THE STUDY OF THE EFFECTS OF METHANOLIC EXTRACT OF *MOMORDICA charantia* ON HISTO-MORPHOLOGY OF KIDNEY AND LIVER AND HAEMATO-BIOCHEMICAL PARAMETERS OF ADULT ALBINO RATS

BY

Umar Isyaku MIKAIL
Ph.D/MED/0029/2009-2010

Ph.D THESIS PRESENTED TO THE DEPARTMENT OF ANATOMY

FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA

OCTOBER, 2016

THE STUDY OF THE EFFECTS OF METHANOLIC EXTRACT OF *MOMORDICA charantia* ON HISTO-MORPHOLOGY OF KIDNEY AND LIVER AND HEMATO-BIOCHEMICAL PARAMETERS OF ADULT ALBINO RATS

BY

Umar Isyaku MIKAIL
BSc. ABU, 1997, MSc. ABU, 2009
Ph.D/MED/0029/2009-2010

**A Ph.D THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN HUMAN ANATOMY
DEPARTMENT OF ANATOMY,
FACULTY OF MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA**

OCTOBER, 2016

DECLARATION

I declare that this thesis titled: Study of the effects Methanolic extract of *Momordica charantia* on histo-morphology of liver and kidney and haemato-biochemical parameters of adult albino rats, has been performed by me in the Department of Human Anatomy, Ahmadu Bello University, Zaria, under the supervision of Professor S.A. Ojo, Professor S.S. Adebisi and Professor S. B. Oladele.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Mikail Umar Isyaku ________________ ________________

Sign *Date*

CERTIFICATION

This thesis entitled: Study of the effects of methanolic extract of Momordica charantia on histo-morphology of liver and kidney and haemato-biochemical parameters of adult albino rats by Mikail Umar Isyaku, meets the regulations governing the award of the degree of Doctor in Philosophy (Ph.D) in Human Anatomy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. S.A. Ojo ________________ ________________
DVM, MSc, Ph.D, MBiol *Sign* *Date*
Department of Veterinary Anatomy
Ahmadu Bello University, Zaria

Prof. S.S. Adebisi ________________ ________________
BSc., MSc. Ph.D *Sign* *Date*
Department of Human Anatomy
Ahmadu Bello University, Zaria

Prof. S. B. Oladele ________________ ________________
DVM, MSc, Ph.D *Sign* *Date*
Department of Veterinary Pathology
Ahmadu Bello University, Zaria

Prof. S.S. Adebisi
Head of Anatomy Department ________________ ________________
Sign *Date*

Prof. Kabir Bala
Dean, School of Postgraduate studies ________________ ________________
Sign *Date*

DEDICATION

This work is dedicated to my beloved Mother, Hajia Asma'u, my loving children and to my late sister Hajiya Mariya.

ACKNOWLEDGEMENTS

My endless gratitude goes to my caring and dedicated supervisors Professor S.A. Ojo, a caring and loving teacher. Sir, I will forever remain indebted to you and your loving family who always welcome me at any time, thank you so much Sir. I will remain grateful and loyal to Professor S.S. Adebisi, sir, may you and your loved ones keep prospering to the highest place in all you do. You care for me; always there for me, I shall forever remember you Sir. My teacher who had also contributed a lot to this work, my supervisor who sit me next to him only to guide and put me through, to polish my work, is Professor S.B. Oladele who shall forever remain in my heart, thank you so much Sir. May the Almighty provide and protect you and your loved ones.

My profound gratitude goes to my young colleague Dr. J.A. Timbuak who spent days and nights to make sure my statistical analysis portion of this work was completed within the shortest time, thank you Doctor, may God, the Almighty take you to the highest level of your endeavor ameen.

I will never forget Dr. Sunday Musa, who has always been there for me since the beginning of this work. You put a lot into my work despite the challenges around you, thank you and may God, the Almighty see you throughout your life.

I am so grateful to Dr. Muhammad Bisala of Department of Veterinary Pathology, who assisted me to read the slide. Also thanks to Malam Adamu, the chief technologist, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences. He guided me toward achieving an excellent extraction of the plant used.

I must extend my gratitude to the staff of embalming unit especially,

Mr. Mamman Bulus and Malam Adamu, who assisted me to keep the animal house clean at all times, during treatment period. I also wish to thank Mr. Peter Akpolu of the histology unit, he assisted me during my slide preparation. I am grateful to all the academic and non teaching staff of Human Anatomy Department who show their concern to me during this work, especially Dr. A. Dahiru, Dr. U.E Umana, Dr.B. Danborno and Dr. W.O. Hamman. I also thank Mr. Dominic Mamman who typed all the work.

Above all, praises go to the Almighty God, the Noble and the provider of health, wealth and peace. May God provide, protect and guide all those who contribute to my progress throughout my life ameen.

TABLE OF CONTENTS

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER SIX

LIST OF FIGURES OF THE RESULTS

LIST OF PLATES

ABSTRACT

Momordica charantia is one of the plants commonly used among the rural dwellers in Northern Nigeria for the treatment of high blood sugar. It is also used as abortifacient for birth control, antilipedemia for the control of obesity and antivirus for the treatment of viral infections. It is also used as anti diarrheal remedy and other abdominal problems. The study became necessary because the rural dwellers in Northern Nigeria abuse the use of this plant. Evaluations of effects of methanolic extract of *Momordica charantia* on kidney, liver, haematology and biochemical parameters of adult albino rats were conducted. In this study twenty adult male rats of mean weight of 270 ±30 gram were used. The animals were grouped into a control group A and treatment groups; B, C and D. LD_{50} was determined to be 1855 mg. The first group of animals having 5 rats (group A) which served as the control was administered orally with distilled water, second group (group B) administered orally with 125 mg/kg body weight, third group (group C) was administered with 250 mg/kg body weight and group D was administered orally with 500 mg/kg body weight. The treatment was for six weeks, weights of the rats were recorded weekly. Haematological and biochemical parameters were determined using standard procedures. The haematology revealed that, there was elevation of white blood cells (WBC), increased eosinophils, lymphocytes and neutrophils. The biochemical analysis revealed that there was

increased creatinine levels and increased aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) among the groups treated with the methanolic extract of *Momordica charantia*. The histo-morphology of the kidney revealed that, there was necrosis of renal tubular cells, congested intertubular spaces and mononuclear cellular infiltration. The histo-morphology of the liver revealed that, there was vacuolation of hepatocytes; focal areas of necrosis, dilated sinusoids with some areas of haemorrhage. The results of this study indicated that, extract of *Momordica charantia* was found to produce mild to severe effects on the rats depending on the dose of the extract administered. Therefore, prolonged administration of the extract of this plant as commonly practiced by the rural dwellers of Northern Nigeria should be discouraged.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Plants have always been very useful source of remedy to several ailments owing to its easy availability and affordability in traditional setting worldwide. Some of these plants that exhibit medicinal properties have been known to help in stabilizing different internal organs in human being and animals, while others have had side effects on the organs probably due to the varying amounts or quantity of toxic matter present in such plants, (Sofowora, 2003). Because of this, toxicity testing in animals is carried out to identify potential hazard of the plant/drugs, this toxicity study helps in determining the upper limits of administration, because not all contents of the plant's extract usually have medicinal property (Ahmed *et al.*, 2001).

In this study, *Momordica charantia* which is herbal plant used traditionally in the Northern Nigeria, in treatments of hyperglycemic conditions such as diabetes mellitus will be considered. *Momordica charantia* also inhibits primary human adipocyte's differentiation by modulating adipogenic gene (Chaturvedi *et al.*, 2006). *Momordica charantia* (MC) also called Bitter melon (BM) is a member of the cucurbitaceae family, it is also known as bitter melon, bitter gourd, balsam pear, all in English; Garahuni or Daddagu in Hausa, Ejinrinwere, Ejirin, Igbole-aja in Yoruba (Abdullahi *et al.,* 2003; Grover and Yadav, 2004).

Momordica charantia is widely grown in tropical areas such as the Amazon, East Africa, Asia, India, West Africa and Caribean. It is used traditionally as both food and medicine (Nadine *et al*., 2005). The plant is a climbing perennial with elongated fruit (the cultivated type) and or small rounded fruits (the wild type). The elongated fruit resembles a warty gourd or cucumber. The unripe fruit is white or green in color and has a bitter taste that becomes more pronounced as the fruit ripens.

It is herbaceous plant with auxiliary tendrils. It grows up to 5meters. It bears simple, alternate leaves 4cm to 12cm across with 3-7deeply separated lobes. Each plant bears separate yellow male and female flowers (Abdullahi *et al*., 2003).

In the northern part of Nigeria, flowering occurs during the months of June and July, while fruiting occurs during the months of September to November (Ananya and Sarmisha, 2010). The fruits have a distinct watery exterior and an oblong shape at both ends. It is hollow in cross-section with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds.

The fruit is mostly eaten green or as it begins to turn yellow (Yibchok *et al.*, 2006).

The seeds, fruits, leaves and roots of the plant have been used in traditional medicine (Abdullahi *et al*., 2003) for microbial infections, sluggish digestion and intestinal gas, menstrual stimulation, wound healing, inflammation, fever reduction, hypertension and as a laxative and emetic (Tropical Plants Database, 2007).

The clinical conditions for which *Momordica charantia* extracts (primarily from the fruits) are currently being used includes: diabetes, dyslipidemia, microbial infection and potentially as a cytotoxic agent for certain types of cancer (Pitchakarn, 2012).

Medicinal uses of *Momordica charantia* is wide, it is also used as antivirus, antibiotic, antitumor, purgative, abortifacient, hypotensive, laxative and vermifuge (Pitchakarn, 2012)..

The plant contains several biologically active compounds chiefly; *Momordicine* I and II. This compound (momordicine) is composed of albumin, globulin, glutelin, aminobutyric acid and oleanolic acids, insulin-like peptide (plant (p) insulin). The P-insulin is structurally and pharmacologically similar to bovine insulin and is composed of two (2) polypeptide chains held together by disulfide bond (Okabe *et al*., 1982: Abdullahi *et al*., 2003).

The plant has numerous other constituents such as proteins (momordin which may have anti-cancer properties), glycosides, saponins and minerals like vitamins A and C and beta-carotene, as well as the minerals iron, phosphorus and potassium. It also contains bioactive substances like momordin, charantin, charantosides, goyaglycosides and momordicoside. It contains some cytotoxic (also called ribosomes-inactive) proteins like momorcharin and momordin (Toshihiro *et al*., 2007).

The most known mechanism of action of this plant is its blood sugar lowering effect. Researches on diabetes has demonstrated that *Momordica charantia* extracts increase glucose utilization by the liver, decrease gluconeogenesis via inhibition of two key enzymes (glucose-6-phosphatase) and improve glucose oxidation through the shunt pathway by activating glucose-6-phosphate dehydrogenase. Extracts of *Momordica charantia* also enhance cellular uptake of glucose, promote insulin release and potentiate its effects and increase the number of insulin producing beta cells in the pancreas of diabetic animals (Yibchok-Anun *et al*., 2006). *Momordica charantia* extracts have been shown to inhibit growth and proliferation of various types of cancer cells in animals and in vitro. This may be attributed to the identification of potent inhibitor of guanylate cyclase which is an enzyme present in high amounts in many types of tumors cells (Manabe *et al*., 2003).

Another research indicates *Momordica charantia* extracts modify the immune response in cancer patients via decreased intestinal secretion of interleukin-7, reduced lymphotcyte number and increase T-helper and natural killer cell population (Manabe *et al.,* 2003).

Momordica charantia extracts have broad-spectrum antimicrobial activities; it has been shown to prevent infection by numerous viruses, bacteria, parasitic organisms and fungi. Although mechanisms have not been determined for all organisms, in the case of viral infection, it is thought that certain bitter melon constituents prevent viral penetration of the cell wall, (Manabe *et al.,* 2003). The immune-stimulating properties of *Momordica charantia* extracts may also contribute to decreased rates of microbial infection observed in animal studies. Animal studies demonstrated that *Momordica charantia* extracts, particularly the saponin fraction, have lipid-lowering effects resulting from inhibition of pancreatic lipase activity and subsequent decreased lipid absorption (Oishi *et al*., 2007).

Another animal study has demonstrated that *Momordica charantia* juice (also called Bitter Melon Juice "BMJ") has inhibitory effect on membrane lipid peroxidation (Ahmed *et al.,* 2001).

Like most bitter-tasting food, bitter melon is said to stimulate digestion and thus help to treat dyspepsia and constipation (Muthaura *et al*., 2007), however, it is suspected to cause heart burn and ulcers (Waako *et al*., 2005), although these negative effects appear to be limited by its action as demulcent and mild inflammation modulator (Nerurkar *et al*., 2006) it was also reported that bitter melon is used to treat gastrointestinal diseases and extracts have shown activity in vitro against the nematode worms especially *Caenorhabditis elegans* (Nadine *et al*., 2005).

Perhaps the best-researched use of bitter melon is to lower blood sugar levels in diabetics. Alcohol extracted charantin from Momordica *charantia* consists of mixed steroids and in animal model of diabetes, which improved glucose tolerance to a degree similar to the oral hypoglycemic agent, tolbutamide (Muthaura *et al*., 2007).

A clinical trial of nine patients with confirmed type 1 diabetes found that subcutaneous injection of *Momordica charantia* extract containing crystallized p-insulin resulted in statistically significant decrease in blood sugar levels compared with controls. Fasting blood sugar was drawn prior to the administration of p-insulin and plasma glucose levels were used to determine the dosage of p-insulin given to each patient. The onset of p-insulin effect was noted 30-60 minutes after administration with peak effect ranging widely from 4-12 hours (Abdullahi *et al*., 2003).

This most resembles long acting (NPH) insulin that demonstrates an onset of 1.5-2 hours, with a peak effect from 4-12 hours after administration oral bitter melon preparation have also been shown to be effective in clinical trials of type 2 diabetes. Welihinda *et al*., (2002), demonstrated a statistically significant improvement in glucose tolerance in type 2 diabetics. Eighteen diabetic subjects (average age 38 years) were given 100ml of bitter melon juice 30 minutes prior to a glucose load. Improved glucose tolerance was observed in 13 of the 18 patients (73%). To serve as a control, a glucose tolerance test was administered to all the 18 subjects using 100ml of water prior to the glucose load (Welihinda *et al*., 2002).

It was also reported that bitter melon helps to prevent or counteract diabetes mellitus type 2. Tea from the leaves of bitter melon is used for this purpose in many Asian countries and Panama (Chaturvedi *et al*., 2004: Sridhar *et al*., 2008). The plant contains substance called *charantin*, which is known to have hypoglycemic effect on normal and diabetic rabbits (Chaturvedi *et al.*, 2004). It was found that *Momordica_charantia* has increased insulin sensitivity, (Virdi *et al*., 2003).

In 2007, a study by the Philippine's Department of Health determined that, a daily dose of 100 mg/kg body of weight of powdered bitter melon is comparable to 2.5 mg/kg body weight of the anti-diabetes drugs called glibenclamide taken twice per day (Ahmed *et al.*, 2009).

Tablets of *Momordica charantia* extract is sold in the Philippines as a food supplement under the trade name charantia, and are exported to many countries (Leatherdale *et al.*, 2008).

Momordica charantia also contains lectin. Lectin has insulin-like activity due to its non-protein specific linking together to insulin receptors. This lectin lowers blood glucose concentration by acting on peripheral tissues and similar to insulin's effect in the brain, which suppresses appetite. This lectin is likely a major contributor to the hypoglycemia effect that develops after eating *Momordica charantia* (Miura *et al.,* 2001).

It has been claimed that *Momordica charantia* bitterness comes from quinine (Grover and Yadav, 2004). Bitter melon is traditionally regarded as useful for preventing and treating malaria in Asia. Tea from the leaves of bitter melon is used for this purpose in Panama and Colombia (Ananya and Sarmisha, 2010).

In northern part of Nigeria, bitter melon is boiled with garlic and onions then serve with honey for the treatment of malaria and cancer prevention (Majekodunmi *et al*., 1990).

In vitro studies have shown that bitter melon extract posse's broad-spectrum antimicrobial activity. Bitter melon extracts inhibits infection and growth of several viruses, including HIV (Jiratchariyakul *et al*., 2001), Herpes Simplex (Bourinbaiar and Lee-Huang, 2006) and Epstein Barr Virus. A preliminary report on the effect of bitter melon extracts in three (3) HIV patients showed a normalization of CD4/CD8 ratios with treatment. It is believed bitter melon extracts inhibit HIV replication by prevent syncytial formation and cell-to-cell infection (Zhang *et al.,* 2002).

In West Africa, especially in Togo, the plant (bitter melon) is traditionally used against viral infections such as chicken pox and measles (Nadine *et al*., 2005). Tests with bitter melon leaves extracts have shown invitro activity against the herpes simplex type I virus, apparently due to unidentified compound other than the momordicins (Toshihiro *et al.,* 2007).

Laboratory test suggests that compounds in bitter melon might be effective for treating HIV infection. As most compounds isolated from bitter melon that impact HIV have either been proteins or lectins, neither of which are well absorbed, it is unlikely that oral intake of bitter melon slows HIV among the infected people (Jiratachariyaku *et al*., 2001). It is possible oral ingestion of bitter melon could upset negative effects of anti-HIV drugs, if a test tube study can be shown to be applicable to humans (Nerurkar *et al.,* 2006).

Bitter melon extracts also appear to inhibit the growth of numerous gram-negative and gram positive bacteria, including Escherichia coli, Salmonella, Shigella (Omoregbe *et al.,* 2006), Staphylococcus Pseudomonas, Streptobacillus and Parasitic organisms Escherichia histolytica and Plasmodium falciparum (Khan and Omoloso, 2008).

Several animal studies using rodent model of diabetics have examined the effect of bitter melon extracts on abnormal lipid parameters. Significant decreases in triglycerides and low diet lipids (LDL) cholesterol and increases in High Diet Lipid (HDL) cholesterol were noted (Ahmed *et.,al.,* 2001: Chaturvedi *et al*., 2004: Dans *et al*., 2007).

In the longest study periods (10 weeks) bitter melon extract was given to normal and streptozotocin-induced type I diabetic rats. Diabetic rats had elevated total cholesterol, triglycerides, phospholipids as well as decreased High diet lipid cholesterol, moderate increase in plasma lipid peroxides and malondialdehyde (signs of increased oxidative stress) also observed. After 10 weeks, diabetic rats receiving bitter melon extracts experienced a normalization all parameters compared to the control rats which were not given the bitter melon extracts (Ahmed *et al.,* 2001; Chaturvedi, 2005).

Two compounds extracted from bitter melon, α-eleostearic acid (from the seeds) and 15, 16 –dihydroxy-α-eleostearic acid (from the fruit) have been found to induced apoptosis of leukemia cells in vitro (Masuko *et al.,* 2008). Diet which contains 0.01% bitter melon oil (0.006% as α-eleostearic acid) were found to prevent azoxymethane-induced colon carcinogenesis in rats (Kohno *et al.,* 2004) Although clinical trials have not been conducted using bitter melon extracts in cancer patients, In vitro studies indicate bitter melon fruits and seeds extracts inhibit the growth of several cancer cells lines, including prostate adenocaranoma (Tabata, 2012), human colon cancer (Caco-2 cells) (Yasui *et al.*, 2005) and highly metastatic breast cancer cell line (Lee-Huang *et al.*, 2000).

1.2 STATEMENT OF RESEARCH PROBLEMS

The abuse of Momordica *charantia* in the northern Nigeria, for the treatments of diabetes mellitus, birth control, cancer, stomach abnormalities and fever, has being going on for many years, and it was observed that people in the rural areas where modern medical facilities cannot satisfy their medical demands are most affected, 80% of these people rely on the traditional medicine. The abuse of *Momordica charantia* has gone far and the knowledge of its side effects is not well understood by the traditional healers and the general population in the rural areas of Northern Nigeria, (Teoh *et al.*, 2010). Since not all contents of the plant's extract usually have medicinal property (Kim *et al.*, 2002). It is then necessary to investigate these side effects in order to provide an important document for the rural settlers and traditional medicine vendors. This would enlighten them on the effects of *Momordica charantia*.

1.3 JUSTIFICATION/ SIGNIFICANCE OF THE STUDY

Traditional medicine in Nigeria like in other developing countries where medical facilities cannot satisfy their national demands plays an important role in combating both human and animal diseases. It was estimated that about 80% of the people who live in the rural areas, rely on the traditional medicine healers for the treatments of their medical problems, using medicinal herbs. (Teoh *et al*., 2010). However, the histo-morphology of liver and kidney and haemato-biochemical effects of this plant have not yet been studied, tested or documented. Most of the information is still in the hands of the traditional healers, therefore this study becomes necessary.

This study is necessary, because there is excessive abuse of *Momordica charantia* plant by the rural dwellers in northern Nigeria for many of their health problems, and there is no documented information about the effect(s) of this plant available for the rural dwellers. Findings from this study will provide vital information about the effects of *Momordica charantia* (bitter melon) on the kidney and liver and on the haemato- biochemical parameters, which could be useful for further study and provide information on the effects of bitter melon on these organs (liver and kidney) and some component of blood to the rural dwellers and companies which produce juice and tea leaves of *Momordica charantia*.

1.4 AIMS AND OBJECTIVES OF THE STUDY

1.4.1 AIMS OF THE STUDY

This study was aimed at evaluating the possible effects of the extract of *Momordica charantia* on the histo-morphology of the liver and kidney, and haemato-biochemical parameters of albino rats after administration of methanolic extract of *Momordica charantia.*

1.4.2 OBJECTIVES OF THE STUDY

The study objectives are:

i. Evaluate the haematological parameters of adult albino rats following oral administration of extract of *Momordica charantia*

ii. Evaluate the biochemical parameters of the adult albino rats following oral administration of extract of *Momordica charantia*.

iii. Evaluate the histo-morphology of the kidney and liver of adults albino rats following oral administration of *Momordica charantia*

iv. Relate the histo-morphological changes of the kidney and liver to haematological changes of adult albino rats following oral administration of *Momordica charantia*

v. Relate the biochemical changes to histo-morphology changes of the kidney and liver of adult albino rats

1.5 RESEARCH HYPOTHESIS

Methanolic extract of *Momordica charantia* has some negative effects on histo-morphology of the kidney and liver and haemato-biochemical parameters of adult albino rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

More than 500 years have elapsed since African slaves arrived America, bringing not only a rich cultural heritage and their traditional folklore, but also herbs and fruits. *Momordica charantia* also called bitter melon had since been identified to be effective for the treatments of fevers, cancers, bacterial and viral infections, fungal infections, stomach problems, antiworm and abortifacient (Grover and Yadav, 2004).

Although the slaves taken to America have been using bitter melon because of its actions in the human and animal bodies, they did not know how bitter melon exerted its medicinal effects. Bitter melon was used then to treat diabetes and malaria without even knowing what active constituents could be the ones exerting these beneficial properties and may not even know that bitter melon contained any active constituents at all (Grover and Yadav, 2004). Now almost, 500 years had passed, and the state of technique has achieved quite an advance being able to identify the active constituents (Hussain, 2001).

The plant *Momordica charantia* (bitter melon) also called bitter gourd, bitter Squash, Karela, Ampalaya, Garahuni, etc. belong to the family cucurbitaceae (Barbieri *et al*., 1979).

Bitter melon has being known for its sugar lowering ability. The use of bitter melon for diabetes has been reported scholars by from Fancy EF Ng TB school of Biomedical Sciences, Chinese University of Hong Kong, (Tabata, 2012). It was later explained recently by scientist who discovered some active constituents present in bitter melon that it was able to act on a similar way as insulin, with the only difference that insulin stops the use of fats as energy source by inhibiting the release of glucagons and bitter melon inhibits key carbohydrate hydrolyzing enzymes a> α- amylase and α- glucosidase (Mahomoodally *et al.,* 2010). The study showed how bitter melon exerted hypoglycemic action and could be studied as an alternative nutritional therapy in the management of diabetes (Mahomoodally *et al*., 2010)

Infact, bitter melon is used as antidiabetic plant because of its hypoglycemic effect (Hazarika *et al.,* 2009). Studies have shown that bitter melon repairs B-cells, increases insulin levels and enhance the sensitivity of insulin (Hazarika *et al.,* 2009).

The active constituents found in bitter melon thought to exert these hypoglycemic properties are namely: charantin, momordenol and momordicilin, the study showed that momordicilin was found as the most active compound in the respective target site (Chaturvedi, 2006).

Bitter melon contains Gurmarin, which is a polypeptide considered to be similar to bovine insulin and has been shown in clinical studies suppressing the neural responses to sweet taste stimuli (Chaturvedi *et al.,* 2005).

Bitter melon has long been a popular part of many Asian vegetable dishes. Though notorious for its bitter taste, bitter melon is rich in iron, potassium, beta-carotene and other nutrients. But aside from its role as a healthy food, bitter melon is especially valued by diabetics for its known anti diabetes properties. The traditional remedy is made by pounding the raw fruits of the bitter melon into liquid, or by boiling the leaves and fruits for a few minutes, the resulting water then drunk as herbal tea (Senanayake *et al.,* 2004).

Bitter melon has some scientific reports that cited its blood sugar lowering benefit. Research worldwide since 1960s cited the plant's key compound, notably polypeptide – P, a plant insulin known to lower blood sugar levels. The plant's insulin-like benefits have long been enjoyed by many diabetics, who include bitter melon as part of their daily diet, either eaten as a vegetable or taken as tea. With traditional use supported by modern scientific validation of the plant's benefits, bitter melon is the most promising plant/herb for diabetes today (Li, 2012).

Oral bitter melon preparations have also been shown to be effective in clinical trials of type-2 diabetes. Meng (2012) demonstrated a statistically significant improvement in glucose tolerance in type – 2 diabetics. Eighteen diabetic human subjects, with average age 38 years were given 100ml bitter melon juice each 30 minutes prior to a glucose load. Improved glucose tolerance was observation in 13 of 18 patients (73%). To serve as a control, a glucose tolerance test was administered to all the 18 subjects using 100ml of water prior to the glucose load (Meng, 2012).

In another uncontrolled trial, two bitter melon extracts were evaluated. Dried fruit powder was given (5g three times daily) to five subjects with diabetes (type unspecified), and seven subjects in a second group received aqueous extract containing 100g fruit per 100ml of water. Those in the group administered with fruit powdered form, experienced an average 25% drop in blood sugar at the end of three-weeks trial, while those in the group administered with the aqueous extract experienced an average 54% drop in blood sugar after three weeks. Glycosylated hemoglobin (HbA1C) examined in seven subjects decreased an average of 17% the three-week trial (Soundararajan, 2012).

A randomized, double-blind, placebo-controlled, three-month trails involving 40 diabetic patients (either newly diagnosed or poorly controlled) examined the effect of encapsulated bitter melon powder on HbA1C. Secondary outcome measures were fasting blood sugar, total cholesterol, and body weight. Bitter melon extract or placebo (two capsules three times daily; exact dosage not disclosed) was administered to 20 patients in each group. While a sight improvement was noted in HbA1C, it was not statistically significant, and no significant improvements were observed in secondary outcomes compared to the control group (Dans *et al.*, 2007). Although the amount given may have been insufficient to effect improvement, this remains purely speculative since the dosage was not disclosed.

Escalating trends of obesity and associated type 2 diabetes has prompted an increase in the use of alternative and complementary function foods. Bitter melon that is traditionally used to treat diabetes and complications has been demonstrated to alleviate hyperglycemia as well as reduce adiposity in rodents. However its effects on human adipocytes remain unknown. (Nerurkar *et al*., 2010).

It was also pointed out by Biyani *et al.* (2003) that diabetes which is disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action. Also it was pointed out that chronic hyperglycemia during diabetes causes glycation of body proteins which in turn leads to secondary complications affecting eyes, kidneys, nerves, and arteries (Fang, 2012). These may be delayed, lessened or prevented by maintaining blood glucose values close to normal. Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues which regulates gastric emptying and inhibitors of intestinal alpha glucosidases like acarbose, miglitol and voglibiose which delay postprandial hyperglycemia. Sulphonylures, the most widely used class of drugs act by closure of ATP dependent channel. Metformin, a biguanide oral antibiotic limits intestinal glucose absorption. These drugs have certain effects like causing hypoglycemia at higher doses, live problems, lactic acidoses and diarrhea. It is apparent that due to the side effects of the currently used drugs, there is a need for a safe agent with minimal adverse effects, which can be taken for long durations (Biyani *et al*., 2003). In addition to the above drugs of synthetic origin, many agents of plant origin are also in use particularly for the treatment of non-insulin dependent diabetes mellitus (NIDDM). Various medicinal plant like *Momordica charantia* is known to possess antihyperglycemic activity (Ooi, 2012). *Momordica charantia* is the most extensively and most widely acclaimed remedy for the treatment of diabetes mellitus since ancient times.

Momordica charantia (Bitter Melon) is also referred as bitter gourd, Karela or Garahuni, as mentioned before, it is a family member of cucurbitaceae. It is commonly used as a traditional remedy for diabetes in India, Asia, Africa and South America. It is commonly consumed as a vegetable in India. The fruit leaves, seeds and roots of bitter melon have been used in the Indian system of medicine for a number of diseases, besides diabetes. The unripe fruits of this plant have been shown to produce a hypoglycemic effect in experimental models following oral administration (Fang, 2012; Ooi, 2012; Meng, 2012; Li, 2012; Pitchakarn, 2012).

Many clinical trials have confirmed the hypoglycemic actions (Fang, 2012). Some investigators have attempted to purify the active fractions from fruits of bitter melon in order to establish the precise mechanism of its hypoglycemic effects (Tabata, 2012). But, most of these studies were restricted to a very short duration and used a single large dose (Biyani *et al*., 2003).

A systematic study of three extracts of bitter melon indicates that a water extract powder at doses as low as 20 mg/kg body weight can reverse alloxan induced hyperglycemia in rats with no toxicity to viscera up to a period of four weeks.

Higher dosages may not only be ineffective but may also cause toxicity to the viscera such as kidney, liver and small intestine (Biyani *et al*., 2003) It was also mentioned that, while bitter melon may have a wide safe therapeutic margin on oral used, a very high dosage of methanolic extract of bitter melon might be potentially deleterious to liver (Ataman and Idu, 2007).

It was reported that bitter melon has antioxidant and chemo-protective activities by lowering slowing metabolism of various carcinogens and or procarcinogens and enhancing the activities of the enzymes that pick off the carcinogenic radicals (Asli and Alaattin, 2007).

Two compound extracted from bitter melon α- eleostearic acid (from the seeds) and 15, 16 – dihydroxy-α-eleostearic acid (from the fruit) have been found to induce apoptosis of leukemia cells in vitro (Masuko *et al.,* 2008) diet containing 0.01% bitter melon oil (0.006% as α-eleostearic acid) were found to prevent azoxymethane-induced colon carcinogenesis in rats (Kohno *et al.,* 2004).

In the United States of America, approximately 127 million adults are overweight (body mass index BMI>25 kg/m^2) while more than 60 million are classified as obese (BMI>30 kg/m^2), and about 9 million as severely obese (BMI>40 kg/m^2). In the last two decades the prevalence of obesity has risen by 40% (from 46.0% to 64.4%) and the prevalence of obesity has increased by 110% (from 14.5% to 30.5%) (Lowest *et al*., 2009). Obesity is also associated with metabolic disorders such as type 2 diabetes which is the second leading preventable deaths in the USA (Scheen, 2000). Adipose tissue is a critical endocrine organ that is innately involved in regulating not only obesity, but also metabolic processes such as, insulin resistance and type 2 diabetes (Gregoire, 2001).

Adipogenesis involves development of preadiposecytes to mature adipocytes with the accumulation of lipid droplets, increase in fat cells size (hypertrophy) as well as increase in cell number or hyperplasia and plays an important role in obesity. Among the various transcription factors that promote preadipocyte differentiation and influence adipogenesis, peroxisome proliferators-activated receptors(PPARY) is considered to be the “master regulator” of adipogenesis

(Wakabayashi *et al.,* 2009). Similarly, lipolysis or lipid mobilization in adipocytes results from breakdown of adipose triacylglycerols into none sterified fatty acids and glycerol which involves not only lipases and involves not only lipases and triacylglyrol hydrolases, but also lipid-droplet coating proteins such as perilipin (Tansey *et al*., 2001). Overall, an appropriate balance between adipogenesis and lipolysis is therefore crucial for the proper functioning of adipose tissue, which plays an important role in obesity and associated metabolic disorders. (Vernochet *et al.,* 2009).

