



Haematopoietic Potential of *Jatropha tanjorensis* Leaf Extract in *Plasmodium berghi-berghi* Infected Mice Treated with *Hippocratea africana* Root Bark Extract

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Abstract: The haematopoietic potential of *Jatropha tanjorensis* leaf extract in *Plasmodium berghi-berghi* infected mice treated with *Hippocratea africana* root bark extract was investigated. Thirty (21) adult mice weighing 27 – 33g were divided into seven (7) groups with three (3) animals in each group. Group 1 served as normal control while Groups 2 – 7 were parasitized with *Plasmodium berghi-berghi* and Group 2 was the test control (parasitized and untreated) group. Group 3 was administered 8mg/kg bw of artemether-lumefantrine for 3 days. Group 4 and 5 received daily, 200mg/kg bw and 300mg/kg bw of extracts of *Hippocratea africana* and *Jatropha tanjorensis* respectively for 4 days. Group 6 received 8mg/kg bw of artemether-lumefantrine for 3 days followed with 300mg/kg bw of *Jatropha tanjorensis* leaf extract for 4 days. Group 7 was treated with 200mg/kg bw of *Hippocratea africana* root extract for 4 days followed by 300mg/kg bw of *Jatropha tanjorensis* leaf extract for 4 days. Significant anti-plasmodial activity was observed with artemether-lumefantrine and *Hippocratea africana* root bark extract administration. *Plasmodium berghi-berghi* infection induced alterations in haematological indices such as decreased RBC count, platelet count, haemoglobin concentration and haematocrit and increased WBC count. Administration of *Jatropha tanjorensis* leaf extract showed improved haematological indices particularly in the red blood cell counts and haemoglobin concentration. *Jatropha tanjorensis* has haematopoietic activity in *Plasmodium berghi-berghi* infected mice treated *Hippocratea africana* root bark extract.

Keywords: *Jatropha tanjorensis*, *Hippocratea africana*, Artemether-Lumefantrine, Antiplasmodial Activity, Haemato-protection

1. Introduction

Malaria is a major pandemic in the tropical regions of the world such as Africa, South America and Asia. Malaria is a parasitic disease transmitted through the bite of a female anopheles' mosquito infected with any of *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* specie. Nigeria accounts for a quarter of all malaria cases in Africa [1]. Artemisinin-base combination

therapies [ACTs] are recommended by the world health organization for effective management of malaria. However, the high cost of these drugs, unavailability and the emergence of drug resistance *Plasmodium falciparum* has left the poor masses completely reliant on natural herbal remedy with little or no cost and is readily available as well as well proven to be effective against malaria [2]. In addition, it has been observed that in the rural communities, patients who may be seeking quick healing tend to combine herbal therapies with conventional drugs for malaria treatment [3].

Nigeria possesses a rich flora diversity made up of edible vegetables and medicinal plants in the southern part of the country which is used for the treatment of malaria [4]. *Hippocratea africana* is one of such medicinal plants known for its antimalarial activity in Nigeria. It is geographically distributed in tropical African countries like Nigeria, Ghana and Senegal [5, 6] and is found to be rich in phytochemicals such as alkaloids, flavonoids, terpenes, tannins, cardiac glycosides and saponins. These phytochemicals are believed to be responsible for various biological activities such as antimicrobial, anti-inflammatory, anticancer and antimalarial activities [2, 5, 7].

Malaria infection and treatment with antimalarial agents have been found to be associated with altered haematological indices particularly lowered red blood cell count, haemoglobin concentration and haematocrit or packed cell volume. This necessitates the common clinical practice of administration of hematinic after treatment of malaria. Traditionally, herbal extracts with haematopoietic potentials are often taken after treatment with antimalarial drugs or herbs. *Jatropha tanjorensis* is a leafy vegetable commonly consumed for its medicinal benefits particularly haematoprotective potential [8]. The plant is rich in polyphenols, saponins, alkaloids and tannins [9]. It is popularly nicknamed 'hospital too far' because of its tremendous medicinal benefits.

