EFFECT OF AQUEOUS EXTRACT OF THE BARK OF *KHA YA SENEGALENSIS* ON PHENYLHYDRAZINE INDUCED ANAEMIA.

BY

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DECLARATION.

I declare that this work is wholly the author's and has not been part of any Publication nor Presentation for a higher degree.

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Date: 12/5/98

CERTIFICATION

This thesis entitled "Effect of Aqueous Extract of the bark of *Khaya senegalensis* on Phenylhydrazine Induced Anaemia" by Ibrahim, Fatimah Oziohu meets the regulation governing the award of the Degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late Father Alhaji Ibrahim Ouda Ejivade.

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ABSTRACT

Phytochcmical screening was conducted using petroleum ether (40 - 60°C), melhanolic and aqueous extract of the stem bark. Both the methanolic and aqueous extracts gave positive result to carbohydrates, saponin glycosides and tannins. In addition, terpenes and anthraquinoncs were found only in the methanolic extract. The petroleum ether extract contained only steroids. Data on the mineral content of the aqueous extract of the bark showed that it is rich in some of the important mineral element needed for the development and functioning of blood. Trial experiment to determine the suitable dose of phcnylhydrazine for induction of anaemia in rats showed that 750mg/kg body weight would give a suitable anaemic condition. /// *vivo* investigation of the effect of oral daily dose (300mg/kg body weight) of the bark aqueous extract on phcnylhydrazine - induced anaemia in rats did not show any significant curative effect ($P > 0.001$). However, the same oral dosage seemed to produce significant ($P < 0.001$) Prophylaltic effect probably by protecting the red blood cell against Phcnylhydrazine destruction. Subcutaneous injection of 30mg/kg body weight on *Trypanosoma brucei* induced anaemia in the rats did not produce anaemia or trypanocidal effect.

CHAPTER ONE

1.1. INTRODUCTION

Anaemia presents a world wide problem and there is no doubt that the incidence is higher in the third world than in the developed countries (Oma, 1991). This is largely due to the presence of some aggravating factors such as poor nutrition, low socioeconomic status, high prevalence of diseases, for example malaria, trypanosomiasis and helminthic infestation. Similarly in women there is a high suceptibility which is super imposed on the increased demand on body due to the pregnancy and the developing foetus (Ong, 1973; Oma, 1991). Anaemia is among the top ten causes of death in childhood. At least half of the children dying from other causes could also be anaemic. A study conducted by Akinkugbe, (1991) revealed that 19.7% of children in a rural population of Nigeria were anaemic. It is an established fact that a chronic anaemic condition if not treated could be fatal. Knowing the physiological functions of blood and the pathological consequences of its shortage, scientists are working hard to find prophylactics and / or curative agents which are inexpensive with minimum side effects. This will help to curtail the prevalence of the disorder in the afore mentioned regions and population groups.

Anaemia is one of the numerous ailments claimed to have been successfully treated with plant materials by the traditional medical practitioners. In Chinese herbal medicine, blood diseases such as malformation of the blood circulatory system, including blood stagnation, anaemia, vericous veins and haemorrhage were reported to have been treated with plant materials (Richard, 1978).

Moreso, the search for a suitable substitute for blood or its derivatives plasma or serum as transfussion materials has engaged the mind of researchers for long. Best and Taylor (1969) reported the use of gum *Accacia* which was introduced during the world war I for treatment of haemorrhage resulting from injuries. The treatment was reported to be highly successful but it was later discovered to result into damages of the liver of the patients. Later, Sofowora, (1979) reported that the crude extract of *Fagara zanthroxylum* has the ability to revert sickled HbAS erythrocyte to normal configuration.

Nigerians only import finished drugs and those that manufacture still import the raw materials thereby consuming a large sum of our hard earned foreign exchange. Furthermore, some of the drugs manufactured are unaffordable by the people who need them most.

About 80% of Nigerians depend on traditional medicine as a major approach to curing diseases. This may be due to the fact that local medicinal plants are considered to be readily available, effective, economical and less complicated method of curing diseases (Awosika, 1991). According to WHO (Fransworth *et al.*, 1985) about 80% of the inhabitant of the world rely chiefly on traditional medicines for their primary health care need and the major parts of traditional therapy involves the use of plant extracts or their active principles.

At present in every country in the world, official health departments are suffering from malaise, which is caused by insufficient medical service to reach their population and increasing specialisation in western medicines with less focus on the whole patient let alone his or her mileu. A possible cure for this malaise is to incorporate the traditional medical services in the formal health care system. This would ensure a more appreciable health care delivery that would benefit the entire population.

Traditional medicine and orthodox Medicine aim at healing or preventing diseases, but one of the major criticisms levied against traditional medical practitioners is that their potions are not standardised nor are they dispensed in strictly regulated quantities (Sofowora, 1979). However, in recent times, increasing interest has been given to scientific studies of our traditional medicinal plants to find substances with potentially useful biological properties as well as to validate the efficacies of such substances (Akubue, 1986; Errki *et al*., 1993). Also there is a need to scientifically show that these medicinal plants do have the Pharmacological activity claimed by its users. These will make them more acceptable to the increasingly skeptical and westernized people of our country.

The plant commonly called mahogany belongs to the genus *Khaya* and the family *Meliaceae*. Of these, *Khaya senegalensis*, mainly a savanna specie is highly reputable for its numerous medicinal values. Some of these claims have been pharmacologically substantiated (Kerharo, 1969; Adesina, 1983; Olayinka, 1992). In some parts of middle belt and western Nigeria a decoction of the bark is being specifically used in the treatment of anaemia, but this has not been scientifically proven. Therefore this work is largely aimed at investigating this claim. However lack of suitable facilities will hinder some analyses especially those involving the phytochemical studies. Such in depth studies will not be part of this work. Similarly, bleeding of the experimental animals may constitute another problem because it is difficult to get enough blood to determine all the necessary haematological parameters for adequate investigation. Therefore the parameters to be used are Haematocrit and body weight.

1.1.2 AIMS AND OBJECTIVES OF THE RESEARCH

This study is designed to:

(i) Investigate the possible anti-anaemic activity of the aqueous extract of the bark of *K. senegalensis* in laboratory rats.

(ii) If effective, to quantify the effective dose of the crude aqueous extract.

(iii) To determine some mineral elements present in the bark of the plant in order to ascertain the presence of important mineral elements with haemophoetic activity.

(iv) To determine the partial phytochemical composition of the crude aqueous extract in order to detect other useful chemicals and possible toxins.

1.1.3. JUSTIFICATION

There are over 5000 local medicinal plants available as gift to this Nation but this gift has not been fully exploited and hence we import finished drugs, their active chemicals or both the raw materials and the technology of producing them (Awosika, 1991). In a bid to save our hard earned foreign exchange and extend medical services to the entire people of the nation, there is need to look inward for a cheap affordable and effective medical system acceptable to the majority of the population. This will also ensure a diversification in the sourcing of our medicaments and a reduction in our dependence on importation of drugs. This project will help in finding some of these objectives.

1.2. LITERATURE REVIEW

1.2.1 The Mahogany Tree

1.2.1a *Khaya senegalensis*

Khaya senegalensis is the dry zone mahogany widely distributed in the savanna regions, recognised by its round evergreen crown of dark shining foliage, pinnate leaves and characteristic round capsule (Keay *et al.*, 1964). In Nigeria it is called "Madachi" (Hausa), "Oganwo" (Yoruba), "Ono" (Ibo), "Aago" (Ebira) and "Ostachi Kpokpo" (Idoma). It is often planted by the road side for shades.

The matured tree on average is about 33.6m high and 3m in girth. It has dense crown and short bole covered with dark grey scaly bark which yield yellowish gum when wounded and dark pink pulp when slashed (John and Maria, 1962). The leaves are more or less eliptical and shortly accuminated at the apex. The stalk of the leaflets bears 3 - 4 pairs of leaves and the individual leaf is about 5 - 8 cm long and 2 - 4cm broad. The flowers have pale green sepals, cream petals and bright red disc beneath the ovary. The flower is brown in conspicuous pinnacle and is about 3cm long and 2cm broad.

1.2.1b OTHER SPECIES OF *KHAYA*

About 20 species of *Khaya* have been described but only the following five are distinct. They are *K. senegalensis, K. grandifoliola, K. nyasica, K. anthothecea* and *K. invorensis*. The species differ ecologically and it's unlikely that more than one or at most two species will be found in a single locality.

Khaya grandifoliola is one of the sources of African Mahogany, which is extensively used in carpentry. In central Africa a decoction of the bark is taken orally to relieve post

partum pains and decoction of root bark for blenorrhagia and as a local application to dermatitis. The finely powdered calcined bark mixed with indigenous salt and palm oil is taken for the relief of hyperacidity (John and Maria, 1962; Dalziel 1967).

In Tanzania and Mozambique the bark of *Khaya nyasika* is an ingredient in a decoction taken for the relief of colds. In Gaza, the crushed seed is boiled to extract the oil which has a bitter taste and rubbed into the hair to kill vermine. The plant has given negative antibiotic test (Daliziel, 1967).

Khaya anthotheeca is said to be poisonous and the bark is bitter and used as febrifuge in Angola. The trees exudate is a yellowish gum resin while the timber is used in making joinery and for other domestic purposes. The wood of *K. invorensis* has been found to contain β-sitosterol as well as resinous material of tannin nature and the bark is bitter.

1.2.2 USES OF *KHAYA SENEGALENSIS*.

1.2.2a Domestic uses of *K. senegalensis*.

Khaya senegalensis and other species of *Khaya* are well known economic plants throughout Africa where they are used in making carpentry, joinery and cabinet making. The Ubanji tribe of West Africa used it for making canoes and in Uganda the bark is used as fish poison (John and Maria, 1962). In Northern Nigeria the oil from the seed is used as vermifuge and the leaves for cattle and camel fodder. In Senegal and Northern Nigeria the bark is used for tanning.

1.2.2b **Medicinal Uses of *K. senegalensis*.**

Khaya sengalensis and other species are widely used as antimalaria herbs throughout West Africa (Zac, 1986) but the chemical(s) responsible for its antimalarial effect is (are) not fully investigated. It is widely used in traditional and Veterinary medical practice for conditions such as treatment of mucous diarrhoea, vermifuge, taenicide and syphilis (Dalziel, 1967). In Nigeria, a decoction of the bark is used as febrifuge, vermifuge, anticonvulsant and as general body tonic among others. Some of these uses have been pharmacologically substantiated (Kerharo, 1969; Adesina, 1983; Olayinka, 1992). Nwafor *et al.* (1995) reported that the aqueous extract of the bark of *K. senegalensis* inhibited indomethacin induced gastric ulceration in rats. Abdulrahman (1991) also reported that the bark of *K. senegalensis* is one of the ingredients used in the treatment of diarrhoea. It has been shown by Uma (1996) that the aqueous extract of the bark has a powerful antimicrobial action. Moreso, from oral tradition a decoction of the bark is found useful in the management of anaemia.

1.2.3 **Phytochemical Screening**.

The Phytochemical screening conducted by Olayinka (1992) revealed the presence of steroids in the petroleum ether extract of the leaf in addition to terpenes in the bark. In the same study the methanolic extracts of the bark showed the presence of glycosides, tannins, terpenes, saponins and anthraquinones while those of leaves showed the absence of terpenes and anthraquinone and a total absence of alkaloids. Nwafor *et. al.* (1995) showed the presence of tannins, saponin, phlobatannins and cardiac glycosides while the ionic analysis revealed that the aqueous extract contains Ca^{2+}, K^{+}, Na^{+} Cl^{-} ions in varying quantities. In addition to some of these chemical components mentioned above, Abdulrahman (1991)

reported the presence of oxalates and the bitter principle calcedrin which is reported to have a strong antiprotozoal effect. The gum exudate of the bark is found to contain various types of polysaccharides (Aspinall and Bhattacharjee, 1970). Nibosterol and its glycoside nibosterin which is the β - D - glycoside of β-sitosterol have been isolated from the gum. Other workers have also shown the presence of a complex mixture of limonoids, methyl angolensate and methyl-6-hydroxyangolensate (Taylor, 1969; Olarewaju *et al.*, 1978).

1.2.4 ACTIVE INGREDIENTS OF MEDICINAL PLANTS.

Generally speaking there exist many pharmacologically active ingredients in one plant product some of them having similar pharmacological activity and have exerting a synergistic effect, some having and antagonistic activity against each other, and some others protecting (chemically or physiologically) the decomposition of the active principles present in the herb (Takagi *et al.*, 1963).

Active ingredients most commonly fall on one of six categories: Alkaloids, glycosides, essential oils, gum and resins, fatty oils and antibiotics. From medical view points alkaloids and glycosides are the most important principles (Sanff, 1978).

1.2.4a **Alkaloids**.

These are diverse group of alkaline compounds with marked physiological activity, their ring structure is usually complex and always contains Nitrogen. The alkaloids include morphine, cocaine, nicotine, quinine and more than 5,500 other compounds with different pharmacological effects. More than 90% of the known alkaloids are found in flowering plants (Sanff, 1978; Trease and Evans, 1989).

1.2.4b **Essential oils**

Many crude drugs are used medicinally because of their volatile oil contents; however, the volatile oil separated from drugs are used as drugs themselves. They usually have various chemical constituents; terpenes derivatives or aromatic compounds often contain alcohol, ketones, phenol, ether, esters and some times sulphur and nitrogen. Many are highly germicidal, this property being due to their volatility and inability to penetrate into protoplasm but they usually are too insoluble in water to be important in medicine as antiseptic. They are valuable in cough drops, mouth washes, gargles, spray and healing ointments (Tyler *et al.*, 1988).

1.2.4c **Glycosides**

Glycosides are group of compounds which when hydrolysed yield components of one of several sugars (glycone) and non sugar component (aglycone). There are eleven (11) types of glycosides classified according to their aglycone moiety. Among the most important in modern medicine include the cardiac glycosides. For example digitalis, cyanogenic glycoside and steroidal sapogenins (Robinson, 1967). The anthraquinone and related glycosides are also useful as stimulant, cathartics and exerts their action by increasing the tones of the smooth muscle in the wall of the large intestine. Plant materials containing anthraquinones are normally used as purgative drug.

