen through a quadruplicate folded muslin cloth. The slimy mucilage was later evaporated in an oven at 50 ºC for 72 h. The amorphous powdered material was stored in a desiccator until used. The same procedure of extraction was repeated but the slimy mucilage was used as such without further modifications.

Granule preparation
The granules were prepared according to documented specifications,[25] with few modifications. Granules containing varying proportion of lactose as bulking agent, 2 %w/v *cissus* gum [*cissus* crude (Craw), *cissus* gum evaporated (Cev)], 5 %w/v *cissus* gum [*cissus* crude (Craw), *cissus* gum evaporated (Cev)], and 10%w/v *Cissus* gum [*cissus* crude (Craw), *cissus* gum evaporated (Cev)] with either 1 or 2 %w/v lubricant (magnesium stearate) were made into granules by wet granulation with water. (Table 1). Lactose, cissus gum, and magnesium stearate were weighed and transferred to a small-scale granulator (Type T2C Wily A, Bachofen AG, Basel, Switzerland) via a mixer. The mixer was set to 100 revolutions per minute (rpm), and the powders were mixed for 10 minutes before adding the granulating liquid. There was continued mixing for an additional 10 minutes until a consisted wet mass was obtained. It was then filtered through a 1.40 mm screen and dried in an oven (Gallenkamp, UK) at 50ºC for 1 hour. The granules obtained were screened through a 1.40 mm screen and a 355 µm sieve and the retained (coarse granules) were obtained and kept for use.

Tablet formulation
A batch, each contains 2 % *cissus* gum with magnesium stearate as lubricant (1 and 2 %), and drug content of 28 % [(artemether (14.286 %) and lumefantrine (85.714 %)]. The batches were made up to 100 % with lactose (Acofarma, Spain) as diluent. The proportionately weighed powders were mixed for 10 minutes in a mixer set to 100 rpm, after which water (20%) was added. Continuous stirring continued for additional 10 minutes to obtain a desirable consistent wet mass which was passed through a 1.40 mm screen and dried in an oven (Gallenkamp, UK) at 50°C for 1 hour, then through a 1.00 mm screen, followed by a 355 µm sieve. After which the retained coarse granules were collected. The recommended arthemeter and lumefantrine dosing schedule ratio was used for rthe formulation.[26]
The load of the upper punch adjusted, the speed of the machine zeroed (0), the upper point and lower point adjusted for a weight of 500 ± 5 % of the granules after many trials. The granules were poured into the distributor of the automatic instrumented single punch tablet press, model CPR-6 (Dott Bonaface and C. Pharmaceutical Machinery Division, Milano, Italy) and the tableting machine connected to the Cosalt Data Acquisition Systems software (Adlink Techonology Inc PCI-9118DG ID:0 Dasylab version 8.00.04).
Ten tablets from each batch were analyzed for the following parameters: maximum force needed to move the upper punch (kN), total positive work done by the upper punch (J), work lost in the elastic return of the pressed mass (J), work lost in the friction forces (J), net compression work remaining in the pressed mass (J), maximum force needed to move the lower punch (kN), total force acting on the lower punch (kN), total force needed to move the upper punch (kN), progressive total work done to move the upper punch (J), progressive work done by friction acting on the upper punch (% of 10 J), frictional force in upper punch (kN) and displacement of upper punch (mm). This formed batch AL2Cev1. Triplicate batches were manufactured, and the average parameters calculated. The same procedures were repeated using artemether-lumefantrine tablet batches (AL2Cev2, AL5Cev1, AL5Cev2, AL10Cev1 and AL10Cev2) containing 2 % *cissus* gum with 2 % lubricant, 5 % *cissus* gum with 1 % lubricant, 5 % *cissus* gum with 2 % lubricant, 10 % *cissus* gum with 1 % lubricant and 10 % *cissus* gum with 2 % lubricant, respectively.

Experimental Animals
Adult Wistar albino mice (15-25 g) of both genders were obtained from the Faculty of Biological Science Laboratory at the University of Nigeria in Nsukka. The animals were housed in steel cages within the facility, with free access to water and standard livestock pellets. All animals used in the experiments were treated in accordance with the National Institute of Health's Guidelines for Laboratory Animal Care and Use (Pub No. 85-23, revised 1985). Before beginning the study, the National Health Research Ethics Committee (NHREC) of the University of Nigeria, Nsukka, granted ethical approval. The Ethical Permission Number issued was NHREC/05/01/2013B.

Parasite
The *Plasmodium berghei* (NK - 65), which is chloroquine sensitive is a rodent parasite that was obtained from the National Institute of Medical Research (NIMR) in Yaba, Lagos, Nigeria. The parasite was kept in the University Animal House Laboratory after a serial inoculation from donor mice to uninfected mice. The experimental animals were inoculated intraperitoneally using a standard inoculum of $1x10^7$ of infested erythrocytes obtained from a donor mouse (0.2 mL) according to standard protocol.[27] The day of the inoculation was referred to as day 0, followed by days D1, D2, and so on.

