ANTIDIABETIC ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *Dichrostachys cinerea (L)Wight et Arn* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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M. Sc/SCIEN/16703/2010-2011

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DECLARATION

The research work presented in this thesis entitled"ANTIDIABETIC**, ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *DICHROSTACHYS CINEREA*(L.) WIGHT ET ARN IN STREPTOZOTOCIN INDUCED DIABETIC RATS"** has been performed by me in the department of Biochemistry under the supervision of Professor S.E. Atawodi and Dr. K.M. Anigo.The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for degree program at any university.

Zaharaddeen Sullubawa Ayuba _____ _____
M.Sc/SCIEN/16703/2010-2011 **Signature** **Date**

CERTIFICATION

This is to certify that the thesis entitled "**ANTIDIABETIC, ANTIOXIDANT AND HEPATOPROTECTIVE EFFECTS OF METHANOLIC EXTRACTS OF DIFFERENT PARTS OF *DICHROSTACHYS CINEREA* (L.) WIGHT ET ARN IN STREPTOZOTOCIN INDUCED DIABETIC RATS**" meets the regulations governing the award of degree of Masters of Science (M.Sc.) of Ahmadu Bello University, Zaria, and is approved for its contributions to knowledge and literary presentation.

Prof. S.E. Atawodi Chairman, Supervisory Committee	_____ Signature	_____ Date
Dr. K.M. Anigo Member, Supervisory Committee	_____ Signature	_____ Date
Prof. I.A. Umar Head, Department of Biochemistry	_____ Signature	_____ Date
Prof. A.Z. Hassan Dean, School of Postgraduate studies	_____ Signature	_____ Date

DEDICATION

I dedicated this research thesis to Almighty ALLAH (S.W.T) for his infinite mercy upon me, to carried out the work successfully.

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ABSTRACT

The study was carried out to investigate antidiabetic, antioxidant and hepatoprotective activities of different parts (Fruits, Leaves, Stem-bark and Roots) of methanol extracts of *Dichrostachys cinerea* (Mimosaceae) in normoglycemic and Streptozotocin induced hyperglycaemic rats at 60mg/kg body weight. Glibenclimide (1mg/kg) was used as a reference standard. Diabetic rats treated with leaves, stem-bark, and roots extracts showed a significant decrease ($p<0.05$) in fasting blood glucose compared with diabetic untreated. Stem-bark extracts showed the most highest decrease in fasting blood glucose after 21 days of the treatment , followed by leaves and roots while fruit extracts showed no significant ($p<0.05$) difference. Furthermore there was significant increase ($p<0.05$) in the packed cell volume (PCV) and body weight of diabetic groups treated with stem-bark, leaves and root extracts except in the diabetic group treated with fruits extract,which showed no significant increase ($p>0.05$). All diabetic treated groups showed significant ($p<0.05$) decrease in malondialdehyde (MDA), significant increase ($p<0.05$) in superoxide dismutase (SOD) and catalase activities with stem-back extracts showing the highest activities in superoxide dismutase and catalase followed by leaves, roots and fruits. All diabetic treated groups showed a significant reduction of aspartate amino transferase (AST) alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities , Total bilirubin (TB) concentration compared to diabetic control .There was no significant ($P<0.05$) change in level of serum urea and creatinine in the treated group administered with stem-barks, leaves and fruit extracts of *Dichrostachys cinerea*. Photomicrograph of the liver and kidney of the rats showed nephrotoxic and hepatotoxic effects of the extract. These studies suggest that various parts of *Dichrostachys cinerea* have antihyperglycemic, antioxidants and ameliorative effects on Streptozotocin induced-diabetes mellitus.

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ABBREVIATIONS AND SYMBOLS

ADA	American Diabetes Association
AGEs	Advance Glycation End Products
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AMP	Adenosine Monophosphate
AST	Aspartate aminotransferase
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
DPP	Diabetes Prevention Program
FPG	Fasting Plasma Glucose
GDM	Gestational Diabetes Mellitus
HCN	Hydrogen Cyanide
H&E	Hematoxylin and Eosin
HLA	Human Leukocyte Antigen
HNF	Hepatocyte Nuclear Factor
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IPF	Insulin Promoter Factor*
MDA	Malondialdehyde
MODY	Maturity-Onset Diabetes Mellitus
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral Glucose Tolerance Test
OECD	Organisation of Economic Cooporation and Development
PG	Plasma Glucose
PPAR- γ	Peroxisome Proliferator-Activated Receptor-γ
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase
STZ	Streptozotocin
TBL	Total Bilirubin

TP	Total Protein
UGDP	University Group Diabetes Program
WSF	Water Soluble Fraction

CHAPTER ONE

INTRODUCTION

Diabetes mellitus is a metabolic disorder that either arrives during the early years of growth (Juvenille diabetes) or later in life or is called as maturity onset diabetes. It is observed as the body's inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. Pancreatic damage resulting in the dysfunction of and cells causes disordered glucose homeostasis. In diabetic individuals the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called as Insulin Dependent Diabetes Mellitus (IDDM) or due to insulin resistance that is termed as Non Insulin Dependent Diabetes Mellitus (NIDDM) (American Diabetes Association, 2009).

The classification of the disease is dependent on the nature of insulin deficiency Type 1 or IDDM, which is attributable to autoimmune responses leading to destruction of insulin producing B cell and type 11 or NIDDM which is attributable to resistance of normally insulin sensitive tissue liver and muscle to insulin in addition to defects in insulin secretion.
Diabetes mellitus has been defined by the WHO on basis of laboratory findings as a fasting Plasma level above 8.0mmol/L or a postprandial level above 11.0mmol/L after oral ingestion of 50g glucose (WHO 1980) Diabetes mellitus is a major global health problem that affects more than 185 million people around the world (Zimmet *et al.,* 2001). This disease is an increasingly prevalent metabolic disorder in humans and is characterised by hyperglycaemia . The Recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030.Many diabetes sufferers in Nigeria depend on local medicinal plants for the treatment of their illness (Onoagbe and Esekheigbe, 1999b) (Onoagbe, *et al*, 1999). And hundreds of such plants are in common use.

A good number of the popularly used Nigerian plants have not been scientifically scrutinised for their efficacy and antidiabetic properties (Onoagbe *et al*, 1999). The present investigation is a continuation of our research efforts aimed at providing the requisite scientific information on the therapeutic value of Nigerian medicinal plants in the treatment of diabetes mellitus. Type 2 Diabetes Mellitus begins with a period of insulin resistance with increased pancreatic insulin secretion. As the disease progresses, pancreatic function weaken and as a result, insulin levels fail to keep up with the body requirements (Inzucchi, 2003).

The presence of free radicals in biological materials was discovered about 50 years ago (Drogue, 2002). Today, there is a large body of evidence indicating that patients in hospital intensive care units (ICUs) are exposed to excessive free radicals from drugs and other substances that alter cellular reduction-oxidation (redox) balance, and disrupt normal biological functions (Kehrer,1993).

Oxidative stress and the damage that it causes have been implicated in a wide variety of natural and pathological processes, including aging, cancer, diabetes, atherosclerosis, neurological degeneration, schizophrenia, and autoimmune disorders, such as arthritis (Betteridge, 2000) Oxidative stress can be derived from a variety of sources and includes events such as the production of reactive oxygen species by mitochondrial oxidative phosphorylation, ionizing radiation exposure, and metabolism of exogenous compounds (Shackelford, *et al*., 2000).

Hepatic abnormalities such as elevations of transaminases and alkaline phosphatase (ALP) are common in diabetes mellitus (Erbey *et al*., 2000, Vozarova *et al.,* 2002, Meybodi *et al.,* 2008, Leed *et al.,* 2009*).* The measurement of these enzymes and other biochemical markers such as albumin, total bilirubin in serum constitutes the liver function tests (Coates, 2011).

Plants are regarded as the pharmaceutical factories of natural origin for most of the drugs used by human beings. Plants medicines are highly important in the lives of human. As India is the largest producer and consumer of the medicinal drugs and is rightly called the botanical garden of the world (Dubey, *et al.*, 2000). Most of the plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan, *et al.,* 2006). Large number of primary metabolites acts as precursors of pharmacologically active metabolites in pharmaceutical compounds for the synthesis of drugs. Biotechnologically derived and synthesized medicines have renewed interest to pay attention on herbalism (Dev, 1997).

Herbal medicine (also known as traditional or indigenous medicine) comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as, the health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being (WHO,2008). The World Health Organization (WHO) estimated that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care (WHO, 2008). In Some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often called complementary and alternative medicine (WHO, 2008). They further describe medicinal plant as any plant in which one or more of its organ contains substances that can be used for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutics properties and constituents have been

established scientifically and plants that are regarded as medicinal but which have not yet been subjected to thorough investigation. Furthermore, WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Based on these definitions, medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (Harborne, 1973; Sofowora, 1996) which are presumably capable of ameliorating or curing disease conditions. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants. Tyler (1999) has reported that plants also contain certain other compounds that moderate the effects of the active ingredients.

D. cinerea belong to the family of fabaceae it is commonly referred to as sickle or sekelbos,Marabou-Thorn,Kalahari Christmas tree and Chainese lantern tree.in Zulu it is called Ugagane,umhezane and Umzilazembe (Pacific Island Ecosystem at Risk) while the local name in Hausa called (Dundu),Yoruba (Kara), Ibo (Ami ogwu).

It is widely distributed throughout Africa and has now encroached upon many overgrown bushveld region. Despite this undesirable ecological impact, it is a valuable medicinal plant (Van Wyk and Gericke , 2003)

D. cinerea trees are already recognised for their high efficient nitrogen fixation activity, uses in traditional medicine and a gum with potential for food and pharmaceutical application (Kambizi and Afolayan ,2001 Mbuna and Mhinzi 2003, Schulze et al 1991,) *D.cinerea* fruits are among the most abundant tree fruits that can be collected and stored for use late in the dry season (Kindness *et al.*, 1999) .

1.2 STATEMENT OF RESEARCH PROBLEM

In 1998, it was estimated that 143 million people worldwide suffer from diabetes (King *et al*, 1998), a figure five times more than the estimates ten years before. This number may probably doubled by the year 2030 (Harris *et al*, 1998). Despite the presence of known anti-diabetic medicines in the pharmaceutical market, diabetes and its related complications continue to be a major medical problem (Ji *et al*, 2006). The pathogenesis of diabetes mellitus and the possibility of its management by the oral administration of hypoglycaemic agents have stimulated greater interest in recent years (David, 2001). A number of plants have shown varying degrees of hypoglycaemic and anti-diabetic activities (Roman-Ramos *et al*, 1995; Grover *et al*, 2002).There are an estimated 285 million adults with diabetes in 2010; this number will continue to increase globally due to an aging population, growth of population size, urbanization and high prevalence of obesity and sedentary lifestyle (Shaw *et al* 2010). Diabetes leads to both premature death and complications such as blindness, amputations, renal disease, and cardiovascular diseases. Studies have shown that there is prevalence of lipid abnormalities in adults with diabetes. Patients with diabetes are likely to have cardiovascular problem and die from these problem (leiter, *et al.,* 2008). Most existing anti-diabetic drugs are toxic and expensive in Nigeria, many people use herbal remedies in treating diabetes.

1.3 JUSTIFICATION

The management of diabetes without any side effects is still a challenge to the medical system as the treatment for diabetes is relatively limited with significant side effects. There is growing interest in the use of natural health products as an alternative approach to current

medications. Plant sources have become a target to explore new drugs and in searching biologically active compounds (Yeh, 2003)

Due to side effects incurred such as weight gain, hypoglycaemia, lactic acidosis, hepatitis, liver damage and gastrointestinal tract disturbance in management of diabetes with synthetic drugs, there is need to search for anti-diabetic agents from natural product that will not have such side effects. Therefore, this work will be undertaken to investigate the antidiabetic antioxidant and hepatoprotective effects of *D. cinerea* (L.) Wight et Arn.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

The aim of this work was to investigate and compare the Antidiabetic, Antioxidant and Hepatoprotective effects of methanol extracts of different parts (Fruits, Leaves, Stem-bark and Roots) of *D. cinerea* (L.) Wight et Arn

1.4.2 Specific Objectives

The specific objectives of this study include:

i. Evaluation of antidiabetic properties of methanolic extract of different parts of *D. cinerea.* (L.) Wight et Arn. on streptozotocin induced diabetic albino rats
ii. Evaluation of antioxidant properties of methanolic extracts of different parts of *D. cinerea* (L.) Wight et Arn. on streptozotocin induced diabetic albino rats
iii. Evaluation of toxicity properties of extract of different parts of *D. cinerea* on albino rats

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorders manifested by abnormally high levels of glucose in the blood. The hyperglycaemia is the result of a deficiency of insulin secretion caused by pancreatic B-cell dysfunction or of resistance to the action of insulin in liver and muscle, or a combination of these.(Albert and Zimmet 2001) it is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2012). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2012)

2.1.1 Pathogenesis of Diabetes Mellitus

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues (Vinicor, 1998). Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. (ADA, 2012)

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome. (ADA, 2012)

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers (ADA, 2012). In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load (ADA, 2012).

The degree of hyperglycemia (if any) may change over time, depending on the extent of the underlying disease process. A disease process may be present but may not have progressed far enough to cause hyperglycemia. The same disease process can cause impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) without fulfilling the criteria for the diagnosis of diabetes (Ganda, 1995). In some individuals with diabetes, adequate glycemic control can be achieved with weight reduction, exercise, and/or oral glucose-lowering agents. These individuals therefore do not require insulin. Other individuals who have some residual insulin secretion but require exogenous insulin for adequate glycemic control can survive without it. Individuals with extensive β-cell destruction and therefore no residual insulin secretion require insulin for survival. The severity of the metabolic abnormality can progress, regress, or stay the same. Thus, the degree of hyperglycemia reflects the severity of the underlying metabolic process and its treatment more than the nature of the process itself. (ADA 2012)

2.1.2 Classification of Diabetes Mellitus

2.1.2.1 *Type 1 Diabetes Mellitus*

Immune-mediated diabetes. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes, type 1 Diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas (Dorman and Bunker, 2000). Markers of the immune destruction of the β-cell include islet cell autoantibodies, autoantbodies to insulin, auto antibodies to GAD (GAD65), and auto antibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these auto antibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These

HLA-DR/DQ alleles can be either predisposing or protective (Grubin et al., 1994; Nepom 1993; Verge et al., 1996). In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life (Mathis *et al.,* 1998).Autoimmune destruction of β–cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia (ADA, 2012).

Some forms of type 1 diabetes have no known etiologies. These are commonly referred to as idiopathic diabetes mellitus. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is

strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go (ADA, 2012).

2.1.2.2 *Type 2 Diabetes Mellitus*

This form of diabetes, which accounts for 90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur, and patients do not have any of the other causes of diabetes listed above or below (WHO, 1999).

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes (Sinha *et al.,* 2002 ,van Dam,2003). Nevertheless, such patients are at increased risk of developing macro-vascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β–cell function been normal. Thus, insulin secretion is

defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (Wild *et al*., 2004).

2.1.3 Other Specific Types of Diabetes

2.1.3.1 *Genetic Defects of the β-cell*

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern (Stride and Hattersley, 2002). Abnormalities at six genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the β-cell. Thus, glucokinase serves as the "glucose sensor" for the β-cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. The less common forms result from mutations in other transcription factors, including HNF-4α, HNF-1β, insulin promoter factor (IPF)-1, and NeuroD1 (; Demenais *et al*., 2003,Frayling *et al*., 2003, Kim *et al.,* 2004 ,Winter 2003).

2.1.3.2 *Genetic Defects in Insulin Action*

There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson-Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia (ADA, 2012).

2.1.3.3 *Drug- or Chemical-Induced Diabetes*

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency (Wylie-Rosett and Vinicor 2001, ADA, 2012).

2.1.3.4 *Viral Infections*

Certain viruses have been associated with β-cell destruction (Todd, 1999). Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease. (ADA, 2012).

2.1.4 Gestational Diabetes Mellitus

For many years, GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Although most cases resolve with delivery, the definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. This definition facilitated a uniform strategy for detection and classification of GDM, but its limitations were recognized for many years. As the ongoing epidemic of obesity and diabetes has led to more type 2 diabetes in women of child bearing age, the number of pregnant women with undiagnosed type 2 diabetes has increased (Coustan and Carpenter, 1998; Ganda, 1995, ADA 2012).

2.1.5 Categories of Increased Risk for Diabetes

In 1997 and 2003, The Expert Committee on Diagnosis and Classification of Diabetes Mellitus (Genuth, *et al.,* 2003) recognized an intermediate group of individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal. These people were defined as having impaired fasting glucose (IFG) [fasting plasma glucose (FPG) levels 100 mg/dl (5.6 mmol/l) to 125mg/dl (6.9mmol/l)], or impaired glucose tolerance (IGT) [2-h values in the oral glucose tolerance test (OGTT) of 140 mg/dl (7.8 mmol/l) to 199

mg/dl (11.0 mmol/l).Individuals with IFG and/or IGT have been referred to as having pre-diabetes, indicating the relatively high risk for the future development of diabetes. IFG and IGT should not be viewed as clinical entities in their own right but rather risk factors for diabetes as well as cardiovascular disease. IFG and IGT are associated with obesity (especially abdominal or visceral obesity), dyslipidemia with high triglycerides and/or low HDL cholesterol, and hypertension. Structured lifestyle intervention, aimed at increasing physical activity and producing 5–10% loss of body weight, and certain pharmacological agents have been demonstrated to prevent or delay the development of diabetes in people with IGT; the potential impact of such interventions to reduce mortality or the incidence of cardiovascular disease has not been demonstrated to date.As A1C is used more commonly to diagnose diabetes in individuals with risk factors, it will also identify those at higher risk for developing diabetes in the future. When recommending the use of the A1C to diagnose diabetes in its 2009 report, the International Expert Committee stressed the continuum of risk for diabetes with all glycemic measures and did not formally identify an equivalent intermediate category for A1C. The group did note that those with A1C levels above the laboratory "normal" range but below the diagnostic cut point for diabetes (6.0 to 6.5%) are at very high risk of developing diabetes. Indeed, incidence of diabetes in people with A1C levels in this range is more than 10 times that of people with lower levels (Edelman *et al*., 2004; Pradhan *et al*., 2007; Sato *et al.*, 2009; Shimazaki *et al*., 2007). However, the 6.0 to 6.5% range fails to identify a substantial number of patients who have IFG and/or IGT. Prospective studies indicate that people within the A1C range of 5.5–6.0% have a 5-year cumulative incidence of diabetes that ranges from 12 to 25% (Edelman *et al.*, 2004; Pradhan *et al.*, 2007; Sato *et al*., 2009; Shimazaki *et al*., 2007), which is appreciably (three- to eightfold) higher than incidence in the U.S. population as a whole (Geiss *et al*., 2006). Analyses of nationally representative data from the National Health and Nutrition

Examination Survey (NHANES) indicate that the A1C value that most accurately identifies people with IFG or IGT falls between 5.5 and 6.0%. In addition, linear regression analyses of these data indicate that among the nondiabetic adult population, an FPG of 110 mg/dl (6.1 mmol/l) corresponds to an A1C of 5.6%, while an FPG of 100 mg/dl (5.6 mmol/l) corresponds to an A1C of 5.4%. Finally, evidence from the Diabetes Prevention Program (DPP), wherein the mean A1C was 5.9% (SD 0.5%), indicates that preventive interventions are effective in groups of people with A1C levels both below and above 5.9% (Knowler *et al*., 2002). For these reasons, the most appropriate A1C level above which to initiate preventive interventions is likely to be somewhere in the range of 5.5–6%.as was the case with FPG and 2-h PG, defining a lower limit of an intermediate category of A1C is somewhat arbitrary, as the risk of diabetes with any measure or surrogate of glycemia is a continuum, extending well into the normal ranges. To maximize equity and efficiency of preventive interventions, such an A1C cut point should balance the costs of "false negatives" (failing to identify those who are going to develop diabetes) against the costs of "false positives" (falsely identifying and then spending intervention resources on those who were not going to develop diabetes anyway).Compared to the fasting glucose cut-point of 100 mg/dl (5.6 mmol/l), an A1C cut point of 5.7%is less sensitive but more specific and has a higher positive predictive value to identify people at risk for later development of diabetes. A large prospective study found that a 5.7% cut point has a sensitivity of 66% and specificity of 88% for the identification of subsequent 6-year diabetes incidence (Droumaguet *et al*., 2006). Receiver operating curve analyses of nationally representative U.S. data (NHANES 1999-2006) indicate that an A1C value of 5.7% has modest sensitivity (39-45%) but high specificity (81-91%) to identify cases of IFP (FPG .100 mg/ dl) (5.6 mmol/l) or IGT (2-h glucose . 140 mg/dl). Other analysis suggests that an A1C of 5.7% is associated with diabetes risk similar to the high-risk participants in the DPP. Hence, it is reasonable to consider an

A1C range of 5.7 to 6.4% as identifying individuals with high risk for future diabetes and to whom the term pre-diabetes may be applied if desired. Individuals with an A1C of 5.7–6.4% should be informed of their increased risk for diabetes as well as cardiovascular disease and counseled about effective strategies, such as weight loss and physical activity, to lower their risks. As with glucose measurements, the continuum of risk is curvilinear, so that as A1C rises, the risk of diabetes rises disproportionately. Accordingly, interventions should be most intensive and follow-up should be particularly vigilant for those with A1C levels above 6.0%, who should be considered to be at very high risk. However, just as an individual with a fasting glucose of 98 mg/dl (5.4 mmol/l) may not be at negligible risk for diabetes, individuals with A1C levels below 5.7% may still be at risk, depending on level of A1C and presence of other risk factors, such as obesity and family history. (ADA, 2012).

2.1.6 Diagnostic Criteria for Diabetes Mellitus

For decades, the diagnosis of diabetes has been based on glucose criteria, either the FPG or the 75-g OGTT. In 1997, the first Expert Committee on the Diagnosis and Classification of Diabetes Mellitus revised the diagnostic criteria, using the observed association between FPG levels and presence of retinopathy as the key factor with which to identify threshold glucose level (Vinicor, 1998). The Committee examined data from three cross-sectional epidemiologic studies that assessed retinopathy with fundus photography or direct ophthalmoscopy and measured glycemia as FPG, 2-h PG, and A1C. These studies demonstrated glycemic levels below which there was little prevalent retinopathy and above which the prevalence of retinopathy increased in an apparently linear fashion. The deciles of the three measures at which retinopathy began to increase were the same for each measure within each population. Moreover, the glycemic values above which retinopathy increased were similar among the populations. These analyses helped to inform a new diagnostic cut

point of ≥126 mg/dl (7.0 mmol/l) for FPG and confirmed the long-standing diagnostic 2-h PG value of ≥200 mg/dl (11.1 mmol/l) (ADA,2012)

2.1.7. Diagnosis of Gestational Diabetes

Gestational Diabetes Mellitus (GDM) carries risks for the mother and neonate. The Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study (Metzger *et al*., 2008), a large-scale (~25,000 pregnant women) multinational epidemiologic study, demonstrated that risk of adverse maternal, fetal, and neonatal outcomes continuously increased as a function of maternal glycemia at 24-28 weeks, even within ranges previously considered normal for pregnancy. For most complications, there was no threshold for risk. These results have led to careful reconsideration of the diagnostic criteria for GDM. After deliberations in 2008-2009, the IADPSG international association of the diabetes and pregnancy study group, an international consensus group with representatives from multiple obstetrical and diabetes organizations, including ADA, developed revised recommendations for diagnosing GDM. The group recommended that all women not known to have diabetes undergo a 75-g OGTT at 24-28 weeks of gestation. Additionally, the group developed diagnostic cutpoints for the fasting, 1-h, and 2-h plasma glucose measurements that conveyed an odds ratio for adverse outcomes of at least 1.75 compared with women with mean glucose levels in the HAPO study (Metzger *et al*., 2010).

Admittedly, there are few data from randomized clinical trials regarding therapeutic interventions in women who will now be diagnosed with GDM based on only one blood glucose value above the specified cutpoints (in contrast to the older criteria that stipulated at least two abnormal values). Expected benefits to their pregnancies and offspring is inferred from intervention trials that focused on women with more mild hyperglycemia than identified using older GDM diagnostic criteria and that found modest benefits (Landon *et al*., 2009)

2.1.8 Oxidative Stress and Diabetic Complications

Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. The metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium. This increased superoxide production causes the activation of 5 major pathways involved in the pathogenesis of complications: polyol pathway flux, increased formation of AGEs (advanced glycation end products), increased expression of the receptor for AGEs and its activating ligands, activation of protein kinase C isoforms, and overactivity of the hexosamine pathway. It also directly inactivates 2 critical antiatherosclerotic enzymes, endothelial nitric oxide synthase and prostacyclin synthase. Through these pathways, increased intracellular reactive oxygen species (ROS) cause defective angiogenesis in response to ischemia, activate a number of proinflammatory pathways, and cause long-lasting epigenetic changes that drive persistent expression of proinflammatory genes after glycemia is normalized ("hyperglycemic memory"). Atherosclerosis and cardiomyopathy in type 2 diabetes are caused in part by pathway-selective insulin resistance, which increases mitochondrial ROS production from free fatty acids and by inactivation of antiatherosclerosis enzymes by ROS. Overexpression of superoxide dismutase in transgenic diabetic mice prevents diabetic retinopathy, nephropathy, and cardiomyopathy. The majority of publications regarding the mechanisms underlying hyperglycemia-induced diabetic vascular damage focus on the 5 major mechanisms indicated above (Giacco and Brownlee, 2010, Ramasamy and Goldberg, 2010).

2.1.9 Management of Diabetes Mellitus

2.1.9.1 *Sulphonylureas*

Sulphonylureas have been extensively used for the treatment of type 2 diabetes for nearly 50 years. They lower blood glucose concentrations primarily by stimulating insulin secretion from the ß cells of the pancreatic islets. By the 1960s several sulphonylureas were available, including tolbutamide, acetohexamide, tolazamide and chlorpropamide, offering a range of pharmacokinetic options. However, doubts about safety were raised in the 1970s. A large US multicentre trial of antidiabetic therapy, the UGDP (University Group Diabetes Program) (Seltzer, 1972) reported apparent detrimental cardiovascular effects of tolbutamide. The UGDP was heavily criticised for perceived methodological failings and its findings were far from being universally accepted. Subsequent observational and randomised clinical studies using sulphonylureas have provided mixed evidence, but a review of the available literature provides little in the way of convincing evidence of cardiovascular toxicity (Krentz, 2003)

A succession of more potent so-called second-generation sulphonylureas emerged in the 1970s and 1980s, for example glibenclamide (glyburide), gliclazide and glipizide. The latest, glimepiride, was introduced in the late 1990s (Evans and Krentz, 1999)

2.1.9.2. α-glucosidase inhibitors

Inhibitors of intestinal α-glucosidase enzymes retard the rate of carbohydrate digestion, thereby providing an alternative means to reduce postprandial hyperglycaemia (Lebovitz, 1998) Acarbose, the first α-glucosidase inhibitor to be marketed, was introduced in the early 1990s. Recently, two additional agents, miglitol and voglibose, have been introduced in some countries (Krentz and Bailey, 2001) The α-glucosidase inhibitors do not cause weight gain, can reduce postprandial hyperinsulinaemia and have lowered plasma triglyceride

concentrations in some studies (Lebovitz, 1998).Their good safety record is a further advantage, but limited gastrointestinal tolerability has substantially limited their use. The relatively high cost of α-glucosidase inhibitors is another consideration that has influenced prescribing. In the UK, acarbose use remains low.

