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Therapeutic Efficacy of Triple Regimen of Artemether, Lumefantrine and *Hippocratea africana* in the Treatment of *Plasmodium berghei* Infected Mice

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Abstract: Combination therapy is fast replacing monotherapy in the treatment of infectious diseases and *Plasmodium* resistance to artemisinin–based combination therapies (ACTs) is an emerging challenge. Our study aimed to evaluate the therapeutic efficacy of combining Artemether-Lumefantrine with crude root bark extract of *Hippocratea africana*, on mice infected with *Plasmodium berghei*. Forty-five albino mice which weighed 30 - 38g were grouped into five with seven mice in each. The mice were inoculated intraperitoneally with *Plasmodium berghei* and kept for seven days for the parasitaemia to develop. A daily single dose of 200mg/Kg body weight of extract of *H. africana* was administered orally for ten days, while therapeutic dose of Artemether-lumefantrine was administered as daily single dose to the relevant groups on the last six days of treatment. A non-parasitized and parasitize untreated groups served as controls. The weights of the animals were recorded before and after treatment. The animals were sacrificed and blood obtained for determination of percentage parasitaemia and the erythrocytes count of the parasitized mice using standard methods. The results showed the mean body weight and percentage body weight changes of parasitized mice treated with combination of ACT plus *H. africana* not statistically different from those of non-parasitized untreated mice. Parasitized mice treated with ACT plus Extract had a significantly ($p < 0.05$) reduced percentage parasitaemia when compared with those treated with ACT only. Treatment with ACT plus Extract also showed a significant increase in parasite clearance (100%) when compared to mice treated with either ACT only (93.10%) or Extract only (82.15%). We concluded that combining artemether, lumefantrine and *H. africana* root bark extract exhibited a good therapeutic efficacy as demonstrated by body weight recovery, parasite clearance and reversion of clinical signs induced by *Plasmodium berghei* parasitaemia. The triple regimen was more efficacious than ACT alone, and therefore, may be a useful regimen in addressing the emerging problem of resistance of plasmodium species to standards ACTs.

Keywords: Drug-herb Combination, *Hippocratea africana*, Artemisinin Therapy, Drug Resistance

1. Introduction

Treatment of malaria continues to pose a big challenge, both to the sufferers and to all categories of health care providers [1]. Combination therapy, whether as polyherbal, synthetic agents or both, is becoming a commoner practice that is fast replacing monotherapeutic approaches in the management of malaria [2]. Different combinations and formulations of chemotherapeutic agents have been designed

and employed in the treatment of clinical entities, especially in the Sub-Sahara Africa [2] WHO recommends Artemisinin-Based Combination Therapies (ACTs) for the treatment of uncomplicated malaria, which entails combining two or more active ingredients with different mechanisms of action, hence making ACTs the most effective antimalarial medicines available today [3]. The ACT artemether-lumefantrine has been shown to be very effective against malaria parasite through its haemolytic action [4]. Artemether interacts with blood components to generate free radicals which may destroy the malaria parasite, while lumefantrine eliminates residual parasites, reduces parasite burden, and resolves clinical symptoms of the disease [4, 5].

The use of medicinal plants in the treatment of malaria is well reported. Concomitant use of WHO recommended artemisinin-based combination therapy (ACT) with medicinal plants extracts is a very common practice in the southern part of Nigeria.

In recent years, malaria has become more difficult to control and treat because *Plasmodium falciparium* has become resistant to available drugs, and mosquitoes that transmit the disease-causing parasites have also become resistant to insecticides [6]. This has led to intensification of the quest for effective treatment modality, especially in the face of challenges of co-infections and concurrent diseases [2]. The reported widespread resistance of *Plasmodium species* to the commonly available anti-malarial drugs has necessitated countries to review and deploy new anti-malarial drug policies to ensure effective management of the disease (1,6). High costs, limited production of artemisinin derivatives, toxicity and other factors limit the use of ACT [7, 8]. In view of the problems associated with antimalarial drug resistance, new drugs or drug combinations are required for effective treatment of malaria.

Plants have always been considered to be a possible alternative and rich source of new drugs. Most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates [9]. There are reports of a renewed interest in indigenous medicine worldwide in the last decade, arising from the realization of the limitations of orthodox drugs [10]. In many developing countries, data available showed that one-fifth of patients use indigenous herbal remedies to treat malaria [11]. It has been observed by Adebayo and Krettli that some of these herbal remedies are used in combination with other medicines [10].

