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Haematinic Potentials of the Leaf Extract of *Aleurites fordii* on Normal and Anaemic Wistar Albino Rats

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Abstract : This study was designed to investigate the effects of ethanolic leaf extract of *Aleurites fordii* on haematological and biochemical parameters of normal and anaemic induced albino Wistar rats. The leaf extract was given per os in graded doses of 100mg/kg, 200mg/kg and 400mg/kg body weight to nine groups of albino Wistar rats (n = 45) of five per group. Anaemia was induced in group 2-6 following intraperitoneal treatment with cyclophosphamide at 30mg/kg b.w for three days. Groups 4,5 and 6 then received 100mg/kg, 200mg/kg and 400mg/kg body weight of methanolic extract of A. fordii while group 3 received blood tonic(Chemiron) following anaemic induction. Group 2 received only the cyclophosphamide. After 7 and 14 days, blood samples were collected for haematological and biochemical analysis. At concentrations of 100, 200 and 400mg /kg body weight of the rats, the extract significantly increased the blood parameters (packed cell volume, haemoglobin level red blood cell count and platelet counts) of groups treated with Cyclophosphamide and also untreated groups after seven and fourteen day. our findings suggest that the ethanolic extract of *Aleurites fordii* posses haematinic potential and could be compared favorably with Chemiron®; a standard haematinic drug in the treatment of anaemia.
Keywords : *Aleurites fordii*, Anaemia, Haematinic, cyclophosphamide, biochemical, haematology.

Introduction

Developing nations are known for their application of herbs in the treatment of various diseases and in boosting blood levels in man[1]. World Health Organization in 2001 estimated that about 80% of people worldwide rely on some sort of traditional medicine for primary health care[2]. Anaemia which is a reduction in haemoglobin and haematocrit is high in frequency in developing countries. Some Africans resort to orthodox methods of treatment due to mitigating circumstances such as high cost of drugs, poverty and poor nutrition[3]. Interestingly, some drugs that are essential in modern medicine, as most prescribed drugs, were initially synthesized from herbs, trees and shrubs in form of plant extracts[4]. In recent times, advances in laboratory and clinical research have improved the value of orthodox medical practice. Quality control and knowledge of active ingredients and chemical composition of plant extracts are currently studied to understand and modify plant content with the view of knowing their interactions and effects on cellular metabolism. Plants are harnessed for their antioxidative, anti-lipid, cholerectic, anti-inflammatory, membrane stabilizing, hepatoprotective, immunomodulatory and haematoprotective property[5].

Aleurites fordii, is a monoecious, deciduous, wood oil milky juice tree found mostly in the tropical country. It is found in China known as Tung oil. In Nigeria, its various uses include in making food such as soups and corn meal and in the making of house hold materials. Traditionally, this vegetable is added as a rich source of mineral and vitamins in sauces and soups. Hence, its name 'Hospital too far'. Already, research has reported the antibacterial effects of the fruit extract while the seed oil is applied externally in the treatment of wounds, burns and parasitic skin infestations [6]. Locally, the use of this plant as a blood tonic has not been validated and this informed this work. Therefore, this study was designed to investigate the effect of the leaf extracts of A*leuritesfordii* on biochemical and haematological parameters of normal and anaemic albino wistar rats.

Materials and Methods

Plant Material

The leaf of *Aleurites fordii* was collected from its natural habitat at Nsukka, Enugu State Nigeria and was authenticated by a taxonomist in the department of the Plant Science and Biotechnology University of Nigeria Nsukkka Campus.

Plant extraction

The plant leaves weighing seven hundred grams was dried at room temperature under shade and pulverized using an electric blender. The pulverized substance was weighed to obtain 1kg powder. It was then extracted with 70% ethanol using Soxhlet extractor. The ethanolic extract was concentrated to dryness using a hot-air oven at 40 ^{0}C.

Acute toxicity testing

The determination of LD50 was done as described by Lorke 1983[7].

Experimental Animals

Forty –five Albino Wistar rats weighing between 150-200gms were selected for the study. They were allowed to acclimatize for two weeks at the Animal Research Laboratory of the University of Nigeria Enugu Campus and were fed with standard pellets and water *ad libitum* under standard conditions. All animals were handled according to recommended international guidelines for the care and management of laboratory animals.

