EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 9
106-111
Year: 2021

HEPATORENAL STATUS OF MALARIA INFECTED MICE TREATED WITH *HIPPOCRATEA AFRICANA* ROOT EXTRACT AND ARTEMETHER-LUMEFANTRINE

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Article Received on 28/06/2021 **Article Revised on 18/07/2021** **Article Accepted on 08/08/2021**

ABSTRACT

Objective: To assess the hepatic and renal status of *Plasmodium berghei* infected mice treated separately and concomitantly with *Hippocratea africana* root extract and Artemether-lumefantrine (ACT). **Methods**: Thirty-five adult mice (30 - 35g) divided into 5 groups of 6 mice each were inoculated with 4.8 x 10^7 *P. berghei* infected red blood cells per ml from a donor mouse with 65% parasitaemia. Dosages of 3mg/Kg body weight of artemether, 18mg/Kg body weight of lumefantrine, and 200mg/Kg body weight of *H. africana* were administered orally for 6 days. A non-parasitized group served as normal control. **Results:** Treatment with ACT and *H. africana* extract separately reduced the serum liver enzymes of parasitized animals significantly ($P < 0.05$) when compared with the parasitized untreated group. The values were, however, significantly ($P < 0.05$) higher than that of the normal control. Concurrent administration of ACT and *H. africana* significantly ($P < 0.05$) lowered the serum liver enzymes of the parasitized animals to levels that were not significantly ($P > 0.05$) different from the non-parasitized group. Total proteins, albumin and globulin fractions of mice parasitized treated separately with ACT and *H. africana* were significantly ($P < 0.05$) increased when compared with the parasitized untreated group. Treatment with ACT and *H. africana* separately significantly ($P < 0.05$) raised the total serum proteins and all the protein fractions of the parasitized mice, while ACT-extract concomitant treatment showed slight just increase ($P > 0.05$) in the serum protein of the mice. . All the treatment groups showed significant reduction in the total bilirubin and fractions. There were significant ($P < 0.05$) increases in electrolytes in all the treatment groups, when compared with parasitized untreated groups Concomitantly treated with ACT and H. africana lowered electrolytes levels in all groups. **Conclusion:** The concurrent administrations of artemether-lumefantrine (ACT) with root bark extract of *H. africana* did not show any evidence of toxicity to the hepatocytes, and did not derange the biosynthetic function of the liver. The concomitant treatment rather restored parasite-induced hepatic function disturbances observed in the untreated parasitized mice.

KEYWORDS: *Hippocrearea africana*, Artemether lumefantrine, malaria, Plasmodium.

INTRODUCTION

Treatment of malaria has been very challenging because of its complex pathophysiology and multiple organs affectation.[1] There is almost no organ in the body that has not been shown to be adversely impacted upon by *Plasmodium species* parasitaemia. Multiple organ failure is a major mode of death in malaria infection.[2] Hepatic and renal dysfunction has been widely reported as common complications of the disease, which is usually associated with poor prognosis, severe anemia, hypotension, and bleeding manifestations, electrolyte imbalance and increased mortality.[2,3] Researchers have shown that hepatic involvement in malaria carries a bad prognosis and majority of deaths in malaria have hepatic failure in combination with other organ dysfunction.[4-6] The incidence of acute renal failure in malaria all over world ranges from 0.57% to 60%, which has been attributed to glomerulonephritis and other haematological, homeostatic, hormonal and immunologic disorders in the kidney.[7-9] Treatment of malarial disease therefore goes beyond parasite clearance. Most patients do not admit that they are well despite parasites elimination from blood, due to these impacts of parasitaemia on the organs.[1] This is the reason many patients, especially in the rural areas, seem to have no faith on the prescription antimalarials drugs, hence embarking on use of herbal remedies, either alone or in combination with prescription drugs,[10] to alleviate their tiredness, anorexia, weakness, diarrhea, jaundice, headache, dizziness and insomnia resulting from organs affectation by the disease.[11,12] Although information on the safety of herbal products is scarce, their use as

alternative and/or complementary medicine is globally popular.[13] The increasing consumptions of medicinal herbs and herbal products globally, cut across social and racial classes in both developing and developed countries.[14-16] World Health Organization (WHO) reported that, about 70% of the world population currently uses medicinal herbs as complementary or alternative medicine.[17] In the USA, nearly 50% of the herb users concomitantly used prescription drugs.[18] Between 60% and 85% native Africans use herbal medicine usually in combination.[19] In Nigeria, researchers reported that pregnant women used both traditional herbal medicine and pharmaceutical drugs with the highest prevalence of concomitant use among nulliparous mothers.[20]

