Haematinic Effects of Aqueous Extract of *Lophira lanceolata* Leaves in Phenylhydrazine- induced Haemato-toxicity in Wistar Rats

Abstract

Aim: The haematinic activity of the aqueous extract of *Lophira lanceolata* leaves was investigated using rat model of phenylhydrazine- induced anaemia.
Methods and Results: Red Blood Cell (RBC) count, Haemoglobin (Hb) concentration and Packed Cell Volume (PCV) were analysed as indices of anaemia. Following phenylhydrazine administration to rats at a dose of 10mg/kg for 8 days, a significant decrease ($P<0.05$) in the haematological parameters was observed indicating anaemia. However, treatment with graded doses (200, 400 and 800 mg/kg) of the aqueous extract of *Lophira lanceolata* leaves produced a significant ($P<0.05$) increase in the RBC count, Hb concentration and PCV time- and dose-dependently.
Conclusion: It was concluded that *Lophira lanceolata* leaves possess haematinic activity, making it useful in the management of anaemia.

Key words: Haematinic, *Lophira lanceolata*, Phenylhydrazine, Haemato- toxicity, Wistar rats

1.0 INTRODUCTION

Anaemia is the most prevalent nutritional deficiency disorder in the world. WHO defines anaemia as the condition in which the haemoglobin content of blood is lower than normal as a result of deficiency of one or more essential nutrients [1]. According to WHO, more than 2 billion people worldwide suffer from anaemia with iron deficiency responsible for 50% of the cases [2]. Anaemia occurs in women of reproductive age anaemia due to menorrhagia while in pregnancy the excess need of iron usually results to anaemia [3]. Iron is important for formation of haemoglobin, myoglobin, cytochrome oxidase, peroxidase and catalase. The total quantity of iron in the body averages 4 to 5 grams, about 65 percent of which is in the form of haemoglobin [4]. A man excretes about 1 mg of iron each day, mainly into the faces. For a woman, the menstrual lost of blood brings the iron loss to a value of about 2mg/day [5].

Through the ages man has learnt to take advantage of the many resources placed at his disposal by nature to meet his essential needs in all fields. As important reserves and sources of abundance, natural resources are indispensable for socio-economic development. According to Gbile [6], the diversity of the flora in Africa partly explains the strength of traditional medicine.

Some Africans resort to orthodox methods of treatment due to mitigating circumstances such as high cost of drugs, poverty and poor nutrition [7]. Many herbs have been used locally for the management of anaemia and one of such plants is *Lophira lanceolata.* Therefore, this study was undertaken to determine the haematinic potentials of the aqueous leaf extract of *Lophira lanceolata* in rats to provide a scientific basis justifying the use of the plant in traditional medicine for the acclaimed treatment of anaemia.

Lophira lanceolata is a tree of the tropical and sub- tropical regions. It is a common tree in Cameroun, Nigeria and Sudan. It often grows gregariously on fallow land at the edge of forests. It is a tree of 8 to 10 m tall, straight or twisted, with leaves alternate, clustered at the end of short straight branches, glabrous, bright and blade oblong-lanceolate. The bark surface is corky grey [8]. *Lophira lanceolata* is used in traditional medicine to treat several illnesses. The decoction of the fresh leaves is administered orally against headaches, dysentery, diarrhoea, cough, abdominal pains and cardiovascular diseases. It is also used on skin to cure wounds [8].

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and drugs

All chemicals/drugs used were purchased locally.

2.1.2 Animals

Adult Wistar rats of either sex weighing 180–220g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

2.2 Methods

2.2.1 Plant Collection and Identification

The leaves of *Lophira lanceolata* were collected from a natural habitat in Okpella Area of Edo State, Nigeria. The plants were identified at the herbarium unit of the Department of Biology, University of Benin, Benin-City, Nigeria and voucher specimens were deposited for future references.

2.2.2 Preparation of Extracts

The leaves of *Lophira lanceolata* were shade- dried for seven (7) days and pulverized using an electric blender. Two thousand (2000) gram of the pulverized leaves was soaked in distilled water for 72- hours. The resulting mixture was filtered using Whatman filter paper (Size No1) and the extract (referred to as LLAE henceforth) was concentrated using a free- dryer.

2.2.3 Acute Toxicity Study

The oral median lethal dose (LD_{50}) of the extract was determined in rats according to the method of Lorke [9].

2.2.4 Experimental Design

A total of 25 adult wistar rats were weighed and divided into 5 groups of 5 animals each and treated as follows:

Group 1: received distilled water (1 ml) daily (normal control),
Group 2: Anaemic and received distilled water (1 ml) daily (anaemic control),
Group 3: Anaemic and received LLAE at 200 mg/kg body weight/day
Group 4: Anaemic and received LLAE at 400 mg/kg body weight/day
Group 5: Anaemic and received LLAE at 800 mg/kg body weight/day

Rats were made anaemic by oral intubations of phenylhydrazine (10 mg/kg body weight) daily for 8 days. Rats that developed anaemia with haemoglobin concentration lower than 13 g/dl were selected for the study.

