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College of Veterinary Medicine

**Anti- anaemic Effect of Aqueous Extract of *Capparis decidua* Stems in AlCl<sub>3</sub>-Induced Anaemia in Wistar Rats.**

الأثر المضاد لفقر الدم للمستخلص المائى لسيقان نبات الطنذب باحداث فقر الدم  
بثالث كلوريد الالمونيوم فى الجرذان.

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# *Dedication*

*Mothers,*

*&*

*Fathers,*

*&*

*Sisters,*

*&*

*Brothers,*

*&*

*Co-colleagues,*

*&*

*Teachers,*

*And*

*Scientist.*

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## Abstract

This is case control study conducted in Sudan University of Science and Technology to investigate the antianemic effect of the aqueous extract of *Capparis decidua* stems against  $AlCl_3$ -induced anaemia in Wistar albino rats.  $AlCl_3$  was given at a dose of 100 mg/kg BW per os daily for 15 days. Treatment of anaemia was done by oral administration of aqueous extract of *C. decidua* stems at a dose of 200mg/kg (low dose) and 400mg/kg BW (high dose) daily for 15 days. Simultaneous oral administration both doses of the extract did not correct the reduction in haematological parameters significantly compared to  $AlCl_3$  group. However, high dose of the extract exert insignificant increase in haematological parameters compared with control rats.

In conclusion, the aqueous extract of *C. decidua* stems has no antianaemic effect by the doses used in this study. This effect could be seen by increasing the dosage of the plant extract.

## Arabic abstract

هذه دراسة حالة والشواهد اجريت في جامعة السودان للعلوم والتكنولوجيا للتقصي عن الاثر المضاد لفقر الدم للمستخلص المائي لجذوع نبات الطنذب وذلك في الجرذان المحدث فقر الدم فيها بواسطة كلورايد الالمونيوم. تم اعطاء كلوريد الالمونيوم بجرعة 100 ملجم/كجم على وزن الجسم فمويا لمدة 15 يوم. عولج فقر الدم عبر التجريع الفموي للمستخلص المائي لجذوع نبات الطنذب بجرعه 200 ملجم/كجم على وزن الجسم ( جرعة منخفضة) وجرعة 400 ملجم/كجم على وزن الجسم (جرعة عالية) فمويا لمدة 15 يوما . النتائج اوضحت ان جرعتي المستخلص لم تصححا الانخفاض في قياسات الدم بشكل معياري مقارنة بمجموعة كلورايد الالمونيوم ، ومع ذلك اظهرت الجرعة العالية للمستخلص زيادة غير معيارية في قياسات الدم مقارنة مع الجرذان الضابطة.

في الخلاصة المستخلص المائي لجذوع نبات الطنذب ليس له اي اثر كمضاد لفقر الدم بالجرعات المستخدمة في هذه الدراسة. هذا الاثر قد يمكن مشاهدته بزيادة جرعة المستخلص النباتي .

## Introduction

Floras comprise various type of plants used in herbalism and some of them have medicinal activates. Medicinal plants have important role when used as drugs in which act as synergistic or supportive or preventive (Hassan, 2012). Till now people in developing countries rely in herbal medicine rather than allopathic medicine because herbal is low in cost and with less side effect (Mahmoud and Gairola, 2013).

Sudan has extensive type of plants due to variable climatic condition, this wide range of plants used in folk medicine to treat both animals and human aliments. (Mohammed *et al.*, 2014).

Anaemia is a worldwide condition which causes significant morbidity (lowered resistance) and mortality. Incidence of anaemia in developing countries is highest; this due to malnutrition, blood sucking parasite and frequent use of drugs (Pingali *et al.*, .2015).

Socially anaemia has adverse outcome among the adult such as reduced life quality and depression. In Sudan the incidence of anaemia among the adult in the eastern Sudan in 2011 was estimated about 36.2 % (Abdullah *et al.*, 2012). Adam *et al.* (2005) reported that the prevalence of anaemia in pregnant women is 73% in Eastern Sudan and the associated risk factor is Malaria.

### Objective:

- The aim of this study is to investigate the anti-anaemic effect of aqueous extract of *Capparis decidua* stems in AlCl<sub>3</sub> induced anaemia in rats.

## Chapter One

### Literature Review

#### 1.1 Definition of anaemia:

Anaemia is defined as deficiency of red blood cell (RBC) or haemoglobin in the blood, which results in the disturbance of oxygen transport.

#### 1.2 Causes of anaemia:

There are three main causes of anaemia as follow:

##### 1.2.1 Blood loss:

For example through heavy menstrual period or gastrointestinal bleeding from an ulcer.

##### 1.2.2 Defective red blood cell production:

Which can result from nutritional or vitamin deficiencies or chronic illness where the bone marrow does not work properly.

##### 1.2.3 Red blood cell destruction:

This may be due to hereditary factor some auto immune disorder or as side effect of some drugs (Sammour *et al.*, 2014)

#### 1.3 Classification of anaemia:

Anaemia is classified according to several variable including aetiology, changes in erythrocyte morphology and degree of bone marrow regenerative response (Adam and Fetman 2001).

##### 1.3.1 Regenerative anaemia:-

###### 1.3.1.1 Blood loss anaemia:

This may be acute or chronic, in acute blood loss anaemia; there is a risk of hypovolemic shock, a fall in red blood cell count, haematocrit and haemoglobin due to haemodilution. Chronic loss does not affect blood volume but lead to iron deficiency anaemia the new red blood cell have little haemoglobin give rise to microcytic hypochromic anaemia .

### **1.3.1.2 Iron deficiency anaemia:**

In iron deficiency anaemia the red blood cells are decreased in number and are microcytic, hypochromic and malformed (poikilocytosis). Iron content of the body is normally kept constant by regulation of the amount absorbed and lost. Iron requirements are increased during infancy, puberty, pregnancy and menstruation. The signs and symptoms of iron deficiency anaemia include fatigue, dyspnoea, angina, tachycardia, epithelial atrophy and dysphagia.

### **1.3.1.3 Haemolytic anaemia:**

This is due to premature destruction of red blood cells with retention of iron in the body the red blood cells are normocytic and normochromic. The cause could be intrinsic or extrinsic to red blood cell. Intrinsic causes defect of red blood cells membrane, haemoglobinopathies and inherited enzyme defect while extrinsic cause include drug, bacteria, toxin, antibodies and trauma in haemolytic anaemia. The erythrocytes have shortened life span, the bone marrow is usually able to compensate by producing new red blood cells. The clinical signs of haemolytic anaemia include pain in bone, jaundice and anaemia.

## **1.3.2 Non regenerative anaemia:-**

### **1.3.2.1 Aplastic anaemia:-**

A primary condition of bone marrow stem cells that result in a reduction of all three haemopoietic cells, with fair replacement of bone marrow. Symptoms include weakness, fatigue, pallor, petechial, ecchymosis, bleeding from nose, vagina, gum, gastrointestinal tract and infections.

### **1.3.2.2 Sickle cell anaemia and thalassemia:**

This happens due to defect in the haemoglobin; the shape of red blood cell is deformed, causing obstruction and tissue hypoxia. The symptoms are anaemia, pain especially in bones and joints, growth and sexual maturation may be delayed, increased susceptibility to infections and hepatosplenomegaly.

### **1.3.2.3Thalassemia:**

Thalassemia is characterized by abnormal decrease in haemoglobin content of erythrocytes (hypochromic), smaller than normal (microcytic) and destruction of RBC (haemolysis). Quantitative defect in haemoglobin result from absent or deficiency in alpha or beta chain of haemoglobin. The main clinical sings of thalassemia include severe anaemia market haemolysis, organ dysfunction due to iron over load, recurrent bone fracture deformity of skull due to expansion of bone marrow, growth retardation, splenomegaly and hepatomegaly (Sammour et al., 2014)

### **1.3.2.4 Pernicious anaemia:**

Pernicious anaemia is result of the inability of the body to absorb vitamin B12 which causes a fall in the number of red blood cells, is actually the end stage of an autoimmune inflammation of the stomach, resulting in destruction of stomach cell by one's own antibodies. This causes decreased secretion of acid and enzymes required to release food bound vitamin B<sub>12</sub>. The general symptoms of pernicious anaemia include weakness, fatigue, upset stomach, abnormal rapid heartbeat, and abnormal yellow coloration of the skin, headache and breathlessness (Acomb and Holden, 2007).

