HAEMOPOIETIC AND HAEMATINIC POTENTIALS OF CRUDE METHANOLIC AND AQUEOUS SEED EXTRACT OF TELFAIRIA OCCIDENTALIS IN ALBINO WISTAR RATS.

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A RESEARCH PROJECT PRESENTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILMENT FOR THE AWARD OF MASTERS DEGREE IN MEDICAL LABORATORY SCIENCES WITH SPECIALIZATION IN HAEMATOLOGY.

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This is to certify that this project was originally carried out by **Duruewuru Hope Obianuju** Registration Number PG/MSC/07/47019 that Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus.

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DEDICATION

This work is dedicated to the Almighty God, and my Lord and Saviour Jesus Christ for all He has done for me.

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To my Father, my Creator, the Maker of the universe, to my Lord and Saviour Jesus Christ, and sweet Holy Spirit, I say thank You for your mercies, love, care and grace that has been upon my life. Thank You Sir for proving to me that with you, I can do all things. I wish to express my unalloyed gratitude to my supervisor Ven. Prof E.O Ukaejiofo for accepting to supervise this work despite his very tight schedule, for his fatherly advice and for all his encouragement throughout the period of this study.

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ABSTRACT

Haemopoietic and Heamatinic potentials of crude methanol and aqueous seed extract of *Telfairia Occidentalis* (Ugu) in albino rats were studied using one hundred and twenty eight rats of mixed sexes ages 2-3 month weighing (150± 20)g. Acute toxicity studies was performed and LD_{50} of the *Telfairia Occidentalis* obtained using standard methods. This work was carried out in the animal house of the University of Nigeria, Enugu Campus. The work was done in two set. The first set, the aqueous seed extract, the rats were divided into nine groups of eight animals each Group A, B_1-B_3, C-G. Group A was the normal control. Group G was the negative control; Group F was the positive control. Groups A, B_1-B_3 were used for haemopoietic potential while groups C-G were used for haematinic potential and were first induced for anaemia using phenylhydrazine for 9 days. At the end of the induction, the animals were given graded extract concentration, Group B_1 ï B_3 received 50, 100 and 200mg/kg body weight respectively also Groups C-E respectively received 50,100 and 200mg/kg body weight of extracts. Group A, the normal control received only feed and water. Group F, the positive control received 100mg/kg body weight of Astymin(a commercially prepared haematinic) while Groups G, the negative control received only feed and water after induction of anaemia. The bloods was collected from the animals on interval of 3days (day 3, day 6 and 9) from the onset of the administration of the extracts and were distributed appropriately into di-potassium EDTA for haematology studies and plain bottles for liver enzymes. The rats were sacrificed after collection of blood, the bone marrow was collected, the liver and kidney extracted for histological studies. The second set was used for the methanol extract, here the seeds were extracted in methanol and the process repeated as in the case of aqueous extract. Results obtained showed that the reticulocytes of the rats that received the various concentration of both methanol and aqueous extract were significantly increased $p<0.05$ while the platelets were only significantly increased $p<0.05$ in aqueous extract. The bone marrow examination also revealed increased cellularity when compared with the control group. In haematinic studies, aneamia was actually achieved but with the administration of the extract the conditions improved. This improvement was gradual as the reticulocytes which were very high on the 3rdday of administration 48.5 ± 3.5 and 26 ± 1.5% for both methanol and ethanol extract respectively gradually reduced by the 9th day 11.0 ± 1.0 and 12.5 ± 0.5% though still much higher than the controls. The Rbcs, Hb and PCV values were 6.1 ± 1.1×10^6/µl, 13.1 ± 2.0g/dl and 41.1 ± 3.0% respectively giving a significant increase (p ’ 0.05) when compared with the positive control 5.1 ± 0.1 × 10^6/µl, 11.6 ± 0.3 g/dl and 35.0 ± 0.9% and negative control values 3.3 ± 0.1 × 10^6/ml, 8.2 ± 4.0 g/dl and 24.9 ± 2.0% in rats that received the various concentrations of the extracts. This effect was observed more in methanolic extract than in the aqueous extract. This study therefore suggests that the seed of *Telfairia occidentalis* (Ugu) has both haemopoietic and haematinic potentials that for hemapoietic potentials, the aqueous extract is better while for haematinic potentials methanol extract is better. It also suggests that this seed can be safely consumed as it has no adverse effect on both the liver and the kidney cells. The serum transaminases were also not affected.

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CHAPTER ONE

INTRODUCTION

HAEMOPOIESIS

This is the process by which cellular elements of blood are formed. The formation of all types of blood cells is generated by a remarkable self - regulated system that is responsible to the demand, put on it. The three main types of cells are the red cell: (erythrocytes), which serve to carry oxygen, the white cells (leucocytes) which function in the prevention and recovery from disease and the thrombocytes which function in blood clotting. The formation of these cells is one of the most active and important processes in the body. Most of the circulating cells live only for a short time and must be replaced in order to maintain life. For instance in the human adult, a red blood cell has an average lifespan of approximately 120 days, 2.5 billion red blood cells have to be produced daily to replace those that are randomly destroyed (Abdounad and Lichtman 2001)

The lifelong production of blood cells occurs in haemopoietic tissue and it involves a very high level of cell turnover demanded by the need to replace mature circulating blood cells at a rapid rate and is necessitated by the limited lifespan of the mature cells. Granulocytes survive for only a few hours (6 to 8hrs) followed by platelets (with a 10 day life span) and erythrocytes survives

for a few months ï 4 months (120 days lifespan) so that some 10^{13} new cells must be replaced each day to maintain steadystate blood counts (Gordon 2002).

In the adult human, the bone marrow is the major site of haemopoiesis and it contains cells that represent the various stages in the development of the various types of cells that is from stem cell pool to differentiation, to lineage selection, to maturation, to function, then to cell death,i.e. apoptosis (Bradford *el-al* 1977). The production of blood cell is largely controlled by afeedback mechanism. When the demand for production of cells of a particular type increases or levels of the cell fall in blood, stimulating substances called cytokines are released. The cytokines stimulate the stem cells to generate new mature blood cells (Frimberger 1998). This occurs in few days required for blood cell maturation. The production of lymphocytes is an exception to this neat process. Many more lymphocytes are generated daily than needed in the blood. Mostlymphocytes are destroyed during development thus lymphopoiesis (production of lymphocytes) is inefficient compared to all other haemopoiesis.

HAEMATINICS

Haematinics on the other hand are the chemical agents/substances that are required for normal erythopoiesis. They can be said to be medicine that increases thehaemoglobin content of the blood. It isused in the treatment of iron deficiency anaemia. It can also be defined as the agent that improves the quality

of blood by increasing the number of erythrocytes and/or the haemoglobin concentration.

ANAEMIA

Anaemia is characterized by the decrease of the red cell (Erslev 2001). It is functionally best characterized by haemoglobin level below normal for age and sex of the sufferer. It is the disorder in which one suffers from tissue hypoxia the consequence of a low oxygen carrying capacity of the blood (Erslev 2001). It can include decreased oxygen ï binding ability of each haemoglobin molecule due to deformity or lack in normal development as in some other types of haemoglobin defects. Because the haemoglobin found inside the red blood cell normally carries oxygen from the lungs to the tissue, anaemia leads to hypoxia (lack of oxygen) in organs and because all human cells depend on oxygen for survival, varying degrees of anaemia can have a wide range of chemical consequences.

Anaemia is the most common disorder of the blood. There are several kinds of anaemia produced by a variety of underlying causes. It can be classified in a variety of ways based on the morphology of red blood cells, underlying etiologic mechanism and discernible clinical spectra (Erslev 2001). The three main classes of anaemia include excessive loss of blood (acutely such as haemorrhage or chronically through low volume loss), excessive blood cell destruction (haemolysis) or deficient red blood cell production

(ineffectivehaemopoiesis). There are two major approaches: the kinetic approach which involves evaluating production, destruction and loss (Gordon 2002) and the morphologic approach which groups anaemia by red cell size. The morphologic approach uses a quickly available and low cost laboratory test (MCV) as itôs starting point.

TELFAIRIA OCCIDENTALIS

Telfairia occidentalis is a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds (Oboh 2007). It is commonly known as flouted Gourd, Fluted pumpkin, and óUguô. The young shoots and leaves of the female young plants are the main ingredients of a popular Nigerian soup Edikang Ikong (Akwaowo *et-al* 2000). The large (up to 5cm) darkened seed is rich in fats and protein and can be eaten whole, ground into power for another kind of soup or made into fermented porridge. The fruits of plant are large, weighing up to 13kg but inedible (Asiegbu 1987).

The main use of *Telfairia occidentalis* is as a leaf and seed vegetable. The tender shoots, succulent leaves and immature seeds are cooked and consumed as vegetable. The leaves can be used alone or together with other vegetables like Okra(*Abelmosculus Caillei*). Immature seeds are cooked or roasted. Seeds can also be fermented for several days and eaten asslurry. The fruit pulp with young seed isoccasionally made into marmalade (Egbekun *et-al* 1998). The rawflour shows better water and fats absorption properties than oil, hence its useful application in baking products *(*Akwaowo *et-al 2001).*

The rind and pulp of the fruit of *Telfairia occidentalis* are used as fodder for livestock while the old stems are macerated to produce fibres that are used as sponge (Akoroda *et-al 1990*).Pregnant woman and patients that are anaemic use the leaf juice to strengthen their blood (Akwaowo *et-al 2000).*

PHYTOCHEMISTRY:

The composition of *Telfairia occidentalis* leaf is comparable to that of other dark green leafy vegetables. The leaves contain Trepenoids, oil, Tannis in very low concentrations, flavonoid, proteins and alkaloids in moderateconcentrations. Carbohydrates and saponins in very high concentrations. It also contains anti-nutrient cyanide ata concentration below toxic level, hence may not affect the bioavailability of the mineral contents *(*Akubue *et-al 1998).*The leaves are very high in mineral content like magnesium (Mg), iron (Fe), potassium (K) carotene (vitamin A) and Ascorbic Acid (Vitamin C) *(*Akpanabiatu *etal 1998).*The high content of mineralnutrients makes the leaf potentially useful as a food supplement. The seeds on the other hand, contain moisture, crude fats, carbohydrates, crudefiber, crude protein and ash. The mineral content includes calcium (Ca), phosphorus (P) and oil *(*Leung*etal 1968).*

The mineral content of the seeds are reported to be very high *(*Agemator *2007).* The seeds are also high in essential amino acids (except lysine), hence can be compared with soya beans meals with 95% biological value

*(*Asiegbu*1987).*Agametor (2007) reported that the seed contains vitamin C in relatively small amount, sodium and potassium in significant levels, calcium necessary for bone growth and muscle neurological function is also present at a significant level. Phosphorous, lack of which results in rickets, and iron required for haemoglobin formation are all present in the seed of *Telfairia occidentalis.*

The fruit pulp has a protein of about 1.0%. The main constituent of the seed oil is oleic acid, stearic acid, palmitic acid and lineoleic acid (*Akwaowo et-al 2001*)

JUSTIFICATION

Though considerable works have been done on the importance and uses of*Telfairia occidentalis*leaf as a blood builder, not much is known about the effects of the seed. Does it have the same properties as the leaf? If yes, does this affect the liver and/or the kidney? This work is therefore aimed, among other things, to find out whether the seed of *Telfairia occidentalis* has any haematinic and/or haemopoietic potentialas the leaf and also whether the consumption of this seed extract has any adverse / toxic effect on the liver and/or kidney.

AIMS AND OBJECTIVES

To determine the haemopoietic and haematinic potential of the crude seed extract of *Telfairia occidentalis.*

To compare the aqueous extract and methanol extract of the seed,whichhas more effect on blood components.

To determine whether any of the extracts (aqueous or methanolic) has any adverse effect on the liver and/or kidney.

CHAPTER TWO

LITERATURE REVIEW

2.0 HERBAL MEDICINE

A herb is plant or plant part used for its scent flavour or therapeutic properties (Gratus *et al* 2009). Herbal medicine products are dietary supplements that people take to improve their health.

Many herbs have been used for a long time for claimed health benefits. They are sold as tablets, capsules, powders, tea extract and fresh or dried plants, however some can cause health problems, some are not effective and some may interact with other drugs taken (1zzo and Ernest 2009).

Herbal medicine also called Botanicals, phytotherapy is a traditional medicine or folk medicine practice based on the use of plant and plant extracts (Kraft 2009).The scope of herbal is sometimes extended to include fungal of bee production as well as minerals and shells of certain animal parts. Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from wide varieties of predators such as insects, fungi and herbivorous mammals. By chance some of these compounds whilst being toxic to plants predators turnout to have beneficial effect when used to treat human diseases, such secondary metabolites are highly varied in structure, many are aromatic substances most of which are phenols or their oxygen substituted derivatives (Joy *et al* 1998).

Indigenous cultures (such as Africans of native American) used herbs in their healing rituals while others developed traditional medicinal systems in which herbal therapies are used. In the 19th century when chemical analysis became available, scientists began to extract and modify the active ingredients from plants, other chemists began making their own versions of plant compounds and overtime the use of herbal medicine declined in favour of synthetic drugs. However herbs are staging a comeback ñHerbal renaissanceò is happening all over the globe. (Ansari, 1993).Recently the WHO estimated that 80% of people rely on herbal medicine for some part of their primary health care (Kraft, 2009).A number estimated to be less than 10% of the total chemical compound in plants manifest their effect on human body by binding to receptor molecule present in the body, such processes are identical to those already well understood for conventional drugs and as such herbal medicine do not differ greatly from conventional drugs in terms of how they work (Altschuler *et al 2007*) . This enables herbal medicines to be in principle just as effective as conventional medicines but also gives the same potential to cause harmful side effects. Many species of herbs used by humans to season food yield useful medicinal compounds. These herbal products today symbolize safety in contrast to the synthetics that are regarded unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic product of the modern age surpassed their importance for a while. However the blind dependence on synthetics is over and people are

returning to naturals with hope of safety and security. A number of herbs however similar to conventional drugs are thought to likely cause adverse effects, furthermore inappropriate formulation or lack of understanding of plants and drug interaction have led to adverse reactions that are sometimes life threatening or lethal (Izzo *et al 2009*).

2.1 MEDICINAL PLANTS:

Plants have been used for medicinal purposes long before recorded history as ancient Chinese and Egyptian papyrus writings described medicinal uses for plants (Manheirmer*, et al 2009*). Plants provide an alternative strategy in search for new drugs. According to world health organization (WHO 1977), a medicinal plant is any plant which in one or more of its organs contain substances that can be used for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants are regarded as medicinal but which have not yet been subjected to thorough investigations. There is rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties . It is likely that plants will continue to be a valuable source of new molecules which may after possible chemical manipulation provide new and improved drugs (Shah*, et al 2006*).Medicinal plants are a therapeutic resources used by the population by the continent specifically for healthcare which may also serve as starting materials for drugs (Sofowora*, 1993*) .

WHO in 2001 further defined medicinal plants as herbal preparations produced by subjecting plant material to extraction, fractionation, purification, concentration, or other physical or biological processes which may be produced for immediate consumption or as basis for herbal products.Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (Harbone, 1973, Sofowora, 1996) which have curative properties. This complex chemical substance of different compositions is found as secondary metabolites in one or more of these plants. Tyler, (1999) has reported that plants also contain other compounds that moderate the effects of the active ingredients and some important chemical intermediates needed for manufacturing the modern drugs e.g eliosgenin, solasodine, b-ionone. Gill, (1952) reported phenolic compound to be present in plants in addition to tannins, alkaloid and flavonoid and that many of these indigenous medicinal plants are used as spices and food plants. Okwu, (1999,2001) in his report stated that many of these plants are sometimes added to the foods meant for pregnant women and nursing mothers for medicinal purposes some of which include; *Cleome rutudosperma, Emilia coccinea, Euphrobia heterophylia, Physcalis brasilensis, Sida acuta, Spigelia anthelmia, Stachytarpheta cayennensis and Tridia procumbens* are extensively used in herbal medicine in south eastern Nigeria.

Okwu, (2001) also noted that steroids and phlobatannins were found to be present in all the plants. These steroidal compounds are of interest and importance in pharmaceutical industries due to their relationship with such compounds as sex hormones hence the leaves of *Cleome rutidosperma* are used as vegetable for expectant mothers or breast feeding mothers to ensure their hormonal balance since steroidal structure could serve as potent starting material in the synthesis of these hormones.

2.2 USES OF MEDICINAL PLANTS

Over three quarters of the worldôs population rely mainly on plant extracts for healthcare. More than, 30% of the entire plant species at one time or the other were used for medicinal purposes. It has been estimated that in developed countries such as the United States of America, plant drugs constitute as much as 25% of the total drugs while in fast developing countries like China and India, the contributions is as much as 80% thus the economic importance of medicinal plant is much more to countries such as India than to the rest of the world. These countries provide two-third of the plants used in modern system of medicine and the healthcare of rural population depends on indigenous system of medicine. The drugs are derived either from the whole plant or from different organs like the leaves, seeds, stems, flowers, bark, root, etc. Some drugs are prepared from excretory plant product such as gum, resin and latex. Even allopathic system of medicine has adopted a number of plant derived drugs.

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatment, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, large segments of the world population still like drugs from plants. Also in many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third worldôs population although many such countries spend 40-60% of their total wealth on drugs and health care (Atal & Kapur 1982). Hence as part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drug will follow in future.

Green plants synthesize and preserve a variety of biochemical products many of which are extractible and used as chemical feed stocks or as raw materials for various scientific investigations. Many secondary metabolites of plants are commercially important and find their use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices,

diverse geographical distribution, labour cost, selection of the superior plants stock and owner exploitation by pharmaceutical industries (Bhakuni*,etal 1971*).

Plants especially those used in Ayurueda can provide biologically active molecules and lead structures for development of modified derivatives with enhanced activity and/or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structures from about 90 species of plants (Aslkar*, et al 1992*). In some cases, the crude extract of medicinal plant may be used as medicaments, on the other hand the isolation and identification of the active principles and elucidation of mechanism of action of a drug is of paramount importance. Hence works in both mixture of traditional medicine and single active components are very important, where the active molecule cannot be synthesized economically, the product must be obtained from cultivation of plant material.

A major lacuna in Ayurveda is the lack of drug standardization information and quality control. Most of the Ayurvedic medicines are in the form crude extracts which are a mixture of several ingredients and the active principles when isolated individually fail to give desired activity. This implies that the activity of the extract is the synergistic effect of its various compounds,(Aiyer,1996).However, in the absence of pharmacopoeia data on the various plant extract, it is not possible to isolate or standardize the active

contents having desired effects. A combination therapy integrating Ayurveda and allopathy whereby the side effect and the undesirable reactions could be controlled can be the thought of. Studies have shown that the toxic of radiation and chemotherapy in cancer treatment could be reduced by Ayurvedic medications and similarly surgical wound healing could be accelerated by Ayurvedic medicines (Ibid). Modern science and technology have essential role to play in the process.

2.3 *TELFAIRIA OCCIDENTALIS* (T.O)

Telfairia occidentalis commonly called ñUguò among the Igbosô (Eastern part of Nigeria), flouted gourd, because of the shape of the fruit, costillda in Spanish, krobonko in Ghana and Gonugbe in Sierra-Leone belong to the family cucurbitacea and is cultivated across the lowlands in humid tropics of west Africa ï Nigeria, Ghana, Sierra-Leone mainly for its nutritional value (Axtel, 1992). It is a popular vegetable all over Nigeria but it is rare in Uganda and absent in the rest of East Africa. It has been suggested that it originated in south-east Nigeria and was distributed by the Igbos who have cultivated this crop since time immemorial (Akoroda, 1990). It is however equally possible that fluted pumpkin was originally wild throughout its current range but wild plants have been harvested to local extinction and are now replaced by cultivated forms. It is so popular that different tribes call it different local names. The Igbos know it as Ugu, the Yorubas call it Uroko or Aporoke, the

Efiks call it Ubong, Urohobo call it Umee, while in Edo it is called Umeke(Akoroda, 1990. Bidafu and Ogusira 1991). The plant is dioecius, perenial and drought tolerant hence it can be cultivated in dry season by irrigation. Propagation is mainly by seed which resides inside a big pod (fruit) which weigh up to 13kg or less. The pod (fruit) which has its shape like a flute can contain as much as 50 seeds depending on the size of the pod. The young shoot and the leaves of the female plant are usually used for cooking porridge, soup, and is the main ingredient for the famous Nigerian Edikang ikang soup. The large dark red seed is rich in fat, protein, carbohydrates and some minerals (Agemator, 2007) and can be eaten whole or ground into powder for another kind of soup or made as fermented porridge.

2.31 CLASSIFICATION AND DESCRIPTION

Its classification to specie level according to Akoroda(1990) is as follows-

Kingdom ---------- Plantae

Division ---------- Magnoliophyta

Class ---------- Magnoliopsida

Order ---------- Cucurbitalis

Family ---------- curbitacae

Genus ---------- Telfairia

Species ---------- *Telfairia Occidentalis*

Telfairia occidentalis is a perennial dioecious herb as already mentioned climbing by coiled often branched tendrils to a height of more than 20 meters, root system ramifying in the top surface of the soil, stem is angular glabrous, becoming fibrous when old. The leaves are arranged spirally, pedated compound with 3-5 leaflets stipules are absent. Petiole (2-) 4-11(-15) cm long, leaflets with underneath 3-veined from near the base. Male inflorescence an auxiliary peduncle up to 25cm long, width at the base of the peduncle are long-pedicellate flower flowering long before the others; female flowers solitary in the leaf axil flowers 5-merous cream colouredpedicel up to 4cm long receptacle campanulate, sepals triangular up to 5mm long, petals free oblong fringed male flowers with 3-stamens two -4-locular, one-2-loccular with 3-celled ovary and 3 large heart-shaped stigmas(Akoroda*, 1990*).

