HYPOGLYCEMIC AND HEPATO-PROTECTIVE EFFECTS OF HYDRO-ETHANOL SILK OF *Zea-Mays* EXTRACT ON ALLOXAN-INDUCED DIABETIC WISTAR RATS

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DECLARATION

I hereby declare that the research work reported in this dissertation entitled "**Hypoglycemic and Hepato-Protective Effects of Hydro-Ethanol Silk of *Zea-Mays* Extract on Alloxan-Induced Diabetic Wistar Rats**" was carried outby me in the Department of Human Physiology, Faculty of Medicine, A.B.U, Zaria, under the supervision of Prof.Aliyu Mohammed and Dr. Yusuf

Tanko. The information derived from the literatures has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree in any institution.

_____ _____

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CERTIFICATION

This dissertation entitled "**Hypoglycemic and Hepato-Protective Effects of Hydro-Ethanol Silk of *Zea-Mays* Extract on Alloxan-Induced Diabetic Wistar Rats**" byMAKAMA Nathan Yerima meet the regulation governing the award of Master of Science (M.Sc.) degree in Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of**Prof. Aliyu Mohammed** and **Dr. Tanko Yusuf**. It is therefore approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this research work to Almighty God for the strength and courage he bestowed on me to carry out this Study, and also to myfirst child, Ethan Nathan Yerima.

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TABLE OF CONTENTS

Title Page ----------i

Declaration ----------ii

Certification ----------iii

Dedication ----------iv

Acknowledgement ----------v

Table of Contents ----------vi

List of Tables ----------xi

List of Plates----------xii

List of Abbreviations----------xv

Abstract ----------xvi

CHAPTER ONE----------1

1.0 INTRODUCTION ----------1

1.1Backgroundof the Study----------1

1.2 Statement of Research Problem----------5

1.3 Justification of the Study----------5

1.4 Aim and Objectives----------5

1.4.1 Aim of the study----------5

1.4.2 Objectives of the study----------5

1.5 Research Hypothesis----------6

CHAPTER TWO----------7

2.0 LITERATURE REVIEW----------7

2.1 Definition of Diabetes Mellitus----------7

2.3. Classification of Diabetes Mellitus----------8

2.3.1 Type 1 diabetes mellitus----------9

2.3.2 Type 2 diabetes mellitus----------9

2.2.3. Gestational Diabetes Mellitus----------10

2.2.4 Other Specific Types Diabetes----------11

2.3. Pathophysiology of Diabetes Mellitus----------12

2.3.1 Impaired Glucose Tolerance and Impaired Fasting Glucose----------16

2.4 Diagnostic Criteria for Diabetes Mellitus----------16

2.5 Complications of Diabetes Mellitus----------17

2.5.1 Acute complication of diabetes mellitus----------17

2.5.2 Chronic Complication of Diabetes of Mellitus----------19

2.6 Managements of Diabetes Mellitus----------26

2.6.1 Life Style Modification----------26

2.6.2 Drugs Used in the Management of Diabetes Mellitus----------28

2.6.3 Herbs and Plants----------31

2.7 The Pancreas----------35

2.7.1 Embryonic development of the pancreas----------35

2.7.2 Endocrine pancreas----------36

2.7.3 Cell types in the pancreatic islet----------37

2.7.4 The Role of pancreatic development in human diabetes----------38

2.8 The liver----------41

2.8.1 The embryonic development of the liver----------41

2.8.2 Microscopic Structure and Functional Activity of the Liver----------42

2.8.3 Blood supply to the Liver----------43

2.8.3 Lobules of the Liver----------43

CHAPTER THREE----------44

3.0 MATERIALS AND METHODS----------44

3.1 MATERIALS ----------44

3.1.1 Plants----------44

3.1.2 Experimental Animals----------44

3.1.3 Chemicals----------44

3.1.4 Other Materials----------44

3.2 METHODS----------45

3.1.5 Weighing----------45

3.2.1 Preparation of Extract----------45

3.2.2Acute toxicity study----------45

3.2.3 Induction of Diabetes mellitus----------47

3.2.4 Experimental Design----------47

3.2.5 Determination of Blood Glucose Levels----------48

3.2.7 Morphological Studies----------48

3.3 Histopathological Analysis----------49

3.3.1 H and E staining method----------49

3.4 Biochemical Studies----------49

3.4.1 Estimation of Alanine amino-transferase (ALT) ----------50

3.4.2 Estimation of Aspartate amino-transferase (AST) ----------50

3.4.3 Estimation of Alkaline phosphates (ALP) ----------51

3.5. Statistical Analysis ----------51

CAPTER FOUR----------52

4.0 RESULTS----------52

4.1 Acute Toxicity Study ----------52

4.2 Effect of aqueous extract of Corn Silk on body weight----------53

4.3 Efffect of aqueous extract of Corn Silk on the Fasting Glucose concentration of normoglycaemic rats----------54

4.4 Effect of aqueous extract of Corn Silk on serum LFT----------56

4.5 Hispathological studies of the pancreas----------58

4.6 Histopathology of the Liver----------64

CHAPTER FIVE----------70

5.1 Discussions----------70

5.2 Acute Toxicity Study----------71

5.3 Body weight----------71

5.4 Blood Glucose Levels----------71

5.5 Liver Function Test----------72

5.6 Histopathology of Pancreas----------73

5.7 Histopathology of the Liver----------74

CHAPTER SIX----------75

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS----------75

6.1 Summary----------75

6.2 Conclusion----------76

6.3 Recommendations----------76

Reference----------78

LIST OF TABLES

Table 3.1: Experimental Design ----------58

Table 4.1: Observation in phase I and II of the acute toxicity study following single Oral administration of aqueous extract of Corn Silk in adult Wistar rats**.** ----------52

Table 4.2: Changes in Body Weight of the Wistar rats on Alloxan induced Diabetes on experimental rats treated with Corn Silk ----------53

Table 4.3: Plasma glucose concentrations (mg/dL) on Alloxan induced Diabetes on experimental rats treated with Hydro-ethanoliccorn silk extract.----------55

Table4.4: Activities of enzymes (ALP, ALT and AST) of the liver of alloxan induced Diabetes on experimental rats treated with Hydro-ethanolic Corn Silk extract.----------67

LIST OF PLATES

Plate 1.1: Corn Plant showing corn silk----------34

Plate 2.1: The pancreas and associated pancreatic islets----------40

Plate 3.1: Experimental design for Acute Toxicity Study----------47

Plate I: A section of the pancreas of the untreated experimental animal (Wistar rat) showing the normal architectureof the pancreas with the beta cells in islet of langerhans (I) (H and E X250)----------59

Plate II: A section of the pancreas of experimental group administred with 60mg/kg of Alloxan without treatment showing severe degranulation of the beta cells with Eosinophilic material in islet of langerhans (I) (H and E X250).----------60

Plate III: A section of the pancreas of the experimental group treated with Alloxan + Corn Silk 0.6 g/kg showing regeneration of beta cells in islet of Langerhans (H and E X250)----------61

Plate IV: A section of the pancreas of the experimental group treated withAlloxan + Corn Silk 0.8 g/kg showing regeneration of beta cells in islet of Langerhans (H and E X250) ----------62

Plate V: A section of the pancreas treated with Alloxan + Gliber group showing the necrotic architecture of the Islet of Langerhans with slight regeneration of beta cells in isletof langerhans (I) (H and E X250). ----------63

Plate VI: Photomicrograph section the control liver tissue showing normal histological appearance of the liver with normal Central Vein (CV), and Hepartocyte (H) ----------65

Plate VII: Photomicrograph of the Diabetic Control group showing the presence of both Microvascular (N) and Macrovascular (S)

Fat droplet. ----------66

Plate VIII: Section of Liver fromAlloxan induced diabetic rat treated with Corn Silk (0.6 g/kg bw) showing Restored liver cytoarchitecture of the liver with and lose membrane of the central vein (CV) (H and E Stain X 250)----------67

Plate IX: Section of Liver from Alloxan induced diabetic rat treated with Corn Silk (0.6 g/kg bw) showing Restored liver cytoarchitecture, lose membrane of the central vein (CV) (H and E Stain X 250).----------68

Plate X: Section of Liver from Alloxan induced diabetic rat treated with Glibenclamide (20mg/kg bw) showing Restored liver cytoarchitecture with fat droplet degeneration (arrow head) and membrane of the central vein (CV) (H and E Stain X 250). ----------69

LIST OF ABBREVIATION

AGEs: Advanced Glycation End Products

ALP: Alkaline Phosphates

ALT: Alanine Amino-Transferase

APUD: Amine Precursor Uptake and Decarboxylation

AST: Aspartate Amino-Transferase

CAD: Coronary Artery Disease

CHF: Congestive Heart Failure

CIDP: Co-existing Chronic Inflammatory Demyelinating Polyneuropathy

cIMT: Carotid intima – media thickness

CVD: Cerebral Vascular Disease

DCCT: Diabetes Control and Complications Trial.

DKA: Ketoacidosis.

DM: Diabetes Mellitus

EDIC: Epidemiology of Diabetes Interventions and Complications Study

EHBD: Extrahepatic Bile Ducts

ESRD: Erythrocyte Sedimentation Rate Disorder

ET: Endothelin

FFA: Free Fatty Acid

cIMT: CaroticIntima Media Thickness

GDM: Gestational Diabetes Mellitus

GLUT-4:	Glucose Transporter 2
IDDM:	Insulin Dependent Diabetes Mellitus
IFG:	Impaired Fasting Glucose
IGT:	Impaired Glucose Tolerance
IHBD:	Intrahepatic Bile Ducts.
IMT:	Intima Media Thickness
MI:	Myocardial Infarction
MODY:	Maturity Onset Diabetes of the Young.
NCDs:	Non Communicable Diseases
PAD:	Pulmonary Artery Disease
PVD:	Peripheral Vascular
RAS:	Rennin Angiotensin System
STM:	Septum Transversum Mesenchyme
T1DM:	Type 1 Diabetes Mellitus
T2DM:	Type 2 Diabetes Mellitus
TNF:	Tumor Necrosis Factor
TRAIL:	Related Apoptosis Inducing ligand.
UKPDS:	United Kingdom Prospective Diabetes Study
WHO:	World Health Organization

ABSTRACT

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases The management of diabetes without side effects is a major challenge in the medical system.Silkof *Zea mays*from a variety of maize sources has been shown to have a range of biological activities and potential health benefits such as hypoglycemia, antioxidant, hypocholesteromic and hepatoprotective activities. The current research is aimed at evaluating theHypoglycemic and Hepato-Protective Effects of Hydro-Ethanol Extract of *Zeamays*on Alloxan-Induced Diabetic Wistar Rats. Twenty Five adult Wistar rats of both sexes were used, with average weight of 150gms and they were randomly divided into five groups. The animals in groups II, III, IV and V were exposed to 160 mg/kg per body weight of the Alloxanintraperitoneally to induce diabetes mellitus; only those rats with blood glucose above 200 mg dl^{-1} were selected for the study. After induction of diabetes, animals of group II, III, IV and V received *Zea mays*silk extract (0.6 g/kg, 0.8 g kg^{-1}) and Glibenclamide (1mg/kg) orally for 14 days respectively, while group I served as control and were administered with normal saline. The therapeutic effects of *Zea mays* on blood glucose, liver function test (ALT, AST, and ALP) were assayed including the histological architecture of the pancreas and liver.The diabetic control group was characterized by a significant Increased ($P \leq 0.05$)in Glucose concentration and increased in the liver function test (ALT, AST, and ALP). This present work indicates that hydro- ethanol extract of*Zea mays*silkpossesses significant hypoglycaemic and hepato-protective activity in vivo by reducing liver function enzymes, initiate regeneration of beta cell in the

pancreas and restore the cyto-architecture of the liver in diabetes induced adult wistar rats within the 14 days of treatment.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Diabetes mellitus (DM) is a chronic metabolic disorder in which blood sugar (glucose) levels are abnormally high (hyperglycemia), due to insufficient production or inappropriate metabolism of insulin (Shoback, 2011). It is a chronic non-communicable disease which generally starts insidiously over a period of long time, and even in the absence of symptoms, hence called as a silent killer. Many individual are accidentally detected as a case of DM when they are investigated for some other reasons like preoperative investigation. It is characterized by a state of hyperglycemia (high blood sugar level) due to insulin deficiency. Insulin is an essential hormone produced by the beta cells of Langerhans of pancreas. It is required for metabolism of glucose; in the absence of insulin, the body cannot metabolize glucose hence it cannot be utilized for body functions leading to a state of chronic hyperglycemia. If this hyperglycemia is not treated in due time it can lead to serious consequences on body like damage nerve (neuritis) and blood vessels (micro-angiopathies and atherosclerosis) (Sampatti, 2016).

In the year 2012, DM was a direct cause of death of 1.5 million people and most of them (80%) belong to low and middle income countries. Asian countries contribute to more than 60% of world's diabetic burden (Shoback, 2011). The prevalence of DM is expected to rise from 285 million in the year 2010 to 438 million in 2030. WHO projects that DM will be the 7th leading cause of death by 2030 (Unwin *et al.,* 2009).

There are two major forms of DM, type 1 DM also previously called Insulin dependent DM (IDDM) where a person is unable to produce insulin. So they require exogenous insulin

injections for life long to survive. Type 2 DM, previously known as Non-Insulin dependent DM (NIDDM), where person is able to produce insulin but not adequate to meet body demands. It is the most common form of DM and the common risk factors attributed to NIDDM are overweight/obesity and sedentary life styles (WHO, 2012). World Health Organization has recently announced that Diabetes Mellitus (Type 2) is a preventable disease with simple life style modifications like diet and regular physical exercise. Diabeties Mellitus (DM) can be controlled and complications can be prevented with early diagnosis and prompt treatment. Early initiation of mass activities is important to prevent the diabetes in order to achieve global sustainable development goal 3 target of prevention of premature deaths and disabilities due to chronic non communicable diseases (NCDs) by one third by 2030 (Unwin *et al.*, 2009).

All sectors of society have a role to play, including governments, employers, educators, manufacturers, civil societies, private sector, the mass media and individuals themselves in this mission of prevention of DM.

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency (Lambert, 2002). This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell mediated autoimmune attack (Rother, 2007). There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe (Rother, 2007).

Type 2 Diabetes Mellitus DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency. This form was

previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (Shoback, 2011), The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor(Seidell, 2000).

Gestational Diabetes occurs when pregnant women without a previous diagnosis of diabetes develop a high blood glucose level. It may precede development of type 2 DM. Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in life (Lawrence *et al*., 2008).Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes (Sattar, 2010).

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia. The classic symptoms of untreated diabetes are loss of weight, polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Cooke, 2008). Symptoms may develop rapidly (weeks or months) in type 1 diabetes; while they usually develop much more slowly may be subtle or even absent in type 2 diabetes.Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. Blurred vision is a common complaint leading to a diabetes diagnosis. A

number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes(Saydah, 2001).

This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) (Wild *et al.*, 2004).All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20). The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease(ERFC, 2010). Diabetes also damages the capillaries (causes microangiopathy) (Boussageon *et al.*, 2011).Diabetic retinopathy, which affects blood vessel formation in the retina of the eye, can lead to visual symptoms including reduced vision and potentially blindness. Diabetic nephropathy, the impact of diabetes on the kidneys, can lead to scarring changes in the kidney, loss of small or progressively larger amounts of protein in the urine, and eventually chronic kidney disease requiring dialysis(Narayan*et al*., 1990). Another risk is diabetic neuropathy, the impact of diabetes on the nervous system - most commonly causing numbness, tingling and pain in the feet, and also increasing the risk of skin damage due to altered sensation (Cukierman, 2005).

Corn silk (*ZeamaysL.*) refers to the stigmas from the female flowers of maize. Fresh corn silk resembles soft silk threads 10-20 cm long that are either light green or yellow-brown in color. Corn silk contains proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids (Abdel-Wahab, 2002). There have been many reports on the biological activities of corn silk constituents. Methanol extracts of corn silk showed an antioxidative activity on the level of lipid peroxidation. Volatiles from corn silk inhibited the growth of Aspergillus flavus, indicating that it has an antifungal activity (Ziringue, 2000).

