TITLE PAGE

ANTIHYPERGLYCEAMIC EFFECT OF N-HEXANE FRACTION OF *Lepidagathis alopecuroides* LEAVES IN ALLOXAN INDUCED DIABETIC RATS

A PROJECT REPORT SUBMITTED IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc) IN PHARMACHOLOGICAL BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA.

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CERTIFICATION

Ukam Endurance Egwu, a postgraduate student with Registration Number PG/M.Sc/17/00078 in the Department of Biochemistry, Faculty of Biological sciences, University of Nigeria, Nsukka, has satisfactorily completed the requirements for the course work and research for the award of Masters of Science (M.Sc.) in Biochemistry (Pharmacological Biochemistry). The work embodied in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

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DEDICATION

This project work is dedicated to God Almighty who in his infinite mercy brought this work to a successful conclusion.

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ABSTRACT

Lepidagathis alopecuroides (Vahl) is a tropical shrub belonging to the family Acanthaceae commonly found in the coastal countries of West Africa. The leaves are used to immobilize fish in many communities of Rivers and Cross River States of Nigeria. The present study was carried out to investigate the antihyperglycemic activity of the n-hexane leaf fraction of *L. alopecuroides* in alloxan induced diabetic rats. A total of eighteen adult mice were used to determine the LD_{50}, while a total of 24 adult Wister rats where used for the evaluation of the antidiabetic effect of the fraction. The animals were divided into six groups, with each group containing 4 rats. Group 1 was the normal control, group 2 was the positive control, group 3 was the standard control, and group 4, 5, and 6 received 100, 200 and 400 mg/kg b.w of the n-hexane leaf fraction respectively. Their blood glucose and insulin levels were assayed. Their serum samples were analyzed after 2 weeks of treatment, to ascertain the alkaline phosphatase (ALP), alanine aminotranferase (ALT) aspartate aminotranferase (AST) superoxide dismutase (SOD) and catalase (CAT) activities. Malondialdehyde (MDA), reduced glutathione (GSH), creatinine and urea serum levels were also tested. Lipid profile (total cholesterol, TAG, LDL and HDL) as well as some haematological indices (RBC, WBC, Hb, and PCV), and the histology of the pancreas were also investigated. The phytochemical screening of the plant leaf showed that alkaloids, terpenoids, flavonoids, and saponins were abundantly present while phenols and tannins were moderately present. Glycosides were slightly present. The LD_{50} of the extract showed no mortality. The body weight of the animals in the treated diabetic groups as well as in the normal control group significantly increased by day 14 when compared to positive control group. The result of the study on blood glucose as well as insulin level revealed that the leaf fraction caused a significant ($p < 0.05$) reduction in hyperglycemia relative to the positive control group. A significant ($p < 0.05$) decrease in insulin level was observed in positive control group when compared to normal control. In contrast, both standard control and the groups that received graded doses of leaf fraction showed a significant ($p < 0.05$) increase in insulin level when compared to positive control. Generally, there were significant ($p < 0.05$) decrease in the activities of ALP, AST, and ALT in the treatment groups when compared to the untreated group. Urea and Creatinine concentration significantly ($p < 0.05$) reduced in the treatment groups when compared to positive control. There were significant ($p < 0.05$) reduction in MDA concentration of the groups that received 200 and 400 mg/kg of leaf fraction when compared to untreated groups. CAT, SOD and GSH level in treatment groups were significantly ($p < 0.05$) higher when compared to positive control. LDL, TAG, and total cholesterol significantly ($p < 0.05$) decrease in the treatment groups when compared to positive control. However, HDL on the other hand increased significantly ($p < 0.05$) in treatment groups when compared to positive control.WBC, RBC, HB and PVC significantly ($p < 0.05$) increased in the treatment groups when compared to positive control. Histology of positive control showed a marked decrease in the number and sizes of pancreatic islets. The islet cells showed a vacuolar degeneration and necrosis, swollen with foamy cytoplasm and eosinophilic necrotic debris when compared to normal control, which showed the normal histomorphology of the endocrine pancreas. There was also a reduction in the number and sizes of pancreatic islets in group 3 and 4. However, group 5 and 6 showed a marked proliferation of the α-cells in the pancreatic islets with less abundant β-cells. The results of this study showed that the n-hexane leaf fraction of *Lepidagathis alopecuroides* significantly reduced the hyperglycemia in alloxan-induced diabetic rats and also restored some altered haematological indices as well as some biochemical parameters in alloxan-induced diabetic rats. The

investigation possibly authenticates the use of *L. alopecuroides* leaf fraction in folkloric treatment of diabetes and its associated complication.