Fruit is a dropping ellipsoid berry measuring 40-95cm x 20-50cm, weighing up to 6kg with 10 prominent ribs, pale green and covered with white bloom wax, fruit pulp yellow, many seeded. Seeds compressed ovoid up to 4.5cm long black or brown- red seedlings with hypogeal germination, developing first taproot and then numerous spreading axillary roots ; epicotyls 5-12cm long cotyledons planoconvex fleshy *(* Akoroda *1990*).

2.32 PHYTOCHEMISTRY

The leaf:

Telfairia occidentalis is an important staple vegetable grown in Nigeria mainly for its edible leaves and seeds but recently it has been discovered that almost every part of the plants is useful. The plant provides luxuriant edible green leaves which are rich in mineral content and vitamins. The leaves are borne on the stem of the plant which has branching long twisting tendrils and they are divided into three fine leaflets with terminal leaflets up to 15cm long. The composition of the leaves has been analyzed by many; According to Akwaowo*, etal (2000),* the leaf composition per 100g edible portion is water 86.4g, energy 147k(47 kcal), protein 2.9g, fat 1.8g, carbohydrates 7.0g.Kayode *et al (2009),*Ladeji *et al (1995*),Oboh and Akindahunsi (2004) in their work showed that *Telfairia occidentalis* also contains some minerals and vitamins which include magnesium, iron, potassium, sodium, phosphorus, calcium, ascorbic acid and carotene; while Oliver (1960), Ladeji *et al*(1995), Longe *et al*(1983); Horsfall and Spiff (2005) andFasuyi (2006) added that this leaf also contains thiamine, Riboflavin, nicotinic acid or pro vitamins, pyridoxine(B_6), Folic acid, Pantothemic acids like Phenol just like other green leafy vegetables. These contents make the leaf of *Telfairia occidentalis* highly nutritious and potentially useful as food supplement. The young leaves have also been shown to contain anti nutrients cyanide at 60mg per 100g dry matter, and tannins at 41mg

per100g dry matter but their concentrations are below toxic level and may not affect the bio- availability of the minerals. (Leung *et al 1968*).

Fasuyi (2006) and Tindal (1968) both recorded that the leaves are also very rich in amino acid profile such as aspartate,glycine, glutamine, histamine, lysine, methionine, tryptopham, cystine, leucine arginine, serine, threanine, phyenylalanine , valine tyrosine and isoleucine.

2.33 THE SEED

The seeds of *Telfairia occidentalis* are highly nutritious and are eaten roosted or boiled like the seeds of breadfruit (*Treculia*). They are also sometimes used as soup thickener (Okoli and Mbediogu 1993).The seeds is very rich in oil especially unsaturated fatty acids which form 61% of the oil (Odoemena and Onyeneke 1998). Leung,*et al* (1968) reported the composition of the seed per 100g edible portion to be: water 6.2g, energy 2280KJ(543Kcal), protein 20.5g, fat 45.0g carbohydrate 23.5g, fibre 2.2g, calcium 84mg and phosphorus 572mg. He also reported a high content of essential amino acids with the exception of Lysine in the seed. Akpanabiatu *et al*(1998) recorded a protein content of 28-37% and oil content of 47-56% of the dry matter with a very high content of mineral. Akubue *et al(1980)* and Taylor *et al* (1983) documented that *Telfairia Occidentalis* seeds are good sources of four minerals required in human nutrition, their report showed that the seed contain 29% oil and 30% protein. Asiegbu (1987) reported that the *Telfairia Occidentalis* seed contains 47% oil

and 31% protein with the protein being markedly deficient in sulphur amino acid. Longe *et al* (1983) reported that fluted pumpkin seeds had 53% fat, 22% protein, 3% fibre, 15% carbohydrates and 2% ash. In his study, Agemator *(2007)* showed that the high content of essential nutrients (crude protein 3.47%, crude fat 31.38%, moisture 10.93%, ash 2.02%, carbohydrate 50.08%, fibre 2.12%, calcium 280µg/g, vitamin A 890iu, vitamin C 0.7µg/g) in *Telfairia Occidentalis* Hook F seeds is enough to be used to supplement other dietary sources. The study showed that the seed of *Telfairia Occidentalis* Hook F is high in carbohydrates, fat and phosphorus and also contains high levels of vitamin A which can supplement other dietary sources. In addition, the oil present in *Telfairia Occidentalis* seed is high in iodine values when compared with that of palm oil. This shows that the oil has high content of unsaturated fatty acids relative to palm oil, indicating that it may be used for cooking or in manufacturing margarine. Akwaowo *et al (2000)* however reported that the main constituents of the *Telfairia Occidentalis* seed oil are oleic acid 37%, stearic and palmitic acid both 21%, linoleic acid 15%, variation between samples was reported to be large. The fruit pulp has a protein content of 1.0%. The roots contain cucubitacine sesquiterpene lactones (Iwuh 1983).

2.4 MEDICINAL PROPERTIES AND USES

Telfairia Occidentalis has been claimed to have many medicinal properties, although many of these claims are yet to be validated by scientific research, most have been validated. Both the leaves and the seeds have been reported to have a high content of protein. The high protein content of leaves of plants especially that of *Telfairia Occidentalis* could have supplementary effect for daily protein requirement of the body. The symptoms of protein energy malnutrition known as kwashiorkor and marasmus were rarely observed among dwellers in region where adequate amount of protein is observed from fruits and leaves of plants rich in protein such as *Telfairia Occidentalis* (Dike *2010,* Kayode *2010,* Kayode *et al 2009).* Fasuyi *and*Nonyerem *(2007)* investigations show that *Telfairia Occidentalis* leaf meal caused increased growth in birds. Adaramoye *et al (2001)* reported that *Telfairia Occidentalis* leaves has hypolipdemic effect and may be useful therapy in hypercholesterolemia. This confirms the work of Eseyin *et al(2005).* The vitamin A content and consumption pattern of some leafy vegetable (*Telfairia Occidentalis* inclusive) among pregnant women in Calabar, Nigeria was investigated by William *et al (2009)*, they observed that *Telfairia Occidentalis* has the highest vitamin A content which is inadequate amount to sustain their vitamin A requirement.

The fruit of *Telfairia Occidentalis* has been utilized in the production of marmalade (Egbekun *et al* 1998). The use of *Telfairia Occidentalis* in reproduction and fertility in traditional medicine is gradually becoming a thing of interest in medical science. A study carried out by Nwagwu and his colleague in 2009 shows that *Telfairia Occidentalis* has the potential to regenerate testicular damage and also increase spermatogenesis. However, more work is required to establish this observation.

2.41 ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTY

Almost all organisms possess antioxidative defence and repair systems that evolved to protect them against oxidative damage. These systems are sufficient to prevent them entirely. However, anti-oxidants supplement or food containing anti-oxidants may be used to help the human body reduce oxidative damage *(*Yang *et al 2002).*

In recent years, there has been a particular interest in the antioxidants and health benefits of phytochemicals in food vegetables. This was as a result of their potential effects on human health (Wei and Shiow, 2001). Many researchers especially in the field of medical sciences have observed free radical scavenging ability and antioxidant property in *Telfairia occidentalis*. The dark green leaves of *Telfairia occidentalis* extracts (such as aqueous and ethanol extracts) have been found to suppress or prevent the production of free radicals and scavenge

already produced free radicals, lower lipid peroxidation status and elevates antioxidant enzymes (such as superoxide dimutase and catalase) both *in vitro* and *in vivo* (Oboh *et al* 2004, 2006, Nwanna and Oboh, 2007, Adaramoye *et al* 2007, Iweala and Obidoa 2009, Kayode et *al* 2009, Kayode et al 2010). Kayode et *al*(2009, 2010) reported that *Telfairia occidentalis* has also been found to ameliorate oxidative brain and liver damage induced by malnutrition in rats. Nwanna and Oboh (2007) reported that hepatoprotective property polyphenol extract of *Telfairia occidentalis* leaves on acetaminophen induced liver damage, Oboh in 2005 had earlier reported that both aqueous and ethanol extract of Telfairia occidentalis leaves protects the liver cells against garliac-induced oxidative damage. However aqueous extracts are more effective than the ethanolic extracts which could be attributed to higher antioxidant activity of the aqueous extract of *Telfairia occidentalis* leaves.Eseyin *et al* 2007, Iweala and oboh (2009) and kayode et al 2009 have all reported the hepatoprotective effects of *Telfairia occidentalis* leaves. The use of the leaves in folk medicine in Nigeria in the treatment of certain diseases in which the participation of reactive oxygen species have been implicated could be as a result of the antioxidant and free radical scavenging ability.

2.42 ANTIMALARIAL AND ANTIMICROBIAL PROPERTIES

Malaria is a potentially life threatening disease in the tropics as it affects over 400 million people yearly. It is responsible for the death of an estimated 10,000 women of reproductive age, and over 1million infants and young children each (Barbian 1989, Mishra *et al* 2003). Drug resistance, increases in the production and circulation of false drugs, cost of newer and effective drugs have been a major factor affecting the poor populace thus making their choice of herbal remedies inevitable and economical. A study by Okonkon *et al 2007* has shown that ethanol root extract of *Telfairia occidentalis*possesses antiplasmodial potential. The blood schizontaocidal activity of the root extract is comparable to that of chloroquine. Oboh *et al 2006* have reported that the ethanolic and aqueous extract of *Telfairia Occidentalis* show inhibitory effect on the growth of some of the commonly encountered enterobacteriacae in Nigeria namely *Escherichia coli*, *Pseudomonas aeroginosa*, *Proteus species* and *Salmonella typhi*, however both extracts did not inhibit the growth of fungi tested which include *AspergillusFlavus,Aspergillus fumigatum*, *Penicillium italium* and *Geotricuum albiedun*. Odoemena and Onyeneke (1998) also reported on the anti bacterial activity of the leaves while Odemena and Essien (1995) reported the anti bacteria activity of the root extracts against *Staphylococcus aureus*, *Streptococcus pyogenes* , *Shigella dysenterae,* and *Klebsiella pneumonia*. Oluwole *et al* 2003 reported on the antinflammatory activities of *Telfairia*

Occidentalis, the young leaves sliced and mixed with coconut water and salt are stored in a bottle and used in the treatment of convulsion in ethanomedcine(Gbile 1986).

2.43 *TELFAIRIA OCCIDENTALIS* AS A BLOOD BOOSTER AND DIABETIC CONTROL AGENT:

In Nigeria, herbal preparations of the plant have been employed in the treatment of anaemia, chronic fatigue and diabetes (Aderlbigbe 1999,Alada, et al 1999 2000, Diana*et al* 2006).

Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections. In anaemia, there is decreased level of circulating haemoglobin, less than 13g/dl in males and 12g/dl in females (Okochi *et al 2003*). In the tropics, where malaria is endemic between 10-20% of the population present less than 10g//dl of haemoglobin. Children are more vulnerable. The *Telfairia Occidentalis* leaves are rich in iron and play a key role in the cure of anaemia. They are also noted for lactating properties and are in high demand for nursing mothers (Okoli and Mgbediogu 1983).

Type 2 diabetes associated with increased oxidative stress which probably results both from excess generation of reactive oxygen and decreased anti-oxidantdefences *(*Baynes *1991,* Tribe and Poston *1996).* In the recent years, it has been known that the most popular factor to increase the free radicals

production in diabetes is the hyperglycaemic status which can induce damage through over production of super oxide radicals in the mitochondria (Brownlee 2001). Super oxide is converted to hydroxyl which can diffuse through membranes and initiate lipoperoxidation. The oxidation of unsaturated lipids has implications not only for atherosclerosis levels but also for stability and integrity of the red cell membranes *(*Steinberg *et al 1989).* Increased levels of lipoperoxidation as evidenced by breakdown products like malondi aldehyde have been found in the erythrocytes and plasma of type 2 diabetic patients. Supplementation with antioxidants is therefore an attractive potential therapy*(*Kayode *2011).* Aqueous extracts of*Telfairia Occidentalis* leaves have been reported to reduce blood glucose level and also have antidiabetic effects in glucose induced by hyperglycaemic and streptozocin (stz) induced diabetic mice *(*Aderlbigbe *et al 1999),* while it did not alter glucose levels in normoglycemic mice. Salmon *et al* 2008 also reported reduced blood glucose levels by *Telfairia Occidentalis* leaves in male rats. Hypoglycemic effects have also been reported by many other researchers *(*Eseyin *et al 2007,*Nwozo *et al 2004).*

Other uses of *Telfairia Occidentalis* include the use of the roots as rodenticide and an ordeal poison (Gill 1992).

2.5 HAEMOPOIESIS

Haematopoiesis can be said to be the process by which cellular elements of the blood are formed i.e. the production of the blood cells. The blood of mammals includes a number of different cell types essential for survival. These cells (formation of which is the most active and important process in the body) includes erythrocytes (red blood cells) that transports oxygen, platelets (thrombocytes) that mediate the clotting of blood and support tissue integrity, neutrophils, eosinophil, basophil and monocyte that are essential to host defence against bacteria, fungi parasites and viruses; T-lymphocytes, natural killer (NK) cells and dendrtic cells all function as antigen-presenting cells and in cell-mediated immunity and B-lymphocytes that are the source of antibodies. Quesenberry and Clovin(2001).

The production of these cell types andtheir levels in the peripheral blood is controlled by multiple humoral and cellular factors that adjust to meet needs. This production occurs in haemopoietic tissues and it involves a high level of cell turnover, demanded by the need to replace mature circulating blood cells at a rapid rate and is necessitated by the limited life span of the mature circulating blood cells. Granulocytes survive for only a few hours, thrombocytes a few days and erythrocytes for a few months so that some 10^{13} new cells must be replaced each day to maintain steady state blood counts (Gordon 1993).

Infections by a variety of micro-organisms result in almost immediate release of mature neutrophils from the marrow storage pool, followed by an increase in the production of granulocytes and usually monocytes until the infectious agents are cleared. Likewise, haemorrhage or acute haemolysis results in rapid release of marrow reticulocytes, followed by a sustained increase in red cell production until the red cell numbers returns to normal. On the other hand, platelet production and release responds to several stimuli including decrease in platelet number, acute anaemia and tissue destruction or inflammation. Quesenberry (2002). The modulation of increased production may occur within different subset of these cells. (Kipps 1997, Plas and Thomas 1998).

Mature cell types are derived from stem cells which is the most important cell in haemopoietic cell production. They differentiate into progenitor cells that are controlled by circulating or membrane-bound cytokines or various adhesion proteins which are phased into circulation following damage to haemopoietic system either by myelotoxic chemotherapy or after stem cell transplantation.

A haematolymphopoietic stem cell is defined as a cell with extensive self-renewal and proliferative potential coupled with the capacity to differentiate into progenitors of all the blood cell lineages namely erythrocytes, neutrophil granulocytes, mast cells, monocytes and macrophages, platelets, B-lymphocytes, T-lymphocytes and natural killer cells and dendritic cells (Quesenberry and Clovin *2001).* Self-renewal refers to the potential to produce

daughter cells with identical character. Nakahata and Ogawa (1982) in their studies with murine and haemapoietic cells indicate that two daughter cells from a primitive undifferentiated cell can have totally different lineages, for instance, one daughter cell giving rise to neutrophils and monocytes, while the other cell may give rise to erythrocytes, megakaryocytes and mast cells. These observations suggest that there is a less-ordered or stochastic system in which the commitment decision to produce different lineages is made with one cell cycle transit, although concordance of daughter cells is still a model in which there is ordered and progressive lineage restriction and differentiation: once the differentiated characteristics are attained, cell renewal potential declines precipitoriously *(*Nakahata and Ogawa, *1982).* The stem cell according to Lemschka *et al (1986)* can therefore be said to be the ability of that single cell to repopulate long-term haematopoiesis in the whole animal. This long term repopulating stem cell can be seen in the repopulation that occurs in patients that are given marrow ablative treatment, allogenic marrow transplant (Iwu *et al 1967,*Abrason *et al 1977).* Moreover, it has also been shown in experiments with mice where unique radiation-induced chromosome abnormalities or retroviral markers have established the capacity of very few cells(approaching one cell) to totally repopulate the lymphohaemapoietic system (Suda *et al 1983).*

2.6 ANAEMIA

Anaemia characterized by decrease in size of red cell mass and functionally best characterized by a haemoglobin concentration below normal can be defined as the decrease in the total number of circulating erythrocytes or decrease in the concentration of haemoglobin in the blood or decrease in haematocrit compared to the normal population (Becks *1992*).

It is a disorder which one suffers from tissue hypoxia, the consequence of a low oxygen-carrying capacity of the blood (Erslev *2001).*This results in decreased oxygen-binding ability of each haemoglobin molecule due to deformity or lack in numerical development as in other types of haemoglobin deficiency. Because haemoglobin (usually found inside the red blood cells) normally carries oxygen from the lungs to the tissues, anaemia leads to hypoxia(lack of oxygen) in organs and because all human cells depend on oxygen for survival, varying degree of anaemia can have a wide range of clinical consequences.

Anaemia is the most common disorder of blood. There are several kinds of anaemia produced by a variety of underlying causes.

2.7 HAEMATINICS

Haematinics are chemical agents/substances that are required for normal erythropoiesis. It can also be said to be an agent that improves the quantity of blood by increasing the number of erythrocytes and or the haemoglobin

concentration. These chemical agents includes iron(Fe), cobalt(co), Zinc(Zn), Vitamins B_{12}folic acid and erythropoietin.

2.71 IRON

Iron is a component of all living organism. It plays an important roleparticularly in electron transfer reactions. About 70% of the iron in the human body is in the form of haemoglobin, in circulating red blood cells which contain 1mg of iron per ml of packed cells. 10 ï 20% is in the storage forms as haemosiderin or ferritin. Ferritin is water soluble while haemosiderin is insoluble in water. 10% is in form of myoglobin, a heam containing protein which is present in the muscles and less than 10% formed in the cytochromes and other iron containing enzymes as transport iron transferrins.

2.72 ERYTHROPOIETIN

Erythropoietin is the primary regulator of erythoid progenitors and is the first to be extensively studied*(*Iscore *et al 1975).* Erythropoietin was found to be active in vivo and invitro *(*Reissman *1950,* Erslev *1953)*and acted via a cell membrane receptor to induce erythroid differentiation *(*DôAndrea *et al 1989)* and to suppress apoptosis or programmed cell death *(*Koury *et al 1989).*

It is one of the most specific of the lympho haematopoietic cytokines acting on erythroid cells while many other cytokines show action on multiple lineages Reissman *(1950)* and Erslev *(1953)* were the first to provide the definitions of

erythropoietin the hormone was later purified, its sequence determined and the gene encoding its production cloned *(*Jacobs *et al 1985).* Koury *et al (1988)* reported that erythropoietin is produced in peritubular interstitial cells while Maxwell *et al (1990*) reported its production to be in the renal, tubular cells.

Hepatocytes and kupffer cells have been implicated as the cell of organ for hepatic erythropoietin *(*Rich *et al 1982).* The tissue oxygen-sensing mechanisms that are linked to erythropoietin elaboration may involve a heme protein (Goldberg *et al 1988).* The response of BFU-E and CFU-E to erythropoietin correlates directly with the presence of erythropoietin receptors. Two classes of erythropoietin receptors have been identified on erythroblast. They are the high and low affinity obtained from the spleens of mice infected with the anaemia strain of friend virus *(*Sawyer *et al 1987).*

2.8 LIVER ENZYMES

The liver is the biggest gland in the human body. It is about 2.8% of the body weight an average of 1.5kg in men and 1.2kg in women. In general, the liver performs more than 500 different functions participating in the regulation of glucose in the blood, accumulating nutrients, vitamins iron, and glycogen producing bile to help breakdown fats. The main function of the liver is the barrier cleansing. This is a powerful filter neutralising toxins which are produced by metabolic reaction that converts toxic compounds which removes and destroys the bacteria. It takes the blow in case of poisoning, drug overdose

and poor nutrition. The liver rarely makes itself felt in the beginning of the disease usually when there are symptoms has been already seriously ill. Removal of liver inevitably leads to death within 1-5 days. However, the liver has huge domestic reserves. It has the amazing ability to recover from injury so that man can survive even after the removal of 70% of the liver tissue.

2.81 THE ENZYMES

The liver produces a huge number of liver enzymes entering directly into the blood. When the liver is damaged, some of the liver enzymes in the blood streams are decreased while others are increased. Liver enzymes are proteins that help to speed up a chemical reaction in the liver. Liver function tests are blood test that are used to evaluate various function of the liver for example; metabolism, storage, filtration and excretion which are often performed by the liver enzymes. Liver enzymes which are found in the normal plasma or serum can be divided into different groups viz:

The transaminases: in this group, we have aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGOT).

Cholestatic liver enzyme: in this group, we have the alanine phosphatase (AP) and gamma glutamyl transferase (GGT). Elevation of these enzymes can indicate the presence of liver disease.

