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Haematinic Properties of Methanolic Stem Bark and Fruit Extracts of *Ficus Sur* in Rats Pre-exposed to Phenylhydrazine-induced Haemolytic Anaemia

Adebayo, M.A[1], Enitan, S.S[2], Owonikoko W.M[2], Igogo, E[1] and *Ajeigbe, K.O[2]

[1]*Department of Pharmacognosy, College of Pharmacy, Igbinedion University Okada, Edo State, Nigeria*
[2]*Department of Physiology, College of Health Science, Igbinedion University Okada, Edo State, Nigeria*

ABSTRACT

The potential effects of a 14-day oral administration of methanolic stem bark and fruit extracts of *Ficus sur* on some haematological parameters in anaemic rats was investigated. Hemolytic anaemia was experimentally induced in rats by daily oral administration of Phenylhydrazine (PHZ) at a dose of 10 mg/Kg for 8 days. Post-induction, 50 mg/Kg, 100 mg/Kg and 150 mg/Kg of methanolic stem bark and fruit extracts of *Ficus sur* were administered to rats in group 1-3 and group 4-6, respectively. Group 7 which received 50 mg/Kg of FeSO4 served as the positive control. Group 8 received no treatment as negative control. The Haematocrit and Haemoglobin concentration and Red Blood Cell Count of rats treated with both methanolic stem bark and fruit extracts of *F. sur* were found to be significantly higher than the negative control, though less than the positive control. However, there was no significant difference between the White Blood Cell Count of all the groups treated with extract and the positive control, except for the negative control in which significant increase was observed. Differential White Blood Cell Count shows the proportion of cells in this order: lymphocytes>neutrophils>monocytes. Calculated Rf value following the thin layer chromatography test of the methanolic stem bark and fruit extracts of *Ficus sur* was 0.8375 cm. Qualitative phytochemical screening of the extracts revealed the presence of tannin, flavonoid, terpenoid and saponin, while alkaloid, glycoside and anthraquinone were absent in both. The results of this study affirm the haematinic properties of *Ficus sur*, hence may be explored in the complementary treatment of anaemia.

Key Words: *Ficus sur, Rats, haematic properties, Haematological parameters, Phenylhydrazine, Haemolytic anaemia.*

*Author for correspondence: *E-mail:* kazeemajeigbe@gmail.com; *Tel: +2348035705220*

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INTRODUCTION

World-wide, anaemia is the commonest red cell disorder associated with several conditions such as nutritional deficiencies, genetic or acquired defects, parasitic infections, blood loss, as well as drug toxicity, affecting people of all ages although the people at greater risk are the elderly, young women of child bearing age and infants. There are over 400 types of anaemia, many of which are rare but in all cases, there is usually fall in haemoglobin concentration below normal values for a person's age, gender, and environment, but not invariably, accompanied by a fall of the red cell count below normal values (Cheesebrough, 2002a; Ogbe *et al*., 2010).

Of interest, is drug-induced haemolytic anaemia— a type of anaemia resulting from an increase in the rate of red cell destruction due to direct toxic action of some drugs and/or chemicals (Dacie, 1967). A number of drugs and chemicals have been documented to cause haemolytic anaemia. They fall into two broad groups: (1) those which regularly cause haemolytic anaemia such as the Phenylhydrazine and Acetylphenylhydrazine and (2) those which cause haemolysis only occasionally or rarely such as the antimalarials (e.g Quinine and Primaquine) and analgesics (e.g Acetylsalicylic

acid and Antipyrine). Phenylhydrazine (PHZ) in particular, is well documented for its ability to produce haemolytic anaemia in rats and humans (Dornfest *et al*., 1983; Ogiso *et al*., 1989; Dornfest *et al*., 1992). Though used as therapy against *Polycythaemia vera* in the past (Falconer, 1933), its use has been terminated due to undesirable effects seen (Toth, 1988). Presently, it is mainly used for experimental induction of anaemia in animals. PHZ is known to decrease Haemoglobin levels, Red Blood Cell count and Packed Cell Volume (Giffin and Allen, 1933).

Several plants used in part or as a whole to manage anaemia have well been documented. Those commonly used in Nigeria include *Parquetina nigrescens, Sorghum bicolor, Terminalia catappa, Trema orientalis, Mangifera indica, Waltheria indica, Theobroma cacao, Harungana madagascariensis, Tetracera alnifolia* and *Detarium microcarpum* among others (Gbadamosi *et al*., 2012).

