

**PROTECTIVE ROLE OF *HARUNGANA MADAGASCARIENSIS* LAM. EX POIR. (HYPERICACEAE)  
ETHANOL LEAVE EXTRACT ON HEMATOLOGY AND SERUM PROTEIN LEVELS IN  
PHENYLHYDRAZINE-INDUCED ANAEMIA**

Nku-Ekpang Okot-Asi T.<sup>1</sup>; Uwagie-Ero Edwin A.<sup>2</sup>; Beshel Favour N.<sup>1</sup>; Asinta Emmanuel L.<sup>1</sup> And  
Nwaehujor Chinaka O.<sup>3,\*</sup>

<sup>1</sup>Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross  
River State, Nigeria

<sup>2</sup>Department of Surgery, Faculty of Veterinary Medicine, University of Benin, Benin City, Edo State,  
Nigeria

<sup>3</sup>Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Cross  
River State, Nigeria

[chinaka\\_n@yahoo.com](mailto:chinaka_n@yahoo.com)

### Abstract

Different parts of *Harungana madagascariensis* (HM) have been used to treat various kinds of diseases in traditional medicine especially around West Africa but no literature has been cited using its ethanol leaf extract to alleviate hemolytic anaemia. The aim of this study therefore, was to investigate the activities of the ethanol leaf extract of HM on some haematological parameters and protein levels in phenylhydrazine (PHZ)-induced anaemic male Wistar rats. Qualitative phytochemical analysis of the leaf extract confirmed alkaloids, tannins, flavonoids, saponnins, anthraquinone, glycosides, etc. and absence of steroids. Acute toxicity test of the extract of HM was done in Wistar rats to 4800 mg/kg. Forty male rats (weighing between 150-230 g) were randomly grouped into 5. Group 1 served as normal control and received distilled water, Group 2 - negative control and animals were given a single dose of PHZ (40 mg/kg) b/w intraperitoneally. Group 3 received HM extract at 1500 mg/kg/day orally only, while Groups 4 and 5 respectively received 1500 mg/kg (low dose, LD) and 3000 mg/kg (high dose, HD) of HM orally after challenge with single dose of 40 mg/kg PHZ intraperitoneally. The treatments lasted for 28 days. Animals were anesthetized post-treatment and blood was collected from each rat through the retrobulbar plexus for estimation of hematological parameters and protein levels respectively. Acute toxicity study showed an LD<sub>50</sub> at 4639.80 mg/kg. In the extract-treated groups, RBC count, PCV, Hb concentration, MCHC, MCH, WBC count, platelet count and plateletcrit were significantly increased ( $p < 0.001$ ) while MCV, MPV, P-LCR, PDW significantly reduced ( $p < 0.001$ ) when compared with the anaemic control group. Serum proteins were significantly increased ( $p < 0.001$ ) in the extract-treated groups compared to anaemic group. In conclusion, the oral administration of ethanol leaf extract of *Harungana madagascariensis* can be said to have anti-anaemic potentials.

**Keywords:** *Harungana madagascariensis* (HM), Anaemia, Haematology, Phenylhydrazine, Serum protein, Wistar rats

## Introduction

Anaemia is a common blood disorder that affects people of all ages, although the people at greater risk are the elderly, young women of childbearing age and the infants (Luka et al., 2014). Anaemia refers to deficiency of haemoglobin in the blood, which can be caused by decreased production of red blood cells, increased destruction of RBC or excessive loss of blood from the body (Sembulingam, 2012). It is a condition that has multiple origins and one of such origin is destruction of red blood cells membrane induced by free radicals (Shetlar and Hill, 1985)

Medicinal plants comprise of plants which have at least one of their parts (leaves, stem, barks or root) used for therapeutic purposes (Bruneton, 1993). They are known to contain substances that are either of therapeutic importance, or are precursors for the synthesis of useful drugs (Sofowora, 1993). Over the years, medicinal plants have been recognized to be of great importance to the health of individuals and communities. These medicinal plants are said to contain biologically active compounds such as enzymes, minerals, vitamins, phytochemicals like alkaloids, flavonoids, anthocyanins, carotenoids, simple phenolic glycosides etc., which have medicinal activities that are either of therapeutic important or are precursors for the synthesis of useful drugs (Olowokudejo et al., 2008). The ready availability and economy of plants as direct therapeutic agents make them more attractive when compared to modern medicines (Agbor and Ngogang, 2005)

