https://doi.org/10.46344/JBINO.2020.v09i05.38

HEMATOLOGICAL AND HISTOLOGICAL EFFECTS OF *FICUS SYCOMORUS* LEAF EXTRACT ON BONE MARROW OF PHENYLHYDRAZINE - INDUCED HAEMOLYTIC ANAEMIA

Finbarrs-Bello Elizabeth, Christian Ejuiwa Mba, Anikwe Vincent Amiobi.

Department of Anatomy, Faculty of Basic Medical Sciences. Enugu State University of Science and Technology, Parklane. Enugu State. Nigeria.

E-mail : elizabeth.finbarrs-bello@esut.edu.ng

ABSTRACT

Anemia contributes to increase morbidity and mortality in most African countries. The populaces have over time relied on herbal remedies within their localities for intervention. *Ficus sycomorus* is one of such plants that have being in use in the treatment of anemia. This study investigated the modulatory effect of *Ficus sycomorus* leaf extract on hematological parameters and histology of the bone marrow on phenlyhydrazine - induced anemia. Thirty six rats of both sexes weighting between of 140-200 grams were grouped into nine groups with four animals in each group. The control group (grp 1) received 0.1ml normal saline (vehicle). Group 2 served as the untreated anemic group which received of 40mg/kg of phenlyhydrazine (PHZ). Groups 3, 4 and 5 were administered low, medium and high doses of extract only (non- anemic), Groups 6, 7 and 8 were induced anemia via PHZ and treated with low, medium and high doses of *Ficus sycomorus* aqueous leaf extract, while group 9 was induced and treated with folic acid as standard drug. These hematological analysis revealed that oral administration of *Ficus sycomorus* aqueous leaf extract has doses dependent enhancing effect on RBC, Hb and PCV comparable with folic acid. The histological indicated that *Ficus sycomorus* leaf extract has a mild dose dependent effect. The implied the extract exhibited erythropoeitic properties in the treatment of the experimental model of induced anemia and consequently justified it folklore use in traditional medicine practice.

Keywords: Anemia, erythropoeisis, bone marrow, Ficus sycomorus.

INTRODUCTION

The local people of different communities have a long history of traditional plant usage for medicinal purposes. This widely used source of treatment in the primary health care system of resource poor communities is due to the fact that these plants are affordable and easily accessible. Medicinal plant is an important element of indigenous medical systems all over the world. The ethnobotanical provides a rich resource for natural drug research and development (Farnsworth, 1990). Natural products have played an important role throughout the world in treating and preventing human diseases. These medicinal products have come from various source materials including terrestrial plants, terrestrial microorganisms, marine organisms, and terrestrial vertebrates and invertebrates (Newman *et al*., 2000).

The World Health Organization (WHO) has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines (WHO, 1993). Medicinal plants are resources of new drugs and many of the modern medicines are produced indirectly from plants. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.

The sycamore fig (*Ficus sycomorus*) is a fig species in the mulberry family, hence its alternative name, fig mulberry. The species dates back to ancient Egyptian times and, despite what its name suggests, it is not actually a maple tree, but a fig species-proper. The tree boasts an enormous, broad crown and can grow up to 20 meters in height. It prefers frost-free, warm climates and can be found growing near rivers, streams and in woods (Marius 2015). The plant is widely distributed in tropical Africa stretching from Senegal to South Africa, Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast. In Nigeria the plant is mostly found in semi-arid regions (Williams *et al.,* 1980).

Ficus sycomorus is used traditionally in the treatment of snake bites, jaundice, chest pains, dysentery, cool, coughs and throat infections (Sofowora, 1993). In northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal infections, jaundice and dysentery (Berg and Corner 2005). The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal infection, jaundice and dysentery (Hassan *et al*., 2007; Aduom *et al*., 2012). The parts of *F. sycomorus* used traditionally for the treatment of tumors and diseases associated or characterized by inflammation include the fruits in different stages of ripening, fresh or dry, tree bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches (Lansky *et al*., 2008). Natives of Aninri Local Government have long used the leaf of this plant traditionally in combination with other plants to treat blood loss cases like

malaria induced anemia. Studies carried out by (Sandabe et al., 2007) observed that *ficus sycomorus* extract can stimulate erythropoiesis in rabbits which is an indication that it may be useful in anemic situations, such as hemolytic anemia. Sycamore is a rich source of minerals e.g. phosphorus, magnesium, calcium and iron (Okoronkwo *et al.,* 2014). Iron is required for red blood cells formation (Elizabeth *et al*., 2016).

Anemia is a condition that develops once blood lacks enough healthy red blood cells or hemoglobin. Anemia affects the lives of over two billion individuals globally, accounting for over 30% percentage of the world's population (Ramesh & Lopamudra, 2010). There are several types of anemia, many of which are rare but in all cases there is a reduction of number of circulating red blood cells and circulating hemoglobin (Holden and Acomb, 2007). Hemolytic anemia is a form of inherited or acquired anemia which results from either intravascular or extravascular RBC destruction (Powers and Silbersyein, 2009). It has numerous external and internal causes ranging from relatively harmless to life-threatening.

Anemia can also be separated into morphological and causal categories (Bruner *et al.,* 1996). The morphological category depends on the size of hemocytes and Hbg concentration, whereas the causal category consists of hypochromic anemia and hemolytic anemia due to hemorrhagic anemia and aplastic anemia and nutritional deficiency of RBCs (iron, vitamin B12, and folic acid) (Seo *et al.,* 1996). Hemorrhagic and hypochromic anemia are cured by supplementing the blood and by nutritional means and are eliminated by investigating the causes for the hemolytic anemia. No special treatments for essential aplastic anemia and other anemias are available currently, and these diseases are treated by restoring bone marrow using crude drugs.