Successful treatment of obesity usually requires multiple interventions such as exercise, diet, behavioral modification and pharmacotherapy (Sheperd, 2003). The escalating trend of obesity as mentioned above has resulted in a revealed interest in the use of functional foods including herbs and alternative medicine (Shewmake and Huntington, 2009). Momordica *charantia* commonly known as bitter melon, is widely cultivated in Asia, Africa and South America is extensively used in folk medicine as remedy for diabetes and complications, especially in India, Southeastern Asia, Africa and South America (Grover *et al*., 2002). Animal studies indicate that bitter melon juice was also effective in reducing weight gain, possibly due to reduced adipose hypertrophy, inhibition of lipogenic genes and increased plasma catecholamines (Chen *et al.,* 2003) and Huang *et al*., 2008).

However effects of bitter melon juice on human adipocyte differentiation has not yet been published.

Nevertheless it was observed that bitter melon juice significantly reduced lipid accumulation and increases lipolysis in primary human adipocytes, with a concomitant reduction in PPARY perilipin and resisting gene expression. This is critical as it laid the foundation of identification of molecular targets and anti obesity effects of bitter melon for clinical trials. (Nerurkar *et al.*, 2008).

Several animal studies using a rodent model of diabetes, have examined the effect of bitter melon extracts on abnormal lipid parameters. Significant decreases in triglycerides and LDL cholesterol and increases in HDL cholesterol were noted in all the studies, (Tansey *et al*., 2004: Charurvedi *et al*., 2005). In the longest study (10 weeks) Momordica *charantia* extracts was administered to normal and streptozotocin induced type-1 diabetic rats. The diabetic rats had elevated total cholesterol triglycerides and phospholipid as well as decreased HDL cholesterol; moderate increase in plasma lipid peroxides and malondialdehyde which is a sign of increased oxidative stress, were also observed. After 10 weeks, diabetic rats receiving Momordica *charantia* extract, experienced a normalization of all parameters compared to the control which were not given the extract (Tansey *et al.,* 2001).

Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins and steroids. One chemical has clinically demonstrated the ability to inhibit the enzyme called guanylate cyclase that is thought to be linked to the cause of psoriasis and also necessary for the growth of leukemia and cancer cells. In addition, a protein found in bitter melon, momordin has clinically demonstrated anticancerous activitiy against Hodgkins lymphoma in animals. Other proteins in the plant alpha- and beta- momorcharin and cucurbitacin B have been tested for possible anticancerous effects (Nerurkar *et al.*, 2008). A chemical analog of these bitter melon proteins has been developed, patented and named MAP-30 its developers reported that it was able to inhibit prostate tumor growth. Two of these proteins alpha-and beta momorcharin have also been reported to inhibit HIV virus in test tube studies in one study. The HIV infected cells treated with alpha and beta momorcharin showed a nearly complete loss of viral antigen while healthy cells were largely unaffected (*Christensen et al*., 2009).

In numerous studies atleast three different groups of constituents found in all parts of bitter melon have clinically demonstrated hypoglycemic properties or other actions of potential benefits against diabetes mellitus. These chemicals that lower blood sugar include a mixture of steroidal saponins known as charantims, insulin-like peptides and alkaloid. The hypoglycemic effect is more pronounced in the fruit of the bitter melon where these chemicals are found in greater abundance (Roffey *et al*., 2007).

The following plant chemicals are all found in bitter melon: alkaloids, charantin, charine, cryptoxanthin cucurbitaccins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodial, galacturonic acids, gentisic acid, goyaglycosides, goyassaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, linoleic acid, momorcharasides, momorcharins, momordenol, momordicins, momordicilin, momordicinin, momordicosides, momordin, vracil, vaccine, v-insulin steroidal glycosides, peptides, polypeptides, ribosomes-inactivating proteins, steroidal glycosides, trypsin inhibitors (Roffey *et al.,* 2007).

To date, close to 100 *in vivo* studies have demonstrated the blood sugar-lowering effect of bitter melon fruit. The fruit has also shown the ability to enhance cells uptake of glucose, to promote insulin release and to potentiate the effect of insulin. In other in vivo studies, bitter melon fruit has been shown to reduce total cholesterol. In one of the studies it was found that elevated cholesterol and triglyceride levels in diabetic rats were returned to normal after 10 weeks of treatment. (Tansey *et al*., 2007).

Several *in vivo* studies have demonstrated the antitumor activity of the entire plant of bitter melon. In a study, a water extract blocked the growth of rat prostate carcinoma, another study reported that a hot water extracts of the entire plant inhibited the development of mammary, tumors in mice. Numerous in vitro studies have also demonstrated the anticancerous and antileukemic activity of bitter melon against numerous cell lines, including liver cancer, human leukemia, melanoma and solid sarcomas (Fang, 2012).

Bitter melon, like several of its isolated plant chemicals, also has been documented within vitro antiviral activity against numerous viruses including Epstein-Barr, herpes and HIV viruses. In an in vivo study, a leaf extract increased resistance to viral infections and had an immune-stimulant effect in humans and animals, increasing interferon production and natural killer cell activity (Nerurkar *et al.*, 2008).

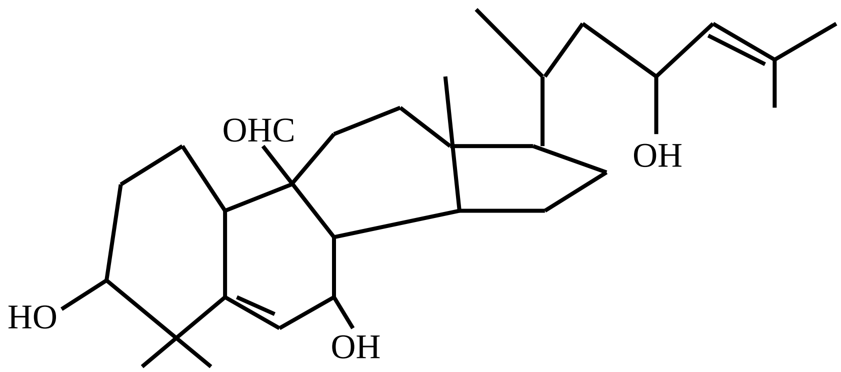
In addition to these properties, leaf extracts of bitter melon have demonstrated broad-spectrum antimicrobial activity. Various extracts of the leaves have demonstrated in vitro antibacterial activities against Eschericia coli, Staphylococcus, Pseudomonas, Salmonella, Streptobacillus and Streptococcus, an extract of the entire plant was shown to have antiprotozoal activity against Entamoeba histolytica. The fruit juice have demonstrated the same type of antibacterial properties and in another study a fruit extract demonstrated activity against the stomach ulcer-causing bacterial Helicobactepylori (Mahomoodally, 2010).

Many *in vivo* clinical studies have demonstrated the relatively low toxicity of all parts of the bitter melon plant when ingested orally. However, toxicity and even death in laboratory animals has been reported when extracts are injected intravenously. Other and leaf (ingested orally) to be safe during pregnancy. The seeds, however, have demonstrated the ability to induce abortions in rats and mice and the root has been documented as a uterine stimulant in animals. The fruit and leaf of bitter melon have demonstrated to have in vivo antifertility effect in female animals and in male animals to affect the production of sperm negatively (Chen and Li, 2005).

2.1 CHEMISTRY OF MOMORDICA *charantia*

As seen above, the plant (momordica charantia) contains several biologically active compounds, chiefly momordicin I and II, and cucurbitacin B. It also contains several bioactive glycodises (including momordin, charantin, charantosides, goyaglycosides, momordicosides) and other terpenoid compounds (including momordicin 28, momordicinin, momordicilin, momordenol and momordol (Sabira *et al*., 2007). It also contain cytotoxic proteins (also ribosome – inactivating protein) such as momorcharin and momordin (Marcelo and Marc, 2002).

2.1 Momordicin I



3,7,23 – trihydroxy cucurbitan – 5, 24 – dien – 19 or momordicin I

(Sabira *et al.*, 2007).

Fig. 2.1: This chemical compound is found in the leaves of the bitter melon and is possibly responsible for its reputed medicinal properties.

2.2 Momordicilin

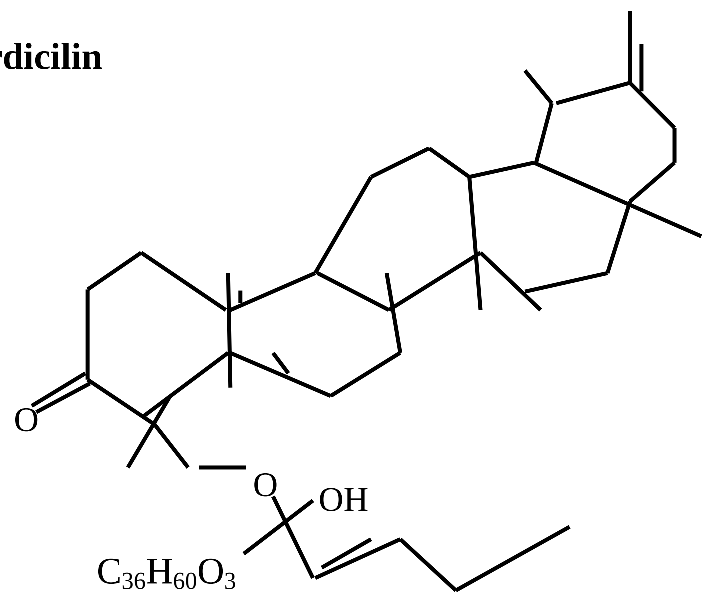
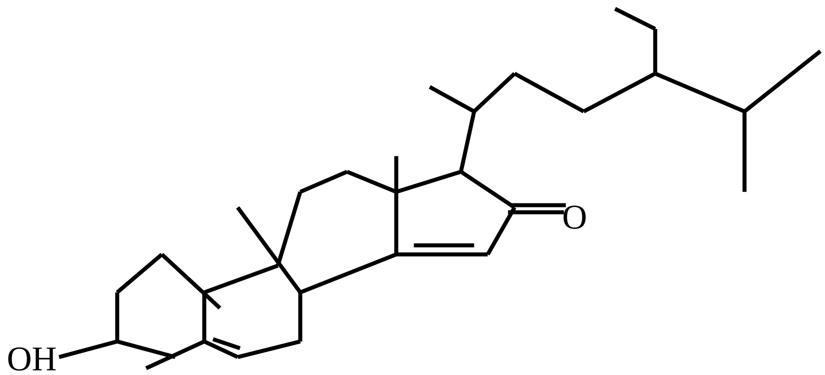


Fig. 2.2: 24- (1-hydroxy, 1-methyl 2- pentenyloxyl) ursan -3-one or momordicilin found in the fresh fruit of bitter melon (Sabira *et al.*, 2007).

This compound is soluble in ethyl acetate and chloroform but not soluble in petrol. It crystallizes as needles and melt at 170-171^{o}c. It was first isolated by Sabira *et al*. (2007).

2.3: Momordenol

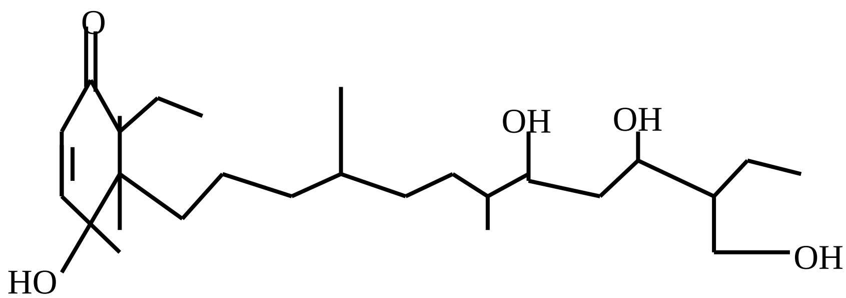


$C_{29}H_{46}O_2$

Fig. 2.3: Momordenol or 3β- hydroxyl-stigmasta-5, 14-dien-16 one A compound found also in the fresh fruit of the bitter melon. It is a natural sterol with chemical formula $C_{29}H_{46}O_2$ (Sabira *et al.*, 2007).

This compound is soluble in ethyl acetate and methanol but not soluble in pure chloroform or petrol. It crystalises as fine needles that melt at 160-161^{o}c. It was also isolated by Sabira *et al*. (2007).

2.4 Momordol



$C_{26}H_{48}O_5$

Fig. 2.4: 1-hydroxy-1,2-dimethyl-2 (8’, 10’-dihydroxy-4’,7’-dimethyl-11-hydroxy methyl – trideca) -3-ethyl-cyclohex-5-en-4 one. The compound with chemical formula $C_{26}H_{48}O_5$ (Sabira *et al.*, 2007).

Found in fresh fruit of bitter melon. The compound is oily liquid, soluble in ethyl acetate and methanol but not in pure chloroform or petrol. It was also isolated by Sabira *et al.*, (2007).

Fig. 2.5: The leaves of *Momordica* Fig. 2.6: Unripe fruit of *Momordica charantia*

Fig. 2.7: Ripened fruit of *Momordica charantia* (wild type)

(Alternative Medicine Review, 2007)

Momordica charantia, a member of the Cucurbitaceae family, is known as bitter melon, bitter gourd, balsam pear, karela, and pare. It grows in tropical areas of the Amazon, East Africa, Asia, India, South America, and the Caribbean and is used traditionally as both food and medicine. The plant is a climbing perennial with elongated fruit that resembles a warty gourd or cucumber. The unripe fruit is white or green in color and has a bitter taste that becomes more pronounced as the fruit ripens. (Oishi *et al.*, 2007)

The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for microbial infections, sluggish digestion and intestinal gas, menstrual stimulation, wound healing, inflammation, fever reduction, hypertension, and as a laxative and emetic. Clinical conditions for which Momordica *charantia* extract (primarily from the fruit) are currently being used include diabetes, dyslipidemia, microbial infections, and potentially as a cytotoxic agent for certain types of cancer. (Ahmed *et al.*, 2001).

Active Constituents

Although they have not been definitively determined, research indicates the primary constituents responsible for the hypoglycemic properties of Momordica are charantin, insulin-like peptide (plant (p)-insulin), cucurbutanoids, momordicin, and oleanolic acids. P-insulin is structurally and pharmacologically similar to bovine insulin and is composed of two polypeptide chains held together by disulfide bonds. Momordica *charantia* also has numerous other constituents including proteins (momordin, which may have anticancer properties), glycosides, saponins, and minerals. (Chaturvedi *et al.,* 2004).

It is also rich in vitamins A and C and beta-carotene, as well as the minerals iron, phosphorus, and potassium.

Mechanisms of Action of *Momordica charantia*

The most well researched Momordica *charantia* mechanism is its blood sugar lowering effect. Research using a validated animal model of diabetes has demonstrated *Momordica* charantia extract increase glucose utilization by the liver, decrease gluconeogenesis via inhibition of two key enzymes (glucose-6-phosphatase and fructose-1,6-bisphosphatase), and improve glucose oxidation through the shunt pathway by activating glucose-6-phosphate dehydrogenase.

Extracts of *Momordica charantia* also enhance cellular uptake of glucose, promote insulin release and potentiate its effect, and increase the number of insulin-producing beta cells in the pancreas of diabetic animals. (Ahmed *et al.*, 2001).

Bitter melon extract have been shown to inhibit growth and proliferation of various types of cancer cells in animals and in vitro. This may be attributed to the identification of a potent inhibitor of guanylate cyclase, an enzyme present in high amounts in many types of tumor cells. Other research indicates

Momordica charantia extract modify the immune response in cancer patients via decreased intestinal secretion of interleukin-7, reduced lymphocyte number, and increased T-helper and natural killer cell populations. (Harinantenaina *et al.*, 2006).

Momordica charantia extracts have broad-spectrum antimicrobial activity, having been shown to prevent infection by numerous viruses, bacteria, parasitic organisms, and fungi. Although mechanisms have not been determined for all organisms, in the case of viral infection it is thought that certain bitter melon constituents prevent viral penetration of the cell wall. The immune-stimulating properties of *Momordica charantia* extract may also contribute to decreased rates of microbial infection observed in animal studies. Animal studies demonstrate *Momordica charantia* extract, particularly the saponin fraction; have lipid-lowering effects resulting from inhibition of pancreatic lipase activity and subsequent decreased lipid absorption. Another study demonstrated Momordica *charantia* juice has an inhibitory effect on membrane lipid peroxidation (Oishi *et al.*, 2007)

Clinical Indications

Diabetes

Perhaps the best-researched use of bitter melon is to lower blood sugar levels in diabetics. Alcohol extracted charantin from *Momordica charantia* consists of mixed steroids, and in an animal model of diabetes it improved glucose tolerance to a degree similar to the oral hypoglycemic agent, tolbutamide.

A clinical trial of nine patients with confirmed type 1 diabetes found that subcutaneous injection of a *Momordica charantia* extract containing crystallized p-insulin resulted in a statistically significant decrease in blood sugar levels compared with controls. Fasting blood sugar was drawn prior to the administration of p-insulin and plasma glucose levels were used to determine the dosage of p-insulin given to each patient. The onset of p-insulin's effect was noted 30-60 minutes after administration, with peak effect ranging widely from 4-12 hours.

This most resembles long acting (NPH) insulin that demonstrates an onset of 1.5-2 hours, with a peak effect from 4-12 hours after administration. Oral bitter melon preparations have also been shown to be effective in clinical trials of type 2 diabetes.

It was demonstrated that a statistically significant improvement in glucose tolerance in type 2 diabetics. Eighteen diabetic subjects (average age 38 years) were given 100mL *Momordica charantia* juice 30 minutes prior to a glucose load. Improved glucose tolerance was observed in 13 of 18 patients (73%). To serve as a control, a glucose tolerance test was administered to all 18 subjects using 100ml of water prior to the glucose load. (Welihinda *et al.*, 2002)

In another uncontrolled trial, two *Momordica charantia* extract were evaluated. Dried fruit powder was given (5 g three times daily) to five subjects with diabetes (type unspecified), and seven subjects in a second group received an aqueous extract containing 100 g fruit per 100 ml of water. Those in the fruit powder group experienced an average 25-percent drop in blood sugar at the end of the three-week trial, while those in the aqueous extract group experienced an average 54-percent drop in blood sugar after three weeks. Glycosylated hemoglobin (HbA1C) examined in seven subjects decreased an average of 17 percent after the three-week trial. (Sarkar *et al.,* 1996).

A randomized, double-blind, placebo-controlled, three-month trial involving 40 diabetic patients (either newly diagnosed or poorly controlled) examined the effect of encapsulated *Momordica charantia* extract powder (Charantia) on HbA1C. Secondary outcome measures were fasting blood sugar, total cholesterol, and body weight.

Momordica charantia extract or placebo (two capsules three times daily; exact dosage not disclosed) was administered to 20 patients in each group. While a slight improvement was noted in HbA1C, it was not statistically significant, and no significant improvements were observed in secondary outcomes compared to the control group. (Harinantenaina *et al.,* 2006).

Although the amount given may have been insufficient to effect improvement, this remains purely speculative since the dosage was not disclosed.

Dyslipidemia

Several animal studies using a rodent model of diabetes have examined the effect of bitter melon extract on abnormal lipid parameters. Significant decreases in triglycerides and LDL cholesterol and increases in HDL cholesterol were noted in all studies.

In the longest study (10 weeks) MC extract was given to normal and streptozotocin-induced type-1 diabetic rats. Diabetic rats had elevated total cholesterol, triglycerides, and phospholipids, as well as decreased HDL cholesterol; moderate increases in plasma lipid peroxides and malondialdehyde (signs of increased oxidative stress) were also observed. After 10 weeks, diabetic rats receiving *Momordica charantia* extract experienced a normalization of all parameters compared to control rats not given the extract.

Microbial Infections

In vitro studies have shown bitter melon extracts and the MAP30 protein analog, isolated from the seeds of *Momordica charantia* extract, possess broad-spectrum antimicrobial activity. *Momordica charantia* extract inhibit infection and growth of several viruses, including HIV, Herpes simplex, and Epstein Barr virus.

A preliminary report on the effect of *Momordica charantia* extract in three HIV patients showed a normalization of CD4/CD8 ratios with *Momordica charantia* treatment. It is believed Momordica *charantia* extract inhibit HIV replication by preventing syncytial formation and cell-to-cell infection. (Sarkar *et al.,* 1996).

Momordica charantia extract also appear to inhibit the growth of numerous gram-negative and gram-positive bacteria, including E. coli, *Salmonella*, *Shigella*, *Staphylococcus*, *Pseudomonas*, *Streptobacillus*, *Streptococcus*, and H. pylori, and parasitic organisms E. histolytica and *Plasmodium* falciparum.

Cancer

Although clinical trials have not been conducted using *Momordica charantia* extract in cancer patients, in vitro studies indicate bitter melon fruit and seed extracts inhibit the growth of several cancer cell lines, including prostate adenocarcinoma, human colon cancer (Caco-2 cells),and the highly metastatic breast cancer cell line MDA-MB 231.

Drug-Botanical Interactions

Due to its hypoglycemic effects, *Momordica charantia* extract may potentiate the effects of insulin and oral hypoglycemic medications. Patients should be advised to closely monitor blood sugar when adding this botanical to a treatment regimen.

Side Effects and Toxicity

Oral ingestion of bitter melon fruit is safe as demonstrated by long-term consumption of the fruit in Asian cultures. Subcutaneous injection of p-insulin extracted from *Momordica charantia* appears to be safe; however, intravenous injection of *Momordica charantia* extract is significantly more toxic and not recommended.

Because bitter melon seeds contain momorcharin, shown to have antifertility effects in female mice, bitter melon seed consumption is not recommended in those seeking to become pregnant.

Dosage

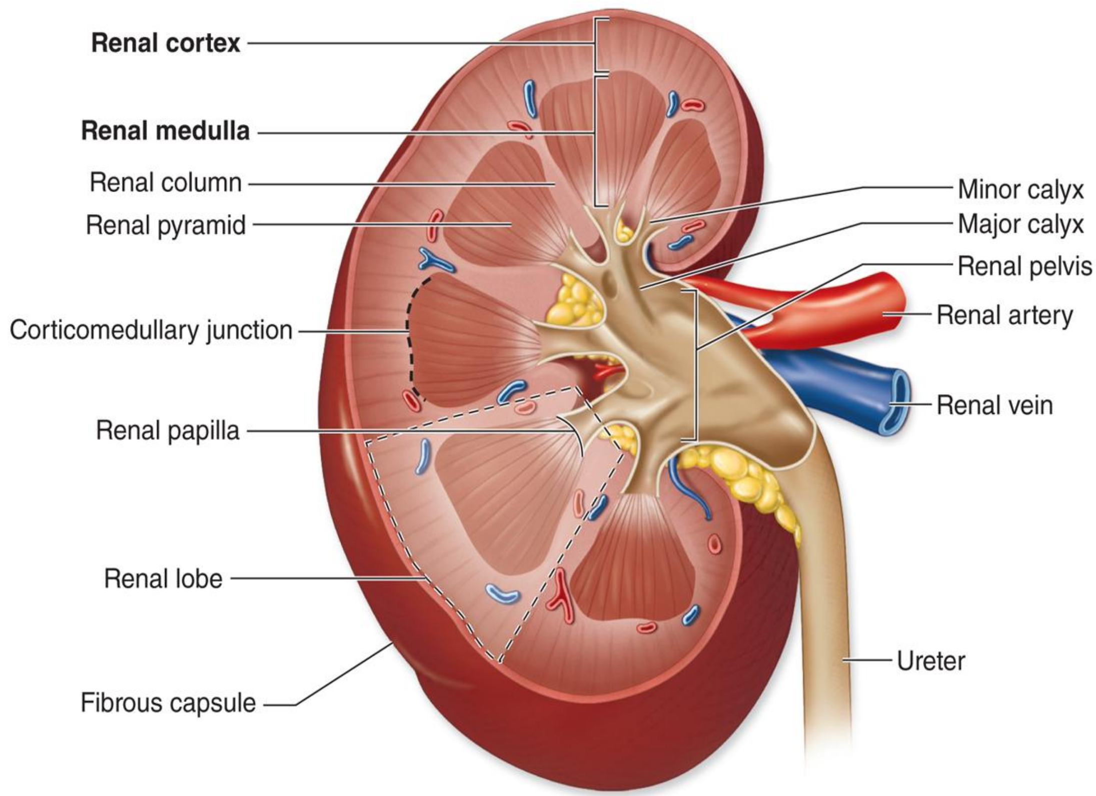
Dosage recommendations depend on the form of bitter melon being consumed. The dose of fresh juice is 50-100 ml but it is extremely bitter and difficult to drink. Although encapsulated dry powder is easier to ingest, the standard dose is 3-15 g daily – a large dose in capsule form. A standardized, encapsulated extract dosage ranges from 100-200 mg three times daily (Sarkar *et al.,* 1996).

Warnings and Contraindications

Because seed extract have been shown to induce abortion in mice and the root is a documented uterine stimulant, use is not recommended in pregnant women or those seeking pregnancy, although the fruit was not found to induce miscarriage, safety in pregnancy has not been established.

2.2 HISTOLOGY OF THE KIDNEY

Each mammalian kidney has a concave medial border called the hilum. It is through this hilum nerves, blood and lymph vessels entered and exit, and ureter exits. It also has a convex lateral surface, both covered by a thin fibrous capsule as shown in the diagram below



(Tisher and Brenner, 2009).

Kidney. Figure 2.2

Each kidney is bean-shaped, with a concave hilum where the ureter and the renal artery and veins enter. The ureter divides and subdivides into several major and minor calyces, around which is located the renal sinus containing adipose tissue. Attached to each minor calyx is a medullary pyramid, a conical region of medulla delimited by extensions of cortex. The cortex and hilum are covered with a fibrous capsule.

Within the hilum the upper end of the ureter expands as the renal pelvis and divides into two or three major calyces. Smaller branches called the minor calyses arise from each major calyx. The area surrounding the renal pelvis and calyces contains adipose tissue (Tisher and Brenner, 2009).

The kidney has an outer cortex which is a darker region with many corpuscles and cross sections of tubules and an inner medulla consisting of straight aligned structures as in the above diagram or figure. In human being, the renal medulla consists of 8-12 conical structures called the renal pyramids, which are separated by extensions from the cortex called the renal columns. Each pyramid plus the cortical tissue at its base and along its sides constitutes a renal lobe. Striations extending from the medulla into the cortex are called medullary rays, these plus the attached cortical tissue are considered lobules. The tip of each pyramid called the renal papilla projects into a minor calyx that collects urine formed by tubules in the pyramid as indicated in the above figure (Hughes, 2005).

Each kidney contained about 1 million functional units called the nephrons, that consist of simple, single-layered epithelium along their entire lengths.

Renal corpuscle is an initial dilated part enclosing a tuft of capillary loops and the site of blood filtration, always located in the cortex.

Proximal tubule is a long convulated part, located entirely in the cortex, with a shorter straight part that enters the medulla.

Loop of Henle (or nephron loop), is in the medulla, with a thin ascending and a thin descending limbs.

Distal tubule, it consists of a thick straight part ascending from the loop of Henle back into the cortex and a convulated part completely in the cortex

Connecting tubule, a short final part linking the **nephron** to connecting ducts. Connecting tubules from several **nephrons** merge to form collecting tubules that then merge as a larger collecting duct. These converse in the renal papilla, where they deliver urine to a mino carlyx. Cortical nephrons are located almost completely in the cortex while juxtamedullary nephrons (about one-seventh of the total) lie close to the medulla and have long loops of Henle. (Tisher and Brenner, 2009)

Connecting tubules from several nephrons merge to form collecting tubules that hen merge as larger collecting ducts. These converge in the renal papilla, where they deliver urine to a minor calyx. Cortical nephrons are located almost completely in the cortex while **juxtamedullary nephrons** (about one-seventh of the total) lie close to the medulla and have long loops of Henle (Lu, 2007).

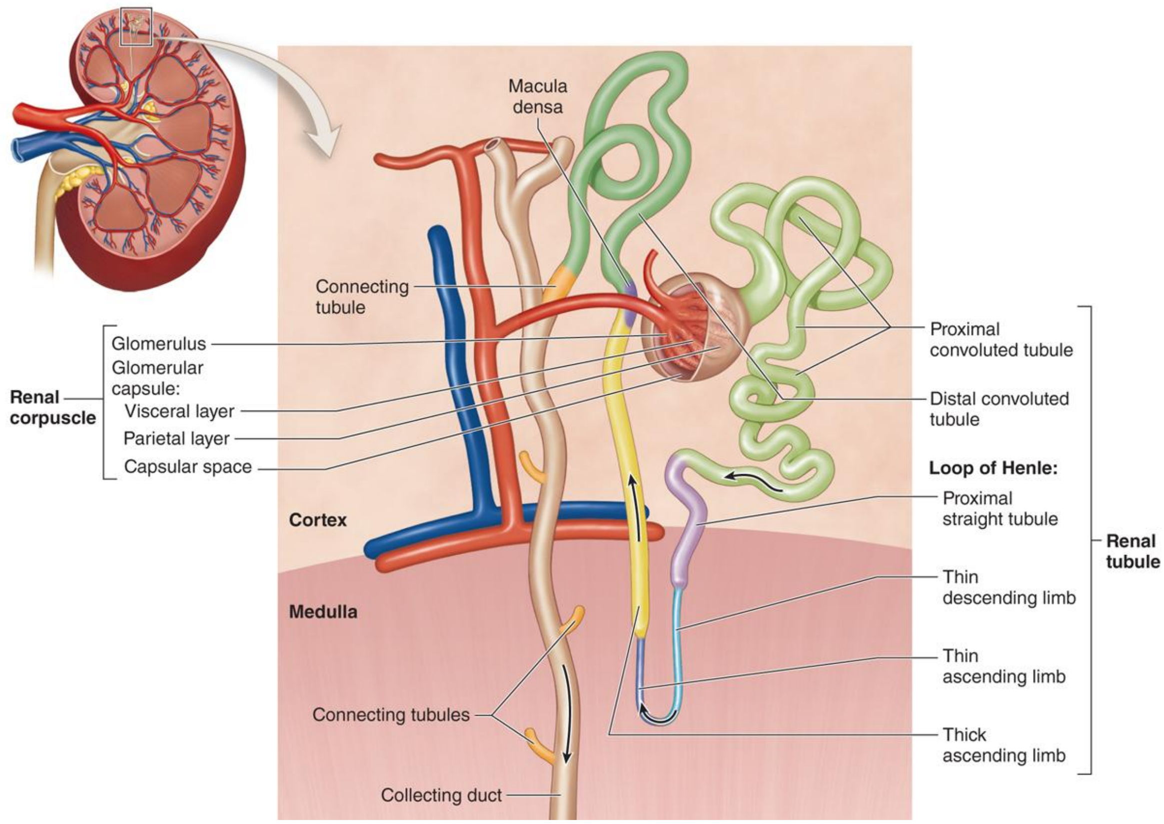
2.2.1 Blood Circulation

As expected for an organ specialized to process the blood, the kidney vasculature is large, well-organized, and closely associated with all components of the nephron. Blood vessels of the kidneys are named according to their locations or shapes.

Each kidney's renal artery divides into two or more seg-mental arteries at the hilum. Around the renal pelvis, these branches further as the interlobar arteries, which extend between the renal pyramids toward the corticomedullary junction. Here the interlobar arteries divide again to form the arcuate arteries that run in an arc along this junction at the base of each renal pyramid. Smaller inter-lobular arteries (or cortical radial arteries) radiate from the arcuate arteries, extending deeply into the cortex. (Remuzzi *et al.*, 1997)

From the interlobular arteries arise the microvascular afferent arterioles, which divide to form a plexus of capillary loops called the glomerulus, each of which is located within a renal corpuscle where the blood is filtered. Blood leaves the glomerular capillaries, not via venules, but via efferent arterioles, which at once branch again to form another capillary network, usually the peritubular cap-illaries profusely distributed throughout the cortex. From the juxtaglomerular corpuscles near the medulla, efferent arterioles do not form peritubular capillaries, but instead branch repeatedly to form parallel tassel-like bundles of capillary loops called the vasa recta that penetrate deep into the medulla in association with the loops of Henle and collecting ducts. Collectively, the cortex receives over 10 times more blood than the medulla. (Remuzzi *et al.*, 1997)

Blood leaves the kidney in veins that follow the same courses as arteries and have the same names (Figure 2.2.2). The outermost peritubular capillaries and capillaries in the kidney capsule converge into small stellate veins that empty into the interlobular veins.



(Tisher and Brenner, 2009).