Hippocratea Africana (Wild) Loess Hippocrateaceae, commonly known as African paddle-pod, is a perennial climber with glabrous hairs, which inhabit the green forests of the Niger Delta and is widely distributed in tropical Africa.[11,21] (Hyde *et al*, 2016; Uwah *et al*, 2021). The root is also used traditionally as an antipoison or and in treating liver diseases 22(Okokon *et al*, 2013). The Ibibios of the Niger Delta region of Nigeria use the root of the plant in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea 23(Okokon *et al*, 2006). Significant quantities of phytochemicals such as alkaloids, cardiac glycosides and flavonoids, tannins and flavonoids have been demonstrated as its major constituents 24(Rajeswari *et al*, 2014). The root of *H. africana* was reported to possess in vivo antiplasmodial activity with LD_{50} of 2.45 g kg^{-1}(Okokon *et al*, 2013).[22]

Artemether-lumefantrine was the first fixed dose combination of an artemisinin derivative with a second unrelated antimalarial compound[25] (Nosten and White, 2007). Artemether is an artemisinin in which the lactone has been converted to the corresponding lactol methyl ether 26(NCBI, 2021). It is a sesquiterpenoid, a cyclic acetal, an organic peroxide, an artemisinin derivative and a semisynthetic derivative[26] (NCBI, 2021). It is used in combination with lumefantrine as an antimalarial for the treatment of multi-drug resistant strains of falciparum malaria. Lumefantrine is an aryl amino-alcohol that is active against all the human malaria parasites, including multi-drug–resistant P. falciparum. It was viewed that the excellent adverse effects profile and recent price reductions are make artemether-lumefantrine an increasingly attractive treatment option[27] (White *et al*., 1999). The present study investigated the effects artemether-lumefantrine and *Hippocratea africana* concurrent administration on indices of hepatorenal function of plasmodium berghei infected mice. Plasmodium berghei has been widely used in studying malaria models in mice.

MATERIALS AND METHODS

Collection and identification of plant materia

Hippocratea africana (Willd) Loes were harvested from its natural habitat and was identified and authenticated by a taxonomist in the Department of Botany and ecological study, University of Uyo, Nigeria. A voucher specimen of the roots of *H. africana* was deposited in the herbarium of the institution with voucher number. The roots were washed with clean water and the bark scrapped with a sharp knife, sun dried and crushed into pellets using a mortar and pestle. The pellets were blended with an electric blender into powder. About 500g of the powdered *H. africana* root bark was blended in 1000ml of 80% ethanol and left overnight for extraction to occur 11(Uwah *et al*, 2021). The mixture was filtered and the filtrate was concentrated *in vacuo* at 40^{o}C to obtain a dry crude extract which could dissolve homogeneously in normal saline.

Mice inoculation with *Plasmodium berghei*

Thirty-five adult mice (30 - 35g) were divided into five groups of six mice each. Infected blood was obtained by cardiac puncture from a chloroform anaesthetized donor mouse, using sterile syringes and needles. About 0.1ml of the infected blood 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 red blood cells, was administered to each mouse intraperitoneally. The inoculum contained 4.8 x 10^7 *P. berghei* infested red blood cells per ml from the donor the mouse with a 65% parasitaemia. A non-parasitized and parasitized untreated group served as normal controls. The mice had free access to food and water and were kept at room temperature of 28.0 ± 2.0^{0}C for the period which the experiment lasted[28] (Adekunle *et al*., 2007; Uwah *et al*, 2020). All the inoculated animals were kept for 6 days for parasitaemia to develop. At the end the sixth day, thick films were made from blood collected through tail puncture of the inoculated mice to ascertain parasitaemia, using the method described by Greewood and Armstrong (1991).[29]

Preparation of antimalarial drugs and plant extract

Artemether-lumefantrine (Coartem brand) containing 20mg of artemether and 120mg of lumefantrine was dissolved in a calculated amount of normal saline (0.9% saline in water). Calculated amount of artemether-lumefantrine, based on therapeutic dosages of 3mg/Kg body weight of artemether and 18mg/Kg body weight of lumefantrine, was sustained 0.5ml of normal saline for each mouse.