Hippocratea africana (Wild) Loess Hippocrateaceae, commonly known as African paddle-pod, inhabits the green forests and is a perennial climber with glaburous hairs and is widely distributed in tropical Africa, reproducing from seeds [12]. In West Africa, the plants is known by various names such as *nnoto* (Akan-Asante, Ghana), *onchom* (Mandyak, Guinea-Bissau), *njabo (li)* (Loko, Sierra Leone) *Rdelbi* (Fula-Pulaa, Senegal) and *kesayso* (Manding-Mandinka, The Gambia) [13]. In Nigeria, it is known. by the names *godayi or gwaďayi* (Hausa), *balandibi* (Fulfulde), *ipungwa* (Tiv), *pọnju òwiwí* (Yoruba) and *mba* (or *eba*) enang enang (Ibibio) [12, 14] The root of the plant is used traditionally by the Ibibio of the Niger Delta region of Nigeria in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea [12, 15]. The root is also used traditionally as an antipoison or antidote to treat liver diseases [16]. The plant has been reported to contain significant quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids, tannins and flavonoids as the major constituents [17]. The root of *H. Africana* has been reported to possess *in vivo* antiplasmodial activity with LD_{50} of 2.45 g kg^{-1} [16]. The blood schizontocidal activity and chemosuppressive effect, both in early and established infection in mice, were comparable to chloroquine at 5mg/kg [16)].

The aim of our study was to evaluate the therapeutic efficacy of combining Artemether-Lumefantrine, a WHO advocated artemisinin-based combination therapy (ACT), with crude root bark extract of *H. africana*, on *Plasmodium berghei* infected mice.

2. Materials and Methods

2.1. Collection and Identification of Plant Material

The roots of *Hippocratea africana* (Willd) Loes were harvested from its natural habitat and was identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Akwa Ibom State, Nigeria. A voucher specimen of the roots of *Hippocratea Africana* was deposited in the University of Uyo herbarium with voucher number. The roots of *H. africana* were washed with clean water and the bark scrapped with a sharp knife, sun dried and crushed with a mortar into pellets. The pellets were blended into powdered form using an electric blender. About 500g of the powdered *H. africana* root bark was blended in 1000ml of 80% ethanol. It was left overnight to achieve a good extraction. The mixture was filtered and the filtrate was concentrated *in vacuo* at 40°C to obtain a dry crude extract which could dissolve homogeneously in normal saline and distilled water.

2.2. Inoculation of Experimental Mice with Plasmodium Berghei

Forty-five albino mice which weighed between 30 - 38g were divided into five groups of seven mice each. About 0.1ml of infected blood obtained from donor mouse was mixed with 10ml of normal saline and 0.2ml of the mixture, equivalent to 0.2ml of blood which containing about 1 x 10^7 *Plasmodium berghei* parasitized erythrocytes, was administered intraperitoneally to each animal. The inoculum consisted of 5 x 10^7 *P. berghei* infested erythrocytes per ml of blood from the donor mouse with a 66% parasitaemia. A non-parasitized group served as normal control. The animals were fed *ad libitum* and kept at room temperature of 28.0±2°C for the period which the experiment lasted [17, 18]. The inoculated animals were kept for eight days for the parasite to develop. On the eighth day, thick films were prepared from blood collected through tail puncture of the parasitized

animals to ascertain parasitaemia using the method described by Greenwood and Armstrong [19].

2.3. Preparation of Antimalarial Drugs

Coaterm brand of Artemether-lumefantrine containing 20mg of artemether and 120mg of lumefantrine was dissolved in a calculated amount of 0.9% saline in water, such that 0.08mg and 0.64mg of artemether and lumefantrine respectively were sustained in 0.5ml of solvent, equivalent to therapeutic doses of 3mg/Kg body weight of artemether and 18mg/Kg body weight of lumefantrine.