Anemia animal models include a low-iron diet, and the other model is induced by vitamin B12 and folic acid deficiency. However, these models require a long induction time; thus, anemia is generally induced by cyclophosphamide, an anti-cancer agent, or by phenylhydrazine (PHZ). This compound is used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. Phenylhydrazine is used for the induction of haemolytic anaemia and the study of its mechanism in rats (Berger 1985a) Exposure to phenylhydrazine may cause damage to red blood cells, potentially resulting in anaemia and consequential secondary involvement of other tissues, such as the spleen and liver (Stern, 1989).

Folic acid (Folate) occurs as yellow crystals which are insoluble in water, but its sodium salt is freely water soluble. Chemically it is *Pteroyl glutamic acid (PGA)* consisting of pteridine + paraaminobenzoic acid (PABA) + glutamic acid (Katzungs, 2015). The primary use of *folic acid* is in treating deficiency states that arise from

inadequate levels of the vitamin. A primary result of *folic acid* deficiency is megaloblastic anemia (large-sized red blood cells), which is caused by diminished synthesis of purines and pyrimidines. This leads to an inability of erythropoietic tissue to make DNA and, thereby, proliferate (Lippincott, 2012).

There are several types of anemia, many of which are rare but in all cases there is a reduction of number of circulating red blood cells and circulating hemoglobin (Holden and Acomb, 2007). Managing hemolytic anemias includes avoiding suspect medications, treating related infections and taking drugs that suppress your immune system, which may be attacking your red blood cells. Depending on the severity of your anemia, a blood transfusion or plasmapheresis may be necessary. These Anemia management modalities increases costs of medical care and lower a person's productivity through a decreased ability to work .Plants have long served as an alternative source of treatment for some diseases. Traditional medicine utilizes *ficus sycomorus* in treatment of blood loss related (Sandabe *et al.,* 2007). though no evidence has been shown to prove this hence, it is imperative to check the effect of *ficus sycomorus* extract on experimentally induced hemolytic anemia in albino wister rats, This study investigates the effect of ethanolic extract of *ficus sycomorus* on Phenylhydrazine-induced hemolytic anemia in albino wister rats.

MATERIALS AND METHOD

Collection and Extraction of Plant Material

The plant material was collected from a farm in Aninri L.G.A Enugu state Nigeria. And identification was done afterwards. The plant materials was washed with distilled water and air-dried under shade for seven days. Thereafter, the materials were pulverized into a fine powder; the dried powder was then submerged in ethanol for 48 hours. After which it was filtered through mesh cloth. The filtrate (ethanolic extract) was left in the open for the ethanol to evaporate to paste. The aqueous extract of *Ficus Sycomorus* was dark green in color and with a peculiar fragrance. The extraction process gave a yield of 32.76 % w/w. This was transferred into a suitable container and kept in the refrigerator at low temperature (4^0C) for the experiment .

Experimental animal

Thirty six male albino Wistar rats weighing (140-200 g) were purchased from the Laboratory Animal Facility of the Department of Anatomy, College of Medicine Enugu State University of Science and Technology and used for the experiments. During the two weeks acclimatization they were kept in clean cages, maintained at normal room temperature and natural daylight/night conditions and were allowed free access to standard commercial pelleted feed and clean drinking water. The study was approved by Animal Experimental ethic committee at Enugu State University of Science and Technology ,College of Medicine.

Induction of anemia

Anemia was induced in rats on day0 by intraperitoneal administration of phenylhydrazine (PHZ) (**P**henylhydrazine Hydrochloride spectrum chemical U.S.A) at 40 mg/kg. Two additional administration of the same dosage were given on day1 at 9am and 6pm as described by (Moreau *et al*., 2012).

A total of thirty six male albino Wistar rats weighing (140 – 200g) was used for the study. They were randomly divided into 9 groups as follows: (Table 1: Administration schedule) The extracts were dissolved in normal saline and treatment was *per os* which lasted for 14 days.

Treatments

GROUP	WHAT WAS ADMINISTRED	DOSAGE
Group 1	Normal control	0.1ml of normal saline
Group 2	Negative control	PHZ only
Group 3	Low dose extract alone	300mg/kg/day
Group 4	Medium dose extract alone	450mg/kg/day
Group 5	High dose extract alone	600mg/kg/day
Group 6	PHZ + low dose extract	300mg/kg/day
Group 7	PHZ + medium dose extract	450mg/kg/day
Group 8	PHZ + high dose extract	600mg/kg/day
Group 9	PHZ + folic acid standard drug	600mg/kg/day

Table 1 showing administrative schedule

Histological Study

This was carried out as described by Finbarrs-Bello et al., 2016. At the end of the experiment the metaphysis from the various groups of rats were collected for histopathology after which they were fixed in 10% formal saline, decalcified in formic acid and dehydrated in ascending grades of ethanol. Thereafter, the tissues were cleared in chloroform overnight, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and sectioned at 5 – 6 microns. The sections were deparaffinized in xylene, taken to water and subsequently stained with Haematoxylin and Eosin (H and E). Selected sections were captured onto a computer with Moticam 2001 camera attached to a Moticam- microscope (Moticam products, London, UK).

Statistical analysis

Data was be analyzed using a one way ANOVA followed with a post hoc test (least square division test) using the SPSS 18 version. Results were presented as mean±SEM and p value of less than 0.05 was accepted as statistically significant.