Secretory enzymes: these are enzymes synthesized in the liver but normally located in the blood plasma, they play physiological role such as enzymes

involved in blood clotting process (AC globulin), cholinesterase. Any damage to the liver reduces their synthesis and activity of these enzymes decreases.

Certain enzymes enter into the blood from the tissues where they perform intracellular function. Some of them are in the cell cytosol (LDH, ALT AST) others are in the mitochondria (GGT, AP etc) and cause damage to the liver. Enzymes from the cells are washed in the blood and their activity increases. ALT and AST are of the greatest diagnostic value. In acute parachymatous hepatitis, ALT increases by 20 ï 30 times more while activity of AST increases several times smaller.

Outside the liver, AST enzymes are often found in the heart, muscle, kidney and brain where it is released into the serum. At the time one of these tissues are damaged for instance with heart attack and muscle disorders, AST level in serum rises hence it is not a highly specific indicator of liver injury.

ALT is primarily found in the liver. Hence as a result of liver injury it is released into the blood stream and often serves as a fairly specific indicator of liver status (Polio *et al 1987).* The high level of AST and ALT in the liver cells can easily damage the numerous liver cells (extensive hepatic necrosis) and can lead to cell death. The higher the ALT levels in the liver, the more the death of cells in the liver. However, ALTs are not always good indicators of how well the liver is functioning (Paliwal *et al 2009*). Only a liver biopsy can reveal the functioning integrity of the liver (Rao *et al 1990*). Diseases that may cause

increased level of ALT and AST liver enzymes include acute viral hepatitis A or B, liver cirrhosis hepatoma etc (Polio *et al 1987).*

Furthermore, certain drugs have been shown to cause liver damage for instance, toxins produced after acetaminophen (brand name ï Tylerol) overdose or other substances capable of producing toxicity in the liver or prolonged collapse of circulatory system(shock), when the liver is deprived of fresh blood bringing oxygen and nutrients (Rahman *et al 1996*). In this situation, ALT and AST serum levels can range anywhere from ten times the upper limits of normal to thousands units/liter (Sanhni and Saxena 2001).

2.9 TOXICITY

Toxicology is a very old concern to humans from the time of stone age to this modern era(Taneminar *et al 2009).* Now it is a separate branch of science and has its own importance. Toxicology deals with the toxicity by chemical or compound by intention or by accidental exposure to living organism. Excess of any compound will be harmful to life and considered under toxicity studies.

In humans, toxicity is a threatening truth more than any disease by any organism as toxic substance are everywhere ï in air, in water and in food *(*Hodgson *2004)*, many compounds which are essential for human welfare, food which are ingested, for nourishments are injurious to us in every manner (ibid). Some are used as food additives and others as daily material.

Liver is a target organ and primary site of detoxification and is generally the major site of intense metabolism (Guyton 2004). As a result, the liver is prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. The liver plays an importance role in metabolism to maintain energy level and structural stability of the body hence any change in the liver systematic definitely affects the complete metabolism of the animal *(*Guyton *2004).* The liver is also the site of bio-transformation by which a toxic compound can be transformed to a less harmful form to reduce toxicity *(*Hodgson *2004).* This however can damage the liver cells and produce hepatotoxicity. Alanine transaminase (ALT), an enzyme that helps to metabolise proteins is usually increased in the liver and subsequently released into the blood stream when the liver is damaged. Aspartate transaminase on the other hand plays a role in metabolism of amino acid alanine. An increase in the level of this enzyme may indicate liver damage or disease.Aspartate transaminase is predominantly found in the skeletal muscles and kidney while Alaninetransaminases is a cystolic enzyme and is more specific for liver damage or disease.

The liver histology was also examined to see whether there is an indication of liver damage or toxicity by ingestion of *Telfairia occidentalis*. The kidney being an organ which functions majorly in filtration and excretion of waste products of metabolism can also in the presence of toxic metabolites accumulate some

toxins which will affect its functionality hence kidney histology of the *Telfairia occidentalis*feed rats will also be examined to establish the presence/absence of any toxin in the kidney cells.

CHAPTER THREE
MATERIALS AND METHODS

3.1COLLECTION OF PLANT MATERIALS

The seeds were collected from the selected markets here in Enugu

ANIMAL COLLECTION:

One hundred and twenty eight (128) albino wistar rats (150 ±200g) were obtained from the Physiology Department of the College of Medicine, Enugu campus of the University of Nigeria. They were kept in clean gauzed cages under standard condition of temperature 28^{o}Cï 30^{o}C and with light / dark periodicity of 12:12 hours in the animal house of the University of Nigeria, Enugu campus. The animals were acclimatized for two weeks and were fed with standard pellets (Top Feed Nigeria) and water ad libitum. All the animals were handled according to international and local guidelines for handling experimental animals throughout the duration of the studies.

3.2 SEED EXTRACTION

The seeds were first removed from the fruit pod; they were carefully extracted from the pulp, inside the fruit pod and washed. The seed hard coat was finally removed to get the endosperm which was then air dried under the shade for 6 weeks and then grinded into powder. 400g of the grinded seed powder were weighed in two places: one was dissolvedin 3,000mls of distilled water while

the other was dissolved in 3,000mls of absolute methanol (aldrich brand) for 48 hours with intermittent vigorous shaking. The suspension was strained with muslin strainer and then filtered with no 1 filter paper (watsmann). The methanol filtrate was then evaporated to dryness on a rotary evaporatorwhile the aqueous was frozen at -82^{o}C and then allowed to thaw. The clean whitish supernatant was removed while the residue (the extract) was allowed to dry in an incubator at 37^{o}C$\pm$2^{0}C. The extracts obtained were stored at4^{o}C $\pm$ 2^{o}C until use. For administration, 10gm of the extracts were dissolved in 50mls of Tween 80 and then made up to 100mls with 2.5% Tween 80.

3.3 ACUTE TOXICITY TESTING (MEDIAN LETHALDOSE)

The acute toxicity study was carried out in rat to determine the 50% lethal dose (LD_{50}) of the extract by intra-peritoneal route using the method of Lorke,(1983) and asdescribed by Okonkon *etal 2009.*

3.4 EXPERIMENTAL DESIGN

One hundred and twenty eight (128) rats weighing between (150 ï 200g) were used for the whole study. The study was in two sets. The first set:aqueous seed study, the animals were divided into nine groups of eight animals each, group A, B_1, B_2,B_3, C, D, E, F,and G. In groups C ï G, the animals were induced for anaemia using phenylhydrazine. These were used for haematinic activity study while B_1 ï B_3were used for haemopoietic activity. Anaemia was not induced in

these animals. Group A is the normal control; the animals were fed with only food and water. There was no induction of anaemia and no extract was administered. Groups B_1 ï B_3 received graded concentration of the extract. In groups B_1, 50mg/kg body weight of extract was administered, Group B_2 100mg/kg body weight of the extracts was administered while group B_3 received 200mg/kg body weight of the extract.

Groups C to E in addition to being induced for anaemia with phenylhydrazine also received graded concentration of the extracts. Group C received 50mg/kg of extract; Group D received 100mg/kgbody weight of extract;Group E received 200mg/kg body weight of extract; Group F was used as the haematinic control, in addition to being induced for anaemia received 100mg/kg body weight of Astymin,(a popular commercially formulated haematinic); Group G was used as the anaemic control, they received only phenylhydrazine, after which they continued with their normal feed and water. The second set was the methanol extraction, the same pattern as described in aqueous only that the extraction was done with methanol only.

2mg/kg body weight of Phenyl hydrazine was administered to groups C-G orally for 9 days in addition to their normal feed and water while Groups A, B_1 ï B_3received their normal feed and water for those 9 days. On the tenth day, 6mls of blood were collected from two rats from each of the cages A, B_1 ï B_3 and C ï G for baseline values. 2mls of blood were collected into tripotassium

ethylenediaminetetra acetic acid (k_3 EDTA) for complete blood count while 4mls was collected into plain tube for enzymes analysis. The blood was collected from retro-orbital plexus of the median cantus of the eye. After collection of blood, the animals were euthanized under anaesthesia and their bone marrow cells were collected from the femur. It was emulsified with a drop of normal saline on a clean grease free slide from where the bone marrow smear was prepared. The dried marrow smear was then fixed in methanol until it was stained. The liver and kidney were extracted and washed in normal saline and then placed in a well labelled universal container, containing 10mls of 10% formol saline (formalin) for histological studies.

Haematological parameters were however carried out the same day using haematology autoanalyser (Sysmex KX ï 21N), while the samples for enzyme studies were spun and then separated and stored at -32^{o}C until it was analyzed. The liver and kidney were taken to Histology Department of the University of Nigeria Teaching Hospital for sectioning and histological studies.

Administration of the extracts commenced after the baseline samples were collected that is on the 9th day after the commencement of phenylhydrazine administration. As already stated, A received no extract, B_1 ï B_2 received graded concentration of the extract that is 50mg/kg bwt, 100mg/kgbwt and 200 mg/kg bwtrespectively, Group F received Astymin (100 mg/kg) haematinic

control,while Group G received no extract, no haematinics after anaemia has been induced only feed and water (anaemic control)

On the third day (D_3), sixth day (D_6)and ninth day (D_9)from the onset of the administration of the extracts, samples of blood were collected from two rats each from each group, their bone marrow samples were collected, and their organs of liver and kidney excised and processed as described above. This procedure was also carried out for methanol seed extracts.

3.5 ANALYTICAL METHODS

3.51 HAEMATOLOGICAL PARAMETERS: Complete blood count was carried out using haematological auto analyzer (Sysmex KX ï 21N); this instrument also was used to enumeratethe reticulocytes and the platelets. The blood films were made and stained with leishmann stain as described by Dacie and Lewis, (2005).

The bone marrow smear which was fixed upon collection was stained with leishmann stain as described by Dacie and Lewis, (2005) Both the bone marrow and the blood films were later examined using x 40 magnification for the bone marrow examination and x 100 magnification for blood films and WBC differential count.

3.52 ENZYME DETERMINATION: Alanine and Aspartate transaminases were analyzed using the end point technique of Reitman and Frankel (1957) with protocol described in Randox Kit (Randox laboratory LTD United Kingdom)

3.53 HISTOLOGICAL EVALUATION OF THE LIVER AND KIDNEY

The formalin fixed liver and kidney tissues were embedded in paraffin wax and microtome sections were stained with Haematoxlyn and Eosin for light microscopy

Statistical analysis was done using SPSS version 17 for multivariance analytical methods.

CHAPTER FOUR

RESULTS

Tables 4.1 -4.2 show the effect of methanolicand aqueous seed extracts of *Telfairiaoccidentalis* (T.O) on serum Transaminases and some haematological parameters on day 3 of administration to rats . The haemoglobin concentrations (Hb) were significantly decreased in all the groups that received various concentrations of the methanolic extract $p<0.05$, while no significant difference was noted in those administered with aqueous extracts. The packed cell volume (PCV) showed significant decrease in both methanolic and the aqueousextract for the rats that received 200mg/kg body weight $p<0.05$.The MCHC were significantly decreased$p<0.01$ in both extract at 100mg/kg,but was increased $p<0.01$ at a concentration of 50 mg/kg in aqueous extract. MCH was also significantly decreased in the aqueousextracts at a concentration of 100mg/kg $p<0.05$. Platelets were significantly decreased $p<0.05$ at 100mg/kg and 200mg/kg body weight in rats fed with methanol extract when compared with the normal control while the reticulocytes were significantly increased in all the concentrations of both methanolic and aqueous extract $p<0.05$.The serum transaminases showed no significant difference in both extracts.

Tables 4.3 ï 4.4 revealthe effect of methanolic and aqueous seed extract of T.O on serum transaminases and some haematological parameters on Day 6 of administration to rats. On the sixth day of administration, the blood collected

revealed a significant decrease $p< 0.05$ in Hb concentration and PCV in all the concentration of methanolic extract administered when compared with the normal control. The reticulocytes showed significant increase $p<0.05$ in all the concentration of extract given when compared the normal control. The MCHC were significantly decreased $p<0.05$ at a concentration of100mg/kg body weight of rats, and $p<0.01$ at a concentration of 200mg/kg body weight of rats when compared with the normal control.The red blood cells (Rbc) were also significantly decreased at 200mg/kg body weight only at $p<0.05$ while theMCH and MCV showed significant increase $p<0.05$ at a concentration of 200mg/kg when compared with the normal control.

In aqueous extract, the Hb and PCV showed significant decrease $p<0.05$ at a concentration of 50mg/kg body weight when compared with the normal control while the reticulocytes were significantly increased $p<0.05$ at a concentration of 50mg/kg and 200mg/kg body weight only. The WBC and platelets only showed significant increase $p<0.05$ at a concentration of 200mg/kg body weight when compared with the normal control.

Tables 4.5 -4.6 show the effect of methanolic and aqueous seed extracts on serum transaminases and some haematological parameters on the 9^{th} day of administration to the rats. The results showed that the Hband PCV were significantly decreased $p<0.05$ in all the rats that received the various concentration of the methanolic extract when compared with the normal control.

The MCHC were also significantly decreased $p<0.01$ in the rats that received 50mg/kg and 200mg/kg body weight when compared with the normal. The MCV on the other hand were increased and decreased $p<0.05$ in the rats that received 50mg/kg and 200mg/kg body weight respectively when compared with the normal control. Also while the platelets and WBC were significantly increased at a concentration of 100mg/kg and 200mg/kg body weight $p<0.05$ and $p<0.01$ respectively when compared with the normal control, the reticulocytes were significantly increased in all the concentrations $p<0.05$ when compared with the normal control. In aqueous extract on day 9 of administration, the Rbcs were significantly decreased $p<0.05$ at the concentration of 200mg/kg body weight when comparedwith the normal control. The MCHC were significantly decreased $p<0.01$ at a concentration of 200mg/kg body weight while the platelets were decreased $p<0.05$ at the concentration of 100mg/kg and 200mg/kg body weight when compared with the normal control. The MCV on the other hand were significantly increased $p<0.05$ at the concentration of 200mg/kg when compared with the normal control while the reticulocytes were significantly increased $p<0.05$ in all the concentrations of aqueous extracts. The transaminases showed no significant difference in both extracts and in all the concentrations.

Tables 4.7 ï 4.12 showed the haematinic effects of the methanolic and aqueous seed extracts of T.O on various days of administration.

Tables 4.7shows theeffect of the methanolic seed extract on serumTransaminases and some Haematological parameters on Day 3 of administration to rats.

The Rbcs and the Hb levels were significantly decreased $p<0.01$ in the rats that received various concentrations of the extract, when compared with the normal control. The positive and negative control were also significantly decreased $p<0.01$ for Rbcs and $p<0.05$ for Hb concentrations. Also significant decrease was observed in the Pcv in all the concentrations of the extract given including the negative control $p<0.05$ when compared with the normal control, however for those that received 50mg/kg body weight when compared with the positive and negative control groups were also significantly decreased $p<0.05$. The MCV and MCH of all the concentrations including the positive and negative controls were significantly increased $p<0.001$ and $p<0.01$ respectively when compared with the normal control. The MCHC were on the other hand significantly decreased in all the concentrations including positive and negative control $p<0.05$ when compared with the normal control. For platelets counts only the group that received 50mg/kg weight and negative control groupshowed significant decrease $p<0.01$ and $p<0.001$ respectively when compared with the normal control. The reticulocyte count of all the groups that received the various concentrations of the extract were significantly increased $p<0.05$ when compared with the positive control, the negative control and the normal control

groups. The positive and negative controls were also significantly increased $p<0.05$ when compared with the normal control. The total WBC count of the groups that received 50mg/kg and 200mg/kg were significantly increased $p<0.05$ when compared with the negative control. The alanine transaminase of the group that received 50mg/kg were significantly increased $p<0.05$ when compared with the positive and negative control while those that received 100mg/kg, 200mg/kg , the positive and negative control were significantly decreased $p<0.05$ when compared with the normal control. While aspartate transaminase were only significantly increased $p<0.05$ in the negative control groups when compared with the normal control groups.

Table 4.8shows the effect of aqueous seed extract of *Telfairia occidentalis* on serum Transaminase and some other haematological parameters of anaemic rats on day 3 of administration. The Rbc and Hb concentration showed significant decrease $p<0.01$ in the rats that received 50mg/kg when compared with the normal control, however in the rats that received 100mg/kg and 200mg/kg, the Rbc and Hb were significantly increased $p<0.05$ when compared with both the negative and positive. The Rbc and Hb of the negative control were significantly decreased $p<0.05$ when compared with the normal control. The PCV in all the concentrations including the positive control were significantly increased $p<0.05$ when compared with the negative control. All were however decreased $p<0.05$ when compared with the normal control. The MCV of the

positive and negative control were significantly increased $p<0.05$ when compared with the normal control but in all the various concentrations of the extract, their MCV were increased atp<0.01 when compared with the normal control and significantly decreased $p<0.01$ and $p<0.05$ respectively when compared with the positive and negative control. The MCH at a concentration of 200mg/kg were significantly decreased with the negative control $p<0.05$ while the MCHCat a concentration of 50mg/kg, the positive and negative control were significantly decreased $p<0.01$ with the normal control. The plateletswere significantly decreased $p<0.01$ at concentration of 50mg/kg, and $p<0.05$ at a concentration of 100mg/kg with the normal control. The negative control were also significantly decreased $p<0.05$ when compared with the normal control. The reticulocytes on the other hand were significantly increased in all the concentrations including the positive and negative control at $p<0.05$, when compared with the normal control. The total WBC of those that received 200mg/kg of extract were significantly decreased $p<0.05$ with the negative control, while those that received 50mg/kg and the positive controlwere significantly increased $p<0.05$ with the normal control. The alanine transaminase of the negative control were significantly decreased $p<0.05$ when compared with the negative and normal control. While in concentration of 100mg/kg and 200mg/kg the aspartate transaminase were significantly increased $p<0.05$ when compared with the positive control, the positive control were also significantly decreased $p<0.05$ with the normal control.

Table 4.9 shows the effect of methanol seed extract of *Telfairia occidentalis* on serum transaminase and some haematological parameter of anaemic rats on day 6 of administration. On day 6 of extract administration, the Hb concentration in all the concentration of extract given were significantly increased $p<0.05$ when compared with the negative control. The Hb of the negative control were also significantly decreased at $p<0.05$ with the normal control. The PCV of the rats that received 50mg/kg, 100mg/kg and the positive control were significantly increased at $p<0.05$ with the negative control while those that received 200mg/kg were increased only at $p<0.01$ with the negative control. The negative control were also significantly decreased $p<0.05$ when compared with the normal control. The MCV of the rats that received 100mg/kg were also significantly increased at $p<0.05$ with the negative and normal control while those that received 200mg/kg and the positive control were significantly increased $p<0.01$with the negative control and $p<0.05$ with the normal control. The MCH of all the concentration of extract given including the positive control were all significantly increased $p<0.05$ when compared with the negative and normal control. In all the concentration of extract given, the platelets were significantly decreased $p<0.05$ when compared with the negative and normal control, however the positive control were decreased at $p<0.01$ with the normal control. In all the concentrations and positive control, the reticulocytes were significantly increased $p<0.05$ with the normal control, however in those that received 100mg/kg and 200mg/kg, the reticulocytes were significantly increased

$p<0.05$ when compared with the positive control while the negative control were only significantly increased $p<0.01$ with the normal control. The total WBC of the groups that received 100mg/kg were significantly increased when compared with the positive control, negative control and normal control. The Neutrophils of those that received 50mg/kg and 200mg/kg were significantly decreased $p<0.05$ with the positive control while their lymphocyteswere significantly increased $p<0.05$ with the positive control. The positive control neutrophils were significantly decreased $p<0.01$ with the negative control and normal control. The aspartate transaminase of the rats that received 50mg/kg, 200mg/kg and the negative control were significantly deceased $p<0.05$ when compared with the normal control while those that received 100mg/kg were significantly increased $p<0.05$ when compared with the positive and negative control. The positive control were significantly decreased $p<0.01$ when compared with the normal control.

Table 4.10 snows the effects of aqueous seed extract of *Telfairia occidentalis* on serum transaminase and some haematolgical parameters on day 6. The Rbc showed significant decrease $p<0.05$ only in the negative control when compared with the normal control. In all the concentration of extract and in positive control group, the Hb concentration showed significant increase $p<0.05$ when compared with the negative control, the negative control was also significantly decreased $p<0.05$ when compared with the normal control. The PCV values

were also significantly increased $p<0.05$ in all the concentration of the extract given and the positive control when compared with the negative control. However those that received 100mg/kg and the positive control were in addition significantly decreased $p<0.01$ when compare with the normal group while the negative control were significantly decreased at $p<0.05$ with the normal. The MCHC on the hand were significantly increased in the groups that received 100mg/kg, $p<0.05$ when compared with the negative control. The platelets were significantly decreased in the groups that received 50mg/kg $p<0.01$ and 200mg/kg $p<0.05$ when compared with the negative control. The negative control were also significantly increased $p<0.05$ when compared with the normal control. The reticulocytes count revealed significant increase in all concentrations of the extract given when compared with the positive control $p<0.01$ and normal control $p<0.05$. However those that received 50mg/kg also showed significant increase $p<0.01$ when compared with the negative control. The total WBC in the group that received 100mg/kg and positive control showed significant increase $p<0.01$ when compared with the negative control while the group that received 200mg/kg and the negative control showed significant decrease$p<0.05$ with the normal control, in differential count, the neutrophils showed significant increase $p<0.05$ in the groups that received50mg/kg, 100mg/kg and negative control, and $p<0.01$ in the groups that received 200mg/kg and the positive control when compared with the normal control. Alanine transaminase revealed significant decrease $p<0.05$ in

the groups that received 50mg/kg and 200mg/kg when compare with the positive control, the groups that received 100mg/kg, the positive and negative control showed significant increase $p<0.05$ with the normal control. The aspartate transaminase in all the concentration of the extract showed significant increase $p<0.01$ and $p<0.05$ respectively with the positive and negative control while both positive and negative control showed significant decrease $p<0.05$ with the normal control.