Figure 2.2.1

Each kidney contains approximately 1 million functional units called nephrons. Each nephron originates in the cortex, at the renal corpuscle surrounding a small tuft of glomerular capillaries. Extending from the corpuscle is the long proximal convoluted tubule which leads to short proximal straight tubule that enters the outer medulla. This tubule continues as the thin descending limb and the thin ascending limb of the nephron's loop of Henle in the medulla. The loop of Henle ends with a thick ascending limb, a straight tubule that reenters the cortex and ends at its thickened macula densa area where it contacts the arterioles entering *the* glomerulus. Beyond the macula densa this tubule is the distal convoluted tubule, the end of which is the short connecting tubule. Connecting tubules from many nephrons merge into cortical collecting tubules and a collecting duct that transports urine to the calyx.

2.2.2 Renal Function: Filtration, Secretion and Reabsorption

All the major functions of the kidneys, the removal of metabolic wastes and excess water and electrolytes from blood are performed by various specialized epithelial cells of the nephrons and collecting systems. Renal function involves specific activities:

- Filtration, by which water and solutes in the blood leave the vascular space and enter the lumen of the nephron;
- Tubular secretion, by which substances move from epithelial cells of the tubules into the lumens, usually after uptake from the surrounding interstitium and capillaries; and
- Tubular reabsorpiion, by which substances move from the tubular lumen across the epithelium into the interstitium and surrounding capillaries.

Along the length of the nephron tubule and collecting system, the nitrate receives various secreted molecules while others are reabsorbed and then enters the minor calyces as urine and undergoes excretion. (Neisen, 2007).

The number of nephrons decreases slightly in older adults, a process accelerated by high blood pressure. If a kidney is donated for transplant (unilateral nephrectomy), the remaining kidney undergoes compensatory growth, with cellular hypertrophy in the proximal parts of the nephron tubules and an increase in the rate of filtration, which allow normal renal function to continue. (Farguhar, 2006).

2.2.3 Renal Corpuscles and Blood Filtration

At the beginning of each nephron is a renal corpuscle, about 200 μm in diameter and containing a tuft of glomerular capillaries, surrounded by a double-walled epithelial capsule called the glomerular (Bowman) capsule (Figure 2.2.1). The internal or visceral layer of this capsule closely envelops the glomerular capillaries, which are finely fenestrated. The outer parietal layer forms the surface of the capsule. Between the two capsular layers is the capsular (or urinary) space, which receives the fluid filtered through the capillary wall and visceral layer. Each renal corpuscle has a vascular pole, where the afferent arteriole enters and the efferent arteriole leaves, and a tubular pole, where the proximal convoluted tubule (PCT) begins.

The outer parietal layer of a glomerular capsule consists of a simple squamous epithelium supported externally by a basal lamina. At the tubular pole, this epithelium changes to the simple cuboidal epithelium that continues and forms the proximal tubule (Figure 2.2.3).

The visceral layer of a renal corpuscle consists of unusual stellate epithelial cells called podocytes, which together with the capillary endothelial cells compose the apparatus for renal filtration. From the cell body of each podocyte several primary processes extend and curve around a length of glomerular capillary. Each primary process gives rise to many parallel, in terdigitating secondary processes or pedicels (L. *pedicellus*, little foot (Figure 2.2.3). The pedicels cover much of the capillary surface, in direct contact with the basal lamina, (Hughes, 2005).

glycoproteins and proteoglycans interact to form a series of openings within the slit diaphragm, with a surface that is negatively charged.

Between the highly fenestrated endothelial cells of the capillaries and the covering podocytes is the thick (300-360 nm) glomerular basement membrane (GBM). This membrane is the most substantial part of the filtration barrier that separates the blood from the capsular space and forms by fusion of the capillary- and podocyte-produced basal laminae. Laminin and fibronectin in this fused basement membrane bind integrins of both the podocyte and endothelial cell membranes, and the meshwork of cross-linked type IV collagen and large proteoglycans restricts passage of proteins larger than about 70 kDa. Smaller proteins that are filtered from plasma are degraded, and the amino acids reabsorbed in the proximal tubule. Polyanionic GAGs in the glomerular membrane are abundant and their negative charges, like those of the slit diaphragms, tend to restrict filtration of organic anions. (Farguhar, 2006).

Filtration, therefore, occurs through a structure with three parts:

- The fenestrations of the capillary endothelium, which blocks blood cells and platelets;
- The thick, combined basal laminae, or GBM, which restricts large proteins and some organic anions; and
- The filtration slit diaphragms between pedicels, which restrict some small proteins and organic anions.

Normally about 20% of the blood plasma entering a glom-erulus is filtered into the capsular space. The initial glomerular filtrate has a chemical composition similar to that of plasma except that it contains very little protein. The glomerular filter blocks filtration of most plasma proteins, but smaller proteins, including most polypeptide hormones, are removed into the filtrate. (Farguhar, 2006).

Capillaries of each glomerulus have a total length of approximately 1 cm and are uniquely situated between two arterioles; afferent and efferent, the muscle of which allows increased hydrostatic pressure in these vessels, favoring movement of plasma across the glomerular filter. The glomerular filtration rate (GFR) is constantly regulated by neural and hormonal inputs affecting the degree of constriction in each of these arterioles. The total glomerular filtration area of an adult has been estimated at 500 cm^2 and the average GFR at 125 mL/min or 180 L/d. Because the total amount of circulating plasma averages 3 L, it follows that the kidneys typically filter the entire blood volume 60 times every day. (Farguhar, 2006).

In addition to capillary endothelial cells and podocytes, renal corpuscles also contain mesangial cells (Gr. *mesos*, in the midst + *angion*, vessel), most of which resemble vascular pericytes in having contractile properties and producing components of an external lamina. Mesangial cells are difficult to distinguish in routine sections from podocytes, but often stain more darkly. They and their surrounding matrix comprise the mesangium (Figure 2.2.4), which fills interstices between capillaries that lack podocytes. Functions of the mesangium include the following:

- Physical support of capillaries within the glomerulus;
- Adjusted contractions in response to blood pressure changes, which help maintain an optimal filtration rate;
- Phagocytosis of protein aggregates adhering to the glomerular filter, including antibody-antigen complexes abundant in many pathological conditions; and

- Secretion of several cytokines, prostaglandins, and other factors important for immune defense and repair in the glomerulus.

2.2.4 Proximal Convoluted Tubule

Cells in many parts of the nephron tubule and collecting system reabsorb water and electrolytes, but other activities are restricted mainly to specific tubular regions. Summary of major function; of parts within nephrons and collecting ducts, along with the histologic features involved in these activities.

At the tubular pole of the renal corpuscle, the simple squamous epithelium of the capsule's parietal layer is continuous with the simple cuboidal epithelium of the proximal convoluted tubule (PCT) (Figures 2.2.3). These long, tortuous tubules fill most of the cortex. PCT cells are specialized for both reabsorption and secretion. Over half of the water and electrolytes, and all of the organic nutrients DCT, distal convoluted tubule; PCT, proximal convoluted tubule; Tal, thick ascending limb.

Histologic features and major functions of regions within renal tubules

(Glucose, amino acids, vitamins, etc), filtered from plasma in the renal corpuscle are normally reabsorbed in the PCT. These molecules are transferred directly across the tubular wall for immediate uptake again into the plasma of the peritubular capillaries.

Transcellular reabsorption involves both active and passive mechanisms, with the cells having a large variety of trans-membrane ion pumps, ion channels, transporters, enzymes, and carrier proteins. Water and certain solutes can also move passively between the cells (paracellular transport) along osmotic gradients through leaky apical tight junctions.

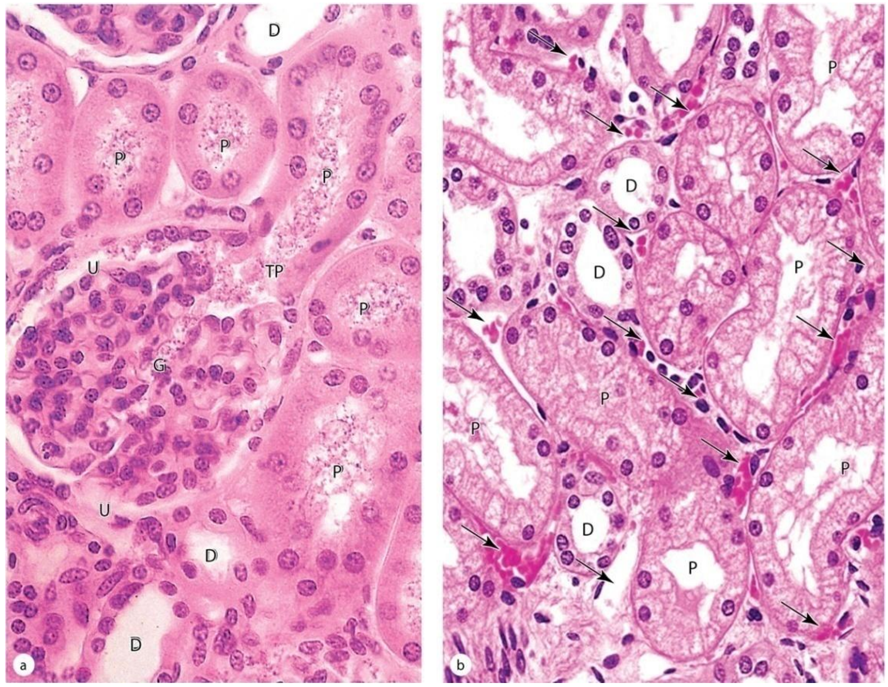
Small proteins in the filtrate are either reabsorbed by receptor-mediated endocytosis and degraded in the cuboidal cells, or degraded by peptidases on the luminal surface. In both cases the amino acids are released at the basolateral cell surfaces for uptake by capillaries.

Conversely, organic anions and cations not filtered in the renal corpuscle (because of the polyanions in the filter or binding to plasma proteins) may be released in the peritubular capillaries, taken up by the cells of the proximal tubules and undergo secretion into the filtrate. Organic anion and cation transporters allow the kidneys to dispose of such substances at a higher rate than by glomerular filtration alone. Because these molecules include important substances (such as bile salts, creatinine, etc) and many antibiotics and other drugs, this process of tubular secretion is of great phar-macologic importance as a key mechanism of drug clearance. (Andrews and Porter, 1984).

The cells of the proximal tubules have central nuclei and very acidophilic cytoplasm because of the abundant mitochondria. The cell apex has very many long microvilli that form a prominent brush border in the lumen that facilitates reabsorption (Figure 2.2.3). Because the cells are large, each transverse section of a PCT typically contains only three to five nuclei. In routine histologic preparations, the long brush border may be disorganized and give the lumens a fuzz-filled appearance. Peritu-bular capillaries are abundant in the sparse surrounding connective tissue interstitium, which fills only about 10% of the cortex.

Ultrastructurally the apical cytoplasm of these cells has numerous pits and vesicles near the bases of the microvilli, indicating active endocytosis and pinocytosis. These vesicles contain the small, reabsorbed proteins that will be degraded in lysosomes, with the amino acids released to the circulation. Proximal tubular cells also have many long basal membrane invaginations and lateral interdigitations with neighboring cells. Both the brush border and the basolateral folds contain the many types of transmembrane proteins that mediate tubilar reabsorption and secretion. Long mitochondria concentrated along the basal invagination supply ATP locally for the membrane proteins involved in active transport. Because of the extensive interdigitations of the lateral membranes, discrete limits between cells of the proximal tubule are difficult to see with the light microscope. (Andrews and Porter, 1984).

Besides their major roles in reabsorption and sedretiori, cells of the proximal tubule also perform hydroxylation of vitamin D and release to the capillaries. Moreover, fibroblastic interstitial cells in cortical areas near the proximal tubules produce erythropoietin, the growth factor secreted in response to a prolonged decrease in local oxygen concentration.



(Tisher and Brenner, 2009).

Fig. 2.2.2 renal cortex: proximal and distal convoluted tubules.

(a) The micrograph shows the continuity at a renal corpuscle's tubular pole (TP) between the simple cuboidal epithelium of a proximal convoluted tubule (P) and the simple squamous epithelium of the capsule's parietal layer. The urinary space (U) between the parietal layer and the glomerulus (G) drains into the lumen of the proximal tubule. The lumens of the proximal tubules appear filled, because of the long microvilli of the brush border and aggregates of small plasma proteins bound to this structure. By contrast, the lumens of distal convoluted tubules (D) appear empty, lacking a brush border and protein.
(b) Here the abundant peritubular capillaries and draining venules (arrows) that surround the proximal (P) and distal (D) convoluted tubules are clearly seen. Both X400. H and E.

TAL of the nephron, which are monitored by cells of the macula densa. Increased ion levels in the lumen cause these cells to release ATP, adenosine, and other vasoactive compounds that trigger contraction of the afferent arteriole, which lowers glomerular pressure and decreases the GFR. This lowers tubular ion concentrations, which turns off the release of vasoconstrictors from the macula densa (Hughes, 2005).

Decreased arterial pressure leads to increased autonomic stimulation to the JGA as a result of baroreceptor function, including local baroreceptors in the afferent arteriole, possibly the JG cells themselves. This causes the JG cells to release renin, an aspartyl protease, into the blood (Figure 2.2.7). There renin cleaves the plasma protein angiotensinogen into the inactive deca-peptide angiotensin I. Angiotensin-converting enzyme (ACE) on lung capillaries clips this further to angiotensin II, a potent vasoconstrictor that directly raises systemic blood pressure and stimulates the adrenals to secrete aldosterone. Aldosterone promotes Na^{r} and water reabsorption in the distal convoluted and connecting tubules, which raises blood volume to help increase blood pressure. The return of normal blood pressure turns off secretion of renin by JG cells (Hughes, 2005).

Pathology of the Kidney

Glomerular diseases constitute some of the major problems in nephrology; indeed, chronic glomerulonephritis is one of the most common causes of chronic renal failure in humans. Glomeruli may be injured by a variety of factors and in the course of a number of systemic diseases. Immunologic diseases such as systemic lupus erythematosus (SLE), vascular disorders such as hypertension and plyarteritis modosa, metabolic diseases conditions such as Fabry disease often affect the glomerular diseases to differentiate them from those in which the kidney is the only or predominant organ involved. The latter constitute the various types of primary glomerulonephritis or, because some do not have a cellular inflammatory component, glomerulopathy (Tisher and Brenner, 2009). However, both the clinical manifestations and glomerular histologic changes in primary and secondary forms can be similar. Both the primary glomerulonephritides and the systemic diseases affecting the glomerulus can result in these syndromes. Thus, a critical point in the clinical differential diagnosis is first to exclude the major systemic disorders, of which the major four are diabetes mellitus, SLE, vasculitis, and amyloidosis (Tisher and Brenner, 2009).

Histologic Alterations

Various types of glomerulonephritis are characterized by one of more or four basic tissue reactions.

Hypercellularity: So-called inflammatory diseases of the glomerulus are associated with an increase in the number of cells in the glomerular tuffs. This hypercellularity is associated with one or combinations of three of the following:

1. Cellular proliferation: of messangial, endothelial, or in certain cases, parietal epithelial cells.
2. Leukocytic infiltration: considering of neutrophils, monocytes and in some diseases, lymphocytes.
3. Formation of crescents: These are accumulations of cells composed of proliferating epithelial cells and infiltrating leukocytes (Tisher and Brenner, 2009)

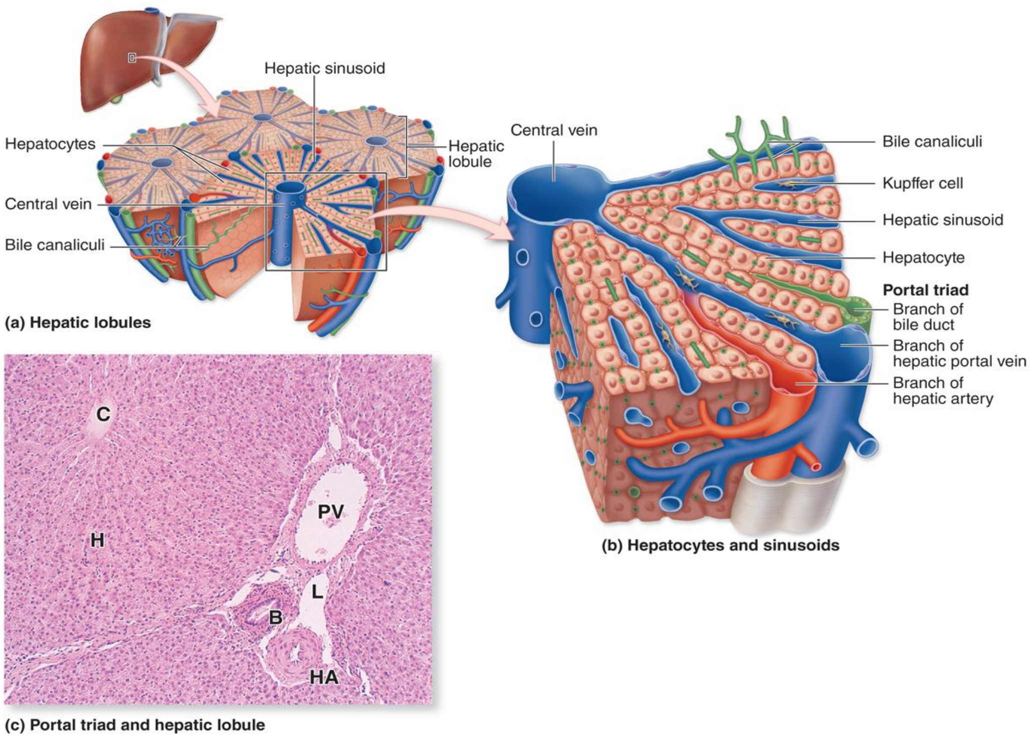
THE GLOMERULAR SYNDROMES	
Acute nephritic syndrome	▪ Hematuria, azotemia, variable proteinuria, oliguria, edema, and hypertension.
Rapidly progressive glomerulonephritis	▪ Acute nephritis, poteinuria, and acute renal failure.
Nephritic syndrome	▪ >3.5gm proteinuria, hypoalbuminemia, hyperlipidemia, lipiduria.
Chronic renal failure	▪ Azotemia → uremia progressing for years.
Asymptomatic hematuria or proteinuria	▪ Glomerular hematuria; subnephrotic proteinuria.

(Tisher and Brenner, 2009).

2.27 HEPATIC LOBULES

The livers unique histologic organization and microvasculature allow hepatocytes to perform their diverse metabolic, exocrine, and endocrine functions. Hepatocytes are large cuboidal or polyhedral epithelial cells, with large, round central nuclei and eosinophilic cytoplasm rich in mitochondria. The cells are frequently binucleated and about 50% of them are polyploidy, with two to eight times the normal chromosome number.

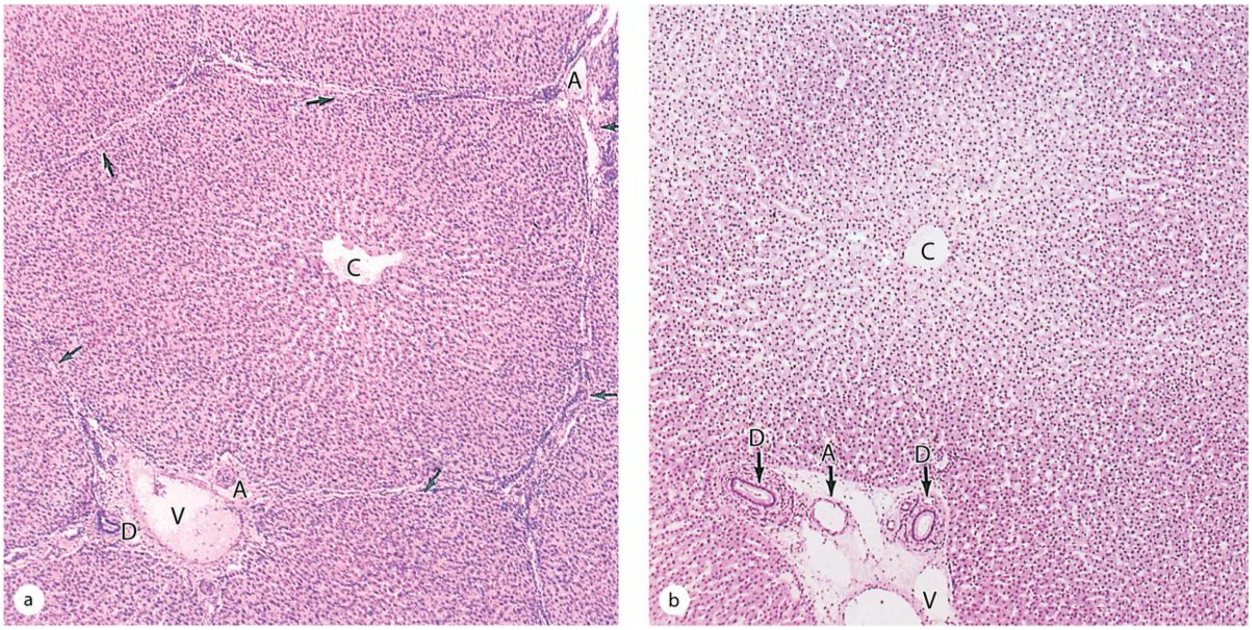
The liver parenchyma is organized as thousands of small (~0.7×2 mm) hepatic lobules in which hepatocytes form hundreds of irregular plates arranged radially around a small central vein (Alison, 2008).



(Alison, 2008)
Fig. 2.3.1
The liver, a large organ in the upper right quadrant of the abdomen, immediately below the diaphragm, is composed of thousands of polygonal structures called **hepatic lobules**, which are the basic functional units of the organ. **(a)** Diagram showing a small **central vein** in the center of a hepatic lobule and several sets of blood vessels at its periphery. The peripheral vessels are grouped in connective tissue of the **portal tracts** and include a branch of the portal vein, a branch of the hepatic artery, and a branch of the bile duct (the **portal triad**).

(b) Both blood vessels in this triad branch as **sinusoids**, which run between plates of **hepatocytes** and drain into the central vein.

(c) Micrograph of a lobule shows the central vein (**C**), plates of hepatocytes (**H**), and in an adjacent portal area a small lymphatic (**L**) and components of the portal triad: a portal venule (**PV**), hepatic arteriole (**HA**), and bile ductule (**B**). X220. H and E.



(Alison, 2008)

Fig. 2.3.2 Hepatic lobule

Cut transversely, hepatic lobules are polygonal units showing plates of epithelial cells called **hepatocytes** radiating from a central venule (**C**). **(a)** Hepatic lobules of some mammals, such as the pig, are delimited on all sides by connective tissue.

(b) In humans these lobules have much less connective tissue and their boundaries are more difficult to distinguish. In both cases peripheral connective tissue of portal areas contains the a portal triad: small bile ductules (**D**), venule (**V**) branches of the portal vein, and arteriole (**A**) branches of the hepatic artery. Both X150. H and E.

plates are supported by a delicate stroma of reticulin fibers (Figure 2.3.2). Peripherally each lobule has three to six portal areas with more fibrous connective tissue, each of which contains three interlobular structures that comprise the portal triad:

- A venule branch of the portal vein, with blood rich in nutrients but low in O_2,
- An arteriole branch of the hepatic artery that supplies O_2,
- One or two small bile ductules of cuboidal epithelium, branches of the bile conducting system.

Most of the peripheral portal areas also contain lymphatics and nerve fibers and in some species (example pigs) extend thin sheets of fibrous connective tissue completely around the lobules, making individual lobules easier to distinguish than in humans (Alison, 2008).

Between all of the anastomosing plates of hepatocytes of a hepatic lobule are important vascular sinusoids that emerge from the peripheral branches of the portal vein and hepatic artery and converge on the lobule's central vein (Figures 2.3.3). The venous and arterial blood mixes in these irregular hepatic sinusoids. The anastomosing sinusoids have thin, discontinuous linings of fenestrated endothelial cells surrounded by sparse basal lamina and reticular fibers. The discontinuities and fenestrations allow plasma to fill a narrow perisinusoidal space (or space of Disse) and directly bathe the many irregular microvilli projecting from the hepatocytes into this space (Figure 2.3.2). This direct contact between hepatocytes and plasma facilitates most key hepatocyte functions that involve uptake and release of nutrients, proteins, and potential toxins (Cousins, 2005).

Two other functionally important cells are found with the sinusoids of hepatic lobules:

- Numerous specialized stellate macrophages, usually called Kupffer cells, are found within the sinusoid lining (Figure 2.3.3). These cells recognize and phagocytose aged erythrocytes, freeing heme and iron for reuse or storage in ferritin complexes. Kupffer cells are also antigen-presenting cells and remove any bacteria or debris present in the portal blood.
- In the perisinusoidal space are hepatic stellate cells (or Ito cells) with small lipid droplets that store vitamin A

Blood always flows from the periphery to the center of 'each hepatic lobule. Consequently, oxygen and metabolites, as well as all other toxic or nontoxic substances absorbed in the intestines, reach the lobule's peripheral cells first and then the more central cells. This direction of blood flow partly explains why the properties and function of the periportal hepatocytes differ from those of the centrolobular cells. Hepatocytes near the portal areas can rely on aerobic metabolism and are often more active in protein synthesis, while the more central cells are exposed to lower concentrations of nutrients and oxygen and are more involved with detoxification and glycogen metabolism, (Goresky and Groom, 2004).

Function of the Liver

As mentioned previously, hepatocytes are highly versatile cells with diverse functions that are reflected in their structure. Abundant rough ER is focused on synthesis of plasma proteins and causes cytoplasmic basophilia, which is often more pronounced in hepatocytes near the portal areas. Abundant smooth ER, distributed more evenly throughout the cytoplasm, contains the enzyme systems for the biotransformation or detoxification of substances in blood, which are then usually excreted with bile. These include enzymes responsible for oxidation, methylation, and conjugation of steroids, baroiturates, antihistamines, anticonvulsants, and other drugs. Under some conditions prolonged presence of drugs can lead to increased amounts of SER in hepatocytes, thus improving the liver's detoxification capacity. Other SER enzymes (glucuronosyl transferases) conjugate bilirubin to glucuronate, rendering it more soluble and facilitating its excretion in bile, (Jugermann and Katz, 2009).

Glycogen granules and small lipid droplets in hepatocytes, and very small electron-dense ferritin complexes (hemosiderin) primarily in the Kupffer cells, respectively mediate temporary storage of glucose, triglycerides, and iron.

Hepatocyte peroxisomes are also abundant and important for oxidation of excess fatty acids, catalase-mediated breakdown of the hydrogen peroxide generated by fatty acid oxidation (by mean of catalase activity), and conversion of excess purines to uric acid. Many Golgi complexes are also present, involved in synthesis of both plasma proteins and bile components. The numerous mitochondria provide energy for all these activities, (Kraus, 2004).

The different categories of hepatocyte functions including secretion of proteins into blood, the exocrine secretion of bile, and the removal of diverse small compounds from blood have led to three ways of considering liver lobule structure, (Kraus, 2004).

- The classic hepatic lobule, with blood flowing past hepatocytes from the portal areas to a central venule, emphasizes the endocrine function of the structure producing factors for uptake by plasma.
- The concept of portal lobules of hepatocytes is more useful when considering the exocrine function of these cells, that is, bile secretion. The portal area has the bile ductule at the center, and bile, moving in the opposite direction as the blood, flows toward it from all the surrounding hepatocytes. The tissue draining bile into each portal area duct is roughly triangular in shape, with the central veins of three classic lobules at its angles, (Kraus, 2004).

- The hepatic acinus, a third way of viewing liver cells, emphasizes the nature of the blood supply to the hepatocytes and the oxygen gradient from the hepatic artery branch to the central vein. In a liver acinus hepatocytes make up an irregular oval or diamond-shaped area extending from two portal triads to the two closest central veins. Periportal hepatocytes nearest the hepatic arteriole, comprising zone I in the acinus, get the most oxygen and nutrients and can most readily carry out functions requiring oxidative metabolism such as protein synthesis. Hepatocytes in zone III, near the central vein, get the least oxygen and nutrients. They are the preferential sites of glycolysis, lipid formation, and drug biotransformations and are the first hepatocytes to undergo fatty accumulation and ischemic necrosis. In the intervening zone II, hepatocytes have an intermediate range of metabolic functions between those in zones I and III. The major activities in any given hepatocyte result from the cell adapting to the microenvironment produced by the contents of the blood to which it is exposed.
- Unlike the salivary glands and pancreas, the liver has a strong capacity for regeneration despite its normal slow rate of cell renewal. Hepatocyte loss from the action of toxic sub; stances triggers mitosis in the remaining healthy hepatocytes in a process of compensatory hyperplasia that maintains the original tissue mass. Surgical removal of a liver portion produces a similar

response in the hepatocytes of the remaining lobe(s). The regenerated liver tissue is usually well organized, with the typical lobular arrangement, and replaces the functions of the destroyed tissue. This regenerative capacity is important clinically because one major liver lobe can sometimes be donated by a living relative for surgical transplant and I full liver function restored in both donor and recipient.

Besides proliferation of existing hepatocytes, a role for liver stem cells in regeneration has been shown in some experimental models. Such cells, often called oval cells, are present among cholangiocytes of the bile canals near portal areas and produce progenitor cells for both hepatocytes and cholangiocytes, (Henderson and Daniel, 2004).

Pathology of the Liver

The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory, and neoplastic insults. In some instances, the disease is primary to the liver, as in viral hepatitis and hepatocellular carcinoma. More often the hepatic involvement is secondary, often to some of the most common diseases in humans, such as cardiac decompensation, disseminated cancer, alcoholism, and extrahepatic infections. The enormous functional reserve of the liver masks to some extent the clinical impact of early liver damage. However, with progression of diffuse disease or strategic disruption of bile flow, the consequences of deranged liver function become life threatening, (Jugermann and Kietzmann, 2006).

Before specific disease are reviewed:

1. General morphologic patterns of hepatic injury,
2. Hepatic formation of bile,
3. Hepatic failure, and
4. Cirrhosis.

From a morphologic standpoint, the liver is an inherently simple organ, with a limited repertoire of responses to injurious events. Regardless of cause, five general responses are seen, (Gitnick, 2009).

Damage from toxic or immunologic insult may cause hepatocytes to take on a swollen, edematous appearance (ballooning degeneration) with irregular clumped cytoplasm and large clear spaces. Alternatively, retained biliary material may impart a diffuse foamy swollen appearance to the hepatocyte (foamy degeneration). Substances may accumulate in viable hepatocytes, including iron and copper. Accumulation of fat droplets within hepatocytes is known as steatosis. Multiple tiny droplets that do not displace the nucleus are known as microvesicular steatosis and appear in such conditions as alcoholic liver disease and acute that displaces the nucleus, macrovesicular steatosis, may be seen in the alcoholic liver or in the livers of obese or diabetic individuals, (Jugermann and Kietzmann, 2006).

Necrosis and Apoptosis

Any significant insult to the liver may cause hepatocyte necrosis. In ischemic coagulative necrosis, the liver cells are poorly stained and “mummified” and often have lysed nuclei. With cell death that is toxic or immunologically mediated, isolated hepatocytes round up to form shrunken, pyknotic, and intensely eosinophilic Councilman bodies containing fragmented nuclei. This form of cell death is the consequence of apoptosis. Alternatively, hepatocytes may asmotically swell and rupture, so-called lytic necrosis, (Henriksen, 2005).

Necrosis frequently exhibits a zonal distribution. The most obvious is necrosis of hepatocytes immediately around the terminal hepatic vein (centrilobular necrosis), an injury that is characteristic of ischemic injury and a number of drug and toxic reactions. Pure midzonal and periportal necrosis is rare; the latter may be seen ineclampsia. With most other causes of hepatic injury, a variable mixture of hepatocellular death and inflammation is encountered, (Shulman, 2004). The hepatocyte necrosis may be limited to scattered cells within hepatic lobules (focal necrosis) or to the interface between the periportal parenchyma and inflamed portal tracts (interface hepatitis), (LaMont and Carey, 2002). With more severe inflammatory injury, necrosis of contiguous hepatocytes may span adjacent lobules in a portal to portal, portal to central, or central to central fashion (bridging necrosis) or most of the liver (massive necrosis) is usually accompanied by hepatic failure.

With disseminated candidal or bacterial infection, macroscopic abscesses may occur, (Carithers, 2002).