2.4. Experimental Design and Treatment of Experimental Animals

Based on already established safety dose of the crude root bark extract of *Hippocratea africana* reported [12, 13, 15], 200mg/Kg body weight of the plant extract was administered orally to respective groups of mice as shown on Table 1. The prepared solution of artemether-lumefantrine were administered orally to the respective group of mice (Table 1), depending on the group mean weight of the animals. The untreated control groups were administered normal saline. Pre-treatment and post-treatment weights of the mice were recorded.

Table 1. *Experimental design for treatment of mice with Artemether-lumefantrine and H. africana root bark extract.*

Group	Treatment	Period of Treatment
I	Non parasitized Control (Normal saline)	Day 1 to Day 10
II	Parasitized Untreated (Normal Saline)	Day 1 to Day 10
III	ACT Only	Day 6 to Day10
IV	*H. africana* Only	Day 1 to Day 10
V	*H. africana* + ACT	Day 1 to day 10 and Day 6 to Day 10 respectively

2.5. Clinical Observation of Mice

All Non-parasitized and Parasitized mice were visually monitored for behavioral changes and signs of illness which include lethargy, piloerection, decreased locomotor activity and diarrhea. Any signs of illness observed were quantified using arbitrary scale and recorded as either absent (–), mild (+), moderate (++) or severe (+++), depending on severity. Pre-treatment and post-treatment weight were recorded.

2.6. Collection of Blood Sample and Parasitaemia Measurement

A drop of blood was collected from the mice by tail puncture and transferred onto the edge of a microscope slide (single, 76 × 26 mm thickness) and drawn evenly across a second slide to make a thin blood film and allowed to dry at room temperature. The smear was stained with Leishman stain. Slides were examined under light microscopy (Vickers Instruments) with oil immersion (x1000 magnification). Parasitaemia was counted based on the Leishman positive bodies which represent the parasitized red blood cells. The Leishman positive cells were counted with the aid of a graticule and hand counter. Five fields of approximately 200 cells each were counted and the parasitaemia was calculated as the percentage of the total red blood cells containing Leishman positive bodies.

2.7. Statistical Analysis

Standard computerized statistical tools were used in the analysis of the results obtained. All data were expressed as mean±standard deviation (SD). Analysis of Variance was used to analyze data, while Student's t-test was used for comparison. Any difference in mean was considered significant at $p < 0$.

3. Results

3.1. Clinical Observations of Pretreatment Parasitized Mice

At the end of treatments, clinical examination of the parasitized untreated mice (group II) were severely lethargy with marked piloerection (Table 2). The mice clustered together at the corner of their cages marked decrease in locomotor activity. The tail and pinnae were markedly paler compared to the normal animals (Group I). The remnant of food in the containers were markedly increased compared with the normal non-parasitized mice, with no evidence of passage of watery stool. Mice treated with combination of artemether-lumefantrine and *H. africana* (Group V) showed negative lethargy and piloerection. There was less decrease in locomotor activities in comparison with parasitized untreated group. Clinical features of treatment groups were as shown in Table 2.

3.2. Effects of Treatments on Mean Body Weights

As shown in Table 3, the parasitized untreated mice (Group II) showed a significant ($p < 0.05$) decreases in the mean body weight and percentage reduction in mean body weight when compared with the mean body weight (MBW) and percentage body weight increases recorded for the normal control (Group I)). The mean body weight changes and the corresponding percentage changes in mean weight of treated groups (III, IV and V) were significantly ($p < 0.05$) increased when compared with the parasitized untreated group (Group II). Test groups treated with Artemether-lumefantrine only (Group III) and *H. africana* only (Group IV) showed significant ($p < 0.05$) decreases in mean body weight and percentage body weight changes when compared with the normal control (Group I). Mean body weight change and the percentage change in body weight recorded for test

group treated with combination of ACT and *H. africana* (Group V) was not statistically different when compared with normal control group (Group I)..

3.3. Result of Treatments effect on Parasitaemia, Parasite Clearance and Mortality

The percentage of parasitaemia before commencement of treatments, after the various treatments and the percentage parasite clearance of the infected mice treated with the various treatments are as shown on Table 4.

There was a significant ($p < 0.05$) increase in the parasitaemia of the parasitized untreated mice at the end of the experiment when compared with the value recorded at the beginning of the treatment. Mean parasitaemia levels recorded for test groups III and IV were significantly reduced ($p < .0.05$) when compared to the value obtained for the parasitized untreated group. Test group V did not record any parasitaemia after the treatment.