*Harungana madagascariensis* (HM) is one of such traditional medicinal plants used in the treatment of anaemia (Gbadamosi et al., 2012). The plant is native to Madagascar, Mauritius and Tropical Africa (i.e. Sudan, Kenya, Tanzania, Rwanda, Cameroon, Burundi, Equatorial Guinea, Congo, Ethiopia, Nigeria, and Malawi) (Orwa et al., 2009). *H. madagascariensis* occurs at medium to low altitudes in evergreen forest, at forest margins and along river and stream banks (Orwa et al., 2009). It has several names among various tribes in Nigeria. Among the Efiks, Hausa, Yoruba and Igbo people in Nigeria, it is referred to as Oton, Alilibar, elepo, and uturu (ururtu) respectively (Adeneye et al., 2008).

The plant *Harungana madagascariensis* have been used in the management of a variety of illness (Liu, 2004). The leaves and root are used traditionally for the treatment of anaemia, nephrosis, malaria, gastro-intestinal disorders and fever (Gbadamosi et al., 2012). Aqueous leaf extract has been shown to have anti-microbial activity on different strains of bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Eschericia coli* and *Salmonella typhi*), thus substantiating its use as an antimicrobial agent and for gastro-intestinal disorders (Okoli et al., 2002). The leaf extract inhibited *Entamoeba hystolytica* growth at a concentration of less than 10µg/ml as described by Tona et al, 2000. The roots extract also exhibited more than 70 % inhibition of acetylcholine and potassium chloride solution-induced contractions on isolated guinea-pig ileum (Tona et al., 2000). *Harungana madagascariensis* is a component of Jubi Formula, a herbal preparation which was found to restore the pack cell volume (PCV) and hemoglobins (Hb) concentration in anemic conditions and have proven to be a potential substitute for blood transfusion (Erah et al., 2003). Lukwa et al., (2001) made an ethnobotanical survey on some herbal remedies used by traditional healers in Mola, Kariba district of Zimbabwe for the treatment and prevention of malaria, and as such, *Harungana madagascariensis* is one of the plants used for the treatment of malaria. Earlier studies of *Harungana madagascariensis* were based on the stem bark (Moulari et al., 2006). Only in traditional medicine and as practiced in West Africa have this plant been used in the treatment of anaemia but no scientific work has been documented on it. Therefore, the aim of this study was to investigate the activities of ethanol leaf extract of HM on some haematological parameters and protein levels in phenylhydrazine(PHZ)-induced anaemic Wistar rats by mimicking ethno-medicinal practices.

## Methods

### Collection of plant material

Fresh leaves of *Harungana madagascariensis* (HM) were collected from the deciduous forest in Ikot-Omin, 8 Miles, in Calabar Municipality Local Government Area of Cross River State, Nigeria in December, 2016. The plant was identified at the Herbarium in Botany Department, University of

Calabar, Nigeria. The leaves were thoroughly rinsed in tap water and thereafter dried in open-air for about 2 weeks, away from direct sunlight. It was then cut into small pieces and pulverized into coarse powder using an electric blender.

#### Extraction

The preparation of the extract was done according to the method described by Adeneye et al. (2008). A 1000 g of the powdered leaves of HM was soaked in 80 % ethanol/water (v/v) in a glass jar, stirred and kept for 48 h. The mixture was then filtered first with a white cotton material, then with Whatman filter paper into a beaker. Thereafter, this was concentrated to dryness using a rotary evaporator (Büchi, Switzerland). The HM ethanol leaf extract yield was 108.43 g.

#### Phytochemical analysis

The ethanol leaf extract of HM was subjected to qualitative phytochemical analysis. A 2 g of the crude extract was weighed and dissolved in 20 ml of distilled water. The solution was screened for the presence and absence of alkaloids, flavonoids, tannins, saponins, terpenes, anthraquinones and glycosides using standard methods (Trease and Evans, 1984; Harbourne, 1998).