Experimental Design

A total of 45 male albino Wistar rats were randomly allotted into nine groups of five rats each. Group one (1) animals served as control and was fed with only water and standard pellets. Groups 2-6 were induced with Cyclophosphamide to produce anaemia at the dose of 30 mg/kg body weight (per os) for three days while groups 7-9 were fed with different doses (100mg/kg b.wt, 200mg/kg b.wt, 400mg/kg b.wt) of the extract.

Induction of Anaemia

Anaemia was induced using 30mg/kg body weight of cyclophosphamide (Fisher scientific company New Jersey USA) intraperitoneally.

Collection of blood and laboratory analysis

Five ml of blood was collected from each rat.. All laboratory procedures are as described by Dacie and Lewis[8].

Statistical Analysis

Data generated was analyzed with Statistical Package for Social Science software version 20 (SPSS-IBM). Data was presented as mean ± standard error of mean. One way analysis of variance was used as the statistical technique followed by Duncan's post hoc for comparism. P values less than 0.05 were considered significant.

Result

The phytochemical screenings are as presented in table1. Tables 2 and 3 shows the result of the quantitative levels of vitamins and mineral content present in the ethanolic leaf extract of *A. fordii*. The acute toxicity study was determined to be 2,400mg/kg b.wt. The baseline studies (table 4) showed the mean values of all parameters to be normal and animals stable. The markedly decrease in the levels of blood parameters of cyclophosphamide treated groups compared to non induced control group was as a result of successful induction of anaemia (table 5). The ethanolic extract of *A. fordii* was orally administered to the treated and non treated rats at different doses of 100, 200 and 400mg/kg body weight. There was significant difference in the levels of RBC, PCV, Hb and platelet counts of all groups that were not treated with cyclophosphamide and those treated with cyclophosphamide when compared to normal group ($p<0.05$). However, group with the highest dose of extract (400mg/kg b.wt)recorded a significant increase in serum enzyme levels ($P<0.05$).. All the groups had normal liver architecture except the groups that received 400mg/kg bwt. of the ethanolic leaf extract of A. fordii (Groups 6 and 9). These groups had vacuolar degeneration and hepatocyte necrosis.

Table 1.The phyto-constituents of the ethanolic extract of *Aleurites fordii.*

Phytoconstituent	**Percent**
Saponins	7.17
Alkaloids	10.66
Flavonoids	41.87
Glycosides	0.21
Tannins	0.18
Carotenoids	0.51
Anthraquinone	Trace

Table 2.Vitamin content of extract of *Alerites fordii*.

Vitamin	**Quantity**
Vitamin A	5756.74 IU
Vitamin B6	6.36mg/100g
Vitamin B9	9.47mg/100g
Vitamin B12	466920 ug/100g
Vitamin C	85.99mg/100g
Vitamin E	56mg/100g

Table3. Quantitative distributions of the mineral content of *A.fordii.*

Mineral	**Quantity**
Copper	0.18mg/100g
Zinc	0.34mg/100mg
Selenium	1.55mg/100g
Iron	3.73mg/100g
Phosphorus	2.23mg/100g