Ficus sur also known as bloom cluster fig or cape fig is a continental Afrotropical plant, from the family Moraceae, used in traditional medicine (Berg, 1991; Hankey, 2003; Lumbile and Mogotsi, 2008). The plant along with many others in this family is important in the traditional treatment of many diseases and ailments. The plant extracts have been reported in the treatment of diarrhea, dysentery, sexually transmitted diseases, infertility, chest ailments, tuberculosis, leprosy, convulsions, skin rashes, mouth sores, rheumatism, pain and wound (Irvine, 1961; Amos *et al*., 2001; Ahmadu *et al*., 2007, Oyeleke *et al*., 2008; Sandabe and Kwari, 2000; Wakeel *et al*., 2004) among many others. They are reported to possess inhibitory activities against the growth and disease inducing activities of some pathogenic microorganisms (Hassan, 2005; Hassan and Almahy, 2005; Oyeleke *et al*., 2008; Sandabe *et al*., 2006; Solomon-Wisdom *et al*., 2011; Stary, 1998; Udobi *et al*., 2008). Although, traditional medicine practitioners in Nigeria use *Ficus sur* stem bark and fruits to treat anaemia, there seems to be limited scientific evidence or validation of the action. This research, therefore, is carried out to establish the haematinic properties of methanolic stem bark and fruit extracts of *Ficus sur* in wistar albino strain rats pre-exposed to phenyhydrazine-induced haemolytic anaemia

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MATERIALS AND METHODS

Plant Materials

The stem bark and mature ripe fruits of *Ficus sur* were collected in the month of April 2014, at the Crown Estate of Igbinedion University Okada and were identified at the Department of Botany, College of Natural and Applied Science, Igbinedion University, Okada, Edo State.

Preparation of plant parts:

Plant parts were prepared according to the method described by Sofowora (1982). Briefly, the stem and fruits were rinsed with distilled water to remove debris and sand and then oven dried, the dried stem and fruits were milled with a blender until they were grounded into powder.

Methanol extraction:

500 g of the powered stem bark and fruits of *Ficus sur* plant were weighed separately into a 3.5 L capacity glass jar and 2.5 L of methanol was added. The mixtures were agitated with a stirrer for 20 minutes, closed and left undisturbed for 72 hours. Afterwards, each of the homogenates was filtered into separate sterile containers using a funnel containing appropriate amounts of sterile cotton wool and later with Whatman No. 1 filter paper. The residue was pressed with a spatula to ensure complete filtration. The methanolic filtrates were transferred into separate clean beaker and concentrated by evaporation to dryness in a water bath for 24 hours. The concentrates were then collected into pre-weighed small glass bottles and stored in the refrigerator prior to use and after daily administration to the experimental animals.

Thin layer chromatography test

Five (5) thin layer chromatography (TLC) aluminum plates coated with silica gel was used, the plated were cut to a size of 4 X 10 cm, a straight line was drawn with a pencil 1 cm vertically from the base of the plates. The plates were flushed with ethanol to clean them. Drops of the methanolic stem bark and fruit extracts of *Ficus sur* were then spotted on the line 1 cm from the base of the TLC plates. Thereafter the spotted plate was dipped into a beaker containing a solvent mixture of methanol and ethyl acetate at a ratio of (8:2) just enough to cover the bottom of the plate and closed. The solvent front ran slowly up the TLC plate and after a suitable time when it is about a centimetre from the top of the tray, the plate was removed and allowed to dry. The plate was then placed in an iodine tank to display how the mixtures have separated; the spots were marked with a pencil and the position of the separated constituents was determined. This procedure was repeated for four different TLC plates and placed in different ratios of solvent mixture of methanol and ethyl acetate (6:4, 5:5, 2:8) ml.

Calculation:

R_f value = Distance travelled by constituent/Solvent front

Qualitative Phytochemical Screening of *Ficus Sur*

The Methanolic stem bark and fruit extracts of *Ficus sur* were subjected to preliminary phytochemical screening for the detection of alkaloid, anthraquinone, tannin, saponin, cardiac glycoside, flavonoid and terpernoid using standard procedures of Trease and Evans (1989) and Sofowora (1982).

Experimental Design

A total of 45 male albino rats weighing 130-150 g were purchased from the Animal Production and Health Department, Federal University of Technology, Akure (Ondo State, Nigeria) and housed in the Central Animal Facility, College of Health Sciences, Igbinedion University Okada (Edo state, Nigeria) separately in well ventilated plastic cages under hygienic conditions, with proper aeration at 25±2ºC, and a relative humidity of 45–50%. The rats were randomly assigned into 9 groups of 5 rats each and fed on standard rat diet (10g/100g body weight) twice daily and tap water *ad libitum*. All studies on animal experimentation were conducted in accordance with the Current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research (ILAR, 1996).