Anti-malarial study
Prophylactic Test
The prophylactic activity of *Cissus populnea* matrixed artemether-lumenfantrine tablet was carried out according to the method described by Peter, 1965.
Eighteen Albino mice were randomly grouped into three (3), with each group having six animals each. Group 1 was administered distilled water (10 ml/kg) orally, Group 2 was administered orally with standard drug Coartem® containing artemether-lumenfantrine (10 mg/kg) while group 3 was administered with artemether-lumenfantrine tablets containing *Cissus populnea* as matrix (10 mg/kg) orally on day 0 which was continued until day 4. On day 4, the animals were inoculated intraperitoneally with 0.2ml of the blood sample containing $1x\ 10^7$ *Plasmodium berghei* from a donor mouse.
Blood sample was collected from the tail of each inoculated experimental animal 72 hours after inoculation, parasitemia was confirmed microscopically, and the percentage inhibition of parasitemia was calculated.
The inhibition of parasitemia (%) was calculated using the formula:

$$\%\text{Parasitemia count in each animal} = \frac{\text{Number of infected RBC}}{\text{Total Number of RBC}} \text{ x } \frac{100}{1}$$

Percentage reduction in parasitaemia count was determined with this formular:

$$\%\text{Reduction in parasitemia } \frac{MPC - MPT}{MPC} \text{x } \frac{100}{1}$$

MPC = Mean parasitemia in control, MPT = Mean parasitemia in treated group.

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The blood collected was also subjected to hematological tests to determine Packed Cell Volume (PCV),[28] Hemoglobin concentration (Hb),[29] Erythrocyte counts (EC),[30] Total leucocyte count (TLC)[30] and Differential Leucocyte Counts (DLC).[31]
Animals from each group were sacrificed, the liver, spleen, kidney, and brain were taken for histopathological assay on day 21.

Suppressive Test
Suppressive activity of artemether-lumefantrine oral tablets containing *Cissus populnea* as a delivery matrix (4-day test) was performed as described in a previous study.[32] Twenty-four Swiss albino mice of varying gender were administered intra-peritoneally (i.p) with 0.2 mL infected blood samples. The mice were grouped into four (4), with six mice in each group. Group 1 were administered with normal saline; they were the control group. Group 2 were administered with standard Coartem® (arthemeter-lumefantrine) while group 3 were administered with artemether-lumefantrine tablets containing *Cissus populnea* (10 mg/kg) as matrix, Group 4 were not inoculated with the parasite (Naïve).
The treatment lasted for four (4) consecutive days. The last group was neither treated nor infected (naïve group). Samples of blood were collected from the tail of each experimental animal 24 hours after the last treatment. The smears were fixed in absolute ethanol, stained with 10% Giemsa, and examined under a microscope. The parasitemia of each mouse was obtained from the number of inoculated and uninoculated blood samples across six fields. The percentage suppression of parasitemia for each treated group was calculated by comparing parasitemia in the infected control group to that of treated mice.

$$\%\text{Parasitemia count in each animal} = \frac{\text{Number of infected RBC}}{\text{Total Number of RBC}} \times \frac{100}{1}$$

Inhibition of parasitaemia was determined using the relationship:
Inhibition of parasitemia (%) = 100{1-(PT/PC)
Where, PT = parasitemia of treated group, PC = parasitemia of control group.
The blood collected was also subjected to hematological tests to determine Packed cell Volume (PCV),[28] Hemoglobin concentration (Hb),[29] Erythrocyte counts (EC),[30] Total leucocyte count (TLC)[30] and Differential Leucocyte Counts.[31]
On the 21st day, two animals from each treatment group were sacrificed, the liver, spleen, kidney, and brain were excised surgically for histopathological assay.

Curative Test
The curative activity of artemether-lumefantrine oral tablets matrixed in *Cissus populnea* gum against established infection was evaluated as described in a previous study.[33] Eighteen (18) mice were intraperitoneally inoculated with 0.2 ml of the diluted blood of the donor mouse containing 1×10^7 infected erythrocytes (Day 0). The mice were allowed free access to water and food. On day 3, the mice were grouped into three (3) groups: Group 1 were administered with normal saline (10 ml/kg), Group 2 were administered with standard Coartem® (artemether-lumefantrine) (10 mg/kg) while group 3 was administered with artemether-lumefantrine tablets containing *Cissus populnea* as matrix (10 mg/kg), Group 4 were not inoculated with the parasite (Naïve).
The treatment continued daily for 5 days. Blood samples collected from the tail of each experimental animal was used to prepare films which was then fixed with absolute methanol stained with Giemsa. Microscopic examination across six fields was used to determine the parasitemia. The percentage of inhibition was also calculated as previously described.
The blood collected was also subjected to hematological tests to determine Packed Cell Volume (PCV),[28] Hemoglobin concentration (Hb),[29] Erythrocyte counts (EC),[30] Total leucocyte count (TLC)[30] and Differential Leucocyte Counts (DLC).[31]
The animals in all the groups were observed for the survival trend and cure for 21 days and they were sacrificed, and their tissues (the liver, spleen, kidney, and brain) examined by histopathological assay.

Histological Assay
These were performed with hematoxylin and eosin procedures as described by Layton and Bancroft (2013).[34] The liver, spleen, kidney, and brain were fixed in 10% formalin saline, grossed, and cut longitudinally into 4 mm thick pieces for histological analysis using standard procedures. The microtome sections were dried on a hot plate then stained with hematoxylin and eosin stains for examination under a microscope.

Statistical analysis
The data was analyzed with One-way ANOVA, followed by Dunnett's multiple comparisons post-hoc test, and GraphPad Prism version 5.0. The result was shown as mean ± SEM (standard error of mean). $P < 0.05$ was regarded as statistically significant.