RESULTS

Hematology

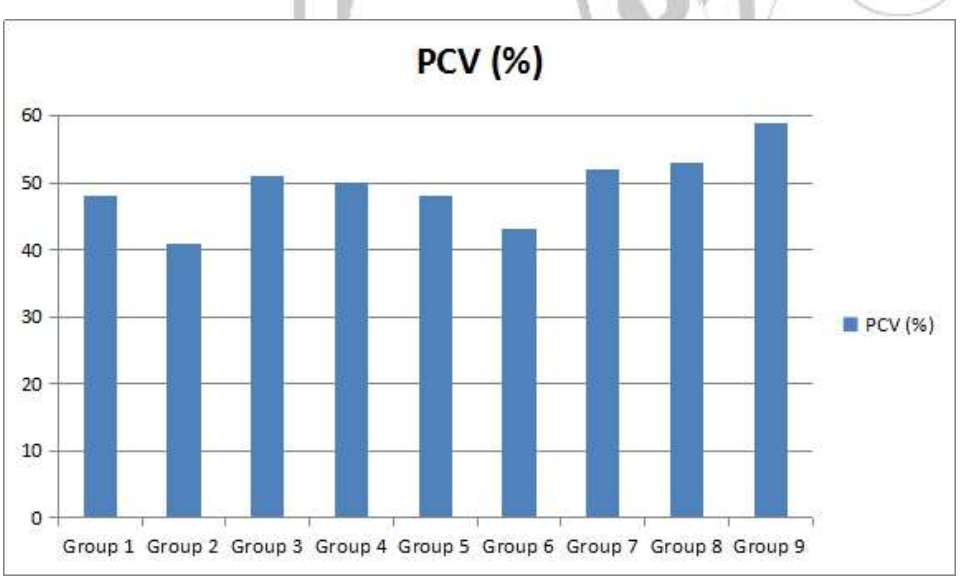

Figure 1: Comparison of Packed Cell Volume: Result showed that there was a significant ($p<0.05$) reduction in % PCV in the negative control when compared to the normal control. While Groups 3, 4, 5, 7, 8 & 9 showed significant increase in % PCV when compared to the normal and negative groups. Group 6

showed a decreased %PCV compared to the normal control but with a slight increase when compared to group 2 (negative control).

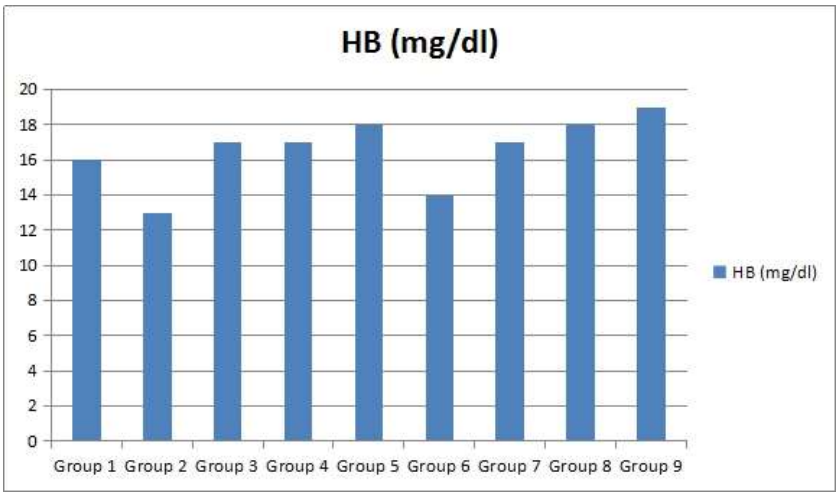

Figure II: Comparison of Hemoglobin Concentration : The negative control group showed a significant ($p<0.05$) reduction in Hb concentration when compared to the normal control. Significant increase ($p<0.05$) in hemoglobin concentration was observed in Groups 3, 4, 5, 6, 7, 8 and 9 when compared to the negative control. However, Hb concentration in Group 6 reduced when compared to Normal control (Group 1)

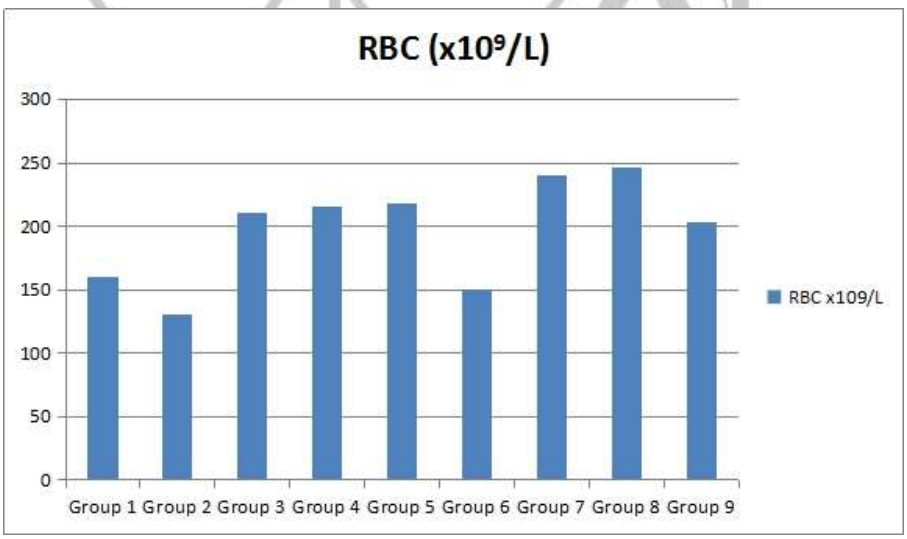

Figure III: Comparison of Red Blood Cell Count: Result showed that there was a significant ($p<0.05$) decrease in RBC count in the negative control group when compared to the control. Group 3, 4, 5, 7, 8, & 9

showed a significant increase in RBC count when compared to the negative and normal control groups. Group 6 however showed a significant increase when compared to the negative control but also a slight decrease when compared to the normal control. Group 7 & 8 where higher when compared to group 9

