

**STUDIES ON ANTIANAEMIC POTENTIAL OF METHANOL
EXTRACT OF RED CREOLE ONIONS (*Allium cepa*)
IN PHENYLHYDRAZINE-INDUCED HAEMOLYTIC
ANAEMIA IN RATS**

BY

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M.Sc/SCIEN/4576/2011-2012**

**A DISSERTATION SUBMITTED TO POSTGRADUATE SCHOOL
AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF MASTER DEGREE (M.Sc) IN BIOCHEMISTRY**

**DEPARTMENT OF BIOCHEMISTRY
FACULTY OF LIFE SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA-NIGERIA**

JANUARY, 2018

DECLARATION

I declare that the research work in the dissertation entitled “STUDIES ON ANTIANAEMIC POTENTIAL OF METHANOL EXTRACT OF RED CREOLE ONIONS (*Allium cepa*) IN PHENYLHYDRAZINE-INDUCED HAEMOLYTIC ANAEMIA IN RATS” has been performed by me in the Department of Biochemistry Ahmadu Bello University, Zaria, under the supervision of Prof. Sani Ibrahim and Prof M.N. Shuaibu. The information derived from the literature has been dully acknowledged in the text and the list of references provided. No part of this dissertation was previously presented for another degree or diploma at any university.

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Signature

Date

CERTIFICATION

This dissertation entitled “STUDIES ON ANTIANAEMIC POTENTIAL OF METHANOL EXTRACT OF RED CREOLE ONION (*Allium cepa*) IN PHENYLHYDRAZINE-INDUCED HAEMOLYTIC ANAEMIA IN RATS” by Amina Zakari meets the regulations governing the award of Master of Science (Biochemistry) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This dissertation is dedicated to my Parents, my Caring husband and my loving kids, for their abundant support, understanding, and love.

ACKNOWLEDGEMENTS

My most sincere gratitude goes to God Almighty for keeping me alive throughout my study period in this institution and also for the gift of knowledge, wisdom and understanding leading to the completion of this research work. In high regards, my heartfelt gratitude goes to my supervisors, Prof. Sani Ibrahim and Prof M.N Shuaibu for their enthusiasm, their valuable contributions, and for creating sufficient time to supervise my research work to completion.

I sincerely thank the Head, Biochemistry Department, and the academic staff of Biochemistry Department for their invaluable contributions in my study. My thanks also goes to the Non-academic and Technical Staff of the Department for their assistance.

Special thanks to Mal. Kabiru Musa of the Department of Pharmacology, Mal. Nasiru and his coworkers of Human Physiology Department, Mal. Ya'u and Aliyu of Animal House, Mal. Bashir of Chemistry Department, for your contributions and practical support at a demanding time made it possible for me to undertake and complete this project.

I express my deep appreciation to my father, Alh. Zakari Saleh in blessed memory, my mother, Haj.Bilkisu, my sister Dr. Fatima, my husband Mal. Abdullahi, my siblings, Aisha, Ramlah, Alameen, Hassana, Bilkisu and Aliyu. My thanks also goes to my Nephew and Nieces Najeeb, Hanifa, and Zainab. I also express my gratitude to all my brothers and sisters.

I acknowledge all the contributions and assistance of my Friends, especially, Hussaina, Mlm Zarah, Hauwa and a colleague Atabo Samuel. To you all, I remain grateful.

ABSTRACT

The antianemic potential of crude methanol extract of red creole onion cultivar was investigated in phenylhydrazine-induced anaemic albino rats. LD₅₀ of the crude methanol extract was found to be greater than 5000mg/kg/b.w. Thirty five (35) apparently healthy albino rats were grouped into seven groups of five (5) rats per group for the determination of the antianemic effect of the crude methanol extract, and groups two (II) to six (VI) were intraperitoneally administered with phenylhydrazine (10mg/kg/b.w/day) for 4 days. The study was carried out for a period of 21 days in which there was subsequent treatment of groups four (IV) to six (VI) with crude methanol extract of red creole onions at three different doses of 200, 400 and 600mg/kg/b.w/day respectively, while Bioferon (0.23ml/kg/b.w) was used as a standard drug for positive control group. Haematological parameters were determined on weekly basis. Non-significant ($p > 0.05$) increase in Hb, PCV and RBC concentrations were observed for all the treated groups at the end of the experiment, however, the RBC of the group that receives the highest dose (600mg/kg/b.w) decreased slightly after two weeks treatment with the extract. Non-significant ($p > 0.05$) decrease in reticulocytes counts were also observed for all the treated anemic groups. The non-significant increase in all the parameters were not comparable with the standard drug (Bioferon) which shows a significant ($p < 0.05$) increase in Hb, PCV and RBC as well as decrease in reticulocytes counts after three weeks treatment with the drug. The phytochemicals found in red creole onion extract include alkaloids, flavonoids, cardiac glycosides and phenolics. Terpenoids, Saponins and cyanogenic glycosides were absent. These results suggest that red creole onion does not possess antianaemic effect, especially as compared with the standard drug.

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CHAPTER ONE

INTRODUCTION

Anaemia is defined as a decrease in the number of red blood cells or less than the normal quantity of hemoglobin in the blood (Iwalewa *et al*, 2009). However, it can include decreased oxygen-binding ability of each hemoglobin molecule due to deformity or lack in numerical development as in some other types of hemoglobin deficiency. Because hemoglobin normally carries oxygen from the lungs to the capillaries, anemia leads to hypoxia in organs. Since all human cells depend on oxygen for survival, varying degrees of anemia has a wide range of clinical consequences. Anaemia is characterized by excessive destruction of erythrocytes at a rate that exceeds the bone marrow's capability to compensate for the blood loss (Holy *et al*, 2015).

Anaemia is one of the clinical conditions that constitute a serious health problem in many tropical countries as a result of the prevalence of different forms of parasitic infections, including malaria (Dacie and Lewis, 1994). In the tropics, due to prevalence of malaria and other parasitic infections, between 10 to 20 % of the population are reported to possess less than 10g/dl of haemoglobin in the blood (Diallo *et al*, 2008).

Hemolytic Anemia is an acquired type of Anemia caused by hemolysis (premature destruction of red blood cells). Autoimmune hemolytic anemia is the primary type, in which antibodies produced by the immune system damage RBCs. The causes of hemolytic anemia is sometimes unknown or associated with disorders such as systemic lupus erythematosus, lymphoma, and paroxysmal nocturnal hemoglobinuria. Other causes are high exposure to certain metals or chemicals (lead, copper, benzene, naphthalene), snake and insect bites, malaria, transfusions, post-surgical complications, and drugs such as methyldopa. In infants, blood group

incompatibility between mother and child or infections in the womb can cause hemolytic anemia. As for its treatment, corticosteroids can be used for autoimmune hemolytic anemia. Blood transfusions is also beneficial in many cases. Various immunosuppressive drugs may be tried, as well as splenectomy. Eculizumab (Soliris) is approved for treatment of paroxysmal nocturnal hemoglobinuria (Alleyne *et al*, 2008)

Phenylhydrazine is used for the induction of haemolytic anaemia, and its mechanism of induction had been studied in many species. Phenylhydrazine and its derivative 2, 4-dinitrophenylhydrazine are toxic agents. Their toxic action has been attributed to their ability to undergo auto oxidation. This increased oxidant potential enables them to oxidize enzymes, membrane proteins and hemoglobin. Phenylhydrazine initiates lipid peroxidation in membrane phospholipids (Jain and Hochstein, 1979), while 2,4-dinitrophenylhydrazine induces lipid peroxidation and other oxidative damage in rabbits (Ologundudu and Obi, 2005; Ologundudu *et al*, 2006a,b) and rats (Maduka *et al*, 2003).

Medicinal plants are useful worldwide in the treatment of diseases. Plants have provided the basis for traditional treatment for different types of disease and still offer an enormous potential source of new chemotherapeutic agents. In a recent report released by the world health organization, more than 80% of the world population still relies on herbal medicines as their primary source of health care. Millions of Africans of all ages rely on herbal medicine for primary health care (Abubakar *et al*, 2005).

Antianaemic effects of plants can be assessed clinically in humans, *in vivo* using animal models or *in vitro* using a variety of test systems. Preliminary testing of a herb in an animal model can give valuable information on the type of extract to be made, a suitable dose, the likely toxic

effects, and of course, the efficacy. This information can then be translated to human studies, although species differences and ethical considerations may be limiting factors. Animal and human studies are essential to determine the ultimate safety and efficacy of a herb or its components when used clinically (Amala, 2006).

Onion (*Allium cepa*), also known as the onion bulb or common onion, is used as a vegetable and is the most widely cultivated species of the genus *Allium*. This genus also contains several other species variously referred to as onions and cultivated for food. Onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season (Fritsch and Friesen, 2002). Onion is easily propagated, transported, and stored. The onion (*Allium cepa* L.) is one of the world's most important vegetable crops. It is grown in a wide range of climatic conditions, from relatively hot and dry areas to fairly cool and humid zones (Tindall, 1983, Messiaen, 1992). Because of its distinctive pungent flavour, the onion is used almost daily as an essential seasoning for a wide variety of dishes and it is, therefore, a major trading vegetable commodity in all urban and rural centres.

Members of the same onion cultivar produce bulb of the same size (either small or big sizes), same texture, same flavour (stronger or mild pungent), same keeping or storing quality, and same shape (either oblate, flat or globular) (Abdalla, 1987). Red, white and yellow are the most common bulb colours observed. Different cultivars can differ in terms of some phytochemicals and active components, for example, all onion cultivars contain quercetin, which is effective in reducing the risk of cardiovascular disease, an anticancer, and an antioxidant, however, there is difference in quercetin levels between cultivars with red and yellow onions having higher concentrations and white having a low concentration (Currah and Proctor 1990).

In Nigeria, the onion producing states are Kano, Kaduna, Jigawa, Sokoto, Plateau, Bauchi and Kebbi states, with Alieru town in Kebbi considered as onions home in Nigeria (Irabor, 2014). The onion cultivars in the northern part of the country include Kaharda, Red creole, koumassa, maikanmakwarwa, Sokoto local (Abubakar and Ado, 2013), Kano red, Kano white, Gindin tasa, Wuyan makorowa, 'Yar yawuri, Fara, Hauke, and Doguwa (Garba, *et al*, 2011). In North-eastern part of the country however, only five species are popular, and they include: Red globe (crayon), Sweet Spanish, Yellow globe, Spring or Scallion and White globe (Ibrahim, 2010).

Red creole onion is a slightly pungent, spicy, purple red onion which is excellent for storing. It originates from United States and it produces mainly single onion with hard, flat and round bulb which is good for cooking or in salads. It is a medium size onion and it does well in hot-dry climate as well as different extremes of cold temperature (Babasaheb, 2004).

Consumption of onions is believed to benefit health in that onions contain phenolics and flavonoids, although most often not used specifically as a medicinal herb, the onion has a wide range of beneficial actions on the body and when eaten (especially raw) on a regular basis will promote the general health of the body. The bulb is antihelmintic, antibiotic, anti-inflammatory, anti-microbial, antirheumatic, antiseptic, antispasmodic, depurative, diuretic, expectorant, fungicidal, hypocholesterolemic, hypoglycemic, hypotensive, stomachic, stimulant, antiviral, antibacterial, tonic, among others, and it may be used both internally and externally for medicinal purposes (Gupta, 1994).

1.1 STATEMENT OF RESEARCH PROBLEM

The incidence of anaemia is higher in the third world than in developed countries due to the presence of many aggravating factors such as poor nutrition, and high prevalence of blood parasites example, Plasmodium, trypanosomes and helminthes infestation (Ogbe *et al*, 2010). Anaemia prevalence remains high in Africa, with an overall incidence of 57% among pregnant women, 48% among non-pregnant women and 68% among preschool children (WHO, 2011). This indicates that women are more susceptible to anaemia during pregnancy due to high demand from the developing foetus (Orna, 1991).

Although there are various drugs used for the treatment of anaemia, they are not affordable to many poor people especially those in the developing countries such as Nigeria. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anaemia, so they depend heavily on plants and herbal products for the treatment of many diseases including anaemia. Due to the fact that anaemia is very common and the incidence is likely to increase in future (Duff, 2008), there is need to prevent it or seek for more cost-effective and better treatment strategies.

1.2 JUSTIFICATION

Anaemia is the most common blood disorder in developing countries, and according to a study more than 30% of the world population experience some degree of anaemia in their life time. The people at greater risk are the elderly, young women of child-bearing age, and the infants (WHO, 2008).

In Nigeria, prevalence of anaemia among women of reproductive age (15- 49) was 48.50 %, while that of children under five was 71.00% as of 2011 (WHO, 2011). Recently, in her

media conference in Lagos, the Nutritional Society of Nigeria (NSN) revealed that 72% of Nigerian children are anaemic (NSN, 2015).