Table 4.11 shows the effect of methanol seed extract of *Telfairia occidentalis*on serum transaminasesand some haematological parameters of anaemic rats on the 9^{th} day administration. On the 9^{th} day of administration, Hb concentration and PCV values were significantly increased $p<0.01$ in all the concentration of the extract given including the positive control when compared to the negative and normal control. The MCV were also significantly increased $p<0.05$ in all the concentration of the extract given together with the positive and negative control when compared with the normal control. In all the concentration of the extract given, the platelets were significantly decreased $p<0.05$ when compared with the normal control. The positive and the negative control were also decreased significantly at $p<0.05$. The reticulocytes were significantly increased in the rats that received 50mg/kg, 100mg/kg $p<0.01$ when compared with the positive and negative control and $p<0.05$ when compared with the normal control. In those that received 200mg/kg, they were

significantly increased $p<0.01$ with the positive control and $p<0.05$ with the normal control. The positive and negative control was still significantly increased at $p< 0.05$ when compared with the normal control. In the alanine transaminase, the rats that received 100mg/kg of extract, the positive and negative control were significantly decreased when compared with the normal control while aspartate transaminase only the positive and negative control showed significant increase $p<0.05$ with the normal control.

Table 4.12 shows the effects of aqueous seed extract on serum transaminases and some haematological parameters of anaemic rats on day 9 of administration. Here the Rbc were significantly decreased $p<0.01$ in rats that received 50mg/kg when compared with the positive and negative control, they were also significantly decreased $p<0.05$ when compared with the normal control, while those that received 100mg/kg were only significantly decreased $p<0.05$ when compared with the positive and normal control. The Hb concentration of those that received 50mg/kg and 100mg/kg were significantly decreased $p<0.01$ when compared with the positive, negative and normal control while in those that received 200mg/kg and the positive control, they were significantly decreased $p<0.05$ with the normal control. The negative control were also significantly decreased $p<0.01$ with the normal control. The PCV in all the groups including the negative and positive control were significantly decreased $p<0.05$ when compared with the normal control. The MCH on the other hand were

significantly increased $p<0.05$ in those that received the various concentrations of the extract including the positive and negative when compared with the normal control. The MCHC were significantly increased $p<0.05$ in those that received 100mg/kg, 200mg/kg and the positive control when compared with the negative control. The platelets showed significant increase $p<0.05$ in those that received 50mg/kg and 100mg/kg when compared with the negative control. The reticulocytes in all concentration were significantly increased $p<0.05$ when compared with the positive and negative control and $p<0.01$ when compared with the normal control, the positive control were significantly increased $p<0.05$ and $p<0.01$ when compared with the negative control and normal control, the negative control were also significantly increased $p<0.01$ when compared with the normal control. The total WBC of those that received 50mg/kg were significantly increased $p<0.05$ when compared with the negative and normal control, while in the 200mg/kg they were significantly increased $p<0.05$ when compared with the positive, negative and normal control while the positive control were significantly increased $p<0.05$ with the normal, the negative control were significantly decreased $p<0.05$. In the differential count only the neutrophils of those that received 200mg/kg were significantly increased $p<0.05$ when compared with the negative control.

Table 4:1: Some haematological parameters and biochemical enzymes of the rat after 3 days administration of methanol seed extract of *Telfairia occidentalis* (T.O)

VARIABLE	ANormal Control	$B_1$50mg/kg wt of Extract	$B_2$100mg/kg wt of Extract	$B_3$200mg/kg wt of Extract
ALANINE TRANSAMINASE Iμ/l	34.0 ± 0.0	41.5 ± 2.5	30.0 ± 0.0	32 .0 ± 2.0
ASPARTATE TRANSAMINASE Iμ/l	40.0 ± 23.0	60.0 ± 14.0	63.0 ± 0.0	64.0 ± 10.0
RBC $X10^6/\mu L$	7.5 ± .6	6.8 ± 0.5	7.8 ± 0.1	7.1 ± 0.2
HB g/dl	12.1± .8	10.8^a± .6	11.9^a±.1	11.5^a ± .0
PCV%	37.1 ± 3.0	38.2 ± 3.5	35.5 ± 2.0	34.8^a± .2
MCVfl	56.4 ± 3.1	56.1± .7	55.6 ± .9	55.9 ±.1
MCH pg	16.6 ± .3	15.9 ±.4	15.3 ± .3	16.2 ± .4
MCHCg/dl	28.1 ± 1.5	28.4 ± 1.0	27.5^b ±.1	29.1 ± .8
PLATELETS $x10^3/\mu L$	804.0± 119.5	857.5 ± 42.5	689.5^a± 62.5	574.0^a± 24.0
RETICS%	1.4 ± 1.0	7.3^a± 1.3	9.6^a± 3.1	8.0^a± 1.0
TWBC $X10^3/\mu L$	9.9 ± 1.3	12.3^a± 1.1	8.2 ± 0.6	10.1 ± 10
Neutrophils %	24.5± 2.5	56.5± 3.5	26.0 ± 4.0	30 .5 ± 2.5
Lymphocytes %	70 .0 ± 3.0	38.5^a± 3.5	70.5 ± 4.0	60.5± .0
Eosinophils %	1.5 ± .5	1.0± 1.0	3.0± 2.5	2.5 ± 2.5
Monocytes %	4.0 ± .0	2.0 ± .0	1.0 ± 1.0	1.5± .5
Basophils %	-	-	-	-

Key a = $p<0.05$ with normal control

b = $p<0.01$

TABLE 4.2 Some haematological parameters and biochemical enzymes of the rats after 3 days administration of aqueous seed extract of T.O.

VARIABLES	A Normal control	B_1 50mg/kg wt of Extract	B_2 100mg/kg wt of Extract	B_3 200mg/kg wt of Extract
ALANINE TRANSAMINASE Iμ/l	75.5 ± 36.5	46.0 ± 2.0	89.5 ± 35.5	39.0[a] ± 0.0
ASPARTATE TRANSAMINASE Iμ/l	117.5 ± 1.5	131.5 ± 8.5	117.5 ± 2.5	90.0 ± 27.00
RBC $X10^6/\mu L$	9.6 ± .4	7.5 ± .1	8.2 ±0.2	6.6 ± 0.6
HB g/dl	11.9 ± .5	11.9 ± .5	12.1 ± .0	11.3 ± .6
PCV%	36.2 ± 2.4	35.0 ± .4	36.6 ± .5	31.9[a] ± 1.8
MCVfl	64.0 ± 2.3	48.3 ± 8.5	56.9 ± 1.8	61.2 ± 3.1
MCH pg	21.9 ± 5.1	15.7 ± .5	14.8[a] ± .3	16.4 ± .1
MCHCg/dl	26.7 ± 1.2	27.1[b] ± .0	25.9 ± 2.5	26.9 ± 1.5
PLATELETS $x10^3/\mu L$	896.0 ± 122	970.0 ± 80	865.0 ± 90	912.5 ± 75
RETICS%	3.5 ± .5	6.3[a] ± .3	6.3[a] ± 1.8	4.8[b] ± .3
TWBC $X10^3/\mu L$	7.40 ± 2.2	11.6 ± .4	6.2± 1.0	8.4 ± 0.0
Neutrophils %	34.0 ± 5.0	35.5 ± 11.5	25.5 ± 2.5	28.0 ± 2.0
Lymphocytes %	64.0 ± 7.0	63.5 ± 10.5	74.0 ± 2.0	71.0± 2.0
Eosinophils %	1.0 ± 0.0	-	-	-
Monocytes %	3.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
Basophils %	-	-	-	-

Key a = p< 0.05

With normal control

b = p< 0.01

TABLE 4.3: Some haematological parameters and biochemical enzymes of the rat after 6 days administration of methanol seed extract of *Telfairia occidentalis* (T.O)

VARIABLE	A Normal control	B_1 50mg/kg wt Extract	B_2 100mg/kg wt Extract	B_3 200mg/kg wt Extract
ALANINE TRANSAMINASE Iμ/l	50.0 ± 11.0	48.0 ± 0.0	54.5 ± 65.5	44.0 ± 10.0
ASPARTATE TRANSAMINASE Iμ/l	103.0 ± 17.0	82.0 ±36.0	130.5 ± 5.5	96.5 ± 22.5
RBC $X10^6/\mu L$	6.9 ± .11	7.9 ± .8	6.5 ± .35	5.8[a] ± .13
HB g/dl	12.5 ± 1.4	10.8 [a] ± 30	10.4[a] ± .0	10.3[a] ± 1.6
PCV%	38.3 ± 2.6	32.9[a] ± 2.2	32.6[a] ± 1.5	30.2[a] ± 1 .6
MCVfl	55.9 ± .5	64.8 ± 8.4	57.1 ± 1 .8	86.5[b] ± 2.6
MCH pg	15.19 ± .2	17.9 ± 2.1	15.6 ± .4	21.2[a] ± .8
MCHCg/dl	28.4 ± .4	28.3 ± .3	26.0[a] ± .8	24.6[b] ± .3
PLATELETS $x10^3/\mu L$	1035.0 ± 65.0	910.0 ± 90.0	695.0[a] ± 81.0	730.5[a] ± 87.5
RETICS%	1.5 ± 0.0	4.0[a] ± .0	5.5[a] ± .5	6.0[a] ± .0
TWBC $X10^3/\mu L$	10.2 ± 2.3	7.7 ± .7	12. 4 ± 1.1	12.2 ± 1.5
Neutrophils %	23.5 ± 1.5	23.5 ± 1.5	30.5 [a] ± .5	32.5 ± 9.5
Lymphocytes %	70.5 ± .5	72.5 ± .5	60.5[b] ± 2.5	16.5 ± 12.5
Eosinophils %	3.0 ± .0	2.0 ± 1 .0	4.0 ±2.0	2.0 ± 1.0
Monocytes %	1.5 ± .5	2.0 ± 1.0	5.0 ± 1.0	4.0 ± 2.0
Basophils %	-	-	-	-

Key a = P< 0.05, b = P< 0.01 when compared with normal control

TABLE 4.4:Some haematological parameters and biochemical enzymes of the rats after 6 days administration of aqueous seed extract of T.O

VARIABLES	A Normal control	B_1 50mg/kg wt Extract	B_2 100mg/kg wt Extract	B_3 200mg/kg wt Extract
ALANINE TRANSAMINASE Iμ/l	39.0 ± 5.0	37.0 ± 7.0	29.0 ± 10.0	34.5 ± 4.5
ASPARTATE TRANSAMINASE Iμ/l	112.0 ± 0.0	112.0 ± 0.0	112.0 ± 0.0	112.0 ± 0.0
RBC $X10^6/\mu L$	7.5 ± .4	5.2[a] ± 0.2	8.1 ± 0.5	7.4 ± 0.0
HB g/dl	14.7 ± .7	11.4[a] ± .2	14.6 ± .6	13.7 ± .5
PCV%	44.2 ± 1.1	34.3[a] ± .3	45.5 ± 1.5	40.5 ± 4 .5
MCVfl	67.4 ± .6	71.7 ± 8.4	63.3[a] ± 1 .7	58.9[a] ± .2
MCH pg	20.1 ± .7	20.2 ± .7	18.1 ± .4	17.8 ± .2
MCHCg/dl	29.7 ± .6	28.4 ± 2.5	31.1 ± 1.1	30.2 ± .8
PLATELETS $x10^3/\mu L$	566.0 ± 23.0	865 ± 86.5	786.0 ± 59.0	908[a].0 ± 135.0
RETICS%	3.5 ± .2	8.4[a] ± 1.9	2.9 ± .2	4.5[a] ± .1.2
TWBC $X10^3/\mu L$	11.1 ± .4	12.3 ± .6	12.6 ± .1	15.7[a] ± .1
Neutrophils %	19.0 ± 1.0	49.0 ± .o	48.5 ± 11 .5	29.0 ± 5.0
Lymphocytes %	79.0 ± .0	50.0 ± 5.0	51.5 ± .11.5	71.0 ± 5.0
Eosinophils %	1.0 ± 0.0	2.0 .0	-	-
Monocytes %	1.5 ± 0.5	-	-	-
Basophils %	-	-	-	-

Key a = P< 0.05when compared with normal control

TABLE 4.5: Some haematological parameters and biochemical enzymes of the rat after 9 days administration of methanol seed extract

VARIABLES	A Normal control	B1 50mg/kg wt Seed Extract	B2 100mg/kg wt Seed Extract	B3 200mg/kg wt Seed Extract
ALANINE TRANSAMINASE Iμ/l	33.5 ± 14.5	34.0 ± 0.0	30.0 ± 0.0	46.5 ± 7.5
ASPARTATE TRANSAMINASE Iμ/l	74.5 ± 11.5	89.0 ± 15.0	134.0 ± 60.0	115.0 ± 0.0
RBC $X10^6$/μL	6.4 ± 0.2	7.7 ± 1.8	5.9 ±0.4	6.7 ± 0.0
HB g/dl	15.0 ± .5	10.4^a± .7	9.9^a± .6	10.3^a± .6
PCV%	44.4 ± 1.3	30.4^a ± .0	38.7^a ± 8.4	34.2^a ± 3.4
MCVfl	58.4 ± .9	63.2^a ± .2	58.9 ± 1.2	55.4^a ± 1.3
MCH pg	16.6 ± .3	16.5 ± 0.0	16.70 ± 0.2	15.5 ± .8
MCHCg/dl	28.3 ± 1.1	26.0^b ± .0	28.5 ± 1.1	26.3^b ± .4
PLATELETS $x10^3$/μL	804.0 ± 15.0	776.5 ± 87.5	1009.0^a ± 134.0	887.5^a ± 13.5
RETICS%	1.8 ± .3	10.0^a ± 0.0	12.5^a ± .3	12.5^a ± 3.0
TWBC $X10^3$/μL	10.05 ± 0.2	11.9 ± .4	15.3^b± 1.6	14.2^b ± 2.3
Neutrophils %	18.5 ± .5	17.5 ± 4.5	26.00 ± 4.0	23.5 ± 3.5
Lymphocytes %	18.5 ± .5	81.0 ± 0.3	73.5 ± 4.5	75.0± 2.0
Eosinophils %	-	-	-	-
Monocytes %	-	2.0 ± 0.0	1.0 ± 0.0	-

Key a = P< 0.05

Withnormal control

b = P<0.01

TABLE 4.6: Some haematological parameters and biochemical enzymes of the after 9 days administration of aqueous seed extract of T.O.

VARIABLES	A Normal control	B_1 50mg/kg wt Extract	B_2 100mg/kg wt Extract	B_3 200mg/kg wt Extract
ALANINE TRANSAMINASE Iμ/l	85.5 ± 24.5	51.0 ± 30.0	92.0 ± 23.0	51.5 ± 3 6.0
ASPARTATE TRANSAMINASE Iμ/l	75.0 ± 29.0	89.0 ± 15	68.5 ± 5.1	56.0 ± 18.0
RBC $X10^6/\mu L$	6.9 ± .0	7.5 ± .4	6.9 ± .1	5.3^a± .3
HB g/dl	11.6 ± .2	12.6 ± .3	11.3 ± .2	11.9 ± .1
PCV%	34.7 ± .3	38.4 ± .6	34.7 ±.2	37.7 ± .7
MCVfl	63.5 ± 3.6	62.8 ± 2.3	63.3 ± .7	71.1a ± 4.8
MCH pg	16.3 ± .9	16.9 ± .7	16.4 ± .5	18.6 ± .9
MCHCg/dl	26.6 ± 1.2	26.8 ± .1	25.9 ± 1.0	25.5^c ± .6
PLATELETS $x10^3/\mu L$	1044.5 ± 93.5	995 ± 147	760^a ± 78	751a ± 221
RETICS%	3.8 ± .3	6.0^a ± .0	5.5^a ± .5	5.8^a ± .3
TWBC $X10^3/\mu L$	12.0 ± .5	10.9 ± 1.11	11.4± 1.3	12.3 ± .1
Neutrophils %	29.0 ± 5.0	18.0 ± .0	30.5 ± .5	26.0 ± 3.0
Lymphocytes %	67.0 ± 5.0	79.5 ± 1.5	67.0 ± .0	73.0 ± 4.0
Eosinophils %	2.0 ± 0.0	3.0 .0	1.5 .5	-
Monocytes %	2.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	-
Basophils %	-	-	-	-

Key a = P< 0.05, b + P< 0.01, c = p<0.001 with normal control

TABLE 4.7: Some haematological parameters and biochemical enzymes the anaemic rats after 3 days administration of methanol seed extract of T.O

.PHENYLYDRAZINE ←——— ———→

VARIABLES	50mg/kg wt Extract	100mg/kg wt Extract	200mg/kg wt Extract	100mg/kg Astymin	With only feed & water	Normal control
ALANINE TRANSAMINASE Iμ/l	36.5^{b} ± 2.5	19.5^{c} ± 3.5	16.0^{c} ± .0	16.0^{c} ± 0.0	19.50^{c} ± 3.5	34.0 ± 0.0
ASPARTATE TRANSAMINASE Iμ/l	57.0 ± 11.0	50.5 ± 12.5	39.0 ± 7.0	47.5 ± 15.5	58.50^{c} ± 4.5	40.0 ± 23.0
RBC $X10^{6}$/μL	3.9^{d} ± .3	3.5^{d} ±.2	3.3^{d} ± .7	33^{d} ± .1	3.3^{d} ±.1	7.5 ± 0.5
HB g/dl	9.8^{d} ± .3	9.5^{d} ± 1.0	9.4^{d} ± 1.0	7.3^{c} ± 1.3	8.2^{c} ± .4	12.4 ± .8
PCV%	$33.4^{a,bd}$ ± .9	28.3^{d} ± 1.9	29.5^{d} ± 3.6	24.5^{c} ± 3.9	24.9^{c} ± 2.0	37.1 ± 2.9
MCVfl	109.5^{e} ± 4.5	126.7^{e} ± 1.8	119.5^{e} ± 5.6	117.1^{e} ± 4.2	117.8^{e} ± 2.5	56.4 ± 3.1
MCH pg	24.7^{d} ± .8	27.3^{d} ± 1.2	25.0^{d} ± .7	24.7^{d} ± .6	25.0^{d} ± .2	16.6 ± .3
MCHCg/dl	22.6^{c} ± .2	$21.^{c}$ ± 1.3	21.1^{c} ± 1.6	21.3^{c} ± 1.2	21.3^{c}±.65	28.1 ± 1.5
PLATELETS $x10^{3}$/μL	386.0^{d} ± 91.0	513.0 ± 76	507.5 ± 5.5	689.5 ± 43.5	$445.^{e}$ ± 67.5	840.0 ± 119.5
RETICS%	28.0^{abc} ± 2.0	$48.5^{a,bc}$ ± 3.5	45.0^{abc} ± 3.5	19.5^{e} ± 5.5	21.0^{c} ± 5.5	1.40 ± 1.0
TWBC $X10^{3}$/μL	12.2^{b} ± 1.4	8.2 ± 0.3	12.8^{b} ± 0.3	12.5 ± 1.0	8.0 ± 0.05	9.9 ± 1.3
Neutrophils %	28.0 ± 5.0	41.5 ± 7.6	33.0 ± 1.0	34.0 ± 2.0	32.0 ± 1.0	24.5 ± 2.5
Lymphocytes %	70.0 ± 5.0	55.5 ± 9.5	65.5 ± .5	63.5 ± 1.5	63.0 ± 11.0	70.0 ± 3.0
Eosinophils %	1.0 ± 1.0	2.5 ± 1.5	1.0 ± 1.0	1.5± 5	2.0 ± 1.0	1.5 ± .5
Monocytes %	1.0 ± 1.0	.5 ± .5	1.0 ± 1.0	5.0 ± .15	.50 ± .5	4.0± .0
Basophils %	-	-	-	-	-	-

Key a = P< 0.05 with positive control
b = P<0.05 with negative control
c = P<0.05 d = P<0.01, e = P<0.001 with Normal control

TABLE 4.8: Some haematological parameters and biochemical enzyme the after 3 days administration of aqueous seed extract of T.O

← PHENYHLDRAZINE →

VARIABLS	With50 mg/kg wtExtract	With 100mg/kg WtExtract	With 200mg/kg wt Extract	with 100mg/kg wt Astymin	With normal feed and water	Normal control
Alanine Transaminase Iμ/l	29.5 ± 3.5	30.0 ± 14.0	37.0 ± 7.0	37.0 ± 7.0	23.0[e] ± .0	75.5 ± 36.5
Aspartate Transaminase Iμ/l	84.5 ± 38.5	112.0[a] ± 8.0	111.0[a] ± 8.0	68. 5[e] ± 5.5	116.0 ± 4.0	117.5 ± 1.5
RBC $X10^6/\mu L$	4.1,[f] ± 1.1	5.4[a,c] ± 0.9	5.7[a,c] ± .1	3.8 ± 4 .0	3.3[e] ±0.1	6.9 ± .4
HB g/dl	9.6,[f] ± 1 .8	10.1[e] ± .0	10.6[a,c] ± .0	9.0 ± 0.0	8.1[e]± .3	11.9 ± .5
PCV%	31.9[cde] ± 3.0	30.4[ce] ± .1	30.8[ce] ± .1	29.8[ce] ± 1.3	25.9[e] ± 2.0	36.2 ± 2.4
MCVfl	81..5[bcf] ± 17.5	80.9[b,c,f] ± 20.3	82.9[b,c,f] ± .1	112.5[e] ± .5	118.0[e] ± 2.0	64.0 ± 2.3
MCH pg	23.9 ± 1.9	20.7 ± 4.8	18.8[c] ± .5	22.2 ± 3.2	24.6 ± .6	21.9 ± 5.1
MCHCg/dl	21.7[f] ± 1.6	24.3 ± 1.9	23.1 ± 1 .0	21.0[f] ± 2.0	21.0[f]±.5	26.7 ± 1.2
PLATELETS $x10^3/\mu L$	618.0[f] ± 1.0	541.5[e] ± 43.5	706.5 ± 95.0	700.0 ± 50.0	451.5[e] ± 11.5	896.0 ± 122.0
RETICS%	17.5[e] ± 2.5	19.0[e] ± 1.0	26.5[f] ± 1.5	21.0 [e] ± 1.0	21.0[e] ± 1.0	9.0 ± 0.0
TWBC $X10^3/\mu L$	12.9[e] ± .2	10.7 ± 1 .9	6.3 [a] ± .1	12.75[e] ± .8	7.9 ± .5	7.4 ± 2.2
Neutrophils %	33.0 ± 10	25.5 ± 2.5	27.0 ± 2.0	32.0 ± 1.0	27.5 ± 2.5	34.0 ± 5.0
Lymphocytes %	66.5 ± 9.5	73.5 ± 1.5	71.5 ± 2.5	65.0 ± 3.0	68.5 ± 1.5	64.0 ± 1.0
Eosinophils %	1.0 0	1.0 ± 0	1.0 ± 0	3.0± 0	2.5 ± .5	1.0 ± 0
Monocytes %	-	1.0 ± 0	2.0 ± 0	1.5 ± 5	1.5 ± .5	3.0 ± 0
Basophils %	-	-	-	-	-	-

Key a = P<0.05, b= P<0.01 with positive control,c = P<0.05 d.=P<0.01 with negative control, e = P<0.05 f = P<0.01 with normal control

TABLE 4.9: Some haematological parameters and biochemical enzymes of the anaemic rats after 3 days administration of methanol seed extract of T.O.