Inflammation

Injury to the liver associated with an influx of acute or chronic inflammatory cells is termed hepatitis. Although hepatocyte necrosis may precede the onset of inflammation, the converse is also the true. Attack on viable liver cells by sensitized T cells is a common cause of liver damage. Inflammation may be limited to the site of leukocyte entry (portal tracts) or spill over into the parenchyma. When hepatocytes undergo necrosis or apoptosis, scavenger macrophages engulf the dead cells within a few hours, generating clumps of inflammatory cells in an otherwise normal parenchyma. Foreign bodies, organisms, and a variety of drugs may incite a granulomatous reaction, (Lands, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 THE MATERIALS

The materials used during this study include; The plant (leaves and stem), Wistar rats (n=20), methanol (5 liters), EDTA bottles (30), plain bottles (30), syringes, animal feed, aluminum cages, plastic bottles for the drinking water of the rats, electronic weighing machine, distilled water, separating glass funnels and evaporating dishes, water bath machines, wooden mortar and pestle, test tubes for phytochemical analysis of the plant, and hand gloves, surgical blades, chloroform and small size plastic bucket for the use as anesthesia medium, glass slides, stains and microscope camera, microtome.

3.2 EXPERIMENTAL ANIMALS

Male adult albino rats (n=20) were purchased from Animal House of Human Anatomy Department, Ahmadu Bello University, Zaria. The rats were randomly selected and used for this study. They were grouped into a control group **A** and treatment groups **B, C** and **D**.

The rats were grouped according to their body weights. The mean body weight of the rats was 270±30g. They were kept in the Animal House of Anatomy Department, Ahmadu Bello University, Zaria for two weeks to acclimatized with the environment according to Grover *et al.* (2002).

3.3 ANIMAL GROUPING

The animals were grouped into a control group **A** and the treatment groups **B, C and D** as follows:

Group A: The control group and has five (5) rats, they were administered orally with distilled water daily for six (6) weeks.

Group B: The animals in this group were five (5) with average body weights in grammes 260±20 g weight **Appendix II**

The rats were administered orally with **125 mg** of the extract for six (6) weeks.

Group C: The rats were also five (5) and their average body weights was 258±20 g and were administered orally with **250 mg** of the plant extracts for six weeks **Appendix III**

Group D: The rats were five (5), their average weight was 264±30 g at the beginning of the study and were administered orally with **500 mg** of the *Momordica charantia* extract for six weeks **Appendix IV**

During this period, the animals were closely monitored and standard environmental conditions plus (+) or minus (-) 25^{O}C was maintained, relative humidity of 60% and 12 hours light and dark cycle were maintained.

The animals were properly fed with growers feed from Vital Feed Company. The animals were housed in plastic cages with stainless steel mesh covers, the floors were covered with saw dust which suck away the urine of the rats. This was changed on daily basis.

There was cross ventilation in the Animal House and was kept clean throughout the study-clean tap water was used for the rats drinks in plastic bottle with stainless steel nozzles while the feed was provided in plastic bowls.

3.4 THE PLANT

The plant was obtained from some gardeners around Shika Dam, Zaria Local Government (LGA) in Kaduna State, Nigeria on the 11th September, 2014. The plant was taken to the Herbarium Unit of the Department of Biological \Sciences, Ahmadu Bello, University, Zaria, for identification and authentification, the voucher number 1139 and the date was 19/10/2014

The collection of the plant took about two (2) weeks and was taken in the early morning and after sun set as was suggested by Sofowora, (2012). These periods before sun rise and after sunset are the best periods because, plants' activities such as photosynthesis are inactivated at these periods and plants chemical constituents are properly maintained.

The plant was identified as follows:

Family: **CUCURBITACEAE**

Momordica Charantia

Hausa: **Garahuni**

Common name: **Balsam pear**

Voucher no.: **1139**

Date of identification**:** 19th October, 2014.

The leaves and stem of the plant were carefully packed into a well ventilated room and spread sparsely for shade drying. Every two (2) days the spread plant was checked and overturned. This process was run for two (2) weeks for the plant to dry.

The leaves and stem of the plant were parked and crushed into powdered by means of wooden mortar and pestle. The powdered form was weighed to be 2.6 kg which was assumed to be enough for the extraction procedures.

3.5 EXTRACTION OF *MOMORDICA charantia*

Maceration method was used for the extraction, 500 g of the powder of the plant was taken and placed into a separating funnel with a clean cotton wool placed at the narrowed part of the funnel to block the passage of the plant into the collecting beaker plate I.

At first, 2 liters of cool methanol was poured onto the 500g in the funnel and allowed to drain for 12 hours plate I. The powder was observed to be greenish which suggested that there were more of plants' constituents remaining, hence the need to put more methanol plate II. The extraction continued for another 12 hours until the powder became pale yellowish. A total of 5.5 liters of methanol was used for the methanolic extraction.

Maceration Method

Plate I: Extraction medium and methanol container with collecting bottle.

Plate II: The extraction medium with cotton blocking the lower neck preventing escape of powder of *Momordica charantia*.

Plate III: Extraction medium tap and collecting bottle.

Plate IV: Water bath for drying of the *Momordica charantia* extract.

The filtrate was collected in a collecting beaker and poured into evaporating dishes which were on water bath machines at $45^{O}C$ **plate IV.**

The filtrate was evaporated and dried for 8 hours. The filtrate was then collected in a glass bottle and weighted.

3.6 WEIGHING OF THE EXTRACT

The dry powder was 500 g (DP)

The empty glass bottle weight was 131.78 g (EB)

The glass bottle with the dry extract was weighted to be 241.39 g (BE)

Weight of extract = weight of bottle with extracts (BE) minus weight of

Empty bottle (EB) = BE – EB => 241.39-131.78 = 109.61g

Therefore the weight of the extract (WE) is 109.61g.

The percentage yield of the plant is equals to weight of the extract (WE) divided by weight of the dry powder (DP) times 100%

$$=> \quad \frac{WE}{DP} = \frac{109.61}{500} \times 100$$

$$= 21.92\%w/v$$

Therefore the percentage yield of this plant is 21.92%w/v

3.7 PHYTOCHEMICAL SCREENING OF *MOMORDICA charantia*

Basic phytochemical screening consists of performing simple chemical tests to detect the presence of alkaloids, tannins, saponins, anthraquinones, cardenolides in the plant's extract. Simple, standard chemical tests have been devised for this study as laid down by Evans (1996) and adopted by Abdullahi *et al.,* 2003

A small portion of the extract about 0.5 g was taken and dissolved in a test tube containing distilled water. A drop of 0.5 ml was taken for each of the tests as shown on **plate V.**

Plate 5: The methanolic extract of *Momordica charantia* dissolved in distilled water contained in each of the test tubes for phytochemical analysis.

3.7.1 Test for Carbohydrates

Molisch Test was used to test for the presence of carbohydrate in this plant. A drop of 0.5ml solution of the extract was dropped in a test tube containing 5 ml Fehling solution and was boiled in a water bath; there was brick-red precipitate which indicates the presence of reducing sugar (Evans and Bicks 1996; Abdullahi *et al.,* 2003).

3.7.2 Test for Glycosides

0.5ml of the extract's solution was dropped in a test tube and 5ml of dilute sulphuric acid was added and boiled on water bath for 10-15 mins. This was then cooled and neutralized with 20% potassium hydroxide (KOH).

It was then divided into two portions: 5 ml Fehling solution was added to the first portion and boiled; a brick-red precipitate was observed which showed the presence of reducing sugar (Evans and Bicks 1996; Abdullahi *et al*., 2003).

3ml of Ferric chloride solution was added to the second portion and was shaken; there was greenish-blue colour. This indicated the presence of phenolic aglycones due to the hydrolysis (Evans and Bicks 1996; Abdullahi *et al.,* 2003).

3.7.3 Test for Free Anthracene Derivatives

Bontrager's test was used for this test. A portion of the extract was placed in a dry test tube, 5ml of chloroform was added and was shaken for at least 5 minutes. This was filtered and the filtrate was added into an equal volume of 10% ammonia solution, and was shaken again; there was presence of bright pink colour in the aqueous upper layer which indicated the presence of free anthraquinones (Evans and Bicks 1996; Abdullahi *et al.,* 2003).

3.7.4 Test for Unsaturated Steroid and Triterpenes

Liebermann – Bucchard Test was used in this test. 3ml of acetic acid anhydride was added to the solution of the extract. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour; blue-green color in upper layer and a reddish colour which indicates the presence of triterpene (Evans and Bicks 1996; Abdullahi *et al.,* 2003)

3.7.5 Test for Unsaturated Sterols

Salkowski Test was used. 2-3 drops of concentrated sulphuric acid was added down the side of test tube containing 2 ml of the extract. Immediately there was colour change at the interphase of the extract and sulphuric acid. After a period of one hour, there was changed in colour to cherry red. This indicates the presence of unsaturated sterols (Evans and Bicks 1996; Abdullahi *et al.,* 2003).

3.7.6 Test for Cardiac Glycoside

Keller-kiliani Test was used. A drop of 0.5 ml of the extract was dropped into 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. It was carefully observed that, there was the presence of a purple-brown ring. This indicates the presence of deoxy sugars. There was also a pale green colour in the upper acetic acid layer, which indicates the presence of cardiac glycosides (Evans and Bicks 1996; Abdullahi *et al*., 2003).

3.7.7 Test for Saponin Glycoside

Frothing test was adopted for this test. About 10 ml of distilled water was added to a 2 ml of the extract and was shaken vigorously and was observed for 30 minutes. A honeycomb froth that persists for 10-15mins indicates the presence of saponins (Evans and Bicks 1996; Abdullahi *et al.*, 2003).

3.7.8 Test for Tannins

Ferric chloride test was used for this test. 3-5 drops of ferric chloride solution was added to 2 ml of the extract. There was precipitation of a greenish-black colour; this indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish-blue precipitate (Evans and Bicks 1996; Abdullahi *et al*., 2003).

Bromine water test was used. Few drops of the bromine water were added to the extracts in a test tube. A buff coloured precipitate indicates condensed tannins while hydrolysable tannins gave none of these at all (Evans and Bicks 1996; Abdullahi *et al*., 2003).

3.7.9 Test for Flavonoids

Shinoda test was used, Sodium hydroxide and ferric chloride tests were used for shinoda test; a drop of 0.5ml of the extract was dissolved in 1-2 ml of 50% methanol in the heated metallic magnesium chips and few drops of concentrated hydrochloric acid were added. There was appearance of red colour which indicates presence of flavonoids (Evans and Bicks 1996; Abdullahi *et al*., 2003).

Sodium hydroxide test: Few drops of 10% sodium hydrochloride was added to a 0.5 ml drop of the extract. Yellow colour appeared and this indicates presence of flavonoid (Evans and Bicks 1996; Abdullahi *et al.*, 2003).

Ferric chloride test: Few drops of ferric chloride were added to a solution 0.5 ml of the extract, a green precipitate indicates presence of phenolic neucles (Evans and Bicks 1996; Abdullahi *et al.*, 2003).

3.7.10 Test for Alkaloids

Mayer's Test was used for this test; A drop of 2-3 ml of the Mayer's reagent was dropped into 2 ml of the extra and a cream precipitate was observed. That indicated presence of alkaloids (Evans and Bicks 1995; Abdullahi *et al*., 2003).

3.8 ADMINISTRATION OF THE EXTRACT TO THE ANIMALS

The animals were weighed just before the commencement of the extract administration. The administration of the extract was done for the period of six (6) weeks.

The extract of *Momordica charantia* was measured by means of electronic measuring machine which measures in milligram (mg). The measured extracts were then transferred to well labelled small size plastic bottles. The bottles where labelled 125 mg, 250 mg and 500 mg of *Momordica charantia*. The corresponding weights were placed in each bottle with same label.

The blood collecting bottles were arranged in accordance with the grouping and labeled as groups A, B, C and D. They were separated to avoid the slightest error of wrong placement of blood tissue.

Five bottles were used on each of the four groups. They were labeled as group A1, A_2, A_3, A_4 and A_5. This was done to all groups. This means each bottle was carrying blood tissue of a specific rat. The blood sample collection bottles used were forty, twenty in each set.

3.9 ACUTE TOXICITY STUDY

The limit test of the extract of *Momordica charantia* was conducted at as high dose as 1000 mg/kg body weight and was relatively found to be safe within 48 hours observation period following oral administration of a single dose to the adult albino rat.

LD_{50} was determined to be 1855 mg (Evans and Bicks 1995; Abdullahi *et al.*, 2003). The choice of the dosages was based on the fact that at high dose of 1855mg of the methanolic extract of *Momordica charantia*, there was no toxicity on treated rats for pilot study was observed. Therefore the animals were exposed to the selected dosages for long period of six (6) weeks for chronic toxicity study as was suggested by Grover and Yadav (2004). The people in the rural areas in Northern Nigeria use this plant in forages, as drinking water and spices for other dishes for a long period of their life time, (Evans and Bicks 1996; Abdullahi *et al.*, 2003)

3.10 ROUTE OF ADMINISTRATION

The extract of *Momordica charantia* is water soluble and was administered by oral route. Robber-caped needles were used to administer the extracts.

The extracts were dissolved in warm distilled water by vigorous shaking, to facilitate dissolution of the extract. The content was immediately taken and administered to the rats.

The period of extract administration took six (6) weeks and was done at a fixed time daily 9:00 am as was done by Atama and Idu (2007).

3.11 ANIMAL SACRIFICE

The rats were humanely sacrificed, blood sample collected was from the heart of the rats, for haematological and biochemical analysis, the liver and kidney were harvested and fixed in neutral buffered formalin solution for histo-morphological study.

3.12 COLLECTION OF BLOOD SAMPLE

Materials used for the collection of blood sample include:

1. Syringes
2. Dissecting set,
3. 10%EDTA bottles (20)
4. Sterilized plain bottles (20)
5. Anaesthesia (chloroform)
6. Anaesthetic medium (plastic bucket with tight cover)

The blood samples were collected from the rats by cardiac puncture, while the rats were under anaesthesia. The rats were anaesthesized with chloroform. The thorax was carefully opened with sharp scissors. The blood was carefully taken by cardiac puncture through the anterior surface of the heart, using sterilized syringes. The blood was divided into two (2) parts; volume of blood taken was about 5ml to 7ml and was immediately transferred to the collecting bottles. The blood taken from each rat was divided into two (2) parts, one part was placed in

10% EDTA bottle to prevent clotting for the haematology while the second part was placed in plain bottle without EDTA for the biochemical analyses of the respective rats as was described by Atama and Idu (2007).

The 10% EDTA bottles were used to prevent clotting of the blood and the plain sterilized bottles were used to allow the blood to clot.

3.13 HAEMATOLOGY

The blood sample collected in 10%EDTA bottles from each rat was used for hematological analysis of red blood cell (RBC), white blood cell (WBC), hemoglobin, lymphocytes, eosinophil and neutrophils counts as according to Sujathan *et al.* (2011).

3.14 BIOCHEMICAL ASSAY

The second part of blood sample was collected without EDTA was centrifuged using centrifuging machine at 1000rpm and the serum obtained was used for biochemical analysis in Ahmadu Bello University, Teaching Hospital, Zaria, for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkalinephosphatase (ALP), Urea, Creatinine and serum protein as was done by (Sujathan *et al.*, 2011).

3.15 HARVESTING OF TISSUES

Materials used for the harvest of the tissue include:

1. Dissecting set,

2. Syringes,
3. Digital weighing balance
4. Sterilized plastic bottles used as tissue fixation media
5. Neutral buffered solution of formalin

On the last day of the administration of methanolic extract of *Momordica charantia*, the animals were weighed before the haematological and histological procedures. The weights were recorded as shown in appendix I-IV.

After collection of the blood for the haematology and biochemical analyses the liver and kidney of the rats were carefully harvested and immediately weighed and fixed in buffered neutral formalin solution which was contained in well labeled plastic containers. The weights of these organs were recorded for statiscal analyses in the table shown in appendix IIX-X as was described by Akhtar *et al*. (2007).

3.16 PREPARATION OF THE TISSUES FOR MICROSCOPIC EXAMINATIONS

Materials used for tissue preparation include:

1. Air tight sample bottle
2. Normal saline
3. Buffered neutral solution of formalin
4. Alcohol (70% ethanol, 90% ethanol, 100% ethanol)
5. Chloroform

6. Paraffin wax
7. Egg albumin
8. Sliding microtome
9. Glass slides and cover slips
10. Oven
11. Water bath
12. Automatic tissue processor (Histokinett Bench Model) and
13. Light microscope.

3.17 STATISTICAL ANALYSIS OF THE RESULTS

The data obtained from these studies were analysed with statiscal package for social science (SPSS) software, minitab Version16.22. One-way analysis of variance (ANOVA) was used to compare data both between and within the groups and Holm Sidak multiple comparison post hoc tests was used. It best compares the level of significance between the control and the test groups. All data were expressed as mean±SE and P□ 0.05 were considered to be significant. Data were presented in both graphs and tables.

CHAPTER FOUR

4.0 RESULTS

The results of white blood cell (WBC) count in this study showed that the group treated with the methanolic extract of *Momordica charantia* had elevated value of the WBC count when compared to the control group (Figure1).

It was observed that the red blood cell (RBC) count of the groups treated with methanolic extract of *Momordica charantia* showed no change in value when compared to the control group. The statistical analyses also showed no significant difference between the treated and the control group (Figure 4.2).

This study also revealed that there was no change in hemoglobin values across the groups treated with methanolic extract of *Momordica charantia* when compared to the control group (Figure 4.3).

On the other hand, there was increase in lymphocytes count across the groups treated with methanolic extract of *Momordica charantia* when compared to the control group (Figure 4.4).

There was increase in eosinophil values across the groups treated with the methanolic extract of *Momordica charantia* when compared to the control group. The statistical analyses indicated that there was significance different between the treated groups and the control groups (Figure 4.5).

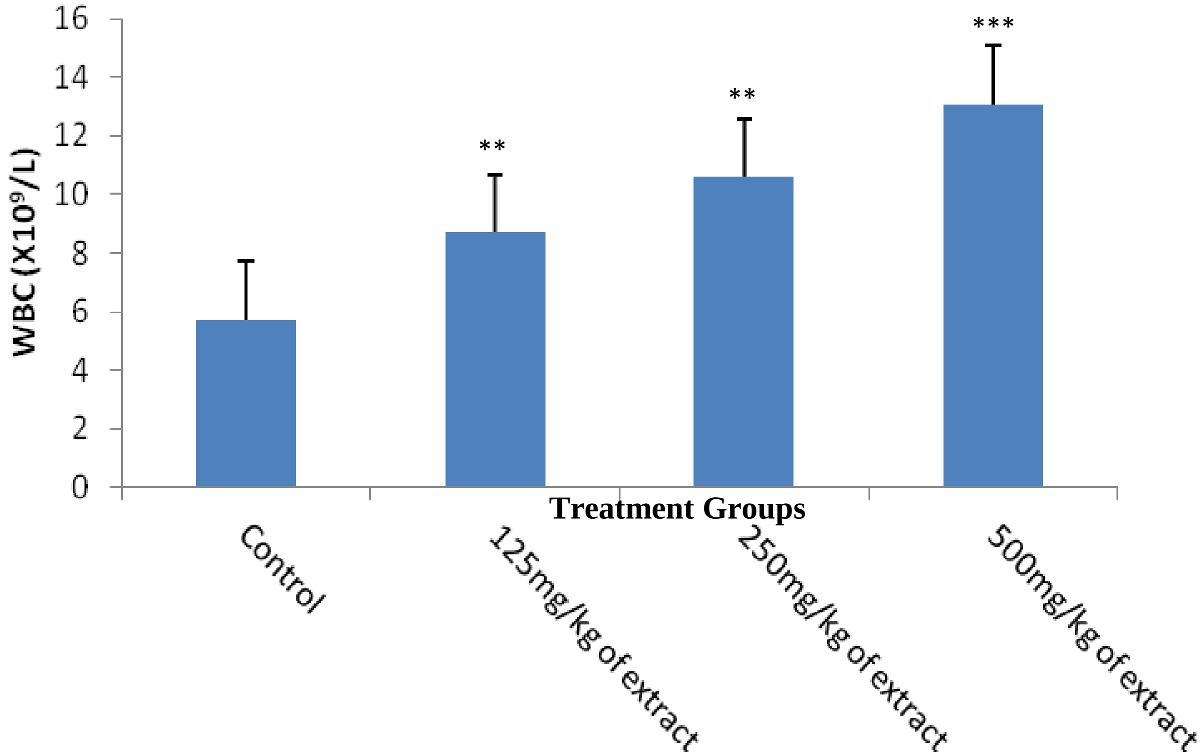


Figure 4.1: The effect of methanolic extract of *Momordica charantia* on white blood cell count in albino rats.

** P<0.027, ***P<0.014 n=5

Mean±SEM (one way analyses of variance was used)

P<0.05

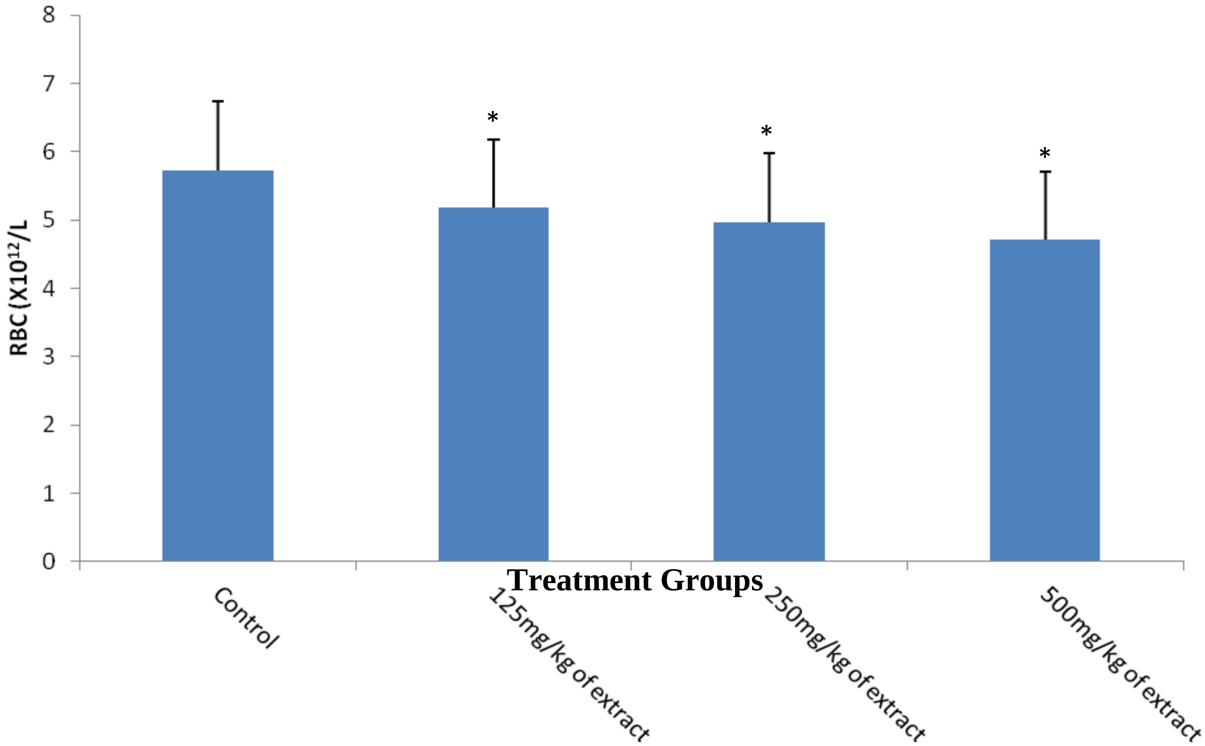


Figure 4.2: The effect of methanolic extract of *Momordica charantia* on red blood cell count in albino rats.

*P>0.038, n=5

Mean±SEM (one way analyses of variance was used)

P>0.05

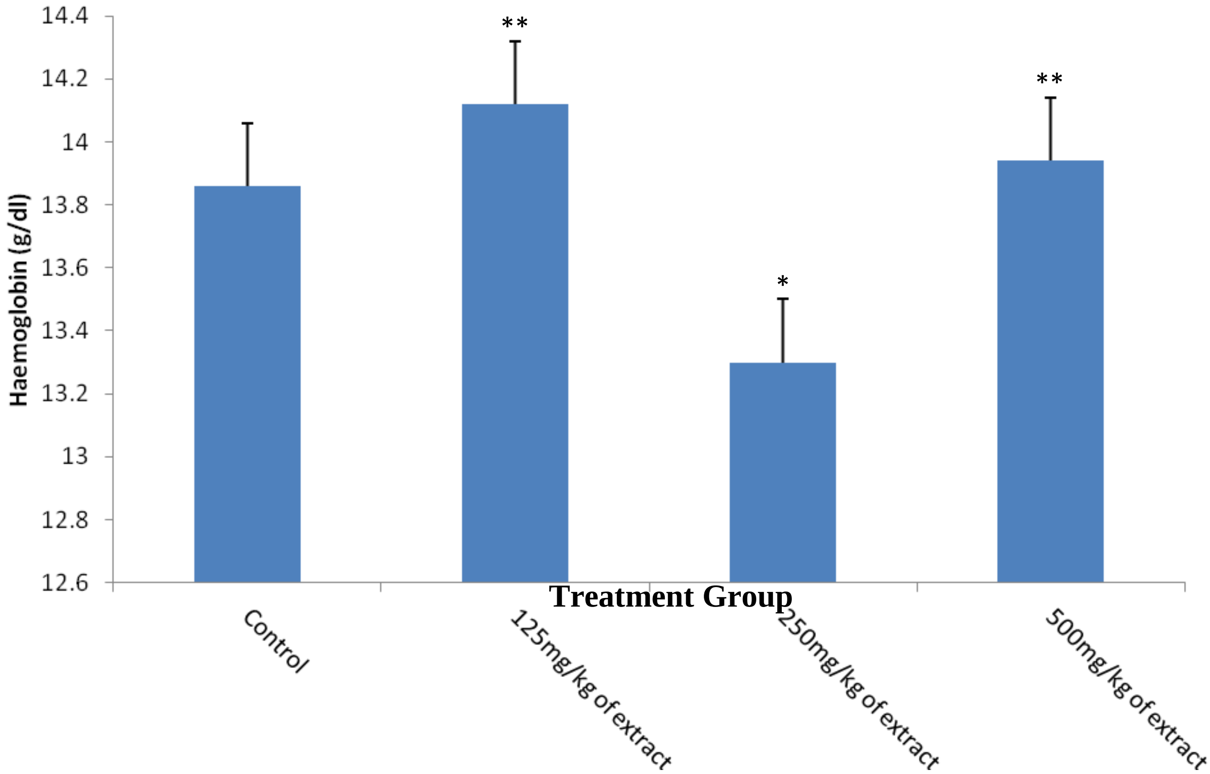


Figure 4.3: The effect of methanolic extract of *Momordica charantia* on haemoglobin concentration in albino rats.

** P>0.866, *P>0.169 n=5

Mean±SEM (one way analyses of variance was used)

P>0.05

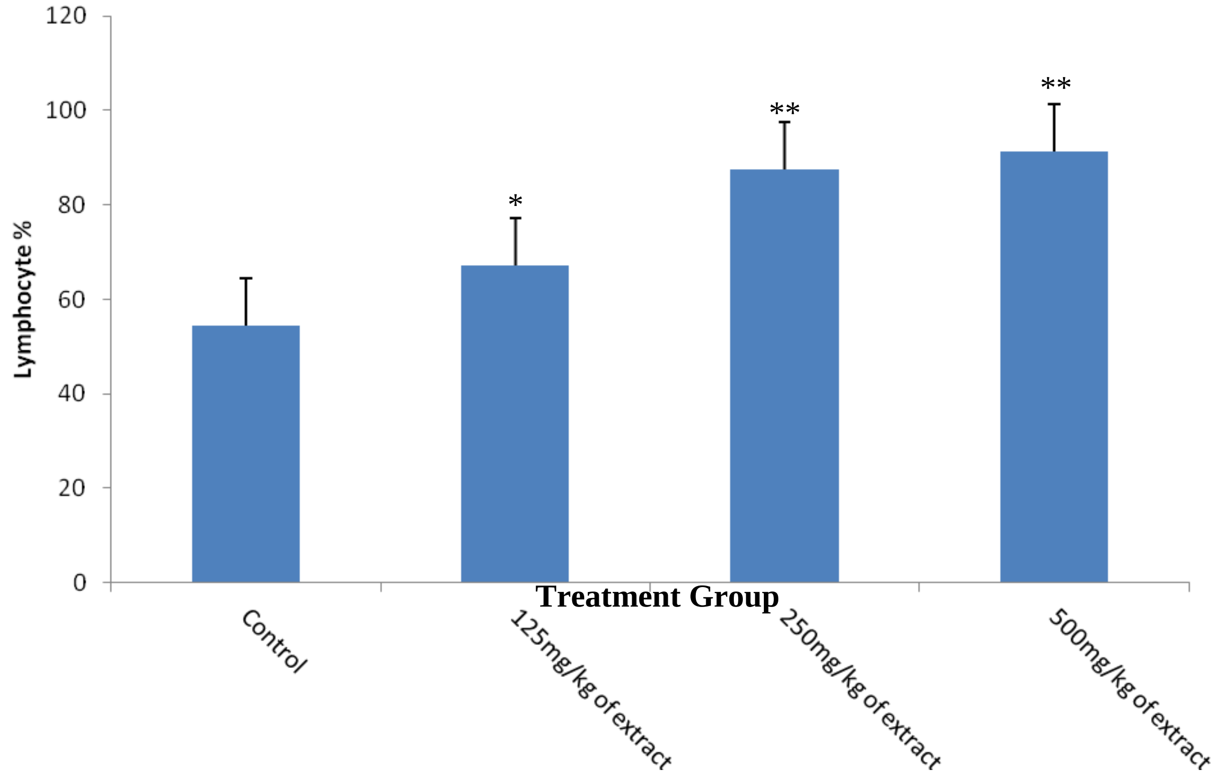


Figure 4.4: The effect of methanolic extract of *Momordica charantia* on lymphocyte count in albino rats.

** $P<0.024$, *$P<0.017$ n=5

Mean±SEM (one way analyses of variance was used)

$P<0.05$

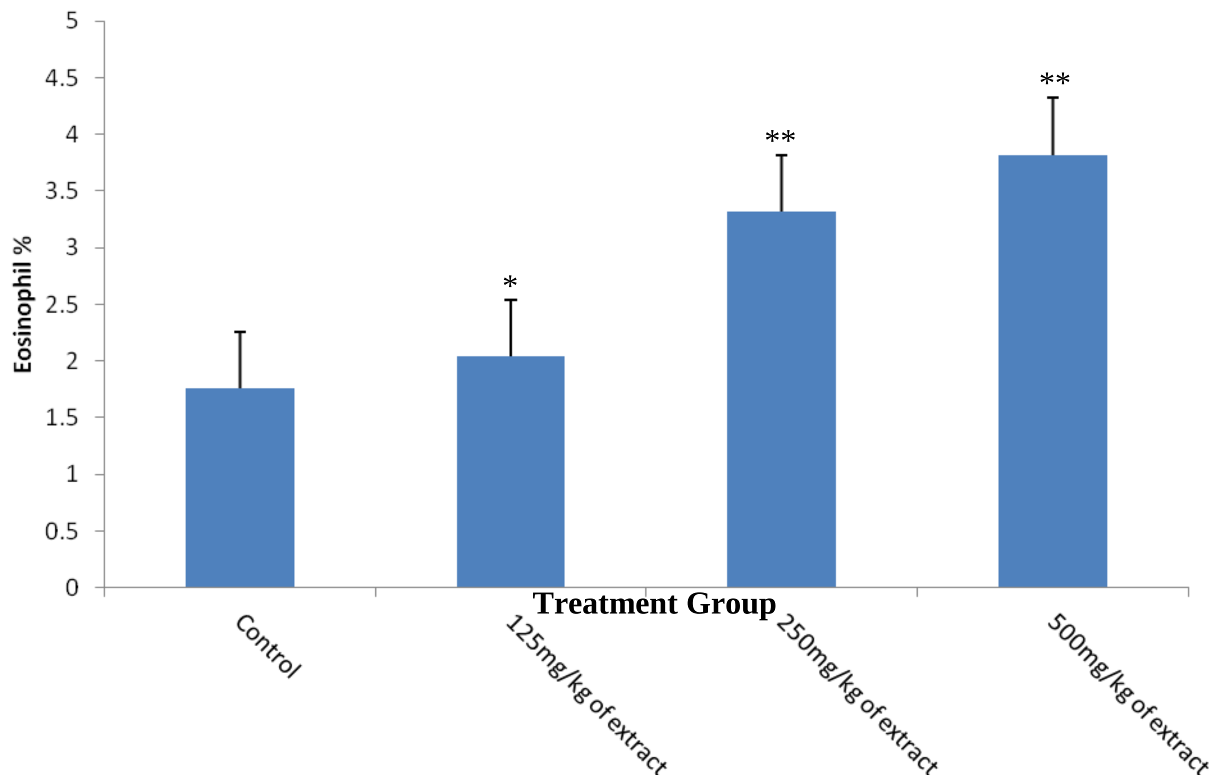


Figure 4.5: The effect of methanolic extract of *Momordica charantia* on eosinophil count in albino rats.