The present study was designed to evaluate the haematopoietic potential of *Jatropha tanjorensis* leaf extract in *Plasmodium berghei-berghi* infected mice treated with *Hippocratea africana* root bark extract.

2. Materials and Methods

2.1. Plant Material

The root of *Hippocratea africana* was collected from Afaha Etok forest in Ibesikpo-Asutan Local Government Area, Akwa Ibom State. It was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo. The voucher number (UUH3394) was given. The roots were gently washed to get rid of debris. The root bark was processed to obtain crude extract according to the method described by Ndem and Basse, [2017] [2]. The root bark was scrapped, cut into small pieces and pulverized. The pulverized sample was macerated in 80% ethanol (Sigma Aldrich) for 72 hours. Within this period, the mixture was shaken thrice in every 24 hours to allow the solvent to solubilize the active phytochemicals. After 72 hours, the clear orange colour supernatant was carefully siphoned off and concentrated to dryness in a water bath at 40°C to obtain the crude extract.

Fresh leaves of *Jatropha tanjorensis* were collected from Uyo metropolis, Akwa Ibom State, Nigeria. The leaves were washed and pulverized using a manual grinder. It was then macerated in water for 6 hours, filtered and the filtrate concentrated to dryness in water bath at 40°C to obtain the crude extract.

2.2. Acute Toxicity of *Jatropha tanjorensis*

The acute toxicity LD₅₀ test for *Jatropha tanjorensis* was carried out using Lorke's method. The aqueous extract of *Jatropha tanjorensis* ranging from 1000mg/kg to 4000mg/kg was administered to 40 mice and observed for 24 hours. The LD₅₀ was calculated to be 1161.89mg/kg.

2.3. Synthetic Drugs

Artemether-Lumefantrine [Coartem[®], Novartis] was obtained from Uchris Pharmacy in Uyo Metropolis in Akwa Ibom State, Nigeria.

2.4. Experimental Animals and Design

Twenty-One (21) male adult mice weighing between 27-33g were used for the study. They were obtained from the Animal House, Department of Pharmacology, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The animals were maintained under standard laboratory conditions and fed with normal rat chow and clean drinking water *ad libitum*. The mice were divided into 7 groups with 3 animals in each group.

Group 1 served as normal control while Groups 2-7 were parasitized with *Plasmodium berghei-berghi* and Group 2 was the test control (parasitized and untreated) group. Group 3 was administered 8mg/kg bw of artemether-lumefantrine for 3 days. Group 4 and 5 received daily, 200mg/kg bw and 300mg/kg bw of *Hippocratea africana* and *Jatropha tanjorensis* respectively for 4 days. Group 6 received 8mg/kg bw of artemether-lumefantrine for 3 days followed with 300mg/kg bw of *Jatropha tanjorensis* for 4 days. Group 7 was treated with 200mg/kg bw of *Hippocratea africana* for 4 days followed by 300mg/kg bw of *Jatropha tanjorensis* for 4 days.

2.5. Malaria Parasite and Inoculation

Malaria parasite, *Plasmodium berghei-berghi* was obtained from the Department of Pharmacology, University of Uyo, Uyo, Nigeria through a donor mouse. The experimental animals in the present study were induced with malaria according to the method described by Ndem and Basse [2]. The parasite was obtained from the donor mouse through cardiac puncture after being anaesthetized with chloroform. The blood was diluted with normal saline and 0.3ml of the infected blood was passage intraperitoneally into each of the mouse with 10⁷ parasitized erythrocytes. The parasites were inoculated for 7 days then the animals were confirmed to be infected with malaria through microscopic examination of blood films from the tail of each mouse.