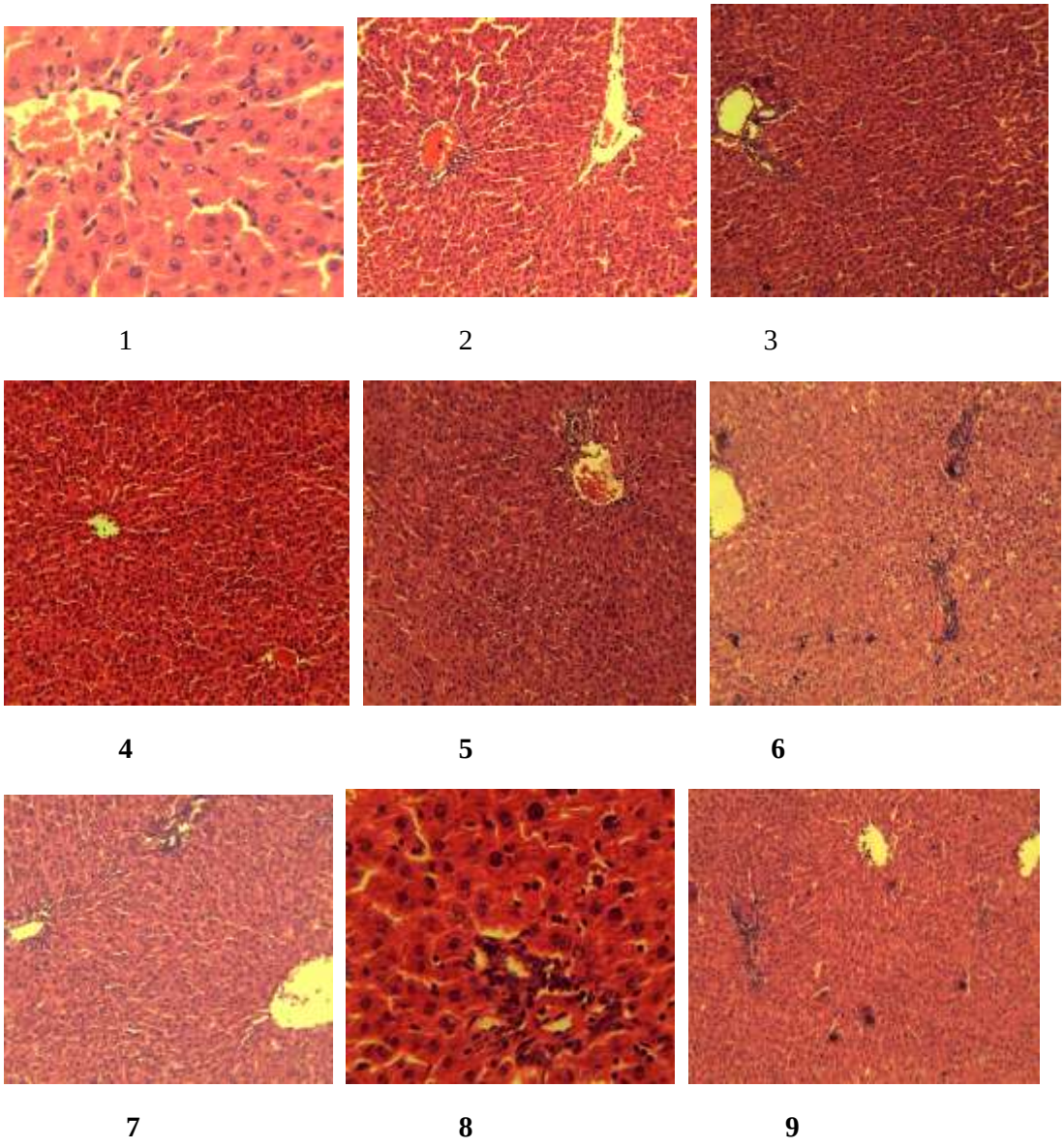

Figure 1 Photomicrograph of histology of liver sections of treated (2-6) and untreated groups(1,7-9) (H and E X100)

Table 4. Baseline mean values of haematological and biochemical parameters of rats fed with *Aleurite. fordii*

