

**THE ROLE OF n-BUTANOL LEAF fraction OF *Telfairia occidentalis* ON SOME
HAEMATOLOGICAL PARAMETERS IN PHENYL-HYDRAZINE INDUCED
ANEMIA IN WISTAR RATS**

BY

SALAWU AKEEM BABASOJI

**HUMAN PHYSIOLOGY
AHMADU BELLOUNIVERSITY
ZARIA, NIGERIA**

SEPTEMBER, 2014

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BY

**SALAWU AKEEM BABASOJI B.Sc (A.B.U) 2004
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**DEPARTMENT OF HUMAN PHYSIOLOGY,
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ZARIA, NIGERIA**

SEPTEMBER, 2014

DECLARATION

I hereby declare that the work in this thesis entitled The Role of n-Butanol Leaf Fraction of *Telfairia Occidentalis* On Some Haematological Parameters in Phenyl Hydrazine Induced Anemia in Wistar Rats was performed by me in the Department of Human Physiology. Under the supervision of Dr. Tanko Yusuf and Dr. Aliyu Mohammed. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Salawu Akeem Babasoji

Name of student

Signature

Date

CERTIFICATION

This thesis entitled: The Role of n-Butanol Leaf fraction of *Telfairia Occidentalis* On Some Haematological Parameters in Phenyl Hydrazine Induced Anemia In Wistar Rats meets the regulation governing the award of the degree of MSc Human Physiology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr T. Yusuf, B.Sc, M.Sc, Ph.D

Chairman, Supervisory Committee.

Signature

Date

Dr A. Mohammed, MB.BS, M.Sc, PhD.

Member, Supervisory Committee.

Signature

Date

Dr A. Mohammed, MB.BS, MSc, PhD.

Head of Department

Signature

Date

Prof. A.Z. Hassan

Dean, School of Postgraduate
Studies

Signature

Date

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ABSTRACT

Anemia is a serious health problem especially in developing countries. In the tropics, rural dwellers had resort to herbal treatment in some cases of anemia. The main aim of this work was to ascertain the role of n-butanol leaf fraction of *Telfairia Occidentalis* on some haematological parameters in phenyl hydrazine induced anemia in wistar rats. Thirty (30) adult Wistar rats were used for this study. All animals were assayed for haematological parameters 1day before the onset of the experiment. The animals were grouped into five groups of five rats each (n =5). Anemia was induced in the Wistar rats by intraperitoneal injection of Phenyl hydrazine Hydrochloride 50mg/kg w/w in DMSO once daily for 3 days. Group i. serves as the negative control group, received 50mg/kg of Phenylhydrazine Hydrochloride and 1ml/kg normal saline (untreated) group ii. serve as Positive control group received Vitamin B12 0.4ml/kg (standard haematinic) intramuscularly, while group iii, iv. and v. received 100mg/kg, 200mg/kg and 300mg/kg body weight of n-butanol leaf fraction of *Telfairia occidentalis* for 2 weeks respectively. Assessment of RBC and WBC was carried out using the newly improved Neubauer counting chamber, PCV using the microhaematocrit reader, Hb using the AO-Hb meter and Bilirubin concentration using colorimetric estimation for the serum bilirubin. There was a significant ($P<0.05$) increase in the level of RBC, PCV, HB and Bilirubin concentration after treatment with the fraction as compared to the control groups respectively. RBC ($5.30\pm 0.0217 \times 10^6/\mu\text{L}$ as compared to $4.78\pm 0.17 \times 10^6/\mu\text{L}$), PCV ($50.60\pm 0.51\%$ as compared to $37.08\pm 0.37\%$), HB ($16.84\pm 0.18 \text{ g/dl}$ as compared to $12.56\pm 0.13 \text{ g/dl}$), WBC ($6.10\pm 0.15 \times 10^9/\text{L}$ as compared to $6.17\pm 0.88 \times 10^9/\text{L}$) and Bilirubin ($18.16\pm 0.08 \mu\text{mol/L}$ as compared to $15.54\pm 0.21 \mu\text{mol/L}$). However, the significant increase obtained from the results of RBC, PCV and Bilirubin was not dose dependent while that of HB and WBC was dose dependent. In conclusion, Intraperitoneal administration of 50mg/kg Phenylhydrazine hydrochloride for 3days decreases the blood parameters below the pre-anemic level (1day before induction) due to the production of reactive oxygen species. Phytochemical screening of this leaf fraction indicates the presence of flavonoids, saponin secoiridoid glycosides and alkaloids, these natural antioxidants could be responsible for reversing the damaging effect of PHZ and thus playing a modulatory role and also maintaining the integrity of the RBC.

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ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

AAKP	American Association of kidney patients
Bw	Body weight
CAT	Catalase
CoA	Coenzyme A
DNA	Deoxyribonucleic Acid
DPG	Diphosphoglycerate
EASE	Estimation Assessment of Substance Exposure
ED	Effective Dose
EDTA	Ethylene-diamine-tetracetic acid
EPO	Erythropoietin
GABA	γ -aminobutyric acid
GLUT1	Glucose transporters 1
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide (GSSG)
H ₂ O ₂	Hydrogen peroxide
HB	Hemoglobin
HBO	Hyperbaric Oxygen
HCT	Haematocrit
HIV	Human Immune deficiency Virus.
LD	Lethal Dose.
LDL	Low Density Lipoprotein

MCV	Mean Copuscular Haemoglobin.
MDA	Malondialdehyde
metHb	Methylhaemoglobin
NHLBI	National Heart,Lungs and Blood Institute.
OHU	Hydroxyl radical
oxyHb	Oxyhaemoglobin
PHZ	Phenylhydrazine
PPm	Packs per Million
PTWI	Provisional tolerable weekly intake
RBC	Red blood cell.
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TC	Transcobalamin
w/v	Weight per volume
WBC	White Blood Cell.
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development (WHO, 2005).

Anemia is a reduction from the normal quantity of circulating hemoglobin in the blood less than 13 g/dl for male and less than 12 g/dl for female adults (Okochi *et al.*, 2003). It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children (Adam *et al.*, 2005).

Hemolytic anemia is a form of anemia due to hemolysis, the abnormal breakdown of Red Blood Cells (RBCs), either in the blood vessels (intravascular hemolysis) or elsewhere in the human body (extravascular). It has numerous possible causes, ranging from relatively harmless to life-threatening (Telford *et al.*, 2003). The general classification of hemolytic anemia is either inherited or acquired. Treatment depends on the cause and nature of the breakdown (Yamoto *et al.*, 1998).

Anemia is the result of a wide variety of causes that can be isolated, but more often coexist. Globally, the most significant contributor to the onset of anemia is iron deficiency so that IDA and anemia are often used synonymously, and the prevalence of anemia has often been used as a proxy for IDA (De Maeyer, 1989). It is generally assumed that 50% of the cases of anemia are due to iron deficiency, but the proportion may vary among population groups and in different areas according to the local conditions (De Maeyer, 1989).

Anemia is an indicator of both poor nutrition and poor health. The most dramatic health effects of anemia is increased risk of maternal and child mortality which have been well documented (Irwin and Kirchner, 2001). In addition, anemia should be built into the primary health care system and existing programmes. These strategies should be tailored to local conditions, taking into account the specific etiology and prevalence of anemia in a given setting and population group (Benoist *et al.*, 2008).

The importance of medicinal plants in traditional healthcare practices, providing clues to new areas of research and in biodiversity conservation is now well recognized. The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed (Ranjan *et al.*, 2010). The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases (Kumar *et al.*, 2009). *Telfairia occidentalis* is a tropical vine grown in West Africa as a leaf vegetable for its edible seeds. Lately the popularity has spanned across other ethnic groups, thereby taking the medicinal and commercial value of the vegetable to a higher level. (Alada, 2000).

1.0.1 Nutritional Content

This blood-building vegetable is known to contain protein, carbohydrate, fat, calcium, iron, magnesium, potassium, calcium, and vitamin such as A, B2, B5, B12 and thiamine.(Harte *et al.*, 1992).



Figure 1.1 Ugu Leaf (*Telfairia Occidentalis*) adopted from *Journal of plant nutrition*, 2011.

T. occidentalis leaves contain 30.5% crude protein, 3.0% crude lipid, 87.3% crude fibre, and 8.4% total ash. The leaves had low level of tannic acid (0.5mg/100 g DM) and oxalate (4 mg/100 g DM), but high level of phytic acid (12 mg/100 g DM) (Oboh, 2005).

1.1 STATEMENT OF RESEARCH PROBLEM

An estimated 30% of the world's population is afflicted with anemia. Anemia in pregnancy is a leading cause of maternal and perinatal deaths in developing countries. In developing countries, anemia affects almost two thirds of the pregnant population. It is also estimated that anemia is responsible for as much as 20% of all maternal deaths in Sub-Saharan Africa (WHO, 2005).

Anemia in pregnancy in Nigeria is not different from other parts of sub-Saharan Africa. In the neighboring republic of Niger, Murray, *et al.*, found that about 47% of breast-feeding women were iron deficient. Harrison, *et al.*, (1989) showed that about 40% of pregnant women are anemic. Isah, *et al.*, (1985) found a prevalence of anemia of 46% in non-pregnant and 52% of pregnant women respectively in Zaria. Iron deficiency was found in 54% and 25% of non-pregnant and pregnant women respectively by Isah, *et al.* Gwarzo, *et al.*, (1994) in Kano found a prevalence of iron deficiency of 17% among pregnant women in Kano. With increase in the prevalence of anemia in developing countries such as Nigeria. It is important to assess the role of n-batanol fraction of *Telfairia Occidentalis* on anemia.

The leaves of *Telfairia Occidentalis* have been reported to possess anit-microbial, anti-fungal properties and also, anti-cholesterolemia (Oluwole, *et al.*, 2003). The young leaves sliced and mixed with coconut water and salt are stored in a bottle and used for the treatment of convulsion in ethno medicine (Gbile, 2003) The leaf extract is useful in the management of cholesterolemia, liver problems and impaired defense immune systems (Eseyin, *et al.*, 2005).

1.2 JUSTIFICATION

Although there are various drugs for the treatment of anemia, they are not accessible to many poor people especially those in the developing countries such as Nigeria. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anemia, so they depend heavily on plants and herbal products for the treatment of diseases and anemia.

As a result of the fact that anemia is very common and the incidence is likely to increase in future, there is need to prevent it or seek for more cost-effective and better treatment strategies (Duff, 2008).

Available studies reveal that the leaf extract of *Telfairia occidentalis* can be used to treat Anemia, convulsion, arteriosclerotic cardio vascular diseases, high blood pressure, hypocholesterolemia, arthritis, liver problems, and inflammatory conditions (Oyolu, 1978).

Most of the studies carried out use aqueous or crude extract of this plant on haematological indices (Ogbe, *et al.*, 2010). There is need for the isolation and identification of the active compounds responsible for the anti-anemic activities.

In this study, we considered the role of n-butanol leaf fraction of *Telfairia occidentalis* on some haematological parameters in phenyl hydrazine induced anemia in Wistar rats.

1.3 HYPOTHESIS

The n-batanol leaf frction of *Telfairia occidentalis* possess anti-anemic properties.

1.4 AIM AND OBJECTIVES

To the role of n-butanol leaf fraction of *Telfairia occidentalis* on some haematological indices in phenylhydrazine-induce anemia in Wistar rats.

1.4.1 SPECIFIC OBJECTIVES

1. To ascertain the role of n-butanol leaf fraction of *T. Occidentalis* on PCV in phenylhydrazine induced anemia in Wistar rats.
2. To ascertain the role of n-butanol leaf fraction of *T. occidentalis* on RBC count in phenyl hydrazine induced anemia in Wistar rats.

3. To ascertain the role of n-butanol leaf fraction of *T. occidentalis* on WBC count in phenyl hydrazine induced anemia in Wistar rats.
4. To ascertain the role of n-butanol leaf fraction of *T. occidentalis* on HB concentration in phenyl hydrazine induced anemia in Wistar rats.
5. To ascertain the role of n-butanol leaf fraction of *T. occidentalis* on Bilirubin concentration in phenyl hydrazine induced anemia in Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

Telfairia occidentalis, commonly called fluted pumpkin is a vegetable which belongs to the family *Cucurbitaceae* is popularly known as ugu in Igbo, Aparoko in Yoruba, and ubong in Efik (Iwu, 1993). Other common names for the plant include fluted gourd and fluted pumpkin (Iwu, 1993).

Though endemic to southeastern Nigeria, *Telfairia* is of local ethno-botanical importance in the folklore and the dietary and cropping systems of Igbos and their neighbours. *Telfairia* has long been important in the internal food trade of Igbos (Bosa and Mgbeogwu, 1973). Like other leaf vegetables, it is of low commercial value but, in some cases, can provide an appreciable cash income to small farm families (Iwu, 1993).

Its leaves, succulent shoots, and seed kernels, constitute the usual ingredients that are popular and regularly consumed in Igbo soups. Soups made of leaf vegetables are essential for consumption of starchy pastes of yam, cassava, or cocoyam, which are frequently consumed in the humid areas of Nigeria (Iwu, 1993). Many good attributes account for the increasing importance of this chief vegetable among 30 to 35 million people in Nigeria (Eseyin, *et al.*, 2007).

The plant is a drought-tolerant, dioecious perennial that is usually grown trellised. The young shoots and leaves of the female plant are the main ingredients of a Nigerian soup, *edikang ikong*. The large (up to 5 cm), dark-red seed is rich in fat and protein, and can be eaten whole, ground into powder for another kind of soup, or made into a fermented

porridge. The fruit of the plant is large, weighing up to 13 kg, but it is inedible (Odoemena and Onyeneke, 1998)

2.1 Vitamin B₁₂

Vitamin B₁₂, a complex water-soluble organic compound that is essential to a number of micro-organisms and animals, including humans. Vitamin B₁₂ aids in the development of red blood cells in higher animals (Brim, *et al.*, 2002).

