THE EFFECTS OF AQUEOUS EXTRACTS OF *Hibiscus sabdariffa*, *Azanza garckeana* AND *Grewia tenax* ON HEMATOLOGICAL PARAMETERS AND CORRECTION OF ANEMIA IN RATS

By

Reem Hassan Ahmed Ali
B.V.Sc. (1993) U. of K.
M.V.Sc. (2000) U. of K.

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Supervisor

Prof. Hashim Mohammed El hadi

Co- supervisor

Prof. Abd El wahab Hassan Mohammed

Department of Physiology
Faculty of Veterinary Medicine
University of Khartoum

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Dedication

I dedicate this work to:

My dear husband

My little daughter

My family

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CONTENTS

List of tables

List of figures

ABSTRACT

This study was carried out to investigate the possible effects of three traditional medicinal plants extracts in the correction of anemia. These plants were: seeds of *Hibiscus. sabdariffa* (Kerkade), fruits of *Azanza. garckeana* (Jakjak) and fruits of the common plant *Grewia . tenax* (Guddeim). Two types of anemia were chosen for the study: hemorrhagic anemia (by using hemorrhagic bled rats) and nutritional anemia (by using nutritionally iron- deficient rats). A constant dose of 2g/kg body weight of aqueous extract is used for the evaluation of the possible effects of these three plants on the two types of experiments on various hematological parameters of anemic rats.

Estimation of iron content of the three plants revealed that *G. tenax* has had the highest value of iron content, and *A. garckeana* extract came second; while *H. sabdariffa* extract has had the least amount of iron content.

The three aqueous extracts of the three plants caused significant-increases in the hemoglobin (Hb), Packed corpuscular volume (PCV) and Red blood cell count (RBC) of the hemorrhagic anemic rats, throughout the first and second weeks of experiments; in addition to that, extracts of *G. tenax* and *H. sabdariffa* continued to have the same favorable effect to the third and fourth weeks of the experiment. However, these extracts did not cause any noticeable effects on other hematological parameters (Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).

Studies carried out on iron-deficient anemic rats revealed that only *G. tenax* and *H. sabdariffa* extracts that had small and equal effects on the hemoglobin levels and red blood cell counts of the rats, but with no effect on other hematological parameters.

The effects of *A. garckeana* extract and *H. sabdariffa* extract on iron absorption were studied by using rat everted gut sac technique. The obtained results were compared with that of a former study carried out on fruits of *G. tenax*.

It was found that, *H. sabdaiffa* extract had better tendency in increasing iron absorption than did the *A. garckeana* extract when compared to the capability of *G. tena*x extract in increasing iron absorption performed in that former study.

ملخص الدراسة

أجريت هذه الدراسة لتقييم تأثيرمستخلصات ثلاث نباتات تستعمل تقليديا كعلاج طبي لفقر الدم في السودان و قد شملت هذه النباتات: بذور نبات الكركدي، ثمار نبات الجغجغ و ثمار النبات المعروف بالقضيم. تم جمع هذه النباتات و حللت محتوياتها مختبريا بتركيز على كميات نسب الحديد في هذه المستخلصات النباتية قد أثبتت احتواء نبات القضيم على أعلى نسبة من الحديد، يليه نبات الجغجغ و أقل نسبة وجدت في نبات الكركدي.

تم اختيار نوعين من انواع الأنيميا لاجراء الدراسة، و هما:

(1) الأنيميا النزفية، و ذلك عن طريق اجراء النزيف لجرذان التجارب.

(2) أنيميا نقص التغذية التجريبي، و ذلك عن طريق استخدام جرذان عرضت لنقص الحديد في الغذاء.

تم استخدام جرعة موحدة من المستخلص المائي لهذه النباتات في هذه التجارب و هي (2 جم/كجم من وزن الحيوان) لتقييم أثرها على مختلف مكونات دم الجرذان المصابة بالأنيميا. تسببت هذه المستخلصات المائية للنباتات الطبية في ارتفاع معنوي أثبت احصائيا (دلالة احصائية) لنسب هيموقلوبين الدم، في كبوس الخلايا وفي عدد كريات الدم الحمراء و قد كان ذلك في حالة الأنيميا النزفية في خلال الأسبوعين الأول و الثاني من اجراء التجارب. بالاضافة الى ذلك، أظهر مستخلصي نباتي القضيم و الكركدي تأثيرا ايجابيا امتد للأسبوع الثالث و الرابع من التجربة.

أثبتت الدراسات التي أجريت على جرذان نقص الحديد، أن مستخلصي نباتي القضيم و الكركدي قد أظهرا تأثيرا ضعيفا و لكن متساويا على مستوى الهيموقلوبين و كذلك على عدد كريات الدم الحمراء في دم تلك الجرذان. لكن لم يكن لديها تأثير واضح على مكونات الدم الأخرى.

تمت دراسة تأثير مستخلصي نباتات الجغجغ و الكركدي على امتصاص الحديد و ذلك عن طريق استخدام تقنية أمعاء الجرذان المعزولة والمقلوبة. قورنت نتائج هذه الدراسة بما سبقها من دراسات وتوافقت مع مستخلص نبات القضيم.

أظهرت النتائج التي وجدت بان مستخلص بذور الكركدي له خاصية في زيادة امتصاص الحديد أفضل من تلك التي وجدت في مستخلص نبات الجغجغ ويرتقي لمعدل ما أظهره مستخلص نبات القضيم في الدراسات السابقة.

INTRODUCTION

Anemia and iron deficiency constitute the most frequent nutritional problems worldwide. Some age groups are more vulnerable to suffer this deficiency, especially as a consequence of increased requirements and/or losses of the mineral. For these reason infants, preschoolers and pregnant and childbearing age women are more susceptible to iron deficiency (INACG, 1977 and WHO, 1993).

Most programs up to date, focused on iron supplementation and /or fortification. However, food supplementation programs are relatively expensive, non compliance and result in complex interaction between supplements and endogenous food components. Thus, a sustainable solution to dietary iron deficiency is required to reduce significantly the prevalence of iron deficiency anemia especially in the third world communities.

Worldwide, different plants were investigated for their role in the treatment and correction of different types of anemia, their iron content and their ability to enhance iron absorption in the body. So herbal medications can be considered as one of the solutions to dietary iron deficiency and can be the replacement for food supplementation programs especially in areas of illiterate populations where natives lack appropriate health care.

Sudan with its vast areas is rich in wild plants that play an important role in the nutrition of different populations especially in rural areas. Not many of these plants are scientifically investigated for their role in treatment of anemia. However a popular indigenous fruit, *Grewia tenax*, locally known as "Guddeim" is the first plant to be studied.

Grewia tenax is available in markets in wide areas in Sudan. It has been previously studied for its ability to treat anemia. It was stated that it increases blood hemoglobin level and increases iron absorption. On the other hand, there were other indigenous plants that were used by natives in folk medicine for the same role, especially in Kordofan States (Western Sudan). Those plants were not exposed to scientific studies to investigate this folkloric assumption. Examples for these plants are: seeds of *Hibiscus sabdariffa* and fruits of *Azanza garckeana*. These plants were studied for other activities and assumptions, however, no studies were found to evaluate their role in the treatment of anemia.

Thus, the current study is directed for investigation of the efficiency of *H. sabdariffa* seeds and *A. garckeana* fruits in the treatment of anemia in comparison to the effects of *G. tenax* fruits in the previous studies.

Objectives of the study:

- To investigate the efficiency of three medicinal plants which are used in folk medicine in the Sudan for the treatment of anemia.
- To evaluate chemical composition of these plants.
- To investigate the tendencies of those plants to increase iron absorption and/ or availability in the body.
- To compare between the anti-anemic effects of the three plants.

LITERATURE REVIEW

1.1. Definition of anemia:

Anemia is defined as a condition in which the number of red blood cells (RBC) or the amount of hemoglobin (Hb) which carries oxygen in them is low as stated by Allan (2008).

Hemoglobin enables the red blood cells to carry oxygen to the lungs and deliver it to all parts of the body. When the number of red blood cells is reduced or the amount of hemoglobin in them is low, an inadequate supply of oxygen in the tissues produces the symptoms of anemia. (Allan, 2008)

1.2. Anemia Overview:

Blood is actually a liquid made up of several different cell types. One of the most important and most numerous cell types is the red blood cell. The purpose of the red blood cell is to carry and deliver oxygen to the body cells. Anemia describes the condition in which the number of red blood cells in the blood is low.

Passmore (1986) stated that anemia is actually a sign of a disease process rather than a disease itself. It is usually classified as either chronic or acute. Chronic anemia happens over a long period of time. Acute anemia happens quickly. The life of the red blood cells is about 120 days. The bone marrow replaces them at a rate which enables their numbers to be maintained for optimum health. For the production of erythrocytes many nutrients are needed e.g. iron, copper, protein, vitamins C, B_{12} and E and folate.

1.3. Anemia in animals:

A deficiency of iron in the diet of animals causes anemia and failure to thrive. It is most likely to occur in newborn animals whose sole source of iron is the milk of the dam, milk being a poor source of iron. However, anemias in animals are classified as hemorrhagic or hemolytic anemia or anemia due to decreased production of erythrocytes. Hemorrhagic

anemia may be due to spontaneous rupture or traumatic injury to large blood vessels. The primary hemorrhagic diseases are less common e.g. abomasal ulcer, pyelonephritis, sweet clover poisoning, massive hook worms.

Hemolytic anemia is caused by diseases like babesiosis, leptospirosis, weeds poisoning, copper poisoning …etc. Hemolytic anemia can be sufficiently severe to cause hemoglobinuria and may result in hemoglobinuric nephrosis and depression of renal function; while anemia due to nutritional deficiency is reversible. In clinical cases of anemia, there are signs of pallor mucus membranes, muscular weakness, depression and anorexia. (Blood *et al*, 1986)

On the other hand, Blood *et al*. (1986) also stated that deposits of iron in the liver of the newborn are insufficient to maintain normal hemopoiesis for more than 2-3 weeks. More than half of the iron in the animal body is found as a constituent of hemoglobin. A relatively small amount is found in myoglobin and in certain enzymes which play a part in oxygen utilization. Iron-deficiency conditions are not common in farm animals except in the very young confined to a milk diet. However, continued blood loss by hemorrhage in any animal may bring about a sub clinical anemia and an associated iron deficiency.

Cattle heavily infested with sucking lice may develop serious and even fetal anemia. The chronic form is characterized by a non-regenerative anemia with sub-normal levels of serum iron and treatment with iron is necessary for an optimal response.

Horses heavily infested with blood- sucking strongylid worms develope often sub-normal hemoglobin levels and respond to treatment with iron.

On occasions veal calves, and possibly young lambs and kids, may also suffer from an iron-deficiency.

Confirmation of diagnosis will depend upon hemoglobin determinations and curative and preventive trials with administered iron.

Treatment of anemia in animals includes blood transfusion and hematinic preparation. Iron administered by mouth or parenterally is in common use. Vitamin B 12 is widely used as a non-hematinic particularly in horses. In extreme cases of anemia, irreversible changes caused by anoxia of kidneys and heart muscle, may prevent complete recovery in spite of adequate treatment.

1.4. Biologic importance of heme:

Iron is a part of heme, which is the active site of electron transport in cytochromes and cytochrome oxidase. Heme is also the site of oxygen uptake by myoglobin and

hemoglobin, thereby providing the means of transporting oxygen to tissues and within muscle cells. Hemoglobin in the root nodules of legumes protects nitrogen-fixing enzymes of symbiotic bacteria from oxidative inactivation; the ammonia formed is important in the synthesis of amino acids and proteins. The amino acids and proteins of legumes are transferred through the food chain to herbivores and thence to humans. Heme is also the active site of peroxidases that protect cells from oxidative injury by reducing peroxides to water. (Maurice *et al*, 1999)

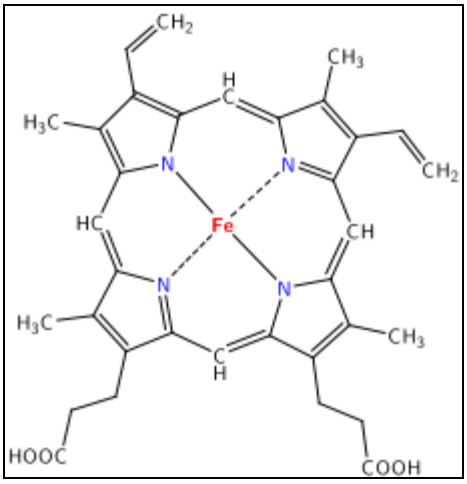

Figure 1. Structure of Heme

1.4.1. Hemoglobin:

The oxygen-carrying molecule in red blood cells is hemoglobin. Hemoglobin is a large, complex molecule. It contains an atom of iron reduced in the ferrus form at its center. Iron attaches itself easily to oxygen atoms. It is the iron in hemoglobin that actually carries oxygen to cells.

Hemoglobin has the following properties. It is the major protein containing substance of the red blood cells. Hemoglobin, along with its cofactor heme, is synthesized in immature red blood cells. Hemoglobin functions as oxygen carrier. It has a molecular weight of 64.500 and is composed of four subunits. Each subunit contains a heme group, which can bind one molecule of O_2.

Another feature of Hb is that it responds to the pH of the blood stream. Acidic pH increases a decrease in the affinity of Hb for O_2 (The Bohr Effect). This effect is useful in promoting the release of O2 in active tissues, where there is increased production of CO_2. The CO_2 enters the red blood cells, where it is converted to carbonic acid by the action of

carbonic anhydrase. The acid dissociates in the cell, inducing release of O_2 to the tissues. The Bohr Effect is dependant on the presence of both forms of globin. (Maurice *et al,*1999)

1.4.2. Myoglobin:

Maurice, *et al* (1999) stated that myoglobin is a monomeric protein with a molecular weight of 16.900. It is a minor protein of muscle and is used for short- term storage of oxygen. Myoglobin binds oxygen more tightly than Hb does. The rate of oxygen release increases when the environment contains very low levels of oxygen.

1.5. Iron:

Iron is the most abundant trace element in the human body (3-4 g). The bulk of iron circulates in the erythrocytes in the form of hemoglobin. Much smaller amounts are carried in the blood bound to a specific plasma protein, transferrin. Each molecule of transferrin can accommodate two molecules of iron. The ability of transferrin to bind iron is called the total iron-binding capacity (TIBC). Transferrin can be estimated from TIBC by the formula:

Transferrin (mg/dl) = TIBC/ 1.45 (Margaret *et al,* 1995)

Ferritin is a water-soluble complex of iron and protein. It is more easily mobilized than hemosedrin for Hb formation. It is present in small amounts in plasma.
Hemosedrin is an insoluble iron-protein complex found in macrophages in the bone marrow, liver and spleen. Un-like ferritin, it is visible by light microscopy in tissue sections and bone marrow films after staining by Perls reaction. (Parveen *et al.* 1999)

In humans, the total quantity of body iron varies with weight, hemoglobin concentration, sex and the size of the storage compartment. Of these, the largest is the iron in hemoglobin.

To some extent, the body's needs for iron can be controlled at the point of absorption and normal iron balance is maintained largely by regulation of iron absorption. Ingested inorganic iron is solubilized and ionized by acid gastric juice, reduced to the ferrous form (Fe II), and chelated. Iron absorption is promoted by substances that form low molecular weight iron chelates, such as ascorbic acid, sugars and amino acids. The mucin of normal gastric juice chelates and stabilizes iron, thereby reducing its precipitation at the alkaline pH of the small intestine.

Absorption may occur at any level of the small intestine but it is most efficient in the duodenum. The divalent or Ferrus form of iron is more readily soluble than the trivalent or Fe (III) because of the low solubility of ferric hydroxides and phosphates at the alkaline pH of intestinal fluid. Thus, Fe (II) traverses more readily the mucous layer to reach the brush border of intestinal epithelial cells. (Maurice *et al,* 1999)

Heme- iron forms the main part of dietary iron and derived from hemoglobin and myoglobin in red or organs meats. Non-heme iron is mainly derived from cereals which are commonly fortified with iron. Heme-iron is better absorbed than non-heme iron whose availability is more affected by other dietary constituents.

To be absorbed, iron contained in heme-proteins must be successfully liberated, first by digestion of the protein, with liberation of heme. Heme is absorbed as such by the mucosal epithelium of the small bowel. Within the cytosol, iron is liberated from protoporphyrin by the microsomal enzyme heme oxygenase which breaks the porphyrin ring yielding Fe (III), biliverdin and CO. Fe (III) is then bound by paraferritin and transported to the serosal side of the cell. Biliverdin is converted to bilirubin, which is transported in plasma to the liver for excretion. The carbon monoxide released by heme catabolism is transported to the lungs for excretion in exhaled air.

Plotting the logarithm of iron dosage against the logarithm of iron absorbed yields a straight line. For each two- fold increment in iron dosage, a 1.6 fold increment in absorption can be anticipated. (Maurice *et al,* 1999)

1.5.1. Factors influencing iron absorption:

- Heme-iron is absorbed better than non-heme iron.
- Ferrous iron is better absorbed than ferric iron.
- Gastric acidity helps to keep iron in the ferrous state and soluble in the upper gut.
- Formation of insoluble complexes with phytate or phosphate decreases iron absorption.
- Iron absorption is increased with low iron stores and increased erythropoeitic activity e.g. bleeding, hemolysis and high altitude.
- There is a decreased absorption in iron overload, except in hereditary hemochromatosis, where it is increased. (Parveen *et al,* 1999)
- In plant foods, phytates- which are salts of inositol hexaphosphates- chelates iron thus rendering it unavailable for absorption by the body. (Harland *et al,l* 1980)

1.5.2. Iron Transport:

In blood or other body fluids, iron is transported by a protein called transferrin. Transferrin binds iron that either is released from intestinal epithelium into the blood or lymph or is secreted from macrophages following degradation of hemoglobin. It distributes and transfers iron throughout the body to wherever it is needed, mostly to erythrocyte precursors in the bone marrow for new hemoglobin synthesis. Transferrin is the normal plasma protein that transports iron between various iron compartments.

The normal concentration of transferrin in plasma is about to 2.2 to 3.5 g/l. Since iron is the natural ligand of transferrin, the plasma concentration of transferrin may be measured by the amount of iron that it will bound (TIBC). Normal TIBC is about 45 to 80 Mmol (250-450 Mg/dl). The amount of iron actually bound to trasferrin is measured as the serum iron concentration or SI.

When transferrin is 100 %, iron absorbed by the intestinal mucosa can not be bound by transferrin , most of this excess iron is deposited in hepatocytes. Only about 3 mg of iron is transferrin bound at any time. Yet turnover is very rapid, as 25-30 mg of iron is transported daily from sites of absorption or release to cells where iron is needed. Normally, 70-90 % of this iron is taken up by the erythropoietic cells of bone marrow for hemoglobin synthesis. Smaller quantities are delivered to other cells for formation of myoglobin, cytochromes, perioxidases or other functional iron proteins. (Maurice *et a*,1999)

1.5.3. Iron re-utilization:

On the other hand, Maurice, *et al* (1999) also reported that the active manner in which the body conserves and reutilizes iron is an important characteristic of iron metabolism. More than 90 % of hemoglobin iron is repeatedly recycled by phagocytosis of old erythrocytes, which occurs chiefly in macrophages of the liver and spleen. Phagocytized red cells are digested at a rate sufficient to release approximately 20 % of the hemoglobin iron within a few hours, and the remainders are more slowly. The iron released by the action of the monocyte-macrophage system is bound to transferrin and is ultimately redistributed. About 40 % of the hemoglobin iron of non-viable erythrocytes reappears in circulating red cells within 12 days. The rate of reutilization varies considerably. The remainder of iron derived from hemoglobin catabolism, enters the storage pool as ferritin or hemosedrin and normally turns over very slowly: approximately 40 % remains in storage after 140 days. When the rate of erythropoiesis increases, however, storage iron may be released more rapidly from the storage pools to plasma transferrin.

Conversely, in the presence of chronic disease such as infection, rheumatoid arthritis or malignancy, the storage iron derived from hemoglobin catabolism is reused much more slowly.

1.5.4. Iron stores:

Maurice, *et al* (1999) also stated that iron is stored in reticuloendothelial cells, hepatocytes and skeletal muscle cells (500-1500 mg). About 2/3 of this is stored as ferritin and 1/3 as hemosedrin in normal individuals. Small amounts of iron are also found in plasma (about 4 mg bound to transferrin) with some in myoglobin and enzymes.

1.5.5. Iron Overload:

Harland and Harland (1980) reported that, an excessive body burden of iron can be produced by greater than normal absorption from the alimentary canal, by parenteral injection or by combination of both. Excess iron is deposited largely as hemosiderin in reticuloendothelial cells or in the parenchymal cells of certain tissues. The site of deposition depends in part on the portal of entry. Excess iron derived from intestinal absorption is carried to tissues, bound by plasma transferrin and transferred to parenchymal and reticuloendothelial cells and developing erythroblasts. In iron overload, serum iron concentrations and transferrin saturation are usually increased and the TIBC may be depressed.

1.6. Types of Anemia:

More than four hundred different kinds of anemia have been identified. Many of them are rare. Some are mild medical problems, while others are moderate or serious. Some are so serious that they may cause death. Isselbacher (1994) summarized the descriptive types and causes of anemia in the following table:

1.6.1. Summary of descriptive types and causes of anemia: [Table 1]

Type	Lab values	Causes
Macrocytic, normochromic	MCV: > 100fl MCHC: 34	Vitamin B_{12} deficiency, folate deficiency, vitamin C deficiency, chemotherapy (megaloblastic marrow); aplastic anemia, hypothyroidism (normoblastic marrow)
Microcytic, hypochromic	MCV: < 80 MCHC: < 30	Iron deficiency, thalassemia, sideroblastic anemia, chronic lead poisoning, anemia of chronic illness
Normocytic, normochromic	MCV: 80–99fl MCHC: 34 + / -2	Iron deficiency (early), chronic disease
MCV: mean corpuscular volume MCHC: mean corpuscular hemoglobin concentration fl: femtoliter (one quadrillionth of a liter)		

1.7. Causes of anemia:

Uthman, (1998) reported that anemia is caused primarily by one of three conditions. The first is bleeding. Bleeding results in the loss of red blood cells from the body. The second condition is a decreased rate of red blood cell production. Red blood cells are not produced as fast as they die off. The third condition is an increased rate of red blood cell destruction. Red blood cells die off faster than they can be replaced by the body. Of these factors,

bleeding is the most common cause of anemia. Bleeding can be a chronic or acute problem. Some common causes of chronic bleeding include:

- Cancer
- Gastrointestinal (digestive system) tumors
- Other diseases and disorders of the digestive system
- Heavy menstrual flow
- Hemorrhoids
- Nosebleeds
- Stomach ulcers
- Long-term alcohol abuse

On the other aspect, acute blood loss is usually the result of:

- Childbirth
- Injury
- A ruptured blood vessel
- Surgery

1.8. Iron deficiency anemia:

Anemia in the developing world is most commonly caused by an iron deficiency, which affects up to 50 percent of the population in some countries. Iron deficiency not only impairs the production of red cells in the blood, but also affects general cell growth and proliferation in tissues like the nervous system and the gastrointestinal tract. Red cells in a patient with iron-deficiency anemia are both microcytic and hypochromic. (Isselbacher, 1994)

Margaret *et al.* (1995) stated that Iron deficiency anemia develops when there is inadequate iron for hemoglobin synthesis. A normal level of Hb is maintained, for as long as possible, after the iron stores are depleted; latent iron deficiency is said to be present during this period.

1.8.1. Causes of iron deficiency anemia:

Parveen *et al.* (1999) has reported the causes of anemia in the following:

- Blood loss.
- Increased demands such as growth and pregnancy.
- Decreased absorption e.g. post gastrectomy.
- Poor intake.

Most iron deficiency is due to blood loss, usually from the uterus or gastrointestinal tract. Premenopausal women are always in a state of precarious iron balance owing to menstruation.

The most common cause of iron deficiency worldwide is blood loss from the gastrointestinal tract resulting from hookworm infestations. The poor quality of the diet, predominantly containing vegetables, also contributes to the high prevalence of iron deficiency in developing countries.

Whereas Margaret *et al* (1995) stated that, in iron deficiency anemia, blood indices will be changed as follows:

- Erythrocyte indices such as MCV, MCH and MCHC are low.
- A decrease in the serum ferritin level is the first biochemical change occurring during the development of iron deficiency. It reflects depletion of iron stores.
- In the intermediate stage of iron deficiency, serum ferritin continues to be low, and in addition, serum iron is low.
- Transferrin increases thus increasing TIBC.

In summary, the sequence of total events in iron deficiency is:

- Depletion of iron stores; serum ferritin decreases.
- TIBC and free erythrocyte protoporphrin increases, iron saturation decreases.
- Serum iron decreases; iron saturation decreases further.
- Hb and PCV levels decrease (i.e anemia)
- Erythrocyte indices (MCV, MCH and MCHC) decrease.

1.9. Hemorrhagic anemia:

This type of anemia is caused by excessive blood loss.

1.9.1. Clinical findings:

Evidence of hemorrhage, indirect-gastrointestinal, depends on the amount of blood lost, period of time during bleeding, and site of hemorrhage. Hemorrhage from multiple sites suggests clotting abnormalities.

1.9.2. Laboratory findings:

* Initially the PCV will be normal because all components are lost in similar proportions. Animal may be in hypovolemic shock.