2.1.9.3 *Meglitinides*

Mechanistically the meglitinides are similar to the sulfonylureas. They interact with binding sites on ATP-dependent potassium channels in the beta-cell membrane. These sites are distinct from those involved in sulfonylurea binding. The binding of the meglitinides results in potassium channel blockade which depolarizes the beta cell and leads to an opening of calcium channels. The resulting increased calcium influx induces insulin secretion. Thus the actions of repaglinide are dependent upon functional beta cells in the pancreatic islets. The ion channel binding mechanism of these drugs is highly tissue selective with low affinity for heart and skeletal muscle. Some studies suggest that nateglinide may have a "faster-on/faster-off" action and thus a lesser effect at lower glucose concentrations. (Krentz and Bailey, 2004).

2.1.9.4 *Insulin Sensitisers*

Insulin resistance is a prominent metabolic defect in most patients with type 2 diabetes (Reaven 1988; Krentz, 2002). Defective insulin action is not confined to glucose metabolism, subtle defects also being demonstrable in the regulation of other aspects of intermediary metabolism (e.g. lipolysis), using appropriate investigative techniques. Many cross-sectional and prospective studies have implicated insulin resistance in the pathogenesis of type 2 diabetes and the related metabolic Syndrome of cardiovascular risk. (Ginsberg, 2000). Therefore, defective insulin action at target tissue level is an attractive therapeutic target in

type 2 diabetes. The biguanides and, in particular, the thiazolidinediones act directly against insulin resistance, and so are regarded as insulin sensitising drugs. (Krentz and Bailey, 2005).

2.1.9.5 *Biguanides*

The finding that *Galega officinalis* (goat's rue or French lilac), historically used as a traditional treatment for diabetes in Europe, was rich in guanidine led to the introduction of several glucose-lowering guanidine derivatives in the 1920s. These early antidiabetic agents were all but forgotten as insulin became widely available and it was not until the late 1950s that three antidiabetic biguanides were reported: metformin, phenformin and buformin. Phenformin was withdrawn in many countries in the 1970s because of a high incidence of lactic acidosis; buformin received limited use in a few countries, leaving metformin as the main biguanide on a global basis. Metformin is the only biguanide available in the UK and, since 1995, the US. Extensive clinical experience with metformin has been complemented by favourable results from the UKPDS. Metformin also enjoys the accolade of being among the least expensive of the oral antidiabetic agents. (Krentz *et al,* 1994; Bailey and Turner, 1996)

2.1.9.6 Thiazolidinediones

Thiazolidinediones improve whole-body insulin sensitivity via multiple actions on gene regulation. These effects result from stimulation of a nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ), for which thiazolidinediones are potent synthetic agonists (Day, 1999). The antidiabetic activity of thiazolidinediones was described in the early 1980s, troglitazone being the first of the class to become available for clinical use. Troglitazone was introduced in the US in 1997, only to be withdrawn in 2000 because of cases of idiosyncratic hepatotoxicity resulting in fatalities. Troglitazone was available in the

UK for only for a few weeks in 1997 before being withdrawn by its distributor as reports of hepatotoxicity accumulated in other countries. To date, two other thiazolidinediones, rosiglitazone and pioglitazone, have not shown the hepatotoxicity that led to the demise of troglitazone. Rosiglitazone and pioglitazone were introduced in the US in 1999 and in Europe in 2000 (Krentz *et al*., 2000) Combination preparations (e.g. thiazolidinedione plus metformin) are also available.

2.2. THE PLANT *D. CINEREA* (L.) WIGHT ET ARN

D. cinerea is a small tree or strongly growing shrub which grows up to eight meters in height and has an untidy growth pattern with thorny branches. It forms impenetrable thickets in overgrazed bushveld (Van Wyk and Gericke, 2003). The feather like leaves show similarities with the leaves of the Acacia species. The characteristic flowers hang in clusters from branches. The curved and coiled seedpods are borne in clusters on long stalks.

D. cinerea (L.) Wight et Arn is a semi-deciduous to deciduous tree up to 7 m tall with an open crown. Bark on young branches green and hairy but dark grey-brown and longitudinally fissured on older branches and stems; smooth on spines formed from modified side shoots. Slash cream coloured to light yellow. Strong alternate thorns, up to 8 cm long, almost at right angles, slightly re curved, grow out of the branches and may bear leaves at the base. Twigs grey brown violet, with prominent light lenticels. Leaves bi pinnate; rachis 4-8 cm, with 5-15 (max. 19) pairs of pinnae, which each bear (min. 9) 12-22 (max. 41) pairs of leaflets; terminal pair of pinnae shorter, dark green, underside pale. Leaflets about 8 x 2.5 mm wide; leaflets and petioles very tomentose and ciliate (Orwa, *et al*., 2009).

2.2.1 Taxonomy of *D. cinerea* (L.) Wight Et Arn

Kingdom	Plant
Order	Fabales
Family	Fabaceae mimosaceae
Genus	Dichrostachys
Species	D.cinerea

FIGURE: I Plant of D.cinerea (L.) Wight et Arn en.wikipedia.org
(540 x 600)

25

FIGURE: II leaves of D. cinerea (L.) Wight et Arn www.mountmorelandconservancy.co.za (219 x 245)

26

FIGURE: III Fruits of D. Cinerea *(L.) Wight et Arn www*.biodiversityexplorer.org (658 x 445)

27

2.2.2 Phytochemistry of *D.cinerea*

Phytochimical studies performed on *D. cinerea* (L.) Wight et Arn extracts have revealed the presence of tannins, sterols and triterpenes, of reductionist compounds, polyphenols, flavonoids as well as of cardiotonic heterosides (Tillement and Albengres, 1977).

Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001 and Cai *et al.*, 2003)

Phytochemical analysis revealed the presence of glycosides in the methanolic extract ,have synthesized and used antioxidants such as (-)-mesquitol from natural sources (*D. cinerea*), the compound demonstrated antioxidant,activity (Hughes *et al* ., 2005)

2.2.3 Medicinal Uses of *D. Cinerea* (L.) Wight et Arn

The Haikum Bushmen of Namibia chews the fresh leaves to treat diarrhoea, toothaches and earache (Van Wyk and Gericke, 2003). It is also applied directly to snakebites (Van Wyk *et al.*, 2002). Extracts of the leaves and bark, as well as powdered bark are used for wound healing (Van Wyk *et al.*, 2002). Infusions of the roots are used to treat abdominal pain, coughs and pneumonia (Van Wyk and Gericke, 2003). Powdered roots are sniffed to curb nose bleeds, whilst the leaves and roots are smoked to relieve head colds, and to treat tuberculosis and for treatment of epilepsy (Van Wyk and Gericke, 2003).

In Sudan, it is used for the treatment of wounds (Eisa *et al.*, 2000) and in Zimbabwe it is frequently used for the treatment of sexually transmitted diseases (Kambizi and Afolayan, 2001).The bark is used to treat dysentery, headaches, toothaches, elephantiasis and acts as a vermifuge. Root infusions are taken for leprosy, syphilis coughs, as an anthelmintic, purgative and strong diuretic. Pounded roots and leaves are used to treat epilepsy. The roots

are chewed and placed on the sites of snakebites and scorpion stings, and the leaves, which are believed to produce a local anaesthesia, are used for the same purpose and also as a remedy for sore eyes and toothache. Leaves are taken as a diuretic and laxative, and used for gonorrhoea and boils; powder from leaves is used in the massage of fractures. The plant is used as a veterinary medicine in India (Bein, 1996).

In Ivory Coast traditional medicine, air-dried powdered stem bark of *Dichrostachys cinerea* is used by inhalation for the treatment of this airways affection (Quattara, 2007). is also used for the treatment of wounds, rheumatism and renal troubles (Eisa Almagboul 2000). Pharmacological report on *D. cinerea* has shown antibacterial effect. (Kambizi and Afolayan 2001) and antiviral. Several authors have shown that the species inhibit protein farnesyl-transferase activity (Jagadeeshwar , Ashok K 2003). Moreover, chemical studies revealed the presence of a new isomer of mesquitol (a main active principle), which showed free-radical scavenging property and a-glucosidase inhibitory activities. (Achidi and Kinnoudo 2008)

Toxicity studies of the methanol extract was evaluated as per OECD guideline 423. The methanol extract was suspended in 0.5% w/v sodium carbomethyl cellulose in distilled water. The animals were fasted overnight, provided only water, after which the methanol extract was separately administered orally at the dose level of 5mg/kg and the animal were oberserved carefully and continuously for any behavioural changes for 72h and for mortality if any up to a period of 14 days ,since no mortality was observed in any animal, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg. The animals were observed for toxic symptoms such as behavioural changes, locomotion, and mortality for 72h (Patel *et. al.*, 2009)

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1Chemicals

Streptozotocin (streptozotocin) and Glibenclamide were purchased from Sigma chemical Company St. Louis U.S.A. and used before the expiry date. All other chemicals and reagents used were of analytical grade

3.1.2 Animals

Male Wistar strain albino rats weighing between 150 -200g were obtained from the animal house, Department of Pharmacology, Ahmadu Bello University, (ABU) Zaria. The animals were kept and maintained in well ventilated cages. Rats were maintained on grower's mash (Vital Feeds Nigeria Ltd) and provided with water *ad libitum.* They were allowed to acclimatize to the laboratory conditions before the treatment.

3.2 METHODOLOGY

3.2.1 Plant Sample Collection and Preparation

The different parts of the plant (Fruits, Leaves, Stem-barks, and Roots) of *D.cinerea* (L.) Wight et Arn were collected from Jangefe villege, Katsina State, and identified at the herbarium unit, Department of Biological Sciences ABU Zaria. The samples were washed with water, shade-dried and then pulverised using mortar and pestle. The powdered samples were stored in airtight containers and properly labelled**.**

3.2.2 Extraction of Plants Parts of *D.cinerea* (L.) Wight Et Arn

The dried 350g plant material of the different parts (roots, stem bark ,fruits and leaves) of plant materials were weighed accurately and placed in soxhlet extraction chamber which suspended above the flask containing 1000cm^3 of 80% methanol and below a condenser.
At the end of the extraction process, the flask containing the methanol extract was removed and evaporated by using rotary evaporator (Harborne, 1973).

3.2.3 Preliminary Phytochemical Screening

3.2.3.1 *Test for Carbohydrates*.

Molisch's Test: Few drops of Molisch reagent was added to a little quantity of each sample in a test tube and a small quantity of concentrated sulphuric acid was allowed to run down the side of the test tube. A purple to violet colour at the interface indicates the presence of carbohydrates (Trease and Evans, 1983).

*3.2.3.2 Test for Cardiac Glycosides***.**

Kella Killiani Test: Each sample was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45 degree, 1 cm^3 of concentrated sulphuric acid was added down the side. A purple ring colour at the interface indicates the presence of cardiac glycosides (Trease and Evans, 1983).

3.2.3.3 Test for Saponins.

Frothing test: Small quantity of extracted sample was dissolved in 10cm^3 of distilled water. This was then shaken vigorously for 30 seconds and was allowed to stand for 30 minutes. A

honey comb formed for more than 30 minutes indicates the presence of saponin (Trease and Evans, 1983).

3.2.3.4 *Test for Steroid and Triterpenes (Liberman-Burchards Test)*

Equal volume of acetic anhydride was added to each sample. Concentrated sulphuric acid (1 cm^3) was added down side the tube. The colour change was observed immediately and later. Red, pink or purple colour indicates the presence of triterpenes while blue or blue-green indicate steroids (Trease and Evans, 1983).

3.2.3.5 *Test for Flavonoids*

Sodium Hydroxide Test: Few drops of aqueous NaOH were added to 5 cm^3 of each sample, a yellow colouration shows the presence of flavanoid (Trease and Evans, 1983).

3.2.3.6 *Test for Tannins*.

Ferric Chloride Test: Extract sample of 0.5 cm^3 was dissolved in 10 cm^3 of distilled water, and then filtered. Few drops of ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicates hydrolysable tannins and green precipitates indicate the presence of condensed tannins (Trease and Evans, 1983).

3.2.3.7 Test for Alkaloids.

Picric Acid Test: Few drops of 1% picric acid solution were added to each sample. Yellow coloured solution indicates alkaloids (Trease and Evans, 1983).

Tannic Acid Test: Few drops of tannic acid were added to each sample. Black precipitate indicates the presence of alkaloids (Sofowora, 1982).

3.2.4 Animals Grouping and Treatment

A total of 60 rats divided into 12 groups of 5 rats each was used in this study. The groups will be as follows:

Group 1:Non-diabetic rats without any treatment (Normal control)

Group 2:Diabetic rats without treatment (Negative control)

Group 3:Diabetic rats treated with roots crude extract

Group 4:Diabetic rats treated with fruit crude extract

Group 5:Diabetic rats treated with leaves crude extract

Group 6:Diabetic rats treated with stem-bark crude extract

Group 7: Diabetic rats treated with Glinbenclamide (Positive)

Group 8:Non-diabetic rats treated with fruit crude extract

Group 9:Non-diabetic rats treated with leaves crude extract

Group 10:Non-diabetic rats treated with stem-bark crude extract

Group 11:Non-diabetic rats treated with roots crude extract

Group12: Non-diabetic rats treated with Glinbenclamide

3.2.5 Induction of Diabetes

Diabetes Mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight of streptozotocin, dissolved in 0.1M fresh cold citrate buffer, (pH 4.5) into overnight fasted rats (Burcelin *et al.,* 1995).

3.2.6 Dose

100mg/kg body weight for the treatment was selected based on acute oral toxicity studies of the methanol extract evaluated as per OECD guideline 423, according to (Kumar *et al* 2013).

3.2.6 Treatment

The extracts were orally administered to the rats daily for a period of 21days, while blood glucose and body weights were estimated weekly. The rats were fasted overnight, anaesthetized with chloroform and sacrificed 24 hours after the last treatment.

3.2.7 Blood Collection and Preparation of Samples

After 21 days, of the treatment, the rats were sacrificed, their blood collected in non-heparinized tubes and allowed to stand for 20 mins to clot ,they were then centrifuged at 3,500rpm for about 15 minutes.The serum obtained was then used for analysis. Storage was done at 4ºC to maintain enzyme activity. Heart, Liver and kidney were collected for histopathological study.

2.3 BIOCHEMICAL ANALYSIS

2.3.1 Blood Glucose Determination

The fasting blood glucose was determined as described by Clark and Lyons, (1962) using glucometer. The percentage of change in this parameter was calculated by applying the following formula proposed by Jiménez *et al.* (1986):

% change of glucose= $(G_x\text{-}G_i/G_i) \times 100$

Where G_x is the glycaemia at time x and G_i is the glycaemia at initial time.

3.3.2 Determination Packed Cell Volume (PCV)

The PCV was determined using heparinized capillary tubes. Blood was collected by a slight cut on the tail tip using a razor blade, into heparinized capillary tubes. They were then centrifuged at 3,000rpm for 10mins. The measurement of PCV (%) was done using microhematocrit method (Schalm *et al.*, 1975).

3.4 ESTIMATION OF SERUM ANTIOXIDANT ENZYMES

3.4.1 Superoxide Dismutase (SOD) Activity

The superoxide dismutase activity was determined by the method of Misra and Fridovich (1972).

3.4.1.1 *Principle* is based on the ability of SOD to inhibit the auto oxidation of epinephrine in alkaline pH

3.4.1.2 *Procedure*

The tissue homogenate was centrifuged at 3,500rpm for 20mins and 1.0 cm^3 of the supernatant was diluted in 9.0 cm^3 of water to make a 1.0 cm^3 in ten dilutions. An aliquot of 0.2 cm^3 of the diluted tissue supernatant was added to 2.5 cm^3 of 0.05M phosphate buffer pH 10.2 to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 cm^3 of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette will contain 2.5 cm^3 of phosphate buffer, 0.3 cm^3 of substrate (adrenaline) and 0.2 cm^3 of water. The increase in absorbance at 480nm was monitored every 30secs for 150secs.

3.3.1.2 Calculation

Increase in absorbance per minute = $\frac{A_3 - A_O}{2.5}$

A_O = absorbance after 30secs

A_3 = absorbance after 150secs

% inhibition = $\frac{\text{increase in absorbance for substrate} \times 100}{\text{increase in absorbance of blank}}$

1 unit of SOD activity will be given as the amount of SOD that causes 50% inhibition of the oxidation of adrenaline to adenochrome during 1 minute.

3.4.2 Determination of Catalase Activity of Samples

Principle: Catalase activity was determined by following the decomposition of H_2O_2 according to Sinha (1971).

The homogenized tissue was centrifuged at 3,500 rpm for 20 mins and 1.0 cm^3 of the supernatant was mixed with 49.0 cm^3 of distilled water to give a 1.0 in 50 dilution of the tissue homogenate.

The assay mixture contained 4.0 cm^3 of H_2O_2 (800 µmoles) solution and 5.0 cm^3 of phosphate buffer in a conical flask. Properly diluted enzyme preparation (1.0cm^3) was rapidly mixed with the reaction mixture by a gentle swirling motion. The reaction was run at room temperature. A 1.0 cm^3 portion of reaction mixture was withdrawn and blown into 2.0 cm^3 dichromate/acetic acid reagent at 60 secs intervals. The H_2O_2 content of the withdrawn sample was determined by taking the absorbance at 570 nm.

3.4.3 Estimation of Lipid Peroxidation

Lipid peroxidation was estimated spectrophotometrically by (Varshney and Kale 1990) and was expressed in terms of malondialdehyde (MDA) formed per mg protein.

3.4.3.1 Principle. At low pH of 2-3 and high temperature (100^0C), malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex, which absorbs maximally at 532 nm.
3.4.3.2 Procedure: Homogenized tissue was centrifuged at 3,500 rpm for 20 mins ,Supernatant (0.4 cm^3) was mixed with 1.6 cm^3 of 0.15M tris KCl buffer to which 0.5 cm^3 of 30% TCA has been added. Then 0.5 cm^3 of 52mM TBA was added and was placed in a water bath for 45min at 80^oC. It will then be cooled in ice and centrifuged at room temperature for 10min at 3000rpm. The absorbance of the clear supernatant will then be measured against reference blank of distilled water at 532nm.

3.4.3.3 Calculation

$$\text{Concentration} = \frac{\text{Absorbance}}{\text{Molar Absorptivity}}$$

Molar Absorptivity = 1.56×10^{5} $LMcm^{-1}$

3.5 ESTIMATION OF EFFECT OF THE EXTRACTS ON LIVER

3.5.1 Aspartate Amino Transferase (AST)

AST was determine using the method of Reitman and frankle(1957)

3.5.1.1 *Principle* is based on monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine.The maximum absorbance at 456nm is proportional to the concentration of AST in the sample.

3.5.2 Alanine Amino Transferase (ALT)

ALT was determined according to the method of Reitman and Frankel(1957) as described by Sini *et al*., (2006).

3.5.2.1 *Principle* Is based on the monitoring of the concentration of pyruvate hydrazone formed with 2.4-dinitrophenyl hydrazine which absorbs maximally at 546nm.The absorbace is proportional to the concentration of ALT in the sample.

3.5.2.2. *Procedure* The assay mixture containing 0.2 cm^3 of sample fraction and 0.5 cm^3 of substrate solution (for AST: aspartate and α-ketoglutarate; for ALT: alanine and α-ketoglutarate) was incubated at 37^oC for 60 min for AST and 30min for ALT. After incubation, 0.5 cm^3 of DNPH solution was added to arrest the reaction, which was kept for 20min at room temperature. To this, 1.0 cm^3 of 0.4N NaOH was added and absorbance was read at 510nm. Activities were expressed as IU/L

3.5.3 Alkaline Phosphatase (ALP)

Activity of alkaline phosphatase (ALP) was determined by a standard method described by Haussament (1977) using Randox® assay kit (Randox Laboratories Limited UK)

3.5.3.1 *Principle:* Alkaline phosphatase is measured by monitoring the concentration of phosphate hydrazone formed with 2, 4-dinitrophenylhydrazine at 405 nm and 37^0C.

$$\text{P-nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{Phosphate} + \text{P-nitrophenol}$$

3.5.3.2 *Procedure:* Micro-pipette was used, exactly 0.01 cm^3 of test serum was dispensed into a 1 cm^3 cuvette followed by the addition of 0.05 cm^3 of the reagent (prethanolamine buffer, $MgCl_2$, p-nitrophenylphosphate). The absorbance of the mixture was immediately taken using a colorimeter at a wavelength of 405 nm, and the timer started simultaneously as the initial absorbance was been recorded. The absorbance was read again at 1, 2, and 3 min. The initial absorbance reading was subtracted from the absorbance readings at 1, 2 and 3 mins (i.e. $A_{1/2/3} - A_{initial}$). The average of the three values obtained was then computed and used to calculate the ALP activity using the formula:

ALP (U/I) = 2760 x ΔA at 405nm/min

3.5.4 Determination of Serum Total Protein (TP) Concentration

The serum TP concentration was determined using the Randox® kit (Randox Laboratories Limited UK) based on the method described by Fine *et al*. (1935).

3.5.4.1 *Principle:* The protein assay is based on a two step reaction of protein with an alkaline copper tartarate solution and Folin reagent leading to colour development. First is the reaction between protein and copper at alkaline pH, and subsequently, the reduction of Folin reagent by the copper-treated protein. Colour formation is due to amino acids that reduce the Folin reagent, yielding reduced species that imparts a characteristic blue colour.

3.5.4.2 *Procedure:* Micro-pipette was used, exactly, 0.02 cm^3 of distilled water, 0.02 cm^3 of standard and 0.02 cm^3 of test serum were dispensed into the test tubes labelled reagent blank, standard and sample respectively, 1 cm^3 of the Biuret reagent was then added to each of the test tubes and the mixture incubated at 25°C for 30 mins in a water bath. After the period of incubation, the absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) were then

read against the reagent blank using a colorimeter at a wavelength of 546 nm and the values recorded. The total protein concentration was then calculated using the formula:

$$\text{Total protein concentration (g/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{concentration of standard}$$

3.5.4 Determination of Serum Albumin (ALB) Concentration

The serum ALB concentration was determined using the Randox® kit (Randox Laboratories Limited UK) based on the method described by Doumas *et al*., (1971).

3.5.4.1 *Principle:* The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3 ' 5, 5' – tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The absorbance of the blue green colour of albumin-BCG-complex at 630 nm is directly proportional to the concentration of albumin in the sample.

3.5.4.2 *Procedure:* Bromocresol green (BCG) 3 cm^3 was dispensed into each of the tubes labelled reagent blank, standard and sample containing 0.01 cm^3 distilled water, 0.01 cm^3 standard albumin and 0.01 cm^3 test serum respectively. The mixture was then incubated for 5 mins at 25°C in a water bath. After the incubation, the absorbance of the sample (A_{sample}) and that of the standard ($A_{standard}$) against the reagent blank were read using a colorimeter at a wavelength of 578 nm. The values obtained were used to calculate the albumin concentration using the formula:

$$\text{Albumin concentration (g/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{concentration of standard}$$

3.5.5 Determination of Serum Total Bilirubin (TBL) Concentration

Serum total bilirubin concentration was determinaed by the methods of Jendrassik and Grof, (1938) and Sherlock (1951) using Randox® kit (Randox Laboratories Limited UK).

3.5.5.1 *Principle:* Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid.

3.5.5.2 *Procedure:* Micro-pipette was used ,exactly, 2 cm^3 of reagent 1 , 29mmol/L of 0.17N (sulphanilic acid) was pipette each into two different test tubes labelled "sample blank" and "sample" followed by the addition of 1 drop (50 μl) of reagent 2, 25mmol/L (nitrite) and the 10 cm^3 of reagent 3 0.26mol/L(caffeine). Two hundred microlitre of the test serum was then dispensed into each of the tubes and the mixture incubated in a water bath for 10 min at 25°C. This was followed by the addition of 10cm^3 of reagent 4, 0.52mol/L (tartrate) and the mixture incubated again at 25°C for 10 mins. The absorbance of the sample (A_{TB}) was then read against the sample blank using a colorimeter at 578 nm wavelength. The total bilirubin concentration was calculated thus:

Total bilirubin (mg/dl) = 10.8 x A_{TB} (578nm)

3.6 ESTIMATION OF KIDNEY FUNCTION PARAMETERS

3.6.1 Determination of Creatinine Concentrarion

The serum creatinine concentration was determined by the method described by (Bartels and Bohmer 1973) using Randox® kit (Randox Laboratories Limited UK).

3.6.1.1 Principle: Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is proportional to the creatinine concentration.

3.6.1.2 Procedure: Exactly, 0.1 cm^3 of creatinine standard solution and 0.1 cm^3 of test serum were dispensed into separate cuvettes labelled standard and sample respectively. This was followed by addition of 1.0 cm^3 of the working reagent (picric acid + NaOH). The absorbances of the mixtures were then taken after 30 secs (A_1) and at exactly 2 minutes later (A_2) using a colorimeter at 492 nm wavelength. The ΔA of the sample or standard was calculated by subtracting A_2 from A_1 i.e. $A_2 - A_1 = \Delta A$ of sample or standard. The values obtained were used for calculating the serum creatinine concentration with the aid of the formula:

$$\text{Creatinine concentration (mg/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{concentration of standard}$$

3.6.2 Determination of Urea Concentration

The serum urea concentration was determined using the Randox® kit (Randox Laboratories Limited UK) based on the method of Berthelot's reaction (Fawcett and Scout, 1960).