1.2Statement of Research Problem

Globally, as of 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double (Wild *et al*., 2004).The management of diabetes without side effects is a major challenge in the medical system.

1.2 Justificationof the Study

Corn silk has been used specifically in China as an antidiabetic agent. Research has been attempted in china on its effects on glyceamic metabolism(Jianyou *et al*.,2009). Exploring the antidiabetic effect of corn silk of maize specie in our locality will help elucidate and established corn silk as a potential antidiabetic agent.

1.4 Aim and Objectives

1.4.1 Aim of the study.

To evaluate the effect of hydroethanolic corn silk extract on blood glucose level, and its liver enzymes nature on alloxan induced diabetic wistar rats.

1.4.2 Objectives of the study

i. To determine the mean lethal dose (LD_{50}) by acute toxicity studies of hydroethanolic corn silk extract.
ii. To evaluate the effects of the extract on blood glucose levels of alloxan induced diabetic wistar rats.
iii. To evaluate the effects of the hydroethanolic corn silk extract on liver enzymes such as Alkaline phosphatase, Alanine aminotransferase and Aspartate aminotransferase of the treated model.

iv. Histopathological studies of Liver and Pancreas of alloxan-induced hyperglycemic models treated with hydroethanolic extract of corn silk, standard drug and normal saline.

1.5 Research Hypothesis.

i. The hydroethanolic extract of Corn silkpossesses hypoglycemic effect on alloxan induced diabetic wistar rats.

ii. The hydroethanolic extract of Corn silk does not possess hypoglycemic effect on alloxan induced diabetic wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder in which blood sugar (glucose) levels are abnormally high (hyperglycemia), due to insufficient production or inappropriate metabolism of insulin. Fasting plasma glucose levels of more than 126 mg/dl on two or more tests on different days indicate diabetes, a random blood glucose level of 200 mg/dl or higher also indicates diabetes (WHO, 1999). The early symptoms of untreated diabetes mellitus are related to elevated blood sugar levels, and excretion of it in the urine causing dehydration. This in turn causes increased thirst and water consumption. The other common symptoms include fatigue, blurred vision, nausea, vomiting, numbness of hands and foot etc (WHO, 1994) the worldwide survey reported that diabetes mellitus (DM) is affecting nearly 10% of the world's population (siddharth, 2001).

Diabetes Mellitus have been projected as the world's main disabler and killer in the next 25 years (Edwin *et al.,* 2006). According to the international diabetes federation, more than one fifth of Indians are diabetic. Therefore, India has been declared as "diabetic capital of the world" at the recent conference in Paris (Murugan and Reddy, 2009), diabetes mellitus is a major cause of disability and hospitalization and its results in significant financial burden (vats *et al.,* 2002).

DM is a serious illness with multiple complications and premature mortality, accounting for at least 10% of the total health care expenditure in many countries (king *et al,* 1998) and is likely to remain a significant threat to public health in the years to come (marx, 2002).

Diabetes mellitus is rated one of the top five most significant diseases in the developed world, and the third "killer" of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality (Li *etal.*, 2004). Despite the considerable progress in the understanding and management of diabetes, with type 2 making up about 90% of the cases. Its incidence is increasing rapidly, and by 2030, this number is estimated to almost double, diabetes mellitus occurs throughout the world, but is most common (especially type 2) in the more developed countries, the greatest increase in prevalence is, however, expected to occur in incidence in developing countriesfollowing the trend of urbanization and lifestyle changes, perhaps most importantly a "western style" diet. This has suggested an environmental (i.e dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation (Wild, *et al*., 20014).

Over time, diabetes can lead to blindness, kidney failure, and nerve damages. These types of damages are the result of damages to small vessels, referred to as microvascular disease (boussageon,*et al*., 2011). It is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to stroke and coronary heart disease. This is referred to as macrovascular disease (Porter and Barrett, 2004). Fasting blood sugar levels should never be higher than 126mg/dl. Hemoglobin A_1C (also called Glycosylated hemoglobin) can be used to diagnose diabetes (Li, *et al.*, 2004).

2.2 Classification of Diabetes Mellitus

The American diabetes association revised the nomenclature for the major types of diabetes into Type 1 (DM 1), Type 2 (DM 2), gestational and other specific types of diabetes. This classification of diabetes is based on etiology(Joao, 2000). The term "diabetes" without qualification, usually refers to diabetes mellitus. The rare disease diabetes insipidus has

similar symptoms as diabetes mellitus, but without disturbances in the sugar metabolism (Pittas and Greenberg, 2003).

2.2.1 Type1 Diabetes Mellitus

Type 1 diabetes mellitus is also referred to insulin dependent diabetes mellitus, childhood – onset diabetes mellitus and juvenile diabetes mellitus.Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children. It is characterized by loss of insulin- producing beta cells of the islets of langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified into immune mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack (Rother, 2007). There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages.

Since it is common among young individuals and insulin hormone is used for treatment. Type 1 diabetes is also referred to as insulin dependent diabetes mellitus (IDDM) or Juvenile Diabetes (Rother, 2007).

2.2.2. Type 2 Diabetes Mellitus.

Type 2 diabetes is the most prevalent form of diabetes, accounting for over 90% of all cases of diabetes (Wild *et al*., 2004).This is referred to adult –onset diabetes, obesity related diabetes, and non insulin dependent diabetes mellitus. It is characterize by insulin resistance which may be combined with relatively reduced insulin secretion. Typically, they do not require insulin to survive but often will eventually need insulin to maintain reasonable

glycemic control, often after many years. The rate of basal hepatic glucose production is excessive, despite plasma insulin concentration that are increased two-fold to four-fold (DeFronzo *et al.,* 1989). These findings provide unequivocal evidence for hepatic resistance to insulin, and this evidence is substantiated by an impaired ability of insulin to suppress hepatic glucose production. Accelerated gluconeogenesis is the major abnormality responsible for the increased rate of basal hepatic glucose production. The increased rate of basal hepatic glucose production is closely correlated with the increase in fasting plasma glucose level (Magnusson *et al.,* 1992). Muscle tissue in patients with type 2 diabetes is resistant to insulin (Bonadonna *et al.,* 1990). Defects in insulin receptor function, insulin receptor-signal transduction pathway, glucose transport and phosphorylation, glucose synthesis and glucose oxidation contribute to muscle insulin resistance. In response to a meal, the ability of endogenous secreted insulin to augment muscle glucose uptake is markedly impaired (Mitrakou *et al.,* 1990). Muscle insulin resistance and impaired suppression of hepatic glucose production contribute almost equally to the excessive postprandial increase in plasma glucose level (Ferrannini *et al.,* 1988) The non responsiveness of the body tissues to insulin is mostly involving receptors insufficient. In type 2 diabetes the predominant abnormally is reduced insulin sensitivity and one of the earlier signs most sustained manifestation is hyperglycemia, which can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver(Richard, 2010).

2.2.3. Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes

is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in life(Wild *et al*., 2004).

Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia(Richard, 2010).

A 2008 study completed in the U.S. found the number of American women entering pregnancy with pre-existing diabetes is increasing(Rother, 2007). In fact, the rate of diabetes in expectant mothers has more than doubled in the past six years(Rother, 2007). This is particularly problematic as diabetes raises the risk of complications during pregnancy, as well as increasing the potential for the children of diabetic mothers to become diabetic in the future (Jennifer , 2007; David and Gardner, 2011).

2.2.4 Other Specific Types Diabetes

This type of diabetes is secondary to manifestation of a primary pathological or disease condition. Depending on the etiology, this includes:

i. Maturity onset diabetes of the young (MODY): Currently 6 monogenetic defects of beta cell function defined with defects in islet cell glucokinase or in various

transcriptions factors such as HNF-1 alpha, HNF-4 alpha. IPF1. The end result is impaired insulin release and hyperglycemia. Autosomal dominant pattern. Onset of hyperglycemia generally before age 25(Richard, 2010).

ii. Genetic defects in insulin action:This is cause by mutant insulin gene. Insulin exhibits impaired receptor binding, and mutation of insulin receptor. Often associated with acanthosis nigricans (thickening and discoloration of skin) and some forms of polycystic ovarian syndrome (uncommon)(Richard, 2010).

iii. Disease of the exocrine pancreas; There must be extensive damage to pancreas for diabetes to occur, causes includes trauma, infection, chronic nectrotizing pancreatitis, pancreatic carcinoma, cystic fibrosis and hemochromatosis (Richard, 2010),

iv. Endocrinopathics:Includes acromegaly, cushing's syndrome, glucagonoma and pheochromocytoma, caused by excess secretion of hormones which antagonize insulin including growth hormone, cortisol, glucagon and epinephrine (Pittas and Greenberg, 2003).

v. Infections:Congenital rubella is the most common virus implicated in the development of diabetes coxsackievirus B, adenovirus, mumps and cytomegalovirus have all been implicated in inducing certain cases of the disease (Pittas and Greenberg, 2003).

vi. Drug/chemical induced diabetes; Many drugs may impair insulin resistance or insulin secretion leading to diabetes in predisposed individuals(Pittas and Greenberg, 2003).

2.3. Pathophysiology of Diabetes Mellitus

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscles and fat cells, but not central nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of

diabetes mellitus. Humans are capable of digesting some carbohydrates, in particular those most common food, starch and some disaccharides such as sucrose, are converted within a few hours to simpler forms, most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body. The rest are passed on for processing by gut flora largely in the colon. Insulin is released into the blood by beta cells (β-cells), found in the islets of langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules or for storage (Harris, *et al.*, 1995).

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Lower glucose levels result both in the reduced release of insulin from the β-cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin. Glucose thus forcibly produced from internal liver cell stores (as glycogen) re-enters the bloodstream; muscle cells lack the necessary export mechanism. Normally, liver cells do this when the level of insulin is low (which normally correlate with low level of blood glucose) (Reaven, 1998). Higher insulin levels increase some anabolic (building up) processes, such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and vice versa. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat-burning metabolic phase). If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not

have its usual effect, so it will not be absorbed properly by those body cells that require it, nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels blood glucose, poor protein synthesis, and other metabolic derangements such as acidosis (Olefsky, 1989; Ningthoujam and Sandhyarani, 2009).

In type 1 diabetes the pancreas cannot synthesize enough amount of insulin hormone as required by the body. The pathophysiology of type 1 diabetes mellitus suggests that it is an autoimmune disease. In which the body's own immune system generates secretion of substances that attack the beta cells of the pancreas. Consequently, the pancreas secretes little or no insulin (Fox *et al.,* 2004), the autoimmune beta cell destruction is thought to be triggered by an environmental event, such as a viral infection. Genetically determined susceptibility factors increase the risk of such autoimmune phenomena, the onset of type1 diabetes mellitus is usually abrupt. It generally occurs before the age of 30years, but may be diagnosed at any age. Type 1 diabetic patient is absolutely dependent on exogenously administered insulin for survivals are highly susceptile to diabetes ketoacidosis. Because the pancreas produces no insulin, glucose cannot enter cells and remains in the bloodstream. To meet cellular energy needs, fat is broken down through lipolysis, releasing glycerol and free fatty acids. Glycerol is converted to glucose for cellular use. Fatty acids are converted to ketone, resulting in increased ketone levels in body fluids and decreased hydrogen ion concentration (pH). ketones are excreted in the urine, accompanied by large amounts of water. The accumulation of ketones in body fluids decreases pH, electrolyte loss and dehydration from excessive urination, and alterations in the bicarbonate buffer system result in diabetic ketoacidosis (DKA). Untreated DKA can result in coma or death (Brian, 2006). In type

2diabetes mellitus, the underlying pathophysiologic defect does not involve autoimmune beta-cell destruction. Rather is characterized by the following three disorders:

i. Peripheral resistance to insulin, especially in muscle cells:
ii. Increased production of glucose by the liver and,
iii. Altered pancreatic insulin secretion.

Increased tissue resistance to insulin generally occurs first and is eventually followed by impaired insulin secretion. The pancreas produces insulin, yet insulin resistance prevents its proper use at the cellular level. Glucose cannot enter target cells and accumulates in the bloodstream, resulting in hyperglycemia (Brian, 2006). In essence, there is normal production of insulin hormone but the body cells are resistant to insulin. Since the body cells and the tissue are non responsive to insulin, glucose remains in the bloodstream (Eriksson, *et al.*, 2003). Although the genetic predisposition to type 2 diabetes is strong, no single genetic defects have been found. In addition to genetic influences, acquired risk factors for type 2 diabetes include obesity, advancing age, and an inactive lifestyle. Type 2 diabetes usually has a slow onset and may remain undiagnosed for years. Approximately half of those who have type 2 diabetes are unaware of their disease. Unfortunately, the insidious nature of the disease allows prolonged periods of hyperglycemia to begin exerting negative effects on major organ system(Richard, 2010).

While in gestational DM the case is different, during early pregnancy, increases in estrogens, progestin and other pregnancy related hormones lead to lower glucose levels, promotion of fat deposition, delayed gastric emptying, and increased appetite. As gestation progresses, however, postprandial glucose levels steadily increase as insulin sensitivity steadily decreases.

For glucose control to be maintained in pregnancy it is necessary for maternal insulin secretion to increase sufficiently to counteract the fall in insulin sensitivity. GMD occurs when there is insufficient insulin secretion to counteract the pregnancy related decrease in insulin sensitivity. Therefore the pathophysiology of gestational diabetes is associated with increased insulin resistance. Most patients with gestational diabetes return to a normoglycemic state after parturition. However, about 30% to 50% of women with history of gestational diabetes will develop type 2 diabetes within 10 years (Brian, 2006).

2.3.1 Impaired Glucose Tolerance and Impaired Fasting Glucose

The conditions know as impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) represent metabolic states lying between diabetes and normoglycemia. People with IFG have increased fasting blood glucose levels but usually have normal levels following food consumption. Those withIGT are normoglycemic most of the time but can become hyperglycemic after large glucose loads. IGT and IFG are not considered to be clinical entities; rather, they are risk factors for future diabetes. The pathophysiology of IFG and IGT is related primarily to increase insulin resistance where as endogenous insulin secretion is normal in most patients. Approximately 30 to 40% of individuals with IGT or IFG will develop type 2 diabetes within 10 years after onset (Brian, 2006).

2.4 Diagnostic Criteria for Diabetes Mellitus

Diabetes mellitus is common characterized by persistent hyperglycemia. The physiological blood glucose levels of a healthy man are 60mg/dl to 110mg/dl fasting glucose and 80mg/dl to 160 mg/dl in the postprandial state (Selvin, 2010).

2.5 Complications of Diabetes Mellitus

The ultimate outcome for all three types of diabetes mellitus is high blood glucose level or hyperglycemia. The disease is characterized by different etiologies but share similar signs, symptoms and complications. Over a period of time, high glucose level in the bloodstream can lead to severe complications. All forms of diabetes increase the risk of long term complications. These typically develop after many years (10- 20), but may be the first symptom in those who have otherwise not received a diagnosis before that time(WHO, 2006).

2.5.1 Some Short-term complication of diabetes mellitus

2.5.1.1 Ketoacidosis

Diabetic ketoacidosis (DKA) is a life-threatening complication of type 1 Diabetes caused by an absolute deficiency of insulin and often revealing the diagnosis (Diabetes Care, 2004). Diabetic ketoacidosis (DKA) is also frequently observed in patients with pre-existing Type 1 Diabetes due to inter-current infections, ischemia, or inappropriate adherence to treatment. This risk may be aggravated when patients are inclined to postpone insulin injections intending to avoid hypoglycemia when they are not eating. Importantly, nausea and vomiting may also be the first symptoms DKA. In addition, patients with DKA typically present with hyperventilation due to respiratory compensation of metabolic acidosis, severe dehydration, and coma, but DKA may also mimic acute abdomen. Laboratory tests reveal a high plasma glucose level and metabolic acidosis, with either increased or decreased potassium values. The treatment of ketoacidosis consists of rehydration with saline and intravenous insulin substation to suppress ketogenesis. Major electrolyte shifts occur because of rehydration and recovering from acidosis, therefore potassium and other electrolytes should be closely monitored and substituted if necessary (Geert *et al*., 2007).