1.2.4d **Saponins.**

These are glycosides characterised by their property of producing frothing in aqueous solution. They are surface active agents which form molecular compounds with many sterols

and have haemolytic effect. Saponins are generally toxic to fungi and bacteria and are therefore of some pharmacological interest (Erich, 1977). The fact that a plant material contains saponin does not make the plant toxic. Saponins elicit their hemolytic effect when small dose is injected into the blood stream, but are comparatively harmless when taken orally (Trease and Evan, 1989).

1.2.4e **Tannins**

Tannins are a variety of phenolic plant constituents having an astringent taste and the ability to tan leather (Robinson, 1967). They are chemically divided into two groups, hydrolysable tannins and nonhydrolysable tannins. The hydrolysable tannins are readily hydrolysed to yield the phenolic acid and the sugar while non-hydrolysable or condensed tannins tend to polymerize, yielding insoluble, usually red coloured product phlobaphen when treated with hydrolytic agents (Tyler *et al.*, 1988). They are employed in medicines as astringents in the gastrointestinal tract and on skin abrassions. In the treatment of burns the proteins of the exposed tissues are precipitated and forms a mildly antiseptic, protective coat under which the regeneration of new tissue may take place.

1.2.4f **Gums and resins**.

Gums are polymers of various rare sugars and resins are oxidation products of essential oils; both are employed as purgative and ointment (Robinson, 1967).

1.2.4g **Fatty oils or lipids**

Esters of fatty acid are used in emulsions and as purgative (Tyler, *et. al.*, 1988)

1.2.5 **Anaemia**

An anaemic condition is one in which there is reduction in the number of erythrocytes, the quantity of haemoglobin, and the volume of packed red cells per 100ml of blood below normal for the age and sex of patients. This occurs when the equilibrium between blood loss (through bleeding or destruction) and blood production is disturbed (Bowman and Rand, 1980; Dorland, 1985).

1.2.5a **Etiology of anaemia**

Anaemia is not a disease in itself but rather a manifestation of an underlying disease process. The correct diagnostic terminology, therefore, requires the inclusion of the pathogenesis (Wintrobe *et al*, 1981; Oma, 1991). There exist diverse etiologies such as iso-autoimmuned disorders, hemolysis associated with haematologic diseases, metabolic disorders haemolysis due to infection; nutritional deficiencies, haemolysis due to physiological and chemical or toxic agents, heriditory spherocytosis and elliptocytosis, paroxisimal nocturnal hemoglobinuria and erythropoietic porphyria among others. (Izak *et al.*, 1966; Ernst, 1970;Douglas and John, 1975; Williams *et al.*, 1977; Hebbel *et al.*, 1981.

1.2.5b **General Symptoms**.

The symptom of anaemia are mainly referable to central nervous systems and the cardiovascular system and the severity of the symptoms corresponds only roughly to the

degree of anaemia. The mental state being characterised by lassitude, weakness and rapid onset of fatigue with shortness of breath and dysponoea on exertion.

In severe anaemia there may be faintness, drowsiness or even clouding of consciousness. Neurological effects include headache, tinitus and paraesthesia (Bowman and Rand, 1980). Other clinical features include skin pallor, cardiac arythmias gastrointestinal symptoms, increased basal metabolic rate, some changes in biochemical composition of the body. (John *et al.*, 1975; Wintrobe *et al.*, 1981; Bunn and Forget, 1984).

1.2.5c. **Types of Anaemia**

1.2.5c (i). **Iron deficiency anaemia**

Iron deficiency anaemia is one of the most import public health problem facing many developing countries. It is the commonest cause of anaemia seen in clinical practice (Oma, 1991); Perry (1964) reported that the most common anaemia occurring during pregnancy result from Iron deficiency. The possible factors that could lead to this disorder include, insufficient Iron intake in the diet, impaired absorption and loss of blood (Oma, 1991). The definitive diagnostic sign are a low erythrocyte count, low blood haemoglobin, low mean corpuscular haemoglobin (MCH) and low mean corpuscular volume (MCV) and normal white blood cell counts and platelets. Oral or parenteral administration of Iron preparatives is adequate in the therapy of anaemia due to Iron deficiency (Bowman and Rand 1980; Oma, 1991).

1.2.5c (ii) **Anaemia due to vitamin B_{12} and Folic acid deficiencies**.

Vitamin B_{12} and folic acid are required for the synthesis of purines and pyrimidine bases and their incorporation into DNA. In adult, erythropoiesis exerts the greatest demand

for these bases, since 10 - 20g of cells are formed per day (Bowman and Rand, 1980). It has been reported by several investigators that increased blood production accompanying haemolytic disease is usually associated with folic acid deficiency (Charinain *et al.*, 1959). A deficiency in either vitamins results in faulty cell division and maturation. In many of such instances the bone marrow shows megaloblastic transformation which reverts to normal following treatment with the appropriate vitamins.

Izak *et al.*, (1966) reported that massive haemorhage and phenylhydrazine induced hemolysis produce profound decrease in folate activity in rats. The rate of cell formation is decreased because of slower and less frequent mitosis than normal, and also because of destruction of some partly matured cell within the bone marrow. There is therefore a megaloblastic macrocytic anaemia. There is further reduction of RBC and haemoglobin concentration due to short life span of macrocytes than erythrocte (Bowman and Rand, 1980). However the MCHC is normal while the MCH is higher than normal/. Plasma bilirubin levels are raised and iron stores are saturated (unless there is an associated Iron deficiency).

1.2.5c (iii) **Sideroblastic anaemia**

A less common type of anaemia termed sideroblastic anaemia is produced by pyridoxine defiency. Sideroblastic anaemia encompases a heterogenous group of disorders characterised by excessive accumulation of Iron due to defective haem synthesis within the mitochondria of normoblast (Nicholls *et al.*, 1984). Verloop and Rademaker (1960) showed that the large number of sideroblast in the patients with sideroblastic anaemia is correlated with the pyridoxin deficiency. The decarboxylase enzyme engaged in the synthesis of

protoporphyrin requires Pyridoxal phosphate as a co-enzyme. Deficiency of the vitamin precusor of the co-enzyme impairs synthesis so that there is an excess of iron available in the developing erythrocyte in relation to the amount of the protoporphyrin for the formation of haem.

In pyridoxin deficiency there is usually a disturbance of tryptophan metabolism as a result an abnormally large amount of xanthuremic acid excreted in the urine particularly after tryptophan administration. The tryptophan tolerance test is the most sensitive method of demonstrating pyridoxin deficiency (Bowman and Rand, 1980).

1.2.5c (iv) **Anaemia of chronic disorder**

Anaemia of chronic disorder is characterised by slightly shortened red cell life span, a disturbed iron metabolism and an impaired compensatory increase in the rate of red cell production (Douglas and John, 1975). This syndrome known as anaemia of chronic disorder is characterized by a low index marrow erythroid: granulocytic (E:G) ratio of 1: 2-3, hypoferremia with low to normal transferine levels, increase red cell protoporphyrin and reduced or absent marrow sideroblasts in the presence of reticuloendothelial Iron stores (Cartwright, 1971). There is normal MCV while the MCH and MCHC are low (Thompson and Proctor, 1987).

The chronic disorders that are frequently accompanied by anaemia include infection of any type and various chronic inflammatory disorders. The former group which include tuberculosis, oesteomyelitis and subacute bacterial endocarditis. The later group is chiefly represented by rheumatoid arthritis but also include other collagen diseases, and malignant

diseases. It has been recognised that non infectious disorders, such as Hodgkins disease or metastatic carcinoma are associated with anaemia (Douglas and John, 1975; Berner, 1983).

1.2.5c (v) **Sickle Cell anaemia**

Sickle cell disease is characterised by a chronic haemolytic anaemia with intermittent crises. The majority of these crises, so called painful or intractive crises due to micro vascular occlusions of widely distributed organs are generally attributed to the abnormal shape and decrease deformability of sickled red blood cells and their inability to pass through small vessel (Vordemeier *et al.*, 1992) Sickle RBC are reported to adhere more readily than normal RBCs to cultured human endothelial cells during both static and flow condition and this enhanced adherence is directly related to the clinical vasocclusive severity (Hebbbel *et al.*, 1981). This occurs in homozygous for the gene coding for 6-val variant of normal β- Chain in (6 - glu). After infancy the blood contains mainly hemoglobin S which can be designated $\alpha_2^A\beta_2^s$; Haemoglobin A is absent (Bowman and Rand, 1980). In deoxygenated state haemoglobin S is much less soluble than normal haemoglobin and the solubility is decreased still further by a slight fall in pH. Consequently when the oxygen tension is low and there is acidosis the haemoglobin S forms crystalline structure and the erythrocyte is deformed from disc into an elongated form generally described as sickled shape.

Another form of haemoglobin called haemoglobin C is closely related to haemoglobin S, containing a variant amino acid at the 6 position in the β - chain. The gene has high incidence in parts of West Africa (Ghana and adjacent countries) Homozygotes for haemoglobin C and S have a mild to moderate form of sickle cell anaemia (Bowman and Rand, 1980).

1.2.5.c (vi) **Thalassemias**

In thalassemia globin chains are structurally normal in contrast to Hbs and Hbc but are produced in inadequate amounts. There are 2 types of thalassemia, the α -thalassemias and β-thalassemias. The α - thalassmias have decreased or absent β - globin chain synthesis while β - thalassemias have decreased or absent α - globin chain synthesis (Berner, 1983). Because normal adult haemoglobin (HbA) is comprised of two α- chains and two β- chains, there are varying degrees of impairment of HbA formation in both types of thalassemia. Thalassemia may be inherited in combination with other Hb variant such as Hbs (Essan, 1979; Berner, 1983). The distribution of β- thalaassemia geographically corresponds fairly well to the areas where malaria was endemic for example Sardinia and Southern Italy.

1.2.5(vii) **Aplastic Anaemia**

The term aplastic anaemia resulting from reduction or loss of hematopoeitic tissue, its replacement by fatty bone marrow mass, is believed to be caused by toxic radiant or immunologic injury to bone marrow stem cell or their micro environment, reducing their capacity for normal cellular renewal (Allan, 1977). It usually extends to all types of precursors of blood cells so that the anaemia is accompanied by leucopenia, thrombocytopenia. Most cases of aplastic anaemia are induced by drugs or chemicals; this effect is termed myelotoxicity.

1.2.6 **Hemolytic Agents and Toxins**

Many drugs and toxins, some haemolytic *in vitro* and some not, produce lysis *in vivo*. Benzols, phenols and many organic solvents used in industries, arsenics, phenylhydrazines,

sulphonamides and phenancetin are some of the agents that are known to produce acute or slowly developing hemolytic anaemias (Emerson *et al.*, 1946; Ponder, 1971; Gordon - Smith, 1980). The way in which some of these substances act is often obscure. In some cases there may be injury to the circulating red cells either resulting directly in haemolysis or in agglutination which is followed by heamolysis. There is evidence that the effect of many drugs is determined to some extent by individuals sensitivity and that it may be related to the state of the animal nutrition (Luzzatto, 1979). Ingestion of fava beans produces severe haemolytic anaemia in a sensitive individual.

Many bacteria produce hemolysis the potency of which may be very dependent on the age of the culture medium. Hemolysis *in vivo* is known to follow infection with hemolytic varieties of *E. coli* and of *Streptococcus hemolyticus* and *Streptococcus validans* (Ponder, 1971)

The malaria parasite and two forms of Bartonella (*Bartonella bacilliforms* which causes oroya fever and *Bartonella muris* that causes anaemia in dogs and rats) produce red cell destruction *in vivo*. In this case the destruction of the cell is almost certainly mechanical (Gutheridge and Coombs, 1977).

1.2.7 **Metabolism of erythrocyte.**

Erythrocytes contain 35% solid materials which is almost all haemoglobin (32%). The remaining solids are proteins and lipids which form the stromal network for the support of the haemoglobin. The membrane is freely permeable to water, Urea, creatinine, HCO_3^-, Cl^-, OH^- and H^+. The transport of Na^+, K^+, Ca^{2+} and perhaps P_i; across the erythrocyte membrane requires energy. The phosphate content of the RBC is 50 - 100 fold greater than in plasma,

most of which is organic, that is, hexose phosphate, triose phosphates, and ATP (Thomas, 1986).

The metabolic fuel of the erythrocyte is glucose which is utilised by glycolysis. The energy of ATP is necessary to preserve the intergrity of the erythrocyte and is also used for the membrane transport of iron (Ernst, 1970). The NADH from glycolysis is used for the conversion of methemoglobin to hemoglobin and for the conversion of pyruvate to lactate. Hexokinase, phosphofructokinase, pyruvate kinase, glyceraldehyde - 3 - phosphate dehydrogenase, and phosphoglycerate kinase are implicated in the control of glycolysis (Thomas, 1986). A major factor in the activation of glycolysis in the erythrocyte is inorganic phosphate. Approximately 10% of the glucose metabolised by the erythrocyte is by hexose monophosphate shunt. The ratio of the oxidized to reduced form of glutathione, present in large amount in the erythrocyte, the amount of H_2O_2 in the RBC break down product of NAD, 2' - Phosphoadenosine diphosphate ribose are implicated in the control of the shunt (Ernst, 1970). The glutathione and H_2O_2 may well control the rate of shunt metabolism by altering the ratio of the reduced to oxidized form of NADP.

1.2.8 **Phenylhydrazine and its mode of action.**

Phenylhydrazine is a toxic nitrogen base compound $C_6H_5NH.NH_2$ made by reduction of benzenediazonium chloride. It is used as a reagent for sugar, aldehyde, ketone and related compound. Phenylhydrazine and its hydrochloride derivative have been extensively used experimentally to study oxidative haemolysis and were previously used for the treatment of polycythaemia vera (Gordon - Smith, 1980).