Results and Discussions

Prophylactic Antiplasmodial Effect of Cissus populnea Formulated Artemether-Lumefantrine Oral Tablets
Figure 1 showed that the group that was administered distilled water (negative control) had the highest mean parasitemia count of 57.68 % indicating no antimalarial activity. The mean parasitemia count in mice infected and treated with *Cissus populnea* formulated artemether-lumefantrine oral tablets was 31.29% (chemo-suppression of 45.75 %) while the standard had a mean parasitaemia count of 26.5% (chemo-suppression of 54.54 %) when compared to control.
Histological sections of the liver, kidney, spleen, and brain showed normal tissue morphology except in the untreated groups where there was observed inflammation of cells (Figures 2-3).

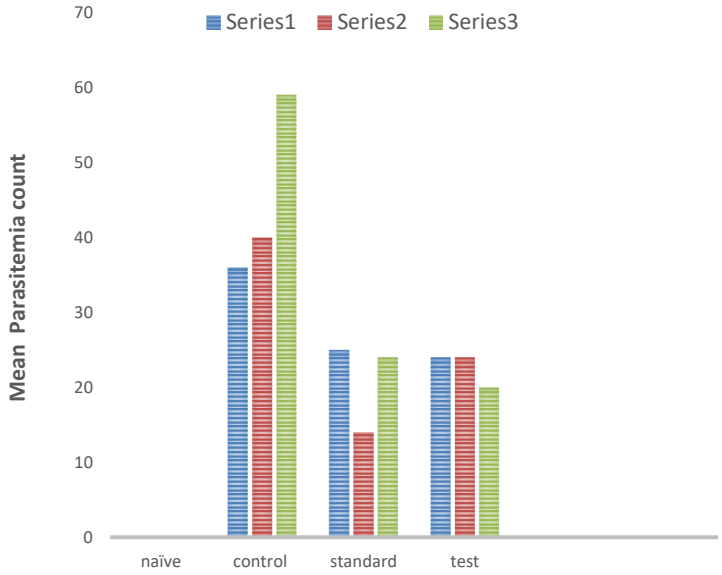

Figure 1: Suppressive, curative, and prophylactic effects of *Cissus populnea* formulated Artemether-Lumefantrine oral tablets
Series 1 =Suppressive effect of *Cissus populnea* formulated Artemether-Lumefantrine oral tablets
Series 2 =Curative effect of *Cissus populnea* formulated Artemether-Lumefantrine oral tablets
Series 3= Prophylactic effect of *Cissus populnea* formulated Artemether-Lumefantrine oral tablets
Control = Normal Saline, Standard = Coartem®, Test = *Cissus populnea* formulated artemether-lumefantrine oral tablets
Bars are expressed as mean ± SEM (n=6)
*** $p < 0.001$ when compared to control
** $p<0.01$ when compared to control

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Table 1: Effect of *Cissus populnea* formulated artemether-lumefantrine on hematological parameters during prophylactic anti-malarial test

	PCV (%)	Hb (g/dl)	RBC ($\chi 10^3$ µl)	WBC ($\chi 10^3$ µl)	L (%)	N (%)	M (%)	E (%)	B (%)
Control	40.75 ± 1.11	10.64 ± 0.88	8.94 ± 0.29	11.28 ± 0.34	58.25 ± 0.85	38.0 ± 0.82	2.5 ± 0.29	1.25 ± 0.25	0.5 ± 0.29
Test	26.00 ± 1.44****	4.83 ± 0.11****	6.70 ± 0.28**	17.67 ± 0.57****	75.5 ± 2.01***	18.75 ± 0.32****	1.75 ± 0.48	3.0 ± 0.41*	0.00 ± 0.00
Standard Drug	26.25 ± 1.03****	5.07 ± 0.35	7.44 ± 0.38	17.29 ± 0.66****	74.0 ± 2.16***	20.0 ± 0.21***	2.25 ± 0.75	3.5 ± 0.65	0.25 ± 0.25

Values are expressed as mean ± SEM, significance represented as *(P<0.05), ** (P<0.01), *** (P<0.001), ****(P<0.0001)
Key:
Test: *Cissus polulnea* formulated artemether-lumefantrine oral tablets, standard: Coartem®, control: distilled water
PCV: Packed cell volume, Hb: Hemoglobin, RBC: Red blood cell, WBC: white blood cell, L: Lymphocytes, N: Neutrophil, M: Monocyte, E: Eosinophil, B- Basophil

Table 2: Effect of *Cissus populnea* formulated artemether-lumefantrine on hematological parameters during suppressive anti-malarial test

	Naïve	Control	Test	Standard
PCV (%)	41.0 ± 1.47	29.0 ± 1.23	35.0 ± 2.04	38.25 ± 2.66
Hb (g/dl)	10.63 ± 0.87	4.12 ± 0.17	5.58 ± 0.43	5.37 ± 0.06
RBC(x10^6µl)	9.39 ± 0.45	8.27 ± 1.01	9.67 ± 0.15	9.76 ± 0.57
WBC(x10^3µl)	11.28 ± 0.31	15.1 ± 1.29	15.17 ± 0.51	13.87 ± 0.61
L (%)	58.25 ± 0.85	66.5 ± 6.91	75.0 ± 2.89	74.0 ± 3.56
N (%)	38.0 ± 0.82	18.25 ± 2.39	18.0 ± 1.63	17.75 ± 2.63
M (%)	2.25 ± 0.25	4.0 ± 0.41	3.75 ± 0.63	4.25 ± 0.63
E (%)	1.25 ± 0.25	5.0 ± 1.47	3.25 ± 0.95	3.75 ± 0.85
B (%)	0.0 ± 0.0	0.25 ± 0.25	0.0 ± 0.0	0.25 ± 0.25

Values are expressed as mean ± SEM, significance represented as *(P<0.05) **(P<0.01) ***(P<0.001) ****(P<0.0001) when compared to naive. L=lymphocyte, N=neutrophil, M=monocyte, E=eosinophil, B=basophil.