2.2.4.1 Haematological investigation

Blood was collected by ocular puncture after overnight fast. The blood was collected before induction of anaemia, after induction of anaemia with PHZ and during 1, 2, and 3 weeks of treatments. The haemoglobin concentration (Hb), red blood cell count and pack cell volume (PCV) were assessed.

2.2.5 Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of

variance (ANOVA) which was followed by Duncan test and difference between means at P > 0.05 were considered significant.

3.0 RESULTS

3.1 Acute Toxicity

The results of acute toxicity studies showed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of aqueous extract of *Lophira lanceolata*. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg.

3.2 Effect of the Administration of Aqueous Extract of *Lophira lanceolata* Leaves on Haematological Parameters of Wistar rats

Tables 1, 2, 3 and 4 shows the changes in haematological parameters of rats treated with phenylhydrazine and the extract. Following the administration of phenylhydrazine (PHZ) for 8 days, the RBC, Hb, and PCV of rats decreased significantly ($P<0.05$) (Table 1) giving rise to macrocytic anaemia. After one week of treatment of the anaemic rats in groups 3, 4, and 5 with *Lophira lanceolata* extract, there was no significant ($P>0.05$) changes in RBC, Hb, and PVC compared to the anaemic control (group 2) (Table 2). However, at 2 week- post treatment, The Hb, RBC and PCV significantly ($P<0.05$) and dose- dependently increased with 800mg/ kg of the extract taking the values to near normal range (Table 3) Similarly, at 3 week- post treatment, the Hb, RBC and PCV significantly ($P<0.05$) and dose- dependently increased but with 800mg/ kg of the extract this time taking the values to the normal range (Table 4). Figures 1-3 shows the changes in Hb, PCV and RBC per group during the 3- week treatment regimen. The Hb of the treated rats did not show significant changes within the first week of the experiment but there was a steady increase up to 3 week- post treatment (Figure 1). Similar observations were made for RBC and PCV (Figures 2 and 3 respectively).

Table 1: Effect of Phenylhydrazine on some Haematological Parameters after 8- days of Administration

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Hb (g/ dl)	16.31±1.11^{b}	11.23±1.11^{a}	12.06±1.13^{a}	11.17±1.23^{a}	11.33±1.25^{a}
RBC (x10^{6}µg /l)	6.29±0.80^{b}	3.29±0.81^{a}	3.07±0.33^{a}	3.05±0.44^{a}	3.07±0.53^{a}
PCV (%)	51.11±2.33^{b}	43.23±3.17^{a}	42.11±1.13^{a}	42.13±2.26^{a}	43.12±3.01^{a}

Data are presented as mean ± SD. Data was analyzed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) within the rows. Group 1: Control received 1ml distilled water. Groups 2-5: received 10mg/ kg phenylhydrazine for 8 days

Table 2: Effect of the Aqueous Extract of *Lophira lanceolata* on the Haematological Parameters of Wistar rats one week- post treatment

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Hb (g/ dl)	16.58±2.01^{b}	11.73±1.01^{a}	12.16±1.13^{a}	12.22±1.78^{a}	12.05±1.33^{a}
RBC (x10^{6}µg /l)	6.67±1.02^{b}	3.03±0.72^{a}	3.67±0.42^{a}	3.53±0.79^{a}	3.77±0.30^{a}
PCV (%)	51.55±2.13^{b}	43.43±2.42^{a}	44.21±1.32^{a}	45.23±3.21^{a}	45.22±2.99^{a}

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) within the rows. Group 1: Normal control and received 1ml distilled water. Group 2: Anaemic control and received 1ml distilled water. Group 3: Anaemic and received 200 mg/ kg LLAE, Group 4: Anaemic and received 400 mg/ kg LLAE, Group 5: Anaemic and received 800 mg/ kg LLAE

Table 3: Effect of the Aqueous Extract of *Lophira lanceolata* on the Haematological Parameters of Wistar rats two week- post treatment

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Hb (g/ dl)	16.98±1.45^{b}	11.43±1.25^{a}	12.12±1.32^{a}	14.28±1.23^{ab}	16.12±2.21^{b}
RBC (x10^{6}µg /l)	6.29±0.80^{b}	3.13±0.52^{a}	3.68±0.48^{a}	4.31±0.99^{ab}	5.43±0.98^{ab}
PCV (%)	51.11±2.33^{b}	42.08±2.44^{a}	44.23±2.32^{a}	48.15±2.73^{ab}	50.45±2.91^{b}