* Splenic contraction delivers high-PCV blood (80%) to the circulation, temporarily increasing the PCV.

* Starting 2-3 hours after onset and lasting for 2-3 days, the blood volume is restored by the addition of interstitial fluid. This causes dilution of the erythrocyte mass and the signs of anemia (reduced PCV, RBC, and Hb) become evident. Plasma proteins are also reduced.

* Platelet numbers usually increase during the first few hours. Persistent thrombocytosis may suggest continued bleeding.

* Neutrophilic leukocytosis commonly occurs by approximately 3 hours post-hemorrhage.

* Signs of increased erythrocyte production (polychromasia, reticulocytosis) become evident by 48-72 hours and reach a maximum approximately 7 days after the onset of hemorrhage. Erythroid hyperplasia is evident in the bone marrow and precedes the changes in the peripheral blood.

* Plasma protein concentration begins to increase in 2-3 days and returns to normal before the PCV, RBC, and Hb.

* The hemogram returns to normal in 1-2 weeks in the dog.

* Thrombocytopenia and subsequent hemorrhage may occur with primary bone marrow failure; the anemia in these cases is non-regenerative.

1.9.3. Causes:

Trauma, Surgery, GI ulcers, Hemostasis defects, Sweet clover, Warfarin, Bracken fern and Factor X deficiency in pups

1.9.4 Characteristics of chronic blood loss:

1.9.4.1 Clinical findings:

1- Anemia develops slowly and hypovolemia does not occur.

2- PCV can reach low values before clinical signs of anemia become obvious because slow onset allows for physiologic adaptation.

1.9.4.2 Laboratory findings:

1. Regenerative response, but less intense than acute blood loss.

2. Hypoproteinemia is usually not noted.

3. Persistent thrombocytosis may be evident.

4. Body stores of iron may become depleted and an iron-lack anemia develops.

1.9.4.3. Causes:

*Parasitism

* Ancylostomiasis

* Strongylosis-equines

* Hemonchosis-ruminants

* Coccidiosis

* Fleas, ticks, lice

* GI ulcers, tumors

* Hematuria

* Vascular neoplasms

* Hemophilia

* Thrombocytopenia

* Vitamin K deficiency

* Differential features between anemias caused by external and internal hemorrhage

* External blood loss prevents reutilization of certain components (iron, plasma, and protein). These may be reabsorbed with internal hemorrhage.

* In internal hemorrhage, some erythrocytes are reabsorbed into lymphatics, particularly when hemorrhage is into body cavities, and the remainders are lysed or phagocytized, and iron and plasma proteins are reutilized. Therefore, the anemia may not be severe and recovery may be faster. (Murray *et al*, 2000).

1.10. Treatment of anemia with drugs:

Experiments carried out by Nenortiene *et al,* (2002) on new born piglets given anti-anemic powder Ferosol-2 were conducted. Calcium, manganese and ascorbic acid were added to Ferosol-2. Manganese and ascorbic acid stimulated erythropoiesis and being strong reductants stabilize iron on storage and in gastrointestinal tract. Iron in Ferosol-2 was found to be well assimilated in gastrointestinal tract of piglets, easily gets into blood and joins albumin of plasma. Experimental data showed increased resorption of iron in Ferosol-2 composition due to ascorbic acid. Ferosol-2 leads to significantly higher amount of hemoglobin and red blood cells in piglets.

Another study conducted by Idoate *et al*, (2003) on the protective effect of 3 different anti-anemic preparations was carried out to compare the gastrodoudenal toxicity caused by the three types in normal and anemic rats administered repeated therapeutic doses. The preparations were ovoalbumin (TM/FMOA), iron protein succinylate and ferrous sulphate. The effectiveness of the preparations in resolving the anemia was similar in the three groups but TM/FMOA exerts a protective effect against the toxicity normally observed of the iron in other formulations in normal and anemic rats, which was attributed to the fact that administration of iron bound to a protein core allows for gradual release of iron.

On the other hand, the response between food supplemented with iron in powdered and iron in syrup both used for the treatment of iron-deficiency anemia was evaluated in two groups of children by Ahmed *et al* (2003). The patients were randomized to receive either iron in syrup form, group (A) or equivalent doses of iron powder sprinkled over food, (group B). Hemoglobin rise in group B was more than in group A, but was statistically non-significant ($P<0.05$). There was small but significant ($P<0.05$) rise in serum ferritin in both groups. There was no significant difference between the two forms of iron administration. The conclusion was that, the powder form of iron is a cost effective and better tolerated

method of iron administration in children and can be considered as an alternate option for the treatment of iron-deficiency anemia in children.

In another study carried out on twenty women with iron-deficiency anemia. Tot' tema drug was prescribed twice daily. Receiving dates confirmed excellent G.I. absorption, which guarantee Tot'tema efficacy. (Milchev *et al*, 2004)

Ghinea (2004) has reported that, Ferro-Folgamma as one of the most indicated medicines in iron-deficiency. Due to its components this medicine has many indications: insufficient alimentary intake concerning iron, folic acid, B_{12} vitamin, vegetarian alimentation, increased needs during growth period, iron-deficiency anemia secondary to chronic hemorrhages, malnutrition, anemias associated with chronic alcohol intake, preventive treatment of iron-deficiency anemia and megaloblastic anemia during pregnancy and lactation.

Bozhinova *et al*, (2004) recommended the utilization of Ferro-Flogamma for treatment and prophylaxis of iron-deficiency anemia in pregnant women, because of its good gastric acceptance, a few side undesired reactions and supplement of folic acid and vit. B_{12} increases the iron resorbtion.

Another study carried out by Vermeer *et al,* (2002) on the efficacy of two iron products in preventing iron-deficiency. A total of 102 newborn piglets from ten litters were treated intramuscularly with 200 mg iron as iron dextran per ml, or 200 mg iron as gleptoferron per ml. Both products could be found between the two formulations. It could be concluded that iron-dextran and gleptoferron can be used with similar effect for anemia prevention in piglets.

1.10.1. Availability of iron in plants:

Harland and Harland (1980) stated that, iron absorption is an issue of continuing interest in the nutritional sciences because of the relatively high frequency of iron deficiency anemia and the remarkably poor efficiency of absorption of most forms of dietary iron.

The availability of the iron in plant foods such as beans, peas, corn, bread and rice is quite poor. It ranges from less than 1 % to 10 %. The availability of iron in the meat is considerably higher than that in plant products. The non-heme iron in meat, fish, chicken and liver may be about 20 % available. The heme-iron of meat may be close to 30 % available. Nearly all of the iron in plants is non-heme iron.

The term availability describes the percentage of the iron in the food which is absorbed and used for physiological purposes, such as red blood cell formation.

The interactions between different foods have sparked some interest. For example, if rice is consumed with orange juice, the orange juice can enhance the absorption of iron in the rice. This effect results from the chelation of the iron by the ascorbate in the juice and the increased absorbability of the iron from the complex.

On the other hand, Sandberg (2002) reported that, if rice is consumed with tea, the tannins in the tea can reduce the absorption of the iron in the rice because the iron in the iron-tannin complex is not readily available.

In general, including meat in the diet can increase the availability of iron from other foods. The mechanism of this effect is not clear. Also, phytic acid has been identified as a major inhibitor of iron absorption in plant foods.

The mineral content of legumes is generally high, but the bioavailability is poor due to the presence of phytate, some legumes also contain considerable amount of iron-binding polyphenols inhibiting iron absorption. It has been demonstrated that nutritional iron deficiency reaches its greatest prevalence in populations subsisting on cereal and legume basal diet.

1.10.2. Example of plants containing iron:

As stated in the **Phytochemeco** database (James *et al*, 1998)

1. *Taraxacum officinale* WIGG.-Dandelion (Leaf) 500-5,000PPm (From Czech Republic)
2. *Echinacea* spp-Coneflower (Root) 700-4,800ppm. (Italia plants)
3. *Artemisia vulgaris* L.-Mugwort (Plant) 1,200-3,900ppm. (Chinese plant)
4. *Physalis ixocarpa* BROT.-Tomatillo (Fruit) 14-2,974ppm. (Mexican plant)
5. *Asiasarum sieboldii* (MIQ.) MAEK. – Siebold's Wild Ginger (Root) 450-2,800ppm. (Japanese plant)
6. *Carthamus tinctorius* L.-Safflower (Flower) 81-2,200ppm. (American plant)
7. *Nyssa sylvatica* MARSHALL- Black Gum (Leaf) 8-1,820 ppm. (American plant)
8. *Schizonepeta tenuifolia* BRIQ.- Ching- Chieh (Plant) 1,700ppm. (Chinese plant)
9. *Amaranthus* sp.-pigweed (Leaf) 23-1,527ppm. (Origion is Himalaya)
10. *Camellia sinensis* (L.)KUNTZE – Tea (Leaf) 189- 1,500 ppm. (South and South East Asia)
11. *Arctium lappa* L. Gobo (Root) 8-1,470ppm. (American plant)
12. *Nepeta cataria* L. - Catnip (Plant) 1,380ppm. (Found in Europe, Africa, Asia and North America)

13. *Juniperus Virginia* L.-Red Cedar (Shoot) 11- 1,320ppm. (American plant)

14. *Polygonum cuspidatum* Slebold & Zucc. Japanese Knotweed (plant) 360-1.300 ppm. (Japanese plant)

15. *Senna occidentalis* (L.) Erwin H. & Barenby- Coffee Senna (Seed) 1.300 ppm. (Pacific Areas)

1.11. Treatment of anemia with plants extracts:

Jin *et al,* (1998) carried out studies to detect the effects of medicinal plants on hemorrhagic anemia. One of these is the effects of hairy root *Huangqi* on hemopoietic system. The oral administration of 5-20 gm/kg per day for 5-12 days could increase the count of RBC and reticulocytes in mice with hemorrhagic anemia raise the hemoglobin, RBC and PCV in mice with hemolytic anemia, enhance the number of WBC and nucleated cells in bone marrow of mice treated with X-ray.

Another study conducted by Hatono *et al,* (2004) revealed that the extract from the root of *Angelica acutiloba Kitagawa*, which is used as herbal medicine in Japan, was found to be clinically effective for postmenstrual blood loss and erythropoietin-resistant anemia. The results suggested the polysaccharides in this plant promote hematopoiesis by activating immature erythroid cells in mice with 5- fluorouracil-induced anemia.

The effect of pollen and propolis produced by bees was studied by using these natural products to the diets and it was found to improve the digestive utilization of iron and the regeneration efficiency of hemoglobin, especially during recovery from an anemic syndrome. Furthermore, when used in iron- deficient rats, these natural products were found to lessen, to a large extent, the adverse effects of iron deficiency and to improve the digestive utilization of calcium and magnesium. (Haro *et al*, 2000)

Feeding 437 children with fermented soya been for six months, significantly decreased the incidence of iron-deficiency anemia from 21.7 % to 1.25 %.The absorption rate(21.8 %) in the children given the diet containing fermented soya was significantly higher (14.2%) than that in children given control diet and about the same as that in the Fe SO_4 group (22.48%). (Qin, 1998)

In another study conducted by Miller, (1998) anemic rats were fed diets containing adequate quantities of all required nutrients except iron, which was supplied by ferrus sulphate and / or corn grain. The results indicated that slopes of the regression lines for

response (hemoglobin concentration to ferrus sulphate intake) were not altered significantly by inclusion of up to 75 % corn in the diets, hence it was concluded that, corn diets do not contain an inhibitor of iron absorption.

Furthermore, Pandit *et al,* (1999) has found that Ayurvedic preparations of metallic iron commonly categorized as different putas of *Louha Bashma* was chemically analyzed and pharmacologically investigated in iron deficiency anemia. The effect of a representative puta viz.50 puta of *Louha Bashma* in the management of induced iron-deficiency anemia in animal model was found to be statistically highly significant ($P< 0.001$) in comparison to the control and standard drug Fefol treated groups.

Previous studies carried out by Sulieman and Eldoma (1994) has reported that *Grewia. tenax* was used to increase haemoglobin level and hence is for the treatment of anemia.

Some plants were found to contain iron. Examples of these are *Bridelia cathartica* and *Lannea stuhlmannii.* Their iron content was determined by using atomic absorption spectrophotometry. Plant extracts were made by wet and dry procedures. The results obtained from both procedures agreed significantly and the average of both methods was taken as the iron content in each plant part. In most cases, the values obtained for the root bark had higher total iron contents of 35.69 and 35.21 mg/100g were found in the root bark of the above two plants respectively. The iron content of the decoctions prepared in the traditional way was low. However, the therapeutic potential of the herbs can not be established on basis of available iron content alone as other factors play a role in the absorption of iron in the body. (Omolo *et al*, 1997)

The effect of administration of aqueous extract of *Paraquetina nigrescens* on hematological parameters was investigated by Agbor and Odetola (2001) in hemorrhagic anemic rats. The anemic rats were administered with 400mg/kg, 800mg/kg and 1600mg/kg of *P. nigrescens* aqueous extract daily for four weeks. Significant ($P< 0.05$), progressive and dose related increases were observed in red blood cell count, hemoglobin, PCV and reticulocyte. While a decrease in white blood cell count was observed in the test rats treated with the extract compared with both normal and bled control rats.

A standard hemoglobin repletion bioassay was used with rats made anemic followed by complete diets containing equivalent amounts of iron as Fe SO4 or one of three different bioengineered rice varieties. Rice diets were as effective as the Fe SO4 diet in replenishing PCV, Hb concentration and liver iron concentrations. The found results suggested that Mendelian and biotechnological approaches to manipulating ferritin expression of seed iron

in rice may contribute to a sustainable solution to global problems of iron deficiency. (Laura *et al,* 2002)

Recently, many medicinal plants were used in the treatment and correction of the various types of anemia. A clinical trial was adopted to observe the therapeutic effect of *shengxuening* (SXN) in treating iron-deficiency anemia. Its effect in the treatment of iron-deficiency anemia was evident. It could improve the iron metabolism, increase levels of Fe, transferrin saturation, serum ferritin and lower levels of TIBC and transferring. Ke *et al,* (2004)

On the other hand, the effect of *Toki-shakuyaku-san* and iron preparation in- vivo in an animal experiment (anemic rats) was evaluated by Akase *et al,* (2004). The results showed trends toward improvement in indices of anemia i.e RBC count, Hb-level, PCV and serum iron. Also improvement of anemic symptoms in the groups treated with the plant only and/ or with iron preparation. It was concluded that by using *Toki-shakuyaku-san* and iron preparation in combination is possible to lessen the adverse reactions such as gastrointestinal symptoms, and that more ameliorative effect on the anemic state can be expected.

1.12. Local plants used in the study:

1.12.1. *Hibiscus sabdarrifa*:

Family: Malvaceae

English name: Roselle

Local name: Kerkade

In folk medicine, the beverage of *H. sabdariffa* is reported to be antiseptic, aphrodisiac, astringent, cholagogue, digestive, diuretic, emollient, purgative, refrigerant, resolvent, sedative, stomachic and tonic. Roselle is a folk remedy for abscesses, cancer, dedility, dyspepsia, dysuria, fever, heart ailments, hypertension, neurosis and scurvy. The drink made by placing the calyx in water, is said to be a folk remedy for cancer. Medicinally, leaves are emollient and are much used in Guinea as a diuretic, refrigerant and sedative. It was found that using electrothermal atomic absorption spectrometry after nitric/ perchloric digestion revealed that *H.sabdarrifa* infusion could supply greater amounts of iron (111± 5 µg/g total, 40.5 % leached). (Wrobe and Urbina, 2002).

Toxicological studies carried out by Akindahunsi and Olaleye (2003) on the aqueous fraction of an aqueous-alcohol extract of *H.sabdarrifa* L. calyces, revealed increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in all treated rat

groups compared to the controls. Prolong usage of this extract (250mg/kg) at 15-dose level could cause liver injury while the effect was mild at small dose levels (1-10). Though, the average consumption of 150-180 mg/kg per/day appears safe; the extract should be taken with caution bearing in mind that higher doses could affect the liver.

Also Rao (1996) has analyzed the seeds of *H.sabdariffa* for their proximate composition. Protein (22.3 %), fat (22.8%) and dietary fibre (42.6%). The seeds were good sources of minerals like phosphorus, magnesium and calcium.

Other study carried out by Rao (1996) revealed that the aqueous extract of *H.sabdariffa* showed a miracicidal and/or cercaricidal activity at a concentration of ≤ 10000 ppm; and it was toxic at 50-100 ppm.

On the other hand, the aqueous extract of the calyces of *H.sabdariffa* induced an estrogen- like activity in immature female rats when injected at 500mg/kg i.p. (El Sheikh *et al,* 1990).

Toxicological studies carried out by Ali *et al.* (1989) for 3 months on the extract of *H.sabdariffa* seeds showed that they had no cytotoxic effect. It was concluded that the refreshing herbal drink made from roselle is safe for human consumption.

Another study on the nutritional properties of *Hibiscus sabdariffa* seeds indicated that the whole seed flour contained 26.28, 20.13, 43.21 and 4.83 % respectively of protein, oil, carbohydrate and minerals. (Askari *et al,*1996)

Further studies on the phytochemical, pharmacological and toxicological properties of *Hibiscus sabdariffa* showed strong in- vitro and iv-vivo anti-oxidant activity. In rats and rabbits, the extract showed antihypercholesterolemic, antinociceptive anti-pyretic, but not anti-inflammatory activities. In rat and man, a strong anti-hypertensive action has been demonstrated. The effects of the calyx extracts on smooth muscles in-vitro are variable, but they mostly inhibit the tone of the isolated muscles. In healthy men, consumption of *H.sabdariffa* has resulted in significant decreases in the urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium and phosphate, but not oxalate. Oil extracted from the plant seeds has shown to have an inhibitory effect on some bacteria and fungi in-vitro. The plant extracts are characterized by a very low degree of toxicity. The LD_{50} of *H.sabdariffa* calyx extract in rats was found to b above 5000mg/kg. A single report has suggested that excessive doses for relatively long periods could have a deleterious effect on the testes of rats. (Abu Tarboush *et al,* 1997)

1.12.1.1. Chemistry of seeds of *H. sabdariffa*: [Table 2]

Ali *et al.* (2005) has summarized the chemistry of seeds of *H. sabdariffa* in the following table:

Moisture	7.6 %
Crude protein	24%
Fat	22.3 %
Fibre	15.3 %
N- free extract	23.9%

1.12.1.2. Component acids of the seed lipids were:

2.1 % myristic, 35.2 % palmitic, 2 % palmitoleic, 3.4 % stearic, 34 % oleic, 14.4 % linoleic and 3 unusual HBr- reacting fatty acids (cis-12, 13-epoxy- cis-9- octadecenoic (12,13- epoxoleic) 4.5 %; sterculic, 2.9 % and malvalic, 1.3 %. (James, 1983)

On the other hand, Salma *et al.* (1979) has reported that the sterols in the seed oil are: 61.3 % ß-sitosterol, 16.5 % campasterol, 5.1 % cholesterol and 3.2 % ergosterol (said to be rare in vegetable oil but the most common mycosterol in most fungi, including yeast).

1.12.2. *Azanza garckeana:*

Family: Malvaceae

English name: African Chewing Gum.

Local name: Jakjak.

James, (1983) repoted *Azanza garckeana* as a valuable edible indigenous fruit tree species that is widely distributed in east and southern Africa. The specific countries where the species is found are Botswana, Kenya, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia and Zimbabwe. Palgrave, (1988) described the fruits of *Azanza garckeana* are divided into 4-5 sections. They are yellowish to brownish green and hairy when mature. Fruits are edible from August and they are the most useful source of *Azanza garckeana.* On the other hand, Mojeremane and Tshwenyane (2004) reported that the fruits are eaten while slightly green or when ripe. They are persistent, therefore are picked on ripening. They can be soaked in a small amount of water to make jelly and can also be boiled and used as relish or made into porridge. Also Connelly *et al.* (1996)

stated that the aqueous and organic fractions from *A. garckeana* showed that this plant had a weak anti-malarial activity. Whereas Roy, *et al* (1992) stated that the solvent fractions of the heart wood of *Azanza garckeana* had yielded six naphthoquinones. These include mansonones E…H., azanzone B. and a new compound A, with structure 3,18 dimethyl 1-7-hydroxy-5-isopropyl 1-1,2-naphthalenedione which has been established by spectroscopic methods.

The edible portion of the wild fruit of *Azanza garckeana* was analyzed for moisture, protein, fat, crude fibre, ash and minerals (Ca, Mg, Fe, P, K and Na).The total carbohydrate and energy contents were also calculated. The highest level of fibre (45.3 %) was found on *A. garckeana.* The lowest level of energy value in *A. garckeana* and it was 810 KJ $100g^{-1}$. (Kalenga *et al*, 1994)

1.12.2.1. Chemical Composition of *Azanza garckeana* : [Table 3]

Saka *et al*, (1994) analyzed the chemical composition of *Azanza garckeana* in the following table:

Chemical composition	Amount
Dry matter	52.8 %
pH	5.96
Ascorbic acid (mg/100 gm of fresh weight)	20.5 (1.8)
Crude protein	12.0 %
Fat	1.1 %
Fibre	45.3 %
Total carbohydrate	35.2 %
Phosphorus	1476 (µg/g)
Calcium	95 (µg/g)
Magnesium	1453 (µg/g)
Iron	84 (µg/g)
Potassium	26190 (µg/g)
Sodium	202 (µg/g)

Figure 2. *Azanza garckeana* Fruits.

Figure 3. *Azanza garckeana* Tree

Figure 4. *Grewia tenax* Fruits

Figure 5.*Hibiscus sabdariffa* Fruits and Seeds

1.12.3. Grewia tenax: (Forsk.) Fiori.:

1.13.3.1. Taxonomy:

Synonyms: *Grewia betulifolia juss., Grewia populifolia vahl*.

Family: Tiliaceae.

Local name: Gudeim**.**

1.12.3.2. Description:

Small tree or shrub, up to 2m high, with dark grey bark and minute white lenticles (which persist only for a very short period). Leaves small, 1.5-4 cm wide, dentate wrinkly, glabrous or pubescent. Flowers white, solitary, axillar, 1.5 cm. Fruit red when ripe, glabrous, smooth, between 1 and 4 lobes, the size of a maize kernel, 21.000 seeds/kg. Distribution: The northernmost of the Grewia species of the Sahel; frequent in the transitional zone to the Sahara but also in the South of Moroco and Algeria. From the Atlantic through semi-arid Africa; to Kenya, Somalia, Zambia. Btsowana, Namibia, on the Arabian Peninsula and Iran to India. (Maydell, 1986)

1.12.3.3. Folk Uses: Fruits used for treatment of malaria and anemia. (El Gazali *et al*, 1998).

Grewia tenax roots, leaves, juice and fruit decoctions have been used in Africa and Southeast Asiatic countries for a variety of medical purposes.

It is an acrid, orange fruit usually eaten raw. Leaves are boiled and eaten as vegetable. In the Sudan it is found in Western, Northern and Southern states. Fruits are eaten fresh, or dried for later consumption. A drink is prepared by soaking the fruit overnight, hand pressing, sieving and sweetening. A porridge called *Nasha*, is also flavored by this drink, by addition of custard and flour.

1.12.3.4. Chemical composition:

The Fruits contains:

Protein (crude)	= 6.3 % (dry)
Fat	= 0.4 % (dry)
Fibre (crude)	= 8.1 % (dry)
Ash	= 4.5 % (dry)

1.12.3.4.1. Carbohydrate (soluble):

Starch= 15.1 % dry, Sucrose= 1.6 % dry, D- glucose= 21.0 % dry and D- Fructose= 24.3 % dry.

1.12.3.4.2. Amino acids:

Aspartic acid= 8.1 g, Threonine= 2.1 g, Serine= 2.4 g, Glutamic acid 6.2 g, Proline= 11.6 G, Glycine= 3.5 g, Alanine= 2.4 g, Valine= 2.8 g, Cysteine=1g, Methionine= 0.7 g, Isoleucine= 2g, Leucine= 3.4 g, Tyrosine= 2.5 g, Phenylalanine= 2.2 g, Lysine= 2g, Histidine= 1.1 g and Arginine= 3.2 g. **(**Abdelmuti *et al*, 1991).

1.12.3.4.3. Other constituents:

Every 100gm of plant contains: 146mg of vitamin (C), 218mg calcium, 174mg magnesium and 1352mg of pottassium. (Rahmatalla, 1999)

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Animals:

Twenty five Wistar albino rats weighing between 140 g and 150 g were used in the hemorrhagic anemia model. While other twenty five male Wistar rats of 21 days of age were used in the nutritional anemia model. All of the rats were obtained from the animal house of Medicinal and Aromatic Plants Research Institute (MAPRI). The animals were acclimatized for 7 days in the Department of Pharmacology and Toxicology of (MAPRI) before experimentation. They were fed *ad. Libitum* on a standard diet prepared in the animal house of the institute.

2.1.2. Chemical analysis of the rat diet:

*** Components:**

-Dry matter: 71.69%

-Ash: 6.25%

-Organic matter: 93.75%

-Crude protein: 3.64%

-Crude Fibre: 3.92%

-Ether extract: 9.08%

-Net free energy: 77.11%

The analysis of the diet was made by: The environment and natural resources Research Institute- Medicinal and Aromatic Plants Research Institute (MAPRI)-National Center for Research-Khartoum-Sudan.