2.6. Collection of Blood Sample

The animals were denied food for 24 hours after the administration of the last dosage of the extracts and drugs but were still allowed water *ad libitum*. They were chloroform anaesthetized and blood samples were obtained by cardiac puncture using sterile needles and syringes into EDTA

containing sample tubes. The blood was used to evaluate haematological indices and thick films were prepared for parasite count microscopically.

2.7. Haematological Assay

Haematological parameters were determined using automated haematological analyzer: Sysmex® Analyzer KX-21N. RBC, HGB, HCT, WBC and Platelets count were estimated in whole blood.

2.8. Statistical Analysis

Statistical analysis was carried out using widows SPSS. One-way analysis of variance (ANOVA) and Least Significant Least Multiple Post Hoc test were employed for comparison to assess statistical significance. All the results are presented as mean ± standard deviation (SD). Probability level <0.05 was considered significant.

3. Results

3.1. Antiplasmodial Activity of Artemether-Lumefantrine and Hippocratea africana Root Bark Extract in Plasmodium berghi-berghi Infected Mice

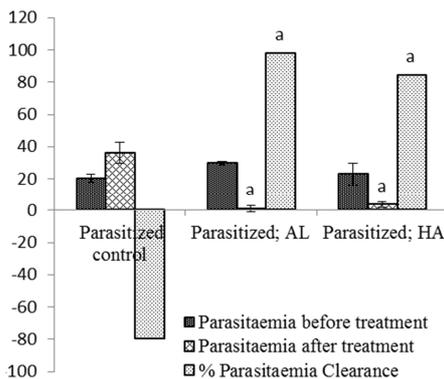


Figure 1. Antiplasmodial activity of artemether-lumefantrine and Hippocratea africana root bark extract in Plasmodium berghi-berghi infected mice. AL = artemether-lumefantrine; HA = Hippocratea africana. a = significantly different when compared to Group I at p<0.05.

Red Blood Cell Count (10⁶/μL)

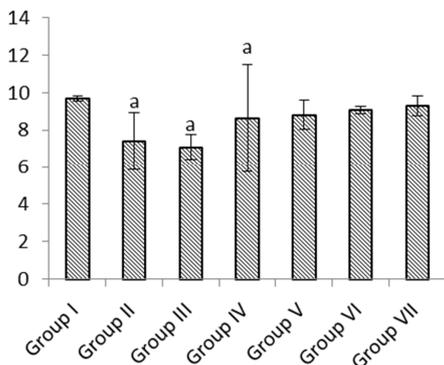


Figure 2. Effect of Jatropha tanjorensis leaf extract on Red Blood Cell count of Plasmodium berghi-berghi infected mice treated with artemether-lumefantrine and Hippocratea africana root bark extract. a = significantly different when compared to Group I at p<0.05.

Parasitaemia was established following the inoculation of Plasmodium berghi-berghi in albino mice. The result of antiplasmodial activity of athermether lumfantrine and ethanol extract of root bark of Hippocratea africana is presented in Figure 1. Significant parasitaemia clearance was observed in groups treated with Hippocratea africana root bark extract and artemether-lumefantrine.

Heamoglobin Concentration (g/dl)

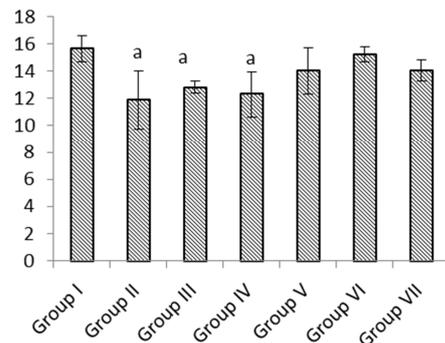


Figure 3. Effect of Jatropha tanjorensis leaf extract on Heamoglobin concentration of Plasmodium berghi-berghi infected mice treated with artemether-lumefantrine and Hippocratea africana root bark extract. a = significantly different when compared to Group I at p<0.05.