### **1.3.2.5 Anaemia of chronic disease:**

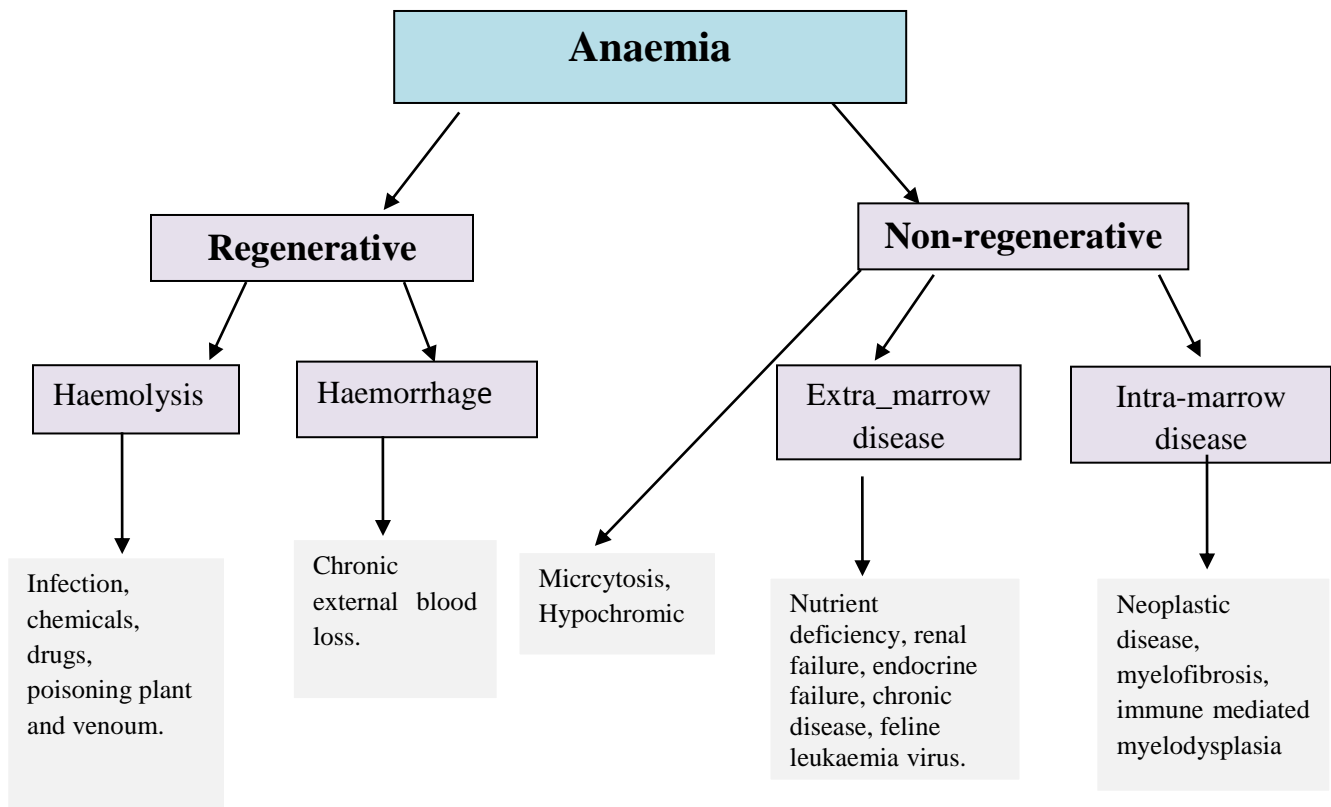
Anaemia of chronic disease is a common disorder associated with wide variety of inflammatory diseases including arthritis , malignancies and inflammatory bowel disease. In some cases they appear to be impaired response to erythropoietin. However , there is also shift of iron from the circulation into the reticuloendothelial system leading to iron restricted erythropoiesis despite normal iron store . (Acomb and Holden, 2007). Table (1) and Fig 1 illustrate different morphological and etiological classification of anaemia.

**Table (1) Morphological and etiological classification of anaemia.**

<b>Erythrocyte size</b>	<b>Haemoglobin content</b>	<b>Etiological classification</b>
Macrocytic	Normochromic	Vitamin B12 deficiency, folic acid deficiency.
Macrocytic	Hypochromic	Haemolysis, haemorrhage.
Normocytic	Normochromic	Anaemia of chronic inflammatory, acute blood loss.
Normocytic	Hypochromic	Early iron deficiency.
Microcytic	Normochromic	Iron deficiency in progression.
Microcytic	Hypochromic	Iron deficiency, chronic external blood loss.

Source: (Fettman and Adams 2001)





**Fig. 1: Flowchart of approach to classification of anaemia.**

Source (Fettman and Adams 2001)

#### **1.4. Signs and symptom of anaemia:-**

The general symptoms include:

- Tiredness and lethargy.
- Inhibit physical exercise and result in reduced mental performance.  
    reduce oxygen carrying capacity of the blood lead to reduced tissue oxygenation and wide spread organ dysfunction .
- Rapid blood loss e.g.; haemorrhage, shock with collapse, and dyspnoea and tachycardia.
- Reduce the amount of haemoglobin when the haemoglobin falls below 7 or 8 g/dl there is almost always compensatory increase in cardiac output.
- Increase respiratory rate (Acomb and Holden, 2007).

#### **1.5 Micronutrients required for erythropoiesis:-**

##### **1.5.1 Haemoglobin synthesis:**

The synthesis of haemoglobin is started in the early stage of erythrocyte production. The first step of haem synthesis comprises the production of aminolevulinic acid from glycine and succinyl-CoA in the mitochondria. It involves pyridoxal phosphate as cofactor and dietary deficiency of vitamin B<sub>6</sub> can result in an anaemia, morphologically and functionally characteristic of decreased haemoglobin synthesis. Aminolevulinic acid is transported to the cytosol through a series of reactions, it is converted into coproporphyrinogen (III) which is then transported back into the mitochondria for derivation of protoporphyrin (IX). Insertion of ferrous iron into protoporphyrin (IX) catalyzed by haem synthesis to produce the haem molecule which is transferred from the mitochondria to the cytosol for condensation with globin chain to produce haemoglobin during this process the iron moiety of haem becomes oxidized to the ferric state and the ferric haem thus formed is inserted into an alpha and beta globin chain. The ferric haem containing globin chain spontaneously combine to form

alpha and beta dimmers , two of which combine in turn to produce mature hemoglobin tetramers.

#### **1.5.1.1 Nutrient deficiencies affecting haemoglobin synthesis:-**

Dietary iron deficiency is un common in adult animal but is routinely observed in neonates that are born with limited iron reserve and that principally consume milk or milk substitutes low in iron contention deficiency is manifested initially as normocytic hypochromic anaemia owing to impaired hemoglobin synthesis impaired hemoglobin synthesis lead to both decreased cell hemoglobin content and additional cell division resulting in smaller mature erythrocyte. Nutrient deficiencies affecting division of erythroid precursor cell.

Vitamin B<sub>12</sub> "cyanocobalamin " is a cobalt containing vitamin required by cell throughout the body for conversion of ribose nucleotides into deoxyribose nucleotides a major step in the formation of deoxyribonucleic acid thus it is an essential nutrient for nuclear maturation and cell division and deficiency of this vitamin result in generalized depression of cellular development and tissue growth because the erythropoietin centers of the bone marrow are among the most rapidly growing and proliferating tissue ,in adequate amount of cyanocobalamin are especially manifested by decrease in erythrocyte production erythrocyte precursors fail to mature properly under condition of vitamin B<sub>12</sub> deficiency and cell proliferation is inhibited.Folic acid "pteroylglutamic acid " folic acid deficiency anaemia are considered rare in most species however naturally occurring foliate antagonists in moldy feeds can block intestinal microbial synthesis of Focalin in herbivores folic acid responsive anaemia may occur in animals treated with synthetic foliate antagonists for their antineoplastic or antimicrobial activities .

### **1.5.1.2 Protein:**

protein in an adequate amount is important for normal rate of haemoglobin synthesis and erythrocyte production, a primary deficiency of protein in the diet or secondary deficiency subsequent to intestinal or urinary protein loss can contribute to the development of anaemia never the less protein deficiency by itself has not been demonstrated as an important cause of anaemia in domestic animals .