Group	Parameters (Mean ± Standard Error of Mean)								
	ALT (iu/L)	**AST ((iu/L)**	**RBC(10^6/µ)**	**PCV (%)**	**HGB (g/dl)**	**Platelet (10^3/µl)**	**TWBC(10^3/µl)**	**Neutro (%)**	**Lympho (%)**
1 (Normal control)	27.60 ± 2.89	63.60 ± 3.44	8.14 ± 0.28	46.32 ± 1.73	13.70 ± 0.58	6.62 ± 0.40	13.34 ± 0.55	29.20 ± 1.62	70.80 ± 1.62
2 (Anaemic no treatment)	28.20 ± 1.77	66.20 ± 5.23	7.92 ± 0.25	44.10 ± 1.27	13.52 ± 0.40	6.83 ± 0.24	13.20 ± 0.93	30.00 ± 2.82	70.00 ± 2.83
3 (Anaemic + chemiron[@])	23.90 ± 1.07	69.00 ± 1.79	7.99 ± 0.27	45.08 ± 1.41	13.10 ± 0.37	6.06 ± 0.22	13.26 ± 0.74	28.40 ± 3.12	71.60 ± 3.12
4 (Anaemic +100mg/kg extract)	25.24 ± 5.66	69.60 ± 3.44	8.22 ± 0.23	47.98 ± 1.46	13.64 ± 0.33	6.19 ± 0.52	12.90 ± 0.94	29.20 ± 3.89	70.80 ± 3.89
5 (Anaemic +200mg/kg extract)	26.60 ± 2.38	68.00 ± 5.79	8.06 ± 0.20	45.54 ± 0.96	13.12 ± 0.19	6.09 ± 0.36	12.46 ± 0.67	28.40 ± 3.19	71.60 ± 3.19
6 (Anaemic +400mg/kg extract)	25.40 ± 0.93	68.40 ± 4.26	8.14 ± 0.29	47.66 ± 0.86	13.34 ± 0.33	6.69 ± 0.47	12.78 ± 0.68	27.60 ± 2.14	72.40 ± 2.14
7 (Normal + 100mg/kg extract)	24.50 ± 2.04	68.60 ± 4.95	8.21 ± 0.44	6.28 ± 1.18	13.74 ± 0.41	6.99 ± 0.24	12.90 ± 0.69	31.60 ± 4.17	68.40 ± 4.17
8 (Normal + 200mg/kg)	25.50 ± 3.00	68.00 ± 3.07	8.16 ± 0.33	45.62 ± 1.06	13.40 ± 0.35	6.11 ± 0.10	13.00 ± 0.53	27.20 ± 3.44	72.80 ± 3.44
9 (Normal + 400mg/kg)	25.40 ± 1.31	69.80 ± 3.48	8.66 ± 0.18	47.94 ± 2.59	13.36 ± 0.55	6.76 ± 0.36	13.06 ± 0.78	32.00 ± 2.61	68.00 ± 2.61

The values are expressed as mean ±SEM.

Table 5. Effect of induction with cyclophosphamide on Mean values serum enzymes and Haematologic indices of rats

	Parameter (Mean ± Standard Error of Mean)								
Group	**ALT (iu/L)**	**AST ((iu/L)**	**RBC (10^6/µl)**	**PCV (%)**	**Hgb (g/dl)**	**Platelet (10^3/µl)**	**TWBC (10^3/µl)**	**Neutro (%)**	**Lympho (%)**
1 (Normal control)	28.40 ± 1.44[a]	65.90 ± 4.32[a]	7.48 ± 0.45[a]	42.50 ± 1.22[a]	12.92 ± 0.51[a]	7.11 ± 0.63[a]	13.70 ± 0.68[a]	31.60 ± 2.99[a]	68.00 ± 3.03[a]
2(Anaemic no treatment)	29.60 ± 2.48[a]	68.10 ± 2.18[a]	5.32 ± 0.37[b]	30.48 ± 1.97[b]	8.70 ± 0.55[b]	5.38 ± 0.28[b]	6.80 ± 0.85[b]	15.20 ± 1.16[b]	26.40 ± 2.48[b]
3 (Anaemicchemiron)	27.20 ± 1.39[a]	69.60 ± 1.72[a]	5.67 ± 0.22[b]	32.04 ± 1.27[b]	9.14 ± 0.34[b]	5.28 ± 0.36[b]	6.16 ± 0.65[b]	14.40 ± 1.21[b]	26.40 ± 2.79[b]
4 (Anaemic 100mg/kg)	29.60 ± 2.07[a]	68.50 ± 2.24[a]	5.57 ± 0.30[b]	31.80 ± 1.41[b]	9.00 ± 0.40[b]	5.14 ± 0.26[b]	6.60 ± 0.34[b]	14.20 ± 1.02[b]	25.20 ± 1.36[b]
5 (Anaemic 200mg/kg	29.70 ± 1.06[a]	69.30 ± 1.09[a]	5.46 ± 0.32[b]	30.40 ± 1.42[b]	8.34 ± 0.28[b]	5.22 ± 0.35[b]	6.28 ± 0.33[b]	14.40 ± 1.60[b]	28.80 ± 1.02[b]
6 (Anaemic 400mg/kg	30.60 ± 1.71[a]	69.00 ± 1.98[a]	5.67 ± 0.38[b]	31.44 ± 2.01[b]	8.56 ± 0.62[b]	5.44 ± 0.44[b]	6.24 ± 0.40[b]	14.20 ± 1.56[b]	26.40 ± 2.14[b]
7 (Normal 100mg/kg)	29.40 ± 1.48[a]	68.50 ± 2.43[a]	7.60 ± 0.31[a]	42.78 ± 1.51[a]	13.00 ± 0.64[a]	6.55 ± 0.51[a]	12.92 ± 0.39[a]	32.40 ± 2.56[a]	67.60 ± 2.56[a]
8 (Normal 200mg/kg)	27.60 ± 1.70[a]	67.20 ± 0.96[a]	7.27 ± 0.47[a]	43.62 ± 1.85[a]	12.92 ± 0.56[a]	6.99 ± 0.39[a]	12.96 ± 0.34[a]	31.20 ± 4.32[a]	68.80 ± 4.32[a]
9 (Normal 400mg/kg)	28.10 ± 2.44[a]	69.00 ± 1.34[a]	7.16 ± 0.38[a]	42.54 ± 1.35[a]	13.06 ± 0.56[a]	6.91 ± 0.28[a]	12.90 ± 0.53[a]	32.00 ± 3.52[a]	68.00 ± 3.52[a]