#### Acute toxicity test

Thirty-six (36) Wistar rats of both sexes (135 – 147 g) were purchased from the Department of Agriculture, University of Calabar, Calabar, Cross River State. They were randomly grouped into 6 groups of 6 animals each. The rats were allowed to acclimatize for 7 days. Five doses of the extract (300 mg/kg, 600 mg /kg, 1200 mg/kg, 2400 mg/kg and 4800 mg/kg), each dissolved in distilled water was administered *per os* to the rats in different groups respectively. The first group served as the control. Animals were monitored closely within the first 24 h post treatment for behavioral changes and death due to toxicity. The median lethal dose (LD<sub>50</sub>) was determined according to the method of Kengni et al. (2013).

#### Experimental animals

A total of forty (40) male Wistar (150-230 g) were used for this experiment. The animals were obtained from the rat colony of the animal houses

of Department of Agriculture, University of Calabar, Nigeria, and Veterinary Surgery, University of Benin, Benin City, Edo State, Nigeria. The rats were acclimatized for 7 days and maintained on standard rat feed (growers feed) and tap water which was made available *ad libitum*. The rats were maintained at an ambient temperature between 28 – 30 °C, humidity of 55 ± 5 %, and standard (natural) photoperiod of approximately 12 h of light (06:30 h–18:30 h) alternating with approximately 12 h of darkness (18:30 h - 06:30 h). The conduct of experiments were approved and in accordance with the approved research guidelines on laboratory animal use of the Faculty of Basic Medical Sciences, University of Calabar, Calabar and Faculty of Veterinary Medicine, University of Benin, Benin City, Edo State, where the animal studies were carried out. All animals were humanely handled and their welfare respected throughout this study as stipulated in the 1964 Helsinki Declaration as amended (Williams, 2008).

#### Determination of haemoglobin (Hb) concentration

Before the induction of anaemia using Phenylhydrazine (PHZ), Hb concentration of the animals was determined using Sahli's apparatus (Balasubramaniam and Malathi, 1992). After the determination of Hb concentration, anaemia was induced by intraperitoneal administration of phenylhydrazine (PHZ) at 40 mg/kg at 48 h interval for two (2) consecutive times (Nku-Ekpang et al., 2015). After the Induction of anaemia, rats with Hb concentration <11.5 g/dl were declared Anaemic (Johnson-Delaney, 1996).

#### Effect of HM on PHZ-induced anemia

The animals were allowed to acclimatize for 7 days. Thereafter, they were randomly divided into 5 groups of 8 rats kept in different cages. The Experiment lasted for 28 days and the groups were as follows:

**Group 1-Normal Control group** received distilled water only

**Group2-Anaemic Control group** was challenged with a single dose of 40 mg/kg phenylhydrazine (PHZ) intraperitoneally and received distilled water only.

**Group3- Extract Treated Group:** received HM extract at 1500 mg/kg/day orally alone

**Group 4- Anaemia + Extract Treated Group (Low Dose)** was challenged with a single dose of 40 mg/kg phenylhydrazine (PHZ) intraperitoneally and received HM extract at 1500 mg/kg daily after challenge

**Group 5 - Anaemia + Extract Treated Group (High Dose)** was challenged with a single dose of 40 mg/kg phenylhydrazine (PHZ) intraperitoneally and received HM extract at 3000 mg/kg daily after challenge

#### Collection and analysis of blood samples

The animals were starved for overnight but had water and were anesthetized using chloroform in an inhalation chamber with 4 % isoflurane (IsoFlo, Abbott Laboratories, Berkshire, UK) regulated with a calibrated vaporizer (Fernandez et al, 2010). Blood samples from each rat were collected via retrobulbar plexus into EDTA and plain sample bottles.