### **1.5.2 Other nutrients:**

production of normal erythrocyte is influenced directly or indirectly by several nutrients that act as coenzymes or cofactors in the synthesis of haemoglobin, metabolic enzymes or other important structural and functional protein these include riboflavin niacin, pantothenic acid , thiamin , biotin and ascorbic acid , antioxidant vitamin A ,B and C play an important role in protecting erythrocyte against oxidative damage from free radicals and their deficiency can contribute to shortened erythrocyte survival in the circulation (Fettman and Adams, 2001).

### **1.6 Diagnosis of anaemia:**

The clinical diagnosis of anaemia is made from the history, physical examination, symptoms, haematologic values and other procedures and finding. The history and physical finding can yield information that may prove valuable in identifying and narrowing the possible cause or causes of anaemia and in lessening the need for ordering expensive special test obtaining a good history requires particularly in regard to diet, drug ingestion, exposure to chemicals, bleeding history, family history of disease, neurologic symptoms, previous medication, jaundice and various underlying disease that produce anaemia. In the physical examination certain features should be evaluated closely such as skin, sternal tenderness, lymphadenopathy, cardiac murmurs, splenomegaly and hepatomegaly. Laboratory procedures complete blood cell count with cell indices to

detect the presence of anaemia the medical technologist performs complete blood count on a haematology cell analyzer to determine the red blood cell count, haemoglobin, haematocrit, red blood cell indices, white blood cell count and platelet count blood smear examination the most important, evaluation of an anaemia is to examine the peripheral blood smear giving particular attention to the red blood cell as to variation in size, shape and colour normal red blood cell on wrights stained blood film are nearly uniform in size. Bone marrow examination in the determination of the type of anaemia, marrow aspiration and biopsy are important procedures other needed laboratory test are complete urinalysis including microscopic examination and faecal analysis with occult blood test and microscopic examination for parasite particular special test may be indicated on the basis of the morphologic type of anaemia present such as serum iron, iron binding capacity and serum ferritin if microcytic hypochromic anaemia is present, the cause of the anaemia must be determined before replacement therapy or supportive therapy (Rodak *et al.*, 2012).

### **1.7 Treatment of anaemia:**

Anaemia is treated by different ways according to its type; two main treatment lines are used:

#### **1.7.1 Degenerative anaemia:**

Iron was used in the treatment of blood loss (haemorrhagic, iron deficiency anaemia) either as oral or parenteral therapy. Oral therapy included different iron salts such as ferrous sulphate and hydrate, ferrous gluconate and fumarate at a dose rate of 200-400 mg daily to correct iron deficiency. Parenteral iron therapy is given to people who tolerate oral iron therapy, these drugs include iron dextran which is given by intramuscular and intravenous routes, and sodium ferric gluconate complex can be used intravenously only. Iron chelators as deferoxamine and deferasirox are used in haemochromatosis (Masters, 2009).

Treatment of haemolytic anaemia is problematic due to difficulty in reverse oxidative damage of erythrocyte, but antioxidant such as N-acetyl cysteine, Vitamin (A, E, C) could be used to reduce oxidative damage (Fettman and Adams, 2001).

Generally haemolytic anaemia needed to increase folic acid and foliate supplement to compensate chronic haemolysis especially in poor diet patient. Auto immune haemolytic anaemia is treated by high dose of corticosteroid drugs or alternatively rituximab .Penicillin V (250mg) twice daily or erythromycin are used as prophylactic antibiotics against pneumococcal infection which causes sickle cell haemolytic anaemia (Acomb and Holden, 2007).

### **1.7.2 Non regenerative anaemia:**

Pernicious anaemia which resulted from malabsorption of vitamin B<sub>12</sub> is treated by oral vitamin B<sub>12</sub> at a dose of 1000 mcg daily. Parenteral injection as cyanocobalamine or hydroxocobalamine, the last one is preferred because of highly protein bounded and therefore remains longer in circulation. The initial therapy consists of 100-1000 mcg of vitamin B<sub>12</sub> intramuscularly daily or every other day for 1-2 weeks. Maintenance therapy should be injected for 1-2weeks to 6 months if nervous signs are appeared (Masters, 2009).

Primary bone marrow disease such as a plastic anaemia and bone marrow failure must be treated by addressing the primary disorder, however, lithium carbonate at dose of 11mg/kg orally twice daily noted to improved haematopoiesis (Fettman and Adams, 2001).

In chronic disease anaemia such as malignancies, arthritis or bowel inflammation and chronic renal failure the treatment mainly directed to treat underlying cause. Erythropoietin and Erythrocyte –stimulating such as epotin alfa, darbepoetin alfa and methoxypolyethylene glycol-Epotin beta used to stimulated erythroid proliferation, differentiation and release of reticulocyte from bone marrow (Masters, 2009).

Treatment of sickle cell anaemia by erythropoietin has been shown to increase foetal haemoglobin. Sickle cell crises treated depend on removal of trigger factor, hydration and effective pain killer to reduce pain. Appropriate antibiotic and strong opioid like morphine is very successful in the treatment of this type of anaemia. (Acomb and Holden, 2007). Table (1) shows summary of various drugs used extensively to treat different types of anaemia.

**Table (2) Summary of anti-anaemic drugs (Masters, 2009).**

<b>Subclass</b>	<b>Mechanism and action</b>	<b>Effect</b>	<b>Clinical application</b>
Iron: Ferrous (sulfate, gluconate, fumarate) Iron dextran Sodium ferric gluconate	Required for synthesis of haem and haem containing protein	Normal haem synthesis, when deficient result in inadequate haem production	Iron deficiency anaemia.
Iron chelators: Deferoxamine Deferasirox	Chelated excessive iron	Reduced acute and chronic iron toxicity	Iron toxicity and Hemochromatosis
VitaminB <sub>12</sub> : Cyanocobalamin Hydroxocobalamin	Enzymatic reactivation converted homocysteine to thionine and metabolize L-methylmalonyl-CoA	For amino acid, and fatty acid metabolism, DNA synthesis	Pernicious anaemia
Erythrocyte-stimulating agent: Epoetin Alfa Darbepoetin Alfa Methoxy polyethylene glycol- Epoetin beta	Erythropoietin Agonist which stimulated red blood cells progeneration	Stimulated erythrocyte proliferation and reticulocyte release from bone marrow	Chronic disease anaemia



## 1.8 Methods of induce anaemia

There are many methods used to induce anaemia experimentally as follows:

**1.8.1 Iron deficiency anaemia:** This type of anaemia induced experimentally by introducing basal diet without iron source (iron deficiency 3-5 mg) for 21 day (Soliman *et al.*, 2010).

**1.8.2 Haemorrhagic anaemia:** Bleeding is successful method to cause haemorrhagic anaemia. This done by successive bleeding from tail vein for 3 days (Marcović *et al.*, 2010).

**1.8.3 Haemolytic anaemia:** This type of anaemia induced by many chemicals such as phenyl hydrazine 2.5% which causes anaemia by activating reactive oxygen group (ROG). It is initially used at a dose of 30 mg/kg with maintenance dose of 10-15 and 20 mg/kg at interval of 30 day within the duration of experiment. The anaemia induced two days after administration of phenyl hydrazine (Ogbe *et al.*, 2010). Phenyl hydrazine can be used also intraperitoneally at a dose of 40mg/kg for 3 successive days (Pingali *et al.*, 2015).

Aluminium chloride ( $AlCl_3$ ) is used extensively to produce haemolytic anaemia. It has anaemic, hepatotoxic, neurotoxic and bone marrow effects. It causes high level of serum haemodialysis in patients which is associated with impaired erythropoiesis. It is used at a dose of 0.5 mg/kg (Osman *et al.*, 2010).

**1.8.4 Pernicious anaemia:** It is induced experimentally by giving vitamin and foliates deficient diet fed for 12 week (Herrman *et al.*, 2009).

**1.8.5 A plastic anaemia:** As reported by Ibrahim (2015) it can be induced by benzene which reduced blood cell and depressed bone marrow, the given dose is 2ml/kg daily for 2 weeks via subcutaneous and oral rout. Cyclophosphamide at a dose of 0.3 mg/kg intraperitoneally also induced a plastic anaemia (Madukwe *et al.*, 2013). Moreover, Carboplatin which is used as chemotherapy can induce anaemia via its toxicity to the bone marrow; this is described by Woo *et al.* (2008) when used

carboplatin as single dose (60mg/kg) it was dissolved in sterile saline before injection in the tail vein.