2.5.1.2 Hypoglycemia

Hypoglycemia is a common problem in type 1Diabetes due to relatively overdosing of insulin in combinations with underestimating the energy expenditure of physical exercise. It may also be related to concomitant medications such as the use of (nonselective) β-blocking agents. Besides the risk of hypoglycemic coma and (very seldom) death, fear of hypoglycemia restrains patients to strive for optimal glycemic control. The risk of hypoglycemia increases with improved glycemic control, autonomic neuropathy, longer duration of diabetes, and the presence of long-term complications (TerBraak *et al*., 2000).

Hypoglycemia is defined as an event with a glucose concentration below 3.9 mmol /l, with symtoms that recovers after glucose normalization. Severe hypoglycemia is defined as any hypoglycemic event requiring help from another person to recover and may be complicated by seizure or coma (Diabetes care, 2005). Classical autonomous warning symptoms include sweating, palpitations, anxiety, hunger, and tremor. When plasma glucose concentration decreases further symptoms can progress toward neuro-glycopenic symptoms consisting of drowsing, paralysis, and cognitive dysfunction precluding appropriate self-management by glucose ingestion. When hypoglycemia remains untreated and blood glucose concentrations further decrease, this ultimately results in coma and/or convulsion. During hypoglycemia the cardiac QT interval is lengthened by sympathoadrenal stimulation directly on the myocardium and decrease serum potassium; this supposedly contributes to (fatal) cardiac arrhythmia during hypoglycemia (Robinson *et al*., 2003; Murphy *et al*., 2004).

2.5.2 Long-Term Complication of Diabetes of Mellitus

Long-term complications in type 1 diabetic patients are traditionally divided into microvascular complications, comprising retinopathy, nephropathy, and neuropathy, and macrovascular damage, including peripheral vascular (PVD), coronary artery disease (CAD), and cerebral vascular disease (CVD). Moreover, musculoskeletal complications and the diabetes foot are clinical entities that are multicausal and/or for which causes are not yet fully understood. Long-term complications are prevalent in any population of type 1 diabetic patients with increasing prevalence and severity in relation to disease duration and glycemic control and other risk factors (Michael, 2005).

The pathogenesis of long term diabetic complications is multi-factorial, complicated, and not yet fully elucidated. In brief, focusing on the role of hyperglycemia, so-called advanced glycation end products (AGEs) and sorbitol are considered to contribute to tissue damage, like protein kinase C in the kidney (Michael, 2005).

2.5.2.1 Microvascular complications

Diabetic microvascular complications are caused by prolonged exposure to high glucose levels. This has been established by large-scale prospective studies for both type 1 Diabetes (T1DM) by the Diabetes control and complications Trial/Epidemiology of Diabetes Interventions and complications study (DCCT/EDIC, 1993) and for type 2 diabetes (T2DM)] (UKPDS, 1998; Murphy *et al*., 2004).

Retinopathy: Diabetic retinopathy is the most widespread cause of visual loss in the Western world and probably the most feared complication of diabetes. It is caused by microangiopathic lesions of the retinal precapillary arterioles, capillaries, and venules. Damage is caused both by microvascular leakage from breakdown of the inner blood-retinal barrier and by microvascular occlusion. Prevalence of retinopathy at 8-10 years after the onset

of diabetes in more recent reports varies between 30 and 60%. This is lower than may be expected from older cohort studies. Data supports the conclusion that this may be attributed to better levels of glycemic control that RE achieved during the last decades (Lecaire *et al*., 2006). It was already known from previous intervention studies that the development and progression of diabetic retinopathy in TID can be prevented by better glycemic control (DCCT, 1993). An important observation from these trials is that retinopathy may actually worsen during the first year of tightened glycemic control. This observation is even more relevant with respect to women planning to become pregnant; this usually calls for intensified insulin treatment in order to achieve adequate glycemic control before conception (Murphy *et al*., 2004).

Although the risk of progression during pregnancy is increased in women with the highest initial HbAlc values and in those with the greatest reduction in HbAlc values, probably factors related to pregnancy per se also contribute. Apart from glycemic control other factors that increase the risk of, or are associated with, retinopathy include diabetes duration, albuminuria and nephropathy, and hypertension. Lowering blood pressure has been shown to decrease the progression of retinopathy in type 2 diabetes. Epidemiological data suggest that the same is probably true for Type 1 Diabetes (TID). Several systems for the classification of the severity of diabetic retinopathy and diabetic macular edema exist. An international group of experts reached consensus about a five-stage disease severity classification that was developed for clinical purposes aiming to improve screening of people with diabetes and in order to facilitate proper communication and discussion among health care providers (Wilkinson *et al*., 2008).

Diabetic Neuropathy: The ultimate consequences of diabetic neuropathy include such grave conditions as impaired mobility, limb amputation, and progressive deformities particularly the feet. Diabetic neuropathies are heterogeneous with diverse clinical manifestations including symmetric polyneuropathy, autonomic neuropathy, radiculopathies, mononeuropathies, and mononeuropathy multiplex. However, not all peripheral neuropathy in diabetes patients is caused by diabetes (Boulton *et al*., 2005). Attention should be paid to other potential causes of neuropathy, such as monoclonal gammopathy, folic acid or vitamin B_{12}deficiency, extensive use of alcohol, and co-existing chronic inflammatory demyelinating polyneuropathy (CIDP). Diabetic polyneuropathy is primarily a symmetrical sensory neuropathy that gradually causes the typical "stocking-glove" sensory loss at progression. Various staging systems for use in research and for clinical purposes exist. Diabetes is the most common cause of neuropathic (Charcot) arthopathy in the western world. A warm, swollen foot in a diabetic patient with long-standing neuropathy without local or systemic signs of infection is consistent with charcot's disease and calls for abstinence from putting weight on the foot until symptoms subside(WHO, 2006).

Major clinical manifestation of diabetic autonomic neuropathy include resting tachycardia, orthostatic hypotension, disturbances of gastrointestinal motility, erectile dysfunction, sudomotor dysfunction, impaired neurovascular function, and hypoglycemic autonomic failure resulting in impaired glucose counter-regulation in case of hypoglycemia. Bladder dysfunction may affect renal function and should be distinguished from worsening nephropathy. Referral to an urologist is warranted to assess the potential need for intermittent self-catheterization (Geet *et al*., 2006).

Nephropathy: In up to 40% of type 1 diabetic patients, diabetic nephropathy may ultimately result in end-stage renal disease (ESRD). ESRD necessitates renal function replacement by hemodialysis, peritoneal dialysis, or renal transplantation if possible. Early stage of nephropathy tend to become clinical detectable about 10-12 years after diagnosis. Annual screening for urinary albumin excretion and monitoring of serum creatinine level are recommended for all patients with diabetes duration of 5 years or longer. Once nephropathy has progressed to persistent macroalbumiria, prognosis worsens: about half of patients with macroalbuminuria develop ESRD within 10 years and 75% within 20 years (Molittch *et al*., 2004).

Risk factors for diabetic nephropathy for which interventions are available include poor glycemic control, high blood pressure, early glomerular hyperfiltration, and smoking. In addition, several studies suggest that genetic susceptibility may play a role with a variety of underlying mechanism, including ACE polymorphism. In the presence of chronic hyperglycemia the risk for diabetes-associated kidney disease seems to be magnified by risk alleles at several susceptibility loci, some of which differ between men and women (Freedman *et al*., 2007). At the time nephropathy is diagnosed, nearly all patients have other signs of microvascular damage, such as retinopathy. Usually, retinopathy precedes the onset of overt nephropathy. Intensive insulin treatment aiming for near-normoglycemia has been demonstrated to delay the onset of microalbuminuria and the progression of microalbuminuria tomacroalbuminuria (DCCT, 1993).

2.5.2.2 *Macrovascular complications*

Macrovascular complications develop in patients with type 1 (T1DM) (Nathan *et al*., 2003; Nathan *et al*., 2005; Turner et al., 1998) and type 2 diabetes mellitus (T2DM) (Holman et al.,

2008; Califf *et al*., 2008). Indeed, diabetes itself accounts for 75 – 90% of the excess coronary artery disease (CAD) risk and enhances the effects of other cardiovascular risk factors. Death from stroke and myocardial infarction (MI) are the leading causes of mortality in type 1 Diabetes Mellitus and Type 2 Diabetes Mellitus (Dale *et al*., 2008). A range of hemodynamic and metabolic factors has been considered responsible for the development and progression of macrovascular disease in diabetes (Calcutt *et al*., 2009). In terms of hemodynamic factors, in particular the hormonal cascade known as the rennin angiotensin system (RAS) has been shown to have pivotal role in diabetes – associated atherosclerosis; however, other vasoactive systems such as the endothelin (ET) and urotensin system (Bousette *et al* ., 2004; Hassan *et al*., 2005) have also been implicated in diabetic macrovascular disease. More recently, a role for novel factors such as tumor necrosis factor (TNF) related apoptosis inducing ligand (TRAIL) and the complement system has also been suggested (Secchiero *et al*., 2004; Ostergaard *et al*., 2005).

Congestive heart failure: The main myocardial energy production is normally based on β-oxidation of free fatty Acid (FFA) (70%) with a smaller contribution from glucose oxidation (30%) and lactate. Free fatty Acid is produce by lipolysis of endogenous cardiac or exogenous stores of triglycerides. Oxidation of free Fatty Acid is an effective supplier of energy in the form of ATP if oxygen supply is sufficient. In conditions with limited oxygen availability, glucose oxidation will provide more energy per mole oxygen and support more work than Free Fatty Acid (Wolff *et al*., 2002). In the person with diabetes, glucose utilization for energy production is substantially lower, about 10%(WHO, 2006). The shift to an even more pronounced β-oxidation of free fatty Acid causes higher oxygen utilization than under normal

circumstances. The major restriction to glucose utilization in the diabetic heart is the slow rate of glucose transport across the sarcolemmal membrane in the myocardium (Garvey *et al*., 1989). The impaired glucose oxidation in the diabetic heart can also result from a decreased rate of phosphorylation of glucose which subsequently limits the entry of glucose into cell. The depressed phophorylation is triggered by the increasing circulating free Fatty Acid (Rodrigues *et al*., 1992). People with diabetes are also known to have increased risk for other disturbances such as reduced myocardial blood flow and blunted hyperkinetic response to myocardial ischemia resulting in diminished myocardial function. Indeed, CHF is an insulin-resistant state with an increased released of non-esterified fatty acids which are taken up in muscular tissue and down regulate glucose uptake and utilization (Swan *et al*., 1997).

Another consequence of hyperglycemia is oxidation stress and activation of processes triggered by an increased level of diacylglycerol and protein kinase C. Besides, many other unfavorable effects of the increased levels of inflammatory cytokines in heart failures patients may enhance insulin resistance (Kannel and McGee, 1979).

Cerebrovascular disease*:* Diabetes predisposes patient to vascular thrombo – occlusive events in a number of ways. There is accelerated atherosclerosis in both large and medium sized vessels. There is disordered endothelial response and the blood of the patient with diabetes is hypercoagulable (Air and Kissela, 2007). Carotid intima – media thickness (cIMT) is a useful proxy for early atherosclerosis, as it is easily measured by Doppler. Intima Media Thickness (IMT) is associated with primary stroke, with an increased relative risk per standard deviation (0.163mm) of 1.57 in patients who had not had a previous stroke (Bots et al., 1997). Atherosclerosis also affects the ability of endothelium to release nitric oxide (NO), a potent vasodilator. The diabetic blood vessel has either reduced NO production or altered

NO metabolism. In addition to its vasodilating affects, NO protects against platelet aggregation and enables the blood vessel to withstand ischemic conditions with predictably poor response to ischemia. The blood of the patient with diabetes is hypercoagulable. Studies have demonstrated increased thrombin generation, increased prothrombin fragments and increased thrombin – antithrombin II complexes (Yamada *et al*., 2000). Furthermore, the elevated prothrombotic levels were significantly associated with macroangiopathic complications.

Thus, taken together, the person with diabetes has a vascular environment that is highly susceptible to thrombo – occlusive complications. With early atherosclerosis and disordered endothelial response, the conditionsnare predisposed to thrombophilia. The blood, in its hypercoagulable state combined with platelets that are highly active in themselves, is far more likely to form clots (Chester *et al*., 1978).

Peripheral vascular disease (PAD): The pathophysiology of PAD in those with diabetes is similar to that of a non – diabetic population. The abnormal metabolic state that accompanies diabetes directly contributes to the development of atherosclerosis. Pro-atherogenic changes include increases in vascular inflammation and alterations in multiple cell types. Both mechanism of atherosclerotic complications are of importance in PAD (gradual narrowing resulting in stenosis and acute thrombosis in existing atherosclerotic lesion). Obviously, the long – term accumulation of lipids in the vessel wall is important and sudden local thrombosis can occur at any time, although in most cases this happens after symptoms (claudication) have developed (Richard *et al*., 2010). To reach the stage of critical limb – threatening ischemia, advanced atheroscelerosis has developed. Often, multiple segments of the arterial tree from the aorta to the foot are affected (stenotic and/or occluded). In people with diabetes, the

atherosclerotic lesions are more peripherally located than in people without diabetes. In order to develop ischemic non – healing ulcers, perfusion in those with diabetes is measurement of toe pressure. A toe pressure below 20 – 25 mmHg signals a poor chance of healing of a peripherally located ulcer (Richard *et al*., 2010).

2.6 Management of Diabetes Mellitus

Diabetes mellitus is chronic disease which cannot be cured except in very specific situations (Nathan *et al*., 2005). Management concentrations on keeping blood sugar levels as close to normal (euglycemia) as possible, without causing hypoglycemia. Depending on the type and stage of the disease, its management can usually be accomplished with life style modification, (diet, and exercise); and use of appropriate medication.

2.6.1 Life Style Modification;

Life style modifications are recommended with the goal of keeping both short term and long term blood glucose levels within acceptable bounds. Patient education, understanding, and participation is vital, since the complications of diabetes are far less common and less severe in people who have well managed blood sugar levels (Pignone*et al*., 2010) . There are roles for patient education, dietetic support, sensible exercise; attention is also paid to other life style factors that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise (Adler, *et al*., 2000).

The ideal diet for diabetic patients remains to be determined. Recommendations generally call for low fat, high carbohydrate diets. The primary purpose of this recommendation is to reduce the risk for coronary heart disease, a major killer of diabetic patients; some investigators also suggest that high carbohydrate diets also improve glucose tolerance, even in patients with non

insulin dependent diabetes mellitus (NIDDM). Another potential advantage of a low fat diet (high percentage of carbohydrate) is that it may promote weight reduction. High fat diets are thought by many investigators to stimulate weight gain, thus in obese NIDDM patients a trial of a low fat weight reduction diet may be worthwhile. However if after an adequate trial of a low fat weight reduction is not achieved, this suggests that the patient is consuming large quantities of carbohydrates. As the patient education institute notes, carbohydrate should make up between 50 and 60 percent of a diabetics caloric intake (Libby, 2011). This is because carbohydrates are rapidly broken down into glucose, so carbohydrate heavy meals can cause a sudden rise in blood glucose levels. Ideally, your carbohydrates should come from vegetables, fruit and whole grains, as these carbohydrates are broken down more slowly, which helps mitigate the effects of carbohydrates on blood glucose levels.