Hematocrit (%)

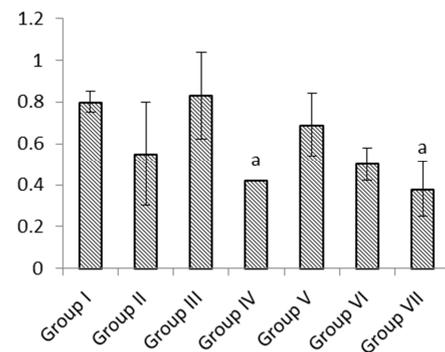


Figure 4. Effect of Jatropha tanjorensis leaf extract on haematocrit level of Plasmodium berghi-berghi infected mice treated with artemether-lumefantrine and Hippocratea africana root bark extract. a = significantly different when compared to Group I at p<0.05.

3.2. Haematopoietic Potential of Jatropha tanjorensis Leaf Extract in Plasmodium berghi-berghi Infected Mice Treated with Hippocratea africana Root Bark Extract

The result of the haematopoietic potential of Jatropha tanjorensis leaf extract in Plasmodium berghi-berghi infected mice treated with artemether-lumefantrine, ciprofloxacin and Hippocratea africana root bark extract is presented in Figures 2-6. Reduced RBC count, PLT count, HGB concentration and HCT were observed as a result of parasite inoculation in the experimental animals. White blood cell counts increased due to malaria induction. Treatment with artemether-lumefantrine, Hippocratea africana resulted in reduced red blood cell count and haemoglobin concentration. However, administration of Jatropha tanjorensis to the

treated groups improved the haematological indices as observed in increased haemoglobin concentration and red blood counts in Groups VI and VII.

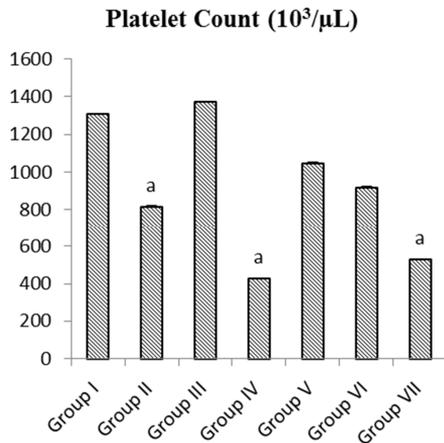


Figure 5. Effect of *Jatropha tanjorensis* leaf extract on Platelet count of *Plasmodium berghei-berghi* infected mice treated with artemether-lumefantrine and *Hippocratea africana* root bark extract. a = significantly different when compared to Group I at $p < 0.05$.

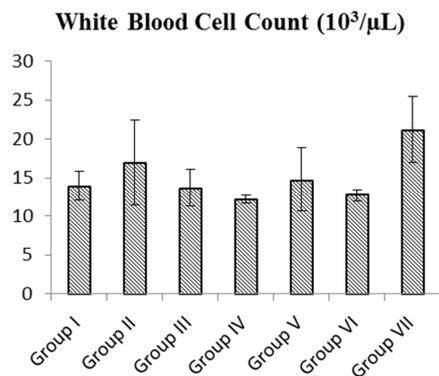


Figure 6. Effect of *Jatropha tanjorensis* leaf extract on White Blood Cell Count of *Plasmodium berghei-berghi* infected mice treated with artemether-lumefantrine and *Hippocratea africana* root bark extract. a = significantly different when compared to Group I at $p < 0.05$.

4. Discussion

Malaria remains one of the most prevalent infectious diseases in the world particularly in Africa. The disease is thought to be the major cause of severe anaemia in at least 50% of people living in malaria endemic [10]. Anaemia is defined as decreased in the number of red blood cells or less than normal haemoglobin concentration. The malaria parasite grows and multiplies first in the liver cells and then in the red blood cells causing lysis of the red blood cells [11]. The present study has shown that the inoculation of the *Plasmodium berghei-berghi* in the experimental animal resulted in anaemia as manifested in lowered red blood cell count and haemoglobin concentration.