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) within the rows. Group 1: Normal control and received 1ml distilled water. Group 2: Anaemic control and received 1ml distilled water. Group 3: Anaemic and received 200 mg/ kg LLAE, Group 4: Anaemic and received 400 mg/ kg LLAE, Group 5: Anaemic and received 800 mg/ kg LLAE

Table 4: Effect of the Aqueous Extract of *Lophira lanceolata* on the Haematological Parameters of Wistar rats three week- post treatment

Treatment	Group 1	Group 2	Group 3	Group 4	Group 5
Hb (g/ dl)	17.26±1.05^{b}	10.86±1.42^{a}	14.12±1.18^{ab}	16.99±1.42^{b}	17.01±2.06^{b}
RBC (x10^{6}µg /l)	6.97±1.01^{b}	3.25±0.63^{a}	5.31±0.41^{ab}	5.81±0.78^{ab}	6.93±1.01^{b}
PCV (%)	51.85±3.26^{b}	41.19±1.32^{a}	46.25±2.43^{ab}	47.31±1.23^{ab}	50.96±2.08^{b}

Data are presented as mean ± SD. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) within the rows. Group 1: Normal control and received 1ml distilled water. Group 2: Anaemic control and received 1ml distilled water. Group 3: Anaemic and received 200 mg/ kg LLAE, Group 4: Anaemic and received 400 mg/ kg LLAE, Group 5: Anaemic and received 800 mg/ kg LLAE

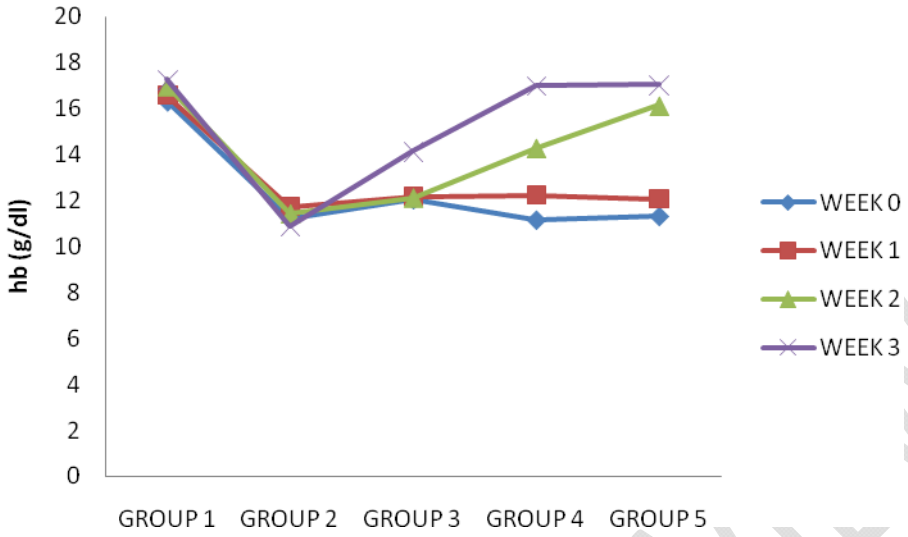

Figure 1: Changes in Haemoglobin concentration (g/dl) across the groups within the 3-week treatment period. Group 1: Normal control and received 1ml distilled water. Group 2: Anaemic control and received 1ml distilled water. Group 3: Anaemic and received 200 mg/ kg LLAE, Group 4: Anaemic and received 400 mg/ kg LLAE, Group 5: Anaemic and received 800 mg/ kg LLAE

