Immunomodulatory Activity of *Haematostaphis barteri* H.F (Anacardiaceae) Leaf Methanol Extract in Swiss Albino Rats

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**Abstract**

The aerial parts of *Haematostaphis barteri* are known to be used for treating many ailments including wounds and sores. The immunomodulatory properties of methanol extract and fractions of *H. barteri* leaf on Delayed-type Hypersensitivity response (DTH), (as well as) some haematological parameters associated with the immune system were evaluated. The methanol extract (ME) at 100, 200, 300 and 400 mg/kg body weight produced significant (p<0.05) inhibition of DTH response after 24 hours in rats by 0.63 ± 0.01a, 0.66 ± 0.01a, 0.70 ± 0.01a and 0.74 ± 0.01a respectively. Treatment of rats with single intraperitoneal injection of Lamuvidine 20 mg after oral administration of extract resulted in an increase in WBC mobilization into the rat’s peritoneal fluid which was significant (p<0.05). The total white blood cell counts (WBC) or total leucocytes (TLC) were higher in the extract treated groups when compared to the control. However, a fall in percentage neutrophils antibody titre were observed with methanol extract suggesting that *H. barteri* may act through a cell mediated mechanism in boosting immune system of the animal, hence justify the use of the plant in boosting blood and immune system in traditional medicine.

**Keywords**

*Haematostaphis barteri*; Immunomodulatory; Hypersensitivity; Total Leucocytes

**Introduction**

*Haematostaphis barteri* is a plant used in northern part of Nigeria, to manage degenerative diseases such as cancer, anemia and hemorrhage. It is known as “blood plum” in English and locally called “JininKafiri” in Hausa language in Nigeria. The plant is mainly distributed in northern Nigeria states of Taraba, Kaduna, Adamawa, Gombe, Kano, etc, Uganda, parts of Ghana, and Ivory Coast [1]. The stem barks was reported to possess antioxidant activity due to presence of secondary metabolites such as tannins, flavonoids, and cardiac glycosides [2].

Immunomodulation means a change in the body’s immune system, which is caused by agents called immunomodulators that activate or suppress its function. It is the adjustment of immune response to the desired level, and involved immunostimulation, immunoprecession, or induction of immunologic tolerance capability. Many proteins, amino acids, and other natural compounds have showed asignificant ability to regulate immune response for example interferon-r steroids [2]. It has been observed that immunodulatory substances from natural sources could play a vital role in disease prevention and treatment,
especially with the increasing global emphasis on natural system and the campaign of a “greening” of the society. Malnutrition and infection diseases have remained a challenge especially in developing nations as they greatly compromise the body’s immune system responses in the affected individual [3].

Inadequate balanced dietary intake, most especially proteins, and diseases are immediate cause of malnutrition and these reinforced one another synergistically. The causes of immunodeficiency include stress, infectious diseases such as acute respiratory tract infections, diarrheal diseases, yellow fever, hepatitis A and E, tuberculosis, and HIV/AIDS [4]. Proper nutrition by individuals strengthen the body immune system and it defiance capabilities. Various allopathic drugs or medicines are used to modulate the immune system. However, these drugs are very expensive for poor people they are not easily accessible. And in, most cases drug are associated with adverse drugs reactions. As a result, the majority of people especially in the rural areas of the developing world turn to the use of alternative herbal medicines from medicinal plants such as Haematostaphis barteri that is widely accepted, accessible cheaper, and assumed to have fencer side effects [5].

In Africa and Asia continents, up to about 80% of the population have been reported to depend on traditional medicine for their daily primary health care needs including immunomodulation or immune boosting. A number of medicinal plants have long been used and reported to boost the immune system or to modulate the immune system in anemic situations, and they are used putatively to treat and prevent various disease conditions worldwide [6]. Haematostaphis barteri is a common herb and has been documented to have various phytochemicals, macronutrient, and micronutrient that contribute to its vast medicinal value including the management of disease such as asthma, bronchitis, mastitis, skin conditions, worm infestations, and HIV/ADS symptoms among others [7]. Its ability to treat most of these conditions has been attributed to the nutritionals and immunomodulatory properties it possesses including its antioxidant and anticancer activity among others [8]. The plant is commonly available and widely used in local communities by traditional medicine practitioners in Nigeria as blood builders or boosters and in the management of various disease condition such as anaemia and as anti-trypanosome agent [9].