The pre and post treatment parasitaemia level in the present study is presented in Figure 1. It was observed that *Hippocratea africana* had parasitaemia clearance of 84% while artemether-lumefantrine had a parasitaemia clearance of

99%. Artemether-Lumefantrine is the world health organizations recommended first choice drug for treatment of malaria due to its high or near total clearance of the parasite. Ndem and Bassey [2], had reported that *H. africana* has significant antimalarial activity and the present study corroborates the report.

Furthermore, antimalarial agents have been reported to aggravate anaemia in malaria cases. Osonuya *et al.*, [12], reported that artemether can aggravate anaemia when administered in malaria infection. The study reported decrease in red blood cell count, haemoglobin concentration and white blood cell count in albino rats. The present study has shown that administration of artemether-lumefantrine decreased red blood cell count and haemoglobin concentration while there was increase in white blood cell count. This observation further adds credence to the common clinical practice of patients treated with antimalarial agents being placed on haematinic after completion of the malaria treatment.

Jatropha tanjorensis leaves are consumed in Nigeria as soups and as tonic with the claim that it increases blood volume [13]. The present study supports this claim as there were observed improved haematological indices following administration of *Jatropha tanjorensis* in parasitized and treated animals. The antimalarial agents used in this study [*Hippocratea africana* and artemether-lumefantrine] were observed to adversely affect and worsen the already altered haematological indices in the experimental animals due to infection with *Plasmodium berghei-berghi*. However, *J. tanjorensis* administration after treatment with the antimalarial agents resulted in increased RBC count, platelet count and haemoglobin concentration while there was a significant decrease in the WBC counts and its differentials when compared to the parasitized control group. The observed changes in haematological indices suggest that *J. tanjorensis* positively modulates these indices in the experimental animals.

In the present study, inoculation of *Plasmodium berghei-berghi* was observed to decrease the total platelet count and this was further aggravated by the administration of *Hippocratea africana* root bark extract but artemether-lumefantrine had no deleterious effect on the platelet count. Administration of *Jatropha tanjorensis* improved the platelet count in the treated animals. Platelets play a role in the clotting of blood. Its reduction constitutes an important cause of generalized bleeding. Some drugs such as non-steroidal anti-inflammatory drugs have been reported to negatively affect platelet and result in platelet dysfunction [14]. Some herbal medicines have also been reported to alter platelet function and coagulation [15, 16].

Haematopoietic potentials of African medicinal plants such as *Telferia occidentalis*, *Citrillus lanatus* spinach and wheatgrass have been reported [17]. Phytochemicals such as beta-carotene, lutein, saponin and quercetin have been reported to be responsible for the observed haematopoietic potentials of these plants. *Jatropha tanjorensis* has been reported to possess arrays of phytochemicals which include

polyphenols, saponins, tannins and alkaloids [9]. These phytochemicals may be responsible for the haematopoietic effect of *J. tanjorensis* observed in the present study. Alkaloids have been reported to inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase which result in the accumulation of cAMP. The accumulated cAMP stimulates the phosphorylation of proteins and synthesis of protein, thereby promoting erythropoiesis [18]. The *Jatropha tanjorensis* leaf extract may have stimulated the kidney to release the erythropoietic factor that converted the blood protein to erythropoietin. The erythropoietin stimulated the production of red blood cells, thereby improving the haematological parameters.

5. Conclusion

The positive haematological parameters observed in this study following the administration of *Jatropha tanjorensis* suggest that *J. tanjorensis* possess haematopoietic property that may be due to the bioactive agents present in the plant. These bioactive agents may have enhanced erythropoietic activity. Further studies will be required to elucidate the active principle in the leaf extract of *Jatropha tanjorensis* that is responsible for the observed haematopoietic activity observed in the present study.

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