The vitamin, which is unique in that it contains a metallic ion, cobalt, Vitamin B₁₂ occurs in several forms, called cobalamins; cyanocobalamin is the principal one used in vitamin supplements and pharmaceuticals. Vitamin B₁₂ was first isolated in 1948 by American chemist Karl Folkers and British chemist Baron Alexander Todd. Vitamin B₁₂ is involved in cellular metabolism in two active coenzyme forms methylcobalamin and 5-deoxyadenosylcobalamin (Bose, *et al.*, 1995). Vitamin B₁₂ cooperates with folic acid (folate) in the synthesis of deoxyribonucleic acid (DNA). A deficiency of either compound leads to disordered production of DNA and, hence, to the impaired production of red blood cells (Gimsing and Nexø, 2003).

Vitamin B₁₂ also has a separate biochemical role, unrelated to folic acid, in the synthesis of fatty acids in the myelin sheath that surrounds nerve cells (Harte, *et al.*, 1992). Vitamin B₁₂ is synthesized by micro-organisms that occur in the lumen (the first stomach chamber) of cows and sheep. From the lumen it is transferred to the muscle and other tissues, which other animals and humans eat. Good dietary sources of vitamin B₁₂ are eggs, meat, and dairy products (Christenen, 1992).

In humans a lack of the vitamin results in defective formation of the papillae (small projections) of the tongue, giving an appearance of abnormal smoothness. A deficiency of vitamin B₁₂ often causes defective function of the intestine, resulting in indigestion and sometimes constipation or diarrhea (Harte, *et al.*, 1992). A very serious effect is degeneration of certain motor and sensory tracts of the spinal cord, if the degeneration continues for some time, treatment with vitamin B₁₂ may not correct it (Bose, *et al.*, 1996).

Initial numbness and tingling of fingers or toes may, without treatment, progress to instability of gait or paralysis. Because vitamin B₁₂ is found in animal but not vegetable foods, strict vegetarians (vegans) who do not eat dairy products, meats, fish, eggs, or vitamin B₁₂-fortified foods may develop a deficiency if they do not receive supplements of the vitamin (Moestrup, *et al.*, 2003).

Deficiency may also result from competition for vitamin B₁₂ by the broad tapeworm or by intestinal bacteria growing in cul-de-sacs or above partial obstructions in the digestive tract. Additional nutritional deficiencies, such as those of folic acid or iron, are likely to develop in such cases, as in primary intestinal diseases such as celiac disease, tropical sprue, or regional enteritis, all of which affect the absorptive capacity of the small bowel (Burger, *et al.*, 1975).

Pernicious anemia, a disease characterized by the impaired production of red blood cells, is caused by the lack of intrinsic factor, a substance that is normally produced by the stomach and binds with vitamin B₁₂, allowing it to be absorbed and used by the body; treatment involves the administration of intramuscular injections of the vitamin (Bothwell and Chalton, 1981).

Vitamin B12 acts as a cofactor in the production of succinyl-CoA and the essential amino acid methionine in the mitochondrial fraction and cytoplasm, respectively (Scott, *et al.*, 1984). Vitamin deficiency is associated with severe neurological and haematological symptoms, identifying the nervous tissue and the bone marrow as the organs most sensitive to vitamin deficiency (Burger, *et al.*, 1975). Because of the high vitamin B₁₂ content in the human liver, this organ traditionally has been considered the major storage site for vitamin B₁₂ (Burger, *et al.*, 1975).

However, a possible role for the rat kidney is suggested by the well known ability of this organ to accumulate the vitamin during states of vitamin surplus. Absorbed B₁₂ is transported to the tissues bound to transcobalamin (TC) (Birn, *et al.*, 2002). Two receptors for the uptake of TC-B₁₂ have been reported in rats, the multiligand, endocytic receptor megalin and a 62 kDa protein of as yet unknown structure Megalin (600 kDa) belongs to the low density lipoprotein (LDL) receptor family and is heavily expressed in kidney proximal tubules (Birn, *et al.*, 2002).

2.2 Phenyl Hydrazine Hydrochloride (PHZ)

Phenylhydrazine (CAS No. 100-63-0) exists as yellow to pale brown crystals or as a yellowish oily liquid. It is sparingly soluble in water and is miscible with other organic solvents. Phenylhydrazine is used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries (Goldstein, *et al.*, 1980). The number of persons potentially exposed to phenylhydrazine or its hydrochloride salt is not known, but it is expected to be small (Giffin and conner, 1929). No personal exposure data were available, although the Estimation and Assessment of Substance Exposure (EASE) model predicted exposure (8-h time-weighted average) to be around 2.3 mg/m³ (0.5 ppm)

(Frost and Hjorth, 1959). In practice, the 8-h time-weighted average exposure will be less than this figure. The limited data on toxicokinetics indicate that phenyl hydrazine is well absorbed by inhalation, oral, and dermal routes and binds readily to haemoglobin in red blood cells (Clark, *et al.*, 1988). Metabolism seems to occur via ring hydroxylation and conjugation, probably with glucuronic acid. Excretion is primarily via the urine (Conner and Giffin., 1929). Phenyl hydrazine is toxic by single exposure via the oral route (LD50 80–188 mg/kg body weight) and is expected to be toxic by the inhalation and dermal routes (data from these routes of exposure are less clear). Phenyl hydrazine has potential for skin and eye irritation (Brooke, 1997).

Anemia is a widespread public health problem with major consequences for human health as well as social and economic development (De Maeyer and Adiels-Tegman, 1985; Benoist, *et al.*, 2008). Estimates of the prevalence of anemia vary widely and accurate data are often lacking, it can be assumed that in resource-poor areas significant proportions of young children and women of childbearing age are anemic (Irwin and Kirchner, 2001). For instance, in Africa more than 100 million children are thought to be anemic (morbidity and mortality particularly in the developing world (Holden and Acomb, 2007). Hence anemia is one of the leading health disorders posing a great threat to global healthcare. Medicinal plants are currently being used in various parts of the world especially in the tropics for the treatment of various forms diseases (Brabin, *et al.*, 2001).

The subtle nature of its presentation means, however, that mild-to-moderate degree of anemia frequently remain undetected and untreated by health care workers (Phillips-Howard, *et al.*, 2003; Schellenberg, *et al.*, 2003).

The cause of anemia is frequently multifactorial, Infectious diseases in particular malaria, helminth infections and other infections such as tuberculosis and HIV/AIDS – are important factors contributing to the high prevalence of anemia in many populations (May, *et al.*, 2000; Nussenblatt and Semba, 2002). For example, *Plasmodium falciparum* malaria-related anemia contributes significantly to childhood mortality and thus preventing and treating anemia in children is of major importance (Nussenblatt and Semba, 2002). Helminth infections, in particular hookworm infections and schistosomiasis, cause blood loss and thus also contribute to the etiology of anemia (Torlesse and Hodges, 2001).

HIV/AIDS is an increasing cause of anemia and anemia is recognized as an independent risk factor for early death among HIV/AIDS-infected individuals (Van Eijk, *et al.*, 2002). Other nutritional deficiencies besides iron, such as vitamin B12, folate and vitamin A can also cause anemia although the magnitude of their contribution is unclear (Semba and Bloem, 2002). Furthermore, the impact of haemoglobinopathies on anemia prevalence needs to be considered among some populations such as ours (George and Tabansi, 2010).

2.3 Anemia among Women

Anemia is characterized by a low level of haemoglobin in the blood. Haemoglobin is necessary for transporting oxygen from the lungs to other tissues and organs of the body (Lamina and Sorunmu., 2003). Anemia usually results from a nutritional deficiency of iron, folate, vitamin B12, or some other nutrients. This type of anemia is commonly referred to as Iron-Deficiency Anemia. Iron deficiency is the most widespread form of malnutrition in the world, affecting more than two billion people (Stolzfus and Dreyfuss, 1998). In India, anemia affects an estimated 50 percent of the population (Seshadri, 1998).

Anemia may have detrimental effects on the health of women and children and may become an underlying cause of maternal mortality and perinatal mortality. Anemia also results in an increased risk of premature delivery and low birth weight (Ngnie, *et al.*, 2007). Early detection of anemia can help to prevent complications related to pregnancy and delivery, as well as child development problems (Van den Broek, 1996). Information on the prevalence of anemia can be useful for the development of health-intervention programmes designed to prevent anemia, such as iron fortification programmes. In India, under the Government's Reproductive and Child Health (Hodges and Tolersse, 2006)

Program, iron and folic acid tablets are provided to pregnant women in order to prevent anemia during pregnancy. Because anemia is such a serious health problem in India, NFHS-2 undertook direct measurement of the haemoglobin levels of all ever-married women age 15–49 and their children under three years of age (Hodges and Tolersse, 2006).

Anemia may be defined as **any condition resulting from a significant decrease in the total body erythrocyte mass**. Measurement of total body RBC mass requires special radiolabeling techniques that are not amenable to general medical diagnostic work (Van den Broek, 1996). Measurements typically substituted for RBC mass determination take advantage of the body's tendency to maintain normal total blood volume by dilution of the depleted RBC component with plasma (Lamina and Sorunmu, 2003).

This adjustment results in decrease of the total blood hemoglobin concentration, the RBC count, and the hematocrit (Ngnie *et al.*, 2007). Therefore, a pragmatic definition of anemia is a state which exists when the hemoglobin is less than 12 g/dL. Anemia may exist as a

laboratory finding in a subjectively healthy individual, because the body can, within limits, compensate for the decreased red cell mass (Van den Broek, *et al.*, 2000).

One must be careful in blindly applying this practical definition of anemia in every case. As the following diagram shows, it is possible to be severely anemic and have a normal hematocrit (and hemoglobin). This occurs when there is rapid hemorrhage, with red cells and plasma being rapidly lost simultaneously, before the body can respond by hiking up the plasma volume (Muoneke, *et al.*, 2011).

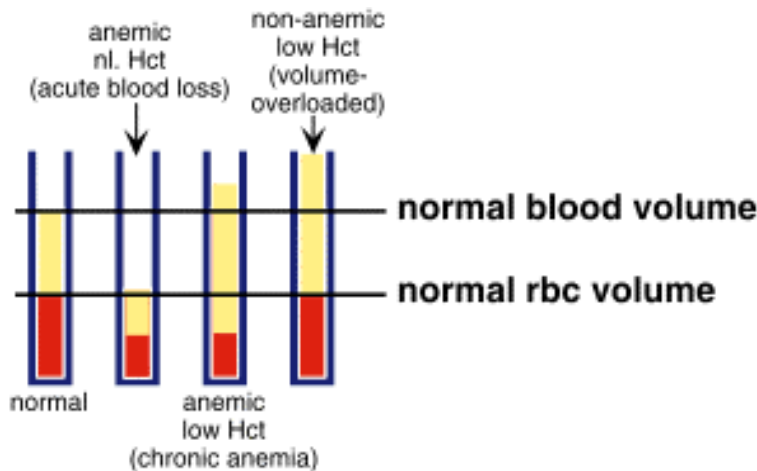


Fig 2.1 Anemia and hematocrit adopted from (WHO, 2005)

The final example in the above diagram illustrates that a person can have a low hematocrit and *not* be anemic. This occurs when a patient is over hydrated, typically as a result of overenthusiastic intravenous fluid therapy.

2.3.1 Decreased Hemoglobin Oxygen Affinity

Increased oxygen extraction of anemic blood by the tissues produces increased concentration of deoxyhemoglobin in the RBC, which stimulates the production of 2,3-diphosphoglycerate (2,3-DPG). 2, 3-DPG shifts the hemoglobin-oxygen dissociation curve

to the right, thus allowing the tissues to more easily strip the hemoglobin of its precious electron-accepting cargo (Murphy and Bremen, 2001)

2.3.2 Redistribution of Blood Flow

In anemia selective vasoconstriction of blood vessels subserving certain nonvital areas allows more blood to flow into critical areas. The main donor sites who sacrifice their aerobic lifestyle are the skin and kidneys (Aluka, *et al.*, 2001). Shunting of blood away from cutaneous sites is the mechanism behind the clinical finding of pallor, a cardinal sign of anemia. Although the kidney can hardly be thought of as a non vital area, it receives (in the normal state) much more blood flow than is needed to meet its metabolic requirements. Although by definition total body red cell mass is decreased in anemia, in the chronically anemic patient the *total blood volume* paradoxically is increased, due to increased plasma volume. It is as if the body was trying to make up in blood quantity what it lacks in quality (Aluka, *et al.*, 2001).

2.3.3 Increased Cardiac Output

The heart can respond to tissue hypoxia by increased cardiac output. The increased output is matched by decreased peripheral vascular resistance and decreased blood viscosity (thinner blood flows more freely than thick blood), so that cardiac output can rise without an increase in blood pressure. Generally, anemia must be fairly severe (hemoglobin < 7 g/dL) before cardiac output rises (Lamina and Sorunmu, 2003).

When the above mechanisms are overwhelmed by the increasing magnitude of the anemia, or when the demands of physical activity or intercurrent illness overwhelm them, a clinical disease state becomes apparent to the physician and to the patient (Lamina and Sorunmu, 2003). The severity of clinical symptoms bears less relationship to the severity of the

anemia than to the length of time over which the condition develops. An acute hemorrhagic condition may produce symptoms with loss of as little as 20% of the total blood volume (or 20% of the total red cell mass). Conversely, anemias developing over periods long enough to allow compensatory mechanisms to operate will allow much greater loss of RBC mass before producing symptoms. It is not terribly uncommon to see a patient with a hemoglobin of 4 g/dL, representing a loss of 70% of the RBC mass, being reluctantly dragged into a clinic by relatives concerned that he or she is looking a bit washed out (Koller, 1982).