[a, b] Values with different superscripts vertically are statistically different ($P \leq 0.05$)

Table 6. Effect of *Aleuritefordii* on Mean values for serum enzymes and blood parameters of rats at 7 days of treatment.

Group	Parameters (Mean ± Standard Error of Mean)								
	ALT (iu/L)	**AST ((iu/L)**	**RBC (10^6/µl)**	**PCV (%)**	**HGB (g/dl)**	**Platelet (10^3/µl)**	**TWBC (10^3/µl)**	**Neutro (%)**	**Lympho (%)**
1 (Normal control)	27.96 ± 1.92^{a}	67.76 ± 2.36^{ab}	8.00 ± 1.49^{a}	47.92 ± 0.60^{a}	13.78 ± 0.31^{a}	6.83 ± 0.45^{acd}	13.26 ± 0.27^{a}	30.20 ± 2.03^{a}	69.60 ± 2.11^{a}
2(Anaemic no treatment)	26.48 ± 1.61^{a}	65.77 ± 2.88^{a}	5.07 ± 0.28^{b}	28.33 ± 2.56^{c}	8.47 ± 0.52^{b}	5.14 ± 0.34^{b}	6.88 ± 0.51^{b}	13.25 ± 0.48^{b}	24.50 ± 0.96^{b}
3 (Anaemicchemiron)	27.48 ± 1.96^{a}	67.94 ± 2.73^{ab}	6.80 ± 1.87^{c}	40.60 ± 1.64^{b}	10.00 ± 0.30^{c}	6.26 ± 0.31^{a}	8.32 ± 0.41^{c}	20.80 ± 1.02^{c}	45.80 ± 3.55^{c}
4 (Anaemic 100mg/kg)	29.24 ± 1.60^{a}	66.94 ± 2.86^{ab}	5.58 ± 1.51^{b}	35.78 ± 1.48^{d}	9.20 ± 0.25^{bc}	5.51 ± 0.13^{b}	6.68 ± 0.57^{b}	21.40 ± 1.03^{c}	44.40 ± 2.25^{c}
5 (Anaemic 200mg/kg	28.40 ± 1.60^{a}	67.90 ± 1.95^{ab}	6.39 ± 1.99^{c}	40.72 ± 1.48^{b}	9.80 ± 0.10^{c}	6.42 ± 1.88^{ac}	7.84 ± 0.43^{bc}	22.00 ± 1.58^{c}	47.80 ± 1.28^{c}
6 (Anaemic 400mg/kg	36.42 ± 1.37^{b}	72.84 ± 1.00^{b}	6.35 ± 1.60^{c}	41.10 ± 1.17^{b}	9.92 ± 0.21^{c}	6.40 ± 0.21^{ac}	7.70 ± 0.35^{bc}	23.20 ± 0.58^{c}	47.00 ± 1.62^{c}
7 (Normal 100mg/kg)	26.84 ± 1.70^{a}	64.88 ± 1.50^{a}	7.80 ± 0.14^{a}	48.16 ± 0.63^{a}	13.44 ± 0.33^{a}	6.40 ± 0.20^{ac}	13.02 ± 0.24^{a}	31.00 ± 2.10^{a}	68.80 ± 2.15^{a}
8 (Normal 200mg/kg)	25.74 ± 1.79^{a}	66.04 ± 1.06^{a}	8.25 ± 0.32^{a}	47.76 ± 1.99^{a}	13.30 ± 0.49^{a}	7.01 ± 0.25^{cd}	13.16 ± 0.48^{a}	32.40 ± 2.46^{a}	67.60 ± 2.46^{a}
9 (Normal 400mg/kg)	34.68 ± 1.93^{b}	69.04 ± 1.79^{a}b	8.02 ± 0.21^{a}	49.74 ± 0.80^{a}	14.30 0.28^{a}	7.24 ± 0.15^{d}	13.32 ± 0.66^{a}	32.60 ± 2.99^{a}	67.40 ± 2.99^{a}