2.6.2.1 Principle: Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured spectrophotometrically.

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

$$NH_3 + \text{hypochlorite} + \text{phenol} \longrightarrow \text{indophenols (blue compound)}$$

2.6.2.2 Procedure: Exactly, 1cm^3 of reagent 1 (sodium nitroprusside [6 mmol/l] + urease [1 g/l]) was pipette into three separately labelled test tubes: reagent blank, standard and sample containing 0.1 cm^3 of distilled water, 0.1 cm^3 urea standard and 0.1 cm^3 test serum respectively. The mixture was incubated at 37°C for 10 mins in a water bath. After the period of incubation 2.5 cm^3 of reagent 2 (phenol [120 mmol/l]) was added to the contents of each test tube followed by 2.5 ml of reagent 3 (Sodium hypochlorite [27 mmol/l]). The mixture was incubated for 15 minutes at 37°C in a water bath. The absorbance of the sample (A_{sample})

and that of the standard ($A_{standard}$) were read against the reagent blank using a colorimeter at wavelength of 546 nm and the readings obtained were used for the calculation of the serum urea concentration with the aid of the formula:

$$\text{Urea concentration (mg/dl)} = \frac{A_{sample}}{A_{standard}} \times \text{concentration of standard}$$

3.6.3 Histopathological Studies:

3.6.3.1 Tissue fixation: Formal-saline 10% will be used to fix the tissues in ice-cold container.

3.6.3.2 Dehydration: The fixed tissues will be dehydrated to remove water from the tissue using a series of graded alcohol (70, 80, 90 & 100%).

*3.6.3.3 Clearing:*The dehydrant will be removed or cleared using xylene or toluene.

3.6.3.4Embeddding : Molten embedding agent (paraffin) will be introduced into the tissue to enable the tissues to be cut into sections.

3.6.3.5.Sectioning: The embedded tissue will be cut into sections with a microtome (a knife).Once sections are cut, they are floated on a warm water bath that helps removewrinkles. They will then be picked up on a glass microscopic slide. The glass slideswill be placed in a warm oven for about 15mins to help the section adhere to the slide.

3.6.3.6 Staining: The slide will be "deparaffinized" by running them through xylene to alcohol to water, before staining is done.This is to allow water soluble dye to penetrate the sections. The routine dye known as Hematoxylin and Eosin (H & E) will be used for staining various cellular components of the pancreas tissue.The pancreas tissue will be stained by Modified Aldehyde Fuchsin Stain technique (Halami, 1952).

3.6.3.7 Mounting/viewing: The stained section on the slide will then be covered with a thin glass to protect the tissue from being scratched in order to provide better optical quality for viewing under microscope. DPX oil will be used on the slide before mounting to improve the refractive index of the slide and then viewed with a magnification (X 250).

3.7 STATISTICAL ANALYSIS

Data obtained was expressed as means ± SD. The statistical significance of differences in degree of anti-diabetic and antioxidant of different parts of *D. cinerea* (L.) Wight et Arn. was compared using Duncan Multiple Range Test (DMRT).

A value of $p < 0.05$ was considered to indicate a significant difference between groups

CHAPTER FOUR

RESULTS

4.1 PRELIMINARY PHYTOCHEMICAL CONSTITUENTS OF *D. CINEREA* (L.) WIGHT ET ARN

Preliminary phytochemical investigation (Table 4.1) of *D.cinerea* revealed the presence of carbohydrates, saponins, tannins, steroids and Triterpenes, Alkaloids, Cardiac glycoside and flavonoids and absence of anthraquinone in fruits, leaf ,stem bark and roots.

4.2 EFFECTS OF METHANOL EXTRACTS OF DIFFERENT PARTS OF *D.cinerea* (L.) Wight Et Arn ON BODY WEIGHT CHANGE

Reduction in body weight (Table 4.2.1) in the Streptozotocin induced diabetic rats shows the changes in the body weight of Streptozotocin-induced diabetic rats. Induction of diabetes following treatment with streptozotocin caused a reduction in body weight in all diabetic groups .The result showed a significant (P>0.05) difference in the body weight change of all treated groups when compared to the negative control. Significant ($p<0.05$) improvement was observed in extracts treated groups and glibenclimide treated group for the period of 21 days of the treatments ,except in the group treated with the fruit and stem bark extracts , while there were no significant ($p>0.05$) decrease in weight in normoglycemic groups treated with the different extracts and the glibenclimide.

Table 4.1 Preliminary Phytochemical constituents of methanol extracts of different part of *D. cinerea* **(L.) Wight Et Arn**

Phytochemical Test	Fruits	Leaves	Stem-Barks	Roots
Carbohydrates	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Cardiac Glycosides	+	+	+	+
Alkaloids	+	+	+	+
Steroids and Triterpenes	+	+	+	+
Anthraquinone	-	-	-	-

Key: + = Present, - = Absent

Table 4.2.1.Effects of Methanolic Extracts of Different parts of *D. cinerea* on Body Weight in Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and diabetic rats treated with 100mg/kg body weight of the extracts.

Treatment	Bodyweight(g)			
n=5	Day (0)	Day(7)	Day(14)	Day(21)
Normal Control	162.20±9.23^{b}	171.00±2.74^{b}	176.20±8.11^{b}	184.80±6.37^{c}
DiabeticUntreated	150.20±14.31^{ab}	152.20±3.70^{a}	150.40±9.86^{a}	148.40±0.89^{ab}
Diabetic + fruit	142.40±7.89^{a}	145.00±14.82^{a}	143.60±16.86^{a}	139.80±29.11^{a}
Diabetic + Leaves	151.20±9.25^{ab}	152.60±11.08^{ab}	159.80±5.63^{ab}	168.80±3.49^{bc}
Diabetic + Stem	152.00±7.58^{ab}	144.00±24.77^{a}	149.20±31.55^{a}	152.60±26.66^{ab}
Diabetic + Roots	144.40±14.60^{a}	140.60±10.90^{a}	144.80±20.38^{a}	158.20±21.34^{ab}
Diabetic + Glib	151.20±5.63^{ab}	154.20±3.27^{a}	161.80±4.55^{a}	168.40±6.27^{bc}

Values are mean ± SD (n = 5), Values are statistically significant compared to control group at p <0.05. Values with different superscript across the rows are significantly different ($p<0.05$)

Table 4.2.2 Effects of Methanolic Extracts of Different parts of *D. cinerea* on Body weight of non-diabetic, normal control rats.treated with 100mg/kg of the extracts.

Treatment	Body weight (g)			
n=5	Day(0)	Day(7)	Day(14)	Day(21)
Normal Control	162.20±9.23^{a}	171.00±2.74^{b}	176.20±8.10^{b}	184.80±6.38^{b}
Non-Diabetic+Fruit	150.20±9.85^{a}	155.60±3.78^{ab}	160.40±2.88^{ab}	166.00±9.77^{ab}
Non-Diabetic+Leaf	159.00±24.57^{a}	166.40±28.95^{ab}	173.20±24.63^{ab}	183.00±17.83^{b}
Non Diabetic+Stem	159.80±20.56^{a}	163.40±17.85^{ab}	157.60±19.11^{ab}	169.20±10.06^{ab}
Non-Diabetic+Root	151.40±8.47^{a}	143.00±10.51^{a}	150.00±6.48^{a}	153.60±9.76^{a}
Non-Diabetic+Glib	153.60±27.04^{a}	152.40±28.40^{ab}	170.00±27.04^{ab}	170.20±25.53^{ab}

Values are mean ± SD (n = 5), Values are statistically significant compared to control group at $p < 0.05$. Values with different superscript across the rows are significantly different ($p<0.05$)

4.3 EFFECTS OF METHANOL EXTRACTS OF DIFFERENT PARTS OF *D.CINEREA* (L.) WIGHT ET ARN ON BLOOD GLUCOSE LEVEL

Table 4.3.1 shows a significant ($P<0.05$) increase in blood glucose level was observed following induction of diabetes when compared to normal control rats group. Oral administration of methanolic extract of fruits, leaves, Stem-bark and root extracts reduced the blood glucose level significantly ($P<0.05$) except for the fruits extract.

Results of antidiabetic study established the scientific basis for the utility of this plant parts in the treatment of diabetes. The methanol extracts of leaves ,stem-bark, root and the standard drug glibenclimide showed significant reduction, ($P<0.05$) in blood glucose levels streptozotocin induced diabetic rats ,stem-bark with the highest reduction has 76.07%, then glibenclimide 53.19%,leaves 47.01%,root 43.10%, while fruits has 2.83% which showed no significant reduction.

Table 4.3.1 Effects of Methanol Extracts of Different parts of *D. cinerea* on Fasting blood glucose in Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Diabetic rats treated with 100mg/kg body weight of the extracts .

Treatment n=5	Fasting Blood Sugar mg /dl Day (0)	Day(7)	Day(14)	Day(21)
Normal Control	83.40±9.58[a]	87.80±5.81[a]	98.00±1.73[a]	99.40±1.51[a]
DiabeticUntreated	369.80±189.20[b]	330.40±121.37[b]	332.80±137.46[b]	334.00±205.0[b]
Diabetic + fruit	304.20±172.09[b]	294.40±116.98[b]	304.40±223.00[b]	295.60±201.0[b]
Diabetic + Leaves	287.20±174.17[b]	202.60±196.52[ab]	149.80±91.46[ab]	151.40±92.11[a]
Diabetic + Stem	346.60±84.97[b]	252.60±141.55[ab]	194.20±131.7[ab]	83.40±27.32[a]
Diabetic + Roots	323.40±87.37[b]	337.00±86.20[b]	245.20±106.8[ab]	184.00±60.05[ab]
Diabetic + Glib	297.40±107.36[b]	242.00±103.70[ab]	197.80±136.7[ab]	139.20±92.14[a]

Values are mean ± SD (n=5), Values are statistically significant compared to control group at $p < 0.05$ .Values with different superscript across the rows are significantly different ($p<0.05$).

Table 4.3.2 Effects of Methanol Extracts of Different parts of *D. cinerea* on Fasting blood glucose level of non-diabetic, normal control, rats treated with 100mg/kg of the extracts

Treatment	Fasting Blood	Sugar mg/dl		
n=5	Day(0)	Day(7)	Day(14)	Day(21)
Normal Control	83.40±9.58^{a}	87.80±5.81^{ab}	98.00±1.73^{d}	99.40±1.52^{c}
Non-Diabetic+Fruit	79.80±10.16^{a}	85.40±5.03^{ab}	85.20±8.73^{ab}	92.60±4.92^{bc}
Non-Diabetic+Leaf	84.00±13.87^{a}	91.40±14.47^{ab}	94.60±8.32^{cd}	84.80±12.30^{ab}
Non Diabetic+Stem	99.40±10.52^{b}	95.40±6.23^{b}	92.40±5.13^{bcd}	84.00±8.46^{ab}
Non-Diabetic+Root	93.60±10.01^{ab}	76.60±14.04^{a}	81.60±5.41^{a}	75.20±5.81^{a}
Non-Diabetic+Glib	89.40±4.09^{ab}	92.20±0.70^{ab}	88.40±3.20^{abc}	78.00±1.87^{a}

Values are mean ± SD (n=5) , Values are statistically significant compared to control group at $p < 0.05$. Values with different superscript across the rows are significantly different ($p<0.05$)

4.4 EFFECTS OF METHANOL EXTRACTS OF DIFFERENT PARTS OF *D.cinerea*(L.) Wight Et Arn ON PACKED CELL VOLUME

Table 4.4.1 Shows leaves, stem and roots extracts including Glibenclamide treated group significant ($p<0.05$) increase the packed cell volume of the diabetic groups when compared to the negative control, while there was no significant increase ($P<0.05$) in the diabetic treated with fruits extracts compared with diabetic control. There was no significant effect of the extracts on normoglycemic group.

Table 4.4.1 Effects of Methanol Extracts of Different parts of *D. cinerea* on Packed Cell Volume in Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and diabetic rats treated with 100mg/kg body weight of the extracs, diabetic rats treated with 100mg/kg of the extracts.

Treatment	Packed cell volume in (%)
Normal Control	47.20 ± 3.56^{ab}
Diabetic Control	43.40 ± 1.14^{a}
Diabetic+Fruits	44.00 ± 4.06^{a}
Diabetic+leaves	48.60 ± 4.45^{b}
Diabetic+Stem-barks	44.80 ± 3.35^{ab}
Diabetic+Roots	45.80 ± 0.45^{ab}
Diabetic+Glib	46.20 ± 1.79^{ab}

Values are mean ± SD (n = 5) for each group Values are statistically significant compared to control group at $p < 0.05$. Values with different superscript down the group are significantly different ($p<0.05$).

Table 4.4.2 Effects of Methanol Extracts of Different parts of *Dychrostachys cinerea* on Packed cell volume of non-diabetic ,normal control rats treated with 100mg/kg of the extracts

Treatment	Packed cell volume (%)
Normal Control	47.20±3.56[a]
Non- Diabetic+Fruit	45.20±3.35[a]
Non-Diabetic+leaf	47.80±1.30[a]
Non-Diabetic+Stem	46.80±2.17[a]
Non- Diabetic+Root	48.60±1.95[a]
Non-Diabetic+Glib	47.97±2.17[a]

Values are mean ± SD (n = 5) , Values are statistically significant compared to control group at $p < 0.05$. Values with different superscript down the group are significantly different ($p<0.05$)

4.5 EFFECTS OF METHANOL EXTRACTS OF DIFFERENT PARTS OF *D.cinerea* (L.) Wight Et Arn ON ANTIOXIDANT PARAMETERS

Table 4.5.1 Shows malondialdehyde levels in tissues of Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Barks and Root extracts of *D.Cinerea* . *D Cinerea* treatment significantly (P< 0.05) decreased the level of MDA in the liver of diabetic rats when compared with diabetic control rats. Including glibenclamide treated diabetic rats. However, the stem-bark extract showed the most significant (p<0.05) reduction in MDA level in the both liver and kidney.

Table 4.5.3 Shows superoxide dismutase activity in tissues of Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Barks and Root Extracts of *D.Cinerea* .there was significant (P<0.05) increase in superoxide dismutase activity in all the groups treated when compared with the diabetic control group in both liver and kidney homogenates and there was no significant (P<0.05) increase in superoxide dismutase activity when normal control group compared with the normoglycemic treated.

One CAT enzyme can convert 40 million molecules of hydrogen peroxide to water and oxygen per second.

Table 4.5.5 Shows Catalase levels in tissues of Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves,Stem- Barks and Roots Extracts of *D.Cinerea* there was significant (P<0.05) increase in catalase activity in all the groups treated when compared with the diabetic control group in both liver and kidney homogenates and there was significant (P>0.05) decrease in catalase activity in groups treated with fruits leaves, root and glibenclimide normal control compared with the normoglycemic treated. While there was no significant difference (p<0.05) in group treated with stem when compared with normal control group.

Table 4.5.1 Effects of Methanol Extracts of Different parts of *D. cinerea* on Malondialdehyde levels in tissues of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and diabetic rats treated with 100mg/kg body weight of the extracts.

Treatment	Malondialdehyde (umol/mg)	
N=5	Liver	Kidney
Normal Control	1.50 ± 0.17^{a}	1.44 ± 0.11^{a}
Diabetic Control	$2.08\pm0.0.22^{d}$	2.28 ± 0.15^{d}
Diabetic + Fruits	1.86 ± 0.20^{cd}	2.20 ± 0.21^{cd}
Diabetic+ leaves	1.80 ± 0.14^{bc}	2.20 ± 0.32^{cd}
Diabetic+ Stem-barks	1.54 ± 0.23^{ab}	1.50 ± 0.07^{ab}
Diabetic+ Roots	1.64 ± 0.11^{abc}	1.72 ± 0.22^{b}
Diabetic+ Glib	1.68 ± 0.23^{abc}	1.98 ± 0.08^{c}

Values are mean ± SD (n=5), Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

4.5.2 Effects of Methanol Extracts of Different parts of *Dychrostachys cinerea* on Malondialdehyde levels in tissues of non-diabetic,normal control rats. treated with 100mg/kg of the extracts

Treatment	Malondialdehyde (umol/mg)	
n=5	Liver	Kidney
Normal Control	1.50 ± 0.17^{a}	1.44 ± 0.11^{a}
Non- Diabetic+Fruit	1.80 ± 0.21^{b}	2.20 ± 0.31^{c}
Non-Diabetic+leaf	1.74 ± 0.29^{ab}	1.76 ± 0.18^{bc}
Non-Diabetic+Stem	1.48 ± 0.19^{a}	1.46 ± 0.32^{ab}
Non- Diabetic+Root	1.52 ± 0.08^{a}	1.62 ± 0.11^{ab}
Non-Diabetic+Glib	1.58 ± 0.13^{ab}	1.70 ± 0.14^{ab}

Values are mean ± SD (n=5) for each group, Values are statistically significant compared to control group at $p < 0.05$ .Values with different superscript down the group are significantly different ($p<0.05$)

4.5.3 Table Effects of Methanol Extracts of Different parts of *D. cinerea* on Superoxide dismutase activity in tissues of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Diabetic rats treated with 100mg/kg of the extracts

Treatment	Superoxide Dismutase (IU/L)	
N=5	Liver	Kidney
Normal Control	2.24 ± 0.32^{e}	2.58 ± 0.22^{b}
Diabetic Control	1.54 ± 0.05^{a}	1.58 ± 0.09^{a}
Diabetic + Fruits	1.62 ± 0.11^{ab}	1.68 ± 0.27^{a}
Diabetic+ leaves	1.68 ± 0.16^{abc}	1.94 ± 0.34^{ab}
Diabetic+ Stem-barks	2.00 ± 0.22^{de}	2.32 ± 0.17^{ab}
Diabetic+ Roots	1.88 ± 0.27^{bcd}	2.04 ± 0.16^{b}
Diabetic+ Glib	1.94 ± 0.25^{cd}	2.10 ± 1.72^{c}

Values are presented as mean ± SD (n=5) for each group,Values are statistically significant compared to control group at $p < 0.05$. Values with different superscript down the group are significantly different ($p<0.05$)

Table 4.5.4 Effects of Methanol Extracts of Different parts of *D. cinerea* on Superoxide dismutase levels in tissues of non-diabetic normal control rats.treated with 100mg/kg of the extracts.

Treatment	Superoxide Dismutase (IU/L)	
n=5	Liver	Kidney
Normal Control	2.24 ± 0.31^{b}	2.58 ± 0.22^{b}
Non- Diabetic+Fruit	1.92 ± 0.27^{ab}	2.04 ± 0.34^{a}
Non-Diabetic+leaf	1.76 ± 2.08^{a}	2.08 ± 0.29^{a}
Non-Diabetic+Stem	2.06 ± 0.13^{ab}	2.32 ± 0.27^{ab}
Non- Diabetic+Root	2.02 ± 0.29^{ab}	2.18 ± 0.25^{a}
Non-Diabetic+Glib	2.08 ± 0.31^{ab}	2.14 ± 0.20^{a}

Values are presented as mean ± SD (n=5) for each group,Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

4.5.5 Effects of Methanol Extracts of Different parts of *D. cinerea* on Catalase levels in tissues of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and diabetic rats treated with 100mg/kg the extracts

Treatment	Catalase (IU/L)	
n=5	Liver	Kidney
Normal Control	54.60 ± 0.87^{a}	50.80 ± 0.51^{d}
Diabetic Control	36.40 ± 1.67^{e}	35.40 ± 0.51^{a}
Diabetic + Fruits	39.40 ± 1.83^{ab}	39.00 ± 1.00^{ab}
Diabetic+ leaves	40.40 ± 0.81^{b}	42.60 ± 1.81^{bc}
Diabetic+ Stem-barks	50.00 ± 0.71^{d}	49.80 ± 0.80^{d}
Diabetic+ Roots	44.80 ± 0.49^{c}	44.80 ± 1.80^{c}
Diabetic+ Glib	47.20 ± 1.59^{d}	43.80 ± 1.93^{c}

Values are presented as mean ± SD (n=5) for each group,Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

4.5.6 Effects of Methanol Extracts of Different parts of *D. cinerea* on catalase levels in tissues of non-diabetic, normal control rats. treated with 100mg/kg of the extracts

Treatment	Catalase (IU/L/)	
n=5	Liver	Kidney
Normal Control	54.60±0.87[c]	50.80±1.66[b]
Non- Diabetic+Fruit	44.00±0.89[a]	44.20±0.66[a]
Non-Diabetic+leaf	47.40±1.23[b]	43.20±0.49[a]
Non-Diabetic+Stem	52.00±0.63[c]	50.60±0.933[b]
Non- Diabetic+Root	47.80±1.39[b]	45.00±0.11[a]
Non-Diabetic+Glib	48.60±1.57[b]	47.00±1.92[a]

Values are presented as mean ± SD (n=5) for each group,Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

4.6 EFFECTS OF METHANOLIC EXTRACTS OF *D.cinerea* ON SERUM LIVER DAMAGE INDICATORS AND LIVER FUNCTION PARAMETERS

Table 4.6.1 Shows AST ALT and ALP levels were significantly ($p<0.05$) decreased in groups treated with methanolic extracts of *D.cinerea* when compared with diabetic control rats except in the ALP levels of diabetic group treated with fruit and stem-bark extracts which is not significantly ($p<0.05$) different from the negative control. The decrease may be due to the ameliorative effects of the root and leaves extracts.

Table 4.6.1 also shows serum concentrations of total protein (TP), albumin (ALB) and total bilirubin (TB) of Streptozotocin-induced diabetic rats and normoglycemic rats are shown in Table 4.6.1 and Table 4.6.2 respectively. There was significant ($P<0.05$) increase in the concentrations of serum TP, while no significant increase in ALB of all the diabetic treated groups. Both Glibenclamide and methanolic extracts of fruits, leaves, stem-bark and root of *D.cinerea* significantly ($P<0.05$) reduced total bilirubin concentration when compared to diabetic control.

Table 4.6.1 Effects of Methanol Extracts of Different parts of *D. cinerea* on Liver Marker Enzymes and Liver Function Parameters level of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Diabetic rats treated with 100mg/kg body weight of the extracts

Treatment n=5	**Liver Maker Enzymes (IU/L) Liver Function Parameters mg/dl**					
	ALT	**AST**	**ALP**	**Total Protein (TP)**	**Albumin (ALB)**	**Total Bilirubin (TB)**
Normal Control	24.00±0.63^{a}	16.60±1.47^{a}	59.00±2.23^{a}	6.90±0.22^{d}	3.36±0.16^{a}	0.41±0.04^{a}
Diabetic Control	41.0±1.75^{e}	28.20±0.37^{e}	71.60±3.08^{b}	5.54±0.36^{a}	2.88±0.19^{b}	0.70±0.12^{d}
Diabetic + Fruits	38.80±1.39^{cd}	24.80±0.97^{cd}	69.60±1.99^{b}	5.80±0.27^{ab}	3.00±0.13^{ab}	0.62±0.10^{cd}
Diabetic+ leaves	27.40±0.51^{ab}	21.20±0.37^{b}	64.20±2.89^{ab}	6.64±0.19^{cd}	3.22±0.27^{ab}	0.44±0.09^{b}
Diabetic+ Stem	38.80±0.58^{cd}	26.80±0.37^{de}	70.80±3.26^{b}	5.92±0.26^{b}	3.12±0.25^{ab}	0.64±0.04^{b}
Diabetic+ Roots	30.60±0.60^{b}	21.60±1.03^{b}	69.40±4.28^{ab}	6.62±0.22^{cd}	3.20±0.25^{ab}	0.46±0.09^{b}
Diabetic+ Glib	37.40±2.06^{c}	23.80±0.80^{bc}	67.40±1.08^{ab}	6.44±0.31^{c}	3.18±0.31^{ab}	0.57±0.04^{cd}

Values are mean ± SD (n=5), Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

4.6.2 Effects of Methanol Extracts of Different parts of *D cinerea* on Liver Marker Enzymes and Liver Function Parameters level of non diabetic normal control rats treated with 100mg/kg of the extracts.

Treatment	**Liver Maker**	**Enzymes(IU/L)**	**Liver Function**	**Parameters mg/dl**		
n=5	**ALT**	**AST**	**ALP**	**Total Protein TP**	**Albumin (ALB)**	**Total Bilirubin (TB)**
Normal Control	24.00±1.41^{a}	16.60±3.29^{b}	59.00±5.00^{a}	6.90±0.02^{b}	3.36±0.04^{a}	0.41±0.04^{a}
Non-Diabetic+Fruit	26.80±1.64^{dc}	18.20±0.45^{ab}	60.20±6.82^{a}	6.80±0.02^{b}	3.34±0.02^{a}	0.44±0.04^{ab}
Non-Diabetic+leaf	31.80±2.28^{cd}	22.40±2.30^{c}	67.20±2.59^{a}	6.16±0.04^{a}	3.26±0.04^{a}	0.57±0.09^{c}
Non-Diabetic+Stem	26.20±2.59^{ab}	18.40±1.51^{ab}	62.20±6.01^{a}	6.80±1.00^{b}	3.32±0.04^{a}	0.46±0.07^{ab}
Non- Diabetic+Root	33.80±1.30^{ab}	24.00±2.35^{c}	67.00±4.71^{a}	5.82±0.16^{a}	3.20±0.16a	0.62±0.09^{c}
Non-Diabetic+Glib	29.29±4.81^{bc}	21.20±4.38^{bc}	64.80±9.52^{a}	6.10±0.07^{a}	3.30±0.04^{a}	0.54±0.09^{bc}

Values are mean ± SD (n=5) ,Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

4.7 EFFECTS OF *D. cinerea* (L.) Wight Et Arn METHANOLIC EXTRACTS ON KIDNEY FUNCTION PARAMETERS IN STEPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

Serum urea and creatinine concentrations after a 21-day oral administration of methanolic fruits leaves, stem-bark and roots extracts of *D.cinerea* to streptozotocin-induced diabetic rats is shown in Table 4.7.1 The result showed a significant ($P>0.05$) reduction in urea concentrations of the group treated with roots, there was significant ($P<0.05$) increase in the groups treated with fruits, leaves, stem-bark and glibenclimide. The urea concentrations of the leaf, stem and Glibenclamide treated groups are not significantly ($p<0.05$) different from the normal control groups. Creatinine concentration is significantly increased in the groups treated with fruits,leaves,stem-bark and glibenclimide when compared with the negative control ,with the exception of group treated root extract which showed significant ($P>0.05$) decrease.

Table 4.7.1 Effects of Methanol Extracts of Different parts of *D. cinerea* on Serum Urea and Creatinine levels in Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and diabetic rats treated with 100mg/kg body weight of the extracts

Treatment	Urea and Creatinine (mg/dl)	
N=5	Urea	Creatinine
Normal Control	11.09 ± 0.80^{a}	0.50 ± 0.04^{a}
Diabetic Control	11.76 ± 0.87^{b}	0.63 ± 0.02^{b}
Diabetic + Fruits	16.45 ± 0.94^{c}	0.74 ± 0.04^{c}
Diabetic+ leaves	16.13 ± 2.24^{c}	0.71 ± 0.04^{cd}
Diabetic+Stem-barks	18.54 ± 0.76^{d}	0.77 ± 0.04^{d}
Diabetic+Roots	11.65 ± 1.03^{ab}	0.53 ± 0.07^{a}
Diabetic+Glib	15.80 ± 2.50^{c}	0.71 ± 0.02^{c}

Values are mean ± SD (n=5) , Values are statistically significant compared to control group at $p < 0.05$.Values with different superscript down the group are significantly different ($p<0.05$)

Table 4.7.2 Effects of Methanol Extracts of Different parts of *D. cinerea* on serum urea and creatinine levels of non-diabetic rats, normal control rats treated with 100mg/kg of the extracts.