It has been known for many years that parenteral injection of phenylhyldrazine or its less toxic ethyl derivative induces a profound haemolytic anaemia. It has been observed that following the administration of Phenylhydrazine, there is a decrease in NADPH/NADP ratio; a decrease in reduced glutathione and an increase in methemoglobin level and mechanical fragility of the red cell. (Ferrali, *et al.*, 1992). It is assumed that the increasing influence of the drug on the rate of oxidation of haemoglobin to methemoglobin renders the protective mechanism (GSH - Peroxidase and catalyse) inadequate.

Ferrali *et al.*, (1992) also observed a rapid release of iron accompanied by methemoglobin formation in mouse erythrocyte incubated with phenylhydrazine. The erythrocyte were depleted of GSH by the short incubation with phenylhydrazine; and the release of iron was accompanied by lipid peroxidation and subsequently haemolysis.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1(a) *Chemicals.*

Unless otherwise stated all reagents used were of analytical grade obtained from British Drug House (BDH) Poole, England.

2.1(b) Equipment

Quick fit soxhlet extractor apparatus, EEL - type flame photometer model M3001, Pye Unicam atomic absorption spectrophotometer model 290B and Hemocytometer model 601MD were used during the experiment.

2.1 (c) Collection and Preparation of the plant materials.

The stem bark of matured *K. senegalensis* was collected from three different locations in Zaria viz: Ahmadu Bello University main campus Samaru, Bassawa and Bomo villages. The preparation was as is done locally whereby samples were pooled, thoroughly washed and sun dried for one week. The dried bark was pound to a coarse powder using wooden mortar and pestle and then stored in a dry container until needed.

2.2 Methods

2.2 (a) Preparation of the aqueous extract of the plant material and determination of its solid content.

Aliquots of 30g of the powdered sample was soaked for 5 minutes in 500ml of cold distilled water. This was then boiled for 15 minutes and the boiled sample was allowed to

cool and settle. The supernatant was decanted and filtered through a thimble. The filtrate was made up to a final volume of 500ml which was stored in a screw corked brown bottle and refrigerated till used.

The solid content of the extract was determined by the method of Aguwa and Mittal (1983). In this, 20ml of the preparation above was evaporated to dryness and then heated to a constant weight on a petri dish. The final weight is taken and solid concentration expressed in mg/ml.

2.2 (b) **Preparation of Methanolic extract of the plant material.**

In this, 100g of the powdered sample was extracted in methanol using soxhlet apparatus. The extract was evaporated to dryness in a rotary evaporator to remove the methanol. The dry methanolic extract was reconstituted in 20% Ethanol to a desired concentration for the treatment.

2.3 **Determination of some mineral content of the plant extract (AOAC 1980).**

2.3.1 Methods used

The methods used to determine the mineral content were Atomic absorption spectrophotometry and flame photometry.

Principle: In atomic absorption spectroscopy, a solution containing the element of interest is passed through a cool flame. In terms of energy, the bulk of element atoms were in the ground state. Radiation of a characteristic wavelength from a suitable hallow cathode lamp is then passed through the flame. The atoms will now absorb some of the radiation and become excited. The resulting decrease in the intensity of the radiation is then measured using a

detector system. The extent of absorption is dependent on the number of the absorbing atoms and the decrease in the intensity is therefore related to the concentration of the element in solution.

In flame photometry, the atoms of the elements were excited by flame and on returning to the ground state the emitted radiation characteristic of the element concerned. The intensity of the radiation emitted is directly proportional to the number of emitting atoms and hence the concentration of the element present.

2.3.2 Sample Preparation

The aqueous extract (5ml) was evaporated to dryness and the dried sample was digested into a solution by mixed acid digestion method (Allen, 1974) as described below. The dried sample was placed in a 300ml kjeldahl digestion flask and to it were added 10ml of 96% concentrated nitric acid, 0.5ml sulphuric acid (98%) and 1ml of 70% perchloric acid. The mixture was then digested initially at moderate heat and later more vigorously until the evolution of white fumes. The digest was allowed to cool, diluted with distilled water, transferred into 50ml volumetric flask and made up to mark with distilled water. A blank determination was carried out. The final solution was subjected to atomic spectroscopic analysis for calcium, magnesium, zinc, Iron, Copper in a pye unicam atomic absorption spectrophotometer, model 290B. The potassium and sodium were analysed for, in an EEL flame photometer since they can not stand the operational temperature of the atomic absorption spectrophotometer without ionisation.

2.4 Quanlitative Phytochemical Studies of *K. Senegalensis*

The powdered samples of plant materials were separately extracted with different solvents namely petroleum ether (60-80°C) and methanolic extracts were obtained by continuos extraction in soxhlet for 24 hours at 100°C while the aqueous extract was by hot percolation. The extracts were evaporated to dryness and stored in a powdered form in a dessicator until required.

2.4.1 General Test for Carbohydrate.

A 1gm portion of each powdered extract was dissolved in 5ml of distilled water and subjected to the Molisch's test. A purple colour indicated the presence of carbohydrate (Trease and Evans, 1984).

2.4.2 General Test for glycosides.

Aliquotes of 0.5g portion of each powdered extract was placed in two test tubes. Dilute sulphuric acid (5ml) was added to one tube and distilled water (5ml) was added to the other. The mixture in both test tubes were heated in a beaker of boiling water for 10 minutes and then allowed to cool. The cooled solutions were made alkaline with 20% potassium hydroxide solution and heated with Fehling's solution for 5 minutes. The formation of a reddish brown precipitate in the test tube containing the powdered extract and sulphuric acid is taken as positive and the solution of the extract in distilled water serves as negative control.

2.4.2 (a) Test for cardiac glycosides.

To extract the glycoside, 0.5g of the powdered drug was boiled with 70% ethanol for 2 minutes. The mixture was cooled and filtered. The filtrate was diluted with an equal volume of distilled water followed by the addition of 3 drops of strong solution of lead subacetate. The mixture was shaken thoroughly and filtered. The filtrate was divided into 2 portions. A portion of the filtrate was extracted in a separatory funnel with 5ml of chloroform. The chloroform (lower layer) was allowed to run off into a small evaporating dish and the solvent carefully removed by gentle heating on a hot plate. The residue mainly glycoside was used for the test.

The residue in the evaporating dish dissolved in 1ml of glacial acetic acid containing a trace of Iron III chloride solution. This solution was carefully poured on the surface of about 1ml of 2M H_2SO_4 already contained in another tube so as to form separate layer. On standing a brown or red colour at the interface is positive for deoxysugars, a pale green or blue colour at the upper layer (due to steroid nucleus) are produced by cardiac glycosides.

2.4.2(b) Test for Steroidal Aglycone.

As in 2.4.2(a) above, 1ml of the alcoholic filtrate was mixed with 1ml of 2% (v/v) solution of 3,5 dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5% (w/v) NaOH solution. A purple-brown colour was produced in the presence of steroidal aglycone.

2.4.2 (c) Test for cyanogenic glycosides.

A little quantity (0.5g) of the powdered bark was soaked in a clean test tube with enough distilled water to cover it. A moist sodium picrate paper was suspended in the neck of the tube trapping it by means of a cork. The closed test tube was placed in the oven at 45°C for 1 hour. A blue black colour indicate the presence of cyanogenic glycosides.

2.4.3 **Test for Anthraquinones.**

Powdered bark (1g) was boiled in 5ml of 10% (v/v) HCl, filtered hot and the filtrate shaken with 10ml of benzene. To the benzene layer was added 10% (v/v) ammonium hydroxide (half the volume of benzene). Pink or violet colour in the ammoniacal layer was taken as an evidence for the presence of combined anthraquinones. Commercial sample of anthraquinone was used in place of the extract for the positive control (Trease and Evans, 1984).

2.4.4 **Test for saponins**

2.4.4(a) Frothing Test for Saponins.

Here 0.5g of the dried plant extract was shaken with 10ml distilled water in a test tube for 1 minute. The mixture was kept aside and observed for persistent frothing for 2 minutes.

2.4.4(b) Blood Haemolysis Test for Saponins.

One gram of the powdered plant extracts was dissolved in 5ml of water. 1ml of this solution was mixed with 1ml of blood sample in normal saline and set aside for 3 hours and observed for haemolysis. A positive control test was carried out using 1% solution of

saponin. For the negative control, 1ml of distilled water was used in place of the extract (Wall *et al.*, 1952).

2.4.5 **Test for Tannins**.

One gram of the powdered extracts each was boiled with 10ml of distilled water for 5 minutes. To 2 ml of the cooled solution, 1 drop of ferric chloride test reagent was added. A blue or green colour was taken as positive.

2.4.6 **Test for Steroid and Terpenes**.

Powdered extract (5g) was dissolved in a small amount of chloroform and subjected to Liebermann Burcard's test. A bluish green or blue colour was taken as positive for steroid, a pink colour for terpenes and a brownish colour was interpreted as negative test (Yen and Yang, 1971). This was repeated with the petroleum ether extract.

2.4.7 **Test for Alkaloids**.

One gram of the dried powdered extract each was mixed with 5ml NH_4OH followed by extraction with 25ml chloroform (in small portions). The chloroform extracts were pooled and evaporated to dryness and the residue obtained was redissolved in 5ml of 0.5M hydrochloric acid. Then two 2ml portions of the acidic filtrate were separately treated with Mayer's and Dragendorff's reagents. Tests were interpreted as positive when turbidity or moderate to heavy precipitate were obtained.

2.5 Animal *in vivo* Experiments

2.5.1 Maintenance of the animals.

White albino rats (Wistar Strain) were obtained from Institute of Agricultural Research (IAR) A.B.U. Zaria. The animals were housed in plastic cages with wire gauze cover and saw dust bedding. They were fed with Sander's feed growers mash and watered on an *ad libitum* basis. The weights of the animals were monitored throughout the period of the experiment on alternate days.

2.5.2 Blood sampling and Analysis

The rats were bled from the tail to measure the packed cell volume (PCV) using microhaematocrit method (Schalm *et al.*, 1975). The blood from each rat was used to fill two heparinised capillary tubes (up to two third of its length), one end of the tube was sealed with Bunsen burner flame and centrifuged at 12,000g for 3 minutes in microhaematcrit centrifuge. Average of 2 determinations was used throughout this project.

2.5.3 a. **Induction of Anaemia**.

2.5.3a(i) Determination of suitable dose of Phenylhdrazine - HCl for the induction of anaemia.

For this investigation, 12 rats were used. They were divided into 4 groups of 3 rats each and housed in separate cages. Each cage labelled I, II, III and IV. Groups I, II, and III were given subentaneous injection of 2.5% (w/v) neutralized Phenylhydrazine - HCl solution at the starting dose of 750, 950 and 1,200mg/kg body weight respectively, Group IV served as control and was given 0.4ml of distilled water subcutaneously. The test groups were given maintenance dose of phenylhydrazine - HCl half the initial dose.

2.5.3a(ii) Chemical Induction.

Anaemia was induced by subcutaneous injection of appropriate volume of 2.5% (w/v) neutralized phenylhydrazine - hydrochloride in the rats. (Harris and Kugler, 1971). The drug (phenylhydrazine - HCl) was given at a starting dose of 750mg/kg body weight and maintenance dose of 350mg/kg body weight on alternate days for duration of the experiment.

2.5.3 b. **Parasitemia induced anaemia**

2.5.3 b (i.) Parasitemia Induction

Trypanosoma brucei brucei was obtained from the Department of Parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The parasite was maintained in mice and caused death at a dose of 10^4 parasite in laboratory mice in 28 - 33 days (chronic infection).

The rats in each group were intraperitoneally inoculated with about 10^2 *T. brucei brucei* obtained from donor mouse's blood. The blood was diluted with Phosphate saline glucose (PSG) and 0.1ml of each was inoculated into healthy rats using 1ml syringe with 26 gauge needle. The level of parasitemia was routinely monitored by wet film preparation from the tail vein on a slide and examined on microscope (x 40 objective lense).

2.5.3b(ii) Counting of parasite.

To count the parasite 5µl of fresh tail blood from the infected Rat was obtained and diluted with about 5µl of PSG on a slide. It was then covered with a cover slip and examined at x 400 magnification. A minimum of 20 fields were examined to observe and count the motile parasites.

Dilution 5µl: 5µl

Dilution Factor (DF) = 2

Total number of parasite in 20 fields = Tp.

∴ Number of parasite per ml of blood = Tp x DF x 10^4.

2.6.1 Determination of the Effect of Different Subcutaneous doses of *K. senegalensis* Extract on Weight and PCV of Rats after Two days of the Onset of Phenylhydrazine - HCl Induced Anaemia.

Anaemia was induced in 12 rats divided into 4 groups (3 rats in each). Each group was given subcutaneous injection of 750mg/kg body weight of 2.5% (w/v) and were treated after 2 days of the onset of anaemia, with the aqueous extract of *K. senegalensis*. Groups I, II and III were treated with 10, 20 and 30 mg/kg of the extract respectively and group IV served as anaemic control that was not treated. Aneamia was not induced in groups V and VI but animals in these groups were treated with 30mg/kg body weight of the extract and 3ml distilled water respectively. The treatments were administered subcutaneously.

2.6.2 Effect of Methanolic and Aqueous Extract of *K. senegalensis* on PCV, Weight and Parasitemia of Rats after Two days of the Onset of *Trypanosome brucei brucei* induced Anaemia.

Twenty - one rats were used and they were divided into 7 groups of 3 rats each Groups I to IV were infected with *T. brucei brucei* as in section 2.5.3(b)(I) while groups V to VII served as control groups. On day 5 after the parasitema was massive (5.5 x 10^6 ± 3.8 x

10^6), Group I was treated with 30mg/kg body weight and Group IV was given distilled water as control. Similarly, group II was given 30mg/kg of methanolic extract (reconstituted in ethanol) and group III 20% ethanol as control. Groups V, VI and VII were not infected but were given 30mg/kg body weight of the aqueous extract, 30mg/kg body weight of the methanolic extracts and distilled water respectively.

2.6.3 Determination of the Effect of Different Oral Doses of the Aqueous Extract of *K. senegalensis* on Phenylhydrazine Induced Anaemia.