When symptoms do develop, they are pretty much what you would expect given the precarious state of oxygen delivery to the tissues, dyspnea on exertion, easy fatigability, fainting, lightheadedness, tinnitus, and headache. In addition, the hyperdynamic state of the circulatory system can produce palpitations and roaring in the ears. Pre-existing cardiovascular pathologic conditions are, as you would expect, exacerbated by the anemia. Angina pectoris, intermittent claudication, and night muscle cramps speak to the effect of anemia on already compromised perfusion. (Massawe, *et al.*, 1999)

Clinical signs of a slowly developed anemia are pallor, tachycardia, and a systolic ejection murmur (Massawe *et al.*, 1999). In rapidly developing anemia as from hemorrhage and certain catastrophic hemolytic anemias), additional symptoms and signs are noted: syncope on rising from bed, orthostatic hypotension i.e., the blood pressure falls when the patient is raised from the supine to the sitting or standing positions) and orthostatic tachycardia (Moestrup, 1996).

2.4. Classification of Anemia

Anemia can be classified by cytometric schemes (i.e., those that depend on cell size and hemoglobin-content parameters, such as MCV and MCHC), erythrokinetic schemes (those that take into account the rates of RBC production and destruction), and biochemical/molecular schemes (those that consider the etiology of the anemia at the molecular level (Mather and Loncar, 2006)

- a. **Cytometric classification:** normochromic, normocytic
- b. **Erythrokinetic classification:** hemolytic
- c. **Biochemical/molecular classification:** DNA point mutation producing amino acid substitution in hemoglobin beta chain

2.4.1 Cytometric Classification

Cytometric parameters are more easily and less expensively measured than are erythrokinetic and biochemical ones, it is most practical to work from the cytometric classification, to the erythrokinetic, and then (hopefully) to the biochemical. Your first job in working up a patient with anaemia is to place the case in one of three major cytometric categories (WHO, 2006).

Normochromic, normocytic anemia (normal MCHC, normal MCV). This is a form of anaemia in which the concentration of hemoglobin in the red blood cells is within the standard range. However, there is insufficient numbers of red blood cells (WHO, 2006). These are also referred to as anemia of inflammatory response is a form of anemia seen in chronic illness eg from chronic infection, chronic immune activation, or malignancy. New discoveries suggest that the syndrome likely largely the results of body's production of hepcidin, a master regulator of human iron metabolism.

- a. **hemolytic anaemias (those characterized by accelerated destruction of RBC's)**
 - b. **anaemia of acute hemorrhage**
 - c. **aplastic anemias (those characterized by disappearance of RBC's precursors from the marrow)**
1. **Hypochromic, microcytic anemia (low MCHC, low MCV).**

These include:

- a. **iron deficiency anemia**
- b. **thalassemias**
- c. **anemia of chronic disease (rare cases)**

2. **Normochromic, macrocytic anemia (normal MCHC, high MCV).**

These include:

- a. **vitamin B₁₂ deficiency**
- b. **folate deficiency**

2.4.2. Erythrokinetic Classification

This is based on the rate of RBC turnover. If this is high, a **normoaregenerative** anemia exists. Such anemias are seen in hemolysis (excess destruction of RBC's or hemorrhage loss of RBC's from the vascular compartment. In these cases, the marrow responds appropriately to anemia by briskly stepping up the production of RBC's and releasing them into the bloodstream prematurely (Koller, 1982).

2.4.3 Reticulocyte Count

A sample of blood is stained with a supravital dye that marks reticulocytes. An increased number of reticulocytes is seen when the marrow is churning out RBC's at excessive speed

(presumably to make up for those lost to hemolysis or hemorrhage). Most labs will report the result of the reticulocyte count in percent of all RBC's counted. A typical normal range is 0.5-1.5 %. Making clinical decisions based on this raw count is somewhat fallacious. A normal person with an RBC count of 5,000,000 /microliter and an absolute reticulocyte count of 50,000 /microliter would have a relative reticulocyte count of 1.0%. An anemic person with 2,000,000 RBC's/microliter and the *same* 50,000 retics/microliter would have an apparently "abnormal" relative reticulocytes count of 2.5 % and could be misdiagnosed as having high turnover.

Clearly, one needs to find some way to correct the raw reticulocytes count so as to avoid this problem. One can easily calculate the **absolute reticulocyte count** (in cells/microliter) by multiplying the RBC count by the relative reticulocyte count. The normal range for the absolute reticulocyte count is 50,000-90,000 /microliter.

2.4.4 Serum Unconjugated Bilirubin and Urine Urobilinogen Concentration

When red cells, at the end of their 120-day life-span, go to the spleen, they are systematically dismantled (Muoneke, *et al.*, 2011). Through a series of biochemical steps, the heme is changed into bilirubin. The bilirubin is greedily scarfed up by the liver, conjugated with glucuronide, taken into the alimentary tract in the bile, and converted to urobilinogen by colonic bacteria. The urobilinogen is excreted in the stool (most of it) or reabsorbed and excreted in the urine (Irwin and Kirchner, 2001).

In cases of accelerated RBC destruction, the capacity of the liver to capture bilirubin is saturated, and the concentration of unconjugated bilirubin in serum increases, occasionally to the point of producing clinical jaundice (Scott, *et al.*, 1984).

Moreover, the increased production of urobilinogen that results is reflected by increased urobilinogen concentration in the urine. Unconjugated bilirubin is not water soluble and therefore will not be excreted in the urine, despite its elevation in the serum.

2.4.5 Serum Haptoglobin Concentration

When an RBC is destroyed, the liberated hemoglobin binds mole-for-mole with a serum protein, haptoglobin. The "purpose" of this reaction is to keep the kidneys from squandering iron free hemoglobin is freely filtered by the glomerulus, but hemoglobin haptoglobin complexes are too big to muscle their way through, so that they are safe to bumble their way back to the reticuloendothelial system where they can be properly disassembled) (Burkhill, 1985). The serum haptoglobin concentration then decreases. Laboratory measurement of haptoglobin is fairly easy and yields useful information to assist in documenting decreased RBC life span (George and Tabansi, 2010). In the case of hemolysis which takes place in the bloodstream (rather than in the RES), so-called **intravascular hemolysis**, additional biochemical phenomena are observed. Free hemoglobin in excess of that which binds haptoglobin is rapidly filtered into the urine (Nordenberg, *et al.*, 1990). What remains in the plasma spontaneously degrades into metheme and globin. A portion of metheme binds albumin to produce a measurable compound, **methemalbumin**, while the remainder binds to a measurable serum protein, **hemopexin**, which then decreases in serum concentration (Irwin and Kirchner, 2001).

2.4.6 Bone Marrow Biopsy

This can be used to directly observe any accelerated production of RBC's. The ratio of the number of myeloid to erythroid precursors (the M:E ratio) tends to decrease in high-production states, and the marrow becomes hypercellular. Marrow biopsy is not usually

performed *just* to measure the M:E ratio, but to answer other hematologic questions that have been raised (Scholl and Hediger, 1994).

The normo regenerative anemia are in contrast to those characterized by inadequate marrow response to the degree of anemia. These are the **hyporegenerative** anemias. In such cases, the reticulocyte production index is decreased. The classic example is aplastic anemia. **Aplastic anemia**, in which there is primary marrow failure to produce enough erythrocyte mass. As you have probably come to expect, the distinction of these categories is not always absolute. For instance, in thalassemia major there is a degree of hemolysis generally associated with the normoregenerative states) and inadequate marrow response to the degree of anemia.

2.4.7 Biochemical Classification

Finally, one should attempt to determine the etiology of the anemia as specifically as possible. In some cases (*e.g.*, iron deficiency), etiologic classification is easily attained; in others (*e.g.* aplastic anemia) the biochemical mechanism of disease may be hopelessly elusive (Shneider, *et al.*, 1976). Generally, biochemical tests are aimed at identifying a depleted cofactor necessary for normal haematopoiesis (iron, ferritin, folate, B₁₂), an abnormally functioning enzyme (glucose-6-phosphate dehydrogenase, pyruvate kinase), or abnormal function of the immune system the direct antiglobulin (Shneider, *et al.*, 1976).

Iron deficiency anemia is caused by insufficient dietary intake or absorption of iron to replace losses from menstruation or losses due to diseases (Scholl and Hediger, 1994).

Iron is an essential part of hemoglobin, and low iron levels result in decreased incorporation of hemoglobin into red blood cells (Scholl and Hediger, 1994). In the United

States, 20% of all women of childbearing age have iron deficiency anemia, compared with only 2% of adult men (WHO, 2006).

The principal cause of iron deficiency anemia in premenopausal women is blood lost during menses. Studies have shown that iron deficiency without anemia causes poor school performance and lower IQ in teenage girls, although this may be due to socioeconomic factors (Scott, *et al.*, 1984). Iron deficiency is the most prevalent deficiency state on a worldwide basis. Iron deficiency is sometimes the cause of abnormal fissuring of the angular (corner) sections of the lips angular stomatitis (Shneider, *et al.*, 1998).

Iron deficiency anemia can also be due to bleeding lesions of the gastrointestinal tract. Fecal occult blood testing, upper endoscopy and lower endoscopy should be performed to identify bleeding lesions (Messawe, *et al.*, 1999). In men and post-menopausal women the chances are higher that bleeding from the gastrointestinal tract could be due to colon polyp or colorectal cancer (Messawe, *et al.*, 1999).

Worldwide, the most common cause of iron deficiency anemia is parasitic infestations, amebiasis, schistosomiasis and whipworm (Burkhill, 1985).

2.4.8 Macrocytic Anemia

Megaloblastic anemia, the most common cause of macrocytic anemia, is due to a deficiency of either vitamin B₁₂, folic acid (or both). Deficiency in folate and/or vitamin B₁₂ can be due either to inadequate intake or insufficient absorption (Burger, *et al.*, 1975). Folate deficiency normally does not produce neurological symptoms, while B₁₂ deficiency does. Pernicious anemia is caused by a lack of intrinsic factor (Birn, *et al.*, 2002).

Intrinsic factor is required to absorb vitamin B₁₂ from food. A lack of intrinsic factor may arise from an autoimmune condition targeting the parietal cells (atrophic gastritis) that produce intrinsic factor or against intrinsic factor itself. These lead to poor absorption of vitamin B₁₂ (Van Eijk, *et al.*, 1999).

Macrocytic anemia can also be caused by removal of the functional portion of the stomach, such as during gastric bypass surgery, leading to reduced vitamin B₁₂/folate absorption. Therefore one must always be aware of anemia following this procedure (Van Eijk, *et al.*, 1999).

- a. Hypothyroidism
- b. Alcoholism commonly causes a macrocytosis, although not specifically anemia. Other types of liver disease can also cause macrocytosis.
- c. Methotrexate, zidovudine, and other drugs that inhibit DNA replication.

Macrocytic anemia can be further divided into "megaloblastic anemia" or "non-megaloblastic macrocytic anemia". The cause of megaloblastic anemia is primarily a failure of DNA synthesis with preserved RNA synthesis, which results in restricted cell division of the progenitor cells (Muoneke, *et al.*, 2011). The megaloblastic anemias often present with neutrophil hypersegmentation (6–10 lobes). The non-megaloblastic macrocytic anemias have different etiologies (i.e. there is unimpaired DNA globin synthesis,) which occur, for example in alcoholism (Macgregor, 1993).

In addition to the non-specific symptoms of anemia, specific features of vitamin B₁₂ deficiency include peripheral neuropathy and subacute combined degeneration of the cord

with resulting balance difficulties from posterior column spinal cord pathology. Other features may include a smooth, red tongue and glossitis (Irwin and Kirchner, 2001).

The treatment for vitamin B₁₂-deficient anemia was first devised by William Murphy who bled dogs to make them anemic and then fed them various substances to see what (if anything) would make them healthy again. He discovered that ingesting large amounts of liver seemed to cure the disease (Harte, 1952). George Minot and George Whipple then set about to isolate the curative substance chemically and ultimately were able to isolate the vitamin B₁₂ from the liver. All three shared the 1934 Nobel Prize in Medicine.

2.4.9 Normocytic Anemia

Normocytic anemia occurs when the overall hemoglobin levels are decreased, but the red blood cell size (mean corpuscular volume) remains normal. Causes include:

- a. Acute blood loss
- b. Anemia of chronic disease
- c. Aplastic anemia (bone marrow failure)
- d. Hemolytic anemia

2.5.0 Dimorphic Anemia

When two or more causes of anemia act simultaneously, e.g., macrocytic hypochromic, due to hookworm infestation leading to deficiency of both iron and vitamin B₁₂ or folic acid or following a blood transfusion more than one abnormality of red cell indices may be seen (Birn *et al.*, 2006). Evidence for multiple causes appears with an elevated RBC distribution width (RDW), which suggests a wider-than-normal range of red cell sizes.

2.5.1 Heinz Body Anemia

Heinz bodies form in the cytoplasm of RBCs and appear like small dark dots under the microscope. There are many causes of Heinz body anemia, and some forms can be drug induced. It is triggered in cats by eating onions or acetaminophen (paracetamol). It can be triggered in dogs by ingesting onions or zinc, and in horses by ingesting dry red maple leaves.

2.5.2 Hyperanemia is a severe form of anemia, in which the hematocrit is below 10%.

Refractory anemia is an anemia which does not respond to treatment. It is often seen secondary to myelodysplastic syndromes (WHO, 2005).

Iron deficiency anemia may also be refractory as a clinical manifestation of gastrointestinal problems which disrupt iron metabolism.