Treatment	Urea and Creatinine(mg/dl)	
n=5	Urea	Creatinine
Normal Control	11.09 ± 0.81^{a}	0.50 ± 0.04^{a}
Non- Diabetic+Fruit	11.65 ± 0.50^{a}	0.58 ± 0.04^{b}
Non-Diabetic+leaf	11.76 ± 0.56^{a}	0.61 ± 0.04^{bc}
Non-Diabetic+Stem	13.45 ± 0.76^{b}	0.68 ± 0.04^{d}
Non- Diabetic+Root	15.80 ± 0.50^{c}	0.71 ± 0.02^{d}
Non-Diabetic+Glib	13.39 ± 1.31^{b}	0.66 ± 0.07^{cd}

Values are mean ± SD (n=5) ,Values are statistically significant compared to control group at $p < 0.05$ Values with different superscript down the group are significantly different ($p<0.05$)

Table 4.8.1 Summary of histopathological studies of Normal control, Diabetic control, Diabetic and Non-diabetic rats treated with various methanolic extracts of *D.cinerea* **.**

Organ	Normal Control	Diabetic Control	Diabetic+ fruits	Non-Diabetic +fruits	Diabetic+ leaves	Non-diabetic+ leaves
Heart	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes
Liver	No Observable pathological changes	Slight lymphocyte hyperplasia	Vascular congestion with slight hepatic necrosis	No Observable pathological changes	No Observable pathological changes	Moderate lymphocyte hyperplasia
Kidney	No Observable pathological changes	Intense lymphocyte hyperplasia	Moderate lymphocyte hyperplasia	Slight glomerular necrosis and slight lymphocyte hyperplasia	Moderate glomerular necrosis and slight lympocyte	Slight glomerular necrosis

Table 4.8.2 Summary Continuation of Histopathological Study

Organ	Diabetic+ stem-barks	Non-Diabetic+ stem-barks	Diabetic+ Roots	Non-Diabetic+ Roots	Diabetic+ Glibenclamide	Non-Diabetic+ Glibenclamide
Heart	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes	No Observable pathological changes
Liver	Vascular congestion with slight necrosis	No Observable pathological changes	No Observable pathological changes	Moderate hepatocellular necrosis	Vascular congestion	Slight Vascular Congestion and slight lymphocyte
Kidney	Glomerular necrosis and slight lymphocyte hyperplasia	Slight lymphocyte hyperplasia and glomerular necrosis	Slight lymphocyte hyperplasia	Slight glomerular and tubular necrosis	Moderate glomerular and tubular necrosis	Moderate glomerular and tubular necrosis

Plates 4.9.1 Showed Photomicrograph of kidney section of Normal control, Diabetic control, Non-diabetic and Diabetic rats treated with fruits, leaves, stem-bark, and root methanol extracts of *D.cinerea*

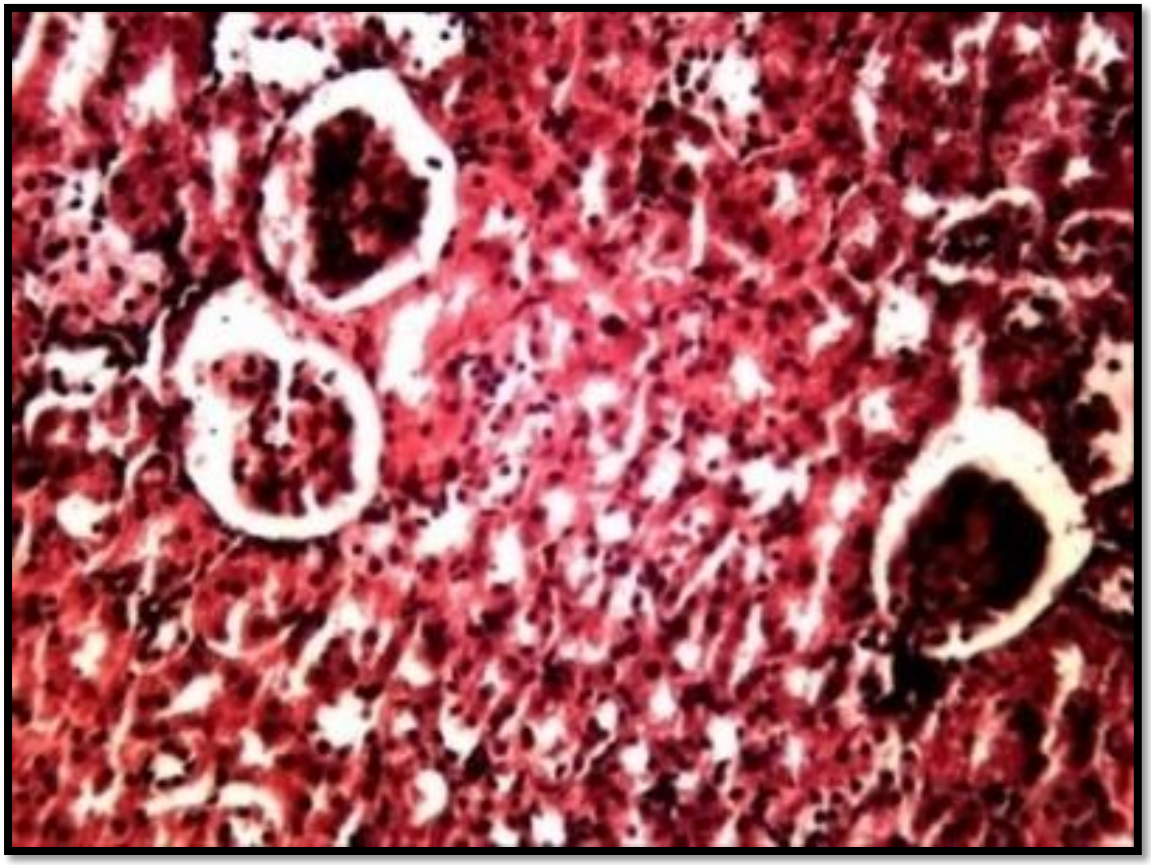


PLATE I: Normal Control, No Observable pathological changes Hematoxylin and Eosin Stain (H & E) (X 250)

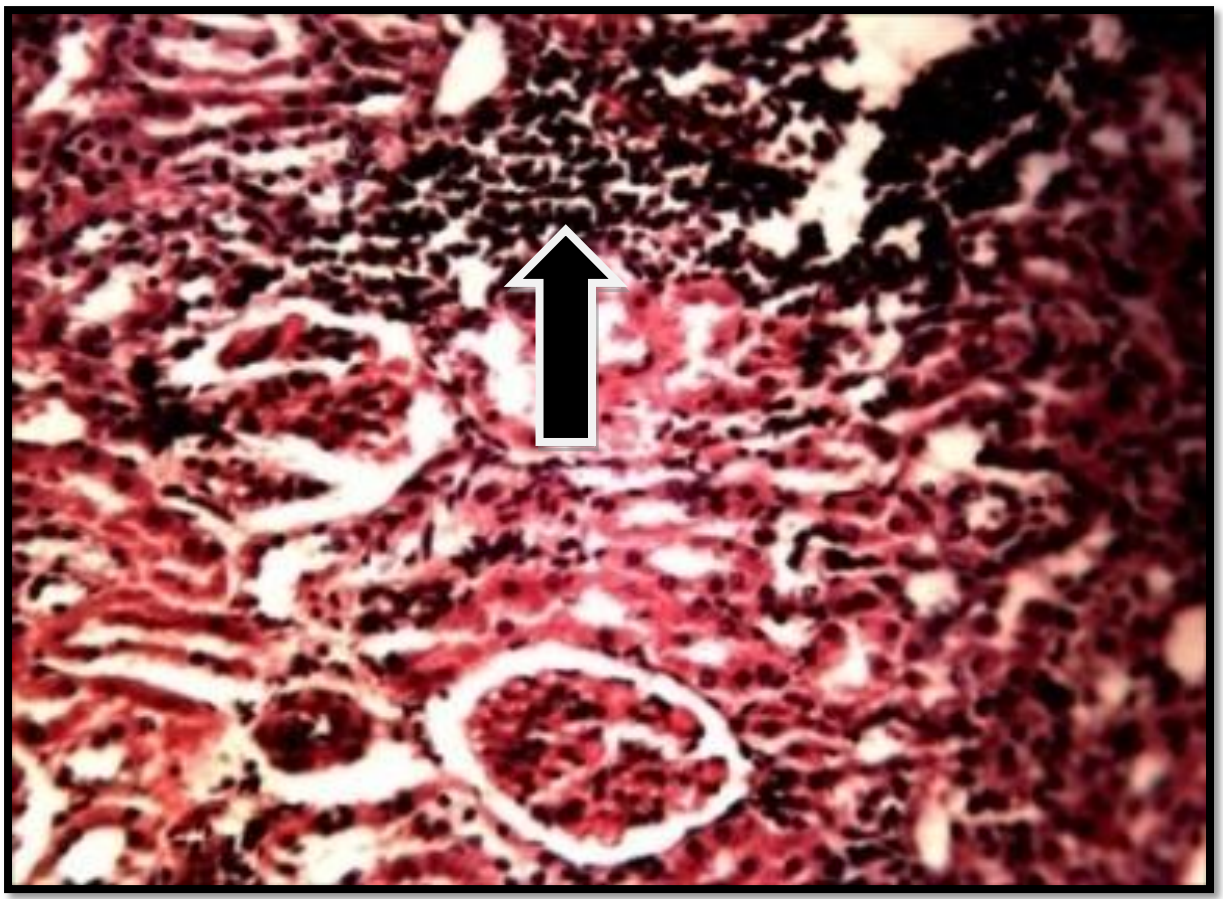


PLATE II: Diabetic+Control ,Intense lymphocytes hyperplasia , Hematoxylin and Eosin Stain (H & E) (X 250)

72

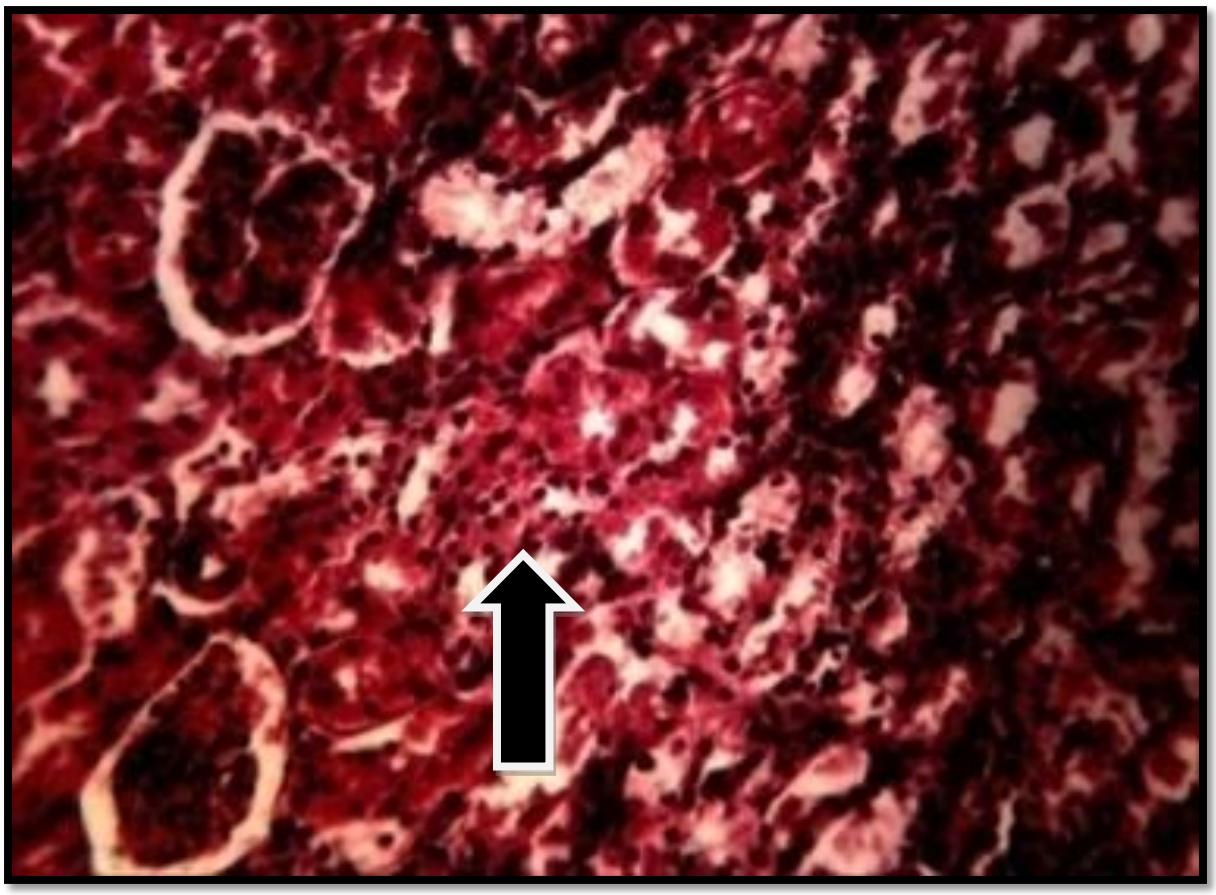


PLATE III:Diabeic + Fruits , Intense lymphocytes Hematoxylin and Eosin Stain (H & E) (X 250)

73

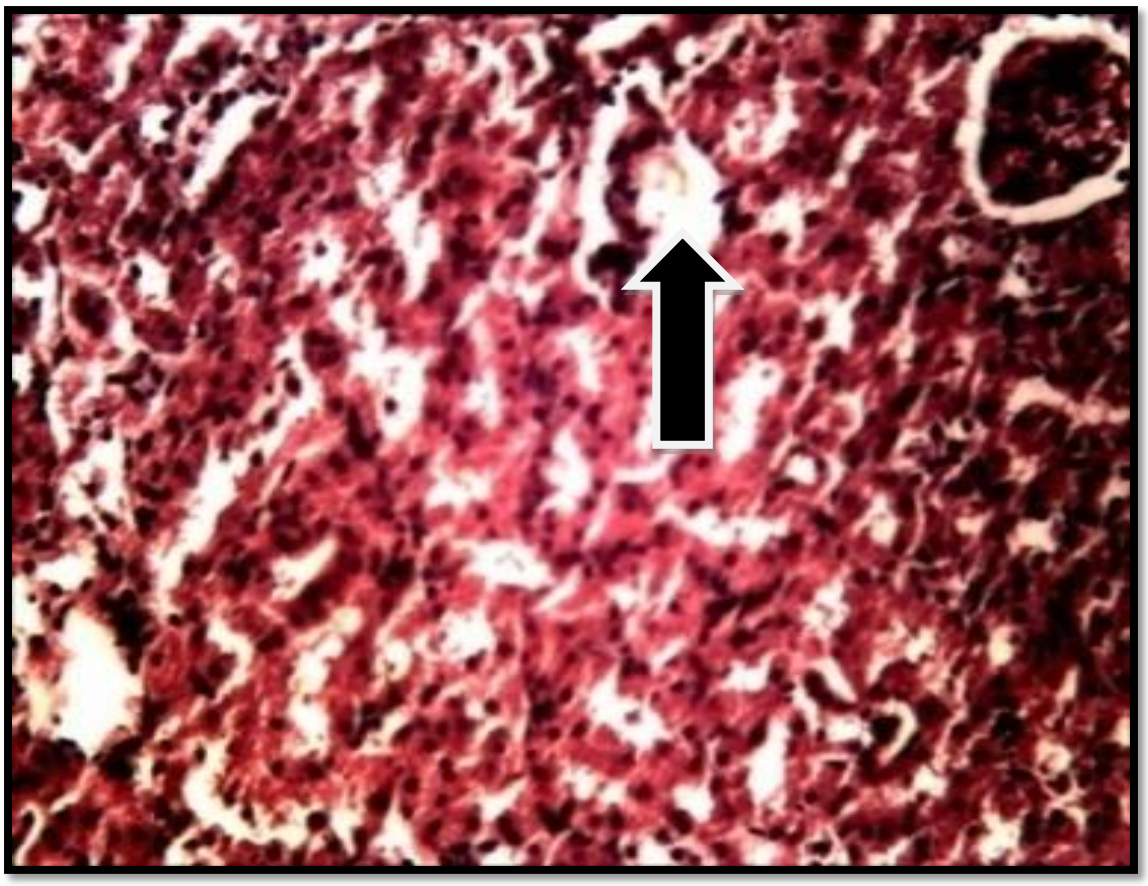


PLATE IV :Non-Diabetic+Fruits Slight glomerular necrosis and lymphocytes hyperplasia Hematoxylin and Eosin Stain (H & E) (X 250)

74

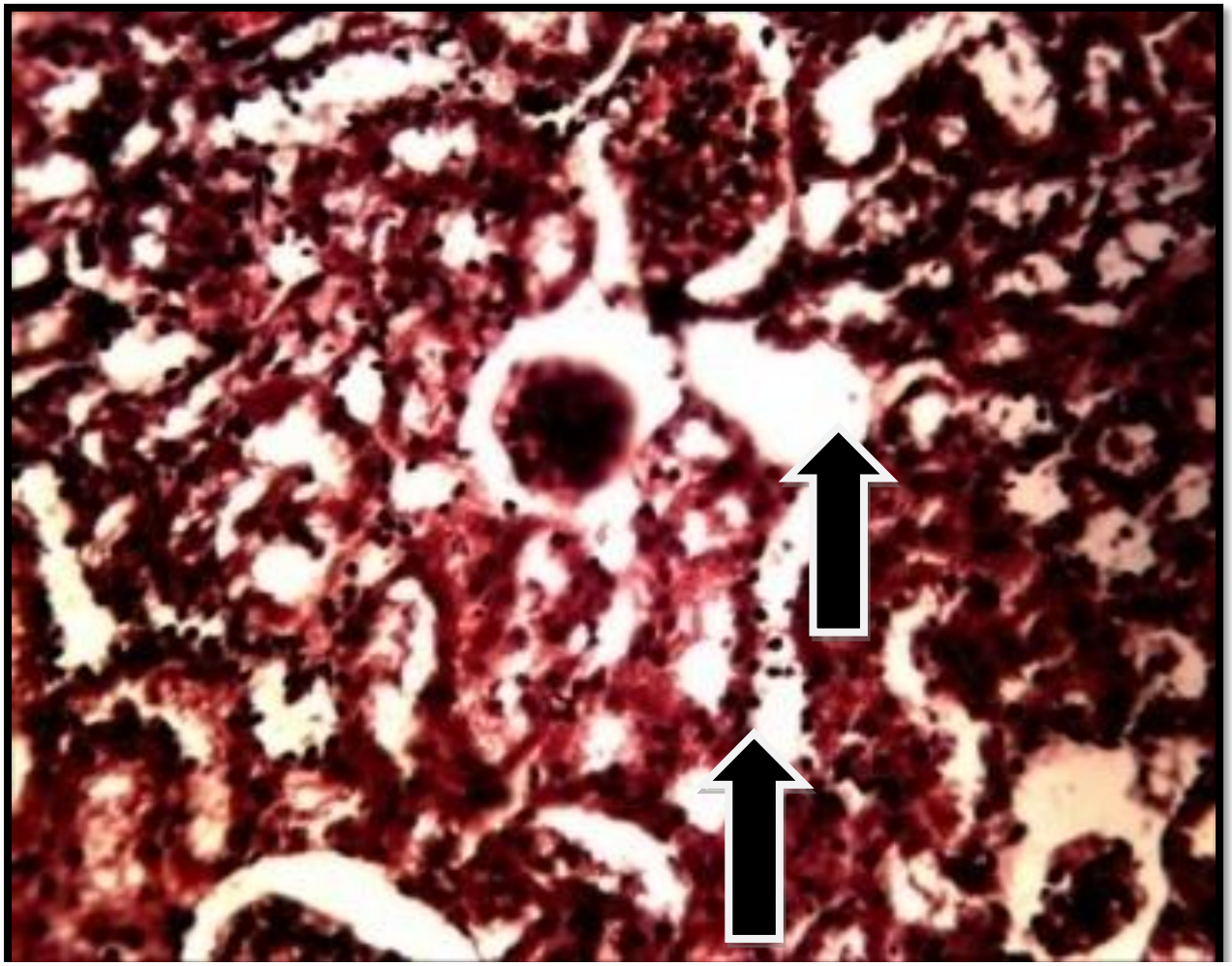


PLATE V: Diabeic+leaves Slight lymphocytes,Moderate Glomerular Necrosis Stain (H & E) (X 250)

75

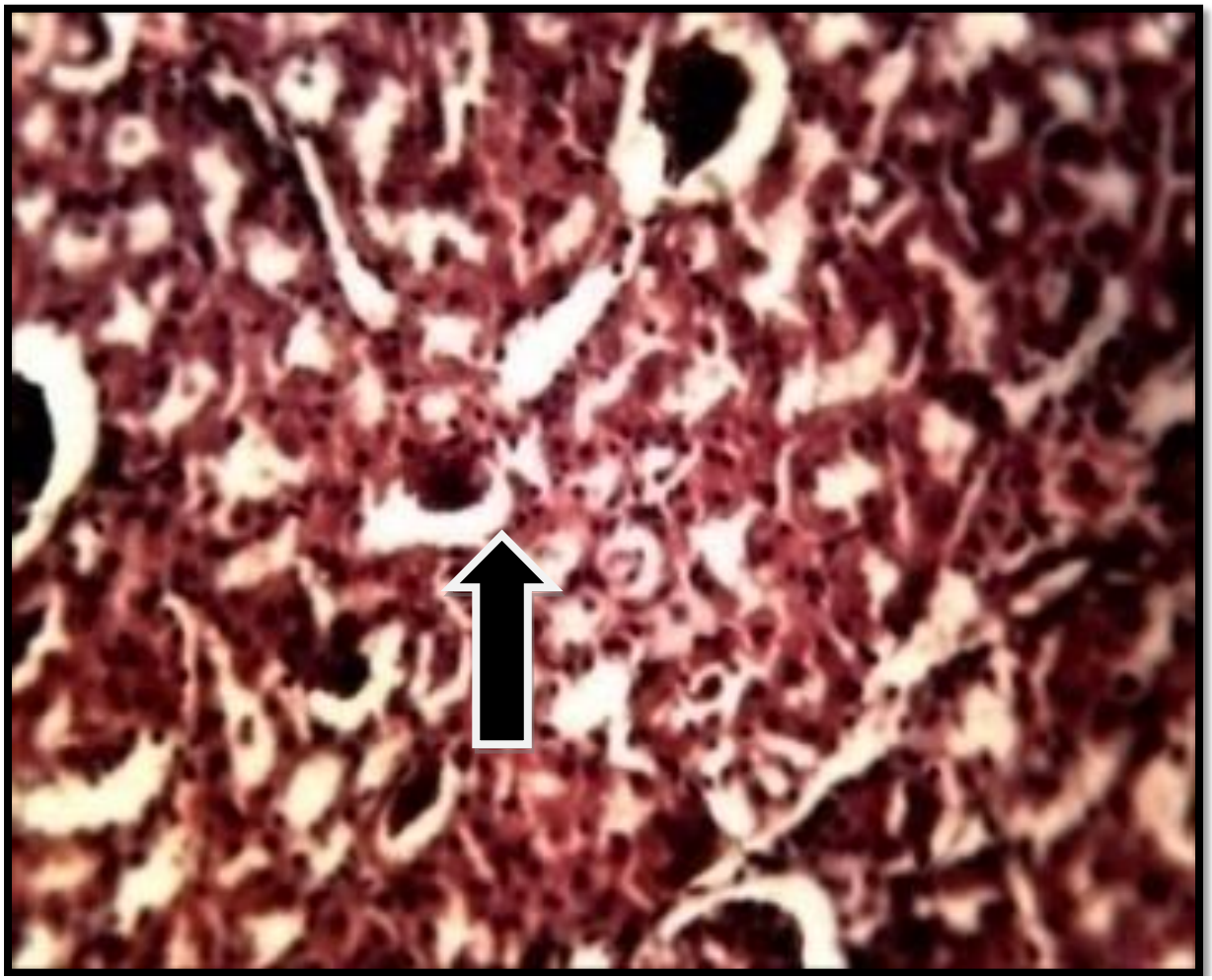


PLATE VI: Non-Diabetic+ leaves, Slight glomerular necrosis. Hematoxylin and Eosin Stain (H & E) (X 250)

76

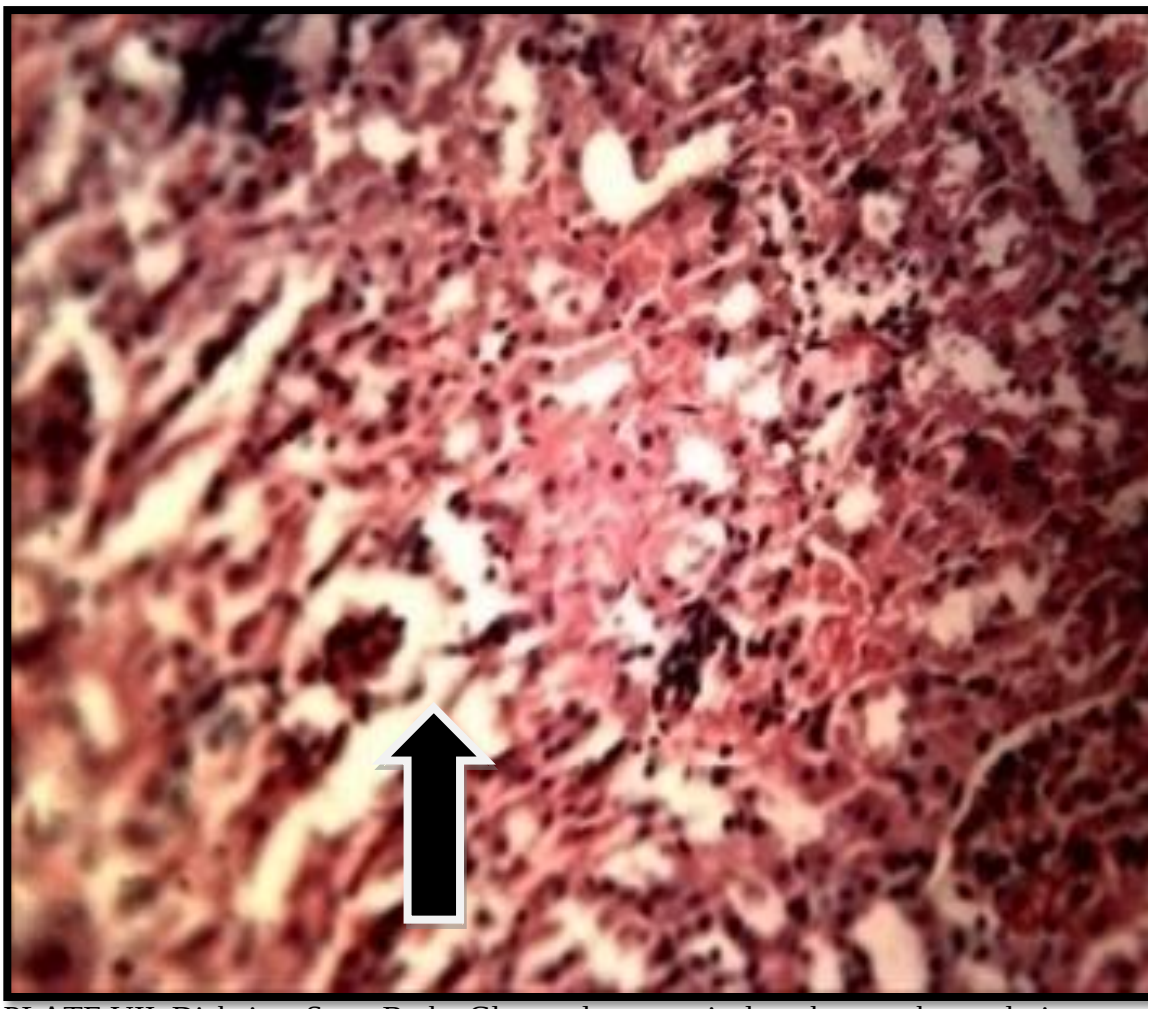


PLATE VII: Diabeic + Stem-Bark, Glomerular necrosis, lymphocytes hyperplasia, Stain (H & E) (X 250)