Table 1:
Experimental protocol for *Ficus sur* treated rats

Groups	Treatments (n=5)
G1	received 50 mg/Kg/B.W./D MSBEFS
G2	received 100 mg/Kg/B.W./D MSBEFS
G3	received 150 mg/Kg/B.W./D MSBEFS
G4	received 50 mg/Kg/B.W./D MSBEFS
G5	received 100 mg/Kg/B.W./D MSBEFS
G6	received 150 mg/Kg/B.W./D MSBEFS
G7	received 50 mg/Kg/B.W./D of $FeSO_4$ (Positive control)
G8	Negative (Anaemic) control
G9	Zero (Non-Anaemic) control

MSBEFS= Methanolic Stem Bark Extract of Ficus sur

Induction phase
Experimental Haemolytic Anaemia: Hemolytic anemia was experimentally induced in the 40 rats by daily oral administration of Phenylhydrazine (PHZ) at a dose of 10 mg/Kg for 8 days as described by Yeshoda (1942) and Berger (1985). Blood samples were obtained from the tail vein just before commencement of oral administration of phenylhydrazine (day 0) and also on the 9th day, 24 hrs after the final dose. While blood smears were made from each blood sample, Haematocrit (Hct) and Haemoglobin concentration (Hb) of the induced rats were determined following exposure to PHZ. Hemolytic anemia was observed on day 9 and was evidenced by decreased PCV and Hb, with blood pictures revealing marked anisocytosis and poikilocytosis. Rats with PCV and Hb level ≤30% and ≤ 10 g/dl, respectively, were considered anaemic and were used for the study, while un-induced 5 rats served as the zero (non-anaemic) control.

Post treatment phase
Blood collection: Overnight, prior to euthanasia, the animals were placed on eighteen (18) hours fasting after the last administration and on the 15th day, all the animals were sacrificed by cervical dislocation as described by Ochei and Kolhatkar (2006). Blood samples were taken from each rat by terminal bleeding from the heart and transferred into a clean EDTA container (thoroughly mixed) ready for haematological investigations.

Haematological studies: Haematological parameters were investigated using standards: Haematocrit (Packed cell volume) was determined by microhaematocrit method as described by Cheesbrough (2002b), Haemoglobin concentration was estimated using the Sahli acid haematin method, as described by Cheesbrough (2002c), Red Blood Cell Count was carried out using visual Cell Count Method as described by Baker *et al.*, (2001a), Total White Blood Cell Count was carried out according to the method as described by Cheesbrough (2002d), while Differential White Blood Cell Count was similarly carried out according to the method as described by Baker *et al.*, (2001b) and Cheesbrough (2002e).

Statistical analysis
All the numerical results were collected from the nine (9) animal groups (controls and extract-treated). Data are presented as mean±SEM and analysed using one way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparisons Test using SPSS-18.0 (Statistical Packages for Social Scientists – Version 18.0) statistical program. P values < 0.05 were considered significant

RESULTS

The percentage yield of the methanolic stem bark and fruit extracts of *Ficus sur* were 8.00% and 10.50%, respectively. While the Calculated R_f value following the thin layer chromatography test of the methanolic stem bark and fruit extracts of *Ficus sur* was 0.8375 cm, where distance travelled by constituent was 6.7 cm and the solvent front was 8 cm.

Preliminary Phytochemical Screening
The qualitative phytochemical screening of the extracts revealed the presence of tannin, saponin, flavonoid and terpenoid, while alkaloid, glycoside and anthraquinone were absent in both extracts (Table 2).

Haematological Parameters
Haematocrit and Haemoglobin concentration: The Mean±SEM Haematocrit and Haemoglobin concentration of both methanolic stem bark (50 mg/Kg: 38.25±1.18%; 10.13±0.59 g/dl, respectively, 100 mg/Kg: 38.50±1.19%; 10.63±0.43 g/dl, respectively, 150 mg/Kg: 48.25±1.03%; 13.63±0.52 g/dl, respectively) and fruit (50 mg/Kg: 47.50±0.96%; 13.25±0.48 g/dl, respectively, 150 mg/Kg: 49.75±1.65%; 13.38±0.38 g/dl, respectively) extracts of *F. sur* at the various concentrations tested were found to be significantly higher and lower than the negative (28.25±1.65%; 8.75±0.48 g/dl, respectively) and positive (60.75±1.44%; 16.25±0.48 g/dl, respectively) control, respectively, at P<0.001, except for the 100 mg/Kg fruit extract (53.25±1.18%; 15.63±0.59 g/dl, respectively) at P<0.05 and P>0.05, respectively. Whereas there was no significant (P>0.05) difference between all the extract treated groups and the zero control, except for the 100 mg/Kg fruit extract at P<0.01 (Table 3).