Onions are known to possess many curative value rights from the ancient time. Recently, some natural nutritionist claimed that onions and juice of onion stalk are good herbal medicine for the treatment of anaemia. Example, according to Rudy S. (2010), onion stimulates blood formation and improves haemoglobin level. Also, Oluwaseun (2015), claimed that eating onions aids in the treatment of anaemia.

A survey of literature revealed that no scientific approach has been made to back this up. Hence the challenge of this investigation is to assess the antianaemic potential of red creole onion cultivar so as to validate the ethnotherapeutic claim. Red creole onion cultivar was chosen because it is a popular standard variety in high demand due to its good keeping quality (Kuepper, 2014) .

1.3 AIM AND OBJECTIVES

The general aim of this research work is to evaluate the anti anaemic effect of methanol extract of red creole onion (*Allium cepa*) in phenyl- hydrazine induced anaemic rats. The specific objectives are to:

1. identify and quantify some of the phytochemical constituents of the crude extract.
2. assess the antianaemic potential of the crude methanol extract of red creole onions.
3. establish the fraction of the crude methanol extract that has the highest antianaemic potential.
4. characterize the most active fraction of the methanol crude extract.

CHAPTER TWO

LITERATURE REVIEW

2.1 ONIONS (*Allium cepa*)

Also known as the bulb onion or common onion, is used as a vegetable and is the most widely cultivated species of the genus *Allium*. It belongs to the Family *Alliaceae*, order *Amaryllidaceae*, and genus *Allium*. This genus also contains several other species variously referred to as onions and cultivated for food. Onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season (Fritsch and Friesen 2002). Because of their bulbs, tubers and rhizomes, these plants are able to survive under harsh conditions, e.g. winter or dryness. The plant has a fan of hollow, bluish-green leaves which arises from a shortened crown stem (stemplate) and the bulb at the base of the plant begins to swell when a certain day-length is reached. In the autumn (or in spring, in the case of over wintering onions), the foliage dies down and the outer layers of the bulb become dry and brittle. The crop is harvested and dried and the onions are ready for use or storage. Some varieties of *A. cepa*, such as shallots and potato onions, produce multiple bulbs (Babasaheb, 2004).

2.1.1. Onion Cultivars

A cultivar is a plant or group of plants selected for desirable characteristics that can be maintained by propagation. It has characteristics that easily distinguish it from any other known cultivar, and to be uniform and stable, the cultivar must retain these characteristics under repeated propagation. Example is the popular ornamental garden plants like roses, camellias e.t.c which are cultivars produced by careful breeding and selection for flower colour and form. Similarly, the world's agricultural food crops are almost exclusively cultivars that have been selected for characteristics such as improved yield, flavour, and resistance to disease. Trees used

in forestry are also special selections grown for their enhanced quality and yield of timber (Brickell, 1992).

International Union for the Protection of New Varieties of Plants, requires that a cultivar be distinct, uniform and stable. To be distinct, it must have characteristics that easily distinguish it from any other known cultivar. To be uniform and stable, the cultivar must retain these characteristics under repeated propagation. Its production generally entails considerable human involvement although in a few cases it may be as little as simply selecting variation from plants growing in the wild (whether by collecting growing tissue to propagate from or by gathering seed (Brickell, 1992). Cultivars generally occur as ornamentals and food crops. *Malus* 'Granny smith' and *Malus* 'Red delicious' for instance, are cultivars of apples propagated by cuttings or grafting, *Lactuca* 'Red sails' and *Lactuca* 'Great lakes' are lettuce cultivars propagated by seeds. Named cultivars of *Hosta* and *Hemerocallis* plants are cultivars produced by micropropagation or division.

Most onion cultivars are about 89% water, 4% sugar, 1% protein, 2% fibre, and 0.1% fat. .Onion cultivars continue to change, every year a newly developed hybrid cultivars are added to the old ones. The hybrid cultivars are highly yielding and more uniform in bulb size than the old cultivars. Red, white and yellow are the most common bulb colours observed. Members of the same onion cultivar produces bulb of the same size (either small or big sizes), same texture, same flavour (stronger or mild pungent), same keeping or storing quality, and same shape (either oblate, flat or globular) (Abdalla, 1987).

The Indian Agricultural Research Institute (IARI), New Delhi, classified onion cultivars into two groups namely, large- sized (5-10cm in diameter) bulbs and small sized (1.2- 2.5 cm) bulbs. The large- sized deshi (local) cultivars are Red globe, white patna, Yadgiri red e.t.c, while the small-sized cultivars include Yellow globe, Queen of Paris, Market gardener e.t.c (Babasaheb, 2004). However, in another classification onion (*allium cepa*) is nowadays considered as

divisible into two large cultivar groups namely common onion group with large, normally solitary bulbs, reproduced from seeds or seed- grown bulblets (sets), and aggregatum group, with smaller several to many bulbs forming an aggregated cluster ,originating from a single mother bulb (Currah and Proctor,1990).

In Nigeria, the onion producing states are Kano, Kaduna, Jigawa, Sokoto, Plateau, Bauchi and Kebbi states ,with Alieru town in Kebbi considered as onions home in Nigeria (Irabor, 2014). The onion cultivars in the northern part of the country include Kaharda, Red creole, koumassa, maikanmakwarwa, Sokoto local (Abubakar and Ado, 2013), Kano red, Kano white, Gindintasa, Wuyan makorowa, 'Yar yawuri, Fara, Hauke, and Doguwa (Garba *et al*, 2011). In North-eastern part of the country however, only five species are popular, and they include: Red globe (crayon), Sweet Spanish, Yellow globe, Spring or Scallion and White globe (Ibrahim, 2010).

Red creole onion is slightly pungent, spicy, purple red onion which is excellent for storing. It originates from united state and it produces mainly single onion with hard, flat and round bulb which is good for cooking or in salads. It is a short day mid-sized onion especially suited for southern climates. It do well in hot-dry climate as well as different extremes of cold temperature (Babasaheb, 2004).

2.1.2 Functional Uses of Onions

Onions are cultivated and used around the world. As a food item, they are usually served cooked, as a vegetable or part of a prepared savoury dish, but can also be eaten raw or used to make pickles or chutneys. They are pungent when chopped and contain certain chemical substances which irritate the eyes. It is an important vegetable crop whose distinctive flavour is appreciated by people throughout the world. One of the advantages of onion is that the bulbs can be harvested and sold either green' in salads, while the mature bulbs are cooked or eaten raw as a vegetable(Lannoy, 2001). Onions are Onions are also used as a thickening agent for curries

providing a bulk of the base. Fresh onion has a pungent, persistent, even irritating taste, but when sautéed, onion becomes sweet and much less pungent. They are cooked in a variety of ways such as fried boiled and roasted (Abayomi and Terry, 2009). The juice of Onions is good for either scalding or burning by fire, water, or gunpowder and used with vinegar, takes away all blemishes, spots and marks in the skin: and dropped in the ears, eases the pains and noise of them. They are very versatile and can be baked, boiled, braised, grilled, fried, roasted, sautéed, or eaten raw in salad. Their layered nature makes them easy to hollow out once cooked. Onion is a major spice item in the Nigerian diet, and ranked among the most important often chopped and used as an ingredient in various hearty warm dishes, and may also used as a main ingredient in their own right, for example in French onion soup or onion chutney. They are also used raw in cold salads. vegetable in Nigeria based on the level of consumption and other uses (Babatola and Lawal, 2000).Mature onion bulbs are eaten raw as salad. They are cooked in a variety of ways such as fried boiled and roasted (Brise *et al*, 1997).

2.1.3 Medicinal Uses of Onions

Onions are not highly nutritious but have health-giving properties partly associated with its high sulphur content (Abayomi and Terry, 2009). Onion is used as a diuretic, expectorant and antiseptic. Onions are highly recommended for people trying to prevent cardiovascular disease, cancer, and infections. They appear to be at least somewhat effective against colds, heart disease, diabetes, and other diseases and contain antiinflammatory, anticholesterol, and anticancer components. Onions contain many active compounds that appear to inhibit the growth of cancerous cells, help combat heart disease, inhibit strokes, lower blood pressure & cholesterol, and stimulate the immune system. Alliums are also antibacterial and anti-fungal, so they can relieve stomach upset & other gastrointestinal disorders (Gupta, 1994). As with Garlic, Onions

help prevent thrombosis and reduce hypertension, according to the American Heart Association. Although most often not used specifically as a medicinal herb, the onion has a wide range of beneficial actions on the body and when eaten (especially raw) on a regular basis will promote the general health of the body.(Benkeblia, 2004) The bulb is anthelmintic, antibiotic, anti-inflammatory, anti-microbial, antirheumatic, antisclerotic, antiseptic, antispasmodic, carminative, depurative, digestive, diuretic, expectorant, febrifuge, fungicidal, hypocholesterolemic, hypoglycemic, hypotensive, lithontripic, stomachic, stimulant, rubefacient, antiviral, antibacterial, tonic, and vermifuge among others (Xiao and Parkin 2007).

For medicinal purposes, onions may be used both internally and externally. Onion (*Allium cepa*) and other alliaceous vegetables are similar in many respects to garlic. Internal use of onion increases circulation and stimulates and warms the body. Onion is effective against many bacteria including *Bacillus subtilis*, *Salmonella*, and *E. coli*. Externally, onion is used in the form of poultices for tumors and earaches. The tear-evoking lachrymatory chemical released when onion is crushed or cut has a stimulating effect on the mucosa and secretory glands of the eyes and nose has resulted in onion being selected for use as a treatment for respiratory problems (Slimestad and Vagen, 2009).

2.2 ANAEMIA

Anaemia means deficiency of hemoglobin in the blood, which can be caused by either too few red blood cells or too little hemoglobin in the cells (Guyton and Hall, 2006) . Some types of anaemia and their physiologic causes are blood loss anaemia, aplastic anaemia, megaloblastic anaemia, and haemolytic anaemia.

2.2.1 Blood Loss Anaemia

After rapid hemorrhage, the body replaces the fluid portion of the plasma in 1 to 3 days, but this leaves a low concentration of red blood cells. If a second hemorrhage does not occur, the red blood cell concentration usually returns to normal within 3 to 6 weeks. In chronic blood loss, a person frequently cannot absorb enough iron from the intestines to form hemoglobin as rapidly as it is lost. Red cells are then produced that are much smaller than normal and have too little hemoglobin inside them, giving rise to microcytic, hypochromic anaemia (Guyton and Hall, 2006).

2.2.2 Aplastic Anaemia

Bone marrow aplasia means lack of functioning bone marrow. For instance, a person exposed to gamma ray radiation from a nuclear bomb blast can sustain complete destruction of bone marrow, followed in a few weeks by lethal anaemia. Likewise, excessive x-ray treatment, certain industrial chemicals, and even drugs to which the person might be sensitive can cause the same effect (Guyton and Hall, 2006).

2.2.3 Megaloblastic Anaemia

The deficiency of vitamin B₁₂, folic acid, and intrinsic factor from the stomach mucosa can lead to slow reproduction of erythroblasts in the bone marrow. As a result, the red cells grow too large with odd shapes called megaloblasts. Thus, atrophy of the stomach mucosa, as occurs in pernicious anaemia or loss of the entire stomach after surgical total gastrectomy can lead to megaloblastic anemia. Also, patients who have intestinal sprue, in which folic acid, vitamin B₁₂, and other vitamin B compounds are poorly absorbed, often develop megaloblastic anemia. Because in these states the erythroblasts cannot proliferate rapidly enough to form normal numbers of red blood cells, those red cells formed are mostly over-sized and have bizarre shapes,

and fragile membranes. These cells rupture easily, leaving the person in dire need of an adequate number of red cells (Guyton and Hall, 2006).

2.2.4 Haemolytic Anaemia

Hemolysis is the destruction or removal of red blood cells from the circulation before their normal life span of 120 days. Hemolysis can be a lifelong asymptomatic condition, it most often presents as anaemia when erythrocytosis cannot match the pace of red cell destruction (Gurpreet *et al*, 2004).

Some different abnormalities of the red blood cells (many of which are hereditarily acquired), make the cells fragile so that the cells rupture easily as they pass through the capillaries, especially through the spleen. Even though the number of red blood cells formed may be normal, or even much greater than normal in some hemolytic diseases, but the life span of the fragile red cell is very short that the cells are destroyed faster than the one formed. This result to serious anaemia, some of these types of anaemia include hereditary spherocytosis, sickle cell anaemia and erythroblastosis fetalis.

In hereditary spherocytosis, the red cells are very small and spherical rather than being biconcave discs. These cells cannot withstand compression forces because they do not have the normal loose, bag like cell membrane structure of the biconcave discs. On passing through the splenic pulp and some other tight vascular beds, they are easily ruptured by even slight compression (Guyton and Hall, 2006).

