IJMPR 2020, 4(6), 147-152

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878

IJMPR

Research Article

SJIF Impact Factor: 5.273

COMBINING *HIPPOCRATEA AFRICANA* ROOT BARK EXTRACT WITH ARTEMETHER-LUMEFANTRINE IMPROVES BIOCHEMICAL INDICES OF RENAL FUNCTION IN *PLASMODIUM BEHGEI* INFECTED MICE

Anthony Fidelis Uwah*[1], Henry Dan Akpan[2] and Blessing Obinaju Effiong[1]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Nigeria.

[2]Department of Biochemistry, Faculty of Science, University of Uyo, Uyo, Nigeria.

Received on: 15/11/2020

Revised on: 05/12/2020

Accepted on: 25/12/2020

***Corresponding Author**

Dr. Anthony Fidelis Uwah

Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Uyo, Nigeria.

ABSTRACT

Many patients have resorted to the use of herbal remedies, either alone or in combination with standard antimalarial drugs, to alleviate their symptoms due to parasitamia. Our study aimed at evaluating the effect of combining Artemether-Lumefantrine, an artemisinin-based combination therapy (ACT), with *Hippocratea africana,* a tropical medical plant, on some biochemical indices of renal function of *Plasmodium berghei* infected mice. Fifty albino mice weighing between 30 - 35g were divided into five groups of ten mice in each. Parasites were got from infected blood of a donor mice. The root extract was obtained using 80% ethanol. Calculated amount of ACT and crude root bark extract of *H, africana* were sustained in normal saline and administered orally to parasitized mice. A non-parasitized and parasitized untreated groups were administered only normal saline. Blood samples and serum were got using standard procedures. Serum electrolytes, creatinine, urea and blood pH were determined. The results showed a significant ($P<0.05$) reduction in serum electrolytes and blood pH, and significant ($P<0.05$) increase in serum urea and creatinine of the parasitized untreated mice when compared to the non-parasitized group. Concomitant treatment of parasitized mice with the *H. africana* extract and ACT significantly ($P<0.05$) raised the values of electrolytes and blood pH, and significantly ($P<0.05$) reduced urea and creatinine to levels comparable to non-parasitized mice. The values of electrolytes, pH, urea and creatinine obtained from ACT-extract combination therapy were significantly ($P<0.05$) different from values for either ACT or *H. africa*na only. We concluded that combining *H. africana* root bark extract with Artemether-Lumefantrine improved biochemical indices and restored renal function in *Plasmodium berghei* infected mice.

KEYWORDS: *Hippocratea africana*, renal function, electrolytes, malaria, herbal remedy.

INTRODUCTION

The impact of malaria parasitaemia on body tissues and organs are enormous. This has posed a huge challenge in the treatment of the disease. Most malaria sufferers still felt unwell after taking standard antimalarial drugs, despite parasite clearance from blood, because of the impact of parasitaemia body organs and systems.[1-5] This is why many patients, especially in the rural areas, seem to have lost faith on antimalarial drugs, hence embarking on use of herbal remedies, either alone or in combination with prescription drugs, to alleviate their symptoms.[6,7]

Derangement in renal function has been reported by several scholars as a serious complication of malaria.[2,8,9,10] Several factors including various chemical mediators, catecholamine release, cytoadherence of parasitized erythrocytes, dehydration, intravascular hemolysis, intravascular coagu-lation, sepsis, hyperbilirubinemia and hyperparasitemia have been implicated in the pathogenesis of renal injuries in malaria. Factor contributing to kidney disease in malaria is hepatic dysfunction, with jaundice and hepatomegaly, through which hyperbilirubinemia can lead to cast nephropathy and acute kidney injury.[8,11,12] Hence, therapy formulation for malaria treatment should necessarily include regimen aimed at relieving parasite-induced renal injuries and derangements in renal function of the sufferer.