Histological Findings

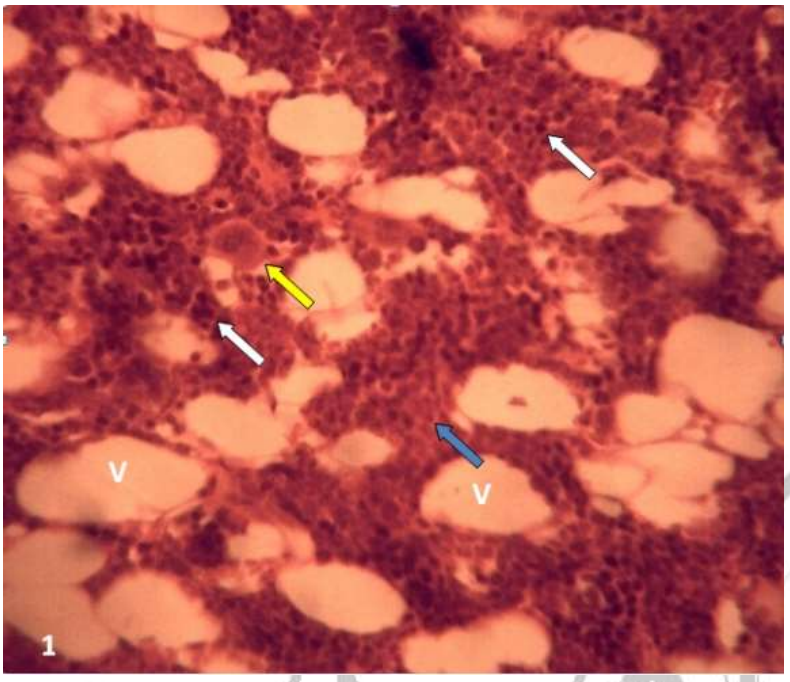

Plate 1: A photomicrograph of group 1 (normal control) administered normal saline only, section of the bone marrow in this group shows the normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity. The hematopoietic tissue consists of a variety of cell types including, the blood cells and their precursors, erythroid and myeloid cells, megakaryocytes, macrophages, and adipocytes represented by their fat vacuoles (V). Areas of myeloid (Blue arrow) and erythroid (White arrow) hematopoiesis as well as megakaryocytes (Yellow arrows) can be identified in the hematopoietic tissue. The erythroid cells/element is seen as round dense and deeply basophilic nuclei without much cytoplasm. The myeloid cells/element has lighter staining nuclei and pink cytoplasm. Megakaryocytes are identified as large cells with multilobated nuclei. H and E x 400.

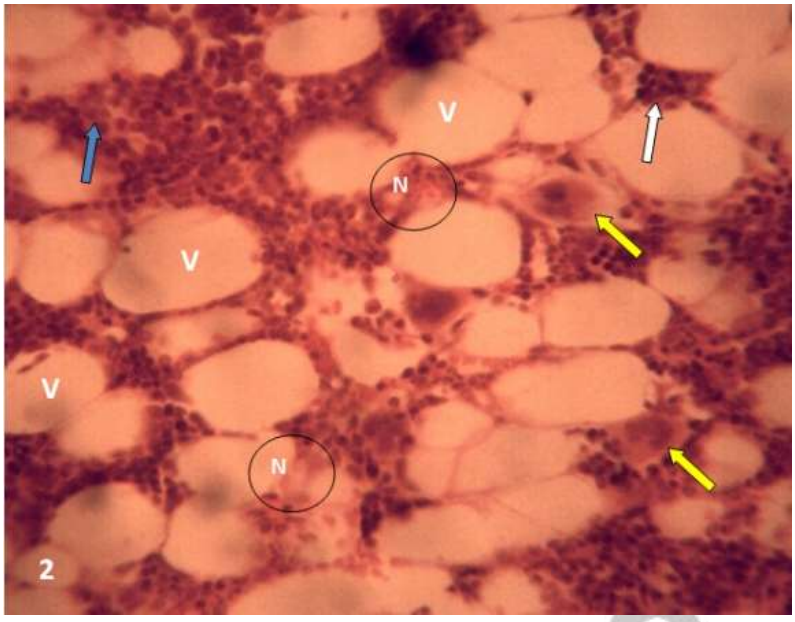

Plate 2: A photomicrograph of group 2 (negative control) administered phenylhydrazine (PHZ) at 40 mg/kg, section of the bone marrow in this group shows an abnormal hematopoietic microenvironment with reduced hematopoietic cellularity replaced with numerous adipose tissue vacuoles (V). Areas of cellular necrosis (N) are also noticed. H and E x 400.