	← PHENYLYDRAZINE →					
VARIABLES	With50mg/kg wtExtract	With 100mg/kg wtExtract	With 200mg/kg Extract	with100mg /kg wt Astymin	With Normal feed and water	Normal control
Alanine Transaminase Iμ/l	36.5 ± 2.5	49.0 ± 5.0	34.5 ± 4.5	39.5 ± 4.5	46.50 ± 7.5	50.0 ± 11.0
Aspartate Transaminase Iμ/l	89.0[e] ± 15.0	119.5[a,c] ± 4.5	66.0[e] ± 20.0	58.5[f] ± 4.5	75.0[e] ± 1.0	103.0 ± 17.0
RBC $X10^6/\mu L$	5.5 ± 2.4	4.9 ± .3	4.8 ± 0.1	5.2 ± .1	6.4 ±.3	6.9 ± .11
HB g/dl	11.7[c] ± .15	11.8[c] ± .4	11.0[c] ± .2	11.6[c] ± .3	10.3[e]± ..25	12..5 ± 1 .4
PCV%	35.5[c] ± 0.0	36.7[c] ± 1.0	33.5[f] ± 1.6	35.0[c] ± .9	30.3[e] ± 2.6	38.3 ± 2.6
MCVfl	85..7[d,f] ± 1.8	100.4[c,e] ± 6.3	89.9[d,e] ± 3.2	87.1[ed] ± 2.1	53. ± 1 .3	55.9 ± .5
MCH pg	21.2[d,f] ± .4	25.7[d,e] ± .5	23.2[d,e] ± .6	22.1[de] ± .3	16.1 ± .2	15.9 ± .2
MCHCg/dl	23.6 ± 1.3	23.9 ± .7	24.5 ± 1.3	25.8 ± .4	27.5 ± 1.4	28.4 ± .4
PLATELETS $x10^3/\mu L$	606.0[c,e] ± 37.0	592.0[c,e] ± 15.0	593.0[ce] ± 15.0	722.5[f] ± 68.5	936.0 ± 15.0	1035.0 ± 65.0
RETICS%	15.0[e] ± 3.0	28.5[c,e] ± 3.5	20.5[e] ± 3.5	12.5[e] ± 2.5	11.0[f] ± 1.0	1.5 ± .0
TWBC $X10^3/\mu L$	10.9 ± 0.3	7.4[a,c,e] ± .2	11.6 +0.6	11.8 ± 1.0	11.9 ± 0.5	10. 2 ± 2.3
Neutrophils %	14.0 [a] ±1.0	34.0 ± 1.0	25.5[a] ± 1.5	42.0 ± 2.0	17.5 ± 4.5	23.5 ± 1.5
Lymphocytes %	83.5[a] ± .5	64.5 ± .5	73.0[a] ± 2.0	51.5[fd] ± 3.5	81.0 ± 4.0	70.5 ± 0.5
Eosinophils %	1.0 ± 0.0	3.0 ± .0	1.0 ± .0	4.0 ± 1.0	1.5 ± .5	3.0 ± 0.
Monocytes %	2.0 ± .0	-	3.0 ± .0	2.5 ± .5	-	1.5 ± .5

Key a =P<0.05, b= P<0.01 with positive control, c = P<0.05 d. P<0.01 with negative control, e = P<0.05 f = P<0.01 with normal control

TABLE 4.10: Some haematological parameters and biochemical enzyme the after 6 days administration of aqueous seed extract of T.O PHENYHLDRAZINE

VARIABLES	With 50mg/kg wtExtract	With100mg/ kg wt Extract	With200mg/ kg wt Extract	With100mg/kg wt Astymin	With normal feed and water	Normal control
Alanine Transaminase Iμ/l	39.0 [a] ± 5.0	75.5[e] ± 36.5	39.0 [a] ± 0.0	65.5[e] ± 4.0	61.5[e] ± 7.5	39.0 ± 5.0
Aspartate Transaminase Iμ/l	112.0[b] ± 0	112.0[b] ± .0	112.0[b] ± 0	32.0[e] ± 6.0	60.0[e] ± 14.0	112.0 ± 0
RBC $X10^6$/μL	5.3 ± 0.3	5.3 ± .1	5.6 ± .2	6.1 ± .2	4.6[e] ±.1	7.5 ± .4
HB g/dl	12.4[c] ± .2	12.5[c] ± .4	13.0[c] ± .2	12.9[c] ± .1	10.3[e]± .3	14.7 ± .7
PCV%	41.1[c] ± 3.9	37.9[f] ± .1	32.5[b] ± .5	37.9[cf] ± 2.1	30.7[e] ± .7	44.2 ± 1.1
MCVfl	90.9 ± 9.8	86.9 ± 3.1	92.8 ± 3.2	88.4 ± 3.9	80.9 ± .1	67.4 ± .6
MCH pg	23.4 ± 3.0	23.7 ± .8	22.9 ± .4	25.3 ± .7	20.8 ± .2	20.1 ± .7
MCHCg/dl	25.7 ± .6	27.2[c] ± 0.0	24.7 ± 1.3	26.3 ± .1	22.6 ± 2.0	29.7 ± .6
PLATELETS $x10^3$/μL	567.0[d] ± 2.0	663.0 ± 156	403.0[c] ± 24.0	617.5 ± 65.5	708.0 [e] ± 13.0	566.0 ± 93.0
RETICS%	9.9[a,de] ± .8	15.8[bce] ± .5	14.1[bce] ± .2	5.3 ± .4	16.4[e] ± .2	3.5 ± .2
TWBC $X10^3$/μL	10.2 ± 3.7	12.9[d] ± .5	6.2[e] ±.3	14.2[d] ± .3	6.5[e] ± .1	11. 0 ± .4
Neutrophils %	43.5[e] ± 25.5	50.5[e] ± 5.5	36.0[f] ± 3.0	28.0[f] ± 2.0	40.0[e] ± 3.0	19.0 ± 1.0
Lymphocytes %	55.0 ± 25.0	49.0 ± 6.0	64.0 ± .3	70.5 ± 2.5	58.0 ± 3.0	79.0 ± .0
Eosinophils %	-	-	-	-	1.0 ± 0 .0	1.0 ± 0.
Monocytes %	-	-	-	-	-	-

Key a =P<0.05 b= P<0.01 with positive control, c = P<0.05 d. P<0.01 with negative control, e = P<0.05 f = P<0.01 with normal control

TABLE 4.21: Some haematological parameters and biochemical enzymes the anaemic rats after 9 days administration of methanol seed extract of T.O.

	← PHENYLHYDRAZINE →					
VARIABLES	With 50mg/kg Extract	With 100mg/kg Extract	With 200mg/kg Extract	with 100mg/kg Astymin	With Normal feed and water	Normal control
Alanine Transaminase Iμ/l	46.5 ± 16.5	12.0[e] ± 0.0	43.0 ± 20.0	16.0[e] ± 0.0	28.5[e] ± 6.0	85.5 ± 24.5
Aspartate Transaminase Iμ/l	75.0 ± 37.0	90.50 ± 27.5	96.5 ± 33.5	123.0[e] ± 3.0	126.5[e] ± 3.0	75.0 ± 29.0
RBC $X10^6/\mu L$	6.1 ± 1.0	5.9 ± 0.5	5.7 ± 0.4	5.8 ± 0 .3	5.7 ±0.2	6.8 ± .03
HB g/dl	12.8[df] ± 1 .0	13.1[df] ± 0.1	12.2[df] ± .5	12.3[df] ± .4	11.9 ± 0.1	11.6 ± .2
PCV%	37.5[df] ± 1.2	40.4[df] ± 2 .0	37.9[df] ± 1.0	37.1[df] ± 1.6	35.1 ± .2	34.7 ± .3
MCVfl	84..7[e] ± 2.0	85.1[e] ± 1.2	83.9[e] ± 4.3	85.7[e] ± 1.0	83.5[e] ± 1.4	63.5 ± 3.6
MCH pg	21.9 ± .4	21.90 ± .40	21.4 ± .6	21.8 ± .3	21.7 ± .1	16.3 ± .9
MCHCg/dl	25.9 ± .2	25.8 ± .8	25.5 ± .6	25.5 ± .1	25.9 ±.4	26.6 ± 1.2
PLATELETS $x10^3/\mu L$	503.0[e] ± 4.4	635.5[e] ± 41.5	704.0[e] ± 50.0	703.0[e] ± 86.0	536.0[e] ± 178	1044.5 ± 93.5
RETICS%	12.5[a,d,e] ± .5	11.0[ade] ± 1.0	10.0[be] ± 1.0	8.8[e] ± .3	9.0[e] ± .0	3.8 ± .3
TWBC $X10^3/\mu L$	11.3 ± 0.5	9.3 ± 1 .0	9.6 ± 0.6	12.1 ± 0.2	10.2 ± 2.4	12. 03 ± 0.5
Neutrophils %	26.5 ± .5	23.5 ± 9.5	22.5 ± 3.5	26.0 ± 1.0	29.0 ± 1.0	29.0 ± 5.0
Lymphocytes %	68.5 ± .5	74.5 ± 9.5	75.5 ± 5.0	72.0 ± 3.0	69.5 ± .5	67.0 ± 5.0
Eosinophils %	2.5 ± .5	-	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0 .0	-
Monocytes %	-2.0 ± 0	2.0 ± 0	3.0 ± 0	2.0 ± 0	1.0 ± 0. 0	-

Key a = P<0.05 b= P<0.01 with positive control, c = P< 0.05, d = P <0.01 with negative control, e = P < 0.05, f = P < 0.01 with normal control

TABLE 4.23: Some haematological parameters and biochemical enzyme the after 9 days administration of aqueous seed extract of T.O.

	← PHENYLHYDRAZINE →					
VARIABLES	With50mg/kg wt Extract	With100mg/kg wt Extract	With 200mg/kg wt Extract	With100mg/kg wt Asytmin	With Normal feed and water	Normal control
Alanine Transaminase Iμ/l	32.0 ± 2.0	30.0 ± 4.0	34.5 ± 4.5	36.0 ± 0.0	23.0^{e} ± 4.0	33.5 ± 14.5
Aspartate Transaminase Iμ/l	68.5^{a} ± 5.5	50.0^{b} ± 4.0	68.5^{a} ± 5.5	123.0^{f} ± 3.0	64.0±10.0	74.5 ± 11.5
RBC $X10^{6}/\mu L$	$3.7^{bd,e}$ ± .2	$4.8^{a,e}$	5.6 ±.2	5.8 ± .0	5.5 ±.0	6.4± .15
HB g/dl	$8.3^{b,d,f}$ ± .2	$8.8^{b,c,f}$ ±2	11.3^{e} ±.5	12.3^{e} ±.4	11.7^{f} ± .0	15.0 ±.5
PCV%	32.9^{e} ± 4.8	31.4^{e} ± 2.2	33.4^{e} ± 1.3	37.1^{e} ± 1.6	35.4^{e} ± .2	44.4 ± 1.3
MCVfl	86.4^{e} ± 1.0	78.1^{e} ± 3.3	73.7 ± 1.5	85.7^{e} ± 1.0	86.8^{e} ± .8	58.4±.9
MCH pg	21.3^{e} ± .2	19.7 ± .5	19.6 ± .7	21.8^{e} ± .3	21.9^{e} ± .3	16.6 ±.3
MCHCg/dl	24.9 ± .1	25.9^{c} ± .4	25.8^{c} ± .4	25.5 ± .1	21.5 ±.5	28.3 ±1.10
PLATELETS $x10^{3}/\mu L$	932.0^{c} ± 36.0	796.0^{c} ± 7.0	626.0 ± 68.0	703.0 ± 86.0	636.0 ± 18.0	804 ±15.0
RETICS%	$12.5^{a,c,f}$ ± .50	$14.5^{a,c,f}$ ± 1.5	$13.0^{ac,f}$ ± 1.0	$8.8^{c,,f}$ ± .3	6.4^{f} ± 1.1	1.8 ± .3
TWBC $X10^{3}/\mu L$	$12.3^{c,e}$ ± .5	$14.1^{a,c,e}$ ± .6	10.7 ± .7	12.1^{e} ± 0.2	7.9^{e} ± .3	10.1 ± .2
Neutrophils %	38.0 ± 13	24.0 ± 3.0	35.0^{c} ± 3.0	26.0 ± 1.0	16.5 ± 1.5	18.5 ± .5
Lymphocytes %	60.5 ± 12.5	74.5 ± 1.5	6.20 ± 3.0	72.0± 3.0	78.5 ± 3.5	81.5 ±.5
Eosinophils %	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 1.0	2.0± 0.0	2.0 ± 0.0	-
Monocytes %	1.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	3.0 ± 2.0	-
Basophils %	-	-	-	-	-	-

Key a =P<0.05 b= P<0.01 with positive control
c =P<0.05 d. P<0.0 with negative control
e = P<0.05 f = P<0.01 with normal control

HISTOLOGY RESULTS

Our results showed that the liver and kidney sections from the control animals and experiment groups at the termination of the research have no structural and cellular changes. Plates I- IX show the representative photomicrograph of the sections of some groups.

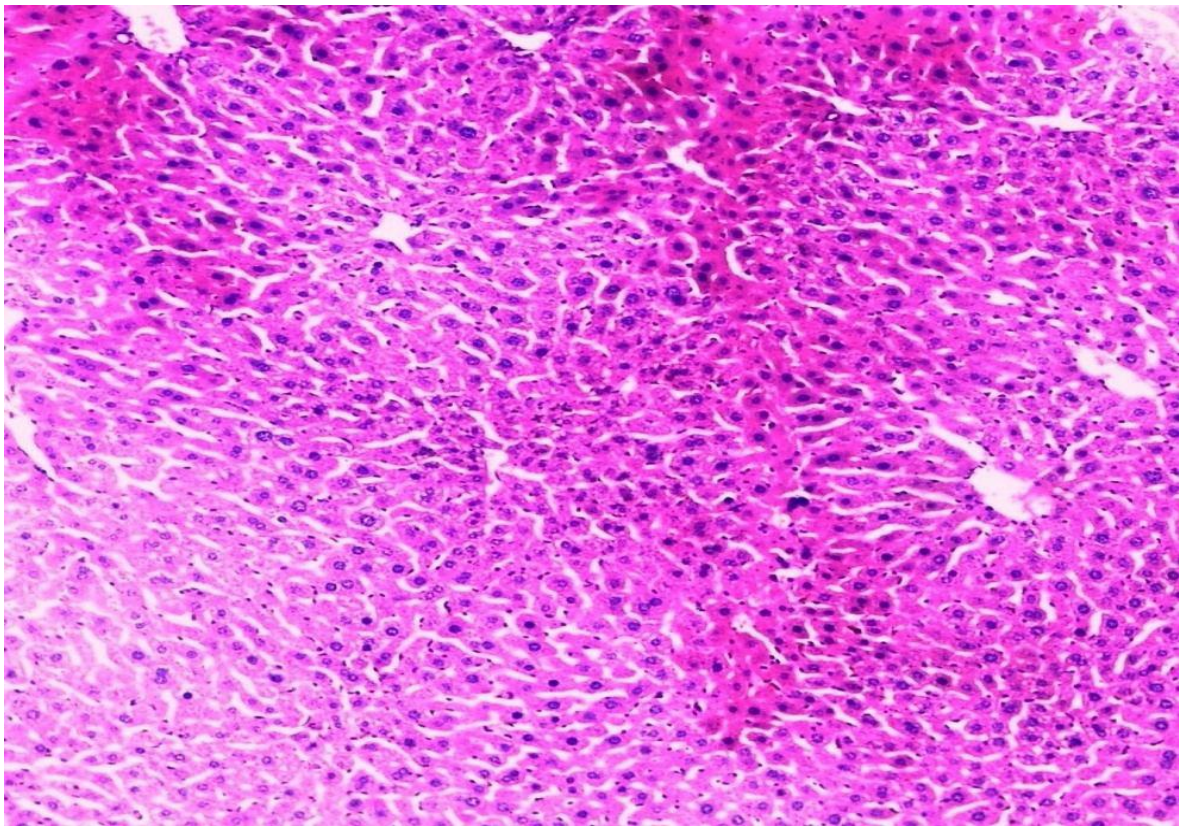

Plate 1: Represents the photomicrograph of liver section of wistar rat (high dose) stained by H&E technique, displaying normal histological features. Hepatocytes are arranged into cords separated by vascular sinusoids. In this image, the cords are the solid substance (mostly bright pink hepatocyte cytoplasm) separated by the clear space of sinusoids. X 400]

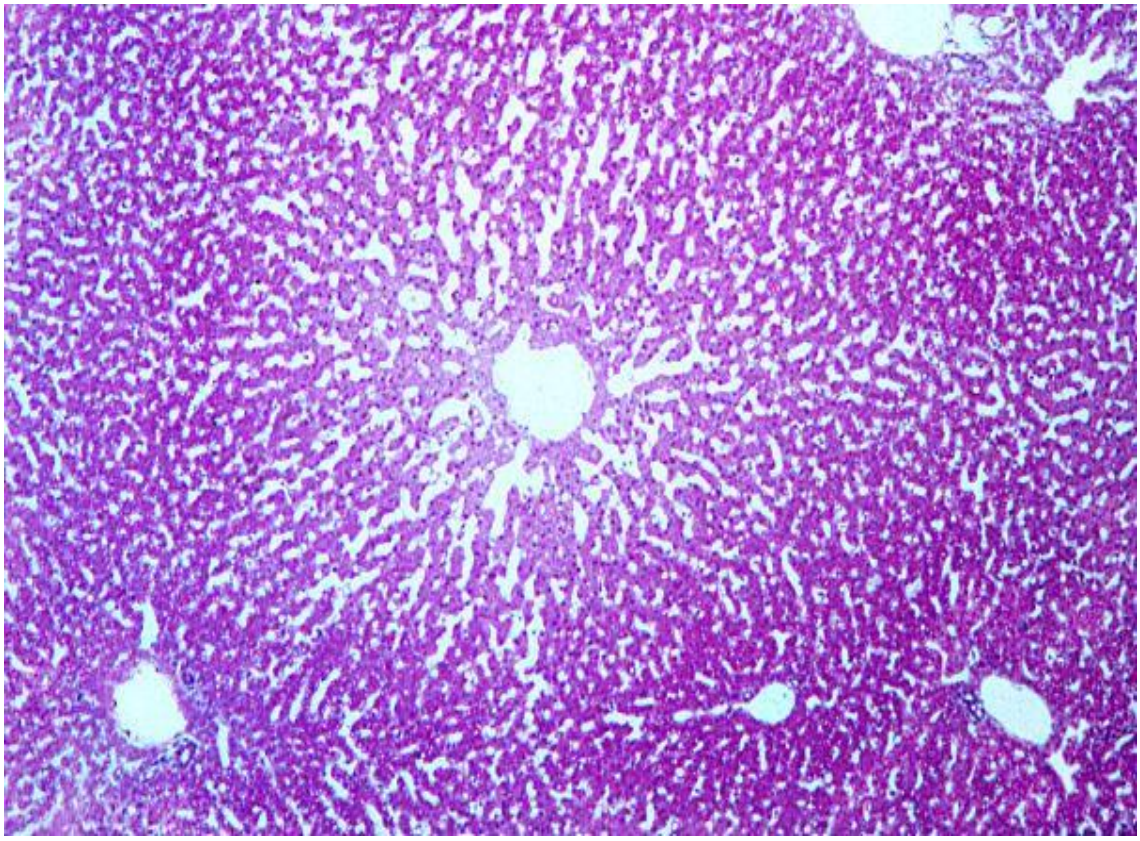

PLATE II: Hepatocytes are arranged into cords separated by vascular sinusoids. In this image, the cords are the solid substance (mostly bright pink hepatocyte cytoplasm) separated by the clear space of sinusoids. Note the central veins which are connected to the sinusoids. (Control).H & E X 50.