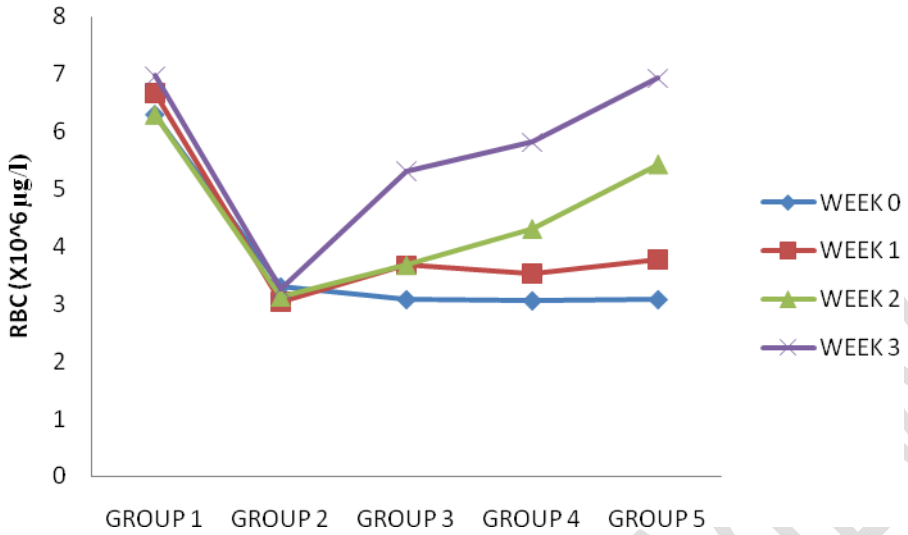

Figure 2: Changes in RBC concentration ($x10^6$µg /l) across the groups within the 3- week treatment period. Group 1: Normal control and received 1ml distilled water. Group 2: Anaemic control and received 1ml distilled water. Group 3: Anaemic and received 200 mg/ kg LLAE, Group 4: Anaemic and received 400 mg/ kg LLAE, Group 5: Anaemic and received 800 mg/ kg LLAE

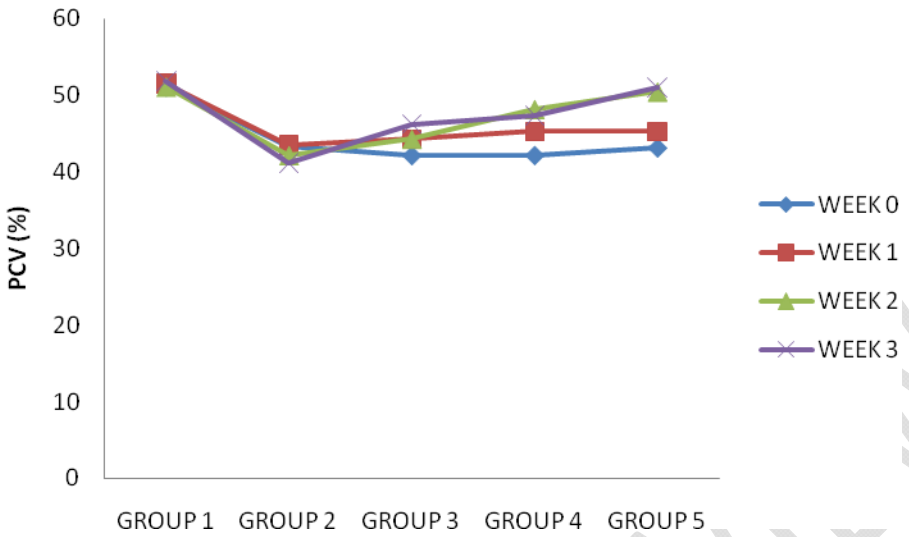

Figure 3: Changes in PVC (%) across the groups within the 3- week treatment period. Group 1: Normal control and received 1ml distilled water. Group 2: Anaemic control and received 1ml distilled water. Group 3: Anaemic and received 200 mg/ kg LLAE, Group 4: Anaemic and received 400 mg/ kg LLAE, Group 5: Anaemic and received 800 mg/ kg LLAE

4.0 DISCUSSION

Phenylhydrazine produces both aryl and hydroxyl radicals when incubated with rat liver microsomes [10] and oxidized by hydrogen peroxide at pH 7.4 and 37^{o}C [11]. The radicals induce oxidative stress in the red cell membrane resulting in haemolysis, which may have resulted via lipid peroxidation [12], [13], [14], [15]. Sub-chronic intoxication of rats with PHZ (10 mg/kg/day for 8 days) results in marked haemolytic anaemia characterised by decreased RBC, Hb and PCV [16]. Similar results were obtained in our study when experimental rats were administered PHZ (Table 1).

The main function of the RBC is the transportation of oxygen to tissues in the body. As such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study PHZ altered the function of RBC by haemolysis characterised by decreased levels of RBC, Hb and PCV. However, our results indicated that the aqueous leaf extract of *Lophira lanceolata* markedly increased the concentration of haemoglobin, red blood cell count and the packed cell volume beginning from 2 week after treatment. It was also observed that the recovery of the treated groups was dose related with the highest dose of 800 mg/kg effecting the highest change. At the third week of the experiment, treatment of anaemic rats with *Lophira lanceolata* increased the RBC, Hb and PCV to near normal values. *Lophira lanceolata* is the well known source of minerals, Sterols, Proteins and other vitamins. These chemical constituents of the plant might be responsible for the haematinic activity.

5.0 Conclusion

The oral administration of the aqueous leaf- extract of *Lophira lanceolata* significantly improved the haematological parameters affected by phenylhydrazine administeration. From this study, it was inferred that *Lophira lanceolata* leaves possess haematinic potential, therefore it could be useful in the management of anaemia.

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