Twelve rats were used and were divided into 6 groups of 2 each. Anaemia was induced in all of them. Groups I to V were treated with 100, 200, 300, 400 and 500mg/kg body weight of *K. senegalsis* aqueous extract respectively by oral administration. Group VI served as control and was only given distilled water orally.

2.6.4 Effect of Oral Administration of 300 mg/kg body weight of the Aqueous Extract of *K. senegalensis* Before and After the onset of chemically induced anaemia.

Twenty five adult male and female rats were used. They were divided into 5 groups of 5 rats each. The rats in each group were further separated based on their sex to avoid pregnancy during the course of the experiment. Each rat was labeled appropriately. Each group labeled in Roman Figure I to V and was treated as follows:

Group I: The appropriate aqueous extract and 2.5% (w/v) phenylhydrazine were given simultaneously from day 1.

Group II: The extract was given on day 2 after the induction of anaemia by phenylhydrazine.

Group III: Anaemia was induced but not treated with the aqueous extract. This is to serve as positive control.

Group IV: Anaemia is not induced and members are only treated with the extract. This serves as negative control.

Group V. This group was only treated with distilled water. This is to serve as control for the possible effect of both the extract and phenylhydrazine.

CHAPTER THREE

3.0 RESULTS

3.1. Phytochemical content.

On table 3.1 is shown the result of the qualitative phytochemical screening conducted using the petroleum ether (40 - 60°C), methanolic and aqueous extract of the stem bark. Present in the methanolic and aqueous extracts were glycosides, saponins, tannins, and carbohydrates while terpenes and anthraquinones were found only in the methanolic extract. The petroleum ether extract contained only steroids. There were no alkaloids and cyanogenic glycosides in all the extracts (see table 3.1).

3.2 Mineral Content.

On table 3.2 is shown, some of the mineral contents of the aqueous extract. Potassium was highest (1780.8 ± 70.9 mg/100g) while copper was lowest (4.2 ± 1.0mg/100g). There is also appreciable amount of calcium, magnesium, sodium, iron and zinc in the extract.

3.3 Suitable dose (mg/kg body weight) of 2.5 w/v phenylhydrazine - HCl for induction of anaemia.

The suitable dose of phenylhydrazine for induction of anaemia was determined from the result of the experiment shown on fig. 3.1. Group I which received 750mg/kg body wt survived and gave a desirable anaemic condition while groups II and III that received 950mg/kg body wt and 1200mg/kg body weight respectively died on days 3 and 4 respectively. The control group maintained its normal PCV and weight. The effect of

Table 3.1 Some Phytochemicals Present in the bark of *K. senegalensis*.

	Alkaloids	Glycoside-s	Cardiac Glycosides		Cyanogenic glycosides	Saponins		Steriods	Terpenes	Tannins	General carbohydrates	Anthraquinones
			with deoxy sugars	with aglycone ring		Blood heamolysis test	Frothing test					
Methanolic etract of the stem bark	-	++	-	+	-	+	++	-	+	+++	+++	++
Methanolic extract of defatted bark	-	+++	-	+	-	+	++	-	+	+++	++	++
Petroleum ether extract of stem bark	-	TN	TN	TN	TN	TN	TN	++	-	TN	TN	-
Aqueous extract of stem bark	-	++	-	+	-	+	++	-	-	+++	+++	-

KEY: +++ = Copiously present.

++ = Moderately present.

\+ = Slightly present.

\- = Absent.

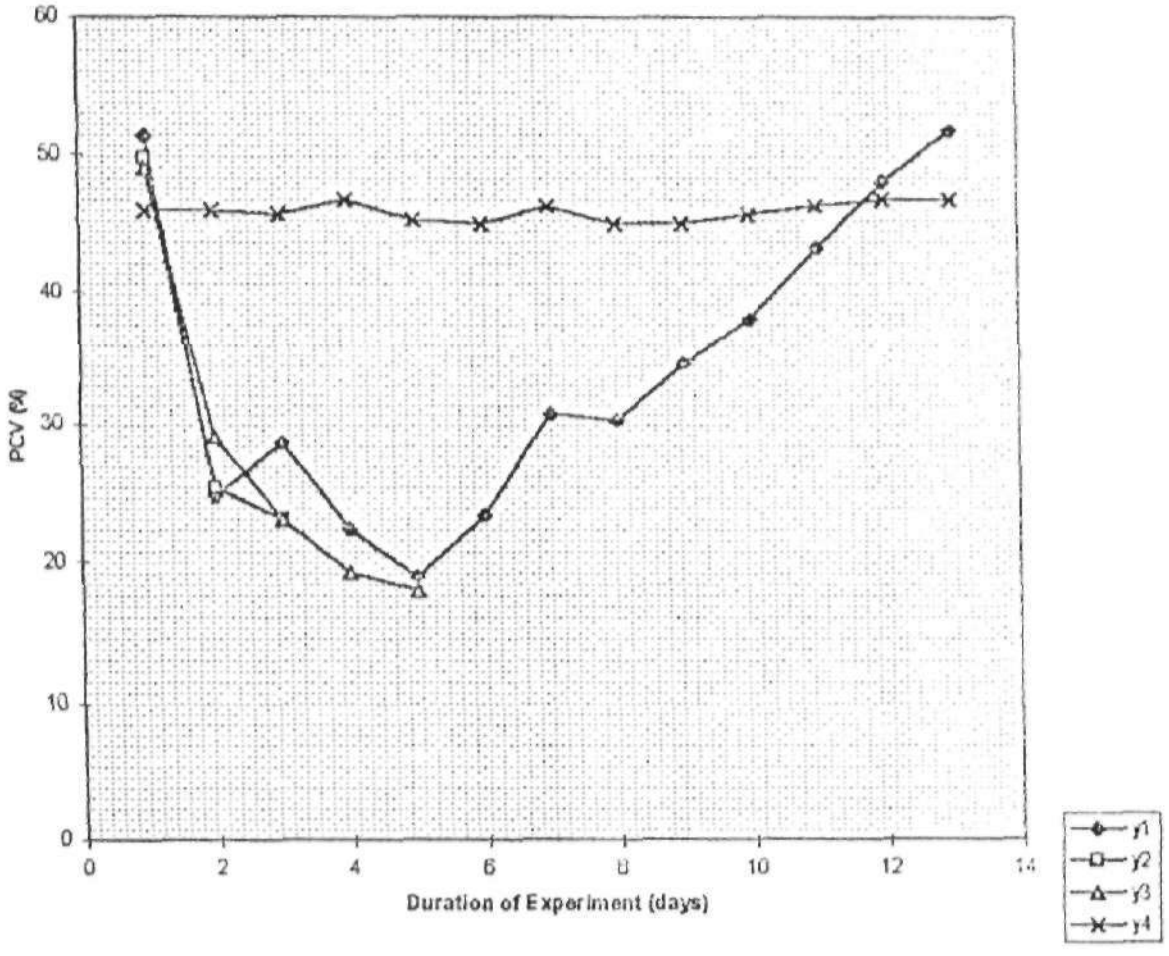
TN = Test not applicable.

Table 3.2: Some mineral content of the aqueous extract of the bark of *K. senegalensis*.

Element	Content (mg/100g)
Calcium	453.3 ± 3.5
Magnesium	230.5 ± 2.6
Zinc	12.4 ± 1.3
Iron	47.3 ± 3.2
Copper	4.2 ± 1.0
Potassium	1780.8 ± 70.9
Sodium	90.5 ± 4.2

Note: The total extract obtained from 30g of dried bark was 500 ml.

Fig 3.1



The effect of various ooses of 2.5% w/v Phenylhydrazine-HCl on PCV of wistar rats

Y1 was given 750mg/kg body weight.
Y2 was given 950mg/kg body weight.
Y3 was given 1200mg/kg body weight
Y4 was given distilled water (Control)

NB: Experimental animals in group Y2 and Y3 died before the end of the experiment.

Each point on the graph represent an average value of 3 determinations (see appendix 1 for standard deviation).

phenylhydrazine on weight is shown on fig 3.2. The patterns of changes in the weights of the animals seem to correlate with that of changes in PCV.

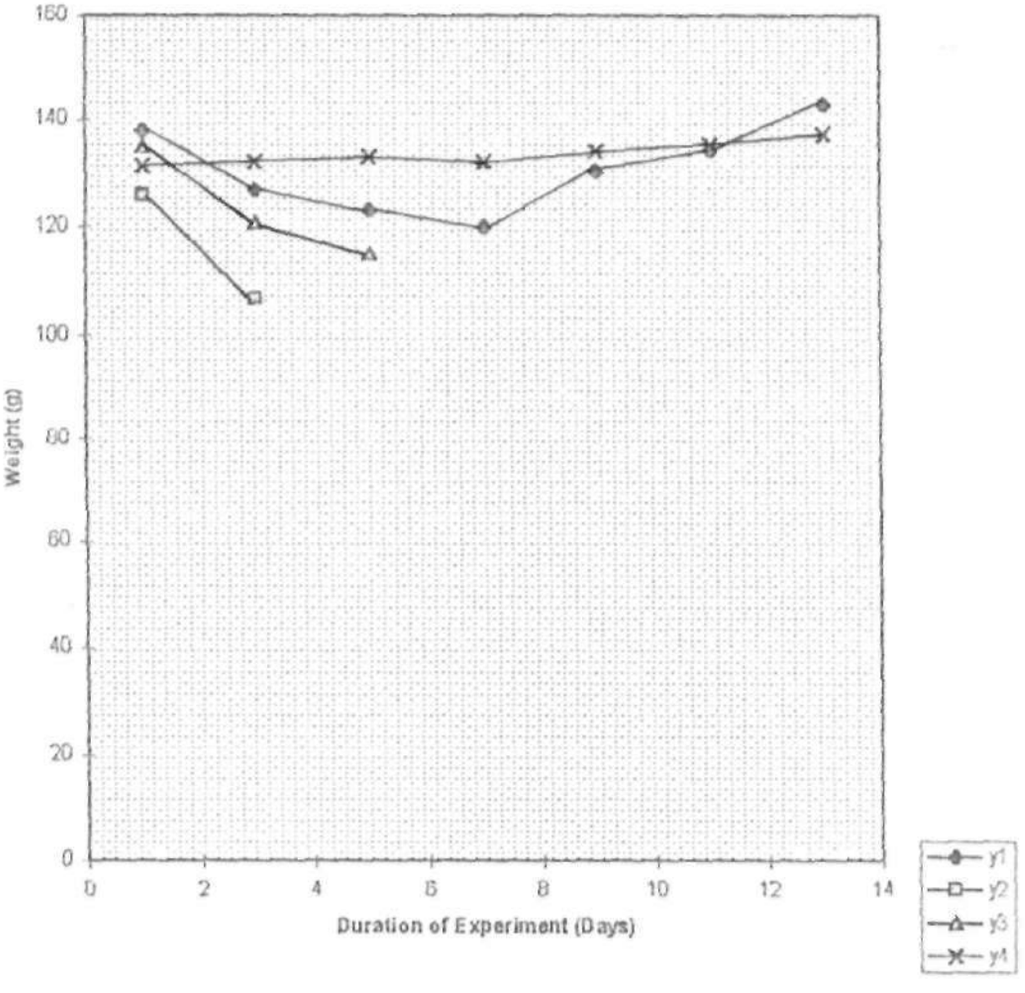
3.4 Effect of different subcutaneous doses of *K. senegalensis* aqueous extract on weight and PCV of rats after 2 days of the onset of Phenylhydrazin - HCl induced anaemia.

All the animals in groups I and II that were given the extract at the dose of 10 and 20mg/kg body weight respectively, died and those of group III with a higher dose (30mg) survived though without any noticeable improvement in the anaemic condition as compared to the control group (group IV) which was anaemic but not treated (figs. 3.3 and 3.4). There was a progressive loss of weight in all the animals treated with phenylhydrazine and the extract. Groups V and VI which were given 30mg/kg body weight of the extract and distilled water respectively, show no changes in their PCVs and weights.

3.5 Effect of different oral doses of *K. senegalensis* Aqueous extract on PCV and weight of rats after 2 days of the onset of Phenylhydrazine induced anaemia.

Oral doses of 100, 200, 300, 400 and 500mg/kg body weight were used to treat 5 groups of anaemic rats (each dose to a group). No improvement was noticed in their anaemic condition, instead there was a progressive and significant weight loss ($P < 0.001$) in all groups except the group III which was given 300mg/kg body weight which showed a tolerable loss of weight (7.1%) and the recovery rate seemed to be faster in that group including the control groups as presented in Figs. 3.5 and 3.6.

Fig3.2.