The parasitized untreated mice recorded significant ($P<0.05$) percentage increase in parasitaemia at the end of experiment in comparison with normal group. The percentage parasitaemia recorded for test groups III, IV and V were significantly ($p < 0.05$) increased when compared with the untreated control group.. Test group V treated with ACT plus *H. africana* extract recorded a significantly ($p < 0.05$) reduced percentage parasitaemia when compared with group III treated with ACT only.

As seen in Table 4, the mean parasites clearance of test groups III, IV and V were significantly ($p < 0.05$) higher when compared to that obtained for parasitized untreated group, though parasite clearance for test group III was significantly higher than that of test group IV.. Test group V showed a significant increase in parasite clearance (100%) when compared to group treated with either ACT only (Group III) or *H. africana* only (Group IV).

Table 2. *Clinical features of Plasmodium berghei infected mice treated with Artemether-lumefantrine and Hippocratea Africana.*

Group	Treatment	Lethargy	Piloerection	Tail/Pinnae pallor	Decreased Locomotor	Diarrhea
I	Normal Control	–	–	–	–	–
II	Parasitized Untreated	+++	+++	+++	+++	–
III	ACT Only	+	+	++	+	–
IV	*H. africana Only*	++	+	+	+	–
V	ACT + *H.africana*	–	–	+	+	–

(–)=Absent, (+)=Mild, (++)=Moderate, (+++)=Severe, ACT=Artemether-Lumefantrine

Table 3. *Mean body weights of Plasmodium berghei infected Mice treated with Artemether-Lumefantrine, Eremomastax speciosa leaf extract and Hippocratea africana root bark extract.*

Group [e]	Treatment	Initial MBW (g)	MBW before treatment (g)	MBW after treatment (g)	MBW change after treatment (g)	% MBW change After treatment
I	Normal Control	31.85±1.85	32.27±1.52	34.40±1.65	2.13±0.41	6.61
II	Parasitized Untreated	31.50±1.34	30.12±2.12	25.65±1.80	-4.47±0.52* [a]	-14.84*
III	ACT Only	30.80±1.55	28.46±1.35	29.53±1.67	1.07±0.74 [a, b]	3.76 [a, b]
IV	*H. africana Only*	30.20±1.10	28.52±1.75	29.08±1.48	0.56±0.05 [a, b]	1.93 [a, b]
V	ACT + *H. africana*	32.45±1.70	29.86±1.09	32.28±1.18	2.46±0.44 [b]	8.10 [b]

e=Mean±Standard Deviation of 6 determinations, a=significantly different when compared with normal control (administered normal saline) at $p < 0.05$, b=significantly different when compared with test group II (parasitized untreated) at $p < 0.05$, ACT=Artemether-Lumefantrine, BW=Body weight, *=Negative change (a decrease).

Table 4. *Parasitaemia, Parasite clearance and mortality in Plasmodium berghei infected Mice treated with Artemether-Lumefantrine, Eremomastax speciosa and Hippocratea Africana.*

Group [e]	Treatment	Before Treatment %	After Treatment %	Percentage Parasitaemia	Parasite Clearance %	% Mortality
I	Normal Control	0.00	0.00	0.00	0.00	0.00
II	Parasitized untreated	23.00±3.12	42.00±5.10	182. 61±2.19	-86.36*	47.82
III	ACT Only	29.50±5.02	2.00±0.05 [a]	6.78±0.10 [a]	93.10 [a]	14.24
IV	*H. africana Only*	21.00±2.80	3.75±0.40 [a]	17.56±1.31 [a,b]	82.15 [a]	28.57
V	ACT + *H. africana*	19.80±5.50	0.00±0.00 [a]	0.00±0.00 [a,b]	100 [a]	0.00

e=Mean±Standard Deviation of 6 determinations, a=significantly different when compared with test group II (Parasitized unt compared with test group III (ACT only) at $p < 0.05$, ACT=Artemether-Lumefantrine, *=percentage increase in parasitaemia

4. Discussions

In Nigeria, several formulations of herbal medicines are used to treat malaria disease. The use of these herbal medicines alongside with the prescription drugs are well reported [20-22]. This is partly due to the challenge of parasite resistance to antimalarials, coupled with the complexity of the disease pathophysiology [23]. Recently resistance of *Plasmodium falciparum* to artemisinins, have documented in five countries, which is reported to manifest in the form of delayed parasite clearance [24].