In sickle cell anaemia, which is present in 0.3 to 1.0% of West African and American black population. The cells have an abnormal type of hemoglobin called hemoglobin S, containing faulty beta chains in the hemoglobin molecule, When these hemoglobin are exposed to low concentrations of oxygen, they precipitates into long crystals inside the red blood cell. These

crystals elongate the cell and give it the appearance of a sickle rather than a biconcave disc. The precipitated hemoglobin also damages the cell membrane, so that the cells become highly fragile, leading to serious anaemia. Such patients frequently experience a vicious circle of events called a sickle cell disease “crisis,” in which low oxygen tension in the tissues causes sickling. This leads to ruptured red cells, causing a further decrease in oxygen tension and still more sickling and red cell destruction. Once the process starts, it progresses rapidly, eventuating in a serious decrease in red blood cells within a few hours and, often to death (Guyton and Hall, 2006).

In erythroblastosis fetalis, Rh-positive red blood cells in the foetus are attacked by antibodies from an Rh-negative mother. These antibodies make the Rh-positive cells fragile, leading to rapid rupture and causing the child to be born with serious anaemia in relation to the Rh factor of blood. The extremely rapid formation of new red cells to make up for the destroyed cells in erythroblastosis fetalis causes a large number of early *blast* forms of red cells to be released from the bone marrow into the blood (Guyton and Hall, 2006).

2.2.5 Classification of Haemolytic Anaemia.

The haemolytic anaemia can be grouped into three categories. (Gupreet *et al*, 2004). Accelerated red blood cells destruction can be caused by the following

- (1) A molecular defect (hemoglobinopathy or enzymopathy) inside the red cell.
- (2) An abnormality in membrane structure and function.
- (3) An environmental factor such as mechanical trauma or an autoantibody.

In intracorporeal types of hemolysis, the patient’s RBC has an abnormally short life span in a normal recipient (with a compatible blood type), while compatible normal RBC lives normally in the patient. The opposite is true in extracorporeal types of hemolysis. Finally, hemolytic disorders can be either inherited or acquired. .(Gupreet *et al*, 2004).

2.2.6 Inherited Hemolytic Anaemia

The inherited hemolytic anemias are due to inborn defects in one of three main components of red cells: the membrane, enzymes, or hemoglobin (Dennis *et al*, 2005). These defects are often known at the genomic level, but their identification still largely depends on their clinical and laboratory manifestations.

2.3 REVIEW OF SOME PLANTS THAT POSSESSED ANTIANAEMIC EFFECTS.

2.3.1 *Jatropha tanjorensis*.

Jatropha tanjorensis (Euphorbiaceae) is a common weed of field crops, bush re-growth, road sides and disturbed places in the higher rainfall forest zones of West Africa. It is commonly called ‘hospital too far’, catholic vegetable, ‘Iyana-Ipaja’ or ‘lapalapa. The leaf is a commonly consumed vegetable in many parts of Southern Nigeria. It is also popular as a natural remedy against diabetes in this region. Phytochemical screening of *J. tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins (Ehimwenma and Osagie, 2007).

The anti- anaemic, acute toxicity and proximate analysis of *J. tanjorensis* was investigated in rabbits. The results revealed that proximate analysis of the sample showed that all the macronutrients were present with protein being the most abundant (41.65%). There was also significant decrease in Packed Cell Volume in groups B, C and D by day 3 of the experiment and subsequent increase by day 14 of the experiment after treatment with aqueous suspension of *J. tanjorensis* leaves. The results suggests that crude extract of *J. tanjorensis* improved the anaemic condition of the treated animals (Groups B, C and D) when compared with the phenyl hydrazine induced but untreated animals (Group E) (Macdonald, *et al*, 2014).

Haemolytic anaemia was diagnosed by a more than 50% reduction in PCV values the rabbits in groups B, C, D and E from the baseline values by three days after phenylhydrazine

administration. Phenylhydrazine has been reported to induce haemolytic anaemia in rabbits at a dose of 30 mg kg⁻¹ body weight two days after administration. The rabbits in group A which served as control and were not induced with anaemia or treated, had PCV, RBC, WBC, Hb, MCV and MCHC concentrations within the normal range throughout the duration of the experiment. The rabbits in group B, C and D which were induced with anaemia had significant ($P<0.05$) decrease in the values for PCV, Hb, RBC, MCV and MCHC concentration by day three of the experiment indicating anaemia, and also a significant increase ($P<0.05$) was also observed for WBC concentration. This group of rabbits was treated by oral administration of 10ml of the aqueous suspension of *J. tanjorensis* leaves powder (10 g/100 ml for group B, 7.5 g/100 ml for group C and 5 g/100 ml for group D) twice daily. Treatment with the plant sample significantly ($P<0.05$) improved the PCV, Hb, RBC, MCV and MCHC levels (Table 3) of the animals in these groups and also significantly ($P<0.05$) reduced the WBC level by day 14 of the experiment bringing it back to acceptable normal value. Rabbits in Group E (test control) were induced with anaemia but were not administered the aqueous suspension of *J. tanjorensis* leaves. They also showed a significant decrease ($P<0.05$) in the values for PCV, Hb, RBC, MCV and MCHC counts and increased WBC count value by day 3 of the experiment. However, there seems to be slight recovery from anaemia by day 14, probably due to decreasing effect of the drug. It has been reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species. However, alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species. Previous phytochemical screening of *J. tanjorensis* leaf revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins. Thus, it appears that the presence of these antioxidants in the plant sample reverse the damaging effect of phenyl hydrazine. (Ehimwenma and Osagie, 2007).

In this study, it can be concluded that the crude extract of *J. tanjorensis* improved the Anaemic condition of the treated animals (Groups B, C and D) when compared with the phenyl hydrazine induced but untreated animals (Group E).

2.3.2 *Telfairia occidentalis* and *Spondias mombin*

Spondias mombin belongs to the family Apacardiaceae, and it is a tropical tree up to 20 m tall, bearing blunt spines or knobs, growing easily from stakes for making fence and enclosure. The common name of *S. mombin* is Yellow mombin, other names include: “caja” in Brazil, Jobo-blanco (Colombia), Baja (Costa Rica), Jobito (panama), Ubo (Peru), while in Northern, Western, and Eastern Nigeria, *S. mombin* is called Isada, Iyeye and Ijinkaraka or Uvuru respectively. *S. mombin* leaves have been reported to be an excellent anthelmintic, anxiolytic, sedative, antiepileptic, antiviral, antibacterial, as well as a common midwife’s remedy during and after birth. In Peru, the leaves and bark powder are applied on wounds and inflammations. *S. mombin* has been shown to contain various constituents, saponins, terpenoids, Fe, Mg, K, among others which are implicated for its numerous pharmacological activities (Adeyemi and Gbolude, 2006). *Telfairia occidentalis* on the other hand, is a vegetable crop and belongs to the family Cucurbitaceae. The common name of *T. occindentalis* is fluted pumpkin, and “ugu” in South eastern Nigeria where it is extensively cultivated. The leaves of *T. occidentalis* are rich sources of Fe, Mg, K, Carotene and Vitamin C for pregnant women and patients suffering from anaemia. (Adjanohoun *et al*,1991). The anti-anaemic activity of aqueous leaves extracts of *Telfairia occidentalis* and *Spondias mombin* were investigated, where the chloramphenicol model for experimental induction of anaemia in rats was employed. Anti-anaemic activity was determined by measuring haematological parameters of packed cell volume (PCV), haemoglobin count (Hb) and white blood cell count (WBC). The PCV and Hb levels of extracts treated rats were significantly ($p < 0.05$) increased compared to control group. The aqueous extract of *T.*

occidentalis produces higher anti-anaemic activity compared to *S. mombin*. The decrease in WBC level was recorded with both extracts. The results obtained in this study showed that aqueous leaves extracts of *T. occidentalis* and *S. mombin* exhibited dose-dependent increase in PVC and Hb. Previous studies have shown that such increase in PCV and haemoglobin count of rats after treatment with extracts were obvious indices for recovery from anaemia. Post administration of extracts of both plants resulted in fall in the WBC values which might be expected recognizing that chloramphenicol which was used to induce anaemia is noted as a high risk drug which obviously affects the immune system of the experimental rodents leading to a fall in WBC. Prolonged administration of the extract beyond 7 days might restore the WBC. This is in agreement with the report that full recovery of experimental animals from biochemical effects of plant extracts could be achieved by chronic administration of the extracts. The anti-anaemic properties of both plants may be connected with reported phytochemical constituents, but the leaves of *T. occidentalis* are richer sources of Fe, Mg, K, carotene and Vitamin C, hence this accounts for its slightly higher anti-anaemic activity than *S. mombin*. It can therefore be concluded that the leaves extracts of *T. occidentalis* and *S. mombin* exhibited anti-anaemic effect in chloramphenicol-induced anaemic rats. This gives credence to the folkloric use of both plants as hematinic (Sylvester, 2016).

2.3.3. *Mukia maderaspatana* (MM) and *Kedrostis foetidissima* (KF)

Mukia maderaspatana (MM) and *Kedrostis foetidissima* (KF) are two plants of Cucurbitaceae family which are commonly used as recipes. *K. Foetidissima* is a climber/prostrate herb very effective in the treatment of asthma, chest pain, urinary tract infection, diarrhea, HIV, small pox and skin diseases (Saravanan and Manokaran, 2012)

M. maderaspatana is a tendril climber/prostrate herb. The plant was reported to have activities such as hepatoprotective, anti-rheumatic, diuretic, stomachic (a digestive tonic), gentle aperients,

antipyretic, anti-flatulent, anti-asthmatic, anti-inflammatory, anti-diabetic and anti-bronchitis. It is also used for tooth ache. Phytochemical investigation of both plants confirmed the presence of polyphenols, flavonoids, alkaloids, tannins and saponins (Saravanan and Manokaran, 2012)

In the experiment, anaemia was induced by administration of phenylhydrazine in the dose of 10 mg/kg for 8 days. The rats with haemoglobin content less than 14 mg/dl were selected for the study. Then animals were divided into 7 groups of 6 animals each. Both extracts were given in the doses of 150mg/kg and 300 mg/kg orally for 4 days. Blood samples were collected from the animals by sinus puncture on 1, 2, 3 and 4th week of the treatment. Blood samples were subjected to the estimation of red blood cells (RBC), white blood cells (WBC) and haemoglobin (Hb). Both the doses of the two plant extracts significantly ($P < 0.01$) enhanced the RBC content from the initial week of treatment to 4th week of treatment when compared to that of standard drug (Vitamin B12). There was no significant change in the WBC content after the treatment of MM extract. But there was less significant ($P < 0.05$) decrease in WBC in Vitamin B12 and KF extract treated groups (Saravanan and Manokaran, 2012)

It has been reported that phenylhydrazine causes oxidative damage to RBC by increasing the formation of reactive oxygen species. Anaemia was observed after 6 days of injection. This seems to be recovery from anaemia in day 9 by the control group given only 0.5% of CMC, probably due to decreasing effect of the drug before a booster dose was given.

In this present study the RBC and Hb contents were increased due to recovery from free radical damage by the anti-oxidant character of MM and KF extracts. WBC content was increased from 1st to 3rd week and then it was decreased because of phenylhydrazine induced anemia is associated with immune activation and recovery. Alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to the cells by free radicals or highly

reactive oxygen species. The differences in anti-anaemic potentials of the plant extracts might be due to the different phytochemicals present especially, the polyphenols (flavonoids) (Saravanan and Manokaran, 2012)

2.4 PHENYLHYDRAZINE

Phenylhydrazine (PHZ) was the first hydrazine derivative characterized by Hermann Emil Fischer in 1875. This compound is used worldwide mainly as a chemical intermediate in the pharmaceutical, agrochemical, and chemical industries. Phenylhydrazine, $C_6H_8N_2$ (see structural diagram below) has a molecular weight 108; it exists as yellow to pale brown crystals or as a yellowish oily liquid, with a freezing point of $19.6^{\circ}C$ and a boiling point of $243.4^{\circ}C$. Phenylhydrazine metabolism seems to occur via ring hydroxylation and conjugation, excretion is primarily via the urine.

Earlier in the 19th century, phenylhydrazine and phenylhydrazine hydrochloride were administered orally (usually around 100-200 mg/day) for the treatment of blood disorders (Giffin and Allen 1933). In some cases, the treatment was effective, while in others, the outcome was fatal example (Giffin and Allen 1928, Giffin and Conner 1929). Phenylhydrazine decreases haemoglobin level, RBC, and PCV, and impairs erythrocyte deformability. It induces reticulocytosis, increased osmotic resistance, free plasma haemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), erythropoietin levels, and extra-medular haematopoiesis in the spleen and liver (Berger, 1985(a), Stern, 1989). This compound can induce vascular dysfunction and haemodynamic disturbance, as well as, decrease in mean arterial pressure and hind-limb vascular resistance. Uncompensated respiratory alkalosis, increased arterial CO_2 tensions and acidosis were found following phenylhydrazine administration (Luangaram *et al*, 2007). Phenylhydrazine-induced anaemia can be used as a

model for the evaluation of its influence on therapeutic effectiveness, for example, antitumour therapy (Golab *et al*, 2002) as a model of reticulocyte research (Xie *et al*, 2002) or erythrocyte senescence under abnormal physiological conditions (Xie *et al*, 2003). Phenylhydrazine-induced anaemia can also be used as a model for the study of haematinic effects plants (Agbor *et al*, 2005, Biswas *et al*, 2005).