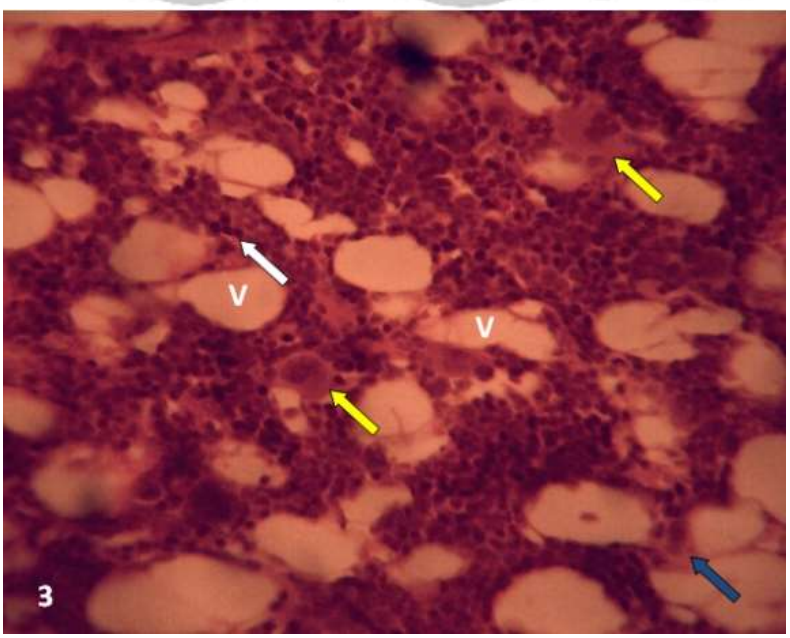

Plate 3: A photomicrograph of group 3 administered low dose extract at 300mg/kg/day , section of the bone marrow in this group shows the normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity. Areas of myeloid (Blue arrow) and erythroid (White arrow) hematopoiesis as well as megakaryocytes (Yellow arrows) and adipocytes represented by their fat vacuoles (V) are identified in the hematopoietic tissue. H and E x 400.

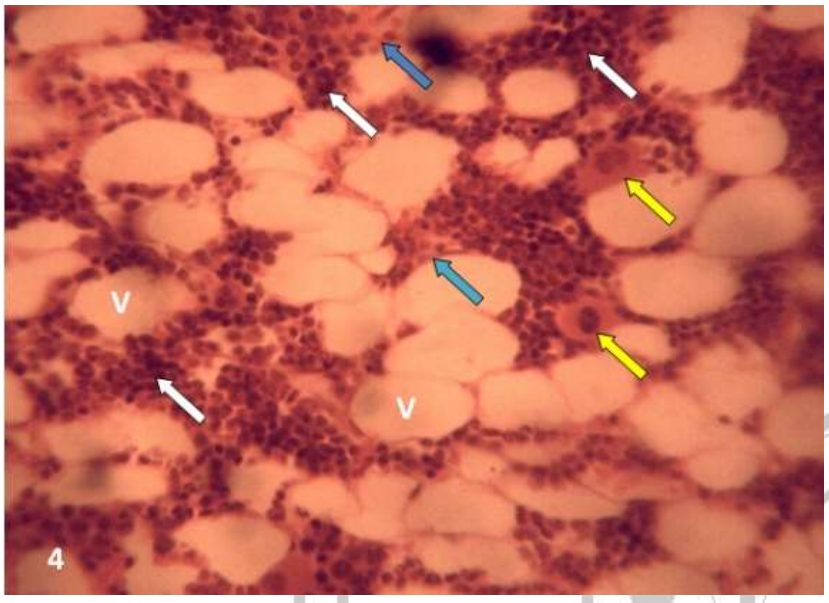

Plate 4: A photomicrograph of group 4 administered medium dose extract at 450mg/kg/day, section of the bone marrow in this group shows a hematopoietic microenvironment with a normal degree of hematopoietic cellularity but with a slight increase in fat vacuoles (V). Areas of myeloid (Blue arrow) and erythroid (White arrow) hematopoiesis as well as megakaryocytes (Yellow arrows) are identified in the hematopoietic tissue. H and E x 400.

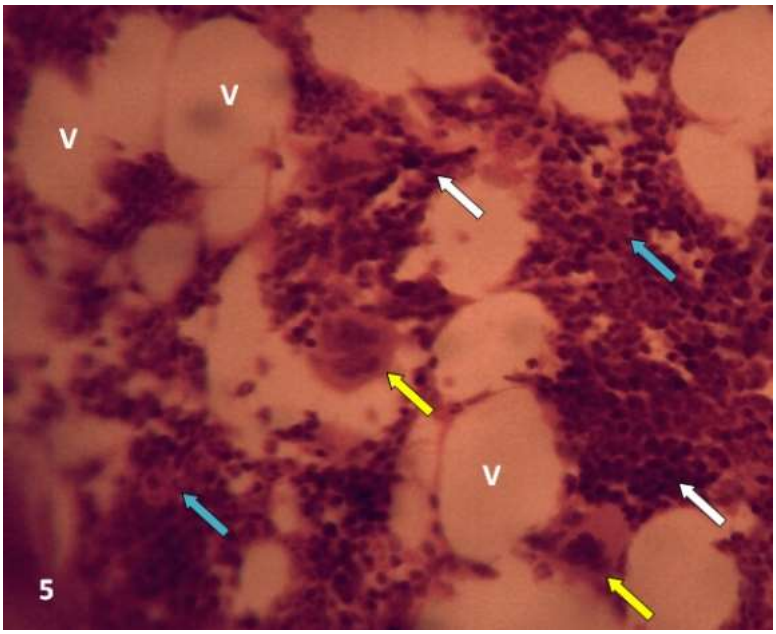

Plate 5: A photomicrograph of group 5 administered high dose extract at 600mg/kg/day**,** section of the bone marrow in this group shows a normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity. Areas of myeloid (Blue arrow) and erythroid (White arrow) hematopoiesis as well as megakaryocytes (Yellow arrows) are identified in the hematopoietic tissue. H and E x 400.