Experimental Design and Treatment of Experimental Animals

The prepared solution of artemether-lumefantrine was administered orally to the respective group of mice, depending on the group mean weight. A dosage of 200mg/Kg body weight of *H. africana* was administered orally which was based on already established safety

dose of the root bark extract[22,30,31] (Ekong *et al*, 2020; Mark *et al*, 2014; Okokon *et al*, 2013). The untreated control groups were administered normal saline. The extracts were administered once daily for six day. Artemether-lumefantrine was also administered concurrently for six days. All the experimental animals had 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, the mice were chloroform anaesthetized and blood sample collected by thoracotomy and cardiac puncture, using sterile syringes and needles. Blood samples collected in plain bottles from where serum was extracted by centrifugation. Liver function was assessed using liver enzymes activities, serum proteins and bilirubin, and blood glucose levels. Electrolytes, urea, creatinine and blood pH levels were use in assessing the renal function of the mice. All biochemical parameters were determined using standard methods.

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 $P < 0.05$.

RESULTS

As shown on Table 1, all the serum liver enzymes assessed, namely AST, ALT and ALP and AST/ALT of the parasitized untreated mice were significantly (P<0.05) raised when compared with the non-parasitized mice. Treatment with ACT and *H. africana e*xtract separately reduced the serum liver enzymes of parasitized animals significantly (P < 0.05) when compared with the parasitized untreated group. The values were, however, significantly (P < 0.05) higher than that of the normal control. Concurrent administration of ACT with extract of *H. africana* significantly (P < 0.05) reduced the serum liver enzymes of the parasitized animals to levels that were not significantly (P > 0.05) different from the non-parasitized group.

As shown on Table 2, there was a significant ($P < 0.05$) reduction in serum total proteins, albumin and globulin fractions of the parasitized untreated mice when compared with the non-parasitized mice. The parasitized untreated mice had significantly ($P < 0.05$) raised total and direct serum bilirubin levels in comparison to the non-parasitized normal control mice. Total proteins, albumin and globulin fractions of mice treated separately with ACT and *H. africana* extract were significantly ($P < 0.05$) increased when compared with the parasitized untreated group.

Treatment with ACT and *H. africana* separately significantly ($P < 0.05$) raised the total serum proteins and all the protein fractions of the parasitized mice, when compared with untreated group. There was slight increase ($P > 0.05$) in the serum protein of mice concurrently treated with extract of *H. africana* and ACT. The total proteins and protein fractions of the mice treated concurrently were not significantly different when compared non-parasitized control. The total bilirubin and bilirubin fractions of parasitized mice untreated significantly ($P < 0.05$) increased when compared to non-parasitized mice. All the treatment groups showed significant reduction in the total bilirubin and fractions.

As shown on Table 3, there were significant increases in the assessed electrolytes and blood pH on parasitized untreated animals when compared with the non-parasitized group, while Serum urea and creatinine significantly ($P < 0.05$) increased. Serum electrolytes significantly increased in all the treatment groups when compared with the untreated parasitized group. There were significant changes between treatment groups and between the treatment groups and non-parasitized group. The same pattern of results were obtained for urea and creatinine.

Table 1: Serum Liver Enzymes and of *Plasmodium berghei* infected Mice treated with Artemether-Lumefantrine and *Hippocratea africana* root bark extract.