Blood samples in EDTA bottles were analyzed using automated haematology cell counter (BC-2800 Mindray auto hematology analyzer, China) for the estimation of some of the haematological parameters which included red blood cell (RBC) count, PCV, Hb concentration, MCV, MCHC, MCH. WBC count (Total WBC count, Differential WBC), Platelet count, Platelet indices (PDW, MPV, P-LCR, Plateletcrit) were analyzed with a Coulter Counter (Marshall Don Graham, 2003)

Blood samples collected into anti-coagulant-free sample bottles were allowed to clot. The resultant serum was collected into pre-labelled eppendorf tubes on ice after centrifugation at 3000 rpm for 10 min and used for determination of total protein, albumin and globulin were assayed by the method of Johnston (1996).

#### Statistical analysis

Data were presented as mean  $\pm$  SEM. Experimental data were analyzed using Analysis of variance (ANOVA) followed by a post HOC test (least square difference {LSD} test) to determine significant differences between means. The analysis

was done with an SPSS 18 statistical package.  $p < 0.05$  was accepted as statistically significant.

#### Results

##### Phytochemical analysis of *H. madagascariensis* ethanol leaf extract

Qualitative phytochemical analysis of *H. madagascariensis* leaf extract showed the presence of alkaloids, tannins, flavonoids, saponins, anthraquinone, glycosides, phenolic compounds, terpenes, anthocyanins and absence of steroids.

Table 1 shows comparison of red blood cell count and red blood cell absolute values while Table 2 and 3 show comparison of total and differential white blood cell count in the different groups, and comparison of platelet count and platelet indices in the different group respectively. Figure 1 compares the effect of *H. madagascariensis* ethanol leaf extract on serum protein, albumin and globulin in normal, treated and anaemic Wistar rats.

#### Discussion

The phytochemical screening of *H. madagascariensis* leaf extract showed the presence of alkaloids, flavonoids, terpenes, saponins, phenols, tannins, glycosides, anthocyanins, anthraquinones which is in line with previous studies that have shown that extract which contain these phytochemical constituents have high antioxidant ability, promote regeneration of tissue, reduce the permeability of blood capillaries and increase their resistance to hemolysis (Bruneton, 1993). The presence of these phytochemicals by their properties justifies the resistance of red blood cells in anaemic, extract-treated groups.

A World Health Organization (WHO) survey indicated that about 70 - 80 % of the world's population rely on non-conventional medicine, mainly of herbal sources, in their primary healthcare (Chan, 2003). Besides, there has been erroneous belief that these medicines are free from adverse effects (Ermst, 2005). Although medicinal plants may produce several biological activities in humans, generally, very little is known about their toxicity and the same applies for HM. The LD<sub>50</sub> of the ethanol leaf extract of HM was high (4639.80 mg/kg body weight) which shows that it is practically non-



toxic and can be recommended to be consumed by humans (Prosper et al., 2012).

PHZ is well known to induce hemolytic anemia and is also known to generate hydroxyl radicals which leads to fragmentation of erythrocytes (Berger, 2007). This is thought to result from the reaction of phenylhydrazine with haemoglobin (Shetlar and Hill, 1985). The accompanying oxidation of phenylhydrazine leads to the formation of a number of products, including benzene, nitrogen, hydrogen peroxides, superoxide anion and the phenyl-radical (Shetlar and Hill, 1985). The products formed depend critically on the conditions of the experiment, especially the amount of oxygen present. This is also in line with an earlier study by Turaskar et al. (2013) and Nku-Ekpang et al., (2015) which showed that PHZ causes oxidative damage to red cells through simultaneous formation of reactive oxygen species (ROS).

The result also showed a significant reduction in RBC count, PCV, Hb concentration in the anaemic control group compared with the normal control group (Table 1). This is in accordance with previous studies that demonstrated that intraperitoneal administration of PHZ decrease Hb concentration, RBC and PCV (Peter et al., 2009; Meena et al., 2014; Nku-Ekpang et al., 2015). This could be due to the presence of phytochemicals rich in antioxidant especially flavonoids which offer protective effect against hemolysis by free radicals (Asgary et al., 2005). It could also be due to the presence of phenols and tannins components of *H. madagascariensis* leaf. Vinson et al., (1995) reported that phenols, flavonoids and tannins are good antioxidant substances which could prevent or control oxidative stress. Also, Odesanmi et al. (2010) reported that tannins can bind to proteins and Carbohydrates (CHO) which are components of the RBC membrane and therefore may prevent breakdown of RBC membrane. This also explains why the RBC was significantly increased in the extract-only-treated group when compared to normal control. Indeed, saponins and alkaloids have shown anti-anaemic properties (Falcone et al., 1997). Alkaloid inhibits cyclic adenosine monophosphate (cAMP) phosphodiesterase thereby accumulating cAMP. This effect stimulates phosphorylation of proteins and synthesis of proteins, which improves

