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 parasite 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 falciparum 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 artesiminin 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 Kretti that some of these herbal remedies are used in combination with other medicines [10].

Hippocratea africana (Wild) 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 scraped 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. Materials and Methods

2.1. Collection and Identification of Plant Material

The roots of Hippocratea africana (Wild) 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 scraped 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 infected 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

Coartem 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

| 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 Day 10                  |
| 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 means±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.

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 * | 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      | 32.71±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              | -4.7±0.52                     | -14.8*                       |
| III     | ACT Only             | 30.80±1.55      | 28.46±1.35               | 29.53±1.67              | 1.07±0.74                     | 3.76±b                       |
| IV      | *H. africana* Only   | 30.20±1.10      | 28.52±1.75               | 29.08±1.48              | 0.56±0.05                     | 1.93±b                       |
| V       | ACT + *H. africana*  | 32.45±1.70      | 29.86±1.09               | 32.28±1.18              | 2.46±0.44                     | 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 * | 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       | 182.6±2.19              | -86.36*             | 47.82       |
| III     | ACT Only                 | 29.50±5.02         | 2.00±0.05        | 6.78±0.10               | 93.10*              | 14.24       |
| IV      | *H. africana* Only       | 21.00±2.80         | 3.75±0.40        | 17.56±1.31               | 82.15*              | 28.57       |
| V       | ACT + *H. africana*      | 19.80±5.50         | 0.00±0.00        | 0.00                    | 100*                | 0.00        |

*e=Mean; Standard Deviation of 6 determinations, a=significantly different when compared with test group II (Parasitized untreated) and 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 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 may 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 possibly due to diminished food intake evidenced by larger quantity of food remnant when compared with that of the normal control. This plasmoidium-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 administration of ACT and extract of H. africana was better either the ACT or plant extract alone. This implies that the ACT-hers 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.

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