The continuous feeding of a high carbohydrate diet to a persistently obese patient with NIDDM may have several untoward effects. For example, it can heighten hyperglycemia, raise plasma triglycerides, and lower high density lipoproteins. In such dietary failures, it may be better to replace carbohydrate wit fat to avoid these responses. Ideally, the fat should not raise the serum cholesterol level, and hence it should be unsaturated. Monounsaturated fatty acids seem preferable to polyunsaturated fatty acids., because polyunsaturated may increase the risk for cancer or promote the oxidation of low density lipoprotein, another potentially atherogenic change, many NIDDM patients, particularly obese patients in the earlier stages of diabetes, tolerate weight- maintenance high- carbohydrate diets without deterioration of glucose, tolerance. However, as their insulin reserve declines, high –carbohydrate diets may further raise glucose levels and a lower –carbohydrate diet seems preferable. The same may be true for non obese patients with NIDDM (Scot, 1991).

Exercise is one of the cornerstone in management of DM, moving toward a more active life is generally inexpensive, convenient, and usually produces great rewards in term of blood glucose control and a general feeling of well being. Daily aerobic exercise for at least 20- 30 minutes is recommended for diabetic patient, in order to boost cardiovascular health and keep healthy weight. And as well moderate intensity exercise helps the cells to respond better to insulin. In addition regular exercise reduces stress, improves concentration, and decrease appetite (Irene*et al*., 2012). Whenever a muscle is actively use, both fatty acids and glucose are burn during and after periods of activities. Once the falling glucose level is sensed by the beta cells in the pancreas, insulin output is reduced giving the cells a rest. This prevents excessive insulin production. In addition the lower insulin levels signal the liver to empty its glucose reserves (glycogen) into blood to supply the muscles with needed energy. As physical activities continues, the liver converts amino acids, lactic acid and fats into glucose to supply the muscles. If an activity continues long enough even the body's fat cells get involve. They compensate for the reduced fatty acids levels in the body by converting their stored triglycerides into fatty acids. Therefore exercise is one of the most effective approach in management of DM, because it lowers blood glucose, lower fatty acids levels in the blood, and reduced the workload of pancreas and also do not cause blood glucose level to fall below normal compared to drugs (Allen and Armstrong, 2012).

2.6.2 Drugs Used in the Management of Diabetes Mellitus

2.6.2.1 *Insulin*

Insulin is used alone or in combination with oral hypoglycemic agents. Augmentation therapy with basal insulin is useful if some beta cell function remains. Replacement of basal-bolus insulin is necessary if beta cell exhaustion occurs. Rescue therapy using replacement is

necessary in cases of glucose toxicity which should mimic the normal release of insulin by the beta cells of the pancreas (Mayfield and White, 2004). Insulin comes in injectable forms-rapid acting, short acting, intermediate acting long acting. The long acting forms are less likely to cause hypoglycemia compared to the short acting forms.

Insulin therapy was limited in its ability to mimic normal physiologic insulin secretion traditional intermediate- and long acting insulins (NPH insulin, lente insulin, and ultralente insulin) are limited by inconsistent absorption and peaks of action that may result in hypoglycemia (Burge and Schade, 1997; Cameron and Bennett, 2009). The pharmacokinetic profiles of the new insulin analogues are distinct from those of the regular insulins, and their onset and durations of action range from rapid to prolong. Currently, two rapid – acting insulin analogues, insulin lispo and insulin aspart, and one long acting insulin analogue, insulin glargine, are available (burge and Schade, 1997; Cameron and Bennett, 2009).

2.6.2.2*Sulfonylureas*

These were the first widely used oral hypoglycemic medications and most widely used drugs for the treatment of type 2 diabetes. They are insulin secretagogues, triggering insulin release by direct action on the channel of the pancreatic beta cells. These drugs are classified into first-generation and second generation agents. Sulfonylureas may also have extra-pancreatic effects, one of which is to increase tissue sensitivity to insulin, but the clinical importance of these effects in minimal. Hypoglycemia and weight gain are some of the side effects of these agents. These agents are effective and inexpensive. The first-generation agents are Tolbutamide, Acetohexamide, Tolazamide, Chlorpropamide and the second-genaration agents are Gilbenclamide, Glipizide (e.g Glucotrol), Glyburide (e.g Diabeta), Glimepiride (e.g Amaryl) and Gliclazide (e.g Diamicron) (Eurich *et al*., 2007).

2.6.2 Biguanides

These reduce hepatic glucose output and increase uptake of glucose, the skeletal muscle. Although, it must not be used in patients with impaired liver and kidney function. The main use for metformin is in the treatment of type 2 diabetes mellitus, especially in overweight people. In addition to suppressing hepatic glucose production, metformin increases insulin sensitivity, enhances peripheral glucose uptake (by phosphorylatingGlucose Transporter -4 (GLUT-4) enhancer factor, increases fatty acid oxidation and decreases absorption of glucose from the gastrointestinal tract. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors (Rendell, 2000; Collier *et al*., 2006; Dell'Aglio *et al*., 2009).

2.6.3 Meglinitides (repaglinide, nateglinide)

Meglinitides help the pancreas to produce insulin and are called "short acting secretagogues. Their mechanism of action is through affecting potassium channel,byclosing potassium channel of the pancreatic beta cells; they open the calcium channel, hence enhancing insulin excystosis (Rendell, 2000)

2.6.4 Thiazolidinediones (glitazones)

This is a nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. Thiazolidinediones reduces insulin resistance by activating peroxisome proliferator activated receptor- gamma (PPAR-y) in the gastrointestinal tract. The side effects of these agents include increased liver enzymes, weight gain, edema, mild anemia (Rendell, 2000).

2.6.5 Alpha-glucose inhibitors (Miglitol, Acarbose)

These are not technical hypoglycemic agents, because they do not have direct effect on insulin secretion or sensitivity. These agents slow down the digestion of starch in the small intestines, so that glucose from starch meal enters the blood stream more slowly, and can be matched more effectively by an impaired insulin response or insensitiuvity. The site of action is in the fats and muscles. It is very effective in the treatment of type 2. Glucosidase inhibitors, decreases postprandial plasma triglyceride levels indiabetes, with major side effects such as diarrhea, abdominal pain, flatulence, and serum levels of transaminases increases at higher doses (Haffner, 2007).

2.6.3 Herbs and Plants:

Some herbs and plant derivatives such as lyceum barbarum (solanaceae) (Zhao *et al*., 2005; Wu *et al*., 2006; Tanaka, *et al*., 2006). Eugenia jambolana (Asteraceae) (Ravi *et al*., 2005; Sharma *et al* ., 2006). Nauclea latifolia leaves (Gidado , *et al.,* 2005) have shown to have antihyperglycemic activity and are in use in treatment of DM. However, many herbs and plants have largely been employed traditionally by native people in the treatment of diabetes and many other diseases in the areas in which they grow, although, Corn silk contains proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids (Abdel-Wahab, 2002). There have been many reports on the biological activities of corn silk constituents. Methanol extracts of corn silk showed an antioxidative activity on the level of lipid peroxidation. Volatiles from corn silk inhibited the growth of Aspergillus flavus, indicating that it has an antifungal activity (Ziringue, 2000).

Corn silk plant is as at these day have inadequate knowledge base on her use.

These include their;

i. Active components
ii. Mechanisms of action,
iii. Minimum potent dose
iv. Minimum lethal dose (Ojowole, 2005).

These are the basis of therapeutic safety, potency and preventive measure against substances toxicity. Many medicinal plants have toxic potential, mostly due to overdose resulting from inadequate knowledge base for their use. This include; cochlospermum planchonii, nim tree leaves etc (Nafiu *et al.,* 2011).

Corn silk (CS)(Stigma maydis) is made from stigmas, the yellowish thread like strands from the female flower of maize.It is a waste material from corn cultivation and available in abundance (Maksimović*et al*., 2005). It has been consumed for a long time as a therapeutic remedy for various illnesses and is important as an alternative natural-based treatment. It has been used as traditional medicine in many parts of the world such as China, Turkey (Maksimović*et al*., 2005), United States and France. It is used for the treatment of cystitis, edema, kidney stones, diuretic, prostate disorder, and urinary infections as well as bedwetting and obesity (Hu*et al*., 2010). It soothes and relaxes the lining of the bladder and urinary tubules, hence reducing irritation and increasing urine secretion (Steenkamp, 2003). Other beneficial treatments of CS include anti-fatigue activity, anti-depressant activity and kaliuretic (Ebrahimzadeh*et al*., 2009). Inaddition, it possesses excellent antioxidant capacity (Ebrahimzadeh*et al*., 2008) and demonstrated protective effects in radiation and

nephrotoxicity (Sepehri*et al*., 2011). However, a recent study showed that there is no antibacterial activity in CS when it was investigated against bacterial species such as Pseudomonas aeruginosa,Klebsiella pneumonia,Staphylococcus aureus, Streptococcus pneumonia,Escherichia coli and Streptococcus pyrogenes (Alam, 2011). In China, it is considered very important medicinal plant in the treatment of prostate problems (Maksimović*et al*., 2005). Meanwhile, the Native Americans used CS to treat urinary tract infections, malaria and heart problems(Maksimović et al., 2005). Although not scientifically proven, CS tea has been claimed to havemany benefits to human health such as lowering blood pressure, decrease prostate inflammation, diabetic and urinary tract infection, edema, obesity and promote relaxation. To date, there are variousCS commercial products for medicinal uses which are available in the market (Wan Rosli*et al*., 2008).

CS is rich in phenolic compounds, particularly flavonoids (Liu*et al*., 2011). It also consists of proteins, vitamins, carbohydrates, calcium, potassium, magnesium and sodium salts, volatiles oils and steroids such as sitosterol and stigmasterol, alkaloids, and saponins (Ebrahimzadeh*et al*., 2008). Due toits potential benefits, there are several studies which reported the pharmacological activities of CS (Liu*et al*., 2011).

This review focuses on the available scientific evidence on potential uses of CS in healthcare including its phytochemical, pharmacological, and botanical description and its toxicological studies (Liu*et al*., 2011).



Figure 2.1 Corn Plant showing corn silk adapted from Li J*et al*., (2011)

2.7 The Pancreas

The pancreas is a retroperitoneal organ, surrounded by a capsule of connective tissue that separates the parenchyma in lobules by means of thin partition walls which contain nerves, lymphatic and blood vessels, and excretory ducts. It is a gland of complex secretion, synthesizing, storing and secreting important hormones and digestive enzymes. Daily, the pancreas secretes about 1,200ml of pancreatic juice, essential for the digestion of carbohydrates, proteins and fats of the foods. The endocrine portion, as a whole, represents only a little proportion of the total volume of the gland and secretes hormones that are mainly involved in the control of the carbohydrate metabolism. Both, endocrine and exocrine components of the pancreas are independent in their functional characteristics as well as in their own pathologic alterations (Eynard *et al*., 2008).

2.7.1 Embryonic development of the pancreas

The pancreas is formed by two buds originating from the endodermal lining of the duodenum. Whereas the dorsal pancreatic bud is in the dorsal mesentery, the ventral pancreatic bud is close to the bile duct. When the duodenum rotates to the right and becomes C-shaped, the ventral pancreatic bud moves dorsally in a manner similar to the shifting of the entrance of the bile duct. Finally the ventral bud comes to lie immediately below and behind the dorsal bud. Later the parenchyma and the duct systems of the dorsal and ventral pancreatic buds fuse.

The ventral bud forms the uncinate process and inferior part of the head of the pancreas. The remaining part of the gland is derived from the dorsal bud. The main pancreatic duct (of Wirsung) is formed by the distal part of the dorsal pancreatic duct and the entire ventral

pancreatic duct. The proximal part of the dorsal pancreatic duct either is obliterated or persists as a small channel, the accessory pancreatic duct (of Santorini). The main pancreatic duct, together with the bile duct, enters the duodenum at the site of the major papilla; the entrance of the accessory duct (when present) is at the site of the minor papilla. In about 10% of cases the duct system fails to fuse, and original double system persist (Sadler and Bridges).

In the third month of fetal life, pancreatic (islets of langerhans) develop from the parenchymatous pancreatic tissue and scatter throughout the pancreas. Insulin secretion begins at approximately the fifth month. Glucagon and somatostain-secreting cells also develop from parenchymal cells. Splanchnic mesoderm surrounding the pancreatic buds forms the pancreatic connective tissue (Liu*et al*., 2011).

2.7.2 Endocrine pancreas

The functional unit of the endocrine pancreas is islet of Langerhans. These spherical structure are formed by tenth to hundreds cell containing many capillary vessels. The islets of Langerhans are scattered in the glands parenchyma surrounded by thin layer of reticular fibers. In the adult human being, there is more than a million of an islet which amounts to 1% of the pancreatic volume. They are mostly in the tail than in the body and head of the pancreas. All the pancreatic endocrine cell types are polarized, with the secretory granules located at the apical domain of the cell, in the vicinity of a fenestrated capillary. As other protein-secreting cells, they have a well-developed rough endoplasmic reticulum and Golgi complex. The cells of the islets are arranged as irregular cords and – with standard hematoxilin-eosin method – has a light staining compared with the surrounding acinar cells (Klover and Mooney, 2004; Eynard *et al*., 2008).

2.7.3 Cell types in the pancreatic islet

i. ***Alpha cell*** *(*or A cell), are about 15-25% of the island cells, homogenously distributed over the gland and mainly around the periphery of the islets. The cytoplasmic granules are relatively large and insoluble in alcohol. These cells in response to cholecystokinin or insulin – produce glucagon, hyperglycemic hormone that opposes the insulin effect inducing hepatic glycogenolysis and the glucose transport toward the blood (Klover and Mooney, 2004).

ii. ***Beta cell*** (or B cell), representing the 50-80% of the island cells, the majority are located toward the periphery, and only some of them in the middle of the island. The granules of B cells are smaller than those of the A cells and are soluble in alcohol. They segregate insulin, hypoglycemic hormone that lowers the blood glucose level by facilitating its entry into the cell and their conversion to glycogen, and also insulin participates in the lipid metabolism. The control of insulin secretion is mainly regulated by the level of blood glucose (Klover and Mooney, 2004; Eynard *et al*., 2008).

iii. ***Delta cell*** (or D cell), complete the 3-10%; they are scattered by the islets and have small cytoplasmic granules containing somatostatin that suppress the endocrine secretion of insulin and glucagon by means of a paracrine mechanism, modulating the activity of Alpha and Beta cells and maintaining the normal glucose level in blood.

iv. ***F cell*** (or PP cell), segregates pancreatic polypeptide, they represent only 1% of the islets cell population, and inhibit the exocrine pancreas (Eynard *et al*., 2008).

Besides the secretion of proteic hormones, the cells of the islets of Langerhans also synthesize others molecules similar to those ofAmine Precursor Uptake and Decarboxylation(APUD)system (Le Douarin and Kalcheim, 1999).

Gap junctions link alpha cells to each other, beta cells to each other, and alpha cells to beta cells. Although experimental proof is lacking, this arrangement may account for synchronous secretory activity. There are also tight junctions between various islet cells. These sites of close apposition or actual fusion of plasma membranes of adjacent cells may affect diffusion of substances into or out of intercellular spaces. This arrangement could either facilitate or hinder paracrine communication between alpha, beta, and delta cells. Blood flows through anastomosing network of capillaries from the center of the islet toward the periphery. This arrangement favors intra islet delivery of insulin from the centrally located beta cells to the peripherally located alpha cells (Maurice, 2010).

2.7.4 The Role of pancreatic development in human diabetes

One beneficial consequences of our improving understanding of pancreatic islet development has been new insights into human diabetes. Short-term energy balance and the control of plasma glucose level depend on the ability of the B cell to sense change in nutrient status and respond with appropriate insulin production and secretion. This fine-tuned capability develops along with morphologic development of the Islets and depends on an adequate number of B cell with precisely functioning gene expression programs placed in the appropriate local environment. Any flaws in pancreatic islet development therefore can potentially lead to impaired secretion and diabetes(Klover and Mooney, 2004).

As a consequence, not surprising, mutations in a number of human genes involved in pancreatic islet development, especially the transcription factor genes can lead to diabetes. In

addition, mutations in the coding sequence of isll and pax4 have implicated in families with later onset diabetes. Analysis of these mutations has given insight into the process by which B cells dysfunction may lead to the development of diabetes ((Klover and Mooney, 2004).