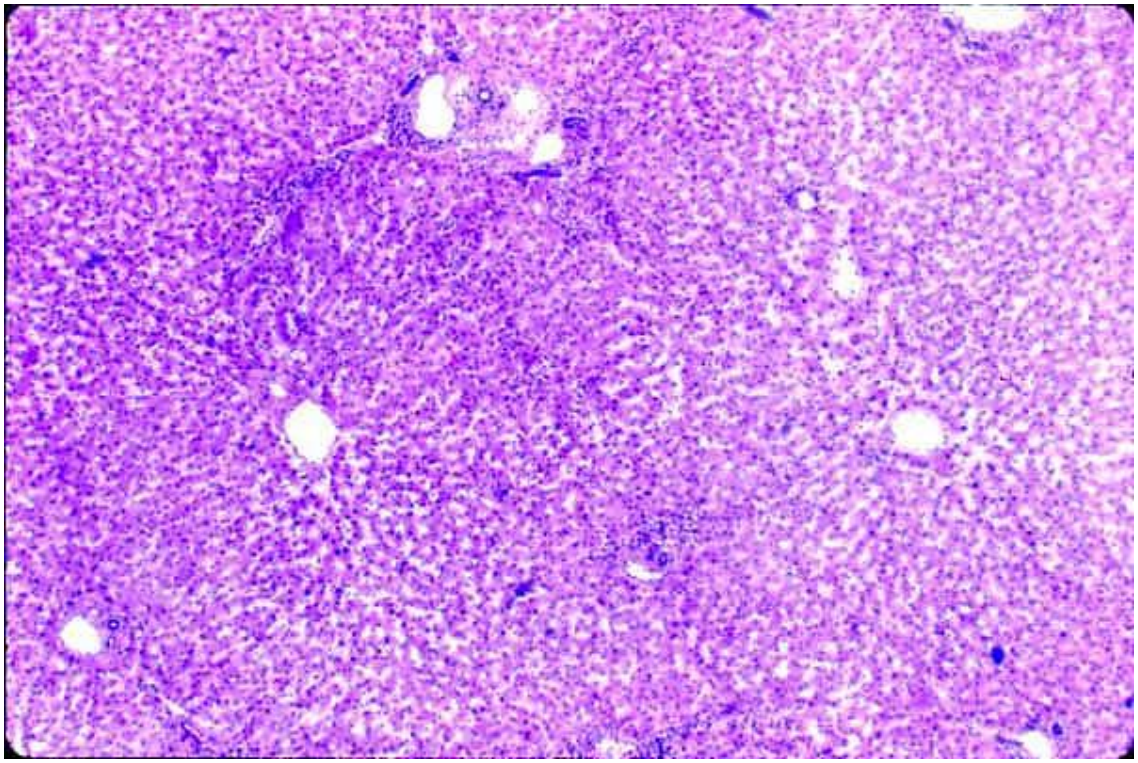

PLATE III: Hepatocytes are arranged into cords, separated by vascular sinusoids. In this image, the cords are the solid substance separated by the clear space of sinusoids (LOW DOSE GROUP) H &E X50.

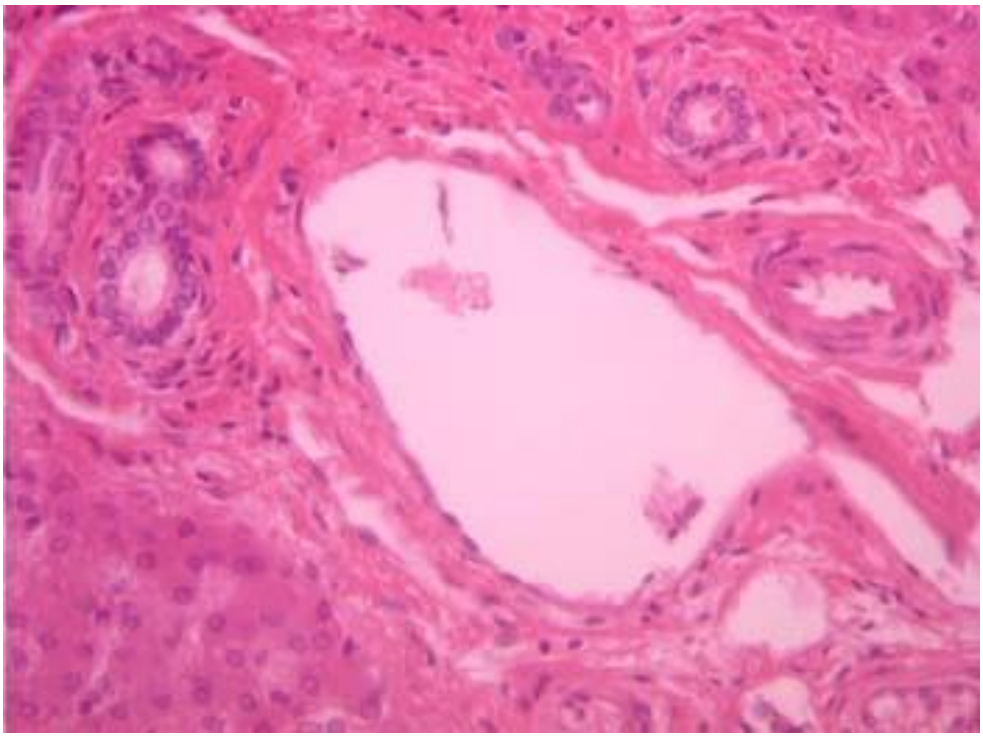

PLATE IV: The portal area with branches of bile duct with simple cuboidal lining; branch of the hepatic artery and hepatic portal vein(HIGH DOSE GROUP).H &E X400.

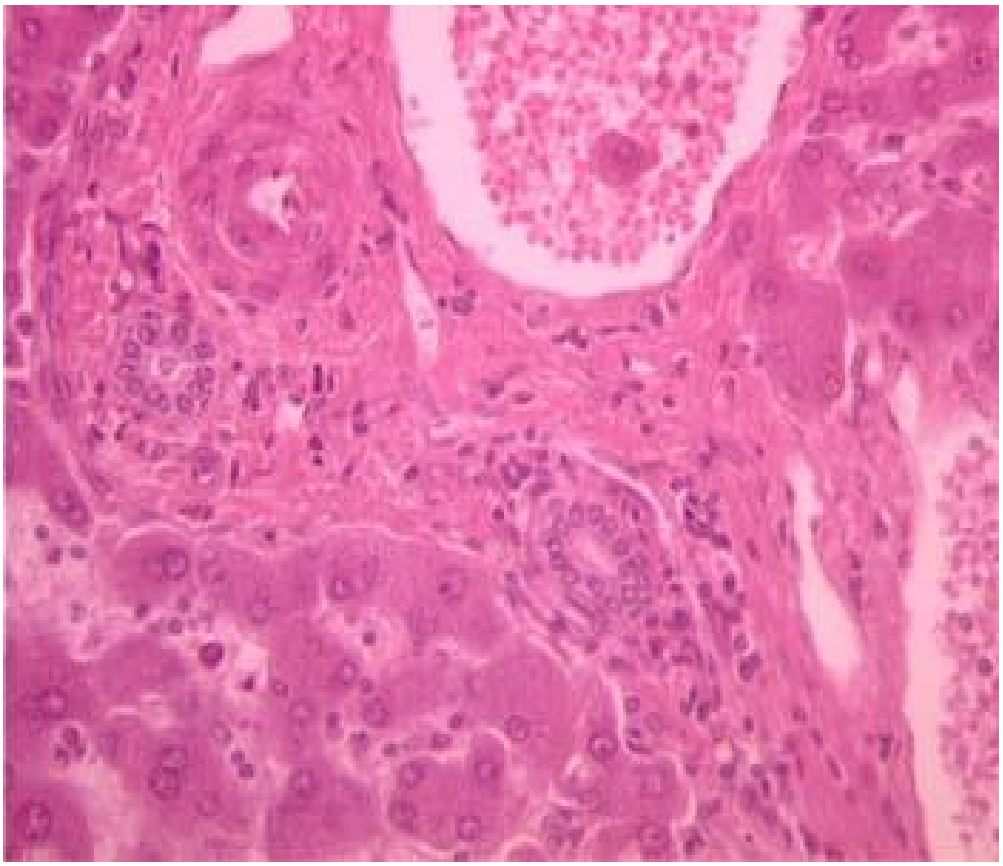

PLATE V: The portal area with branches of bile duct with simple cuboidal lining; branch of the hepatic artery and hepatic portal vein(CONTROL GROUP) H&E X400.

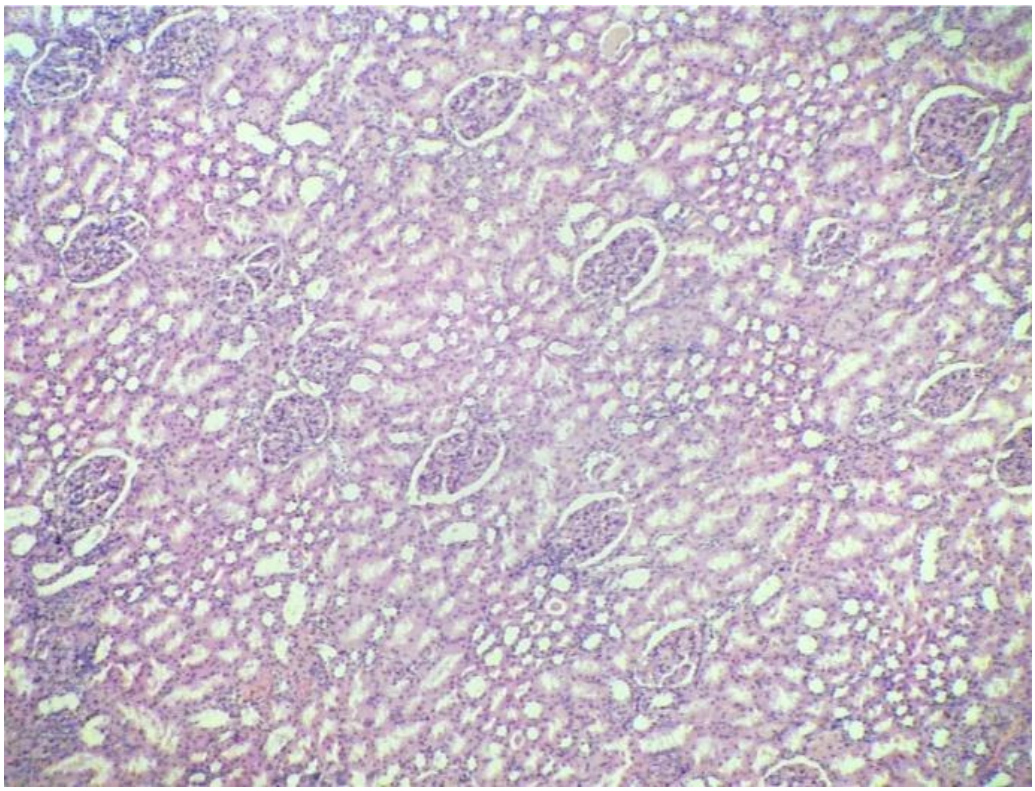

PLATE VI : Glomeruli appear as round solid speckled structures. Each is surrounded by a clear space. The round cross sections of tubules fill the regions between glomeruli. Each tubule has an open center (CONTROL GROUP) H & E X100

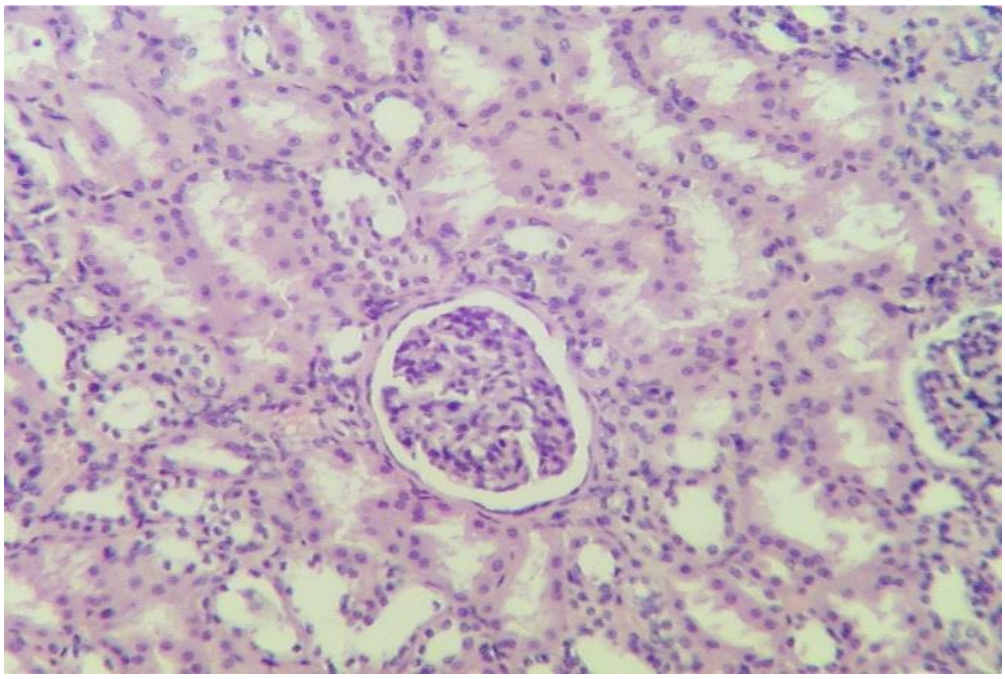

PLATE VII : Glomerulus appears as round solid speckled structure. It is surrounded by a clear space. The round cross sections of tubules fill the regions around glomerulus. Each tubule an open center (HIGH DOSE GROUP) H & E X400

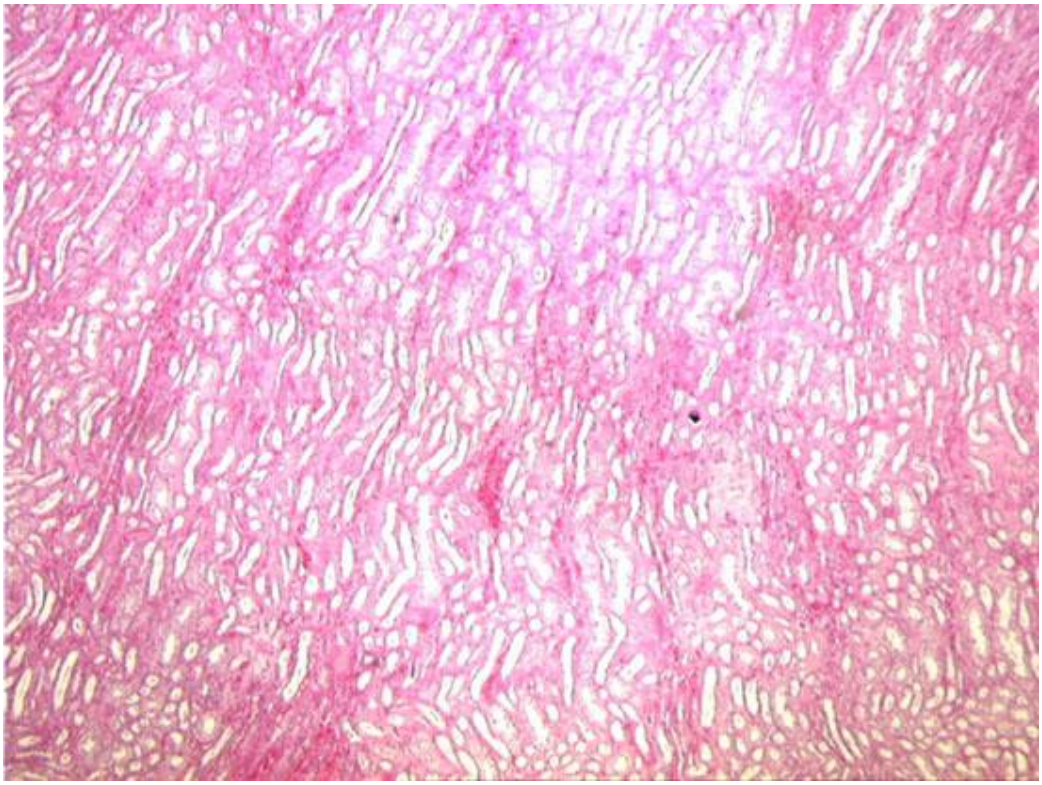

PLATE.VIII : Here the renal medulla is shown to consist solely of tubular profiles, many of which are cut in longitudinal section (HIGH DOSE) X 100.

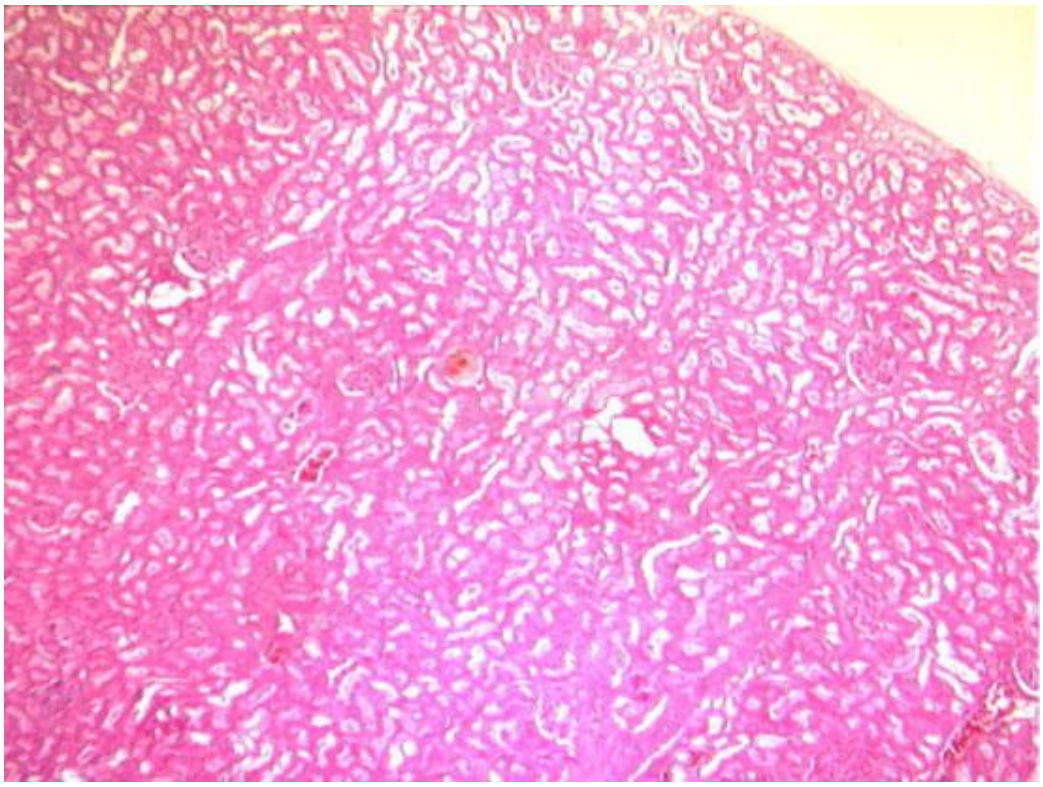

PLATE IX :Note that the cortex consists of renal corpuscles embedded in a matrix of renal tubules.(CONTROL GROUP) X100.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

This work was done to determine the haemopoietic and haematinicpotentials of the seed extract of *Telfairia occidentalis* (T.O).Serum transaminases were also determined to check its effects on Liver enzymes. The histology of the liver and the kidney were also examined to check whether it has any adverse effect on their cells.

The aqueous extract revealed normal haematological parameters on day 3 of administration, however the red cell indices (the MCH,MCHC) showed significant decrease on the groups that received 100mg/kg body weight, their reticulocytes counts were however increased significantly indicating increased haemopoiesis. In the methanol extract at day three, the HB was significantly decreased in all the concentrationsof extract given while the PCV decreased onlyin rats that received 200mg/kg weight. The reticulocytes on the other hand were also significantly increased in all the various concentrations. This increase in reticulocytes count shows that there is effective erythopoiesis, to compensate the drop in haemoglobin while the drop noticed in methanol extract maybe as a result of the solvent used hence aqueous extract is to be preferred. This is similar to the work of Nwanna and Oboh (2006) who reported that the aqueous extract of T.O are more effective than ethanolic extract in terms of antioxidant activity.

