Republic of Iraq
Ministry of Higher Education&
Scientific Research
University of Kerbala /College of
Veterinary Medicine
Department of Physiology,
Biochemistry and Pharmacology

The effect of Moringa leaves powder on iron metabolism indices in male rat with Induced chronic renal failure

A Thesis

Submitted to the Council of the College of Veterinary Medicine ,
University of Kerbala
in Partial Fulfillment Of the Requirement For the Degree of Master of Science in Veterinary Medicine / Physiology

By

Afiaa Layth Darweesh Al-Juhaishi

Supervised by

Asst. Prof. Dr. Wafaa Kadhim Jasim

2020 A.D **1442 A.H**

بسم الله الرحمن الرحيم

{وَقُلْ رَبِّ زِدْنِي عِلْمًا }

صدق الله العلي العظيم

(سورة طه/الايه 114)

Certification of examination committee

We, the examining committee, certify that after reading this thesis and have examined the student **Afiaa Layth Darweesh Al-Juhaishi** in it's contents, and that in our opinion is adequate as a thesis for degree of master in Sciences of Veterinary Medicine /Phyisology.

Asst.prof
Dr. Ayyed Hameed Hassan
College of Veterinary Medicine/ University of Kerbala
(Chairman)

Asst.prof
Dr. Ali Abdulzahra Al-fahham
College of Nursing/
University of Kufa
(member)

Asst.prof
Dr. Muntdhur Mohammad cani
College of Pharmacy/
University of Kerbala
(member)

Asst.prof.
Dr. Wafaa Kadhim Jasim
College of Veterinary Medicine/ University of Kerbala
(member and Superviser)

Approved by the concile of the college of Veterinary Medicine /University of Kerbala

Asst.prof.
Dr. Wafaa Kadhim Jasim
Head of department of physiology ,biochemistry and pharmacology

prof.
Dr. Wefak J.Albazi
The Dean of the College

Date : / /2020

Supervisor Certification

I certify that this thesis entitled "**The effect of Moringa leaves powder on iron metabolism in Male rat Induced chronic renal failure**" was prepared under my supervision at the College of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the degree Master Science in Veterinary Medicine/Physiology
.

Asst. Prof.
Dr. Wafaa Kadhim Jasim
(**Supervisor**)

The recommendation of the department
In the view of the available recommendation, I forward this thesis for debate by the examining committee

Asst. Prof.
Dr. Kadhim Salih Kadhim
Vice dean for higher Studies and Scientific Affairs
College of Veterinary Medicine
University of Kerbala

Certification of Linguistic Evaluator

I certify that thesis entitled "**The effect of Moringa leaves powder on iron metabolism in Male rat Induced chronic renal failure**" for the student **Afiaa Layth Darweesh Al-Juhaishi** was linguistically reviewed by me and the necessary correction has been made. Thus, it is linguistically ready for examination.

Linguistic Evaluator **Signature**

Asst. lecturer

Dhia.K. Nile ……

Dedication

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving Mother , my kind sister Afnan and my smart brother Taha whose words of encouragement and push for tenacity ring in my ears.

I also dedicate this dissertation to my husband, Ahmed who has supported me throughout the process. I will always appreciate all they have done for helping me to complete this research.

Afiaa

Acknowledgments

First of all, I would like to thank Allah, for giving me wisdom and bliss Thank you God for all the good that has happened in my life.A special thanks to my supervisor **Asst.Prof. Dr. Wafaa Kadhim jasim** for her guidance and advisement through the period of the project countless hours of reflecting and reading.My utmost gratitude to Asst. Prof. Dr. Haider AL-Karrawy and Asst. Prof. Dr. Ayyed Hameed Al Mossawi/College of Veterinary Medicine in Kerbala for his help .Great thank to DR.MohammedAbd –Alkadum /College of Veterinary Medicine in Kerbala for his help . Finally I am also indebted to all persons who exerted any effort in helping me in this work and I had forget their name.

Afiaa

Abstract

Our study was performed at college of Veterinary Medicine/University of Kerbala. It is performed during the period from1 november 2019 to 1 February 2020) .The present study was designed to investigat, the relation between Erythroferrone (ERFE) in iron homeostasis and erythropoietic activity in anemia related with induced chronic renal failure in male rats via investigation the correlation between the ERFE and other hormones such as erythropiotein ,hepcidin ,ferritin ,estimation the Hb,PCV,RBC , serum iron,determination the of kidney functions test (creatinine ,urea), and histopathological changes in kidney ,liver and spleen.

Sixty male rats were randomly divided into (4/groups) for 42 days , the first group (GI) control negative was with dimethyl sulfoxide (DMSO) by interaperitoneally for 4 weeks ,The second group (GII) is the positive control was administrated with DMSO by interaperitoneally for 4 weeks and then moringa leaf powder given at dose rang 5% for 2 weeks with diet, the third group (GIII) was administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure and fourth group (GIV) adenine were administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure and the given moringa oleifera leaf powder at dose 5% for 2 weeks with diet .

The results showed there was a significant elevation in the serum urea , serum creatinine,serum ferritin and serum hepcidin in (GIII) adenine treated group and statistically significant decrease in red blood cells count ,pacted cell volum and Hemoglobin in adenine group (GIII) in addition to decrease serum erythropoietin, erythroferrone and iron in comparsion with the other groups ,after moringa oliferia leaves administration we observe that there was a significant reduction in serum

urea , creatinine , ferritin and hepcidin in (GIV) treated group and statistically significant ($p \leq 0.05$) increase in RBC count ,PVC and HB in Forth group (GIV) in addition to serum erythropoietin, serum erythroferrone and serum iron in comparing to (GIII) group .

Histological changes in kidney , spleen and liver demonstrate that adenine treated group was damaged atrophied and degeneration especially the renal tubules that have the adenine crystalline precipitation in add to inflammatory infiltrate ofinflammatory in comparing to control group howere after moringa olifera leaves administration the tissues are able minimize the inflammatory condtion but not fully recoverd .

This study explains the induction of renal failure by adenine and how can chronic renal failure results in anemia and the ablitity of erythrobiotein hormone effect in a positive and negative correlation with the studied biomarkers also explains how can moringa olifera leaves powder works on improvement of anemia

List of contents

List of figures

List of tables

List of abbreviations

Abbreviation	Meaning
CBC	Complete blood count
ERFE	Erythroferrone
EPO	Erythropioteine
CRF	Chronic renal failure
µmol/L	Micromole per litter
ng/ml	Nano gram per millimeter
PCV	Packed cell volume
RBCs	Red blood corpusles
Hb	Hemoglobin
H&E stain	Hematoxylin and Eosin stain
WBC	White blood cell

ROS	Reactive oxygen species
mg/dl	Milligram per deciletter
mmol/L	Millimoles per litter
µl	microlitter
ml	Milliter
Δ	Delta
(O.D)	Optical density
EpoR	Erythropoietin receptor
JAK2	Junns kinase 2
STAT5	Signal transducer and activator of transcriptional protein
PIK3	Phsphoinositide 3 kinase
MAPK	Mitogen activated protein kinase
SOCS	Cytokin signaling
cp	centipoise
DMSO	Dimethyle sulfoxide
sr	Strontium
TMB	Tetramethyl benzidine
HRP	Horseradish peroxidase
GFR	Glomerular filtration rate

Chapter one
Introduction

Chapter one ---------- Introduction

1.Introduction

Anemia commonly occurs in people with chronic kidneydisease(CKD)—the permanent, partial loss of kidney function. Healthy kidneys produce a hormone called erythropoietin (EPO) which is a chemical produced by the body and released into the blood to help trigger or regulate particular body functions prompts the bone marrow to make red blood cells Which then carry oxygen through out the body**(Silverberg et al.,2001)**. When kidneys are diseased or damaged, they do not make enough EPO. As a result, the bone marrow makes fewer red blood cells, causing anemia. When blood has fewer red blood cells, it deprives the body of the oxygen it needs**(Eliopoulos et al.,2006).**

Erythroferrone is a hormone that control iron metabolism through its actions on hepcidin**(Coffey and Ganz,2018)**it is produced in erythroblasts, which proliferate when new red cells are synthesized.

This process is governed by the renal hormone, erythropoietin **(Jelkmann ,2007)**. The mechanism of action of Erythroferrone is to inhibit theexpression of the liver hormone, hepcidin. By suppressing this, ERFE)increases the function of the cellular iron export channe (Ferroportin ,This then results in increased iron absorption fromthe intestine and mobilization of iron from stores **(Boshuizen et al.,2018)**,Which can then be used in the synthesis of hemoglobin in new red blood cells Moringa has variously biological activities such as reducing hyperglycemia ,anti-inflammatory, anti-diabetic, antimicrobial, anticancer and antioxidant.In fact it is believed that the Moringa has many benefits based on its

nutrition. The ratio of grams per gram, Moringa leaves dry powder contains 25 times more iron than spinach **(Gopalakrishnan et al.,2016).** in which iron is one of the therapeutic agent for anemia which can compensate for the loss of the hemoglobin**(Mahima et al.,2014)** The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein**.(Ogbe and Affiku ,2020)**

The present study was designed to investigats the relation between the effect of Moringa leaves powder on iron metabolism indices in male rat with Induced chronic renal failure via performing the following objectives:

1- Determining serum erythoferrone , erythopiotein , hepcidin , ferritin and iron in male rats with induced anemia and renal failure .

2-estimating the RBC,PCV,Hb

3-determining the kidney fuction test creatinine and urea.

4-Evaluating the effect of the powder of Moringa Oleifera leaves as anti-anemia related with chronic renal failure in male rats .

3-Histopathological changes in kidney ,liver and spleen due to renal failure .

Chapter Two
Litreture review

Chapter two----------literature review

2-literature review

2 .1.Anemia

It is a case that related to variety of causes such as pathogenic neoplasm , different kinds of contagion, immunity defensive disorder and ,inflammatory disorders like rheumatism ; It's a condition that displays reduction in number of erythrocytes or hemoglobin level which is associated with cellular respiration.**(Ganz and Nemeth,2011) .**

Anemia is alterative in risk between patients, according to the chronic condition that based on, it most cases are slight in severity, involved patients show progression of the case marks like exhaust, pallor in addition tolack seriousness often at an improper time, shallow respiration, tachycardia, excitation, thoracic tenderness and many other signs **(Weiss and Goodnough ,2005).**

These sings may happen in each person who has resembling cases of anemia, the usual conditions anemia tend to appear in the previous condition rather than the available condition which may be slight or fair anemia,in few conditions ,anemia of chronic disorder are harsh and may lead to bad prognosis(**Guralnik et al.,2004)**

2.2. Causes Of Anemia

The target etiology of anemia related to chronic disorder are different ,it may be related to reduction in red blood cells lifespan of health ,further more the synthesis of erythrocytes may be defective wither it caused by the process of production or the hormonal control such as erythropoietin,or it may be associated with reduction in the erythrocytes

carrying quality of the oxygen or it caused by cancerous neoplastic unit may release such a materials that hinder the erythrocyte development ,such as tumor cells may infect the bones (**Kim et al.,2014)**

In spite of providing enough stock in the cells ,iron is crucial element that is present in the constitutions of our bodies in addition to it assist in cells proper operation and development , Ii is available in multiple sorts of nutrition which is raw meat ,chicken ,eggs plus green fibers**(Hurrell and Egli ,2010).**

Hepcidin is a hormone that is synthesis in hepatic cells that assist in control on biotransformation of iron ,which is essential in improvement of anemia related to chronic disease , Many studies suggest that special cytokines such as (interleukin-6) encourage on hepcidin synthesis **(Kim et al.,2014)** further more hepcidin are able to synthesizd positively in case of inflammation in a specific process that is not associated with interleukin-6 , Extreme levels of hepcidin results in catch iron inside tissues However dropping in iron levels results in hemoglobin synthesis so that anemia occurs; it suggests that hepcidin is a fundamental element that effects the progression of anemia related to chronic diseases (**Ferrucci et al.,2010).**

2. 3. Anemia associated with other chronic conditions

Iron is essential for hemoglobin synthesis, which acts as transporter for oxygen demands **(Heeney and Andrews ,2004)**

In case of chronic conditions related to anemia is the elevation of the absorption and release of iron inside tissue units which results in drop in activated iron level which is responsible for hemoglobin synthesis, reduce levels of activated iron results in restriction of hemoglobin progress it leads to decrase in oxygen level supplied to whole tissues (**Weiss and**

Goodnough ,2005). Iron low levels that's correlated to anemia is a usual disorder in which patients suffer from deficiency in iron; thus, it is unable to synthesis sufficient amount of erythrocytes in order to transport oxygen in circulation (**Goodnough et al.,2010**),Iron insufficiency disorder shows tiredness debility ,pallor ,shallow respiration ,headaches , cool extremities , excitation ,random cardiac output in addition for raise in incidence of infection **(Higdon et al., 2009)**

Iron disorder related to anemia may be developed from hemorrhage or poor food complementary iron with sufficient demands or failure in uptake of iron from the gut canal **(Ganz and Nemeth,2011).** Anemia related to chronic disease and iron insufficiency are mixed up as due to both of them are related to reduce in blood transporting iron, Anemia that caused by chronic renal diseases are correlated to erythropoietin insufficiency from previous defect to renal units **(Theurl et al.,2009)**.

Anemia resulted of inflammation is a usual character of inflammatory conditions such as chronic renal diseases ,contagions ,specific types of pathogenic neoplasm , normocytic normochronic anemia with reduced red blood cells survival period and even though the sufficient concentrations of erythropoietin ,irregular iron distribution is recognized in anemia related to inflammation and it is demonstrate by reduction of iron in blood stream with full iron stock ; however, it results in reduction of iron requirements for erythopoiesis**(Kautz et al.,2014) .**

Studies have also discovered that patients with anemia related to chronic conditioning unequal in spread of iron in circulation so it can't be uptake for synthesizing further erythrocytes**(Cavill et al.,2006**

2.3. 1 Pathophysiology of Anemia

Pathophysiology of the anemia related to chronic kidney disease can be classified into four descriptive ways:

1) Erythropoietin reduction**(Weiss and Gasche,2010).** 2) Decrease in erythrocyte life span**(Weiss and Goodnough ,2005) .** 3)Increase in the blockers or poisonous products that suppress erythrocytes production **(Means,2003**) . 4) Hemorrhage due to thrombocytes failure that exist in urea blood execrations **(Valeri et al.,2007).**

Erythropoietin hormone is essential hormone for red blood cell production ,without which erythropoiesis does not take place. **(Weiss and Gasche,2010)**About 90% of erythropoietin hormone is usually synthesized in renal tubules **(Jelkmann,2004)**

And only 10% of it is synthesized in the hepatic cells .**(Minamishima and Kaelin ,2010)**

As soon as kidney failure progress highest erythropoietin production is probably sharp ,whenever erythropoietin synthesis is encouraged by reduction of oxygen supply related to anemia or any else reasons of defective oxygen transfer see (figure 2-1) ,erythropoietin encourage erythroid precursor ,raise in hemoglobin formation which results in immature reticulocytes to move from bone marrow to the blood stream ,the common role of anemia in regular set of erythropoietin releasing method that results in raise in red blood cells production , hence kidney disorders will hinder this sets which leads to moderate erythropoietin feedback to anemic motivation **(Weiss and Goodnough ,2005) .**

Hepatic cells release hepcidin hormone, which is the essential factor for iron uptake and cellular transport , it's work through linking to iron exporter(ferroportin) results in entrance inside the cells and hydrolyzed of ferroportin by the lysosomes ,below the effect of the increased hepcidin levels ferroportin is reduced from cell barrier ,iron is held in

cells that conduct iron to the circulation so that iron level in the circulation is reduced ,which results in inflammatory conditions**(Ganz ,2011)** in contrast when hepcidin release is drop ,settlement of the ferroportin at the cellular membrane allow in uptake of nutritive iron from large intestine ,facilitating in the freeing of iron from macrophages that utilize aged red blood cells in order to release iron from liver cells **(Balogh et al.,2004)** .

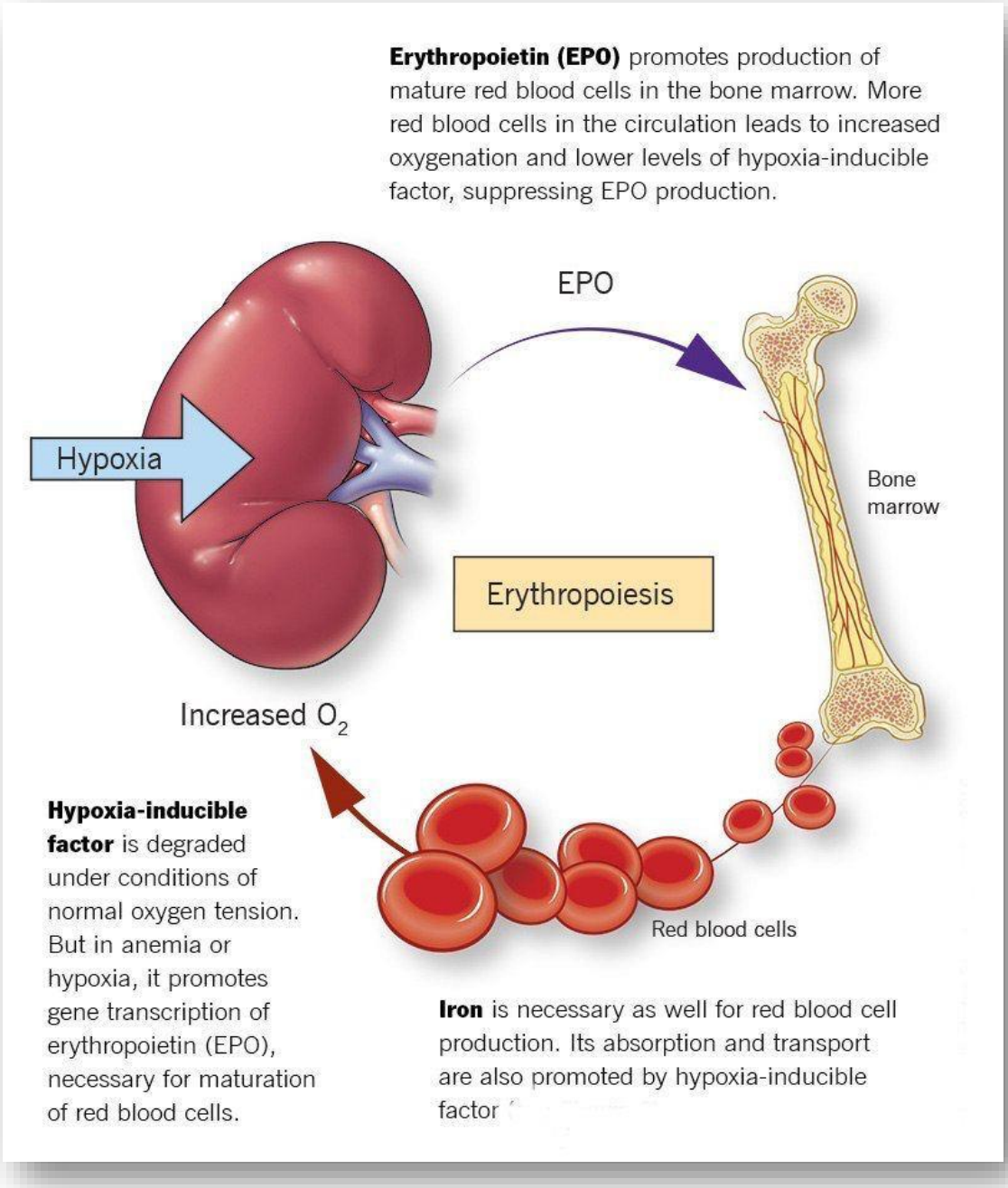


Figure (2-1) erythropoietin production is triggered by hypoxia

(Nakhoul and Simon ,2016)

2.3.2 The role of hepcidin in iron absorbtion

Hepcidin generation is enhanced by proinflammatory cytokine especially interleukin-6 ,last studies show that erythroferrone controlling hepcidin reduction throughout erythrocyte generation process under the influence of external or internal erythropoietin ,it discovered that low amount of erythroferrone in mice results in unable to inhibit hepcidin levels lead to hindrance in healing from blood losses related to anemia**(Kautz et al.,2014).** ERFE is a protein hormone synthesized by erythroblast in bone marrow ,it can block hepcidin effect there for level of iron grow which is necessary for hemoglobin production ,a first it's detected in mice encoded by the mouse sequence (FAM132b) .The similar sequence in man kind which is (FAM132B) ,it can be generated and released by erythroblast ;this gen was earlier discovered in mouse striated myocytes referred to as (myonectin) which binds to lipid balance.**(Koury,2015)**

The iron pathway controlled by the effect of ERFE on hepcidin both in man and mice ,it's synthesized in bone marrow which it's multipling rapidly when free erythrocytes are produced like follow bleeding at body required adequate levels of iron (this mechanism is controlled by the kidney hormone erythropoietin **(Kautz et al.,2014)** .The pathway starts from blockage of hepatic hormone(hepcidin) **(Kim and Nemeth, 2015)** which is controlled by the kidney hormone(erythropoietin) then erythroferrone enhances the role of ferroportin which leads to facilitated iron uptake from the gut and release of iron storages ; hence, it will be available for hemoglobin production into fresh erythrocytes ,reduction in the coding sequence of ERFE results in a little development in hemoglobin and defect in hepcidin regression that caused by hemorrhage further more it will restrict healing from anemia **(Lawen,2015)**.It has additional function as imyonectin , even though it can allow lipid absorption within lipid and liver cells .**(Seldin et al.,2012)**.

2.4. Renal Failure

Renal failure is a sort of kidney dysfunction in which the kidneys are no longer able to filter and clean blood; this can cause unsafe levels of waste products to build up. **(Wijeysundera et al.,2006)** . Etiology of renal failure is sorted by hypovolemia, obstruction of urinary tract, muscle collapse or special types of medications (**Serra et al.,2008).**Complications of renal failure includes uremia, high blood potassium, heart disease, high blood pressure and uremia (**Hovater etal.,2008)**.

2.4.1 Chronic Renal Failure

Chronic kidney disease is a case of renal failure that is developed unders pecific conditions in which there is gradual loss of kidney function forprolonged periods (**Remuzzi et al.,2002).** Mainly chronic kidney failure may result from dysfunction of blood vessels ,nephron parts and inferior urinary tract ,in spite of wide variations of diseases which result in chronic renal failure; the final outcome which is necessary is identical in reducing amount of working nephrons, Sequel of chronic kidney disease results in mild response for anemia, heart and blood vessels disorders as a result from previous kidney dysfunction ultimate fatality(**Kieffer et al.,2016).** One of the most well-known sings of chronic kidney disease is anemia in which complications develop as anemia progress in pathogenisity**(Polzin,2011).** Hepcidin is closely related with anemia that is caused by kidney dysfunction ,hepcidin are the main managers of iron homeostasis in addition it can develop its role by acting on ferroportin which is the main iron gate ,hepcidin cause internalization and degeneration of ferroportein as it produces rise in iron level inside the cells ,when digestible iron insufficiently absorbed; hence, the iron

level would fall in blood circulation that leads to iron insufficiency disorder .(**Kieffer et al.,2016)**

2.4.1.1 Effect of renal failure on body fluid

Kidneys are principle organs in filtration of blood by removing waste product of our natural metabolism that is not used by the body , which includes salts ,acids, hormones ,amino-acids or drugs In order to keep homeostasis renal release of water and electrolytes should equal to fluid uptake (**Blantz et al.,2002)**.

However effect of renal failure on body fluid rely on water and dietary consumption in addtion to degree of renal damage as so it may produce imbalance between extracellular fluids which lead to generalized edema ,acidosis and high levels of nonprotein-hydrogen waste and so urea ,creatinine and uric acid are recognized as metabolic product from the chronic renal failure ,The upkeep of a relatively constant level and a stable structure of the body fluids is crucial for homeostasis .Some of the most prevalent and important problems inclinical medicine appear because of defects inthe control systems that keep this constancy ofthe body fluids **(Schrier ,2006)** .

Fluid absorption andexecration a remaintained During Steady-State conditions ,The relative permanence of the body fluids is noticeable because there is sequential exchange of materials with the exterior environment also within the various compartments of the body. like there is a highly unstable fluid absorbed that must be carefully correspond by equal output from the body to reduce body fluid levels from imbalance**.(Rehrer ,2001)**.

2.4.1.2 effect of renal fluid on water balance

Water enters the body by two major sources: it is swallowed in the shape of liquids or water in the diet; both normally add about 2100 ml/day to the body fluids, and it is made in the body by the oxidation of carbohydrates, adding about 200 ml/day. This offers a total water absorbe of about 2300 ml/day . absorbers of water; although is highly variable between different individuals and even between the same person on different days, depending on weather, habits, and percent of physical activity**(Taniguchi et al.,2012)** .