This present study was carried out in order to evaluate immune boosting potential of H. barteri in vivo rats model to justify its ethnomedicinal claim as an immune and blood boosting remedy.

**Materials and Methods**

**Materials**

The following materials were used in this research work: methanol leaf extract of Haematostaphisbarteri, 30 Swiss albino rats weighing between 100-150 g, CD4 machine, rotary evaporator, water bath, methanol, desiccator and others.

**Methods**

**Plant Collection and Identification**

The Haematostaphis barteri leaves were collected in the Sabon-Dale Village Bali, Taraba State in January 2017, due to its abundance in the Village and its proximity. The plant was identified by a taxonomist in the Department of Science Laboratory Technology (Biology Unit), Federal Polytechnic Bali, with a voucher specimen number of ANA004 deposited at the herbarium of Biology Unit in the department.

**Preparation and Extraction of Plant Material**

The leaves were collected and air-dried under shade until constant weight was achieved. They were then pounded into fine powder using a mortar and pestle. The extraction procedure was carried out at the Chemistry laboratory of Science Laboratory Department using cold maceration technique. 300g of the powder was weighed on an electronic weighing balance, and was then soaked in 800 ml of methanol in an amber-colored bottle for two days with occasional shaking.

The mixture was filtered on the third day using a gauze cloth, and a fine filtrate was attained using Whatman No 1 filter paper (9 cm wide) in a Buchner funnel. The filtrate was then concentrated using a Bilchiortoratory vapor R-200 (BilchiLaboratechnik, Flawil, Switzerland) into a semi-solid extract in an oven set at 50 °C.

The dry extract was stored at 4 °C in desiccators until the immunomodulatory experiment bioassays were ready to be carried out. The percentage yield of the extract was determine. Concentrated stock solution of the Haematostaphisbarteri was prepared by dissolving various weights (5, 10, 20 and 30 g) of leaf extract in 40 mL of distilled water to make a working concentration of 50mg /mL. Control drugs were cyclophosphamide and Lamovidine (20 mg) [10].
Drug Administration
Normal saline was used as negative control, cyclophosphamide was used as an immunosuppressant drug and Lamovidine BP (20 mg) tablet was used as positive control because of its ability to boost immune system especially in HIV and Hepatitis B patients, and administered via intraperitoneal route (i.p) to the rats. All chemical and reagents used were of analytical grades, and were checked for purity prior to use there were no impurities and did not expired before the experiment.

Laboratory Animals Used in the Study
Thirty (30) disease-free Swiss albino rats aged between 6 and 8 weeks of opposite sex were randomized into five experiment groups (N=30, n=5). They were maintained under standard laboratory conditions and temperature (25°C±1°C) and light /dark cycle (12 hours light: 12 hour dark cycle). They were fed with rat pellets and clean water *ad libitum*. Male rats were kept in separate cages from their female counterparts, before and during the time of the study to avoid conception during the study time. The animals were acclimatized for 2 weeks before the experiment was carried out. The protocol used was in compliance with the International Bio-safety Guidelines [11], and the International guidelines for the care and use of laboratory animals in Biomedical Research [12].

Group Treatment and Dosing of Animals
Rats in the normal control group (Group I) received normal saline throughout the 2 weeks of study period. Rats in the group (Group II-VI) were treated with an immunosuppressant drug (cyclophosphamide) at class of 200 mg /kg body weight (b.w) on day zero of the study prior to treatment by intraperitoneal injection [13]. However, Group II rats were given Lamuvidine 20 mg (as positive control drug), while Groups III-VI were given extract doses of *H. barteri* at 100, 200, 300, and 400 mg/kg body weight respectively.