World Health Organization (WHO) advocated for the use of artemisinin-based combination therapy (ACT) in treating malaria.[13] Artemether-lumefantrine was the first fixed dose combination of an artemisinin derivative with a second unrelated antimalarial compound and is highly active against all Plasmodium species.[14]

The use of herbal remedies alongside with prescription drugs in treating ailments is popular globally. it was reported that about 40% of the patients in primary care clinics in the United States of America believed that taking prescriptions medications and herbal remedies together was more effective than taking either alone, and that nearly 50% of the herb users in USA concomitantly used drugs.[6] A reported study showed that between 60% and 85% native Africans use herbal medicine usually in combination.[15] It was also reported that pregnant women in Nigeria used both herbal medicines and pharmaceutical drugs, with the highest prevalence of concomitant use among nulliparous mothers.[16] Many herbal products have been used and proven efficacious in the treatment of malaria.[17,18,19,20]

Hippocratea Africana is a perennial scandent shrub, with altitude range up to 1250 m found, in riverine forest and thickets as climbers.[21] The root is also used traditionally as an antipoison or antidote to treat liver diseases.[22] 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.[23,24] The root bark has been reported to possess *in vivo* antiplasmodial activity with LD_{50} of 2.45 g kg^{-1}, with demonstrated blood schizontocidal activity, both in early and established infection at oral doses of 200 to 600 mg/kg/day in mice,[25] The root bark is widely used in the treatment of malaria in the southern part of Nigeria, where it thrives in forests.

The study aimed at evaluating the effect of combining artemether-lumefantrine with ethanolic extract of the root bark of *H. africana* on the some biochemical indices of renal function in mice infected with *Plasmodium berghei.*

MATERIALS AND METHODS

Collection and identification of plant material

The roots of *Hippocratea africana* (Willd) Loes were harvested from the forest, identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Akwa Ibom State, Nigeria, where a voucher specimen were deposited in the herbarium. The roots were washed with clean water and the bark scrapped with a sharp knife, sun dried and crushed with a mortar into pellets. The root bark pellets were blended into powder with an electric blender. The powdered sample was stored in a dried, airtight container at room temperature.

Inoculation of experimental mice with *Plasmodium Berghei*

Fifty albino mice with weight between 30 - 35g were divided into five groups, each having ten mice. A donor mouse was anaesthetized using chloroform and infected blood obtained by thoracotomy and cardiac puncture using sterile syringes and needles. Infected blood volume of 0.1ml was mixed with 10ml of normal saline, from where 0.2ml of the mixture (equivalent to 0.2ml of blood which contained about 1 x 10^7 *Plasmodium berghei* parasitized erythrocytes) was injected into each animal intraperitoneally. The inoculum contained 5 x 10^7 *P. berghei* infested erythrocytes per ml from the donor mouse with a 66% parasitaemia. A non-parasitized group served as normal control. The animals had free access to Guinea grower feed and were kept at room temperature of 28.0 ± 2^0C for the period which the experiment lasted.[26] All the inoculated animals were kept for seven days for the parasite to fully develop. At the end the seventh day, thick films were made from blood by tail puncture of the parasitized mice to ascertain parasitaemia, using the method described by Greewood and Armstrong.[27]

Preparation of antimalarial drugs and plant extract

A popular brand of Artemether-lumefantrine (Coartem) containing 20mg of artemether and 120mg of lumefantrine was dissolved in a calculated amount of normal saline. Weights of 0.08mg and 0.64mg of artemether and lumefantrine respectively were sustained in 0.5ml of solvent, equivalent to 3mg/Kg body weight of artemether and 18mg/Kg body weight of lumefantrine. The extract was stored in the refrigerator at 4 ^{o}C and used in this study. One kilogramme of the powdered *H. africana* was blended in 2000ml of 80% ethanol. It was left overnight to achieve a better extraction. The mixture was passed through a sieve cloth to separate the filtrates from the residue. The filtrate was concentrated in vacuo at 40°C to obtain a dry crude extract. The dried crude extract, which dissolved completely in water to produce homogenous solution, was stored in the refrigerator and for used for the experiment.

Experimental design and treatment of experimental animals

Calculated amount of prepared solution of artemether-lumefantrine were administered orally to the respective group of mice, depending on the group mean weight of the animals. A dosage of 200mg/Kg body weight of *H. afrcana* was administered orally, based on already established safety dose of the plant root bark extract.[25,28,29] The untreated control groups were administered normal saline. The extracts were administered once daily for ten days, Artemether-lumefantrine was administered in two divided doses twice a day on the last three days of treatment. All the experimental animals has free access to normal rat chow and water *ad libitum* throughout the treatment period.

Collection of blood sample and biochemical analysis

At the end of treatments, all the animals were chloroform-anaesthetized. Blood samples for biochemical analyses were collected into well-labeled plain sample bottles for serum separation. The serum was obtained by centrifugation of clotted blood in a MSE table top centrifuge at 4,000 rpm for 10 minutes. The separated sera were stored in a refrigerator at 4°C until required for analysis. Serum electrolytes (sodium,

potassium, chloride and bicarbonate), urea and creatinine were determined using standard laboratory procedures.