** P<0.047, *P<0.031 n=5

Mean±SEM (one way analyses of variance was used)

P<0.05

The result of the biochemical analysis revealed that neutrophils counts across the groups treated with methanolic extract of *Momordica charantia* had increased values when compared to the control group and the statistical analyses showed significant difference between the methanolic extracted treated groups and the control group (Figure 4.6).

The biochemical analyses of sodium showed that there was no increase or decrease in sodium values across the groups treated with methanolic extract of *Momordica charantia* compared to the control group. The statistical analyses also showed no significant difference between the treated groups and the control (Figure 4.7).

Biochemical analyses of potassium in this study showed that there was no increase or decrease in potassium concentration among the groups treated with methanolic extract of *Momordica charantia* when compared with the control group. The statistical analyses indicated that there was no significance difference between the treated groups and the control group (Figure 4.8).

The result of analysis of urea concentration across the treated groups showed no significant difference when compared to the control group (Figure 4.9).

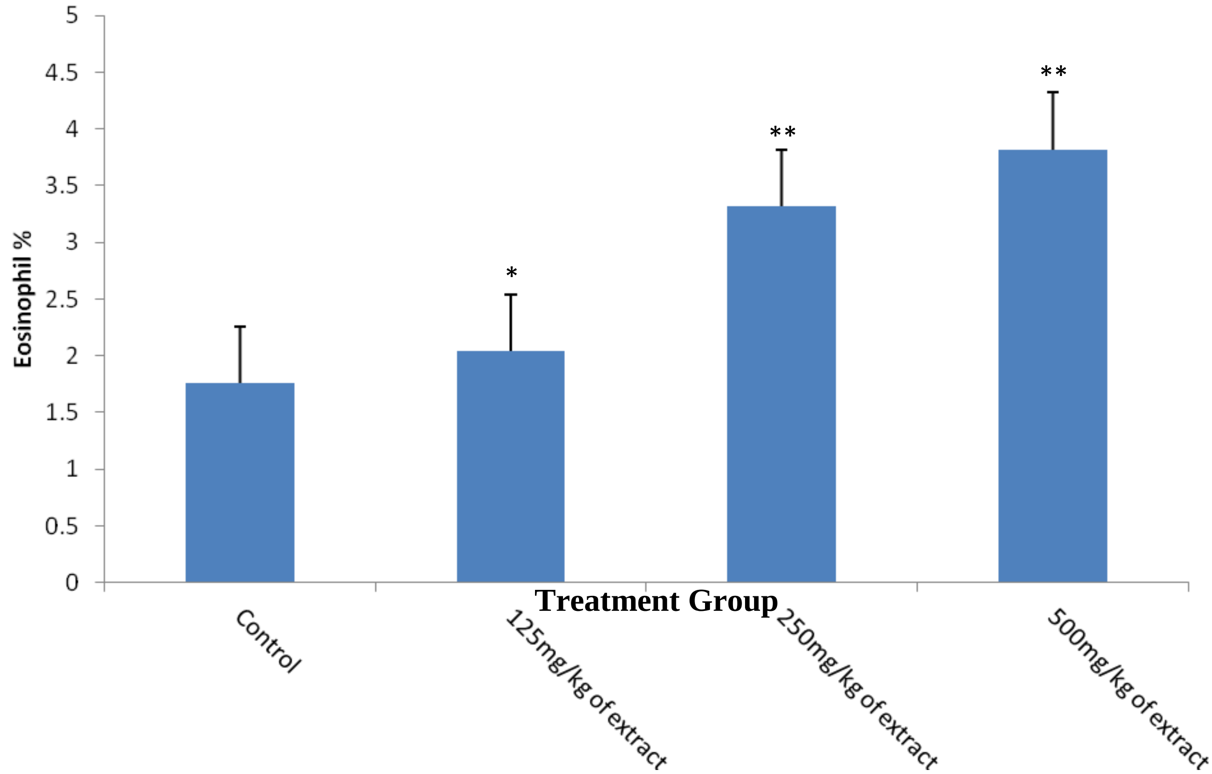
The biochemical analysis of chlorine in this study indicated that there was no statistical difference between the treated groups and the control group (Figure 4.10).

The biochemical analysis of creatinine level of the groups treated with the methanolic extract of *Momordica charantia* showed no statistical difference when compared with the control group (Figure 4.11).

The biochemical analysis of the kidney and liver enzymes aspartate aminotransferase (AST) in this study showed increase in concentration of this enzyme across the groups treated with extract of *Momordica charantia* when compared to the control group (Figure 4.12).

Similarly, the result of biochemical analysis of the kidney and liver enzyme, alanine aminotransferase (ALT), of the treated groups showed increased in concentration of this enzyme when compared to the control group (Figure 4.12).

It was also observed that the result of biochemical analysis of the kidney and liver enzyme called alkaline phosphatase (ALP) of the groups treated with the methanolic extract of *Momordica charantia* had increase in concentration of this enzyme compared to the control group (Figure 4.12).



P<0.05 Figure 4.6: The effect of methanolic extract of *Momordica charantia* on neutrophil count in albino rats.

** P<0.005, *P<0.045 n=5

Mean±SEM (one way analyses of variance was used)

P<0.05

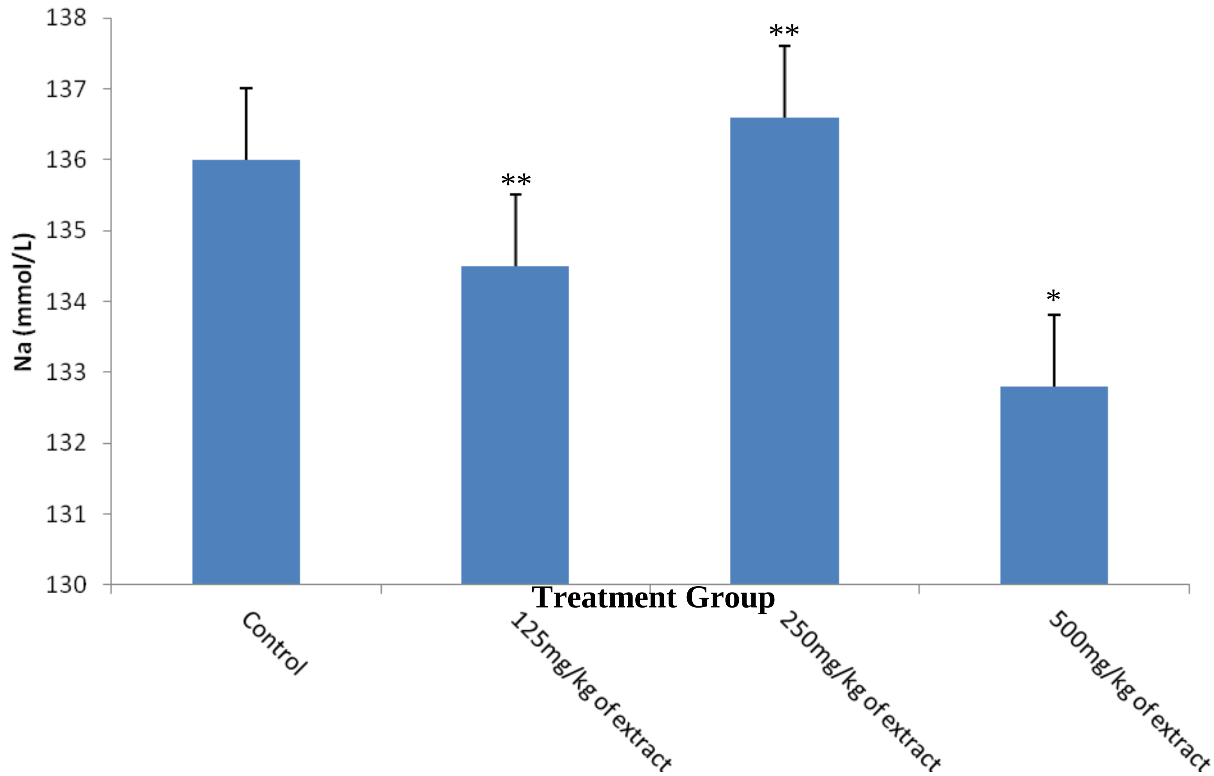


Figure 4.7: The effect of methanolic extract of *Momordica charantia* on Sodium levels in albino rats.

** P>0.412, *P>0.183 n=5

Mean±SEM (one way analyses of variance was used)

P>0.05

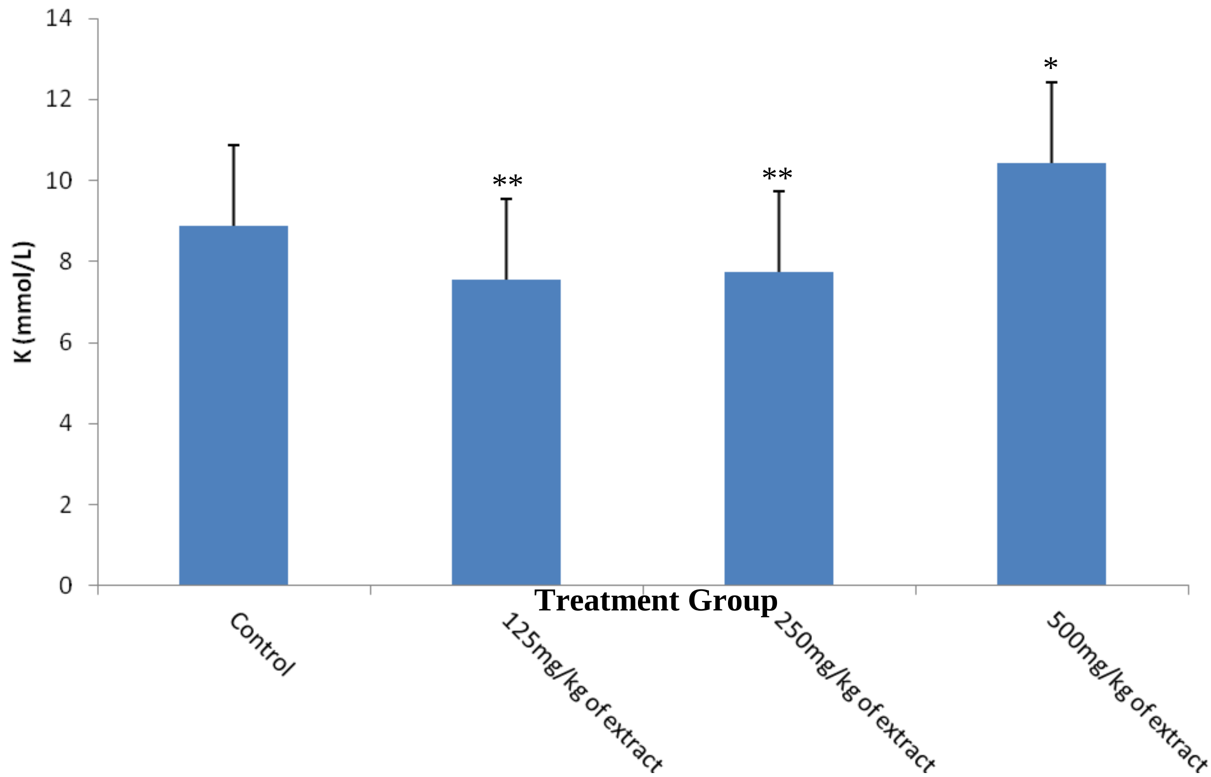


Figure 4.8: The effect of methanolic extract of *Momordica charantia* on Potassium levels in albino rats.

** P>0.164, *P>0.215 n=5

Mean±SEM (one way analyses of variance was used)

P>0.05

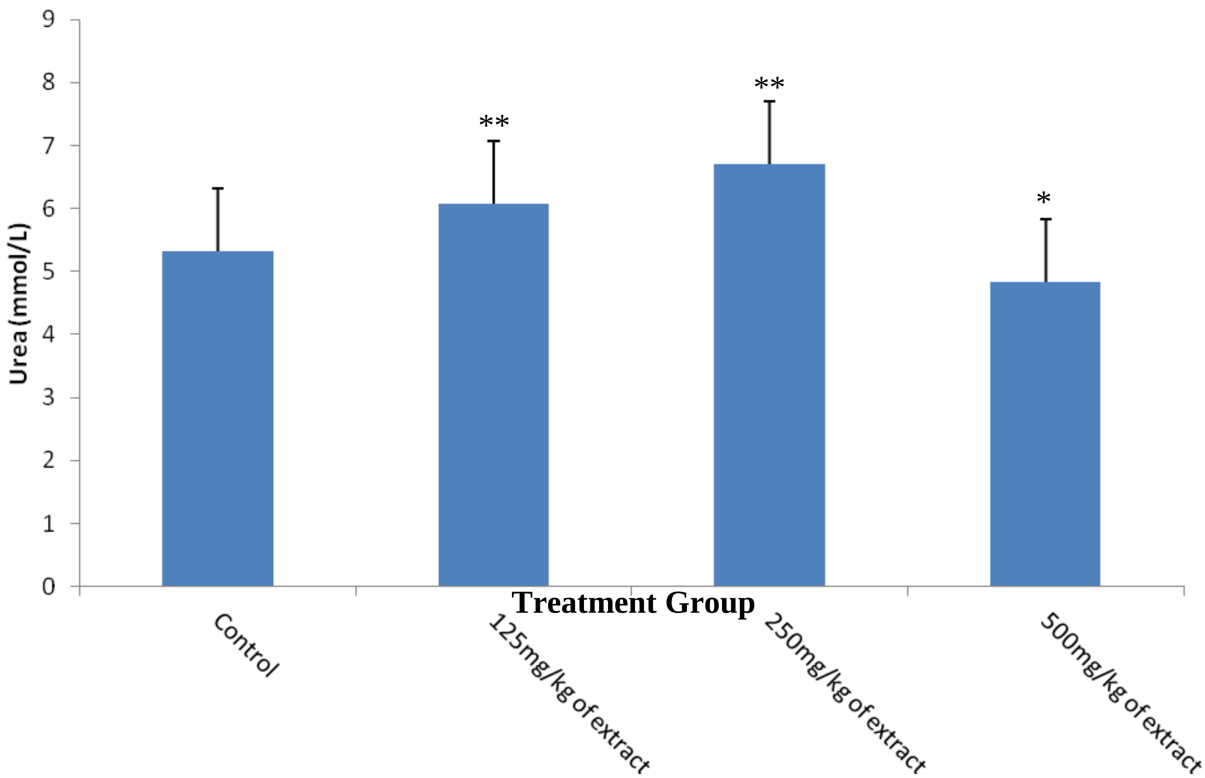


Figure 4.9: The effect of methanolic extract of *Momordica charantia* on Urea levels in albino rats.

** P>0.587, *P>0.532 n=5

Mean±SEM (one way analyses of variance was used)

P>0.05

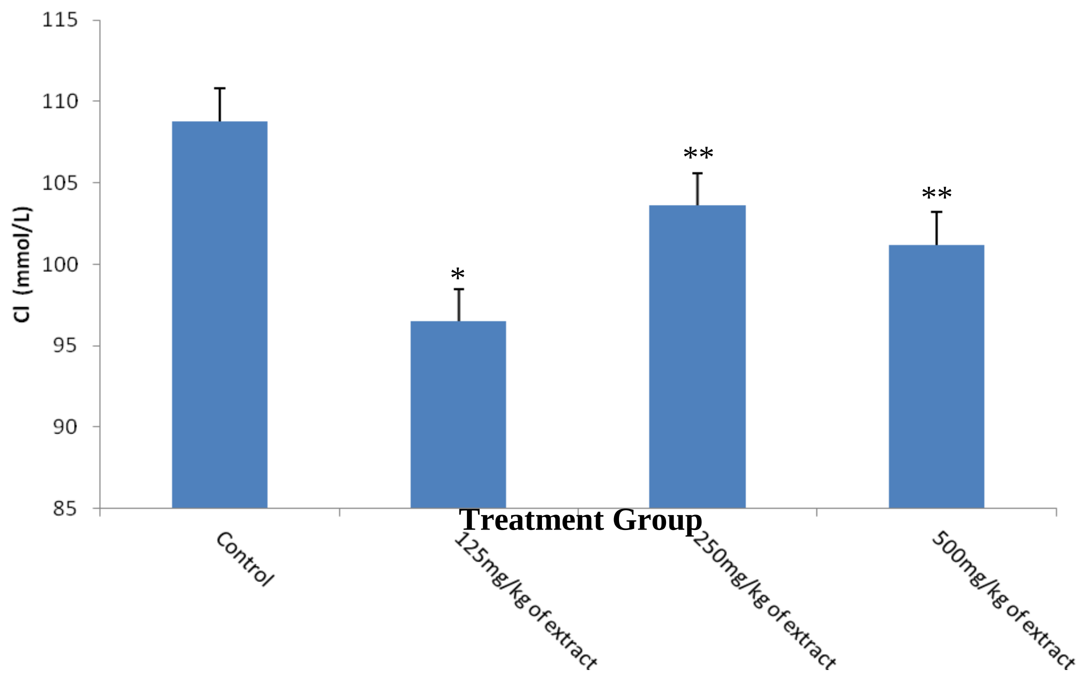


Figure 4.10: The effect of methanolic extract of *Momordica charantia* on Chlorine levels in albino rats.

** P>0.287, *P>0.165 n=5

Mean±SEM (one way analyses of variance was used)

P>0.05

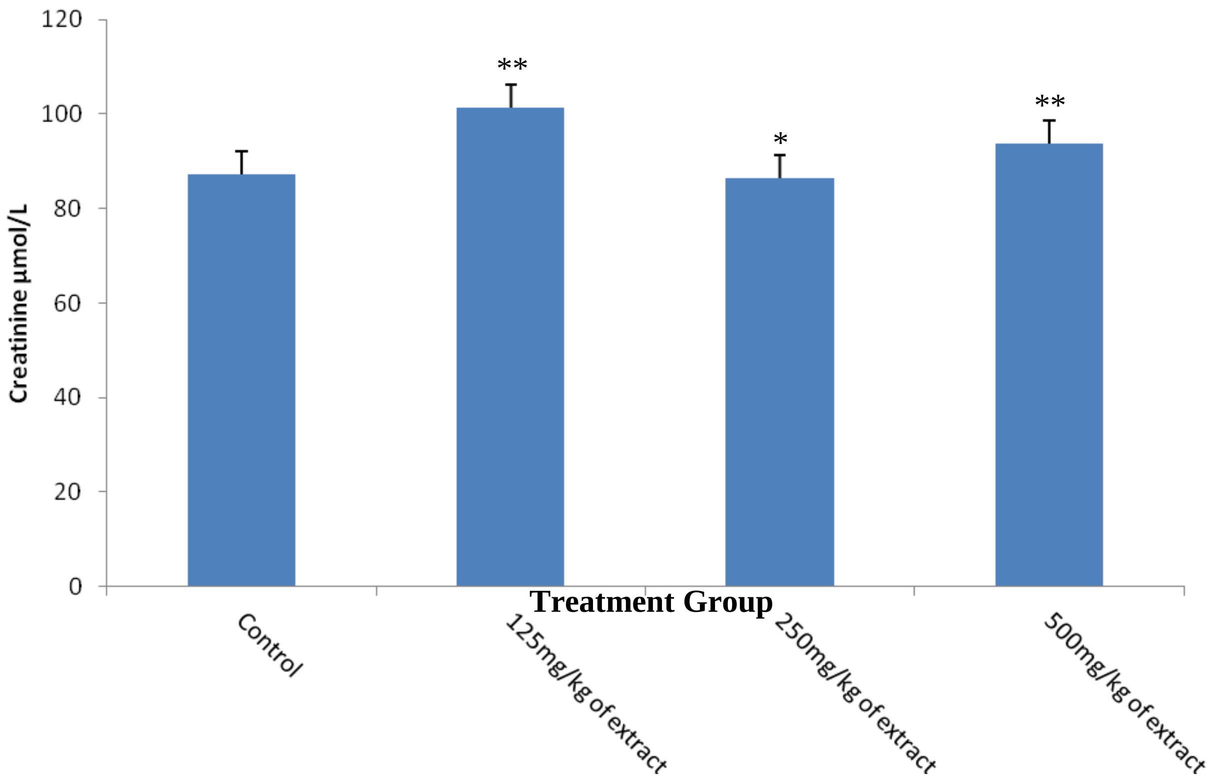


Figure 4.11: The effect of methanolic extract of *Momordica charantia* on Creatinine levels in albino rats.

** $P<0.048$, *$P<0.035$ n=5

Mean±SEM (one way analyses of variance was used)

$P<0.05$

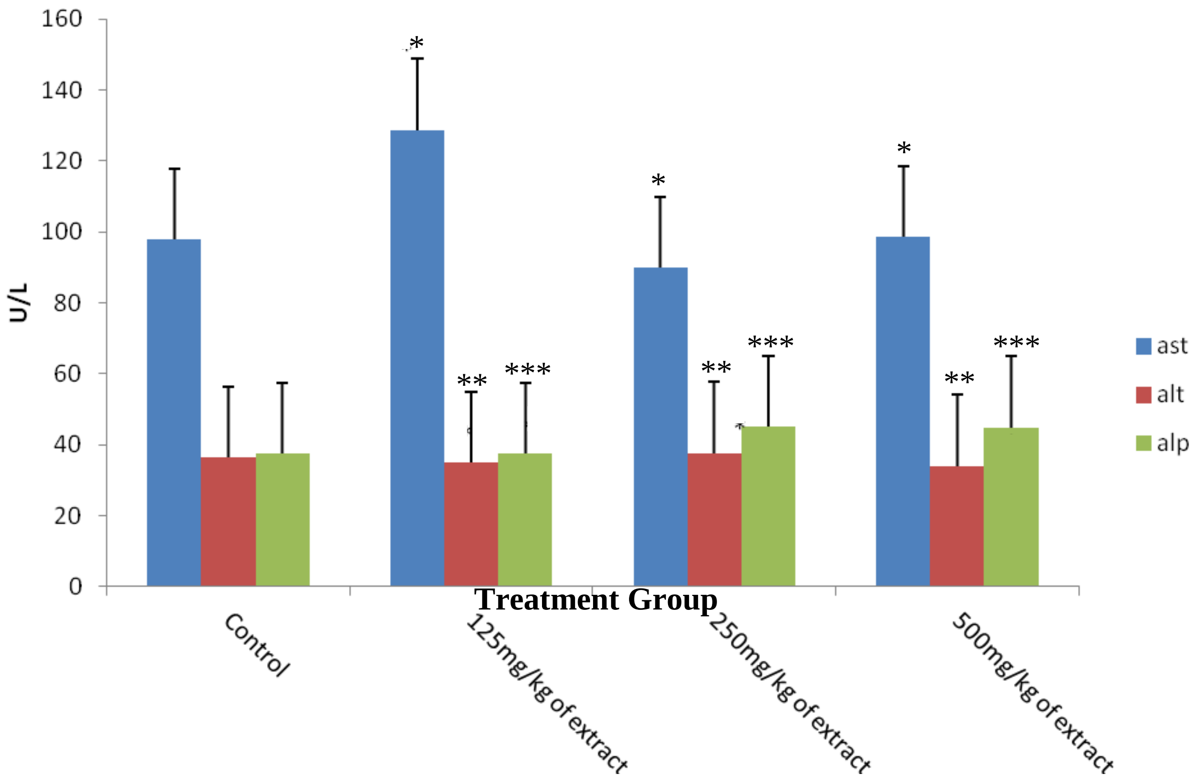


Figure 12: The effect of methanolic extract of *Momordica charantia* on Liver/ kidney enzymes (AST, ALT and ALP) level in albino rats.

***P<0.008, ** P<0.036, *P<0.022   n=5

Mean±SEM (one way analyses of variance was used)

P<0.05

The result of histology

lesions, such as congested intertubular spaces, focal area of necrosis of renal tubular and epithelial cells and widened glomerular spaces (**Plates IV and V)**.

This study of the kidney of control group treated with distilled water showed normal histology as shown in (Plate I).

The result of histology of liver of the group of rats treated with 125 mg/kg body weight of methanolic extract of Momordica *charantia* showed some necrosis of renal tubular epithelial cells (Plates II and III).

The result of histology of the kidney of the group of rats that received 250 mg/kg body weight of methanolic extract of Momordica *charantia* had more revealed that the group of rats that were administered with 500 mg of the extract of Momordica *charantia* had more severe lesions when compared to the other treated groups. The histology showed that there were more congested intertubular spaces, the focal area of necrosis were more in this group. Some of the lumen in this group contained eosinophilic materials. There was distortion of renal architecture (Plates VI and VII).

The histology of the liver of the control group treated with distilled water showed normal hepatic architecture and no lesion was observed (Plates VIII and IX).

The histology of the liver of rats treated with 125 mg/kg body weight of methanolic extract Momordica *charantia* showed some vacoulation of hepatocytes, necrosis at some areas and dilated hepatic cords (Plates X and XI)

This study revealed that there were more lesions in the liver of rats which were administered with 250 mg/kg body of the methanolic extract of Momordica *charantia*. There were more focal areas of necrosis and more vacuolated hepatocytes and dilated hepatic cords (Plates XII and XIII).

It was also observed that there were more lesions in the liver of rats which were administered with 500 mg/kg body weight than the other groups administered with the lower doses. It showed more focal area of necrosis, more vacoulation of hepatocytes and more dilated hepatic cords (Plates XIV and XV).

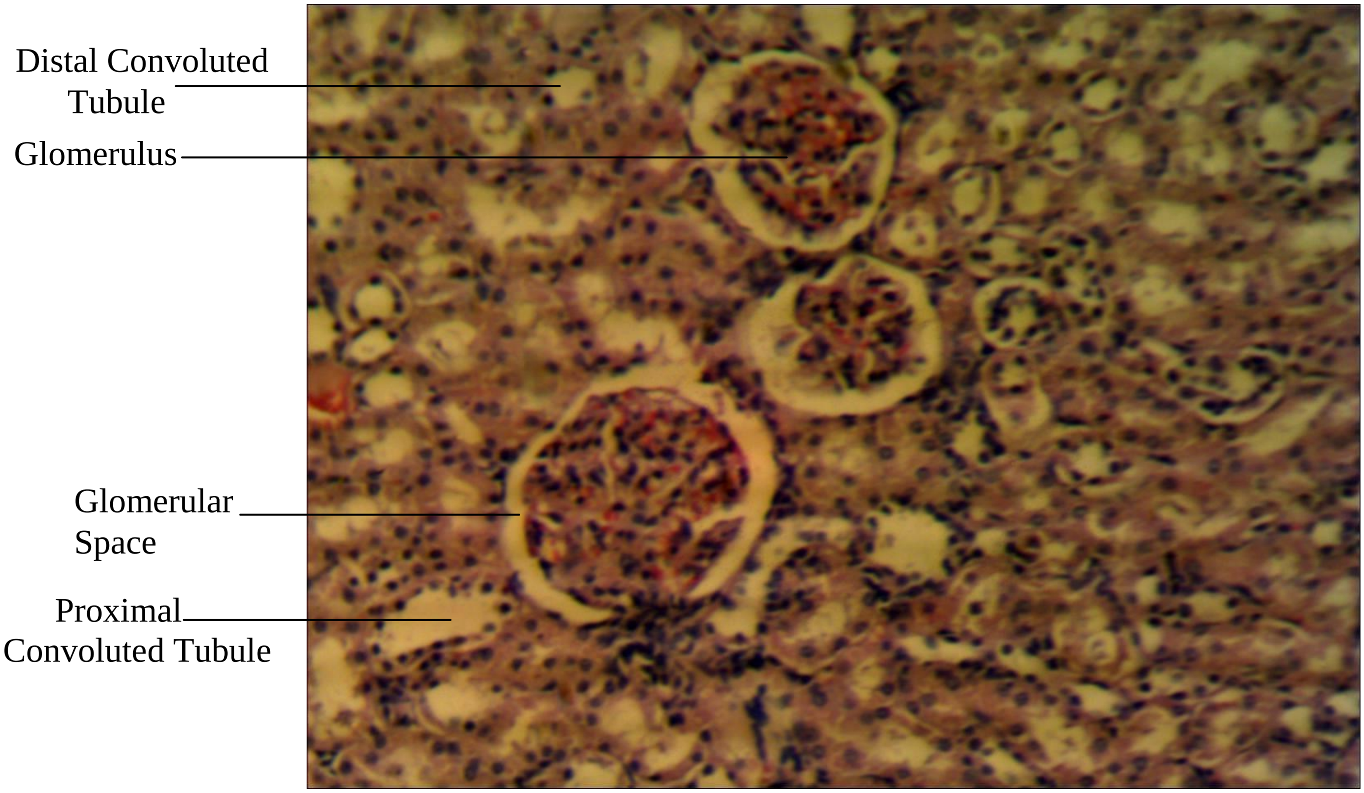


PLATE I: A cross section of kidney cortex of control group administered with distilled water X250 showing normal Histology (Hematoxyline and Eosin).