2.4.1 Phenylhydrazine and Haemolytic Anaemia

Exposure to many chemicals including the administration of some drugs has been associated with red blood cell destruction (Beutler, 2001). Haemolytic anaemia is part of the clinical syndrome associated with intoxication. Chemicals can cause haemolysis by interacting with sulfhydryl groups, inhibition of various enzymes, immune mechanisms, fragmentation of erythrocytes as they pass through the platelet-fibrin mesh or by unknown or poorly defined mechanisms. In haemolytic anaemia, erythrocytes have a shortened life-span. Xie *et al*, (2002) found that mixing either nucleated (from cold blooded animals) or non-nucleated erythrocytes with phenylhydrazine, turned them to green-brown. This study also discovered that inclusion bodies (now termed Heinz bodies) were formed in erythrocytes exposed to phenylhydrazine and that a number of compounds closely related to phenylhydrazine, including N-acetylphenylhydrazine, could induce similar effects. Yeshoda (1942) induced anaemia in rats following a single phenylhydrazine intraperitoneal administration at a dose of 20 mg/kg b.w. (aqueous solution), the erythrocyte concentration lowered to about 50% and haemoglobin level to about 60% of normal values in the course of 4 days.

Phenylhydrazine is used for the induction of haemolytic anaemia and the study of its mechanism in many species, such as rabbit (Hoppe-Seyler, 2005, Brugnara and Defranceschi 1993, Nakanishi *et al.*, 2003, Xie *et al*, 2003), rat (Yeshoda 1942, Berger 1985), Mouse (Golab *et al*,

2002, Paul *et al*, 1999), Calf (Sharma *et al*, 1991), Chicken (Datta *et al*, 1990), Duck (Rigdon, 1953), Rainbow trout (Gilmour and Perry 1996, McClelland *et al*, 2005), *Xenopus* (Twersky *et al*, 1995), Goldfish (Murad and Houston 1992), and *in vitro* also in both rat and human erythrocytes (McMillan *et al*, 1998, Pokhrel and Lau-Cam 2011, Claro *et al*, 2006). Phenylhydrazine injected in a dose of 90 mg/kg /b.w. to 8 weeks old rats causes a decrease to 45% of normal erythrocyte concentration and packed cell volume to 53% on day 3, reticulosis to 4.75% on day 7, an increase in the mean cell volume to 170 and the highest increase from zero values in the count of erythrocytes on day 7. There was both erythrocytes and Heinz bodies increase as well as 60% increase in mean cell haemoglobin on day 3 (Berger 1985). The osmotic fragility increased over three days after anaemia induction (Berger 1985, Redondo *et al*, 1995). This may be a consequence of reticulocytosis. The elevation of MCH seems to be induced by a free plasma haemoglobin increase (Berger 1985, Criswell *et al*, 2000).

Reticulocyte counts are more sensitive than erythropoietin levels in predicting erythroid changes (Criswell *et al*, 2000). Hyper segmented neutrophils were observed rarely, phagocytosing blood lymphocytes were frequent on day 3 (Berger 1985). Lymphocytes with butterfly-shaped nucleolus were also found (Berger 1985). Phenylhydrazine treatment induces hyper-cellularity (Berger, 1985) with erythroid hyperplasia (Berger, 1985, Criswell, *et al*. 2000).

Haemolytic anaemia induced by phenylhydrazine in rats may be detected based on hepatic changes in the expression of a subset of genes, *Alas2*, *beta-gloEraf*, *Hmox1*, *Lgals*, and *Rhcd*, that are mechanistically linked to haematotoxicity (Rokushima *et al*, 2007). These small genes subset were deregulated in all the severe haemolytic conditions, some of which were considered to be involved in hepatic events characteristic of haemolytic anaemia, such as haemoglobin synthesis, heme-metabolism and phagocytosis elevation. As haemolytic anaemia can be induced

also following phenacetin administration (Berger, 1985), identical genes are also expressed (Rokushima *et al*, 2007). The availability of iron in a diet may modulate the regenerative response (Burkhard *et al*, 2001). Intensity of induced anaemia depends on age (Berger 1987, Naeshiro *et al*. 1998). The deepest anaemia was found in haemoglobin level, red blood cell counts and packed cell volume and reticulocytosis in old rats, while the highest increase in mean corpuscular volume (MCV) was found in young adult animals (Berger, 1987). This could be due to the lower phenylhydrazine resistance of mature red blood cells in older animals.

2.4.2 Mechanism at Cellular Level

Phenylhydrazine increases reactive oxygen species (ROS) and lipid peroxidation, and decreases glutathione (GSH), and these effects are reversed by N-acetyl cysteine, a known ROS scavenger (Hill and Thornalley, 1982, Amer *et al*, 2004). Haemolytic anaemia has long been known to be caused by uptake of erythrocytes by macrophages in the spleen and translocation of phosphatidylserine from the inner to the outer of plasma membrane. It has also been identified as a signal for phagocytosis of cells under programmed death by macrophages. Phenylhydrazine generates reactive oxygen specie within both human and rat erythrocytes, and no evidence for lipid peroxidation or phosphatidyl serine externalization was detected (DeJong *et al*, 1997, McMillan *et al*, 2005).

Reactive oxygen specie production was associated with extensive binding of oxidized and denatured haemoglobin to the membrane cytoskeleton. Thus, phenylhydrazine-induced haemolytic injury seems to be derived from oxidative alterations to red blood cell proteins rather than to membrane lipids (McMillan *et al*. 2005).

Vitamins C and E contribute to the decrease in oxidative stress caused by phenylhydrazine *in vitro* (Claro *et al*, 2006). These vitamins inhibited Heinz bodies and methemoglobin formation

but did not protect against glutathione depletion by phenylhydrazine. Quercetin, an antioxidant bio-flavanoid compound, also suppresses reactive oxygen and nitrogen species, and partially protects reduced glutathione (GSH), malondialdehyde levels (Luangaram *et al*, 2007). Melatonin as a free radical scavenger protects against phenylhydrazine-induced oxidative damage to cellular membranes (Karbownik *et al*, 2000).

Phenylhydrazine- induces heinz body formation and oxidative degradation of spectrin without any cross-linking of membrane proteins, thus both these findings impair erythrocyte deformability (Hasegawa *et al*, 1993). Formation of phenyl radicals and the replacement of heme with phenyl-substituted protoporphyrins, causes the destabilisation of hemoglobins to induce heinz bodies and haemolytic anaemia with phenylhydrazine (Nakanishi *et al*, 2003). Phenylhydrazine treatment increases the transport rates in Na-K pump, Na-H exchange, Na-Li exchange, and K-Cl cotransport *in vivo*, while a decrease in Na-K pump, Na-H exchange, Na-Li exchange and increase K-Cl cotransport were found in rabbit red cells (Brugnara and Defranceschi 1993).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

3.1.1 Sample Collection and Identification

Onion was harvested from vegetables production site in Hayin Lambu, (Kaduna state, Nigeria), during cold season, that is in November. The cultivar type was identified in the Department of Plant Sciences, and authenticated at the Herbarium unit, Botany Department, Ahmadu Bello University, (A.B.U) Zaria. A voucher specimen number (423) was deposited with the Herbarium.

3.1.2 Chemicals/ Reagents

Phenylhydrazine was purchased from Sigma-Andrich Chemical Company (St. Louis U.S.A) and all other chemicals and reagents used were of analytical grade.

3.1.3 Experimental Animals

A total of Forty-Eight (48) healthy male and female albino rats (150-200g) were used for this study. They were kept in the animal house of Pharmacology Department of ABU Zaria, in well-ventilated cages and allowed to acclimatize for two weeks before the commencement of the study. They were fed on commercial rat feed, called vital feeds growers pellets produced by Grand Cereals Nigeria limited, and water *ad libitum*.

3.2 METHODS

3.2.1 Preparation of Crude Methanol Extract

A crude methanol extract of the sample was prepared. The outer scales of the bulbs were carefully removed. Edible parts of the bulb was washed under running tap water and then with distilled water to remove surface impurities. A portion (500g) was chopped and

homogenized using laboratory blender. The homogenate was extracted (at room temperature) with 2 litres of methanol for 48hrs in a large amber bottle with occasional stirring. The crude extract was filtered using Whitman No 1 filter paper, and the filtrate obtained was concentrated by gentle evaporation using water bath, at a regulated temperature of 45⁰ C (Trease and Evens 2002). Extract obtained was reconstituted in normal saline and was kept in a sterile sealed containers for subsequent usage.

3.2.2 Determination of LD₅₀ of Crude Methanol Extract

A portion of the crude extract was used for the determination of LD₅₀. The test was conducted using the method described by Lorke, (1983) using 13 rats. It was conducted in two phases. In the first phase, nine rats (9) rats were divided into 3 groups of 3 rats each and they were treated with the crude methanol extract of onions at doses of 10mg, 100mg and 1000mg/ kg body weight orally. They were observed for 48 hours for any mortality. In the second phase, the remaining 4 rats were divided into 4 groups of one rat each, and they were treated with the extracts at doses 1000, 1600, 2900mg/kg and 5000mg/kg body weight orally. They were observed for another 48 hours for any mortality. The LD₅₀ of the crude extract was evaluated at the end of the study.

3.2.3. Qualitative Phytochemical Screening of Crude Methanol Extract.

Test for Terpenoids

Salkowski's Test: Exactly 0.5g of the extract was dissolved in 2ml of chloroform and few drops of concentrated H₂SO₄ was added to form a lower layer. Absence of reddish brown colour at the interface indicates that terpenoids are absent.

Test for Alkaloids

Dragendoff's Test: Another 1 ml of extract was treated with a few drops of Dragendroff's reagent. Appearance of an orange precipitate indicate the presence of Alkaloids (Jaliwala *et al*, 2011).

Test for Cyanogenic Glycosides

Guignard Test: 5.0g of the extract was mixed with 20ml of water and heated in water bath with sodium picrate paper. Absence of various shades of red on the sodium picrate paper within 15mins, indicates that cyanogenic glycosides are absent.

Test for Saponins

Froth test: Exactly 0.5g of the extract was mixed with 10ml of distilled water in a test tube. The tube was shaken vigourously and allowed to stand for 30 mins. Absence of honey comb froth in the tube indicates that saponins are absent.(Trease and Evans, 2008).

Test for Flavonoids

Schinoda's Test: Small amount of magnesium chips were added to 2ml of the extract in a testtube, followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, colour indicate the presence of flavonoids.

Test for Phenols

Ferric Chloride Test: Extracts (2g) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Cardiac glycosides

Keller-killani method: 50mg of the extract was dissolved in 2ml glacial acetic acid containing 2 drops of 2% solution of ferric chloride. The resultant mixture was filtered through Whitman No 1

filter paper and added into another test tube containing 2ml conc H_2SO_4 . Formation of brown ring at the interface indicates the presence of cardiac glycosides.

3.2.4. Quantitative Determination of Phytochemicals in Crude Methanol Extract of Red Creole Onions.

Alkaloid Determination (Harborne 1998)

Procedure: Five (5) g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The extract was filtered and then concentrated on a water bath to one-quarter of the original volume. Ammonium hydroxide solution was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. It was the alkaloid content of the extract.

Determination of Cardiac Glycosides (Onwuka 2005)

Procedure: Exactly 25g of the extract was weight into 250ml sterile bottle and 200ml of 70% ethanol was added. Bottle was covered and shaken vigorously, and then allowed to stand for 6 hours at room temperature. The solution was filtrated through whatmann No 1 filter paper and transferred into 1L volumetric flasks. 500ml of distilled water was added, followed by 100ml of 12.25% lead acetate. Volume was made to 800ml with distilled water and was vigorously shaken and leave to stand for 10mins. 200ml of 4.77% disodium hydrogen phosphate solution was added. The resultant solution was filtered to give a clear filterate and the filterate was evaporated to dryness and weighed. Such is the amount of cardiac glycosides present in the sample.

Flavonoid Determination

Exactly 10g of the plant sample was extracted four times with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.(Soni and Sosa, 2013)

Determination of Total Polyphenol Contents

The total polyphenol content of the plant extracts was estimated using Folin-ciocalteu phenol reagent according to the method described by (Singleton *et al*, 1999).

An aliquot of 0.1 ml of 0.5 mg of extract dissolved in 1 ml methanol was transferred into test-tubes and the volumes made up to 0.5 ml with distilled water. Then, 0.25 ml of Folin-ciocalteu phenol reagent (10% v/v in distilled water) and 1.25 ml of 20% aqueous Sodium carbonate solution were added. The tubes were vortexed and allowed to stand for 40 minutes (mins) before taking absorbance at 725 nm against a blank solution containing 0.1 ml of the solvent instead of the extract. The determination was done in triplicate.

The standard curve was prepared using 0.1-0.5 mg/ml solutions of Gallic acid in methanol and the total phenolic content was expressed as Gallic Acid Equivalent (GAE) in milligrams per gram of dry matter of sample.