4.1. Effects of the Treatments on Clinical Signs and Behavior

The pretreatment parasitized mice manifested physical signs consistent with Plasmodium berghei infection. The clumping together of the mice at the corner of the caged, piloerection, reduced locomotor activity and reductions in food intake were clinical manifestation of hypothermia, malaise and anorexia associated with Plasmodium berghei parasitaemia in mice [25]. Paleness of the pinnae and tail of the parasitized mice may be as result of reduced haemoglobin and other haematological imbalances, which correlates with the reports of other scholars [26, 27]. Plasmodium berghei infection in mice is one of the well-employed animal models in malaria research, and this includes analyses on the severe pathology associated with malaria infections [25]. It was reported that Plasmodium berghei parasitaemia is associated with hypothermia and not fever [8]. Reduction in both food intake and body temperature was shown to be associated with an increased turnover in the brain of 5-hydroxytryptamine (5-HT, serotonin), a putative neurotransmitter [28]. Administration of ACT with the plant extract to the parasitized mice resulted in reversal of the clinical signs associated with the infection. Reversion of the clinical signs of parasitaemia by the triple regimen was better than observed for either ACT or the plant extract only. This implies that there nay be a synergic action between the ACT and the plant extract in either suppressing or reversing the impact of the parasites on body organs.

4.2. Impact of Treatments on Body Weights of Mice

Plasmodium berghei infected untreated mice exhibited significant loss in body weights probably due to diminished food intake evidenced by larger quantity of food remnant when compared with that of the normal control. This plasmodium-induced weight loss is consistent with the earlier findings of other scholars [3, 29, 30]. Concomitant treatment of the infected mice ACT and *H. africana* root bark extract resulted in a significant weight recovery. The triple combination therapy yielded a better weight recovery than either ACT or H. africana extract alone. The observed weight recovery was likely due to reversal of the deleterious effects of parasitaemia on the animals (3). Some phytochemicals from the extract probably worked synergistically with the ACT to prevent body weight loss or induce body weight gain by unknown mechanisms.

4.3. Antiplasmodial Efficacy of Treatments

The root bark extract of *H. africana* alone demonstrated antiplasmodial activity that was comparable to artemether-lumefantrine. The root bark extract of *H. africana* was earlier reported to demonstrate significant antiplasmodial activity shown by higher parasite clearance and the dose-dependent suppression of parasitaemia that was greater than that of 5mg/kg body weight chloroquine prophylactic efficacy in P. berghei infected mice [31, 15]. The observed therapeutic efficacy of *H. africana* may be due to the active phytoconstituents especially alkaloids and flavonoids demonstrated in the herb [31].

Antiplasmodial activity of artemether-lumefantrine plus H. africana demonstrated 100% parasite clearance within the period of the experiment. Parasites clearance by administation of ACT and extract of *H. africana* was better either the ACT or plant extract alone. This implies that the ACT-herbs combination may be a better treatment modality for plasmodium infection in comparison to either standard ACT. This combinations form a triple regimen that may address the emerging problem of resistance to standard ACTs. Certain phytoconstituents in the extract may have potentiated the schizonticidal and chemosuppressive effects of the ACT [32]. Methoxylated flavones artemetin and casticin were reported to demonstrate synergistic action with Artemisinin, and flavanoids present in Artemisia annua was considered to probably contribute to the antimalarial action of extracts or herbal teas prepared from this species [32]. Hence, the observed increased antiplasmodial activity due to the added H. africana root bark extract may be due to synergistic action of the phytocontituents with ACT.

5. Conclusions

We concluded from our study that the triple regimen of artemether, lumefantrine and *H. africana* root bark extract exhibited a good therapeutic efficacy in the treatment of *plasmodium berghei* infection in experimental mice, as demonstrated by an excellent antiplasmodial activity, body weight recovery and reversion of clinical signs of the disease induced by *Plasmodium berghei* parasitaemia. From the data obtained from our study we concluded that the drug-herb combination therapy had a better cidal effect on *plasmodium berghei* and was more efficacious than the artemether-lumefantrine alone. The ACT-*E. speciosa* combination therapy, therefore, may be a useful regimen in addressing the emerging problem of resistance of plasmodium species to standards ACTs.

