PARASITAEMIA REDUCTION AND MODERATELY IMPROVED HAEMATOLOGICAL INDICES POTENTIALS OF HIPPOCRATEA AFRICANA ROOT BARK EXTRACT IN PLASMODIUM BERGHEI-BERGHEI INFECTED MICE

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ABSTRACT

The effect of Hippocratea africana root bark extract on parasitemia concentration and haematological indices of Plasmodium berghei-berghei induced mice was investigated. Thirty-five albino mice weighing between 17g to 29g were divided into 7 groups with 5 mice in each group. Group 1 served as normal control while Groups 2 – 7 were parasitized with P. berghei-berghei obtained from a donor mouse with a parasitaemia of 5 x 10⁷. Group 2 served as infected control while Groups 3, 4 and 5 were orally administered 100mg/kg, 200mg/kg and 300mg/kg of H. africana respectively daily for 4 days. Groups 6 and 7 received therapeutic doses of chloroquine and artemisunate respectively. There was significant reduction in the parasitaemia of the extract treated groups, with 200mg/kg of H. africana showing the highest clearance of 89.8%. Artemisunate showed 100% clearance being the standard drug for malaria treatment. Haematological indices of plasmodium infected mice were severely altered before treatments compared with the normal control. The results of the study showed that WBC counts in the extract treated groups increased non-significant (p > 0.05) except in Group 5 compared with Group 2. Non-significant (p > 0.05) increase in RBC counts, HGB concentration and HCT levels were observed in the extract treated group compared with parasitized untreated Group 2. MCH and MCHC levels changed non-significantly in the treated groups compared with Group 2 while platelet counts were increased non-significantly in the extract treated groups when compared with the parasitized untreated group. The ethanol extract of root bark of Hippocratea africana has a potent antimalarial activity against Plasmodium berghei-berghei and does not negatively affect the haematological indices of the
infected mice beyond the derangement already induced by the parasite but moderately improves it.

**KEYWORDS:** Hippocratea africana, Malaria, Plasmodium berghei-berghei, Haematology, Antiplasmodial Activity.

**INTRODUCTION**
Malaria is one of the commonest infectious diseases and is widespread in tropical and subtropical regions of the world including parts of America, Asia and Africa. The disease is known to be caused by a single-celled protozoa parasite called plasmodium and transmitted through anopheles mosquito. Anemia, fever, nausea and flulike illness are the common manifestation of malaria with coma and death reported in severe cases.\(^1\) Development of drug resistant by plasmodium species and the resistance of anopheles mosquitoes to many insecticides have compounded the control of malaria in infested area.\(^2\) The incidence of resistance, side effects and toxicity of conventional antimalarial drugs like sulphadoxine-pyrimethamine, mefloquine and in recent times, the artemisinin combination therapies\(^3,4\) have further complicated the management of malaria. Unavailability and unaffordability have also been identified as hindrances in assessing orthodox drugs\(^5\) thereby fueling the need for alternative source of antimalarial agents especially in low income economy of developing or underdeveloped countries.

Herbal medicine has been a major aspect of alternative and traditional medicine in the world. Herbs contain active constituents with myriads of medicinal potentials making them a common target for alternative source of drugs.\(^6\) Medicinal plants are commonly used in developing countries for the treatment and management of several disease conditions and they have played a key role in healthcare management around the world. Among the herbal medicine that has been studied is the root bark extract of Hippocratea africana.

Hippocratea africana is called “eba enen-enan” by the Ibibios and Efiks of Southern Nigeria. The plant is valued and highly utilized in the rural areas especially because of its medicinal properties. Almost all parts of the plants (fruit, seeds, leaves, stem bark and roots) have been shown to have medicinal properties. The root of the plant is used locally in the management of several conditions including fever, malaria, body pains, diabetes and diarrhea.\(^7\) The plant is reported to be rich in various phytochemicals which are responsible for its numerous medicinal properties including analgesic, anti-inflammatory and antipyretic activity.\(^8\)
antiulcerogenic activity\textsuperscript{[9]} and antidiarrheal activity\textsuperscript{[10]} The effect of the root bark extract of 
\textit{Hippocratea africana} on biochemical parameters including lipid profile,\textsuperscript{[5]} liver enzymes,\textsuperscript{[11]} indices of renal function,\textsuperscript{[12]} cardiac enzymes\textsuperscript{[13]} male reproductive hormone\textsuperscript{[14]} and seminal fluid evaluation\textsuperscript{[15]} has been reported. The various medicinal and toxicological studies on \textit{H. africana} attributes the observed effects to the presence of a single phytochemical or a synergy of two or more of the various phytochemicals present in the plant.\textsuperscript{[5]}