erythropoiesis (Magnani et al., 1986). Also, flavonoids have anti-anaemic potential and veinotonic properties, which protects the blood capillaries (Bruneton, 1999). The anti-anemia potential and haemoglobin restoring effect of ethanol leaf extract of *H. madagascariensis* as suggested by the data in this study could be attributed in part to its phytochemical constituents. The significant increase in MCV and decrease in MCH and MCHC in the anaemic control group when compared to the normal control group indicates pernicious and megaloblastic anaemia (Sembulingam, 2012). The RBC in this group are macrocytic and hypochromic. RBCs are larger in size. Since the MCHC is less, the cells are pale (less colored) (Sembulingam, 2012). This is attributable to deficiency of vitamin B<sub>12</sub>, folic acid and iron which are necessary factors for erythropoiesis (Ibu, 2005). The administration of the extract of *H. madagascariensis* in the extract-treated groups reversed this deteriorating effect (Table 1).

The extract treated groups showed a significant increase in the total WBC compared to the anaemic control group (Table 2). This could be attributed to the significant increase in lymphocyte level in the extract-treated groups being that lymphocytes are predominant in rats.

The significant decrease in platelet count in the anaemic control group compared with the normal group (Table 3) suggests that the process of clot-formation (blood coagulation) will be prolonged resulting in excessive loss of blood in the case of injury (Guyton and Hall, 2006). The result also showed a significant reduction in PDW, P-LCR, MPV and significant increase in plateletcrit in the extract-treated groups compared with anaemic control group. This is in accordance with studies that have shown that platelet count is inversely proportional to PDW, P-LCR, MPV and directly proportional to plateletcrit which is a good indicator or aid in the differential diagnosis of conditions associated with abnormal platelet counts (Babu and Basu, 2005).

The serum proteins showed a significant decrease in total protein, albumin and globulin in the anaemic control group compared with the normal control group and a significant increase in the extract-treated groups compared with the anaemic control group (Fig. 1). The reduction in serum proteins in the

anaemic control group leads to decrease in erythropoiesis and thus, potentiates anaemia (Menon, 1965; Subramanyan, 1970). Reduction in albumin leads to derangement of vital functions such as metabolism, cell growth and haematopoiesis (Upadhyay, 1971).

In conclusion, oral administration of ethanol leaf extract of *H. madagascariensis* can be said to have anti-anaemic potentials. Experiments are however on-going to determine the mechanism of this observed anti-anaemic action to determine if another biochemical pathway different from our positive control is explored. We also suggest that *in vitro* bio-assay guided fractionation should be explored as an effective way of isolating the active anti-anaemic principle(s) from *H. madagascariensis* before more animal studies are performed.

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**Table 1.** Comparison of red blood cell count and red blood cell absolute values.