On the day 6 and day 9 of administration the haemopoietic activities showed almost the samepattern with that of day three, the HB and the PCV were decreased in all the concentration of the methanolic extract, the MCHC was also decreased on day 6 while on day 9, the decrease was notedonly inthose that received 50mg/kg weightand 200mg/kg weight.On both days the reticulocytes were significantly increased in all the concentrations, the total Rbc showed significant decrease on day 6 at 200mg/kg concentration. In the aqueousextract on the other hand all the parameters remained normal on day6 and 9 except for those that received 50mg/kg weight which showed significant decrease in the Rbc, Hb, PCV on day 6. Their MCVwere also reduced at 100mg/kgwt and 200mg/kgwt. The reticulocytes were significantly increased on both day 6 and day 9. The increase in reticulocyte counts in all the rats that received the extracts both methanol and aqueous shows that there is increased erythopoiesis as this increase in reticulocytes were not observed in those of normal control. From the bone marrow film we observed increased megakaryocytes (20 ï 22 megakaryocytes per field) and increased M: E ratio in both methanol and aqueous extract. Though this increased were not observed in the peripheral blood picture maybe due to short period of study, this observations indicated that the extract actually has haemopoietic potentials. Both the aqueous and the methanolic extract however had no effect on the serum transaminasesandtheir histology report revealed nodamage to both the liver and the kidney cells hence the seed of T.O can be conveniently consumed without harm and also for the

purpose haemopoiesis. This is in linewith the finding of the Agametor 2007 who reported that the seed of T.O has enough iron to support haemoglobin formation.

Another set of animals were induced for anaemia before the administration of the extract to check the haematinic activities of the seed ofT.O, can the seedextract of T.O be used in place of commercially prepared haematinics like Astymin?

According to Alada (2000) and Diana (2006) T.O leaf has been employed in the treatment of anaemia. In this study we tried to evaluatewhether T.O seed also has the same effect. On the day 3 of the administration of the methanol extract, the Hb level where all decreased compared with the normal control$p<0.05$, the PCV and Rbcs were also significantly decreased $p<0.01$ when compared with the normal control. The PCVof the groups that received 50mg/kg however showed significant increase $p<0.05$ when compared with the positive control. This increase in PCV could be attributed to the extracts given since the value were also higher than those of the positive controls.The decrease in the Rbc, Hb and PCVvalues may be due to the anaemia induced, since the animals are yet recovering from the effect. This also accounts for the low values recorded in positive control. The MCVand MCH were significantly increased at $p<.0.01$, this could beas a result of the induced anaemia. Phenylhydrazine induces anaemia by haemolysis, this could have caused the bloating of the red cell in

readiness for the eventual lysis hence the MCHC on the other hand was decreased significantly$p<0.05$. This decrease is also expected since the Hb and the PCV values were decreased, the reticulocytes were also significantlyincreased. The haemolytic activities of the phenylhydrazine could have triggered off the production and releasing of the young red cells to ameliorate the apparent anaemia. In the transaminases with the exception of groups that received 50mg/kg, all the other groups were significantly decreased$p<0.05$ for Alanine while the Aspartate showed no significant difference. In the aqueous extract on the other hand the Hb, PCV and Rbc showed significant decrease $p<0.05$ in all the groups that received the extract when compared with the normal control but showed significant increase when compared with the positive and negative control. This shows that the groups that received the extracts adjusted faster than the other groups including the positive control hence the seed of T.O could actually affect the blood level positively and this effect is more prominent in aqueous extract. The MCV were also significantly increased in the groups that received 100mg/kg and 200mg/kg but were also significantly decreased when compared with the positive and negative controls. This shows that the quantity of the extracts also affected the MCV for the red cell volume to have reduced more than those that received Astymin and those that were fed with only feed and water. The reticulocytes were significantly increased in all the groups. This agrees with the work of Erslev 2000 which stated that under anaemic conditions especially caused by

haemolysis, there is spontaneous production of immature cells which are released to the blood stream to compensate the anaemic condition. The Alanine transaminase were all significantly reduced when compared with the normal control while the groups that received 50mg/kg were significantly reduced when compared with the positive control. The Aspartate transaminase on the hand was significantly increased in the groups that received 100mg/kg and 200mg/kg when compared with the positive control, but with normal control there was no significant difference. Both the methanolic and aqueous extract actually affected the Alanine transaminasethough the effect of the methanol extract was more when compared with the aqueous but this effect was not enough to cause injury to the liver cells.

On Day 6 of the administration of the methanolic seed extract, the Hb and Rbc showed no significant difference with the normal, however the Hb negative control were significantly lower than the rest. This indicates that the various concentration of the extract actually ameliorated the anaemic condition while the negative control was rather slow in recovery. The PCV on the other hand, though normal, the negative control was still lower when compared with both the normal control and all the other groups that received extract and Astymin. In aqueous, the Hb, Rbc and Pcv show no difference with the normal control in all the groups except the negative control which was significantly lower in the three parameters. The MCV of all the groups in both methanol and

aqueousextract were significantly higher than the normal control. This shows that the anaemia has been fully taken care of, even as at the 6^{th} day of administration. The MCH in methanol extract were significantly higher in the groups that received the extracts and Astymin while in aqueous extract, there was no significant difference.The MCHC on the other hand were significantly decreased in all the groups that receivedmethanolic,aqueous extract and Astymin. The negative control showed no significant difference while only at a concentration of 100mg/kg body weight in aqueous extract did we observe a significant increase $p<0.01$ with the negative control.Comparing the results from the Rbcs,Hb, MCV, MCH and MCHC the seed extracts could be said to have helped the rats recover from the anaemia, this positive effect was more in methanolic extract than with the aqueous extract. This however did not agree with the previous work of Nwanna and Oboh (2006) which observed a better effect in aqueous extract than with ethanol though their work was on leaves.

The total WBC of the groups that received 100mg/kg in methanolic extract were significantly lower than the positive control, thenegative control and the normal control $p<0.05$. The differential count showed neutrophils of the groups that received 50mg/kg to be significantly lower than the normal control and the positive control while the groups that received 100mg/kg weight and 200mg/kg weight were significantly higher than the positive control. Also the groups that received 50mg/kg weight and 100mg/kg weight showed higher lymphocytes

than the positive control while that of positive control was significantly lower than the normal control. In the aqueous extract, the WBC count of the groups that received 200mg/kg were significantly lower than the positive control while the positive control were higher than the normal, the negative control were lower than the normal. The neutrophils of all the groups were significantly higher than the normal control.Could the ingestion of the seed of T.O actually increase the neutrophils of an individual? Then in cases of neutropennia, the intake of the T.O seed could be recommended.

The platelets count on the 6^{th} day shows statistically significant decrease in all the group that received methanolic extracts and Astymin when compared with the negative control, $p<0.05$ while in aqueous extract only the groups that received 50mg/kgand 100mg/kg were significantly lower than the negative control. This shows that both the aqueous and methanolic extract of this seed could have a depleting effect on platelets.The reticulocytes of all the groups in both methanolic and aqueous extract were also significantly increased $p<0.05$ however those that receivedthe extracts were higher than both negative and positive control. This showsthat although all the groups were recovering from anaemia, those that received the extract recovered at a much faster rate than even those that received the Astymin, hence the extract could beused as a haematinic. In methanolic extract the ALT showed no significant difference in all the groups; however the AST showed significant decrease in all the

groupsincluding the positive and negative controls $p< 0.05$ except in the groups that received 100mg/kg when compared with the normal control.In aqueous extract the ALT of the groups that received 50 mg/kg weight and 200mg/kg weightwere significantly decreased when compared with the positive control $p<0.05$, the positive control were also significantly higher than the normal control however all the groups that received extract showed no significantdifferencewith the normal control. The AST also showed no significance difference when compared with the normal control, however the positive and negative control showed significant decrease $p<0.05$ both with the normal control and with all the groups that received the extract. From this result it could be deduced that this seed actually has no ill effect on the liver since the ALT and AST levels were not altered by the administration of the extracts. This agrees with the work of Eseyin et *al* (2007), Iweala and Oboh(2009) and Kayode *et al* (2009) who reported that T.O leaves has hepatoprotective activities.

On day 9, administration of the methanolic extract, the Rbc became normal with the normal control, the Hb, and PCV were significantly increased in all the groups with the exception of the negative control. This shows that this extract actually improved the blood level in rats. The MCV and the MCHof all the groups appeared slightly increased with the normal but this was drastically reduced when compared with Day 3 and Day 6 values. The MCHC appeared

normal also. The reticulocytes though still increased were also reduced when compared with Day 3 and Day 6 value. The platelets on the other hand were still decreased when compared with the normal control.The negative and positive controls were also reduced hence this reduction could not have been caused by the extract received. The total WBC of the groups that received 100mg/kg and 200mg/kgappeared significantly decreased $p<0.05$ though their differential counts appeared normal. The aqueous extract on day 9 on the other hand showed significant decreased on the Rbc Hb, and PCV. The positive and negative controls showed no difference in RBC but in Hb and Pcv, they were significantly decreased. The groups that received 50mg/kgalso showed significant decrease when compared with the positive and negative control. The MCV were significantly increased in all the groups while the MCH and MCHC were significantly decreased. The reticuloytes were also significantly increased $p<0.05$ though the positive and negative controls were much lower than the groups that received the extract. This shows that the seed of T.O can actually be used in boostingblood level and that the methanolic extraction is to be preferred in terms of haematinicpotentials. This however does not agree with the findings of Nwanna and Oboh(2006) which preferred aqueous extract than the ethanolic extract though they worked on the antioxidant activities. The WBCS were significantly increased in groups that received 50mg/kg and 100mg1/kg. The positive control was also significantly increased while the negative control was reduced. The differential countshowed no significant difference. The ALT

showed no significant difference in aqueous extracts, but in methanolic extracts it was significantly decreased in all the groupsp<0.05including the positive and negative control.While the ASTwere significantly decreased when compared with the positive control in aqueous extract, it showed no significance difference with normal control in methanolic extract. This shows that the liver has not been affected as the level of ALT and AST were still within the normal range.

The histology of the liver and the kidney of both the controls and the test groups revealed normal cell morphology thus showing that the ingestion of the seed extract of T.O has no adverse effect on both organs.

CONCLUSION

In conclusion, the methanolic and aqueous seed extract of T.O caused some increase in bone marrow megakaryocytes, M:E ratio and reticulocytes counts in peripheral blood film within 3 ï 9 days of extract administration while increase in the Hb and PCV values were observed between the 6^{th} and 9th day of administration. This implies that the extract has both haemopoietic and haematinic potentials.

However the methanol extracts has more haematinic properties while the aqueous extract has more haemopoietic properties. This impliesthat the T.O

seed could be used to improve the blood level as its leaves is also been used. However some other investigation may be required.

RECOMMENDATION

Increased megakaryocytes in the bone marrow did not correspond to the decrease in platelet noted in the peripheral blood, more work should therefore be carried out to determine at what points these megakaryocytes appears in the peripheral blood film. The duration of administration should also be increased and the extract should be purified to determine the actual haematinic component present in the seed.

REFREENCE

Abdound, C.N., and Lichtmma, M.A., (2001): structure of marrow and Hematopoietic micro environment in Williamôs Haematology sixth Ed, Mc Grow Hills pp 29-35

Abrasion, S., Miller, R.G., and Philips, R.A., (1992): The identification in Adult bone marrow of the pluripotent and restricted stem cells of myeloid and lymphoid system. Journal of Experimental Medicine 115:1567.

Adaramoye, O.A., Achiem, J., Akintayo, O.O., and Fafunso, M.A., (2007): Hypoplipidemic effects of *Telfairia occidentalis* (Fluted pumpkin) in rats fed cholesterol ï rich diet. Journal of Medicinal Foods 10:330336.

Aderlbigbe,A.O.,Lawal,B.A.S., and Oluwagbemi., (1999): The antihyperglycaemic effects of *Telfairia occidentalis* in Mice. African Journal of Medical Sciences 28:171-175.

Agemator, C. (2007): (*Telfairia occidentalis hook F*)seed; A nutritionalist assessment .Election Journal of Environmental Agricultural Food Chemistry 6: 1787 ï 179318.

Aiyer, M.N., Kolammal, M.J., (1960): Pharmacology of Ayruvedic drugs: Trivandum nos-4-9.

Akorada, M.A., (1990): Ethanobotany of *Telfairia occidentalis* (Cucurbitaceae) among Igbos of Nigeria Economy batony 44:29-39.

Akpanabiantu, M.T., Bassey, N.B., and Udosen, E.O., (1998): Evaluation of some mineral and toxicants in some Nigerian soup meals. Journal of Food Composition and Analysis. 11(4) 292 - 297

Akubue, P.J., Kar, A., and Nwacheta, F.N., (1980): Toxicity of extracts of roots and leaves for *Telfairia occidentalis*. Plant Medica 88:339-343.

Akwaowo, E.U., Ndon, D.A., and Etuk, E.U., (2000): Minerals and anti-nutrients in fluted pumpkin (*Telfairia occidentalis* Hook F) Food chemistry 70(2) 235-240.

Alada, A.R.A., (2000): The Haematological effects of *Telfairia occidentalis* diet preparation. African Journal of Biomedical Research 3:185-186.

Altsuchuler, J.A., Casella, S.J., Curtis, R.M., (2007): The effects of cinnamon ion on all adolescent with type 1 diabetes. Diabetes core 30(4):806-818.

Ansari, A.A., (1993): Threatened Medical Plant from Madahauu Forest of Garahkpur. Journal of Economic and Taxonomic Botany 17(10) 2-11.

Asiegbu, J.E., (1987): Some biological evaluation of fluted pumpkin.

Aslker, L.V., Kakkarr, K.K and Charkrle, O.J., (1992): Second supplement to glossary of Indian medicinal plants with active principles part 1 (A-K) 1965-8 Publications and information directorate(CSIR) New Delhi pp 18-20.

Asu, J.M., Kawin, B., Minior, P., Mitchael, J.N., (1966): vitamin B_{12} concentration in human tissue Nature 210:1264.

Atal, C.K., Kapur, B.N., (1982): Cultivation and utilization of medicinal plants CSR, RRL, JAMMUL. TWI Indian 727,

Axtel, B.L., (1992): Minor oil crops. FAO Agricultural services, Bulletin no 94, FAO of United Nations.

Babior, M .B., (2001): Megaloblastic anaemia in: Williams haematology sixth EdôMc Graw hill P P 425 -445.

Baggott, J.E., Vaughn, W.H and Hudson, B.B., (1986): inhibition of 5- Amino midazole 4-carboxamide ribotide transform, lase adenosine deaminase and 5 adenylate deaminase by 5- aminoimida zole -4- carboxamideriboide and rebotide Biochemistry Journal 236:193.

Bailey, A.S., Jang, S., Afentoulis, M., (2004): Transplanted adult haemopoietic stem cells differentiated into functional endothelial cells. Blood 103:13-15.

Baynes, J.W., (1991): Perspective in diabetes: Role of oxidative stress in development complications in diabetes. Diabetes, 40:405-412

Ballard, H .S., andLindenbaun , J.,(1974):Megaloblastic anaemia complicating hyper alimentation therapy. American Journal of Medicine 56:740.

Bandyopadhyay, U., Das, D., and Bannerji, K.R. (1999); Reactive oxygen species, Oxidation damage and pathogens. Current science: 658.

Barbican, B.J., (1989): Malaria in pregnancy, its importance and control part 1, postgraduate Doctor, 11:57-59.

Beck, S.W., (1992): Erythopoiesis and blood anaemia in: Clinical Haematology (William S B., edited) Blood M I T press Cambridge, London. 5th Edition 24_35.

Beutler, E., (2001): Anaemia due to nutritional deficiency In: William haematology sixth Ed, PP 471 -47524. Beutler, F., Fairbanks, V.F., and Fanley, J.E., (1963): Clinical disorders of iron metabolism gruneand Stratton New York.

Beutler, F., Fairbanks, V.F., and Fanley, J.E., (1963): Clinical disorders of iron metabolism gruneand Stratton New York.

Bhakuni, D.S., Dhar, M.L., Dhawan, B.N., Gupta, H., Srimal, R.C., (1971): Screening of Indian plants for biological activity, part 111 Indian Journal of Experimental Biology 2:91.

Bidfu, G.O., and Ogunsina, A.O., (1991): Clinical composition of Kernel from some species of cucurbitaleae grown in Nigeria. Plants food, Human nutrition 41:35-44.

Boning. D., and Enciso, G., (1987): Haemoglobin. Oxygen affinity in anaemia. Blut 54:36.

Borelti, F .S., Buchler , P . W., Agnillo , F. ,(2009) Sequestration of extra cellular haemoglobin with haptoglobuin complex decreases its hypertensive and oxidative effects in dogs and guinea pigs .Journal of clinical investigation .119 (8) 2271 -80.

Bradford, G .B., Williams , B., Rossi ,R., Bertoncello ,T . , (1977) cycling and turn overs in the primitive haemopoietic stem cell compartment. .Experiment Haematol 25: 445.

Brownlee, M., (2011): Biochemistry and Molecular cell biology of diabetic complications nature 414:813- 820.

Butterwoths, C.F., jnr; santini , R., Jr and Frommeyer W.B., Jr. (1963); the pteroylglatenate components of American diets as determined by chromatograph filtration. Journal of Chemical Investigation 42:1929.

Chang, H .S. , Yamato, O. , Sakai , Y. ,Yamasaki, M. ,andMaede , Y. , (2004) : Acceleration of super oxide generation in poly morph nuclear leucocytes and inhibition of platelet aggregation by alklen Yl Thiosulphates derived from onions and garlic in dogs and humans .Prostaglandins , leukrienes and essential fatty acid . Journal of Clinical Investigations 70 (1): 77 ï 83

Conrad, M.E., Umbert, J.N., and Moore, E.G., (1991): A role for mucin, the absorption of inorganic iron and other metal cations. A study in rats Gastroenterology 100-129.

Cox, E.V., Sheard, N.F., and Kellehr, J.F., (1992): Treatment of non-deficiency anaemia complicated by scurry and folic acid deficiency. Nutrition review 50:134.

Dacie, J.U., Lewis, S.M., (2005): Practical haematology, Churchill livingstone, Edinburgh, United Kingdom, 9th edition pp 420-422.

DôAndrea, A.D., Lodish, H.F., and Wonge, G.G., (1989): Expression cloning of murine erythropoietic receptor.Cells 57:277.

Devigilis, S., Congia, M., and Turco, M.P., (1998): Depletion of trace elements and acute toxicity induced by desferroxamine in patients with thalassamia. Arch D child 63:250.

Diallo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku., and Aklikokou, K., (2008): Effects of Tectona grandis on phenylhydrazine induced anaemia in rats. Fitoterapis 79, 332 -336

Dike, M.C. (2010): Proximate phytochemical and nutrient composition of some fruits seeds and Leaves of some plant species at Umudike Nigeria. ARPNS of Agricultural Biological science 5: 7 ï 16.

Dina, O.A., Adedapo, A.A., Onyinloye, O.P., and Saba, A.B., (2006): Effects of *Telfairia Occidentalis* extract on experimental induced anaemia in domestic animals. African Journal Biomedical Research 3 : 181 ï 183.

Eaves, A.C., and Eaves, C.J., (1984): Erythopoiesis in culture . Clin Haematol 13 : 371

Eckmekeigolu, C., Ekeyetag, J., Markti, W., (1996): A ferric reductase activity is found in rush border membrane vesicles isolated from $CaCO_2$ cells. Journal of Nutrition 126:2299.

Egbekun, M.N., Nda-suleiman, E.O., and Akinyeye, O., (1998): Utilization of fluted pumpkin fruit(*Telfairia occidentalis*) in marmalade manufacturing. Plant food Hum. Nutr. 52:171-176.

Elchner, E.R., and Hillman, R.S., (1993): Effects of alcohol on serum folate level .Journal of Clinical Investigation 52; 584.

Emeson, P.M., and Wilkinson, J.A., (1966): Lactate dehydrogenase in diagnosis and assessment of response to treatment of megaloblastic anaemia .British Journal of Haematology 12: 678.

Erslev, A.J., (2001): Clinical manifestations and classifications of Erythrocyte disorders In: Williams haematology sixth E.d, M.E Graw Hills. pp 369-374.

Erslev, A .J., (1971) : Feedback circuits of stem cells differentiation. American journal of pathology 65:629

Erslev, A .J., (1959): Control of red cell product ion . Annals of Revolutionary medicine 11: 315

Erslev, A.J., (1953): Humoral regulation of red cell production. Blood 8: 349

Eseyin. O.A., Ebona, P., Arit, E., Arnold, I., and Emmanuel, O., (2007): Hypoglycaemic effects of seed extracts of *Telfairia occidentalis* in rats. Pakistan Journal of Biotechnology 5:49-53.

Fairbanks, V.F., Beutler, E., (2001): Iron metabolism in Williams Haematology sixth edition. Mc Graw-hills pp 295-304.