References

[1] Kinfu G, Gebre-Selassie S, Fikrie N. Therapeutic Efficacy of Artemether-Lumefantrine for the Treatment of Uncomplicated Plasmodium falciparum Malaria in Northern Ethiopia, Malaria Research and Treatment, 2012; 2012: 1-6 https://doi.org/10.1155/2012/548710.

[2] Uwah AF, Ndem JI, Akpan SJ. Combining Artesunate-Amodiaquine and Ciprofloxacin Improves Serum Lipid Profile of Mice Exposed to Plasmodium Berghei Berghei, International Journal of Biomedical Research, 2014; 5 (8): 474-476. DOI: 10.7439/ijbr.

[3] Chris-Ozoko CLE, Naiho AO, Gbagbeke KO, Odafiagwuna P. Effect of Malaria Parasitaemia and Antimalarial/Antioxidant Treatment on Body Weight and Some Reproductive Hormones of Male Mice. Anatomy and Physiology: Current Research, 2020; 2 (320): 1-5. Doi: 10.35248/2161-0940.20.10.320.

[4] McKoy MG, Kong-Quee Iii P, Pepple DJ. In vitro effects of co-incubation of blood with artemether/lumefantrine & vitamin C on the viscosity & elasticity of blood. The Indian Journal of Medical Research, 2016; 143 (5): 577-580. DOI: 10.4103/0971-5916.187105.4.

[5] Djimdé A, Lefèvre G. Understanding the pharmacokinetics of Coartem, Malaria Journal. 2009; 8 (Suppl 1): S [4]. https://europepmc.org/article/pmc/pmc4989830.

[6] Bloland PB, Ettling M, Meek S. Combination therapy for malaria in Africa: hype or hope? Bulletin of the World Health Organization, 2000; 8 (12): 1378–1388.

[7] Haynes RK.. Artemisinin and derivatives: the future for malaria treatment? Current Opinion in Infectious Diseases, 2001; 14: 719–726.

[8] Malomo SO, Adebayo JO, Olorunniji FJ. Decrease in activities of cation ATPases and alkaline phosphatate in kidney andliver of artemether treatead rats. Nigerian Society for Experimental Biology Journal, 2001; 1: 175–182.

[9] Ibrahim HA, Imam IA, Bello AM, Umar U, Muhammad S, Abdullahi SA. The Potential of Nigerian Medicinal Plants as Antimalarial Agent: A Review. International Journal of Science and Technology, 2012; 2 (8): 602-208.

[10] Adebayo JO, Krettli AU. Potential antimalarials from Nigerian aplants: a review Journal of Ethnopharmacology, 2011; 133: 289–30.

[11] Willcox M, Bodeker BG. An overview of ethnobotanical studies on plants used for the treatment of malaria. In: Willcox M, Bodeker G, Rasoanaivo P, editors. Traditional Medicinal Plants and Malaria. Boca Raton: CRC Press, 2004; pp. 187–97.

[12] Ekong US, Udoh DI, Alozie MF, Udofa EJ, Akpaide N I. Investigation of the In vivo Antidiarrhoeal Activity of Hippocratea africana Root Extracts by Model Infection and Protection Test in Mice against Bacterial Isolates from Infectious Diarrhoeal and Gastroenteritis Patients in Uyo, Nigeria. Nigerian Journal of Pharmaceutical and Applied Science Research, 2020; 9 (3): 71-82.

[13] Mark, H., Bart, W., Petra, B. & Meg, C. P. (2014). Hippocratea africana (Willd.) Loes. Flora of Zimbabwe: Species Information: Zimbabwe Flora Team Plus, Nyanga. Retrieved September 2, 2016 from: http://www.zimbabweflora.co.zw/species_id=137110.

[14] Uwah AF. Therapeutic Efficacy And Toxicological Effects Of Concomitant Administration Of Artemether-Lumefantrine With Hippocratea Africana Root And Eremomastax Speciosa Leaf Extracts On Normal And Plasmodium Berghei Infected Mice, A Ph. D Thesis, Department of Medical Laboratory Sciences, University of Calabar, 2017; 85-89.