RESULTS

The results of serum electrolytes, urea, creatinine and blood pH measured to assess renal function were as shown on Table 1. For electrolytes, the sodium level of 112.20 ± 1.09 mmol/L was recorded for the parasitized untreated group was significantly ($p < 0.05$) decreased in comparison with 143.60 ± 2.15 mmol/L obtained for the non-parasitized untreated group. Test groups III and V treated with ACT only and ACT plus *H. africana* extract respectively recorded significantly ($p < 0.05$) increased sodium levels in comparison with the parasitized untreated group, but were non-significantly ($p > 0.05$) decreased when compared with the non-parasitized control. Test group treated with *H. africana* only showed significant changes in sodium level. Serum potassium level of 2.90 ± 0.39 mmol/L obtained for the parasitized untreated group was significantly ($p < 0.05$) decreased when compared with 5.92 ± 0.23 mmol/L recorded for non-parasitized untreated group. Potassium levels of test groups III, IV and V were significantly ($p < 0.05$) increased in comparison with the parasitized untreated group, but not statistically different when compared with the non-parasitized untreated control group.

For serum chloride, the value of 70.20 ± 1.52 mmol/L recorded for parasitized untreated group was significantly ($p < 0.05$) increased in comparison with 110.40 ± 1.87 mmol/L recorded for non-parasitized control. The values recorded for test groups III and IV were significantly ($p < 0.05$) increased in comparison with the parasitized untreated group, but significantly ($p < 0.05$) decreased when compared with the non-parasitized control group. Serum Chloride of the test group V treated with combination of ACT and extract was significantly ($P < 0.05$) increased in comparison with the parasitized untreated group, but not statistically different from the non-parasitized control group. The serum bicarbonate value of 12.88 ± 0.46 mmol/L obtained for the parasitized untreated group was also significantly ($p < 0.05$) decreased in comparison with the 23.86±0.51 mmol/L recorded for the non-parasitized control. The value recorded for test group III respectively was significantly ($p < 0.05$) higher than that of the parasitized untreated group, but significantly ($p< 0.05$) lower than the non-parasitized control. Test groups IV and V recorded values which were significantly ($p < 0.05$) increased in comparison with the parasitized untreated group, but not statistically different from the non-parasitized control group.

The Serum creatinine level of 62.15 ± 0.52 µmol/L recorded for the parasitized untreated group was significantly ($p < 0.05$) increased when compared with 34.47 ± 0.16 µmol/L obtained for the non-parasitized control group. Values recorded for test group IV was significantly ($p < 0.05$) decreased in comparison with the parasitized untreated group, but significantly increased when compared with the non-parasitized control group. Test groups III and V recorded serum creatinine values significantly ($p > 0.05$) reduced in comparison with the parasitized untreated group, but not statistically different the non-parasitized control group. Serum urea level of 1.47 ± 0.05 mmol/L recorded for parasitized untreated mice group was significantly ($p < 0.05$) increased when compared with 0.71 ± 0.06 mmol/ L obtained for the non-parasitized untreated group. Test groups III and IV recorded urea values that were significantly ($p < 0.05$) lower than the value obtained for the parasitized untreated group, but were significantly ($p < 0.05$) higher when compared with values recorded for non-parasitized control. Urea level of test group V treated with combination of ACT and extract was significantly ($P < 0.05$) increased in comparison with the parasitized untreated group, but not statistically different from the non-parasitized control group.

The blood pH value of 6.88 ± 0.05 for parasitized untreated group was a significantly ($P < 0.05$) decrease when compared to 7.33 ± 0.05 recorded for non-parasitized untreated group. All the treated groups had a significant ($P < 0.05$) increase in blood pH in comparison with parasitized untreated group but statistically nit different from the non-parasitized control.

Table 1: Serum electrolytes, urea, creatinine and blood pH of *Plasmodium berghei* infected mice treated with Artemether-Lumefantrine and *Hippocratea africana* root bark extract.