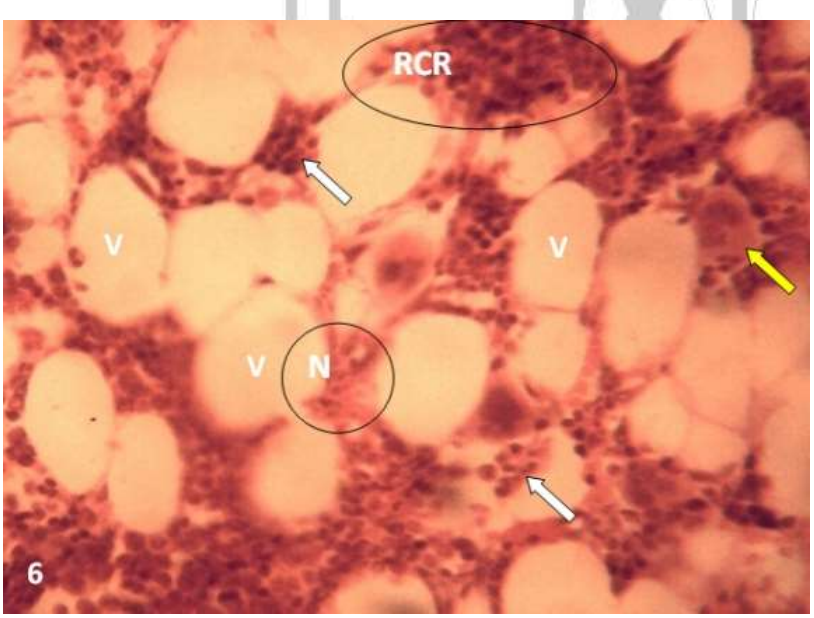

Plate 6: A photomicrograph of group 6 induced with 40mg/kg PHZ and treated with low dose extract at 300mg/kg/day, section of the bone marrow in this group shows a hematopoietic microenvironment with

reduced hematopoietic cellularity, numerous adipose tissue vacuoles (V) replacing the cellular areas, areas of mild cellular necrosis (N) and a region of cellular regeneration (RCR) are also noticed. Generally, the cells appear pale and poorly stained, indicating a decrease in hematopoietic activity but a few areas of erythroid cells/element (white arrows) also indicate cellular recovery/regeneration. H and E x 400.

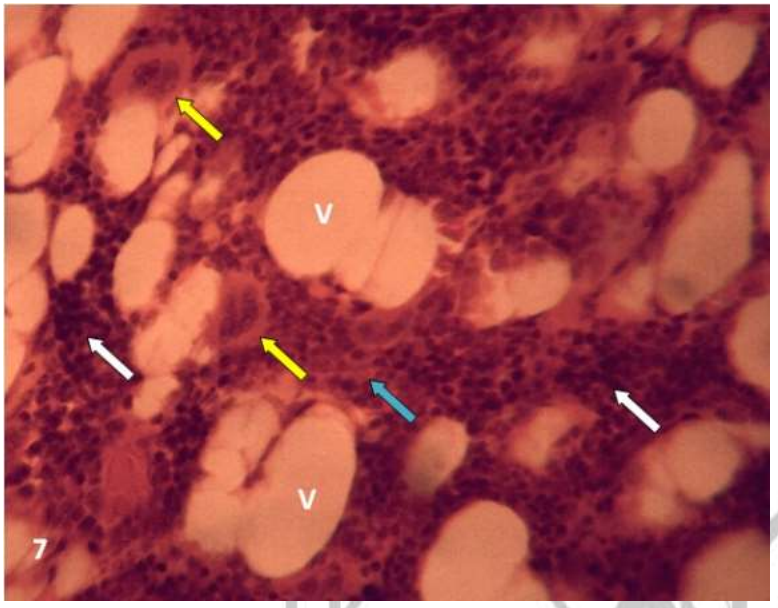

Plate 7: A photomicrograph of group 7 induced with 40mg/kg PHZ and treated with medium dose extract at 450mg/kg/dayThe histology section of the bone marrow in this group shows a normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity and adipose tissue vacuoles (V). H and E x 400.

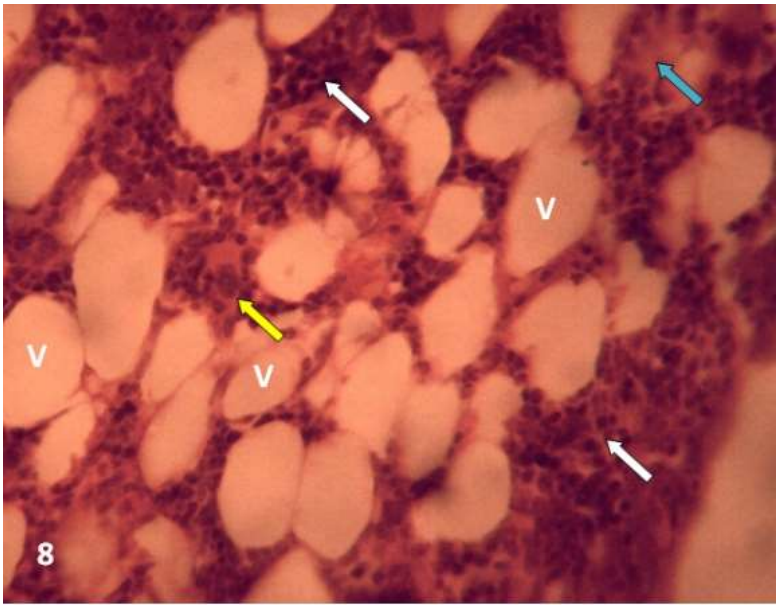

Plate 8: A photomicrograph of group 8 induced with 40mg/kg PHZ and treated with high dose extract at 600mg/kg/day, section of the bone marrow in this group shows a hematopoietic microenvironment with a normal hematopoietic cellularity but increased adipose tissue vacuoles (V). H and E x 400.