Result obtained from the haematological study revealed significant decrease in PCV, Hb, RBC. These abnormal values observed in the plasma of the infected mice suggests anemia resulting from the destruction of erythrocytes (RBCs). The breakdown of the erythrocytes may involve antibody-dependent cell mediated cytotoxicity (ADCC),[35] or Opsonin-independent killing mechanism.[36] Acute anemia in *P.berghei* infested mice as revealed in this study could be due to increased myeloid cell production resulting from immune response of the cells to parasitic infection resulting to inhibition of erythroid development.[37] In humans, anemia develops in patients with severe malaria due to *P. falciparum*, rapidly because of the destruction of RBCs by the mononuclear phagocyte system in the spleen, in response to foreign parasite antigen on the surface of the RBCs.[38] and the maturation of parasites into schizonts and its release from the RBCs.[39]
There was also increased leucocyte and white blood cell count in the mice that were treated with *Cissus populnea* formulated artemether-lumefantrine oral tablets (Table 1) which suggests the presence of an infection. These abnormal values (elevated total and differential white blood cells; leucocytes, neutrophils, and basophils) could be due to inflammatory responses of the cells to the *Plasmodium berghei* in the plasma of the mice. Also, the bye-products of hemoglobin catabolism disrupts the innate immune responses[39] influencing the production of pro-inflammatory cytokines and chemokines.[33]

Suppressive Antiplasmodial Effect of Cissus populnea Formulated Artemether-Lumefantrine Oral Tablets
In standard suppressive antimalarial screening test, a value of $\geq$ 30 % chemo-suppression in parasitemia following treatment makes a drug to be considered active.[40] Findings from the suppressive antiplasmodial effect of *Cissuss populnea* matrixed Artemether-lumefantrine oral tablet revealed that *Cissuss populnea* matrixed Artemether-lumefantrine oral tablet exerted a mean parasitaemia count of 15.22 ±2.06 % (60.99% chemo suppression) on day 4 after infection while the group treated with standard drug gave a mean parasitemia count of 15.86 ± 2.41 % (59.35% chemo-suppression) when compared with control (39.02 ± 2.56%) (Figure 1). There was zero parasitemia count in the naïve group as they were not infected with the plasmodium. This implies that the matrixed *Cissus populnea* matrixed Artemether-lumefantrine oral tablet possessed a better suppressive antiplasmodial effect than the standard drug.
However, the mice that were treated with the standard drug had better hematological parameter than the matrixed tablet as revealed in this study. There was a significant decreased in PCV, Hb, RBC and increased WBC count and leucocyte count in the mice that were treated with *Cissus populnea* formulated artemether-lumefantrine oral tablets (Table 2). These haematological parameters obtained could be due to the adverse effect of the matrixed tablet on the treated mice.
Histology sections of the kidney, liver, brain, and spleen showed normal tissue morphology indicating the ability of the matrixed drug to clear parasite damages to the cells, however, inflammation and proliferation of cells was observed in the untreated group (Figure 4-5).

Curative antiplasmodial effect of Cissus populnea formulated artemether-lumefantrine oral tablets
Result obtained from the curative antiplasmodial activity study revealed that the *Cissuss populnea* matrixed Artemether-lumefantrine oral tablet produced a reduction in parasitemia count in the mice; there was also a reduction in parasitemia count in the artemether-lumefantrine group (Positive group) as shown in Table 1. However, the artemether-lumefantrine oral tablet (control) appears to have a better curative antiplasmodial activity compared to the matrixed tablet as revealed by the chemo-suppression values of 68.40 % and 37.35% respectively. The respective mean parasitaemia counts for the control, test, and standard drugs were 40.70 %, 25.5 % and 12.86 % (Figure 1). The test and standard groups had significantly (p< 0.01, 0.001) chemo-suppression activities of 37.35 % and 68.40 % respectively when compared to control. Histology study revealed that the liver, kidney, spleen was normal except with untreated groups where there was observed inflammation of cells (Figures 6-7). Hematologic study revealed that there was no remarkable variation in the results obtained from the treatment group and the control group(p>0.05) (Table 3).