Table 2:
Qualitative Phytochemical Screening of Methanolic Stem Bark and Fruit Extracts of *Ficus sur*

Phytochemical	**Stem Bark**	**Fruit**
Tannin	+	+
Saponin	+	+
Alkaloid	-	-
Anthraquinone	-	-
Flavonoid	+	+
Terpennoid	+	+
Glycoside	-	-

+ = Present; - = Absent

Red Blood Cell Count and White Blood Cell Count
The Mean±SEM Red Blood Cell Count of methanolic stem bark (50 mg/Kg: 4.37±0.01 X1012 Cell/l; 100 mg/Kg: 5.21±0.14 X1012 Cell/l; 150 mg/Kg: 5.76±0.06 X1012 Cell/l) and fruit (50 mg/Kg: 5.93±0.05 X1012 Cell/l; 100 mg/Kg:

6.28±0.03 X1012 Cell /l; 150 mg/Kg: 5.99±0.04 X1012 Cell/l) extracts of F. sur at the various concentrations tested were found to be significantly higher than the negative control (2.81±0.12 X1012 Cell/l) at P value <0.001. All the doses of the stem bark extract tested were significantly lower than the positive control (6.37±0.07 X1012 Cell/l) at P<0.001, while only the 50 mg/Kg fruit extract in particular was significantly lower than the positive control at P value <0.05. Meanwhile, there was no significant difference (P>0.05) between the fruit extract at higher doses and the positive control. There was no significance difference (P>0.05) between the Mean±SEM White Blood Cell Count of both the methanolic stem bark (50 mg/Kg: 6.62±0.84 $X10^9$ Cell/l; 100 mg/Kg: 7.18±0.40 $X10^9$ Cell/l; 150 mg/Kg: 9.44±0.43 $X10^9$ Cell/l) and fruit (50 mg/Kg: 10.01±0.37 $X10^9$ Cell/l; 100 mg/Kg: 10.72±0.33 $X10^9$ Cell/l; 150 mg/Kg: 7.30±0.46 $X10^9$ Cell /l) extracts treated groups and the positive control (8.55±0.27 $X10^9$ Cell /l), except for the negative control (18.35±0.13 $X10^9$ Cell/l) in which significant increase (P<0.001) was observed.

There were no significant differences between the Mean±SEM White Blood Cell Count of the zero control (6.29±1.02 $X10^9$ Cell /l) and the 50 mg/Kg stem, 100 mg/Kg stem and 150 mg/Kg fruit extracts, except for the 150 mg/Kg stem, 50 mg/Kg and 100 mg/Kg fruit extracts at P value of <0.01, <0.01 and <0.001, respectively (Table 3).

Table 3:
Effects of Methanolic Stem Bark and Fruit Extracts of *Ficus Sur* on some Haematological Parameters of Anaemic Rats

Group	Treatment	HCT (%)	HB (g/dl)	RBC Count ($X10^{12}$ Cell /l)	WBC Count ($X10^9$ Cell /l)
1	50 mg/Kg Stem	38.25±1.18$^{c,\ c,\ d}$	10.13±0.59$^{c,\ c,\ d}$	4.37 ±0.01$^{c,\ c,\ d}$	6.62±0.84$^{d,\ c,\ d}$
2	100 mg/Kg Stem	38.50 ±1.19$^{c,\ c,\ d}$	10.63±0.43$^{c,\ c,\ d}$	5.21 ±0.14$^{c,\ c,\ c}$	7.18 ±0.40$^{d,\ c,\ d}$
3	150 mg/Kg Stem	48.25 ±1.03$^{c,\ c,\ d}$	13.63 ±0.52$^{c,\ c,\ d}$	5.76 ±0.06$^{c,\ c,\ c}$	9.44 ±0.43$^{d,\ c,\ b}$
4	50 mg/Kg Fruit	47.50 ±0.96$^{c,\ c,\ d}$	13.25±0.48$^{c,\ c,\ d}$	5.93±0.05$^{c,\ a,\ c}$	10.01 ±0.37$^{d,\ c,\ b}$
5	100 mg/Kg Fruit	53.25±1.18$^{a,\ d,\ b}$	15.63±0.59$^{a,\ d,\ b}$	6.28 ±0.03$^{c,\ d,\ c}$	10.72±0.33$^{d,\ c,\ c}$
6	150 mg/Kg Fruit	49.75±1.65$^{c,\ c,\ d}$	13.38±0.38$^{c,\ c,\ d}$	5.99 ±0.04$^{c,\ d,\ c}$	7.30 ±0.46$^{d,\ c,\ d}$
7	FeSO4 (50 mg/Kg)	60.75±1.44	16.25±0.48	6.37±0.07	8.55±0.27
8	Negative (Anaemic) control	28.25±1.65	8.75±0.48	2.81±0.12	18.35±0.13
9	Zero (Non-Anaemic) control	44.50 ±1.56	13.25±0.32	4.41 ±0.14	6.29 ±1.02