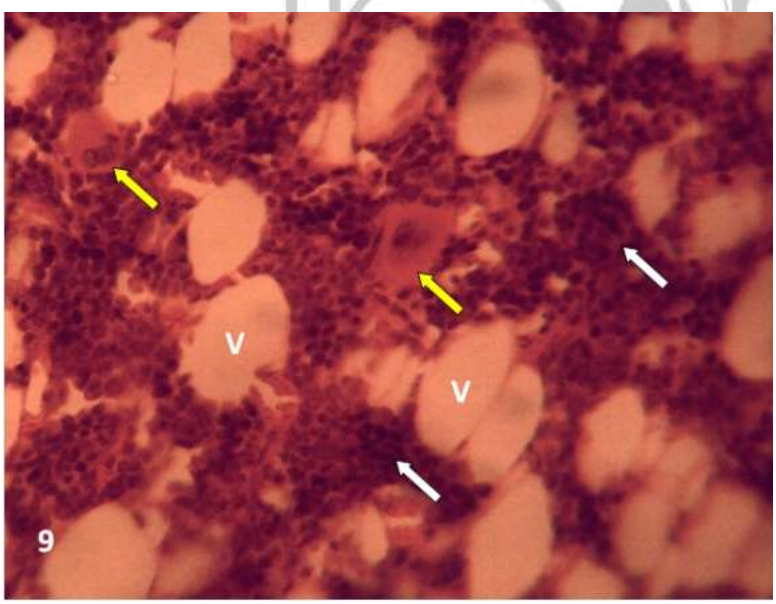

Plate 9: A photomicrograph of group 9 induced with 40mg/kg PHZ and treated with standard drug (folic acid) at 600mg/kg/day, section of the bone marrow in this group shows a normal hematopoietic

microenvironment with a normal degree of hematopoietic cellularity and adipose tissue vacuoles (V). H and E x 400.

DISCUSSION

The present study was aimed at evaluating the effect of *Ficus sycomorus* crude extract on phenylhydrazine-induced haemolytic anaemia in albino wistar rats. Our findings indicate significant result in morphological and haematinic parameters which includes histological examination, blood parameters, RBC, haemoglobin concentration and PCV. In anaemic modelled groups showed a significant decrease when compared with normal control group. This could be due to toxicity caused by PHZ by the involvement of aryl and hydroxyl radicals it generates. It could also be due to poor affinity of oxygen to haemoglobin molecules since the tendency of haemoglobin to bind to oxygen

enhances blood flow to the tissues (Ganong, 2005). In extract only and extract-treated groups, there was also a significant increase in these parameters when compared with the control and anemic groups. This could be due to the phytochemical constituents in the extract and also presence of minerals and vitamins like Iron. These constituents are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow. The effect of oral administration of *Ficus sycomorus* aqueous leaf extract irrespective of the dose has the tendency to increase blood parameters such as RBC, Hb, & PCV. as well as alleviate blood disorders. It will be useful in the treatment of anemia since traditional medicine has become highly integrated in the world of medicine today. However, caution should be taken when administering the extract as it could lead to polycythemia or abnormal high blood cell production when taken in high doses.

The histological findings, shows the proportion of hematological cells to fats cell (adipocytes) in the sections obtained from the control and treated groups. The extract groups showed more hematological cells (greater cellularity) compared to the control. In details, normal control group that was administered normal saline showed the normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity. The hematopoietic tissue consists of a variety of cell types including, the blood cells and their precursors, erythroid and myeloid cells, megakaryocytes, macrophages, and adipocytes represented by their fat vacuoles (V). In contrast, the negative control group, that was administered phenylhydrazine only showed an abnormal hematopoietic microenvironment with reduced hematopoietic cellularity replaced with numerous adipose tissue vacuoles (V). Areas of cellular necrosis (N) were also

noticed. This is in agreement with (Beutler, 2001), which shows that phenylhydrazine induces the destruction of red blood cells by oxidation stress and many joint changes at cellular levels resulting in haemolytic- anaemia.

The histology section of the bone marrow in the extract only group showed a normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity. Areas of myeloid (Blue arrow) and erythroid (White arrow) hematopoiesis as well as megakaryocytes (Yellow arrows) are identified in the hematopoietic tissue. These support the fact that Sycamore is a rich sources of minerals e.g. phosphorus, magnesium, calcium and iron (Okoronkwo *et al.,* 2014) and Iron is required for red blood cells formation (Elizabeth et al., 2016).

The Phenylhydrazine group treated with extract showed a normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity and adipose tissue vacuoles when compared to the negative control group. However the low dose treated group showed a lesser degree of reduced hematopoietic cellularity, numerous adipose tissue vacuoles (V) replacing the cellular areas, areas of mild cellular necrosis (N) and a region of cellular regeneration (RCR) are also noticed. Generally, the cells appear pale and poorly stained, indicating a decrease in hematopoietic activity but a few areas of erythroid cells/element (white arrows) also indicate cellular recovery/regeneration. These indicate that *Ficus sycomorus* leaf extract stimulated erythropoiesis in accordance with studies carried out by (Sandabe *et al.,* 2007). However at low dose *Ficus sycomorus* extract seems not to be effective enough to enhance full recovery from anemia.