Group [e]	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	AST/ALT
I	Non-parasitized Control	86.80 ± 8.87	27.20 ± 2.39	82.40 ± 7.16	3.19 ± 0.24
II	Parasitized Untreated	229.20 ± 23.32 [a]	56.20 ± 5.93 [a].	151.80 ± 6.02 [a]	4.08 ± 0.24 [a]
III	ACT Only	103.60 ± 4.84 [a,b]	40.60 ± 3.65 [a]	100.20 ± 4.38 [a]	2.20 ± 0.37 [a,b]
IV	*H. africana* Only	107.20 ± 6.80 [a,b]	35. 20 ± 4.44 [b]	94.40 ± 3.51 [b]	3.31 ± 0.46
V	ACT + *H. africana*	92 .20 ± 2.77 [a,b]	31.80 ± 6.2 [b]	89.27 ± 3.96 [b]	3.09 ± 0.27

e = Mean ± Standard Deviation of 6 determinations, a = significantly different compared with normal control (administered normal saline) at P < 0.05, b = significantly different when compared with test group II (parasitized untreated) at P < 0.0. 5, ACT = Artemether-Lumefantrine.

Serum proteins and Serum bilirubin of *Plasmodium berghei* infected mice treated with artemether-lumefantrine and *Hippocratea africana* root bark extract.

Group [e]	Treatment	Total protein g/dl	Albumin g/dl	Globulin g/dl	Albumin/ Globulin	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
I	Non-parasitized Control	5.36±0.32	3.88±0.79	1.70±0.29	2.28±0.38	0.08 ± 0.02	0.04 ± 0-01
II	Parasitized Untreated	3.18±0.55[a]	2.66±0.23 [a]	0.82±0.31 [a]	3.24±0.74	0.20 ± 0.02 [a]	0.34 ± 0.03 [a]
III	ACT Only	5.10±0.10 [b]	3.18±0.56 [b]	1.38±0.48 [b]	2.39±0.59	0.11 ± 0.04	0.05 ± 0.02 [b]
IV	*H. africana* Only	4.99±0.51 [b]	3.42±0.40	1.50±0.37 [b]	2.86±1.08 [b]	0.14 ± 0.02 [a, b]	0.62 ± 0.07[a, b]
V	ACT + *H. africana*	5.74±0.27 [b]	3.92±0.62 [b]	1.60±0.27 [b]	2.44 ±0.27	0.14 ± 0.04 [a]	0.47 ± 0.04 [a, 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.

Serum electrolytes, urea, creatinine and glucose of *Plasmodium berghei* infected mice treated with artemether-lumefantrine, *Eremomastax speciosa* leaf extract 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 $_{p}$H
I	Non-parasitized Control	143.60 ± 2.15	5.92 ± 0.23	110.40 ± 1.87	23.86 ± 0.51	34.470.16	0.71±0.06	7.33±0.05
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 [b]	18.50±0.57 [b]	35.82±0.23 [b]	0.87±0.07 [b]	7.23±0.03 [b]
IV	*H. africana* Only	131.20±1.89 [b]	5.18±0.32 [b]	101.20±1.82 [b]	20.62±0.25 [b]	48.62±0.33	0.88±0.04 [b]	7.28±0.03 [b]
V	ACT + *H. africana*	134.20 ±2.43 [b]	4.94±0.42 [b]	103.00 ±2.14 [b]	25.80±0.47 [b]	38.89 ±0.65 [b]	0.87±0.06 [b]	7.23±0.03 [b]

e = Mean ± Standard Deviation of 6 determinations, a = significantly different when compared with normal control group (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.

DISCUSSIONS

Malaria is a parasitic infection of global public health importance caused by *Plasmodium* species. It involves a complex pathophysiology and multiple organs affectation. The present study evaluated the hepatorenal status of *Plasmodium* berghei concurrently treated artemether-lumefantrine and *Hippocratea africana*. The effect of the various treatments on the biosynthetic function of the liver was assessed by measurement of serum proteins. Serum bilirubin was used to assess the transport and metabolic function, while serum liver enzymes were used in assessing any injury to hepatocytes[32,33] (Thapa and Walia, 2007; Krans and Cafasso, 2015). It has been established that metabolism of chemicals occur largely in the liver, which is thought to accounts for the organ's susceptibility to metabolism-dependent, drug-induced injury[34] (Kaplowitz, 2004) Serum electrolytes, creatinine, urea and blood pH were used to determine effects of the various treatments on renal functions were assessed using[35,36] (NKF, 2017; Margolis, 2015).