2.1.3. Plants:

Seeds of *Hibiscus sabdariffa* were collected from the farm of (MAPRI) at Khartoum State where the plant was cultivated. While fruits of *Azanza garckeana* were collected from Kordofan State in western part of Sudan. The *Grewia tenax* fruits were purchased from local markets in Khartoum state. The three plants were authenticated by the taxonomists in (MAPRI).

2.2. Methods:

2.2.1. Method for determination of iron content of *H. sabdariffa*, *Azanza garckeana*, and *Grewia tenax* plants:

The fruits of *A. garckeana*, seeds of *H. sabdariffa* and fruits of *G. tenax* were subjected to chemical analysis in order to evaluate the amount of some elements contained in them. Those elements were: iron, sodium, magnesium and calcium. The analysis was carried out by using atomic absorption spectrophotometeric (PERKIN-ELMER-2380.

As stated by Allen, (1989) 0.5 g of air-dried sample of each of the three plants was weighed into acid washed porcelain basins. The samples were ignited at 550^0C for 2 hours in a muffle furnace. Then they were cooled and 5 ml of 20 % HCl were added to each sample. They were covered with watch glass and heated on a steam bath for 15 minutes. To each sample, 1 ml of HNO_3 was added, evaporated to dryness and heating was continued for 1 hour to dehydrate silica. 1 ml of 20 % HCl was added, swirled to dissolve the residue and then diluted to 10 ml with water and warmed to complete dissolution. The samples were then filtered through a No. 44 filter paper into a 50 ml volumetric flask and diluted to volume. These samples were then read by using an atomic absorption spectrophotometer.

2.2.2. Plant extraction:

Plants of the study were dried in shades and coarsely ground. Then they were soaked in distilled water for overnight. The concentration of each extract was 50 % w/v. The aqueous extract of each plant was minced, filtered and kept at 4˚C for daily administration. This method of extraction was chosen according to the folk use of each plant in traditional medicine. Dose used in this study is 2g/kg body weight for each extract referring to the starting plant material.

2.2.3. Preparation of diets:

Two types of rats diets were used in these experiments. The first diet (A) contained 39.15 mg Fe/kg diet. The source of iron in this diet was from animal protein. While diet (B), the iron deficient diet, contained 26.7 mg Fe/kg diet and the source of iron was from plant origin only.

2.2.2.1. Determination of iron content in the diet:

1 gm of the ground powder of the rats' diet was weighed in a crucible and inserted in an oven of 500 ºC for 3 hours. 50 ml of 20 % HCl were added and then filtered in a volumetric flask. Washed with distilled water and then the volume was completed to 50 ml. All the organic matter is then converted to inorganic ions (Stewart, 1989). The samples were then taken to be read by atomic absorption, Perkin- Elmer, 2380.

2.2.4. Hemorrhagic anemia model:

The rats used in the hemorrhagic anemia experiments were divided into three groups of 5 rats in each group and labeled (A), (B), (C_1), (C_2) and (C_3). The first group (A) was the normal un-bled group. They received distilled water only and served as the control un-bled group. Groups (B), (C_1), (C_2) and (C_3) were bled 30 % off their total blood volume which is 5-7 ml in each rat as described by the (Trevor and Robinson, 1988).

24 hours after bleeding from orbital plexus of rats as described by Khanna *et al*. (1992), hematological parameters of the bled rats were determined and thus considered as the baseline of the hematology values.

Immediately after bleeding, the second group (B) was administered with distilled water and served as the control bled group. While bled groups of (C_1), (C_2) and (C_3) received a daily dose of 2 g/kg body weight of the aqueous extract of the studied plants.

Animals in group (C_1) were administered with 2g/kg body weight of the aqueous extract of *Hibiscus sabdariffa* seeds. While animals in group (C_2) received 2g/kg body weight of *Azanza garckeana* aqueous extract. Group (C_3) has received 2g/kg body weight of *Grewia tenax* aqueous extract.

The administration of each extract continued for 28 consecutive days. Hematological parameters were measured on days 7, 14, 21 and 28 of experiment.

Hematological parameters studied were: Hb level, PCV level, MCV, MCH, MCHC and RBC count by using hematology auto-analyzer (Huma-Plus).

2.2.5. Nutritional anemia model:

In the nutritional -anemia model, rats of 21- days of age were fed with iron deficient diet (26.7 mg Fe/kg diet) for 28 consecutive days and continued feeding for another 4 weeks after the beginning of the experiment.

The iron deficient rats were divided into four equal groups of five rats each. The first iron deficient group of rats (A), were given distilled water only and served as control iron deficient group. The second (B_1), third (B_2) and fourth (B_3) iron deficient groups were administered with 2g/kg body weight of the aqueous extracts of: *Hibiscus sabdariffa*, *Azanza garckeana* and *Grewia tenax* respectively.

The last group of rats (C) were fed with a normal iron-content diet (39.15 mg Fe/kg diet) and served as a control group with the normal diet.

Hematological parameters studied were: Hb level, PCV level, MCV, MCH, MCHC and RBC count by using hematology auto-analyzer (Huma-Plus).

2.2.6. Study of the effects of aqueous extracts of *H. sabdariffa* and *A. garckeana* on iron absorption:

2.2.6.1. Protocol of extraction:

500 gm of each plant were ground and sifted. Boiling water was added and they were separately agitated for 90 minutes. The two plants were steeped for 4 hours at 4◦C and then filtered with a vacuum pump. They were evaporated and freeze-dried. 20 mg of each extract was used in the experiment.

2.2.6.2. Treatment of animals:

Experiments were carried out on male Wistar albino rats weighing between 150 and 200gm. They had free access to water but food was withdrawn 24 hour prior to experimentation.

Animals were sacrificed by cervical dislocation and 3 cm length pieces of duodenum were immediately extracted, stripped of adhering tissue and cleaned with a Ringer solution (9%) contained the following salts(mmol/L) containing the following salts:

NaCl (0.154), KCl (0.0034), HCO_3Na (0.0024) and $CaCl_2$ (0.021).

2.2.6.3. Everted gut sacs:

The everted gut sacs were hanged in an incubatory medium containing 200Mmol/L of $FeSO_4$. In the first preparation, there were the physiological solution and the $FeSO_4$ only, and it was kept as a control preparation. In the second preparation there was 20mg/ml of *H. sabdariffa* extract. In the third preparation there was 20mg/ml of *A. garckeana* extract. The three preparations were maintained at 37◦C and bubbled with O_2.

2.2.6.4. Iron determination:

Iron concentration was measured by using PERKIN- ELMER- 2380 atomic absorption spectrophotometer after 1, 5 and 15 minutes following the addition of the extracts and the $FeSO_4$ solution.

2.2.7. Statistical analysis:

One-way analysis of variance (ANOVA) test was used for the analysis of data of this study by using SPSS version 10 program. The ANOVA test allows determining if one given factor has a significant effect on any of the groups under study.

All data in the study were expressed as Mean ± SD.

P-value

Indicates the probability of getting a mean difference between the groups as high as what is observed by chance. In this study, P value was considered significant at $P \leq 0.05$.

Dunnett's test compares group means. It is specifically designed for situations where all groups are to be pitted against one "**Reference**" group. Its goal is to identify groups whose means are significantly different from the mean of this reference group. This test was applied in this study.

3. RESULTS

3.1. Determination of iron and copper contents of *H. sabdariffa*, *Azanza garckeana*, and *Grewia tenax* plants:

In this study, it was found that *G. tenax* had the highest amount of iron 8.8 mg Fe/ 100 g pulp, while *A. garckeana* fruits contained 6.8 mg/100 g and the least amount of iron was found in *H. sabdariffa* (4.6 mg/100 gm pulp). On the other hand, the copper content was found to be 0.65 mg/100g in *H. sabdariffa,* 0.36 in *G. tenax* and 0.2 in *A. garckeana.* Hence, it was obvious that *G. tenax* had the highest amount of iron in the three plants, *A. garckeana* better than *H. sabdariffa*. These results may affect the use of the three plants in the treatment of iron-deficiency anemia with consideration of other factors affecting the absorption and bioavailability of iron.

Table 4.

Determination of iron and copper contents of *H. sabdariffa*, *Azanza garckeana*, and *Grewia tenax* plants:

Groups	Iron content (mg/ 100 g pulp)	Copper content (mg /100 g pulp)
Hibiscus sabdariffa	**4.6**	**0.65**
Azanza garckeana	**6.8**	**0.2**
Grewia tenax	**8.8**	**0.36**

3.2. Effect of the tested extracts on hematological parameters of anemic rats

3.2.1. The effect of *H. sabdariffa* aqueous extract on hemorrhagic anemic rats:

Administration of 2g/kg of *H. sabdariffa* aqueous extract to hemorrhagic anemic rats caused remarkable changes in their blood parameters. Those changes were found to be most obvious in terms of hemoglobin level and/or PCV values. This extract caused a distinguished increase of 26 % in the hemoglobin level of the hemorrhagic anemic rats after one week of treatment. That increase was compared to 12 % increase in the control bled rats and to a decrease of 10.9 % in the control unbled group. The increasing effect of the extract continued to be of 40 % after two weeks treatment compared to an increase of only 13.3 % in the control bled group. While the control unbled rats showed a decrease of 11 % in this week.

In the third and fourth weeks of treatment, the increasing effect of the extract on the Hb level was slightly lower from that presented in the second week. It was estimated as 34 % and 35 % in those two weeks respectively, compared to 29.4 % in the control bled rats and to 4 to 3.3 % in the control unbled group (table 5. and figure 6.)

The PCV values showed similar changes in the Hb- levels. The extract caused remarkable increases in the PCV values throughout the period of treatment. These increases were estimated as 29 %, 39 %, 33 % and 33 % caused by *H. sabdariffa* extract in the first, second, third and fourth weeks of treatment respectively. The control bled rats showed much lower increases throughout the period of experimentation. These increases were reported as 9.8 %. 2.4 %, 31 % and 21.6 % in the first, second, third and fourth weeks of experiment respectively. While the control unbled rats showed decreases of 14 and 18.6 % in the first two weeks of experiment and slight increases of 0.6 and 2.7 % respectively. Table 6. and figure 7.

H. sabdariffa extract caused marked increases in the RBC counts of hemorrhagic anemic rats. These increases were estimated as 26.4 %, 36.9 %, 32.1 % and 32.6 % in the four weeks of the experiment respectively. These effects of the extract were compared to decreases in the control unbled group and an increase of less that 1 % in the first two weeks of experiment in the control bled group. Table 7. and figure 8.

The effect of *H. sabdariffa* extract on MCV levels of the anemic rats was reported as mild increases in the levels throughout the four weeks of the treatment. These increases of the treated group were estimated as 1.00, 0.72, 1.1 and 0.42 % in the four weeks respectively while the control groups have shown nearly similar increases in the MCV values. They were reported in the control bled group as 10, 1.3, 0.8 and 2.3 % increases in the MCV values in the four weeks of the experiment respectively (table 8.)

H. sabdariffa extract showed low increases in the MCH and MCHC levels of the anemic rats. In the first week, it caused decreases of 1.2 % in the MCH values and of 3 % in the MCHC levels in the anemic rats compared to an increase of 12.8 % in the MCH levels and an increase of 2.3 % in the MCHC level of the control bled group.

In the second week, the MCH level of the treated group was slightly increased (0.5 %) compared to an increase of 11.7 % in the control bled group. However, the MCHC level of the treated group remained un-changed comparable to an increase of 10.3 % in the bled untreated group (table 9.)

These alterations in the MCH and MCHC values continued to the last two weeks of the treatment. Evaluation of the MCH values revealed increases of 0.4 % and 1.2 % in the MCH levels of the treated group, compared to increases of 11.6 % and 3.7 % of the control bled group in the third and fourth weeks respectively. While changes in the MCHC levels were estimated as a decrease of 0.5 and an increase of 0.9 % in the treated group compared to increases of 10.1 % and 1.3 % in the control bled group at these last two weeks of the experiment respectively (table 10.)

3.2.2. The effect of *H. sabdariffa* extract on iron- deficient rats:

H. sabdariffa did not cause recognizable effects on the hemoglobin levels of the anemic, iron-deficient rats. The increase in the hemoglobin level was 0.96 % in the first week compared to an increase of 0.16 % caused by the control iron-deficient group. This increase in the hemoglobin level caused by *H. sabdariffa* extract was statistically significant at $P< 0.05$ when compared to the control normal diet group.

In the second week, there were slight decreases in the treated and control groups. It was found to be 2.9 % in the treated group, 3.8 % in the control iron-deficient group and 3.7 % in the control with the normal iron-content diet group. Table 11. and figure 9.

The increases in the PCV values in the first week were 1.9 % in the treated group, 0.6 % in the control iron-deficient group whereas, the normal diet group showed a decrease of 2.4 % in the PCV level.

In the second week, the three groups showed variable decreases in their PCV levels. It was estimated as 0.3 % in the treated group, 2.3 % in the control iron-deficient group and 7.3 % in the control with the normal iron- content diet. Table 12. and figure 10.

The MCV levels were increased slightly in the first and second weeks. These increases were 0.17 % and 0.7 % caused by *H. sabdariffa* group in the first and second weeks respectively. However, these increases were not statistically significant. The control iron-deficient group showed increases of 2.5 % and 1.7 % in those two weeks respectively. Table 13.and figure 11.

There were decreases in the MCH levels of *H. sabdariffa* group estimated by 0.75 % and 1.8 % in the first two weeks of experimentation. While the control iron-deficient group showed slight increases of 2.1 % and 0.1 % in the same weeks. Table 14. and figure 12.

Treated and control iron-deficient groups showed slight decreases in their MCHC levels. These decreases were lower in the control iron-deficient rats estimated by 0.4 % and 1.5 % in the first and second weeks respectively. However, the *H. sabdariffa* group has shown decreases of 0.8 % and 2.5 % in the same weeks. These differences in the MCHC levels were found to be statistically significant at $P < 0.05$ when compared to the two control groups. Table 14. and figure 12.

The extract caused a slight increase in the RBC count in the first and third weeks of the experiment (1.6 % and 4.6 % respectively). These increases were met by decreases in the control iron-deficient rats (1.8 % and 2.4 % respectively). However, these increases were not statistically proven. Table 13.

Table 5.

The effect of aqueous extract of *Hibiscus sabdariffa* on Hb- level (g/dl):

Weeks	Control un-bled group	Control bled group	*Hibiscus sabdariffa* group
Week 0	13.46 ± 0.46	10.6 ± 1.56	9.8 ± 1.4
Week 1	12 ± 1.96	11.88 ± 0.67	12.4 ± 1.02
Week 2	11.98 ± 0.31	12 ± 0.21	13.76 ± 0.82
Week 3	14 ± 2	15.4 ± 0.55	13.16 ± 0.8
Week 4	13.9 ± 0.5	13.72 ± 0.55	13.28 ± 1.1

(All data are expressed in mean ± SD)

Table 6.

The effect of aqueous extract of *Hibiscus sabdariffa* on PCV level (%):

Weeks	Control un-bled group	Control bled group	*Hibiscus sabdariffa* group
Week 0	44.34 ± 4.01	35.28 ± 5.5	39.94 ± 2.9
Week 1	38.14 ± 6.5	38.72 ± 3.2	43.02 ± 2.13
Week 2	36.1 ± 0.78	36.14 ± 0.55	41.32 ± 2.13
Week 3	44.62 ± 1.56	46.22 ± 1.76	41.32 ± 2.4
Week 4	45.52 ± 1.7	45.02 ± 2.1	41.18 ± 2.6

(All data are expressed in mean ± SD)

Table 7.

The effect of aqueous extract of *Hibiscus sabdariffa* on RBC count ($\times 10^6$ µL):

Weeks	Control un-bled group	Control bled group	*Hibiscus sabdariffa* group
Week 0	7.58 ± 0.78	5.97 ± 1.02	5.04 ± 0.5
Week 1	5.88 ± 0.1	6.03 ± 0.28	6.37 ± 0.26
Week 2	6.1 ± 0.1	6.03 ± 0.28	6.9 ± 0.26*
Week 3	7.53 ± 0.29	7.75 ± 0.46	6.66 ± 0.37
Week 4	7.61 ± 0.27	7.44 ± 0.51	6.68 ± 0.4

(All data are expressed in mean ± SD)

*: significant at $P < 0.05$

Table 8.

The effect of aqueous extract of *Hibiscus sabdariffa* on MCV level hemorrhagic anemic rats (fL):

Weeks	Control un-bled group	Control bled group	*Hibiscus sabdariffa* group
Week 0	58.58 ± 0.91	59.24 ± 1.75	61.44 ± 0.21
Week 1	65.04 ± 9.3	65.22 ± 2.66	62.64 ± 2.6
Week 2	59.18 ± 1.8	60.02 ± 2.04	61.88 ± 1.9
Week 3	59.28 ± 1.57	59.72 ± 1.89	62.06 ± 2.5
Week 4	59.84 ± 1.34	60.58 ± 1.61	61.7 ± 2.6

(All data are expressed in mean ± SD)

Table 9.

The effect of aqueous extract of *Hibiscus sabdariffa* on MCH level (Pg):

Weeks	Control un-bled group	Control bled group	*Hibiscus sabdariffa* group
Week 0	17.88 ± 1.5	17.82 ± 0.77	19.68 ± 0.8
Week 1	20. 46 ± 2.9	20.1 ± 1.75	19.44 ± 0.98
Week 2	19.62 ± 0.69	19.9 ± 0.78	19.78 ± 0.8
Week 3	18.52 ± 2.29	19.88 ± 0.56	19.76 ± 0.84
Week 4	18.26 ± 0.44	18.48 ± 0.61	19.9 ± 1.9

(All data are expressed in mean ± SD)

Table 10.

The effect of aqueous extract of *Hibiscus sabdariffa* on MCHC (g/dl) level:

Weeks	Control un-bled group	Control bled group	*A. garckeana* group
Week 0	30.52 ± 2.1	30.1 ± 0.7	32 ± 1.1
Week 1	31.46 ± 4.4	30.78 ± 2.18	31.02 ± 0.44
Week 2	33.18 ± 0.33	33.14 ± 0.21	32 ± 0.73
Week 3	31.32 ± 3.85	33.14 ± 0.21	31.85 ± 0.56
Week 4	30.54 ± 0.15	30.48 ± 0.43	32.28 ± 2

(All data are expressed in mean ± SD)

Table 11.

Effect of *Hibiscus sabdariffa* aqueous extract on Hb level of iron-deficient rats (g/dl):

	Normal diet group	Deficient diet group	*Hibiscus sabdariffa* group
Week 0	13.72 ± 0.19	12.18 ± 0.59	12.52 ± 0.86
Week 1	13.32 ± 0.44 *	12.2± 12.2	12.64 ± 1.1 *
Week 2	12.72 ± 0.31	11.72 ± 0.68	12.16 ± 0.95
Week 3	13.9 ± 0.52	12.04 ± 1.13	12.74 ± 0.65

(Data are expressed in Mean SD)

*: statistically significant at $p < 0.05$

Table 12.

Effect of *Hibiscus sabdariffa* aqueous extract on PCV level of iron-deficient rats (%):

	Normal diet group	Deficient diet group	*Hibiscus sabdariffa* group
Week 0	42.9 ± 1.36	36.76 ± 2.04	38.46 ± 2.6
Week 1	41.88 ± 1.93	37 ± 3.21	39.18 ± 3.5
Week 2	39.76 ± 1.24	35.9 ± 2.5	38.34 ± 3.2
Week 3	42.44 ± 1.6	36.08 ± 3.9	39.24 ± 2.5

(Data are expressed in Mean ± SD)

Table 13.

Effect of *Hibiscus sabdariffa* aqueous extract on RBC count of iron-deficient rats ($\times 10^6/\mu L$):

Weeks	Normal diet group	Deficient diet group	*Hibiscus sabdariffa* group
Week 0	7.062 ± 0.36	6.61 ± 0.3	6.69 ± 0.41
Week 1	6.822 ± 0.27	6.492 ± 0.5	6.8 ± 0.51
Week 2	6.66 ± 0.22	6.358 ± 0.52	6.6 ± 0.52
Week 3	7.128 ± 0.22	6.446 ± 0.68	7 ± 0.48

(Data are expressed in Mean SD)

Table 14.

Effect of *Hibiscus sabdariffa* aqueous extract on MCV (fL) level of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*Hibiscus sabdariffa* group
Week 0	60.3 ± 2.3	55.6 ± 0.86	57.48 ± 1.5
Week 1	61.42 ± 2.96	56.98 ± 1.03	57.58 ± 1.56
Week 2	59.7 ± 0.7	56.52 ± 1.55	57.88 ± 1.7
Week 3	59.54 ± 0.7	55.98 ±1.26	56.12 ± 1.3

(Data are expressed in Mean ± SD)

Table 15.

Effect of *Hibiscus sabdariffa* aqueous extract on MCH level of iron-deficient rats (Pg):

Weeks	Normal diet group	Deficient diet group	*Hibiscus sabdariffa* group
Week 0	19.48 ± 0.81	18.44 ± 0.36	18.72 ± 0.46
Week 1	19.54 ± 0.87	18.82 ± 0.33	18.58 ± 0.83
Week 2	19.1 ± 0.4	18.46 ± 0.49	18.38 ± 0.38
Week 3	19.5 ± 0.35	18.72 ± 0.75	18.24 ± 0.56

(Data are expressed in Mean ± SD)

Table 16.

Effect of *Hibiscus sabdariffa* aqueous extract on MCHC level of iron-deficient rats (g/dl):

	Normal diet group	Deficient diet group	*Hibiscus sabdariffa* group
Week 0	31.98 ± 0.78	33.16 ± 0.76	32.54 ± 0.6
Week 1	31.84 ± 0.52 *	33.02 ± 0.59	32.28 ± 1.2 *
Week 2	32.02 ± 0.63 *	32.66 ± 0.54	31.74 ± 0.69*
Week 3	32.76 ± 0.18	33.42 ± 0.8	32.48 ± 0.5

(Data are expressed in Mean ± SD)