Some of the water losses cannot be accurately regulated. like, there is a continuous loss of water by evaporation from the respiratorytract and execreted through the skin; both account for about700 ml/day of water loss under normal circumstances . This is termed *insensible water loss* , the insensible water loss through the skin occurs individuallyof sweating and is present even in individuals who are born without sweat glands; the average water loss by diffusion through the skin is about 300 to 400 ml/day. This loss is reduced by the cholesterol-filled cornified layer of the skin, which offers a barrier against huge loss by diffusionThe amount of water lost by sweatingis highly changeable , depending on physical activityand climate temperature. The level of sweat normally about 100 ml/day, but in very hot climate or during heavy exercise, water loss in sweat sometimes increases to 1 to 2 L/hour. This would rapidly

Minimize the body fluids if absorbtion were not alsoraised by activating the thirst **.(Lawson and Holt ,2007)**

Only a small amount of water(100 ml/day) in normally lost in the feces. This can be Raised to several liters a day in persons with severediarrhea. For this reason, severe diarrhea can be life threatening if not treated within a few days.(**Rabbani et al.,2004)**

The rest water loss from the body comes in the urine elimination by the kidneys.There are several mechanisms that manage the rate of urine excretion. In fact, the most important methods by which the body keeping a balance between water absorption and execration, as well as a homeostasis as between intake and output of most electrolytes in the body, is by managing the rates at which the kidneys excrete these substances. For example, urine volume can be as low as 0.5 L/day in a dehydrated person or as high as20 L/day in a person who has been drinking hugeamounts of water.**(Mirza et al.,2009)**

This fluctuation of intake is also true for many of the electrolytes of the body, such as sodium, chloride, and potassium. In some people, sodium absorption may be as low as 20 mEq/day, whereas in others, sodium absorption may be as high as 300 to 500 mEq /day. The kidneys are faced with the dutyof adjusting the output rate ofwater and electrolytes to equal precisely the administered of these substances, as well as keeping balance for massive losses of fluids and electrolytes that happen in certain disease states**.(Aperia,2001)** .

Most popular reasons behind the chronic kidney disease are diabetes mellitus ,increase in blood pressure and glomerulonephritis.The susceptibility appears one of five adults with hypertention and one of three in adults with diabetes having CKD.**(Atkins ,2005)** . Hypertrophy in addtion to the functional modifications which results in vascular resistance and tubular reabsorbtion in these nephrons are considered as one of the adaptive alternations in the renal system as attempt to get over chronic renal failure massive damage

Polycystic kidney disease ,moreover, atherosclerosis of the renal arteries and nephrosclerosis results in damage to the renal vasculature because of chronic renalfailure **(Wilson,2004)**.

Nephrosclerosis with glomerulosclerosis results in reduction in about 10% in the working nephrone, this sequential damage is shown by fall in renal blood flow and GFR **(Caetano et al.,2001)**.Diabetes mellitus in addtion to hypertension are considered as main reasons of end stage renal disease, which is a sequel of chronic renal failure **(Hsu et al.,2008)** Pyelonephritis or interstial nephritis may occur from vascular,glomerular or tubular injury which leads to nephrons damage by either bacterial infection or drugs .the risk of chronic kidney disease is not only it lead to renal dysfunction; also it may be developed to heat disorders **(Hogg et al.,2003)**

2.5. Methods of Induction of Renal Failure in Rats

Routes of administration are generally classified by the site at which the substance is applied ,two major routes were been used based on the target action are enteral (system-wide effect) however it is delivered through the gastrointestinal tract either by drinking water or by mixed with diet (**Ali et al.,2010)** ,Or Parenteral administrationwhich can be done by injection(intraperitonal) **(Al Za'abi et al.,2015**), regardless to methods of administration adenine Generally is metabolized after entrance into 2,8-dihydroxyadenine which deposit in renal tubules forming (crystals) which lead to injury in the renal tissue (**Diwan et al.,2018)** ,many researches considered the injectable method more acceptable than the other methods in which it prevent the possible interaction with other materials may be present in administered diet . **(Ali et al.,2014)**. Induction of renal failure by ligation which is applied on renal parenchyma that is considered most cheapest methods of induction of renal failure **(Perez-Ruiz et al.,2006)**. Strontium

administration can induced chronic renal failure by addting SrCl2 to drinking water **(Schrooten et al.,2003)**.

2.5.1 Adenine Chronic Renal Failure Induction

After induction of chronic renal failure by adenine is converted to 2,8-dihydoxyadenine ,which deposit and solidified in proximal convoluted renal tubules ,in rodents the incidence of kidney failure is marked by blood in urine ,increase of phosphate in blood corresponding to hyperparathydroidism in addition to kidney anemia based on the length of periods of adenine administration ,its risk came from its linking with amount of concentration over the period of the time in constant levels (**Tamura et al.,2009)**

After elimination of adenine from nutrition following two weeks of administration parts of the sings are improved in addition to restoration of the normal condition relatively **.(Abellán et al.,2019).**

chronic kidney diseased show increase concentrations of microbial waste product such as indoxyl sulfate with p-cresol sulfate, accumulation of these products in large quantities may results in decease kidney execration in spite of it not obvious till now with additional reasons may interact with this process such as raise in bowel exchange(**Kieffer et al.,2016) .** In consideration thatAdenine is a nucleobase (purine derivative) it is one of the four bases of DNA structure with chemical composition (C5H5N5) (figure 2-2)

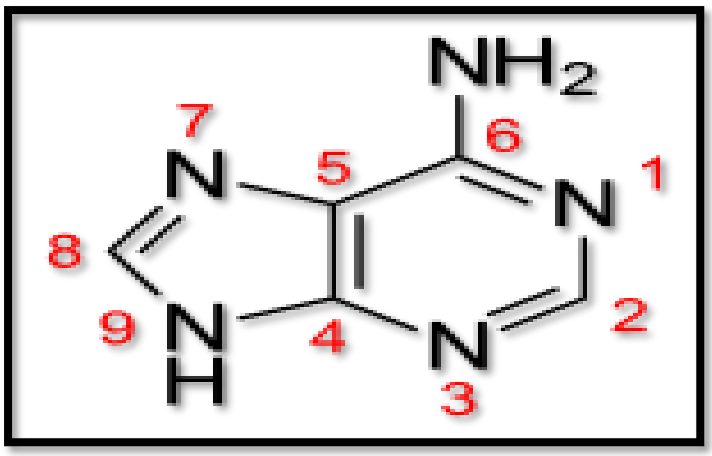


Figure (2-2) Adenine Chemical Structure
(Soad et al.,2010)

Appearance is white to light yellow ,adenine can make numerous tautomers which is regarded equivalent (**Plützer and Kleinermanns,2002)** .

2.6. Moringa

Kingdom : plantae
Order: brassicales
Family : moringaceae
Species : *M.oleifera*

(**Mishra et al.,2011)** . It is a tiny fast-growing permanentor deciduous tree that usually heightens as high as 9 m, with a tender, white wood and corky and gummy bark. Roots have the flavor of horseradish. Leaves Elongation cracked leaves, 30-75 cm long cheif axis and its branch are connected , The leaves are finely hairy, green and almost smooth on the high surface, paler and hairless below, with red-tinged fin-veins, with entire (not toothed) edges, and are rounded or blunt-projects at the tip

and short-pointed at the base. The branches are finely rough and green. bloom are white, scented in large axillary below panicles, pods are pendulous, ribbed, seeds are 3-angled**(Gupta,2010)** (figure2- 3) ,Approximately the huge distribution in India then came in wider distribution in tropic area of Africa ,Arabia ,south east Asia and south America (**Yaméogo et al.,2011**).It is able to breed better in moist or hard bare regions; however ,it is little affected by dehydration**(Anwar et al.,2007)** .also it can be eaten up as prophylaxis periodically in Ghana in addition to West Africa,Moringa have multi-roles in nature usually the therapeutic and non therapeutic benefits ,the non therapeutic such using nut in disturb water because of the purifying activity (**Asiedu-Gyekye et al.,2014)**.

Moringa haS wide Vareity of pharmaceutical uses as rhizome can assit in antilithic,carminiative,anit-inflammatory **(Anwar et al.,2007).**

the stalk of the plant is rich with nourishing elements such as vitamin, minerals, protein ,carbohydrate in addition to potassium,calcium,iron,amino and fatty acid plus variability of glycoside bounds **(Fahey,2005)** . Further more Anti-oxidant activity in which the small cotyledon infusion appears serious decline in DPPH radicals (2,2-diphenyl-1-picrylhydrazyl)when phenols present in wide range in infusion constitute the better response would gotten (**Sreelatha and padma,2009)**

one of most serious problems in our cell metabolism is free radical such as reactive oxygen species(ROS);it is highly risk factor that its accumulation leads to damage biologic tissues causing various diseases like diabetes ,cancer, inflammation ; its mechanism of action depends on mutation change in DNA segment ,one the biggest reasons that initiate this free radical problem is the food additives. For example butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) inspite of

their activity in preservation as antioxidant can lead to formation of cancerous changes in our biological system ,moringa oleifera came as alternative solution for this problem which is first natural compound second it is harmless antioxidant **(Fitriana et al.,2016)**

Moringa has been used for curing properties such as dermal infection ,anemia ,anxiety , asthma ,black head ,blood impurities ,catarrh ,bronchitis and chest congestion.**(Khawaja eta al.,2010)**

It is considered as effective medicinal cure notably at huge doses results in reducing the occurrence of tumors or is; niazimicin and glucomoringin are able to suppress tumorogenesis which are one of the bioactive constitutes of moringa. Infact niazimicin are better Glycosiltransferase than current glycosiltransferase inhibitor(**Pangastuti et L.,2016**) as Niazimicin was belived in its ablity to reduce tumor growth by application in two forms of study first one inside the lab.which, submit the (4-(a-l-rhamnosyloxy)benzyl isothiocyana, niazimicin and b-sitosterol-3-o-b-d- glucopyranoside)considered as effective parameters in contrast to another study involved out the lab.Environment which regards cancer growth practicing on mice derm analysis showed niazimicin get 50% have been reduced in addition of susptiblity of papilloama about 80% .(**Abdull Razis et al.,2014)**

In Asia and Africa moringa cotyledon are essential additives for breast nourishing moms and baby **(Fuglie,2001**) and many other materials such as nitrile compound ,mustard oil, benzyl glycosides ,phenolic glycosides flavonoid glycoside thiocarbamate glycoside with amino acid are extracted (**Farooq et al.,2012)**.

In Philippines described as 'moms univalent buddy' because of the employment for the development of breast nursing and anemia (**Estrella et al.,2000).** Moringa include active ingrediants such as dimeric cationic proteins with molecular wight 13Kda and isoelctronic point beteen 10 and

11 having coagulation nature in purifiying turbid water greater than alum **(Ndabigengesere et al.,1995)** , in addition to phenolic acid**,** isothyocyanate, tannins ,saponins and flavinoids that can be forund in many parts of the plant rather than the leaves only **(Vergara-Jimenez et al.,2017)**

The Stalk extract shows many component such as moringine which is alkaloid similar tobenzylamine that appears to inhibit the occurrence of hyperglycemia in alloxan-induced diabetic rats (**Bour et al.,2005)**

Moringa infusion analysis contains 4 absolute constituents which (niazinin A,niazinin B,niazimicin plus niazinin A+B) and had appeared constant reducing in blood pressure; it is believed in that due to the calcium blocking stimulation activity (**Paikra,2017)**. Moringa capable on dual performance in phase l and phase ll enzymatic activity results in progress in concentrations of hepatic cytochrome b5 ,cytochrome p450 and gluthione-s-transferase (GST) in fact it can suppress the cancer progress activity(**Sharma et al.,2012).**

Nut provides hepatic oxidation,prevents increasing in the blood pressure by specialized element such as thiocarbamate and isothiocyanate ,reduces fever,antimicrobial effect ;it can be eaten as raw food or additive with deserts moringa is plenty of monosaccharide gluconsinolates and isothiocyanates (**Lalas and Tsaknis ,2002)**.

Advantages of moringa appear by its ability to inhibit mineral poising in broad aspect and arsenic poising in specific aspect**(Chattopadhyay et al.,2011)**.

figure (2-3) Moringa oleifera nutritive value
(Moyo et al.,2011)

2.6.1.blood and fluid hemostatic balance

Blood includes both extracellular fluid (the fluid in plasma) and intracellular fluid (the fluid in the red blood cells). Although, blood is regarded as a separate Fluid compartment due to it is contained in achamber of its own, The blood volume is important in the manage of cardiovasculardynamics. The amount blood volume of adults is about 7 percent of body weight, or about 5 liters. About 60 percent of the blood is

plasma and 40 per cent is red bloodcells, but these values can differ considerably in different persons, depending on sex type, weight, and other factors **(McLuckie and Bihari ,2000)**.

Blood is a complex liquid, whose viscosity is different with flow, A fluid's viscosity is evaluated relatively to water. with electrolytes and organic molecules (like proteins) to water increase viscosity from 1.0 cp to _1.4 cp. Cells, chiefly red blood cells (RBCs) have the Major impact with viscosity elevation at a greater-than-exponentialrate with hematocrit (**Winslow,2002)**.

A hematocrit that falls lower than a normal range appears anemia which is more obviously interms of hemoglobin (Hb) levels, although NormalHb values for males range from 13.5–17.5 g/dL,12.0–16.0 g/dL in females (**Thavendiranathan et al.,2005).**

RBCs elevating resistance to the vessel wall, in fact its flow through capillaries measuring only 2.5 _m in diameter, which is surprising given that these blood cells are typically pictured as 8-_m diameterDisks ,however rising RBC numbers build up blood viscosityyet resistance to flow. individuals living at high altitude are shown a physiologic polycythemiastimulated by lowering atmospheric O2concentrations **(Slaghekke et al.,2010) ,**however rising RBC production may assist in counterbalance for reduced O2 supply which is another sort of anemia (hypoxia) that may be generalized or locally.(**Brauner and Wang, 1997)**

Anemic hypoxiahappen when the oxygen carrying capacity of the blood is reduced, and thus, this defect is chiefly associated with the blood. This describes why its lower hemoglobin molecules (or oxygen-binding sites) are ready for binding oxygen. The most common cases occur with reduced hematocrit or anemia are When the hemoglobin levels inside RBCs fall down in fact it also lowers the ability of the blood to carry oxygen(**Pittman,2011**) ,the trade off is build up workload on the heart,

restricting the altitude at which humans can comfortably existto about 5,000 m. On the other hand Increased electrolytic retention such as Naand water through pregnancy results in maternal plasma volume to raise by 40%–50%. Red blood cell (RBC) production is not parallels with the rapid expansion of blood volume, increasing by only 25%–35%. The distance of periods between volume expansion and RBC production results in a physiologic anemia of pregnancy (**Sifakis and Pharmakides ,2000)**. However anemia lower total O2-carrying capacity, there are obvious physiologic gains due to it reduces blood viscosity, which, as so, reduces shear stress.High-velocity flow increasesshear stresson the vascular lining in pregnant women to the point where it could become damaging. Shear stress is comparable to both blood velocity and viscosity **(Dobrica and Fillon ,2009**) ,Because of hematocrit is the main determinant of blood viscosity, anemia lowers stress levels and minimize the risk of vascular endothelial damage,which is considered as one of our bodies compensatory mechanisms for maintains of existence(**Hebbel etal.,2009)**. Anemia may produce defect in mental state ;the symptoms of anemia are different in severity and duration , depending on the sort of anemia and how serious it is (**Brady,2007)**.

2.6.2. Effect of Moringa on anemia

It is believed that leaves and pods of Moringa oleifera are a great value resource and many publications show that the protein, vitamin and mineral content is extraordinary , among them, iron, which is an essential trace element for the evolution of vital body functions. The differences in the average levels of iron may influence the health, its insufficiency produces anemia because of iron deficiency. To keep the average level

of iron, we must take care of our diet and consume iron rich diet , regardless of its source, Iron uptake depends on its oxidation state whether it was heme- iron or non-hem iron , Vitamin C may assist in iron absorption .(**Suzana et al.,2017)**

Avoidance and treatment of anemia due to iron lack could be very simple through making a balanced nutrition, with iron and vitamin C rich food , Moringa oleifera may be an alternative new way to prevent and treat iron deficiency because of its nutrients that can be provided to the human body(**Romero et al.,2016)**

Iron lack is the most ordinary nutritional disorder around the world and accounts relatively one-half of anemia cases,The diagnosis of iron deficiency anemia is confirmed by the performance of low iron stores and a hemoglobin rangesFurthermore, decrease in red blood cell production due to insufficient iron stores in the body (**Short and Domagalski ,2013)**

It is the most common dietary disorder which records for approximately one-half of anemia cases is Iron deficiency, Usually every condition of iron-deficiency anemia can be treated with supplementation(**Nzengu-Lukusa et al.,2016**), further more change in diet. Iron-rich nutrition like fish and leafy greens like spinach are also considered to elevate iron intake **(Pieracci ,et al.,2014)** ,Moringa oleifera is a wealthy source of iron, include 3x the quantity of iron in a serving size as spinach. **Moringa** also contains over 90 other vitamins, minerals, and other essential nutrients **to** provide your body can works as efficiently and normally as possible. It's no wonder moringa oleifera is considered "The Miracle Tree."(**Daba ,2016**) Usually someone who has been suffering from anemia, it's may want to give moringa oleifera a dose. It has been shown to increase iron levels, has awide of benefits, and is simple and

easy to employ. We personally advise our 100% pure moringa oleifera capsules, which have 800mg of pure moringa oleifera leave extract and are packed with iron, vitamin B12, and 88 other vitamins and minerals (**Iskandar et al.,2015)**.

Moringa oleifera is said to protect against starvation due to its increased protein constitution in add to nourishing supplementations,the plants natural composition is important in determination of nutritive ration in man and livestock**(Anjorin et al.,2010)**. In addtition it is considered as hopeful medicine for anemia mostly that related to reduction in iron levels ,its newly taken up may be helpful in resistance of starvation ,mainly in babies and lactating women ,many area like India, Senegal , Benin and Zimbabwe (**Fahey,2005) .**

Chapter Three Materials and Methods

Chapter three…….Materials and Methods

3. Materials and Methods

3.1. Chemicals

Through table below (3-1) whole chemical agents and their deliverers that are applied.

Table (3-1): Chemicals and kits were used in this study

No.	Chemical agents	Source
1	Adenine powder	Sigma Aldrich company(USA)
2	Chloroform	Noorbrok,England
3	Creatinine colorimetric kit	SPECTRUM company Egypt
4	DMSO	LOBA chemie
5	Eosin-hematoxylin stain	Merck,germany
6	Formalin 10%	TEBIA company.USA
7	Rat erythroferrone (FAM32B)ELISA Kit .	Biocellular Company (china)
8	Rat erythriopiotein ELISA Kit	Biocellular Company (china)
9	Rat Ferritin (FE) ELISA Kit.	Biocellular Company(china)
10	Rat Hepcidin (Hepcidin) ELISA Kit.	Biocellular Company(china)

11	Rat serum iron (SI) ELISA Kit.	Biocellular Company(china)
12	Moringa oleifera leaves	Alkafeel Nurseries Group.
13	Trichrome Staine Kit (Modified Masson's)	ScyTek Laboratories ,Inc./U.S.A
14	Urea kit	SPECTRUM company Egypt

3.2. Instruments

The tools that are applied in this research and their deliverers are shown In Table (3-2)

Table (3-2) :Instruments with their suppliers

No.	**Tools type**	**source**
1	automated hematology analyzer	China
2	Analytical sensitive balance	Sartorius /Germany
3	Digital camera (canon)	Toup cam/china
4	EDTA tube	Jordan
5	Electric centrifuge(80-2)	China
6	Electric grinder	China
7	ELISA biotek	USA
8	Eppindrofe tube	Biolabse/ England
9	Freezer	Denka/china
10	Gel tube	Gorden
11	Glasses	China

12	Gloves	Malaysia
13	Incubator	Faithful /Malaysia
14	Insulin syringes	Italy
15	Rack for blood standing	China
16	Light microscope	Lieca/china
17	Masks	China
18	Micropipette	Biobase/ China
19	Optical microscope	Italy
20	Semi-auto chemistry analyzer	GENEX XCHEM-S1/USA
21	Sterile syringes	China
22	Test tube	China

3.3 Animals of the study

The study performed during the period from (1 november 2019 to 1 February 2020) .Mature rat hold in animal house for adaptation in period ranging from two to three weeks then the study actual performance began starting.Sixty adult male rats (rattua albicans) aged (2 -3months) weighting 170-200) gm were obtained from the animal house in Collage

of veterinary medicine of University of AL-Qadesiah .They were set in the animal house of (collage of Pharmacy ,University of Kerbala with standard environment conditions temperature (25-28 C̊)and dark /light cycle 12:12 h/day .

3.4 Prepration of Moringa Oleifera leaves powder

Fresh moringa oleifera leaves are collected from Alkafeel Nurseries Group Washed and dried at room temperature then grained by the electric grinder into powder (**Mun'im et al.,2016**) , 5% of moringa oleifera leave powder was mixed with the usual nutrition ration of crushed feed diet Mixed with tap water then made in shap of cokies then bake in the oven for 15 minuts 180̊C (**Yang et al.,2006 a)**

3.5 The Experimental design

Sixty male rats were divided randomly into four groups (15/group) as shown in figure (3.1):

1- Group (GI) negative control were administrated with DMSO by interaperitoneally for 4 weeks then leaft without treatment for 2 weeks .

2- Group (GII) was the positive control which was administrated with DMSO by interaperitoneally for 4 weeks, and then moringa leaf powder given at dose rang 5% for 2 weeks with diet **(Yang,et al.,2006 a)**

3-Group (GIII) adenine was administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure dissolved by DMSO (**Rahman et al.,2018)** then leaft without treatment for 2 weeks

4- Group (GIV) adenine was administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure dissolved by DMSO (rahman,et al.,2018) and the given moringa oleifera leaf powder at dose 5% for 2 weeks with diet (**Yang et al.,2006 a)**

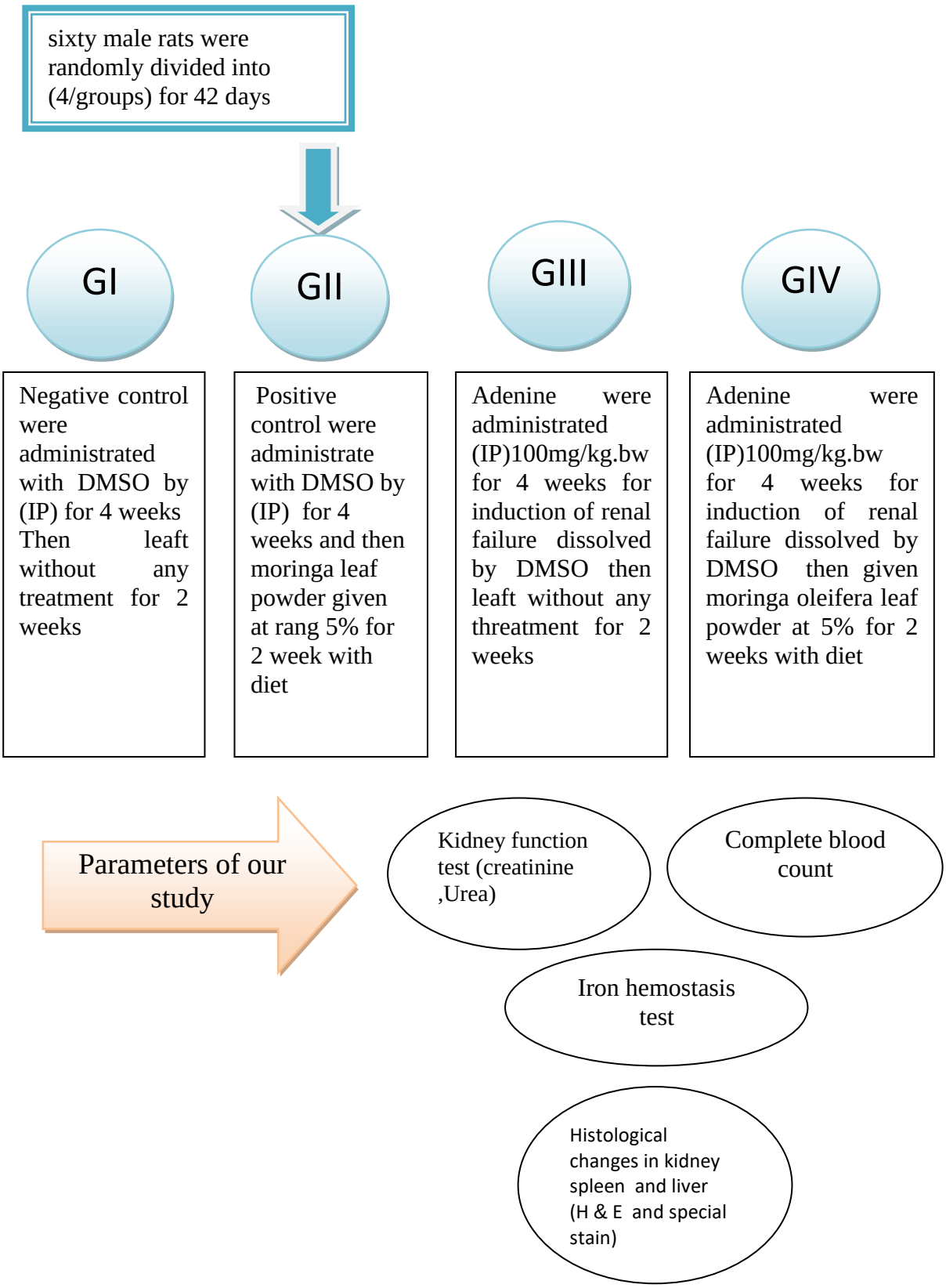


Figure (3.1) :represented experimental design

3.6 Blood collection and tissue preparation

Experimental animals (rats) get anaesthetized by putting them in covered jar include cotton rinsed with chloroform to be sedated for the next step which is blood via cardiac pincture in sterile syringes by needle prick in the heart draining 5ml of blood carefully , then separation of the blood collected into 1 ml drained in EDTA tube for the analysis of iron homeostasis tests quickly separate the blood in the centrifuge at 3500rpm in 15 minutes and then set at eppendrof tube , while the rest of the blood drained into two separated parts ;about 2ml set in gel tube it is left about half hour at room temperature for properly agglutinated ,then it would be separated at centrifuge at 3000 rpm for fifteen minutes to get the serum apart in eppendrof tube, both of samples are hold in freezer at -20℃ ,while the remaining of the blood drained into EDTA - tube for hematological tests . Liver, kidney and spleen were eradicated by abdominal surgical incision , the vital organs which is kidney ,liver and spleen are transformed in to formalin (10%) to be ready for histological examination

3.7 Biochemical parameters

3.7.1 Kidney function test

3.7.1 .1 Estimation of serum urea concentration

Serum urea was measured by (SPEECTRUM-urea kit,Egypt-IFUFCC40) by Semi-auto chemistry analyzer (**Jing et al.,2018**) ,as shown appendix(I)

Principle

Colorimetric determination of Urea activity is obtained according

To the following reactions:

1. Urea is hydrolyzed in the presence of water and urease to produce Ammonia and carbon dioxide

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

2. In the presence of glutamate dehydrogenase (GLDH) and reduce Nicotinamide adenine dinucleotide (NADH), the ammonia combines with*α*-ketoglutarate (α-KG) to produce L- glutamate

$$2NH_4 + 2\ \alpha\text{-KG} \xrightarrow{\text{GLDH}} 2\ \text{L-Glutamate}$$
$$+ \qquad\qquad +$$
$$2NAD + H_2O \qquad\qquad 2NADH$$

The rate decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 578nm.