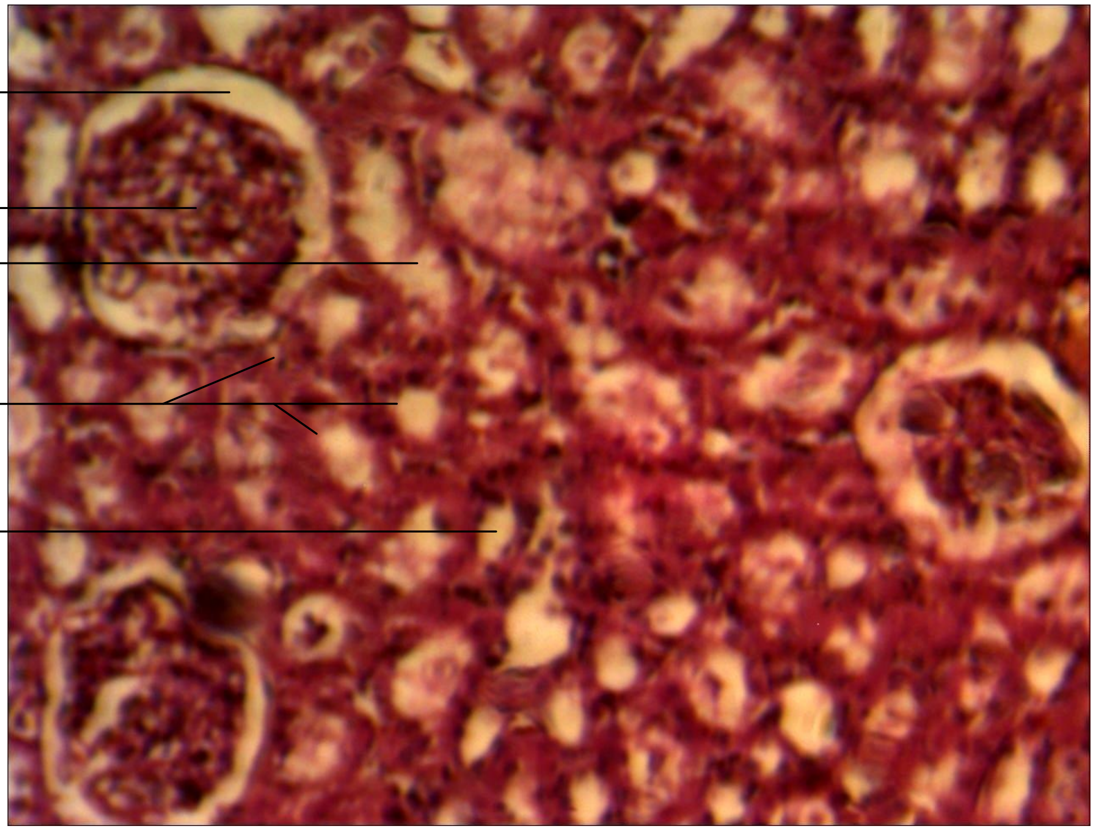


PLATE II: A cross section of kidney cortex treated with 125mg/kg body weight of Momordica *charantia* (methanolic extract) showing some necrosis of renal tubular epithelial cells (Hematoxyline and Eosin) × 250

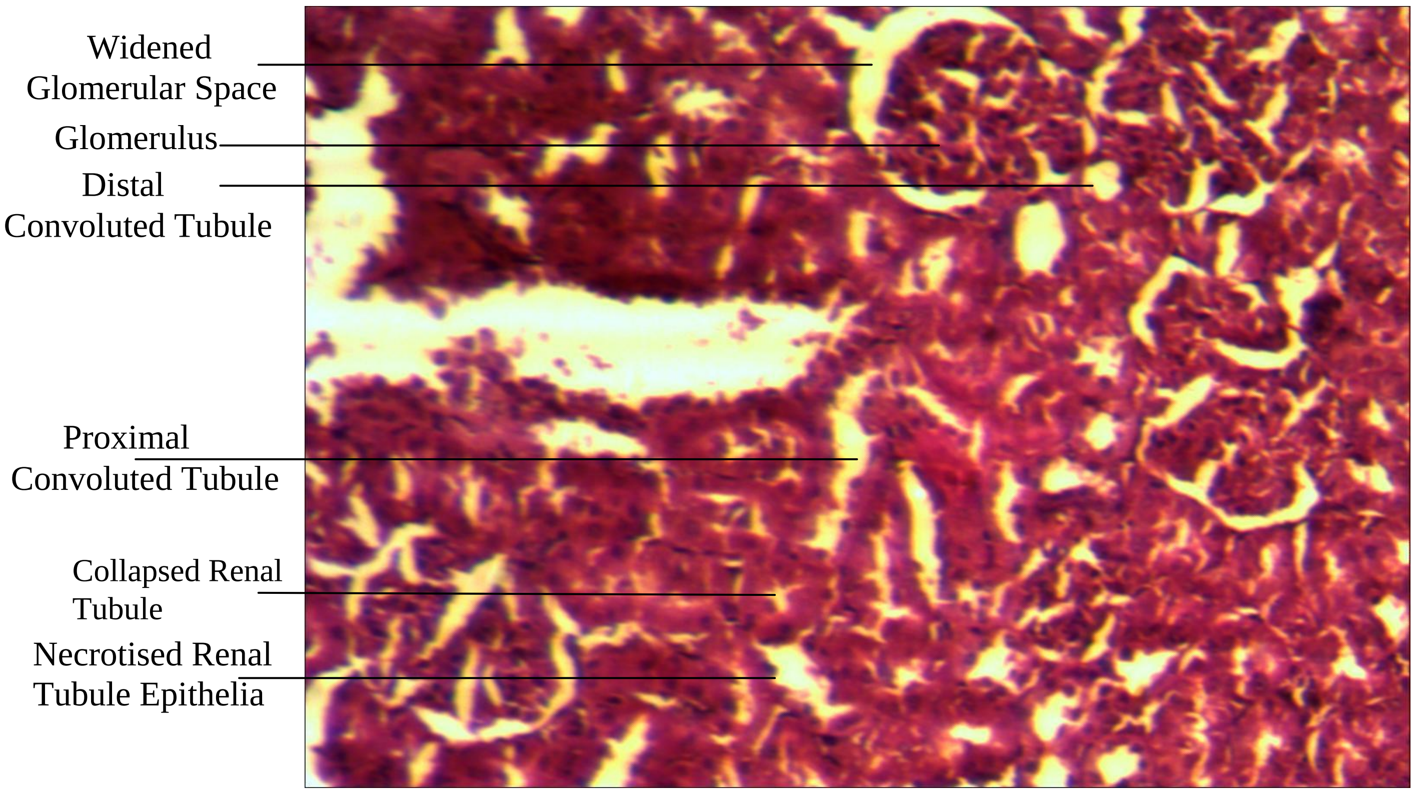


PLATE III: A cross section of kidney cortex treated with 125mg/kg body weight of Momordica *charantia* (methanolic extract) showing congested intertubular spaces focal area of necrosis of renal tubular epithelial cells (Hematoxyline and Eosin stain) × 250.

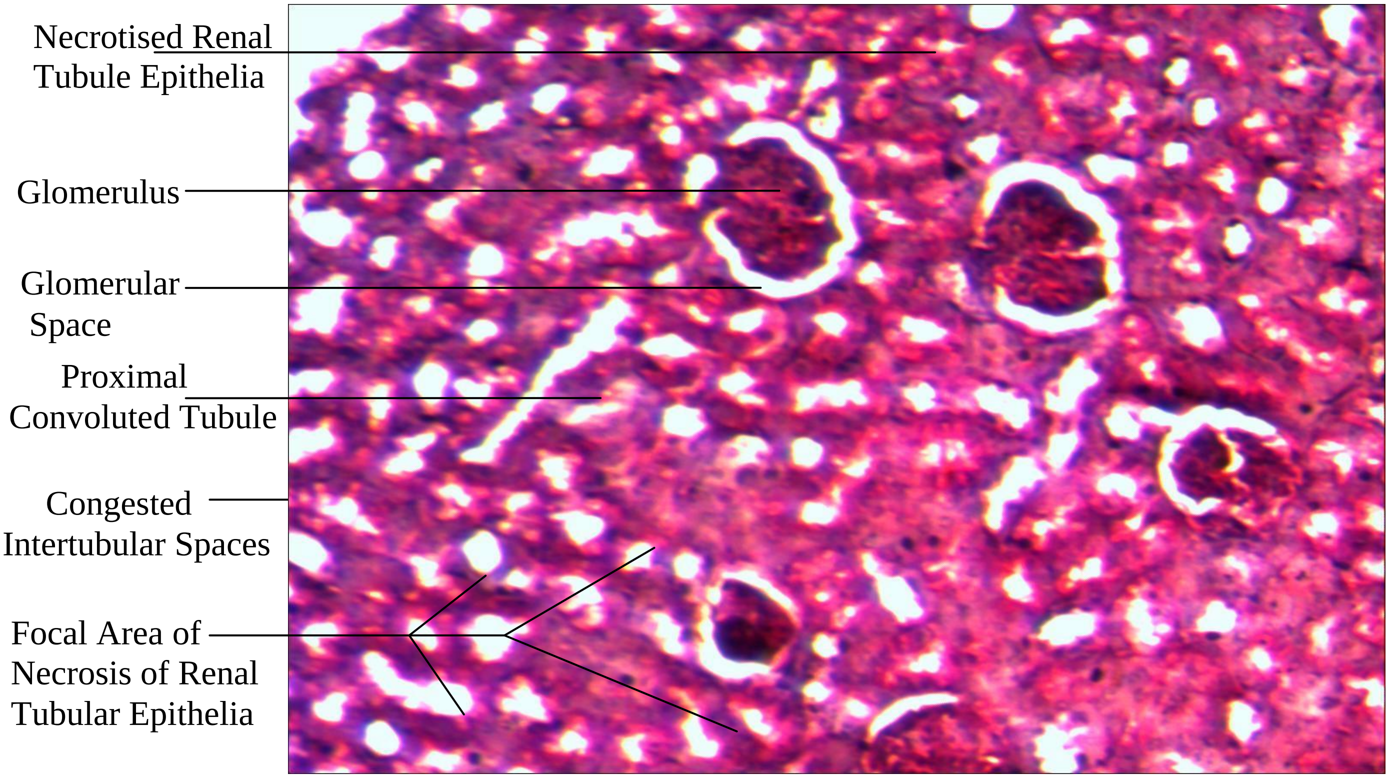


PLATE IV: A cross section of kidney cortex treated with 250mg/kg body weight of Momordica *charantia* showing congested intertubular spaces focal area of necrosis of renal tubular epithelial cells (Hematoxyline and Eosin) × 250

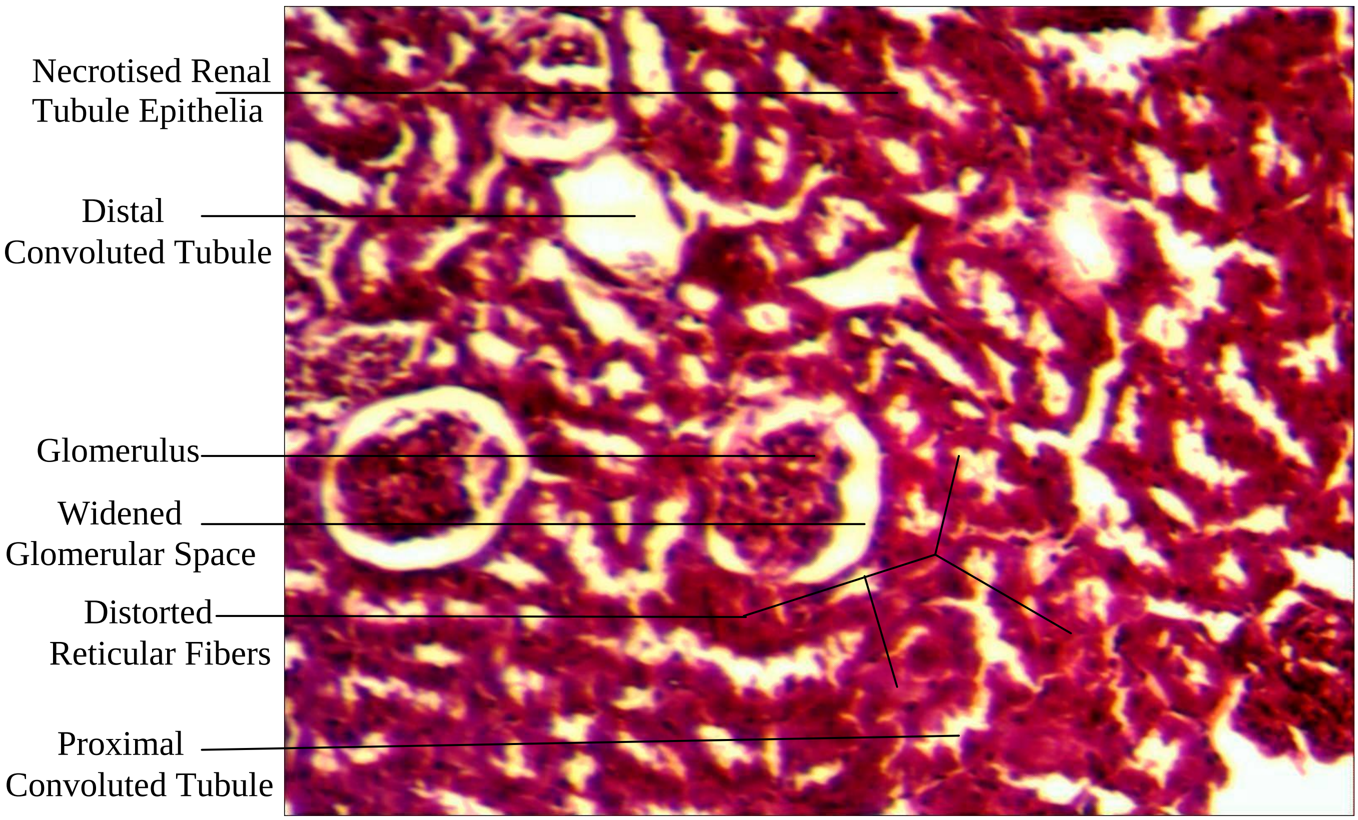


PLATE V: A cross section of kidney cortex treated with 250mg/kg body weight of Momordica *charantia* showing congested intertubular spaces focal area of necrosis of renal tubular epithelial cells (Hematoxyline and Eosin) × 250

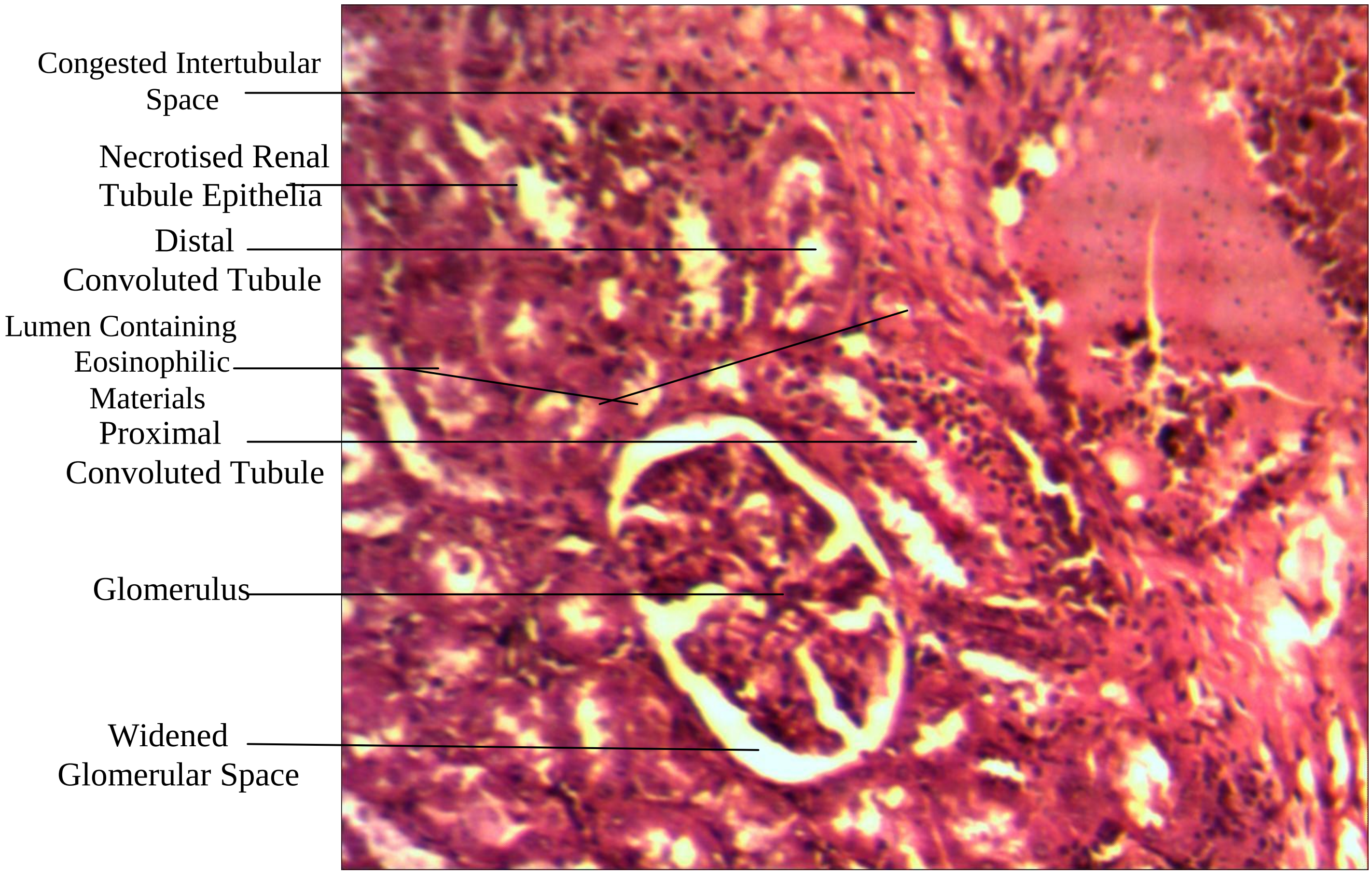


PLATE VI: A cross section of kidney cortex treated with 500mg/kg body weight of Momordica *charantia* showing congested intertubular spaces focal area of necrosis of renal tubular epithelial cells some of the lumen contain eosinophilic materials (Hematoxyline and eosin) × 250

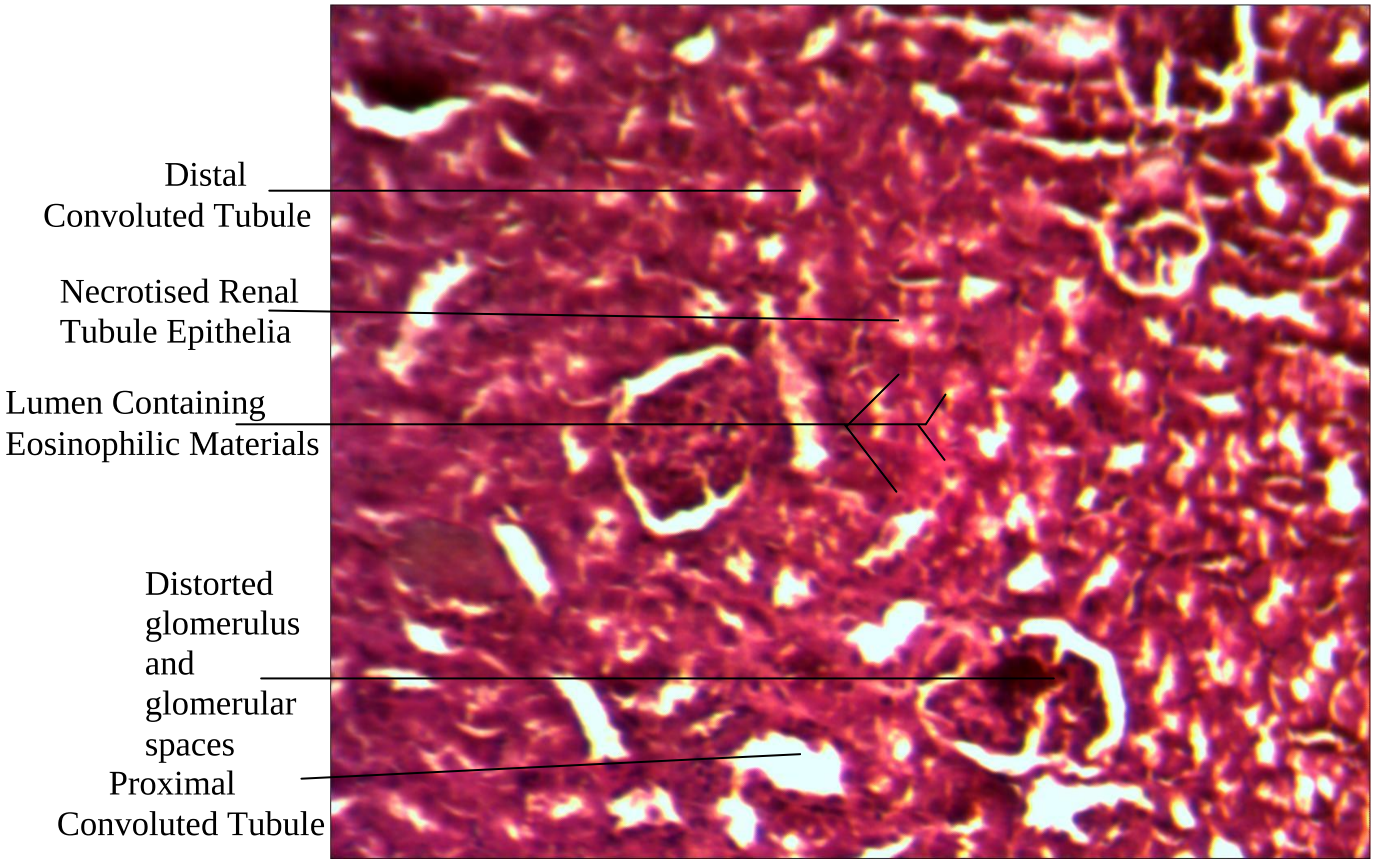


PLATE VII: A cross section of kidney cortex treated with 500mg/kg body weight of Momordica *charantia* (methanolic extract) showing congested intertubular spaces focal area of necrosis of renal tubular epithelial cells some of the lumen contain eosinophilic materials (Hematoxyline and Eosin) × 250

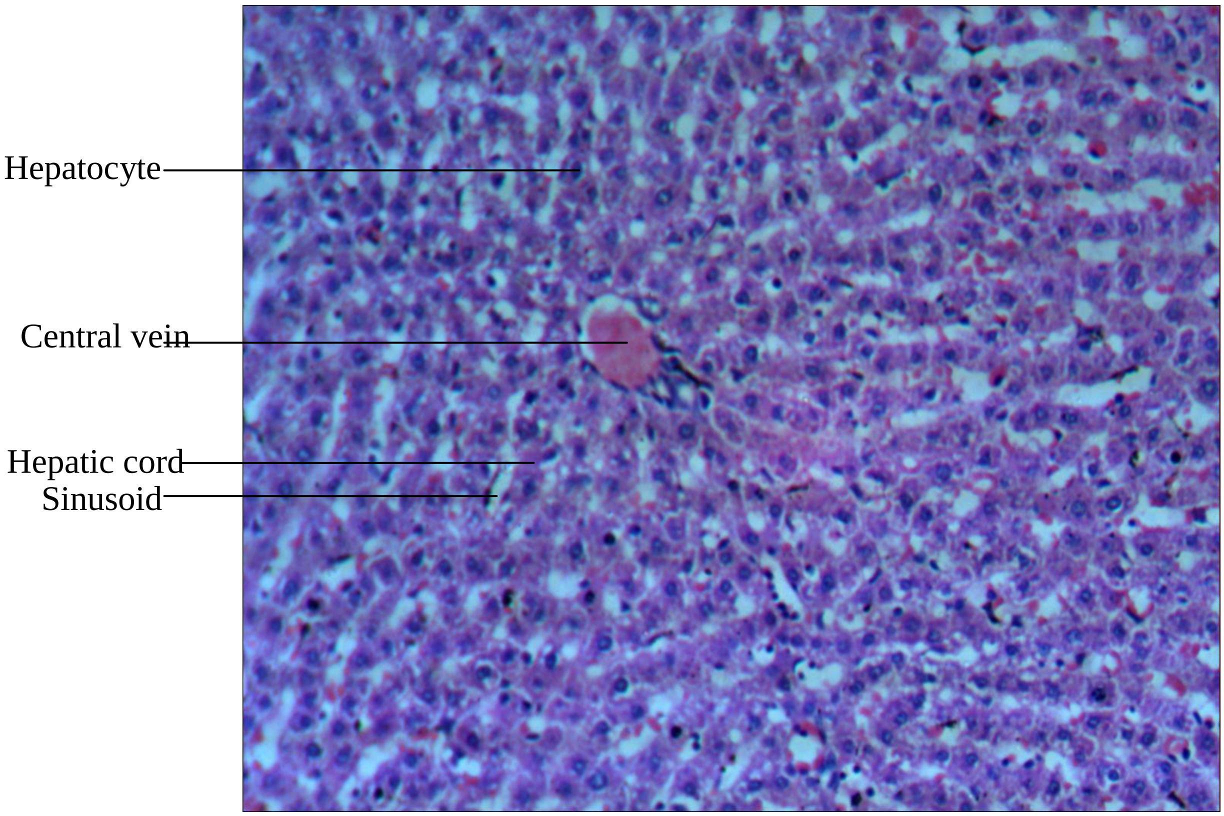


PLATE VIII: A cross section of liver administered with distilled water (control group) showing normal histology (Hematoxyline and Eosin) × 250

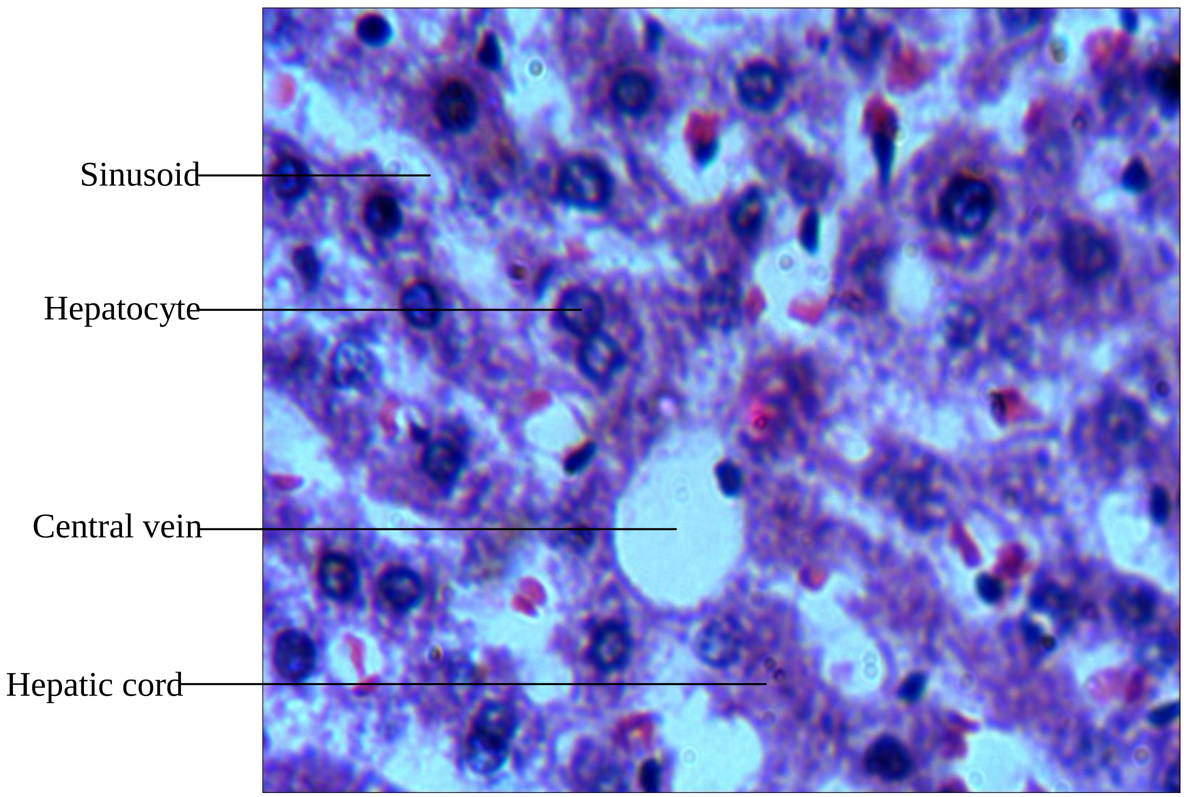


PLATE IX: A cross section of liver administered with distilled water (control group) showing normal histology (Hematoxyline and Eosin stain) × 400

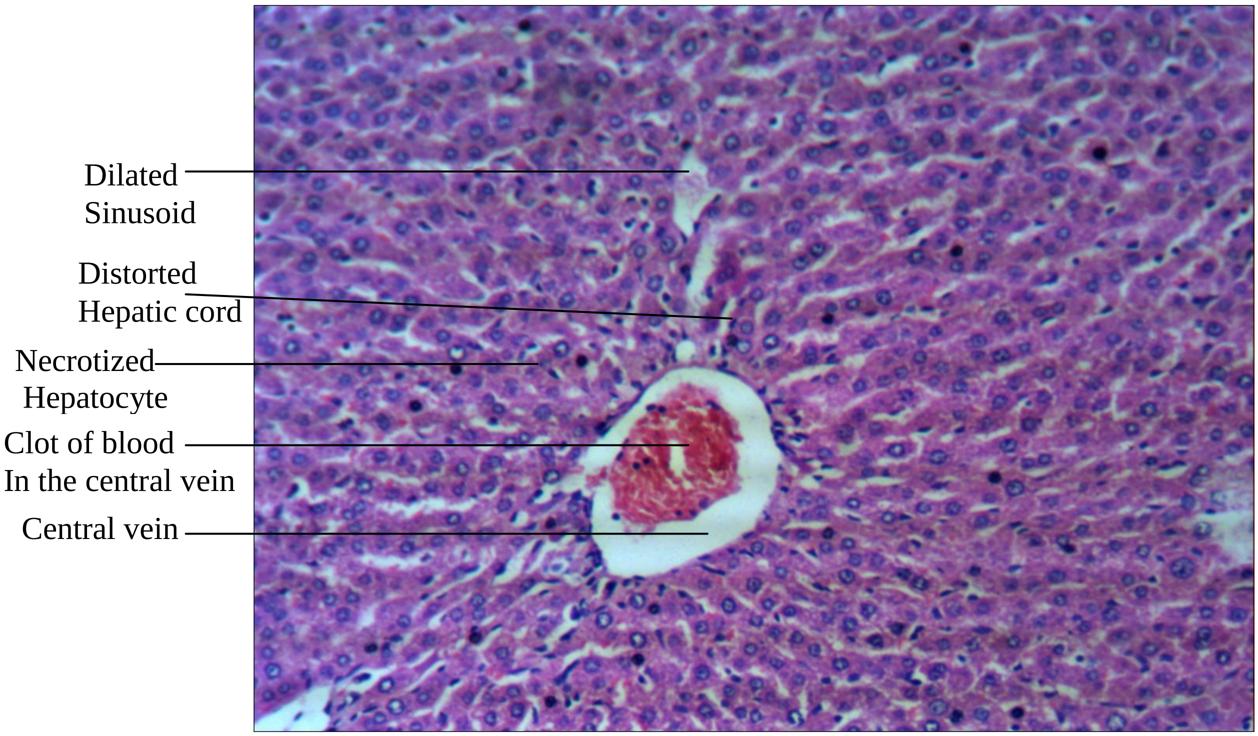


PLATE X: A cross section of liver treated with 125mg/kg body weight of Momordica *charantia* showing dilated central vein, dilated sinusoid and distorted hepatic cords (Hematoxyline and Eosin) × 250

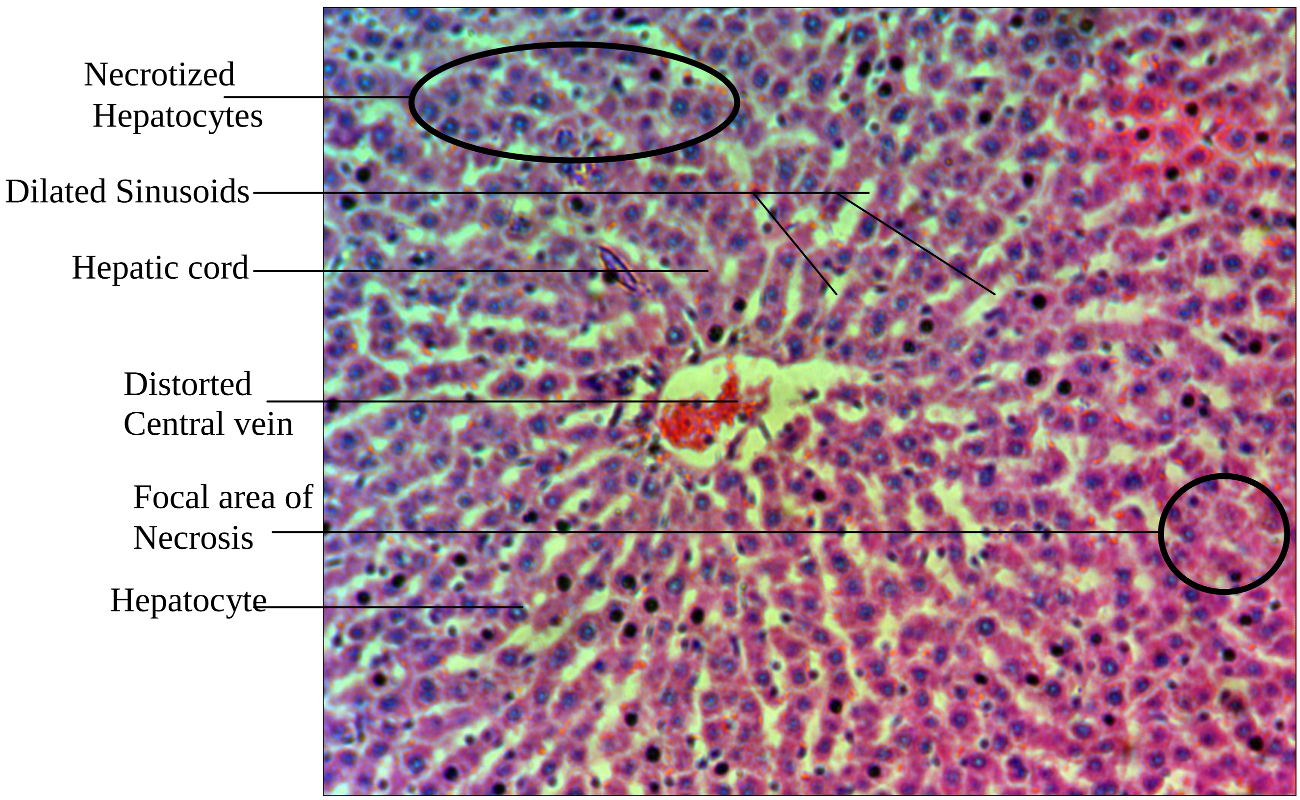


PLATE XI: A cross section of liver treated with 125mg/kg body weight of Momordica *charantia* (methanolic extract) showing focal area of necrosis, dilated sinusoids and distorted central vein (Hematoxyline and Eosin) × 250