77

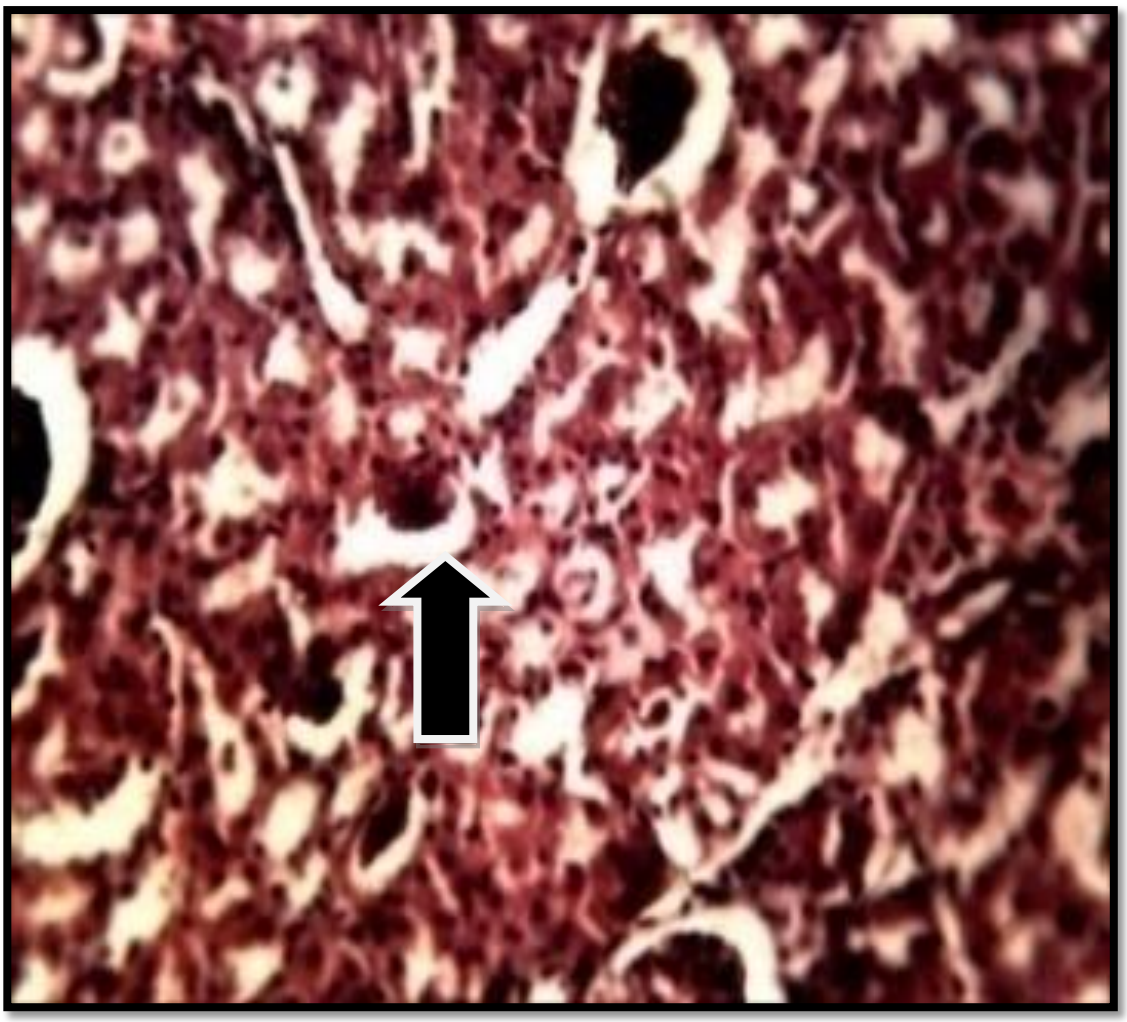
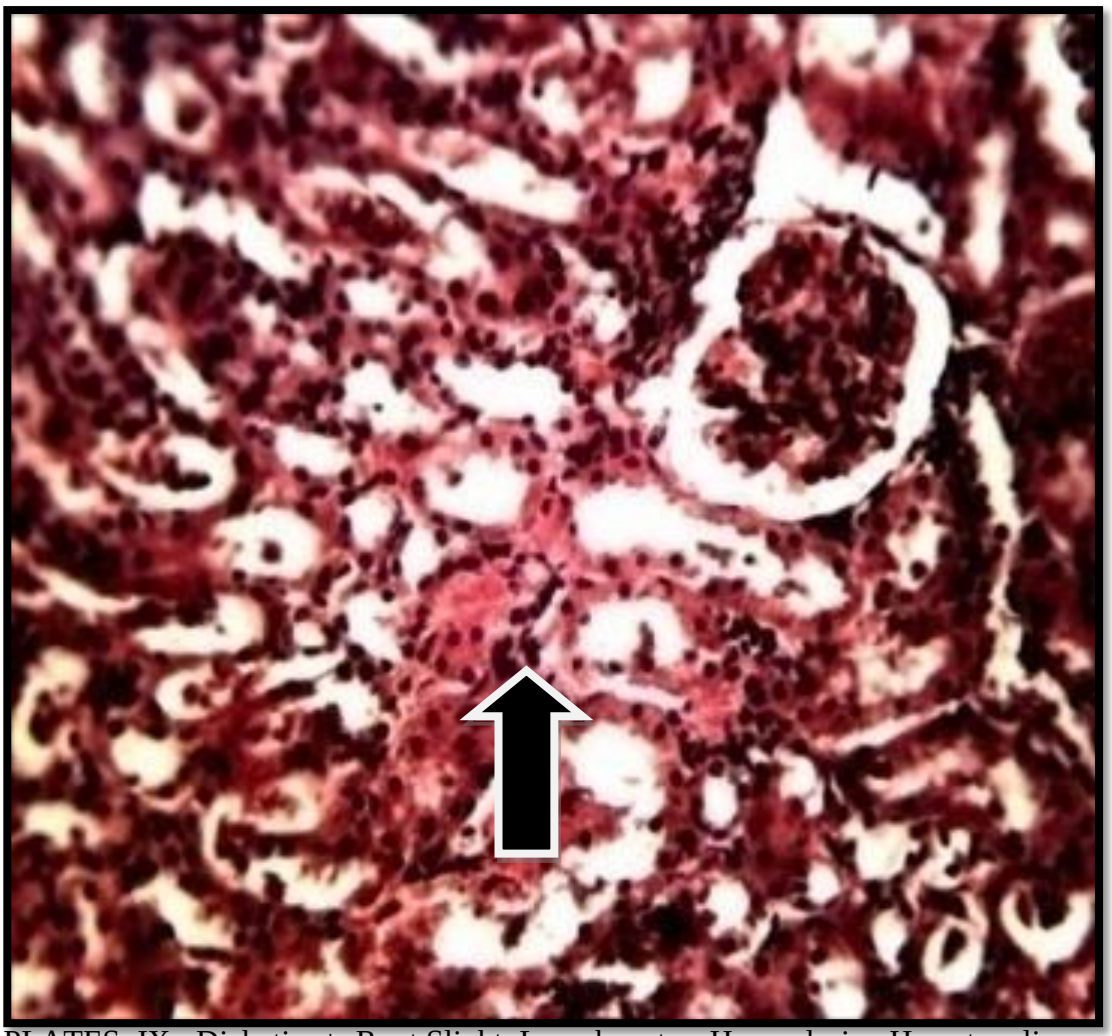


PLATE VIII :Non-Diabetic+Stem-Bark ,Slight lymphocytes hyperplasia and glomerular necrosis. Hematoxylin and Eosin Stain (H & E) (X 250)

78



PLATES IX: Diabetic + Root,Slight Lymphocytes Hyperplasia. Hematoxylin and Eosin Stain (H & E) (X 250)

79

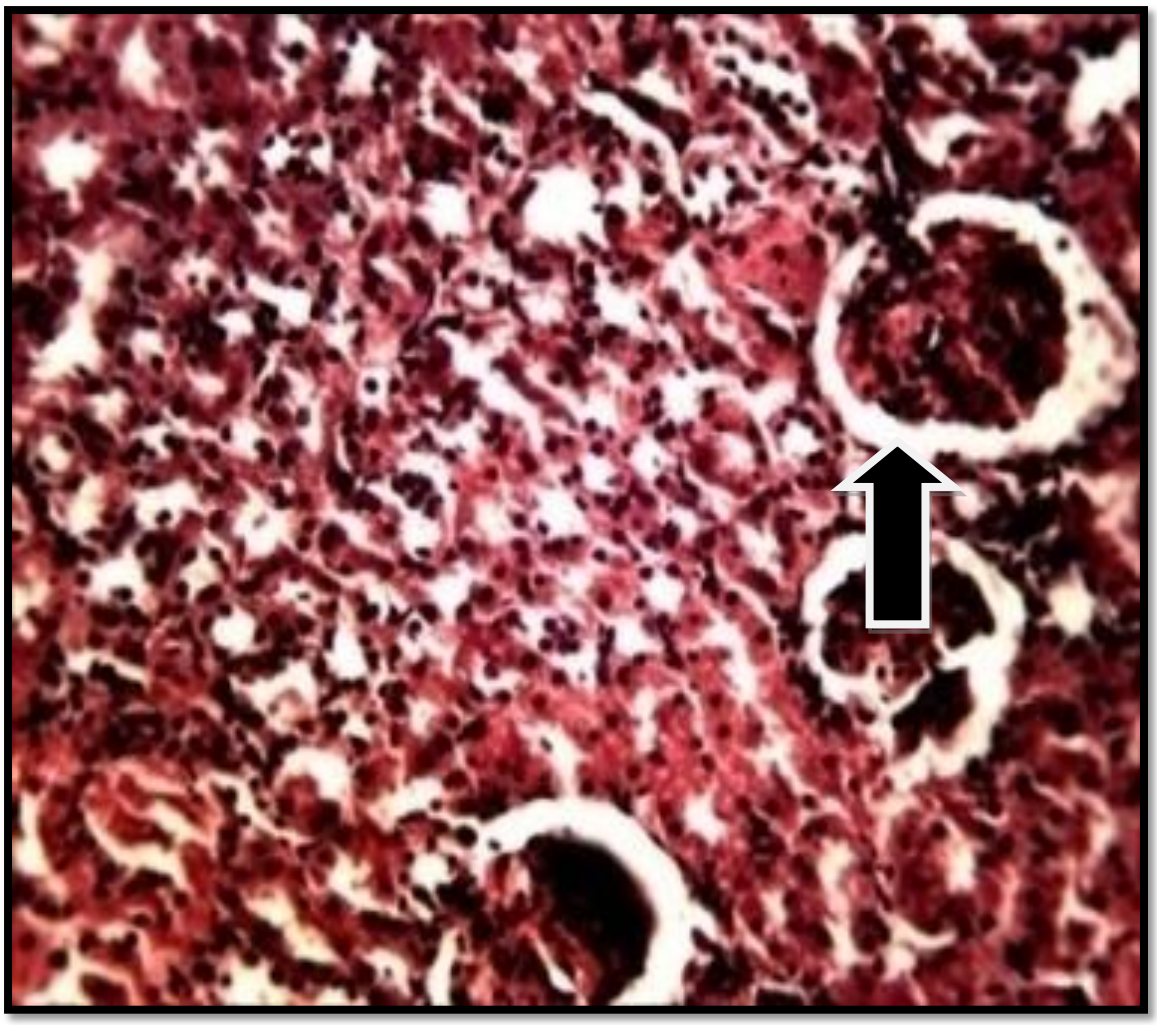


PLATE X: Non-Diabetic+Root , Slight glomerular necrosis, Hematoxylin and Eosin Stain (H & E) (X 250)

80

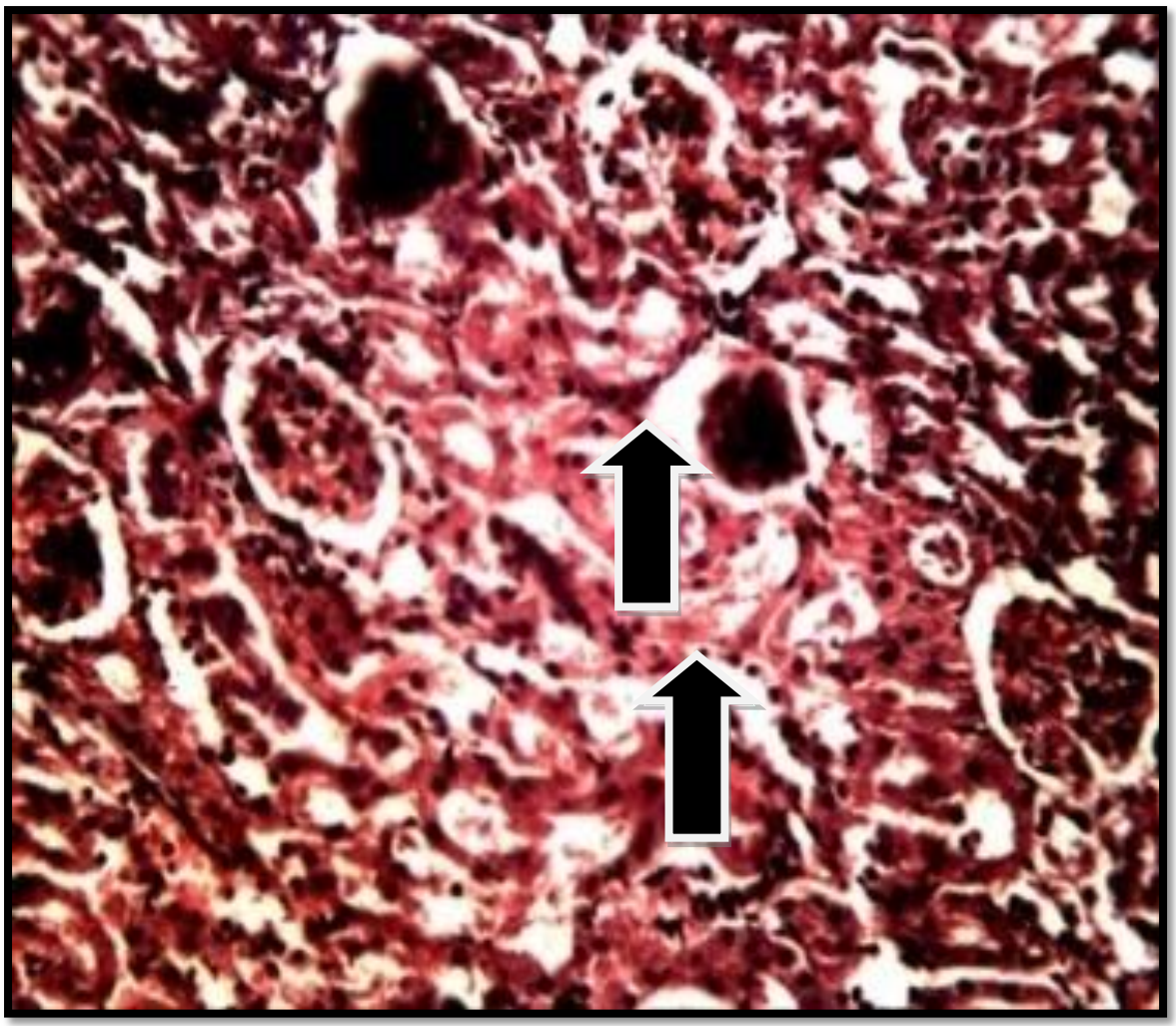


PLATE: XI Diabetic + Glibenclamide ,Slight Glomerular and tubular Necrosis. Hematoxylin and Eosin Stain (H & E) (X 250)

81

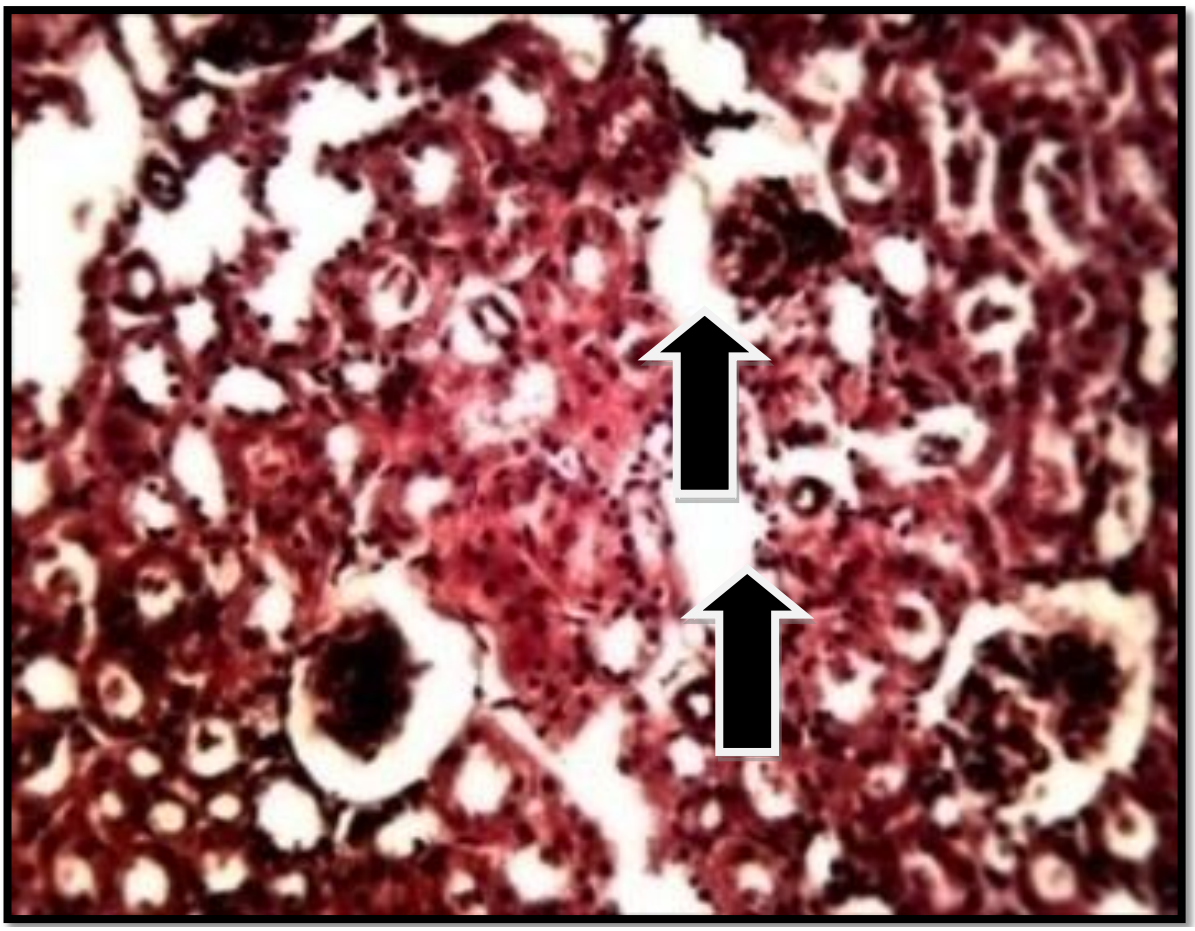


PLATE: XII Non-Diabetic + Glibenclamide Moderate Glomerular and Tubular Necrosis. Hematoxylin and Eosin Stain (H & E) (X 250)

82

Plates: 4.9.2 Photomicrograph of liver section of Normal control, Diabetic control , Non – diabetic and Diabetic treated with fruits, leaves, stem-bark, and root methanol extracts

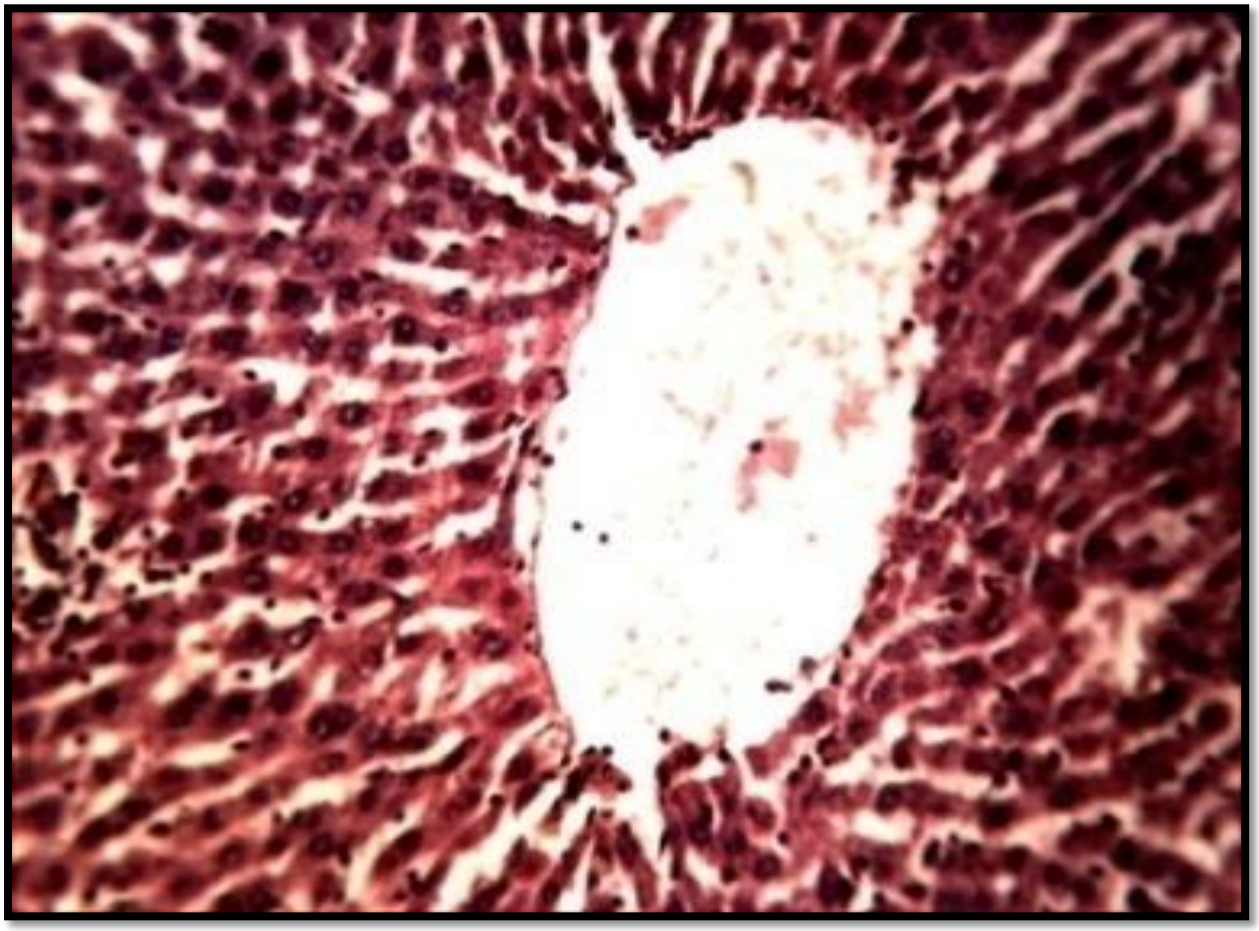


PLATE XIII: Normal Control, No Observable Pathological Changes. Hematoxylin and Eosin Stain (H & E) (X 250)

83

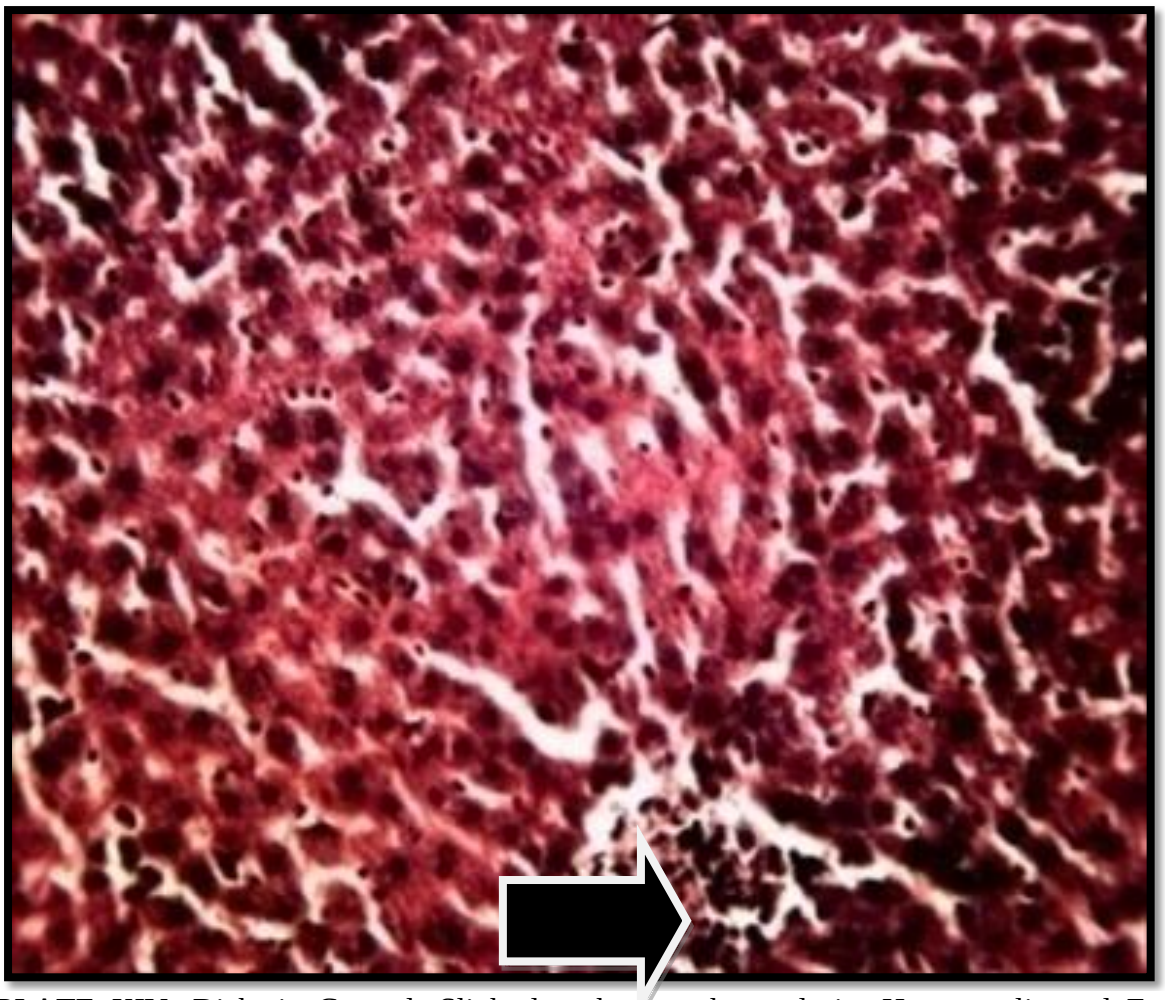


PLATE XIV: Diabetic Control, Slight lymphocytes hyperplasia. Hematoxylin and Eosin Stain (H & E) (X 250)