[15] Okokon JE, Ita JE, Udokpoh AE. The in vivo antimalarial activities of Uvariae chamae and Hippocratea africana. Annals Tropical Medical Parasitology, 2006; 100: 585-590.

[16] Okokon JE, Nwafor PA, Charles U, Dar A, Choudhary MI. Antioxidative burst and hepatoprotective effects of ethanol root extract of Hippocratea africana against paracetamol-induced liver injury, 2013; 51 (7): 872-880.

[17] Rajeswari K, Kuma AR, Rathinam SKM. Phytochemical and anti diarrhoeal activity of Hippocratea africana roots Indian Journal of Research in Pharmacy and Biotechnology 2014; 2 (4): 1357.

[18] 1Adekunle AS, Adekunle OC, Egbewale BE. Serum Status of Selected Biochemical Parameters in Malaria: An Animal Model. Biomedical Research, 2007; 18 *(2)*: 109-113.

[19] Greenwood B. M., Armstrong J. R. M. (1991) Comparison of two simple methods for determining malaria parasite density. Transactions Royal Society Tropical Medicine and Hygiene; 85: 186-188.20.

[20] Gardiner P, Graham RE, Legedza AT, Eisenberg DM, Phillips RS. Factors associated with dietary supplement use among prescription medication users. Archive of Internal Medicine, 2006; 166: 1968–1974.

[21] Kennedy J, Wang CC, Wu CH. Patient Disclosure about Herb and Supplement Use among Adults in the US. Evididence Based Complementary Alternative Medicine, 2008; 5: 451–456.

[22] Gharoro EP, Igbafe AA. Pattern of drug use among antenatal patients in Benin City. Nigeria Medical Science Monitor. 2000; 6: 84–87.

[23] Giveon SM, Liberman N, Klang S, Kahan E. Are people who use 'natural drugs' aware of their potentially harmful side effects and reporting to family physician? Patient Educ. Couns. 2004; 53: 5–11 DOI. 10.1016/S0738-3991(03)00241-6.

[24] World Health Organization (WHO). Antimalarial Drug Efficacy and Resistance, World malaria report 2015, WHO press, Geneva, Switzerland 2015; pp 49 -51.

[25] Franke-Fayard B, Fonager J, Braks A, Khan SM, Janse CJ. Sequestration and Tissue Accumulation of Human Malaria Parasites: Can We Learn Anything from Rodent Models of Malaria? PLoS Pathogenesis, 2010; 6 (9): e1001032.

[26] Uwah AF, Ndem JI, Peter AI. Artesunate-Amodiaquine and Ciprofloxacin Combination Improves Biochemical and Histological Markers of Renal Function of Malaria Infected Mice. Indo American Journal of Pharm Research, 2014; 4 (07).

[27] Boon NA, Colledge NR, Walker BR, Hunter JAA Davidson's Principle and Principle of Medicine. 20th edn. Elsevier: Churchill Livingstone. 2006; pp. 445-485.

[28] Dascombe MJ, Sidara JY. The Absence of Fever in Rat Malaria is Associated with Increased Turnover of 5-Hydroxytryptamine in the Brain. In: Milton A. S. (eds) Temperature Regulation. Advances in Pharmacological Sciences. Birkhäuser, Basel 1994; pp 47-52 https://doi.org/10.1007/978-3-0348-8491-4_8.

[29] Uraku AJ. Hepatoprotective effects of Plasmodium berghei infected swiss mice treated with some plant extracts. J Pharm Allied Health Sci. 2016; 6: 1-7.

[30] Shimada M, Hirose Y, Shimizu K. et al. Upper gastrointestinal pathophysiology due to mouse malaria Plasmodium berghei ANKA infection. Trop Med Health 47, 18 (2019). https://doi.org/10.1186/s41182-019-0146-9.

[31] Ndem JI, Eteng MU, Uwah AF. Effect of Hippocratea africana Root Bark Extract on Lipid Profile of Female and Male Albino Wistar Rats. Journal of Scientific Research and Reports, 2015; 3 (19): 2574-2583.

[32] Rayo CCM, Croft SI, Phillipson JD. Natural product as sources of antiprotozoal drugs: Current opinion in anti-infective investigational. Drugs, 2002; 2, 47-62.