Increase in serum liver enzymes. The extent of rise collaborated with WHO's report that in malaria infection, aminotransferase liver enzymes may be elevated up to 10-fold[37] (Cheaveau *et al*, 2019).[38] Rochar *et al* (2006) reported that Malarial hepatitis is characterized by hyperbilirubinemia and elevated transaminase enzymes of up to more than 3 times the normal levels. Generally, the impact of malaria on liver enzymes has been unclear.[37] (Cheaveau *et al*, 2019). Drug development trials have shown conflicting results[39-41] (Phylo *et al*, 2016; McCarthy *et al*, 2011; Sulyok *et al*, 2017). Adherence of parasitized red blood cells to the liver capillaries endothelium leads to the blockade of intrahepatic channels, causing changes in blood flow resulting in ischaemia and necrosis of the liver cells and release of enzymes into blood[37,42] (Cheaveau *et al*, 2019; Baheti *et al*, 2003).

Treatments of parasitized mice with artemether-lumefantrine (ACT) and with extract of *H. africana* significantly reduced the liver enzymes levels, but were still significantly higher than that of the normal animal. Upon concurrent administration of ACT and *H. africana* extract, the liver enzymes returned to values not significantly different from the normal animals. Combination of ACT and extract further reduced the liver enzymes to within normal levels. These imply that the extract alongside with the ACT synergistically protected the hepatocytes against injuries by the Plasmodium berghei infection. Hepatoprotective effect of plant extracts and herbal products have also been well documented[43,44] (Thabrew and Hughes, 1996; Vandenbergh, et al 1995).

Plasmodium berghei infected mice showed significant reduction in serum total proteins, albumin and globulin, with a significant increase in albumin to globulin ratio. These trends collaborated earlier reports by various scholars[28,45,46] (Adekunle *et al*, 2007; Chidoka and Tochukwu, 201; Uwah *et al*, 2014). It implies that the

parasites may have interfered with protein uptake and biosynthesis function of the liver. Moreover, a study had shown that the parasite has the ability to take up serum albumin which is important for intraerythrocytic growth and differentiation[47] (Tahir, *et al*, 2003). The increases in total and direct bilirubin may have resulted from parasite-induced perturbation in the transport and metabolic function of the hepatocytes. Concurrent administration of ACT with the *H. africana* extract significantly raised serum proteins. These imply that the concomitant administration probably restored amino acid uptake and protein biosynthesis function of the liver hitherto disturbed by parasites infection.

Untreated Plasmodium berghei infected mice showed a significant reduction in serum electrolytes assessed, namely, sodium, potassium, chloride and bicarbonate. There was a significant reduction also in blood pH of the parasitized mice, while serum urea and creatinine significantly increased. These derangements in renal functions indices in plasmodium parasitaemia correlated with earlier reports by other scholars[2,28,46,48] (Nagaraj *et al*, 2018; Uwah *et al* 2014; Adekunle *et al*, 2007; Singh *et al*, 2015). Rise in blood urea and creatinine in malaria have been attributed to dehydration and hypovolaemia associated with the disease[49] (Nand *et al*, 2001). Some scholars also opined that certain disease conditions and xenobiotics may lead to a derangement of one or more electrolytes[50] (Crook, 2007). Concurrent administration of artemether-lumefantrine with the plants extract effectively restored parasite-induced derangements in serum electrolytes, urea, creatinine and blood pH in the Plasmodium berghei infected mice. This infers that combined administration of the ACT and the plant extracts was efficacious in relieving plasmodium-induced electrolytes imbalances. The restorative effect of the combination treatment was better than that of either the ACT or the plant extract alone. The exact mechanisms by which the concomitant administration restored these biochemical indices of renal functions are not known. However, synergistic modifications of electrolyte absorption, metabolism and excretion are among the most frequent implicated restorative mechanisms[51,52] (Ma, 2007; Perazella, 2000).

CONCLUSIONS

We concluded from the study that the concurrent administrations of artemether-lumefantrine (ACT) with root bark extract of *H. africana* did not show any evidence of toxicity to the hepatocytes, and did not derange the biosynthetic function of the liver. The concomitant treatment rather restored parasite-induced hepatic function disturbances observed in the untreated parasitized mice. The concurrent ACT – herb extract treatment was also efficacious in relieving plasmodium-induced electrolytes imbalances, uraemia and lactic acidosis, hence restoring renal function of parasitized animals by unknown mechanism.

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