Haematological indices provide information regarding the status of bone marrow activity and haemolysis.\textsuperscript{[16]} They can be used to determine the extent of deleterious or beneficiary effect of foreign compounds including plant extracts on the blood constituents.\textsuperscript{[17]} It can also be used to explain blood selected functions of chemicals/plant extract.\textsuperscript{[18]} White blood cell count (WBC) is the total count of the number of white blood cells per liter of blood. It is increased in inflammation, infections and leukaemia. It also decreases in conditions such as bone marrow defect and due to some drugs. A total count of red blood cells per liter of blood refers to the red blood count (RBC) and is increased in over-production state of the bone marrow (polycythemia, chronic oxygen deprivation) and is decreased in anemia. Hematocrit (HCT) is the total volume of the red blood cells in the blood while the mean corpuscular haemoglobin (MCH) is the measure of amount of haemoglobin per red blood cell and mean corpuscular haemoglobin concentration (MCHC) is the amount of haemoglobin per liter of fluid in each cell.

Several conditions can lead to alteration in haematological parameters.\textsuperscript{[19]} Malaria has been observed to be associated with low platelets, WBCs and lymphocyte count.\textsuperscript{[20,21]} Furthermore, antimalarial drugs have been proven to result in red blood cell destruction leading to haemolysis of both parasitized and un-parasitized red blood cell in the circulation.\textsuperscript{[22]} Several studies have attempted to evaluate the effect of some xenobiotics including plant extracts on haematological parameters; some have little or no toxic haematologic effect while some have shown altered haematological indices, which some however subside some days after administration.\textsuperscript{[23,24]}

Earlier study by Ndem \textit{et al.}\textsuperscript{[25]} had concluded that the root bark extract of \textit{H. africana} has favorable effects on haematological indices of normal albino rats; Increasing RBC counts and haemoglobin concentration at 100 and 200 mg/kg body weight although high doses may predispose to anaemia. Considering the use of the plant in the treatment of malaria, it is pertinent to evaluate if its haematopoietic potential can ameliorate the haematological
derangement associated with plasmodium infection. The present study achieves this through the evaluation of haematological indices of *Plasmodium berghei-berghei* infected mice treated with ethanol root bark extract of *H. africana*.

**MATERIAL AND METHODS**

**Plant Sample**

The plant part (root) 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 (UUH1455) was given. The roots were gently washed to get rid of debris. 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 45°C to obtain the crude extract.

**Experimental Animals**

Thirty-five (35) albino mice weighing between 17g - 29g used for the study were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. The animals were divided into seven groups of five (5) mice per group. They were housed in a ventilated room in standard laboratory cages under standard laboratory conditions and allowed to acclimatize for 7 days before the study. The animals were fed grower’s mash (guinea feed) and water were provided *ad libitum* throughout the experimental period.

**Induction of Malaria Parasite**

30 mice were infected with malaria parasite (*Plasmodium berghei-berghei*). The parasite was obtained from a donor mouse through cardiac puncture after being anaesthetized with chloroform. The blood was diluted with normal saline and 0.3 ml of the infected blood was passage intraperitoneally into each of the mouse with $10^7$ 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.
Experimental Design
Thirty-five (35) albino mice weighing between 17g - 29g used for the study were divided into 7 groups of 5 mice per group and treated as follows:
Group 1 – Normal control
Group 2 – Parasitized control
Group 3 – Parasitized, treated with 100mg/kg body weight of *H. africana* daily for 4 days.
Group 4 – Parasitized, treated with 200mg/kg body weight of *H. africana* daily for 4 days.
Group 5 – Parasitized, treated with 300mg/kg body weight of *H. africana* daily for 4 days.
Group 6 – Parasitized, treated with therapeutic regimen of Chloroquine (5 mg/kg body weight)
Group 7 – Parasitized, treated with therapeutic regimen of Artesunate (4 mg/kg body weight)

Blood Collection
The animals were denied food for 24 hours after the administration of the last dosage of the extract and drug 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.

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 using this analyser. White blood differentials were also determined as well as red blood cell count.

Statistical Analysis
All the results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed for comparison to assess statistical significance using widows SPSS. Probability level < 0.05 was considered significant.