Group[e]	Treatment	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)	Creatinine (µmol/L)	Urea (mmol/L)	Blood pH
I	Non-parasitized Untreated	143.60 ± 2.15 [b]	5.92±0.23 [b]	110.40±1.87 [b]	23.86±0.51 [b]	34.470.16 [b]	0.71±0.06 [b]	7.33±0.05 [b]
II	Parasitized Untreated	112.20±1.09 [a]	2.90±0.39 [a]	70.20±1.52 [a]	12.88±0.46 [a]	62.15±0.52 [a]	1.47±0.05 [a]	6.88± 0.05[a]
III	ACT Only	142.80±3.11 [b]	4.84±0.33 [b]	101.40±1.13[a,b]	16.50±0.57[a,b]	35.82±0.23 [b]	0.87±0.07[a,b]	7.23±0.03 [b]
IV	*H. africana* Only	131.20±1.89 [a,b]	5.18±0.32 [b]	101.20±1.82[a,b]	20.62±0.25 [b]	48.62±0.33[a,b]	0.88±0.04[a,b]	7.28±0.03 [b]
V	ACT + *H. africana*	138.20 ±2.43 [b]	5.14±0.42 [b]	107.00 ±2.14 [b]	22.80±0.47 [b]	38.89 ±0.65 [b]	0.79±0.06 [b]	7.23±0.03 [b]

e = Mean ± Standard Deviation of 10 determinations,
a = significantly different when compared with non-parasitized untreated group (administered normal saline) at $p < 0.05$, b = significantly different when compared with parasitized untreated group at $p < 0.05$,
ACT = Artemether-lumefantrine

DISCUSSION

Biochemical indices used in accessing the effects of the various treatments on renal function were serum electrolytes, creatinine, urea and blood pH.[30,31] The concentrations of various electrolytes in the body fluids are maintained within a narrow ranges. Certain disease conditions and xenobiotics may lead to a derangement of one or more electrolytes.[32,33] The *Plasmodium berghei* infected untreated mice showed a significant reduction in serum electrolytes and blood pH of the parasitized mice. There was, however, a significant increase in serum urea and creatinine. These *Plasmodium*-induced derangements in electrolytes, serum and creatinine correlated with earlier reports by Uwah *et al*.[34] Adekunle *et al*.[26] and Singh *et al.*[35] Deranged renal functions such as rise in blood urea and creatinine in malaria has been attributed to dehydration and hypovolaemia.[2] Parasitized red cells adhere to healthy red blood cells, platelets and capillary endothelium, leading to formation of rosettes and clumps, which impair microcirculation, and probably contributing to kidney injury, in association with hemodynamic instability.[8] Hence, electrolytes imbalances and Acute kidney injury is a common complication in severe malaria and is associated with increased mortality.[36,37] Acidosis seen in Plasmodium-infected mice in this study was reported as a common complication of severe malaria and identified as the single most important prognostic feature of the disease.[38] The observed acidotic perturbations in pH of the blood of parasitized untreated mice may result from gross derangements in the electrolyte profile, which may have adverse consequences on biochemical processes and mechanisms. In *Plasmodium* parasitaemia, acidosis has been primarily attributed to lactate generated from anaerobic glycolysis as a result of microcirculatory impairment caused by sequestered parasitized red cells.[39,40] Metabolic acidosis is interrelated with kidney injury, since renal dysfunction causes accumulation of acids normally excreted or metabolized by the kidney.[41] Metabolic acidosis in severe malaria is therefore a strong prognostic factor for a fatal outcome.

The results of the present study showed that Concomitant treatment of parasitized mice with Artemether-Lumefantrine and *H. africana* significantly relieved parasite-induced acidotic perturbation in the hydrogen ion concentration (pH). Co-administration of artemether-lumefantrine with the ethanolic extract of *H. africana* effectively restored parasite-induced derangements in serum electrolytes, urea, creatinine and blood pH in the *Plasmodium berghei* infected mice. Parasite-induced hypokalaemia and hypochloraemia were better relieved by the concomitant treatment in comparison with treatment with ACT only. The drug-herb combination therapy also corrected parasite-induced derangements in serum bicarbonate and urea better than ACT only. The exact mechanisms by which the concomitant administrations restore these biochemical indices of renal function are not known. However, synergistic modifications of electrolyte absorption, metabolism and excretion are among the most frequent implicated restorative mechanisms.[42,43] Moreover, synergistic antiplasmodial efficacy as reported by Uwah.[44] may have contributed in restoring hemodynamic activities and improve renal microcirculation. Studies have shown that root bark extraxct of *H. africana* is very safe and is used commonly in treatment of fever, pains and other ailments.[25,29,44]

CONCLUSIONS

From our results we concluded that co-administration of artemether-lumefantrine and root bark extract of *H. africana* was efficacious in relieving plasmodium-induced electrolytes imbalances and acidosis, restoring the tested biochemical indices of renal function in mice. Although the exact mechanism of action is a subject for further research, the action may be due to synergistic modifications of electrolyte absorption, metabolism and excretion, enhanced parasite clearance and reviving of renal microcirculation. The combination therapy may be a good triple regimen for treatment of severe malaria with renal complications.