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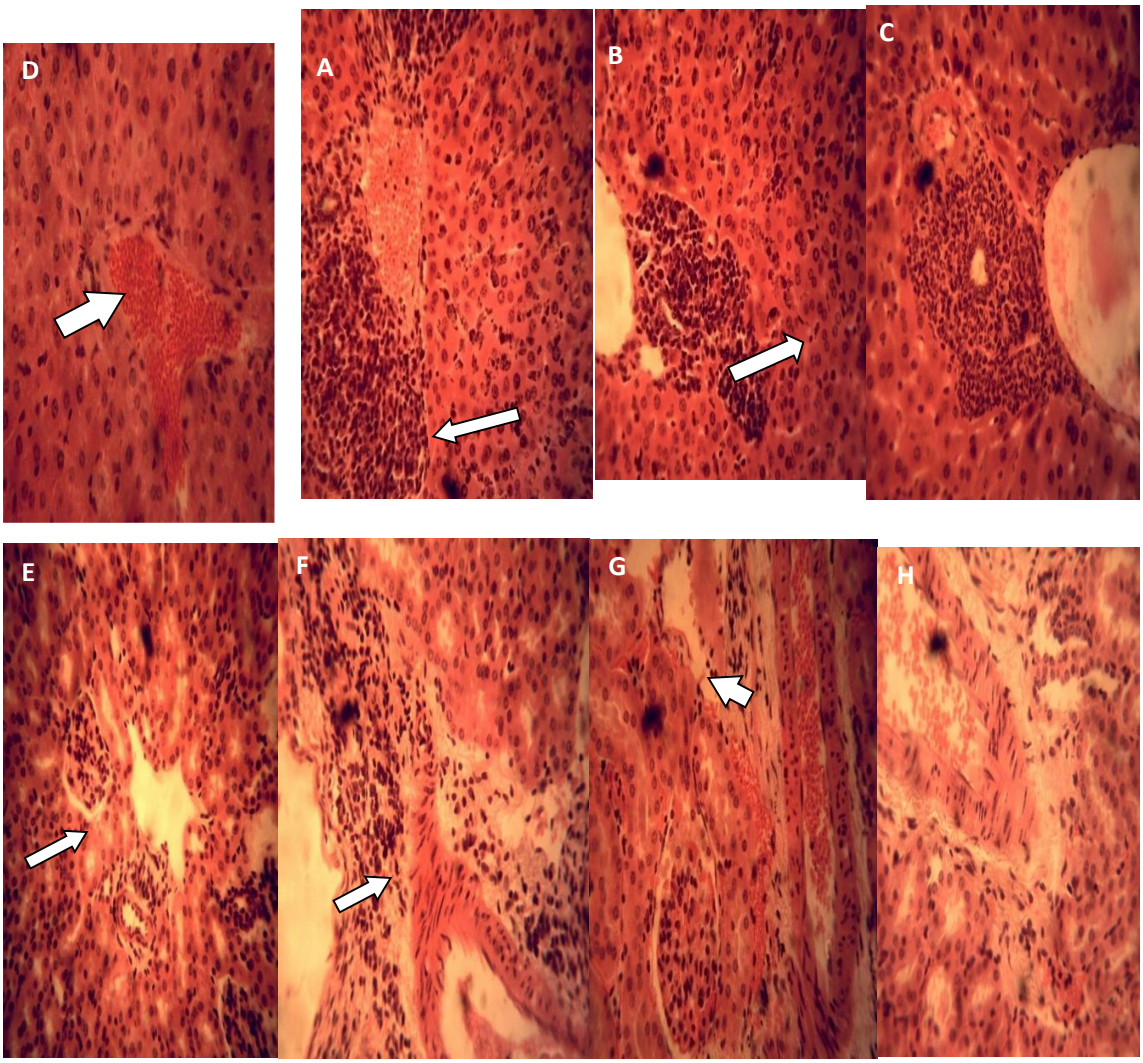

Figure 2: Photomicrograph of the liver showing periportal inflammatory cells infiltration and inflammation round the blood vessels of the renal medulla.

A-D shows Photomicrograph of the liver showing periportal inflammatory cells infiltration

A (administered test drug), B (administered standard drug), and C (infected untreated) while the liver section in D (uninfected untreated) is normal. H and E x400.

E-H shows Photomicrograph of the kidney tissues showing E (administered test drug), F (administered standard drug), and G (infected untreated) while the kidney section in H (uninfected untreated) is normal. H and E x400

Figure 2: Photomicrograph of the liver showing periportal inflammatory cells infiltration and inflammation round the blood vessels of the renal medulla.
A-D shows Photomicrograph of the liver showing periportal inflammatory cells infiltration
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E-H shows Photomicrograph of the kidney tissues showing E (administered test drug), F (administered standard drug), and G (infected untreated) while the kidney section in H (uninfected untreated) is normal. H and E x400

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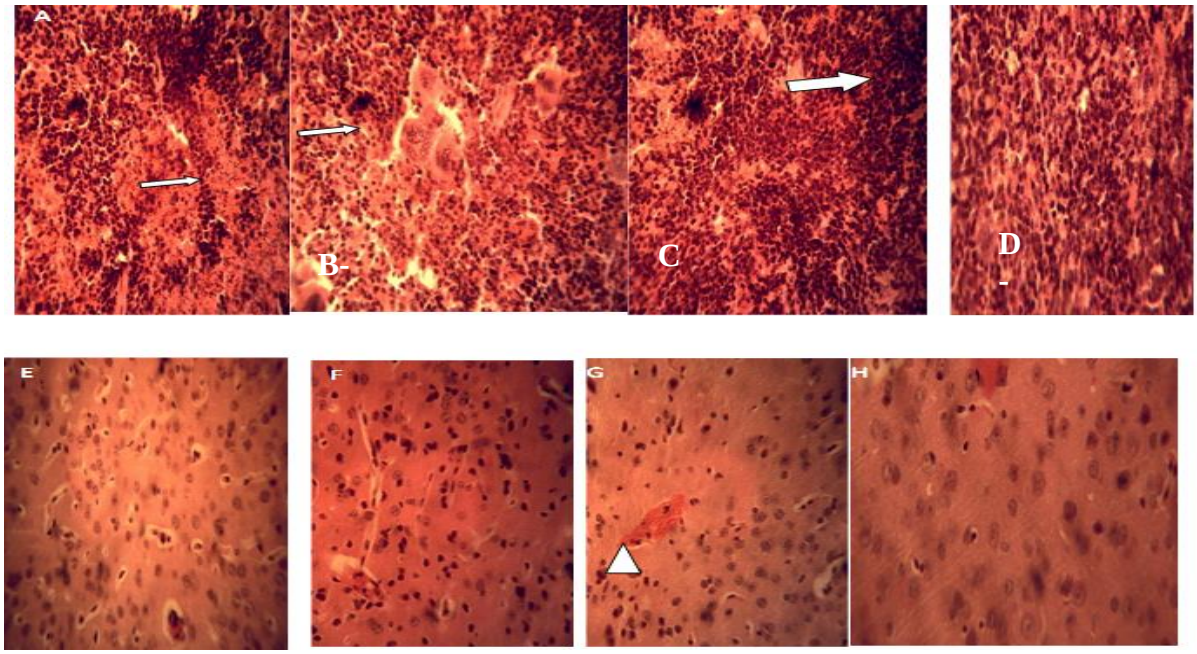