2.5.3 Grading of Anemia

WHO Grading of anemia:

- Grade 1 (Mild Anemia): 10 g/dl - cutoff point for ages
- Grade 2 (Moderate Anemia): 7-10 g/dl
- Grade 3 (Severe Anemia): below 7 g/dl

National Cancer Institute Grading of Anemia:

- Grade 0 (within normal limits) 12.0–16.0 g/dl for women and 14.0–18.0 g/ dl for men
- Grade 1 (Mild) 10 g/dl to levels within normal limits
- Grade 2 (Moderate) 8.0–10.0 g/dl
- Grade 3 (Severe) 6.5–7.9 g/dl
- Grade 4 (Life threatening) <6.5 g/dl

2.5.4 Causes of Anemia

Broadly, causes of anemia may be classified as impaired red blood cell (RBC) production, increased RBC destruction (hemolytic anemias), blood loss and fluid overload (hypervolemia). Several of these may interplay to cause anemia eventually. Indeed, the most common cause of anemia is blood loss, but this usually doesn't cause any lasting symptoms unless a relatively impaired RBC production develops, in turn most commonly by iron deficiency (WHO, 2002).

Impaired production

Disturbance of proliferation and differentiation of stem cells. Pure red cell aplasia. Aplastic anemia, affecting all kinds of blood cells. Fanconi anaemia is a hereditary disorder or defect featuring aplastic anaemia and various other abnormalities.

- i. Anemia of renal failure, by insufficient erythropoietin production
- ii. Anemia of endocrine disorders

Disturbance of proliferation and maturation of erythroblasts

- i. Pernicious anemia is a form of megaloblastic anemia due to vitamin B₁₂ deficiency dependent on impaired absorption of vitamin B₁₂.
- ii. Anemia of folic acid deficiency. As with vitamin B₁₂, it causes megaloblastic anemia
- iii. Anemia of prematurity, by diminished erythropoietin response to declining hematocrit levels, combined with blood loss from laboratory testing. It generally occurs in premature infants at 2 to 6 weeks of age.
- iv. Iron deficiency anemia, resulting in deficient heme synthesis
- v. thalassemias, causing deficient globin synthesis
- vi. Congenital dyserythropoietic anemias, causing ineffective erythropoiesis

vii. Anemia of renal failure (also causing stem cell dysfunction)

Other mechanisms of impaired RBC production

Myelophthistic anemia or myelophthosis is a severe type of anemia resulting from the replacement of bone marrow by other materials, such as malignant tumors or granulomas.

Myelodysplastic syndrome anemia of chronic inflammation.

2.5.6 Increased Destruction of Red blood Cell

Anemias of increased red blood cell destruction are generally classified as hemolytic anemias. These are generally featuring jaundice and elevated LDH levels.

2.5.7 Intrinsic Factor

Intracorpuseular abnormalities, where there the red blood cells have defects that cause premature destruction. All of these, except paroxysmal nocturnal hemoglobinuria, are hereditary genetic disorders (Murphy and Breman, 2001).

Hereditary spherocytosis is a hereditary defect that results in defects in the RBC (Murphy and Breman., 2001). Cell membrane, causing the erythrocytes to be sequestered and destroyed by the spleen. Hereditary elliptocytosis, another defect in membrane skeleton proteins (May, *et al.*, 2000). A betalipoproteinemia, causing defects in membrane lipids Enzyme deficiencies Pyruvate kinase and hexokinase deficiencies, causing defect glycolysis (Irwin Kirchner, 2001). Glucose-6-phosphate dehydrogenase deficiency and glutathione synthetase deficiency, causing increased oxidative stress Hemoglobinopathies, Sickle cell anemia Hemoglobinopathies causing unstable hemoglobins Paroxysmal nocturnal hemoglobinuria (Semba and Bloem, 2002).

2.5.8 Extrinsic Factor

(Extracorporeal) abnormalities Antibody-mediated Warm autoimmune hemolytic anemia is an anemia caused by autoimmune attack against red blood cells, primarily by IgG (Irwin and Kicner, 2001). It is the most common of the autoimmune hemolytic diseases. It can be idiopathic, that is, without any known cause, drug-associated or secondary to another disease such as systemic lupus erythematosus, or a malignancy, such as chronic lymphocytic leukemia (CLL) (Semba and Bloem, 2002).

Cold agglutinin hemolytic anemia is primarily mediated by IgM. It can be idiopathic or result from an underlying condition. Rh disease, one of the causes of hemolytic disease of the newborn.

2.5.9 Mechanical Trauma to Red Cells

Microangiopathic hemolytic anemias, including thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. Infections, including malaria, heart surgery etc.

2.6. Autoimmune Haemolytic Anemia

A number of autoimmune disorders are grouped under the rubric autoimmune hemolytic anaemia. All result from the formation of autoantibodies against red blood cells, an event that can lead to hemolysis (Macgregor, 1983). The autoantibodies sometimes appear after infection with the bacterium *Mycoplasma pneumoniae*, a rather uncommon cause of pneumonia (Benoist, 2002). In that case the autoantibodies are directed against certain antigens that are present on red cells, and they are probably induced by a similar antigen in the microbes (Lackrit, *et al.*, 1995).

Auto antibodies directed against a different antigen of red blood cells are often produced in persons who have been taking the antihypertensive medication alpha methyldopa for several months, the reason for autoantibody development in such cases is unknown (Lackrit, *et al.*, 1995). Other drugs, such as quinine, sulfonamides, or even penicillin, very occasionally cause hemolytic anemia. In such cases it is thought that the drug acts as a hapten that is, it becomes bound to a protein on the surface of red blood cells, and the complex becomes immunogenic (Benoist, 2002).

The auto antibodies that form against red blood cells are categorized into two groups on the basis of their physical properties. Auto antibodies that bind optimally to red blood cells at 37 °C (98.6 °F) are categorized as warm-reacting. Warm-reacting auto antibodies belong primarily to the IgG class and cause about 80 percent of all cases of autoimmune hemolytic anemia. Auto antibodies that attach to red blood cells only when the temperature is below 37 °C are called cold-reacting.

They belong primarily to the IgM class (WHO, 2002). Cold-reacting auto antibodies are efficient at activating the complement system and causing the cell to which they are bound to be destroyed. Nevertheless, as long as the body temperature remains at 37 °C, cold-reacting autoantibodies dissociate from the cell and hemolysis is not severe (Ileobachi and Meniru, 1990).

2.6.1 Blood Loss

- i. Anemia of prematurity from frequent blood sampling for laboratory testing, combined with insufficient RBC production.
- ii. Trauma or surgery, causing acute blood loss

- iii. Gastrointestinal tract lesions, causing a rather chronic blood loss
- iv. Gynecologic disturbances, also generally causing chronic blood loss
- v. From menstruation, mostly among young women Fluid overload

Fluid overload (hypervolemia) causes decreased hemoglobin concentration and apparent anaemia.

- i. General causes of hypervolemia include excessive sodium or fluid intake, sodium or water retention and fluid shift into the intravascular space.
- ii. Anemia of pregnancy is anemia that is induced by blood volume expansion experienced in pregnancy.

2.6.2 Treatments of Anemia

Iron deficiency from nutritional causes is rare in men and post-menopausal women. The diagnosis of iron deficiency mandates a search for potential sources of loss such as gastrointestinal bleeding from ulcers or colon cancer (Marsh *et al.*, 1990). Mild to moderate iron-deficiency anemia is treated by oral iron supplementation with ferrous sulfate, ferrous fumarate, or ferrous gluconate. When taking iron supplements, it is very common to experience stomach upset and/or darkening of the faeces. The stomach upset can be alleviated by taking the iron with food, however, this decreases the amount of iron absorbed. Vitamin C aids in the body's ability to absorb iron, so taking oral iron supplements with orange juice is of benefit (Harte, *et-al.*, 1992). Vitamin supplements given orally (folic acid) or intramuscularly (vitamin B-12) will replace specific deficiencies (Scott, *et al.*, 1984).

In anemia of chronic disease, anemia associated with chemotherapy, or anemia associated with renal disease, some clinicians prescribe recombinant erythropoietin, epoetin alfa, to stimulate red-cell production (Scott, *et al.*, 1984).

In severe cases of anemia, or with ongoing blood loss, a blood transfusion may be necessary. Doctors attempt to avoid blood transfusion in general, since multiple lines of evidence point to increased adverse patient clinical outcomes with more intensive transfusion strategies (Sherry, *et al.*, 1997). The physiological principle that reduction of oxygen delivery associated with anemia leads to adverse clinical outcomes is balanced by the finding that transfusion does not necessarily mitigate these adverse clinical outcomes (Sherry, *et al.*, 1997).

In severe, acute bleeding, transfusions of donated blood are often lifesaving. Improvement in battlefield casualty survival is attributable, at least in part, to the recent improvements in blood banking and transfusion techniques (Nordenberg, *et al.*, 1990). Transfusion of the stable but anemic hospitalized patient has been the subject of numerous clinical trials (Soh, *et al.*, 2004).

Treatment of exceptional blood loss (anemia) is recognized as an indication for hyperbaric oxygen (HBO) by the Undersea and Hyperbaric Medical Society. The use of HBO is indicated when oxygen delivery to tissue is not sufficient in patients who cannot be transfused for medical or religious reasons (Ernest, 2005). HBO may be used for medical reasons when threats of blood product incompatibility or concern for transmissible disease are factors. The beliefs of some religions (ex: Jehovah's Witnesses) may require they use the HBO method (Enerst, 2005).

2.6.3 Anemia and Cardiovascular System

There are numerous effects of anemia on the cardiovascular system. According to the U.S. National Library of Medicine and the National Institutes of Health, iron deficiency is one of the principle causes of anemia, although certain medical conditions, including sickle cell anemia and cancer, can also cause anemia (Diekes, *et al.*, 2005). The human body needs iron to produce hemoglobin, which carries oxygen from the lungs to the body's tissues and organs. Anemia is particularly challenging on the cardiovascular system.

2.6.4 Anemia and Oxygen Delivery

Decreased oxygen delivery to the tissues and organs is one of the main effects of anemia on the cardiovascular system (WHO, 2005). According to the American Association of Kidney Patients (AAKP), while anemia is characterized by decreased oxygen delivery to the tissues, muscle tissue is particularly affected. The National Heart Lung and Blood Institute (NHLBI), a division of the National Institutes of Health, states that a person with anemia has a lower than usual number of red blood cells (the red blood cells don't contain enough hemoglobin). In both situations, a person's body does get enough oxygenated blood, which may cause fatigue or other symptoms (WHO, 1992). Over time, states the NHLBI, decreased oxygen delivery by the cardiovascular system can cause heart and brain damage, along with damage to the body's other organs, and in some cases, anemia may even cause death (WHO, 1992).

2.6.5 Anemia and Hematocrit

Anemia affects a person's hematocrit, a measure of the percentage of the volume of whole blood, including plasma, that's made up of red blood cells. According to the National Institutes of Health, a person's hematocrit depends on the number of red blood cells and the

size of his red blood cells (Aimaku and Olayemi, 2003). The NHLBI adds that hematocrit quantifies the amount of space red blood cells take up in a person's blood, and that a low hemoglobin or hematocrit is a sign of anemia (Alada, 2000). The National Anemia Action Council, an online resource for anemia patients and their caregivers, states that anemia is categorized as mild, moderate or severe based on how far a person's hematocrit levels are below the normal range (Ilobachie and Meniru, 1990). Normal hematocrit levels for men are 39 percent or higher, which means that, in a blood sample, 39 percent or more of the blood sample's volume should be red blood cells. The normal hematocrit values for non-pregnant women are 36 percent or higher (Irwin and kiirchner, 2001).

2.6.6 Anemia on Cardiac and Respiratory Adjustment

The cardiac and respiratory adjustments in chronic anemia and their clinical manifestations have been reviewed. When the oxygen carrying capacity of the blood is diminished, an adequate supply of oxygen to the tissues is maintained by an increased cardiac output, an increased velocity of blood flow, and a relatively more complete abstraction of the oxygen from the blood as it passes through the capillaries (Burkhill, 1985). With the increased blood flow, the average peripheral resistance is decreased but the state of the small blood vessels is not uniform everywhere, the blood flow in the hands and kidneys, for instance, may be reduced, while that of other parts of the body is increased (Murphy and Breman, 2001). The total oxygen consumption of the body in anemia is not strikingly altered. The blood volume generally is slightly reduced but the plasma volume is normal. (Lamina and Sorunmu, 1998)

The deviations from the normal values vary from patient to patient, but generally are definite when the hemoglobin values are less than 50 per cent and are greatest at the lowest levels of hemoglobin concentration.(Cuervo and Mohammed, 2003).

The close interrelationship between the cardiovascular and respiratory systems is exemplified by the coincident changes in the respiratory system in anemia (Chukwubelu and Obi, 1979). The rate and depth of respiration often are increased together with a lowering in the vital capacity and its subdivisions, the reserve and complemental air volumes. The residual air is somewhat increased (Lamina and Sorunmu, 1998).These deviations from the normal are similar to those observed in pulmonary congestion or edema and denote a loss of elasticity and expansibility favoring the occurrence of exertional dyspnea. The arterial blood saturation is usually normal at rest but, during exertion, a significant lowering becomes apparent (George and Tabansi, 2010).

The importance of hemoglobin in the transport of carbon dioxide is reviewed; the decreased availability of hemoglobin as a buffer in carbon dioxide transport in anemia is compensated by the increased ventilation of the blood in the lungs, rendering the arterial blood somewhat alkalotic (Burhill, 1985).

In the anemia due to blood loss, malnutrition, chronic infection, uremia, or leukemia, the blood carbonic anhydrase activity is parallel to the decrease in hemoglobin level leading to a deficiency not only of oxygen carrying capacity but also a decreased ability to absorb carbon dioxide from the tissues and to release it in the lungs (Ogunbode, 2003)

The following factors, many of which are closely interrelated, are operative in the production of dyspnea in anemic patients, the increased respiratory minute volume, the

decreased vital capacity and its subdivisions, the abnormalities in carbon dioxide transport and dissociation, the reduced arterial oxygen capacity and the decreased blood oxygen saturation during effort, and the frequently observed elevated blood lactic acid values (Massawe, *et al.*, 1999).