3.2.5 Experimental Design

3.2.5.1 Induction of Anaemia: Haemolytic anaemia was induced in the rats using Phenylhydrazine by daily intraperitoneal administration at the established dose of 10mg/kg body weight, for four days (Dhakar, 2012). Rats with haemoglobin concentrations less than 13g/dl after the induction of Phenylhydrazine were considered to be anaemic. Anaemia was successfully induced to all the groups of rats that were treated with phenylhydrazine.

3.2.5.2 Animal Grouping: A total of 35 albino rats were used for this experiment. The rats were divided into seven (7) groups, with five (5) rats per group and they were appropriately treated for a period of twenty-one (21) days. Haematological investigations were carried out on weekly basis and changes in haematological parameters were observed and recorded.

Normal control (NC): Normal rats fed with normal feed and water only.

Negative control (Neg C): Anaemic rats fed with normal feed and water only.

Positive control (Pos C): Anaemic rats given normal feed and a standard drug. (Bioferon 0.23ml/kg / body weight)

Low dose (LD): Anaemic rats given normal feed and 200mg/kg/ b.w of methanol extract of red creole onions.

Medium dose (MD): Anaemic rats given normal feed and 400mg/kg/ b.w of methanol extract of red creole onions.

High dose (HD): Anaemic rats given normal feed and 600mg/kg/b.w of methanol extract of red creole onions.

Extract control (EC): Normal rats given normal feed and 200mg/kg/b.w of the methanol extract. (Extract control)

3.2.5.3 Haematological Investigations

Packed cell volume (PCV): Packed cell volume was determined using microhematocrit method as described by Dacie and Lewis (1997) using microhematocrit centrifuge. The PCV determination was done by obtaining blood from a slightly cut rat tail and allowing the blood to flow through a capillary tube to more than two-third of the tube. The ends were sealed off using plasticine. The capillary tubes were placed in the centrifuge and centrifuged for 15 min at 3,000 rpm. The capillary tubes were then placed on the microhematocrit and by this, the respective PCV were determined.

Hemoglobin Concentration: Haemoglobin concentration (Hb) was carried out using the cyanomethaemoglobin method of Dacie and Lewis, (1997).

Red Blood Cell and Reticulocytes Count: These were estimated using improved Neubauer counting chamber (Dacie and Lewis, 1997).

The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated employing the formula of Schalm *et al*, (1975).

Mean Cell Volume: The mean cell volume (MCV) was calculated as follows :

$$MCV = 10 \times \frac{\text{Hamatocrit}}{\text{Red Blood Cell Count}}$$

Mean Corpuscular Hemoglobin :Mean Corpuscular Hemoglobin (MCH) was calculated as follows:

$$MCH = 10 \times \frac{\text{Haemoglobin}}{\text{Red Blood Cell Count}}$$

Mean Corpuscular Hemoglobin Concentration (MCHC): It was calculated as follows:

$$MCHC = \frac{\text{Haemoglobin}}{\text{Hamatocrit}} \times 100$$

The rest of the objectives, that is, to establish the fraction of the crude methanol extract that has the highest antianaemic potential and to characterize the most active fraction of the crude extract were not carried out because the antianaemic potential of the crude extract was found to be non- significant.

3.3 Statistical Analysis.

Results were expressed as Mean $\pm$ Standard Deviation (SD), except otherwise stated. The data was analyzed using analysis of variance (ANOVA) and the differences between means were compared using the Duncan multiple range test .P values less than 0.05 were considered significant.(P < 0.05).

CHAPTER FOUR

RESULTS

4.1 Lethal Dose Determination.

Tables 4.1 and 4.2, shows the lethal dose determination of methanol extract of red creole onions (*Allium cepa*) in albino rats for 48 hrs. There was no mortality within 48 hours after oral administration of 10, 100 and 1000 mg/kg/ body weight of extract to the rats in the first phase. Oral administration of 1000, 1600, 2900 and 5000 mg/kg /body weight of the extract to a different set of rats in the second phase, did not cause any mortality within 48 hrs, just like in the first phase of the experiment. This indicates that the administration of methanol extract of red creole onions orally to albino rats up to the dose of 5000mg/kg/ body weight did not record any mortality as shown in Tables 4.1 and 4.2, thus the LD₅₀ of the extract was considered to be greater than 5000mg/kg /body weight.

Table 4.1: Lethal Dose Determination of Methanol Extract of *Allium cepa* in Albino Rats for 48 hrs (First Phase)

First phase	Dose (mg/kg)	Number of Rats	Number of Death after 48 hrs	% Mortality
Group 1	10	3	0	0
Group 2	100	3	0	0
Group 3	1000	3	0	0

Table 4.2: Lethal Dose Determination of Methanol Extract of *Allium cepa* in Albino Rats for 48 hrs (Second Phase)

Second phase	Dose(mg/kg)	Number of Rats.	Number of Death after 48 hrs	% Mortality
Group 1	1000	1	0	0
Group 2	1600	1	0	0
Group 3	2900	1	0	0
Group 4	5000	1	0	0

LD₅₀ is greater than 5000mg/kg body weight.

4.2. Phytochemical Contents of Methanol Extract of Red Creole Onions (*Allium cepa*).

The result from Tables 4.1 and 4.2 showed the qualitative and quantitative phytochemical composition of methanol extract of red creole onion respectively. The qualitative phytochemical screening of red creole onions showed the presence of alkaloid, flavonoids, cardiac glycoside and phenols, while saponins, terpenoids and cyanogenic glycosides were absent. The quantity of each phytochemical was determined in which flavonoid was found to be the highest followed by cardiac glycosides and alkaloid. Total phenolics was found to have the least quantity.

Table 4.3: Some Phytochemicals Detected in the Methanol Extract of Red Creole Onions (*Allium cepa*)

Phytochemicals	Chemical tests	Remarks
Alkaloids	Dragendorff's	+
Flavonoids	Schinoda	+
Cardiac glycosides	Keller-killani's	+
Phenols	Ferric chloride test	+
Cyanogenic glycosides	Guignard test	—
Terpenoids	Salkowski's	—
Saponins	Frothing	—

Key: + Present — Absent

Table 4.4: Concentration of Some Phytochemicals in Methanol Extract of Red Creole onions (*Allium cepa*)

PHYTOCHEMICALS	CONCENTRATIONS (mg/g)
Alkaloid	9.46±0.52
Flavonoids	20.12±0.82
Cardiac glycosides	11.59±0.35
Total phenolics	4.36±0.48

The values represents mean± S.D for triplicate determinations.

4.3 Effect of Methanol Extract of Red Creole Onions on some Hematological Parameters of Phenylhydrazine- induced Anaemic Rats.

The effect of daily doses of methanol extract of red creole onions on Haemoglobin concentrations of phenylhydrazine-induced anaemic rats is presented in Figure 4.1. The result showed that there was no significant ($p>0.05$) increase in Hb concentrations after 3 weeks treatment with the crude extract, and this non- significant increase is comparable with the negative control group which is not treated at all. Upon calculation of the percentage increase, the methanol extract of red creole onions caused only 3.32%, 2.75%, and 8.79% increase for the groups treated with low dose (200mg/kg/b.w), medium dose (400mg/kg/b.w) and high dose (600mg/kg/b.w) respectively over a period of 3 weeks, and this is comparable with the negative control group which experienced decrease in Hb concentration by 3.08%.

However, upon treatment with standard drug, the positive control group showed a significant ($p<0.05$) increase in Hb concentration. The group recorded 79.52% increase in Hb concentration after three weeks treatment with the standard drug (Bioferon). The normal control group maintained a stable Hb concentration over 3 weeks. (19.58 ± 0.45 , 19.66 ± 0.41 and 20.04 ± 0.19 g/dl respectively).

Figure 4.2 showed that there was no significant ($p>0.05$) increase in packed cell volume (PCV), and the percentage increase showed only 0.71%, 0.80%, and 0.59% after three weeks treatment with low dose, medium dose and high dose respectively. However, the percentage increase is not comparable with the positive control group which were treated with the standard drug, because the group recorded a significant ($p<0.05$) increase of 37.85% after three weeks treatment with the standard drug (Bioferon).

The result of RBC as shown in figure 4.3 is similar to that of Hb and PCV, because non-significant ($p>0.05$) increase in RBC concentrations were observed for the groups treated with low dose, medium dose and high dose of the extract respectively over a period of 3 weeks, with slight deviation in the group that received high dose at second week, in which the RBC slightly decreased. The groups that were treated with low dose, medium dose and high dose of the extract recorded only 7.40%, 7.44% and 0.58% increase respectively, which is statistically non-significant compared with the positive control group with recorded a significant ($p<0.05$) increase of 64.71%.

The result of reticulocytes count as shown in figure 4.4 is also similar in which there was non-significant ($p>0.05$) decrease in reticulocytes over three weeks which is not comparable to the decrease in the group treated with standard drug which recorded a significant ($p<0.05$) decrease of 32.86%.

Figure 4.5 shows a non-significant ($p>0.05$) reduction of Mean cell volume (MCV) for all the treated groups because the highest percentage decrease recorded for MCV after 3 weeks treatment with the 3 doses of the extract is just 11.17%. However upon calculation of the percentage decrease of positive control group that received standard drug, the group recorded a significant ($p<0.05$) decrease by 24.72%. The same thing with figure 4.6 in which there was a non-significant ($p>0.05$) reduction in mean corpuscular haemoglobin (MCH) for all the treated groups, with 5.10% recorded as the highest percentage of reduction for all the treated groups after three weeks of treatment. However the group that received standard drug, that is the positive control group recorded a significant ($p<0.05$) decrease by 21.29%.

Figure 4.7 shows a non-significant ($p>0.05$) increase in mean corpuscular haemoglobin concentration (MCHC) for all the treated groups, because the highest percentage increase

recorded for MCHC after 3 weeks treatment with the 3 doses of the extract is just 6.88%. However upon calculation of the percentage increase of positive control group that received standard drug, the group recorded a significant ($p < 0.05$) increase by 24.48%. All these non-significant ($p > 0.05$) increase and decrease of the haematological parameters as the case may be, were an indication that, the levels of recovery from anaemia upon treatment with the crude extract of red creole onions, were statistically non-significant.

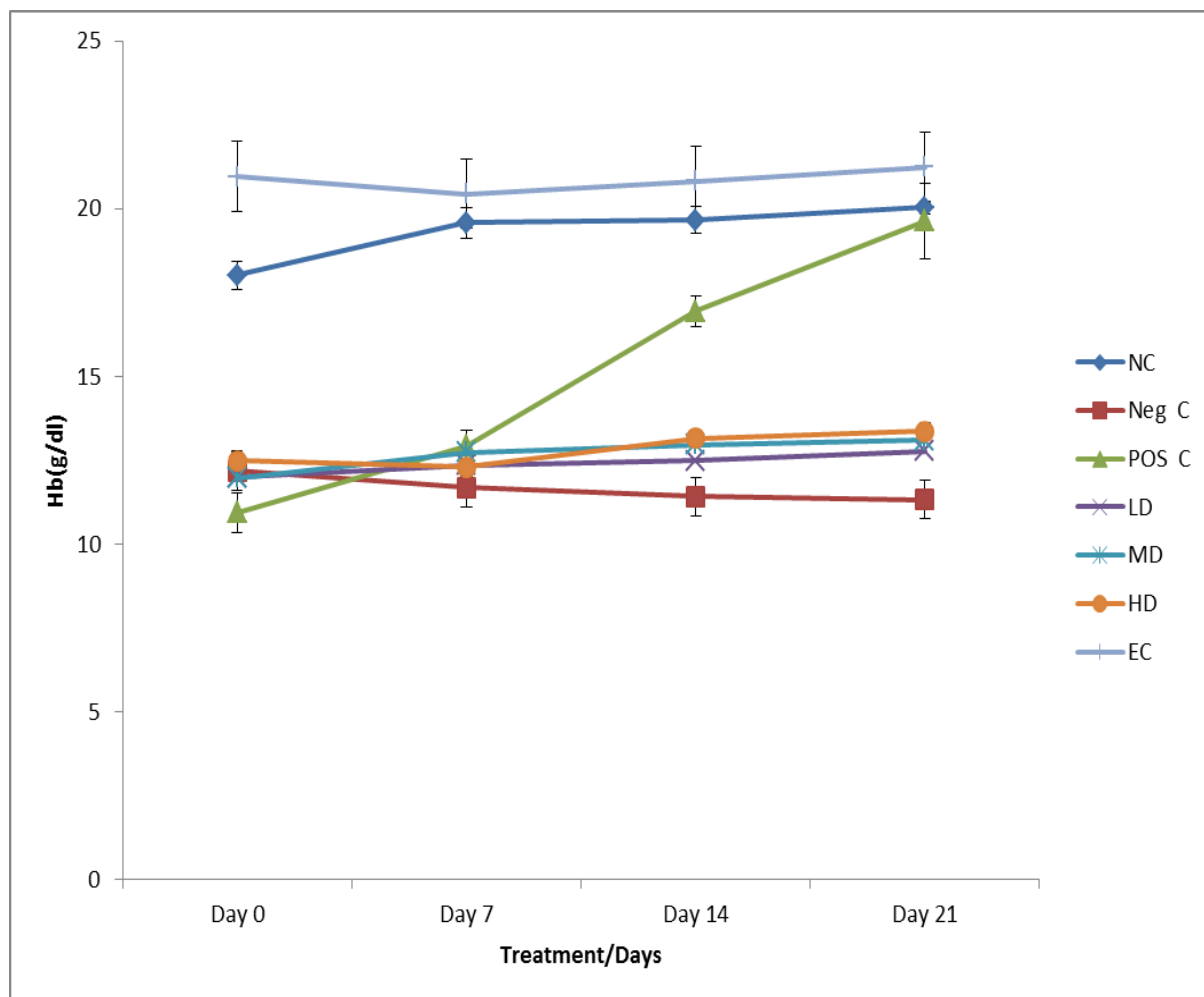


Figure 4.1: Mean Changes in Haemoglobin Concentrations of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control .