REFERENCES

1. Centre for Diseases Control and Prevention (CDC) Severe Malaria. *Global Health,* 2010; 13: 439-450.
2. Nand N, Aggarwal H, Sharma, M. & Singh, M. Systemic Manifestations of Malaria, *Journal, Indian Academy of Clinical Medicine,* 2010; 2(3): 190-194.
3. Haldar K, Murphy SC, Milner DA, Taylor TE. Malaria: mechanisms of erythrocytic infection and pathological correlates of severe disease. Annu Rev Pathol, 2007; 2: 217–249.
4. Planche I; Krishna S. Severe malaria: metabolic complications. *Current Molecular Medicine,* 2006; 6(2): 141–153.
5. Trampuz A, Jereb M, Muzlovic I, Prabhu RM. Clinical review: severe malaria, *Critical Care,* 2003; 7*(4)*: 315–323.
6. Kuo GM, Hawley ST, Weiss LT, Balkrishnan R, Volk, RJ. Factors associated with herbal use among urban multiethnic primary care patients: a cross-sectional survey. *BMC Complementary Alternative Medicin.,* 2004; *4*: 18.
7. Dibong S, Mpondo M, Ngoye A, Priso R. Modalities of exploitation of medicinal plants in Doula's region. *American Journal of Food and Nutrition*, 2011; 1(2): 67-73.
8. Silva GB, Pinto JR, Barros EJG, Farias GMN, and Daher EDF. Kidney involvement in malaria: an update *Revista do Instituto de Medicina Tropical de São Paulo,* 2017; 59 – e53.
9. Kanodia K V, Shah P R, Vanikar A V, Kasat P, Gumber M, Trivedi H L. Malaria induced acute renal failure: A single center experience. Saudi J Kidney Dis Transpl, 2010; 21: 1088-91.