The symptoms and signs exhibited by anemic patients, including palpitation and breathlessness on exertion, tachycardia, cardiac dilatation and hypertrophy, are described. In addition to an apical systolic murmur, other systolic and diastolic murmurs are occasionally heard. The arterial blood pressure is frequently lowered in anemia; the venous pressure is generally within the limits of normal (Scholl and Hediger, 1994). Electrocardiographic abnormalities occur in approximately one-quarter of anemic patients but are minor and not specific in character (Ogunbode, 2003).

The occurrence of angina pectoris, congestive failure, and intermittent claudication in some patients with the development of anemia, and disappearance of these conditions as the anemia is alleviated, is discussed with particular reference to the underlying physiologic mechanisms (Lamina and Sorunmu, 2003).

2.6.7 Anemia and Cancer

Anemia in cancer patients is multifactorial and may occur as either a direct effect of the cancer, as a result of the cancer treatment itself, or due to chemical factors produced by the cancer. The clinical symptoms of anaemia vary according to the individual's capacity to respond to blood loss or reduced red cell production (Burkhill, 1985). The haematological features in anaemic patients depend on the different types of malignant disease (Burkhill, 1985). Clinical and laboratory evaluation, and examination of the bone marrow can provide

important diagnostic clues in many cases. Decisions are commonly made based on subjective consideration rather than on objective data (Ileobachie and Meniru, 1990).

Blood transfusion involves many hazards, some of which may be reduced or avoided. Erythropoietin (EPO) treatment has been found to be effective in preventing anaemia and in reducing the need for blood transfusions, although it would be useful to identify high-risk patient subgroups who would benefit most from this expensive treatment (Adam, *et al.*, 2005).

In advanced cancer patients the use of blood transfusion should be evaluated on an individual basis, according to the presence of distressing symptoms and life expectancy. These measures are unlikely to have an effect in irreversible and progressive bleeding state (Benoist, *et al.*, 2002).

2.6.8 Anemia and Pregnancy

Anemia in pregnancy is common and it is most often caused by an iron deficiency. Iron is a mineral that everyone needs. Pregnant women need more iron for a variety of reasons. The biggest reason is that iron helps your body make new blood to carry the oxygen and nutrients to the baby during pregnancy (Lamina and Sorunmu, 1998). By the end of pregnancy you will have a 30-50% increase in your blood in your body than when you began the pregnancy. Your need for iron will increase 100% over your requirements pre-pregnancy (Lamina and Sorunmu, 1998).

Anemia affects millions of people worldwide. The National Center for Health Statistics estimated that 3.4 million Americans are living with anemia. Nevertheless the actual anaemic individuals may be even greater as anemia is often under-diagnosed and under-treated (WHO, 2006).

2.6.9 Anemia and the Kidney

Patients with chronic kidney disease, normochromic normocytic anemia mainly develops from decreased renal synthesis of erythropoietin (Amin and Faizul, 2004). The anaemia becomes more severe as the GFR (glomerular filtration rate) progressively decreases. No reticulocyte response occurs, red blood cell survival is decreased, and there is an associated increased bleeding tendency due to uraemia-induced platelet dysfunction (Nguyen, *et al.*, 1998).

Iron deficiency is also common in patients with chronic kidney disease (Benoist, *et al.*, 2008). The iron deficiency may be absolute, often due to poor dietary intake or sometimes occult bleeding, or functional, when there is an imbalance between the iron requirements of the erythroid marrow and the actual iron supply (Benoist, *et al.*, 2008). Iron deficiency leads to a reduction in formation of red cell haemoglobin, causing hypochromic microcytic anemia. Other causes for anemia in chronic kidney disease include the presence of uraemic inhibitors (eg parathyroid hormone, inflammatory cytokines), reduced half-life of circulating blood cells, and deficiencies of folate or vitamin B12 (Nexo and Gimsing, 2003).

2.7 Anemia and the Liver

Anemia of diverse etiology is a common complication of chronic liver diseases. The causes of anemia include acute or chronic gastrointestinal hemorrhage, and hypersplenism secondary to portal hypertension (Murphy and Bremen, 2001). Severe hepatocellular disease predisposes to hemorrhage because of impaired blood coagulation caused by deficiency of blood coagulation factors synthesized by hepatocytes, and/or thrombocytopenia. Aplastic anemia, which is characterized by pancytopenia and

hypocellular bone marrow, may follow the development of hepatitis (Irwin and Kirchner, 2001). Its presentation includes progressive anemia and hemorrhagic manifestations (Nielsen, *et al.*, 2001).

Hematological complications of combination therapy for chronic viral hepatitis include clinically significant anemia, secondary to treatment with ribavirin and/or interferon. Ribavirin-induced hemolysis can be reversed by reducing the dose of the drug or discontinuing it altogether. Interferons may contribute to anaemia by inducing bone marrow suppression (Allison, 1991). Alcohol ingestion is implicated in the pathogenesis of chronic liver disease and may contribute to associated anemia. In patients with chronic liver disease, anemia may be exacerbated by deficiency of folic acid and/or vitamin B12 that can occur secondary to inadequate dietary intake or malabsorption (Allison, 1991).

Chronic liver diseases frequently are associated with hematological abnormalities. Anemia of diverse etiology occurs in about 75% of patients with chronic liver disease (Alada, *et al.*, 2002). A major cause of anemia associated with chronic liver disease is hemorrhage, especially into the gastrointestinal tract. Patients with severe hepatocellular disease develop defects of blood coagulation as a consequence of endothelial dysfunction, thrombocytopenia, deficiencies of coagulation factors and various associated disorders (Benoist, *et al.*, 2008).

In severe hepatocellular disease, decreased synthesis of liver-produced plasma proteins leads to reduced serum levels of several blood clotting factors (Ramanujam, *et al.*, 1992). Hemorrhage may occur as a complication of chronic liver disease because of a lack of one or more liver-produced blood clotting factors, thrombocytopenia, and/or defective platelet

function (Rapazzo, *et al.*, 1976). Hemorrhage in such patients may also occur from esophageal or gastric varices secondary to portal hypertension. The biosynthetic pathways of blood coagulation factors II, VII, IX and X are within the hepatocyte and are dependent on vitamin K. Low serum levels of these factors are associated with prolongation of the prothrombin time (PT) (Scneider, *et al.*, 1984). When attributable to hepatocellular disease, they are not improved by administration of vitamin K, correction of the associated impaired blood coagulation necessitates infusion of preparations of the deficient factors (Scott, *et al.*, 1984).

Splenomegaly, which is usually caused by portal hypertension in patients with chronic liver disease, may lead to secondary hemolysis, an increase in plasma volume, macrocytosis and megaloblastic anemia. Alcohol, a common etiologic factor of chronic liver disease, is toxic to the bone marrow (Semba and Bloem, 2004). Alcoholics often develop secondary malnutrition, a manifestation of which may be anemia caused by folic acid deficiency. In some patients, bone marrow failure and aplastic anemia develop after an episode of hepatitis. Finally, anemia is a recognized complication of treatment of chronic hepatitis C with a combination of interferon and ribavirin, anemia in this context is predominantly caused by ribavirin-induced hemolysis (Rapazzo, *et al.*, 1970)

The frequent association of anemia with chronic liver disease and/or hepatocellular failure provides a rationale for examining the role of the liver in the formation and destruction of erythrocytes. Indeed, the liver itself may be implicated in a variety of different mechanisms that contribute to the development of anemia in patients with chronic liver disease (Schneider, *et al.*, 1976). This paper provides an overview of anemia that may complicate chronic liver diseases and the mechanisms responsible.

The frequent association of anemia with chronic liver disease and/or hepatocellular failure provides a rationale for examining the role of the liver in the formation and destruction of red blood cells. Indeed, a variety of different mechanisms may be implicated in the development of anemia in patients with liver disease (Scott, *et al.*, 1984)

2.8. Portal Hypertension and Anemia

Acute gastrointestinal hemorrhage is a common and potentially serious complication of portal hypertension. It is usually caused by rupture of an esophageal varix. Hemorrhage caused by this mechanism is the second most common cause of mortality in patients with cirrhosis (Scholl and Hedger, 1994). In such patients, a ruptured esophageal varix is the cause of approximately 70% of episodes of upper gastrointestinal hemorrhage (Scott, *et al.*, 1984).

Acute hemorrhage may induce severe hypovolemia and subsequently secondary iron deficiency anemia. The initial aim of treatment is correction of hypovolemia and restoration of stable hemodynamic function; minimal values for mean arterial pressure and for hemoglobin of 80 mmHg and 8 g/100 mL, respectively, should be maintained (Ramanujam, *et al.*, 1992). Initially, gelatin-based colloids or solutions of human albumin may be infused to correct hypovolemia. However, infusions of packed erythrocytes in plasma are ideal in this context since such infusions have the potential of correcting, not only hypovolemia, but also secondary anemia (Rapazzo, *et al.*, 1970)

In some patients with cirrhosis, chronic hemorrhage into the gastrointestinal tract occurs. Esophageal and gastric varices and/or portal hypertensive gastropathy may be associated

with slow chronic loss of blood into the gut and development of chronic iron deficiency anemia (Irwin and kirchner, 2001)

Additional treatment with oral iron supplementation is indicated for iron deficiency anemia caused by chronic blood loss. In some cases of advanced chronic liver disease, intravenous iron formulations may be administrated to increase plasma levels and tissue deposits of iron (Birn, *et al.*, 2002).

Hypersplenism secondary to portal hypertension is another mechanism of anemia in patients with chronic liver disease. Hypersplenism is associated with splenomegaly. In addition to chronic liver disease, thrombosis of the splenic vein may also be a cause of an increase in pressure within the portal venous system, which can lead to secondary hypersplenism (Macgregor, 1963). The main characteristics of hypersplenism are those attributable to pancytopenia. Hemolytic anemia occurs because of intrasplenic destruction of erythrocytes (Irwin and Kirchner, 2001). Destruction of megakaryocytes and leukocyte precursors results in thrombocytopenia and leukopenia Symptoms and signs of hypersplenism are influenced by the primary underlying disease; they include abdominal pain and/or discomfort, and, in advanced cases, gastrointestinal hemorrhage secondary to portal hypertension (Mouneke, *et al.*, 2011).

2.9 Aplastic Anemia

Aplastic anemia associated with liver disease is characterized by development of pancytopenia and hypocellular bone marrow in relation to the occurrence of hepatitis (Nussenblatt and Semba, 2002).

The main feature of this syndrome is injury to or loss of pluripotent hematopoietic stem cells, in the absence of infiltrative disease of the bone marrow (Nordenberg, *et al.*, 1990)

Hepatitis-associated aplastic anemia (HAA) has been defined as a variant of aplastic anemia, which occurs concurrently with an increase in the serum level of alanine aminotransferase to at least five times the upper limit of the reference range (Cuervo and Mohammed, 2003). Severe marrow aplasia may be induced by hepatitis viruses, such as hepatitis B virus and hepatitis C virus (HCV), and also by other viruses, such as human immunodeficiency virus, Epstein-Barr virus, transfusion-transmitted virus and echovirus (Murphy and Breman, 2001).

Parvovirus B19 commonly infects pro-erythroblasts and may induce transient red-cell aplasia, particularly in patients with chronic hemolytic anemia (Nordenberg, *et al.*, 1990). It has been postulated that viruses and/or antigens, through the mediation of γ interferon or the cytokine cascade, induce lymphocyte activation and ultimately apoptotic death of hematopoietic cells in the bone marrow (Nordenberg, *et al.*, 1990).

Clinical presentation includes symptoms and signs related to pancytopenia, such as pallor, fatigue, hemorrhagic manifestations, progressive anemia, and bacterial infections. The diagnosis of HAA is suggested by a complete blood count, which reveals pancytopenia (including anemia) together with absolute reticulocytopenia (Nguyen, *et al.*, 1998). A bone marrow biopsy typically reveals hypocellularity that affects red and white cell precursors and megakaryocytes; residual hematopoietic cells appear morphologically normal (Koller, 1982).

2.9 Alcohol, Liver Disease and Anemia

Alcohol is implicated in the pathogenesis of chronic liver disease; it may contribute to anemia secondary to its direct effects on the liver and also to other diverse mechanisms. Scheme, in patients with alcoholic liver disease, depicting how different effects of alcohol may contribute to anemia (Irwin and Kirchner, 2001).

Markers of iron overload tend to be higher among those who consume more than two alcoholic drinks per day than among non-drinkers, after adjusting for potential confounding factors. Consumption of alcohol appears to be associated with an approximately 40% reduction in the risk of development of iron deficiency anemia (Muoneke, *et al.*, 2011).

Folic acid and vitamin B12 deficiencies develop frequently in patients with cirrhosis. These deficiencies may be related to inadequate food intake or intestinal malabsorption (Semba and Bloem, 2002). They are suspected when examination of a blood film reveals hypersegmented cells and oval macrocytes, in addition to round macrocytes characteristic of chronic liver disease. When anemia is caused by these deficiencies, the mean corpuscular volume is increased and bone marrow shows megaloblastic erythropoiesis (Allison, 2001).

Anemia due to folic acid deficiency may result, not only from a lack of folic acid in the diet, but also the weak antifolate action of ethanol. Folic acid deficiency is the most common cause of a low hematocrit in hospitalized patients who are alcoholics (Newmark, *et al.*, 1970).