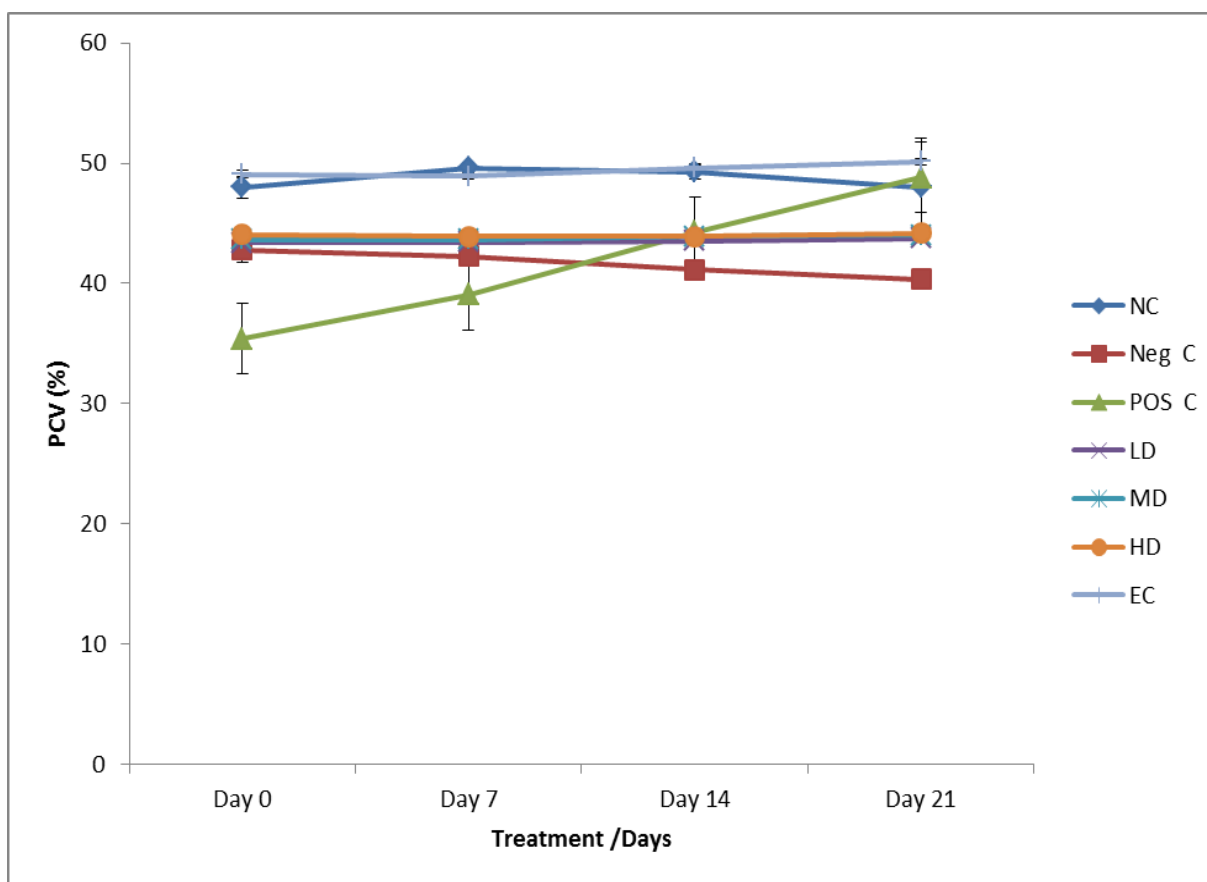


Figure 4.2: Mean Changes in Packed Cell Volume of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control .

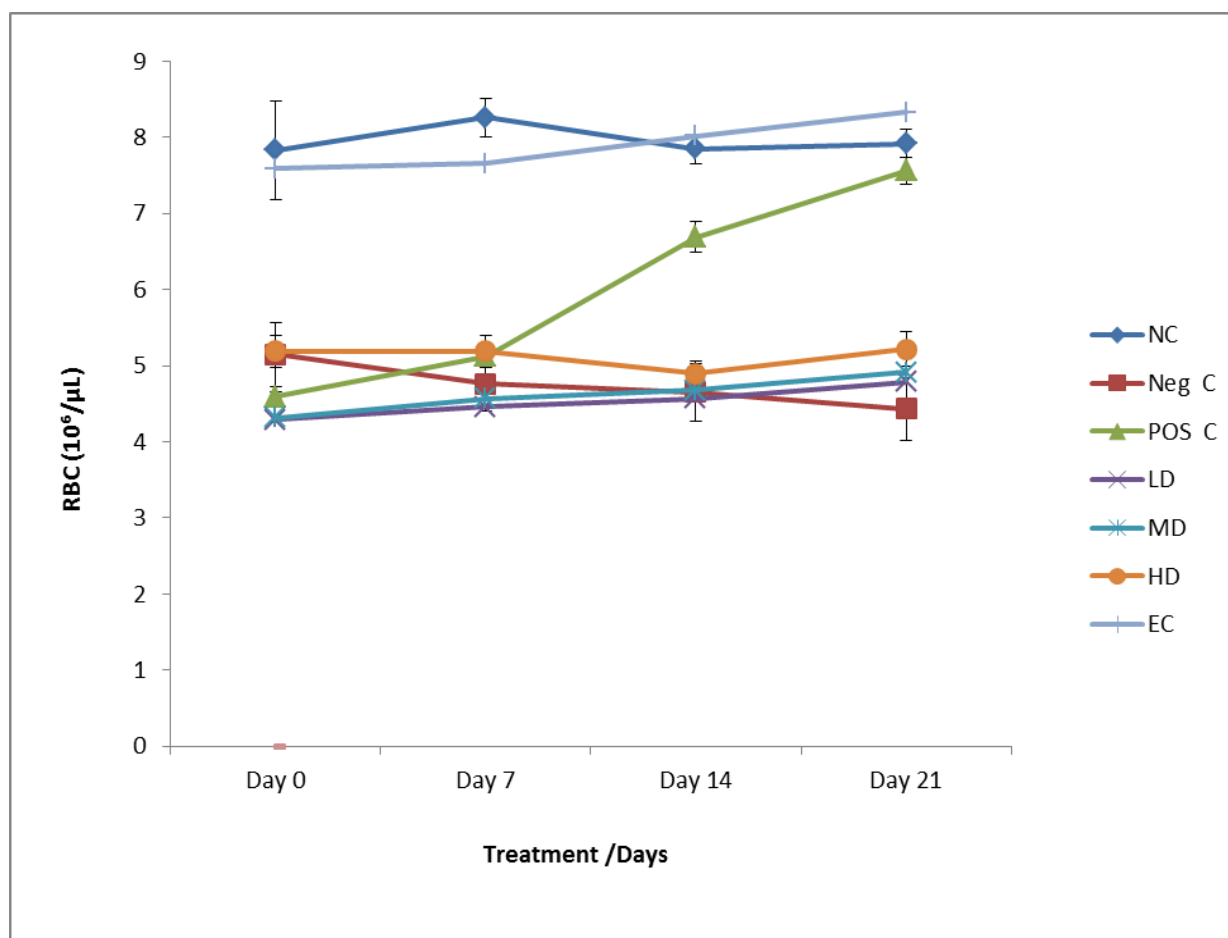


Figure 4.3: Mean Changes in Red Blood Cells Count of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control .

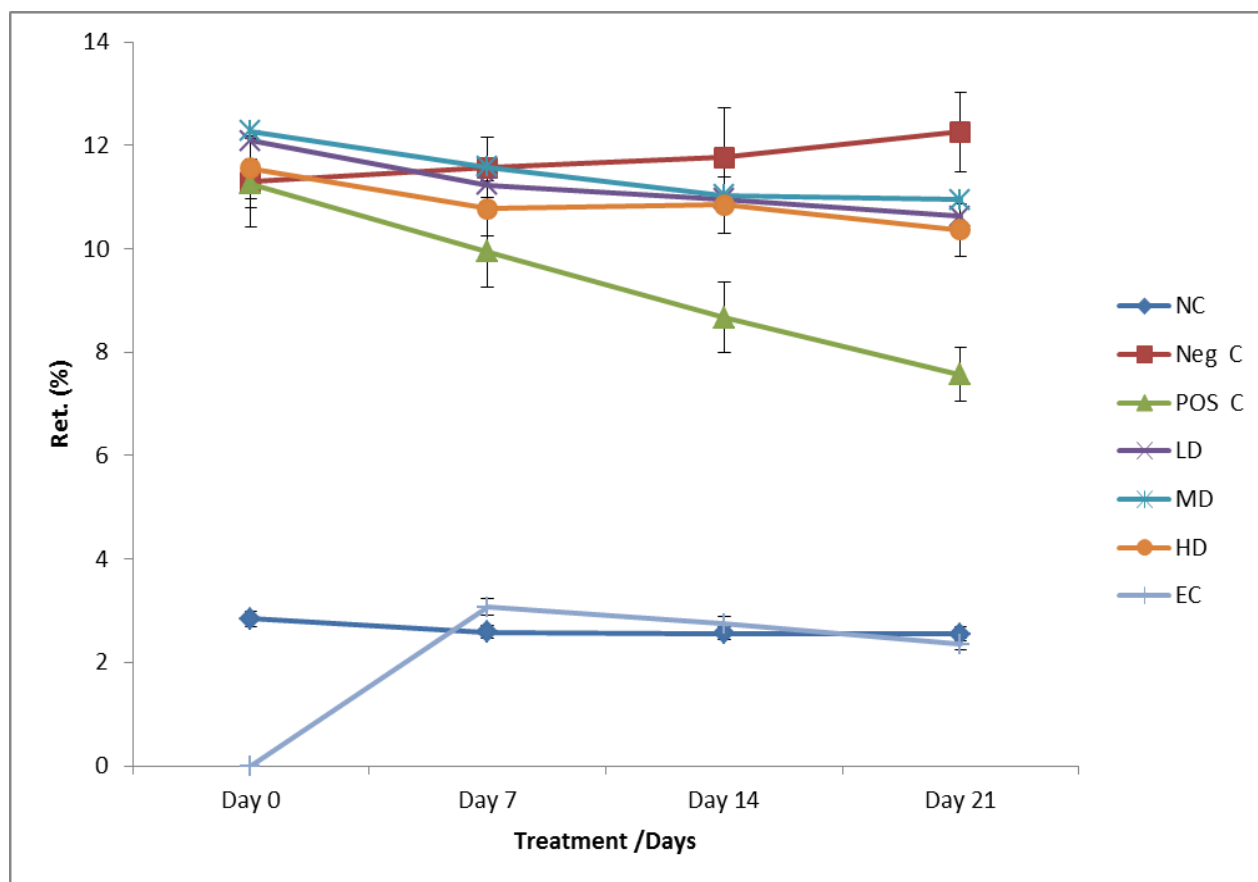


Figure 4.4: Mean Changes in Reticulocytes Count of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control