Each value represents Mean ± SEM of five rats per group. Values of Extract Treated Group differ significantly from the positive control, negative control and zero control at 5 percent level (a = P<0.05), 1 percent level (b = P<0.01), 0.1 percent level (c = P<0.001) and d= P>0.05 (Not Significant).
***HCT** = Haematocrit (%), **HB** = Haemoglobin concentration (g/dl), **RBC Count** = Red Blood Cell Count*

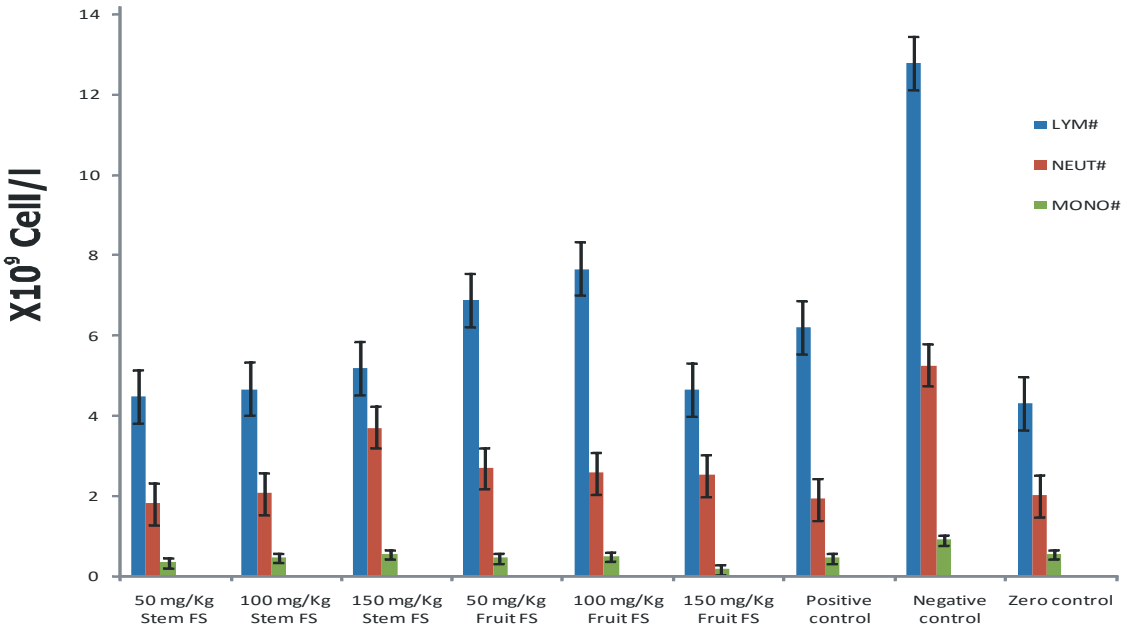

Figure I:
Effect of Methanolic Stem Bark and Fruit Extracts of *Ficus sur* on Differential White Blood Cell Counts in albino Wistar rats.
*Each vertical bar represents Mean ± SEM of four rats per group. **LYM#** = Absolute Lymphocyte Count (...x109 Cell/l), **NEUT#** = Absolute Neutrophil Count (...x109 Cell/l), **MONO#** = Absolute Monocytes Count (...x109 Cell/l), FS =Ficus sur.*