3.7.1.2 Estimation of serum creatinine concentration:

Creatinine concentration in serum was determined by using a special kit SPECTRUM –creatinine kit ,Egypt-IFUFCC10, by using device spectrophotometer sesil,England measured by semi-auto chemistry analyzer **(Dunn et al.,2004)**As shown in appendix(II)

Principle

Creatinine reacts with picric acid in alkaline solution to form a colored complex (**Tietz ,1986**).

NaOH

Creatinine + picrate —→ yellow red complex

3.7.2 Hematological parameters

3.7.2.1 Complete blood corpuscles

A complete blood count (CBC) is a blood test used to measure overall blood cell by automated hematology analyzer **(George-Gay and Parker ,2003)**As shown appendix (III)

3.7.3 Iron homeostasis

3.7.3.1 Estimation of rat serum erythriopiotein ELISA Kit.

This examination was done by preparing process from Biocellular Company(china) by using enzyme-linked immunosorbent assay method to determine the concentrations of erythroipiotein in rat serum (**Kulikov et al.,2015**) as shown in appendix (IV)

3.7.3.2 Estimation of rat erythroferrone (FAM32B) ELISA Kit.

This examination was done by preparing process from Biocellular Company(china) by using enzyme-linked immunosorbent assay method to determine the concentrations of FAM32B in rat serum (**El Gendy et al.,2018**) as shown appendix (V)

3.7.3.3 Estimation of Rat Serum Ferritin (FE) ELISA Kit.

This examination was done by preparing process from Biocellular Company(china) by using enzyme-linked immunosorbent assay method to determine the concentrations of FE in rat serum **(Watanabe et al.,2001**) as shown appendix (VI)

Principle of the assay

The kit assay Rat FE level in the sample, use Purified Rat FE antibody to Coat microtiter plate wells, make solid-phase antibody, then add FE to the wells,Combined antibody which With HRP labeled, become antibody-antigen-enzyme-antibody complex, after washing Completely, AddTMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acidSolution and the color change is measured spectrophotometrically at awavelength of 450 nm. The concentration of FE in the samples is then Determined by comparing the O.D. of the samples to the standard curve .

3.7.3.4 Estimation of Rat serum iron (SI) ELISA Kit

This examination was done by preparing process from Biocellular Company(china) by using enzyme-linked immunosorbent assay method to determine the level of serum iron (SI) in serum of rats . (**Guo et al.,2019**) as shown appendix (VII)

Principle of assay

The kit assay Rat SI level in the sample Purified Rat SI antibody to Coat microtiter plate wells, make solid-phase antibody, then add SI to the wells,Combined antibody which With HRP labeled, become antibody-antigen-enzyme-antibody complex, after washing Completely, AddTMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acidsolution and the color change is measured spectrophotometrically at a wave length of 450 nm. The concentration of SI in the samples is then determined by comparing the O.D. of the samples to the standard curve.

3.7.3.5 Estimation of Rat Serum Hepcidin (Hepcidin) ELISA Kit .

This examination was done by preparing process from Biocellular Company(china) by using enzyme-linked immunosorbent assay method to determine the concentrations hepcidin in rat serum. **(Abbasi et al.,2013**) as shown in appendix (VIII)

Principle of the assay

The kit assay Rat Hepcidin level in the sample Purified Rat Hepcidin Antibody to coat microtiter plate wells, make solid-phase antibody, then addHepcidin to the wells, combined antibody which With HRP labeled, becomeantibody-antigen-enzyme-antibody complex, after washing Completely, AddTMB substrate solution,TMB substrate becomes blue color At HRPenzyme-catalyzed, reaction is terminated by the addition of a sulphuric acidsolution and the color change is measured spectrophotometrically at awavelength of 450 nm. The concentration of Hepcidin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

3.8 Histological study

The liver ,kidney and spleen of each animal were quickly removed and preserved in 10% neutral formalin buffer preparation of histological study according to (Mescher method ,(2010) as shown in appendix (IX)

Statistical Analysis

Data were analyzed using the software package SPSS version24.00 where one way (ANOVA) was used to assess the significant changes between the groups' results. The data were expressed as mean ± standard Error (SE). In addition the correlation between parameters were

performed by pearson correlation coefficients (r). A p-value >0.05 was (considered significant **(Hau et al.,2002)** .

Chapter Four
Results

Chapter four ……Results

4.Result

4.1 Effect of Moringa oleferia Leaves Powder on Some Serum Kidney function tests in male rats with Induced CRF

4.1.1 Urea Concentration in serum

The results in table (4-1) clarified there were a significant ($p \leq 0.05$) elevation in the serum urea in (GIII) adenine treated group in comparison with other groups.

On the other hand, combined adenine with moringa (GIV) in the same table showed a significant ($p \leq 0.05$) reduction comparing to (GIII) but not –significant ($p \geq 0.05$) as compared to control group (GI).

4.1.2 Creatinine Concentration in serum

Table (4-1) illustrated there was statistically significant ($p \leq 0.05$)increment in serum creatinine in adenine treated group (GIII) as compared to other groups.

As the same table showed combined adenine plus moringa (GIV) caused a significant ($p \leq 0.05$) decrement of serum creatinine comparing to (GIII) but it's value reachs close to value recorded in the control group (GI).

Table (4.1)Effect of Moringa Oleifera Leaves powder on Some Serum Kidney Function Tests in Male rats with Induced CRF

groups / parameters	GI Negative Control	GII positive Control + moringa leaves	GIII Adenine	GIV Adenine+ moringa
Urea **mg/dl**	45.00 ±2.92 C	55.50±3.403 BC	99.68 ±6.13 A	69.20 ±5.75 B
Creatinine **mg/dl**	0.45±0.084 B	0.60±0.057 B	2.56 ±0.209 A	0.86 ±0.091 B

-Value are expressed as mean ± ES

-Number of rats in each group =6

-Different letters represent significal ($p \leq 0.05$) difference between group

4.2Effect of Moringa Oleifera leaves powder on Hematological parameters in male rats with induced CRF

There was statistically significant ($p \leq 0.05$) decrease in RBC count, PVC and HB in adenine group (GIII) in comparsion with the other groups (Table) (4.2).

Also table (4.2) showed that combined moringa with adenine (GIV) caused significant ($p \leq 0.05$) increase in RBC count, PVC and Hb in comparing with (GIII)

Table(4-2) effect of Moringa Oleifera leaves powder on Complete Blood Corpuscles in male rats with Induced CRF

groups / parameters	GI Negative Control	GII positive Control + moringa leaves	GIII Adenine	GIV Adenine+ Moringa
RBC Count (cell*10^{12}/ l)	7.43±0.43 B	8.49±0.25 A	5.69±0.23 C	7.29 ±0.32 B
PCV %	40.83±0.82 AB	42.66±0.66 A	35.66±0.33 C	39.50±0.67 B
Hb (mg/dl)	15.25±0.45 A	15.72±0.41 A	9.06±0.34 C	13.65±0.64 B

-Value are expressed as mean ± SEM

-Number of rats in each group =6

-Different letters represent significal ($p \leq 0.05$) difference between groups

4.3 Effect of Moringa Oleifera Leaves powder on Iron Homeostasis Parameters in Male rats with Induced CRF

4.3.1 Erythropoietin

There was statistically significant ($p \leq 0.05$) decreases of serum erythropoietin in adenine treated group (GIII) in comparison with the other treated groups.Table (4.3)

Also table (4-2) showed combined moringa with adenine (GIV) ameliorate the serum erythropoietin but it's value not reached to that recorder in control group (GI).

4.3.2 Erythroferrone

Table (4-3) showed that the serum erythroferrone a significant decrease ($p \leq 0.05$)in adenine treated group (GIII) as compared to other groups . The results in table (4-3) also revealed that rat treated adenine plus moringa (GIV) caused significant ($p \leq 0.05$) increase comparing to (GIII) but it's level still significantly decrease compared to control group (GI).

4.3.3 Ferritin

Depending on the results clarified in table (4-3) there were a significant ($p \leq 0.05$) elevation in serum ferritin in adenine treated group (GIII) in comparson with other groups. According to the table (4-3) thecombine adenine with moringa group (GIV) caused a significant ($p \geq 0.05$) decrease in comparison with (GIII) but not –significant ($p > 0.05$) in comparing to the control group (GI).

4.3.4 Serum Iron

A significant ($p \leq 0.05$) reduction in table (4-3) of serum iron in (GIII) adenine treaded group (GIII) comparing to the other groups . Also the same table revealed a significant ($p \leq 0.05$) elevation in the group treated adenine plus moringa (GIV) Comparing to (GIII) but not significant ($p > 0.05$) as compared to the control group (GI).

4.3.5 Hepcidin

Table (4.3) illustrated there were a significant ($p \leq 0.05$) increment in this parameter in adenine treated group (GIII) Comparing to other groups .

Combined adenine with moringa (GIV) in the same table caused significant ($p \leq 0.05$) decrement of serum hepcidin comparing to (GIII) group, but reach close to value recorded in the control group (GI) .

Table (4-3) Effect of Moringa Oleifera leaves powder on Iron homeostasis Parameters in male rats with induced CRF

Groups / Parameters	GI Negative Control	GII positive Control + moringa leaves	GIII Adenine	GIV Adenine+ moringa
Erythropoietin ng/ml	9.88 ±0.30 A	11.07±0.602 A	4.10 ±0.367 C	7.50 ±0.439 B
erythoferrone ng/ml	19.66 ±1.516 A	18.04 ±1.593 AB	10.23 ±0.426 C	15.52 ±1.025 B
Ferttin ng/ml	6.60 ±0.174 B	6.80 ±1.133 B	9.98 ±0.837 A	7.49 ±.0183 B
Serum iron µmol/L	8.19 ±0.090 A	9.59 ±0.264 A	6.41 ±0.495 B	8.26 ±0.454 A
hepcidin ng/ml	19.25 ±0.456 B	19.60 ±0.622 B	23.09 ±1.101 A	19.64 ±1.363 B

-Value are expressed as mean ± ES

-Number of rats in each group =6

-Different letters represent significal ($p \leq 0.05$) difference between groups

4.4 Correlation between measured parameters

Pearson's correlation coefficient (r) among all studied parameters was presented in table 3.Erythropoietin recorded high significant ($P<0.001$) positive correlation coefficient with erythroferron, iron, RBCs count,

PCV and Hb. On the other hand, erythropoietin was negatively correlated with ferritin, hipcidine, urea and creatinine.

Erythroferron and Iron exhibited the same pattern of erythropoietin in its correlations with other studied parameters except that it was non-significant ($P>0.05$) correlation coefficient of erythroferron with each of iron and PCV ,

In contrast, strong negative correlation coefficient($P<0.001$) was found among serum ferritin and erythropoietin, erythroferron, iron, RBCs and Hb, but it shows strong positive correlation coefficient($P<0.001$) with each of urea and creatinine

Hipcidin shows significant correlation coefficient which resembles that registered to ferritin with other parameters, but it was non-significant between hipcidin and ferritin .

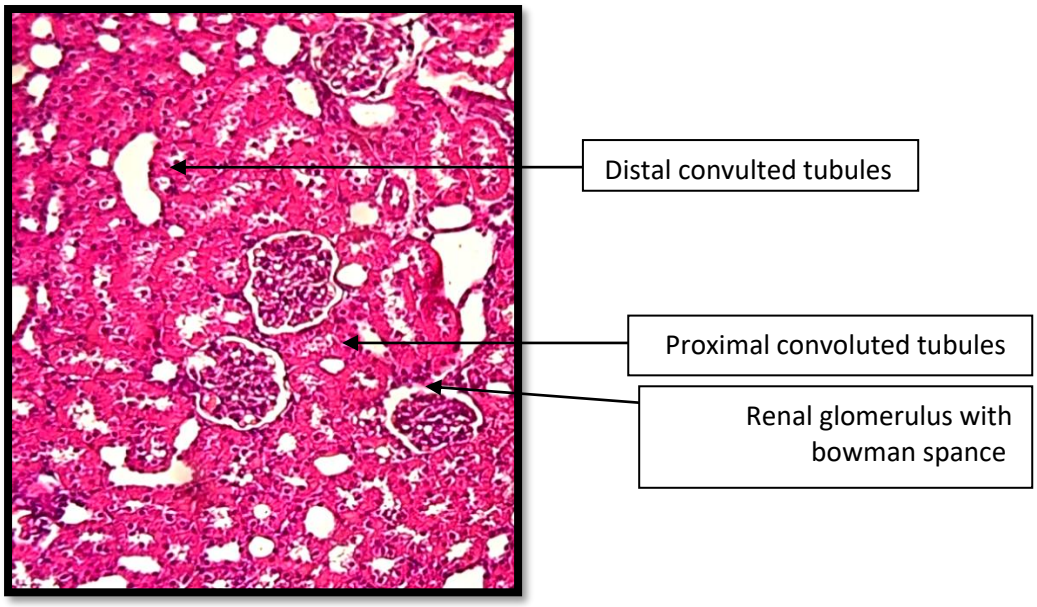
Table (4-4) Pearson's correlation coefficient (r) among all studied parameters

	erythropoietin	*erythroferron*	*ferritin*	*iron*	*hepcidin*	*urea*	*creatinine*	*RBC*	*PCV*	*Hb*
erythropoietin	1	.77**	-.54**	.53**	-.54**	-.78**	-.79**	.73**	.50*	.89**
erythroferron		1	-.51*	.19	-.42*	-.71**	-.74**	55**	.39	.69**
ferritin			1	-.51*	.37	.68**	.67**	-.48*	-.34	-.62**
iron				1	-.34	-.42*	-.51*	.73**	57**	.60**
hepcidin					1	.37	.61**	-.51**	-.27	-.55**
urea						1	.82**	-.71**	-.36	-.84**
creatinine							1	-.69**	-.52**	-.90**
RBC								1	.56**	.74**
PCV									1	.55**
Hb										1

4.5 Histological changes

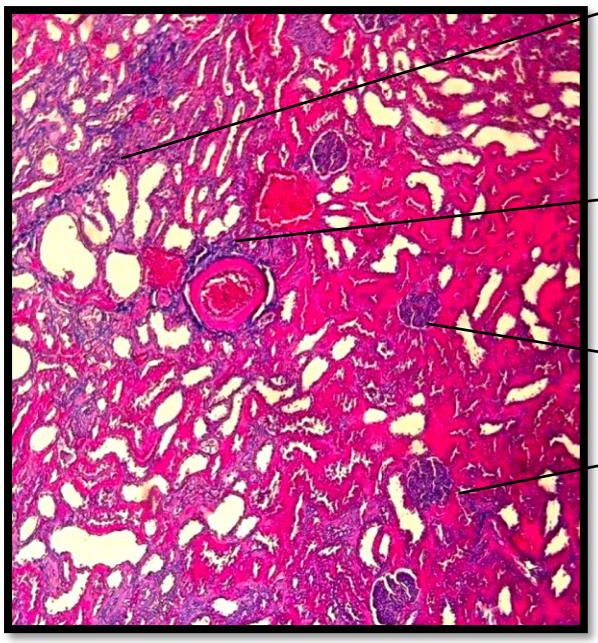
4.5.1 Kidney

Control group histological section appears normal in outlines defined renal tubules with glomeruli are found in figure (4-1)



Figur (4-1) Normal kidney with renal glomerulus , bowman capsular space , normal convoluted tubules stain with(H&E) (10X)

In adenine treated group (GIII) of male rats show inflammatory infiltrate of inflammatory cells with glomeruli and degenative changes occurrence in addidition to desquamation and flattening of epithelial cells, fibrin erxtravasation into bowoman's capsule as in figure (4-2), and necrotic lesion in renal glomeruli in figure (4-3) stained with masson’s trichrome stain stain , the adenine crystals formation in which it appear precipitated in the walls of the renal tubules with cell infiltration in Figure (4-4) stain with hematoxylin and eosin stain .



Fiuger (4-2) : Chronic tubulointerstitial nephritis, with precipitation of adenine material and a moderate number of cellular infiltration of inflammatory cells ,most of the renal tubules showed s desquamation and flattening and fibrosis of epithelial cells,also swelling glomeruli and decrease visceral epithelial cells. (Masson's trichrome stain) (40X)

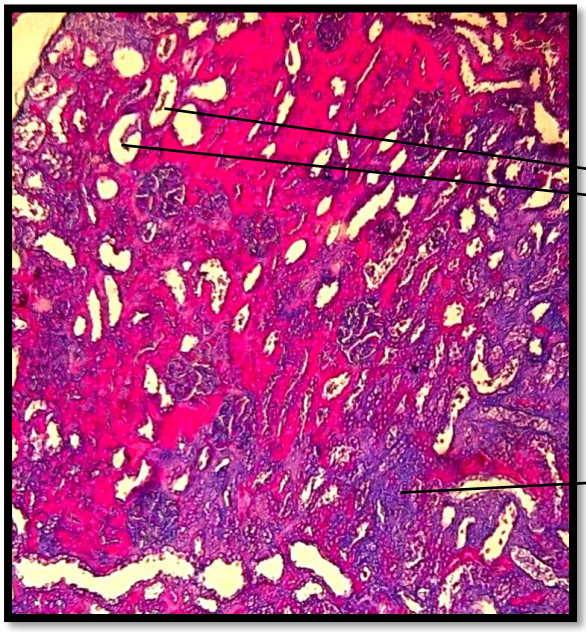
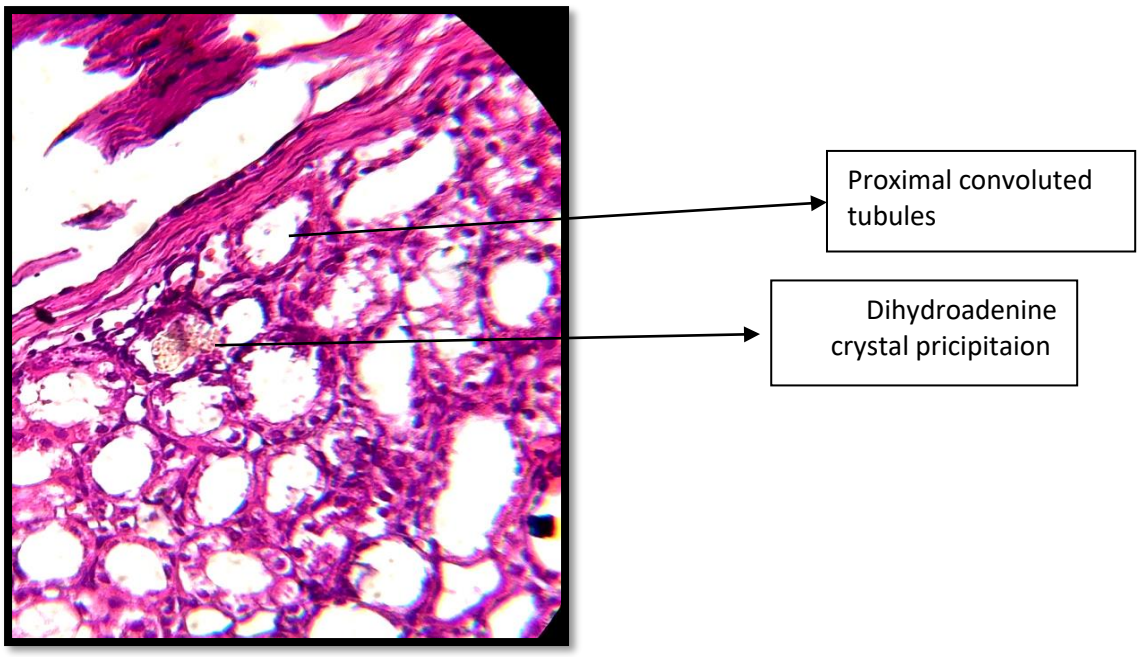


Figure (4-3) we notice that there is inflammatory lesions and necrosis of renal glomeruli in addition to adenine material precipitation stain with (Masson's trichrome stain) 40X



Fiuger (4-4): White arrows show the adenine dihydroadenine crystal formation in which it appear precipitated in the walls of the renal tubules with H&E staine (40X)

In adenine with moringa treated group we can notice that lesser extent of inflammatory cells reduce in swelling but necrosis and desqumation are obviously clear in Figure (4-5)

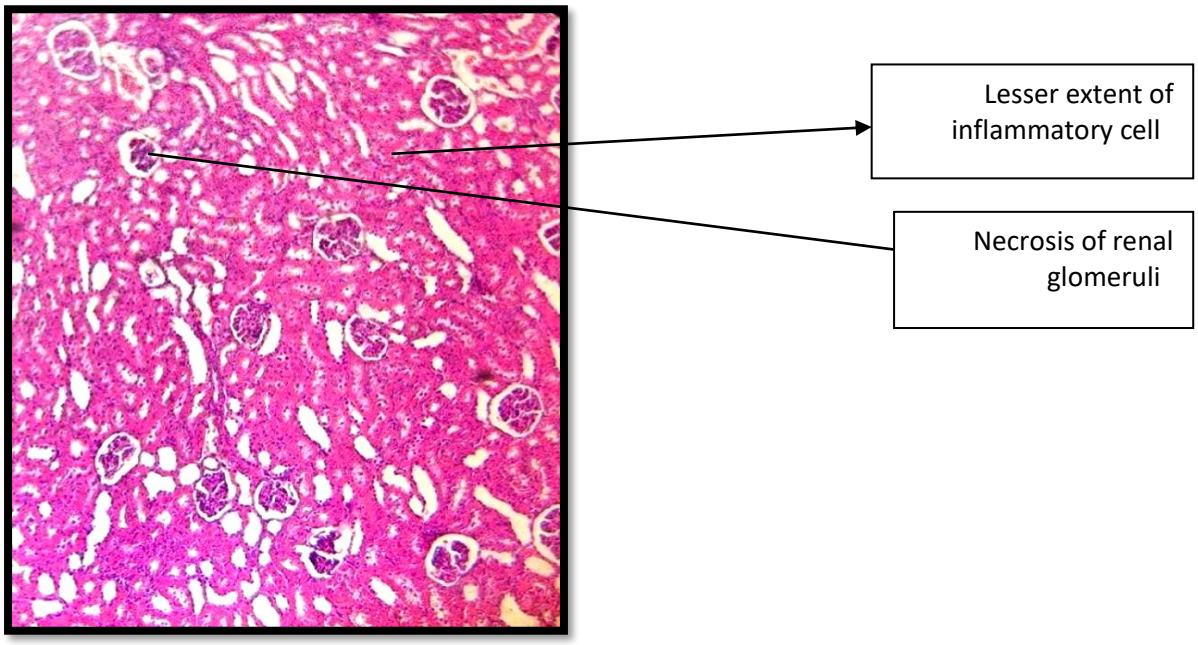
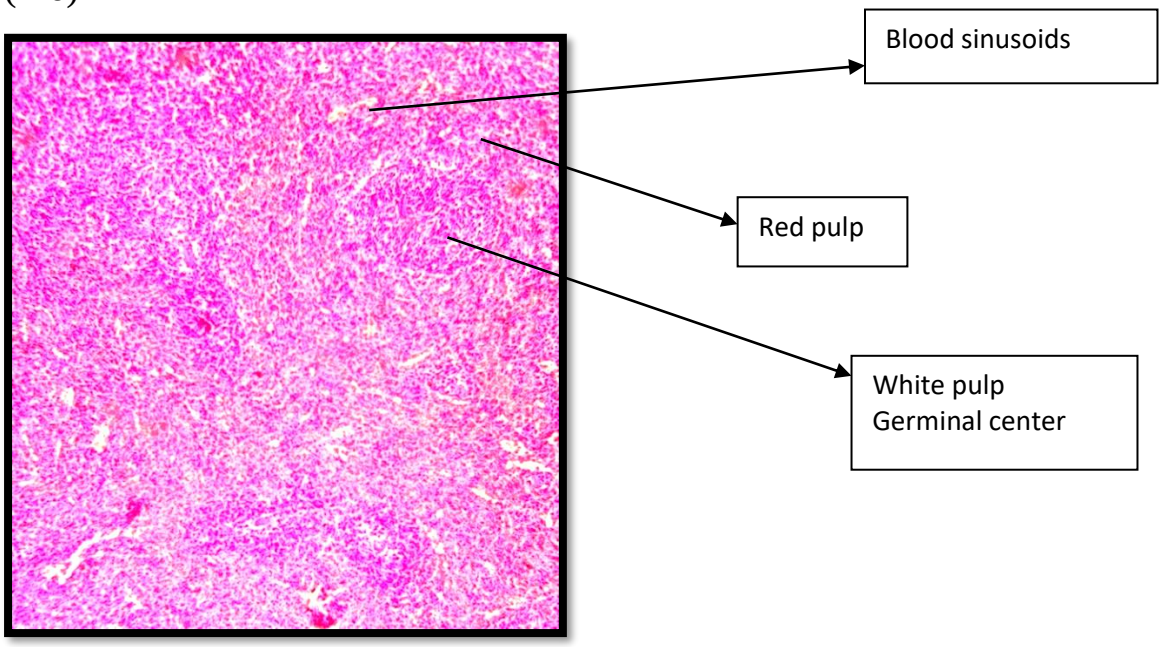