	RBC ( $\times 10^{12}/L$ )	PCV (%)	Hb(g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Normal control	6.76 $\pm$ 0.04 <sup>c</sup>	43.31 $\pm$ 0.25 <sup>c</sup>	14.63 $\pm$ 0.06 <sup>c</sup>	64.05 $\pm$ 0.51 <sup>c</sup>	21.64 $\pm$ 0.15 <sup>c</sup>	33.76 $\pm$ 0.08 <sup>c</sup>
Anaemic control	5.23 $\pm$ 0.04 <sup>***</sup>	38.58 $\pm$ 0.16 <sup>***</sup>	9.25 $\pm$ 0.17 <sup>***</sup>	73.84 $\pm$ 0.31 <sup>***</sup>	17.70 $\pm$ 0.20 <sup>***</sup>	23.95 $\pm$ 0.34 <sup>***</sup>
Extract only	8.50 $\pm$ 0.05 <sup>***,c</sup>	42.98 $\pm$ 0.37 <sup>c</sup>	14.48 $\pm$ 0.11 <sup>c</sup>	50.58 $\pm$ 0.42 <sup>***,c</sup>	17.03 $\pm$ 0.12 <sup>***</sup>	33.70 $\pm$ 0.10 <sup>c</sup>
Ane + Ext. (LD)	6.20 $\pm$ 0.04 <sup>***,c,z</sup>	41.05 $\pm$ 0.27 <sup>***,c,z</sup>	15.88 $\pm$ 0.16 <sup>***,c,z</sup>	66.21 $\pm$ 0.54 <sup>c,z</sup>	25.64 $\pm$ 0.29 <sup>***,c,z</sup>	38.66 $\pm$ 0.50 <sup>***,c,z</sup>
Ane + Ext. (HD)	6.70 $\pm$ 0.20 <sup>c,z,b</sup>	42.15 $\pm$ 0.37 <sup>***,c,x,z</sup>	15.60 $\pm$ 0.15 <sup>***,c,z,b</sup>	63.34 $\pm$ 2.06 <sup>c,z,b</sup>	23.41 $\pm$ 0.55 <sup>***,c,z,b</sup>	37.04 $\pm$ 0.45 <sup>***,c,z,b</sup>

Values are expressed as mean + SEM, n = 8. \*\*\* = significantly different from normal control at p<0.001; c = significantly different from anaemic control at p<0.001; x = significantly different from extract treated at p<0.05; b = significantly different from anaemic + Ext. (LD) at p<0.001; z = significantly different from anaemic + Ext. (LD) at p<0.05

**Table 2:** Comparison of total and differential white blood cell count in the different groups.

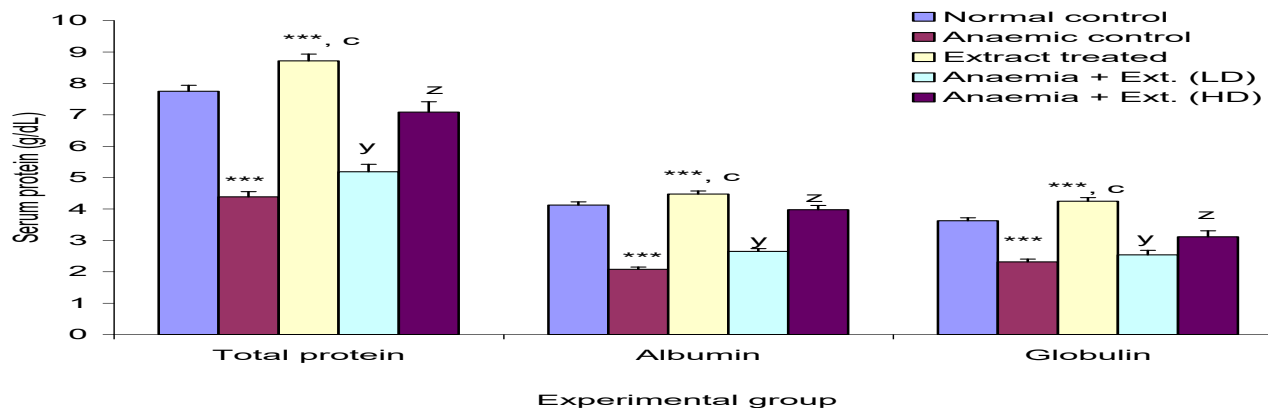
	WBC ( $10^9/L$ )	Neutrophil (%)	Lymphocytes (%)	Monocytes (%)	Eosinophil (%)	Basophil (%)
Normal control	14.33 $\pm$ 0.06 <sup>c</sup>	31.49 $\pm$ 0.04 <sup>c</sup>	68.41 $\pm$ 0.07	3.19 $\pm$ 0.06 <sup>c</sup>	0.52 $\pm$ 0.00 <sup>c</sup>	0.43 $\pm$ 0.01
Anaemic control	8.59 $\pm$ 0.06 <sup>***</sup>	24.34 $\pm$ 0.05 <sup>***</sup>	67.20 $\pm$ 0.07 <sup>***</sup>	7.36 $\pm$ 0.02 <sup>***</sup>	2.28 $\pm$ 0.04 <sup>***</sup>	0.31 $\pm$ 0.01 <sup>***</sup>
Extract only	12.50 $\pm$ 0.17 <sup>***,c</sup>	19.33 $\pm$ 0.05 <sup>***,c</sup>	70.18 $\pm$ 0.03 <sup>***,c</sup>	8.13 $\pm$ 0.03 <sup>***,c</sup>	1.20 $\pm$ 0.03 <sup>***,c</sup>	0.38 $\pm$ 0.02 <sup>***,c</sup>
Anaemia+Ext. (LD)	9.36 $\pm$ 0.07 <sup>***,c,z</sup>	21.48 $\pm$ 0.14 <sup>***,c,z</sup>	66.33 $\pm$ 0.04 <sup>***,c,z</sup>	7.38 $\pm$ 0.04 <sup>***,c,z</sup>	1.59 $\pm$ 0.02 <sup>***,c,z</sup>	0.61 $\pm$ 0.02 <sup>***,c,z</sup>
Anaemia + Ext. (HD)	10.14 $\pm$ 0.11 <sup>***,c,z,b</sup>	22.49 $\pm$ 0.03 <sup>***,c,z,b</sup>	68.05 $\pm$ 0.05 <sup>***,c,z,b</sup>	7.38 $\pm$ 0.08 <sup>***,c,z</sup>	1.63 $\pm$ 0.07 <sup>***,c,z</sup>	0.59 $\pm$ 0.04 <sup>***,c,z</sup>