Figure 3: Photomicrographs of the spleen and brain.
Photomicrograph of the spleen (black arrows) groups A (administered test drug), B (administered standard drug), and C (infected untreated) while the spleen section in D (uninfected untreated) is normal. Note also widespread megakaryocytes and other hemopoietic precursor cells within the red pulp (white arrows) in C. H and E x400.
Photomicrograph of the brain (white arrows) groups E (administered test drug), F (administered standard drug), and G (infected untreated) while the brain section in H (uninfected untreated) is normal note also the congestion of cerebral blood vessels (arrowhead) in G. H and E x400

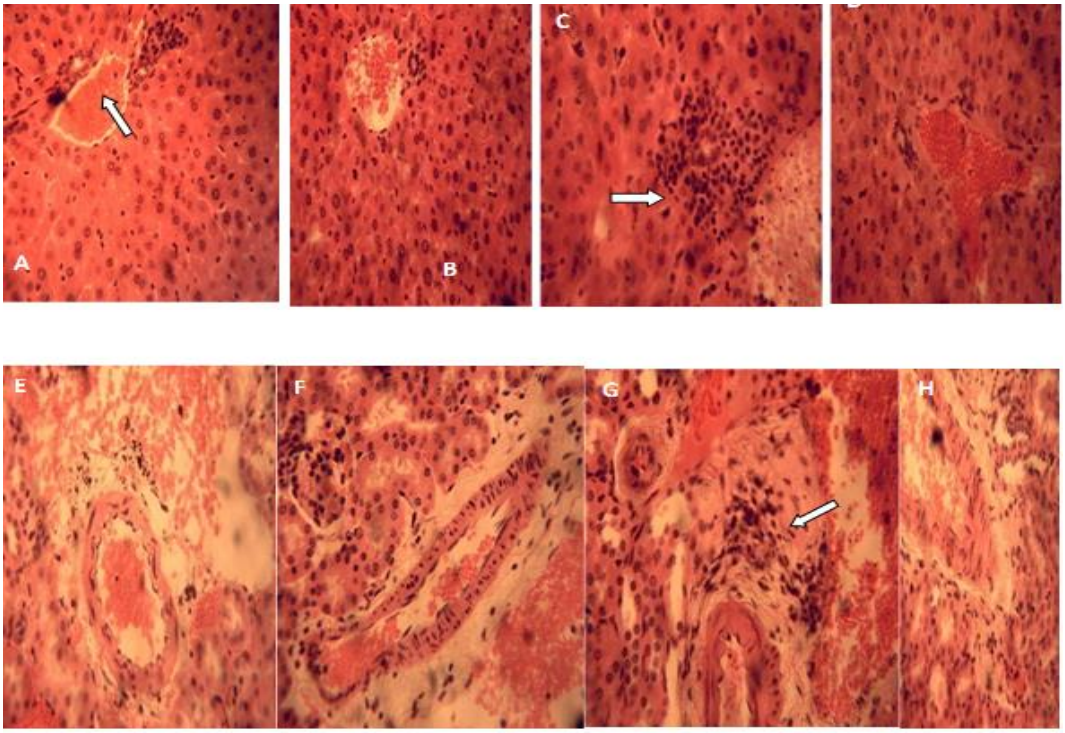

Figure 4: Photomicrographs of liver and the kidney tissues a
Photomicrograph of the liver (white arrows) showing periportal inflammatory cells infiltration in groups A (administered test drug) and C (infected untreated) while the liver section in B (administered standard drug) and D (uninfected untreated) are apparently normal. H and E x400.
Photomicrograph of the kidney tissues showing inflammation around the blood vessels of the renal medulla (white arrows) in groups C (infected untreated) while the kidney section in A (administered test drug), B (administered standard drug) and D (uninfected untreated) are normal. H and E x40