Figure (4-5) : The black glomerular of kidney are reduced in inflammation condtion but cellularity of the glomerular remain necrotic . H&E staine (40x)

4.5.2 spleen

Well defined spleen normal health Structure in the Control group male rats ,Red pulp and white pulp germinal center are clearly normal with peripherally red pulp positioned and centrally located white pulp. Figuer (4-6)



Fiuger (4-6) :Structure of spleen in the Control group male rats ,Red pulp blood sinusoids and white pulp germinal center. The spleen showed peripherally positioned red pulp and centrally located white pulp. (H&E)(10X magnification power)

In adenine group lymphoid follicles with germinal centers are seen, Stromal cells are spindle to polygonal in shape and have eosinophilic cytoplasm and ovoid nuclei with euchromatic chromatin, Necrosis of splenic constituents is Stained with hematoxylin and eosin stain characterized by cell swelling, condensation and dissolution of the nucleus, and cell lysis with accumulation of abundant eosinophilic cytoplasmic and karyorrhectic nuclear debris in Figure (4-7) randomly distributed of multiple, focal aggregates of inflammatory cells that are seen in the spleen, typically are not grossly apparent, other associated

changes, such as red pulp degeneration, necrosis, pigmentation, or vascularchanges in (4-8)

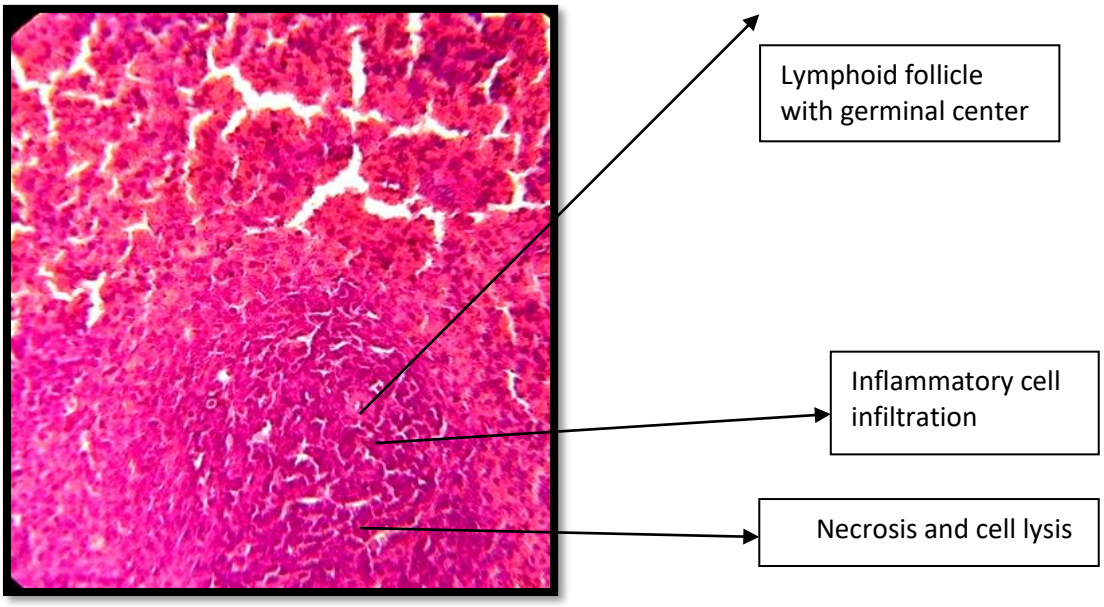


Figure (4-7) :lymphoid follicles with germinal centers are seen (black arrow), Necrosis of splenic constituents is characterized by cell swelling, condensation and dissolution of the nucleus, and cell lysis with accumulation of inflammatory cells .H&E staine (40x)

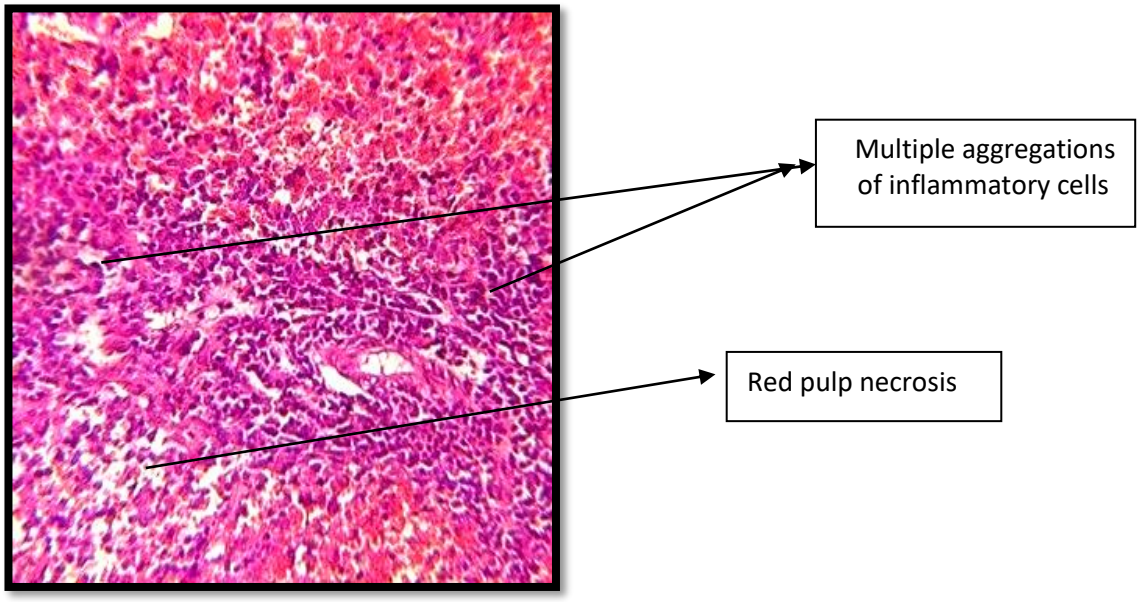


Figure (4-8) : The white arrow showed multiple, focal, randomlydistributed aggregates of inflammatory cells that are seen in the spleen, typically are not grossly apparent, other associated changes, such as red pulp degeneration, necrosis, pigmentation, or vascularchanges. H&E staine (40x)

Spleen in adenine with moringa treated group are becoming lesser extent for inflammatory cells infiltration with an increase in hematopoietic cells,multi-lineage blood cells from a small pool of hematopoietic stem or progenitor cells predominantly erythrocytic series, occurs occasionally in the red pulp in (4-9)

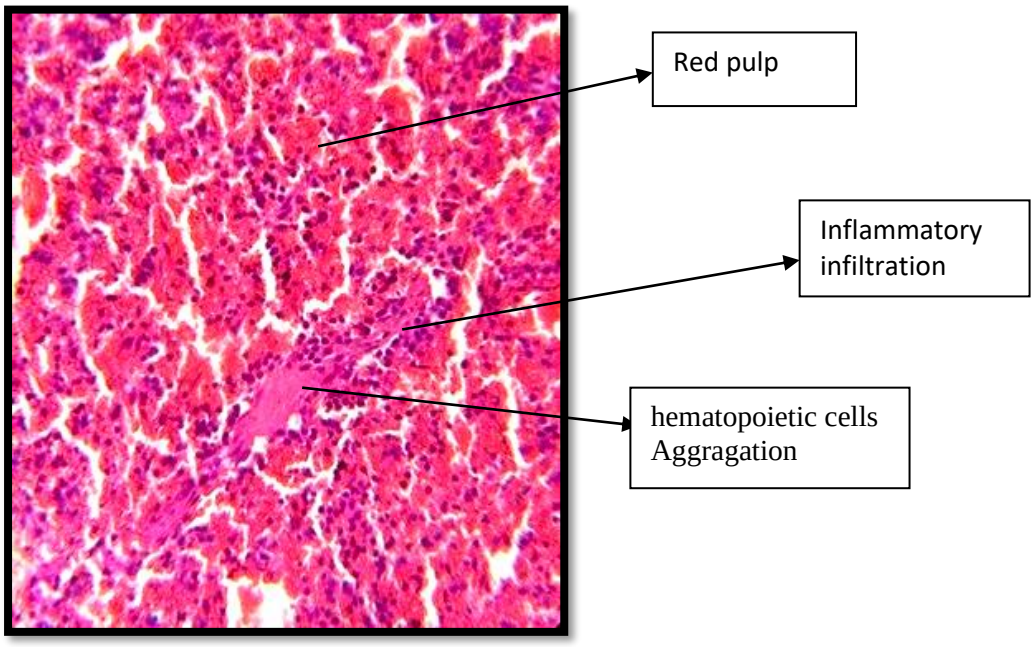
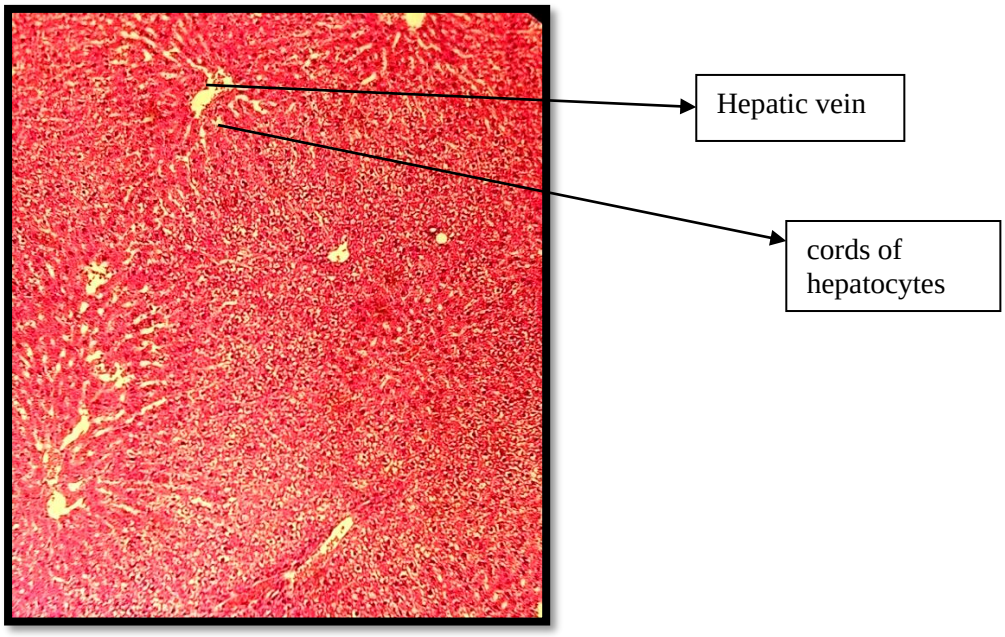


Figure (4-9) :Spleen in adenine and moringa treated group low in inflammation aggregation with an increase in hematopoietic cells,multi-lineage blood cells from a small pool of hematopoietic stem or progenitor cells H&E Staine (40x)

4.5.3 liver

Normal liver showing a central vein and zone surrounding them by cords of hepatocytes and separated by sinusoids in control treated group in male rats (4-10)



Fiuger (4-10) : Normal liver in the control group ,arrow showing central vein and zone surrounding them by cords of hepatocytes and separated by sinusoids. (H&E)(10X magnification power)

In adenine treated group hematoxylin and eosin stain appear a gathering of inflammatory cells, may be accompanied by evidence of hepatocellular necrosis, degeneration of cells, in addition to vascular injury as in figure (4-11)

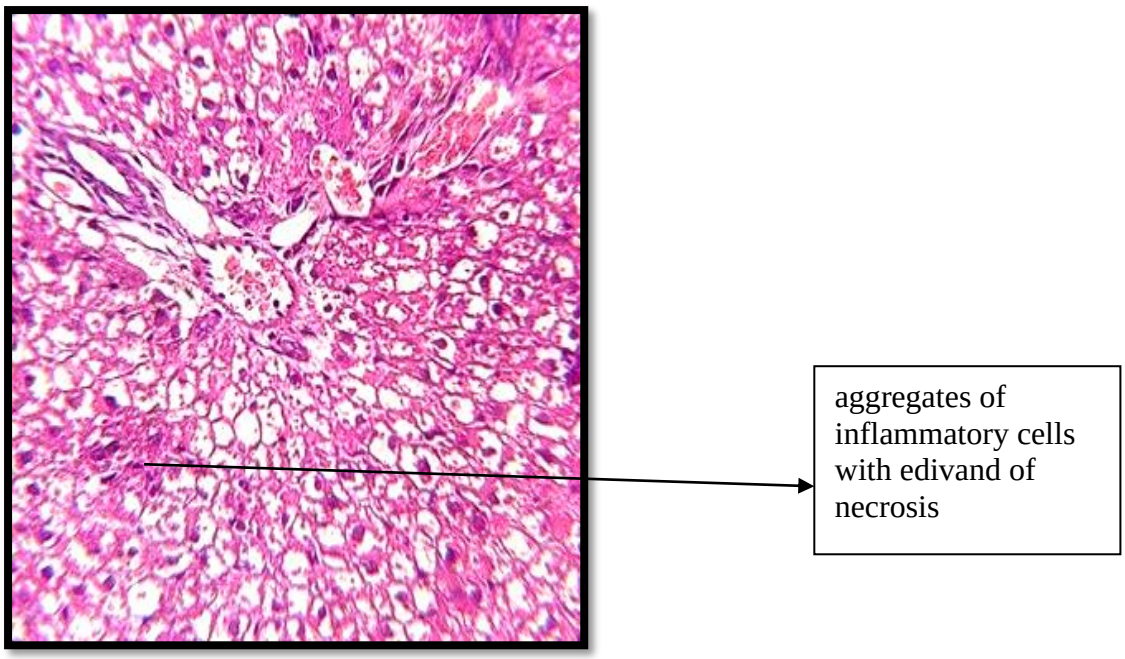


Figure (4-11) in adenine induced chronic renal failure white arrow showed randomly distributed aggregates of inflammatory cells that are seen in the liver these inflammatory cell aggregates may be accompanied by evidence of hepatocellular necrosis, inflammatory cells with other evidence of an inflammatory process, degeneration of cells, and evidence of vascular injury .H&E staine (40x)

In figure (4-12) Adenine with moringa treated group show Liver to be retained in normal appearance with centrally vein position and normal hepatocyte. Stain with hematoxylin and eosin stain.

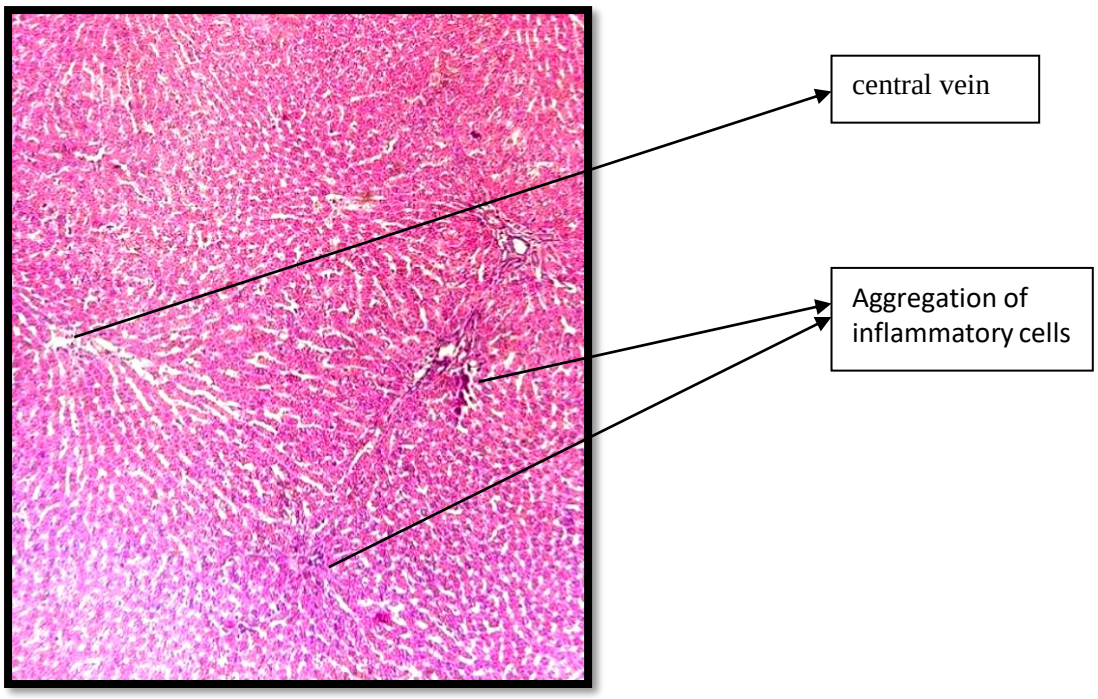


Figure (4-12) : Liver in adenine and moringa treated group appearl small amount of inflammatory cells this picture showed central vein with few aggregates of infimmation . H&E Staine (40x)

Chapter Five

Discussion

5. Discussion

5.1 Effect of Moringa Oleifera Leaves Powder on Some Serum on kidney function tests in male rats with Induced CRF

5.1.1 Urea concentration in serum

The result in table (4-1) showed a significant increase in serum urea in adenine treated group as compare with other groups.The present study agreed with result conducted by **Ikizler et al.,(2004) ; D'Apolito et al.,(2010) ;Alatriste et al.,(2014)** and **Bouatra et al.,2013)**

Urea nitrogenous compounds usually released from the blood by the kidney into the form of urin ,renal dysfunction results in accumulation of urea in blood circulation as it is obvious in chronic renal failure conditions in high levels .

The urea cycle occurs when deamination pathway takes place, after the liver degredate amino acids to produce ammonia which is very toxic, furthermore, it can be highly fatal if it is accumulated in the body;good thingcarrier molecules and enzymes in the liver rapidly change it to urea. The urea cycle takes two molecules of ammonia and one molecule of carbon dioxide, to form one molecule of urea and create one molecule of ornithine for the cycle to start again.(**Kajimura et al.,2006)**

moringa oleifera leaves administration works on develop of the results that show the fall in urea concentrations better than before a significant

decrease in serum urea in group (GIV) combined adenine with moringa in comparsion with adenine group .This agrees with the results of Moringa oleifera in supplement nutrition prevent the nickel-induced changes to the urea concentrations which provide protection toward nickel-induced nephrotoxicity(**Adeyemi, and Elebiyo, 2014).**

5.1.2 Creatinine concentration in serum

The data in table (4-1) showed a significant increase in serum creatinine in adenine treated group as compared to other groups .The result of present study agrees with results of **Syme et al.,(2006);Lassnigg et al.,(2008)** and **Herget-Rosenthal et al.,(2004)**

When there is kidney failure or kidney damage, the kidneys are unable to filter waste effectively ,as though ,it will lead to increase in creatinine concentrations in the bloodCreatinine is a product released by muscles from the degredation of a compound named creatine. Creatinine is cleared from the body by the kidneys, which filter high levels of it from the blood and cleared it into the urine. **(Cirillo et al ,. 2006**).

Creatine is protion of the cycle that generate energy required to contract muscles; Both creatine and creatinine are made by the body at a relatively constant rate, Since nearly all creatinine is filtered from the blood by the kidneys and cleared into the urine, blood concentrations are commonly a good marker of performance rate of the kidney (**Ishikawa et al., 2010).**

When GFR suddenly fall by 50%, the kidneys will shortly filter and release only half as much creatinine,resulting in aggregation of creatinine in the body fluids and increasing in plasma levels **(Waikar, and Bonventre, 2009).**

Furthermore GFR reduced to one-fourth normal, plasma creatinine would rise to approximate 4 times normal, and a fall in GFR to one-eighth normal would increase plasma creatinine to 8 times normal so, under

constant conditions the creatinine release rate match therate of creatinine manufacturing , although the normal rate of creatinine excretion take place at the raise of high plasma creatinine levels **(Avesani et al ., 2004).**

A significant decrease inserum creatinine concentrations in group adenine and moringa comprasion with adenine group which agrees with **(Ejerblad et al.,2006 ;Ali et al.,2013)**

that the disturbance of plasma electrolyte imbalance may influence the pH, osmolality, and blood volume which may lead to renal dysfunction or antherbody disturbances. *M.oleifera* has to own diuretic effect (**Mbikay, 2012).** The balance of normal level of plasma sodium and potassium by *M. oleifera* is regarded with therapeutic potential having antimicrobial effect which include the flavinoids material server as antimicrobial . the development of renal tissues we think because of qurectin material as active ingredient in moringa leaves may help in improvement of the results and body condition in general (**Anwar et al.,2007**).It appears that , at 10 and 15% *M. oleifera* administration may assist in maintaining balance in blood plasma electrolyte levels

(**Adeyemi and Elebiyo, 2014; Vergara-Jimenez et al.,2017)**

5.2.Effect of Moringa Oleifera leaves powder on Complete Blood Corpuscles in male rats with Induced CRF

The data in table (4-1) showed a significant($p \leq 0.05$) decrease in RBC count ,PVC and HB in adenine group as compared with other groups .The results of study agree with result of **Amin et al.,(2014)**

Which occur ,Through dialysis HB concentrations are decreased to critical levels ,that results in anemia (**Dorgalaleh et al.,2013)** happen during anemia condition in patients with acute and chronic renal failure

results revealed reduction in RBC count and HB levels and (**Tomosugi et al.,2006**)It is occur due to pathogenisis of renal anemia .