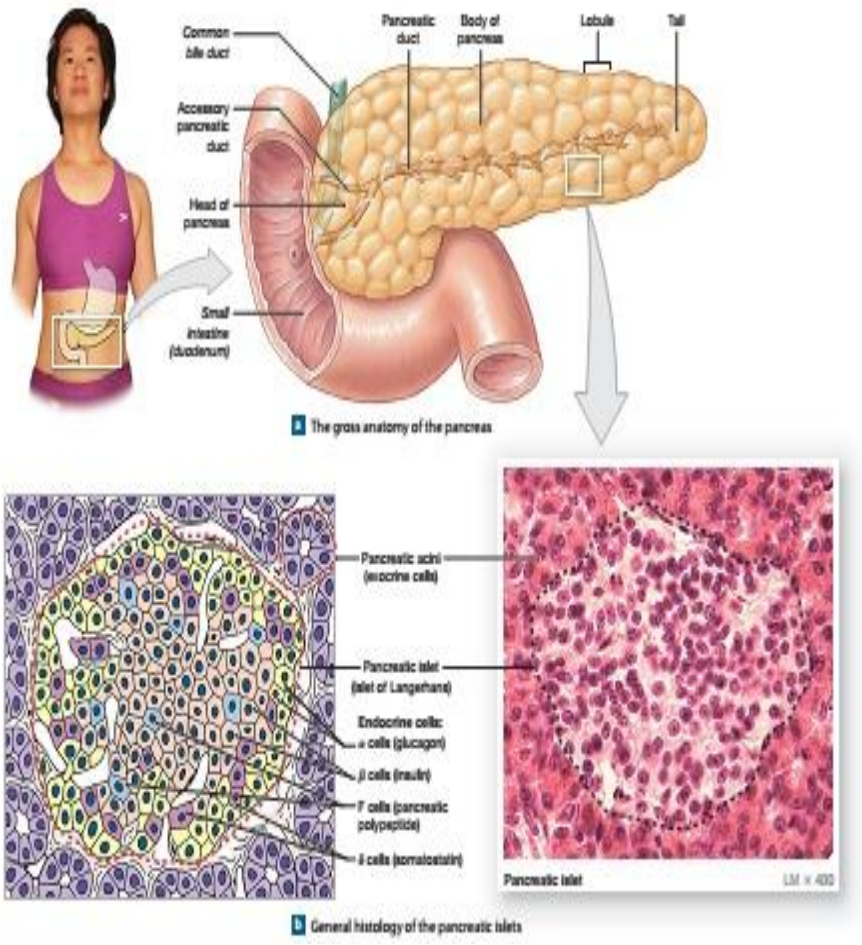


Figure 2.2. Diagram showing the pancreas adapted from Frederic *et al*., (2012)

2.8 The liver

The liver is the largest mass of glandular tissue in the body and the largest internal organ, weighing approximately 1,500g and accounting for nearly 2.5% of adult body weight. It is laocated in the upper right and partially in the upper left quadrants of the abdominal cavity, protected by the ribcage. The liver is enclosed in a capsule of fibrous connective tissue (Gisson's capsule); a serouse covering (visceral peritoneum) surrounds the capsule, except where the liver adheres directly to the diaphragm or the other organs (Michael and Wajaech. 2011).

2.8.1 The embryonic development of the liver

Hepatocytes are the principal cell type in the liver accounting for 70% of the mass of the adult organ. Hepatocytes, along with biliary epithelial cells (BECs; also known as cholangyocytes) are derived from the embryonic endoderm, while the stromal cells, stellate cell, kuppfer cells and blood vessels, are of mesodermal origin.

The endoderm germ layer is established during gastrulation and form a primitive gut tube that is subdivided into foregut, midgut and hindgut. Fate mapping studies in the mouse embryo at embryonic day 8.0 of gestation indicate that the embryonic liver originates from the ventral foregut endoderm (Tremblay and Zaret, 2005). The first morphological sign of the embryonic liver is the formation of the hepatic diverticulum, an out-pocket of thickened ventral foregut epithelium adjacent to the developing liver at week time nine (Handelsman, 2007). The anterior portion of the hepatic diverticulum gives rise to the liver and intrahepatic biliary tree, while the posterior portion forms the gall bladder and extrahepatic bile ducts. At week 9.5, the hepatic endoderm cells, known as hepatoblasts delaminate form the epithelium and invade the adjacent septum transversum mesenchyme (STM) to form the liver bud (Houssaint, 1980; Le

Douarin, 1975; Medlock and Haar, 1983). The STM contributes fibroblasts and stellate cells of the liver. Between week 10-15 the liver bud undergoes a period of accelerated growth as it is vascularized and colonized by hematopoietuc cells to become the major fetal hematopoitic organ.

The hepatoblast are bi-potential and those reading residing next to the portal veins becomes BECs that will line the lumen of the intrahepatic bile ducts (IHBD), while the majority of hepatoblasts in the parenchyma differentiate into hepatocytes (Shiojiri, 1984; Lemaigre, 2003). The maturation of functional hepatocytes and the formation of a biliary network connected to the extrahepatic bile ducts (EHBD) are gradual. Beginning at week 13 this process continues until after birth to generate the characteristic tissue architecture of the liver.

2.8.2 Microscopic Structure and Functional Activity of the Liver

The liver is anatomically divided by deep grooves into lobes (the right and left lobes) and two smaller lobes (the quadrate and caudate lobes). This anatomic division has only topographic importance because it relates lobes of the liver to other abdominal organs. Division into unctional or surgical segment that correspond to the blood supply and bile drainage is more clinically important (Michael and Wajaech, 2011).

In the embryo, the liver develops as an endodermal evagination from the wall of the foregut (specially the site that becomes the duodedenum) to form the hepatic diverticulum. The diverticulum proliferates, giving rise to the hepatocytes, which become arranged in cellular (liver) cords thus forming the parenchyma of the liver. The original stalk of the hepatic diverticulum becomes the common bile duct. An outgrowth from the common bile duct forms the cystic diverticulum that gives rise to the gallbladder and duct (Michael and Wajaech, 2011).

2.8.3 Blood supply to the Liver

The liver receives approximately 25% of the entire cardiac output or about 1ml of blood each minute for every gram of liver tissue. The afferent blood supply to the liver enters at the portal heptis (Kurt, 1991).

Portal Vein: About 75% of the liver blood is supplied by the portal vein, which drains the gastrointestinal tract and, therefore, carries nutrient-rich blood. The smallest branches of the portal vein enter the sinusoids (Kurt, 1991).

Hepatic Artery: The remaining 25% of the liver's blood is supplied by the hepatic artery. Branches of the hepatic artery follow the trabeculae, supplying the connective tissue element with blood. Small branches enter the portal canals, carrying blood to the capillary beds and to the cells of the portal canal (Kurt, 1991).

Although a few small branches of the hepatic artery drain directly into sinusoids most capillary beds derived from the hepatic artery drain into the venules carrying blood to portal veins, which carry the drainage to sinusoids. From the sinusoids, blood flows into central veins and then into hepatic veins which merge with the inferior Vena Cava(Kurt, 1991).

2.8.3 Lobules of the Liver

The liver has several grossly visible lobes demarcated by deep fissures and prominent connective tissue septa. Each lobe contains many lobules (acini), which are the basic functional units of the liver. Since the liver parenchyma in humans appears continuous with no distinct boundaries between lobules (Kurt, 1991).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plants material.

Fresh corn silk was collected around Zaria, Kaduna State of northern Nigeria, by september. The identification of the plant was carried out at the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zariawith a voucher number 971.

3.1.2 Experimental Animals;

A total of 50 wistar rats of both sexes were purchase from experimental animal house of the Department of Human Physiology Ahmadu Bello University Zaria. The health status of the animal was properly monitored before been used for this study. The animals were maintained under normal laboratory conditions of humidity, temperature and light for 7 days prior to the experiment, and allowed free access to food and water.

3.1.3 Chemicals;

Alloxan was been purchased from (sigma chemical company St. Louis U.S.A), glibenclamide and all other chemicals and reagents used was of analytical grade.

3.1.4 Other Materials include;

Distilled water, Normal Saline, digital weighing machine, a pair of scissors, cages and feeds, syringes and needles, spatula, measuring cylinder, dissecting set, dissecting board, cannula, mortal and pestle.

3.2 METHODS

3.1.5 Weighing.

Before the administration,the animals wereweighed with a Galleme Mettler Balance (P162,Switzerland) to determine the respective doses of drugs to be administered to every rat.

3.2.1 Preparation of Extract:

The corn silk of the maize plant was air-dried and made into powder using pestle and mortar. The air- dried powdered plant material was extracted with 10% aqueous and 90% ethanol using soxhlets apparatus, for a high yield.The solvent was removed in-vacuo to yield a residue to be referred to as the Hydro-ethanolic extract. The corn silk used for extraction weighs 5kg and the extract yield was 73.8g

3.2.2Acute toxicity study.

Acute toxicity was determined using the method described by Lorke (1983) and was used to determine the oral median lethal dose (LD_{50}). The study was carried out in two phases. In phase 1; nine (9) rats of either sex were randomly divided into three groups of three rats per group. The animals in the three groups were administered the extract via a cannula with single graded doses (10, 100 and 1000 mg/kg body weight respectively) orally. In phase 2; based on the results of the phase 1 study, three (3) rats were divided into three groups of one rat per group and were administered orally in single graded doses 1600, 2,900 and 5,000 mg/kg body weight of the extract. In each of the two phases of the study, the rats were kept under the same conditions and observed for signs of toxicity and mortality for 24 hours. The LD_{50} was then calculated from the results of the final phase as the square root of the product of the lowest

lethal dose and the highest non-lethal dose, i.e the geometric mean of the consecutive doses which 0 and 100% survival rats were recorded (figure 3.1).

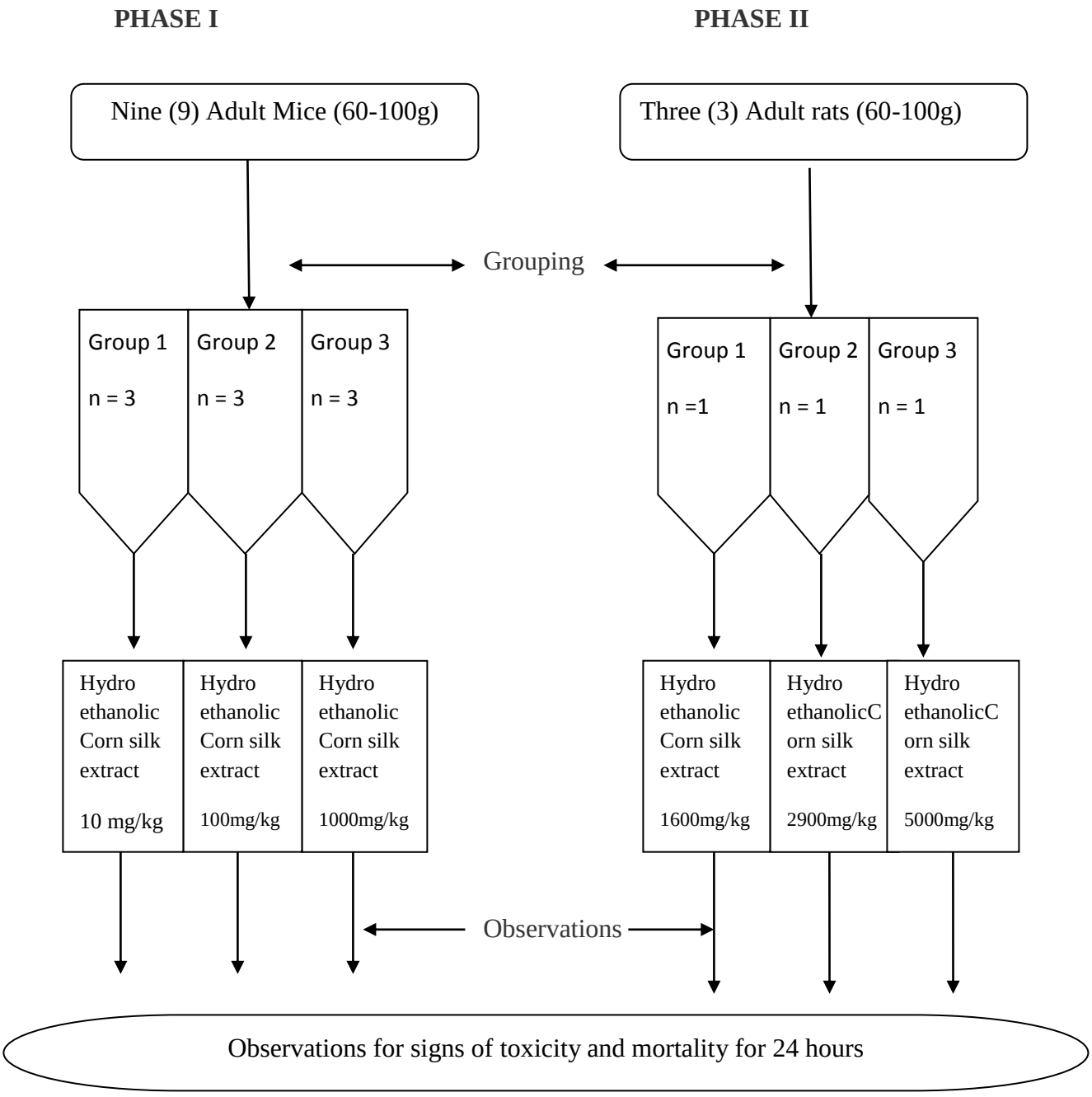


Figure 3.1 Experimental design for Acute Toxicity Study

3.2.3 Induction of Diabetes mellitus

Hyperglycemia was induced by single intraperitoneal injection of alloxan monohydrate(Sigma St. Louis, M.S., U.S.A) at a dose of 150mg/kg body weight (dissolved in 0.9% cold normal saline solution) into 16-18 hrs fasted rats (Katsumata *et al*.,1999). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally for 6hrs.The animal models were administered for the next 24 hours with 5% glucose solution to prevent hypoglycemia (Dhandapani *et al.,* 2002). After 72 hours of alloxan-injection, bloodsamples were collected from the tail vein of the rats. Rats having fasting blood glucose level greater than 200mg/dl were selected for the study.

3.2.4 Experimental Design.

The experimental wistar rats wereweighing between 150-250g and were randomly divided into 5 groups having 5 animals each. The study was carried out on normoglycemic group, Alloxan induced diabetic wistar rats with normal saline orally, and two groups of the hyperglycemic experimental model were administered with two different doses of Hydro-ethanolic extract orally oncedaily for 15 days. The doses were chosen with respect to the lethal dose determined by the method described by Lorke (1983). The experiment were conducted as shown in table 3.1 below

Table 3.1 Animal Grouping, concentration and frequency of administration of Alloxan and Corn Silk

GROUPS	TREATMENT(ORAL ADMINISTRATION)	DURATION
GROUP1	Normal Saline	14 days
GROUP2	Diabetic Control Group	14 days
GROUP3	Diabetic treated with 0.6g kg^{-1} of Corn Silk	14 days
GROUP 4	Diabetic treated with 0.8g kg^{-1} of Corn Silk	14 days
GROUP 5	Diabetic treated with glibenclamide(1mg/ kg)	14 days

3.2.5 Determination of Blood Glucose Levels.

Fasting blood glucose levels was determined at interval of three (3) days interval of administration so as to monitor degree of its effect. This was done using the glucose oxidase principle (Beach and Turner,1958) with a digital glucometer (Accu-Chek Advantage, Roche Diagnostics, Germany).The result was then expressed in mg/dl (Rheney and Kirth, 2000).

3.2.6Morphological Studies.