### **1.9 Aluminum Chloride AlCl<sub>3</sub>**

Aluminium, (Al), is ubiquitous in the environment. Physically it is amber to light pale yellow, almost clear liquid and it is stable at normal temperature and pressure. However, little is known about possible effect of Al as trace element in animals and human in normal condition (Dlugasek *et al* 1999 and Buraimoh *et al.*, 2011).

The principle mechanisms of absorption of Al is poorly understood. After ingestion, the systemic transfer of aluminium is small but it is greatly affected by the ingestion of certain dietary agents like citrate, that complex with the metal in the intestinal lumen or transiently alters the permeability of the mucosa. The small bowel and colon absorb aluminium passively and paracellularly but stomach dose not (Whitehead *et al.*, 1997).

#### **1.9.1 Toxicity of AlCl<sub>3</sub>:**

Recently it is clear that when Al is mobilized from soil by acid rain, it poses hazard to all exposed organs (Buraimoh *et al.*, 2011). The ionic form of Al has no serious biological role, but when accumulate in the body it can induce several clinical disorders such as neurotoxicity, hepatotoxicity, bone marrow diseases and anaemia .It also has direct effect on haematopoiesis and its high levels in serum of hemodialysis patients were associated with impaired erythropoiesis and iron (Fe) deficiency anaemia. Furthermore, Aluminium is known to disrupt cellular functions by perturbing Fe homeostasis (Osman *et al.*, 2014). Aluminium chloride also increases protein carbonyl group concentration. The long term aluminium intoxication of rats and mice besides other harmful effects causes an increase in oxidative stress and accumulated in bone and liver (Dlugaszek *et al.*, 2000 and Kowalczyk *et al.*, 2003).

### **1.9.2 Mechanisms of action of AlCl<sub>3</sub> to induce anaemia:**

Several mechanisms have been proposed for the AlCl<sub>3</sub> - induced anemia, but the exact mechanism is unknown. The proposed mechanisms appear to involve reduction in haeme biosynthesis and Fe metabolism in rats and also reduction of reactive oxygen synthesis in bone marrow (Osman *et al.*, 2014).

### **1. 10 Anti anaemic plants:**

Ogbe *et al.* (2010) studied the antianemic potential of three plant extract (*Mangifera indicica*, *Telfairia occidentalis*, *Amaranthus hybridus*) on phenyl hydrazine-induced anaemia in rabbit. Treatment of anaemia was done with ethanolic extract of *M. indica* stem bark, aqueous leaves extract of *T.occidentalis* produced significant (p<0.05) antianemic effect. The aqueous of *A. hybridus* only produced a minimal antianemic effect, reflected by a significant increase (P<0.05) in haemoglobin concentration. Phytochemical analysis of plant extracts detected saponins, tannins, cardiac glycosides, flavonoids and alkaloid in the 3 extracts. The result showed that *M. indicica* , and *T.occidentalis* have antianemic effect.

The potential ability of *Azania garckeana* fruits aqueous extract on iron deficiency anaemia was evaluated using iron deficient rats and determination of iron absorption capability of plant in averted gut of Wistar albino rats (Ream *et al.*, 2011). The extract of fruits was given 2g/kg body weight to iron deficient rats for 3 weeks caused slight alterations on haematological parameters of nutritionally iron rats except on red blood cells counts of these animals. Phytochemical analysis of the plant extract revealed the presence of moderate contents of iron element and various phytochemical like triterpenes, flavonoides, and saponins. The results revealed that extract has potential effect in increasing iron absorption rather than being a rich source of iron and different doses of the extract can be used to establish its stability in inducing favorable erythropoietic properties.

The antianemic effect of *Justicia secunda* was investigated by Moswa *et al.* (2012). This plant was reported to treat anaemia in Cinglese folk medicine. The study bearing upon the level of this plant, the extensiveness' of its use and its iron contents. Appropriate questionnaire was used for survey and iron was detected by atomic absorption spectrometry with prior acid digestion. The result indicated that the plant is very well known and extensively used by population of Kinshasa and medical staff of medical centers of Kinshasa. On the other hand that it's containing high iron level this might justify its use as an antianemic plant.

The anti-anaemic effect of *Jatropha tanjorensis* was carried out in rabbits by Idu *et al.* (2014). Anaemia was induced by 2.5% phenyl hydrazine HCL at a dose of 30 mg/kg with a maintenance dose of 15 mg/kg-body weight of the same drug given after 2 days of administration of the first dose. Plant material was given at different dosage (10, 7.5 and 5 g/100 ml) P.O twice daily. Blood samples were taken at day 3, 7 and 14 to evaluate various haematological parameters. The results suggests that crude extract of *J. tanjorensis* improves the anaemic condition of the treated animals when compared with the phenyl hydrazine untreated animals.

The antianaemic effect of *Entandrophragma angolense* seed was evaluated in rats. Anaemia was induced using phenylhydrazine at a dose 40 mg/kg IP for two days. The extract of seeds was given daily for 28 days at dose of 200 mg/kg orally. The results revealed that the *E. angolense* increases significantly RBC count at 4<sup>th</sup> week compared with untreated anaemic group and haemoglobin level increased significantly in the first week of treatment (Aurelie *et al.*, 2015).

Veena *et al.* (2015) reported the anti-anemic effect of *Azadirachta indica* (neem leave) combined with *Emblica officinalis* (amla) in phenyl hydrazine induced anaemia in rats. Rats divided into 7 groups. The control rats were given normal saline and the other groups were given 60 mg/kg B.w of phenyl hydrazine 2 days to induce anaemia. Ferrous sulphate (0.012mg/kg) was used as a standard drug. A.

*indica* leaves aqueous extract was administered at a dose of 200 and 400 mg/kg B.w. Two groups of animals were treated by combination of aqueous extract of *A. indica* leaves and *E. officinalis* fruits at a dose of 200 and 400mg/kg B.w orally for 15 days. The combination of *A. indica* and *E. officinalis* produced a significant anti-anemic activity which is indicated by the significant reduction of haematological parameter.

### **1.11 *Capparis decidua***

#### **1.11.1 Taxonomy and Classification**

Order: Capperales

Family: Capparaceae

Genus: *Capparis*

Species: *Decidua*

Vernacular name: Tundub, sodad, murkheit, kursan (Orwa *et al.*, 2009).

#### **1.11.2 Distribution of the plant:**

It is commonly found in dry places in Sind, Baluchistan, Western Rajput Ana, Deccan Peninsula, Egypt, Socotra, Arabia (Verma *et al.*, 2011). In Sudan it is found in Al Abassiya and Abu Gubieha (El Gazali *et al.*, 1987).

#### **1.11.3 Botanic Description:**

It is a bushy shrub in dense tufts (4.5m) high or occasionally small tree with many green vine like apparently leaf less branches, hanging bundles, bark turns whitish, grey colonies with age but most branches and twigs are glossy dark green. Small light brown spines occurs in pairs on the twigs at each node, leaves are very minute (2mm long), short life span on young shoots, fruit is pink red in small group long the leaf less shoot in axils of spine. Fruits are small many seeded avoid or sub globules slightly mucronate pink berry of the size and shape of cherry, becoming blackish when dry (Orwa *et al.*, 2009). (Fig.2 and 3)



**Fig. 2.** *Capparis decidua* tree.



**Fig. 3.** *Capparis decidua* branches

#### **1.11.4 Traditional uses:**

*Capparis decidua* has been used as carminative, tonic, emmenagogue, and aphrodisiac, to improve the appetite and for rheumatism. It is used in cough and asthma and the top shoots and young leaves are made into powder and used as blister. They are also used in boils eruption and swelling and as antidote in poison. They are very efficient in relieving toothache when chewed. Stems and leaves are used for treatment of pyorrhoea. The fruit are astringent and useful in cardiac troubles. Fruit are eaten either green or ripe useful in facial paralysis and solves problem of enlarged spleen and to kill intestinal worms. Root powder is taken in liver problem (Singh *et al.*, 2011).