- : statistically significant at P< 0.05

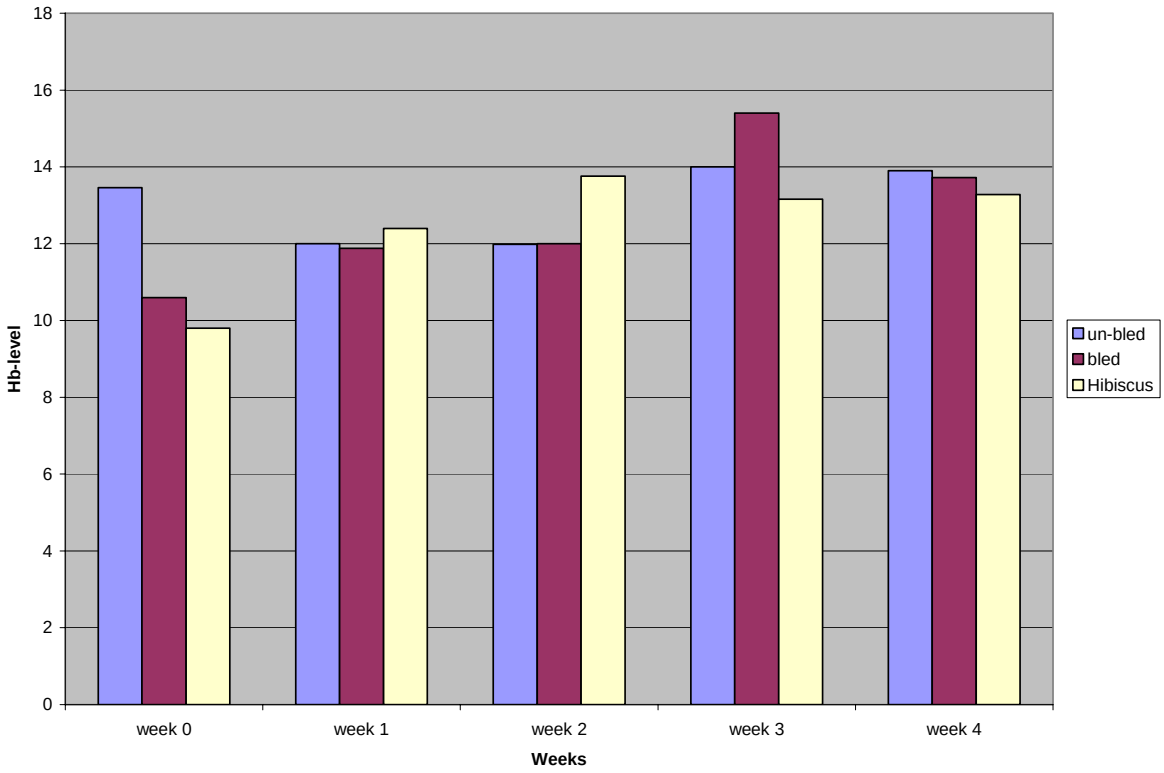

Figure 6. Effect of *H. sabdariffa* on Hb-level of hemorrhagic anemic rats

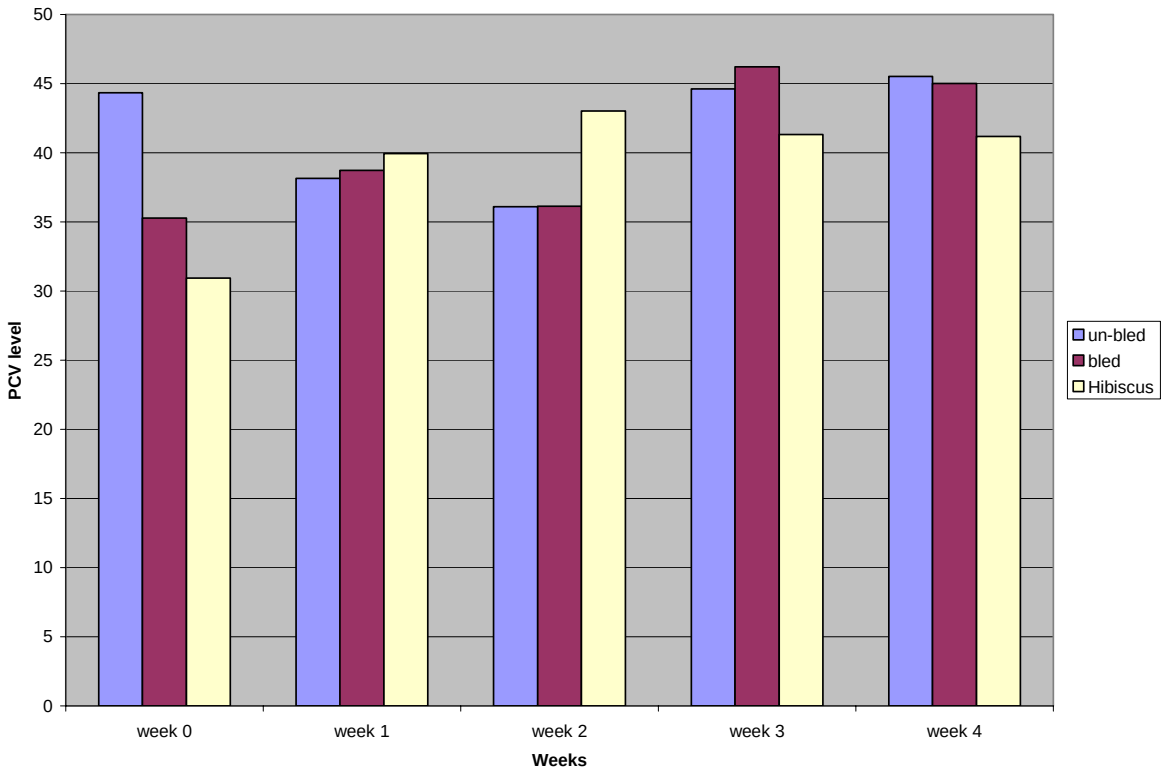

Figure 7. Effect of *H. sabdariffa* on PCV level of hemorrhagic anemic rats

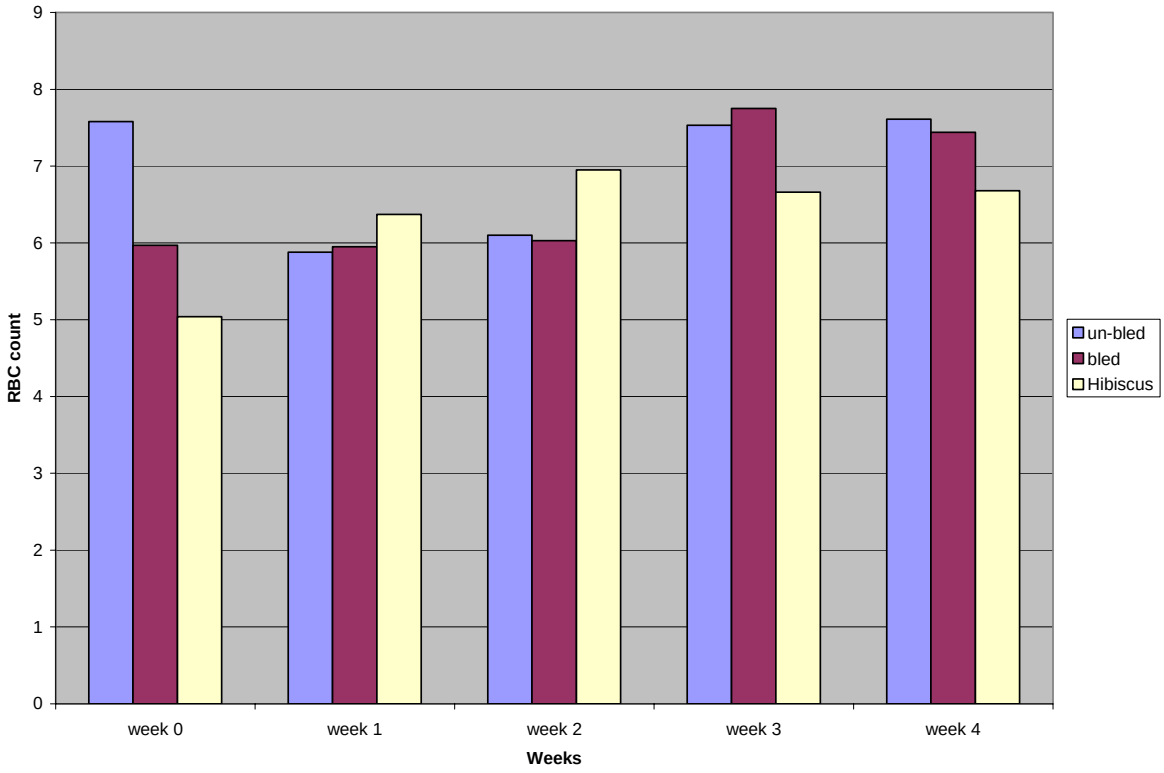

Figure 8. Effect of *H. sabdariffa* on RBC count of hemorrhagic anemic rats

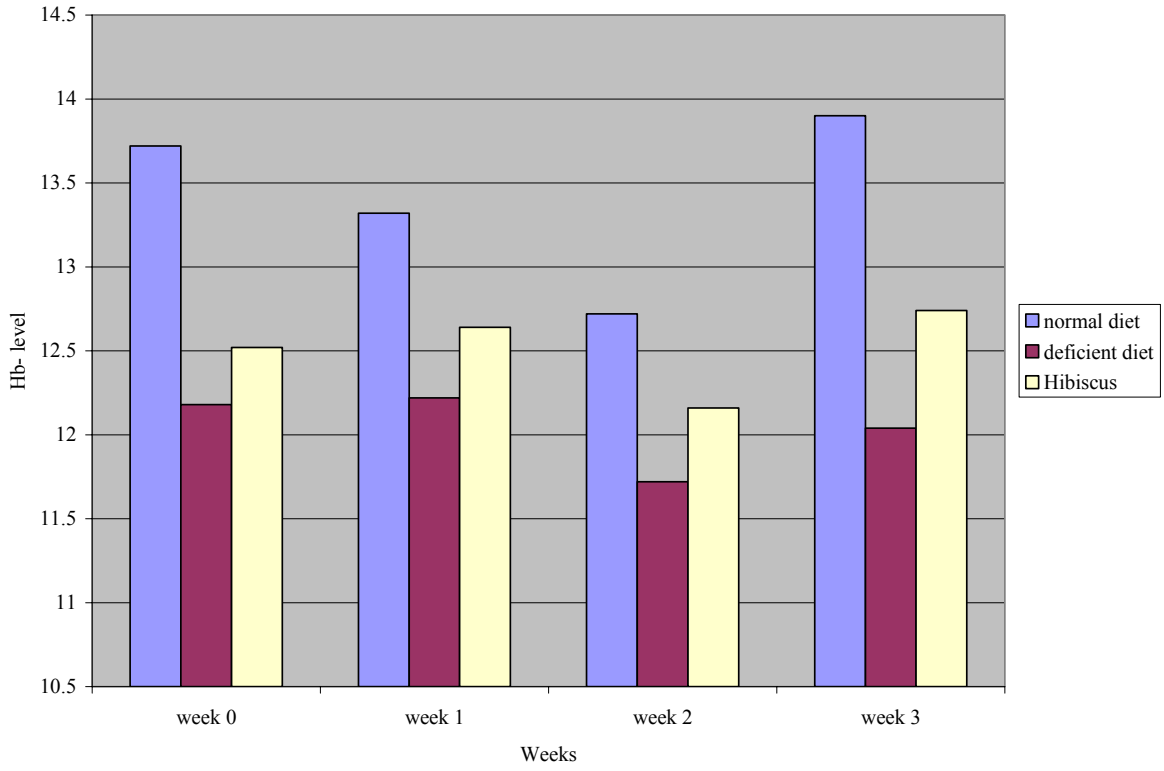

Figure 9. Effect of *H. sabdariffa* on Hb- level of iron-deficient rats

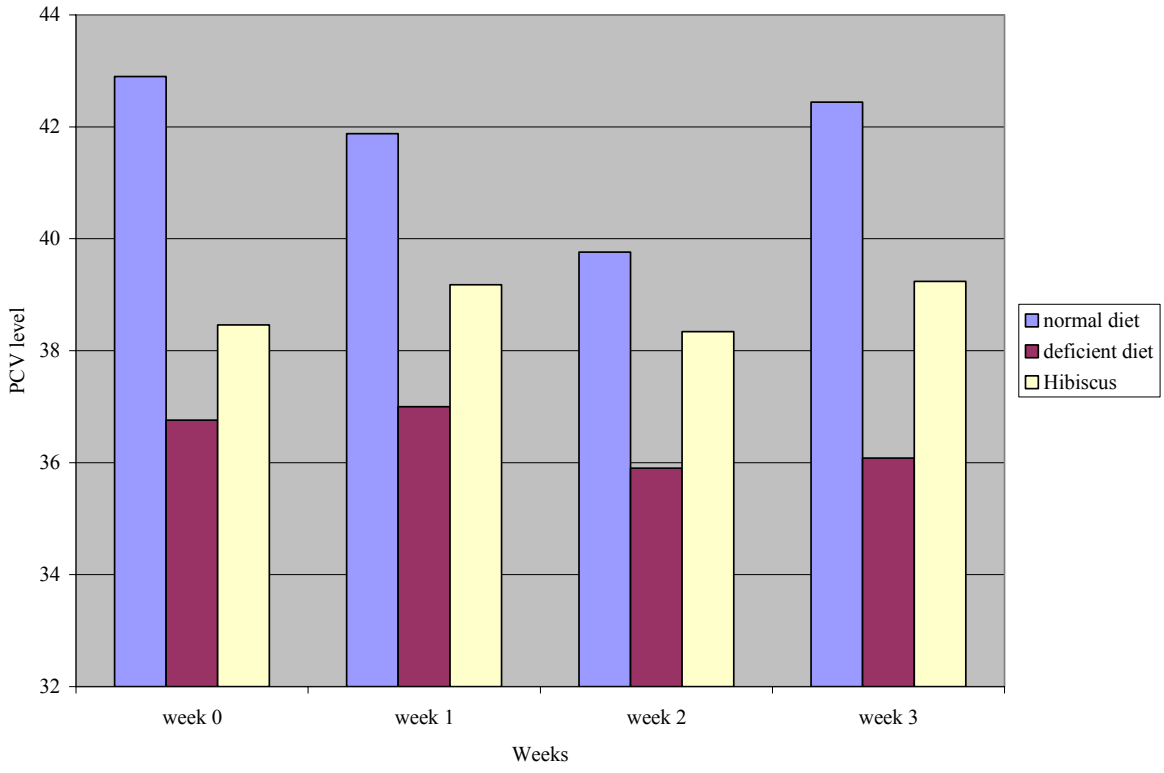

Figure 10. Effect of *H. sabdariffa* on PCV level of iron-deficient rats

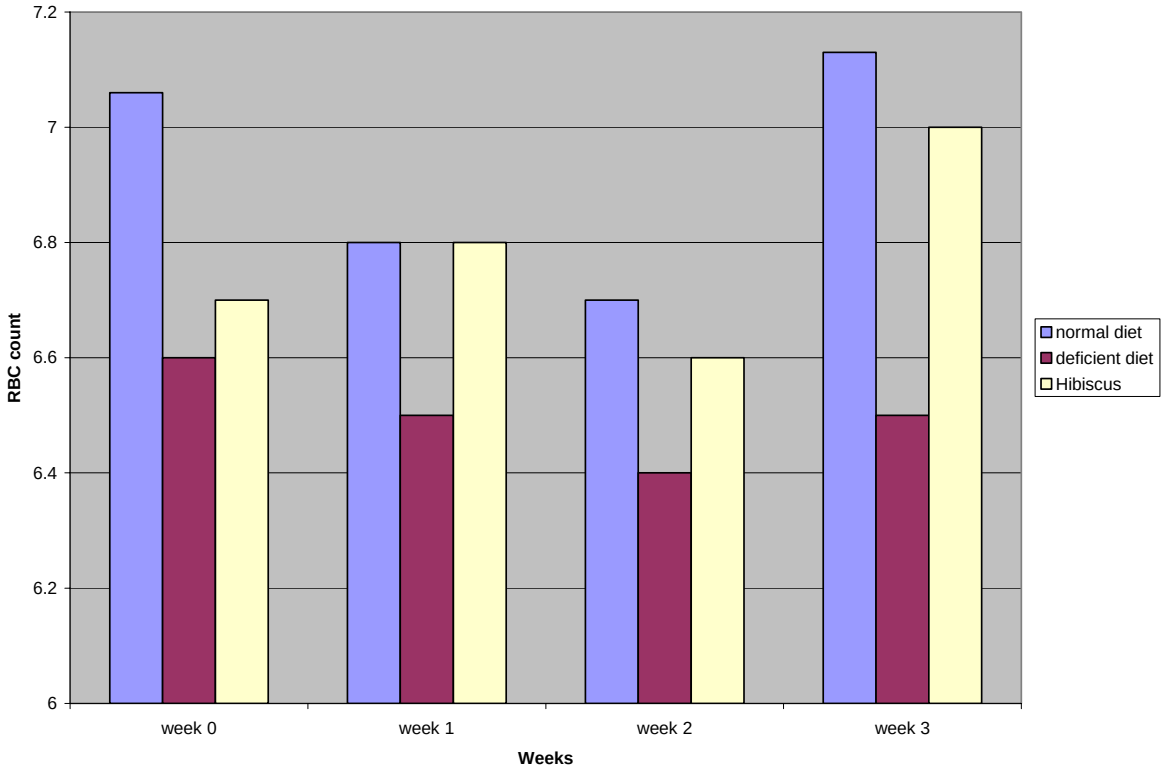

Figure 11. Effect of *H. sabdariffa* on RBC count of iron-deficient rats

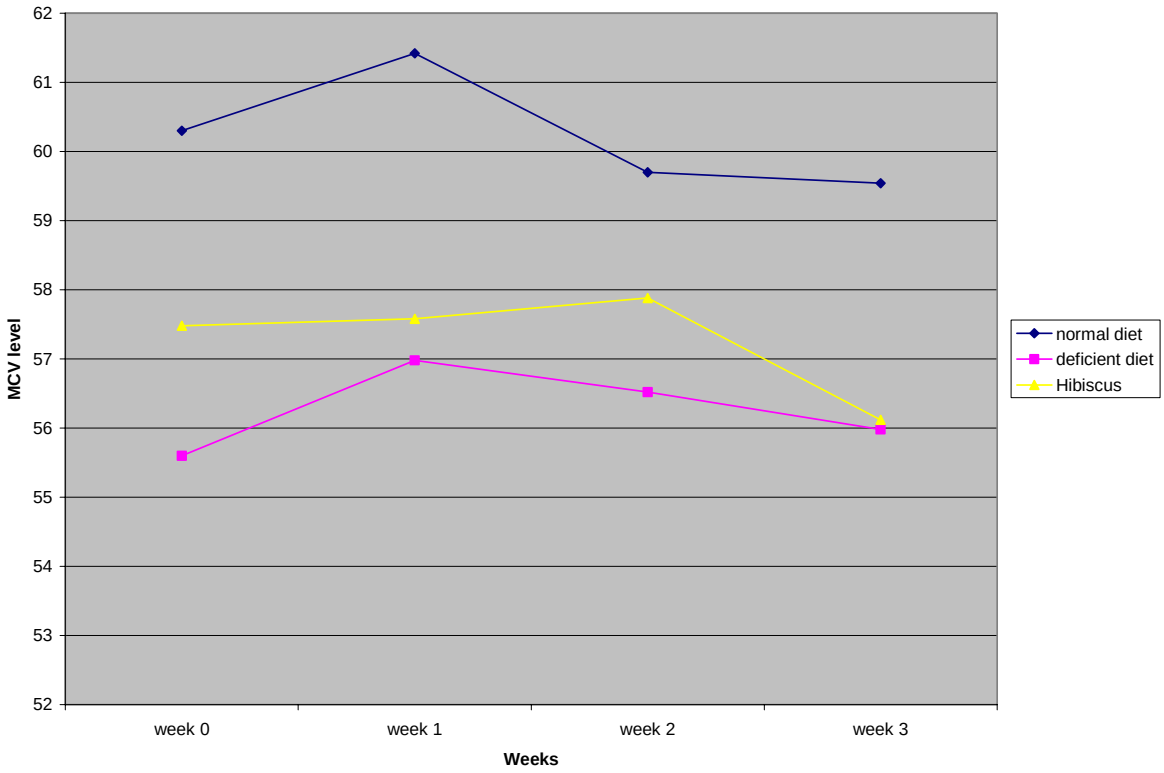

Figure 12. Effect of *H. sabdariffa* on MCV level of iron-deficient rats

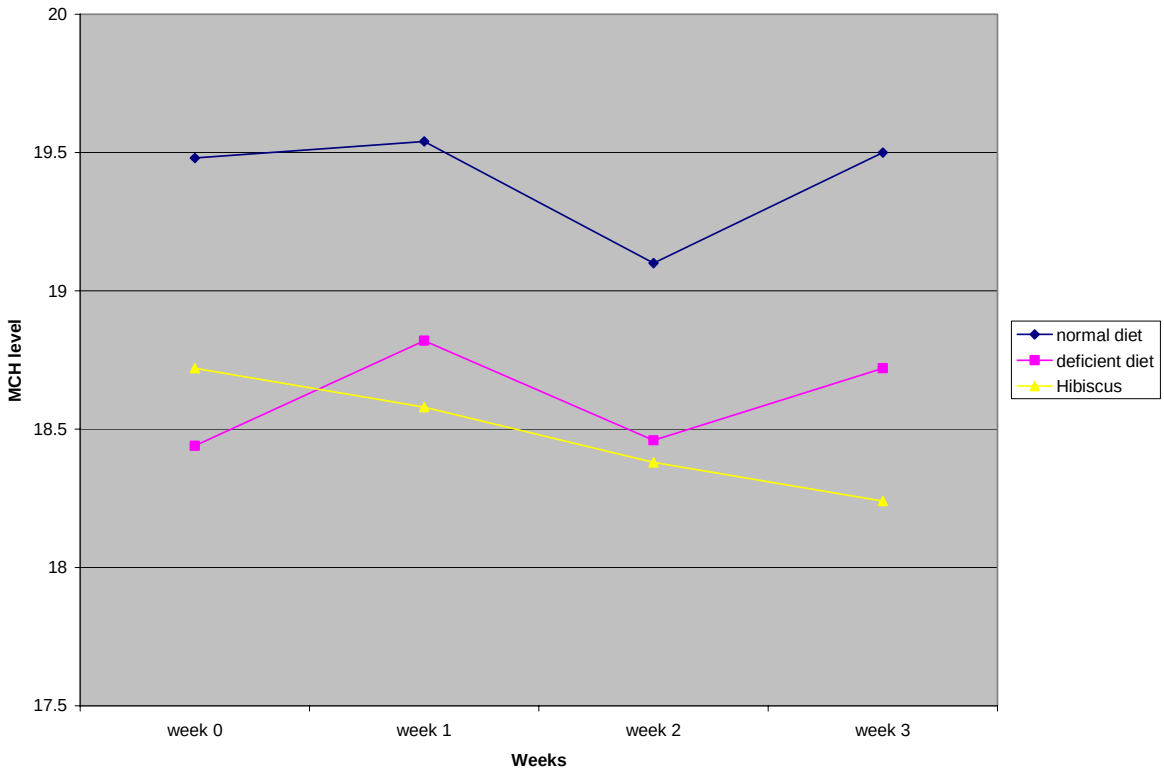

Figure 13. Effect of *H. sabdariffa* on MCH level of iron-deficient rats

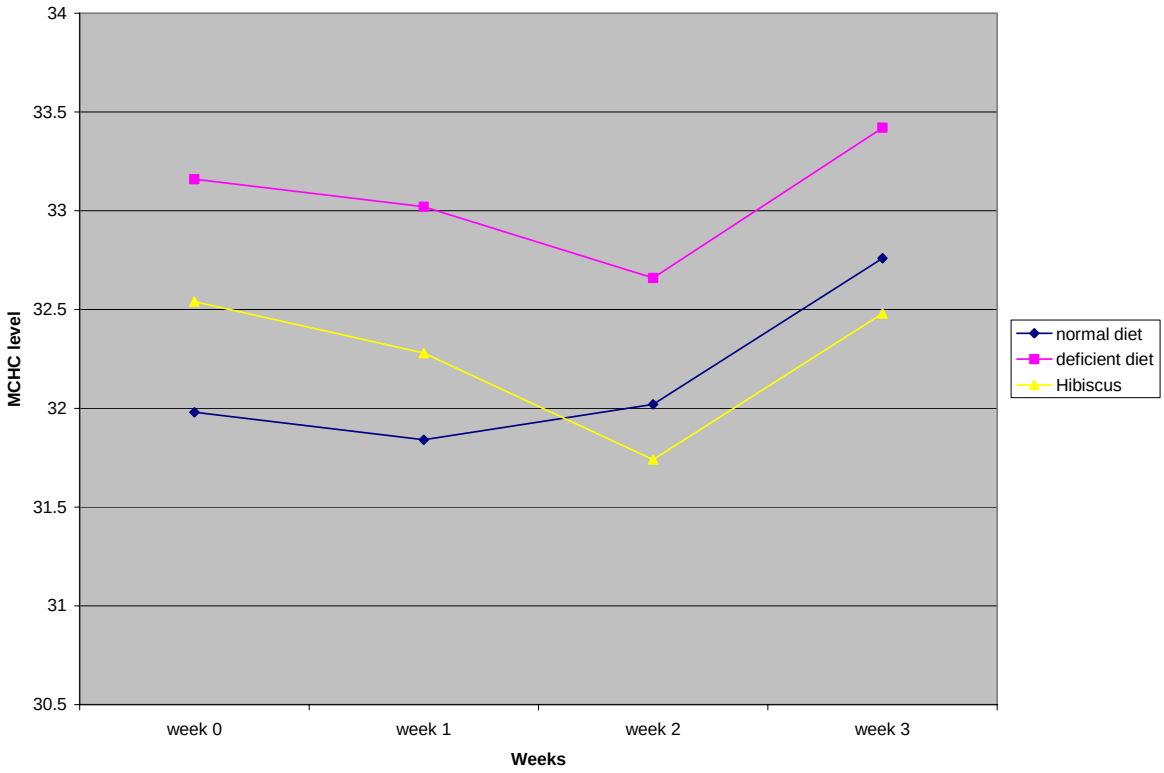

Figure 14. Effect of *H. sabdariffa* on MCHC level of iron-deficient rats

3.2.3. The effect of *A.garckeana aqueous* extract on hemorrhagic anemic rats:

It was found that administration of (2g/kg) of *Azanza garckeana* aqueous extract caused remarkable changes in the blood indices of hemorrhagic anemic rats. This extract caused an increase in the Hb level of the hemorrhagic anemic rats on the first and second weeks of treatment (27.5 % and 39.4 % of increase respectively) compared to the control groups (bled and un-bled). This increase was statistically significant at $P< 0.05$; results are shown in table 17. and figure 15.

On the third week of treatment, the treated group and the control bled group attained normal hemoglobin levels compared with the control un-bled group.

The *A. garckeana* extract showed non significant increases in the PCV levels as attained in the hemoglobin levels. The increases in the PCV values were found to be 32.8 % and 43.8 % in the first and second weeks of treatment respectively compared to that of the control bled group (statistically significant at $P< 0.05$).

In the third week, the PCV levels of the treated group and the control un-bled group were nearly the same.

In the fourth week, all of the groups (treated and controls) attained normal levels of PCV. Table 18. and figure 16.

The red blood cell count was affected largely by the administration of the *A. garckeana* extract. It caused a significant increase ($P < 0.005$) in the first and second weeks of treatment that was estimated by 29.4 % and 36 % respectively. However, on the third and fourth weeks of treatment, the three groups showed stable and nearly equal levels of red blood cell counts (table 19. and figure 17.)

The values of the mean cell volume (MCV) in the control bled group were elevated remarkably (10.1 %) in the first week of experiment. However, the *A. garckeana* group showed a much lower increase in the MCV values that was estimated by 2.4 %. The control un-bled group showed an increase of 11 % in the first week.

In the fourth week, the MCV values of the control bled group were elevated slightly by 2.3 %, whereas, in the treated group the MCV values has returned to the same level that was shown before treatment. Table 20.

The control bled and un-bled groups showed variable increases in the mean cell hemoglobin values (MCH) in the first week of the experiment. These increases were estimated as 12.8 % and 14.4 % respectively.

The *A. garckeana* extract caused a reduction in the MCH level (1.26 %), however, this was not statistically significant.