However after Moringa oleifera leaves administration we can see clear result that describes by increasing in RBC count value with little extent for PVC count and Hb count in (GIV) group moringa oleifera is rich with high nutritional supplements such as (iron ,calcium and vitamins) which is one of the essential component in synthesis of hemoglobin in red blood cells.(**Sajidu et al.,2005)**

furthermore it is affluent with antioxidants that may assist in maintainance of activity of RBC in various body metabolisms in addition to minimizing the risk of hypochromic anemia (**Sreelatha and Padma, 2009**; **Ghebreselassie et al.,2011)**

5.3 Effect of Moringa Oleifera Powder on Iron homeostasis parameters in male rats with Induced CRF

5.3.1 Erythropoietin

The result in table (4-3) showed significant($p \leq 0.05$) decrease of serum erythropoietin in adenine group .The present study agrees with results conducted by **Di Iorio et al.,(2003) ; Hayashi et al.,(2000)** and **Drüeke and Eckardt ,(2002**)

There is fall in levels of EPO in the adenine induced CRF in table(4-3) that reflect the incidence of renal dysfunction

Erythropoietin is a glycoprotein cytokine produced chiefly by the kidney in response to cellular hypoxia; it initiates red blood cell production (erythropoiesis) in the bone marrow ,

Erythropoietin is generated by interstitial fibroblasts in the kidney in close relation with the peritubular capillary and proximal convoluted

tubule. It is also produced in perisinusoidal cells in the liver.(**Tam et al.,2006)**

Usual conditions of cellular hypoxia resulting in raising in levels of EPO include any anemia, and hypoxemia ,Renal dysfunction results in reduction in EPO concentrations that lead to ,Low EPO levels cause red blood cell count to fall and anemia to develop.(**Tong and Nissenson ,2001)** Generally cases with kidney disease will posses anemia which occur in early in the course of kidney disease and grow worse as kidneys fail and can no longer make EPO.(**Palazzuoli et al.,2006)**

There is great improvement in result after moringa oleifera leaves uptake that shown in group (GIV) in same table (4-3). That agrees with results conducted by**(Campana and Myers, 2001)** moringa oleifera may assist in keeping further homeostatic balance by supporting Erythropoietin to fasten its effects by linking to the erythropoietin receptor (EpoR) , EPO link to the erythropoietin receptor on the red cell progenitor surface and activates a JAK2 signalling cascade.This starts the STAT5, PIK3 and Ras MAPK pathways .This makes differentiation, survival and growth of the erythroid cell ,SOCS1, SOCS3 and CIS are also stated which appear as negative regulators of the cytokine signal.(**Hodges et al.,2007) .**

5.3.2 Erythroferrone

The data in table (4-3) showed a significant($p \leq 0.05$) decrease in adenine group as compared to other groups .The results of present study agree with result of **Vallet (2018); Honda et al.,(2016) and Cucuianu et al.,2014)**

Because of Chronic renal failure and as a result of renal dysfunction their would be a disturbance in the hormones manufactured by the kidneys like erythropoietin which is regulate erythropoiesis in bone marrow in which this process involved in absorption of dietary iron

(Cody et al.,2001) .

since one of the most essential element of red blood cell manufacture is hemoglobin that is obtained from iron stores in the body **(Coates, 2014**),yet anemia occurs because erythroferone release is restricted and become in low concentrations that because of hepcidin hormone release from it stimulate the absorption of iron from alimentary track(**Kautz et al., 2014).**However in moringa treated adenine induced CRF group (GIV) show elevation in levels of erythroferrone see table

(4-3) (**Ndong et al.,2007),**Though moringa oleifera leaves provide a natural source for iron (**Koury and Haase,2015**) in addition to other supplements calcium ,minerals ,vitamins which assist in maintenance of biological homeostasis for iron insufficiency , More ever, it contains essential amino acid that simply enters in various body performance ; in fact, protein provides basic integrity in whole body constituents starting from simple carriers to complex units (**Moyo et al.,2011)**

5.3.3 ferritin

In the present study and according to table (4-3) there is sharp elevation in serum ferritin ($p \leq 0.05$) in adenine group as compared to control group these results agree with **Gülçelik and Kayataş ,(2002); Le et al.,(2008);**and **Tbahriti et al.,(2013)**

Chronic renal failure incidence probably assists in occurrence of anemia

Ferritin is an intracellular protein that holds iron and releases it in a controlled manner. In humans, it works as a buffer against low iron levels and iron overload, Ferritin is present in most tissues as a cytosolic protein(**Snow et al.,2011**);

However , small amounts are released into the serum where it's chief role as an iron carrier ,Plasma ferritin is also an indirect sign of the total

amount of iron stored in the body; serum ferritin is used as a diagnostic test for iron-deficiency anemia(**Wang et al.,2010)**

In fact chronic renal failure resulting in disturbances in hormonal released from kidney which later may effect onerythropoiesis ,when erythropoietin is the chief regulator of the red blood cells synthesis so as a result their would be reduction in this process, yet anemia occurs (**Khan and Amedia ,2008)**

However, body needs pure sores of iron to compensate that defect which causes increased levels of ferritin in the blood in the present study, yet moringa is considered as a natural source of iron in the living nature **(Saini et al.,2014)**

Free iron is toxic to cells as it represents as a catalyst in the establishment of free radicals from reactive oxygen species via the Fenton reaction. since vertebrates have complex set of protective mechanisms to bind iron in various tissue types(**Dixon and Stockwell,2014**), moringa oleifera came with additional advantage as it contains a natural source of antioxidant levels; further more, 25% than other dietary supplement(**Verma et al.,2009)**

And according to table (4-3) in the present study adenine induced chronic renal failure with moringa (GIV) show clear improvement in levels of ferritin ($p>0.05$) which is significantly decreased as in comparson to adenine induced chronic renal failure group (GIII) due to it may diminish before been used by the target cells whether it execrated with waste materials or not fully absorbed from alimentary tract or other related conditions (**Hutchinson et al.,2007)**

5.3.4 Serum Iron

In table(4-3) there is reduction in adenine treated group in comparison to other groups in the created study which agrees with **Phan et al.,(2013); Aggarwal et al.,(2003) and Srai et al.,(2010)**

Iron lack is the most ordinary nutritional deficiency around the world, although if the lack of iron is not sufficiently compensated it may results in a state of latent iron deficiency , which eventually leads to iron-deficient anemia (**Killip et al.,2007)**

Anemia tends to start to develop in the initial stages of CKD, Anemia could get worse as CKD developed **(Yamaguchi-yamada et al.,2004).**
In compaired when renal system having chronic renal insufficiency they do not generate adequate EPO as so, the bone marrow produce fewer red blood cells resulting in anemia.(**Spandou et al.,2006)**
Adult human includes about 4 grams (0.005% body weight) of iron, mainly hemoglobin and myoglobin,which perform essential roles in metabolism, oxygen transport by blood and oxygen storage in muscles **(Zingg et al.,2002).**

Iron-having proteins that take place in transport, storage and used of oxygen; further more ,Iron proteins are participate in electron transfer However about three quarters of hemoglobin remains in constant levels, but this percent is equal to about one milligram of iron being absorbed each day, as the human body needs to recycle its own hemoglobin for the iron reduced levels (**Coates,2014)**

Although ,there is significant improvement in the values of the adenine - moringa treated group (GIV), we can see vigorous elevation ($p \leq 0.05$) in serum iron levels , due to moringa oleifera leaves probably assist for the reduced amount of iron in addition to moringa; it probably works through dietary factor to provide non-heme iron(**Idohou-Dossou et al.,2011**)
through by which After absorption enter in series of biological regulations ,The chief element of this regulation is the protein transferrin, that binds iron ions that have been absorbed from

the duodenum and transport it in the blood to cells.(**Conrad and Umbreit ,2002)**

Transferrin, includes Fe^{3+} in the middle of a distorted octahedron, linked to one nitrogen, three oxygen's and a chelating carbonate anion that traps the Fe^{3+} ion , it has such a great stability constant that it is quite efficient at taking up Fe^{3+} ions even from the most stable complexes.

(**Conrad and Umbreit ,2000)**

At the bone marrow, transferrin is reduced from Fe^{3+} and Fe^{2+} and kept as ferritin to be incorporated into hemoglobin. (**Yang et al.,2002)**;

however ,not all nonheme iron sources are absorbed sometimes it may depend on keeping a homeostasis between absorption inhibitors and enhancers which depends on iron condition. **(Theil,2011)**

In addition many other factor attributed to iron absorption such as ascorbic acid which can be found in the moringa leaves (**Abdel-Latif et al.,2018**) because of it's capacity to reduce iron from ferric to ferrous as well as it's efficiency to chelate iron; we should notice that vitamin C is the chief enhancer of iron absorption in vegetarian diet

(Teucher et al., 2004).

Moringa oleifera leaves are loaded with wide variety of nutritive elements like ascorbic acid manganese, iron and selenium fatty acids were observed with α-Linolenic acid Vitamin E had the highest levels of 77 mg/100 g than beta-carotene, which had 18.5 mg/100 g in the dried moringa leaves.(**Moyo et al.,2011)** that may help in improve anemia particularly in addition to general aspect that may be related to similar instances .

5.3.5 Hepcidin

Table (4.3) showed a significant($p \leq 0.05$) increase in serum hepcidin in adenine group as compare with other groups. The results of the present study agree with **Malyszko et al .,(2006); Ribeiro et al.,(2016) and El-Shafie et al.,(2015**)

Hepcidin is a main regulator of the access of iron into the blood stream in mammals ,Hepcidin gene transcription is encouraged by the dual effects first of hepatic iron stores and second the amount of plasma holotransferrin (iron-saturated transferrin), carried through iron of bone morphogenetic proteins (BMP) that influence on BMP receptors and the related Smad pathways.**(De Domenico et al.,2007 a)**

The hepcidin gene promoter includes BMP-responsive factor that links nuclear Smad complexes to strongly elevate transcription,the levels of the BMP ligand (in mice mainly BMP-6) show to be managed by hepatic iron stores.(**Paul et al.,2008)**

Elevation holotransferrin levels also potentiate the BMP receptor signaling by a partially defined mechanism include transferrin receptors 1 and 2 and HFE (a membrane protein that cooperated with transferrin receptor 1) (**Ramey et al.,2009)**

The main act of HFE was recognized by finding that it is mutated in the most prevalent type of hereditary hemochromatosis, a condition in which iron balance is dysregulated.(**Bridle et al.,2003**)

BMP signaling also relies on hemojuvelin, a glycophosphatidylinositol-linked, iron-related co-receptor for BMPs. **(Xia, et al.,2008**)

By infections or any other inflammatory conditions hepcidin release is potentially intended , results in characteristic hypoferremia of inflammation.(**Ganz, 2003)**

In progressed renal diseases iron metabolism appears severely disrupted by Varity number of mechanisms **(Babitt and Lin,2012**) , However

iron lack is already discovered in most of non–dialysis-dependent patients with chronic renal disease ; Iron deficiency is possibly a consequence of reduced iron uptake that results from high hepcidin levels also elevated iron losses, usually from gastrointestinal hemorrhage High hepcidin levels are caused partially by inflammation related to pathogenesis of Varity kidney diseases (**Ganz and Nemeth, 2016**)

However Great changes in the expression of liver hepcidin can be noticed in moringa treated with adenine (GIV) which appears significant reduction($p \leq 0.05$) in hepcidin levels in table(4-3) the iron in Moringa oleifera leaves would overcome the act of anemia and encourage the expression of iron-responsive genes rather than conventional iron supplements.(**Airaodion et al.,2019**)

Since Hepcidin is a chief manager of iron metabolism ,it would suppress iron transport by linking to the iron export channel ferroportin which is located on the basolateral surface of gut enterocytes and the plasma membrane of reticuloendothelial cells (macrophages) **(De Domenico et al.,2007 b)**.

By suppressing ferroportin, hepcidin prevents enterocytes from permit iron into the hepatic portal system, as a result reducing nutritional iron absorption. (**Aeberli et al.,2009)** iron deficiency and iron deficiency anaemia are the main causal aspects of the failure to uptake iron adequately from the gut system(**Zimmermann et al.,2008**) ,since hepcidin increasd levels block enteral absorption

(Sonnweber et al.,2012) ,yet moriga oleifera are able to give the answers by which Moringa provide a host of vitamins and minerals in addition to iron Vitamin C , Folic Acid , Vitamin B12(**Idohou-Dossou et al.,2011)**

Iron is notably hard for the body to be absorb especially from through duodenal lining site , so it is need to eat foods that include vitamins in addition to iron supplement that will help body retain iron ,whether the attempt to prevent anemia or considerably increase iron concentrations , adding Moringa to diet is essential.(**Yang et al.,2006 A)**

5.3.6 Correlation between some parameters

Erythropiotein list highly ($P<0.001$) positive correlation with erythoferrone ,iron, RBCs count, PCV and Hbv in table (4-4) ,although it is negatively correlated with kidney function test(**Wagner et al.,2011)**

Many reports show that iron is essential element in all body active mechanisms since it is used in energy production and tissue respiration(**Theil and Goss ,2009**),yet the increase in urea and creatinine results in accumulation in waste product in usual compensatory mechanisms of homeostatic balance yet chronic kidney failure is developed which agrees with(**Dziedzic et al.,2003).**

erythropoietin was negatively correlated with ferritin, hipcidine in table(4-4**)(Teke et al.,2017)**,due to that iron reduced levels in the body would result in ferritin proteins to be free unbounded with iron(Shi, et al.,2008) also low levels of iron would stimulate hepcidin elevation in order to secrete iron from it stores in the body and that occurs because of chronic renal dysfunction in the first place (**Eleftheriadis et al .,2009)**

Erythroferrone, generate in erythroblasts, it appears as inhibiting hepcidin ,and so offer more free iron for hemoglobin synthesis in case of stress erythropoiesis.**(Kautz et al.,2014)**

Hepcidin manufacturing and release by the liver is under the control of iron levels within macrophages, which assist in release of hepcidin in to

circulation by various proteins such as hemojuvelin , transferrin receptor 2**(Formanowicz et al.,2013)**

Within special conditions where the hepcidin concentrations are abnormally elevated like renal failure , serum iron reduced because iron become arrested within macrophage and **liver(Ribeiro et al.,2016)**;further more , reduction in duodenal iron uptake , this generally leads to anemia due to insufficient amount of serum iron levels that is required for erythropoiesis**(Kato,2010)** .

5.4 histological changes

5.4.1 Kidney

In adenine treated group (GIII) inflammatory infiltration,degenerative changes and interstitial fibrosis are observed , dihydroadenine crystal present in urinary tubules in comparson to control group in figure (4-1) the development of inflammation started when adenine given in huge doses that exceed the ability of the body clearing system to eliminate the waste materials , it tends to accumulate into the excreted systems to find possible way to get out especially renal system therefore inflammation is possible idea in these cases especially when adenine crystal is manufactured **(Eto et al.,2005)**

Infiltration of inflammatory cells (**Boon et al.,2015** ; **Hayashi, et al.,2017)** which acts as a sgin of renal ischemia (renal injury), atrophied and flattening of epithelial cells(**Diwan et al.,2013**), which are developed from the releasing of high levels of serum creatinine (**Faurschou et al.,2006) ,**chronic inflammation may result in degenative changes occurrence (**Al Za'abi et al.,2018**) ,as in figure (4-2)stain with

(Masson’s trichrome stain) shrinking and necrotizing in figure (4-3) lesion are observed (**El-Saft and Mohammed ,2017)**

Multiple capillary basement membrane breaks with extravasations fibrin into Bowman's space as well as break in Bowman's capsule(Hayashi, et al.,2017) glomerulare fibin deposition are important mediator of renal injury because of highly toxic substance and pro-inflammatory cytokine explain the infiltration inflammatory cells into renal tubules adenine uptake in high doses resulting in CRF development that results in limit the nitrogenous waste product to be execrated which in turn occluding of renal tubular system results in glomerulus to decrease visceral epithelial cells **(Fujii ,et al.,2007)**

xanthine dehydrogenase is substrate which can oxidize adenine into low solubility thus lead to renal damage (**Nemmar et al.,2016**) as so it tends to precipitate in renal tubules in form of crystals stain with hematoxylin and eosin stain in Figure (4-4) which agrees with (**Diwan et al.,2018)**

in adenine with moringa treated group(GIV) lesser extent of ivasion of inflammatory cells in kidney showed necrosis and degeneration are present in Figure (4-5) , antioxidant effect of moringa oliera leaves results in reducing the oxidative stress(**Sreelatha and Padma ,,2009**), in which the serum creatinine is reduced which is muscle metabolism by product and serum urea which is the nitrogenous waste product ,as so the combination of these products rather than inflammation resulting in sever renal dysfunction that (**Amin et al.,2014)**

moringa came as a cure therapy because of its protective effect which is rich in antioxidant that minimizes the free radical accumulation by series of reactions that lead to renal damage and ischemia (**Sreelatha and Padma ,,2009**),in addition moringa are rich with vitamine c that assists in getting rid of free radicals by which it metabolizes the nitrogen oxide

(NO) to become after long way of reactions into hydrogen peroxide to water and hydrogen by the catalase ,phenolic acide in moringa olifera leaves works on the scavenginge superoxide, as so free radical formation is reduced**(Yang et al.,2006 b)**

5.4.2 spleen

Adenine treated group (GIII) stain with hematoxylin and eosin stain in comparson to control group that appears normal in histology Figure (4-6) we can notice inflammatory lesions demonstrated by lymphoid follicles with germinal centers are seen(4-7), red pulp degeneration, necrosis, pigmentation other associated changes, such as or vascularchanges in (4-8) in the created study agree with **Akchurin et al.,(2019**) and **Kang,et al.,(2020)**Spleen white pulp forming the lymphatic tissue,red pulp composed of sinuses last with venules that anastmosis with venis and splenic cords**(Steiniger et al.,2011)**Cavities inside stroma related to splenic capillaries in the red pulp which is infiltrate with red and white blood cells ,damaged or defective blood cells are not to be able to be cross fromsplenic cord to reterun to circulation but remind in red pulp**(Pivkin et al ,2016)**.

Toxins from chronic renal failure waste product may affect indirectly on the spleen act **(Shunmin et al.,2003)** by which creatinine and urea can under go by the action of infiltration in the red pulp the diseased or damaged cells **(Kara et al.,2009)** that loaded with adenine accumulate in the splenic stroma (**Fujimori et al.,2004**) in Figure (4-7) Spleen histological section in adenine with moringa treated group (GIV) become little filtration with inflammatory cell with an increase in hematopoietic cells,multi-lineage blood cells from a small pool of hematopoietic stem or progenitor cells predominantly erythrocytic series, occurs occasionally in

the red pulp in (4-9) in the created study ,by which moringa antioxidant effect and protective effect for the red cells we hypothesized the quercetin material in this plant as antioxidant may support the activity of the red cells from being damaged or diseased with long lasting integrity **(Owolabi et al.,2014).**

5.4.3Liver

In adenine treated group appear a gathering in the liver this inflammatory cell aggregates may be accompanied by evidence of hepatocellular necrosis, degeneration of cells, in add to vascular injury in(4-11) in comparson to control group Normal liver showing a central vein and zone surrounding them by cords of hepatocytes and separated by sinusoids in (4-10) in the created study that agrees with **(Fujimori et al.,2004; Saad et al.,2018)**Sever renal failure prognosis developed into end –stage renal failure that effects indirectly on hepatic activity as a result of pro-inflammatory cytokines release and free radicals
(Lhotta,2002; Yeung et al.,2014)
Adenine administration in cases of induction of chronic renal failure may result in disturbance in uric acid metabolism that lead to rise in creatinine concentrations and blood urea nitrogen(**Wang et al.,2019)**
Adenine with moringa treated group show Liver looks with few scattered inglammatory cells appear with centrally vein position stain with hematoxylin and eosin stain Figure (4-12) as moringa oliefria leaves are rich in antioxidant**(El-bakry et al.,2016)** in addition to high nutritive values that minimize the risk of free radical we hypothesized that polyphenols such asflavinoids and phenolic acids may assist in formation and support the natural metabolic pathways in add to reduction toxins released from hepacto- renal injury (**Soliman et al.,2020**).

Chapter Six
Conclusions & Recommendations

6.1 Conclusions

From the created study result we get the following conclusions :

1. Moringa leaves powder at 5% can improve the Hb, PCV, RBC concentrations , serum iron ,Frritin content in blood which can improve the concentrations of urea and creatinine .
2. Erythroferrone hormone serum levels are positively correlated with erythropoietin level,which is regulated the release of hepcidin in high levels of iron in blood circulation and in contrast decrease the hepcidin levels in anemia related with induced chronic renal failure in male rats

6.2 Recommendations

1. Further researches on the moringa oliferia roles and possibility of it's active ingediant toward the body system vital processes

2. Determination to the real combination between erythopiotein and erythropiosis stimulation process

3. Further investigations of moringa with liver biomarker such as albumin levels

References

Abbasi, **M. H.; Fatima, S.; Naz, N.; Malik, I. A.& Sheikh, N**. (2013). Effect of nerium oleander (NO) leaves extract on serum hepcidin, total iron, and infiltration of ED1 positive cells in albino rat. BioMed research international, 2013.

Abdel-Latif, M.; Sakran, T.; Badawi, Y. K. & Abdel-Hady, D. S. (2018). Influence of Moringa oleifera extract, vitamin C, and sodium bicarbonate on heat stress-induced HSP70 expression and cellular immune response in rabbits. Cell Stress and Chaperones, *23*(5): 975-984.

Abdull Razis, **A. F.; Ibrahim, M. D.& Kntayya, S. B.** (2014). Health benefits of Moringa oleifera. Asian Pacific Journal of Cancer Prevention, *15*(20): 8571-8576.

Abellán, C. M.; Mangold-Gehring, S.; Micus, S.; Beddies, G.; Moritz, A.; Hartmann, E. & Eitner, F. (2019). A Novel Model of Chronic Kidney Disease in Rats: Dietary Adenine in Combination with Unilateral Nephrectomy. Kidney Diseases, *3*(3): 135-143.

Adeyemi, O. S.& Elebiyo, T. C. (2014). Moringa oleifera supplemented diets prevented nickel-induced nephrotoxicity in wistar rats. Journal of nutrition and metabolism, 2:8.

Aeberli, I.; Hurrell, R. F. & Zimmermann, M. B. (2009). Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable with normal weight children. International journal of obesity, *33*(10): 1111-1117.

Aggarwal, H. K.; Nand, N.; Singh, S.; Singh, M.& Kaushik, G. (2003). Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. The Journal of the association of physicians of india, 51: 170-174.

Airaodion, A. I.; Ogbuagu, U.; Ogbuagu, E. O.; Ekenjoku, J. A. & Airaodion, E. O. (2019). Protective Effect of ethanolic leaf extract of Moringa oleifera on haematological indices of rats fed with crude oil-treated diet. International Journal of Bio-Science and bio-technology, 11(8): 84-92.

Akchurin, O., Patino, E., Dalal, V., Meza, K., Bhatia, D., Brovender, ShanZhu, Y., Rundles,C., Perelstein ,E., JuhiKumar,Choie.E., & Rivella, S. (2019). Interleukin-6 contributes to the development of anemia in juvenile CKD. *Kidney international reports*, *4*(3), 470-483.

Al Za'abi, M.; Al Busaidi, M.; Yasin, J.; Schupp, N.; Nemmar, A. & Ali, B. H. (2015). Development of a new model for the induction of chronic kidney disease via intraperitoneal adenine administration, and the effect of treatment with gum acacia there on. American journal of translational research, 7(1): 28.

Al Za'abi, M.; Al Salam, S.; Al Suleimani, Y.;Manoj, P.; Nemmar, A., & Ali, B. H. (2018). Gum acacia improves renal function and ameliorates systemic inflammation, oxidative and nitrosative stress in streptozotocin-induced diabetes in rats with adenine-induced chronic kidney disease. Cellular Physiology and Biochemistry, *45*(6): 2293-2304.

Alatriste, **P. V. M.; Arronte, R. U.; Espinosa, C. O. G. & Cuevas, M. D. L. Á. E.** (2014). Effect of probiotics on human blood urea levels in patients with chronic renal failure. Nutricion hospitalaria, *29*(3): 582-590.

Ali, **B. H.; Al-Salam, S.; Al Husseni, I.; Kayed, R. R.; Al-Masroori, N.; Al-Harthi, T. & Nemmar, A**. (2010) A . Effects of Gum Arabic in rats with adenine-induced chronic renal failure. Experimental Biology and Medicine, 235(3): 373-382.

Ali, B. H., Al-Salam, S., Al Za'abi, M., Waly, M. I., Ramkumar, A., Beegam, S., ... & Nemmar, A. (2013) B. New model for adenine-induced chronic renal failure in mice, and the effect of gum acacia treatment thereon: comparison with rats. *Journal of pharmacological and toxicological methods*, *68*(3), 384-393.

Ali, B. H.; Madanagopal, T. T.; Ramkumar, A.; Boudaka, A.; Tageldin, M. H. & Nemmar, A. (2014). Some physiological and histological aspects of the gastrointestinal tract in a mouse model of chronic renal failure. Journal of pharmacological and toxicological methods, 69(2): 162-166.

Ali, B. H.;Adham, S. A.; Al Za'abi, M.;Waly, M. I.;Yasin, J.;Nemmar, A., &Schupp, N. (2015). Ameliorative effect of chrysin on adenine-induced chronic kidney disease in rats. PLoS One, 10(4).

Amin, N.**; Mahmood, R. T.; Asad, M. J.; Zafar, M.& Raja, A. M.** (2014). Evaluating urea and creatinine levels in chronic renal failure pre and post dialysis: a prospective study. Journal of cardiovascular disease, 2(2): 1-4.

Anjorin, T. S.; Ikokoh, P.& Okolo, S. (2010). Mineral composition of Moringa oleifera leaves, pods and seeds from two regions in Abuja, Nigeria. International Journal of agricuture & Biology, *12*(3) 431-434.

Anwar, **F.; Latif, S.; Ashraf, M.& Gilani, A. H**. (2007). Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21(1): 17-25.

Aperia, A. (2001). Regulation of sodium/potassium ATPase activity: impact on salt balance and vascular contractility. Current hypertension reports, 3(2): 165-171.

Asiedu-Gyekye, I. J., Frimpong-Manso, S. A. M. U. E. L., Awortwe, C., Antwi, D. A., & Nyarko, A. K. (2014). Micro-and macroelemental composition and safety evaluation of the nutraceutical Moringa oleifera leaves. *Journal of toxicology*, *2014*.

Atkins, **R. C.** (2005). The epidemiology of chronic kidney disease. Kidney international, *67*: S14-S18.

Avesani, **C. M.; Draibe, S. A.; Kamimura, M. A.; Cendoroglo, M.; Pedrosa, A.; Castro, M. L.& Cuppari, L.** (2004). Assessment of body composition by dual energy X-ray absorptiometry, skinfold thickness and creatinine kinetics in chronic kidney disease patients. Nephrology Dialysis Transplantation, 19(9): 2289-2295.

Babitt, J. L.; & Lin, H. Y. (2012).Mechanisms of anemia in CKD. Journal of the American Society of Nephrology, *23*(10): 1631-1634.