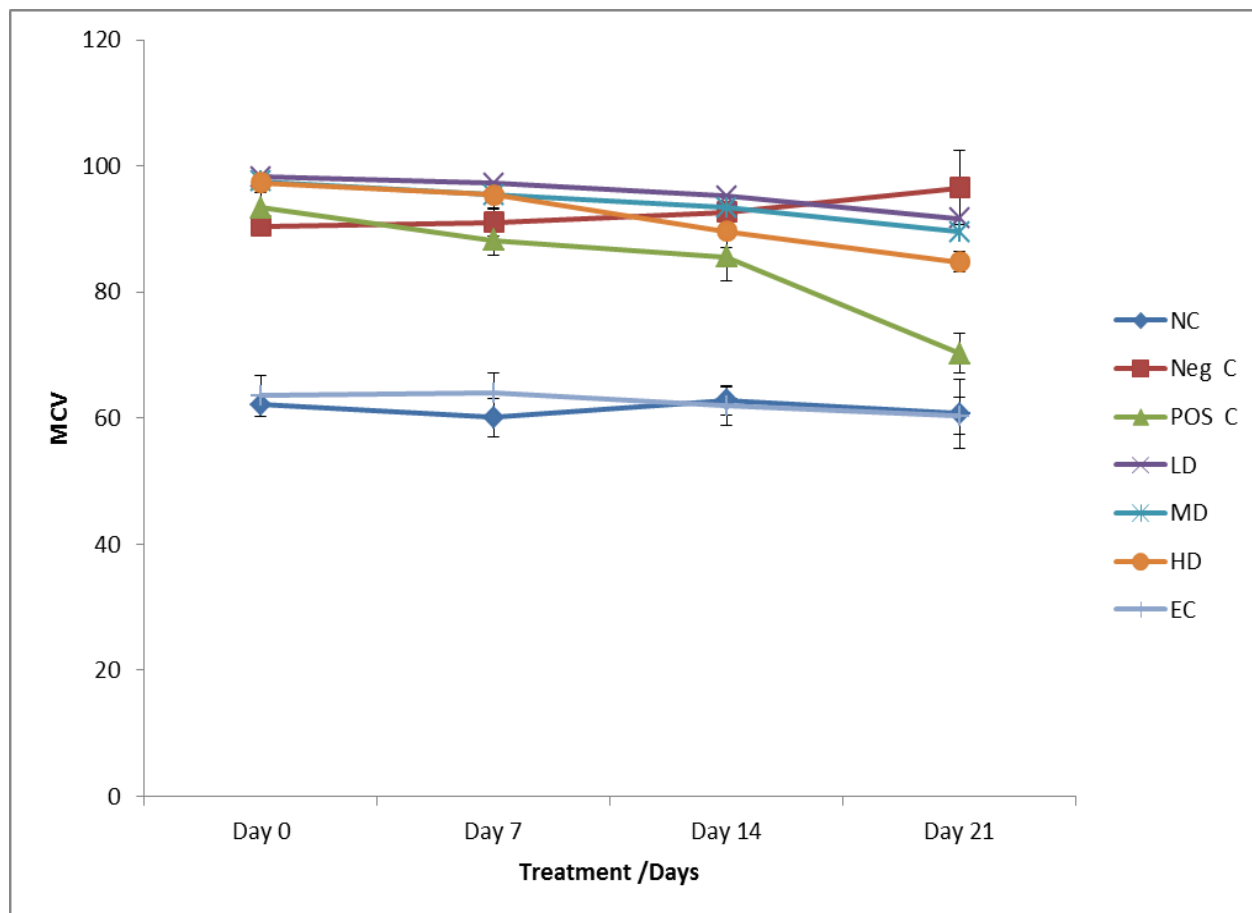


Figure 4.5: Mean Changes in Mean Cell Volume of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control

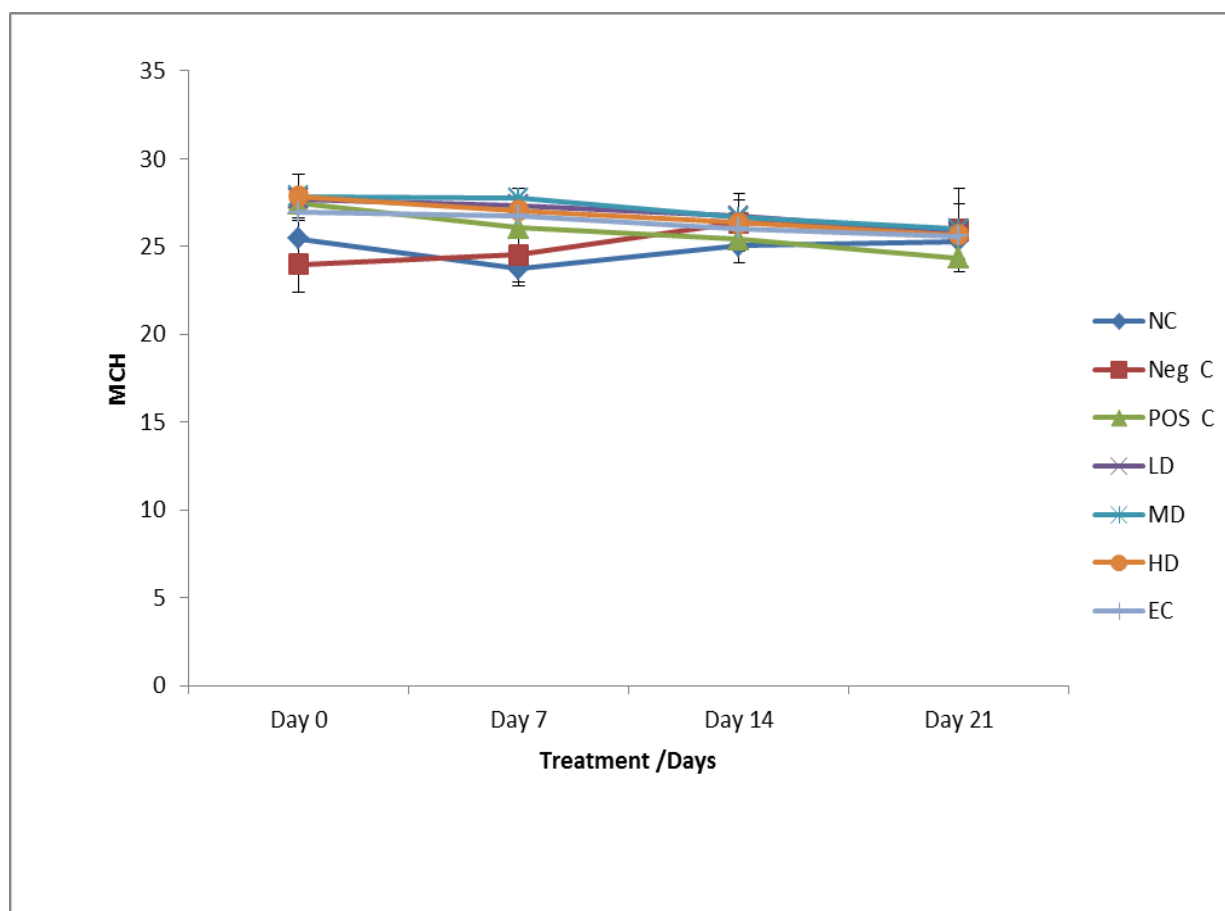


Figure 4.6: Mean Changes in Mean Corpuscular Haemoglobin of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control

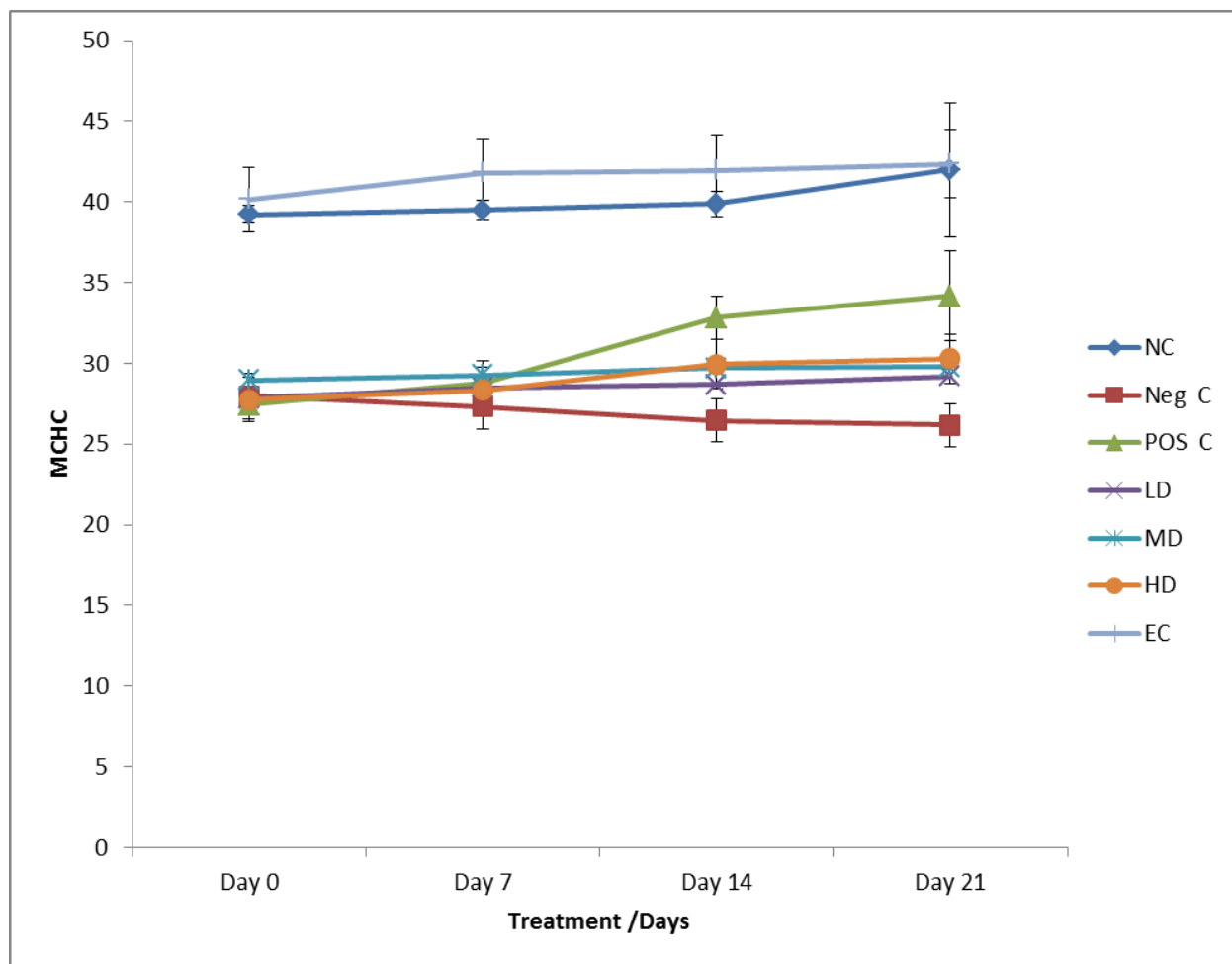


Figure 4.7: Mean Changes in Mean Corpuscular Haemoglobin Concentrations of Phenylhydrazine - Induced Anaemic Albino Rats after Three weeks Treatment with different Doses of Crude Methanol Extract of Red Creole Onions (*Allium cepa L.*) and Bioferon.

NC: Normal control, Neg C: Negative control, POS C : Positive control, LD: Low dose, MD: Medium dose , HD: High dose, EC: Extract control

CHAPTER FIVE

DISCUSSION

Anaemic rats in different groups were administered with methanol extract of red creole onions (*Allium cepa*) and standard antianaemic drug (Bioferon 0.23ml/kg/b.w) and the effect of the extract and drug on certain hematological parameters were studied. The results of this present study demonstrated that the administration of methanol extract of red creole onions non-significantly ($p>0.05$) increased the Hb concentrations (figure 4.2), PCV (figure 4.3), and RBC concentrations (figure 4.4). The crude extract also non-significantly ($p>0.05$) decreased the reticulocyte counts (figure 4.5) in the groups treated with different doses of the extract for twenty one days. However following administration of standard antianaemic drug, the positive control group that were treated with the drug experienced a significant ($p<0.05$) increase or decrease as the case may be, in all the hematological parameters. This may suggest that red creole onions does not possess any antianaemic effect.

The increase in the haematological indices exhibited by the test extract, even though statistically non-significant, might be associated with the phytochemical contents of the red creole onions. Preliminary phytochemical screening carried out on the crude extract in this study indicated that red creole onions contain flavonoids, alkaloids, glycosides and phenolic compounds. Glycosides have been documented to increase the proliferation abilities of bone marrow cells (Gao *et al*, 1992, Li *et al*, 2011), Alkaloids enhance the restoration of haematopoiesis (Bulter,1989), and Flavonoids are recognized for their antioxidant properties (Kandaswami *et al*, 1994, Manikandan *et al*, 2006). However, for red creole onions, all these effects are statistically not enough to conclude that the plant possess antianaemic potential.

The finding of this study does not agree with the claim of some natural nutritionist like Rudy .S. (2010) and oluwaseun (2015), which claimed that eating onions aids in the treatment of anaemia. However, it is possible that those who made such claims have used a different onion cultivar not red creole onion. Different cultivars can differ in terms of some phytochemicals and active components, for example, all onion cultivars contain quercetin, which is effective in reducing the risk of cardiovascular disease, an anticancer, and an antioxidant, however, there is difference in quercetin levels between cultivars, with red and yellow onions having higher concentrations and white having a low concentration (Currah and Proctor 1990), thus, it is possible that another onion cultivar can possess a significant antianaemic effect, which red creole onion does not.