#### **1.11.5 Chemical constituents:**

Numerous phytoconstituents have been identified and isolated from different parts of *C. decidua* which include alkaloids, glycosides, terpenoids, sterols, flavonoids, phenols and fatty acids. The flowers and fruits contain series of hydrocarbons aliphatic alcohols and P-carotene. The seeds contain glycocaprin an isothiocyanate glycoside (EL Ghazali *et al.*, 1987).

#### **1.11.6 Nutritional values:**

The fruit of *C. decidua* is rich in carbohydrate (71%), protein (15-18%), fats (5%), and crude fibre (1%), Ca (20%), Mn (2%), Fe (2%), and Zn (4%). *C. decidua* was found to be the richest source of  $\beta$ -carotene. The present  $\beta$ -carotene (14%) is sufficient to meet the requirement of vitamin A. Fruit is a rich source of vitamin C (Joseph and Jini, 2011).

#### **1.11.7 Pharmacological uses:**

*C. decidua* is used for its medicinal value in the treatment of different disorders. Verma *et al.* (2011) reviewed the pharmacological uses of *C. decidua* such as anti-inflammatory, laxative, anti-diabetic, anti-helminthic, anti-bacterial and astringent. It is used in colic pain, flatulence, anorexia, respiratory disorder, skin diseases, and

general weakness. It is also reported to possess beneficial effect in rheumatism, asthma, liver disorder, hypercholesterolemia and hypertension.

#### **1.11.8 Toxicity of *Capparis decidua*:**

*Capparis decidua* had a significant cytotoxic effect on mice bone marrow cell, but it has little effect on their chromosome structure (Shal *et al.*, 1989).



## **Chapter Two**

### **Materials and Methods**

#### **2.1 Plant material**

*Capparis decidua* stems were obtained from Arkaweit area Khartoum State. The plant material was identified by botanists in Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Khartoum, Sudan. The stems of the plant were cleaned and dried under shed and powdered.

#### **2.2 Extraction (aqueous extract)**

A known weight of the powdered plant material was extracted using infusion method. The filtrate was freeze and dried using a freeze drier apparatus (Harborne, 1983).

#### **2.3 Experimental animal**

Twenty Wistar albino rats (66-150g) were used. The animals were obtained from Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan. Rats were kept in cages under standard condition in the Laboratory Animal's House in the College of Veterinary Medicine, Sudan University of science and Technology (SUST), Hilat Kuku, Khartoum, Sudan. Were provided with a diet (meat, flour, salt (NaCl), water) and free access to water. They were kept for 5 days to acclimatize to laboratory condition.

#### **2.4 Experimental design**

Rats were divided into four groups of five rats each as follows:

- **Group 1:** served as control and kept untreated for two weeks.
- **Group 2:** served as control negative, rats were given aluminium chlorid ( $AlCl_3$ ) at a dose of 100 mg/kg orally daily for two weeks.

- **Group 3:** served as test plant1, (low dose), animals were dosed with AlCl<sub>3</sub> (100 mg /kg) P.O daily and concurrently treated with *C. decidua* stem aqueous extract at a dose of 200mg/kg P.O daily for two weeks.
- **Group 4:** served as test plant2, (high dose), rats were given AlCl<sub>3</sub> (100mg/kg) P.O daily for two weeks, and at same time treated with *C. decidua* at a dose of 400 mg/kg P.O daily for two weeks.

## **2.5 Blood samples collection**

Blood samples were collected into EDTA tubes by puncturing of retro-orbital plexus using capillary tubes twice at day 0 and day 15. The blood was analyzed immediately for various haematological parameters.

### **2.5.1 Haematological parameters**

White blood cells (WBCs), Red blood cells (RBCs), Haemoglobin concentration (Hb), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were analyzed using automatic analyser (SYSMEX auto analyzer. KX-2N, Spain).

## **2.6 Body weight**

Body Wight was recorded during the experimentation at day 0, and then weekly for 2weeks.

## **2.7 Statistical Analysis**

Statistical analysis was performed using software statistical package for Social science (SPSS) version 15. Multivariate analysis was used for the analysis of data .The data were expressed as mean  $\pm$ SDM (Mendenhall, 1971).

## Chapter Three

### Results

#### 3.1 Haematological parameter:

##### 3.1.1 Change in white blood cells counts (WBCs):

After 15 days of treatment with  $\text{AlCl}_3$  and low dose (200mg/kg BW) of aqueous extract of *C. decidua* stems there were significant decrease in WBC counts compared with the control group. There was no significant difference in WBCs count in rats that administrated high dose (400mg/kg BW) of the extract compared to control rats. The result is present in table (3).

##### 3.1.2 Change in red blood cells count (RBCs):

RBC count was significantly decreased ( $p < 0.05$ ) in  $\text{AlCl}_3$  group and low dose (200 mg/kg BW) of *C. decidua* aqueous extract when compared with the control rats. However, high dose (400mg/kg) of the extract showed insignificant decrease in RBCs count compared to control rats and insignificant ( $P > 0.05$ ) increase in RBCs count compared to  $\text{AlCl}_3$  group and low dose of the extract (Table 4).

##### 3.1.3 Change in haemoglobin concentration (Hb):

There were significant differences in haemoglobin concentration in  $\text{AlCl}_3$  and low dose of the extract when compared with control group. However, high dose of the extract showed insignificant decrease in Hb concentration when compared with control group and insignificant increase in Hb concentration when compared with low dose of the extract and  $\text{AlCl}_3$  group. The results are shown in table (5).

##### 3.1.4 Packed cell volume (PCV):

PCV was significantly decreased ( $p < 0.05$ ) in  $\text{AlCl}_3$  group and rats given low dose of the extract compared with control rats. However, high dose (400 mg /kg) of *C. decidua* stems aqueous extract showed insignificant decrease in PCV when

compared with control and insignificant increase when compared with AlCl<sub>3</sub> group.  
The result is presented in table (6).

**Table (3):** Effect of administration of aqueous extract of *C. decidua* stems on WBC count in AlCl<sub>3</sub>-induced anaemia in rats.

Groups	WBCs×10 <sup>3</sup> μl (Mean±SD)	
	Day 0	Day 15
<b>Control</b>	7.06±2.27	12.42±3.64 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	9.30±1.45	6.54±3.12 <sup>b</sup>
<b>Low dose</b>	6.16±2.07	4.86±1.80 <sup>b</sup>
<b>High dose</b>	6.38±1.56	12.03±3.84 <sup>a</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

**Table (4):** Effect of administration of aqueous extract of *C. decidua* stems on RBC count in AlCl<sub>3</sub>-induced anaemia in rats.

Groups	RBC ×10 <sup>6</sup> cells/μl (Mean±SD)	
	Day 0	Day 15
<b>Control</b>	7.01±0.37	7.43±0.41 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	7.96±0.17	6.70±0.52 <sup>b</sup>
<b>Low dose</b>	7.25±0.59	6.25±0.47 <sup>b</sup>
<b>High dose</b>	7.36±0.47	6.90±0.39 <sup>a b</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

**Table (5):** Effect of administration of aqueous extract of *C. decidua* stems on Hb concentration in AlCl<sub>3</sub>-induced anaemia in rats.

Groups	Haemoglobin (g/dl) (Mean±SD)	
	Day 0	Day 15
<b>Control</b>	12.48±0.98	14.46±0.82 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	14.04±0.34	12.98±1.08 <sup>b</sup>
<b>Low dose</b>	12.78±1.01	12.18±0.86 <sup>b</sup>
<b>High dose</b>	13.26±0.62	13.63±0.51 <sup>ab</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

**Table (6):** Effect of administration of aqueous extract of *C. decidua* stems on PCV percentage in AlCl<sub>3</sub>-induced anaemia in rats.

Groups	PCV% (Mean±SD)	
	Day 0	Day 15
<b>Control</b>	39.84+_2.82	43.56+_2.28 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	44.82+_0.65	37.16+_2.75 <sup>b</sup>
<b>Low dose</b>	40.62+_3.55	36.10+_2.99 <sup>b</sup>
<b>High dose</b>	41.68+_2.55	39.83+_2.78 <sup>ab</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).



### **3.1.5 Mean corpuscular volume (MCV):-**

The rats that dosed AlCl<sub>3</sub> exhibited significant (P<0.05) decrease in the mean corpuscular volume (MCV) when compared with control and test groups. Rats which received low dose (200mg/kg) and high dose (400mg/kg) of the extract revealed insignificant changes in MCV values when compared with control rats. The result is shown in table (7).