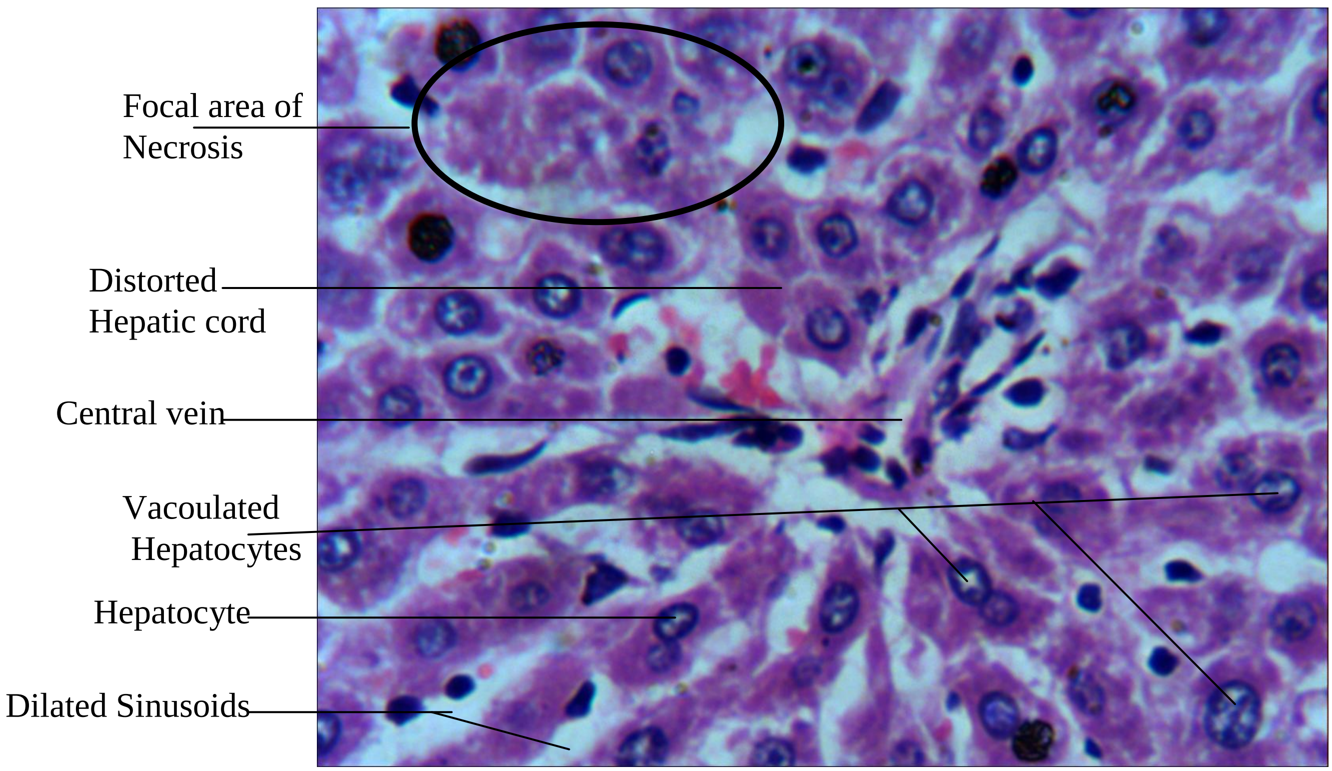


PLATE XII: A cross section of liver treated with 125mg/kg body weight of Momordica *charantia* showing congested vacoulation of hepatocyte, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) × 400

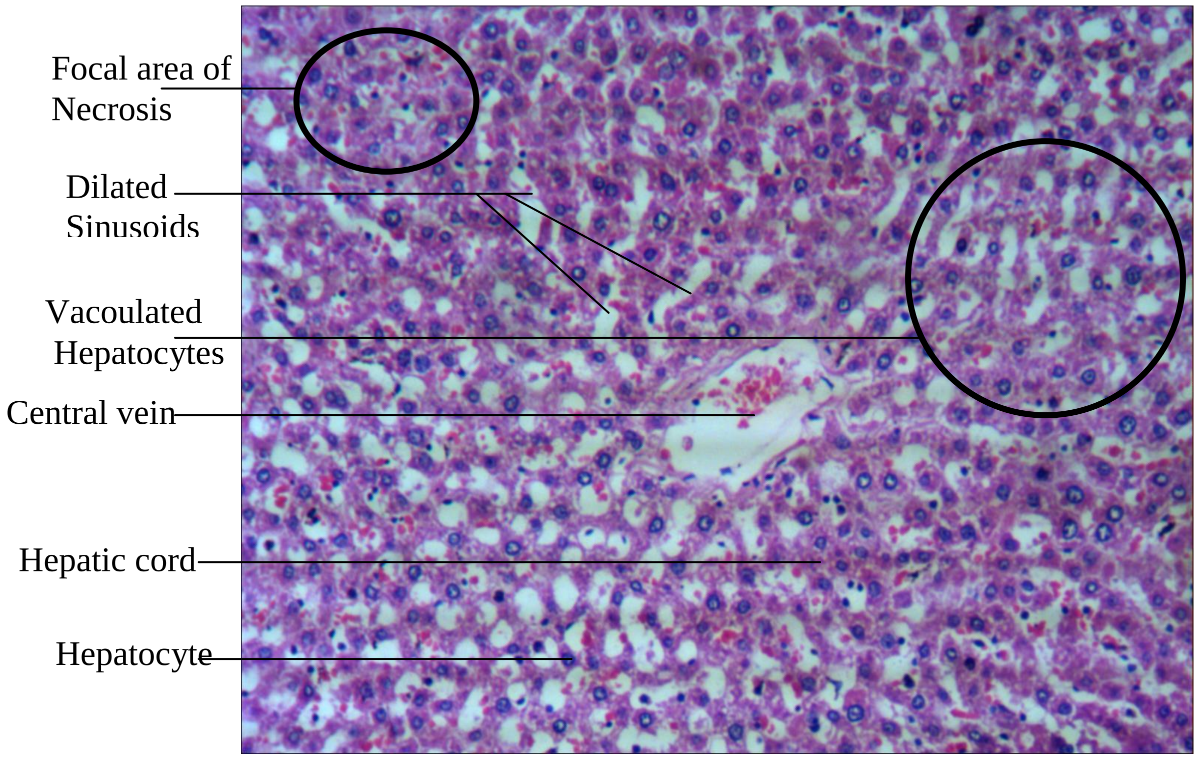


PLATE XIII: A cross section of liver treated with 250mg/kg body weight of Momordica *charantia* showing area of vacoulation of hepatocytes, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) × 250

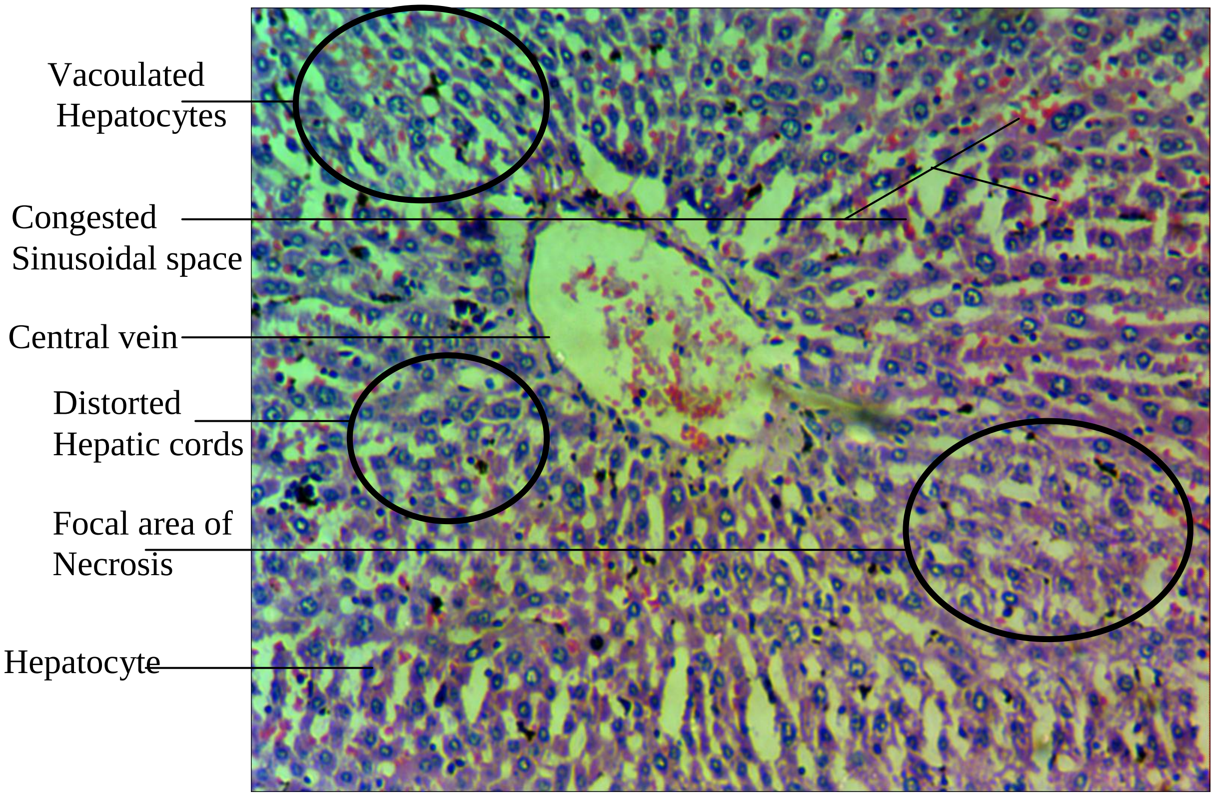


PLATE XIV: A cross section of liver treated with 500mg/kg body weight of Momordica *charantia* showing area of vacoulation of hepatocytes, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) × 250

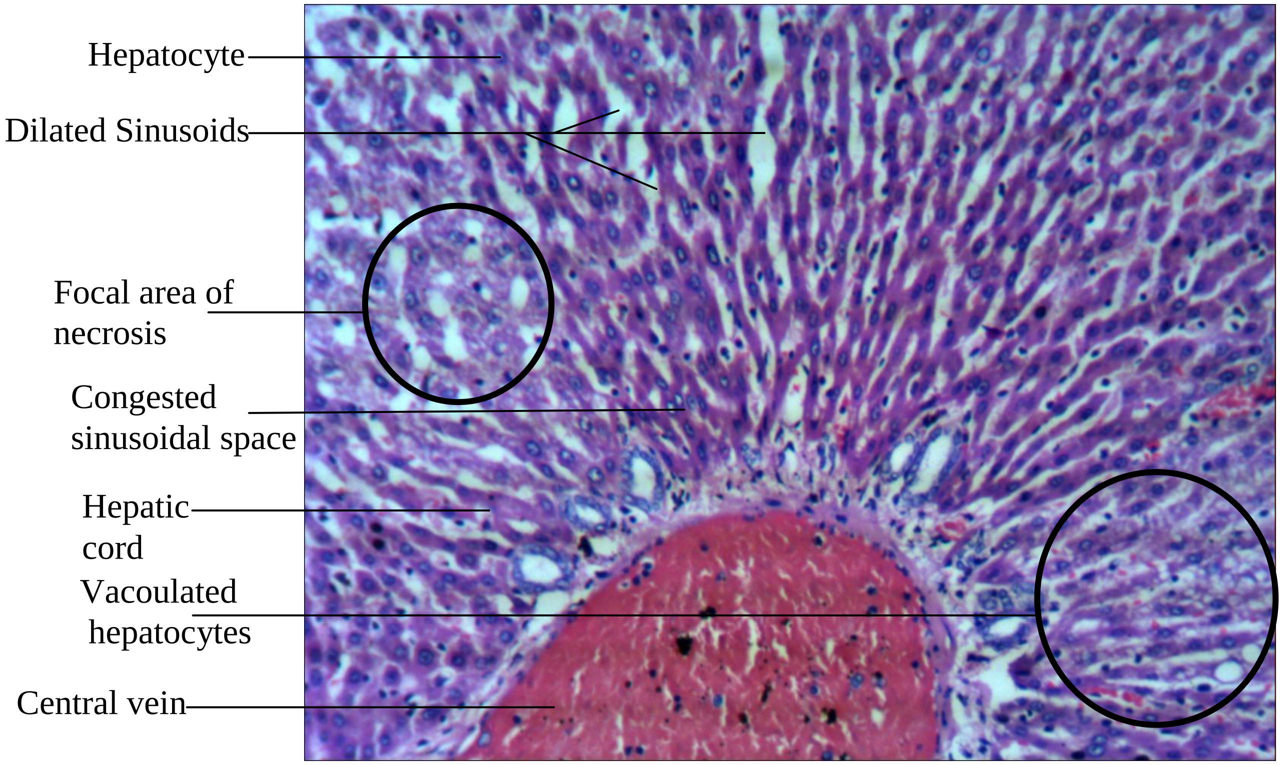


PLATE XV: A cross section of liver treated with 500mg/kg body weight of Momordica *charantia* showing area of vacoulation of hepatocytes, focal area of necrosis and dilated sinusoids (Hematoxyline and Eosin) × 400

CHAPTER FIVE

5.0 DISCUSSION

In this study, it was observed that the WBCs count had elevated levels across the treated groups. The elevated levels ($P<0.05$) of white blood cells (WBCs) in many conditions indicate infection, stress, inflammation, trauma, allergy or certain disease in an organ, tissue or the whole organism (Shcrier, 2010). The methanolic extract of *Momordica charantia* at higher dose in this study might have caused some toxic effects on the body system of the treated rats, an

d consequently, the elevation of white blood cells (WBCs). This is because the white blood cells (WBC) are known to play an important role in animal body’s immune system. They search the blood for invading viruses, bacteria, and fungi. When foreign virus or bacteria enters the blood, the white blood cells (WBCs) or leukocytes recognize and destroy the invading particle before it causes disease (Shcrier, 2007).

The red blood cells (RBCs) count in this study indicates no significant difference between the treated and the control groups. It is believed that when red blood cells (RBCs) count drops to lower than the normal range, it indicates anemia. Anemia has many causes including, low level of certain vitamins or iron, blood loss or due to an underlying condition (Sachs, 2007).

In this study, the percentage of lymphocyte was found to be elevated. It is believed that when there is increasing antigen in the body, the immune systems trigger the release of lymphocytes and elevate the number of lymphocytes in the body (Pepin *et al.*, 2007). Lymphocytes are one of the main types of immune cells. They are divided into B-lymphocyte and T-lymphocytes.

B-lymphocyte produces antibodies proteins (gamma globulins)

that recognize foreign substances (antigen) and attach themselves to them. They are programmed to make one specific antibody. When B-lymphocytes attach to an antigen (foreign body), they mark it for destruction and become powerless. T-lymphocytes then attacked the target cells and destroy it. Other T-lymphocytes are programmed to be able to destroy targeted cells on direct contact.

In this study neutrophil levels were found to be elevated. Neutrophils are a type of white blood cell that body uses to fight infections. High neutrophil levels often result from cases of infection, but other medical conditions and certain drugs can raise their levels as well. For example, physical and mental stress can also cause increase in neutrophil levels. High neutrophil levels do not usually indicate a serious immune problem and are considered to be harmless. In most cases, the levels return to normal after sometimes (Anderson *et al.*, 2004).

Increase in serum sodium (Na^+) concentration is seen in conditions with water loss in excess of salt, as in profuse sweating, severe diarrhoea or vomiting, polyuria (as in diabetes mellitus or insipidus), and inadequate water intake. Some drugs also cause elevation of sodium, such as steroids, with mineralocorticoid activity, carbenoxolone, guanethidine, methyldopa sodium bicarbonate (Murano and Bick, 1990). In this study, feed and water were given to the rats *ad ibitum*; and they were housed in a conducive environment, consequently there was no adverse effect that could result in water loss or sweating.

In this study, the sodium concentration was found to be within the normal range. The statistical analysis showed that, the P-value is greater than 0.05 ($P>0.05$). Therefore, there was no significant difference between the control and the treated groups. On the other hand, decrease in sodium is seen in conditions characterized by intake of free water or hypotonic solutions, as may occur in fluid replacement following sweating, diarrhea, vomiting, and diuretic abuse.

The values obtained from the biochemical analysis of K^+ suggested that, there was no significant increase or decrease in concentration of potassium. The P-value was ($p>0.05$) across the groups. Increase in serum potassium (K^+) is seen in conditions characterized by excess destruction of cells, with redistribution of K^+ from the intracellular and extracellular compartment, as in massive haemolysis, crush injuries, hyperkinetic activity, and malignant hyperpyrexia (Murano and Bick, 1990). On the other hand, decreased renal potassium K^+ excretion is seen in acute renal failure, some cases of chronic renal failure, Addison's disease, and other sodium-depleted conditions. Hyperkalemia due to excess of K^+ intake is usually iatrogenic (Murano and Bick, 1990). There was no indication of any of these conditions on the rat in this study studied, except for mild renal tubule necrosis.

The biochemical analysis of this study indicated that the chlorine level varies among the groups, but statistically it showed that the variation was not significant, because the p-value was greater ($p>0.05$). Therefore, chloride levels are at normal range. Adequate chloride helps to maintain proper fluid and electrolyte balance in the body (Pepin *et al.*, 2007).

In this study, it was observed that the creatinine levels were higher (p□ 0.05) among the rats treated with the methanolic extract of *Momordica charantia* when compared to the control group (**Appendix VI)**. Since creatinine is the metabolic waste product of muscle in the body, and mainly filtered out of the body via kidney (Lugaresi *et al*., 2013). The mild lesions seen on the histology of the kidney of the groups administered with 125 mg, 250 mg, and 500 mg/kg body weights of the extracts might have affected the rate of excretion of creatinine from the kidneys as indicated by the biochemical analysis in Fig. 4..

In this study, it was found that the levels of nzymes such as aspartate aminotransferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) of the treated groups had increased levels when compared to the control group. The statistical analysis of their values showed that there was marked elevation of (AST) among the groups which were treated with the extract of *Momordica charantia*. The increase is dependent on the dosages administered to the rats. For example, the group that received the highest dosage of 500mg/kg body weight had higher levels of AST (aspartate aminotransferase, ALT (alanine aminotransferase) and ALP (alkaline phosphatase). This suggests that, there were some assaults on the liver, as these enzymes are biomarkers of liver disease. This conforms to the histological changes observed in both the liver and kidney of this study. Similar findings were reported by Beri and Shrier (2010).

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are the liver enzymes, which are biomarkers of liver health. When these two enzymes are found to be elevated instead of alkaline phosphatase, it indicates or favour liver cell necrosis. When alkaline phosphates (ALP) are elevated, a problem with bile flow is most likely present. This problem leads to the elevation of bilirubin. This condition is associated with severe liver disease, or gall bladder disease. Therefore, once there is elevation of ALP, bilirubin test can be useful liver function test.

The results of histology of the kidney of this study also showed that the rats administered 125 mg/kg body weight of *Momordica charantia* methanolic extracts of *Momordica charantia* had some necrosis of renal tubular epithelial cells. This condition impaired the function of the kidney such as reabsorption of vital minerals and proteins from the filtrate (Wakabayashi *et al.*, 2009).

The result of the histology of the kidney of rats in the group administered 250 mg/kg body weight of *Momordica charantia* revealed that there were congested inter tubular spaces, which suggested that there was localized assault at the level of the renal tubules, this was confirmed by the biochemical analysis of the kidney enzyme as shown in Fig. 4.12. It is believed that passage of the filtrate may not be possible and if situation continues for a long time, it may affect the other healthy tubules, subsequently causing generalized congestion of the kidney which may lead to the renal failure (Scheen, 2000). There was focal area of necrosis of renal tubular epithelium. The clinicopathologic entity of this condition causes destruction of tubular epithelial cells thereby suppressing the renal function. It is believed that if this condition becomes generalize or more severe, it leads to acute renal failure, by reducing the urine volume to less than 400ml (Farquhar *et al.*, 2006).

The histology of the group which was administered 500 mg/kg body weight of the methanolic extract of *Momordica charantia* revealed that there were more congested intertubular spaces and more focal areas of necrosis of renal tubular epithelium. Some of the lumen contained eosinophilic materials. The histology showed that administration of extracts of *Momordica charantia* causes some assaults on the renal tubules as shown by the biochemical analysis in Fig. 4.12. Although it is believed that such assaults as ischemic, toxic tubular injury (leading to tubular necrosis and renal failure) and inflammatory reactions of the tubules and interstitium (leading to nephritis) could sometimes be reversed by proper management of the conditions but in many cases, they lead to the death of the organism (Farquhar *et al*., 2006).

Tubular cell injury involves tubular epithelial cells. These cells are particularly sensitive to ischaemia and also to toxins. Several factors predispose the tubules to toxic injury such as organic acid, antibiotic (multiple), severe glomerular disease, acute tubule-interstitial nephritis (occurring as a result of hyper sensitivity to drugs), massive infection (pyelonephritis) especially when accompanied by necrosis.

In general, the histological changes observed in this study suggest that the intake of this plant has some effects on the functional unit of the kidney (the nephron). The renal insults observed histologically by biochemical analysis in this study, become more severe with increasing dosage, therefore prolonged use or administration of this plant as practiced by the rural dwellers in Northern Nigeria may exposed them to the above lesions observed.

It was observed that the methanolic extract of *Momordica charantia* administered to the rats in all the treatment groups has caused some mild to more severe effects, for example the liver of group of rats which were administered with 125 mg/kg body weight had vacoulation of hepatocytes. The vacoulation of hepatocytes (also called ballooning degeneration) is a hepatic damage caused by ingested toxic or immunologic insult to the liver. The hepatocytes become swollen, edematous in appearance with irregularly clumped cytoplasm and clear spaces like vacoules within hepatocytes. This type of condition could be caused by toxic chemicals such as some drugs, iron and copper, accumulation of fat droplets (known as steatosis) Similar lesions were observed by Jungermann and Kietzmann (2006). The focal areas of necrosis were also observed with dilated sinusoids, distorted hepatic architecture and congested liver. In liver pathology, any significant insult to the liver may cause hepatocyte necrosis. It can be characterized by poorly stained mummified hepatocytes with sometimes lysed nuclei at some parts (Carithers, 2002).

Necrosis of hepatocytes frequently exhibits a zonal distribution as observed in the groups administered with 250 mg/kg body weight and 500mg/kg body weights. In general, the effects of the extract of this plant are all dosage dependant, i,e., the higher the dosage, the more severe the effects on the organs studied.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

The study was conducted on liver and kidney of albino rat to evaluate effects of extract of *Momordica charantia* on twenty (20) male adult albino rats with average weight 27 ±30 mg. They were divided into four (4) groups. Groups A, B and C were the treatment groups which were administered with 125mg/kg body weight, 250 mg/kg body weight and 500mg/kg body weight of the extract of *Momordica charantia*, respectively. Group D served as the control group and were administered with distilled water for six weeks. Blood samples were collected in 10% EDTA bottles and in sterilized bottles without EDTA for haematology and biochemical assay, respectively. white blood cells (WBCs), red blood cells (RBCs), heamoglobin, lymphocytes, eoisinophil, and neutrophils were determined from the blood placed containing 10% EDTA while sodium Na^{+}, potassium K^{+}, chlorine, urea, creatinine and ALT, AST, ALP levels which were determined from the blood sample without EDTA.

Kidney and liver were also harvested immediately after blood collection. The tissues were fixed in buffer solution of formation. Haematoxylin and Eoisin (H and E) technique was used for the staining to reveal the general structure of the processed tissues.

The data collected from this study was analysed using SPSS version 20, ANOVA was used to compare the data within and between the groups. All data were expressed as mean ± SEM and p<0.05 were considered to be significant.

The results showed lesions on the liver and the kidneys such as necrosis, distortion of the kidney and liver architectures. The statistical analysis revealed that there was increased levels of AST, ALT, ALP, White Blood Cells, lymphocytes eosinophils and neutrophils.

6.2 CONCLUSIONS

The following conclusions were arrived at after the administration methanolic extract of *Momordica charantia*:

- There were elevated levels of white blood cells across the treated groups when compared with the control group.
- There were higher creatinine levels in the treated groups than the control group.
- There were increased values of ALT, ALP and AST in the treated groups than the control group.
- Renal tubular necrosis was observed in the treated groups as opposed to normal kidney structures in the control group.
- Hepatic vacoulation and necrosis were observed in the treated groups as opposed to normal architecture of liver cells in the control group.

In general, the oral administration of extract of *Momordica charantia* has produced some negative effects on histology of the kidney and liver, and also on haematology and biochemical parameters of adult albino rats, ranging from mild to severe lesions. The effects increases with increasing dose, that is, the effects are dose dependant. The lesions observed in the kidney affect mostly the nephron. Necrosis of both nephron and hepatocytes were observed. Therefore, administration of this plant for a long period should be discouraged.

6.3 RECOMMENDATIONS

i. Further studies should be conducted using extract of the plant with lower dosage and for a longer period of time
ii. Chronic toxicity test should be carried out lasting 90 days
iii. Further haematology and biochemical analyses should be done using the extract for a longer period of time.
iv. Aqueous extract of this plant should be use to conduct similar study
v. Further histological studies should be conducted using electron microscope
vi. Traditional healers should be encouraged to be going for screening of this medicinal plant before use.
vii. Companies which produce juice and tea leaves of *Momordica charantia* should be enlightening their consumers of the possible negative effects of *Momordica charantia*.

REFERENCES

Abdullahi, M., Muhammad, G., and Abdulkadir, N.U. (2003). *Medicinal and economic plants of Nupeland*, Jube Evans Books and Publisher, ISBN 978-33921-9-0, pg 95.

Ahmed, I., Lakhani, M.S., Gillett, M. (2001). Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats. *Diabetes Research on Clinical Practice.* 51:155-161.

Ahmed, N., Hassan, M.R., Halder, H., Bennoor, K.S. (2009). Effect of *Momordica charantia* (karolla) extracts on fasting and postprandial serum glucose levels in NIDDM patients. *Bangladesh Medical Research Council Bulletin*, 25:11-13.

Akhtar E.L., Gregoire F.M., Scheen A.J. (2007). Antiobesity Pharmacotherapy in the Management of Type 2 Diabetes . *Medical Journal on Diabetes and Metabolic Disorders*, 16:114-124

Al L., Azad, K., Hassan, Z., Mosihuzzaman, M., Nahar, K., Nasreen, T., Nur-e-Alam, R. (1995). Characterization of hypoglycemic effects of trigonella foenum graesum seed. *Planta Medical Journal*, 61: 358-360.

Alison, M.R. (2008). Regulation of hepatic growth. *Physiological Review*, 66: 499.

Ananya, P., and Sarmistha, S. R. (2010). Medical uses and molecular identification of two *Momordica charantia* varieties – A Review: *Electronic Journal of Biology*, 6 (2): 43-51.

Anderson, R.J., Gabow, P.A. and Gross, P.A. (2004). Urinary chloride concentration in acute renal failure. *Miner Electrolyte Metabolism,* 10:92-97.

Andrews, P.M., and Porter, K.R. (2004), A scanning electron microscopic study of the nephrons. *American Journal of Human*, 140(230):1280-1287.

Andrews, P.M., and Porter, K.R. (1984), A scanning electron microscopic study of the nephrons. *American Journal of Anatomy*, 190:81.

Anila, L., and Vijayalakshmi, N.R. (2000). Beneficial effects of flavonoids from Sesamum indicum, *Emblica officianalis* and *Momordica charantia*. *Phyto Therapeutic Research Journal*, 14:592-595.

Asli, S., and Alaattin, S. (2007), Antioxidant and chemoprotective properties of *Momordica charantia* fruit extract. *African Journal of Biotechnology*, 6(3):273-277.

Atama, J.E and Idu, M. (2007). Histopathologic effects of mathanolic extract of *Momordica charantia* leaves on the liver of Wister rats. *Trends in Medical Research Journal*, 2(4): 176-184.

Azuma, K., Katsukawa, F., Oguchi, S., Murata, M., Yamazaki, H., Shimada, A. and Saruta, T. (2003). Correlation between serum resistin level and adiposity in obese individuals. *Obesity Research Journal*, 11:997 – 1001.

Barbieri, L., Lorenzoni, E. and Stirpe, F. (1979). Inhibition of protein synthesis *in vitro* by lectin from *Momordica charantia*. *Journal of Ethnopharmacology* 18(2): 633-635.

Barger, A.C., and Herd, J.T. (1999). The hepatic circulation, *New England Journal of Medicine,* 284-289.

Berl, T. and Schrier, R.W. (2010), Disorders of water homeostasis, Philadelphia, Lippincott Williams & Wilkins. 11(4):360.

Biyani, M.K., Banavalikar, M.M., Suthar, A.C., Shahani, S., Sivakamis, and Jaspreet, V. (2003), Antihyperglycemi effects of three extracts from Momordica charantia, *Journal of Ethnopharmacology*, 88:107-111.

Boucheir, I.A.D. (2000). The medical treatment of gallstones. *Annual Review of Medicine. 31:59.*

Bourinbaiar, A.S., Lee-Huang, S. (2006). The activity of plant derived antiretroviral proteins MAPH30 and GAP31 against herpes simplex virus. *Biochemistry and Biophysics Research Community Journal*. 219:923-929.

Brassett, C., and Elliis, H. (1991). Transportation of the viscera. *Clinical Anatomy*, 7:801-807.

Carithers, R.L., Jr, (2002). Alcoholic hepatitis and cirrhosis. In Kaplowtz N. (ed): *Liver and biliary diseases*. Baltimore, Williams and Wilkins, 4 (4): 334-346.

Chan, L.L., Chen, Q., Go, A.G., Lam, A.K. and Li, E.T. (2005). Reduced adiposity in bitter melon – fed rats is associated with increase lipid oxidative enzymes activities and uncoupling protein expression. *Journal of Nutrition*, 135: 2517-252.

Chaturvedi, P., George, S., Milinganyo, M. and Tripathi, Y.B. (2004). Effect of Momordica *charantia* on lipid profile and oral glucose tolerance in diabetic rats. *Journal of Phytotherpeutic Research*. 18:954-956.

Chaturvedi, P. (2005). Role of *Momordica charantia* in maintaining the normal lipids and glucose in diabetic rats fed a high-fat and low-carbohydrate diet. *Journal of Biomedical Science*, 63:124 – 126

Chaturvedi, P. (2006). Anti-diabetic potential of *Momordica charantia*: multiple mechanisms behind the effects. *Journal of Traditional Medicine*, 90 (2): 221-227.

Chen, Q., Chan, L.L.Y. and Li, E.T.S. (2003). Bitter melon (*Momordica charantia*) reduces adiposity, lowers serum insulin and normilizes glucose tolerance in rats fed with high diet. *Journal of Nutrition*, 133:1088-1093.

Chen, Q. and Li, E.T. (2005). Reduces adiposity in bitter melon (*Momordica charantia)* fed rats is associated with lower tissue triglyceride and higher plasma catecholamines. *Journal of Nutrition*, 93:747-754.

Cheow, P. O., Zaitun, Y. and Tengku-Aizan H. (2009). *Momordica charantia* for type-2 diabetes mellitus. *The Cochrane Collaboration*, 2:13-16.

Chi-I, C., Chiy-Rong, C., Yu-Wen, L., Hsueh-Ling, C. and Yo-Chia, C. (2008). Cucurbitane-type triteropenoids from the stems of *Momordica charantia*. *Journal of Natural Products*, 71(8): 1327-133019.

Cousins, R.J. (2005). Absorption, transport, and metabolism of copper and zinc: special reference to metallothionine and ceruloplasmin. *Physiological Review*. 65:238.

Dans, A.M., Villarruz, M.V. and Jimeno, C.A. (2007). The effect of *Momordica charantia* capsule preparation on glycemic control in type 2 diabetes mellitus need further studies. *Journal of Clinical Epidemiology*. 60:554-559.

Dietrich, L. (1983). A new approach to acute toxicity test. *British Journal of Toxicology*, 6: 5.

Earnest, C.P., Snell, P.G., Rodriguez, R., Almada, A.L. and Mitchell, T.L. (1995). The effect creatine monohydrate ingestion on anaerobic power indices, muscular strength and body composition. *American Journal of Physiology* ,153(2):207-209.