Determination of Complete Blood Count (CBC)
Two (2) ml of fresh blood was drawn by intra-ventricular puncture from each of the animals in groups I, II, III, IV, V and VI on the 14th day into ethylene-diamine-tetra-acetic acid (EDTA) containers. It was then analyzed at the Sancta Maria Clinic Bali (USAID/Fhi360 affiliate) using CD4 machine for antibody counting and Mindray (3800 BC model) machine for the complete and differential blood cell counts.

Determination of Neutrophils Adhesion
On day the 14th day after administration of the extract, peritoneal fluids from rats in GroupI, II, III, IV, V and VI were obtained by ventricular puncture and incubated to analyzed for total leucocytes white blood cell counts (WBC) or TLC and differential blood cell count with 80mg/mL of nylon fibers for 15 min at 37°C. The incubated blood samples were then analyzed for TLC and PLC. The product of TLC and % neutrophils was given as the neutrophils index (NI) of blood samples. % neutrophils adhesion was calculated as follows:

\[ \%NI = \left( \frac{NIu - NIt}{NIu} \right) \times 100 \]

Where, 
NIu = neutrophils index of untreated blood samples,
NIt=neutrophils index of treated blood samples.

Determination of Delayed-Type Hypersensitivity (DTH) Responses
On day 7 of the study, all the Group III, IV, V and VI rats were primary injected by intraperitoneal injecting. 1 mL suspension containing 1x10^8 of sheep red blood cells (SRBC) was administered into the right hand footpad .The contractual left paw also received on equal volume of 0.1% phosphate buffer saline (PBC). The administration of *Haematostaphis barteri* methanol leaf extracts was continued until the 14th day. On the 14th day, the animals were challenged by subcutaneously injecting 0.1x10^8 SRBCS into the left hand footpad of the rats. The exert of delayed type hypersensitivity (DTH) response in the rats was determined by measuring the footpad thickness of the right hand paw and the left hind paw and expressed as mean percent increment in thickness/edema as shown below:

\[ \% DTH = \frac{LFca - RFcr \times X}{LFca} \times 100 \]

Where, 
LFca = Left footpad challenged with antigen
RFcr = Right footpad of control [14].

Statistical Analysis
The data obtained were expressed as mean ± SEM (standard error mean). Results were analyzed by one–way ANOVA followed by Dennett’s test (Graph pad prism software version 7, 2016). The value of p < 0.05 (at α equal to 95%) was considered statistically significant.
Results
The results obtained from this study were recorded in tables and graphs as shown below: Table 1:

<table>
<thead>
<tr>
<th>Animal (mg/kg)</th>
<th>Initial weight</th>
<th>WAI</th>
<th>WAT</th>
<th>Weight Gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I NC</td>
<td>17</td>
<td>25.32</td>
<td>17.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Group II (10mg/kg)</td>
<td>15.5</td>
<td>13.52</td>
<td>15.52</td>
<td>0.02</td>
</tr>
<tr>
<td>Group III (100mg/kg)</td>
<td>24.0020.62</td>
<td>24.52</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>Group IV (200mg/kg)</td>
<td>23.4</td>
<td>19.12</td>
<td>25.32</td>
<td>1.92</td>
</tr>
<tr>
<td>Group V (300mg/kg)</td>
<td>24.52</td>
<td>15.12</td>
<td>19</td>
<td>2.1</td>
</tr>
<tr>
<td>Group VI 400mg/kg</td>
<td>20.21</td>
<td>18.2223.40</td>
<td>3.19</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Effect of Various Concentrations of *H. Barteri* leaf Methanol Extract on Neutrophils Adhesion of Swiss Albino Rats