In the third week, the MCH of the control bled group continued to increase by 11.6 % while the *A. garckeana* group, caused a decrease of 3.6 %. Table 21.

On the other hand, the *A.garckeana* caused a reduction in the MCHC level on the second week of treatment (3.4 %). While in the control bled rats, there was an increase of 10.3 %.

In the third week, the reduction in the treated group continued and this reduction was 7.4 %, whereas, the increase in the control bled group was found 10.1%. Table 22.

3.2.4. The effect of *A. garckeana aqueous* extract on iron-deficient rats:

In the second type of experiments, the effect of *A. garckeana* aqueous extract was studied in nutritionally iron- deficient rats. After determining the basal hematological values of the nutritionally anemic rats, the treatment with the extract commenced.

The Hb level, PCV, MCV, MCH, MCHC and RBC counts were determined on days 7, 14 and 21 of the treatment. Multiple comparisons were made between the treated group, the control un-treated group and another control group that was fed with a normal iron content diet (39.15 mg Fe/ kg diet).

The *A. garckeana* extract did not show any obvious alterations in the hematological parameters of the nutritionally iron deficient rats. By contrast, this extract was found to cause disadvantageous effects in the second and third weeks of treatment. It caused a decrease of 15.2 % in the Hb values in the second week compared to 3.8 % decrease in the Hb values of the control iron deficient rats. This unfavorable effect continued to the third week when a decrease of 5.3 % was observed in the treated group, compared to a decrease of 0.12 % in the control group that was fed with the iron deficient diet. In this week, the control rats fed with the normal diet showed a decrease of 1.3 % in the Hb level. Table 23. and figure 18.

Despite the above results, the extract possessed advantageous effects on the RBC counts of the anemic rats, although this effect was shown lately in the third week of

treatment. It caused an increase of 2.8 % in the RBC count compared to an increase of 1 % in the normal diet rats and a decrease of 2.4 % in the iron deficient ones. However, this increase in the treated group was not statistically significant. Table 25. and figure 23.

Evaluating other blood indices, revealed that *A. garckeana* extract had caused an increase of 2.1 % in the MCV level in the second week of treatment, compared to an increase of 1.7 % attained by the control iron deficient group. However, this increase attained by the treated group was not consistent because it changed to a decrease of 4.2 % in the third week of treatment. Table 26. and figure 20.

The MCH levels of the treated group showed decreases of 5.1 % and 8.3 % in the second and third weeks of treatment respectively, compared to increases of 0.1 % and 1.5 % in the control iron- deficient group.

These decreases in the MCH values were followed with decreases in the MCHC levels. The extract caused decreases of 6.6 % and 4.3 % in the second and third weeks of treatment respectively compared to 1.5 % decrease and 0.8 % increase in the control iron deficient rats in the same weeks. Table 27. and figure 21.

Table 17.

The effect of aqueous extract of *Azanza garckeana* on Hb level of (g/dl):

Weeks	Control un-bled group	Control bled group	*A. garckeana* group
Week 0	13.46 ± 0.46	10.6 ± 1.56	10.92 ± 1.86
Week 1	12 ± 1.96	11.88 ± 0.67	13.92 ± 1.12
Week 2	11.98 ± 0.31	12 ± 0.21	15.22 ± 1.67
Week 3	14 ± 2	15.4 ± 0.55	14.28 ± 1.34
Week 4	13.9 ± 0.5	13.72 ± 0.55	14.5 ± 0.9

(All data are expressed in mean ± SD)

Table 18.

The effect of aqueous extract of *Azanza garckeana* on PCV level (%):

Weeks	Control un-bled group	Control bled group	*A. garckeana* group
Week 0	44.34 ± 4.01	35.28 ± 5.5	33.28 ± 5.95
Week 1	38.14 ± 6.5	38.72 ± 3.2	44.18 ± 2.05
Week 2	36.1 ± 0.78	36.14 ± 0.55	47.86 ± 3.8
Week 3	44.62 ± 1.56	46.22 ± 1.76	46.88 ± 3.13
Week 4	45.52 ± 1.7	45.02 ± 2.1	43.58 ± 2.47

(All data are expressed in mean ± SD)

Table 19.

The effect of aqueous extract of *Azanza garckeana* on RBC count ($\times 10^6$µL):

Weeks	Control un-bled group	Control bled group	*A. garckeana* group
Week 0	7.58 ± 0.78	5.97 ± 1.02	5.72 ± 0.9
Week 1	5.88 ± 0.1	6.03 ± 0.28	7.4 ± 0.31*
Week 2	6.1 ± 0.1	6.03 ± 0.28	7.78 ± 0.58
Week 3	7.53 ± 0.29	7.75 ± 0.46	7.76 ± 0.38
Week 4	7.61 ± 0.27	7.44 ± 0.51	7.44 ± 0.62 *

(All data are expressed in mean ± SD)

* : significant at $P < 0.05$

Table 20.

The effect of aqueous extract of *Azanza garckeana* on MCV level (fL):

Weeks	Control un-bled group	Control bled group	*A. garckeana* group
Week 0	58.58 ± 0.91	59.24 ± 1.75	58.04 ± 3.24
Week 1	65.04 ± 9.3	65.22 ± 2.66	59.78 ± 2.77
Week 2	59.18 ± 1.8	60.02 ± 2.04	61.56 ± 3.32
Week 3	59.28 ± 1.57	59.72 ± 1.89	60.44 ± 3.13
Week 4	59.84 ± 1.34	60.58 ± 1.61	58.74 ± 2.45

(All data are expressed in mean ± SD)

Table 21.

The effect of aqueous extract of *Azanza garckeana* on MCH level (Pg):

Weeks	Control un-bled group	Control bled group	*A. garckeana* group
Week 0	17.88 ± 1.5	17.82 ± 0.77	10.08 ± 1.34
Week 1	20. 46 ± 2.9	20.1 ± 1.75	18.84 ± 1.43
Week 2	19.62 ± 0.69	19.9 ± 0.78	19.56 ± 1.58
Week 3	18.52 ± 2.29	19.88 ± 0.56	18.4 ± 1.16
Week 4	18.26 ± 0.44	18.48 ± 0.61	19.56 ± 1.07

(All data are expressed in mean ± SD)

Table 22.

The effect of aqueous extract of *Azanza garckeana* on MCHC level (g/dl):

Weeks	Control un-bled group	Control bled group	*B. garckeana* group
Week 0	30.52 ± 2.1	30.1 ± 0.7	32.84 ± 0.69
Week 1	31.46 ± 4.4	30.78 ± 2.18	31.46 ±1.11
Week 2	33.18 ± 0.33	33.14 ± 0.21	30.42 ± 0.83
Week 3	31.32 ± 3.85	33.14 ± 0.21	30.42 ± 0.83
Week 4	30.54 ± 0.15	30.48 ± 0.43	33.26 ± 0.66

(All data are expressed in mean ± SD

Table 23.

Effect of *A.garckeana* aqueous extract on Hb level of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*A.garckeana* group
Week 0	13.72 ± 0.19	12.18 ± 0.59	12.42 ± 0.48
Week 1	13.32 ± 0.44	12.2± 12.2	12.1 ± 1.1
Week 2	12.72 ± 0.31	11.72 ± 0.68	10.525 ± 0.79
Week 3	13.9 ± 0.52	12.04 ± 1.13	11.76 ± 1.83

(Data are expressed in Mean SD)

Table 24.

Effect of *A.garckeana* aqueous extract on PCV level of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*A. garckeana* group
Week 0	42.9 ± 1.36	36.76 ± 2.04	37.92 ± 1.6
Week 1	41.88 ± 1.93	37 ± 3.21	36.88 ± 3.6
Week 2	39.76 ± 1.24	35.9 ± 2.5	34.475 ± 2.1
Week 3	42.44 ± 1.6	36.08 ± 3.9	37.4 ± 4.1

(Data are expressed in Mean SD)

Table 25.

Effect of *A.garckeana* aqueous extract on RBC count of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*A.garckeana* group
Week 0	7.062 ± 0.36	6.61 ± 0.3	6.698 ± 0.45
Week 1	6.822 ± 0.27	6.492 ± 0.5	6.498 ± 0.58
Week 2	6.66 ± 0.22	6.358 ± 0.52	5.9725 ± 0.51
Week 3	7.128 ± 0.22	6.446 ± 0.68	6.886 ±0.7

(Data are expressed in Mean SD)

Table 26.

Effect of *A.garckeana* aqueous extract on MCV level of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*A.garckeana* group
Week 0	60.3 ± 2.3	55.6 ± 0.86	56.64 ± 1.56
Week 1	61.42 ± 2.96	56.98 ± 1.03	56.72 ± 1.56
Week 2	59.7 ± 0.7	56.52 ± 1.55	57.85 ± 2.9
Week 3	59.54 ± 0.7	55.98 ±1.26	54.28 ± 1.48

(Data are expressed in Mean SD)

Table 27.

Effect of *A.garckeana* aqueous extract on MCH level of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*A.garckeana* group
Week 0	19.48 ± 0.81	18.44 ± 0.36	18.58 ± 0.54
Week 1	19.54 ± 0.87	18.82 ± 0.33	17.9 ± 1.1
Week 2	19.1 ± 0.4	18.46 ± 0.49	17.675 ± 1.75
Week 3	19.5 ± 0.35	18.72 ± 0.75	17.04 ± 1.4

(Data are expressed in Mean SD)

Table 28.

Effect of *A.garckeana* aqueous extract on MCHC level of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*A.garckeana* group
Week 0	31.98 ± 0.78	33.16 ± 0.76	32.76 ± 0.45
Week 1	31.84 ± 0.52	33.02 ± 0.59	31.58 ± 1
Week 2	32.02 ± 0.63	32.66 ± 0.54	30.6 ± 3.2
Week 3	32.76 ± 0.18	33.42 ± 0.8	31.34 ± 1.9

(Data are expressed in Mean SD)