### **3.1.6 Change in mean corpuscular haemoglobin (MCH):**

There were insignificant changes in MCH values in AlCl<sub>3</sub> group, low dose (200 mg/kg BW) and high dose (400mg/kg BW) of the extract when compared to control group. This result is presented in table (8).

### **3.1.7 Change in mean corpuscular haemoglobin concentration (MCHC):**

MCHC value was significantly increased (P<0.05) in AlCl<sub>3</sub> group when compared with control group. Rats that given low and high doses of *C. decidua* aqueous extract showed insignificant changes in MCHC value when compared with both control and AlCl<sub>3</sub> group. The result is given in table (9).

### **3.2 Change in body weight:**

There were no significant difference in the body weight between test groups (AlCl<sub>3</sub>, low and high doses of *C. decidua* aqueous extract and the control rats at day 0 and day 7. After 15 days of treatment, AlCl<sub>3</sub> group showed significant decrease in the body weight when compared with the control rats.

Low and high doses of the extract produced insignificant increase in body weights compared with control rats and insignificant decrease in body weights when compared to AlCl<sub>3</sub> group (P>0.05). The result is presented in table (10).

**Table (7):** Effect of administration of aqueous extract of *C. decidua* stems on MCV value in AlCl<sub>3</sub>-induced anaemia in rats.

<b>Groups</b>	<b>MCV fl (Mean±SD)</b>	
	<b>Day 0</b>	<b>Day 15</b>
<b>Control</b>	56.76±1.01	58.62±0.58 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	56.36±0.50	55.44±0.59 <sup>b</sup>
<b>Low dose</b>	55.94±1.14	57.68±1.52 <sup>a</sup>
<b>High dose</b>	56.66±0.56	57.70±1.29 <sup>a</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

**Table (8):** Effect of administration of aqueous extract of *C. decidua* stems on MCH value in AlCl<sub>3</sub>-induced anaemia in rats.

<b>Groups</b>	<b>MCH pg (Mean±SD)</b>	
	<b>Day 0</b>	<b>Day 15</b>
<b>Control</b>	17.6±0.53	19.48±0.44 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	17.64±0.28	19.36±0.38 <sup>a</sup>
<b>Low dose</b>	17.62±0.28	19.80±0.48 <sup>a</sup>
<b>High dose</b>	18.04±0.65	19.80±0.70 <sup>a</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

**Table (9):** Effect of administration of aqueous extract of *C. decidua* stems on MCHC value in AlCl<sub>3</sub>-induced anaemia in rats.

<b>Groups</b>	<b>MCHC g/dl(Mean±SD)</b>	
	<b>Day 0</b>	<b>Day 15</b>
<b>Control</b>	31.32±0.67	33.20±0.57 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	31.32±0.59	34.92±0.89 <sup>b</sup>
<b>Low dose</b>	31.48±0.31	33.78±0.99 <sup>ab</sup>
<b>High dose</b>	31.84±0.84	34.30±1.34 <sup>ab</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

**Table (10):** Effect of administration of aqueous extract of *C. decidua* stems on body weights in AlCl<sub>3</sub>-induced anaemia in rats.

Groups	Body Weight (g) (Mean±SD)		
	Day 0	Day7	Day 15
<b>Control</b>	83.5±14.53 <sup>a</sup>	117.0±28.94 <sup>a</sup>	131.8±29.10 <sup>a</sup>
<b>AlCl<sub>3</sub> group</b>	91.8±6.30 <sup>a</sup>	92.2±8.93 <sup>a</sup>	97.8±8.34 <sup>b</sup>
<b>Low dose</b>	95.6±22.37 <sup>a</sup>	109.0±26.97 <sup>a</sup>	124.8±27.73 <sup>ab</sup>
<b>High dose</b>	93.00±21.78 <sup>a</sup>	95.6±23.22 <sup>a</sup>	100.6±19.18 <sup>ab</sup>

Key: Values are expressed means within the same column with different superscripts are significantly different at p<.05(N=5).

## Chapter Four

### Discussion

In the present study the effect of the aqueous extract of *C. decidua* stems was investigated against  $\text{AlCl}_3$  induced anaemia in rats. The plant is used extensively by tradition to treat various conditions such as inflammation, liver diseases and cardiac problems (Mahmoud and Gairola, 2013).

In this study anaemia was induced by using  $\text{AlCl}_3$  at a dose of 100 mg/kg orally in rats. The results revealed significant reduction in different haematological parameters (RBCs count, Hb, PCV, MCV, MCH and MCHC) compared with control rats, this is in agreement with Al-Hashem (2009) and Al-Qayim *et al* . (2014).

The reduction in RBCs, PCV, Hb concentration indicate the possible haemolytic effect of aluminum chloride. Haemolytic action of this element is due to changes in the cell membrane of the red blood cells. The reduced level of hemoglobin can be associated with hemolysis or disturbances in heme biosynthesis as a result of inhibiting the link of iron with heme and a drop into activity of enzymes taking part in heme biosynthesis, mainly dehydratase of delta-aminolevulonic acid (ALA-D) (Al-Hashem, 2009).

Oral administration of the aqueous extract of *C. decidua* at a dose of 200 mg/kg (low dose) failed to protect the animals from anaemia induced by  $\text{AlCl}_3$  compared to the control rats. The high dose of the extract seem to be better than the low dose, due to occurrence of insignificant increase in some haematological parameters such as RBCs count, PCV and Hb concentration when compared with the control rats, although theses parameters were also found to be insignificantly changed when compared with negative control group.

In respects of weight of the rats, there was a significant reduction in body weight after induction of anaemia by  $AlCl_3$ . The decrease of body weight is one of the indications of anaemia, this is due to lack of appetite in anaemic rats. This observation is in agreement with the finding of Aurelie *et al.*, (2015).

Although *C. decidua* is rich in phytoconstituents such as alkaloid, glycoside, steroid, vitamin and phenols and also contains high level of ions such as Ca, Mn, Fe and Zn. (Mishra *et al.*, 2007 and Singh *et al.*, 2011). It has potent antioxidant activity (Verma, 2011 and Ul-Haq *et al.*, 2011). It is also contains several nutrients such as fatty acid, protein, carbohydrate and vitamins (Mishra *et al.*, 2007, Baloda and Bangarwa, 2010, and Joseph, 2011). However there was insignificant increase in body weight at rats were dosage with plant extract (Low and high dose) these results might be due to decrease in appetite caused by anaemia (Adebiyi *et al.*,2013).

## **Conclusion**

In conclusion, the injection of  $\text{AlCl}_3$  to rats produced a haemolytic anaemia characterized by reducing haematological parameters. This study indicated that the aqueous extract of *Capparis decidua* stems has no significant antianaemic activity by the dosages used.

Reports showed that *C. decidua* contain important elements, vitamins and phytoconstituents which if used in recommended doses could be useful in the treatment of anaemia.

## **Recommendations**

It is recommended that

- Prolong experimentation time of the study.
- Increase the dose of *C. decidua* stems aqueous extract.
- Study the antianaemic effect of other parts of *C. decidua*.



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## **Appendix**

**Appendix (1):**

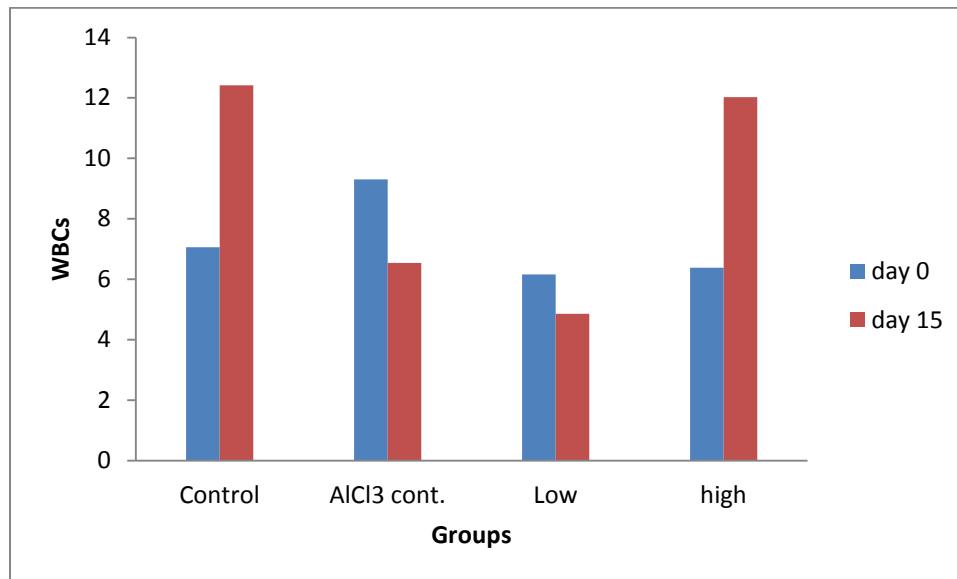


Figure (3): Effect of administration of aqueous extract of *C. decidua* stems on WBC count in  $\text{AlCl}_3$ -induced anaemia in rats.