The histological section of the folic acid treated group showed a normal hematopoietic microenvironment with a normal degree of hematopoietic cellularity and adipose tissue vacuoles (V). But hematological results showed that *Ficus sycomorus* was more effective compared to the standard group. The RBC level of group 7 and 8 treated with medium and high dose extract respectively was greater than RBC level of group 9 treated with standard dose of folic acid. *Ficus sycomorus* leaf extract stimulates erythropoiesis, producing more blood when taking as a supplement and can serve as an alternative herbal remedy to treat anemia in albino wistar rats and possibly in humans. *Ficus sycomorus* leaf extract can be recommended as alternative medicament for anemia or as hematinic in the bone marrow.

Acknowledgements

Cordial thanks to Mr Rex of histology laboratory for providing the technical and experimental supports.

Competing Interests

Authors declared they have no competing interests

Authors' Contributions

Elizabeth Finbarrs-Bello designed and wrote the protocol for the study, AV wrote the first draft of the manuscript. Authors CEM managed the analyses of the study. 'Authors CEM and EFB managed the literature searches. All authors read and approved the final manuscript

Funding: Self funded

REFERENCES

Aduom O.A., Micheal B.O. and Mohammad I.S. (2012): Phytochemicals and hypoglycaemi effect of methanol stem-bark extract of *Ficus sycomorus* Linn (Moraceae) on alloxan induced diabetic Wistar albino rats. *African Journal of Biotechnology* 11(17):4095- 4097.

Berg C.C. and Corner E.J.H. (2005). Moraceae (*Ficus*). In: Nooteboom, H.P. (ed.), Flora Malesiana 17(2): 1–730. Leiden.

Beutler, E. (2001). Hemolytic Anemia Due to Chemical and Physical Agents. In: Williams Hematology, Beutler, E., B.S Coller, M.A. Lichtman, T.J. Kipps and U. Seligsohn (Eds.) 6th Edn., McGraw-Hill, New York, pp: 676:205-210.

Berger J (1985a.): Screening of toxic-haemolytic anaemia in laboratory rats: a model of phenylhydrazineinduced haemolysis. Haematologia 18:193– 200.

Bruner AB, Joffe A, Duggan AK,Casella JF and Brandt J (1996).Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls.348:992-996.

Bancroft, J.D. and Stevens, A. (2002). Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh. 16-64.

Finbarrs-Bello E, N. J. Nto, I. T. Ikele, M. I. Sani and V. Atuadu (2016). Haematopoietic Enhancing Effect of Ethanolic Seed Extract of *Citrullus lanatus* (Watermelon) on Bone Marrow of Wistar Rats. *European Journal of Medicinal Plants 17(3): 1-7, 2016 Article no.EJMP.30452 ISSN: 2231-0894, NLM ID: 101583475.*

Farnsworth, N.R. (1990) The Role of Ethno Pharmacology in Drug Development. Ciba Foundation Symposium 154. Bioactive Compounds from Plants. John Wiley & Sons, Baffins Lane, Chichester (England), 2-21.

Ganong WF (2005). Review of Medical Physiology 22nd ed.
Lange medical books/Mc Graw-Hill New York. Pp. 515-517.

Hassan S.W., Lawal M., Muhammad B.Y., Umar R.A., Bilbis L.S and Saidu Y. (2007**)**: Effects of anthraquinone glycosides and aqueous ethanol extracts of *Ficus sycomorus* L.

(Moraceae) on rat liver and kidney functions. *Asian Journal of Biochemistry* 2(2):136- 141.

Holden J, Acomb C (2007). Anaemia In: Walker R Whittlesea C editors. Clinical Pharmacy and Therapeutics New York: Churchill Livingstone.699-701.

Lansky, E. P., Paavilainen, H. M., Pawlus, A. D. and Newman, R. A. (2008). *Ficus spp*.(fig):Ethnobotany and potential as anticancer and anti-inflammatory agents. *Journal of Ethnopharmacology*, 119: 195- 213.

Newman, D.J., Crag, G.M. and Snader, K.M. (2000) The Influence of Natural Products upon Drug Discovery. Natural Product Reports, ***17***, 215-234.

Okoronkwo, C.U.1, Ogwo, P.A2. Udensi, E.A1., And Agu, R.O1 (2014)., Nutritional and Phytochemical Composition of Utu (Icacina Senegalensis) and Sycamore (Ficus Sycomorus). Seeds. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 7 Ver. III PP 49-53*

Prakash, P. and Gupta, N. (2005) Therapeutic Uses of *Ocimum sanctum* Linn (Tulsi) with a Note on Eugenol and Its Pharmacological Actions: A Review. *Indian Journal of Physiology and Pharmacology*, 49, 125-131.

Sofowara (1993) A Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd., Ibadan, Nigeria, 289-300.

Sandabe UK, Onyelili PA, Chibuzo GA (2006). Phytochemical screening and effects of aqueous extract of Ficus sycomorus L. (Moraceae) stem bark on muscular activity in laboratory animals. J. Ethnopharmacol,; 104: 283-285.

Sandabe, Umar Kyari, abdurrahaman, Fanna, goniri, Bukar And baba, Usman (2007). The Combined Effects Of Aqueous Extract Of Ficus Sycomorus L. (Moraceae) Stem Bark And Nigella Sativa L. (Ranunculaceae) Seeds On Hematological And Biochemical Parameters In Rabbits. Animal Research International 4(3): 753 – 757.

Stern A (1989) Drug-induced oxidative denaturation in red blood cells. Semin. Hematol. 26:301–306.

WHO (1993) Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. Manila.

Williams, C.N., N.Y. Chew and J.A. Rajaratnan, (1980). Tree Crops of the Wetter Regions of the Tropics. Interstate, New York, pp: 93.