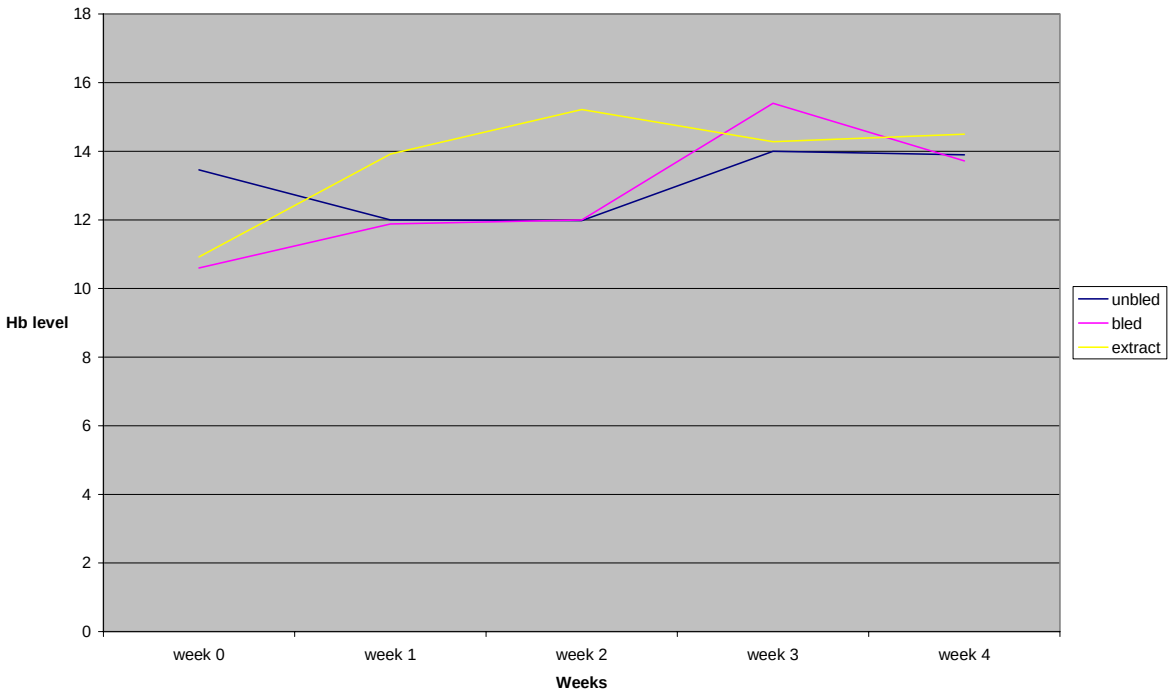

Figure 15. Effect of *A. garckeana* on Hb- level of hemorrhagic anemic rats

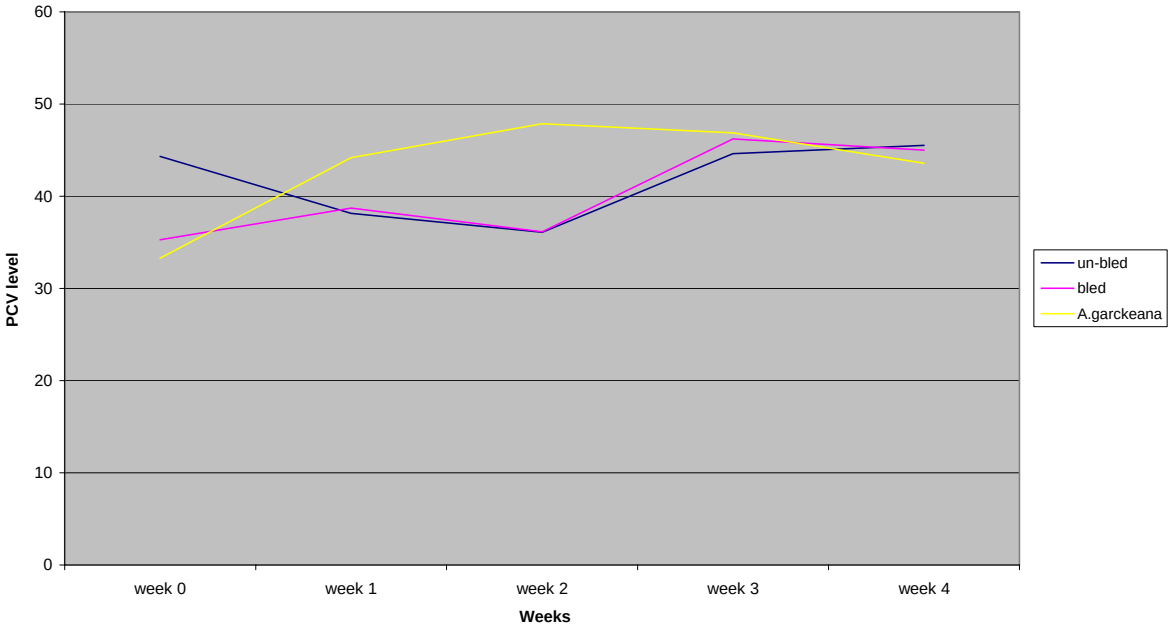

Figure 16. Effect of *A. garckeana* on PCV level of hemorrhagic anemic rats

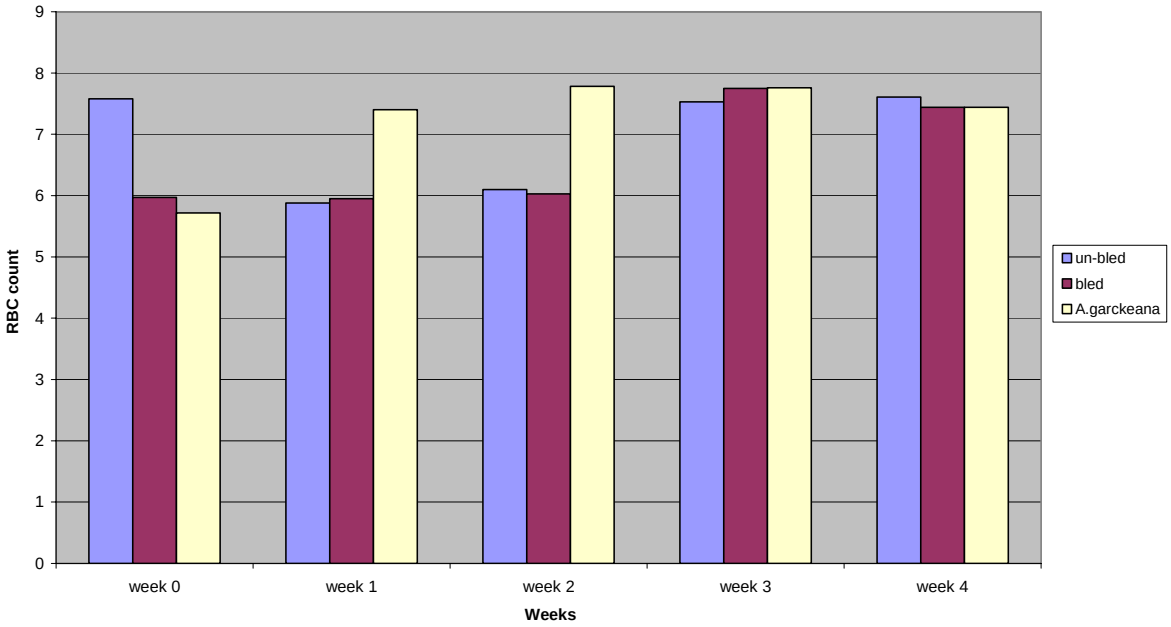

Figure 17. Effect of *A. garckeana* on RBC count of hemorrhagic anemic rats

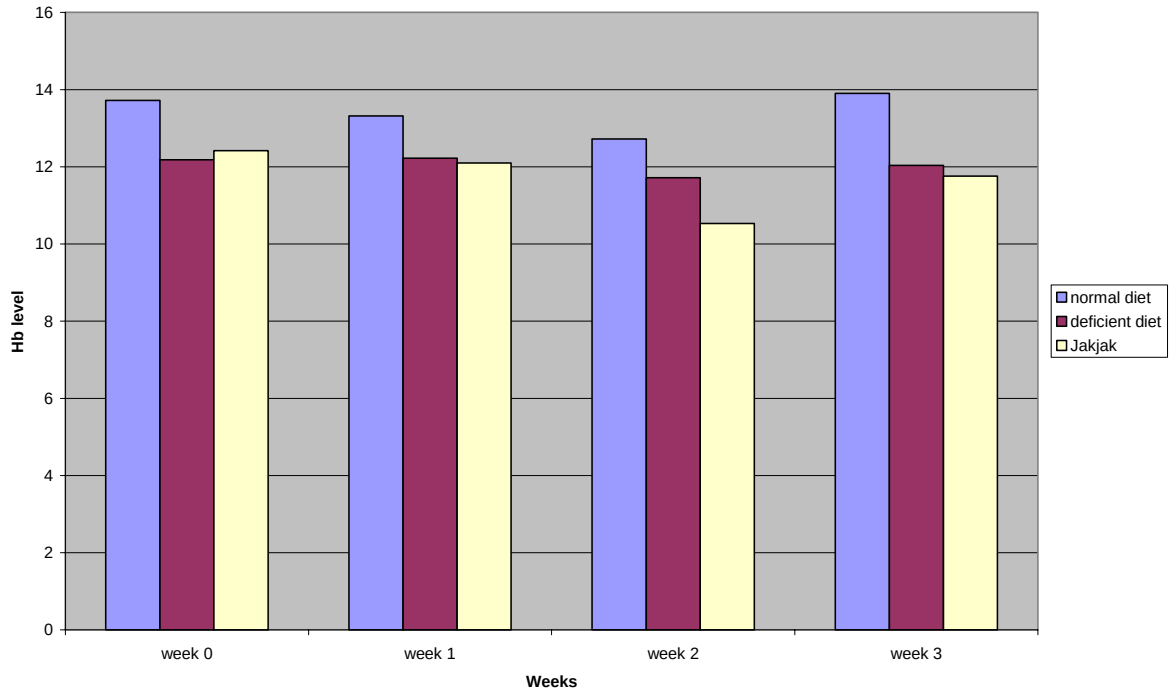

Figure 18. Effect of *A. garckeana* on Hb-level of iron-deficient rats

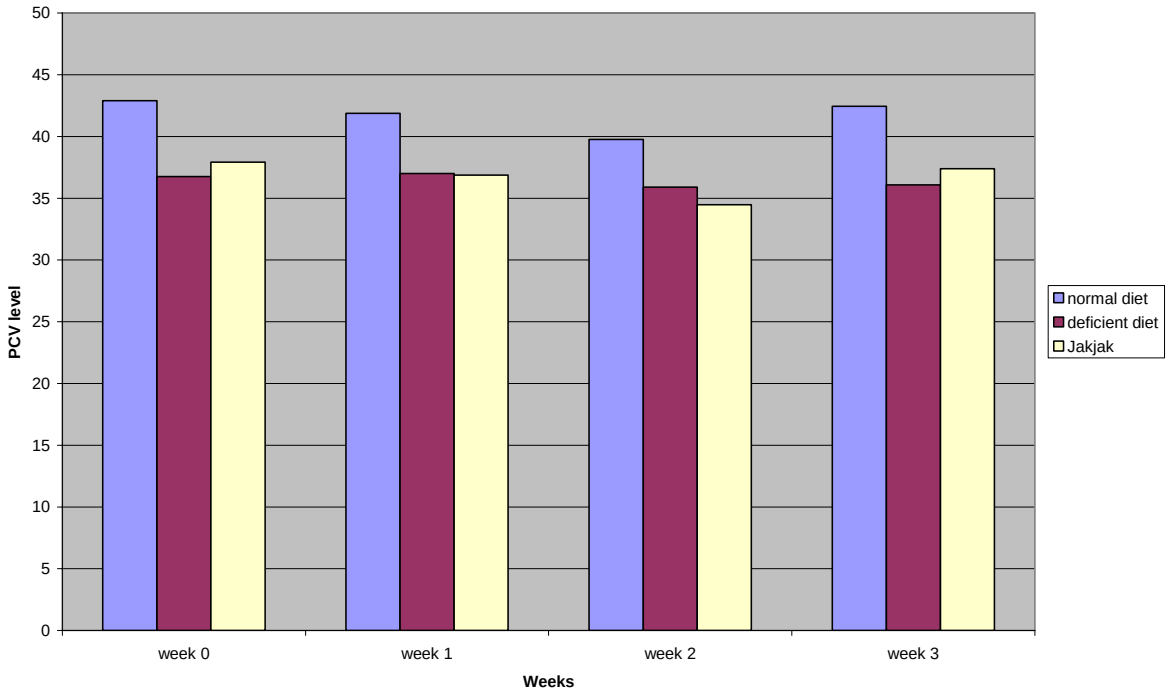

Figure 19. Effect of *A. garckeana* on PCV level of iron-deficient rats

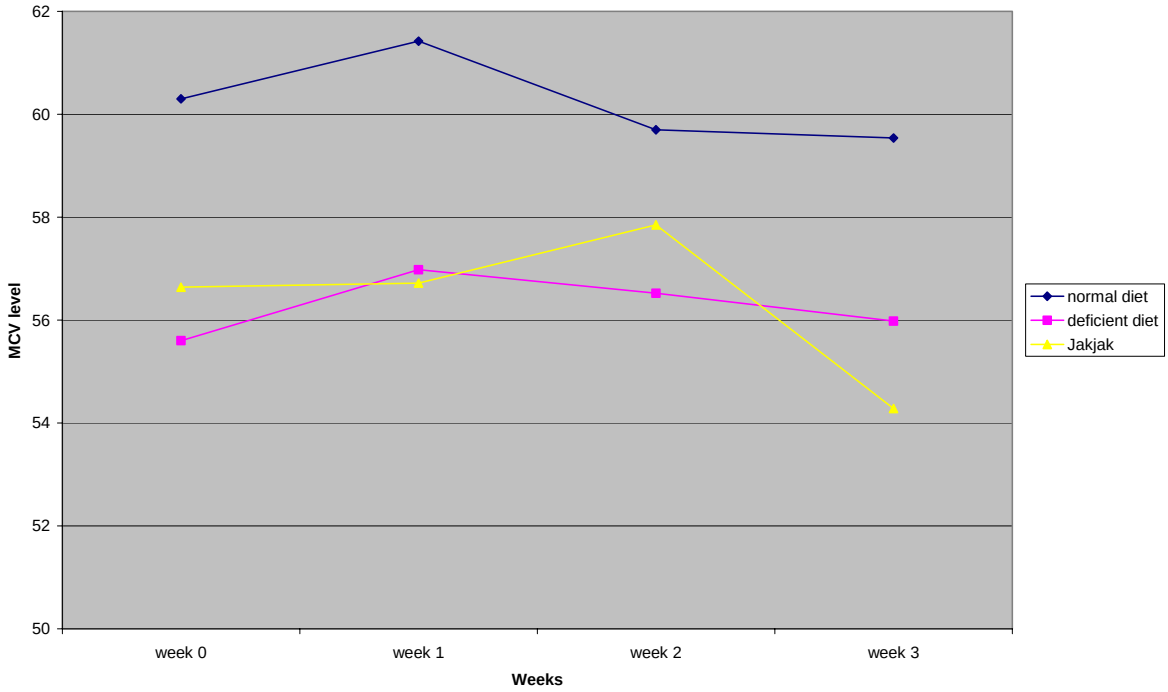

Figure 20. Effect of *A. garckeana* on MCV level of iron-deficient rats

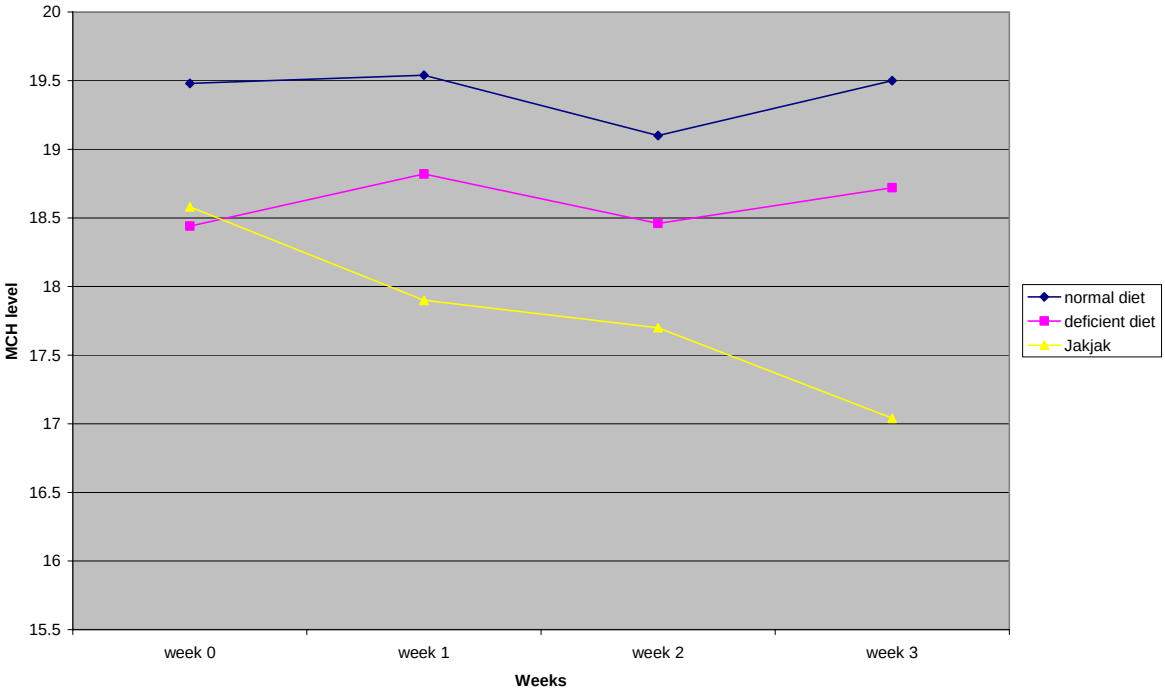

Figure 21. Effect of *A. garckeana* on MCH level of iron-deficient rats

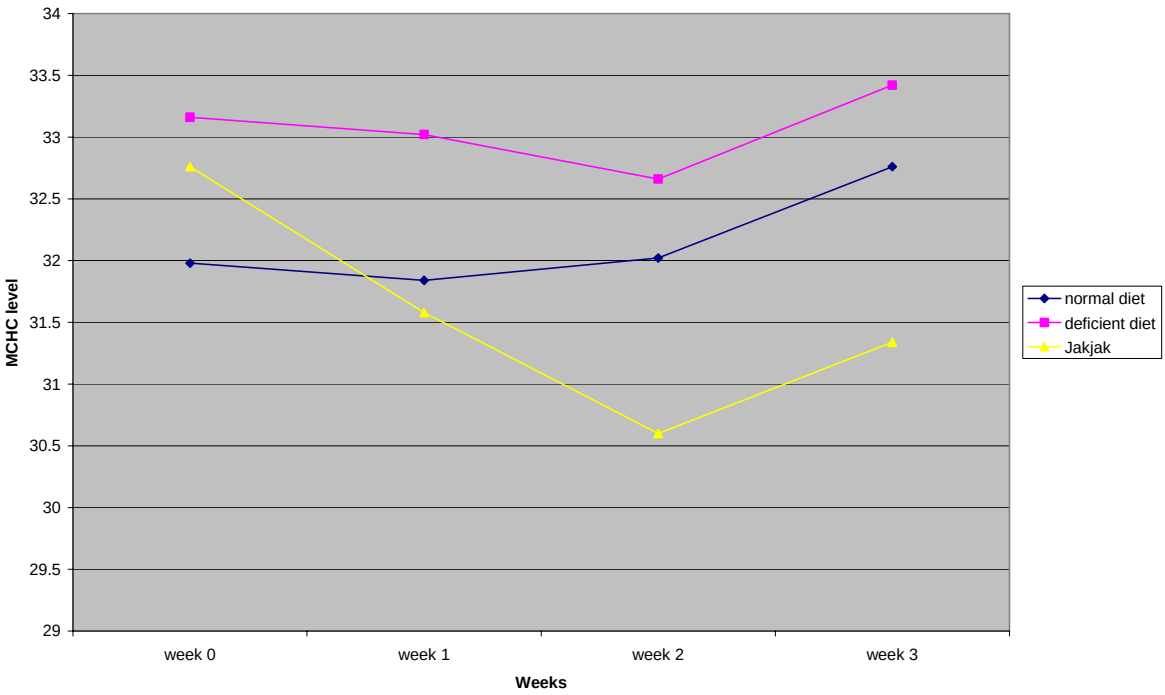

Figure 22. Effect of *A. garckeana* on MCHC level of iron-deficient rats

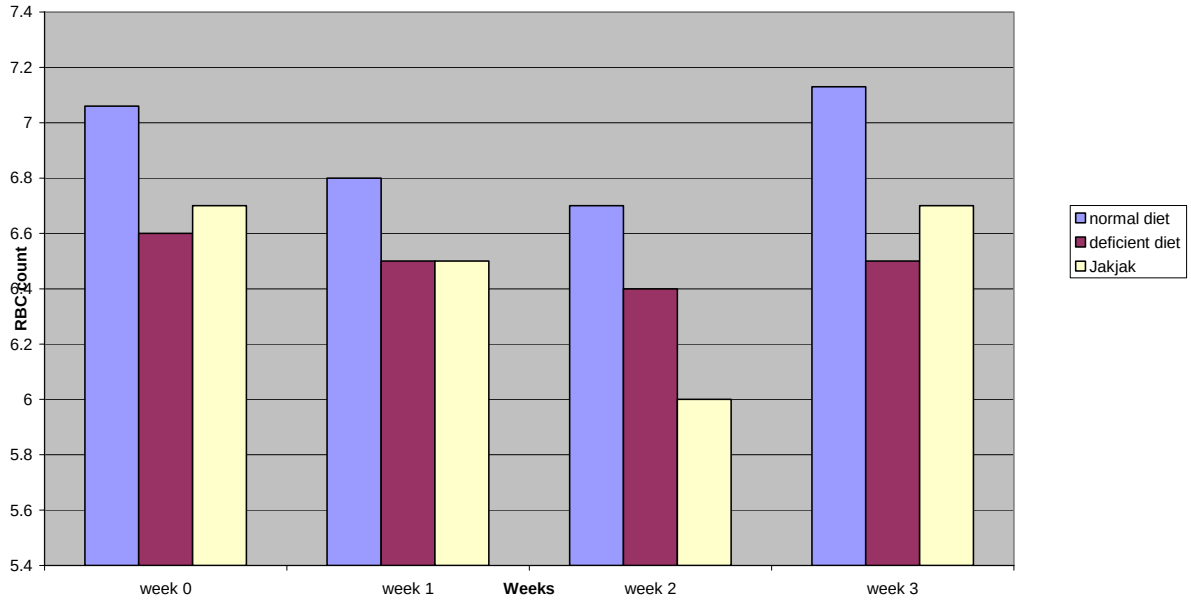

Figure 23. Effect of *A. garckeana* on RBC count of iron-deficient rats

3.2.5. The effect of *Grewia tenax* aqueous extract on hemorrhagic anemic rats:

Administration of the aqueous extract of *Grewia tenax* to hemorrhagic anemic rats caused remarkable changes in their blood indices throughout the four weeks period of treatment.

The aqueous extract of *G. tenax* caused remarkable increases in the hemoglobin levels of the bled anemic rats throughout the experiment. These increases were estimated as 26.5 %, 40.4 %, 34.4 % and 35.5 % in the first, second, third and fourth weeks of the experiment respectively, (Table 29. and figure 24.

These increases in the Hb levels of the treated rats coincided with other increases showed in their red blood cell counts.

It was found that administration of *G. tenax* to the hemorrhagic anemic rats has caused increases of 26.4 %, 36.9 %. 32 % and 32.5 % in their blood cell counts in the first, second, third and fourth weeks of treatment respectively, table 30. and figure 25.

However, the extract has been found to cause much less increases in the PCV values of these animals compared to those showed in the hemoglobin estimations and/or red blood cell counts. The increases in the PCV levels were estimated as 7.7%, 3.46 %, 3.46%4 and 3.1 % in the first, second, third and fourth weeks of treatment respectively, table 31. and figure 26.

It was found that *G. tenax* aqueous extract has shown small increases in the MCV levels of the hemorrhagic anemic rat (table 32.) These increases were estimated as 2 %. 0.7 %, 1 % and 0.4 % increases on weeks 1, 2, 3, and 4 of the treatment respectively.

On the other hand, *G. tenax* aqueous extract has shown small detectable changes in the MCH and MCHC values of these anemic bled rats. Tables (33. and 34.)

These changes were expressed as a decrease of 1.2 % in the first week of treatment. This decrease was followed by small increases of 0.5%, 0.4% and 1.1 % shown on the second, third and fourth weeks of the experiment respectively.

On the same pattern, *G. tenax* has caused a decrease of 3.2 % in the MCHC level in the first week of treatment whereas it has caused no change in the MCHC values of the second week of treatment. Then it was followed by a decrease of 0.5 % in the third week and an increase of 0.88 % in the last week of the experiment.

3.2.6. The effect of *Grewia tenax* aqueous extract on iron- deficient rats:

It was found that, administration of the aqueous extract of *G. tenax* to nutritionally anemic rats (iron- deficient rats) has caused remarkable changes in their blood indices. These changes were compared to those occurred to a group of control un -treated group that was also nutritionally anemic.

In the first week of treatment, *G. tenax* has caused an increase of 0.96 % in the Hb level of the anemic rats compared to an increase of only 0.16 % in the control un-treated group. However, this increase has changed to a decrease of 3 % in the second week of treatment in the *G. tenax* group compared to a decrease of 3.9 % in the control group in the same week. Table 35. and figure 27.

In the third week, the aqueous extract of *G. tenax* has caused an increase of 1.8 % compared to an increase of 1.2 % in the control rats.

The changes in the PCV values were found to follow almost the same pattern of changes that were shown in the first week; there were increases of 1.9 % and 0.65 % in the *G. tenax* and control groups respectively. While in the second week, there were decreases of 0.3 % and 2.4 % in the treated and control groups respectively. In the third week, an increase of 2 % was detected in the *G. tenax* group compared to an increase of 1.9 % in the control un-treated group. Table 36. and figure 28.

Studies on the effect of the *G. tenax* aqueous extract on the red blood cell counts of the iron-deficient rats has shown that the extract has caused an increase of 1.6 % in the first week of treatment compared to a decrease of 1.8 % in the un-treated animals. Table 37. and figure 29.

In the second week, a decrease of only 1.4 % in the *G. tenax* group was detected comparable to a decrease of 4 % in the control group.

In the third week, while an increase of 4.6 % has been shown in the *G. tenax* group, a decrease of 2.5 % was detected in the control group.

G. tenax aqueous extract has shown small un-recognizable changes in the MCV levels of the anemic iron-deficient rats. The extract has caused small increase of 0.17 % in the MCV of the nutritionally anemic rats in the first week of treatment. The increase was found to be slightly higher in the second week (0.7 %) compared to a decrease of 0.14 % in the anemic un-treated group. However, in the third week of treatment there was a decrease of 2.4 % shown in the *G.tenax* group that was compared to an increase of 0.7 % in the anemic un-treated animals. Table 38. and figure 30.

Administration of *G. tenax* aqueous extract to nutritionally deficient rats has caused consistent decreases in the MCH levels of the rats throughout the three weeks of treatment on table 39. These decreases were 0.75 %, 1.9 % and 2.6 % shown in the first, second and third weeks respectively.

On the other hand, the control anemic group has shown increases of 2.1 %. 0.1 % and 1.4 % in the three weeks of treatment respectively.

The effect of *G. tenax* aqueous extract on the MCHC levels of the iron- deficient rats was small and inconsistent. This extract has shown decreases of 0.8 %, 2.5 % and 0.18 % throughout the first, second and third weeks of experimentation respectively. These decreases were compared to other decreases of 0.4 % and 1.5 % and an increase of 0.8 % in the three weeks of experimentation in the anemic un-treated group on table 40. and figure 31.

Table 29.

The effect of aqueous extract of *Grewia tenax* on Hb level (g/dl):

Weeks	Control un-bled group	Control bled group	*Grewia tenax* group
Week 0	13.46 ± 0.46	10.6 ± 1.56	9.8 ± 1.4
Week 1	12 ± 1.96	11.88 ± 0.67	12.4 ± 1.02
Week 2	11.98 ± 0.31	12 ± 0.21	13.76 ± 0.82
Week 3	14 ± 2	15.4 ± 0.55	13.16 ± 0.8
Week 4	13.9 ± 0.5	13.72 ± 0.55	13.28 ± 1.1

(All data are expressed in mean ± SD)

Table 30.

The effect of aqueous extract of *Grewia tenax* on RBC count ($\times 10^6/\mu L$):

Weeks	Control un-bled group	Control bled group	*Grewia tenax* group
Week 0	7.58 ± 0.78	5.97 ± 1.02	5.04 ± 0.5
Week 1	5.88 ± 0.1	6.03 ± 0.28	6.37 ± 0.26
Week 2	6.1 ± 0.1	6.03 ± 0.28	6.9 ± 0.26
Week 3	7.53 ± 0.29	7.75 ± 0.46	6.66 ± 0.37
Week 4	7.61 ± 0.27	7.44 ± 0.51	6.68 ± 0.4

(All data are expressed in mean ± SD)

Table 31.

The effect of aqueous extract of *Grewia tenax* on PCV level (%):

Weeks	Control un-bled group	Control bled group	*Grewia tenax* group
Week 0	44.34 ± 4.01	35.28 ± 5.5	39.94 ± 2.9
Week 1	38.14 ± 6.5	38.72 ± 3.2	43.02 ± 2.13
Week 2	36.1 ± 0.78	36.14 ± 0.55	41.32 ± 2.13
Week 3	44.62 ± 1.56	46.22 ± 1.76	41.32 ± 2.4
Week 4	45.52 ± 1.7	45.02 ± 2.1	41.18 ± 2.6

(All data are expressed in mean ± SD)

Table 32.

The effect of aqueous extract of *Grewia tenax* on MCV level (fL):

Weeks	Control un-bled group	Control bled group	*Grewia tenax* group
Week 0	58.58 ± 0.91	59.24 ± 1.75	61.44 ± 0.21
Week 1	65.04 ± 9.3	65.22 ± 2.66	62.64 ± 2.6
Week 2	59.18 ± 1.8	60.02 ± 2.04	61.88 ± 1.9
Week 3	59.28 ± 1.57	59.72 ± 1.89	62.06 ± 2.5
Week 4	59.84 ± 1.34	60.58 ± 1.61	61.7 ± 2.6

(All data are expressed in mean ± SD)

Table 33.

The effect of aqueous extract of *Grewia tenax* on MCH level (Pg):

Weeks	Control un-bled group	Control bled group	*Grewia tenax* group
Week 0	17.88 ± 1.5	17.82 ± 0.77	19.68 ± 0.8
Week 1	20. 46 ± 2.9	20.1 ± 1.75	19.44 ± 0.98
Week 2	19.62 ± 0.69	19.9 ± 0.78	19.78 ± 0.8
Week 3	18.52 ± 2.29	19.88 ± 0.56	19.76 ± 0.84
Week 4	18.26 ± 0.44	18.48 ± 0.61	19.9 ± 1.9

(All data are expressed in mean ± SD)

Table 34.

The effect of aqueous extract of *Grewia tenax* on MCHC level (g/dl):

Weeks	Control un-bled group	Control bled group	*Grewia tenax* group
Week 0	30.52 ± 2.1	30.1 ± 0.7	32 ± 1.1
Week 1	31.46 ± 4.4	30.78 ± 2.18	31.02 ± 0.44
Week 2	33.18 ± 0.33	33.14 ± 0.21	32 ± 0.73
Week 3	31.32 ± 3.85	33.14 ± 0.21	31.85 ± 0.56
Week 4	30.54 ± 0.15	30.48 ± 0.43	32.28 ± 2

(All data are expressed in mean ± SD)

Table 35.

Effect of *Grewia tenax* aqueous extract on Hb level of iron-deficient rats (g/dl):

Weeks	Normal diet group	Deficient diet group	*Grewia tenax* group
Week 0	13.72 ± 0.19	12.18 ± 0.59	12.52 ± 0.86
Week 1	13.32 ± 0.44	12.2± 12.2	12.64 ± 1.1
Week 2	12.72 ± 0.31	11.72 ± 0.68	12.16 ± 0.95
Week 3	13.9 ± 0.52	12.04 ± 1.13	12.74 ± 0.65

(Data are expressed in Mean ± SD)

Table 36.

Effect of *Grewia tenax* aqueous extract on PCV level of iron-deficient rats (%):

Weeks	Normal diet group	Deficient diet group	*Grewia tenax* group
Week 0	42.9 ± 1.36	36.76 ± 2.04	38.46 ± 2.6
Week 1	41.88 ± 1.93	37 ± 3.21	39.18 ± 3.5
Week 2	39.76 ± 1.24	35.9 ± 2.5	38.34 ± 3.2
Week 3	42.44 ± 1.6	36.08 ± 3.9	39.24 ± 2.5

(Data are expressed in Mean ± SD)

Table 37.

Effect of *Grewia tenax* aqueous extract on RBC count ($\times 10^6$/µL) of iron-deficient rats:

Weeks	Normal diet group	Deficient diet group	*Grewia tenax* group
Week 0	7.062 ± 0.36	6.61 ± 0.3	6.69 ± 0.41
Week 1	6.822 ± 0.27	6.492 ± 0.5	6.8 ± 0.51
Week 2	6.66 ± 0.22	6.358 ± 0.52	6.6 ± 0.52
Week 3	7.128 ± 0.22	6.446 ± 0.68	7 ± 0.48

(Data are expressed in Mean ± SD)

Table 38.

Effect of *Grewia tenax* aqueous extract on MCV level of iron-deficient rats (fL):

Weeks	Normal diet group	Deficient diet group	*Grewia tenax* group
Week 0	60.3 ± 2.3	55.6 ± 0.86	57.48 ± 1.5
Week 1	61.42 ± 2.96	56.98 ± 1.03	57.58 ± 1.56
Week 2	59.7 ± 0.7	56.52 ± 1.55	57.88 ± 1.7
Week 3	59.54 ± 0.7	55.98 ±1.26	56.12 ± 1.3

(Data are expressed in Mean ± SD)

Table 39.

Effect of *Grewia tenax* aqueous extract on MCH level of iron-deficient rats (Pg):

Weeks	Normal diet group	Deficient diet group	*Grewia tenax* group
Week 0	19.48 ± 0.81	18.44 ± 0.36	18.72 ± 0.46
Week 1	19.54 ± 0.87	18.82 ± 0.33	18.58 ± 0.83
Week 2	19.1 ± 0.4	18.46 ± 0.49	18.38 ± 0.38
Week 3	19.5 ± 0.35	18.72 ± 0.75	18.24 ± 0.56

(Data are expressed in Mean ± SD)

Table 40.

Effect of *Grewia tenax* aqueous extract on MCHC level of iron-deficient rats (g/dl):

Weeks	Normal diet group	Deficient diet group	*Grewia tenax* group
Week 0	31.98 ± 0.78	33.16 ± 0.76	32.54 ± 0.6
Week 1	31.84 ± 0.52	33.02 ± 0.59	32.28 ± 1.2
Week 2	32.02 ± 0.63	32.66 ± 0.54	31.74 ± 0.69
Week 3	32.76 ± 0.18	33.42 ± 0.8	32.48 ± 0.5

(Data are expressed in Mean ± SD)