84

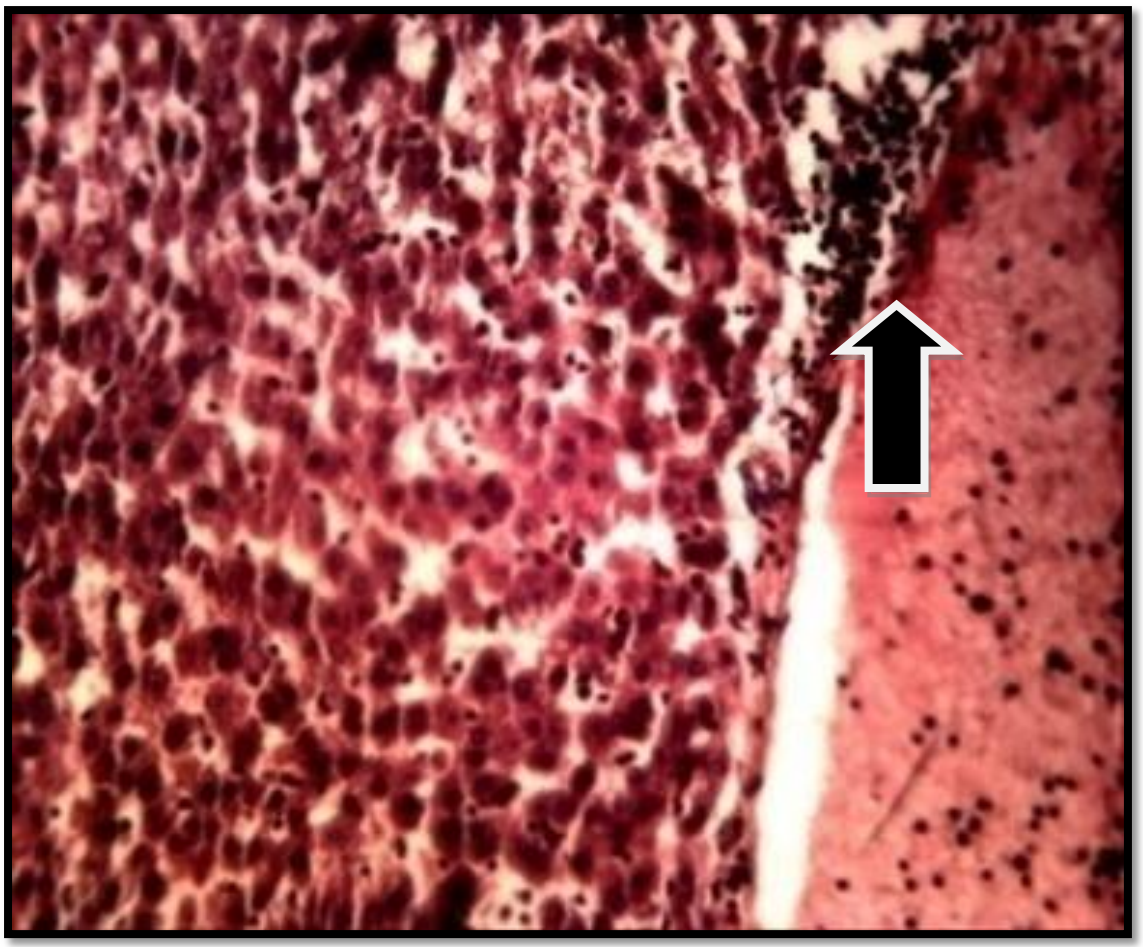


PLATE XV:Diabetic+Fruits ,Vascular congestion with Slight hepatic necrosis. Hematoxylin and Eosin Stain (H & E) (X 250)

85

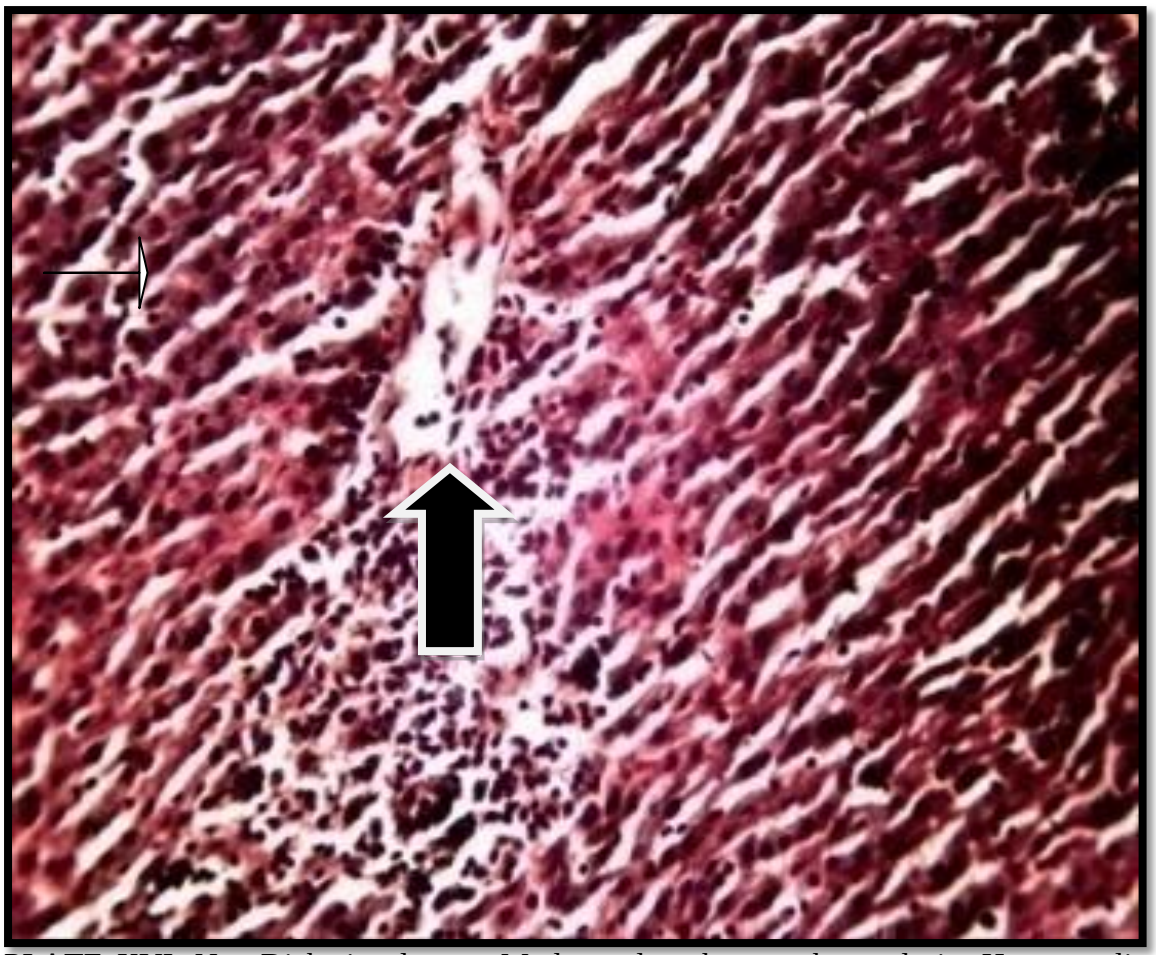


PLATE XVI: Non-Diabetic+ leaves, Moderate lymphocytes hyperplasia. Hematoxylin and Eosin Stain (H & E) (X 250)

86

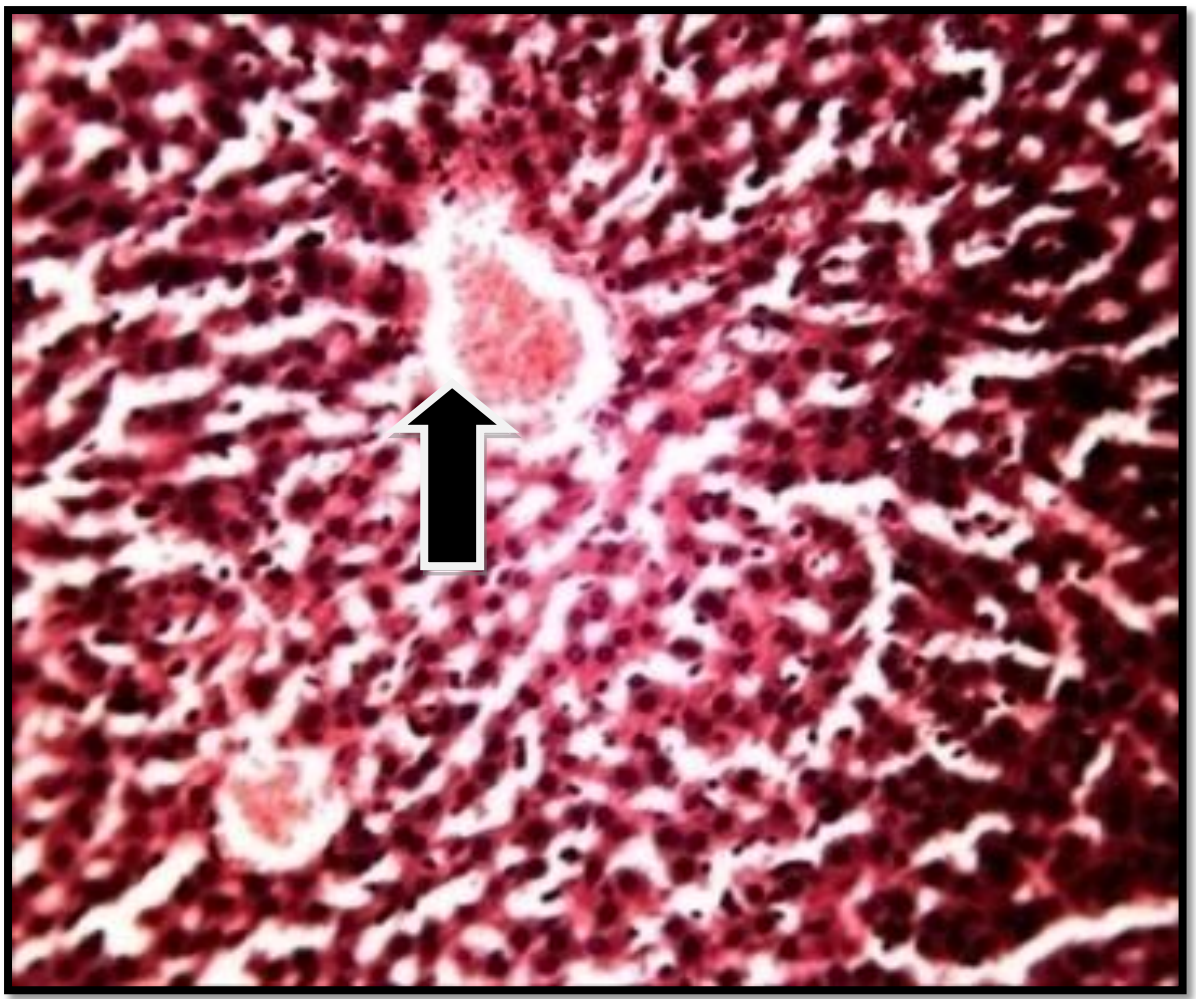


PLATE XVII:Diabetic+Stem-Bark ,Vascular congestion with Slight hepatocellular necrosis Hematoxylin and Eosin Stain (H & E) (X 250)

87

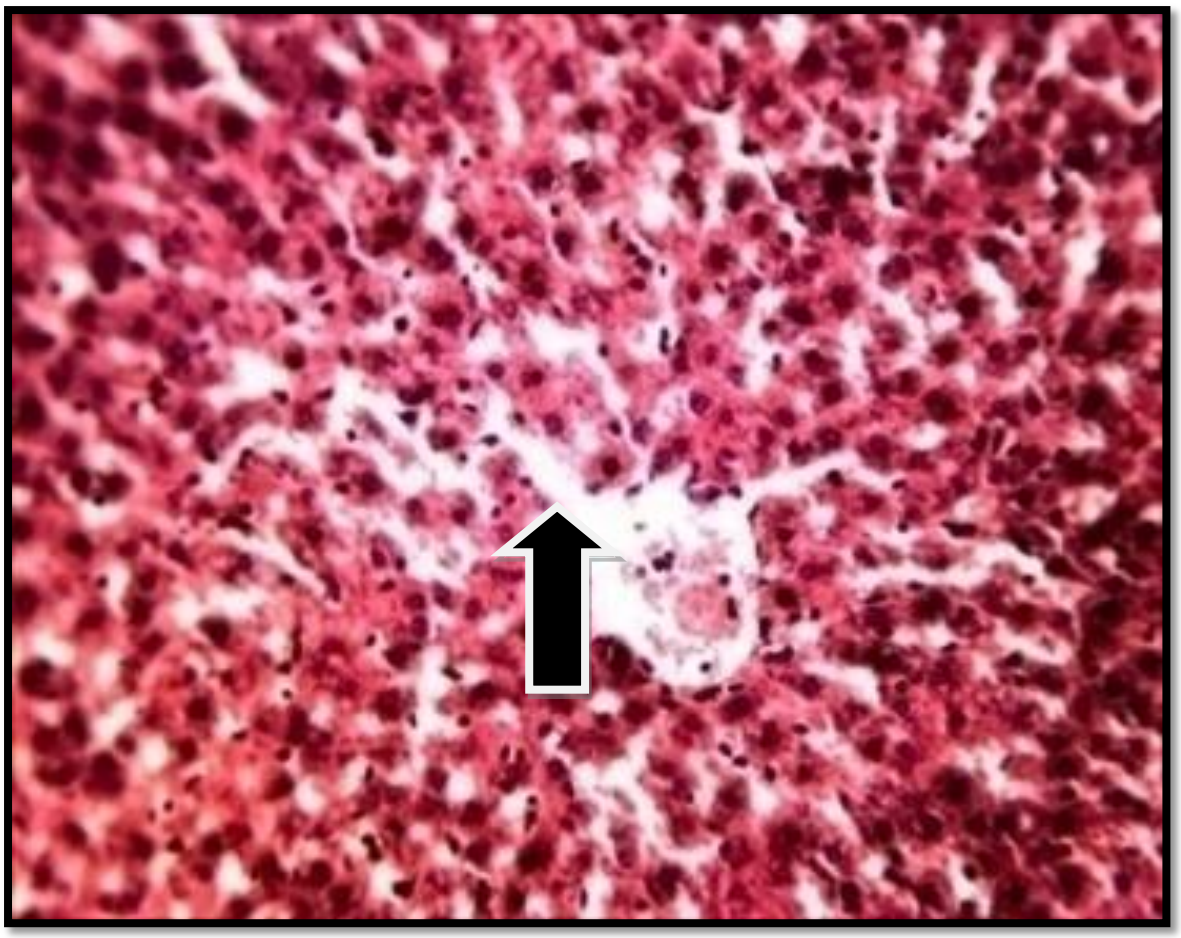


PLATE XVIII: Non-diabetic+roots ,Moderate hepatocellular necrosis. Hematoxylin and Eosin Stain (H & E) (X 250)

88

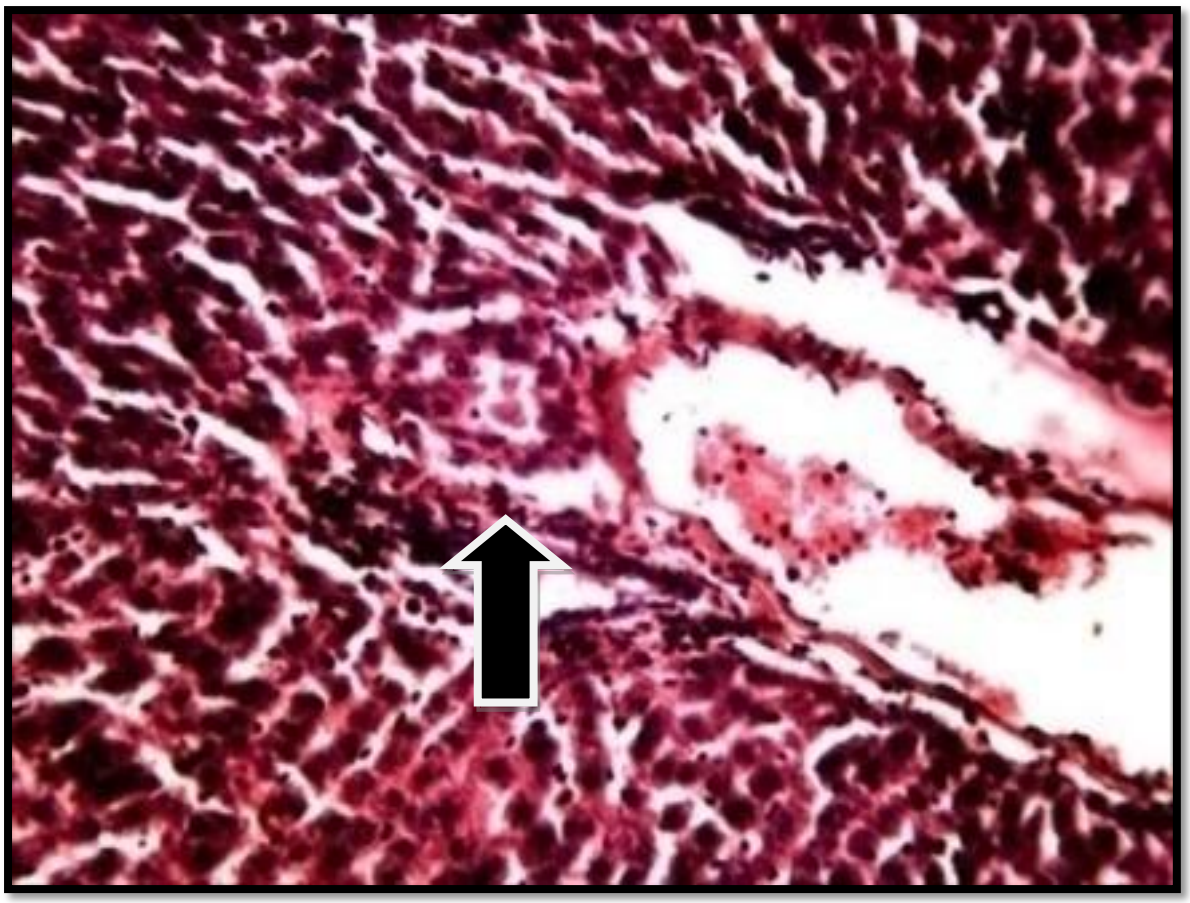


PLATE X1X :Non Diabetic+Glib ,Slight vascular congestion and slight lymphocytes hyperplasia.Hematoxylin and Eosin Stain (H & E) (X 250)

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CHAPTER FIVE

DISCUSSION

The bark of *D. cinerea* has been used as a traditional medicine for the treatment of diabetes mellitus since time immemorial and they claim for its promising activity. The preliminary phytochemical screening of methanol fruits, leaves, stem-bark and root extracts of D.Cinerea revealed presence of carbohydrates,sponins, flavonoids, tannins, steroids and triterpenoids, alkaloids, and cardiac glycoside while anthraquinone was absent in all the extracts (Johnstone, *et al.,* 2012)

Results of antidiabetic study established the scientific basis for the utility of this plant parts in the treatment of diabetes. The methanol extracts of leaves ,stem-bark, root and the standard drug glibenclimide showed significant reduction, ($P<0.05$) in blood glucose levels of streptozotocin induced diabetic's rats ,stem-bark with the highest reduction has 76.07%, then glibenclimide 53.19%,leaves 47.01%,root 43.10%, while fruits has 2.83% which showed no significant reduction. There was a significant reduction ($P<0.05$) in normoglycemic groups in accordance to (Digbijay *et al.,* 2013)

methanol extract of *D.cinerea* could reverse the hyperglycaemic condition in diabetic rats and brought about hypoglycaemic action because blood glucose once lowered by the extract did not increase again throughout experiment when compared to untreated control. The possible mechanism of action of the extract may be due to promoting the insulin release from the undestroyed β-cells or its action may be insulin like(Chandola *et al* 1980) .

The hypoglycemic activity of *D.Cinerea* can be attributed to the presence of phytochemicals such as flavoniods, saponin, tannins, glycosides, alkaloids that were revealed by qualitative phytochemical screening. Studies on medicinal plants that possess blood glucose lowering effect have shown that the effect is due to the presence of these phytochemicals (Oliver 1980, Mukherjee *et al*. 2006).

The decrease in body weight in diabetic rats clearly shows a loss or degradation of structural proteins due to diabetes as the structural proteins are known to contribute to body weight. The structural proteins are known to contribute for the body weight. Ravi *et al.* 2004 reported that the characteristic loss of body weight associated with STZ-induced diabetes is due to excessive breakdown of tissue proteins and an increased muscle wasting in diabetes. Soval *et. al.,* 2007 reported that in the absence of insulin, protein production is not favoured. Additionally, several studies showed that the levels of serum total protein were decline in diabetic animals (Surana *et al* 2008, Yokozawa *et al* 2008, Gupta and Gupta 2009). And the decrease in weight particularly in group treated with fruits and stem bark which may be due to distinct metabolic renal alterations are demonstrable in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Bhavapriya *et al.,* 2001).

There was significant improvement in PCV in all the groups treated with the extracts leaves, stem-bark, and root and fruits but the improvement in fruit extracts was not significant .These improvements in PCV fallowed due to the subsequent blood glucose lowering effects by the different plants extracts. FBG and HbA1c are the sole indicators of glucose metabolism in diabetic patients (Akinloye *et al.*, 2007). Decreases in (PCV) and (Hb) levels in diabetic rats have been reported (Shevchenko and Alfimov, 1995; Azeez *et al.,* 2010) indicating suppression of haemopoiesis. Diabetes-related anaemia was reported in advanced uraemia of diabetic nephropathy; however, diabetes affects the haematological system in several ways (Jones and Peterson, 1981).

The levels of aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found to be increased in diabetic rats after 21 days. It has been reported that patients with type 2 diabetes have higher incidence of liver function test

abnormalities than normal individuals. Mild chronic elevations of transaminases AST and ALT are often reflected underlying insulin resistance.

Table 4.6.1 shows serum TP, ALB, and TB concentrations of control study. Treatments had significant ($P<0.05$) increase in serum ALB and TP in all the groups treated, and also significant decrease ($P<0.05$) of TB in diabetic groups treated with, leaves, roots, and glibenclimide , when compared to normal control group.

Diabetes mellitus is associated with oxidative stress and this leads to depletion in the activities of the enzymic and non-enzymic antioxidant defense system. The antioxidant defense parameters investigated in this study are superoxide dismutase (SOD), catalase and malondialdehyde (MDA). Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of cells and protect pancreatic islets against the cytotoxic effects of STZ (Alvarez *et al.* 2004; Coskun *et al.*, 2005).

SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. SOD is the most efficient catalytic enzyme; its activity is only limited by the frequency of collision between itself and superoxide. group.

Catalase (CAT). They are an important antioxidant defence containing heavy metals in nearly all cells exposed to oxygen CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen. CAT has one of the highest turnover numbers of all enzymes;

Antioxidant activity against SOD and CAT showed a significant result the activities may be suggested due to presence of different class of phytoconstituents like triterpenoids, tannins and phenolic compounds, flavonoids etc. in the methanol extract, flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A_2 (Li *et al*., 2003) .This

property may explain the mechanisms of anti-oxidative action of *D. cinerea*. Flavonoids serve as health promoting compounds as a results of their anion radicals (Wanda *et al.,* 2010). *D. cinerea* was also found to contain saponins known to produce inhibitory effect on inflammation (Just *et al.,* 1998). The presence of these phenolic compounds in *D. cinerea* extracts contribute to its antioxidative properties and thus the usefulness of this plant in herbal medicament.