**Appendix (2):**

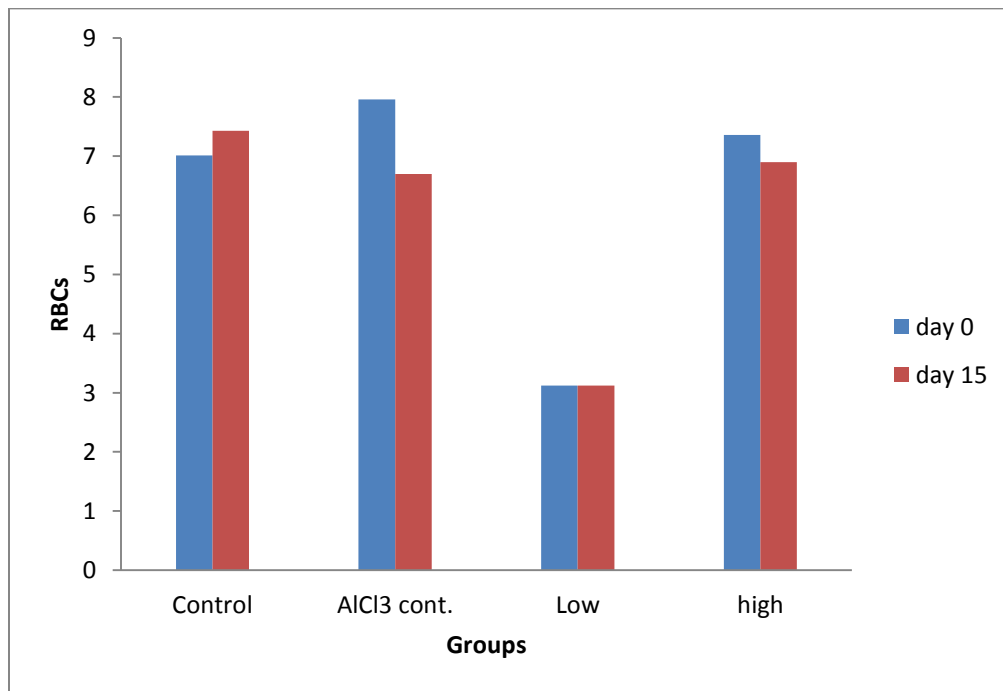


Figure (4): Effect of administration of aqueous extract of *C. decidua* stems on RBC count in AlCl<sub>3</sub>-induced anaemia in rats.

**Appendix (3):**

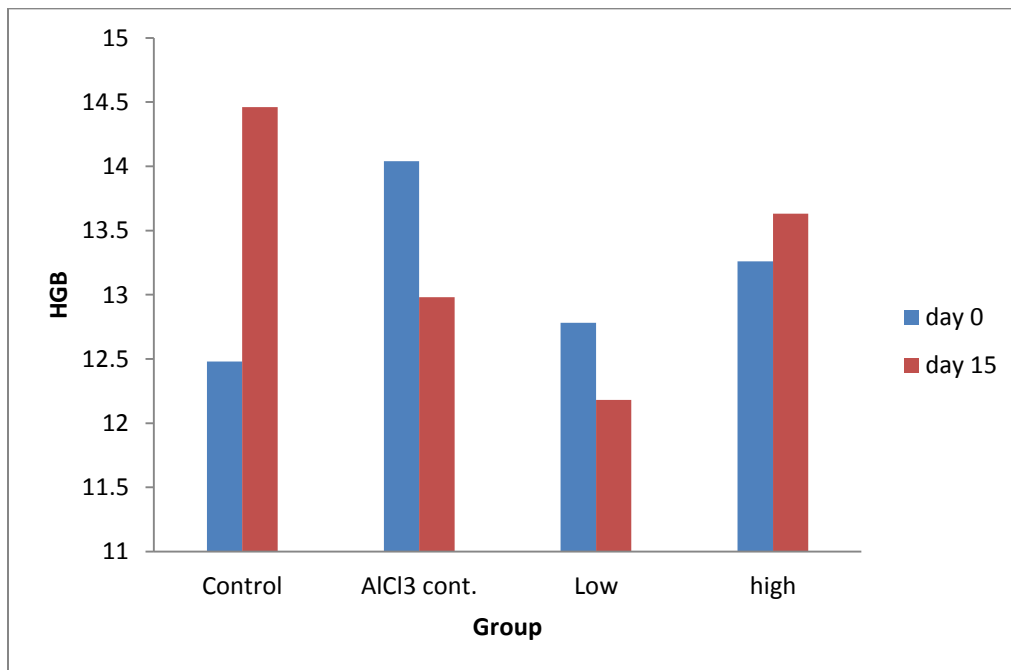


Figure (5): Effect of administration of aqueous extract of *C. decidua* stems on Hb concentration in AlCl<sub>3</sub>-induced anaemia in rats.

**Appendix (4):**

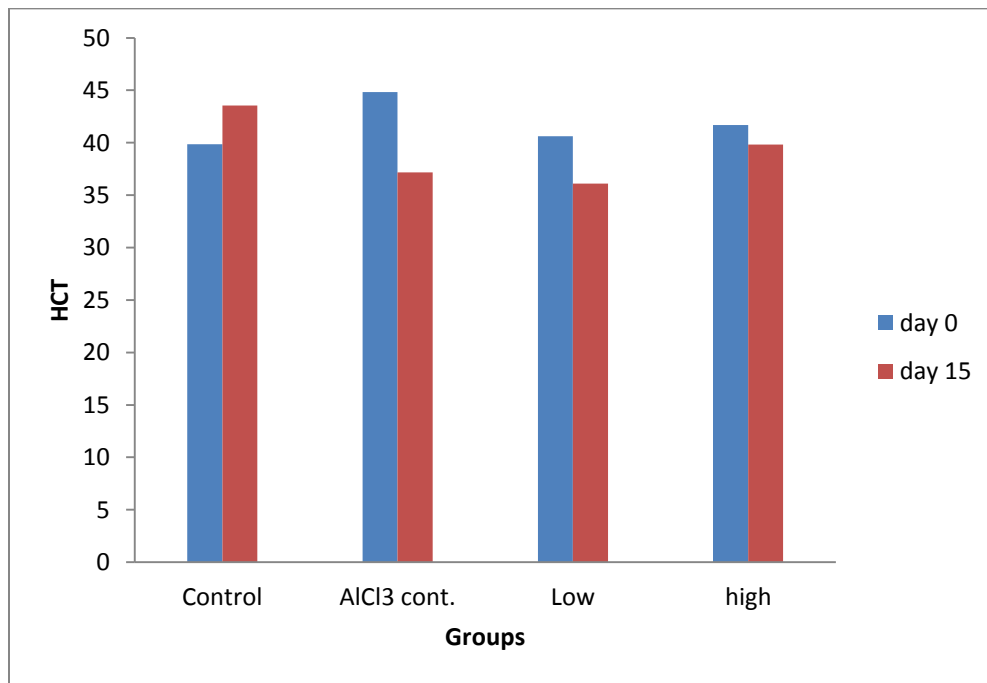


Figure (6): Effect of administration of aqueous extract of *C. decidua* stems on PCV percentage in AlCl<sub>3</sub>-induced anaemia in rats.