Parenterally administered Vitamin B₁₂ not only corrects anemia caused by vitamin B12 deficiency, but may also induce improvement in the peripheral neuropathy that are

associated with this deficiency. Supplements of vitamins A, B and C may be administered empirically to patients with advanced alcoholic disease (Nexo and Gimsing, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection and Identification of Plant Materials

Fresh leaves of *Telfairia occidentalis* was collected around Zaria, northern Nigeria. The identification of the plant will be at the Herbarium unit of the department of Biological Science, Ahmadu Bello University, Zaria, where a specimen voucher was be deposited for future reference.

3.1.1 Method of Extraction

The leaves of the plant was air-dried and made into powder using pestle and mortar. 200g of the leaves were weighed and poured in a conical flask and extracted with 2 Litres of methanol and then concentrated to dryness. The extract is then dissolved in water and partitioned with hexane and then ethyl acetate and finally n-butanol to get the n-butanol fraction of the extract.

3.1.2 Animals

A total of 30 male Wistar rats was used for this study. The animals were purchased from the Animal House, Federal College of Animal Health, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The rats were housed in the Animal House of the Department of Human Physiology Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria. The animals were kept in cages under normal environmental temperature and were fed with standard pellet diet and water given *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment.

3.2 Chemical

All chemicals used were of analytical grades Phenyl hydrazine hydrochloride purchased from Steve Morre chemical company Zaria and produced by Fischer Chemical Company USA. Vitamin B12 (cyanocobalamin) was purchased from Famek pharmaceutical store Samaru zaria manufactured by Green Field Pharmaceutical company, China.

3.3 Acute toxicity study

The intraperitoneal median lethal dose (LD₅₀) of the extract in wistar rats was conducted according to the method of Lorke (1983). In the initial phase, 3 groups of three rats each was treated with the extract at doses of 10, 100 and 1000mg/kg body weight intraperitoneally and observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one rats was injected intraperitoneally with four more specific doses of the extract based on the result of the first phase. The LD₅₀ was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

3.4 Experimental Design

3.4.1 Animal groupings

The animals were grouped into 5 groups containing 5 rats each. All groups were assayed for hematological parameters before onset of experiment.

Group I (n = 5)

Received 50mg/kg phenyl hydrazine and was given distilled water (1ml/kg).

Group II (n=5)

Received 50mg/kg phenyl hydrazine and treated with vitamin B12 (0.4ml/kg).

Group III (n=5)

Received 50mg/kg phenylhydrazine and 100mg/kg of *Telfairia occidentalis* leaf fraction according to their body weight for 2 weeks.

Group IV (n= 5)

Received 50mg/kg phenyl hydrazine and treated with 200mg/kg of *Telfairia Occidentalis* leaf fraction according to their body weight for 2 weeks.

Group V (n=5)

Received 50mg/kg phenyl hydrazine and treated with 300mg/kg of *Telfairia occidentalis* leaf fraction according to their body weight for 2weeks.

3.4.2 Induction of Anemia

Anemia was induced by the method described by Harris and Kugler (1971) and was modified by Sanni *et al* (2005). Anemia was induced in all the rats by intraperitoneal administration of 2.5% phenyl hydrazine hydrochloride w/w in Dimethylsulfoxide (DMSO) at a dose of 50 mg kg⁻¹phenyl hydrazine (PHZ) once daily for 3 days to induce Anemia.

3.5 Sample Collection

Three blood samples were collected from each animal. The first and second collection was from ventral coccygeal vein (1 ml) and the final, via cardiac puncture (2 ml). The blood was analyzed to access for Pack Cell Volume (PCV), White Blood cell count (WBC), Red blood Cell count (RBC) at the department of Human Physiology Laboratory, Ahmadu Bello University, Zaria. The Bilurubin test was carried out at the Chemical Pathology Laboratory of Ahmadu Bello University Teaching hospital Shika Zaria.

Determination of hemoglobin concentration was carried out according to the method described by Jain (1993) using the cyano methaemoglobin method. Packed cell volume (PCV) was carried out using the micro hematocrit centrifugation method according to Alexander and Griffiths. (1991) Red blood cell (RBC) count, White blood cell (WBC) count and count were estimated by visual means using the new improved Neubauer counting chamber according to Dacie and Lewis (1986).

3.6 Determination of Heamoglobin

AO Hb-Meter. This is a specialized type of colorimeter for the convenient evaluation of haemoglobin content of the blood. This instrument compares the absorption of light by the haemoglobin in a layer of haemolyzed blood of carefully defined depth, with the absorption of a standardized glass wedge. In order that the setting of the wedge be sensitive, only to light of colour which is strongly absorbed by the heamoglobin used (Jain, 1993).

3.7 Determination of PCV or Haematocrit

Blood is collect by the process of capillary attraction, allows the blood to be drawn into the capillary by capillary tubes provided (Heparinized) leaving at least 15mm unfilled. The tube is then carefully sealed with plasticin and centrifuged for 5 minutes and the the reading were taken using the micro haematocrit reader.(Allexander and Griffith,1991).

3.8 Biochemical Assays

Bilirubin (direct and total) concentration was determined using Diazo reagents according to Van den Bergh's reaction, while total protein was estimated by Biuret' method using a spectrophotometer (Winsten and Cehelyk, 1969).

3.9 Determination of Bilirubin Concentration

Colorimetric estimation of serum bilirubin by coupling with diazotized sulphanilic acid. The direct van den Bergh reaction, in aqueous medium, yields the 'direct' bilirubin, i.e. the amount of water-soluble bilirubin glucuronide conjugates. Upon prior addition of methanol to solubilize free bilirubin, the total bilirubin is found, and by this indirect van den Bergh reaction the 'indirect' bilirubin, i.e. the amount of unconjugated bilirubin, may be calculated as the difference between the total and direct levels. (Winsten and Cehelyk., 1969).

3.10 Data Analysis

Data obtained from the study were expressed as mean \pm SEM. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests of Tukey using SPSS statistical tool version 19. Values of $p < 0.05$ was considered statistically significant.

CHAPTER FOUR

4.0 RESULT

Table 4.1 The role of n-butanol leaf fraction of *T. occidentalis* on RBC, in PHZ induced anemia in Wistar rats. ($\times 10^6/\mu\text{L}$)

		1DAY BEFORE	3 DAYS AFTER	2 WKS.AFTER
GROUPS	INDUCTION OF	INDUCTION OF	INDUCTION OF	TREATMENT
	ANAEMIA	ANAEMIA	ANAEMIA	WITH FRACTION
i.	CONTROL (WATER 1ml/kg) + PHZ (50mg/kg)	4.48±0.19	3.48±0.34	5.17±0.03
ii.	VIT B ₁₂ 0.4ml/kg + PHZ (50mg/kg)	4.63±0.10	3.11±0.32	5.33±0.07 ^a
iii.	100mg/kg FRACTION + PHZ (50mg/kg)	4.72±0.18	3.72±0.18	5.76±0.13 ^a
iv.	200mg/kg FRACTION + PHZ (50mg/kg)	4.10±0.72	3.22±0.16	5.31±0.07 ^a
v.	300mg/kg FRACTION + PHZ (50mg/kg)	4.78±0.17	2.98±0.05	5.30±0.02 ^a

a = significance (P< 0.05) as compared to the control groups

The result of the effect of n-butanol fraction of *T. occidentalis* on Red blood cell indicates a significant increase ($P < 0.05$) after 2 weeks in Red blood cell level amongst the treated group as compared to the control group, 100mg/kg extract ($5.76 \pm 0.13 \times 10^6/\mu\text{L}$) as compared to the control group ($4.72 \pm 0.18 \times 10^6/\mu\text{L}$), also 200mg/kg extract ($5.31 \pm 0.07 \times 10^6/\mu\text{L}$) as compared to the control group ($4.10 \pm 0.72 \times 10^6/\mu\text{L}$) and 300mg/kg ($5.30 \pm 0.02 \times 10^6/\mu\text{L}$) as compared to the control group ($4.78 \pm 0.17 \times 10^6/\mu\text{L}$) but there is significant increase ($p < 0.05$) in the group treated with vitamin B12 as compared to the control group (5.33 ± 0.07 as compared to $4.63 \pm 0.10 \times 10^6/\mu\text{L}$). However, the fraction increases the RBC count above the pre-anemic level (before induction) and also after induction (3 days after induction) respectively. The result obtained from this study showed that the significant increase amongst the treated groups were not dose dependent.

Table 4.2 Showed role of n-butanol leaf fraction of *T. Occidentalis* on WBC in PHZ induced anemia in Wistar rat ($\times 10^9/L$)

GROUPS	1 DAY BEFORE INDUCTION OF ANAEMIA	3 DAYS AFTER INDUCTION OF ANAEMIA	2WKS AFTER TREATMENT WITH FRACTION
i. CONTROL (WATER 1ml/kg) + PHZ (50mg/kg)	9.48±0.16	8.38±0.25	6.17±0.88
ii. VIT B ₁₂ 0.4ml/kg + PHZ (50mg/kg)	10.14±0.16	9.88±0.26	4.62±0.42 ^a
iii. 100mg/kg FRACTION + PHZ (50mg/kg)	8.88±0.18	7.88±0.18	5.76±0.13 ^a
iv. 200mg/kg FRACTION + PHZ (50mg/kg)	7.88±0.76	6.88±0.76	5.06±0.46 ^a
v. 300mg/kg FRACTION + PHZ (50mg/kg)	8.28±0.17	8.00±0.33	6.10±0.15 ^a

a = significance (P< 0.05) as compared to the control groups.

The result of the role of n-butanol leaf fraction of *T. Occidentalis* on White blood cell indicates a significant decrease ($p < 0.05$) after 2 weeks in white blood cell count in amongst the treated groups as compare to control group, 100mg/kg ($5.76 \pm 0.13 \times 10^9/L$) as compared to the control group ($8.88 \pm 0.18 \times 10^9/L$) and also 200mg/kg ($5.06 \pm 0.46 \times 10^9/L$) as compared to the control group ($7.88 \pm 0.76 \times 10^9/L$) and 300mg/kg ($6.10 \pm 0.15 \times 10^9/L$) as compared to the control group ($8.28 \pm 0.17 \times 10^9/L$). Also there is a significant decrease in the group treated with vitamin B₁₂ ($P < 0.05$) as compared with the control group (4.62 ± 0.42 as compared to $10.14 \pm 0.16 \times 10^9/L$). However, the fraction decrease the WBC count below the Pre-anemic level (1 day before induction) and also after induction (3 days after induction). The result obtained from this study showed that there was a significant decrease in the WBC count amongst the treated group and this decrease is not dose dependent. Table 4.2

Table 4.3 Showed The Role of n-butanol leaf fraction of *T. occidentalis* on PCV in PHZ induced anemia in Wistar rats. (%).

GROUPS	1 DAY BEFORE	3 DAYS AFTER	2 WKS AFTER
	INDUCTION OF ANEMIA	INDUCTION OF ANEMIA	TREATMENT WITH THE FRACTION
i. CONTROL (WATER 1ml/kg) + PHZ (50mg/kg)	38.02±0.66	28.80±0.58	54.20±1.56
ii. VIT B ₁₂ 0.4ml/kg + PHZ (50mg/kg)	39.02±0.37	26.40±1.03	51.00±0.71 ^a
iii. 100mg/kg FRACTION + PHZ (50mg/kg)	37.60±0.51	25.40±2.27	46.40±2.84 ^a
iv. 200mg/kg FRACTION + PHZ (50mg/kg)	37.80±0.58	25.40±0.81	47.40±1.96 ^a
v. 300mg/kg FRACTION + PHZ (50mg/kg)	37.08±0.37	21.40±1.08	50.60±0.51 ^a

a = significance (P < 0.05) as compared to the control groups

The result of the effect of n-butanol leaf fraction of *T. Occidentalis* on the Pack cell volume indicate a significant increase ($P < 0.05$) after 2 weeks in the total PCV level amongst the treated groups as compared to the control groups. 100mg/kg n-butanol fraction ($46.40 \pm 2.84\%$) as compared to the control group ($37.60 \pm 0.51\%$). Also For 200mg/kg n-butanol fraction ($47.40 \pm 1.96\%$) as compared to the control group ($37.80 \pm 0.58\%$), and also 300mg/kg ($50.60 \pm 0.51\%$) as compared to the control group ($37.08 \pm 0.37\%$). There is a significant increase ($P < 0.05$) in the PCV level in the group treated Vitamin B12 ($50.10 \pm 0.71\%$) as compare to the control group ($39.02 \pm 0.37\%$). However, the fraction increases the PCV level above the pre-anemic level (1 day before induction) and also after induction (3 days after induction). The result obtained from this study showed that, there was a significant increase in the PCV level amongst the treated group and this increase is dose dependent. Table 4.3