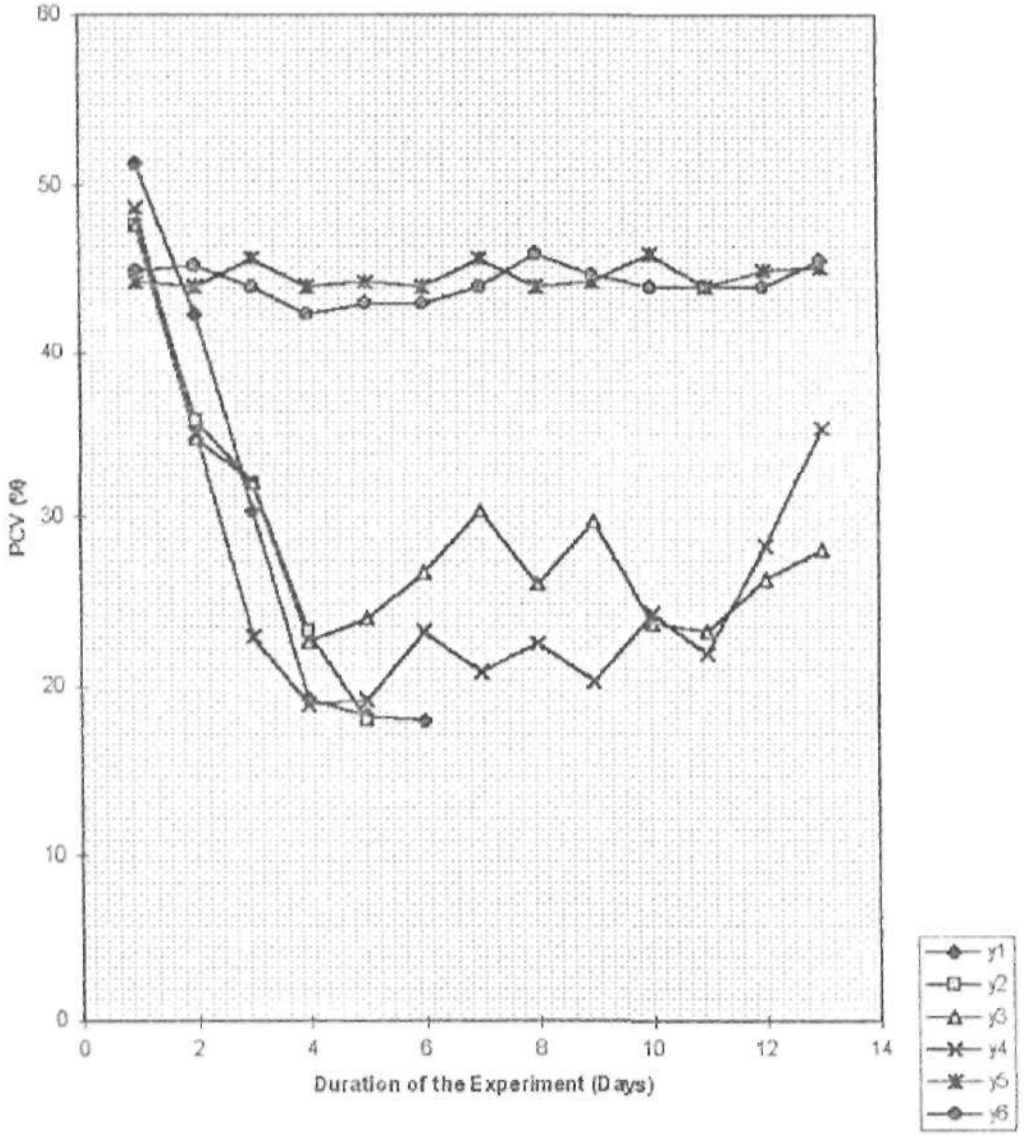


The effect of various doses of 2.5% w/v of phenylhydrazine-HCl on weight of wistar rats .

Y1 was given 750mg/kg body weight
Y2 was given 950mg/kg body weight.
Y3 was given 1200mg/kg body weight
Y4 was given distilled water (Control).

NB: Experimental animals in Y2 and Y3 died before the end of the experiment. Each point on the graph represent an average value of three determinations (see appendix 1)

Fig 3.3.

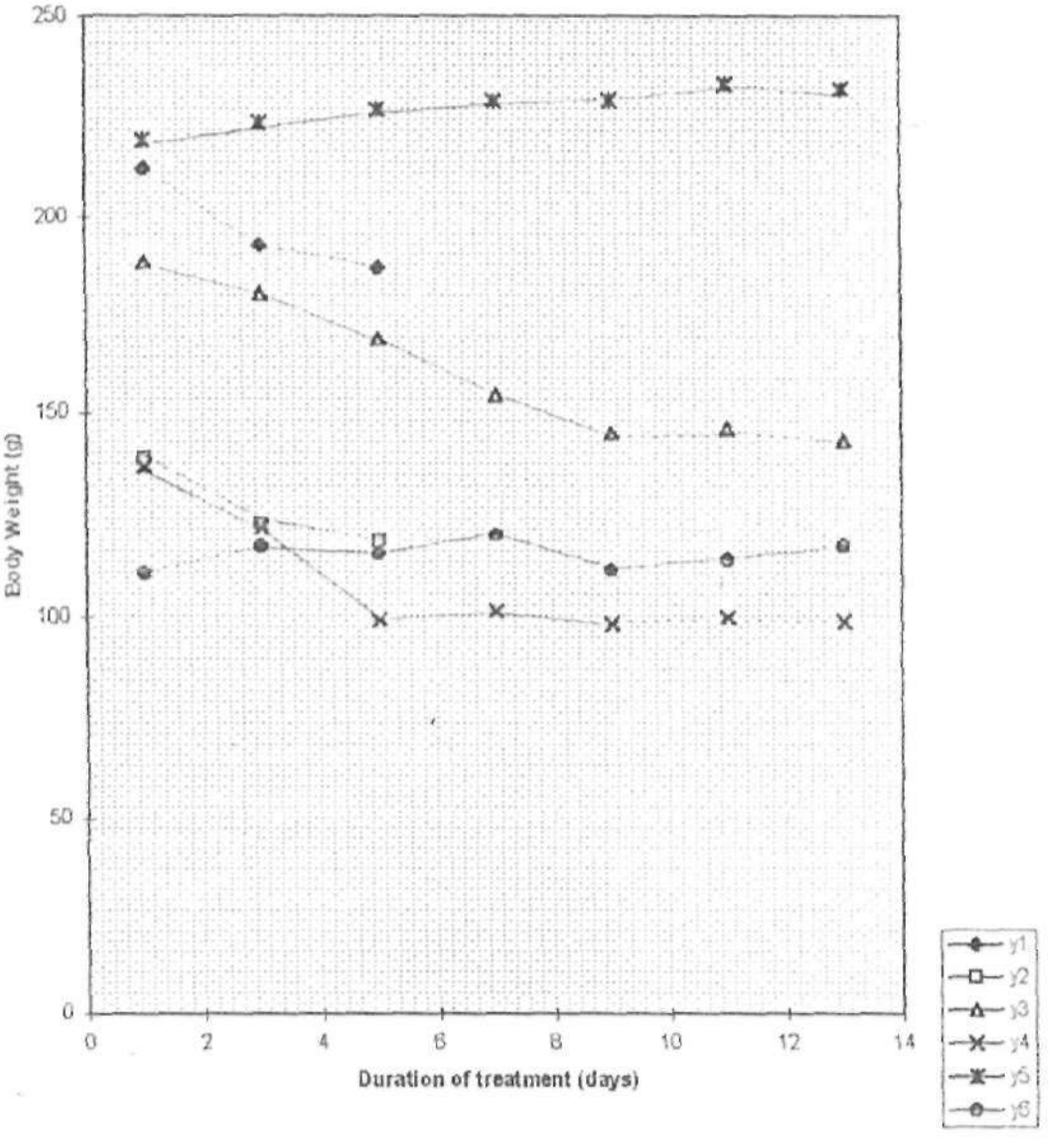


The effect of various doses of aqueous extract of *K. senegalensis* subcutaneously administerd to the experimental rats on PCV from day 2 after the onset of Phenylhydrazine induced anaemia.

Y1 was given 10mg/kg body weight. Y2 was given 20mg/kg body weight.
Y3 was given 30mg/kg body weight. Y4 anaemic but not treated.
Y5 not anaemic but treated with the extract. Y6 was given distilled water (Normal control)

NB: Each point on the graph represent an average value of three determinations (see appendix 2)

Fig 3.4.

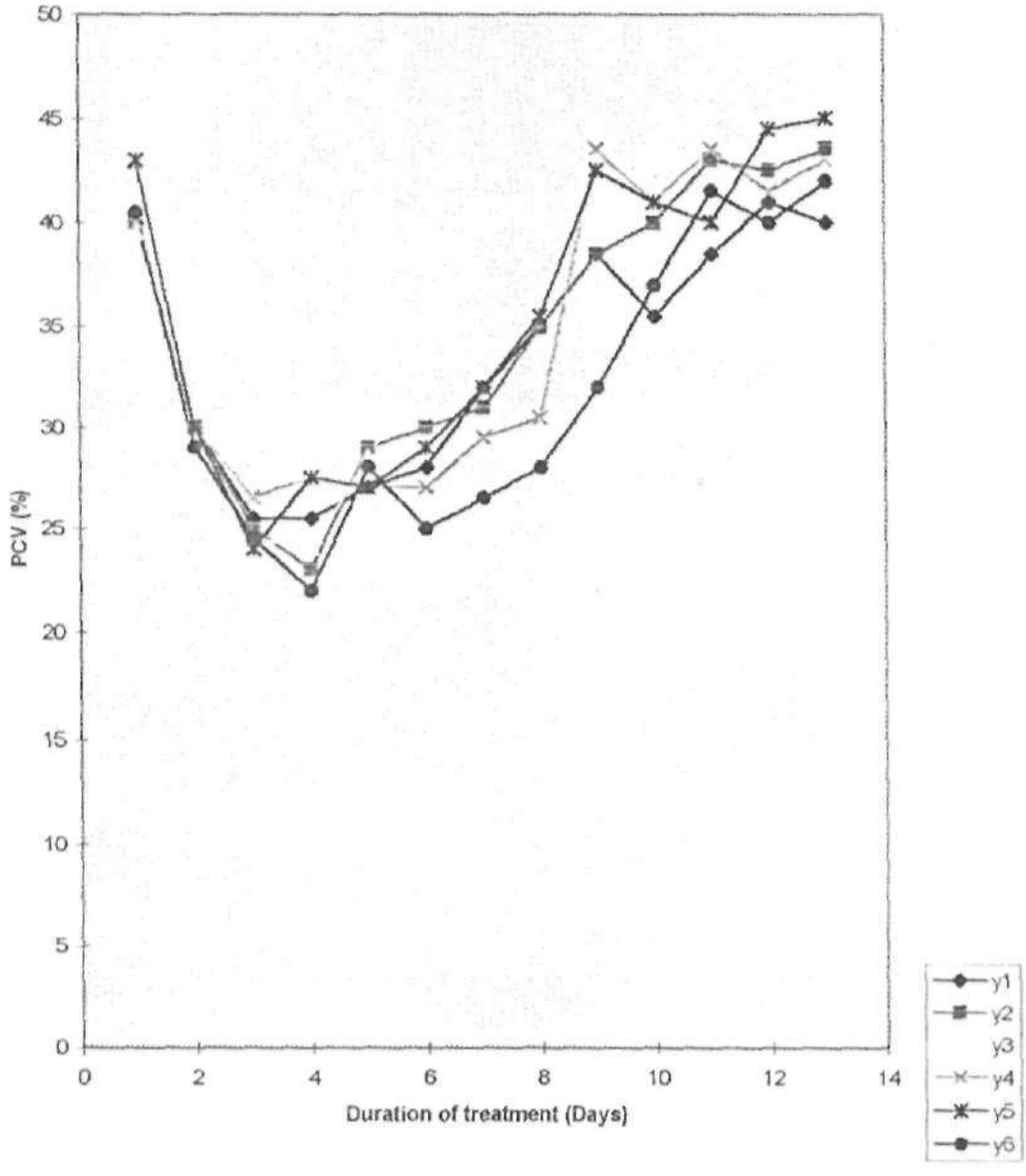


Different subcutaneous doses of aqueous extract of *K. senegalensis* on weight of rats after 2 days of the onset of phenylhydrazine-induced anaemia.

Y1 was give 10mg/kg body weight Y2 was given 20mg/kg body weight
Y3 was given 30mg/kg body weight Y4 anaemic but not treated
Y5 not anaemic but treated Y6 was given distilled water (Normal control)

NB: Each point on the graph represents an average value of 3 determinations (see appendix 2)

Fig. 3.5

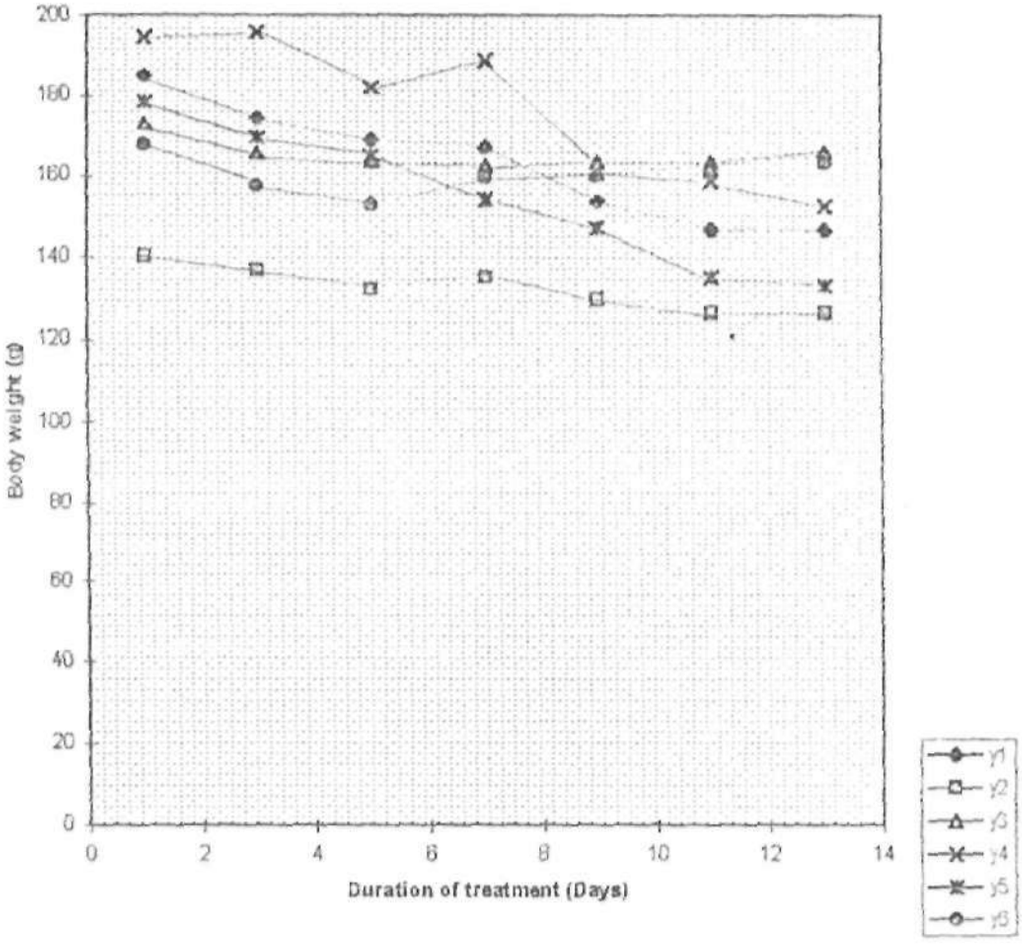


The effect of various oral doses of aqueous extract of *K. senegalensis* on PCV of rats 2 days after the onset of phenylhydrazine induced aneamia.