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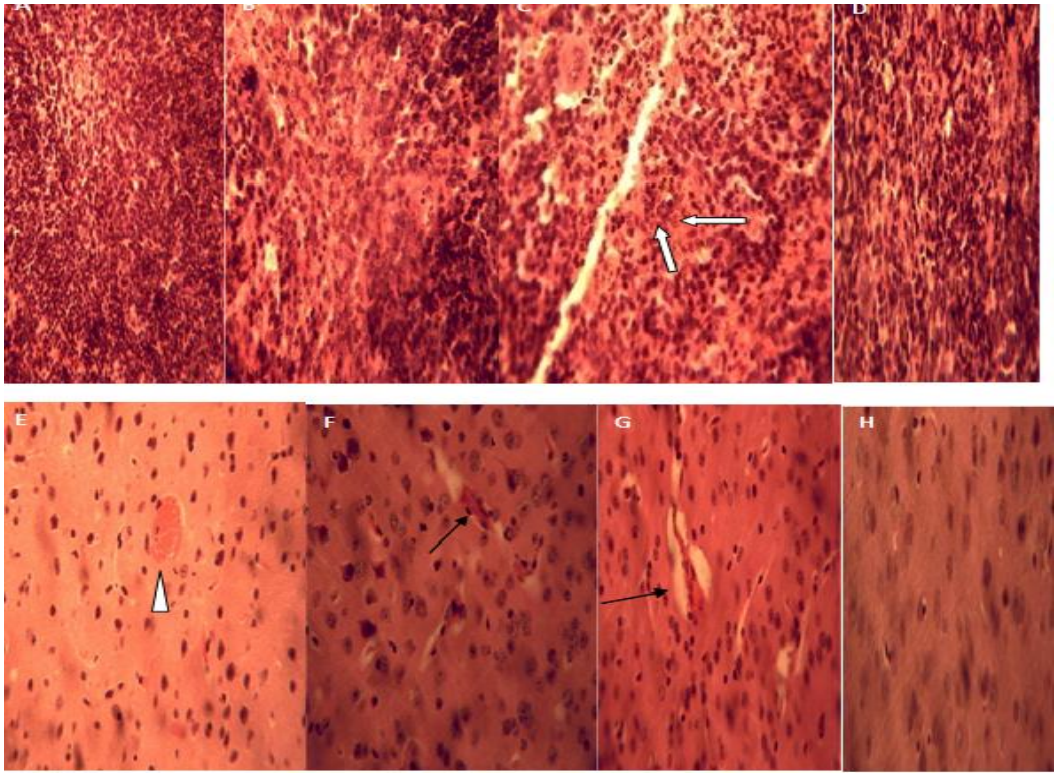

Figure 5: Photomicrograph of the spleen and Photomicrograph of the brain
Photomicrograph of the spleen showing haemozoin pigments seen in the splenic sinusoids within the red pulp (white arrows) in group C (infected untreated) while the kidney section in A (administered test drug), B (administered standard drug) and D (uninfected untreated) are normal. H and E x400. Photomicrograph of the brain showing sequestration of parasitized red blood cells in the micro-vessels (arrows) of the cerebral tissue in groups E (administered test drug), F (administered standard drug), and G (infected untreated) while the brain section in H (uninfected untreated) is normal. Note also the congestion of cerebral blood vessels (arrowhead) in E. H and E x400.

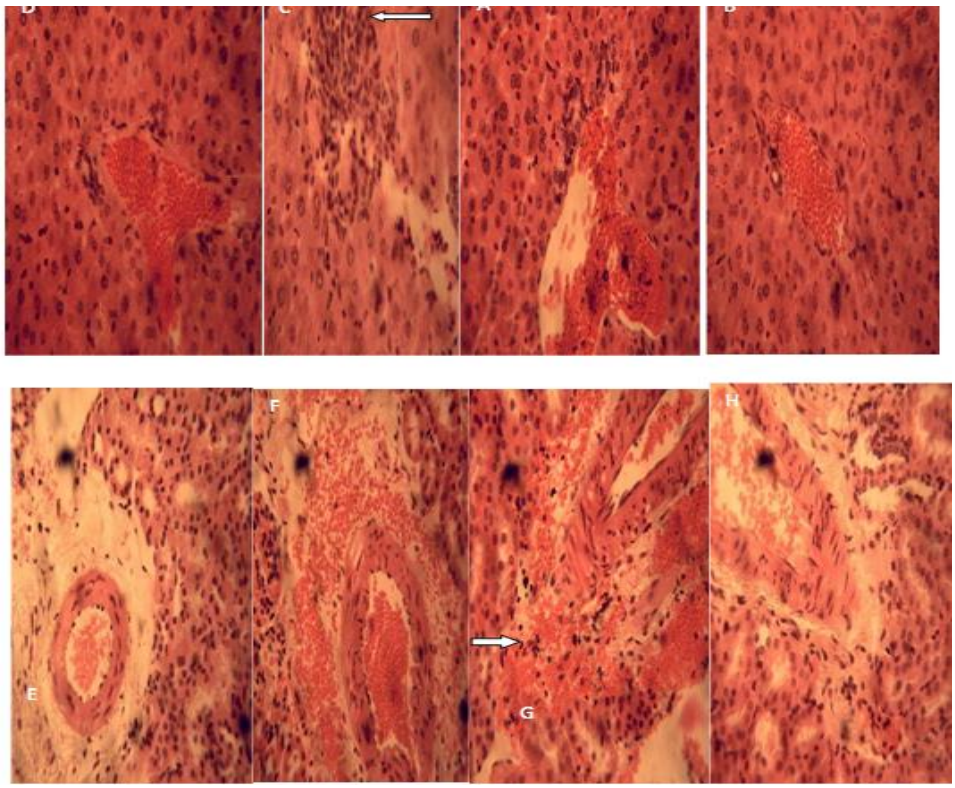

Figure 6: Photomicrograph of the liver and kidney tissues.
Photomicrograph of liver showing periportal inflammatory cells infiltration (white arrows) in groups C (infected untreated) while the liver section in A (administered test drug), B (administered standard drug) and D (uninfected untreated) are apparently normal. H and E x400.