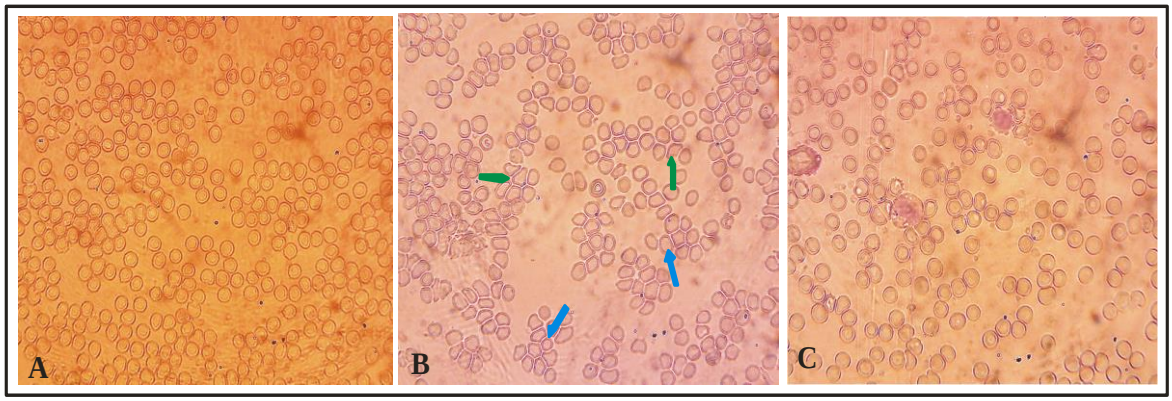

Plate 1:
Photomicrographs of blood film of rat in the zero control group (A) showing normocytic normochromic red cells), negative control group (B) showing marked poikilocytosis (Green arrow) and anisocytosis (Blue) and positive control group (C) showing majority of the red cells to be normal (X40).

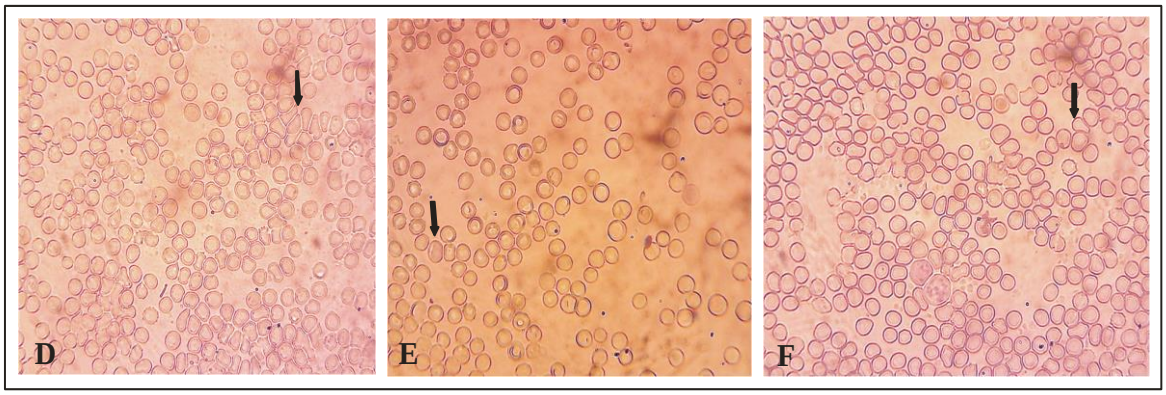

Plate 2:
Photomicrographs of blood film of rat in the 50 mg/Kg (D), 100 mg/Kg (E), and 150 mg/Kg (F) Stem bark extract group showing fewer abnormal red cells (black arrow) compared to the negative control (X40).

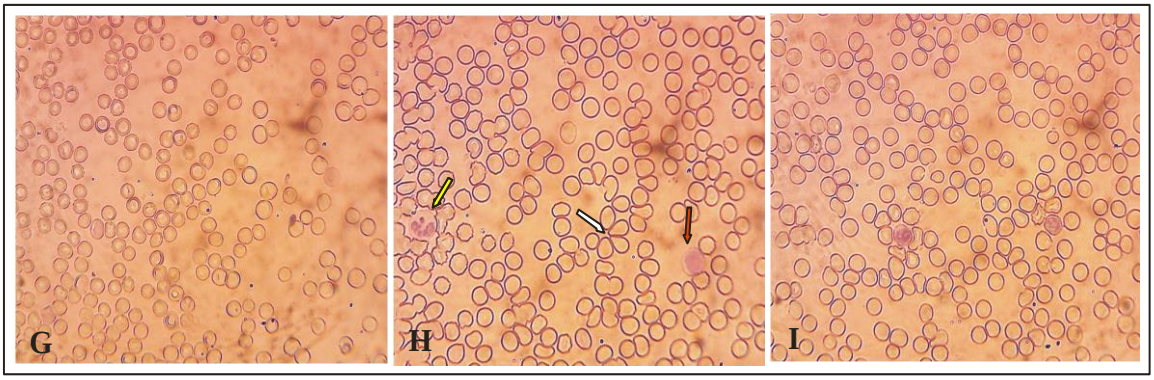

Plate 3:
Photomicrographs of blood film of rat in the 50 mg/Kg (G), 100 mg/Kg (H), and 150 mg/Kg (I) Fruit extract group showing majority of the red cells population to be normal compared to the negative control. Take note of prominent Monocyte (Yellow arrow), Tear drop cells (Feature of extramedullary erythropoiesis - White arrow) and lymphocyte (Red arrow) in the photomicrograph of the 100 mg/Kg (H) Fruit extract group (X40).