Y1 was given 100mg/kg body weight Y2 was given 200mg/kg body weight
Y3 was given 300mg/kg body weight Y4 was given 400mg/kg body weight
Y5 was given 500mg/kg body weight Y6 was given distilled water (Control)

NB: Each point on the graph represents an average value of 2 determinations (see appendix 3).

Fig.3.6



The effect of oral daily doses of aqueous extract of K. senegalensis on weight of rats 2 days after the onset of phenylhydrazine induced anaemia .

Y1 was given 100mg/kg body weight
Y2 was given 200mg/kg body weight.
Y3 was given 300mg/kg body weight
Y4 was given 400mg/kg body weight
Y5 was given 500mg/kg body weight
Y6 was given distilled water (Normal control)

NB: Each point on the graph represent an average value of 2 determinations (see appendix 3).

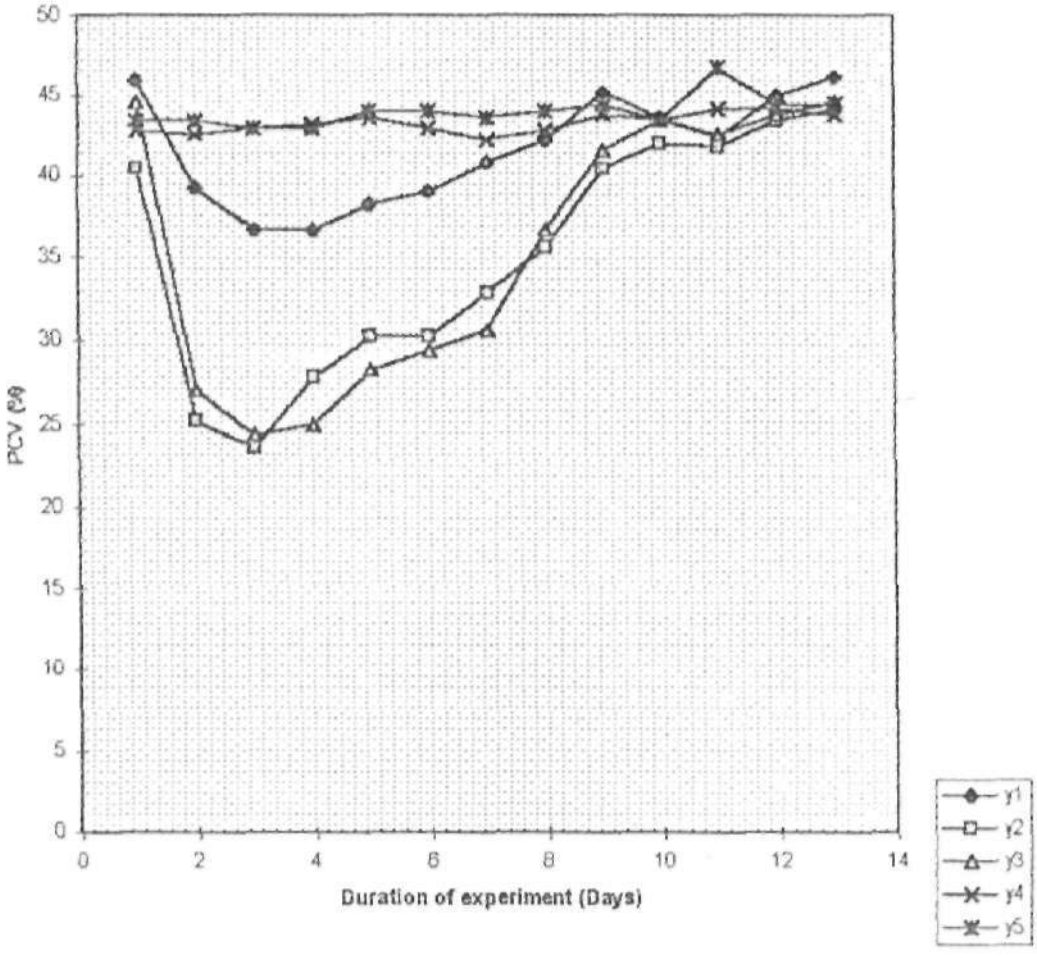
3.6 Effect of oral daily dosage (300mg/kg body weight) of the aqueous extract of the bark of *K. senegalensis* on PCV and weight of rats.

Group I which was given the extract simultaneously with phenylhydrazine show a little decrease in PCV which was not up to the anaemic level. This being significantly different from group II which was anaemic and treated on day II after the onset of anaemia and group III which served as anaemic untreated control. The PCV of animals in group II started increasing from day four when the animals started the oral dose of the extract on day two after the onset of anaemia and there was no further decrease in the PCV up to the end of the experiment (fig. 3.7). Animals in groups IV and V maintained their normal PCV all through while group III being given only Phenylhydrazine served as anaemic untreated control to all other groups.

3.7 Effect of subcutaneous administration of 30mg/kg body weight of the aqueous and methanolic extract of *K. senegalensis* on trypanosome induced anaemia.

Both aqueous and methanolic extracts (30mg/kg body weight) were used to treat trypanosome induced anaemia in experimental rats. The treatment started on day 5 after the infection that is when the parasitemia became massive and the animals anaemic. Of the seven groups, the infected rats from groups II, III and IV started dying as from day eight to eleven with the exception of group I which was treated with the aqueous extract. Animals in this group only started dying on day 22. Groups V, VI and VII which were not infected but treated with aqueous methanolic extracts and distilled water respectively lived and maintained their normal body weight and PCV (figs 3.9 and 3.10).

Fig 3.7



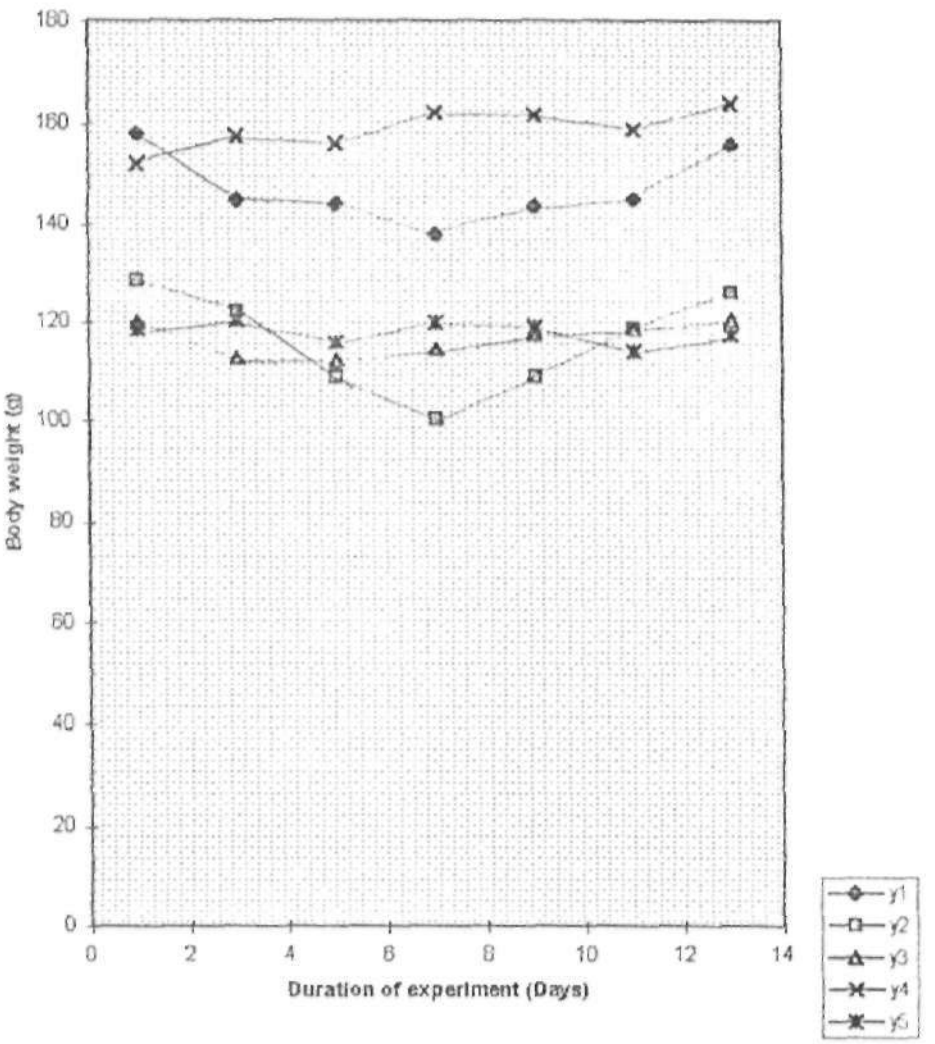
Effect of oral daily doses (300mg/kg body weight) of aqueous extract of the bark of *K. senegalensis* on PCV of rats treated on different days after phenylhydrazine administration.

Y1 was given extract + phenylhydrazine simultaneously from day 1
Y2 was given extract on day 2 after the onset of anaemia.
Y3 was given only phenylhydrazine-HCl (Control)
Y4 was given only the extract (Control)
Y5 was given distilled water (Normal control)

Changes in PCV of phenylhydrazine induced anaemic rats treated as described above.

NB: Each point on the graph represents an average value of 5 determinations (see appendix 4)

Fig. 3.8

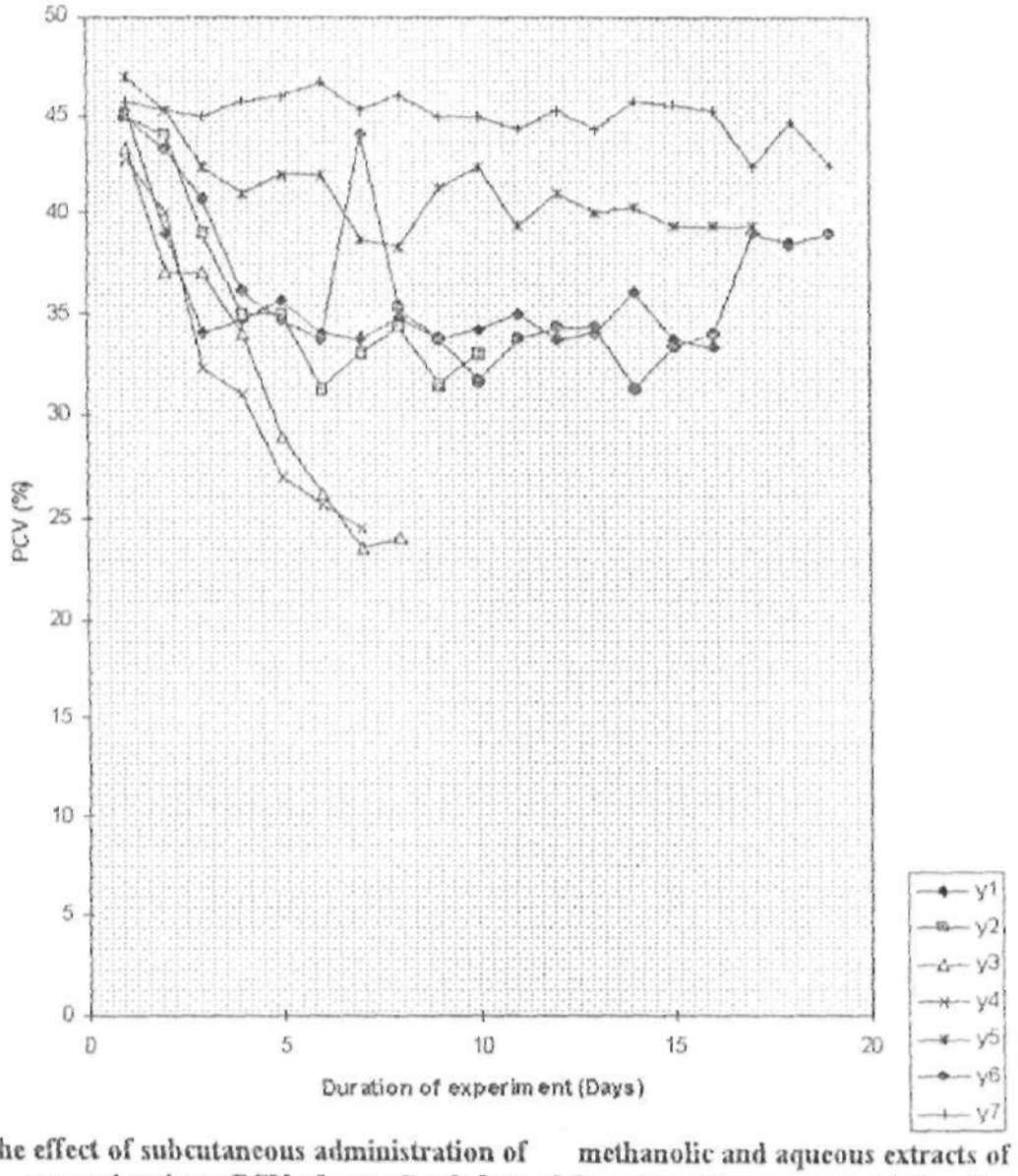


The effect of oral daily doses of the aqueous extract of *K. senegalensis* on weight of rats treated on different days after phenylhydrazine administration .

Y1 was given extract + phenylhydrazine simultaneously from day 1.
Y2 was given extract on day 2 after the onset of anaemia.
Y3 was given only phenylhydrazine (Control)
Y4 was given only the extract (Control)
Y5 was given distilled water (normal control)

NB: Each point on the graph represents an average value of 5 determinations (see appendix 4).