The experimental animals were weighed weekly. On day 15 the experimental animals were humanely sacrificed following chloroform anesthesia. Blood samples were after sacrifice were collected, centrifuge using centrifuging machine at 1000 rpm and the serum obtained was used for liver enzymes analysis: Alanine amino-transaminase (**ALT**), Aspartate amino-

transaminase (**AST**), alkaline phosphate (**ALP**). Liver and Pancreas were harvested, and processed for histopathological analyses.

3.3 Histopathological Analysis

Histological study was performed by mr Bamidele .D following a midline laparotomy to remove the liver and pancreas. Tissues were immediately fixed in Neutral Buffer Fomalin (NBF) for two days and hydrated by passing them through descending grades of alcohol (100%, 95%, 90%, and 70%) for three minutes each,and subsequently embedded in paraffin. Microtome was used in sectioning the tissues at 5µm.

3.3.1 H and E staining method

The methods of H and E, staining technique were carried out by de-waxing the tissue in two changes of xylene for three (3) minutes each, hydrated by passing them through descending grades of alcohol (100%, 95%, and 70%) for three minutes each, and then stained in Harris haematoxyline for ten minutes, washed in tap water to remove excess stain. The slides was then flooded with acid alcohol for few seconds for differentiation and counter stained with Eosin for three minutes. Tissues were then mounted with cover slips using a mounting media. Sections of the tissues were viewed under light microscope and photomicrographs were taken using digital Am scope (MD 900) photo/microscope.

3.4 Biochemical Studies

Using a syringe, blood was obtained from the heart by cardiac puncture and transferred into plane sterilized centrifuge tubes and allowed to clot after which it was centrifuged and the serum used for biochemical assay of liver enzymes i.e. alanine amino-transferase (ALT),

aspartate amino-transferase (AST), and alkaline phosphates (ALP)using Reitman and Frankel method (1959).

3.4.1 Estimation of Alanine amino-transferase (ALT)

Preparation of reagents

Reagent 1: Buffer reagent

Reagent 2: Enzymes reagent

Mix 4 parts (4ml) of Buffer reagent with 1 parts (1ml) of Enzymes reagent.

Procedures: 100µL of the sample was added to 1000µL of working reagent. The mixture was mixed and incubated at 37^0C for 60 seconds. The decreased in absorbance was measured after every 30 seconds at 340nm for 2 minute and calculated. ΔA/min (ΔA/30sec. X 2) ALT (SGPT) U/L=ΔA/min. x 1746

3.4.2 Estimation of Aspartate amino-transferase (AST)

Preparation of reagents

Reagent 1: Tris 121mmol/l, L-aspartate 362mmol/L, malate dehydrogenase 460 U/L, lactate dehydrogenase 660U/L, sodium hydroxide 255mmol/L, pH 7.8.

Reagent 2: NADH 1.3mmol/L, 2-oxoglutarate 75mmol/L, sodium hydroxide 148mmol/L, sodium azide 9.5g/L.

A single working reagent may be prepared by mixing four parts R1 with one part R2. The working reagent is stable for four weeks at 2-8^0C.

Procedures: 100µL of working reagent was added into 100µL of sample; the mixture was shaken and allowed to stand for 60 seconds at 37^0C.The absorbance decrease was measured after every 60 seconds at 340nm for 3 minute. ΔA/min.

AST (U/l)=ΔA/min. X 1746.

3.4.3 Estimation of Alkaline phosphates (ALP)

Preparation of reagents

Reagent 1: Buffer reagent

Reagent 2: Substrate reagent

A single working reagent may be prepared by mixing four parts R1 Buffer reagent with one part R2 Substrate reagent.

Procedures: 20µL of the sample was added into1000µL of working reagent. The mixture was shaken and incubated at 37^0C for 60 seconds. The absorbance increase was measured every after 30 seconds at 405nm for 2 minute ΔA/min.ALP (U/l)= A/min. X 2720.

3.5. Statistical Analysis

The data obtained from these studies were analysed with statistical package for social science (SPSS) software, version 20 (IBM, USA), using one-way analysis of variance (ANOVA) and Turkey's (for anti-hyperglycaemic study) multiple comparison post hoc test which compared the level of significance between the control and test groups. All data were expressed as mean ± SD and $P \leq 0.05$ were considered as significant.

CHAPTER FOUR

4.0 RESULTS

The hydro-ethanolic extract obtained from Corn Silk was a sticky and jelly like dark brown solid residue with a mild sweet smell. The extract was readilyand completely soluble in water. A weight with a yield of 9.93 % was obtained.

4.1 Acute Toxicity Study

No signs of toxicity or mortality were recorded at all doses of Corn silk in both phases of the experiment during the acute (24 hours) observation period following the single dose oral administration in rats (table 4.1). The absence of death at doses up to 5000 mg/kg showed that the LD_{50} of the extract was greater than 5000 mg/kg body weight.

Table 4.1 Observationsin Phase I And II Of The Acute Toxicity Study Following Single Oral Administration of Hydro-Ethanolic Extract of Corn Silk in Adult Wistar Rats.

Acute toxicity study phase I and II				
Group	**Treatment**	**Dose (mg/kg)**	**Signs of toxicity**	**No of deaths**
PHASE I				
1	Corn Silk	10	None	0/3
2	Corn Silk	100	None	0/3
3	Corn Silk	1000	None	0/3
PHASE II				
1	Corn Silk	1600	None	0/1
2	Corn Silk	2900	None	0/1
3	Corn silk	5000	None	0/1

Phase I: n = 3; Phase II: n = 1

4.2 Effect of Hydro-ethanolic Extract of Corn Silk on Body Weighs.

Table 4.2 showed that there is significant decrease in body weight in the Diabetic control group and significant increase in body weight was observed in the entire treated group compare to the control group.

Table 4.2. Changes in Body Weight of the Wistar Rats on Alloxan induced Diabetes on experimental rats treated with Corn Silk

Groups	Initial Weight	Final Weight	Change in B.W
Normoglycemic	142.80 ± 9.22	154.00 ± 9.72	11.20 ± 1.02^{bc}
Diabetic + Normal Saline	139.20 ± 19.37	143.80 ± 9.35	4.60 ± 0.81^{a}
Diabetic + 600mg/kg CS	145.20 ± 9.14	161.00 ± 9.40	15.80 ± 0.74^{cd}
Diabetic + 800mg/kg CS	129.20 ± 4.54	147.80 ± 3.71	18.60 ± 1.86^{d}
Diabetic + 5mg/kg Glibenclamide	132.80 ± 4.13	142.80 ± 3.71	9.60 ± 1.03^{b}

n = 5; data analyzed using one –way ANOVA followed by turkey multiple comparison post hoc test. Values along the same columns with different superscripts a, b, c and d are significantly different ($p \leq 0.05$).

4.3 Efffect of Hydro-ethanolic extract of Corn Silk on the Fasting Glucose concentration of alloxan induced diabetic wistar rats.

Table 4.3 shows that there is no significant ($p \geq 0.05$) difference in the entire treated group compared with the Diabetic control group on day 0, 1 and 3. Significant ($p < 0.05$) reduction in blood glucose levels were observed in all the treated groups on day 5, 7 and 9. Higher significant reduction was observed in the group that was treated with the higher dose (0.8g/kg) of the corn silk compared with the rest of the treated group and the diabetic control group. The least significant reduction of glucose concentration was observed in the glibenclamide treated group compare with Corn silk treated group.

Table 4.3 Plasma Glucose Concentrations (Mg/Dl) on Alloxan Induced Diabetes on Experimental Rats Treated With Hydro-ethanolic Corn Silk Extract.

GROUPS	DAY O	DAY 1	DAY 3	DAY 5	DAY 7	DAY 9
Normoglycemic	94.40 ± 2.50^{a}	96.60 ± 4.49^{a}	95.20 ± 2.25^{a}	92.60 ± 3.17^{a}	99.40 ± 0.68^{a}	97.20 ± 2.47^{a}
Diabetic + Normal Saline	579.20 ± 16.26^{b}	568.00 ± 18.24^{b}	556.80 ± 19.60^{b}	554.60 ± 16.94^{d}	561.60 ± 16.91^{d}	561.80 ± 18.76^{d}
Diabetic + 600mg/kg	581.20 ± 16.42^{b}	563.80 ± 7.07^{b}	551.20 ± 17.89^{b}	459.60 ± 10.36^{bc}	382.00 ± 18.84^{b}	340.20 ± 16.19^{b}
Diabetic + 800mg/kg	594.00 ± 16.20^{b}	572.80 ± 5.91^{b}	500.60 ± 13.91^{b}	409.40 ± 24.02^{b}	361.80 ± 8.37^{b}	318.40 ± 11.45^{b}
Diabetic + Glibenclamide (5mg/kg)	594.00 ± 4.00^{b}	576.40 ± 8.44^{b}	555.00 ± 21.32^{b}	491.40 ± 14.59^{cd}	449.40 ± 18.71^{c}	405.00 ± 16.43^{c}

n=5; data analyzed using one-way ANOVA followed by turkey multiple comparison post hoc test. Values along the same colum with different superscripts ^{a,b,c} and ^{d} are significantly different ($p \leq 0.05$)

4.4 Effect of hydro ethanolic extract of Corn Silk on serum biochemical parameters

Table 4.4 shows that there is significant ($p \leq 0.05$)increased in the serum level of AST, ALT and ALP in the Diabetic Control Group. The reduction observed in the entire treated group was not significant ($p \leq 0.05$)in the serum level of the AST and ALT while the serum level of ALP was significantly reduced in the entire treatment group compare with the diabetic control group.

Table 4.4 Activities of enzymes (ALP, ALT and AST) on the liver of alloxan induced Diabetes on experimental rats treated with Hydro-ethanolic Corn Silk extract after 15 days.

GROUPS	AST(U/L)	ALT(U/L)	ALP(U/L)
Normoglycemic	23.40 ± 6.03^{a}	19.80 ± 3.17^{a}	13.45 ± 2.40^{a}
Diabetic + Normal Saline	50.20 ± 4.94^{b}	35.60 ± 4.12^{b}	31.20 ± 2.58^{b}
Diabetic + 600mg CS	45.60 ± 0.68^{b}	32.00 ± 2.39^{ab}	14.87 ± 0.74^{a}
Diabetic + 800mg CS	40.80 ± 2.06^{b}	24.40 ± 2.93^{ab}	11.37 ± 1.50^{a}
Diabetic + Gliberclamide (5mg/kg)	40.60 ± 3.48^{b}	24.60 ± 4.31^{ab}	12.16 ± 1.69^{a}

ALP – alkaline phosphatase, ALT – alanine transaminase and AST – aspartate transaminase n=5. Values along the same row with different superscripts ^{a,b,c} and ^{d} are significantly different ($p<0.05$).

4.5 Hispathological studies

Multiple sections of pancreas of the adult wistar rats from eat group were taken and studied for any histological changes after 15 days. The cellular integrity and architecture were intact in the normal control group which showed normal appearance of pancreatic lobules and cells. The islets were normal in size, shape and number (plate I). Pancreatic sections of hyperglycemic control rats showed that alloxan caused severe necrotic changes of pancreatic islets. Study of pancreas of treated hyperglycemic groups (0.6 g/kg and 0.8 g/kg of Corn silk) showed a progressive dose dependent regeneration features. But the glibenclamide treated group showed a less regeneration of pancreatic cells (plate V).

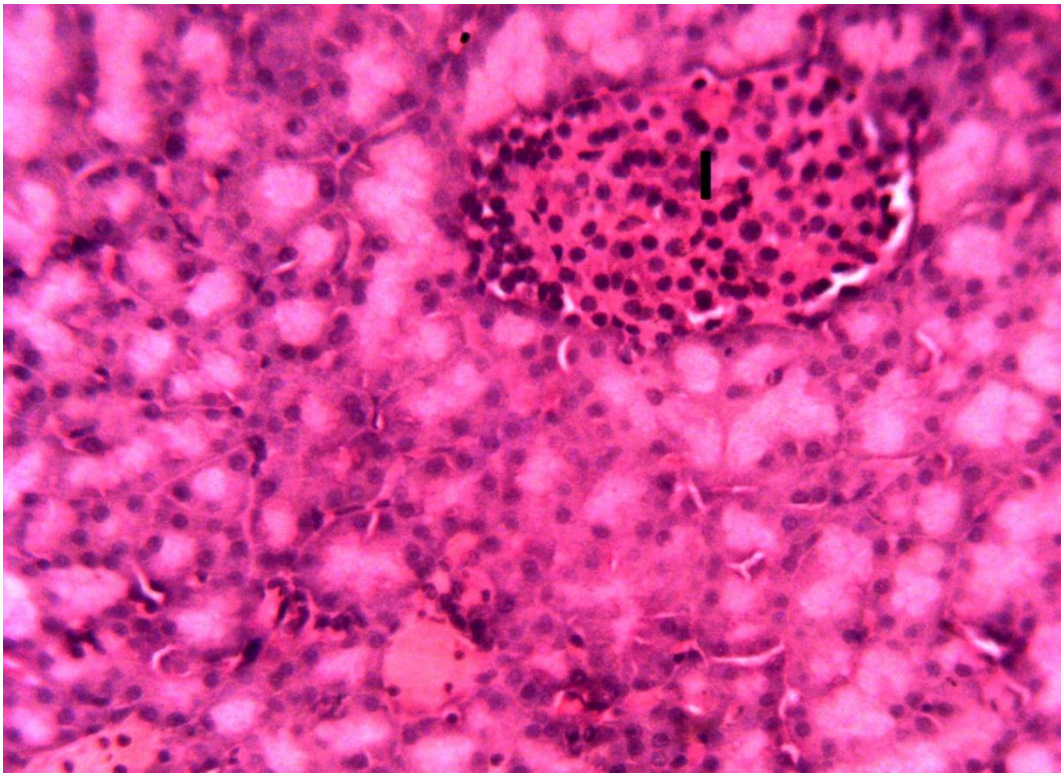


Plate I:A section of the pancreas of the control experimental animal (Wistar rat) showing the normal architectureof the pancreas with the beta cells in islet of langerhans (I) (H and E X250).

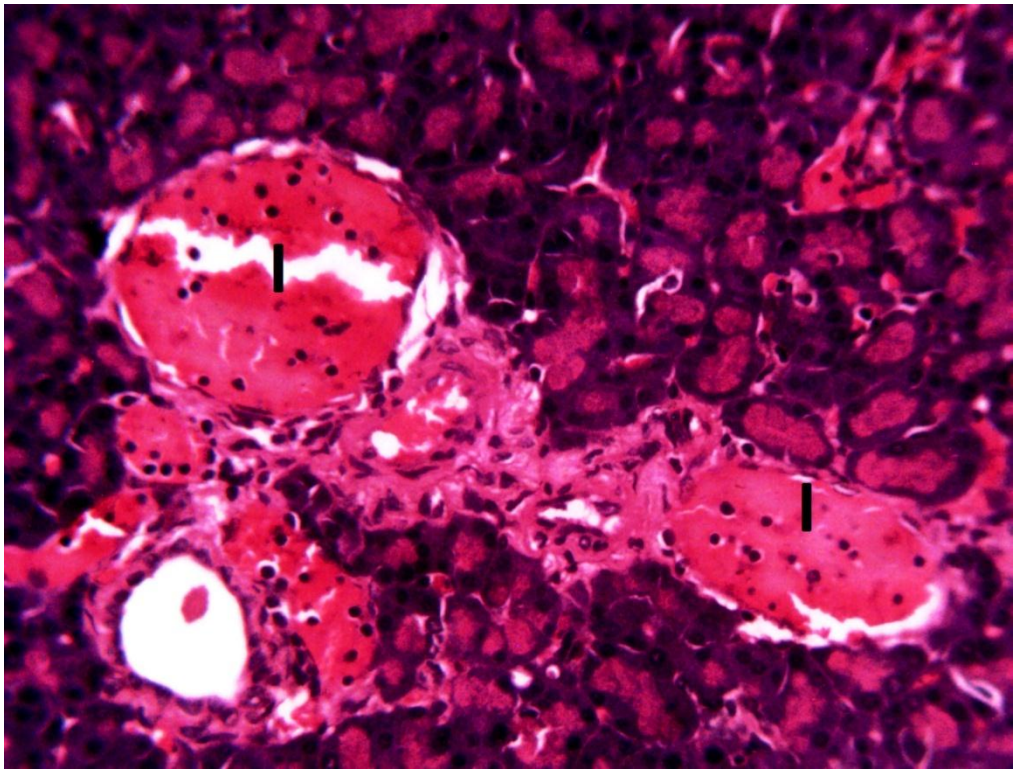


Plate II: A section of the pancreas of experimental group administred with 60mg/kg of Alloxan without treatment showing severe degranulation of the beta cells with Eosinophilic material in islet of langerhans (I) (H and E X250).