Decrease in creatinine level, distinct metabolic renal alterations are demonstrable in experimental diabetes, leading to a negative nitrogen balance, enhanced proteolysis and lowered protein synthesis (Bhavapriya *et al.* 2001) Changes in protein metabolism include a reduced uptake of amino acids by tissues, a higher rate of proteolysis and a fall in protein synthesis, leading to an increase in the production of urea by the liver (Felig *et. al.,* 1995) The overload of urea, glucose and other compounds in the kidney, together with renal vascular changes arising from the increased glycosylation of blood proteins, can damage the kidney and thus promote a loss of protein in the urine (Viberti *et al.,*1994)

Creatinine is the major waste product of creatine metabolism. In the kidney, it is filtered by the glomerulus and actively excreted by the tubules. Moreover, free creatinine appears in the blood serum (Stevenes *et al,*2006) urea is the principal waste products of protein catabolism. Urea is synthesized in the liver from ammonia, produced as a result of the deamination of amino acids. High-serum creatinine level is also the marker of muscle wastage (Luo and Luo 2009). Kidney function tests help to determine if the kidney is performing their task adequately. The diabetic rats had increased levels of creatinine and urea which are considered as significant markers of renal function and this is in agreement with the present result. (Ozsoy *et al* 2006)

The serum urea and creatinine concentrations of the normoglycemic study groups are presented in Table 4.6.2 The result showed no significant ($P>0.05$) change in serum urea

concentrations of the groups treated with fruits and leaves, except in the group treated with the stem-bark, root and glibenclimide extracts where the concentration is significantly ($p<0.05$) increased when compared to the normal control. Creatinine concentration is significantly increased in all non diabetic groups treated with extracts of *D.cinerea.*

Diabetic nephropathy is the largest single cause of end-stage renal failure worldwide. Despite the available modern therapies of glycaemic and blood pressure control, many patients continue to show progressive renal damage. Nephropathy is defined as partial loss of function of kidney associated with nephrotic syndrome, glomerulosclerosis, persistent albuminuria, declining glomerular filtration rate (GFR), elevated arterial blood pressure and fluid retention (Blickle *et al.,* 2007). Accumulation of glycogen in the kidney tubules as a result of the ensuing hyperglycaemia is thought to be responsible for the progression of disease in diabetic nephropathy (Ramesh ., 2006).The intention of this study was to evaluate the ability of fruits ,leaves, stem-bark,and root extracts of *D Cinerea* to protect against changes that occur in the kidney as a result of diabetes mellitus, however, we observed that different parts of *D Cinerea* are not protective against STZ induced morphological and biochemical changes in the kidney but rather slightly worsens it, causing hyperplasia, tubular and glomerular necrosis.

Hyperplasia of renal tubule epithelial implies an increase in the number of lining cells but without proliferation beyond the well defined, basement-bound integrity of the individual tubule structure. in both cases, hyperplasia may involve a cluster of tubule cross-sections because of the extention of the proliferative response along the convolutions of a single tubule segment(Hard and Neal, 1992)

Different parts of *D Cinerea treatment* caused elevation of renal function indices and loss of normal kidney architecture compared to non diabetic rats. The results of kidney biochemistry showed that STZ diabetic rats had a significant elevation in urea and creatinine levels

indicative of renal cell injury There was a dose related increase in urea and creatinine which would indicate renal injury. *D.Cinerea* at the doses of 100 mg/kg showed slightly worse features of toxicities compared to that seen with the diabetic control. The results of this study show that *D.Cinerea* in its crude state should be used with immense caution if at all in the treatment of diabetes. STZ-induced diabetes in rats had been shown to be associated with functional and/or morphological changes in the kidney (Alderson *et al* 2004). All structural changes in kidneys resulting from STZ administration in rats can thus be attributed to altered metabolism in diabetes.(Rasch ,1980) Present observations on the kidney sections showed minimal diffuse mesangial thickening and feature of mild chronic and non-specific interstitial nephritis and only very few glomeruli appear normal, indicating a progressive damage in streptozotocin induced diabetic rats, which increased with the duration of time and the severity of hyperglycaemia. Progressive glomerulosclerosis associated with decreased kidney function, resulting in end stage renal failure is the major finding in diabetic nephropathy

The present study was designed to observe the effects of STZ-induced diabetes on the liver. Elevated activities of serum aminotransferases are a common sign of liver diseases and are observed more frequently among people with diabetes than in general population (Arkkila *et al*.; 2001).We observed accumulation of lipid droplets in the cytoplasm of hepatocytes. This change was reminiscent to the formation of fatty liver. It could be due to the increased influx of fatty acids into the liver induced by hypoinsulinemia and the low capacity of excretion of lipoprotein secretion from liver resulting from a deficiency of apolipoprotein B synthesis (Ohno *et al*. 2000) Hyperlipidemia could be another factor for fatty liver formation. Our findings of fatty liver formation are in agreement with the findings of (Ohno *et al* 2000.; Merzouk *et al*. 2000). The glycogen content of the hepatocytes in the liver of STZ-treated animals was markedly decreased (Thulesen *et al*., 1997; Das *et al*. 1996). It could be due to

the displacement of glycogen in the cytoplasm of hepatocytes as a consequence of accumulation of lipid droplets (Thulesen *et al.* 1997)

The increase in aminotransferases levels may be due to the cellular damage in the liver caused by STZ-induced diabetes. Although ALT is also present in mitochondria and cytosol, the mitochondrial form is low in activity and is very unstable. The detailed mechanism by which enzymes are released from the cytosol and mitochondria of hepatocytes is not completely known.

Very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood (Garella 1997)

CHAPTER SIX

SUMMARY CONCLUSION AND RECOMMENDATIOS

6.1 SUMMARY

The results obtained from this study are quite promising and comparable with the reference standards used. The most significant findings of this study are that the methanolic stem-bark, ,leaves and root extracts of *D. cinerea* have shown beneficial effect not only on decreasing blood glucose level, but is also possess antioxidant activity.

D.Cinerea at the doses of 100 mg/kg showed slight toxicities in kidney and liver. Therefore results of this study showed that *D.Cinerea* in its crude state should be used with immense caution if at all in the treatment of diabetes.

6.2 CONCLUSION

Antidiabetic and antioxidant potential of methanolic extracts of *D.cinerea* were investigated in streptozotocin-induced diabetic and normoglycaemic rats using standard methods. The following conclusions were drawn from this study

1. Phytochemicals such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids and triterpenes are present in *D. cinerea* which have earlier been reported to constitute the active metabolic part of hypoglycaemic plants.
2. Methanol extracts of *D.cinerea* showed ameliorative effect on diabetic rats as revealed by the percentage change in fasting blood glucose, packed cell volume, body weight.
3. *D.cineres* showed maximum antioxidant activity with the stem-bark extract, followed by the leaf and root respectively. All the extracts exhibited significant ($p<0.05$) increase in catalase activity.
4. The hepatotoxicity and nephrotoxicity effects of the extracts were highest with the Stem-bark extract, followed by the roots and leaves extracts.

6.3 RECOMMONDATION

i. Further research should explore the exact antidiabetic mechanism(s) of actions of each of the extract that is responsible for the pharmacological response.

ii. In addition, comprehensive pharmacological investigations should be carried out to assess the likely toxicological effects of this antidiabetic plant.

iii. *D.cinerea* can be very useful in the management of diabetes mellitus since the methanolic extracts obtained from different parts of the plant showed both antidiabetic and antioxidant potential. The use of *D. cinerea* in the management of diabetes should therefore be encouraged at appropriate dose.

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APPENDICES

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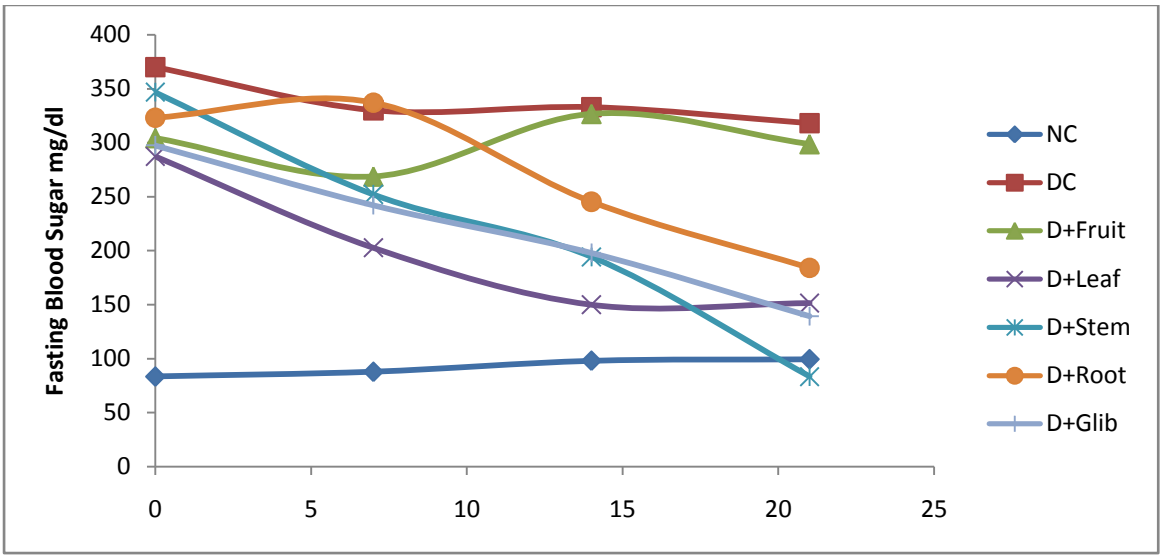

Figure 1.1 Fasting blood glucose level of Normal Control, Diabetic Control and Diabetic treated treated with Glibenclamide 1mg/kg and Streptozotocin-induced diabetic rats treated with 100mg/kg methanolic Fruits, Leaves, Stem-Bark and Roots extracts of *D. Cinerea*

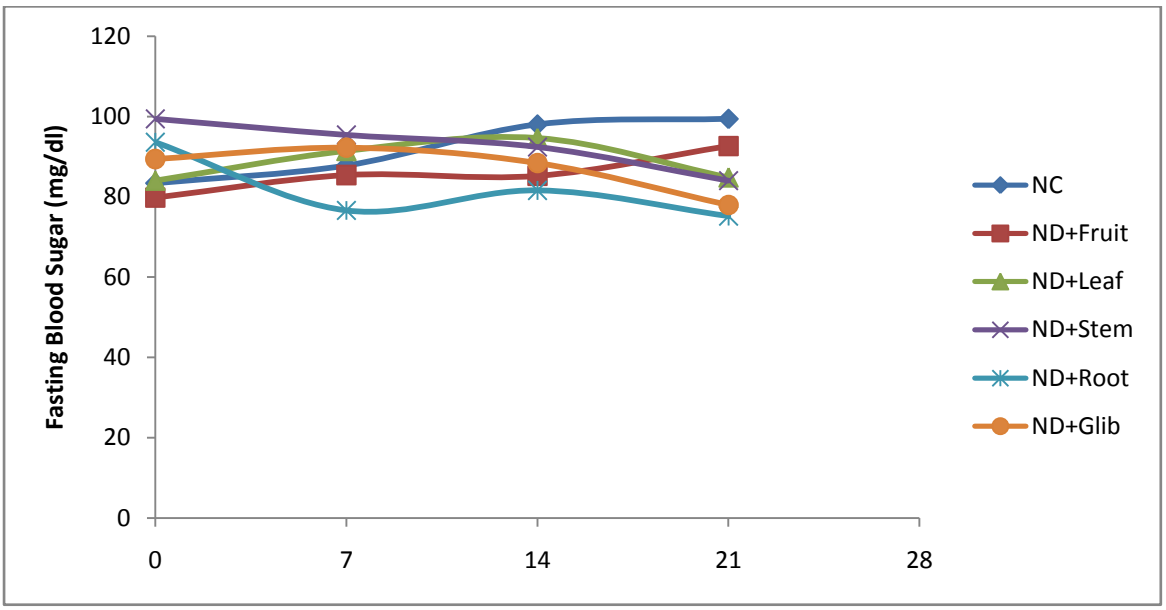

Figure1.2 Fasting blood glucose level of Normal Control and Normoglyceamic treated with 100mg/kg methanolic Fruits, Leaves, Stem-Back and Roots Extracts of *D.Cinerea* (n=5)

APPENDIX 2.0

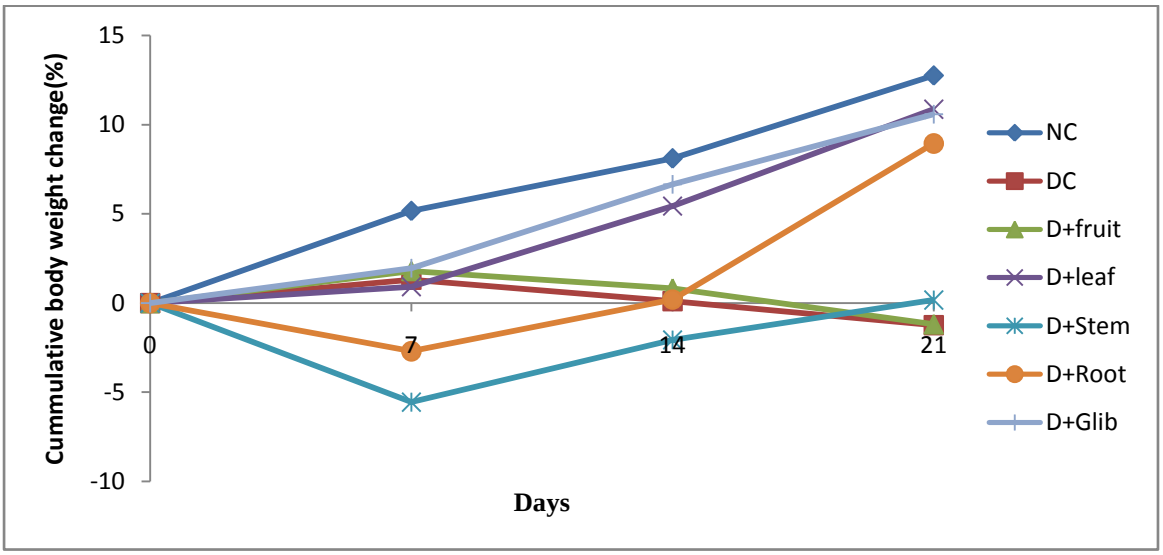

Figure 2.1.Cummulative Body weight change of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)

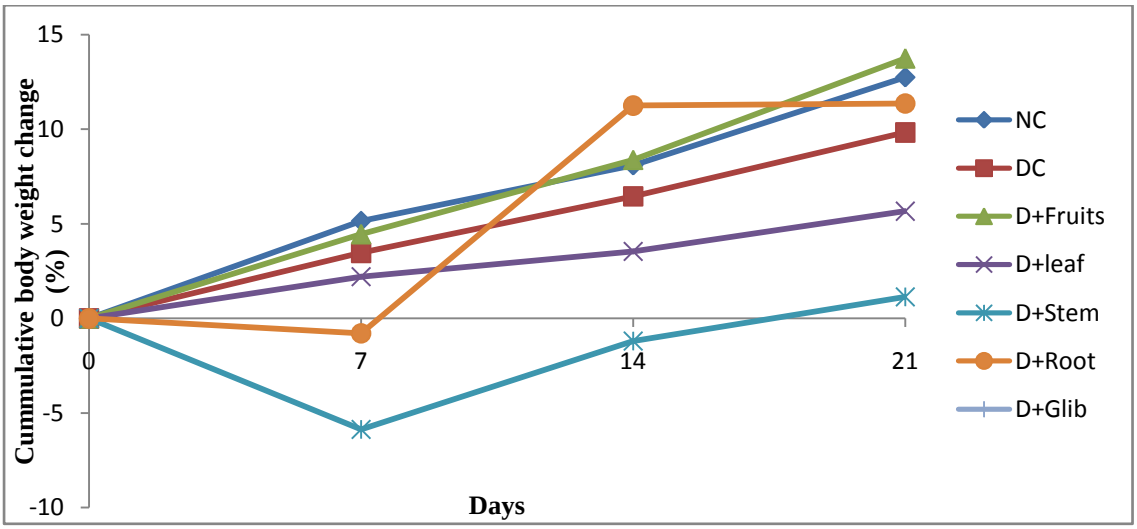

Figure 2.2 Cummulative Body weight change of Normoglyceamic rats treated with 100mg/kg Methanolic ,Fruits,Leaves, Stem-bark and Roots Extracts of *D.Cinerea*

APPENDIX 3.0

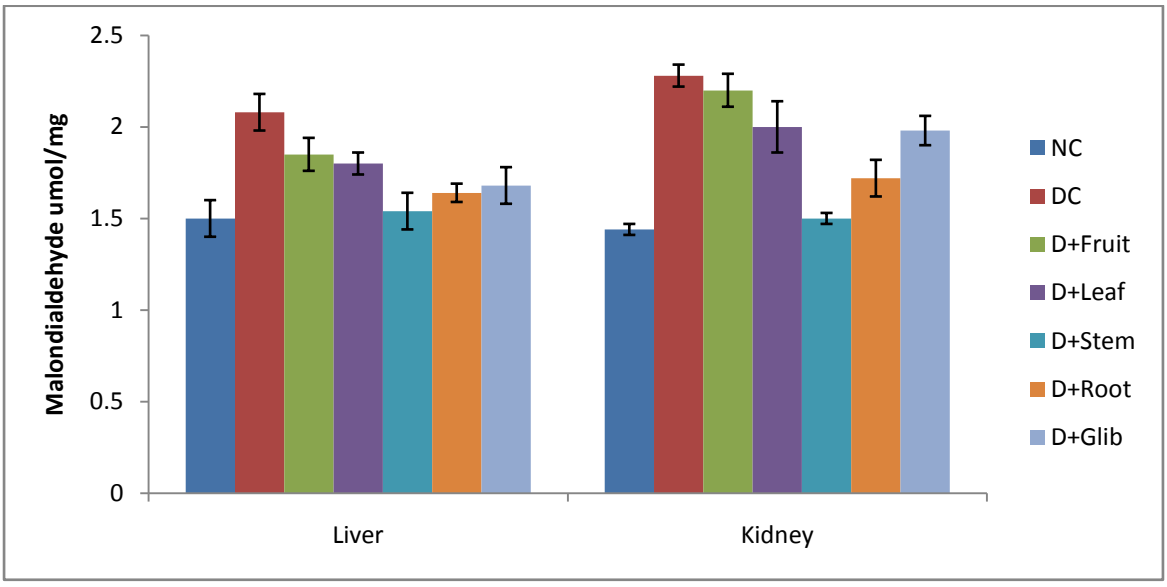

Figure 3.1 Malondialdehyde levels in tissues of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Root extracts of *D.Cinerea* (n=5)

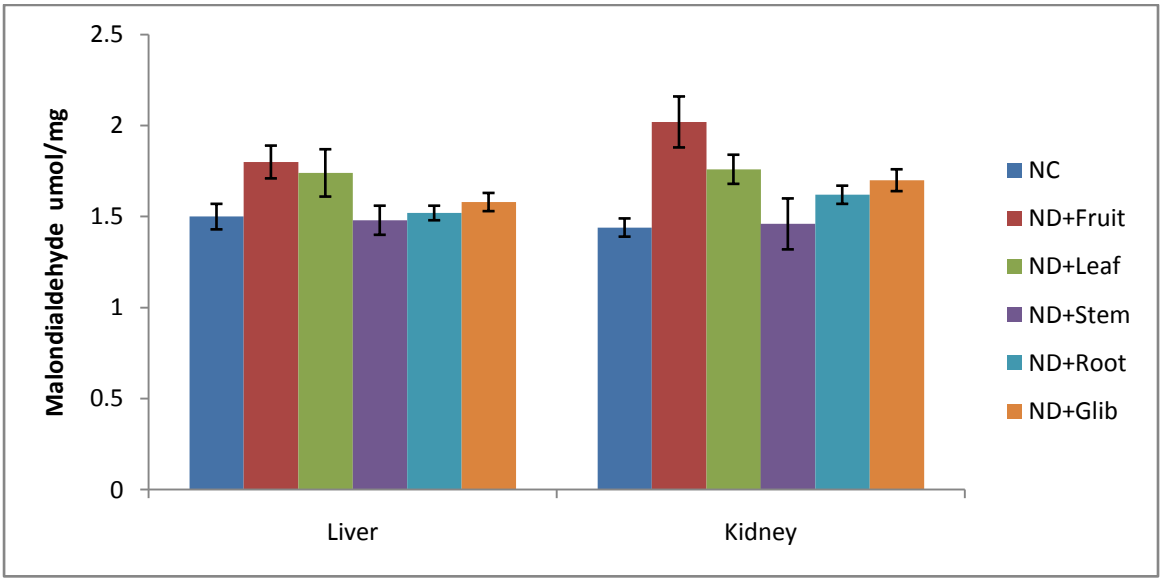

Figure 3.2 Malondialdehyde levels in tissues of Normoglyceamic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots extracts of *D.Cinerea* (n=5)

APPENDIX 4

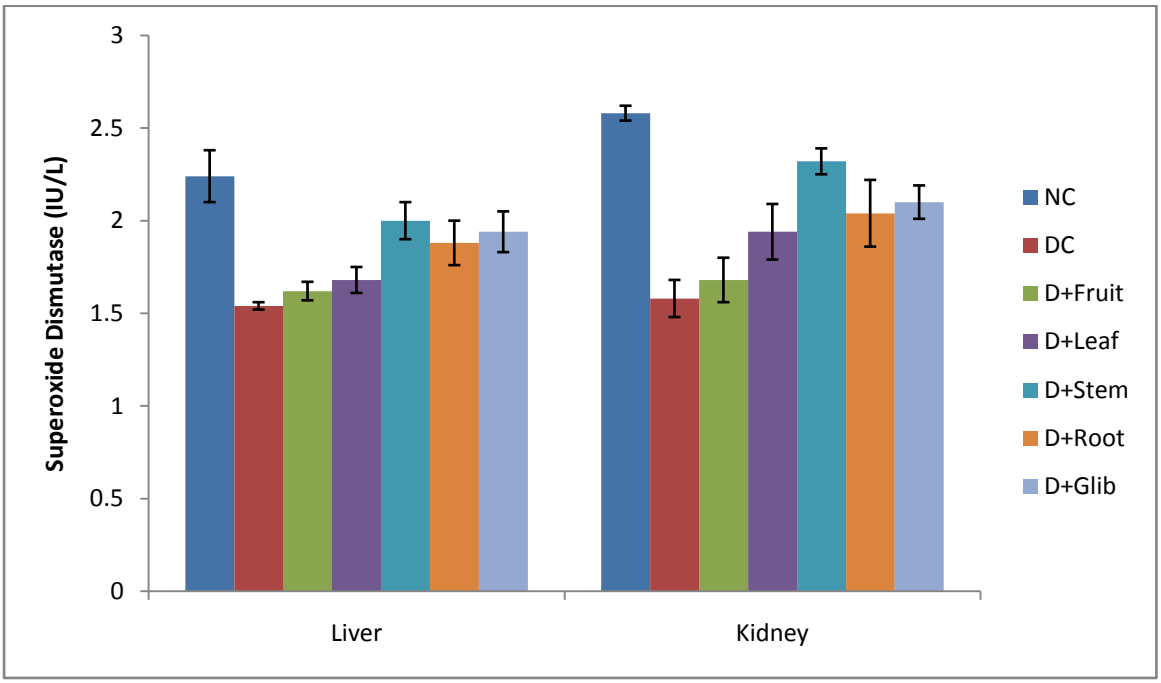

Figure 4.1 Superoxide dismutase levels in tissues of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Root Extracts of *D.Cinerea* (n=5)

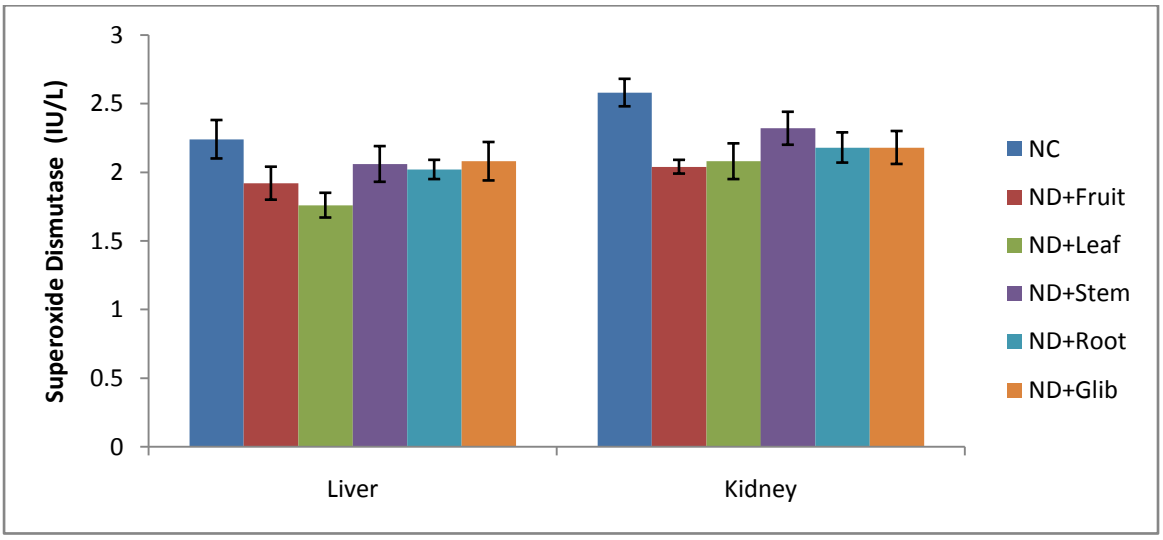

Figure 4.2 Superoxide dismutase levels in tissues of Normoglyceamic rats treated with 100mg/kg Methanolic Fruits,Leaves,Stem-Bark and Roots Extracts of *D. Cinerea* (n=5)

APPENDIX 5.0

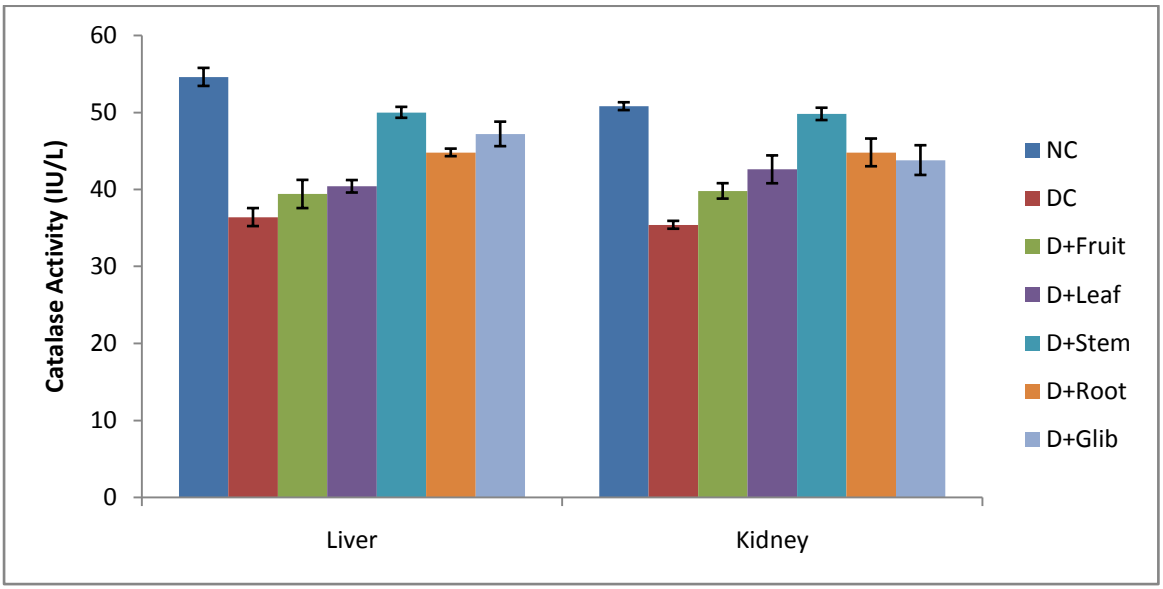

Figure 5.1 Catalase levels in tissues of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves,Stem- Bark and Roots Extracts of *D.Cinerea* (n=5)