[a, b,c,d] Values with different superscripts vertically are statistically different ($P \leq 0.05$) when compared to normal control.

Table 7. Effect of *Aleuritefordii* on the Mean values of serum enzymes and blood parameters of rats at 15 days of treatment.

Group	Parameters (Mean ± Standard Error of Mean)								
	ALT (iu/L)	**AST ((iu/L)**	**RBC(10^6/µl**	**PCV (%)**	**Hgb (g/dl)**	**Platelet (10^3/µl)**	**TWBC(10^3/µl)**	**Neutro (%)**	**Lympho (%)**
1 (Normal control)	26.60 ± 1.87^{a}	68.54 ± 1.91^{a}	8.05 ± 0.21^{ad}	48.78 ± 0.56^{a}	13.50 ± 0.41^{ad}	7.02 ± 0.38^{a}	13.18 ± 0.72^{a}	28.80 ± 3.20^{a}	71.20 ± 3.20^{a}
2(Anaemic no treatment)	28.67 ± 2.19^{a}	64.73 ± 0.82^{a}	4.41± 1.91^{b}	22.80 ± 1.36^{b}	7.00 ± 0.38^{b}	4.68 ± 0.24^{b}	4.83 ± 0.26^{b}	16.00 ± 2.08^{b}	25.33 ± 3.52^{b}
3 (Anaemicche miron)	26.40 ± 1.35^{a}	66.82 ± 2.19^{a}	7.74 ± 1.95^{ac}	47.76 ± 0.43^{a}	13.28 ± 0.29^{ac}	6.79 ± 0.32^{a}	13.20 ± 0.37^{a}	29.00 ± 1.02^{a}	71.00 ± 1.00^{a}
4 (Anaemic 100mg/kg)	27.80 ± 1.69^{a}	68.00 ± 1.36^{a}	7.04 ± 0.25^{c}	43.60 ± 1.13^{c}	12.62 ± 0.18^{c}	6.53 ± 0.33^{a}	12.40 ± 0.81^{a}	30.00 ± 2.39^{a}	70.00 ± 2.38^{a}
5 (Anaemic 200mg/kg	29.00 ± 1.42^{a}	67.60 ± 1.51^{a}	7.59 ± 0.22^{ac}	47.32 ± 0.55^{a}	13.30 ± 0.24^{ac}	6.68 ± 0.22^{a}	13.32 ± 0.24^{a}	29.80 ± 2.20^{a}	70.20 ± 2.20^{a}
6 (Anaemic 400mg/kg	38.90 ± 1.15^{b}	80.40 ± 1.32^{b}	7.58 ± 0.20^{ac}	47.64 ± 0.31^{a}	13.44 ± 0.24^{acd}	6.77 ± 0.20^{a}	12.56 ± 0.79^{a}	28.80 ± 3.07^{a}	71.20 ± 3.07^{a}
7 (Normal 100mg/kg)	25.36 ± 0.96^{a}	64.68 ± 3.40^{a}	8.23 ± 0.16^{ad}	49.64 ± 0.54^{ad}	13.42 ± 0.21^{acd}	6.75 ± 0.36^{a}	13.00 ± 1.94^{a}	30.80 ± 1.88^{a}	69.20 ± 1.88^{a}
8 (Normal 200mg/kg)	25.62 ± 1.45^{a}	63.24 ± 3.34^{a}	8.28 ± 0.36^{ad}	49.32 ± 0.94^{ad}	13.98 ± 0.25^{ad}	7.08 ± 0.19^{a}	13.14 ± 0.35^{a}	28.80 ± 1.50^{a}	71.20 ± 1.50^{a}
9 (Normal 400mg/kg)	34.58 ± 1.46^{c}	75.94 ± 2.05^{b}	8.57 ± 0.36^{d}	50.32 ± 0.92^{d}	14.18 ± 0.47^{d}	7.06 ± 0.51^{a}	13.22 ± 0.48^{a}	27.80 ± 5.67^{a}	72.20 ± 2.53^{a}