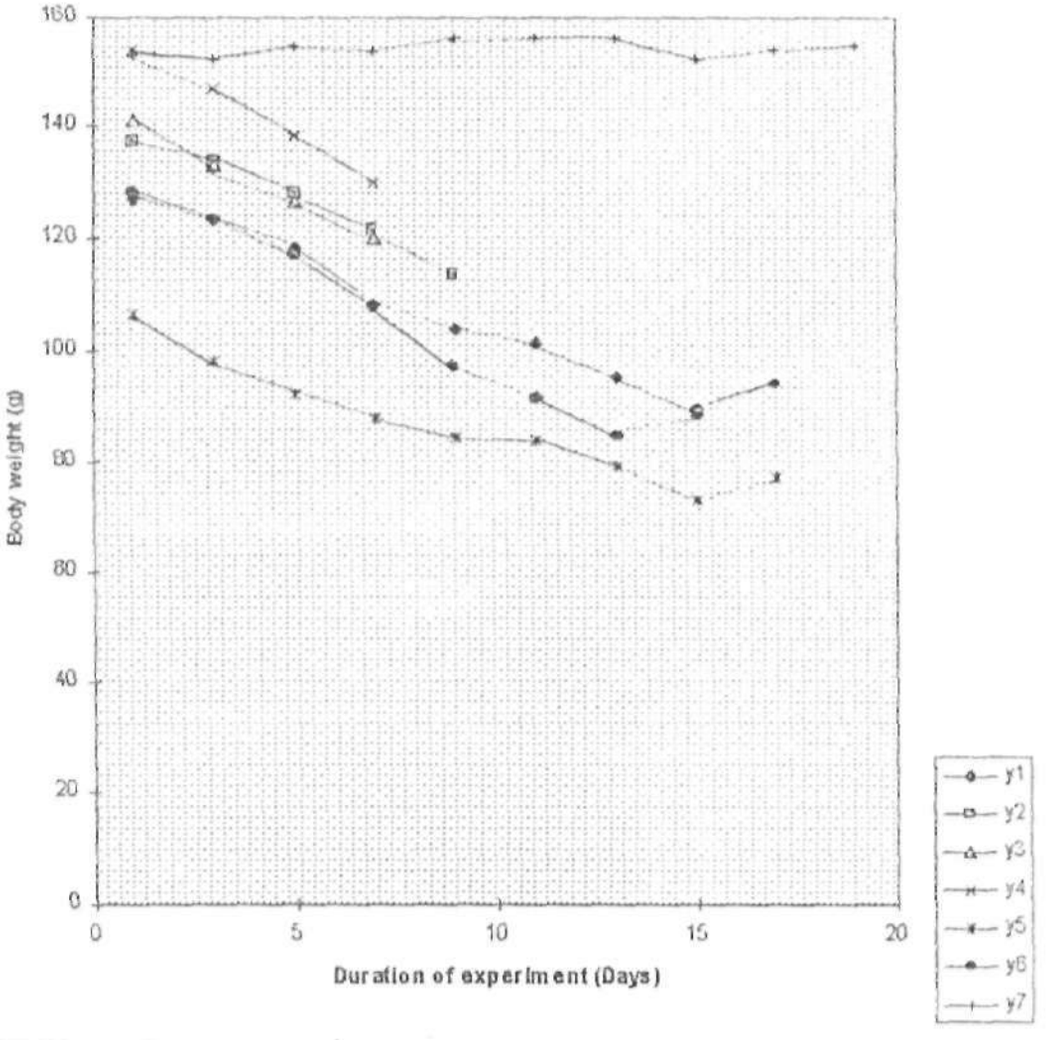
Fig 3.9



The effect of subcutaneous administration of methanolic and aqueous extracts of *K. senegalensis* on PCV of rats after 2 days of the onset of trypanosome induced anaemia .

Y1 was given 30mg/kg body weight of the aqueous extract
Y2 was given 30mg/kg body weight of methanolic extract
Y3 was given 20% ethanol
Y4 was given distilled water
Y5 was not infected but given 30mg/kg body weight of aqueous extract
Y6 was not infected but treated with 30mg/kg body weight of methanolic extract
Y7 was not infected but given only distilled water (Normal control)

Fig. 3.10



The effect of subcutaneously administered methanolic and aqueous extracts of *K. senegalensis* on the weights of rats after 2 days of the onset of trypanosome induced anaemia

Y1 was given 30mg/kg body weight aqueous extract
Y2 was given 30mg/body weight of methanolic extract
Y3 was given 20% ethanol
Y4 was given distilled water
Y5 was not infected but treated with aqueous extract
Y6 was not infected but treated with methanolic extract
Y7 was not infected but given distilled water (Normal control)
Some of the animals started dying before the end of the experiment.

NB: Each point on the graph represent an average value of three determinations (see appendix 5).

CHAPTER FOUR

4.1 DISCUSSION.

In the *in vivo* experiment, the pattern of anaemia induced in the experimental rats by phenylhydrazine which was given subcutaneously at the dose of 750mg/kg body weight in the rats conforms with the result of Harris and Kugler (1971) because the PCV was reduced by 62%. This was higher than 45 - 50% reduction reported by Bowman and Rand (1980). There fore, subcutaneous administration of 750mg/kg body weight would induce severe anaemia (fig. 3.1 and 3.2). The animals that received 950mg/kg body weight died a day earlier than those that received 1200mg/kg body weight showing that the death of the animals is not totally dose related. There could be a critical lethal concentration of phenylhydrazine and/or other contributing factors leading to the death of the animals.

Having established the dose that would induce anaemia, a subcutaneous administration of the aqueous extract of *K. senegalensis* was probed for a possible cure. This was done in a different set of experiment and the result shown in (figs. 3.3 and 3.4). Groups I and II which were given 10 and 20mg/kg body weight respectively of *K. senegalensis* extract on day 2 after the onset of anaemia died on day 6 of the onset of the experiment. This may be due to the combined haemolytic effect of phenylhydrazine and that of a small concentration of saponin present in the extract which is known to cause severe haemolysis when injected into the blood stream (Jenkins *et. al.*, 1957). This could also account for the progressive loss of weight observed in those groups treated with phenylhydrazine and a low dosage of the extract. A different situation may occur when a higher dose is injected and that may be the reason why the animals in group III that was given 30mg/kg body weight survived the

experiment, even though they did not show any improvement in their anaemic conditions as compared to the various control groups (figs. 3.3 and 3.4).

Furthermore, 30mg/kg body weight of both methanolic and aqueous extracts of *K. senegalensis* were tested on *Trypanosoma brucie brucei* induced anaemia. The animals infected with *T. brucei brucei* did not respond to the treatment of the extracts given subcutaneously. But group I that received the aqueous extract lived longer than those treated with the methanolic extract (figs. 3.9 and 3.10). It may be that the aqueous is supportive to some extent in prolonging the lives of the trypanosome infected animals though not active against the parasite. Similarly the methanolic extract may have other toxic agents absent in the aqueous extract. Skin abscesses was one of the major side effects observed in the animals treated with subcutaneous injection of both extracts. This could be due to the presence of tannins which is liable to denature the protein on the injection site (Errki, *et. al.*, 1993). The saponin of the extract could also be implicated since saponins are highly toxic when injected into the blood stream. Therefore subcutaneous route of administration of the extract is not the best route of dispensing it. The methanolic and aqueous extracts may not be useful in the treatment of trypanosome induced anaemia since it is neither active against the parasite nor in improving the resulting anaemia associated with the disease. In the same vane, various oral doses 100, 200, 300, 400 and 500mg/kg body weight of the extract tested on some groups of experimental rats did not show any significant ($P > 0.001$) improvement on the anaemic conditions of the animals (figs. 3.5 and 3.6). The progressive weight loss in some of the experimental animals could be due to interference of the extract with the feeding pattern of the rats since oral administration of high dose of saponin containing remedies could cause bloating thereby reducing the apetite of the animals (Trease and Evans, 1992). However,

those in group III that received 300mg/kg body weight of the extract tolerated it. That might have been responsible for the faster recovery of the animals from their anaemic conditions as compared to the control group ($P > 0.001$). Therefore oral administration of 300mg/kg body weight of the aqueous extract of *K. senegalensis* could be a good dosage which is well tolerated by rats.

Finally, it was observed that the simultaneous oral administration of 300mg/kg body weight of the aqueous extract and subcutaneous injection of phenylhydrazine did not produce anaemia. This was significantly different ($P < 0.001$) from the control in which anaemia was induced but not treated (figs. 3.7 and 3.8). This may suggest a possible protective effect of the extract against phenylhydrazine action on the red cells though the extract was not tested exclusively on the red cells to ascertain this possible protective effect of it.

The aqueous extract of *K. senegalensis* did not produce anti anaemic effect in this study. However, the result of the mineral analysis showed the presence of some important mineral elements that could be useful in the formation and physiological function of blood. The calcium concentration was found to be 453.3 ± 3.5mg/100g dry weight of the aqueous extract. This could provide substantial part of the recommended daily allowance of 1200mg for adults (Hamilton and Whitney, 1979). The absorption of cobalamine (vitamin B_{12}) through the intestinal wall depends on calcium and therefore has a role to play in preventing vitamin B_{12} deficiency anaemia (Thomas, 1986).

Magnesium content of the extract was 230.5 ± 2.6, a value close to the recommended daily allowance which ranges from 350 - 450mg (Oyenuga, 1968). The extract could be a good source of magnesium. Most dietary sources of zinc contain less that 5.0mg/100g (Andrew, 1979). The value of zinc obtained from the aqueous extract of *K. senegalensis* was

12.4 ± 1.3mg/100g showing that it contains adequate amount of zinc. More importantly the recommended daily allowance of iron is 10 to 18mg and the extract was found to contain 47.3 ± 3.2mg/100g dry weight of the aqueous extract which is much higher than values obtained for most fruits and vegetable considered to be very rich sources of iron (Guthrie, 1983). Therefore, the extract should be considered a good source of iron. Copper was detected at the level of 4.2 ± 1.0mg/100g which is higher than 2 to 5mg/100g supplied by a normal diet considered to be adequate for copper supply.

Potassium content of the extract was found to be 1780.8 ± 20.9mg/100g which is much higher than 500mg/100g reported to be adequate supply from food stuff (Oyenuga, 1968). The extract contains 90.5 ± 4.2mg/100g of sodium, a value higher than 50mg/100g of some diet considered to be low sodium food. However, the optimal intake of sodium and potassium remain to be established (Hui, 1992). Sodium and potassium play vital roles in regulating the osmotic pressure in the intracellular and extracellular fluid and to maintain appropriate acid-base balance. It is however noteworthy, that the effect of all these mineral elements depend on their bioavailability which is not studied in this work. In other words, presence of electrolytes and other haemopoietic mineral elements may play a role in cases of anaemia induced by a sudden loss of large quantity of blood.

In the phytochemical screening, the total absence of alkaloids observed in all the extract of *K. senegalensis* is in support of the observation of Adesina (1983), Olayinka (1992) and Nwafor *et. al.* (1995), using different parts of the plant to conduct the screening. Therefore, *K. senegalensis* does not contain alkaloid and may not exhibit any alkaloidal effect. The presence of glycosides particularly the cardiac glycosides with aglycone ring in the extract could explain the tonic effect of the aqueous extract on cardiac muscles reported by Keay, *et.*

al., (1964). Saponins another major component of *K. senegalensis* reported in this work is in agreement with the report of Olayinka (1992). Saponin glycosides are known to have haemolytic effect but this effect is only elicited when a small dose is injected into the blood stream as seen elsewhere in this thesis (Jenkins, *et. al.*, 1957). Since saponins do not cause haemolysis when taken orally (Spector, 1956), the extract may be useful in the treatment of saponin sensitive fungal and bacterial infections of the gastro-intestinal tract. The petroleum ether (40 - 60°C) extract contains steroids but the specific type was not identified. The methanolic extract contains small quantity of terpenes which may produce germicidal effect. The high tannin content observed in all the extracts (except petroleum ether extract), is in line with the result obtained by Adesina (1983). Olayinka (1992) also reported the presence of condensed tannin as the only type of tannin present in the extract. The main action of tannin is an astringent one and *K. senegalensis* contain the condensed type of tannin, this being less toxic than the hydrolysable tannins. Tannins are not absorbed from the gut to any extent so that their effect when taken orally will be confined to the gut wall; tannins are known to be highly haemostatic and thus find effective use as wound remedies. Also, they have anti-inflammatory effect that are of use where local infection has set in (Awosika, 1991). Therefore, *K. senegalensis* may be useful in these conditions.

4.2 CONCLUSION AND SUGGESTION FOR FURTHER WORK

4.2.1 CONCLUSION

K. senegalensis is a well known plant in Nigeria and other parts of Africa for its numerous medicinal and other uses. Although it is extensively used in the middle belt region of Northern Nigeria as anti-anaemic agent, results obtained from this research project did not conclusively and scientifically support its use for this purpose. This is because it did not show any significant ($P > 0.001$) curative effect in both chemically and parasitically induced anaemia.

The aqueous extract contains substantial amount of calcium, magnesium, iron and copper. Therefore, it could serve as good source of these mineral elements that are needed in the body for formation and physiological functions of blood. The presence of potassium and sodium in the extract could also provide enough electrolytes for the maintenance of osmotic pressure of the body fluid. The above two facts might contribute to ameliorating acute anaemia due to sudden loss of blood.

Other compounds contained in the extracts are: saponins, terpenes and tannins, which are known to be active against various parasites some of which could cause anaemia.

4.2.2 SUGGESTIONS FOR FURTHER WORK

Based on the findings of the present work, the following is being suggested:

1. The lack of anti-anaemic property of the extract observed in this work needs to be confirmed using a different experimental approach.

2. *In vivo* and *in vitro* tests are required to probe if the extracts has any effect on other GIT or blood parasites in order to know if the extract is useful for the treatment of other aneamia causing infections.
3. It is also expedient to precisely determine the bioavailablity of the mineral elements present in the extract. This is to find out if some of the mineral elements have a role in ameliorating anaemia.

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APENDIX I: Determination of the suitable dose (in ml) of 2.5% (w/v) Phenylhydrazine - HCl to induce anaemia in wistar Rats.