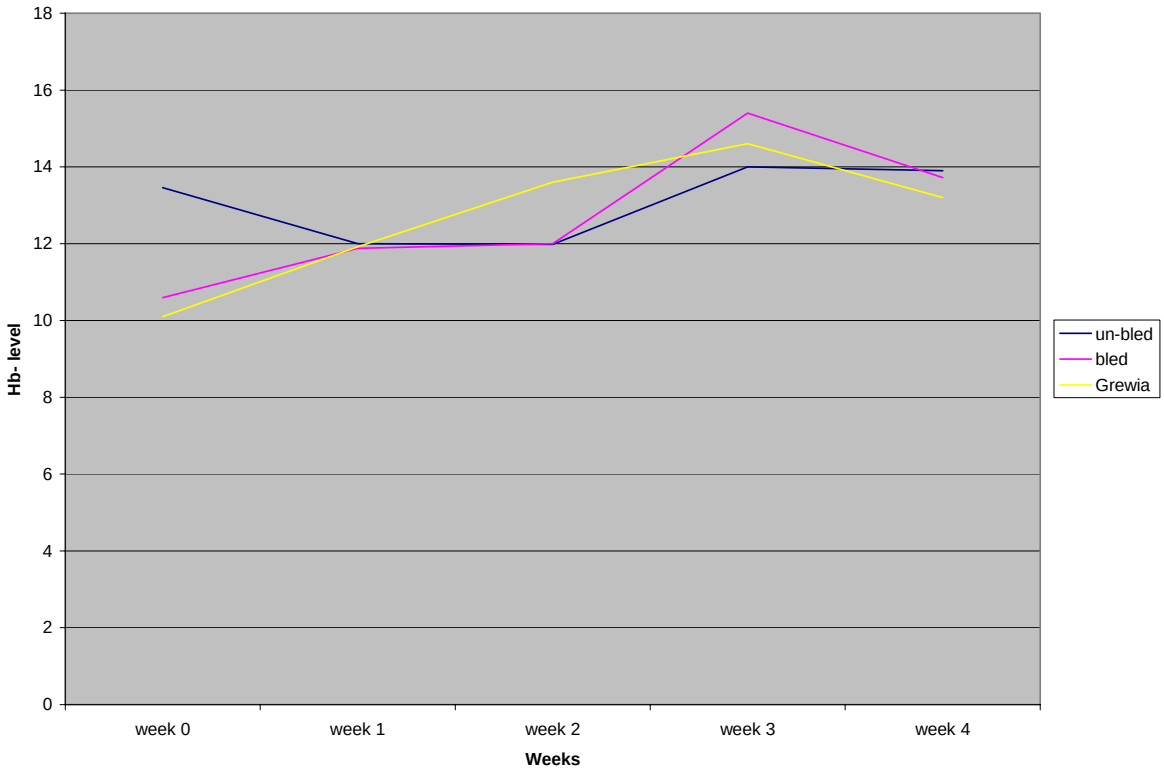

Figure 24. Effect of *G. tenax* on Hb-level of hemorrhagic anemic rats

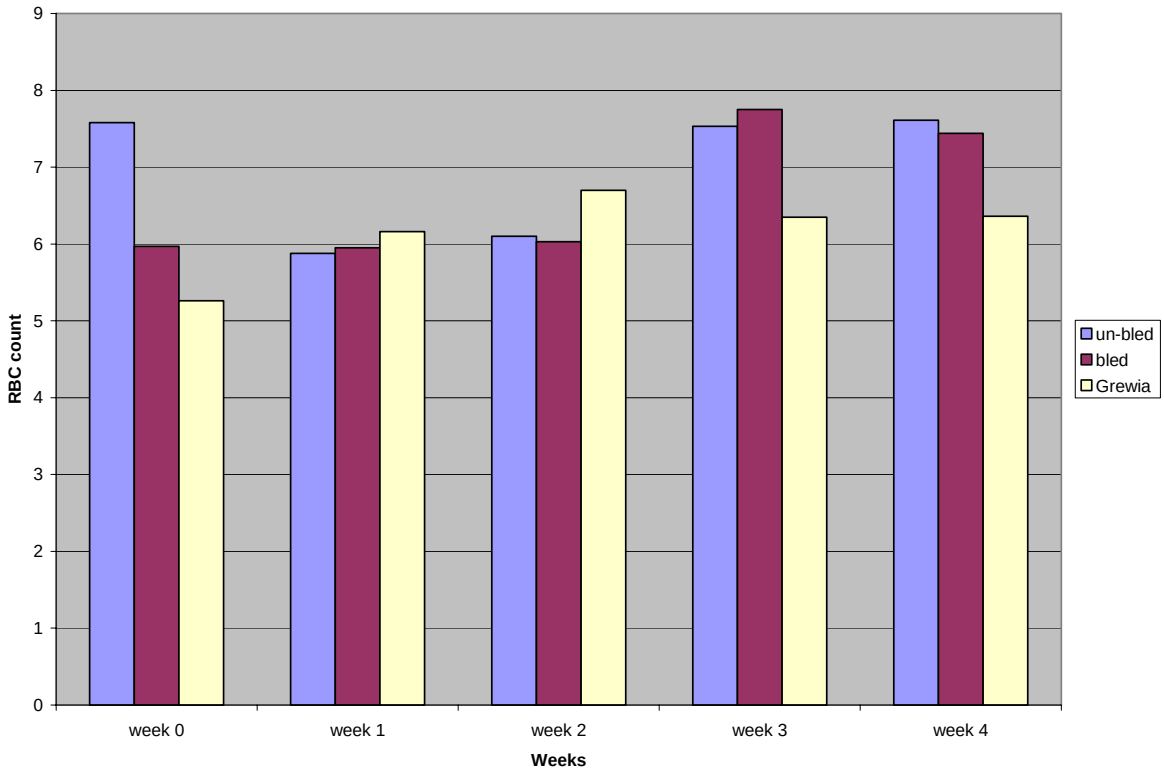

Figure 25. Effect of *G. tenax* on RBC count of hemorrhagic anemic rats

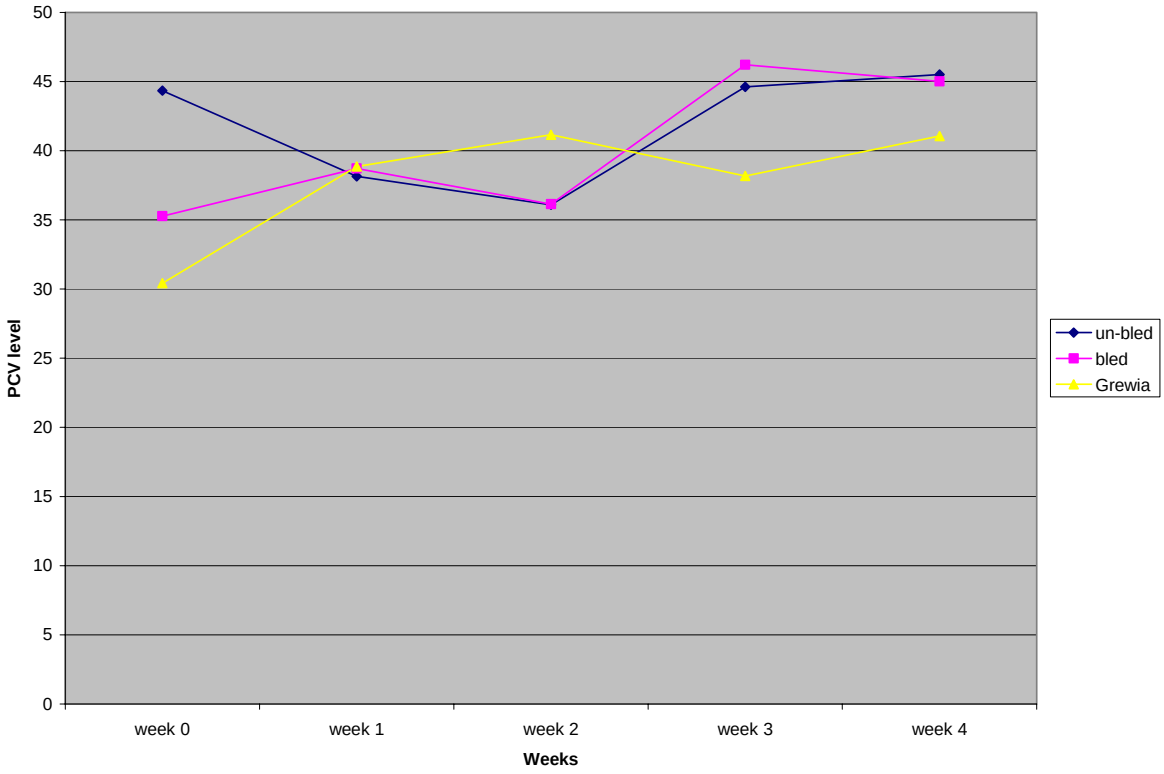

Figure 26. Effect of *G. tenax* on PCV level of hemorrhagic anemic rats

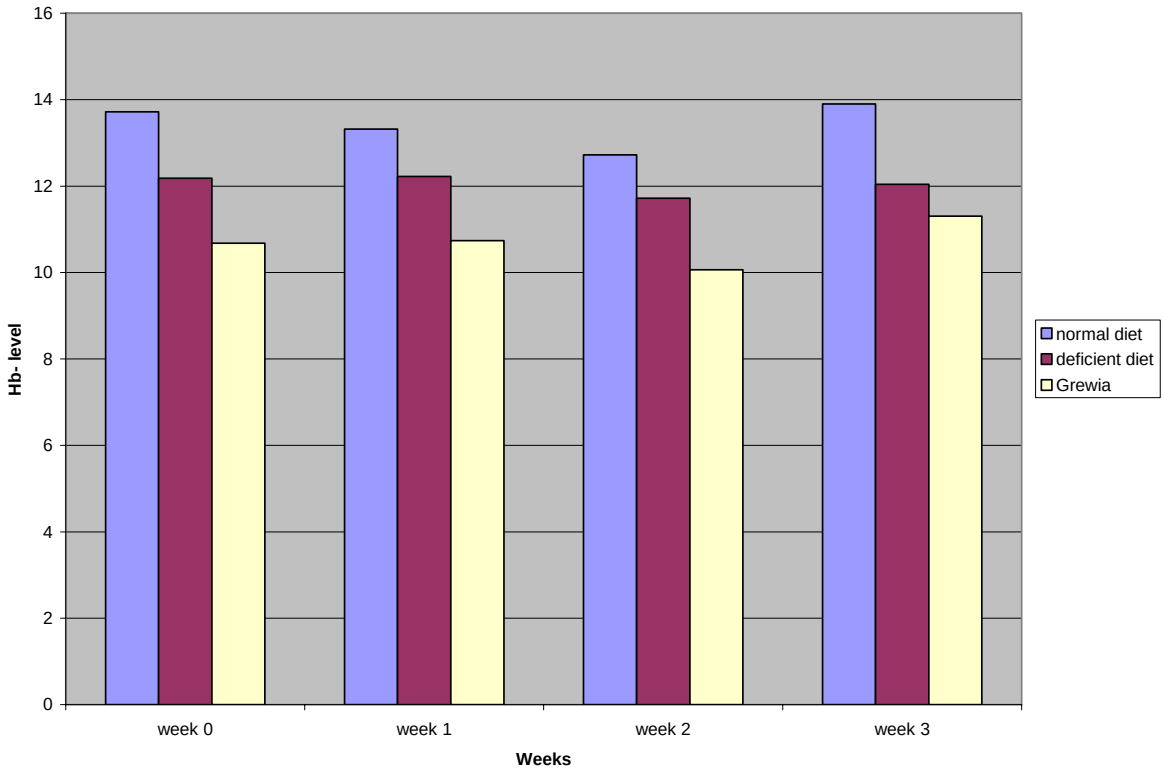

Figure 27. Effect of *G. tenax* on Hb- level of iron- deficient rats

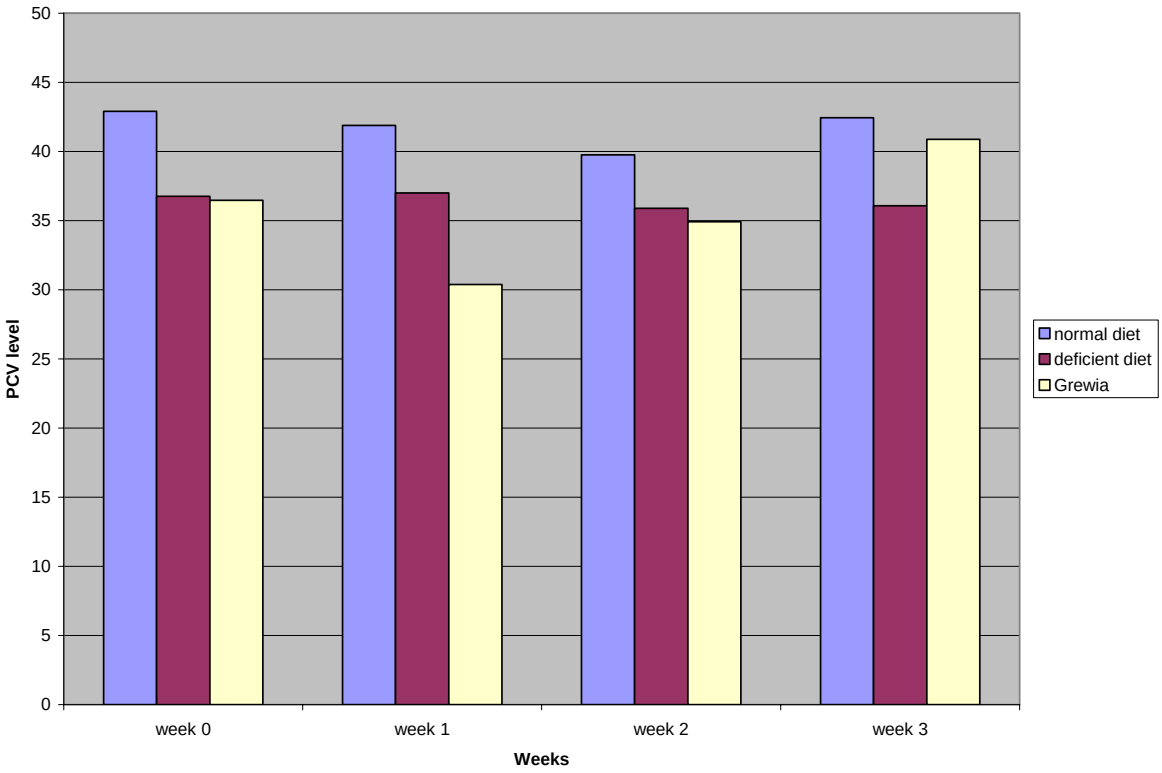

Figure 28. Effect of *G. tenax* on PCV level of iron- deficient rats

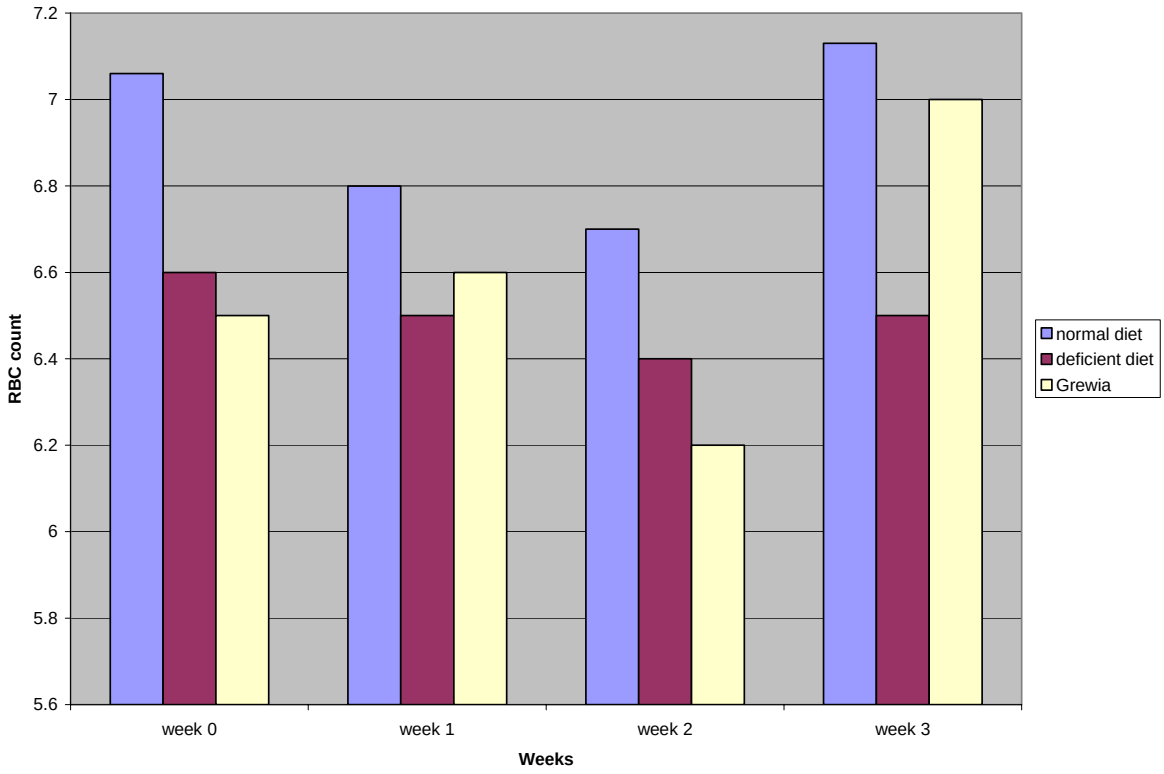

Figure 29. Effect of *G. tenax* on RBC count of iron- deficient rats

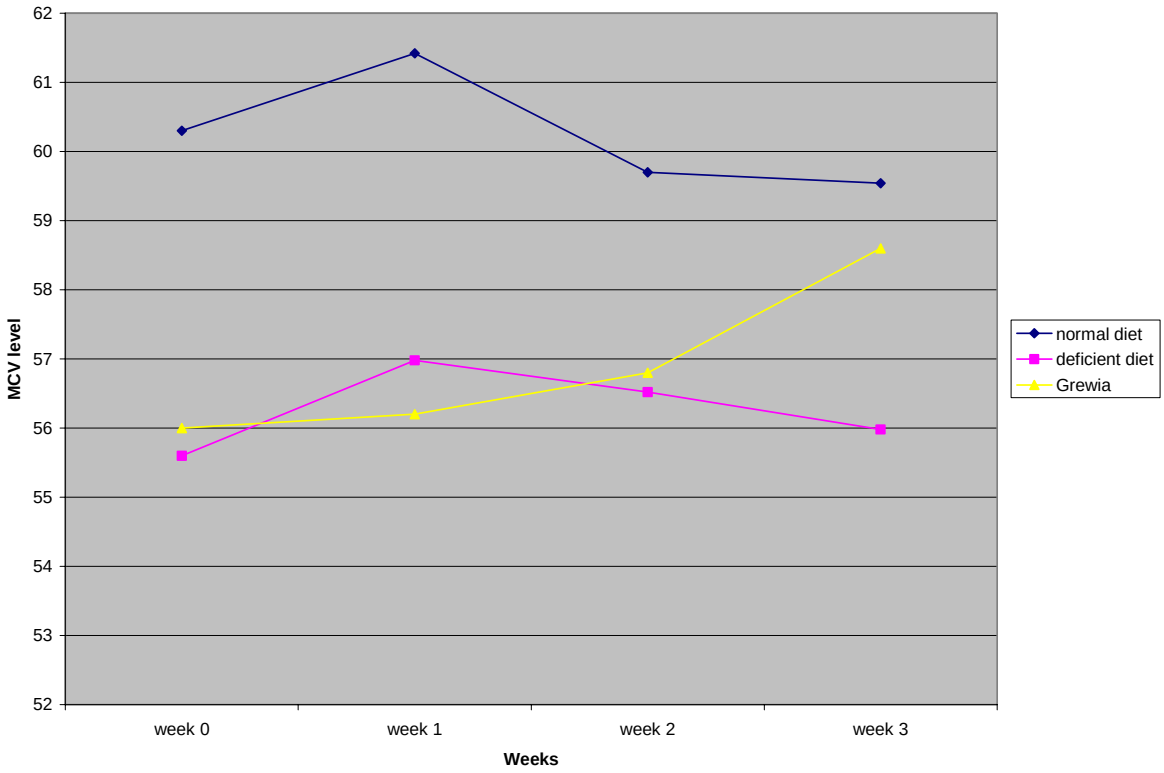

Figure 30. Effect of *G. tenax* on MCV level of iron- deficient rats

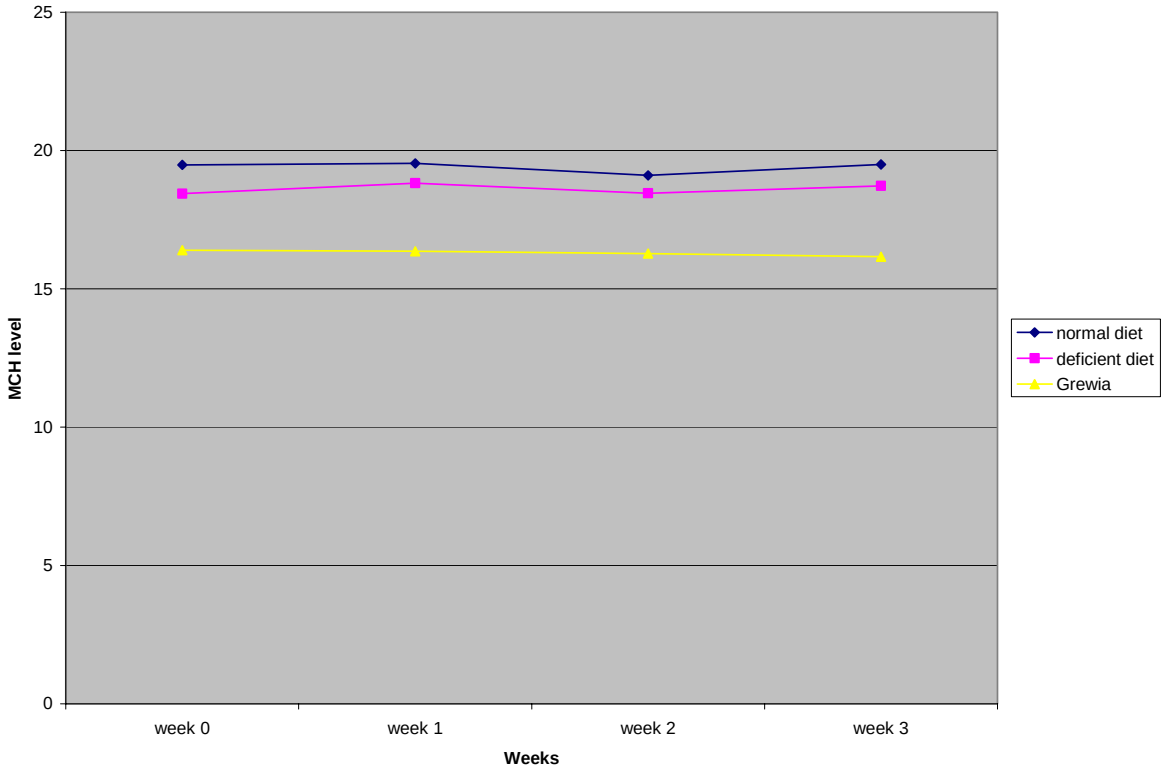

Figure 31. Effect of *G. tenax* on MCH level of iron- deficient rats

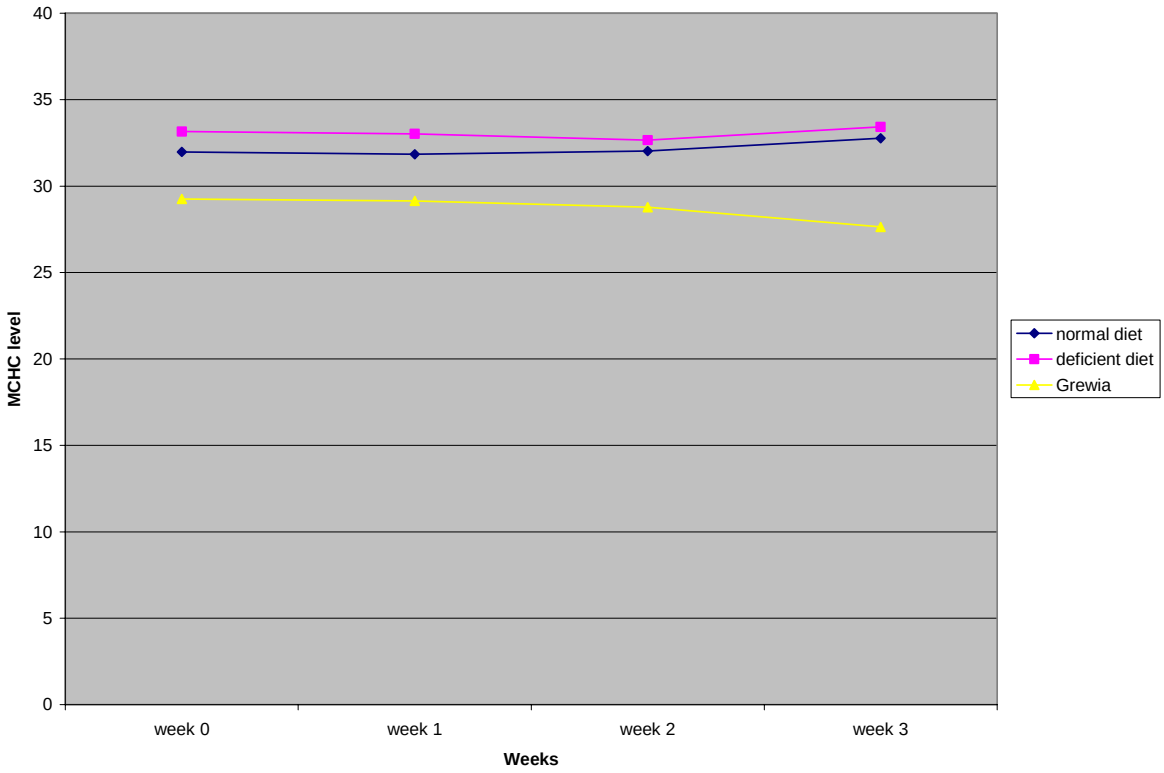

Figure 32. Effect of *G. tenax* on MCHC level of iron- deficient rats

3.3. Study of the effects of aqueous extracts of *H. sabdariffa* and *A. garckeana* on iron absorption:

In this study, the control group has shown an increase in the iron concentration that was estimated by 130.31Mmol/L, 141.30 Mmol/L and 152.84 Mmol/L at incubation times of 1, 5 and 15 minutes after the addition of $FeSO_4$ solution.

20 mg/ml of *H. sabdariffa* extract has shown a decrease in the iron concentration measured as 127.25Mmol/L in the first minute and this decrease continued to the fifth minute then changed to an increase of 150.84 Mmol/L after 15 minutes past the addition of the $FeSO_4$ solution.

In the third group, 20mg/mlof *A. garckeana* extract caused a decrease in the iron concentration estimated by 128.82 Mmol/L in the first minute of incubation time. However, the extract caused increases of 142.33 and 149.9 Mmol/L in the other five and fifteen minutes of incubation times, respectively as shown in table 41. and figure 33.

Table 41.

Effect of *H. sabdariffa* and *A. garckeana* extracts on iron concentration content by using everted gut sac method:

Groups	1 minute	5 minute	15 minute
Control	130.31 Mmol/L	141.30 Mmol/L	152.84 Mmol/L
H. sabdariffa extract	127.25	127.93 Mmol/L	150.84 Mmol/L
A. garckeana extract	128.82 Mmol/L	142.33 Mmol/L	149.90 Mmol/L

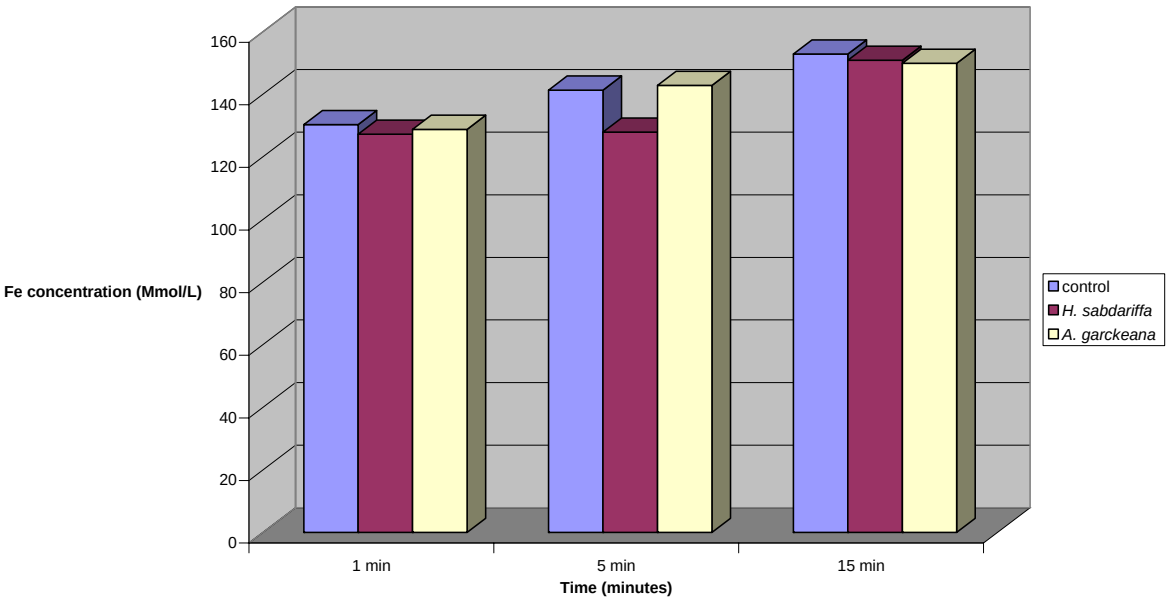

Figure 33. Effect of *H. sabdariffa* and *A. garckeana* on iron absorption

4. DISCUSSION

4.1. Effect of the tested extracts on hematological parameters of anemic rat

4.1.1. The effect of *H. sabdariffa* aqueous extract on hemorrhagic anemic rats:

The daily administration of 2 g/kg body weight of the aqueous extract of *H. sabdariffa* seeds has been continued for four weeks successfully. This extract has been found to cause clear changes in hematological parameters of the hemorrhagic anemic rats. This extract caused remarkable, yet not significant- increases in the hemoglobin, PCV and RBC levels of the anemic bled rats. The increases in the latter three parameters has started in the first week of the experiment and continued throughout the 4 weeks period. However, the increase in terms of the RBC count was slight and lower than that observed in the hemoglobin and PCV levels.