Baigent, C.; Burbury, K. & Wheeler, D. (2000). Premature cardiovascular disease in chronic renal failure. *The Lancet*, *356*(9224): 147-152.

Balogh, A.; Derzbach, L.& Vasarhelyi, B. (2004). Hepcidin, the negative regulator of iron absorbtion. Orvosi hetilap, *145*(30) 1549-1552.

Blantz, R. C.; Deng, A.; Lortie, M.; Munger, K.; Vallon, V.; Gabbai, F. B. & Thomson, S. C. (2002). The complex role of nitric oxide in the regulation of glomerular ultrafiltration. Kidney international, *61*(3): 782-785.

Boon, A. C.; **Lam, A. K.;Gopalan, V.; Benzie, I. F.;Briskey, D.;Coombes, J. S., & Bulmer, A. C.** (2015). Endogenously elevated bilirubin modulates kidney function and protects from circulating oxidative stress in a rat model of adenine-induced kidney failure. Scientific reports, *5*, 15482.

Boshuizen, M.; Binnekade, J. M.; Nota, B.; van de Groep, K.; Cremer, O. L.; Tuinman, P. R .& Juffermans, N. P. (2018). Iron metabolism in critically ill patients developing anemia of inflammation: a case control study. Annals of intensive care, 8(1): 1-8.

Bouatra, S.; Aziat, F.; Mandal, R.; Guo, A. C.; Wilson, M. R.; Knox, C .& Dame, Z. T. (2013). The human urine metabolome. PloS one, 8(9).

Bour, S.; **Visentin, V.; Prevot, D.; Daviaud, D.; Saulnier-Blache, J. S.; Guigne, C. & Carpene, C**. (2005). Effects of oral administration of benzylamine on glucose tolerance and lipid metabolism in rats. Journal of physiology and biochemistry, 61(2): 371-379.

Brady, P. G. (2007). Iron deficiency anemia: a call for aggressive diagnostic evaluation. Southern medical journal , 100(10):966-7.

Brauner, C. J. **& Wang, T.** (1997). The optimal oxygen equilibrium curve: a comparison between environmental hypoxia and anemia. American Zoologist, *37*(1): 101-108.

Bridle, K. R.; Frazer, D. M.; Wilkins, S. J.; Dixon, J. L.; Purdie, D. M.; Crawford, D. H. & Ramm, G. A. (2003). Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homoeostasis. The lancet, 361(9358): 669-673.

Caetano, E. R. S. P.; **Zatz, R.; Saldanha, L. B.& Praxedes, J. N**. (2001). Hypertensive nephrosclerosis as a relevant cause of chronic renal failure. Hypertension, *38*(2): 171-176.

Campana, W. M.; **& Myers, R. R.** (2001). Erythropoietin and erythropoietin receptors in the peripheral nervous system: changes after nerve injury. The Federation of American societies for experimental biology journal, 15(10): 1804-1806.

Cavill, I.; Auerbach, M.; Bailie, G. R.; Barrett-Lee, P.; Beguin, Y.; Kaltwasser, P .& Wilson, K. (2006). Iron and the anaemia of chronic disease: a review and strategic recommendations. Current medical research and opinion, 22(4): 731-737.

Chattopadhyay, S.; **Maiti, S.; Maji, G.; Deb, B.; Pan, B. & Ghosh, D.** (2011). Protective role of Moringa oleifera (Sajina) seed on arsenic-induced hepatocellular degeneration in female albino rats. Biological trace element research, 142(2): 200-212.

Cirillo, M.; Laurenzi, M.; Mancini, M.; Zanchetti, A. & De Santo, N. G. (2006). Low muscular mass and overestimation of microalbuminuria by urinary albumin/creatinine ratio. Hypertension, *47*(1): 56-61.

Coates, T. D. (2014).Physiology and pathophysiology of iron in hemoglobin-associated diseases. Free Radical Biology and medicine, 72: 23-40.

Cody, J. D.; **Daly, C.; Campbell, M. K.; Donaldson, C.; Grant, A.; Khan, I. & MacLeod, A. M**. (2001). Recombinant human erythropoietin for chronic renal failure anaemia in pre-dialysis patients. Cochrane Database of Systematic Reviews, (4).

Coffey, **R. & Ganz, T.** (2018). Erythroferrone: An erythroid regulator of hepcidin and iron metabolism. HemaSphere, *2*(2).

Conrad, M. E. & **Umbreit, J. N**. (2000). Iron absorption and transport—an update. American journal of hematology, *64*(4): 287-298.

Conrad, M. E. & **Umbreit, J. N.** (2002).Pathways of iron absorption. Blood Cells, Molecules, and diseases, *29*(3): 336-355.

Cucuianu, A.; **Patiu, M.; Trifa, A. P.; Tomuleasa, C. & Dima, D.** (2014). Redistribution of iron towards deposits in erythroblastopenic

anemia as a consequence of decreased erythroferrone production. Medical hypotheses, *83*(5): 530-532.

D'Apolito, M.; **Du, X.; Zong, H.; Catucci, A.; Maiuri, L.; Trivisano, T .& Brownlee, M**. (2010). Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. The Journal of clinical investigation, 120(1): 203-213.

Daba, M. (2016). Miracle tree: A review on multi-purposes of Moringa oleifera and its implication for climate change mitigation. Earth science & climatic change, *7*(4).

De Domenico, I.; **Ward, D. M. & Kaplan, J**. (2007) A. Hepcidin regulation: ironing out the details. The Journal of clinical investigation, 117(7): 1755-1758.

De Domenico, I.; Ward, D. M.; Langelier, C.; Vaughn, M. B.; Nemeth, E.; Sundquist, W. I .& Kaplan, J. (2007) B. The molecular mechanism of hepcidin-mediated ferroportin down-regulation. Molecular biology of the cell, 18(7): 2569-2578.

Diwan, V.; Brown, L., &Gobe, G. C. (2018).Adenine-induced chronic kidney disease in rats. Nephrology, *23*(1): 5-11.

Diwan, V.;**Mistry, A.;Gobe, G., & Brown, L**. (2013). Adenine-induced chronic kidney and cardiovascular damage in rats. Journal of pharmacological and toxicological methods, 68(2): 197-207.

Dixon, S. J.& Stockwell, B. R. (2014). The role of iron and reactive oxygen species in cell death. Nature chemical biology, *10*(1): 9.

Dobrica, M. B.; & Fillon, M. (2009).About the validity of Reynolds equation and inertia effects in textured sliders of infinite width. Proceedings of the Institution of mechanical engineers, journal of engineering tribology, 223(1): 69-78.

Dorgalaleh, A.; **Mahmudi, M.; Tabibian, S.; Khatib, Z. K.; Tamaddon, G. H.; Moghaddam, E. S .& Moradi, E**. (2013). Anemia and thrombocytopenia in acute and chronic renal failure. International journal of hematology-oncology and stem cell research, 7(4): 34.

Drüeke, T. B.;& Eckardt, K. U. (2002). Role of secondary hyperparathyroidism in erythropoietin resistance of chronic renal failure patients. Nephrology dialysis transplantation, 17(suppl_5): 28-31.

Dunn, S. R.; Qi, Z.; Bottinger, E. P.; Breyer, M. D.& Sharma, K. (2004). Utility of endogenous creatinine clearance as a measure of renal function in mice. Kidney international, *65*(5): 1959-1967.

Dziedzic, T.; Szczudlik, A.; Klimkowicz, A.; Rog, T. M. & Slowik, A. (2003). Is mannitol safe for patients with intracerebral hemorrhages? Renal considerations. Clinical neurology and neurosurgery, *105*(2): 87-89.

Ejerblad, E., Fored, C. M., Lindblad, P., Fryzek, J., McLaughlin, J. K., & Nyrén, O. (2006). Obesity and risk for chronic renal failure. *Journal of the american society of nephrology*, *17*(6), 1695-1702.

El Gendy, F. M.; **EL-Hawy, M. A.; Shehata, A. M. & Osheba, H. E.** (2018). Erythroferrone and iron status parameters levels in pediatric patients with iron deficiency anemia. European journal of haematology, 100(4): 356-360.

El-bakry, K.;**Toson, E. S.; Serag, M., &Aboser, M**. (2016). Hepatoprotective effect of Moringaoleifera leaves extract against carbon tetrachloride-induced liver damage in rats. World journal of pharmaceutical science , 5, 76-89.

Eleftheriadis, T.; **Liakopoulos, V.; Antoniadi, G.; Kartsios, C. & Stefanidis, I.** (2009). The role of hepcidin in iron homeostasis and anemia in hemodialysis patients. In Seminars in dialysis, 22(1): 70-77.

Eliopoulos, N.; Gagnon, R. F.; Francois, M. & Galipeau, J. (2006). Erythropoietin delivery by genetically engineered bone marrow stromal cells for correction of anemia in mice with chronic renal failure. Journal of the American society of nephrology, 17(6): 1576-1584.

El-Safti, F. E. N. A., **& Mohammed, S. A. (**2017).Light and electron microscopic studies of chronic renal failure using an adenine rat model. Menoufia medical journal, 30(1): 271.

El-Shafie, A. M.; **El-Mashad, G. M.; Hegran, H. H. & El-Deeb, M. M.** (2015). Serum hepcidin level in children with chronic renal failure either on hemodialysis or on conservative therapy. Menoufia medical journal, 28(2):571.

Estrella, M. C. P.; **Jacinto Bias III, V.; David, G. Z.; & Taup, M. A.** (2000). A double-blind, randomized controlled trial on the use of malunggay (Moringa oleifera) for augmentation of the volume ofbreastmilk among non-nursing mothers of preterm infants. The Phillippine journal of pediatrics, 49(1): 3-6.

Eto, N.; Miyata, Y.;Ohno, H., & Yamashita, T. (2005). Nicotinamide prevents the development of hyperphosphataemia by suppressing intestinal sodium-dependent phosphate transporter in rats with adenine-induced renal failure. Nephrology dialysis transplantation, 20(7); 1378-1384.

Fahey, J. W. (2005). Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for life journal, 1(5): 1-15.

Fanelli, C.;Noreddin, A., &Nunes, A. (2017). Inflammation in Nonimmune-Mediated Chronic Kidney Disease.In *Chronic* Kidney Disease-from Pathophysiology to Clinical Improvements.IntechOpen.

Farooq, F.; Rai, M.; Tiwari, A.; Khan, A.A. and Farooq, S.(2012) **:**Medicinal properties of Moringa oleifera: An overview of promising healer**.** Medicinal plants research**,** 6 (27): 4368–4374.

Faurschou, M.;**Starklint, H.;Halberg, P., & Jacobsen, S**. (2006). Prognostic factors in lupus nephritis: diagnostic and therapeutic delay increases the risk of terminal renal failure. The Journal of rheumatology, 33(8): 1563-1569.

Ferrucci, L.; **Semba, R. D.; Guralnik, J. M.; Ershler, W. B.; Bandinelli, S.; Patel, K. V .& Ganz, T.** (2010). Proinflammatory state, hepcidin, and anemia in older persons. *Blood*, The Journal of the American Society of Hematology, 115(18): 3810-3816.

Finney, H.; Newman, D. J. & Price, C. P. (2000). Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. *Annals of clinical biochemistry*, *37*(1): 49-59.

Fitriana, W. D.; **Ersam, T.; Shimizu, K. & Fatmawati, S.** (2016). Antioxidant activity of Moringa oleifera extracts. Indonesian Journal of Chemistry, 16(3):297-301.

Formanowicz, D.; **Kozak, A.; Głowacki, T.; Radom, M. & Formanowicz, P.** (2013). Hemojuvelin–hepcidin axis modeled and analyzed using Petri nets. Journal of biomedical informatics, 46(6): 1030-1043.

Fried, W. (1972).The liver as a source of extrarenal erythropoietin production. Blood, 40(5): 671-677.

Fuglie, L.J.(2001) . "The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics" *in* The Miracle Tree: The Multiple Attributes of Moringa, CTA Publisher,The Handbook of environmental chemistry book series 77:172.

Fujii, S.; **Zhang, L., &Kosaka, H.** (2007).Albuminuria, expression of nicotinamide adenine dinucleotide phosphate oxidase and monocyte chemoattractant protein-1 in the renal tubules of hypertensive Dahl salt-sensitive rats. Hypertension research, 30(10): 991-998.

Fujimori, H.; Ozaki, K.; Matsuura, T.; Matsushima, S.;Narama, I., & Pan-Hou, H. (2004). Effect of Iron Lactate Overloading on Adenine Nucleotide Levels and Adenosine 3′-Monophosphate Forming Enzyme in Rat Liver and Spleen. Biological and pharmaceutical bulletin, 27(9): 1371-1375.

Ganz, T. & Nemeth, **E**. (2011).Hepcidin and disorders of iron metabolism. Annual review of medicine, 62: 347-360.

Ganz, T. & **Nemeth, E.** (2016, March). Iron balance and the role of hepcidin in chronic kidney disease. In Seminars in nephrology ,36(2): 87-93.

Ganz, T. (2003).Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood, 102(3): 783-788.

Ganz, T. (2011).Hepcidin and iron regulation, 10 years later. Blood, The Journal of the American Society of hematology, 117(17): 4425-4433.

George-Gay, B. & Parker, K. (2003).Understanding the complete blood count with differential. Journal of perianesthesia nursing, 18(2): 96-117.

Ghebreselassie, D.: **Mekonnen, Y.: Gebru, G.: Ergete, W. & Huruy, K.** (2011). The effects of Moringa stenopetala on blood parameters and histopathology of liver and kidney in mice. Ethiopian Journal of health development, 25(1): 51-57.

Goodnough, L. T.; Nemeth, E.& Ganz, T. (2010). Detection, evaluation, and management of iron-restricted erythropoiesis. The Journal of the american society of hematology, 116(23): 4754-4761.

Gopalakrishnan, L.; **Doriya, K. & Kumar, D. S. (**2016). Moringa oleifera: A review on nutritive importance and its medicinal application. Food science and human wellness, 5(2):49-56.

Gülçelik, N. E. & **Kayataş, M.** (2002). Importance of serum ferritin levels in patients with renal failure. Nephron, 92(1), 230-231.

Guo, Y. P.; **Tang, B. S.; Liu, H. L.; Huang, J. J.; Xu, Q.; Sun, Q. Y. & Guo, J. F.** (2019). Impaired iPLA2β activity affects iron uptake and storage without iron accumulation: An in vitro study excluding decreased iPLA2β activity as the cause of iron deposition in PLAN. Brain research, 1712: 25-33.

Gupta, R. K. (2010). Medicinal and Aromatic plants. CBS publishers and distributors, 234: 499.

Guralnik, J. M.; **Eisenstaedt, R. S.; Ferrucci, L.; Klein, H. G. & Woodman, R. C.** (2004). Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood, 104(8): 2263-2268.

Hau, M., Kneitz, C., Tony, H. P., Keberle, M., Jahns, R., & Jenett, M. (2002). High resolution ultrasound detects a decrease in pannus vascularisation of small finger joints in patients with rheumatoid arthritis receiving treatment with soluble tumour necrosis factor α receptor (etanercept). *Annals of the rheumatic diseases*, *61*(1), 55-58.

Hayashi, S.;**Oe, Y.;Fushima, T.; Sato, E.; Sato, H.; Ito, S., & Takahashi, N.** (2017). Protease–activated receptor 2 exacerbates adenine–induced renal tubulointerstitial injury in mice. Biochemical and biophysical research communications, 483(1): 547-552.

Hayashi, T.; **Suzuki, A.; Shoji, T.; Togawa, M.; Okada, N.; Tsubakihara, Y.& Hori, M.** (2000). Cardiovascular effect of normalizing the hematocrit level during erythropoietin therapy in predialysis patients with chronic renal failure. American journal of kidney diseases, 35(2): 250-256.

Hebbel, R. P.; Vercellotti, G. M.& Nath, K. A. (2009). A systems biology consideration of the vasculopathy of sickle cell anemia: the need for multi-modality chemo-prophylaxis. Cardiovascular & Haematological Disorders-Drug Targets (Formerly current drug targets-cardiovascular & hematological disorders, 9(4): 271-292.

Heeney, M. M. **& Andrews, N. C**. (2004). Iron homeostasis and inherited iron overload disorders: an overview. Hematology/oncology clinics, 18(6): 1379-1403.

Herget-Rosenthal, S.; Marggraf, G.; Hüsing, J.; Göring, F.; Pietruck, F.; Janssen, O.& Kribben, A. (2004). Early detection of acute renal failure by serum cystatin C. Kidney international, 66(3): 1115-1122.

Higdon J; **Drake, VJ.; Wessling-Resnick M**, (2009).'Micronutrient Information Center – Iron', Linus Pauling Institute, Oregon, USA

Himmelfarb, J.; Evanson, J.; Hakim, R. M.; Freedman, S.; Shyr, Y. & Ikizler, T. A. (2002). Urea volume of distribution exceeds total body water in patients with acute renal failure. Kidney international, 61(1):317-323.

Hodges, VM.; **Rainey, S. Lappin ,TR. Maxwell, AP**. (2007). "Pathophysiology of anemia and erythrocytosis". Critical Reviews in Oncology/hematology. **64** (2): 139–58.

Hogg, R. J.; Furth, S.; Lemley, K. V.; Portman, R.; Schwartz, G. J.; Coresh, J.& Eknoyan, G. (2003). National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for

chronic kidney disease in children and adolescents: evaluation, classification, and stratification. Pediatrics, 111(6): 1416-1421.

Honda, H.; Kobayashi, Y.; Onuma, S.; Shibagaki, K.; Yuza, T.; Hirao, K. & Shibata, T. (2016). Associations among erythroferrone and biomarkers of erythropoiesis and iron metabolism, and treatment with long-term erythropoiesis-stimulating agents in patients on hemodialysis. PLoS one, 11(3).

Hovater, M. B.; **Olteanu, D.; Welty, E. A.& Schwiebert, E. M.** (2008). Purinergic signaling in the lumen of a normal nephron and in remodeled PKD encapsulated cysts. Purinergic signalling, 4(2): 109-124.

Hsu, C. Y.; **Ordonez, J. D.; Chertow, G. M.; Fan, D.; McCulloch, C. E. & Go, A. S.** (2008). The risk of acute renal failure in patients with chronic kidney disease. Kidney international, 74(1): 101-107.

Hurrell, R.& Egli, I. (2010). Iron bioavailability and dietary reference values. The American journal of clinical nutrition, 91(5): 1461S-1467S.

Hutchinson, C.; **Geissler, C. A.; Powell, J. J.; & Bomford, A**. (2007). Proton pump inhibitors suppress absorption of dietary non-haem iron in hereditary haemochromatosis. British Society of Gastroenterology , *56*(9): 1291-1295.

Idohou-Dossou, N.; Diouf, A.; Gueye, A. L.; Guiro, A. T.& Wade, S. (2011). Impact of daily consumption of Moringa (Moringa oleifera) dry leaf powder on iron status of Senegalese lactating women. African journal of food, agriculture, nutrition and development, 11(4).

Ikizler, T. A.; **Sezer, M. T.; Flakoll, P. J.; Hariachar, S.; Kanagasundaram, N. S.; Gritter, N .& Jonathan Himmelfarb for the PICARD Study Group.** (2004). Urea space and total body water measurements by stable isotopes in patients with acute renal failure. Kidney international, 65(2): 725-732.

Ishikawa, J.; Hoshide, S.; Eguchi, K.; Schwartz, J. E.; Pickering, T. G.; Shimada, K. & Kario, K. (2010). Masked hypertension defined by ambulatory blood pressure monitoring is associated with an increased serum glucose level and urinary albumin-creatinine ratio. The Journal of clinical hypertension, 12(8): 578-587.

Iskandar, I.; **Hadju, V.; As' ad, S. & Natsir, R.** (2015). Effect of Moringa oleifera leaf extracts supplementation in preventing maternal anemia and low-birth-weight. International journal of scientific and research publications, 5(2): 1-3.

Jelkmann, W. (2004).Molecular biology of erythropoietin. Internal medicine, 43(8): 649-659.

Jelkmann, W. (2007). Recombinant EPO production—points the nephrologist should know .Nephrology Dialysis Transplantation, 22, : 2749–2753

Jing, W.; **Jabbari, B. & Vaziri, N. D.** (2018). Uremia induces upregulation of cerebral tissue oxidative/inflammatory cascade, down-regulation of Nrf2 pathway and disruption of blood brain barrier. American journal of translational research, 10(7): 2137.

Kajimura, M.; **Walsh, P. J.; Mommsen, T. P.& Wood, C. M**. (2006). The dogfish shark (Squalus acanthias) increases both hepatic and extrahepatic ornithine urea cycle enzyme activities for nitrogen conservation after feeding. Physiological and Biochemical Zoology, 79(3): 602-613.

Kang, L.; **Miao, J. X.; Cao, L. H.; Miao, Y. Y.; Liu, H.; Xiang, L., & Song, Y. G.** (2020). Total glucosides of herbaceous peony (Paeonialactiflora Pall.) flower attenuate adenine-and ethambutol-induced hyperuricaemia in rats. Journal of ethnopharmacology, 113054.

Kara, M.;Tellioglu, G.;Sehirli, O.;Yildar, M.;Krand, O.; Berber, I., &Titiz, I. (2009). Evaluation of gadolinium pre-treatment with or without splenectomy in the setting of renal ischemia reperfusion injury in rats. Renal failure, *31*(10): 956-963.

Karim, N. A. A.; Ibrahim, M. D.; Kntayya, S. B.; Rukayadi, Y.; Hamid, H. A.& Razis, A. F. A. (2016). Moringa oleifera Lam Targeting Chemoprevention. Asian Pacific Journal of cancer prevention, 17(8): 3675-3686.

Kato, A. (2010). Increased hepcidin-25 and erythropoietin responsiveness in patients with cardio–renal anemia syndrome. Future cardiology, 6(6): 769-771.

Kautz, L.; Jung, G.; Nemeth, E. & Ganz, T. (2014). Erythroferrone contributes to recovery from anemia of inflammation. The Journal of the american society of hematology, 124(16): 2569-2574.

Khan, S. & **Amedia Jr, C. A**. (2008).Economic burden of chronic kidney disease. Journal of evaluation in clinical practice, 14(3): 422-434.

khawaja, TM. ; **Taheria, M. and Ikram, UK**. (2010).moringaoleifera:a natural gift –a review .Journal of pharmaceutical sciences ,2:775-81.

Kholif, A. E.; Gouda, G. A.; Morsy, T. A.; Salem, A. Z. M.; Lopez, S. & Kholif, A. M. (2015). Moringa oleifera leaf meal as a protein source in lactating goat's diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. Small ruminant research, 129:129-137.

Kieffer, D. A.; **Piccolo, B. D.; Vaziri, N. D.; Liu, S.; Lau, W. L.; Khazaeli, M .& Adams, S. H.** (2016). Resistant starch alters gut microbiome and metabolomic profiles concurrent with amelioration of chronic kidney disease in rats. American journal of physiology-renal physiology, 310(9): F857-F871.

Killip, S.; **Bennett, J. M. & Chambers, M. D**. (2007). Iron deficiency anemia. *American family physician*, *75*(5):671-678.

Kim, A.& Nemeth, E. (2015).New insights into iron regulation and erythropoiesis. Current opinion in hematology, 22(3): 199.

Kim, A.; **Fung, E.; Parikh, S. G.; Valore, E. V.; Gabayan, V.; Nemeth, E.& Ganz, T.** (2014). A mouse model of anemia of inflammation: complex pathogenesis with partial dependence on hepcidin. The Journal of the american society of hematology, 123(8): 1129-1136.

Klinkhammer, B. M.;Djudjaj, S.;Kunter, U.;Palsson, R.;Edvardsson, V. O.;Wiech, T., & Moeller, M. J. (2020). Cellular and Molecular Mechanisms of Kidney Injury in 2, 8-Dihydroxyadenine Nephropathy. Journal of the american society of nephrology, 31(4): 799-816.

Koury, M. J. (2015). Erythroferrone: a missing link in iron regulation. The hematologist, *12*(1): 10.

Koury, M. J.& Haase, V. H. (2015). Anaemia in kidney disease: harnessing hypoxia responses for therapy. Nature reviews nephrology, 11(7): 394.

Kulikov, V. P.; Tregub, P. P.; Kovzelev, P. D.; Dorokhov, E. A. & Belousov, A. A. (2015). Hypercapnia--alternative hypoxia signal incentives to increase HIF-1α and erythropoietin in the brain. Patologicheskaia fiziologiia i eksperimental'naia terapiia, (3): 34-37.

Lalas, S.& **Tsaknis, J.** (2002). Extraction and identification of natural antioxidant from the seeds of the Moringa oleifera tree variety of Malawi. Journal of the american oil chemists' Society, *79*(7): 677-683.

Lassnigg, A.; **Schmid, E. R.; Hiesmayr, M.; Falk, C.; Druml, W.; Bauer, P. & Schmidlin, D**. (2008). Impact of minimal increases in serum creatinine on outcome in patients after cardiothoracic surgery: do we have to revise current definitions of acute renal failure?. Critical care medicine, 36(4): 1129-1137.

Lawen, A. (2015). Is erythroferrone finally the long sought-after systemic erythroid regulator of iron. World journal of biological chemistry, 6(3): 78.