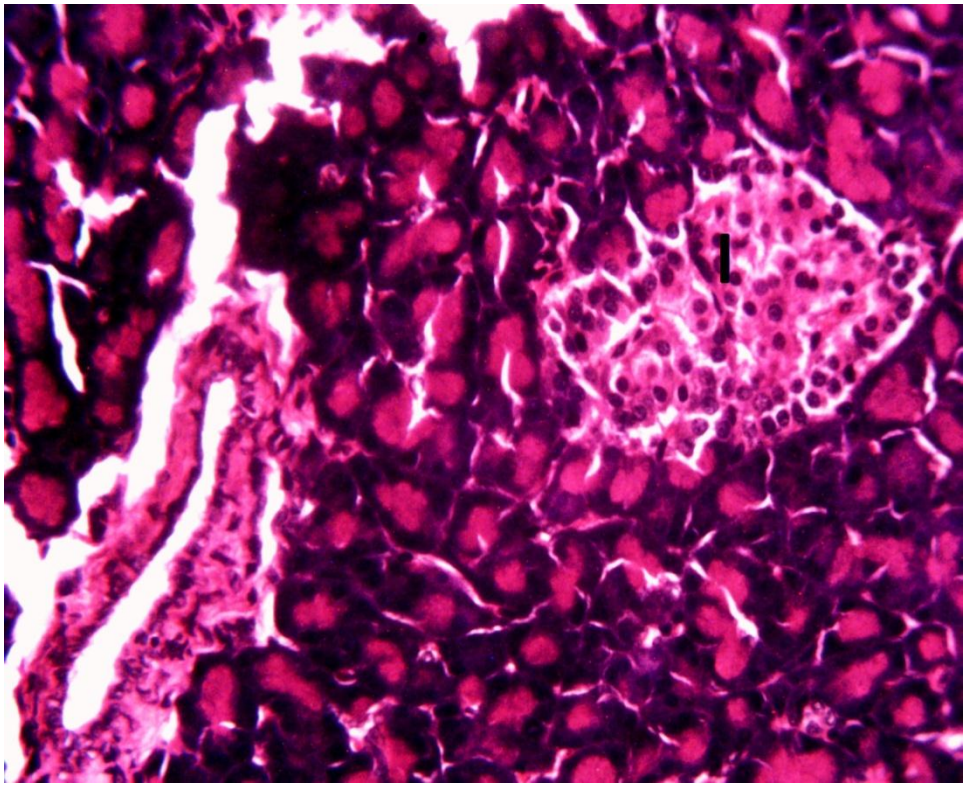


Plate III: A section of the pancreas of the experimental group treated with Alloxan + Corn Silk 0.6 g/kg showing regeneration of beta cells in islet of Langerhans (H and E X250)

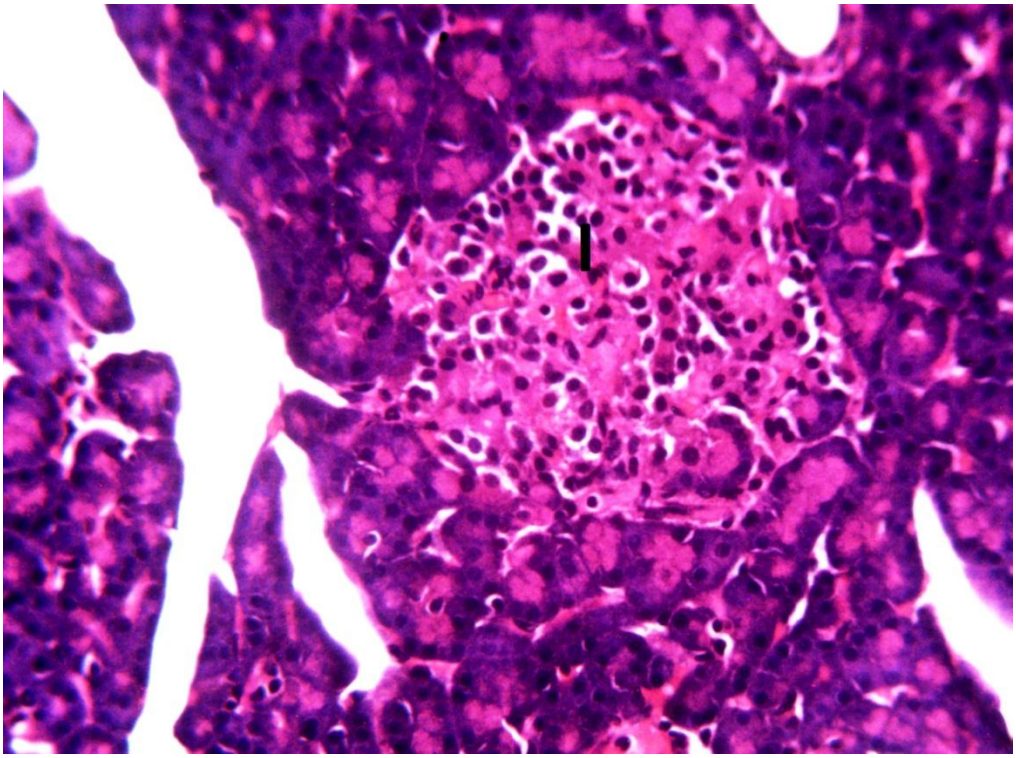


Plate IV: A section of the pancreas of the experimental group treated with Alloxan + Corn Silk 0.8 g/kg showing regeneration of beta cells in islet of Langerhans (H and E X250)

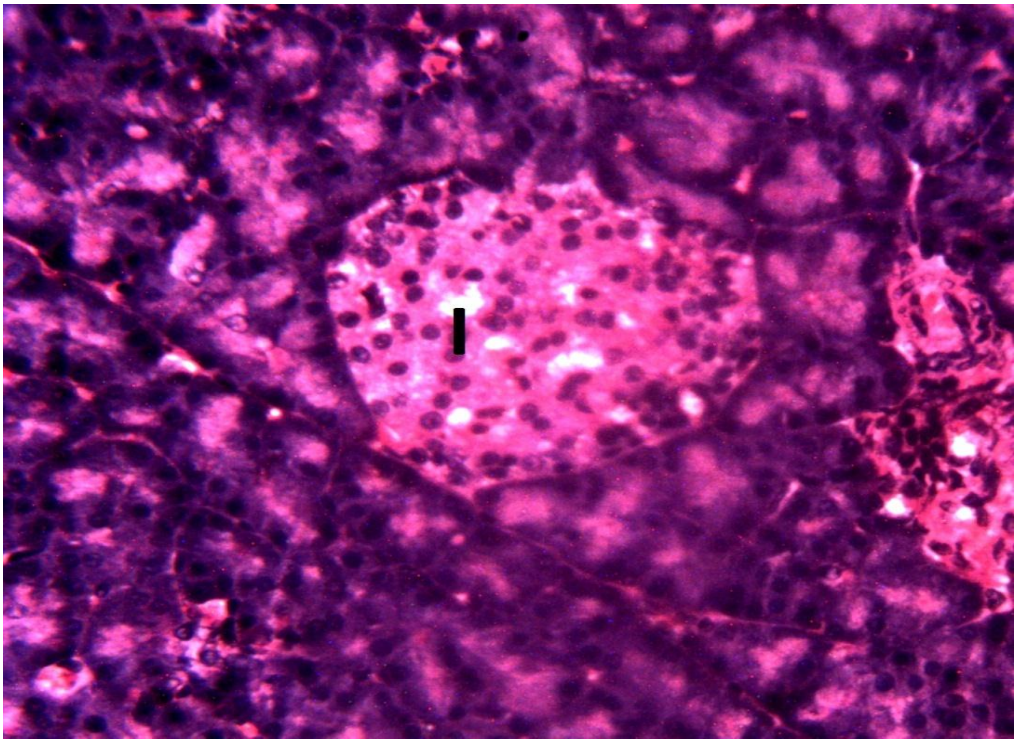


Plate V: A section of the pancreas treated with Alloxan + Gliber group showing the necrotic architecture of the Islet of Langerhans with slight regeneration of beta cells in isletof langerhans (I) (H and E X250).

4.6 Histopathology of the Liver

Multiple sections of liver of the adult wistar rats from each group were taken and studied for any histological changes. The cellular integrity and architecture were intact in the normal control group which showed normal appearance of the hepatocytes in the liver lobule are radially disposed and are arranged like the bricks of a wall. These cellular plates are directed from the periphery of the lobule to its center and anastomose freely, forming a labyrinthine and spongelike structure. The space between these plates contains capillaries, the liver sinusoids.

The histopathological findings obtained in this study showed that Alloxan was hepatotoxic as seen in the liver section characterized by area of vacuolation due to fatty degeneration and necrotic changes, with microvascular and macrovascular fat droplet (Plate 4.7).

Corn Silk when administered therapeutically to rats attenuated the changes associated with Alloxan toxicity by reducing fatty generation, vacuolation of the hepatocytes and necrotic changes thereby restoring almost normal architecture of the liver. This was dose-dependent as treatment with 0.8 g/kg (Plate VIII) showed better result than the 0.6 g/kg of the corn silk and Glibenclamide Treated group (1 mg/kg) as shown in the Plates of tissue slide bellow.

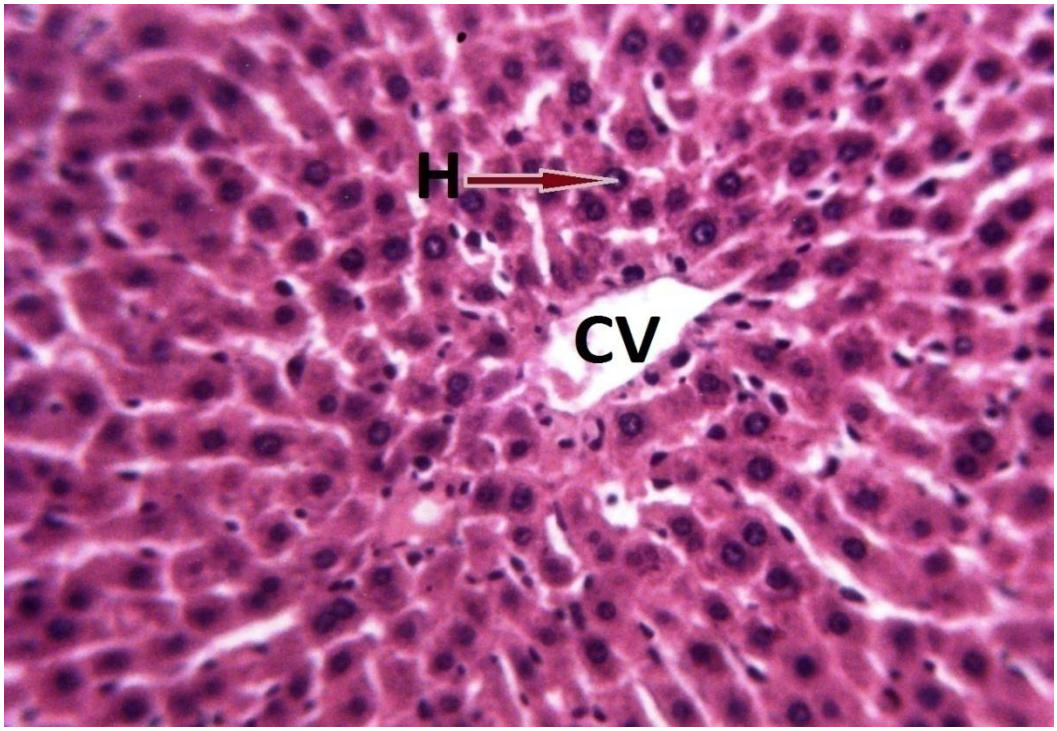


Plate VI: Photomicrograph section the control liver tissue showing normal histological appearance of the liver with normal Central Vein (CV), and Hepartocyte (H)

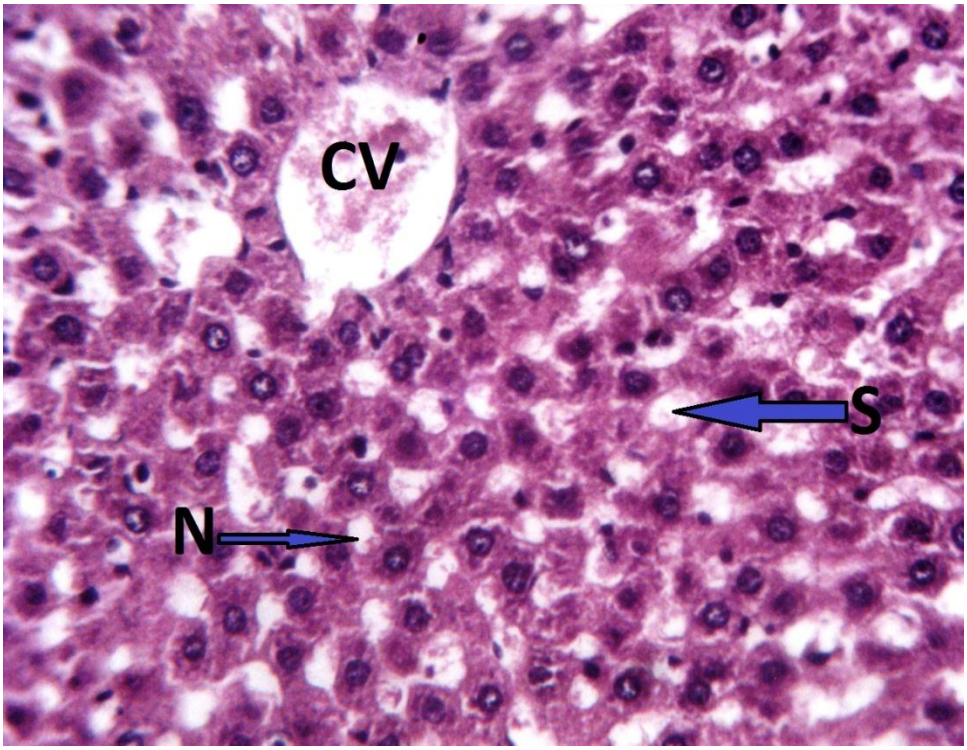


Plate VII; Photomicrograph of the Diabetic Control group showing the presence of both Microvascular (N) and Macrovascular (S) Fat droplet.