Differential White Blood Cell Count

Generally, the absolute lymphocytes and neutrophils counts were significantly ($P<0.001$) reduced in both the methanolic stem bark (50 mg/Kg: 4.49±0.61 $X10^9$ Cell/l; 1.80±0.13 $X10^9$ Cell/l, respectively; 100 mg/Kg: 4.67±0.46 $X10^9$ Cell/l; 2.05±0.14 $X10^9$ Cell/l, respectively; 150 mg/Kg: 5.18±0.13 $X10^9$ Cell/l; 3.71±0.28 $X10^9$ Cell/l, respectively) and fruit (50 mg/Kg: 6.88±0.36 $X10^9$ Cell/l; 2.69±0.01$X10^9$ Cell/l, respectively; 100 mg/Kg: 7.66±0.34 $X10^9$ Cell/l; 2.56±0.20 $X10^9$ Cell/l, respectively; 150 mg/Kg: 4.65±0.29 $X10^9$ Cell/l; 2.50±0.23 $X10^9$ Cell/l, respectively) extracts of *F. sur* when compared to the negative control (12.78±0.32 $X10^9$ Cell/l; 5.26±0.27 $X10^9$ Cell/l, respectively), but no statistical

differences (P>0.05) were observed when compared to the positive (6.20±0.12 $X10^9$ Cell/l; 1.91±0.30 $X10^9$ Cell/l, respectively) and zero (4.31±0.66 $X10^9$ Cell/l; 1.99±0.52 $X10^9$ Cell/l, respectively) control, except for the 50 mg/Kg and 100 mg/Kg fruit extracts in which the Absolute lymphoctyes counts were significantly higher than the zero control at P<0.01 and P<0.001, respectively.

Meanwhile, the absolute neutrophils count of the 150 mg/Kg stem extract was significantly higher than the positive and zero control, but lower than the negative control at P<0.01. On the other hand, the absolute monocytes counts of the stem and fruit extracts show no statistical differences (P>0.05) when compared with the positive and zero control, but, there were irregular differences when compared with the negative control. However, no presence of eosinophils and basophils was observed in the blood smears from all the rats used in this study (Figure 1). While no significant (P>0.05) differences were observed between the stem bark and fruit extracts at the highest dose (150 mg/Kg) tested for all the studied parameters, significant differences were observed both at 50 mg/Kg and 100 mg/Kg doses at P<0.01 and P<0.001, respectively. 100 mg/Kg Fruit extract prove to be the most effective while 50 mg/Kg stem prove to be the least effective

DISCUSSION

In the present study, we examined and compared the potential effects of oral administration of methanolic stem bark and fruit extracts of *Ficus sur* at different concentrations on some haematological parameters in experimental rats pre-exposed to Phenylhydrazine (PHZ). From results obtained, it could be said that Phenylhydrazine selectively destroyed mature red blood cells as evident by marked poikilocytosis and anisocytosis seen in the photomicrograph of blood smear of the negative control rats. According to Abramson (2004), a blood smear is particularly important in the diagnosis of acute hemolysis induced by oxidant damage. There are morphologic abnormalities that are critical in the differential diagnosis of anaemia and that can be determined only from a blood smear. In the hemolytic anaemia, the detection of variations in red cell shape and size is of considerable diagnostic importance (Shattil, 2003; Barbara, 2005).

The marked decreased in HCT, Hb and RBC Count observed in the anaemic rats agrees with the blood pictures and confirms the haemolytic property of phenylhydrazine. Sub chronic intoxication of rats with PHZ has been documented to result in a marked haemolytic anaemia characterized by decreased RBC, Haemoglobin and PCV. The result agrees with already existing literature (Cruz, 1941; Yeshoda, 1942; Jain and Hochstein, 1980; Kinuta *et al*., 1995; Unami *et al*., 1996) that phenylhydrazine induces haemolytic anaemia by selectively destroying matured red blood cells through oxidative stress, denaturation of red cell haemoglobin, membrane phopholipids and enzymes involved in energy metabolism leading to marked decrease in HCT, Hb and RBC Count observed in the rats after administration. On the other hand, significant increase observed in the haematological profile of the positive control group confirms the haematopoietic stimulatory property of ferrous sulphate as a standard hematinic.