Lawson, D. S.& Holt, D. (2007). Insensible water loss from the Jostra Quadrox D oxygenator: an in vitro study. *Perfusion*, *22*(6):407-410.

Le, T. D.; **Bae, S.; Hsu, C. E.; Singh, K. P.; Blair, S. N.& Shang, N**. (2008). Effects of cardiorespiratory fitness on serum ferritin concentration and incidence of type 2 diabetes: evidence from the Aerobics Center Longitudinal Study (ACLS). The review of diabetic studies: RDS, 5(4): 245.

Lhotta, K. (2002, July). Beyond hepatorenal syndrome: glomerulonephritis in patients with liver disease. In Seminars in nephrology, 22(4) : 302-308.

Mahima, A. R.; **Mandil, R.; Verma, A. K. & Kumar, V.** (2014). Nutritional Potentials of Moringa olifera Leaves in Uttar Pradesh, India. Research journal of medicinal plants, 8: 283- 289.

Malyszko, J.; **Malyszko, J. S.; Pawlak, K.& Mysliwiec, M**. (2006). Hepcidin, iron status, and renal function in chronic renal failure, kidney

transplantation, and hemodialysis. American journal of hematology, 81(11): 832-837.

Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. Frontiers in pharmacology, 3: 24.

McLuckie, A.& Bihari, D. (2000). Investigating the relationship between intrathoracic blood volume index and cardiac index. Intensive care medicine, 26(9): 1376-1378.

Means, J. R. (2003).Recent developments in the anemia of chronic disease. Current hematology reports, *2*(2): 116-121.

Mescher, A. L. (2010). Junqueira, s basic histology text and atlas.*12th Ed*.1-5.

Minamishima, Y. A. & Kaelin, W. G. (2010).Reactivation of hepatic EPO synthesis in mice after PHD loss. Science, 329(5990): 407-407.

Mirza, N.; Marson, A. G.& Pirmohamed, M. (2009). Effect of topiramate on acid–base balance: extent, mechanism and effects. British journal of clinical pharmacology, 68(5): 655-661.

Mishra, G.; **Singh, P.; Verma, R.; Kumar, S.; Srivastav, S.; Jha, K. K., & Khosa, R. L.** (2011). Traditional uses, phytochemistry and pharmacological properties of Moringa oleifera plant: An overview. Der Pharmacia Lettre, 3(2): 141-164.

Moyo, B.; **Masika, P. J.; Hugo, A.& Muchenje, V**. (2011). Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. African Journal of Biotechnology, 10(60): 12925-12933.

Mun'im, A.; **Puteri, M. U. & Sari, S. P.** (2016). Anti-anemia effect of standardized extract of Moringa oleífera Lamk.leaves on aniline induced rats. Pharmacognosy Journal, 8(3).

Nakhoul, G.& Simon, J. F. (2016). Anemia of chronic kidney disease: Treat it, but not too aggressively. Cleveland clinical journal of medicine , *83*(8) 613-624.

Ndabigengesere, A., Narasiah, K. S., & Talbot, B. G. (1995). Active agents and mechanism of coagulation of turbid waters using Moringa oleifera. *Water research*, *29*(2), 703-710

Ndong, M.; Uehara, M.; Katsumata, S.; Sato, S.& Suzuki, K. (2007). Preventive effects of Moringa oleifera (Lam) on hyperlipidemia and hepatocyte ultrastructural changes in iron deficient rats. Bioscience, biotechnology, and biochemistry, 71(8):18261833.

Nemmar, A.;Karaca, T.;Beegam, S.;Yuvaraju, P.;Yasin, J.;Hamadi, N. K., & Ali, B. H. (2016). Prolonged pulmonary exposure to diesel exhaust particles exacerbates renal oxidative stress, inflammation and DNA damage in mice with adenine-induced chronic renal failure. Cellular physiology and biochemistry, 38(5): 1703-1713.

Nitsch, D.; **Dietrich, D. F.; von Eckardstein, A.; Gaspoz, J. M.; Downs, S. H.; Leuenberger, P. & Probst-Hensch, N. M**. (2006). Prevalence of renal impairment and its association with cardiovascular risk factors in a general population: results of the Swiss SAPALDIA study. Nephrology Dialysis Transplantation, 21(4): 935-944.

Nzengu-Lukusa, F.; **Yuma-Ramazani, S.; Sokolua-Mvika, E.; Dilu-Keti, A.; Malenga-Nkanga, B.; Shuli, J. B .& Ahuka-Mundeke, S.** (2016). Iron deficency and anemia among donors in Kinshassa. The pan african medical journal, 23: 174-174

Ogbe, A. O.& Affiku, J. P. (2020). Proximate study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. Journal of Microbiology, Biotechnology and food sciences, 9(4): 296-308.

Owolabi, J. O.;gounsola, A. O., & Fabiyi, O. S. (2014).Histological assessment of Moringaoleifera ameliorative activities on lead toxicity in the spleen of adult Wistar rats. World journal of life sciences and medical research, 3(2): 63.

Paikra, B. K. (2017). Phytochemistry and pharmacology of Moringa oleifera Lam. Journal of pharmacopuncture, 20(3): 194.

Palazzuoli, A.; Silverberg, D.; Iovine, F.; Capobianco, S.; Giannotti, G.; Calabrò, A.& Nuti, R. (2006). Erythropoietin improves

anemia exercise tolerance and renal function and reduces B-type natriuretic peptide and hospitalization in patients with heart failure and anemia. American heart journal, 152(6): 1096-e9.

Pangastuti, A.; **Amin, I. F.; Amin, A. Z.& Amin, M.** (2016). Natural bioactive compound from Moringa oleiferaagainst cancer based on in silico screening. Jurnal teknologi, 78(5).

Paul, B. Y.; Hong, C. C.; Sachidanandan, C.; Babitt, J. L.; Deng, D. Y.; Hoyng, S. A.& Peterson, R. T. (2008). Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nature chemical biology, 4(1): 33.

Phan, O.; Maillard, M.; Peregaux, C.; Mordasini, D.; Stehle, J. C.; Funk, F. & Burnier, M. (2013). PA21, a new iron-based noncalcium phosphate binder, prevents vascular calcification in chronic renal failure rats. Journal of pharmacology and experimental therapeutics, 346(2): 281-289.

Perez-Ruiz, L., Ros-Lopez, S., Cardús, A., Fernandez, E., & Valdivielso, J. M. (2006). A forgotten method to induce experimental chronic renal failure in the rat by ligation of the renal parenchyma. *Nephron Experimental Nephrology*, *103*(3), e126-e130.

Pieracci, F. M.; **Stovall, R. T.; Jaouen, B.; Rodil, M.; Cappa, A.; Burlew, C. & Moore, E. E.** (2014). A multicenter, randomized clinical trial of IV iron supplementation for anemia of traumatic critical illness. Critical care medicine, 42(9): 2048-2057.

Pittman, R. N. (2011). Oxygen transport in normal and pathological situations: defects and compensations. In Regulation of Tissue Oxygenation.Morgan & Claypool Life Sciences.National center for biotechnology information, 7:7

Pivkin, I. V.;Peng, Z.;Karniadakis, G. E.; Buffet, P. A.; Dao, M., & Suresh, S. (2016). Biomechanics of red blood cells in human spleen and consequences for physiology and disease. Proceedings of the national academy of Sciences, 113(28): 7804-7809.

Plützer, C.& Kleinermanns, K. (2002). Tautomers and electronic states of jet-cooled adenine investigated by double resonance spectroscopy. Physical chemistry chemical physics, 4(20): 4877-4882.

Polzin, D. J. (2011).Chronic kidney disease in small animals. Veterinary clinics, 41(1):15-30.

Rabbani, G. H.; Teka, T.; Saha, S. K.; Zaman, B.; Majid, N.; Khatun, M.& Fuchs, G. J. (2004). Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea. Digestive diseases and sciences, 49(3): 475-484.

Rahman, A.; **Yamazaki, D.; Sufiun, A.; Kitada, K.; Hitomi, H.; Nakano, D.& Nishiyama, A**. (2018). A novel approach to adenine-induced chronic kidney disease associated anemia in rodents. PloS one, 13(2).

Ramey, G.; Deschemin, J. C. & Vaulont, S. (2009). Cross-talk between the mitogen activated protein kinase and bone morphogenetic protein/hemojuvelin pathways is required for the induction of hepcidin by holotransferrin in primary mouse hepatocytes. Haematologica, 94(6):765-772.

Rehrer, N. J. (2001). Fluid and electrolyte balance in ultra-endurance sport. Sports medicine, *31*(10): 701-715.

Remuzzi, G.; **Ruggenenti, P. & Perico, N.** (2002). Chronic renal diseases: renoprotective benefits of renin–angiotensin system inhibition. Annals of internal medicine, 136(8): 604-615.

Ribeiro, S.; **Garrido, P.; Fernandes, J.; Rocha-Pereira, P.; Costa, E.; Belo, L.& Santos-Silva, A.** (2016). Liver iron is a major regulator of hepcidin gene expression via BMP/SMAD pathway in a rat model of chronic renal failure under treatment with high r H u EPO doses. Biofactors, *42*(3): 296-306.

Romero, R. C.; **Corrales, V. H. D. & Montaño, G. T.** (2016). Aspectons importants de moringa oleifera: und alternative paratratar la anemia por deficiencia de hierroimportant aspects of moringa oleifera: an alternative to treat anemia due to iron dificiency. Biotecnia, *18*(1): 3-9.

Saad, E. A.; **El-Gayar, H. A.; El-Demerdash, R. S. &Radwan, K. H.** (2018). Frankincense administration antagonizes adenine-induced chronic renal failure in rats. Pharmacognosy magazine, *14*(58): 634.

Saini, R. K.; Manoj, P.; Shetty, N. P.; Srinivasan, K. & Giridhar, P. (2014). Dietary iron supplements and Moringa oleifera leaves influence the liver hepcidin messenger RNA expression and biochemical indices of iron status in rats. *Nutrition research*, *34*(7): 630-638.

Sajidu, S. M.; **Henry, E. M. T.; Kwamdera, G. & Mataka, L.** (2005). Removal of lead, iron and cadmium ions by means of polyelectrolytes of the Moringa oleifera whole seed kernel. *WIT transactions on ecology and the environment*, *80*: 8

Schrier, R. W. (2006). Body water homeostasis: clinical disorders of urinary dilution and concentration. *Journal of the american society of nephrology*, *17*(7): 1820-1832.

Schrooten, I., Behets, G. J., Cabrera, W. E., Vercauteren, S. R., Lamberts, L. V., Verberckmoes, S. C., ... & D'Haese, P. C. (2003). Dose-dependent effects of strontium on bone of chronic renal failure rats. *Kidney international*, *63*(3), 927-935.

Seldin, M. M.; **Peterson, J. M.; Byerly, M. S.; Wei, Z. & Wong, G. W.** (2012). Myonectin (CTRP15), a novel myokine that links skeletal muscle to systemic lipid homeostasis. *Journal of biological chemistry*, *287*(15): 11968-11980.

Serra, A.; Romero, R.; Lopez, D.; Navarro, M.; Esteve, A.; Perez, N. & Ariza, A. (2008). Renal injury in the extremely obese patients with normal renal function. *Kidney international*, *73*(8): 947-955.

Sharma, V.; Paliwal, R.; Janmeda, P. & Sharma, S. (2012). Chemopreventive efficacy of Moringa oleifera pods against 7, 12-dimethylbenz [a] anthracene induced hepatic carcinogenesis in mice. *Asian pacific journal of cancer prevention*, *13*(6): 2563-2569.

Shi, H.; Bencze, K. Z.; Stemmler, T. L. & Philpott, C. C. (2008). A cytosolic iron chaperone that delivers iron to ferritin. *Science*, *320*(5880): 1207-1210.

Short, M. W.& **Domagalski, J. E.** (2013). Iron deficiency anemia: evaluation and management. American family physician, *87*(2): 98-104.

Shunmin, L.;**Xiaozhou, Z.;Yihou, Z., &Wuyong, Y.** (2003). Effects of Spleen-Strengthening and Kidney-Invigorating Prescription on Nutritional Status in Rats with Chronic Renal Failure. *Journal of* guangzhou university of traditional chinese medicine, *20*(3): 230-232.

siedu-Gyekye, I. J.; **Frimpong-Manso, S. A. M. U. E. L.; Awortwe, C.; Antwi, D. A.& Nyarko, A. K. (**2014). Micro-and macroelemental composition and safety evaluation of the nutraceutical Moringa oleifera leaves. *Journal of toxicology*, *2014* :13.

Sifakis, S. & Pharmakides, G. (2000).Anemia in pregnancy. *Annals of the new york academy of sciences*, *900*(1): 125-136.

Silverberg, D. S.; **Blum, M.; Agbaria, Z.; Deutsch, V.; Irony, M.; Schwartz, D.& Iaina, A**. (2001). The effect of iv iron alone or in combination with low-dose erythropoietin in the rapid correction of anemia of chronic renal failure in the predialysis period. *Clinical nephrology*, *55*(3): 212-219.

Silverberg, D. S.; **Wexler, D.; Blum, M.; Tchebiner, J. Z.; Sheps, D.; Keren, G. & Schwartz, I.** (2003). The effect of correction of anaemia in diabetics and non-diabetics with severe resistant congestive heart failure and chronic renal failure by subcutaneous erythropoietin and intravenous iron. *Nephrology dialysis transplantation*, *18*(1): 141-146.

Slaghekke, F.; **Kist, W. J.; Oepkes, D.; Pasman, S. A.; Middeldorp, J. M.; Klumper, F. J.& Lopriore, E**. (2010). Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. *Fetal diagnosis and therapy*, *27*(4): 181-190.

Snow, C. L.; **Martineau, L. N.; Hilton, R. J.; Brown, S.; Farrer, J.; Boerio-Goates, J.& Watt, R. K.** (2011). Ferritin iron mineralization

proceeds by different mechanisms in MOPS and imidazole buffers. *Journal of inorganic biochemistry*, *105*(7): 972-977.

Soad, M. M. I.**; Lobna, T. S. & Farahat, M. M**. (2010). Vegetative growth and chemical constituents of croton plants as affected by foliar application of benzyl adenine and gibberellic acid. *Journal of american science*, *6*(7).

Soliman, M. M.;Aldhahrani, A.;Alkhedaide, A.;Nassan, M. A.;Althobaiti, F., & Mohamed, W. A. (2020). The ameliorative impacts of Moringaoleifera leaf extract against oxidative stress and methotrexate-induced hepato-renal dysfunction. *Biomedicine & pharmacotherapy*, *128*, 110259.

Sonnweber, T.; Ress, C.; Nairz, M.; Theurl, I.; Schroll, A.; Murphy, A. T. & Kaser, S. (2012). High-fat diet causes iron deficiency via hepcidin-independent reduction of duodenal iron absorption. *The Journal of nutritional biochemistry*, *23*(12): 1600-1608.

Spandou, E.; **Tsouchnikas, I.; Karkavelas, G.; Dounousi, E.; Simeonidou, C.; Guiba-Tziampiri, O. & Tsakiris, D**. (2006). Erythropoietin attenuates renal injury in experimental acute renal failure ischaemic/reperfusion model. *Nephrology dialysis transplantation*, *21*(2): 330-336.

Srai, S. K.; Chung, **B.; Marks, J.; Pourvali, K.; Solanky, N.; Rapisarda, C.& Sharp, P. A**. (2010). Erythropoietin regulates intestinal iron absorption in a rat model of chronic renal failure. *Kidney international*, *78*(7): 660-667.

Sreelatha, S.& Padma, P. R. (2009). Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. *Plant foods for human nutrition*, *64*(4): 303.

Steiniger, B.; **Bette, M., &Schwarzbach, H**. (2011). The open microcirculation in human spleens: a three-dimensional approach. *Journal of histochemistry& cytochemistry*, *59*(6): 639-648.

Suzana, D.; Suyatna, F. D.; Andrajati, R.; Santi, P. S. & Mun'im, A. (2017). Effect of Moringa oleifera leaves extract against hematology and blood biochemical value of patients with iron deficiency anemia. *Journal of Young Pharmacists*, *9*(1): S79.

Syme, H. M.; Markwell, P. J.; Pfeiffer, D. & Elliott, J. (2006). Survival of cats with naturally occurring chronic renal failure is related to severity of proteinuria. *Journal of veterinary internal medicine*, *20*(3); 528-535.

Tam, B. Y.; Wei, K.; Rudge, J. S.; Hoffman, J.; Holash, J.; Park, S. K. & Jiang, S. (2006). VEGF modulates erythropoiesis through regulation of adult hepatic erythropoietin synthesis. *Nature medicine*, *12*(7): 793-800.

Tamura, M.; **Aizawa, R.; Hori, M. & Ozaki, H**. (2009). Progressive renal dysfunction and macrophage infiltration in interstitial fibrosis in an adenine-induced tubulointerstitial nephritis mouse model. *Histochemistry and cell biology*, *131*(4): 483-490.

Taniguchi, H.; **Sasaki, T. & Fujita, H.** (2012). Preoperative management of surgical patients by "shortened fasting time": a study on the amount of total body water by multi-frequency impedance method. *International journal of medical sciences*, *9*(7): 567.

Tbahriti, H. F.; **Meknassi, D.; Moussaoui, R.; Messaoudi, A.; Zemour, L.; Kaddous, A .& Mekki, K.** (2013). Inflammatory status in chronic renal failure: The role of homocysteinemia and pro-inflammatory cytokines. *World journal of nephrology*, *2*(2): 31.

Teke, H. U.; Cansu, D. U.; Yildiz, P.; Temiz, G. & Bal, C. (2017). Clinical significance of serum IL-6, TNF-&alph;, Hepcidin, and EPO levels in anaemia of chronic disease and iron deficiency anaemia: The laboratory indicators for anaemia.Biomedical research , 28 : 6

Teucher, T. ; **Olivares, & Cori.** (2004). Enhancers of iron absorption: ascorbic acid and other organic acids. International journal for vitamin and nutrition research, 74(6): 403-419.

Thavendiranathan, P.; Bagai, A.; Ebidia, A.; Detsky, A. S. & Choudhry, N. K. (2005). Do blood tests cause anemia in hospitalized patients? The effect of diagnostic phlebotomy on hemoglobin and hematocrit levels. *Journal of general internal medicine*, *20*(6): 520-524.

Theil, E. C. & Goss, D. J. (2009). Living with iron (and oxygen): questions and answers about iron homeostasis. *Chemical reviews*, *109*(10): 4568-4579.

Theil, E. C. (2011). Iron homeostasis and nutritional iron deficiency. *The Journal of nutrition*, *141*(4): 724S-728S.

Theurl, I.; Aigner, E.; Theurl, M.; Nairz, M.; Seifert, M.; Schroll, A. & Wroblewski, V. J. (2009). Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. *Blood, The Journal of the american society of hematology*, *113*(21): 5277-5286.

Tietz, N. W. (1986). Blood urea and creatinine concentrations. *Textbook* 6)(*of Clinical Chemistry*, 1-386.

Tomosugi, N.; **Kawabata, H.; Wakatabe, R.; Higuchi, M.; Yamaya, H.; Umehara, H. & Ishikawa, I.** (2006). Detection of serum hepcidin in renal failure and inflammation by using ProteinChip System. The American Society of Hematology, *108*(4): 1381-1387.

Tong, E. M.& **Nissenson, A. R.** (2001, March).Erythropoietin and anemia. In *seminars in nephrology,* 21(2) 190-203).

Torti, F. M.& Torti, S. V. (2002). Regulation of ferritin genes and protein. *Blood, The Journal of the American society of hematology*, *99*(10):3505-3516.

Valeri, C. R.; Khuri, S. & Ragno, G. (2007). Nonsurgical bleeding diathesis in anemic thrombocytopenic patients: role of temperature, red blood cells, platelets, and plasma-clotting proteins. *Transfusion*, *47*:206S-248S

Vallet, N. (2018). The role of erythroferrone in iron metabolism: From experimental results to pathogenesis. *La Revue de medecine interne*, *39*(3): 178-184.

Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive components in Moringa oleifera leaves protect against chronic disease. *Antioxidants*, *6*(4), 91.

Verma, A. R.; Vijayakumar, M.; Mathela, C. S. & Rao, C. V. (2009). In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. *Food and Chemical Toxicology*, *47*(9): 2196-2201.

Wagner, M.; **Alam, A.; Zimmermann, J.; Rauh, K.; Koljaja-Batzner, A.; Raff, U. & Schramm, L**. (2011). Endogenous erythropoietin and the association with inflammation and mortality in diabetic chronic kidney disease. *Clinical journal of the american society of nephrology*, *6*(7): 1573-1579.

Waikar, S. S. **& Bonventre, J. V**. (2009).Creatinine kinetics and the definition of acute kidney injury. *Journal of the american society of nephrology*, *20*(3): 672-679.

Wang, K.; **Wu, J.;Xu, J.;Gu, S.; Li, Q.; Cao, P., &Zeng, F**. (2018). Correction of anemia in chronic kidney disease with angelica sinensis polysaccharide via restoring EPO production and improving iron availability. *Frontiers in pharmacology*, *9*, 803.

Wang, L.; **Fan, R.;Geng, F.;Gao, Y., & Huang, Q**. (2019). Protective effect of crude polysaccharide from Pao-Tian-Xiong derived from monkshood, against chronic renal failure in mice. *Tropical Journal of Pharmaceutical research*, *18*(6).

Wang, W.; **Knovich, M. A.; Coffman, L. G.; Torti, F. M. & Torti, S. V.** (2010). Serum ferritin: past, present and future. *Biochimica et Biophysica acta (BBA)-general subjects*, *1800*(8): 760-769.

Watanabe, K.; Yamashita, Y.; Ohgawara, H.; Sekigughi, M.; Satake, N.; Orino, K. & Yamamoto, S. (2001). Iron content of rat serum ferritin. *Journal of veterinary medical science*, *63*(5): 587-589.

Weiss, G.& Gasche, C. (2010).Pathogenesis and treatment of anemia in inflammatory bowel disease. *Haematologica*, *95*(2): 175.

Weiss, G. & **Goodnough, L. T.** (2005).Anemia of chronic disease. *New England Journal of medicine*, *352*(10): 1011-1023.

Wijeysundera, D. N.; **Karkouti, K.; Beattie, W. S.; Rao, V. & Ivanov, J**. (2006). Improving the identification of patients at risk of postoperative renal failure after cardiac surgery. *Anesthesiology: The Journal of the american society of anesthesiologists*, *104*(1): 65-72.

Wilson, P. D. (2004). Polycystic kidney disease. *New england journal of medicine*, *350*(2): 151-164.

Winslow, R. M. (2002). Blood substitutes. *Current opinion in hematology*, *9*(2): 146-151.

Xia, Y.; **Babitt, J. L.; Sidis, Y.; Chung, R. T. & Lin, H. Y**. (2008). Hemojuvelin regulates hepcidin expression via a selective subset of BMP ligands and receptors independently of neogenin. , *The Journal of the American Society of Hematology*, *111*(10): 5195-5204.

Yamaguchi-Yamada, M.; manabe, N.; Uchio-yamada, K.; akashi, N.; goto, Y.; miyamoto, Y.& miyamoto, H. (2004). Anemia with chronic renal disorder and disrupted metabolism of erythropoietin in ICR-derived glomerulonephritis (ICGN) mice. *Journal of veterinary medical science*, *66*(4): 423-431.

Yaméogo, C. W.; Bengaly, M. D.; Savadogo, A.; Nikiema, P. A., & Traore, S. A. (2011). Determination of chemical composition and nutritional values of Moringa oleifera leaves. *Pakistan Journal of nutrition*, *10*(3): 264-268.

Yang, J.; Goetz, D.; Li, J. Y.; Wang, W.; Mori, K.; Setlik, D.& Barasch, J. (2002). An iron delivery pathway mediated by a lipocalin. *Molecular cell*, *10*(5):1045-1056.

Yang, R. Y.; Chang, L. C.; Hsu, J. C.; Weng, B. B.; Palada, M. C.; Chadha, M. L. & Levasseur, V. (2006 a). Nutritional and functional properties of moringa leaves from germplasm, to plant, to food, to health. *Moringa leaves: Strategies, standards and markets for a better impact on nutrition in Africa. Moringanews, Conservative dentistry and endodontic journa ,George fox journal , Paris*.2006:1-9

Yang, R. Y.; Tsou, S. C.; Lee, T. C.; Chang, L. C.; Kuo, G. & Lai, P. Y. (2006 b). Moringa, a novel plant rich in antioxidants, bioavailable iron, and nutrients .American chemical society , 17:224-239

Yeung, C. K.; Shen, D. D.;Thummel, K. E., &Himmelfarb, J. (2014). Effects of chronic kidney disease and uremia on hepatic drug metabolism and transport. *Kidney international*, *85*(3): 522-528.

Zimmermann,M. B., **Zeder, C., Muthayya, S., Winichagoon, P., Chaouki, N., Aeberli, I., & Hurrell, R. F**. (2008). Adiposity in women and children from transition countries predicts decreased iron absorption, iron deficiency and a reduced response to iron fortification. *International journal of obesity*, *32*(7), 1098-1104.