<table>
<thead>
<tr>
<th>Animal (mg/kg)</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>Neutrophils Adhesion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (normal saline)</td>
<td>115±3.04</td>
<td>161±3.02</td>
<td>102.4±2.02</td>
</tr>
<tr>
<td>Group II (Lamuvidine; 20)</td>
<td>78±2.01*</td>
<td>64±2.01*</td>
<td>22.2±1.04*</td>
</tr>
<tr>
<td>Group III (HBLME;100)</td>
<td>71±1.02*</td>
<td>52±2.01*</td>
<td>28.4±1.04*</td>
</tr>
<tr>
<td>Group IV (HBLME;200)</td>
<td>65±2.01*</td>
<td>47±1.02*</td>
<td>32.1±2.01a</td>
</tr>
<tr>
<td>Group V (HBLME;300)</td>
<td>62±2.01*</td>
<td>40±1.02*</td>
<td>37.3±2.01*</td>
</tr>
<tr>
<td>Group VI (HBLME; 400)</td>
<td>58±2.01*</td>
<td>38±1.02a</td>
<td>40.1±1.02b</td>
</tr>
</tbody>
</table>

HBLME (*H. barteri* leaf methanol extract), *p <0.05* when compared to the normal saline group, while *b* p <0.05 when compared to the cholesterol group, n=5, numbers proceeded by the same alphabets are statistically significant at p<0.05.

From the table 2 above, neutrophils profile of the mice decreased in dose dependent fashion except in Group I that increased in all the treated groups after five weeks. These results were comparable with the normal control as well as the standard drug (Lamovidine; 20 mg) Group II.

Table 3: Effect of Different Doses of *H. Barteri* leaf Methanol Extract on the of Humoral Antibody Response to SRBC as Determined by Hemagglutination Antibody Titres in Swiss Albino Rats

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mean Hemagglutination Antibody titre±SEM HA units/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Normal saline</td>
<td>2.82±0.04a</td>
</tr>
<tr>
<td>Group II Lamuvidine 20mg/kg</td>
<td>3.82±0.04a</td>
</tr>
<tr>
<td>Group III HBLME 100 mg/kg</td>
<td>3.92±0.02b</td>
</tr>
<tr>
<td>Group IV HBLME 200 mg/kg</td>
<td>5.52±0.02b</td>
</tr>
<tr>
<td>Group V HBLME 300 mg/kg</td>
<td>7.62±0.02b</td>
</tr>
<tr>
<td>Group VI HBLME 400mg/kg</td>
<td>10.8±2.01a</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean for five rats, numbers followed by the same alphabets are statistically significant at p<0.05 (one-way ANOVA).
Blood parameters increase showed increase in antibody production in the rats. Table 5:

Table 4: Effect of H. barteri Leaf Methanol Extract on Blood Parameters of Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Group 1</th>
<th>100mg/kg Group 2</th>
<th>200mg/kg Group 3</th>
<th>300mg/kg Group 4</th>
<th>400mg/kg Group 5</th>
<th>Chemiron Group 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>29.5</td>
<td>19.6</td>
<td>20.4</td>
<td>30.2</td>
<td>33.8</td>
<td>28.5</td>
</tr>
<tr>
<td>MCV</td>
<td>30.1</td>
<td>36.5</td>
<td>35.4</td>
<td>33.5</td>
<td>32.1</td>
<td>30</td>
</tr>
<tr>
<td>PCV</td>
<td>34.2</td>
<td>34.5</td>
<td>33.1</td>
<td>32.2</td>
<td>31</td>
<td>29.2</td>
</tr>
<tr>
<td>MCH</td>
<td>37.7</td>
<td>28.2</td>
<td>22.2</td>
<td>20.1</td>
<td>18</td>
<td>36.9</td>
</tr>
<tr>
<td>MCHC</td>
<td>41.1</td>
<td>5</td>
<td>6.4</td>
<td>7.2</td>
<td>6.4</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard error of the mean for five rats, numbers followed by the same alphabets are statistically significant at p<0.05 (one-way ANOVA).

Discussion

In table 1 above, anemia is induced in the rats as a result of decreased packed cell volume and other blood parameters, however, there was significant weight gain in the animals as a result of treatment of the rats with H. barteri extract in vivo (Table 1). This showed that the leaf extract of the plant possessed blood building and immune modulator properties in the rats. Neutrophils are polymorph nuclear lymphocytes produced daily by the body in large quantities produced by the bone marrow each day and reside mainly in the peripheral vasculature [15]. The multi-lobe nucleus aids neutrophils in movement through tight gaps formed between other cells or within narrow pores in the extracellular matrix (ECM); the option for the cell to align portions of its nucleus in a linear fashion facilitates neutrophils migration from the blood into tissue better than cells with larger spherical nuclear regions [16,17].