$^{a, b,c,d}$Values with different superscripts vertically are statistically different ($P \leq 0.05$)

Discussion

In traditional medicine, most herbal drugs are made from locally available leaves and are employed as treatment to various ailments. The aim of this work is to determine the haematinic potential of the leaf extract of *Aleuritefordii*. The result of this study revealed that the groups induced with cyclophosphamide (Groups 2-6) showed significant decrease ($P<0.05$) in blood parameters when compared to the control group (Group I). This observation can be attributed to the immunosuppressive potential of cyclophosphamide. Cyclophosphamide is an alkylating agent and functions by preventing cell division, induction of aplastic anaemia and is used primarily as an effective drug against some cancers [9].

The group that was treated with the standard haematinic drug (Group3) and those treated with different doses of the extract (Groups 4-6) showed a gradual increase in the values of the blood parameters such that by the 14^{th} day of treatment with extract, there was no significant difference ($P>0.05$) in the mean values of the blood parameters when compared to the normal control group (Group I) and the non induced groups (Groups 7-9). This may probably be due to the phyto-constituent of the leave extract. The phytochemistry revealed the presence of saponins, alkaloids, flavonoids and carotenoids which are known to have anti-inflammatory and immune-stimulating activity, antimicrobial properties and ability to scavenge free radicals [10,11]. Carotenoids are known antioxidants and precursor to vitamin A which are involved in cell differentiation and also in the synthesis of glycoprotein and also in the growth and development of bones [12,13]. Interestingly, cyclophosphamide induced reduction in blood indices in animals that received only cyclophosphamide (Group 2) but the blood indices were not restored to normal counts even after the discontinuation of the drug after three days and throughout the duration of the experiment. However, upon continuous administration of standard haematinic drug to group 3 and the extract (at 100, 200 and 400 mg/kg, body weight) to the anemic groups, all the haematological indices in the drug treated groups were restored to almost normal counts. Furthermore, the vitamin and mineral content (vitamin C, A, B, copper, selenium) found in the extract is indicative of its richness in antioxidants [14]. Iron and vitamin are necessary factors for haemopoiesis[15,16]. Also, haemoglobin, a transporter of oxygen is quite dependent on iron for its production [17].

The result of the liver enzyme markers (ALP, AST) agrees with the overall result since they are known indicators of liver damage. There was no significant difference ($p>0.05$) in the mean values of AST, ALP, ALT total and conjugated bilirubin of all the groups when compared to the normal control. However, group 2 which was the cyclophosphamide control group and the groups that received 400mg/kg body weight dose showed indications of liver injury. The liver is highly sensitive to toxic agents and increased concentration of drugs may cause liver injury even for known herbal/medicinal drugs [18,19]. The results of the serum enzyme test were further confirmed by the observations made following the histopathological studies. All the groups except those that were administered 400mg/kg of the extract (Groups 6 and 9) had normal liver architecture when compared to the control group (Group1). Groups 6 and 9 that were given high dose of the extract had mononuclear leukocyte infiltration, vacuolar degeneration and hepatocyte necrosis.

In conclusion, the ability of the ethanolic extract of *A. fordii* to compare favorably with Chemiron$^{@}$, a standard haematinic drug in the treatment of anaemia induced by cyclophosphamide validates the use of the leaves of this plant for haematopoietic activity. However, about 200mg/kg b.w is suggestive of the safest dose of improving and restoring haemopoietic process.

Disclosure statement/Conflict of interest statement

We do not have any conflict of interest to declare.

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