Zingg, A.; Felber, B.; **Gramlich, V.; Fu, L.; Collman, J. P. & Diederich, F.** (2002). Dendritic Iron (II) Porphyrins as Models for Hemoglobin and Myoglobin: Specific Stabilization of O2 Complexes in Dendrimers with H-Bond-Donor Centers. *Helvetica chimica acta*, *85*(1): 333-351.

Appendix

APPENDIX

appendix(I) estimation of serum urea concentration

Procedure

Wave length :578 nm

Wave length	340
Optical path	1 cm
Sample reagent	1: 100
Reagent volume	I ml
Sample volume	10 µl
Reagent blank limited	Low 0.9 AU High 2.0 AU
Sensitivity	0.9 mg/dl (0.15 mol/L)
Linearity	300mg/dl (49.8mmol/L)

	Standard	specimen
Reagent	1 ml	1 ml
Standard	10 µl	------
Specimen	--------	10 µl

Mix , and after 30 second read the absorbance Al of standard or specimen. Exactly 1 minute later ,read the absorbance A2 of standard or specimen.

Calculation
Δ A Specimen = A1 specimen –A2 specimen
Δ A standard = A1 standard –A2 standard

Serum urea concentration (mg/dl) = Δ A Specimen
_____
Δ A standard

Where n=50.0 mg/dl (8.33 mmol/L)

appendix(II) estimation of serum creatinine concentration

Procedure
Let stand reagent and specimen at room temperature

Pipette in well identified test tube	blank	Standard	Sample
Distilled water	0.5	-------	--------
Standard 2mg/dl	-------	0.5	-------
Trichloroactic acid 1.2mol/L	0.5	0.5	--------
supernatant	--------	-------	1 ml
Reagent mixture (picric acid +NaOH)	1 ml	1 ml	1 ml

Mix and let stand for 20 minute at 20-250 C measure the absorbance of specimen and standard against reagent blank at 246 nm.

Calculation

(A of specimen)
Creatinine (mg/dl)=—→2(standard concentration)

(A of standard)

appendix (III)complete blood count

Procedure

The sample is collecteddrawing the blood into a tube containing an anticoagulant typically (EDTA) to stop it from clotting The testing is typically performed by an automated analyzer Analysis begins when a well mixed blood sample is placed on a rack in the analyzer. The instrument utilizes flow cells, photometers and apertures to analyze different elements in the blood. On board the analyzer, the sample is diluted and aspirated into at least two different channels, one of which is used to count red blood cells and platelets, the other to count white blood cells. Additional channels may be used for differential white blood cell counts and specialized measurements of platelets.

Calculation

Blood cell counting occurs by flow cytometry, in which a very small amount of the specimen is aspirated, diluted and passed through an aperture and a flow cell. Sensors count and identify the number of cells passing through the aperture using two main principles: electrical impedance and light scatteringImpedance-based cell counting operates on the Coulter principle, which measures the drop in current as cells pass through an aperture to count cells and calculate their sizes. Because red blood cells, white blood cells and platelets have different average sizes, this technique allows the three types of cells to be differentiated.
Light scattering techniques direct a laser at individual cells and determine cellular size and complexity by measuring the amount of light scattered at different angles. Forward scatter, which refers to light scattered between 0 and 10 degrees of the beam's axis, correlates with cellular size, while side scatter (light scattered at a 90 degree angle) correlates with cellular complexity. White blood cells, red blood cells and platelets, as well as individual types of white blood cells, can be distinguished based on light scattering characteristics .

appendix (IV) Estimation of rat serum erythriopiotein

Assay procedure

1.Prepare all reagents before starting assay procedure .it is recommended that all standards and samples be add in duplicate to the microelisa stripplate .
2.add standard : set standard well,testing sample well, add standard 50µ to standard well.
3.add sample: add testing sample 10µ then add sample diluents 40µ to testing sample well: blank well doesn't add anything.
4.add 100µ of HRP-conjugate reagent to each well ,cover with an adhesive strip and incubate for 60 minute at 37℃
5.aspirate each well and wash ,repeating the process four times for a total five washes .wash by filling each well with wash solution (400µ) using a squirt bottle ,manifold dispenser or outowasher .complete removal of liquid at each step is essential to good performance .after the last wash ,remove any remaining wash solution by aspirating or decanting .invert the plate and blot it against clean paper towels .
6.add chromogen solution A 50µ and chromogen B 50µ to each well. Gently mix and incubate for 15 minute at 37℃. **protect from light**
7.add 50µ stop solution to each well .the color of the well should change from blue to yellow .if the color in the well is green or the color change doesn't appear uniform ,gently tap the plate to ensure thorough mixing .
8.read the optical density (O.D) at 450nm using microtitter plate reader with 15 minute.

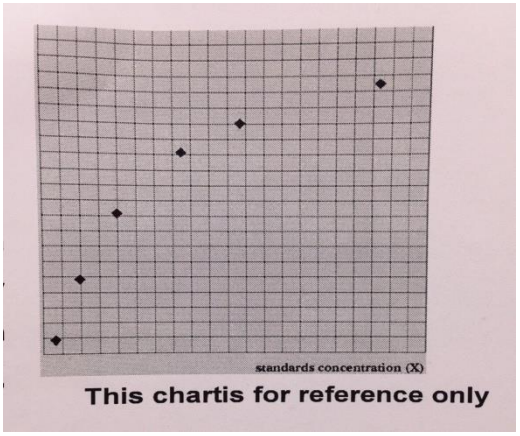
Calculation the results

1.this standard curve is used to determine the amount in unknown sample.the standard curve is generated by plotting the average O.D.(450 nm)obtained for each of six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2.first ,calculate the mean O.D. value for each standard and sample.all O.D. values ,are subtracted by the mean value of the zero standard before result interpretation .construct the standard curve using graph paper on statistical software .
3.to determine the amount in each sample,first locate the O.D. value on the Y-axis and read the coreresponding concentration .

4.any variation in operator,pipetting and washing technique,incubation time or temperature ,and kit age can causes variation in result .each user should obtain their own standard cure

5.detection range: 1-80ng/ml .the sensitivity by this assay is 1.0ng/ml

6.standard curve



appendix (V) Estimation of rat erythroferrone (FAM32B) ELISA Kit.

Assay procedure

1.Prepare all reagents before starting assay procedure .it is recommended that all standards and samples be add in duplicate to the microelisa stripplate .
2.add standard : set standard well,testing sample well, add standard 50µ to standard well.
3.add sample: add testing sample 10µ then add sample diluents 40µ to testing sample well: blank well doesn't add anything.
4.add 100µ of HRP-conjugate reagent to each well ,cover with an adhesive strip and incubate for 60 minute at 37℃
5.aspirate each well and wash ,repeating the process four times for a total five washes .wash by filling each well with wash solution (400µ) using a squirt bottle ,manifold dispenser or outowasher .complete removal of liquid at each step is essential to good performance .after the last wash ,remove any remaining wash solution by aspirating or decanting .invert the plate and blot it against clean paper towels .
6.add chromogen solution A 50µ and chromogen B 50µ to each well. Gently mix and incubate for 15 minute at 37℃. **protect from light**

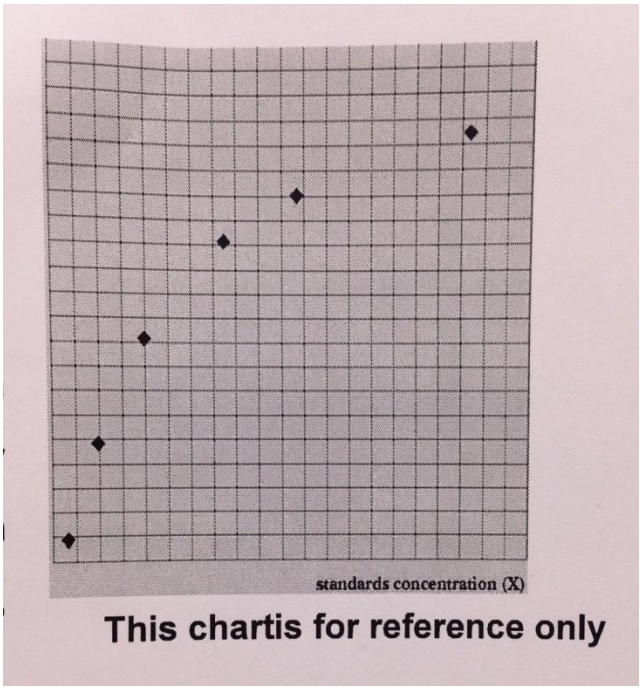
7.add 50µ stop solution to each well .the color of the well should change from blue to yellow .if the color in the well is green or the color change doesn't appear uniform ,gently tap the plate to ensure thorough mixing .
8.read the optical density (O.D) at 450nm using microtitter plate reader with 15 minute.

Calculation the results

1.this standard curve is used to determine the amount in unknown sample.the standard curve is generated by plotting the average O.D.(450 nm)obtained for each of six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2.first ,calculate the mean O.D. value for each standard and sample.all O.D. values ,are subtracted by the mean value of the zero standard before result interpretation .construct the standard curve using graph paper on statistical software .
3.to determine the amount in each sample,first locate the O.D. value on the Y-axis and read the coreresponding concentration .
4.any variation in operator,pipetting and washing technique,incubation time or temperature ,and kit age can causes variation in result .each user should obtain their own standard cure

5.detection range: 1-80ng/ml .the sensitivity by this assay is 1.0ng/ml

6.standard curve



appendix (VI) Estimation of Rat Serum Ferritin (FE) ELISA Kit

Assay procedure

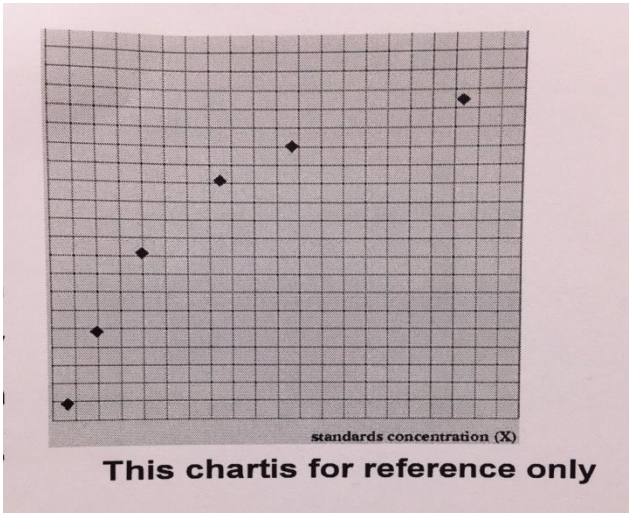
1. Add standard: Set Standard wells, testing sample wells. Add standard 50μl
to standard well.
2.add sample：Set blank wells separately (blank comparison wells don't add
sample and HRP-Conjugate reagent, other each step operation is same).
testing sample well. add Sample dilution 40μl to testing sample well, then add
testing sample 10μl (sample final dilution is 5-fold), add sample to wells , don't
touch the well wall as far as possible, and Gently mix.
3.add enzyme：Add HRP-Conjugate reagent 100μl to each well, except blank
well.
4.Incubate: After closing plate with Closure plate membrane ,incubate for 60
min at 37℃.
5.Configurate liquid: 20-fold wash solution diluted 20-fold with distilled water
and reserve

6.washing：Uncover Closure plate membrane, discard Liquid, dry by swing,
add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by
pat.
7.color：Add Chromogen Solution A 50ul and Chromogen Solution B to each
well, evade the light preservation for 15 min at 37℃
8.Stop the reaction：Add Stop Solution 50μl to each well, Stop the reaction(the
blue color change to yellow color).
9.assay：take blank well as zero , Read absorbance at 450nm after Adding
Stop Solution and within 15min.

Calculate

Take the standard density as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard density
and the OD value ,with the sample OD value in the equation, calculate the sample density

,



appendix (VII) Estimation of Rat serum iron (SI) ELISA Kit.

Assay procedure

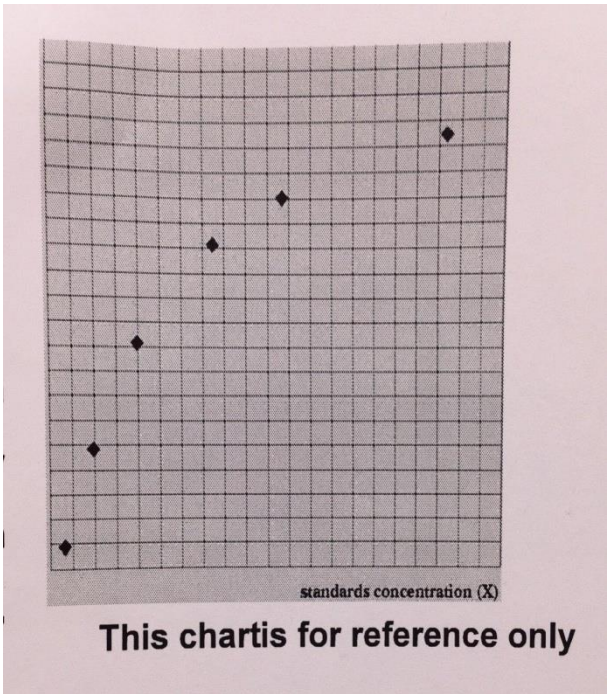
1. Add standard: Set Standard wells, testing sample wells. Add standard 50μl
to standard well.
2.add sample：Set blank wells separately (blank comparison wells don’t add
sample and HRP-Conjugate reagent, other each step operation is same).
testing sample well. add Sample dilution 40μl to testing sample well, then add
testing sample 10μl (sample final dilution is 5-fold), add sample to wells , don’t

touch the well wall as far as possible, and Gently mix.
3.add enzyme：Add HRP-Conjugate reagent 100μl to each well, except blank
well.
4.Incubate: After closing plate with Closure plate membrane ,incubate for 60
min at 37℃.
5.Configurate liquid: 20-fold wash solution diluted 20-fold with distilled water
and reserve.

6.washing：Uncover Closure plate membrane, discard Liquid, dry by swing,
add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by
pat.
7.color：Add Chromogen Solution A 50ul and Chromogen Solution B to each
well, evade the light preservation for 15 min at 37℃
8.Stop the reaction：Add Stop Solution 50μl to each well, Stop the reaction(the
blue color change to yellow color).
9.assay：take blank well as zero , Read absorbance at 450nm after Adding
Stop Solution and within 15min .

Calculate

Take the standard density as the horizontal,
the OD value for the vertical ,draw the standard
curve on graph paper, Find out the corresponding
density according to the sample OD value by the
Sample curve, multiplied by the dilution multiple,
or calculate the straight line regression equation
of the standard curve with the standard density
and the OD value ,with the sample OD value in
the equation, calculate the sample density,



appendix (VIII) 5 Estimation of Rat Serum Hepcidin (Hepcidin) ELISA Kit

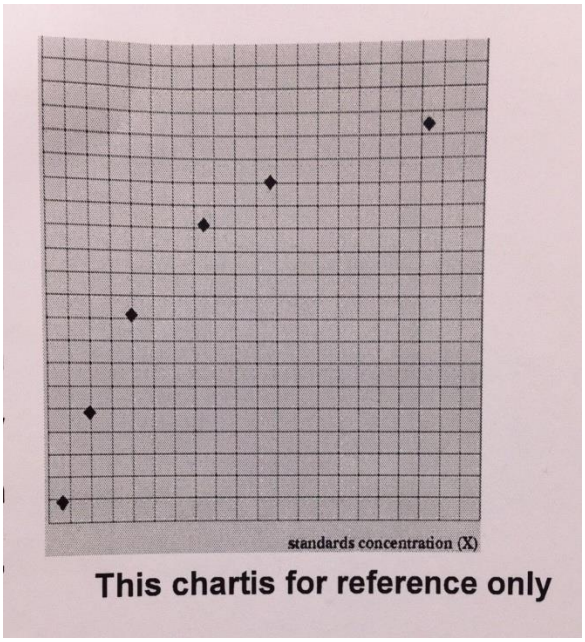
Assay procedure

1. Add standard: Set Standard wells, testing sample wells. Add standard 50μl
to standard well.
2.add sample：Set blank wells separately (blank comparison wells don't add
sample and HRP-Conjugate reagent, other each step operation is same).
testing sample well. add Sample dilution 40μl to testing sample well, then add
testing sample 10μl (sample final dilution is 5-fold), add sample to wells , don't
touch the well wall as far as possible, and Gently mix.
3.add enzyme：Add HRP-Conjugate reagent 100μl to each well, except blank
well.
4.Incubate: After closing plate with Closure plate membrane ,incubate for 60

min at 37℃.
5.Configurate liquid: 20-fold wash solution diluted 20-fold with distilled water
and reserve.
6.washing：Uncover Closure plate membrane, discard Liquid, dry by swing,
add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by
pat.
7.color：Add Chromogen Solution A 50ul and Chromogen Solution B to each
well, evade the light preservation for 15 min at 37℃
8.Stop the reaction：Add Stop Solution 50μl to each well, Stop the reaction(the
blue color change to yellow color).
9.assay：take blank well as zero , Read absorbance at 450nm after Adding
Stop Solution and within 15min.

Calculate

Take the standard density as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density



Assay range
3.75 ng/mL - 120 ng/mL

appendix (IX) Histological study

Histological Technique(E & H) stain

The kidney , liver and spleen of each animal were quickly removed andrapidly weighed then prepared for histological study according toMescher method,(2010) with aid of the light microscope as the following steps:

* Fixation

The specimen fixated in the formalin 10 % for 24 – 48 hours.

* Washing and dehydration

After fixation the specimens washed with water to remove the fixative in order to avoid the interaction between the fixative and staining materials used later. By dehydration the water had been completely extracted from

fragments by bathing them successivelyin a graded series of of ethanol and water (70 %, 80 %, 90 %, and100 % ethanol).

* Clearing

Bathing the dehydrated fragments in solvent (xylene) for 30 –60 minutes, this step was repeated 3 times .As the tissues clearing,they generally became transparent.

* Infiltration and embedding

Once the tissue fragments were impregnated with the solvent,they were placed in melted paraffin in an oven, typically at 52℃.The heat causes the solvent to evaporate, and the space within the tissues becomes filled with paraffin

* Sectioning

After holdes from the oven, the specimen let at room temperature to be solid and removed from their containers in order to sectioning they were put in the rotary microtome and were sliced by themicrotome, a steel blade into sections 5 micrometers thick . The sections were floated on water bath (50 – 55 o C) , then transferred into glass slides coated with Mayers albumin as adhesive substanceand left to dry.

* Staining

The histological sections of the studied organs were stained withHematoxylin - Eosin stain.

Staining Procedure

1. Deparaffinize sections, 2 changes of xylene, 10 minutes each.
2. Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.
3. 95% alcohol for 2 minutes and 70% alcohol for 2 miuntes.
4. Wash briefly in distilled water.
5. Stain in Harris hematoxylin solution for 8 minutes.
6. Wash in running tap water for 5 minutes.
7. Differentiate in 1% acid alcohol for 30 seconds.
8. Wash running tap water for 1 minute.

9. Bluing in 0.2% ammonia water or saturated lithium carbonate solution for 30 seconds to 1 minute.
10. Wash in running tap water for 5 minutes.
11. Rinse in 95% alcohol, 10 dips.
12. Counterstain in eosin-phloxine solution for 30 seconds to 1 minute.
13. Dehydrate through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.
14. Clear in 2 changes of xylene, 5 minutes each.
15. Mount with xylene based mounting medium

Trichrome stain (modified masson's) procedure

The procedure was done according to Trichrome Stain Kit

(Modified Masson's) ScyTek Laboratories, Inc./ U.S.A.

Procedure

1.Mordant in Bouins solution , microwave 1 minute , allow to strand 15 minute

2.wash in running tap water to remove the picric acid ,5 minutes.

3.weigerts working hematoxylin ,10 minute.

4.Blue in running tap water for 5minute , rinse in distilled water.

5.Biebrich scarlet for 5 minute

6.Rinse in distilled water

7.phosphotungistic/phosphomolyboic acid for 10 minute . discard solution

8.Transfer directly into Anilline blue for 5 minutes.

9.Rinse in distilled water

10. 1% Acetic acid for 1minute ,discard solution ,rinse in distilled water .

11.Dehydrate , clear, and coverslip.

Conventional method :Mordant in Bouins solution ,60 C° for 1 hour

جمهورية العراق
وزاره التعليم العالي والبحث العلمي
جامعه كربلاء /كلية الطب البيطري
فرع الفسلجة والكيمياء الحياتية والادوية

تاثير مسحوق اوراق المورينغا على مؤشرات ايض الحديد في ذكور الجرذان المصابه بالفشل الكلوي المزمن المستحدث

رسالة مقدمة الى
مجلس كلية الطب البيطري
وهي جزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري /الفسلجة

من قبل
افياء ليث درويش الجحيشي

باشراف
الاستاذ المساعد الدكتور
وفاء كاظم جاسم

2020م **1441هـ**

الخلاصة

تمت دراستنا في كلية الطب البيطري/ جامعه كربلاء .اجريت هذه الدراسة خلال الفتره الممتده من الاول من شهر تشرين الثاني لسنة 2019 الى الاول من شهر شباط سنة 2020 ,وقد صممت هذه الدراسة للتحقق من دور الارثروفيرون في توازن الحديد ونشاط الكريات الحمر في فقر الدم المرتبط بالفشل الكلوي المستحدث في ذكور الجرذان عن طريق دراسة العلاقة بين الارثروفيرون والهرمونات الاخرى مثل الارثروبايوتين ,الهبسدين ,الفرتين ,فحص كريات الدم الحمراء الكامل ,الحديد في المصل و فحص وظائف الكلية (الكرياتنين ,اليوريا) بالاضافه لدراسة التغيرات التنسجية المرضية في التقطيع النسجي لكل من الكلية , الطحال والكبد .

تم تقسيم ستين جرذ من الذكور عشوائيا الى(4 /مجموعات) لمدة 44 يوما ,تم اعطاء المجموعة الاولى (GI) الضابطة مع ثنائي مثيل السلفوكسيد حقن عن طريق البريتون لمده4 اسابيع والمجموعه الثانية (GII) الضابطة مع ثنائي مثيل السلفوكسيد حقن عن طريق البريتون لمده 4 اسابيع ومن بعدها تم اعطاء مسحوق اوراق المورنكا بجرعة تراوحت 5% لمدة اسبوعين مع النظام الغذائي ,المجموعة الثالثه (GIII) و تم حقن الادنين في البريتون بجرعة 100مجم/كجم من وزن الجسم لمده 4 اسابيع لاحداث الفشل الكلوي والمجموعة الرابعة (GIV) تم حقن الادنين في البريتون بجرعة بجرعه 100مجم/كجم لمده 4 اسابيع لاحداث الفشل الكلوي ومن بعدها تم اعطاء مسحوق اوراق المورنكا بجرعة تراوحت 5% لمدة اسبوعين مع النظام الغذائي .

اضهرت النتائج وجود ارتفاع معنوي ($p\leq0.05$) في اليوريا في الدم والكرياتنين في الدم والفرتين في المصل والهبسدين في المصل في المجموعه المعالجة بالادنين (GIII)

وانخفاض معنوي ($p\leq0.05$) في تعداد كريات الدم الحمراء وخلايا لدم المضغوط و خضاب الدم في مجموعه الادنين (GIII) بالاضافه الى مصل الارثروباوتين ومصل الاريثروفيرون ومصل الحديد بالمقارنه مع المجموعات الاخرى ,بعد اعطاء مسحوق اوراق المورنكا اوليفيرا ,نلاحظ وجود انخفاض كبير ($p\leq0.05$) في مصل اليوريا , الكرياتنين في الدم ,الفرتين في المصل والهبسدين في المصل ايضا في المجموعه المعالجة (GIV) وزياده ذات دلاله احصائية ($p\leq0.05$) في عدد كريات الدم الحمراء ,خلايا الدم المضغوط ,خضاب الدم في مجموعه الادنين (GIV) بالاضافه الى مصل الارثروباوتين ومصل الارثروفيرون ومصل الحديد بالمقارنة مع (GIII)

تظهر التغيرات النسجية في الكلية والطحال والكبد ان المجموعة المعالجة بالادنين تضررت بشكل كبير من الضمور والتنكس والنخر خاصه الانابيب الكلوية التي تحتوي على ترسبات

الادنين البلورية بالاضافه الى الارتشاح الالتهابي لخلايا الارتشاح الالتهابي مقارنة مع مجموعة السيطرة بعد اعطاء اوراق المورنكا اوليفيرا ,تكون الانسجه قادرة على الرجوع لشكلها الطبيعي وتنضيمها المحدد جيدا , واضهرت النتائج انخفاضا كبيرا ($p\leq0.05$) في اليوريا في الدم والكرياتنين في الدم والفرتين في المصل والفرتين في المصل وزياده ذات دلاله احصائيه($p\leq0.05$) في عدد كريات الدم الحمراء وكل من جميع خلايا الدم المضغوط ,خضاب الدم في مجموعه الادنين والمورنكا (GIV) بالاضافه الى مصل الارثروباوتين ومصل الارثروفيرون ومصل الحديد بالمقارنة مع المجموعات الاخرى .

توضح هذة الدراسة تادور العلاجي المهم لمسحوق اوراق المورنكا كمضاد لفقر الدم ودوره المضاد للاكسده كونه غنيا بمضادات الاكسده .