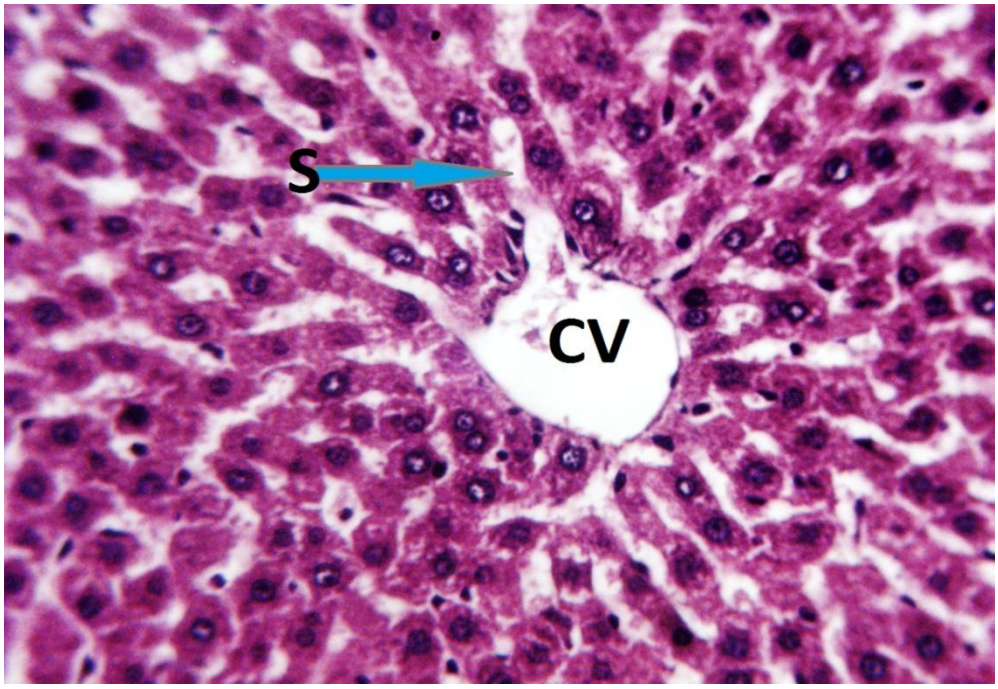


Plate VIII Section of Liver fromAlloxan induced diabetic rat treated with Corn Silk (0.6 g/kg bw) showing Restored liver cytoarchitecture of the liver with and lose membrane of the central vein (CV) (H and E Stain X 250).

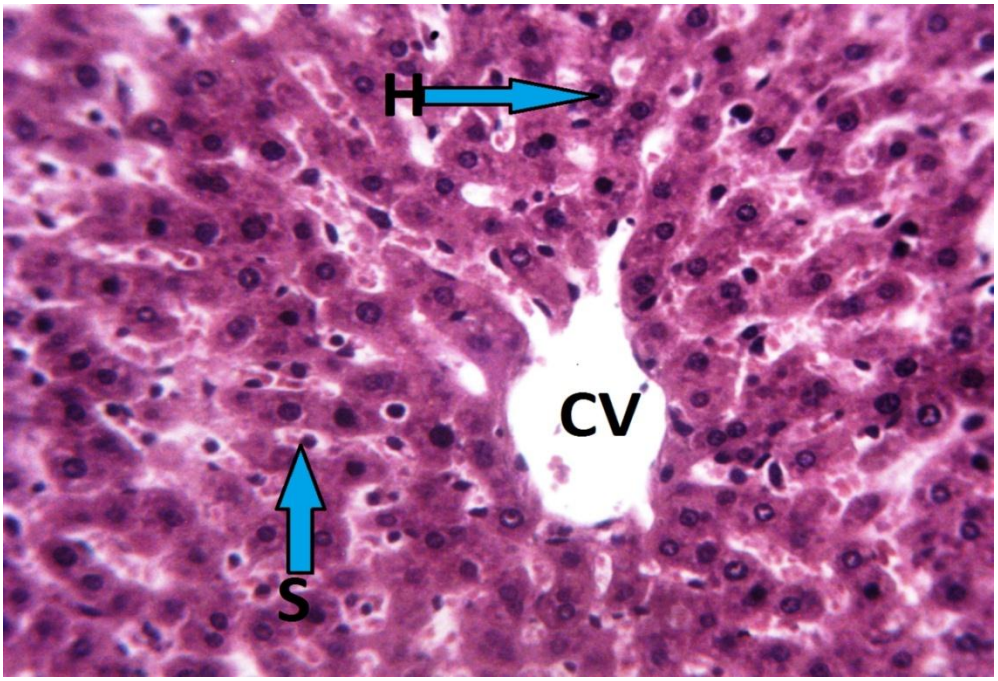


Plate IX: Section of Liver from Alloxan induced diabetic rat treated with Corn Silk (0.6 g/kg bw) showing Restored liver cytoarchitecture, lose membrane of the central vein (CV) (H and E Stain X 250).

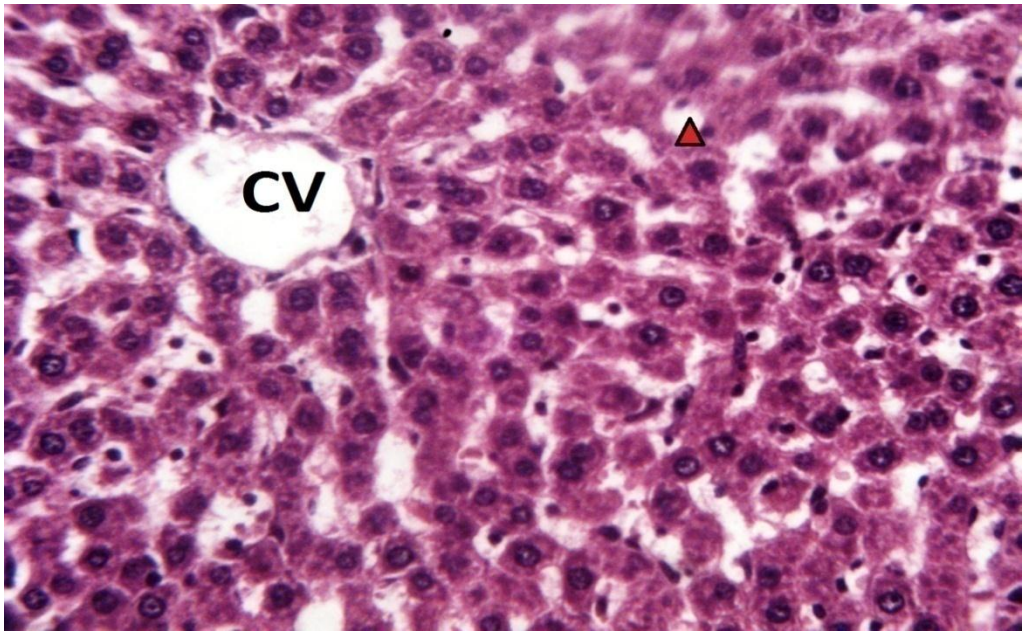


Plate X: Section of Liver from Alloxan induced diabetic rat treated with Gliber (20mg/kg bw) showing Restored liver cytoarchitecture with fat droplet degeneration (arrow head) and membrane of the central vein (CV) (H and E Stain X 250).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion

The present studies were designed to investigate the beneficial effects of corn silk on the Alloxan-induced diabetic pancreas and liver compared to age-matched control.As for its physiologicalactivities, corn silk has been reported to have antioxidant (Hu and Deng, 2011), anti-inflammatory (Wang*et al*., 2012), anti-diabetic (Zhao *et al*., 2012), anti-fatigue (Hu *et al*., 2010), and neuroprotective effects (Kan *et al*., 2011). In particular, its diuretic action is well known (Velazquez *et al*., 2005).

Presently,there is an increased demand for the development of new and novel antidiabetic drugs, which are capable not only in reducing blood glucose level in the body but also in inducing insulin secretion and sensitivity from the endocrine pancreas with minimal side effect. Moreover, the drugs must also improve the β-cell functions, induce its proliferation and suppress its apoptosis. The discussion of this study covers the beneficial effects of Corn silk in diabetic conditions compared to control.

Corn silk contains proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids. Base on folk remedies, corn silk has been used as an oral antidiabetic agent in China for decades. However, the hypoglycemic activity of it has not yet been understood in

terms of modern pharmacological concepts. The purpose of this study is to investigate the effects of corn silk on glycaemic metabolism.

5.2 Acute Toxicity Study

The extract was well tolerated by the animals when administered orally, no sign of acute toxicity like restlessness or seizures were observed over the period of observation. The absence of death in the groups of Wistar rats treated with the extract at 5000mg/kg Corn silk body weight suggests that the extract is practically non-toxic acutely and is safe in Wistar rats (Matsumura, 1975; Tijani *et al*., 2008).

5.3 Body weight

Body and organ weight changes serve as an indicator of adverse effects (Raza *et al*., 2002).At end of the experiment, a significant increase in body weight was observed in the groups of animal treated with corn silk when compared with the other control group of the experiment. This might be as a result of corn silk effect on GLP-1 which initiates their effects by reducing gastric emptying, appetite and enhancing satiety (Bloomgarden, 2004; Kaushal *et al*., 2006; Murphy and Bloom, 2006; Mudaliar and Henry, 2010).

5.4 Blood Glucose Levels

The extract had a remarkable effect on the plasma glucose levels. Corn silk significantly reduced blood glucose levels of treated rats in day 5, 7 and 9 compared to hyperglycaemic control, an effect which is dose dependent. The plant extract at the doses tested decreased the plasma glucose concentration more than the standard reference drug, Glibenclamide, the anti-hyperglycaemic activity of Corn Silk appear to be related to a number of investigation which

have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids possess hypoglycemic properties in various experimental animal model (pulok *et al*., 2006). This hypothesis stipulates that plant which contain terpenoid or flavonoids posses hypoglycemic activities in diabetic and normal mammal. One or more of the other chemical constituents of the plant especially flavonoid may have a role in the anti- hyperglycemic action of the plant extract (Aragao *et al*., 2007). The mechanisms by which Corn Silk reduces blood glucose still remain unclear, consideringthat Corn silk consists of predominantlyflavonoids and terpenoids (Hasanudin*et al*., 2012), its hypoglycemic effect might be connected to the high levels of flavonoid in Corn Silk. Glibenclamide is one of the most frequently prescribed oral hypoglycemic agents (Nathan*et al*., 2003). It exert its action mainly by stimulating insulin secretion and also reduces hepatic glucose production resulting in reduced blood glucose (Rendell, 2004). However, the decrease in blood glucose concentration exhibited by Corn silk is greater than that elicited by Glibenclamide in this study.

5.5 Liver Function Test

Furthermore, the liver function test (LFT) study showed elevated serum liver enzymes (AST, ALT and ALP) in hyperglycemic control compared treated groups and normal. These increases may be due to the leakage of these enzymes from the liver cytosol into the blood stream and /or change in the permeability of liver cell membranes (Ogbonnia *et al*., 2008). ALT is predominantly found in the liver and an increased plasma concentration is an indicator of liver damage while ASTis not only located in the liver but also in the heart and kidney and elevation in their plasma concentrations indicate liver and possible heart damage and also

high level of ALP is a sign of possible liver cholestasis (Ogbonnia *et al*., 2008). There were significant reduction in the level ALP in extract and Glibenclamide treated hyperglycaemic groups, while the reduction observed in AST and ALT was not significant compared with that of the diabetic treated group.From the serum biochemical result of this study, Corn silk appear safe at low and moderate doses (0.6 g/kg and 0.8 g/kg) this is evident in the observed histopathological changes in the liver tissue. The absence of significant increase in the activity normally increases at the earlier phase of hepatic injury and the liver is capable of repairing itself. This suggest that possible cholestasis may have not occurred at the dose levels tested since a rise in plasma ALP level is usually a characteristic finding in cholestatic liver disease (kaneto *et al*., 1996).

5.6 Histopathology of Pancreas

Pancreas is the primary organ involved in regulating the organism dietary and energetic via glucose concentration in the blood and in response to elevated blood glucose, insulin secretion (Sharma *et al*., 2010). In this study, the pancreatic B-cells were destroyed. Alloxan is one of the usual substances used for the induction of Diabetic Mellitus. Alloxan has a destructive effect on the beta cells of the pancreas (Ogbonnia *et al*., 2008). Histopathological study of hyperglycaemic untreated rats showed degeneration of pancreatic islet cells, which was due to alloxan used in this study. This probably gave rise to insulin deficiency, insulin deficiency (Diabetes mellitus) causes excessive elevation of blood glucose and underutilization of glucose leading to hyperglycemia (Standl *et al*., 2003). The histopathological study of hyperglycemic treated groups indicated increased number of beta cells, potentiation of insulin secretion from surviving beta cells of the islets of langerhans and decrease of blood

glucosehave been reported following consumption of some plants extracts (Standl *et al*., 2003). Corn silk may have some chemical components that exert regenerative effects on beta cells, stimulate these cells to produce more insulin or may have some insulin-like substances, induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of pancreas. The restorative effect of corn silk on the islet cells of hyperglycaemic treated rats followed a dose dependent manner and its potency of the regeneration of beta-cells is more than that of Glibenclamide.

5.7 Histopathology of the Liver.

The findings obtained in the histopathological studies revealed a normal appearance of parenchyma and liver extracellular matrix – hepatocytes are arranged in cords located between the sinusoidal capillaries and oriented radially to the terminal venula. Hepatocytes have polygonal shapes, most mononucleate. We noted the presence of rare apoptotic bodies and extramedullary hematopoietic groups, no evidence of pathological lesions was found (Plate 4.1 and 4.11). Steatosis, necrosis or degenerative changes in the Diabetic control group, glycogenic hepatopathy showing glycogen rich hepatocytes with distinct membrane, glycogenated nuclei, giant mitochondria and loss of cellular boundaries indicating necrosis which may be due to a relative lack of insulin, and insulin resistance. This means that Non alcoholic fatty liver disease (NAFLD) co-exist with Diabetes Mellitus. This is in line with the histology of a patient with a diabetic mellitus type 1(Arief and Swan, 2011), as it was established that hepatic fat accumulation is a well-recognized complication of diabetes and excess glycogen accumulation in the liver is seen in 80% of diabetic patients. Glycogen synthesis in the liver is impaired in diabetes due to defective activation of glycogen synthase.

The findings obtained in the histopathological studies revealed a normal appearance of parenchyma and liver extracellular matrix – hepatocytes are arranged in cords located between the sinusoidal capillaries and oriented radially to the terminal venula. Hepatocytes have polygonal shapes, most mononucleate. We noted the presence of rare apoptotic bodies and extramedullary hematopoietic groups, no evidence of pathological lesions was found (Plate 4.6). Diabetic Control group showing the presence of both microvascular and macrovascular fat droplet agrees with the work of Arief and swan in (2011) in which they discovered that non alcoholic fatty liver disease co- exist with Diabetes Mellitus and it also establish that fat accumulation is a well recognized complication of diabetes (Arief and Swam, 2011).

Section of Liver fromAlloxan induced diabetic rat treated with Corn Silk (0.6 g/kg and 0.8 g/kg b.w) showed Restored liver cytoarchitecture of the liver with and lose membrane of the central vein. The potency of the restored liver is more active in the extract treated group which is dose dependent in favor of the higher dose. The treatment group treated with Glibermaclide, the cyto-architecture of the liver is also restored with the presence of few microvascular and macrovascular fat droplet.The findings presented in this work are encouraging and substantiate the search for newer pharmacophores in silk of *zea mays* behind the hypoglycemic.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1Conclusion

It can therefore be concluded from the results of this study that silk of *zea mays* may be relatively safe to consume. The data from this work have shown that the silk of *zea mays* extracts has anti-hyperglycemic effect on diabetic rats, reduced liver function enzymes, initiate regeneration of beta cell in the pancreas and restore the cyto-architecture of the liver in diabetes induced adult wistar rats.

6.2Recommendation

Based on the findings of this work, the following were recommended;

a) There is need to investigate themolecular mechanisms of the silk of *zea mays* extract.

b) Lastly, further studies should be done to investigate anti-hyperlipidemic activity of the silk of *zea mays*.

c) There is also need to evaluate the anti-oxidant properties of the silk of *zea mays***,** knowing that an anti-oxidant has the potency the ameliorate the complication that comes with diabetes mellitus.

6.4 Contribution to Knowledge.

I. The study helps us to understand that *zea-mays* (Silk)have hypoglycemic potency, which is more effective at 800mg/kg.

II. It also help to know that *zea mays* (Silk) can ameliorate the side effect of non alcoholic liver fatty disease induced by diabetes mellitus through the restoration of the liver cytoarchitecture back to its normal state.

III. The study made us to understand that Silk of *zea mays* has the potentials to regenerate beta cells.

IV. The study also made us to understand that Silk of *zea mays* is safe for consumption because of its non toxicity in the liver

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