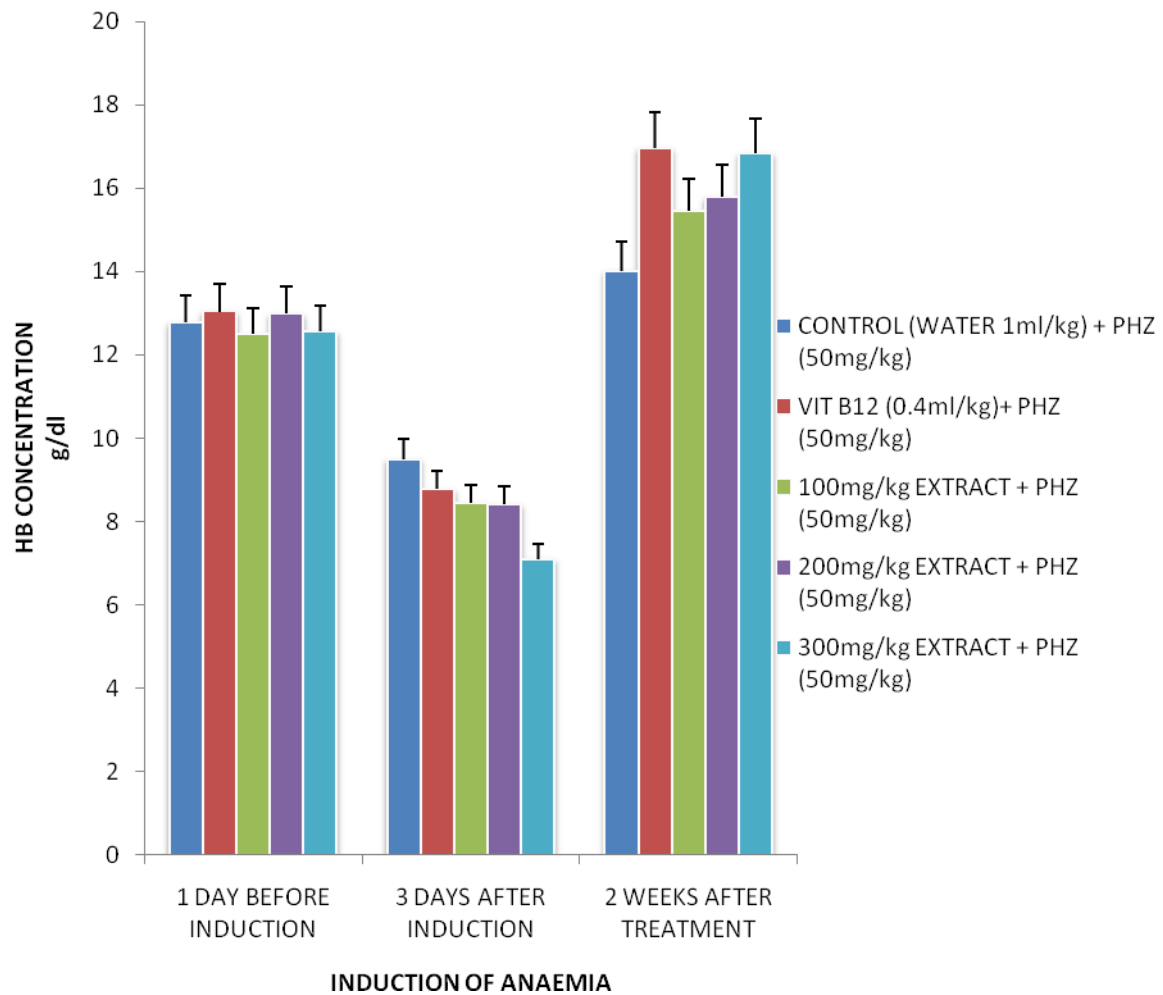


Fig 4.1 Showed the Role of n-butanol leaf fraction on Hb level in Phenyl hydrazine induced anemia in Wistar rats ($P < 0.05$)

The result of the role of n-butanol leaf fraction of *T. Occidentalis* on hemoglobin count indicate a significant increase ($P < 0.05$) in pack cell volume amongst the treated groups as compared to the control groups. 100mg/kg n-butanol fraction (15.44 ± 0.94 g/dL) as compared to the control group (12.50 ± 0.17 g/dL). Also For 200mg/kg n-butanol fraction (15.78 ± 0.56 as compared to the control group (12.98 ± 0.27 g/dL), and also 300mg/kg (16.84 ± 0.18 g/dL) as compared to the control group (12.56 ± 0.13 g/dL). There is a significant increase ($P < 0.05$) in the hemoglobin count in the group treated with Vitamin B12 (16.96 ± 0.23 g/dL) as compare to the control group (13.04 ± 0.13 g/dL). However, the fraction increases HB count above the pre-anemic level (1 day before induction) and also after induction (3 days after induction). The result obtained from this study showed that there was a significant increase amongst the treated group and this increase is dose dependent. Fig 4.1

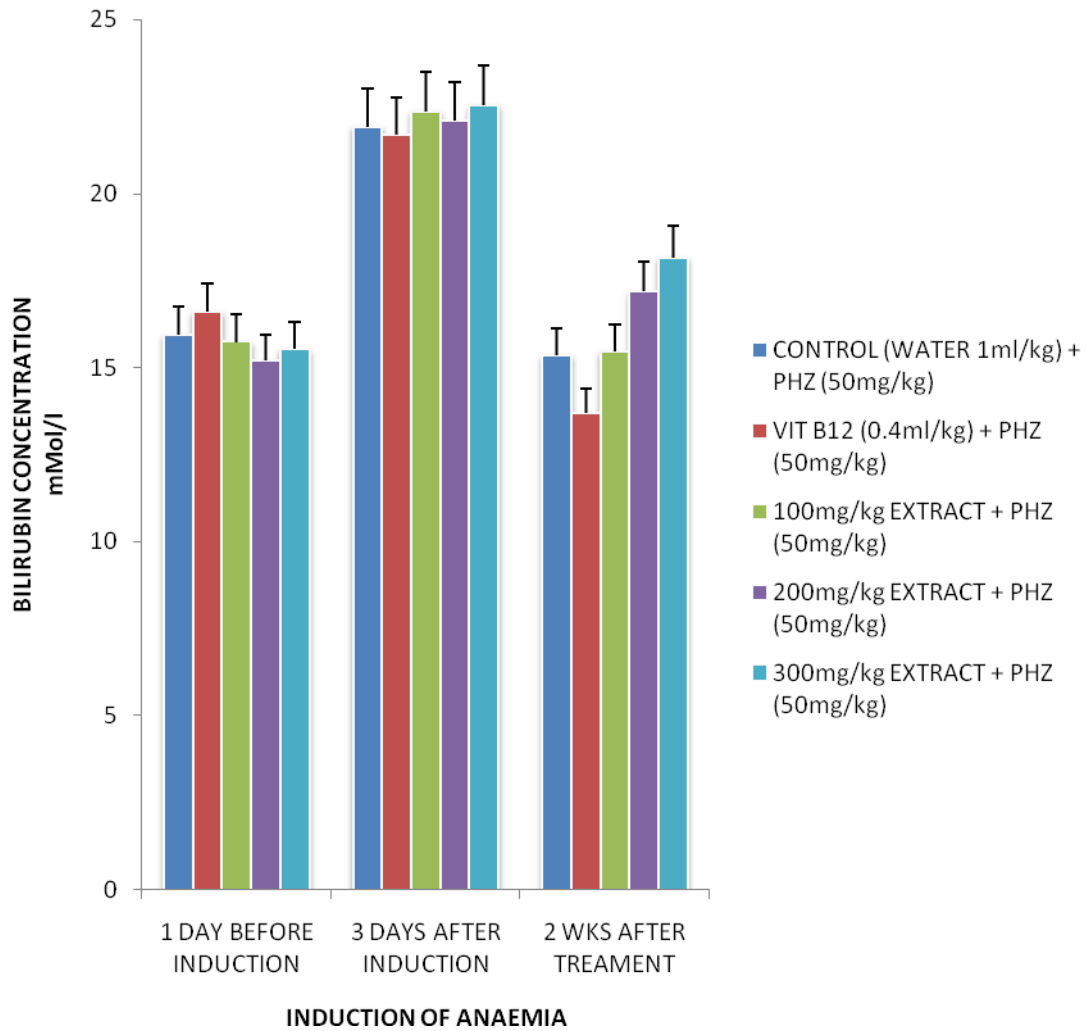


Fig 4.2 Showed The Role n-butanol leaf fraction of *T. Occidentalis* on total bilirubin Concentration in phenyl hydrazine induced anemia in Wistar rats ($P < 0.05$)

The result of the effect of n-butanol leaf fraction of *T. Occidentalis* on the Pack cell volume indicate a significant increase ($P < 0.05$) after 2 weeks in the total bilirubin level amongst the treated groups as compared to the control groups. 100mg/kg n-butanol fraction (15.46 ± 0.46 mmol/L) as compared to the control group (15.74 ± 0.31 mmol/L). Also For 200mg/kg n-butanol fraction (17.18 ± 0.07 mmol/L) as compared to the control group (15.20 ± 0.26 mmol/L), and also 300mg/kg (18.16 ± 0.08 mmol/L) as compared to the control group (15.54 ± 0.21 mmol/L). There is a significant increase ($P < 0.05$) in the Bilirubin level in the group treated Vitamin B12 (13.70 ± 0.20 mmol/L) as compared to the control group (15.360 ± 0.12 mmol/L). However, the fraction increases the bilirubin concentration above the pre-anemic level (1 day before induction). The result obtained from this study showed that there was a significant increase amongst the treated group and this increase is dose dependent (Fig 4.2).

CHAPTER FIVE

5.0 DISCUSSION, SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Discussion

This study reports the role of n-butanol leaf fraction of *T. occidentalis* on some haematological parameters in phenylhydrazine induced-anemia in wistar rats.

Anemia was diagnosed by a more than 40% reduction in PCV values of all rat groups from the baseline values. Similarly, the Hb concn, PCV values and RBC counts were significantly decreased from the baseline values 3 days after administration of phenyl hydrazine hydrochloride, indicating that the chemical could effectively induce anemia in wistar rats at the given dosage. Phenyl hydrazine hydrochloride has earlier been used to induce anemia in rats (Bowman and Rand, 1980).

PHZ increases reactive oxygen species (ROS) and lipid peroxidation, and decreases glutathione (GSH); these effects are reversed by N-acetyl cysteine, a known ROS scavenger (Hill and Thornalley, 1982; Clemens, *et al.*, 1984; Amer, *et al* 2004).

Haemolytic anemia has long been known to be caused by uptake of erythrocytes by macrophages in the spleen and translocation of phosphatidylserine from the inner to the outer of the plasma membrane has been identified as a signal for phagocytosis of cells under programmed death by macrophages. PHZ generates ROS within both human and rat erythrocytes no evidence for lipid peroxidation or phosphatidyl serine externalization was detected (de Jong *et al.*, 1997, McMillan, *et al.*, 2005).

ROS production was associated with extensive binding of oxidized and denatured haemoglobin to the membrane cytoskeleton. Thus, PHZ-induced haemolytic injury seems

to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids (McMillan, *et al.*, 2005). Vitamins C and E contribute to the decrease in oxidative stress caused by PHZ *in vitro* (Claro, *et al.*, 2006). They inhibited Heinz bodies and methemoglobin formation but they did not protect against GSH depletion by PHZ. Quercetin, an antioxidant bio flavanoid compound, also suppresses reactive oxygen and nitrogen species, and it partially protects reduced glutathione (GSH), malondialdehyde levels (Luangaram, *et al.*, 2007).

Melatonin as a free radical scavenger protects against phenylhydrazine-induced oxidative damage to cellular membranes (Karbownik, *et al.* 2000). PHZ induces Heinz body formation and oxidative degradation of spectrin without any crosslinking of membrane proteins, both these findings impair erythrocyte deformability (Hasegawa, *et al.* 1993). Formation of phenyl radicals and the replacement of haem with phenyl-substituted protoporphyrins, causes the destabilisation of haemoglobins to induce Heinz bodies and haemolytic anaemia with PHZ (Nakanishi, *et al.*, 2003).

PHZ treatment increases the transport rates in Na-K pump, Na-H exchange, Na-Li exchange, and K-Cl cotransport *in vivo*, while a decrease in Na-K pump, Na-H exchange, Na-Li exchange and increase K-Cl cotransport were found in rabbit red cells (Brugnara and Defranceschi, 1993).

The results of haematological tests indicated that treatment with n-butanol leaf fraction of *T. occidentalis* significantly elevates ($P < 0.05$) all the parameters of the anemic Wistar rats on after 2 weeks (Table 4.1.0 – 4.1.4) except for the white blood cells which is reduced. On the other hand, the standard heamatinic (Vitamin B12) produced a significant elevation ($P <$

0.05) haemoglobin concentration, Pack cell volume and the Red blood cells concentration (fig 4.3).

The results obtained from this work after treatment with n-butanol leaf fraction of *T Occidentalis* indicates a significant increase ($P < 0.05$) in the RBC count, PCV level, Hb concn and also Bilirubin concentration, except for the WBC count which was decreased after 2 weeks of treatment. The significant increase in HB concentration, Bilirubin concn and PCV was dose dependent, However, RBC count and WBC count were not dose dependent.

This increase in some of these haematological parameters agrees with the findings of (Ogbe *et-al.*, 2010). Findings agree with previous report that aqueous extract of *T. occidentalis* leaves has haematinic effect (Dina *et al.*, 2000). The effect of *T Occidentalis* on an experimentally induced anemia in domestic rabbits. (Ezekiel *et al.*, 2009) Elemental composition and anti-anaemic properties of *T Occidentalis*. (Teddy *et al.*, 2012). The effects of pumpkin extract on routine haematological parameters in acetone-induced oxidative stress in albino rats.

5.2 SUMMARY AND CONCLUSION

In summary, the result obtained from this study showed that Intra-peritoneal administration of 50mg/kg Phenylhydrazine hydrochloride for 3 days induces hemolytic anemia in the Wistar rats by decreasing some hematological parameters, such as the Red blood cell (RBC), white blood cell (WBC), pack cell volume (PCV), Hemoglobin count (Hb) and also increases total serum bilirubin concentration below the pre-anemic level (before induction). However, n-butanol leaf fraction of *Telfairia occidentalis* and vitamin B12 has a modulatory potentials by increasing the blood parameters due to the presence of natural antioxidants (saponin, Flavonoids and secoridoid glycosides) in the fraction. This is in line with the traditional use of *Telfairia occidentalis* in Folkloric medicine.