Since the antianaemic effect of red creole onions was found to be statistically non-significant, there is no need to fractionate the crude extract, determine the fraction with highest antianaemic effect and determine the bioactive components of the most active fraction, thus objectives 3 and 4 were not carried out.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

It can be concluded, based on the result of this study that methanol extract of red creole onions does not exhibit antianaemic effect in phenylhydrazine- induced anaemic rats . This is due to the non-significant increase or decrease as the may be in the haematological parameters of all the groups that were treated with the crude methanol extract of red creole onions, compared with the group that was treated with standard antianaemic drug, which experienced significant change in all the parameters.

6.2 RECOMMENDATIONS

1. Antianaemic activity of other onion cultivars, apart from red creole onions should be tested, perhaps this may validate the claim.
2. Antianaemic activity of red creole onion should be tested at doses higher than 600mg/kg/body weight.
3. The antianaemic activity should be tested using different extract, like aqueous onion extract.

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Appendix 1: Haematological indices of Phenylhydrazine induced haemolytic anaemic rats treated with methanol extract of red creole onions and bioferon on day zero(0).

	Normal Control	Negative Control	Positive Control	Low Dose	Medium Dose	High Dose	Extract Control
Hb(g/dl)	18.01±0.42 ^c	12.19±0.73 ^a	10.94±0.59 ^{ab}	11.99±0.49 ^a	11.97±0.63 ^a	12.48±0.59 ^{ab}	20.95±0.24 ^d
RBC(10 ⁶ /μL)	7.83±0.65 ^c	5.15±0.40 ^b	4.59±0.07 ^{ab}	4.29±0.52 ^a	4.32±0.56 ^a	5.12±0.22 ^b	7.59±0.42 ^c
PCV(%)	47.99±0.91 ^c	42.78±0.20 ^a	35.40±0.48 ^a	43.37±0.31 ^b	43.63±0.31 ^{ab}	44.09±0.26 ^b	49.09±0.31 ^c
Ret.(%)	2.84±0.59 ^a	11.29±0.88 ^c	11.26±0.47 ^c	12.09±0.63 ^c	12.28±1.02 ^c	11.55±0.49 ^{bc}	2.81±0.81 ^a
MCV	62.12±1.15 ^a	90.45±2.43 ^d	93.37±2.49 ^d	98.23±0.12 ^d	97.51±1.03 ^d	97.38±2.22 ^d	63.54±1.23 ^a
MCH	25.45±0.03 ^a	23.98±1.25 ^b	27.46±1.57 ^b	27.71±1.96 ^b	27.87±2.0 ^b	27.85±2.0 ^b	26.95±2.52 ^a
MCHC	39.23±0.52 ^c	27.98±0.77 ^a	27.45±0.52 ^a	27.83±0.88 ^a	28.95±0.55 ^a	27.75±0.83 ^a	40.15±0.51 ^c

Appendix 2: Haematological indices of Phenylhydrazine induced haemolytic anaemic rats treated with methanol extract of red creole onions and bioferon on day seven(7).

	Normal Control	Negetive Control	Positive Control	Low Dose	Medium Dose	High Dose	Extract Control
Hb(g/dl)	19.58± 0.45 ^a	11.68±0.40 ^b	12.92±0.49 ^b	12.36±0.66 ^b	12.74±0.34 ^{ab}	12.29±0.7 ^b	20.44±0.57 ^a
RBC(10 ⁶ /μL	8.26±0.25 ^d	4.76±0.13 ^a	5.12±0.14 ^b	4.46±0.12 ^a	4.57±0.09 ^a	5.19±0.21 ^b	7.66±0.36 ^c
PCV(%)	49.58±0.49 ^{cd}	42.27±0.31 ^a	39.07±0.47 ^b	43.44±0.62 ^b	43.64±0.31 ^b	43.89±0.20 ^b	48.91±0.28 ^c
Ret.(%)	2.59±0.33 ^a	11.57±0.58 ^c	9.94±0.69 ^c	11.23±1.06 ^b	11.58±0.75 ^b	10.78±1.5 ^b	3.07±0.74 ^a
MCV	60.09±2.16 ^a	91.03±3.33 ^d	88.25±2.47 ^c	97.25±3.12 ^d	95.41±2.02 ^d	95.38±2.2 ^d	63.95±2.81 ^a
MCH	23.73±1.13 ^a	24.53±1.25 ^b	26.07±1.57 ^b	27.35±1.96 ^c	27.74±2.05 ^c	27.04±2.03 ^c	26.76±2.02 ^b
MCHC	39.48±0.62 ^c	27.31±0.97 ^a	28.78±1.32 ^b	28.48±1.38 ^b	29.26±0.85 ^{bc}	28.34±1.86 ^a	41.79±1.31 ^d

Appendix 3: Haematological indices of Phenylhydrazine induced haemolytic anaemic rats treated with methanol extract of red creole onions and bioferon on day 14(fourteen).

	Normal Control	Negative Control	Positive Control	Low Dose	Medium Dose	High Dose	Extract Control
Hb(g/dl)	19.66±0.4 ^c	11.41±0.33 ^a	16.94±0.45 ^b	12.49±0.64 ^a	12.97±0.78 ^a	13.15±0.71 ^a	20.81±0.75 ^c
RBC(10 ⁶ /μL)	7.85±0.19 ^c	4.65±0.13 ^b	6.69±0.20 ^c	4.57±0.09 ^a	4.68±0.13 ^b	4.90±0.16 ^b	8.02±0.38 ^c
PCV(%)	49.28±0.65 _b	41.11±0.21 ^a	44.27±0.27 ^a	43.52±0.28 ^a	43.93±0.31 ^a	43.90±0.20 ^a	49.59±0.22 ^b
Ret.(%)	2.56±0.45 ^a	11.77±0.96 ^c	8.67±0.68 ^b	10.96±1.04 ^b	11.04±0.84 ^c	10.85±1.48 ^b	2.74±0.35 ^a
MCV	62.82±1.1 ^a	92.65±2.51 ^d	85.59±3.77 ^b	95.23±2.37 ^d	93.37±2.49 ^d	89.61±2.46 ^{bc}	61.92±2.98 ^a
MCH	25.06±1.1 ^c	26.36±1.30 ^c	25.41±1.66 ^c	26.74±1.88 ^c	26.66±1.66 ^c	26.34±1.75 ^c	26.02±2.18 ^c
MCHC	39.88±0.8 ^c	26.47±0.78 ^a	32.84±1.02 ^b	28.69±1.34 ^a	29.72±1.84 ^a	29.95±1.60 ^a	41.97±1.57 ^{cd}

Appendix 4: Haematological indices of Phenylhydrazine induced haemolytic anaemic rats treated with methanol extract of red creole onions and bioferon on day twenty one (21).

	Normal Control	Negative Control	Positive Control	Low Dose	Medium Dose	High Dose	Extract Control
Hb(g/dl)	20.04±0.19 ^c	11.32±0.36 ^a	19.64±1.13 ^c	12.77±1.30 ^b	13.09±0.76 ^b	13.37±0.59 ^b	21.23±0.70 ^c
RBC(10 ⁶ /μL)	7.92±0.19 ^c	4.43±0.21 ^a	7.56±0.17 ^b	4.79±0.28 ^a	4.91±0.07 ^a	5.22±0.22 ^{bc}	8.33±0.42 ^c
PCV(%)	48.03±4.06 ^b	40.31±0.21 ^a	48.81±0.82 ^b	43.75±0.47 ^a	43.99±0.25 ^a	44.15±0.16 ^a	50.14±0.34 ^c
Ret.(%)	2.55±0.13 ^a	12.26±0.77 ^c	7.56±0.52 ^b	10.63±0.99 ^b	10.95±0.71 ^b	10.36±1.40 ^b	2.35±0.50 ^a
MCV	60.72±5.89 ^a	96.55±3.75 ^{bc}	70.29±3.17 ^b	91.68±5.52 ^{bc}	89.53±1.62 ^{bc}	84.73±3.15 ^{bc}	60.35±3.09 ^a
MCH	25.31±0.59 ^a	25.95±1.77 ^{bc}	24.36±2.36 ^b	25.82±2.71 ^{bc}	25.98±1.62 ^{bc}	25.66±1.52 ^{cb}	25.59±2.14 ^a
MCHC	42.00±4.17 ^a	26.17±0.87 ^{bc}	34.17±2.65 ^b	29.18±2.79 ^c	29.78±1.83 ^{bc}	30.29±1.34 ^{bc}	42.35±1.59 ^a

Appendix 5: Percentage changes(s) in haemoglobin concentration of phenylhydrazine-induced anaemic albino rats after 3 weeks treatment with different doses of crude methanol extract of red creole onions (*Allium cepa* L.) and Bioferon.

Groups	Initial mean(g/dl)	Final mean(g/dl)	Change Value	% Change
200mg/kg/b.w	12.36 ± 0.66 ^a	12.77 ± 1.30 ^a	0.41 ± 0.64	3.32 ↑
400mg/kg/b.w	12.74 ± 0.34 ^a	13.09 ± 0.76 ^a	0.35 ± 0.42	2.75 ↑
600mg/kg/b.w	12.29 ± 0.79 ^a	13.37 ± 0.59 ^a	1.08 ± 0.20	8.79 ↑
Positive Control (standard drug)	10.94 ± 0.59 ^a	19.64 ± 1.13 ^c	8.70 ± 0.54	79.48 ↑
Negative Control	11.68 ± 0.40 ^a	11.32 ± 0.36 ^a	0.36 ± 0.04	3.08 ↓

Values are means ± SD. Values with different superscript down the columns are significantly different (P ≤ 0.05).

↑ Increase ↓ Decrease.

Appendix 6: Percentage changes(s) in Packed cell volume of phenylhydrazine-induced anaemic albino rats after 3 weeks treatment with different doses of crude methanol extract of red creole onions (*Allium cepa* L.) and Bioferon.

Groups	Initial mean(g/dl)	Final mean(g/dl)	Change Value	% Change
200mg/kg/b.w	43.44 ± 0.62 ^a	43.75 ± 0.47 ^a	0.31 ± 0.15	0.71 ↑
400mg/kg/b.w	43.64 ± 0.31 ^a	43.99 ± 0.25 ^a	0.35 ± 0.06	0.80 ↑
600mg/kg/b.w	43.89 ± 0.20 ^a	44.15 ± 0.16 ^a	0.26 ± 0.04	0.59 ↑
Positive Control (standard drug)	35.40 ± 0.48 ^a	48.80 ± 0.82 ^c	13.40 ± 0.34	37.85 ↑
Negative Control	42.78 ± 0.31 ^a	40.31 ± 0.21 ^a	2.47 ± 0.10	5.77 ↓

Values are means ± SD. Values with different superscript down the columns are significantly different (P ≤ 0.05).

↑ Increase ↓ Decrease.

Appendix 7: Percentage changes(s) in Red blood cells count of phenylhydrazine-induced anaemic albino rats after 3 weeks treatment with different doses of crude methanol extract of red creole onions (*Allium cepa* L.) and Bioferon.

Groups	Initial mean(g/dl)	Final mean(g/dl)	Change Value	% Change
200mg/kg/b.w	4.46 ± 0.12 ^a	4.79 ± 0.28 ^a	0.33 ± 0.16	7.40 ↑
400mg/kg/b.w	4.57 ± 0.09 ^b	4.91 ± 0.07 ^b	0.34 ± 0.02	7.44 ↑
600mg/kg/b.w	5.19 ± 0.21 ^b	5.22 ± 0.22 ^b	0.03 ± 0.01	0.58 ↑
Positive Control (standard drug)	4.59 ± 0.07 ^a	7.56 ± 0.17 ^c	2.97 ± 0.10	64.71 ↑
Negative Control	5.15 ± 0.13 ^a	4.43 ± 0.21 ^a	0.72 ± 0.08	13.98 ↓

Values are means ± SD. Values with different superscript down the columns are significantly different (P ≤ 0.05)

↑ Increase ↓ Decrease

Appendix 8: Percentage changes(s) in Reticulocytes count of phenylhydrazine-induced anaemic albino rats after 3 weeks treatment with different doses of crude methanol extract of red creole onions (*Allium cepa* L.) and Bioferon.

Groups	Initial mean(g/dl)	Final mean(g/dl)	Change Value	% Change
200mg/kg/b.w	11.23 ± 1.06 ^a	10.63 ± 0.99 ^a	0.60 ± 0.07	5.34 ↓
400mg/kg/b.w	11.58 ± 0.75 ^b	10.95 ± 0.71 ^b	0.63 ± 0.04	5.44 ↓
600mg/kg/b.w	10.78 ± 1.51 ^a	10.36 ± 1.40 ^a	0.42 ± 0.11	3.90 ↓
Positive Control (standard drug)	11.26 ± 0.69 ^a	7.56 ± 0.52 ^c	3.70 ± 0.17	32.86 ↓
Negetive Control	11.29 ± 0.58 ^b	12.26 ± 0.77 ^b	0.97 ± 0.19	8.59 ↑

Values are means ± SD. Values with different superscript down the columns are significantly different (P ≤ 0.05). ↑ Increase ↓ Decrease.