RESULTS
The results of antiplasmodial activity of ethanol root bark extract of *Hippocratea africana* and its effect on haematological indices of *Plasmodium berghei-berghei* induced mice are presented in Table 1 and 2 respectively.
Table 1: Antiplasmodial Activity of Ethanol Root Bark Extract of *Hippocratea africana* (HA).

<table>
<thead>
<tr>
<th>Groups and Treatment</th>
<th>Parasitaemia Before Treatment</th>
<th>Parasitaemia After Treatment</th>
<th>% Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: Normal Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2: Parasitized - no treatment</td>
<td>18.00 ± 4.0</td>
<td>34.00 ± 10.19</td>
<td>-</td>
</tr>
<tr>
<td>3: Parasitized + 100mg/kg bw of HA</td>
<td>25.00 ± 6.32</td>
<td>12.00 ± 9.79</td>
<td>52.00</td>
</tr>
<tr>
<td>4: Parasitized + 200mg/kg bw of HA</td>
<td>19.60 ± 5.70</td>
<td>2.00 ± 0.40</td>
<td>89.80</td>
</tr>
<tr>
<td>5: Parasitized +300mg/kg bw of HA</td>
<td>16.20 ± 4.02</td>
<td>4.60 ± 1.97</td>
<td>71.61</td>
</tr>
<tr>
<td>6: Parasitized + Chloroquine (5 mg/kg bw)</td>
<td>20.00 ± 5.25</td>
<td>12.00 ± 4.78</td>
<td>40.00</td>
</tr>
<tr>
<td>7: Parasitized + Artesunate (4 mg/kg bw)</td>
<td>27.40 ± 7.00</td>
<td>0.00 ± 0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Value presented as Mean ± Standard Deviation. n = 5

Table 2: The effect of ethanol extract of *H. african* root bark extract on haematological indices of *Plasmodium berghei-berghei* infected mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1: Normal Control</th>
<th>Group 2: Parasitized – No Treatment</th>
<th>Group 3: Parasitized + 100 mg/kg bw of HA</th>
<th>Group 4: Parasitized + 200 mg/kg bw of HA</th>
<th>Group 5: Parasitized + 300 mg/kg bw of HA</th>
<th>Group 6: Parasitized+ Chloroquine (5 mg/kg/ bw)</th>
<th>Group 7: Parasitized + Artesunate (4 mg/kg bw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10^3/UL)</td>
<td>6.32 ± 1.92</td>
<td>20.38 ± 1.36^a</td>
<td>24.78 ± 7.98^a</td>
<td>22.98 ± 4.91^a</td>
<td>19.30 ± 1.82^a</td>
<td>10.98 ± 3.15^b</td>
<td>31.44 ± 5.48^ab</td>
</tr>
<tr>
<td>RBC (x10^6/UL)</td>
<td>7.31 ± 1.14</td>
<td>2.27 ± 0.37^a</td>
<td>1.95 ± 0.50^a</td>
<td>3.12 ± 0.01^a</td>
<td>2.29 ± 0.82^a</td>
<td>6.19 ± 0.75^b</td>
<td>2.20 ± 0.64^a</td>
</tr>
<tr>
<td>HBG (g/dl)</td>
<td>11.28 ± 0.93</td>
<td>3.64 ± 0.63^a</td>
<td>3.48 ± 0.97^a</td>
<td>4.00 ± 0.76^a</td>
<td>3.36 ± 1.20^a</td>
<td>10.54 ± 0.54^b</td>
<td>4.28 ± 0.61^a</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>48.24 ± 3.88</td>
<td>15.55 ± 2.87^a</td>
<td>16.35 ± 3.86^a</td>
<td>19.68 ± 3.60^ab</td>
<td>15.66 ± 2.58^a</td>
<td>45.40 ± 2.51</td>
<td>22.90 ± 2.58^a</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>66.64 ± 6.79</td>
<td>73.10 ± 1.69^a</td>
<td>87.03 ± 2.84^a</td>
<td>79.73 ± 18.39^a</td>
<td>73.66 ± 5.03^a</td>
<td>74.24 ± 9.49</td>
<td>101.45 ± 22.18^ab</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>15.62 ± 1.90</td>
<td>15.85 ± 0.19</td>
<td>16.90 ± 0.73</td>
<td>16.10 ± 3.01</td>
<td>22.12 ± 2.32^ab</td>
<td>17.18 ± 1.78</td>
<td>20.48 ± 3.71^ab</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>23.42 ± 1.06</td>
<td>22.05 ± 0.66</td>
<td>20.95 ± 0.97</td>
<td>20.23 ± 1.37^a</td>
<td>21.82 ± 1.98</td>
<td>23.26 ± 1.26</td>
<td>18.82 ± 0.78^ab</td>
</tr>
<tr>
<td>PLT (x10^3/UL)</td>
<td>1116.60 ± 61.99</td>
<td>390.75 ± 17.58^a</td>
<td>374.00 ± 154.86^a</td>
<td>587.25 ± 129.79^a</td>
<td>683.40 ± 131.09^a</td>
<td>1470.60 ± 131.09^a</td>
<td>647.40 ± 87.00^a</td>
</tr>
</tbody>
</table>