5.3 RECOMMENDATIONS

Based on the findings of the study, the following may be recommended

- The need for the experiment to be conducted using sophisticated modern devices e.g Automated analyzing machine, that can determine the Mean Corpuscular Haemoglobin concentration (MCHC) and reticulocytes count, using a single drop of blood.
- Further studies should be carried out to isolate the active component responsible for the haematinic activity.
- The need to isolate each of the components of the fraction and conduct experiment with each of them and then compare findings.

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APPENDIX I: The Role n-butanol leaf fraction of *T Occidentalis* on total bilirubin level (mmol/L) in PHZ anaemia in Wistar rats

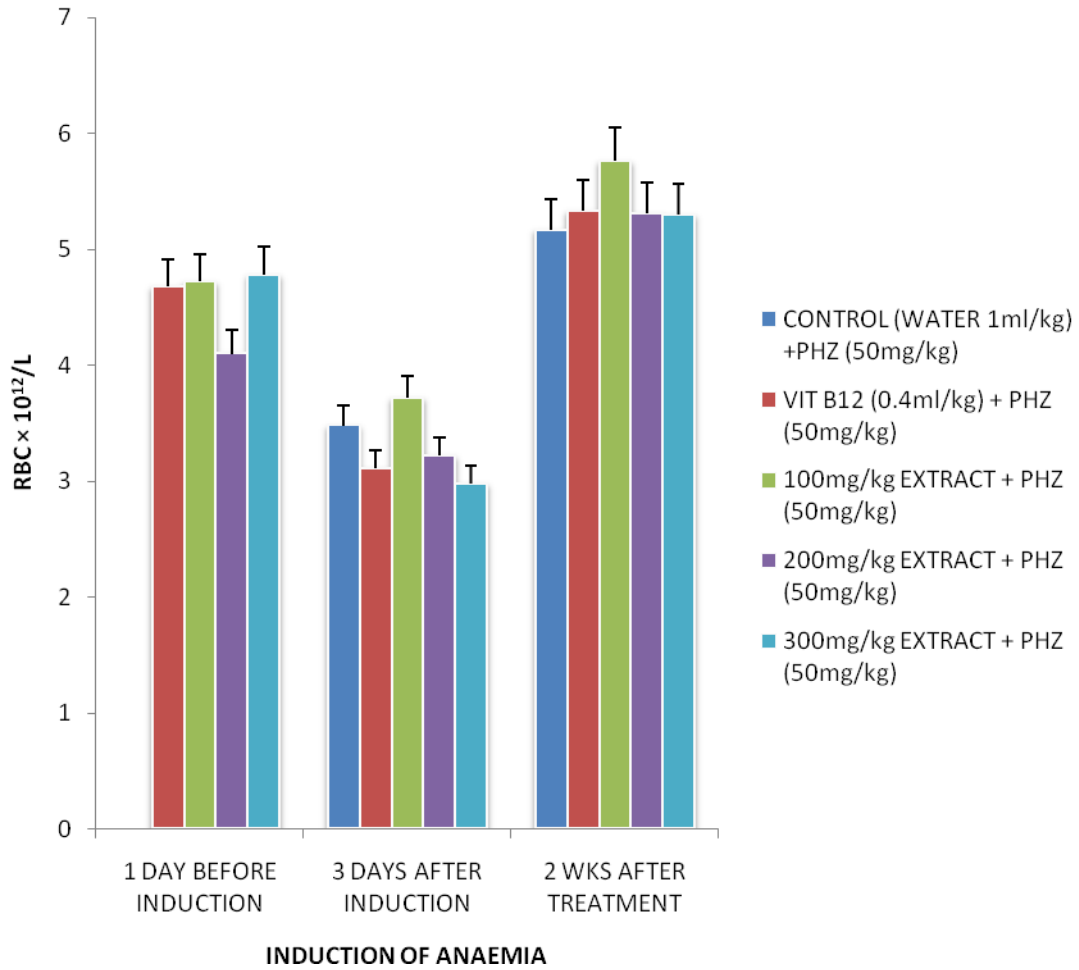
GROUPS	1 DAY BEFORE INDUCTION OF ANAEMIA	3 DAYS AFTER INDUCTION OF ANAEMIA	2 WKS AFTER TREATMENT WITH FRACTION
i. CONTROL (WATER 1ml/kg) + PHZ (50mg/kg)	15.94±0.45	21.92±0.5	15.36±0.12
ii. VIT B ₁₂ 0.4ml/kg + PHZ (50mg/kg)	16.60±0.40	21.68±0.6	13.70±0.20 ^a
iii. 100mg/kgT + PHZ (50mg/kg)	15.74±0.31	22.36±0.6	15.46±0.46 ^a

iv.	200mg/kg EXTRACT + PHZ (50mg/kg)	15.20±0.26	22.08±0.5 1 ^a	17.18±0.07 ^a
v.	300mg/kg EXTRACT + PHZ (50mg/kg)	15.54±0.21	22.54±0.3 9 ^a	18.16±0.08 ^a

a = significance (P< 0.05) as compared to the control groups

APPENDIX 11: Modulatory effect of n-butanolic leaf fraction on *T. Occidentalis* on RBC

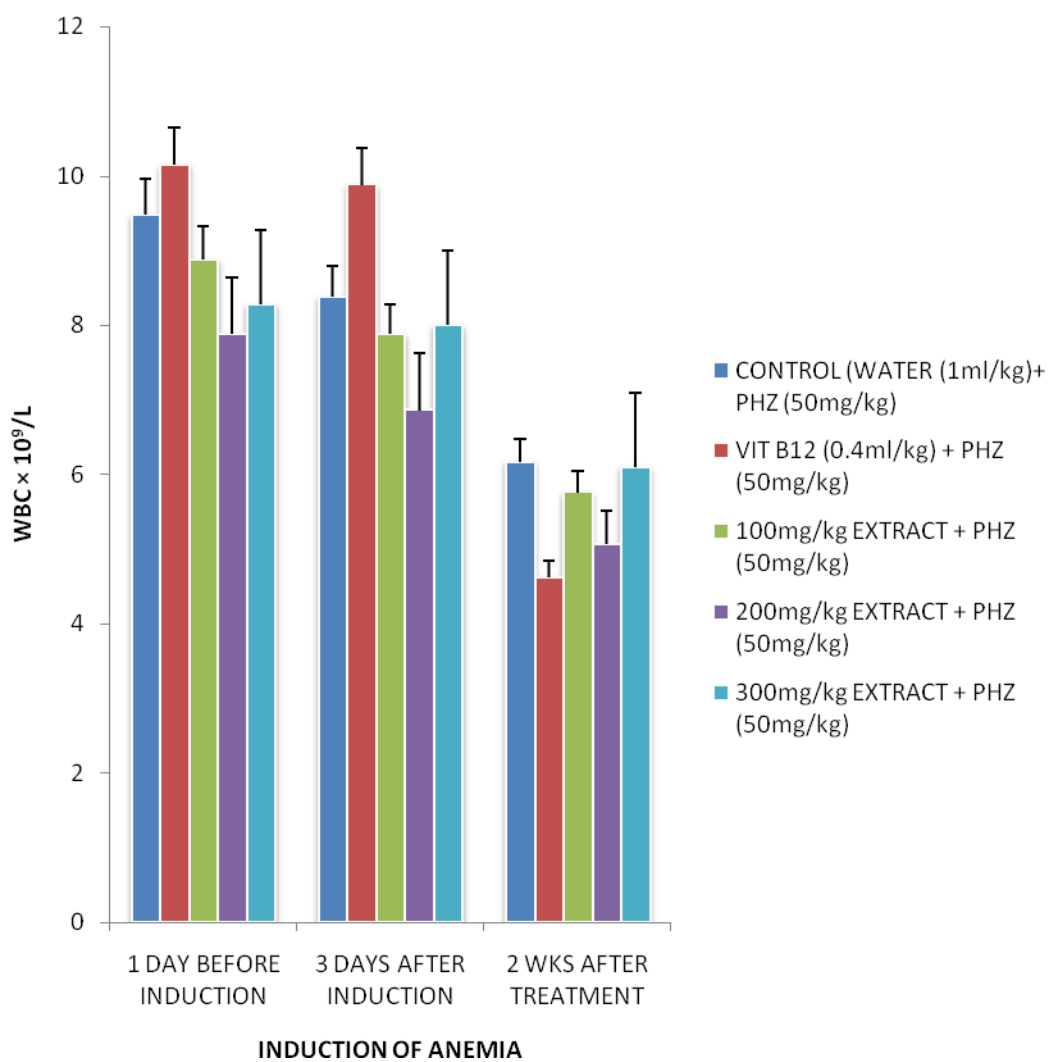
level in phenyl hydrazine induced anaemia in Wistar rats (P<0.05)



APPENDIX III: The role of n-butanol leaf fraction of *T. Occidentalis* on Haemoglobin count, in PHZ induced anemia in Wistar rats.

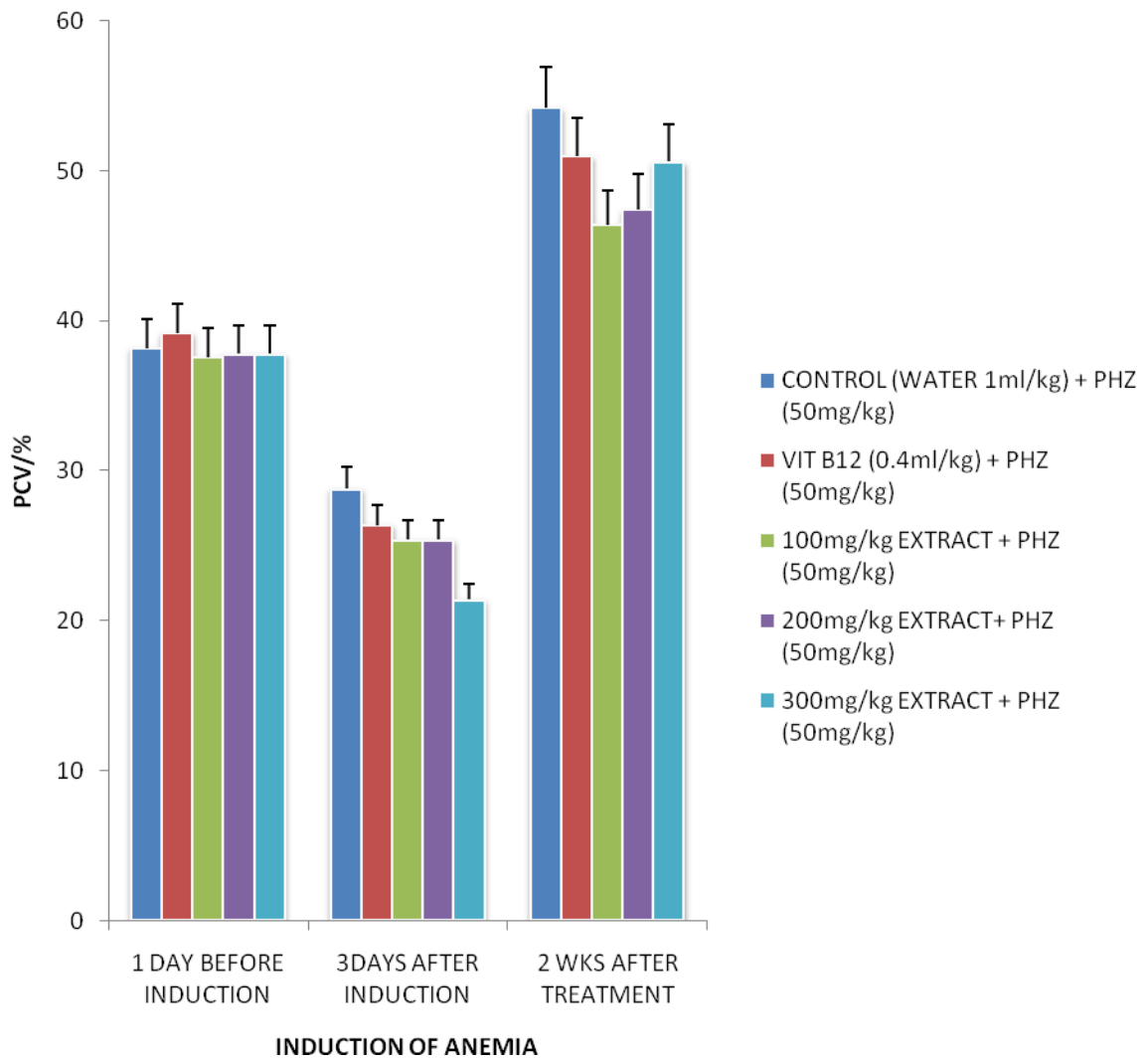
GROUPS	1 DAY BEFORE INDUCTION OF ANEMIA	3 DAYS AFTER INDUCTION OF ANEMIA	2 WKS AFTER TREATMENT WITH FRACTION
i. CONTROL WATER 1m/kg + PHZ (50mg/kg)	12.78±0.22	9.50±0.20	18.02±0.52
ii. VIT B ₁₂ 0.4ml + PHZ (50mg/kg)	13.04±0.13	8.78±0.33	16.96±0.23 ^a
iii. 100mg/kg FF a = significance (P< 0.05) as compared to the control groups (50mg/kg)	12.50±0.17	8.44±0.75	15.44±0.94 ^a
iv. 200mg/kg FRACTION + PHZ (50mg/kg)	12.98±0.27	8.42±0.28	15.78±0.56 ^a
v. 300mg/kg FRACTION + PHZ (50mg/kg)	12.56±0.13	7.10±0.36	16.84±0.18 ^a

APPENDIX IV Showed role of n-butanol leaf fraction of *T. Occidentalis* on WBC in PHZ induced anemia in Wistar rat ($\times 10^9/L$)



APPENDIX V: The role of n-butanol leaf extract of *T. Occidentalis* on PCV

in PHZ induced anemia in Wistar rats



a = significance ($P < 0.05$) as compared to the control groups