Elias, H., and Caro, L.G. (2005), Re-examination of the mammalian liver, *Journal of Human Biology, England*, 109(8):901 – 912.

Eltoum I., Fredenburgh J. Myers R.B. and Grizzle W.E (2001). Introduction to the theory and practice of fixation of tissues. *Journal of Histotechnology*, 24; 173-190

Evans G., and Bicks R.L (1996). Simplified method of phytochemical analysis. *Journal of medical laboratory technology*. *10; 7-13.*

Fang, E. (2012). "In vitro and in vivo anticarcingenic effects of RNase MC2, a ribonuclease isolated from dietary bitter gourd, toward human liver cancer cells." *International Journal of Biochemistry and Cell Biology*. 44(8): 1351-60.

Fang, E.F., and Nigs, T.B (2012). *Momordica charantia* is a cornucopia of health: A review of its credited anti-diabetic, anti-hiv and anti-tumor properties. *Journal of School of Biomedical Sciences*, the Chinese University of Hong Kong, Shatin.

Farquhar, M., Wilson, C.B., and Kalluri, R. (2006). The heymann nephritis antigenic complex; megalin (gp330) and RAP. *Journal of American Society of Nephrology*, 6:35.

Garcia, A., Sekowski, A., Subramanian, V. and Brasaxmle, D. (2003). The central domain is required to target and anchor perilipin A to lipid droplets. *Journal of Biochemistry*, 278:625-635.

Gitnick, G. (2009). *Modern concepts of acute and chronic hepatitis*. New York, Plenum Publishing Corporation.

Goresky, C.A. and Groom, A.C. (2004). Microcirculatory events in the liver and the spleen. Handbook of physiology. *American Physiological Society*. 2:689

Gregoire, F.M. (2006). Adipocvyte differentiation from fibroblast to endocrine cell. *Journal of Experimental Biology and Medicine*, 226: 997-1002.

Grover, J.K., and Yadav, S.P. (2004), Pharmacological actions and potential uses of Momordica charantia: a review. *Journal of Ethnopharmacology*, 93(1): 123-132.

Grover, J.K., Yadav, S. P and Vats, V. (2002). Medicinal plants in India with anti-diabetic potential. *Journal of Ethnopharmocology*, 81:81-100.

Harada, M., Udagawa, N., Fukasawa, K., Hiraoka, B.Y. and Mogi, M. (1986). “Inorganic pyrophosphatase activity of purified bovine pulp alkaline phosphatase at physiological pH". *Journal of Dental Research*, 65(2): 125–7.

Harinantenaina, L., Tanaka, M. and Takaoka, S. (2006). *Momordica charantia* constituents and antidiabetic screening of the isolated major compounds. *Chemical Pharmacology Bulletin (Tokyo)*; 54:1017-1021.

Harlan, L. (2008). Haematology and biochemical analysis of Wister rats, developed at Wister rats institute, Pennsylvania, 8520 Allison Pointe Blvnd Suite 400.

Hazarika, R., Pavida, P., Neog, B., Yadav, R.N. (2009). Binding energy calculation of GSK-3 protein of human against some anti-diabetic compounds of *Momordica charantia*. *Book of Health Benefit of Bitter Melon*, 6-8.

Henderson, J.R. and Daniel, P.M. (2004). Capillary beds portal circulations. *Handbook of physiology*. *American Physiological Society*. 1035.

Henriksen, J.H. (2005). Cirrhosis; ascites and hepatorenal syndrome. Recent advances in pathogenesis. *Journal of Hepatology*, 23(1): 25-30.

Huang, H.L., Hong, Y.W., Wong, Y.H., Chen, Y.N., Chyuan, J.H., Huang, C.J. and Chao, P.M. (2008). *Momordica charantia* inhibits adipocyte hypertrophy and down regulates lipogenic gene expression in adipose tissue of diet induced obese rats. *British Journal of Nutrition*, 99:230-239.

Hughes, J. (2005). The polycystic kidney disease 1 (PKD1) genenencodes a novel protein with multiple cell recognition domains. *National Genetic Journal*, 10:151.

Hussain, H.S.N. (2001). Plants in Kano ethomedicine: screening for antimicrobial activity and alkaloids. *International Journal for Pharmacognosis*. 29(1): 51-6.

Jelalu, K., Denny, M., John, W.H. and Ray, K. (2008). Veterinary laboratory medicine: Laboratory manual and review on clinical pathology: *Elsevier Health Sciences*. 2; 227-237

Jesada, P., Sutawadee, C., Motonobu, G., Weena, J., Mitsuru, S., and Artiwan, S. (2007). New approach for extraction of charantin from *Momordica charantia* with pressurized liquid extraction separation and purification. Technology, 52(3):416-422.

Jiratchariyakul, W., Wiwat, C. and Vongsakul, M. (2001). HIV inhibitor from Thai bitter gourd. *Journal Planta Medical*, 67 (4): 350-353.

Jungermann, K., and Katz, N. (2009). Functional specialization of different hepatocyte populations. *Physiological Review*, 69: 708.

Jungermann, K., and Kietzmann, T. (2006). Zonation of paranchymal and nonparanchymal metabolism in liver. *Annual Review of Nutrition Journal*, 16: 179-203.

Khan, M.R. and Omoloso, A.D. (2008). *Momordica charantia* and allium sativum: board spectrum antibacterial activity. *Korean Journal Pharmacology*. 29:155-158.

Khanna, P., Jain, S.C., Panagariya, A. and Dixit, V.P (1981). Hypoglycemic activity of polypeptide–p from a source. *Journal of Natural Production*, 44: 648-655.

Kim, J.H. (2002). Induction of apoptosis by Momordin I in promyelocytic leukemia (HL-60) cells. *Anticancer Research Journal*, 22(3): 1885-9.

Kirsch, J.F., Eichele, G., Ford, G., Vincent, M.G., Jansonius, J.N. and Gehring, H. (1984). "Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure". *Journal of Molecular Biology,* 174 (3): 497–525.

Kohno, H., Yasui, Y., Suzuki, R., Hosokawa, M., Miyashita, K. and Tanaka, T. (2004), Dietary seed oil rich in conjugated linolenic acid from bitter melon inhibits Azoxymethane-induced rat colon caracinogenesis through elevation of colonic PPARY expression and alteration of lipid composition. *International Journal of Cancer*. 110:896-901.

Kraus, F.N. (2004). Hormonal regulation of hepatic gluconeogenesis. *Physiological Review*. 64:170.

Krawinkel, M.B., and Kedin, G.B. (2006). Bitter gourd (bitter melon) a dietary approach to hyperglycemia, *Nutritional Journal of Biomedical Japan,* 64: 331-337.

Krawinkel, M.B., Kedina, G. B. (2006). *Momordica charantia*: A dietary approach to hyperglycemia. *Nutrition Review*, 64(7): 331-337.

Kulkarni, R.D., and Gaitonde, B.B. (1962). Potentiation of tolbutamide action by Jasabhasma and *Momordica charantia. Journal of Indian Medical Association*, 50:715-719.

LaMont, J.T. and Carey, M.C. (2002). Cholesterol gallstone formation. 2. Pathobiology and pathomechanics. *Progressive Liver Disease Bulletin*, 10:165-191.

LaRusso, N.F. (2004). Proteins in bile: how they get there and what they do. *American Journal of Physiology*. 247:G199.

Lands, W.E.M. (2005). Cellular signals in alcohol-induced liver injury: a review. *Alcohol Clinical Experimental Research*, 19:928-938.

Lee-Huang, S., Huang, P.L. and Sun, Y. (2000). Inhibition of MDA-MB-231 human breast tumor xenografts and HER2 expression by anti-tumor agents GAP31 and MAP30. *Anticancer Research*. 20:6533-659.

Leatherdale B.A., Panesan R.K., Singh G., Atkins T.W. and Bailey C.J., (2008). Improvement in glucose tolerance due to *Momordica charantia*. *British Medical Journal*, 282, 1824-1834.

Li, C. (2012). *Momordica charantia* extract induces apoptosis in human cancer cells through caspase- and mitochondria-dependent pathways. *Evid Base Complement Alternate Medicine*, 2012: 261971.

Low, S., Chin, M.C. and Deurenberg-Yap, M. (2009). Review on epidemic of obesity. *Journal of Medical Academic Singapore*, 38:57-59.

Lugaresi, R., Leme, M., De, S., Painelli, V.T., et, al. (20013). Does long-term creatine supplementation impair kidney function in resistance – trained individuals consuming a high-protein diet? *Journal of the International Society of Sports Nutrition*,10 (1): 1-1.

Lu, W. (2007). Perinatal lethality with kidney and pancreas defects in mice with a targeted PKD-1 mutastion. *National Genetic Journal*, 17:179.

Mahomoodally, M.F., Subratty, A.H., Gurib-Fakim, A., Choudhary, M.I. and Nahar, K.S. (2010). Traditional medicinal herbs and food plants have the potentials to inhibit key carbohydrate hydronizing enzymes in vitro and reduce post prandial blood glucose peaks in vivo. *Journal of Traditional Medicine*, 93: 1010-110.

Majekodunmi, F., Yoshio, T., Hiroyasu, Y., Hikaru, O. and Tatsuo, Y. (1990). New cucurbitane triterpenoids from *Momordica charantia. Journal of Natural Products*, 53(6):1491-1497.

Mall, F.P. (1960): A study of the structural unit of the liver *American Journal. Anatomy*, 5:227.

Manabe, M., Takenaka, R., Nakasa, T. and Okinaka, O. (2003). Induction of anti-inflammatory responses by dietary *Momordica charantia* L. (bitter gourd). *Bioscience Biotechnology and Biochemistry Journal*, 67:2512-2517.

Marcelo, O., and Mac, B. (1992). Nar. Oxford Journals.org/cgi.reprint. Momodin II, a ribosome inactivating protein from *Momordica balsamina*, is homologous to other plant protects. *Nucleic Acids Research*, 20 (17): 4662.

Marcelo, O., and Marc, B. (2002). Momordin II, a ribosome inactivating protein from Momordica charantia, is a homologous to other plants proteins. *Journal of Nucleic Acids Research*, 20(17):4662.

Masuko, K., Mayumi, O.K., Yukari, A., Chizuko, Y., and Mitsuru, Y. (2008). Alpha eleostearic acid its dihydroxy derivatives are the major apoptosis-inducing components of bitter melon. *Journal of Agriculture and Food Chemistry*, 56(22):10515-10520.

Meng, Y. (2012). “Presentation of an antitumor and antivirus agent; chemical modification of MMC and MAP30 from *Momordica charantia* L. with covalent conjugation of polyethyelene glycol.” *International Journal of Nanomedicine*. 7:3133-42.

Miura, T., Itoh, C., Iwamoto, N., Kato, M., Kawai, M., Park, S.R. and Suzuki, I. (2001). Hypoglycemic activity of the fruit of the *Momordica charantia* in type-2 diabetic mice. *Journal of Nutrition Science Vitaminol*, 47(5): 340 – 344.

Murano, G., and Bick, R.L. (1990). Basic concept of hemostasis and thrombosis: clinical laboratory evaluation of thrombohemorrhagic phenomina. *Journal of Hematology,* 8:227 – 233.

Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Omar, S.A. and Guantia, A.N. (2007). Antimalaria activity of some plants traditionally used in treatment of malaria. *Journal of Ethnopharmacology*. 112(3): 545-551.

Nadine, B., Mesanvi, G., Koffi, A., Jim, H., Komlan, D. S., Kossi, K., and Thor, J. A. (2005), Ethnomedicinal uses of *Momordica charantia* (cucurbitaceae) in Togo and relation to its phytochemistry and biological activity. *Journal of Ethnopharmacology*, 96(1-2):49-55.

Nerurkar, A.M., William, D., Torres, R. A. and Lau, C. (2010). Escalating trends of obesity and associated type-2 diabetes. *Journal of Biomedical Science*, 9: 44-49.

Nerurkar, P.V., Lee, Y.K. and Linden, E.H. (2006). Lipid lowering effects of Momordica charantia in HIV-1 protease inhibitor. Treated human hepatoma cells HepG2. *British Journal of Pharmacology*, 148(8):1156-1164.

Nerurkar, P.V., Lee, Y.K., Motosue, M., Adeli, K. and Nerurkar, V,R. (2008). *Momordica charantia* reduces plasma apolipoprotein B-100 and increases hepatic insulin receptor substrate and phosphoinositide-3 kinase interactions. *British Journal of Nutrition*, 100:751-759.

Nerurkar, P.V., Pearson, L., Efird, J.T., Adeli, K., Theriault, A.G. and Nerurkar, V.R. (2005). Microsomal triglyceride transfer protein gene expression and ApoB secretion are inhibited by bitter melon in HepG2 Cells.

Nielsen, E.G. and Couser, W.G. (2007). *Immunologic renal diseases*. New York Lippincott-Raven.

Okabe, H., Miyahara, Y., and Yamauci, T. (1982). Studies on the constituents of Momordica charantia L. *Chemical Pharmacology Bulletin,* 30(12):4334-4340.

Olaniyi ,A.A. (1975). A neutral constituents of *Momordica charantia*. Lloydia, School of Pharmacy, University of Ife, Ile-Ife, Nigeria. 38(4):361-362.

Ooi, C. (2012). *Momordica charantia* for type 2 diabetes mellitus. Cochrane database system review. 15(8): CD007845.

Oishi, Y., Sakamoto, T. and Udagawa, H. (2007). Inhibition of increases in blood glucose and serum neutral fat by *Momordica charantia* saponin fraction. *Bioscience Biotechnology and Biochemistry Journal*. 71:735-740.

Omoregbe, R.E., Ikuebe, O.M.and Ihimire, I.G. (2006). Antimicrobial activity of some medicinal plants extracts on *Escherichia coli*, *Salmonella paratyphi* and *Shigella dysenteriae*. *African Journal of Medical Science*. 25:373-375.

Pepin, M.N., Bouchard, J., Legault, L. and Ethier, J. (2007). Diagnostic performance of fractional excretion of urea and fractional excretion of sodium in the evaluations of patients with acute kidney injury with or without diuretic treatment. *American Journal of Kidney Disease*, 50:566-573.

Pitchakarn, P. (2012). Kuguacin J, a triterpeniod from *Momordica charantia* leaf modulates the progression of androgen-independent human prostate cancer cell line, PC3.*Food Chemistry Toxicology*, 50(3-4): 840-7.

Raman, A., and Lau, C. (1996). Anti-diabetic properties and phytochemistry of Momordica charantia. *Phytomedicine Journal*, 2:349-362.

Rehmuzzi, G., Rugenmenti, P., and Benigni, A. (1997). Understanding the nature of renal disease progression. *Kidney International Bulletin*. 51:2.

Roffey, B.W., Atwal, A.S, Johns, T. and Kubow, S. (2007). Water extracts from *Momordica charantia* glucose uptake and adiponectin secretion in 3T3-L1 adipose cells. *Journal of Ethnopharmacology*, 112:77-84.

Sabira, B., Mansour, A., Bina, S., Siddiqui, A. K., Zafar S.S., and Mohammed A. (2007), Triterpenes, a sterol, and a monocyclic alcohol from *Momordica charantia*. *Phytochemistry*, 44(7):1313-1320.

Sachs, L. (2007). The molecular control of blood cell development. *American Journal of Science*, 238:1374.

Scheen, A.J. (2000). Adipocyte differentiation from fibroblast to endocrine cell. *Journal of Experimental Biology and Medicine*, 226:997-1002.

Schrier, R.W. (2007). Decreased effective blood volume in edematous disorders: What does this mean? *Journal of American Society of Nephrologists*, 18:2028-2031.

Sarkar, S., Pranava, M., and Marita, R. (1996). Demonstration of the hypoglycemic action of Momordica charantia in a validated animal model of diabetes. *Pharmacological Research*, 33:1-4.

Schrier, R.W. (2010). Fluid administration in critically ill patients with acute kidney injury. *Clinical Journal of American Society of Nephrologists*, 5:733-739.

Schrier, R.W. (2010). *Renal sodium excretion, edematous disorders, and diuretic use.* Philadelphia, Lippincott Williams & Wilkins.

Senanayake, G.V., Maruyama, M. and Sakono, M. (2004). The effects of Momordica charantia (bitter melon) extracts on serum and liver lipid parameters in hamsters fed with cholesterol-free and cholesterol-enriched diets. *Journal of Nutrition Science Vitaminol*, 50:253 – 257.

Sheperd, T.M., (2003). Effective management of obesity, *Journal of Family Practice*, 52:1-13.

Shewmake, R.A. and Huntington, M.K. (2009). Nutritional treatment of obesity. *Journal of Primary Health*, 36:357-377.

Shi, Y., Liu, Y., Murdin, A., Raudonikiene–Mance, A. B., Yu, Z., Fantus, G. and Liu, P. (2008). Chlamydophila pneumoniae inhibits differentiation of progenitor adipose cells and impairs insulin signaling. *Journal of Infectious Diseases*, 197:439 – 448.

Shulman, H.M. (2004). Venoocclusive disease of the liver after marrow transplantation: Histological correlates of clinical signs and symptoms. *Hepatology Journal*, 19:1171-1181.

Sofowora, A. (2003). *Medicinal plants and traditional medicine in Africa, 2:213-221*.

Sofowara A. (2012). Medicinal plants and traditional medicine in Africa*, (Rep. and updated version, 2012)*

Soundararajan, R. (2012). Antileukemic potential *Momordica charantia* seed extracts of human myeloid leukemic HL60 cells. *Evid Base Complement Alternate Medicine*, 2012:732404.

Sridhar, M. G., Vinayagamoorthi, R., Arul, S.V., Bobby, Z. and Selvaraj, N. (2008), Bitter melo (Momordica charantia) improves insulin sensitivity by increasing skeletal muscle insulin-stimulated IRS-1 tyrosine phosphorylation in high – fat fed rats. *British Journal of Nutrition*, 99 (4):806-812.

Sujathan, K., Karamala, S.L., Anjaneyulu, H.Y., Chandra, S., Rao T.S., Sreeni, V.D. and Amravathi, P.P. (2011). Haematobiochemical changes of lead poisoning and amelioration with ocimum sanction in Wistar albino rats. *Veterinary World*, 4 (6): 260-263.

Tabata, K. (2012). Kuguaglycoside C. a constituent of *Momordica charantia*, induces caspase-independent cell death of neuroblastoma cells. *Cancer Science*. 103(12): 2153-8.

Tan, M.J., Ming, J.Y., Nigel, T., Cordula, H.B, and Chang, Q.K (2008). Antidiabetic activities of bitter melon in associated with activation of the AMOK pathway. *Journal of Chemistry and Biology*, 15(3): 263-2719.

Tansey, J.T., Sztalryd, C., Gruia-Gray, J., Roush, D.L., Zee, J.V., Gavrilova, O., Reitman, M.L., Deng, C.X., Li, C., Kimmel, A.R. and Londos, C.(2001). Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Journal of Natural Science Academy*, 98: 6494-6499.

Tansey, J.T., Sztalryd, C., Hlavin, E.M., Kimmel, A.R. and Londos, C. (2004). The central role of perilipin in lipid metabolism and adipocyte lipolysis. UBMB Life, 56:379-385.

Tennekoon, K.H., Jeevathayaparan, S., Angunawala, P., Karunanayake, E.H., and Jayasinghe, K.S. (1994). Effect of Momordica *charantia* on key hepatic enzymes. *Journal of Ethnopharmacology*. 44(2):93-7.

Teoh, S.L., Azian, A.L. and Das, S. (2010). Histological changes in the kidneys experimental of diabetic rats fed with *Momordica charantia* extract. *Romanian Journal of Morphology and Embryology*, 51:91-95.

Tisher, C., and Brenner, B.M. (2009). *Renal pathology, with clinical and pathological correlations*, 3rd ed. Philadelphia, J.B. Lippincott.

Toshihiro, A., Naoki, H., Harukuni, T., Motohiko, U., Hiroyuki, A., Yuchi, T., Yumiko, K., Takashi, S., and Hoyoku, N. (2007), Cucurbitane-tupe triterpenoids from the fruits of *Momordica charantia* and their cancer chemopreventive effects. *Journal of Natural Products*, 70:1233-1239.

Tropical plant database (2007). Raintree nutrition. http://rain-tree.com/bitmelon.htm.

Uche-Nwachi, E.O., and McEwen, C. (2009). Teratogenic effect of the water extract of bitter gourd (*Momordica charantia*) on the Sprague Dawley rats. *African Journal of Traditional Complement Alternative Medicine*. 15:7(1):24-33.

Umek, R.M., Friedman, A.D. and McKnight, S.L. (1991). CCAT-enhancer binding protein a component of a differentiation switch. *Journal of Biomedical Science*, 51:288–292.

Valsamakis, G., McTernan, P.G., Chetty, R., Al Daghri, N., Field, A., Hanif, W., Barnett, A.H. and Kumar, S. (2004). Modest weight loss and reduction in waist circumstance after medical treatment are associated with favorable changes in serum adipocytokines. *Metabolism Research Journal*, 53:430–434.

Vernochet, C., Peres, S.B., Davis, K.E., McDonald, M.E., Qiang, L., Wang, H., Scherez, P.E. and Farmer, S.R. (2009). Genetic regulation of repression of selected visceral white adipose genes during the induction of the brown phenotype in white adipocytes by PPAR agonists. *Journal of Molecular Biology*, 10:1128-1139.

Vidhya, S. and Mohan, V. (2002). Rosiglitazone-useful drug has side effects. *Journal of the Association of Physicians India*. 50:615.

Virdi, J., Sivakami, S., Shahani, S., Suthar, A.C., Banavalikar, M.M. and Biyani, M.K. (2003), Antihyperglycemic effects of three extracts from *Momordica charantia*. *Journal of Ethnopharmacology*, 88(1):107–111.

Visarata, N., and Ungsurungsie, M. (1981), Extracts from *Momordica charantia*, *Journal of Pharmaceutical Biology*. 19(2-3):73-80.

Waako, P.J., Gumede, B., Smith, P., Folb, P.I. (2005). "The in vitro and in vivo antimalaria activity of cardiospermum halicacabum L. and Momordica foetida schumch. Et thonn". *Journal of Ethnopharmacology*, 99(1): 137-43.

Wakabayashi, K., Okamura, M., Tsutsumi, S., Nishikawa, N.S., Tanaka, T., Sakakibara, I., Kitakami, J., Ihara, S., Hashimoto, Y. and Hamakubo, T. (2009). The peroxisome proliferation-activated receptor gamma/retinoid X-receptor alpha heterodimer targets. *Journal of Molecular Biology*, 29:3544-3555.

Welihinda, J., Karunanayake, E.H., Sheriff, M.H. and Jayasinghe, H.S. (2002). Effect of *Momordica charantia* on the glucose tolerance in maturity onset diabetes. *Journal of Ethnopharmacol*, 17:277-282.

Wilson, C.B. (2006). Renal response to immunological injury. In Brenner, B.M., Rector, F. (eds): *The kidney*, 5th ed. Philadephia. W.B. Sauders, 1253-1391.

Wisse, E. (1970). An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *Journal of Ultrastructures Res*earch, 31:125.

Wisse, E., and Elias, H., (2008). An electron microscopic study of the fenestrated endothelial lining of the sinusoids of the liver of rat. *Journal of Cell Biology*, 51:126.

Yasui, Y. (2005). Bitter gourd seed fatty acid rich in 9c, 11t, 13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPARgamma in human colon cancer Caco-2 cells. Prostaglandins Leukot. *Essential Fatty Acids*. 73(2): 113-9.

Yobchok-Annu, S., Adisakwattana, S. and Yao, C.Y. (2006). Slow acting protein extract from fruit pulp of *Momordica charantia* with insulin secretagogue and insulinomimetic activities. *Biology and Pharmacology Bulletin*, 20: 112 – 113.

Zhang, H., Halbleib, M., Ahmad, F., Manganiello, V.C. and Greenberg, A. (2002). Tumor necrosis factor-a stimulates lipolysis in differentiated human Adipocyte through activation of extracellular signal-related kinase and elevation intracellular CAMP. *Journal of Diabetes*, 5:2929–2935.

APPENDIXI

Group A 125 mg/kg Body Weight

S/NO	RATS	1ST	2ND	3RD	4TH	5TH	6TH
1.	I →	243	238	232	228	229	222
2.	II →	251	241	239	240	240	233
3.	III →	244	233	231	232	231	229
4.	IIII →	256	252	252	248	239	240
5.	IIIII →	258	255	250	245	246	240

Appendix II

Group B 250 mg/kg Body Weight

	RATS	WEIGHTS/WEEK					
S/NO		**1ST**	**2ND**	**3RD**	**4TH**	**5TH**	**6TH**
1.	I →	218	203	200	201	201	200
2.	II →	251	248	244	241	238	236
3.	III →	230	225	223	222	222	221
4.	IIII →	251	245	243	242	240	249
5.	IIIII →	253	250	251	248	248	239

Appendix III

Group C 500 mg/kg Body Weight

WEIGHTS/WEEK							
S/NO	**RATS**	**1ST**	**2ND**	**3RD**	**4TH**	**5TH**	**6TH**
1.	I	258	250	253	248	248	247
2.	II	250	249	249	247	239	238
3.	III	244	244	238	231	236	233
4.	IIII	243	240	235	228	221	219
5.	IIIII	251	247	243	243	239	235

Appendix IV

Group D Control

S/NO	WEIGHTS/WEEK						
	RATS	**1ST**	**2ND**	**3RD**	**4TH**	**5TH**	**6TH**
1.	I	240	242	242	240	245	244
2.	II	260	263	264	262	264	264
3.	III	315	313	317	316	318	317
4.	IIII	283	283	287	288	288	289
5.	IIIII	227	229	224	227	228	229

Appendix V

Table 1 Haematological parameters for methanolic extract

Variable	**Group A (n=4) Mean±SEM**	**Group B (n=5) Mean±SEM**	**Group C (n=5) Mean±SEM**	**Group D (n=5) Mean±SEM**	**p**	**p**
WBC (X109/L	**7.48±1.528**	**7.66±1.287**	**10.48±1.714**	**9.34±1.428**	**0.027**	**□0.05**
RBC (X1012/L	4.70±0.512	4.97±0.347	5.182±0.42	5.732±0.36	0.38	>0.05
HGB (g/L	14.1±0.518	13.3±0.64	13.94±0.175	13.86±1.169	0.866	>0.05
HCT (%)	24.3±2.906	24.4±1.598	26.62±2.201	28.8±2.174	0.472	>0.05
MCV(FL)	48.8±1.357	49.3±0.435	51.52±1.291	50.3±1.415	0.425	>0.05
MCH(Pg)	34.8±4.527	27.3±2.625	27.56±2.246	24.1±1.59	0.111	>0.05
MCHC(g/L)	66.8±8.053	55.5±5.007	54.04±4.969	47.9±2.663	0.147	>0.05
PLT(X109/L)	**505.0±87.87**	**688.0±64.21**	**427.8±33.39**	**468.0±4.86**	**0.047**	**<0.05**
Lymp(%)	**72.5±4.101**	**64.9±3.06**	**68.56±4.368**	**81.36±1.7**	**0.024**	**<0.05**
Neut(%)	**22.4±1.988**	**31.2±2.715**	**29.62±3.95**	**16.88±1.067**	**0.005**	**<0.05**
Eosin(%)	2.58±0.392	3.38±0.64	1.62±0.551	1.76±.655	0.147	>0.05
Mono(%)	**0.52±0.224**	**0.26±0.26**	**0.20±0.20**	**0.00±0.00**	**0.034**	**□0.05**
RDW-CV(%)RDW-SD(FL)	17.8±0.879	16.8±0.593	16.12±0.403	15.5±0.266	0.071	>0.05
RDW-SD (fl)	28.2±1.491	28.1±3.53	28.6±1.123	25.48±1.048	0.318	>0.05
MPV(FL)	7.00 ±0. 27	7.12±0.412	7.94±0.32	7.9±0.235	0.117	>0.05
PDW(FL)	15.4±0.307	16.2±0.402	16.02±0.203	15.7±0.12	0.289	>0.05

Appendix VI

Biochemical parameters for methanolic extract

Variable	**Group A (5) Mean±SEM**	**Group B (5) Mean±SEM**	**Group C (5) Mean±SEM**	**Group D (5) Mean±SEM**	**P**	**p**
Na	136±1.183	136.6±1.122	132.8±2.478	134.1±1.183	0.412	>0.05
K	7.55±0.924	7.74±0.61	10.42±1.401	8.88±0.576	0.164	>0.05
Cl	96.5±0.188	103.6±1.208	101.2±2.01	108.8±2.853	0.287	>0.05
Ho3	17.0±2.55	18.6±1.288	14.6±1.166	14±1.924	0.233	>0.05
Urea	6.075±1.776	6.7±0.906	4.84±0.978	5.32±0.301	0.587	>0.05
Creatinine	**101.25±13.48**	**86.4±4.63**	**93.6±0.68**	**87.2±4.58**	**0.048**	**□0.05**
Ast	**128.75±22.27**	**89.8±11.60**	**98.6±9.11**	**97.8±7.51**	**0.022**	**□0.05**
Alt	**35±2.739**	**37.6±3.187**	**34±1.975**	**36.4±0.927**	**0.036**	**□0.05**
Alp	**37.5±7.03**	**45±9.576**	**44.8±4.641**	**37.4±7.312**	**0.008**	**□0.05**
Tp	8.35±0.556	8.36±0.387	8.12±0.568	7.96±0.16	0.897	>0.05
Alb	5.5±1.323	5.8±0.663	6.0±0.707	6.0±0.707	0.974	>0.05

Appendix IIX

TABLE OF ORGAN-BODY RATIO OF LIVER

Data source: Data 1 in Notebook 1

Dependent Variable: liver-body ratio

Normality Test: Passed (P = 0.616)

Equal Variance Test: Passed (P = 0.415)

Group Name	N	Missing	Mean	Std Dev	SEM
125 mg/Kg of Extract	4	0	4.476	0.967	0.483
250 mg/Kg of Extract	5	0	4.710	0.482	0.215
500 mg/Kg of Extract	4	0	4.217	0.612	0.306
Control	5	0	4.077	0.764	0.342

Source of Variation	DF	SS	MS	F	P
Between Groups	3	1.146	0.382	0.744	0.544
Residual	14	7.187	0.513		
Total	17	8.333			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.544).the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.090).

Appendix IX

ORGAN-BODY RATIO OF LEFT KIDNEY

Dependent Variable: left kidney-body ratio

Normality Test: Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.573)

Group Name	N	Missing	Mean	Std Dev	SEM
125 mg/Kg of Extract	4	0	0.368	0.0729	0.0364
250 mg/Kg of Extract	5	0	0.410	0.0804	0.0360
500 mg/Kg of Extract	4	0	0.437	0.0612	0.0306
Control	5	0	0.427	0.226	0.101

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.0114	0.00380	0.206	0.890
Residual	14	0.258	0.0184		
Total	17	0.269			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is no statistically significant difference (P = 0.890).

Appendix X

ORGAN-BODY RATIO OF RIGHT KIDNEY

Dependent Variable: right kidney-body ratio

Normality Test: Passed (P = 0.740)

Equal Variance Test: Passed (P = 0.901)

Group Name	N	Missing	Mean	Std Dev	SEM
125 mg/Kg of Extract	4	0	0.390	0.0727	0.0363
250 mg/Kg of Extract	5	0	0.427	0.0489	0.0219
500 mg/Kg of Extract	4	0	0.365	0.0570	0.0285
Control	5	0	0.396	0.0804	0.0360

Source of Variation	DF	SS	MS	F	P
Between Groups	3	0.00908	0.00303	0.694	0.571
Residual	14	0.0610	0.00436		
Total	17	0.0701			

The differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference (P = 0.571).

Appendix XI

Table 1: Organ-body weight ratio

Organ-body weight ratio	Control Mean±SEM (n-5)	125 mg/Kg of Extract Mean±SEM (n-4)	250 mg/Kg of Extract Mean±SEM (n-5)	500 mg/Kg of Extract Mean±SEM (n-4)
Liver-body	4.07±0.34	4.48±0.48	4.71±0.22	4.22±0.31
Left kidney-body	0.43±0.10	0.37±0.04	0.41±0.04	0.44±0.03
Right kidney-body	0.40±0.03	0.39±0.04	0.43±0.02	0.37±0.03

$p>0.05$ (Differences in all means not statistically significant for all organs)