Value presented as Mean ± Standard Deviation. n = 5. ^a = Significantly different from Group 1 (Normal control) at p < 0.05; ^b = Significantly different from Group 2 (Parasitized, not treated) at p < 0.05.
DISCUSSION

Herbal medicine is highly utilized and dependent upon in the developing countries. Different parts of *Hippocratea africana* are widely used in Nigeria and other developing nations in the management of various conditions. The present study validates the use of the root bark of *H. africana* in the treatment of malaria and the resulting haematological alterations accompanying its utilization in a plasmodium infected model. The ethanol root bark extract of *H. africana* has been shown to possess a potent antiplasmodial activity against *Plasmodium berghei-berghei* especially at dose of 200mg/kg body weight with parasite clearance of 89.8%. This value is higher than the percentage clearance of chloroquine (one of the standard antimalarial drugs used in this study). However, artesunate showed 100% parasite clearance in this study (Table 1). The result corroborates earlier report by Okokon et al.,[7] which concluded that *H. africana* has antiplasmodial activity exerted by phytochemicals in the plant including alkaloids and tannins.

The result obtained in this study shows that the extract appears to have slight modification of some haematological indices. Comparison of normal control group and malaria infected groups showed significant increase in indices including WBC count and MCV while RBC count, HGB concentration, HCT level and platelet count decreased significantly. The results show normal immunological response following infection with malaria parasite. Infection with malaria parasite have been reported to be associated with deranged haematological indices.[21]

The WBC count of the malaria infected mice treated with the extract showed significant increase (p < 0.05) when compared to normal control. The WBC count of the extract treated group was not significantly (p > 0.05) different from the negative control implying that the extract did not elicit further immunological response leading to synthesis of more white blood cells. However, artesunate, the standard drug in malaria treatment is observed to show a WBC count significantly higher than that of the negative control group. This adds credence to reports that antimalarial agents elicit immunological response resulting in increased WBC count [20]. Reports have been documented that extracts of plants result in increased white blood cell generation due to immunological responses where these extracts are regarded as xenobiotics in the system. Ethanol extract of leaves of *Nauclea latifolia* has been reported to increase white blood cell count in albino Wistar rats while the aqueous extract of the same plant showed no such response.[18]
The red blood cell counts and haemoglobin concentrations of the extract and drug treated groups in this study showed significant decrease when compared to the normal control. Malaria parasites have been known to cause lysis of red blood cells hence reduced haemoglobin concentrations in infected host.\textsuperscript{[20]} Furthermore, metabolism of drugs has been known to generate free radicals which leads to lipid peroxidation of cell membrane lipids resulting in cell lysis.\textsuperscript{[26]} The present result on RBC count, haemoglobin concentrations and hematocrit confirm these facts. There was no significant difference between the RBC count, HGB concentration and the HCT of the extract treated group and the negative control group. This shows the non-deleterious effect of the extract on these indices. Some plant extracts have been reported to significantly decrease RBC count and HGB concentration in animal models through cell lysis and suppression of red blood cell synthesis.\textsuperscript{[27]} The non-significant changes observed in the extract treated groups when compared to the negative control shows that the extract did not contribute to the already deleterious effect of the plasmodium parasite on these blood indices.

Platelets are known to regulate the clotting of blood and their decrease are implicated in generalized bleeding. The platelet counts of the parasitized animals treated with ethanol root bark extract of \textit{H. africana} was increased when compared to the negative control group. The thrombocytosis associated with parasite infestation in this study was improved upon the administration of the extract, however, the standard drugs showed a more improved condition. The mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations of the extract and drugs treated groups were not significantly altered when compared to the negative control implying a non-involvement of the extract or the drugs in the alterations of these indices from the normal control.

In conclusion, root bark extract of \textit{Hippocratesia africana} possesses antimalarial activity against \textit{Plasmodium berghei-berghei} and does not negatively affect the haematological indices of the infected mice beyond the derangement that was already induced by the parasite but moderately improves it.

REFERENCES