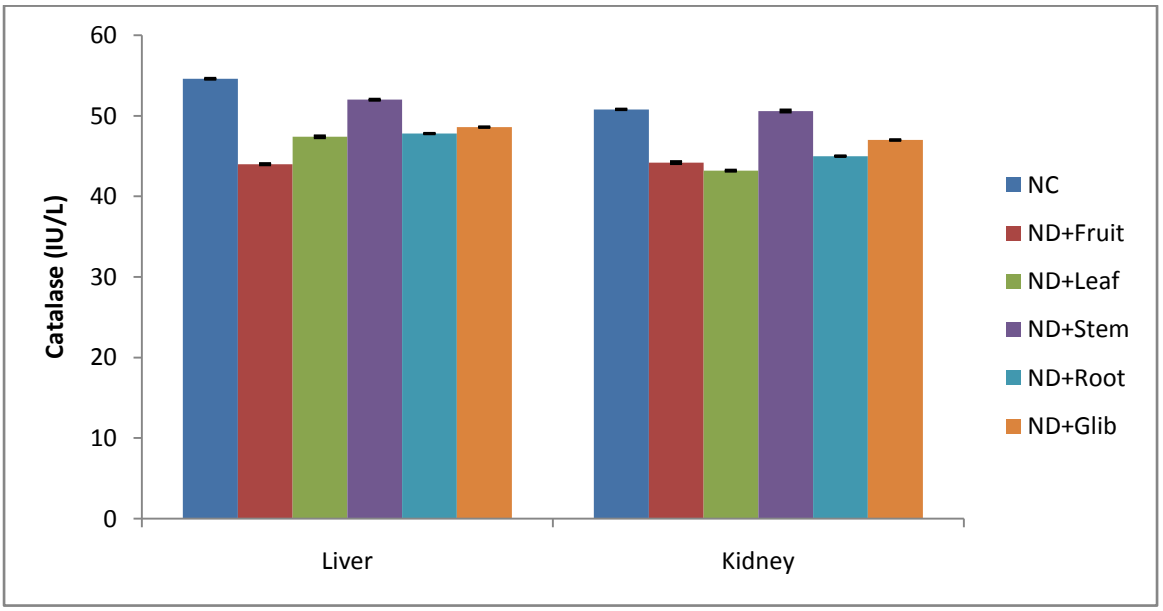

Figure 5.2 Catalase levels in tissues of Normoglyceamic treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)

APPENDIX 6.0

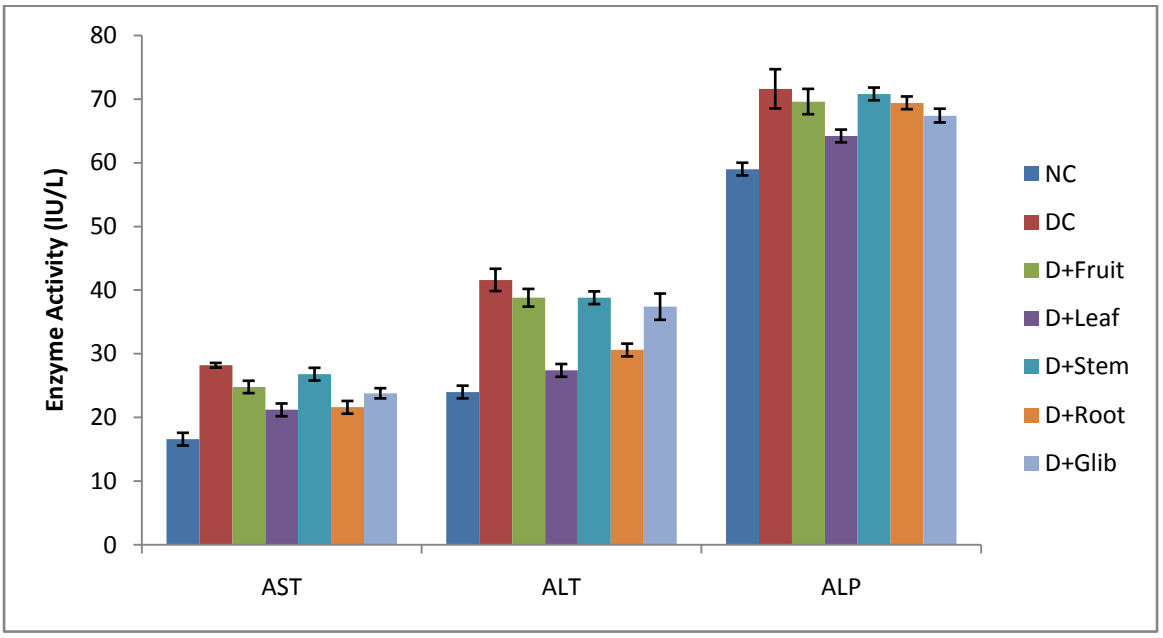

Figure 6.1 Liver marker enzymes level of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Streptozotocin induced rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)

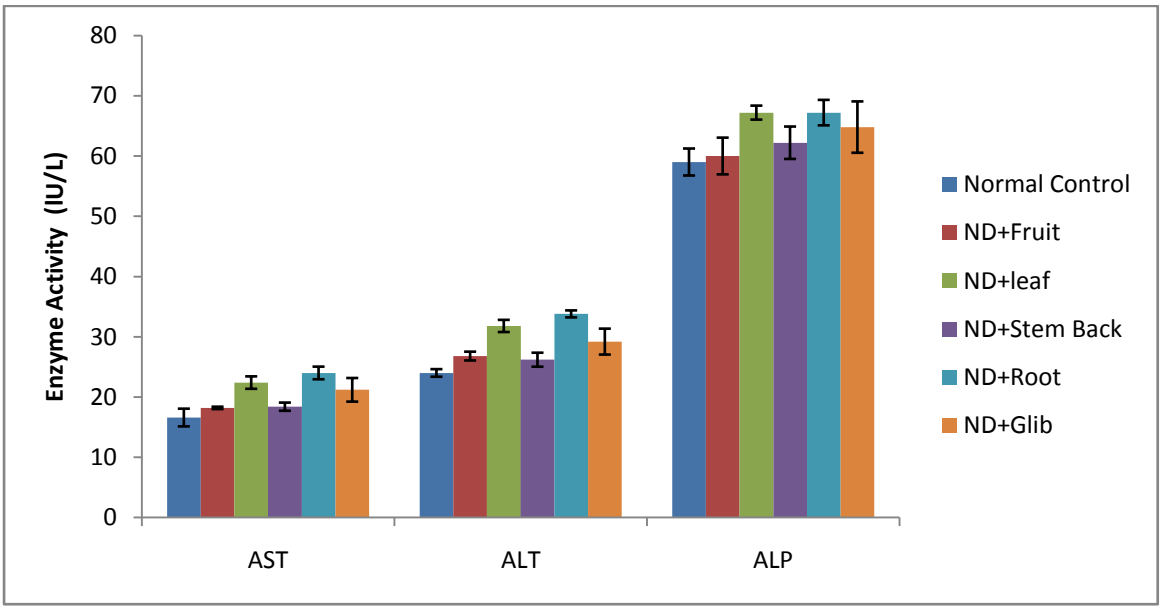

Figure 6.2 Liver marker enzymes level of Normoglyceamic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)

APPENDIX 7.0

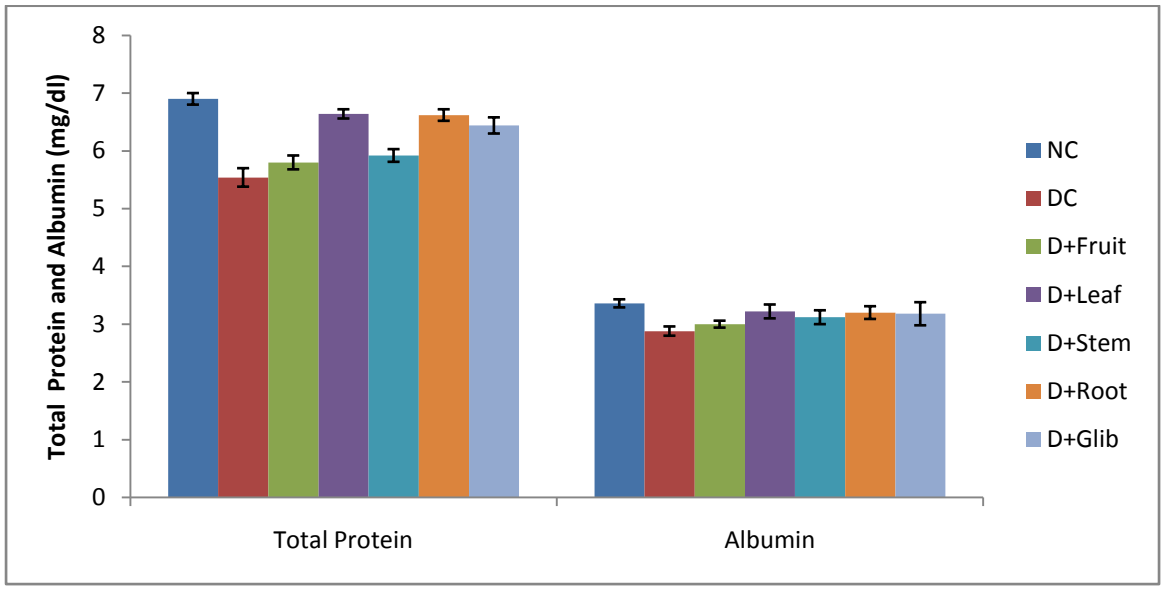

Figure 7.1 Total protein and albumin levels of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots extracts of *D.Cinerea* (n=5)

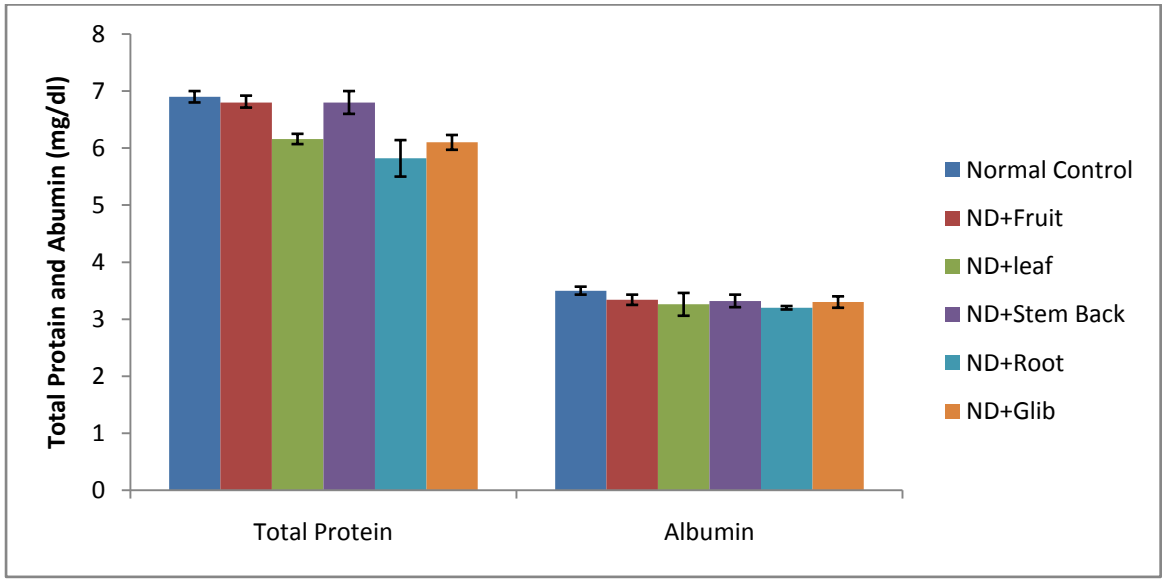

Figure 7.2 Total protein and albumin levels of Normoglyceamic treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)

APPENDIX 8.0

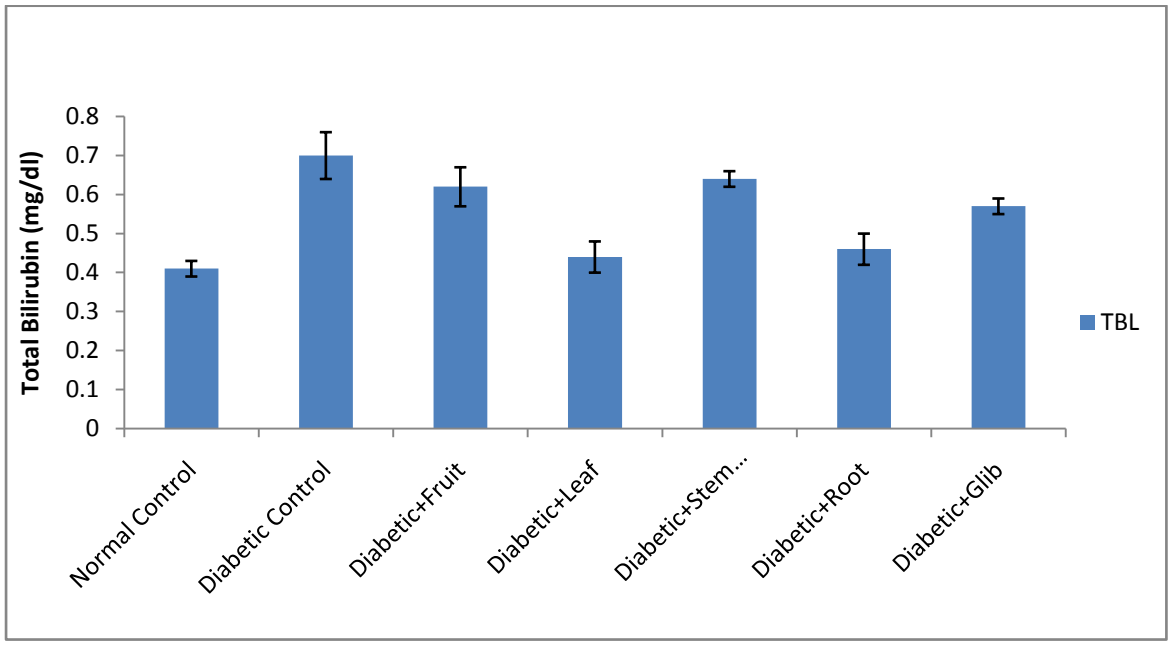

Figure 8.1 Serum Bilirubin levels of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Streptozotocin-induced diabetic rats treated with 100mg/kg methanolic Fruits, leaves, Stem-Bark and Roots extracts of *D.Cinerea* (n=5)

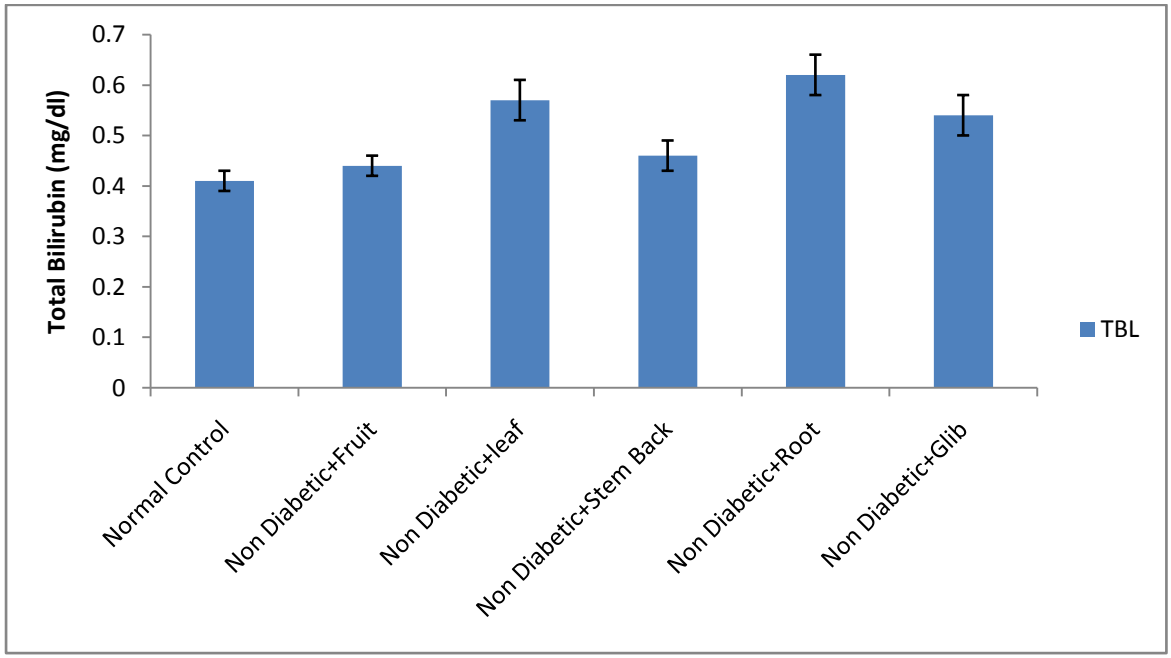

Figure 8.2 Serum bilirubin levels of Normoglyceamic treated with 100mg/kg Methanolic Fruits,Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5) Urea

APPENDIX 9.0

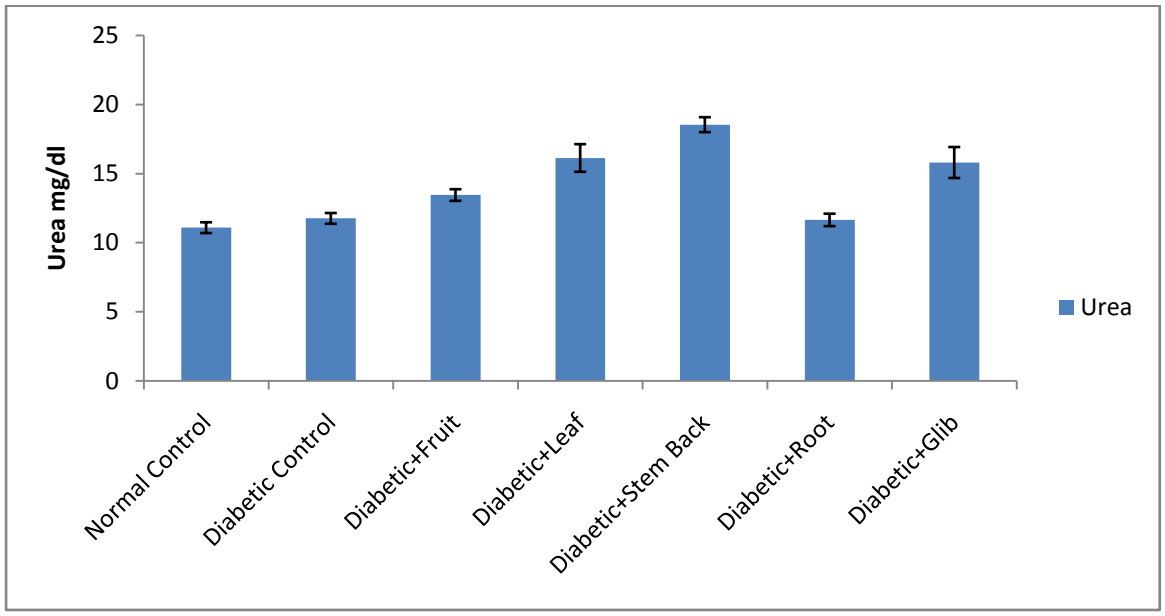

Figure 9.1 Serum urea levels of Normal Control, Diabetic Control, Diabetic treated with Glibenclamide 1mg/kg and Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark and Roots extracts of *D.Cinerea* (n=5)

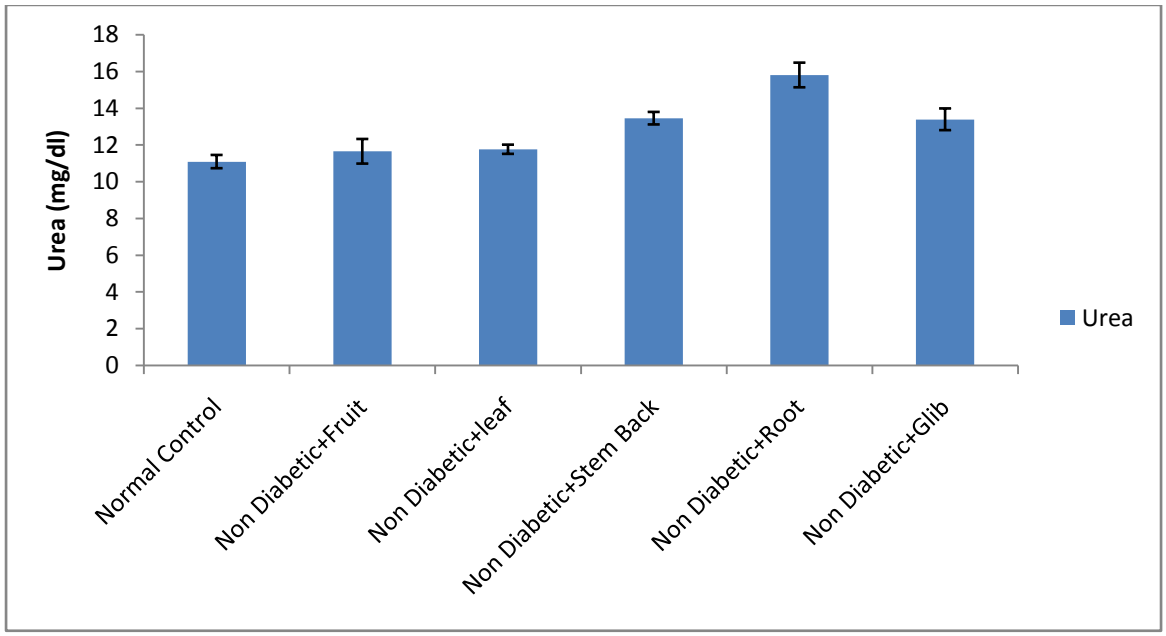

Figure 9.2 Serum urea levels of Normoglyceamic treated with 100mg/kg Methanolic Fruits, Leaves, Stem-Bark sand Roots Extracts of *D.cinerea* (n=5)

APPENDIX 10.0

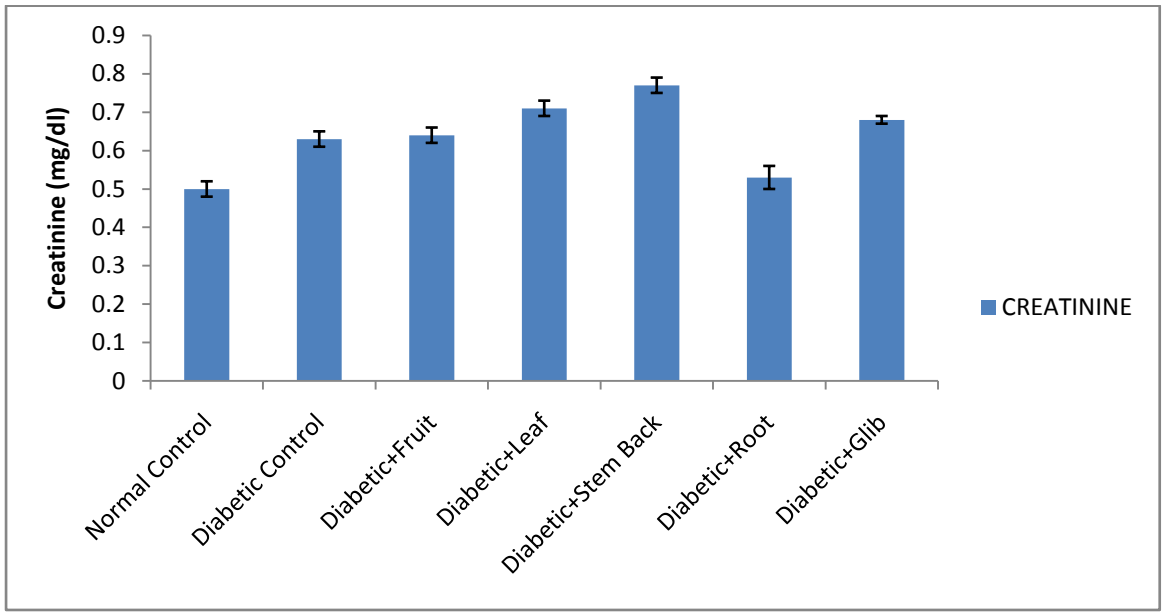

Figure 10.1 Serum creatinine levels of Streptozotocin-induced diabetic rats treated with 100mg/kg Methanolic Fruits, Leafs,Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)

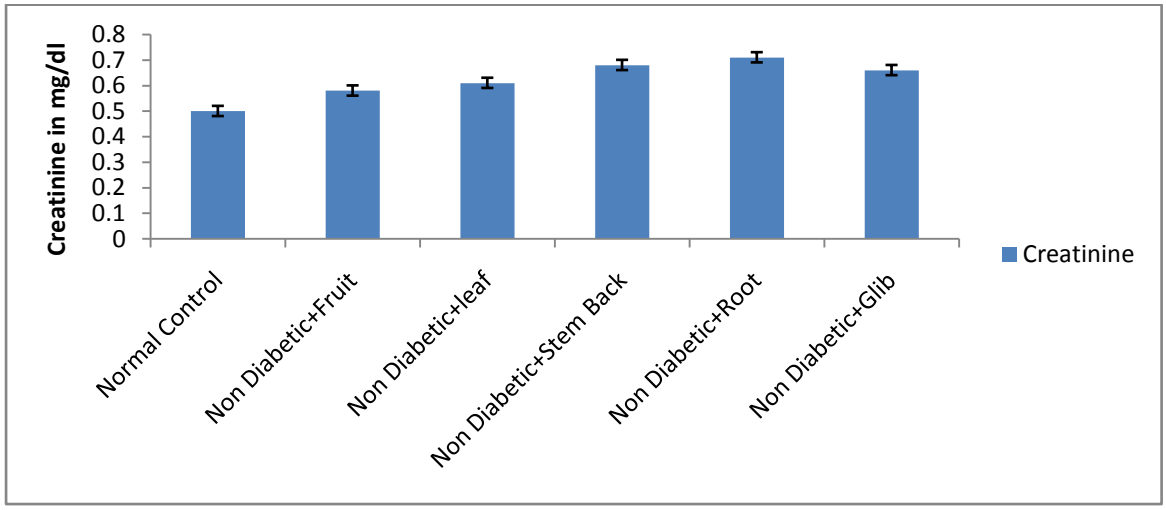

Figure.10.2 Serum creatinine levels of Normoglyceamic treated with 100mg/kg Methanolic Fruits,Leaves, Stem-Bark and Roots Extracts of *D.Cinerea* (n=5)