			Duration of the Experiment (Days)												
Group	Dosage (ml)	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13
I	0.4	PCV (%)	51.3±3.4	24.7±2.6	28.6±2.6	22.3±1.2	19.0±0.8	23.3±0.5	30.7±1.7	30.3±1.3	34.7±2.1	38.0±1.6	43.3±2.4	48.0±1.6	51.7±4.6
		Weight (g)	138.0±6.2		126.7±2.5		123.0±2.5		120.0±0.8		130.3±2.1		134.3±1.7		143.0±5.0
II	0.5	PCV (%)	49.7±2.0	25.3±1.7	23.0±0.8	*									
		Weight (g)	125.7±3.3		106.7±2.9	*									
III	0.6	PCV (%)	49.0±2.9	29.0±1.4	23.0±1.6	19.2±1.2	18**	*							
		Weight (g)	135.0±4.1		120.7±3.3		115**	*							
IV	Control 0.5ml of water	PCV (%)	46.0±0.8	46.0±1.4	45.7±0.5	46.7±0.5	45.3±1.2	45.0±0.0	46.3±1.2	45.0±0.8	45.0±0.8	45.7±1.9	46.3±0.5	46.7±1.2	46.7±0.5
		Weight (g)	131.3±2.6		132.0±1.6		133.0±2.2		132.0±2.2		134.0±1.4		135.3±2.1		137.3±2.1

Key:

* Death of the experimental animals

** Death of 2 of the 3 experimental animals.

Each value represent the mean ± standard deviation.

As shown above, group I - III were treated with various dose (in ml) of Phenylhydrazine and group IV was given 0.5ml of distilled water.

APPENDIX II: Effect of different subcutaneous doses (mg solid content/body wt) of *K. senegalensis* aqueous extract on weight and PCV of rats after 2 days of the onset of phenylhydrazine - HCl induced anaemia.

			Duration of the Experiment (Days)												
Group	Dosage (ml)	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13
I	10	PCV (%)	51.3±2.6	42.3±4.0	30.3±1.2	19.3±1.7	18.3±1.2	18**							
		Weight (g)	212.7±14.3		193.0±8.0		187.7±6.8								
II	20	PCV (%)	47.7±2.1	35.7±3.3	32.0±1.6	23.3±2.1	18.0±1.6	*							
		Weight (g)	138.7±5.3		123.0±2.2		118.8±3.4	*							
III	30	PCV (%)	47.7±1.8	34.7±2.1	32.0±2.2	22.7±1.2	24.0±1.4	26.7±1.2	32.3±1.2	26.0±2.4	29.7±1.2	23.7±1.7	23.3±2.1	26.3±1.7	28.0±1.6
		Weight (g)	138.3±9.5		180.3±6.9		168.7±2.6		154.6±4.8		145.0±3.6		146.2±9.0		145.3±6.3
IV	ANT	PCV (%)	48.7±2.6	35.8±2.4	23.0±1.6	19.0±1.0	19.2±1.3	23.3±1.6	20.9±1.3	22.6±1.8	20.3±1.6	24.3±3.0	22.0±1.8	28.3±1.8	35.3±1.6
		Weight (g)	136.7±3.4		122.0±1.6		99.5±1.5		101.7±3.3		98.7±1.5		100.0±1.6		99.3±1.5
V	NAT	PCV (%)	44.3±3.3	44.0±1.4	45.7±1.7	44.0±1.4	44.3±3.3	44.0±0.8	45.7±0.9	44.6±3.3	44.7±2.1	46.0±1.4	44.0±0.8	45.0±2.4	45.2±1.5
		Weight (g)	219.7±10.7		221.0±11.6		227.3±12.3		229.3±10.2		229.0±10.6		233.7±11.1		232.3±8.7
VI	NCD	PCV (%)	45.0±2.4	45.3±1.2	44.0±0.8	42.3±0.5	43.0±0.5	43.0±0.8	44.0±1.4	46.0±1.6	44.7±1.7	44.0±2.2	44.0±2.2	44.0±1.6	45.6±0.5
		Weight (g)	110.7±4.2		117.3±3.1		115.7±4.8		120.3±3.9		111.7±5.9		114.0±4.5		117.7±3.3

Key: Group I - III = Were given the doses of *K. senegalensis* extract as indicated in the table.
Group IV - (ANT) = Control where the anaemic rates were not treated.
Group V - (NAT) = Control where the rats are not anaemic but treated
Group VI - (NCD) Control where the rats are not anaemic and only treated with distilled water.

* Death of all the experimental animals
** Death of 2 of the 3 experimental animals.
Each value represent the mean standard deviation.

APPENDIX III; Effect of different oral doses of aqueous extract of *K. senegalensis* on PCV and on weight of rats 2 days after the onset of phenylhydrazne - HCl induced anaemia

			Duration of the Experiment (Days)												
Group	Dosage (ml)	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13
I	100	PCV (%)	40.0±0.5	30.0±1.0	25.5±0.5	25.5±3.5	27.0±2.0	28.0±1.0	32.0±2.0	35.0±0.0	38.5±0.5	35.5±0.5	38.5±0.5	41.0±1.0	40.0±2.0
		Weight (g)	185.0±2.0		174.5±2.5		169.0±8.0		167.0±3.0		153.5±3.5		146.5±6.5		136.5±12.5
II	200	PCV (%)	40.0±0.0	30.0±0.0	25.0±0.0	23.0±1.0	29.0±1.0	30.0±0.0	31.0±1.0	35.0±1.5	38.5±1.5	40.0±0.0	43.0±2.0	42.5±2.5	43.5±1.5
		Weight (g)	140.0±3.0		136.5±0.5		132.0±2.0		135.0±4.0		129.5±1.5		126.5±0.5		124.5±0.2
III	300	PCV (%)	39.5±0.5	27.5±1.5	24.5±0.5	24.0±2.0	28.5±1.5	33.5±5.5	35.0±5.0	39.5±2.5	44.0±4.0	42.0±3.0	44.5±2.5	47.5±2.5	46.0±1.0
		Weight (g)	173.0±3.0		165.5±3.5		163.5±4.5		160.5±4.5		163.0±7.0		163.0±3.0		165.5±2.5
IV	400	PCV (%)	40.0±0.0	30.0±0.0	26.5±1.5	27.5±2.5	27.0±0.0	27.0±0.0	29.5±0.5	30.5±1.5	45.5±5.5	41.0±1.0	43.5±1.5	41.5±1.5	43.0±0.0
		Weight (g)	194.5±5.5		196.0±6.5		182.0±5.0		188.0±4.0		160.5±10.5		158.5±10.5		152.5±7.5
V	500	PCV (%)	43.0±3.0	30.0±2.0	24.0±0.0	27.5±1.5	27.0±2.0	29.0±0.0	32.0±0.5	35.5±1.0	42.5±2.5	41.0±1.0	40.0±0.0	44.5±1.5	45.0±0.5
		Weight (g)	178.5±2.5		169.5±2.5		165.0±2.5		154.0±21.5		147.0±0.5		135.0±0.5		133.0±2.5
VI	Control H_2O	PCV (%)	40.5±0.5	29.0±0.0	24.5±0.5	22.0±1.0	28.0±1.0	25.0±0.0	26.5±3.5	28.0±0.0	32.0±2.0	37.0±3.0	41.5±1.5	40.0±1.5	42.0±0.2
		Weight (g)	167.5±2.5		157.5±0.5		152.6±3.0		159.5±2.5		159.6±1.0		161.0±1.0		163.0±3.0

Group I - V were anaemic and treated with various doses of the extract as indicated above

Group VI served as normal control and received only water

Each value represent the mean standard deviation.

APPENDIX IV: Effect of subcutaneous administration of 30mg/kg body weight of the aqueous and methanolic extracts of *K. senegalensis* on trypanosome induced anaemia in rats.

		Duration of the Experiment (Days)																
Group	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
I	PCV (%)	45.32.0	39.00.8	34.00.8	34.70.5	35.72.6	34.01.6	33.70.7	34.81.2	33.73.0	34.20.5	35.00.8	33.70.9	34.00.8	36.00.0	33.70.5	33.31.2	
	Weight (g)	126.77.7		123.38.1		118.35.7		108.36.2		104.010.4		101.35.8		95.33.8		88.51.5		
	Paracetamol	Nil	Nil	6.4[a] 2.5[a]	2.24[a]2.03[a]	3.74.4[b]	3.3[b] 4.7[b]	3.7[c] 4.4[c]	4.0[c] 4.2	8.0[b] 2.7[b]	5.6[c] 3.8[c]	3.6[b] 4.5[b]	7.0[b] 4.2[b]	4.0[c] 4.2[c]	5.0[c] 4.9[b]	10[a]* *		
II	PCV (%)	45.01.63	44.01.6	39.00.8	35.00.8	35.00.8	31.31.3	33.02.2	34.30.9	31.51.5	33**	*						
	Weight (g)	137.36.1		135.74.5		128.02.4		121.31.9		113.51.5*								
	Paracetamol	Nil	Nil	2.4[a] 1.9[a]	5.7[a] 3.6[a]	5.5[a]3.8[b]	1.4[a] 1.9[a]	2.0[a] 2.2[a]	6.7[c] 4.7[c]	10[a] 0	*							
III	PCV (%)	43.32.9	37.01.4	37.02.9	34.03.7	28.08.0	26.31.2	23.50.5*	24**	*								
	Weight (g)	141.02.9		135.05.0		126.34.9		120.05.0	*									
	Paracetamol	Nil	Nil	8.7[a] 1.8[a]	3.5[b] 4.6[b]	3.9[a]4.3[b]	4.0[c] 4.2[c]	10[a] 0*	10[a]* *	*								
IV	PCV (%)	42.72.1	40.0.0%	32.32.1	31.03.7	27.01.4	25.70.9	24.51.5	*									
	Weight (g)	154.07.5		146.70.5		138.35.4		129.50.5										
	Paracetamol	Nil	Nil	2.2[a] 2.0[a]	6.9[c] 4.4[c]	3.7[a]4.3[b]	7.0[c] 4.2[c]	10[a]	*									
V	PCV (%)	47.06.2	45.35.7	42.36.2	41.05.4	42.05.7	42.05.9	38.74.9	38.33.4	41.33.4	42.34.0	39.34.2	41.06.2	40.03.2	40.34.6	39.31.2	39.30.9	39.32.4
	Weight (g)	106.38.2		98.36.2		92.376		87.712.6		84.312.2		84.08.5		79.38.2		73.37.5		77.05.0
	Paracetamol	Nil		Nil		Nil		Nil		nil		Nil		Nil		Nil		Nil
VI	PCV (%)	45.01.6	43.32.4	40.72.5	36.02.9	34.71.2	33.71.7	34.02.2	35.31.2	33.71.9	31.71.2	33.70.5	34.31.7	34.31.2	31.31.2	33.31.2	34.02.0	39.00.8
	Weight (g)	127.76.1		125.34.8		117.36.8		107.77.4		97.02.8		91.32.1		84.74.1		89.33.3		94.04.3
	Paracetamol	Nil		Nil		Nil		Nil		Nil		Nil		Nil		Nil		Nil
VII	PCV (%)	45.71.7	45.31.7	45.01.4	45.70.5	46.00.8	46.70.8	45.31.9	46.01.4	45.01.4	45.01.4	44.30.9	45.32.1	44.31.2	45.70.5	45.53.5	45.31.2	42.31.7
	Weight (g)	153.03.7		152.05.2		154.73.7		153.72.5		156.82.2		156.04.3		156.03.6		152.34.0		154.04.3
	Paracetamol	Nil		Nil		Nil		Nil		Nil		Nil		Nil		Nil		Nil

NOTE: Each value represent the mean standard deviation.

Group I: Infected and treated with aqueous extract.

Group II: Infected and treated with methanolic extract. (dissolved in 20%).

Group III: Infected and given only 20% ethanol.

Group IV: Infected and was given only distilled water.

Group V: No infection but treated with aqueous extract.

Group VI: No infection but treated with methanolic extract.

Group VII: Not infected but given only distilled water.

* Death of experimental animals.

APPENDIX V: Effect of oral daily dosage (300mg/kg body weight) of the aqueous extract of the bark of *K. senegalensis* on PCV and weight of the rats treated on different days after phenylhydrazine administration.

		Duration of the Experiment (Days)												
Group	Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13
I	PCV (%)	46.01.8	39.22.0	36.61.4	36.61.4	38.20.8	39.02.2	40.81.0	42.21.2	45.21.0	43.62.0	42.42.0	45.06.6	46.21.5
	Weight (g)	158.24.1		145.09.8		144.210.6		139.011.4		143.610.2		145.28.8		156.09.7
II	PCV (%)	40.41.6	25.21.6	23.61.2	27.81.9	30.23.0	30.21.6	32.82.7	35.62.9	40.41.4	42.21.7	41.81.2	43.40.8	44.21.5
	Weight (g)	128.45.8		122.47.4		108.814.6		100.417.2		108.813.3		118.810.4		126.09.7
III	PCV (%)	44.62.6	27.00.9	24.40.8	25.01.1	28.21.6	29.43.6	30.64.1	36.63.4	41.62.1	43.41.7	42.61.5	43.81.9	44.60.9
	Weight (g)	120.071.4		112.822.9		112.225.3		114.624.5		117.623.2		118.823.9		120.621.6
IV	PCV (%)	42.82.0	42.61.7	43.01.9	43.21.5	43.60.5	43.01.9	42.21.8	42.81.2	43.81.6	43.41.6	44.22.6	44.22.5	43.81.2
	Weight (g)	152.25.8		157.66.4		156.27.2		162.212.2		161.64.0		159.010.2		163.810.1
V	PCV (%)	43.42.1	43.42.3	43.02.0	43.00.6	44.01.6	45.01.6	43.61.5	44.01.3	44.40.8	43.41.0	46.81.8	44.41.3	44.53.5
	Weight (g)	118.818.9		120.417.8		116.019.3		120.016.4		119.017.5		114.215.5		117.518.3±

Note:
Group I received the extract and phenylhydrazine simultaneously from day 1
Group II was given the extract on day 2 after the onset of anaenia
Group III served as control and was given only phenylhyrazine- HCl
Group IV served as control and received only the extract
Group V served as control and received only distilled water
Each value represent the mean ± standar4d deviation.