**Appendix (5):**

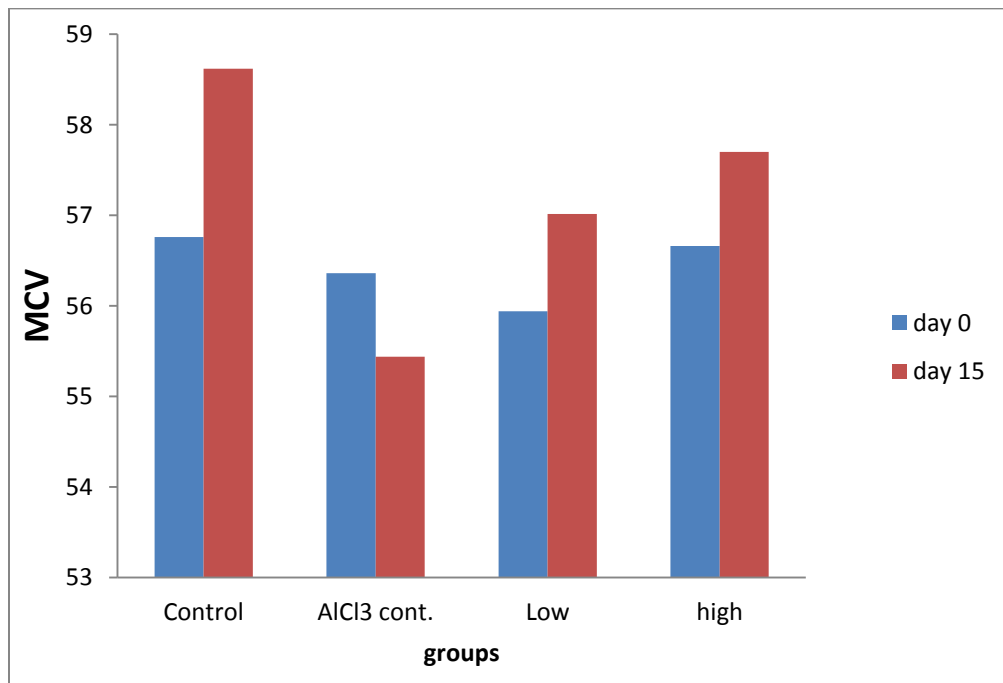


Figure (7): Effect of administration of aqueous extract of *C. decidua* stems on MCV value in AlCl<sub>3</sub>-induced anaemia in rats.

**Appendix (6):**

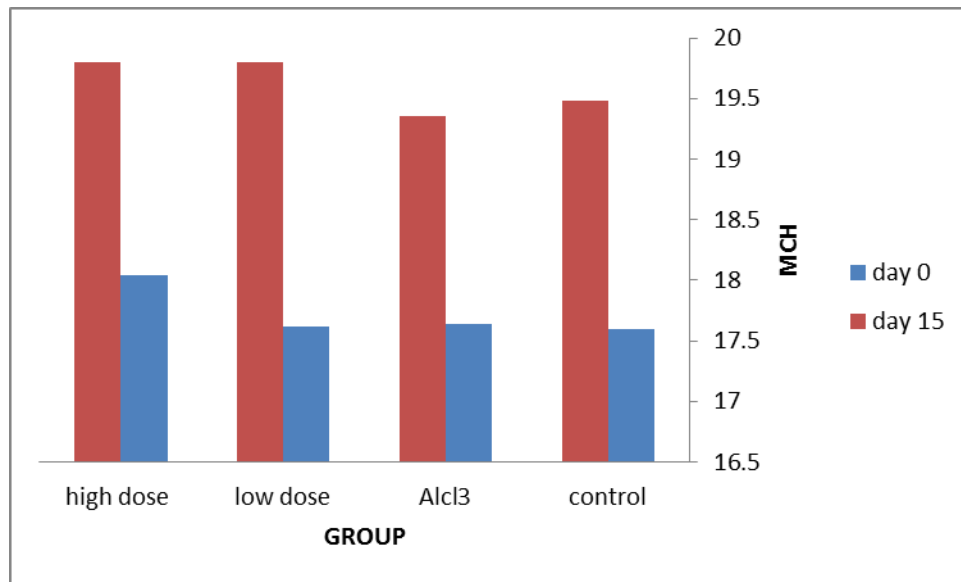


Figure (8): Effect of administration of aqueous extract of *C. decidua* stems on MCH value in AlCl<sub>3</sub>-induced anaemia in rats.

**Appendix (7):**

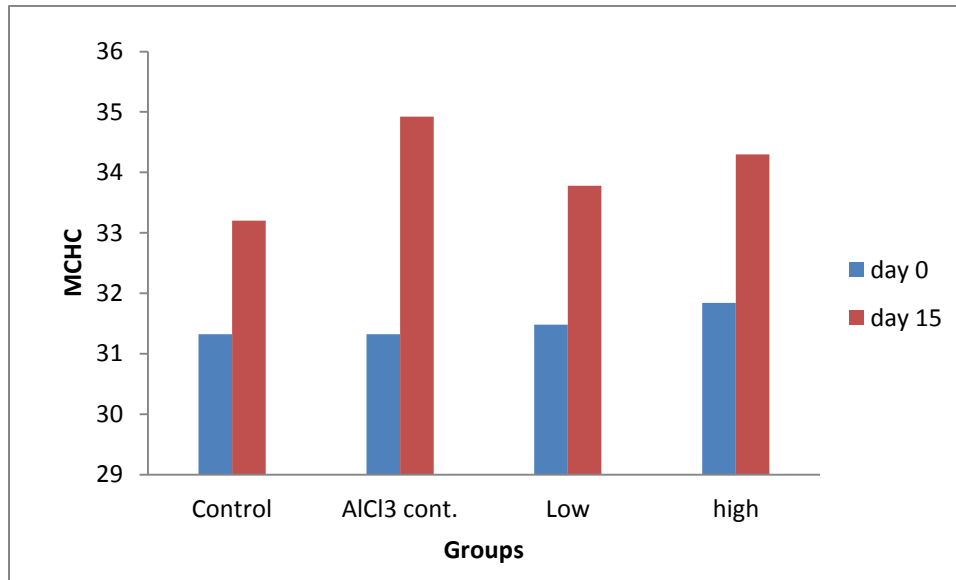


Figure (9): Effect of administration of aqueous extract of *C. decidua* stems on MCHC value in AlCl<sub>3</sub>-induced anaemia in rats.

**Appendix (8):**

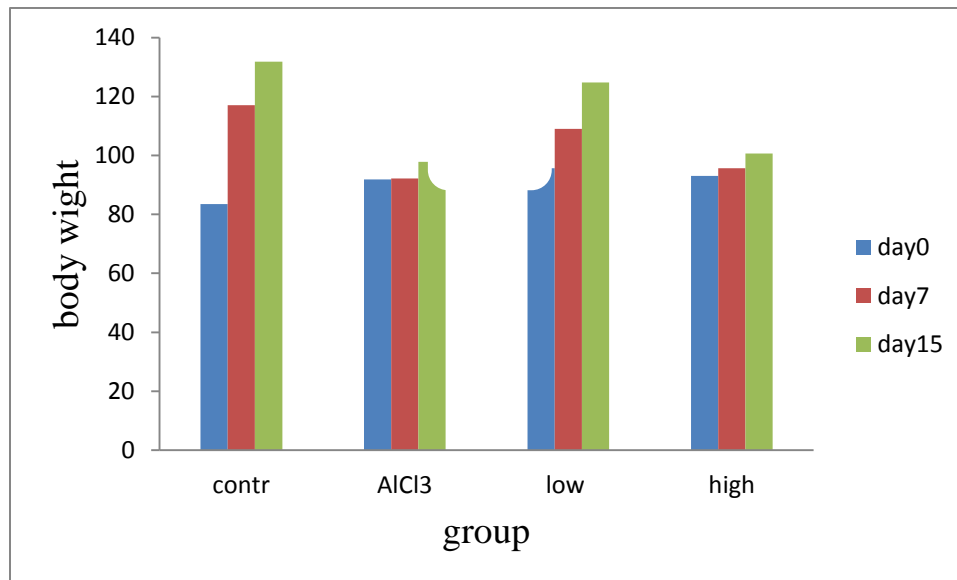


Figure (10): Effect of administration of aqueous extract of *C. decidua* stems on body weights in  $\text{AlCl}_3$ -induced anaemia in rats.