10. Mackintosh CL, Beeson JG, Marsh K. Clinical Features and Pathogenesis of Severe Malaria. *Trends Parasitology,* 2004; 20: 597-603.
11. Filho ACA, Lacerda MVG, Okoshi K, Okoshi MP. Malaria and Vascular Endothelium *Arq Bras Cardiol,* 2014; 103(2): 165–169.
12. Barsoum RS. Malarial acute renal failure. *Journal of American of Society of Nephrology,* 2000; 11: 2147-2154.
13. World Health Organization (WHO). Treatment of severe malaria, *Guidelines for the treatment of malaria*, 3rd ed. World Health Organization Press, 2015; 71 – 75.
14. Nosten F, White NJ. Artemisinin-Based Combination Treatment of Falciparum *Malaria American Journal of Tropical Medicine and Hygiene,* 2007; 77(6): 181-192.
15. van Wyk BE, van Rheede MC, Smith GF (1995). Geographical variation in the major compounds of Aloe ferox leaf exudate. *Planta Medica,* 1995; 61(3): 250-253.
16. Gharoro EP, Igbafe AA. Pattern of drug use among antenatal patients in Benin City. *Nigeria Medical Science Monitor*, 2000; *6*: 84–87.
17. Edith A, Folade M, Ogbole O, Okpako L, Akinboye, D. In vivo antimalarial and cytotoxic properties of Annona senegalensis extract. *African Journal of traditional and Complementary and Alernative medicine*, 2006; 3(1): 137- 141.
18. Guede NZ, Lengo M, Frederic G, Bernard B, Philippe G. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *Journal of Ethnopharmacology*, 2005; 98(3): 281-285.
19. Muregi FW, Chhabra SC, Njagi EN, Lang`at-Thoruwa CC, Njue WM, Orago ASS et al. *In vitro* antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *Journal of Ethnopharmacolog,* 2003; 84: 235-239.
20. Ogbonna DN, Sokari TG, Agomuoh AA. Antimalarial Activities of Some Selected Traditional Herbs from South Eastern Nigeria Against *Plasmodium* Species. *Research Journal of Parasitology,* 2008; *3*: 25-31.
21. Hyde MA, Wursten BT, Ballings P, Coates Palgrave M. *Flora of Zimbabwe: Species information: Hippocratea africana var. richardiana.* Retrieved 13 September, from http://www.zimbabweflora. co.zw/speciesdata_/species137110, 2016.
22. 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.
23. 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.
24. Ita BN, Ngochndo R. Fatty acids of Hippocratea africana root: Antioxidant and anti-microbial activity International Journal of Pharmaceutical Sciences and Research, 2014; 5(7): 2732-2737.
25. Okokon JE, Ita JE, Udokpoh AE. The *in vivo* antimalarial activities of *Uvariae chamae* and *Hippocratea africana*. *Annals Tropical Medical Parasitology,* 2006; 100: 585-59.
26. Adekunle AS, Adekunle OC, Egbewale BE. Serum Status of Selected Biochemical Parameters in Malaria: An Animal Model. *Biomedical Research,* 2007; 18(2): 109-113.
27. Greenwood BM, Armstrong JR. Comparison of two simple methods for determining malaria parasite density Trans R Soc Trop Med Hyg, 1991; 85(2): 186-8.
28. 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 Report.,* 2015; 3(19): 2574-2583.
29. Ndem JI, Eteng MU, Akpanabiatu MI, Uwah AF, Otitoju O, Akpanyung. Effect of *Hippoeratea Africana* Root Bark Extract on some Biochemical Indices of Male and Female Albino Wistar Rats. *Journal of Pharmacognosy and Phytotherapy*, 2013; 5(4): 72-76.
30. National Kidney Foundation (NKF) (2017) Tests to Measure Kidney Function, Damage and Detect Abnormalities, https://www.kidney.org. Retrieved, October 2017.
31. Margolis S. Urinary System Test. *The Johns Hopkins Consumer Guide to Medical Tests, Remedy Health Media. Retrieved October, 23, 2017. from: http://www.health communities.com.*
32. Crook MA. The kidneys. In M. A. Crook (Ed.), *Clinical chemistry and metabolic medicine* (8th ed.). Britian: Brookpower, 36-57.
33. 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).
34. Uwah AF, Ndem JI, Akpan EJ. Combining Artesunate-Amodiaquine and Ciprofloxacin Improves Serum Lipid Profile of Mice Exposed to Plasmodium Berghei Berghei. *International Journal of Biomedical Research,* 2014; 5(8): 447- 449.
35. Singh G, Urhekar AD., Singh, R., Maheshwari, U. & Samant, P. Alteration in biochemical parameters in malaria patients *Plasmodium falciparum* vs. *Plasmodium vivax Journal of Microbiology And Antimicrobial Agent,* 2015; 1(1): 13-15.
36. Conroy AL, Hawkes M, Elphinstone RE, Morgan C, Hermann L, Barker KR, et al. Acute kidney injury is common in pediatric severe malaria and is associated with increased mortality. Open Forum Infect Dis., 2016; 3: 046.
37. Boon NA, Colledge NR, Walker BR. Lipid metabolism. *Davidson's Principles and Practice of*

Medicine. 20th edition. USA: Elsevier, 2006; 445-451.

38. Allen SJ, O'Donnell A, Alexander ND, Clegg JB. Severe malaria in children in Papua New Guinea. QJM, 1006; 89: 779-88.
39. Sriboonvorakul N, Ghose A, Hassan MMU, Hossain MA, Faiz MA, Sasithon P S, Chotivanich K, Sukthana Y, Leopold SJ, Plewes K, Day NPJ, White NJ, Tarning J & Dondor AM Acidosis and acute kidney injury in severe malaria, *Malaria Journal,* 2018; 17(1): 1475-2875.
40. Herdman MT, Sriboonvorakul N, Leopold SJ, Douthwaite S, Mohanty S, Hassan MM, et al. The role of previously unmeasured organic acids in the pathogenesis of severe malaria. Crit Care, 2015; 19: 317.
41. Day NP, Phu NH, Mai NT, Chau TT, Loc PP, Chuong LV, et al. The pathophysiologic and prognostic significance of acidosis in severe adult malaria. Critical Care Medicine, 2000; 28: 1833–40.
42. Ma R, Kong A, Chan N, Tong P, Chan J. Drug-induced endocrine and metabolic disorders. *Drug Safety*, 2007; *30*: 215-245.
43. Perazella, M. Drug-Induced Hyperkalemia: old culprits and new offenders. *American Journal of Medicine*. 2000; 109(4): 307-314.
44. Uwah AF. Therapeutic efficacy and toxicological effects of co-administration of artemether-lumefantrine, *Hippocratea africana* root and *Eremomastax speciosa* leaf extracts on non-parasitized and *plasmodium berghei* infected mice. A Doctorate thesis carried out in the Chemical Pathology Unit Department of Medical Laboratory Sciences College of Medical Sciences University of Calabar, Nigeria, 2018; 150-185.