These findings might possibly favour the traditional claim of using *H. sabdariffa* extract in the treatment of anemia. Also it was reported that, these findings coincide with previous studies conducted on the same plant; however, these findings were carried out on the aqueous extract of the calyces (Adigun *et al*, 2006).

In the above mentioned study, 200 and 400 mg/kg of the aqueous extract of the calyces were administered to normal Wistar albino rats for 2 weeks. The extract has caused significant elevations in hemoglobin (P= 0.004) and PCV (P= 0.03) values. However, these beneficial effects of the extract were not sustained at higher doses (1000 mg/kg body weight).

On the other hand, *H. sabdariffa* aqueous extract of the seeds was found to cause small increases in the MCV, MCH and MCHC levels of the hemorrhagic anemic rats. However, these increases were not recognizable and not significant.

4.1.2. The effect of *H. sabdariffa* aqueous extract on iron-deficient rats:

Despite its effect on the hemorrhagic anemic rats *H. sabdariffa* extract of the seeds, did not cause the same effect on nutritionally iron-deficient animals. However, it has caused slight increase in the hemoglobin level in the first week of experiment that was not consistent because it was dropped in the second week. Also the extract was found to cause a slight increase in the MCV level accompanied by decreases in the MCH and MCHC levels.

These results might confirm the beneficial effects of *H. sabdariffa* extract in counteracting the blood loss caused by the hemorrhagic anemia. However, it was found that it may not have the same positive action on the hematological parameters of nutritional anemia. This might be explained on the amount of iron content in the seeds of *H. sabdariffa* is not sufficient enough to counteract the deficiency in iron in the nutritional anemic animals.

These effects of *H. sabdariffa* extract on hemorrhagic anemic and iron-deficient rats might be compared with previous study conducted on the anti-anemic effect of the aqueous extract of *Sorghum bicolor* (Oladiji *et al*, 2007).

The study of the effect of aqueous extract of *Sorghum bicolor* was carried out on 21 days old Wistar albino rats maintained on deficient and sufficient diets for 6 weeks before administration of the extract for a period of 7 days. It was found that the aqueous extract of *Sorghum bicolor* had produced significant increase in hemoglobin, PCV and RBC count in the iron sufficient and iron- deficient groups ($P< 0.05$). It was suggested that administration of *Sorghum bicolor* extract has restored the anemic condition in the iron deficient animals and thus lend credence to its use folklore medicine in the management of anemia.

4.1.3. The effect of *A. garckeana* aqueous extract on hemorrhagic anemic rats:

A. garckeana is widely used by the natives in Western Sudan for nutritional purposes. This study has aimed to evaluate the efficiency of *A. garckeana* aqueous extract in treating anemia as has been assumed in the traditional use of this plant. Efforts were directed to assess any beneficial effects that may be shown after administration of the extract to anemic rats in two experimentally different models of anemia. In the first trial, the effect of 2g/kg of *A. garckeana* aqueous extract was studied on the hematological parameters of

hemorrhagic anemic rats. This extract showed beneficial effects on some of the hematological parameters. It caused increments in the hemoglobin and PCV levels of anemic bled rats. These increments were found to be remarkable and convenient in the first and second weeks of treatment when compared to the increase in the hemoglobin and hematocrit levels of the control bled rats at the same period of time. However, in the third week all the three groups under study (treated and controls) attained normal and nearly equal values of hemoglobin and PCV levels.

This recovery time to the normal hematological values was shorter than fourth week's time that was reported in a previous study conducted on the aqueous extract of *Ligaria cuneifolia* (Dominighini *et al*, 2004). Another advantage caused by administration of *A. garckeana* extract is expressed in red blood cell count of the hemorrhagic anemic rats in the first and second weeks of treatment. This result favors the claimed hypothesis in the folk medicine that *A. garckeana* aqueous extract could have possible enhancing erythropoietic properties.

Observed changes in hemoglobin and PCV levels and RBC count were correlated with other blood indices e.g. MCV levels.

In the first week of the experiment, the control bled group showed elevated level of MCV which may be due to acute blood loss (macrocytosis i.e. macrocytic anemia). However, the extract was found to counteract the elevation in the MCV level and thus, the treated group showed lower levels of MCV comparabed to the control bled rats.

On the other hand, the extract did not show obvious alterations in the MCH and MCHC values throughout the study. Thus the MCHC values remained in the normal level which may be another evidence for macrocytic anemia.

The effect of *A. garckeana* extract may be compared with another effect established in a study carried out formerly on *Nigella orientalis* plant (Kokdil *et al*, 2006). It was found that administration of 1 ml/kg/day of *Nigella orientalis* fixed oil to Wistar Kyoto rats for 28 days, caused alterations in their hematological parameters. *N. orientalis* caused an increase in the MCV concentrations and a decrease in MCHC values when compared to the levels with of a control group that was administered with distilled water only during the study. On the other hand, *Nigella segetalis* was found to cause a significant increase in the hematocrit levels similar to the effect exerted by the *A. garckeana* extract.

However, the effect produced by *A. garckeana* extract was found to be similar to those produced from daily administration of *Khaya grandifoliola* extract (Bumah, *et al* 2005). The therapeutic efficacy of a crude water extract of *Khaya grandifoliola* has been

established in mice. This study was designed to assess the effect of the extract on the red blood cells and bone for 7 days, 3 weeks and a recovery period of 3 weeks. Daily administration of the extract showed a general pattern of significant ($P < 0.5$) increases in the red blood cell (RBC) count, PCV, hemoglobin and plasma iron content in the groups administered with the extract after 7 and 21 days when compared with control rats.

4.1.4. The effect of *A. garckeana* aqueous extract on iron-deficient rats:

When the effect of the aqueous extract of *A. garckeana*, was studied in nutritionally iron- deficient anemic rats, it was found that this extract did not cause any beneficial effects in terms of the hematology parameters(Hb levels and/or PCV levels). However, this extract was found to cause an increase in the red blood cell count of the anemic rats (3%) expressed in the third week of treatment. This increase was higher than that attained by the control rats that were fed with the normal iron- content diet. Thus, the *A. garckeana* extract caused increments in the red blood cell counts in both types of anemia, hemorrhagic and iron-deficient anemias. Another observation is the increase in the MCV values that was caused by the extract in the second week of treatment.

Results obtained after administration of *A. garckeana* extract to the iron- deficient rats revealed that this extract will have no advantageous effect in terms of MCH and/ or MCHC levels. It conversely caused lowering effects in those latter parameters when compared to the levels attained by the control group.

On conclusion, *A. garckeana* extract was found to have stimulating erythropoietic properties when used in hemorrhagic anemia model. However, these stimulating effects were not found in iron- deficient anemia model, except of a minor increment showed in the red blood cell count of the treated group. Thus, further investigations should be conducted in this plant with other types of extracts and/or different doses so as to establish its stability in inducing favorable erythropoietic properties and its usefulness in the treatment of anemia as claimed in the folk medicine.

4.1.5. The effect of *Grewia tenax* aqueous extract on hemorrhagic anemic rats:

Fruits of *Grewia tenax* are used widely in Sudan for purposes of treatment of anemia. A previous study conducted by Rahmatalla (1999) on the pulp juice extract of *Grewia tenax*, showed its beneficial effect on two groups of volunteers (doses of 100 gm and 150 gm respectively). *Grewia tenax* showed significant increases in terms of Hb and PCV levels of the second group after 3 weeks period of treatment. Also chemical analysis of *Grewia tenax* showed that it is a rich source of iron and vitamin C (average 9.1 mg Fe and 117 mg vitamin C/ 100 gm pulp). Hence, *Grewia tenax* is included in this study as a reference plant and to compare its effect in the correction of induced anemia with that produced by *Azanza garckeana* and *Hibiscus sabdariffa* aqueous extracts.

This study has elucidated that administration of *Grewia tenax* extract has caused favorable changes in many blood indices of hemorrhagic anemic rats. This extract has caused remarkable increases in the hemoglobin, PCV and red blood cell count levels of the anemic rats and also small increases in the MCV levels. But this effect was not detected in the MCH and MCHC values of the rats. Thus, these favorable effects of *Grewia tenax* in correction of hemorrhagic anemia supports a previous study carried out on the direct effect of *G. tenax* on iron absorption by using everted gut sacs of rats (Khemiss, 2006). In this study, it was reported that the addition of aqueous extract of *G. tenax* at different concentrations to incubated freshly prepared rat everted gut sac has favored significantly iron transfer from the mucus side towards the serous one. The maximum of iron absorption has occurred in the presence of 10 mg/ml of the extract and 5 minutes of incubation time in stomach, duodenum and jejunum.

Those findings may explain the positive effect of aqueous extract of *G. tenax* on blood indices of hemorrhagic anemic rats obtained in the present study.

4.1.6. The effect of *Grewia tenax* aqueous extract on iron-deficient rats:

The effect of *G. tenax* on nutritionally iron-deficient rats was less recognizable than that in the hemorrhagic ones. The extract has caused small increases in the Hb and PCV levels of iron-deficient rats that appeared in the first and third weeks of treatment. On the other hand, the effect of *G. tenax* extract on the red blood cell counts has

been detected as an increase in their levels when compared to the levels of the control untreated animals.

Conversely, the extract did not show any favorable increasing effects on the MCV, MCH or MCHC levels of the iron deficient rats. Instead, there were small decreases appeared in the parameters of those animals.

Thus, this study revealed that the aqueous extract of *G. tenax* could correct the blood indices in cases of acute blood loss as found in hemorrhagic anemia and this might be due to its effect on iron absorption in the gut but these effects are not shown in cases of nutritional anemia when there is no enough iron to make all the red blood cells the body needs.

4.2. Study of the effects of aqueous extracts of *H. sabdariffa* and *A. garckeana* on iron absorption:

It is well known that, maximum iron uptake is normally detected in the duodenum and maximum of iron uptake occurs at 15 minutes of incubation time. Thus, in this study the duodenum was the tissue that was chosen for studying the effects of *H. sabdariffa* and *A. garckeana* extracts on direct iron absorption. These effects are also compared to that of *G. tenax* extract. In a former study, it was found that *G. tenax* extract caused maximum iron uptake at 5 minutes of incubation time and registered at the duodenum at a dose of 10 mg/ml. However, *Grewia tenax* extract used at a dose of 20mg/ml has favored iron transfer. An increase in the absorption was detected at 5 minutes and 15 minutes of incubation times. This decrease was not significant at 1 minute of the incubation time.

In the current study, 20 mg/ml of *H. sabdariffa* and 20 mg/ml of *A. garckeana* extracts caused decreases in the iron concentration at the first and fifth minutes of incubation for the *H. sabdariffa* extract and at the first minute only for the *A. garckeana* extract. However, these decreases were not proven statistically.

Comparison made between the three extracts revealed that the *G. tenax* extract has had the best capability of iron transfer among the three extracts after 5 minutes of incubation time. Followed by the extract of *H. sabdariffa* with less capability of iron absorption at the same incubation time. The least is the *A. garckeana* extract which showed less iron uptake at the first incubation minute and that effect was transient disappeared at the fifth minute of incubation.

5. CONCLUSION

This study aimed to investigate and explore the efficiency of three medicinal plants extracts on hematological parameters of two different types of anemia. Also it aimed to confirm the claim for the use of these extracts in the folk medicine for the treatment and correction of anemia.

From all these studies conducted, it is found that the three plants extracts of *G. tenax*, *A. garckeana* and *H. sabdariffa* have had the capabilities to increase hematological parameters in induced hemorrhagic anemia. However, the extract of *A. garckeana* has showed this capability in the first and second weeks of treatment only i.e. transient effect.

The three extracts showed the tendency to increase the haemoglobin levels and red blood cell counts of the bled animals (except for last weeks in case of *A. garckeana* extract).

Studies conducted on iron-deficient anemia, showed that *G. tenax* and *H. sabdariffa* caused small and equal effects on the increases in the Hb-levels of the iron-deficient animals in the first week of treatment only and the effect was combined with equal small effect on the red blood cell counts in the first and third weeks of treatment.

The above findings were confirmed by the fact that *G. tenax* extract has had the highest amount of iron. Whereas *H. sabdariffa* had the less amount of iron than did the *A. garckeana* extract but it seemed that iron contained in the former plant was more available. Another confirmation for this study is the findings of the everted gut sac studies. *A. garckeana* being the least extract in capability of iron transfer compared to capability of *H. sabdariffa* and / or *G. tenax* extracts.

These results may support positively the use of these three plants extracts in folk medicine for the correction of anemia.

Recommendations:

- This study investigated one dose of each plant, so it is recommended that higher doses must be studied to evaluate the whole effect of these plants.
- Types of extracts other than the aqueous one can be investigated.
- Detailed pharmacological and toxicological studies must be carried out before these plants can be introduced as anti-anemic agents.

6. REFERENCES

Abdelmuti O. M. S.; (1991). Biochemical and nutritional evaluation of famine foods of the Sudan. Famine foods database. Robert Freedman.

Abu Tarboush H.M.; Ahmed S.A.B. and Al- Kahtani H.A. (1997). Some nutritional and functional properties of Kerkade (*Hibiscus sabdariffa*) seed products. Cereal Chemistry 74:3, 352-355.

Adigun M.O.; Oguddipe O.D.; Anetor J.I. and Odetunde A.O. (2006). Dose dependant changes in some hematological parameters during short-term administration of *Hibiscus sabdariffa* calyx aqueous extract (Zobo) in Wistar albino rats. African Journal of Medical Sciences 35 (1): 73-7.

Agbor A.G. and Odetola A. A. (2001). Hematological studies of *Paraqutina ingrescens* on hemorrhagic anemic rats. African journal of medicine and medicinal plants 30 (1-2): 105-9.

Ahmed P.; Mahmoad A.; Aziz S. and Azimm W. (2003). Comparison of response between foods supplemented with powdered iron and in iron syrup form for iron deficiency anemia. J. Coll. Physicians Surgery 13 (7): 402-4.

Akase T.; Onodera S.; Matsushita R.; Akase T. and Tashiro S. (2004). A comparative study of laboratory parameters and symptoms affected by Toki-shkuyaku-san and iron preparation in rats with iron-deficiency anemia. Biological and Pharmaceutical Bulletin 27 (6): 871-8.

Akindahunsi A. A. and Olaleye M. T. (2003). Toxicological investigation of aqueous methanolic extract of the calyces of *Hibiscus sabdariffa* L. Journal of Ethnopharmacology 89(1): 161-4.

Ali B.H.; Al Wabel N. and Blunden G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L. Phytotherapy Research 19 (5): 469- 75.

Ali M. B.; Salih W. M. and Humida A. M. (1989). An estrogen-like activity of *Hibiscus sabdariffa* L. (Roselle). Fitoterapia 60 (6): 547-548.

Allan E. Lichtin (2008). The Merck Manual of medical information. 2nd Home edition. Merck and Co. Incorporation.

Allen. S. E. (1989). Chemical analysis of ecological materials. 379-400. 2 th edition. Blackwell Scientific Publications.

Askari A.; Mirza M. and Solangi M.S.P. (1996). Toxicological studies of herbal beverage and seeds extract of *Hibiscus sabdariffa* L. (Roselle). Pakistan Journal of Scientific and Industrial Research 39(1-4): 38-42.

Blood D. C.; Radostis O. and Handerson J. (1986). Veterinary Medicine. A textbook of cattle, sheep, pigs, goats and horses. 6 th. Edition. Bailliere Tindall. London.

Bozhinova S.; Penkov V. and Bogdanova A. (2004). Ferro- Folgamma a drug of treatment and prophylaxis of iron-deficiency anemia in pregnant women. Akush Ginekol (Soffia) 43 (3): 27-31.

Bumah V. V.; Essien E. U.; Agbedahunsi J. M. and Eka O. U. (2005). Effects of *Nigella orientalis* and *Nigella segetalis* fixed oils on blood biochemistry in rats. Phytotherapy research 20 (1): 71-5.

Connelly M. P.; Fabiano E.; Patel I. H.; Kinyanjui S. M.; Mberu E. K. and Watkins W. M. (1996). Anti malarial activity in crude extracts of Malawian medicnal plants. Annual Tropical Medicine Parasitology 31 (1): 4171- 4172.

Dominighini A.; Ferrero M.; Mengarelli G.; Ronco M.T.; de Lujan Alvarez M.; Wagner M.; Gurni A.; Carnovale C. and Luquita A. (2004). Hemorrhagic changes induced by aqueous extract of Ligaria cuneifolia: treatment by two different administration routes. Clinical Hemorrhagic Microcircle31 (2): 113-21.

El Ghazali, G.E.B., Khalid, H.E., El Tohami, M.S., Abdalla, W. E., Yagi, S.M.A. (1998). Medicinal Plants of the Sudan, part IV, page 127"Medicinal Plants commonly used in Khartoum State", MAPRI, NCR, Khartoum (in Arabic).

El Sheikh S.H.; Bashir A.K.; Suliman S.M. and El Wassila M. (1990). Toxicity of certain Sudanese plant extracts to cercariae and miracidia of Schistosoma mansoni. International Journal of Crude Drug Research 28:4 (241-245).

Ghinea M.M. (2004). Treatment of iron-deficiency anemia with Ferro-Folgamma. Rom. J. Inter. Med. 42 (1) 225-30.

Harland, B. F. and Harland, J. (1980). Fermentative reduction of phytate in rye white and whole wheat breads. Cereals Chemistry 57 (3): 226-229.

Haro A.; lopez- Aliaga I.; Lisbona F.; Barrionuevo M.; Alferez M. J. and Campos M.S. (2000). Beneficial effect of pollen and/or proplis on the metabolism of iron, calcium, phosphorus and magnesium in rats with nutritional ferropenic anemia. Journal of Agriculture and Food Chemistry 48 (11): 5715-22.

Hatono R.; Takano F.; Fushia S.; Michimata M.; Tanaka Kazama I.; Suzuki M. and Matsubara M. (2004). Water-soluble extracts from *Angelica acutiloba Kitagawa* enhance hematopoiesis by activating immature erythroid cells in mice with 5- fluorouracil-induced anemia. Experimental Hematology (10): 918-24.

Idoate Gastearena M.A.; Gil A.G.; Azqueta A.; Coronel M.P. and Gimeno M. (2003). A comparative study on the gastroduodenal tolerance of different anti-anemic preparations. Human Experimental Toxicology 22 (3): 137-41.

International Anemia Consultative Group (INACG). (1977). Guidelines for the eradication of iron deficiency anemia. A report of the International Nutritional Anemia Consultative Group. Washington, DC: The Nutrition Foundation.

Isselbacher, K. J. (1994). *Harrison's Textbook of Internal Medicine,* 13th edition. New York: McGraw-Hill.

James A. D. (1983). Handbook of Energy Crops. (Unpublished work).

James A. D. and Beckstrom-Sternberg M.S. (1998). Phytochemico data base.

Jin R.; Chen C.; Sun Z.; Zhang X.; Xie H.; Li Y.; Liu D and Hu Z. (1998). Effects of hairy root huangqi hemopoietic function. Zhong Yao Cai 21 (1): 26-9.

Kalenga J. D.; Saka and Jerome D. M. sonthi (1994). Nutritional value of edible fruits of indigenous wild trees in Malawi. Forest Ecology and Management 64 (2-3): 245-248.

Ke Y.F.; Wei.K.M.; Zheng J.X.; Pu. J. and Liang W. (2004). Study on treatment of iron-deficiency anemia by *shengxuening*. Zhongguo Zhong Xi Yi Jie He Za Zhi 24 (10): 893- 6.

Khanna, A.K.; Chandar,R.; Kapoor, N.K.; Singh,C. and Srivastava A. K. (1992). Hypoglycaemic activity of T.chebulain in rats. Fitoterapia LXIV: (4) 315-356.

Khemiss, F.S.; Ghoul-Mazgar; A. A. Moshtagie and D. Saidane (2006). Study of the effect of aqueous extract of *Grewia tenax* fruit on iron absorption by everted gut sac. Journal of Ethnopharmacology 103 : 90-98.

Kokdil G.; Tamer L.; Ercan B.; Celic M. and Atik U. (2006). Effects of Nigella orientalis and N. segetalis fixed oils on blood biochemistry in rats. Phytotherapy research 20 (1): 71-5.

Laura E. M. ; Fumio T.; Fumiyuki G.; Toshihiro Y.; Elizabeth C. T. and John L. B. (2002). Transgenic rice is a source of iron for iron-depleted rats. Journal of nutrition 132: 957-960.

Margaret D. S.; Cathrine C. and Judith A. G. (1995). Nutrition Assessment (A comprehensive guide for planning intervention). 2nd edition- Aspen Publishers, Inc. Gaithersburg, Maryland. Pages 216-217.

Maurice E. S.; James A. O.; Moshe S. and A. Cathrine R. (1999). Modern Nutrition in health and disease. 9 th edition. Lippincot Williams and Wilkins.

Maydell H. J. (1986). Trees and Shrub of the Sahel. Their characteristics and uses puslished by Eschborn -296-7

Milchev N.; Ivancheva K. and Paskaleva V. (2004). Treatment of iron-deficiency anemia with ferrous gluconate. Akush Ginekol (Sofiia) 43(Suppl 3): 45-8.

Miller J. (1987). Effects of dietary corn on hematological response of anemic rats to ferrus sulphate. Journal of nutrition 108 (1): 33-9.

Mojeremane W and Tshwenyane S. O. (2004). *Azanza garckeana*: A valuable edible endigenous fruit tree of Botswana. Pakistan journal of nutrition 3 (5):246-267.

Murray P.; Barron E.; Pfaller M. and Tenover F. (2000). Immunology and Pathology. 14: 1479-84. Clorado State University. 3rd edition.

Nenortiene P.; Sapragoniene M.; Stankevicius A.; Matusevicius P. and Daunoras G. (2002). Preparation, analysis and anti-anemic action of peroral powders with ferrous oxalate. Ferosol-2. Medicina (kaunas) 38(1):69-76.

Oladiji A.T; Jacob T.O. and Yakubu M.T (2007). Anti-anaemic potentials of aqueous extract of Sorghum bicolor (L.) moench stem bark in rats. Journal of Ethnopharmacology 111(3):651-6.

Omolo O.J.; Chhabra S.C. and Nyagah G. (1997). Determination of iron content in different parts of herbs used traditionally for anemia treatment in East Africa. Journal of Ethnopharmacology 58(2): 97-102.

Palgrave, K.C.(1988). Trees of southern Africa. .Struik Publishers. Cape Town .Pages 49-67

Pandit S.; Biswas T.K.; Debnath P.K.; Saha A.V.; Chowdhury U.; Shaw B.P.; Sen S. and Mukherjee B. (1999). Chemical and pharmacological evaluation of different ayurvedic preparations of iron. Journal of ethnopharmacology 65 (2): 149-56.

Parveen K. and Michael C. (1999). Clinical Medicine. 4th edition. W. B. SAUNDERS. Pages 360-368.

Passmore, R. and Eastwood, M.A. (1986). Human Nutrition and Dietetics. 8th edition. Churchhill Livingstone: Edinburg, London, Melbourne, New York. p.p. 117, 332, 514.

Qin H.S. (1989). A study on the effect of fermented soya bean in preventing iron deficiency anemia in children. Zhonghua Yu Fang Yi Xue Za Zhi 23 (6): 352-4.

Rahmatalla H.M. (1999). Consumption patterns and anti-anemic properties of *Grewia tenax.* (MSc.Thesis; U. of K.).

Rao P.U. (1996). Nutrient Composition and biological evaluation of mesta (*H. sabdariffa*) seeds. Plant foods for human nutrition 49: 1, 27-34.

Roy M. L. and Shirley I.M. (1992). O-Naphthoquinones from the heart wood of Azanza garckeana. Phytochemistry 31(1): 4171-4172.

Saka J. J. D. M. and Maghembe J. A. (1994). The Nutritional value of edible fruits of indigenous wild trees of Malawi. Forest Ecology and Management 64: 245-248.

Sandberg A.S. (2002). Bioavailability of minerals in legumes. British Journal of Nutrition 88 Suppl. 3: 281-5.

Sulieman, M.S. and Eldoma, A. M. (1994). Marketing of non-wood forest products (excluding the Gum Arabic) in Sudan. Forest National Corporation (FNC), Ministry of Agriculture, Animal Wealth and Natural Resources- Khartoum, Sudan. p.p. 3-10, 20, 30-36.

Trevor B. P. and Ruth R. (1988). Handbook on the care and management of laboratory animals. 6 the edition. Longman Scientific and Technical. Page 320.

Vermeer J. E.; Kuijpers A.H. and Elbers A.R. (2002). Comparison of the efficacy of two different iron-supplements for anemia prevention in piglets. Tijdschr Diergeneeskd 127 (4): 110-4.

WHO-UNICEF. Indicators and strategies for iron deficiency and anemia programs. World Health Organization Technical Report (1993).

Wrobel K. and Urbina (2000). Determination of total aluminum, chromium, copper, iron, manganese and nickel and their fractions leached to the infusions of black tea, green tea, *Hibiscus sabdarrifa* and IIex paraguariensis (mate) by ETA-AAS. Biological Trace Element Research 78 (1-3): 271-80.

APPENDIX