From our study, the plant extract has significant effect on neutrophils in dose-dependent fashion. This result further revealed that plant has the capability of modulating the immune system and boosting blood in the rats. Neutrophils have been viewed as swift, short-lived effector cells of the immune system, which serve solely to perform phagocytises, recruit other effector cells and commit cell suicide via apoptosis. Moreover, neutrophils have been considered the major innate immune cells responsible for tissue damage and harm to the host tissues; however, this is far from a complete picture, as neutrophils have even more flexibility to adjust to the prevalent micro environmental conditions in a distressed tissue (Table 2) [18, 19].

Blood is a reflector of the overall animal health and provides important profiles for the toxicological impact on animal tissues. The result of our hematological study revealed significant decreases in red blood cell (RBC), packed cell volume (PCV), haemoglobin concentration (HB), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV) (p≤0.05) values of the treated mice when compared with the control (Table 4). The non significant effects of the extract on RBC could mean that the balance between the rate of production and destruction of red blood corpuscles (erythropoiesis) was not affected negatively [20].

MCV and MCHC relate to individual red blood cells while HB, RBC, and PCV are associated with the total population on red blood cells. The absence of observable...
significant effect of the extract on these parameters may be an indication that neither the incorporation of haemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells was altered [21]. This obviously contradicts the results in table 4 above where progressive decrease in these parameters were witnessed in all the treated groups. An adequate haemoglobin percentage is needed for the normal physiology of animals, which depends on the erythrocyte count. *H. barteri* may have induced inhibition of RBC formation that reduces the RBC counts and leads to a decrease in Hb contents which was observed in the animals. The leaf methanol extract of *H. barteri* showed a dose-dependent in delayed type hypersensitivity response after 24 hours in all the treated groups when compared to the control groups (Table 5).

The dose dependent and reduction in number of RBC as seen from our study may occur due to the hemolytic activity of the extract on the animals or it could also be as a result of a suppressive action of the extract on erythropoiesis as well as the presence of Cyanogentic glycosides and saponins in the extract as was reported by Ukwubile et al. [22] as saponins are class of glycosides and are known to cause haemolysis of red blood cells.

**Conclusion**

This study showed that the administration of methanol extract of *H. barteri* significantly increases the weights of the animals. The mean body weight of animals increased in a dose dependent manner. Also from the result obtained, it could be seen that the extract have significant effect on PCV, HB, RBC, MCV and MCHC following the administration of *H. barteri* extract, but there is significant increase on WBC. Alterations in weight and hematological parameters observed in the present study point to selective toxicity of *H. barteri* leaf extract on the immune system of experimental animals. Hence, the plant possessed immune modulator property in rats but may not be perfectly safe as an oral medication at the doses investigated because of the presence of some glycosides such as cyanogenic glycosides and saponins, as well as alkaloids, in the leaf extract of the plant. Therefore, for ethno-medicinal prescription, it is recommended that ways to remove these poisonous glycosides from the leaf of the plant during extraction should be studied to guarantee the use of the leaf extract in disease management traditionally.

Figure 5: Medicinal Plant Research Laboratory for Preliminary Screening of Plant Extract

Figure 6: Immunology Laboratory Where Most of the Experiments Were Carried Out

Figure 7: Reflotron Plus Apparatus for the Analysis of Some Biochemical Parameters
Acknowledgement

The authors are thankful to Mr Livinus Tam of Sancta Maria Clinic, Miss Rita Taikongo and Mr Bulus Auta of University of Jos, for their individual helps in this research.

Ethical Issue

The animals used in this research work were in accordance with animal in research guidelines of the National Health Institute and the guidelines of animal use of the University of Jos Nigeria.

References