Although there seems to be limited information regarding the effects of these extracts on blood parameters particularly in rats, the result of this present work agrees with that of Akomas *et al*., (2014) who used ethanolic leaf extract of *Ficus sur* to improve the haematological profile of diabetic rats at a concentration of 150 and 300 mg/Kg. The outcome of this present study shows that the methanolic stem bark extract of *Ficus sur* appear to exhibit a haematopoietic activity in a dose dependent manner, with the highest concentration tested (150 mg/Kg) proving to be most effective.

HCT, Hb and RBC Count are particularly important for the diagnosis of anaemia in humans and most animals. The haematological parameters of the extract treated rats investigated in this study fall within the normal range of values as reported by Mitruka and Rawnsley (1977); Ihedioha *et al*., (2004). Besides, the treated groups had higher values for these parameters than the negative and zero control. This agrees with the photomicrographs of the blood smears taken from the extracts treated groups which show that the population of deformed red blood cells were significantly reduced when compared to that of the negative control (anaemic) rats. This indicates that a more efficient erythropoiesis occurred following administration of the extracts and without doubt, the anaemic status of the treated rats was improved upon suggesting that methanolic extracts of *Ficus sur* possess inherent-anti-anaemic properties which may be due to its ability to improve bone marrow functions, a major site for erythropoiesis (Orhue, *et al*., 2008).

The study further revealed that there was no significant difference between the White Blood Cell Count of both the methanolic stem bark and fruit extracts treated groups and the positive control, except for the negative control in which significant increase was observed. The marked peripheral leucocytosis observed in the negative control group is typical and characteristic of haemolytic anaemia and is comparable to those of diabetic rats reported by Akomas *et al*., (2014).

In most clinical situations, when a WBC count is requested, it is usual to perform also a differential WBC count in order to provide information on the proportion of the different white cells present in circulating blood (Cheesbrough, 2002e; Tatfeng and Enitan, 2012; Enitan *et al*., 2012). Of all the white blood cell population counted, the lymphocytes were the mostly proliferated cells, followed by the neutrophils and lastly the monocytes. Eosinophils and basophils were entirely absent in the study. The extract when administered to anaemic rats appear to have the potential to stimulate production of more lymphocytes than any other cell line type and it is indicative of the immunological process involved in PHZ-induced hematotoxicity. Though there was marked lymphocytosis, the outcome of this present work show that extracts of *F. sur* could stimulate different blood cell lines to proliferate, thus exhibiting a hallmark effect in the haematopoietic micro-environment. Therefore, it may be found useful in the improvement and maintenance of the haematological status of both anaemic and non-anaemic animals, respectively.

The outcome of this present study also suggests that *F. sur* can enhance extramedullary erythropoiesis as evident by

presence of tear-drop cells in the blood picture taken particularly from the 100 mg/Kg fruit extract treated rats. Presence of tear-drop cells in periphery blood film is a feature of extramedullary erythropoiesis. Stimulation of splenic erythropoiesis by *F. sur* components is most likely mediated by hypoxia which steps up the production of erythropoietin.

Phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new agents from higher plants (Sofowora, 1993). Our qualitative phytochemical screening of the stem bark and fruit extracts indicated the presence of tannin, saponin, flavonoid, terpenoid, while alkaloid, glycoside and anthraquinone were absent in both extracts. This present work agrees with previous studies by Hassan and Almahy (2005); Oyeleke *et al*., (2008); Adebayo-Tayo and Odeniyi (2012), except for alkaloid, glycoside and anthraquinone. Interestingly, tannin and saponin were present in both plant parts studies, unlike the findings of Solomon-Wisdom *et al*., (2011) where certain phytochemicals may be found in only one part of the plant and not in the other. It is worthy of note that biological activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, and nutrient concentrations of the soil as well as the extraction method (Enitan *et al*., 2014). The relationship between chemical composition of plants and geographical location has been documented. Adeshina *et al*., 2010, reported that the geographical location of the plant influences the amount of phytochemicals present in it. This justifies the need for further investigation. The presence of phytochemical constituents in the methanolic extracts of *Ficus sur* indicates that the plant could be used in a multitude of beneficial ways than already studied.

The outcome of this current study further confirms the haemato-stimulatory and anti-anaemic potentials of these extracts and therefore can serve as haematinics. No doubt, good and effective haematopoietic function can be enhanced and maintained by the use of micronutrients found in plants (Feder, 2008). From the data generated in this study, it can be concluded that methanolic extract of *Ficcus sur* at different concentrations tested have varied stimulating effects on the haematological parameters investigated.

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