Fasuyi, A.O., (2006): Nutritional potential of some tropical vegetable meals: chemical charactization and functional properties. African journal of Bio-Technology 5: 49-53.

Fasuyi, A.O., and Nonyerem, A.O., (2007): Biochemical nutritional and implications of *Telfairia occidentalis* leaf meal as protein supplement in broiler starter diets. African Journal Biotechnology., 6:1055-1063

Finch, C.A., Eubelbeissk, K., Cook, J.D., (1970): Ferro kinetics in man medicine (Baltimore) 49:17

Finney, D.J., (1971): Profit Analysis cambridge university press 303pp. Food and Nutrition Board: Natinal Reseach council.

Frimberger, A.E, Stering, A .L., Tuft, R . A., (1998): Anin vitro model of haematopoietic stem cell cloning .Blood 92: 5859.

Fuchs, C.J., Tienboon, P., and Linpisam, S.,(1996): Nutritional factors and thalassamia major Arch Dis child 74:224.

Gbile, Z.O., (1986): Ethobony taxonomy and conservation of medicinal plants. In: State of medical plant research in Nigeria sofowora A, (ed). University of Ibadan press, Ibadan, Nigeria.

Gills, I.S.,(1992): Ethano medical uses of plants in Nigeria. Uniben press, University of Benin, Benin city, Edo state, Nigeria pp 228-229.

Godwin, H.A., and Rosenbeg, I.H., (1975): Comparative studies of the intestinal absorbtion of Hipteroylmonoglutamase and (^{3} H) Pteroylheptaglutamate in man. Gastroenterology 69: 364.

Goldberg, M.A., Dunning, S.P., and Bunn, H.F., (1988): Regulation of blood erythropoietin gene evidence that blood oxygen sensor is a heme protein. Science 242 : 1412.

Gordon, N.Y., Marley, S.B., and Davidson, R.I., (2002) contact mediated inhibition of human haemopoietic progenitor cell, proliferation may be conferred by stem cells antigen CD34.Blood Haematology Journal 1:77-78.

Grantham, M.C., Gregor, S., and Ani, C., (2001): Iron deficiency anaemia, re-examining the blood Nature and magnitude of blood public. Health problem Journal of Nutrition 131 (2) 6495 ï 6685.

Grasbeck, R.., (1959): calculations on vitamin B2 turn over in man, scand Journal of Clinic Laboratory Investigation 11:250.

Gratus, G., Wilson, S., Greenfield, S.M.., (2009): The use of Herbal medicine by people with cancer, a qualitative study complements. Alternative medicine 9:14

Gregory, C.J., and Eaves, A.C., (1978): Three stages of erythropoiesis progenitor cell differentiation distinguished by a number of physical and biological properties Blood 51:527

Guyton, A.C., and Hall, J.E., (2001): Atextbook of medical physiology, 9th Ed, prism on print Ltd, Bangalore Indian pp xliii ï 1148.

Haltrman, J.S., Kaczorowski., J.M Aligner., C.A Auinger, P., Sziliagyi. P.G., (2001): Iron deficiency and cognitive achievement among school aged children and adolescent in united states. Paediatrics 107 (6): 1381 ï 1386.

Harborne, J.B., (1973): Phytochemical methods. A guide to modern technique of plants analysis 1st Edition Chapman and Hall London ISBN: 0412572609.

Heyssel, R.M., Bozian, B.C., Darby, W.C., (1960): Vitamin B_{12} turnover in man, the assimilation of vit B12 daily dietary requirements American Journal Clinical Investigation 18: 176

Hill, R.J., and Levin, J., (1989): Regulation of thrombopoiesis their biochemistry and physiology, Blood cells 15 : 141

Hillman, R.S., Finch, C.A., (1967): Erythropoietin normal and abnormal. SeminHaematology 4:327.

Hodges, R.E., Baker, E.M., Hood, J., Sauberlich, H.E., and March, S.C.,(1969): Experimental scurvy in man American journal of clinical nutrition 22:535

Hodgson, E., (2004): A textbook of modern toxicology 3rd edition, John Wiley and Sens inc. New Jersey pp 203-2011.

Horsefall, Jr. M., and Spift, I.A., (2006): Equilibrium absorption study of Al,CO_3^+ and Ag^{2+} in Aqueous solution of fluted pumpkin (*Telfairia occidentalis* Hook F) waste biomass Acta chemical solvent 52:124-181.

Hubers, H.A., Beghin, Y., Pootrakul, P., Einsphar, D., Finch, C.A., (1990): Intact transferring receptors in human plasma and their relation to erythropoiesis Blood 75:102.

Ideguchi, H., Ishikawa, A., and Futata, Y., (1194): A comprehensive scheme for blood schematic investigations of hemolytic Anaemia. Annals of clinical science 24 (5): 412 -421.

Iscore, N.N., (1975): Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture: Experimental Hematology 3:32.

Iweala, E.E.J., and Obidoa, O., (2009): Some biochemical haematological and histological responses to a long term consumption *Telfairia occidentalis* supplemented diet in rats. Pakistan Journal of Nutrition 8:1199-1230.

Iwuh, M.W., (1983): Traditional Igbo Medicine. Institute of African studies, University of Nigeria Nsukka.

Iwu, A.M., Till, J.E., Siminovitch, I., and Mc Cullah, E.A., (1967): A cytological study of the capacity for differentiation of normal haemopoietic colony- forming cells. Journal of cell physiology 69:172.

Izzo, A.A., Ernest, C., (2009): Interactions between Herbal medicine, systematic and prescribed drugs: an updated systematic review drugs 69(13):1777-98.

Jacobs, A., (1968): Gastric factor in absorption Lancet 1:1313.

Jacobs, K., Shoemaker, C., and Rudersdorf, R., (1988): Isolation and characterization of genomic and DNA clones of human erythropoietin. Nature 313:806.

Joy, P., Thomas, J., Matthew, S., and Kama, P., (1988): Kerala Agricultural University, Aromatic and medicinal plant research sechan,, Odakkali Asamannoor P.O, Ernakulam district kerals India P.N 683549.

Karft, K., (2009): Complementary/Alternative medicine in the context of prevention of disease and maintenance of healthy preview, medicine

Kayode, O.T., Kayode, A.A., and Odetola, A.A., (2009): Therapeutic effects of *Telfairia occidentalis* on protein energy malnutrition induced liver damage. Research journal of Medicinal plant 80-92.

Kipps, T.J., (1997): Human B-cell biology. Internal Revolution Immunology 15:243.

Koury, M.J., and Bondurant, M.C., (1989): A model of erythropoietin action. Blood 74(suppl-l): 489.

Koury , S .T. ,Bondurant , M .C. ,and Koury ,M . K., (1988) : Localisation of erythropoietin synthesizing cells in murine kidney by in situ hybridization Blood 7 :527.

Lacombe, C., and Dasilva, J.L., (1988): Pertublar cells are the site of erythropoietin synthesis in murine hypoxic kidney. Journal of Clinical Investigation 81:620.

Ladeji, O., Okoye, Z.S.C., and Ojobe, T., (1995): Chemical Evaluation of the nutritive value of leaves of fluted pumpkin (*Telfairia occidentalis*): Food chemistry, 53:353-355.

Landom, M.J., Eyre, D.H., and Hyten, F.E., (1975): Transfer of foliate to the foetus. British Journal Obstetrics and Gynaecology 82:12.

Lemischka, I.R., Raulet, D.A., and Mulligan, R.C., (1986): Developmental potential and dynamic behaviour of haemapoietic stem cells. Cells, 45:917.

Leung, W.T.W., Busson, F., and Jardin, C., (1968): Food composition table for use in Africa. FAO Rome Italy pp 306.

Lieber, C.S., (1980): Metabolism and metabolic effects of alcohol. Seminar Haematology17:85.

Longe, O.G., Ravinu, G.O., and Fetnoa, B.L., (1983): Nutritious value of fluted pumpkin (Telfairia occidentalis). Journal of Agriculture Food chemistry. 31: 982-992.

Manheirmer, E., and Wieland, S., (2009): Collaboration for Traditional Chinese medicine therapies. Journal of Alternative complementary Medicine 15(9): 10001-14.

Martin Reudon, E., Walt, S.M., (2003): Stem cell plasticity: British journal of Haematolgy 122:877-91

Maxwell, A.P., Lappoin, T.R.J., and Johnson, C.F., (1990): Erythropoietin producing in kidney tublar cells. British journal of Haematology 74:335.

McDonald, T.P., (1988): Thrombopoietin: its biology purification and characteristics. Experimental Haematology 16:201.

McFarland., and Libre, E., (1963): Abnormal leucocyte response in alcoholism. Annals of internal medicine 59:865.

Mc Leod, D.L., Shreeve, M.M., and Axelrad, A.A., (1974): Improved plasma culture system for production of erythrocytic colonies in vitro quantitative assay method for cfu-E Blood 44:517.

Mishra, S.K., Mohapartra, S., and Mohanty S.Y., (2006): Jaundice in Falciparum Malaria. JIACM., 4:12-3.

Miyake, T.,Kung, C.K.H., and Goldwasser, E., (1972) : Purification of human erythropoietin. Journal of Biological chemistry 352: 55-58.

Mody, R.J., Brown, P.L., and Westabler, D.S., (2003): Refractory iron deficiency as the primary clinical manifestation of celiac disease. Journal of paediatric. Haematology/ oncology 25 (2): 169 -72.

Mollin, D.L., and Himes, J.D., (1964): Observation on the nature of pathogenesis of anaemia following partial gastroectomy, Proc R. soc Med .57:575.

Murray, J.F., Johnson, B.C., (1963): The circulatory effects of haematocrit variations in normovolemic and hypovolemic dogs. Journal of Clinical Investigations 42: 1150.

Nakahta, T., Ogawa, M., (1982): Origin of murine haemopoietic colonies with apparent restriction to granulocyte macrophage megakaryocytes (GMM) differentiation. Journal of cell physiology 111:29

Nakahata T., and Ogawa M., (1982): Haemapoietic colony-forming cells in umbilical cord blood with extensive capability to generate mono and multi potential haemapoietic progenitors. Journal of Clinical Investigation 70:134.

Nwangwa, E.K., Mordi, J., Ebeye, O.A., and Ojieh, A.T., (2007): Testicular regenerative effects induced by extracts of *Telfairia occidentalis* in rats. Carderno de Pesquisa,Serie boil., 19:27-35

Nwanna, E.E., and Oboh, G., (1982): Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidetalis* (Fluted pumpkin) leaves on acetaminophen induced liver damage. Pakistan J. of Biological science 10: 2682 -2687.

Nwozo, S.O., Adaramonye, O.A., and Ajaiyeoba., (2004): Anti diabetic and hypolipidaemic studies of *Telfairia occidentalis* on alloxan induced diabetic rabbits. Nigeria Journal Production medic 8:45-47.

Oboh, G., Nwanna, E.E., and Elusiyan, C.A., (2006): Antioxidant and antimicrobial properties of *Telfairia occidenalis* (fluted pumpkin) leaf extract Journal of Pharmacology and Toxicology 1:167-175).

Oboh, G., and Akindahunsi, A.A., (2004): Changes in ascorbic total phenol and antioxidant activity of sun dried commonly consumed green leafy vegetable in Nigeria. Nutritional Health 18:29-36.

Odoemena, C.S and Onyeke, E.C., (1998): Lipids of fluted pumpkin (Telfairia occidentalis) seeds proceedings of the 1st African conference on Biochemistry of lipids (ACBC'98).

Okochi, V.I., Okpuzor, A.J., and Alli, L.A., (2003): Comparison of an African herbal formula with commercially available haematinics. African Journal of Technology; 2:237-240.

Okoli, B.E., and Mgbeogu, C.M., (1983): Fluted pumpkin, West African vegetable crop Economic Botany 37:145-149.

Okwu, L.E., (2001): Evaluation of the chemical properties of indigenous spices and flavoring agents. Global journal of pure applied sciences 7(3); 455-459.

Okwu, L.E., (1999): Flavoring properties of species on cassava fufu: African Journal of roots tubers crops. 3(2):19-21

Oliver, Deôs, S.C., (1980): Chemical constituents of vegetable drugs. In: medicinal plants in Nigeria, published by Nigeria College of Arts and science and Technology Ibadan pp 15.

Oluwole, F.S., Falode, A.O., and Ogundipe, O.O., (2008): Anti-inflammatory effects of some common Nigerian Vegetables. Nigeria Journal of physiology science 18:25-38.

Packman, C.H., (2008): Haemolytic anaemia due to warm antibodies. Blood Revolution 22 (1): 17 ï 31.

Paliwal, A., Gurjar, R.K., and Sharma, H.N., (2009): Analysis of liver enzymes in albino rats urelers strees Y ï Cyhatothin and Nuvan toxicity. Biology and medicine 1(2) 70-73.

Penning, J.A.T., Young, B.E., Wilson, D.B., Johnson, R.D., Vander Van, J.E., (1963): Mineral content of food survey 1982-1984. Journal American Dietary Assessment 86:876.

Plas, D.R., and Thomas, M.C. (1988): Negative regulations Of antigen receptor in lymphocytes.Journal of molecular medicine 76:585.

Polio, G., Albano, E., Diazain, M.U., (1987): The role of lipid preoxidase in liver damagechemistry and physics of lipid 45:117 - 142

Post, R.M., and Desforges, J.F., (1963): Thrombocytopennia and alcoholism Anal of internal medicine 68:1230

Pritchard, J.A., Scott, D.E., and Whalley, P.J., (1970): Infants of mothers with megaloblastic anemia due to foliate deficiency. JAMA 211:1984.

Quesenberry, P.J., and Clovin, G.A., (2001): Hematopoietic stem cell progenitor cells and cytokines. In Williamsôs Hematology Sixth Ed. Mc Graw Hills PP 15- 174.

Raffin, S.B., Woo, C.H., Roost, K.T., and Price, D.C., (1975): Intestinal absorption of hemoglobin iron-heme cleveage by mucosalheme oxygenase .Journal of Clinical Investigation 54; 1344

Rahman, M.F., Siddiqui, M.K.J., and Jamil, K., (2001): Effects of repacide (Azadiractita indica) on aspartate and alanine transferase profile in subchloric study with rats. Human and Experimental Toxicology 20:43:249.

Rahman, M.F, Siddiqui, M.K.J., and Mustafa M.,(1996): Effects repeated oral administration on repacide (Azadirachta Indica) on some hematological and biochemical parameters in rats. Indian Journal of Toxicology 20:43; 249.

Rao, C.V., and Banerji, S.A., (1990): Effects of feeding polychlorinated b; phenyl (Aroclor 1260) on hepatic enzymes in rat.Indian Journal of Experiment Biology 28; 149-151.

Reissman, K.R., (1950): Studies on mechanism of rats during hypoxia Blood 5:372.

Reitman, S., and Frankel, S., (1957): A colorimeric method for determination of serum glutamic oxaloacetic and glutamic pyruvic acid transaminase. American Journal of Chemical Pathology 1: 56-63.

Reuler, J.B., Broudy, V.C., and Coonely, T.G., (1985): Adult scurvy JAMA 253:805

Rich, I.N., Hart, W., and Kubarak, B., (1982): Extra renal erythrpoietin production by macrophases .Blood 60: 1007.

Ricketts, c., Cavill, I., Napier, J.A., and Jacobs, A., (1997): Ferrokinetics and erythopoiesis in man an evaluation of ferrokinetic measurement British Journal of Haematology 35:41.

Shahni, K., Saxena, Y., (2001): Toxic effect of diefethialone on the liver of swiss albino mice muomuslns .u.p Journal of zoology21 (1): 47-52.

Salman, T., Olayaki, L.A., and Oyeyemi, W.A., (2008): Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. African Journal of Biotechnology 7;2299-2303.

Sawyer, S.T., Krantz, S.B., and Goldwasser, E., (1987): Binding and receptor Mediated endocytosis of erythropoietin in freud virus infected erythroid cells. Journal of Biological Chemistry 262:5554.

Schwartz, R.E., Reyers, M., Kodie, L., (2002): Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocytr-like cells. Journal of clinical investigations 109:(1291-302)

Selhub, J., Nakamura, S., Carone, F.A., (1987): Renal flolate absorption and the kidney folate binding pistein II. Micro infusion studies. American Journal of Physiology 252: F 757.

Shah, J.S., Shah, M.B., Goswam, S.S., Santani, D.D.,(2006): Mechanism of action anti-ulcer activity of bark of extract of manikara hexandra against experimentally induced gasrric ulcers in rats. Pharmalognosy magazine 2:40-45.

Sofowora, A., (1993): Medicinal plants and Traditional medicine in African 2nd ed. spectrum Ltd. Ibadan Nigeria pp 112 ISBN 978246-219.

Srivastara, A.V., Raina, R., and Chavdhery, R.k., (1989): Acute toxicity and biochemical alterations in rats after single oral experiment to dicholorovous. Pesticides 2:35-49.

Steinberg, S.F., (1984): Mechanism of foliate homeostasis. American Journal Physiology246:9319.

Sties, T.E., Bailey, L.B., Scott, K.C., Toth, J.P., and Fisher, W.B., (1997): The kinetics modeling of folate metabolism through use of chronic administration of dietary labeled folic acid in men. Journal of Clinical Nutrition 65:53.

Stokes, P.L., Melikian, V., leening, R.L., Portmen-Graham, H., Blair, J.A., and Cooke, W.T., (1975): Folate Metabolism in surveying. American Journal of Nutrition. Nutri 28.

Suda, T., Suda, J., and Ogawa M., (1983):Single cell origin of mouse haemapoieitic colonies expressing multiple lineages in variable combinations . Proc Natural Acad Sciences USA 80:6689

Sullivan, L.W., and Herbert, V., (1963): Suppression of haemopoiesis by ethanol. Journal of Clinical Investigation 43: 2048.

Taneminar, I., Apaliwan Rkaurja H.M., and Sharmia., (2009): Toxicolgy, Human perspective Biology and Medicine vol (2) 70-73.

Taylor, O.O.A., Fetuga, B.L. Oyenuya, V.A., (1983): Accumulation of mineral elements in five tropical leafy vegetables as influenced by nitrogen fertilization and age. Scienta Horticulture 18:312-332.

Thorlling, E.B., and Erslev, A .J., (1968): The tissue tension of oxygen and its relation to hematocritand erythropoiesis. Blood 31:33.

Trease, G., and Evans, S.M., (2002): Pharmagnosy 15th Ed. English language book society. Bailieve Trinidad London pp 23-67.

Trentin, J.J., (1971): Determination of bone marrow stem cell differentiation by stroma haemopoetic induced micro environment (HIM) (1971): American Journal of pathology 65. 621.

Tribe, R.M., and Poston, L., (1996): Oxidative stress and lipids in diabetes, a role in endothelium vasodilators dysfunction. Vasmed 1:196-206.

Tyler, V.E., (1999): Phyto medicines, back to future. Journal of Natural production 62:1589-1592.

Ugwu, C.E., Alumara, E.O., and Ezeanyika, L.U.S., (2011): *Telfairia occidentalis* reduces cholesterol levels in rats. African Journal of Biochemistry research vol 5(1)opp 2832.

Van Campen, D., (1973): Enchancement of iron absorption ligated segments of rats intestine by histidine cyesteine and lysine. Effects of removing ionizing groups of stero-isomerism-Journal Nutrition 103:139.

Wantable, F., Nakana, Y., Sadio, H., Tamura, V., and Yamanaka, H., (1992): Cytochrome b / cytochrome b reductase complex in rat liver microsomes has NADPH-linked aqua cobalamin reductase activity. Journal of Nutrition 122:940.

Wei, Z., and Shiow, Y., (2001); Antioxidant activities of phenol compounds in selected herbs. Journal of Agriculture food chemistry 49:5166:5170

Whitehead, V.M., Comty, C.H., Posen, G.A., and Kay, M., (1986); Homeostasis of folic acid in patients undergoing maintainance haemodialysis. N Eng J Med 279:970.

WHO (2008): Worldwide prevalence of anaemia 1993 -2005 , Geneva WHO ISBN 978 9241 59 6657.

WHO (2001): Geneva legal status of traditional medicine and complementary/alternative: A world review. WHO pp 129-143.

WHO (1977): Resolution- promotion and development of training and research in traditional medicine WHO documents NO: 30:49-59.

Williams, I.O., Parker, I.I.O., and Swanson, J., (2009): Vitamin A content of southeastern Nigerian vegetable dishes, their consumption pattern and contribution to vitamin A requirement of pregnant women in calabar urban Nigeria. Pak. J.Nutr, 8:100-1004

Wu, A.M., Till, J.e., Siminovatich, L., and Mcullah, F.A., (1967): A cytological study of the capacity for differentiation of normal hemopoietic colony-forming cells. Journal of cell physiology 69:172.

Yang, J.H., Lin, H.C., Mau, J.I.,(2002): Antioxidant properties of several mushrooms. Food chemistry 77:229-225

Yuzbasiyan-Gurka, V.A., Brener, G.J., Vander, A.J., Gunther, M.J and Prassad, A.S., (1989): Net renal tublar reabsorbtion of zinc in healthy man and impaired handling in sickle cell anaemia. American Journal of Haematology 3:87.

Zipori,, D., (1992). The renewal and differentiation of hemapoietic stemcell In Williamsôs Hematology Sixth Ed. Mc Graw Hills PP 15- 174.