Values are expressed as mean + SEM, n = 8; \*\*\* = significantly different from normal control at p<0.001; c = significantly different from anaemic control at p<0.001; x = significantly different from extract treated at p<0.05; b = significantly different from anaemic + Ext. (LD) at p<0.001; z = significantly different from anaemic + Ext. (LD) at p<0.05

**Table 3:** Comparison of platelet count and platelet indices in the different group.

	Platelet ( $10^9/l$ )	PDW (fl)	MPV (fl)	P-LCR (%)	PCT (%)
Normal control	42.75±0.49	8.66±0.24	7.43±0.10	10.24±0.19	0.49±0.01
Anaemic	40.63±0.18***	11.50±0.35***	9.58±0.12***	13.73±0.17***	0.20±0.01***
Extract only	42.00±0.60 <sup>a</sup>	7.58±0.13** <sup>c</sup>	7.30±0.10 <sup>c</sup>	10.16±0.18 <sup>c</sup>	0.51±0.01 <sup>c</sup>
Anaemia + Ext. (LD)	41.50±0.60	7.54±0.13** <sup>c,z</sup>	6.55±0.10*** <sup>c,z</sup>	9.49±0.10*** <sup>c,z</sup>	0.41±0.02*** <sup>c,z</sup>
Anaemia + Ext. (HD)	41.00±0.33*	8.08±0.13 <sup>c,z</sup>	7.46±0.10*** <sup>c,z</sup>	9.86±0.12*** <sup>c,z</sup>	0.51±0.01 <sup>c,b</sup>

Values are expressed as mean + SEM, n = 8. \*\*\* = significantly different from normal control at p<0.001; c = significantly different from anaemic control at p<0.001; x = significantly different from extract treated at p<0.05; b = significantly different from anaemic + Ext. (LD) at p<0.001; z = significantly different from anaemic + Ext. (LD) at p<0.05

**Figure 1.** Comparison of serum protein, albumin and globulin

**Figure 1:** Comparison of serum proteins in the different groups. Values are expressed as mean + SEM, n = 8.

\*\*\* = significantly different from normal control at p<0.001;  
c = significantly different from anaemic control at p<0.001;  
y = significantly different from control; anaemic control;  
extract treated at p<0.001;  
z = significantly different from all group at p<0.001;