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Photomicrograph of the kidney tissues showing hemorrhages and few inflammatory cells around the blood vessels of the renal medulla (white arrows) in groups G (infected untreated) while the kidney section in E (administered test drug), F (administered standard drug) and H (uninfected untreated) are normal. H and E x400

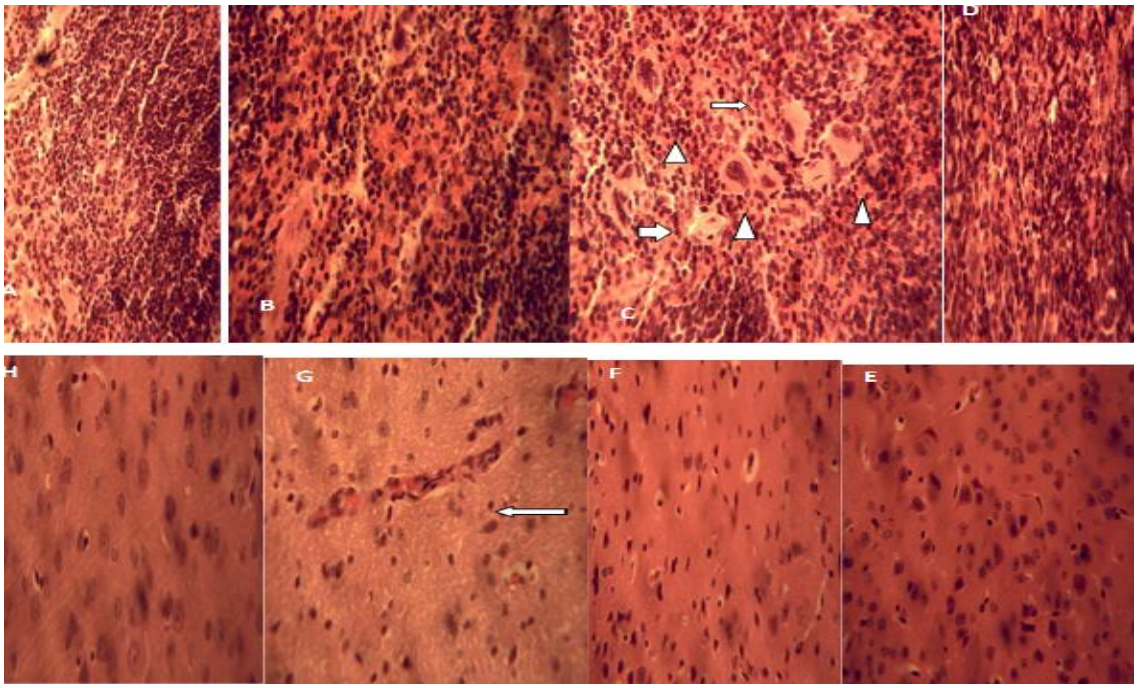

Figure 7: Photomicrograph of the spleen and brain.
Photomicrograph showing haemozoin pigments seen in the splenic sinusoids within the red pulp (white arrows) with many megakaryocytes (arrowhead) in group C (infected untreated) while the kidney section in A (administered test drug), B (administered standard drug) and D (uninfected untreated) are normal. H and E x400.
Photomicrograph of the brain showing sequestration of parasitized red blood cells in the micro-vessels (arrows) of the cerebral tissue in groups G (infected untreated) while the brain section in E (administered test drug), F (administered standard drug) and H (uninfected untreated) are normal. H and E x400.

Table 3: Effect of *Cissus populnea* formulated artemether-lumefantrine on hematological parameters during curative anti-malarial test.

	Naïve	**Control**	**Test**	**Standard**
PCV (%)	41.00 ± 1.47	24.25 ± 1.65	32.50 ± 2.0	30.25 ± 1.44
Hb (g/dl)	10.64 ± 0.88	4.04 ± 0.27	6.14 ± 0.2	6.56 ± 0.16
RBC ($\times$ **10**^{6}µL)	9.39 ± 0.45	7.64 ± 0.79	8.93 ± 0.39	9.26 ± 0.52
WBC (x10^{3}µl)	11.28 ± 0.31	17.90 ± 1.07	15.20 ± 1.92	12.15 ± 1.5
L (%)	58.25 ± 0.85	79.75 ± 0.85	72.75 ± 1.55	74.00 ± 4.02
N (%)	38.00 ± 0.82	13.00 ± 1.47	19.00 ± 0.41	14.74 ± 3.30
M (%)	2.25 ± 0.25	2.75 ± 0.63	3.74 0.25	3.00 ± 0.41
E (%)	1.50 ± 0.25	4.50 ± 1.04	4.50 ± 1.85	7.75 ± 0.63
B (%)	0.00 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.75 ± 0.25

Values are expressed as mean ± SEM, n= 6, L=lymphocyte, N=neutrophil, M=monocyte, E=eosinophil, B=basophil.

Conclusion

Artemether lumefantrine matrixed in *Cissus populnea* provides a basis for new antimalarial therapy since it possessed a prophylactic and suppressive effect against Plasmodium spp. The histopathological analysis of the matrixed drug on different organs showed no observable damage in any of the organs. Therefore, incorporation of Cissus populnea gum in artemether-lumefantrine based tablets possessed promising antimalarial potential with minimal histological and hematological effects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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