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LIST OF ABREVIATIONS

ABA: Abcisic acid

ADA: American Diabetes Association

ALP: Alkaline Phosphatase

ALT: Alaline Aminotransferase

ANOVA: Analysis of variance

AST: Aspartate Aminotransferase

BDCP: Bioresources Development and Conservation program

BGs: Biguanides

CAT: Catalase

DNA: Deoxyribonucleic acid

EDTA: Ethylene Diammine Tetra-acetic acid

GA: Gibberellic acid

GAD: Glutamic acid decarboxylase

GLUT 2: Glucose Transporter 2

GPx: Glutathione peroxidase

GR: Glutathion reductase

HDL: High density lipoprotein

IDDM: Insulin Dependent Diabetes Mellitus

LD: Lethal dose

LDL: Low density lipoprotein

MDA: Malondialdehyde

NADPH: Nicotinamide adenine dinucleotide hydrogen

NBT: Nitroblue terazolium

OHD: Oral hypoglycemic drug

PUFAs: Poly unsaturated fatty acids

SDS: Sodium Dodecyl Sulphate

Se: Selenium

SOD: Superoxide dismutase

SU: Sulphonylurea

TBA: Thiobarbituric acid

TCA: Trichloroacetic acid

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CHAPTER ONE

INTRODUCTION

Diabetes Mellitus (DM) is a common disorder characterized by the presence of chronic hyper glycemia that results from defects in insulin secretion, insulin action or both (Maryam and Rahimi, 2015). DM is a prevalent disease affecting the citizens of both developed and developing countries (Mashud *et al.,* 2010). It is predicted that about 366 million people are likely to be diabetic by the 2030 (WHO, 2011). It is a major health concern because it is frequently associated with debilitating health complications such as blindness, kidney function failure, cardiovascular diseases, amputations, and subsequently death. The classic symptoms of untreated diabetes are weight loss, polyuria. Other symptoms include blurry vision, headache, fatigue, slow healing of cuts and itchy skin (WHO, 2018). The therapeutic cure for diabetes mellitus has remained elusive despite the discovery of an array of medications that can ameliorate the outcome of the disease. Therefore, it has become imperative to investigate alternative sources of medicament, especially those that are cheap and easily sourced. In traditional practices, medicinal plants are used to control diabetes mellitus in many African countries. In Nigeria, herbal therapies occupy a very special position in health delivery, especially among the rural populace. Easy accessibility and low cost of treatment enhance patronage (Iwueke *et al.*, 2008). Recent findings revealed that over 80 % of the African population relies on medicinal plant species for their primary healthcare (Ngbolua *et al.*, 2016). The hypoglycemic effects of some medicinal plants used as antidiabetic remedies have been reported (Odoemena *et al.*, 2010; Maryam and Rahimi, 2015).

Lepidagathis alopecuroides (Vahl) is a tropical shrub belonging to the family Acanthaceae commonly found in the coastal countries of West Africa. The leaves are used to immobilize fish in many communities of Rivers and Cross River States of Nigeria. In Rivers State it is used for quick kill of hardy fish like mudskippers (Obomanu *et al.*, 2007) and in Cross River State it is normally applied in pools to kill mostly catfishes and tilapias constituting the major source of dietary protein (Ekanem *et al.*, 2003). Local fishermen applies finely ground parts of the leaves of this plant on mudflats of tidal water at low tide period to stupefy mudskippers and other mud dwelling fishes in parts of Rivers and Cross River states, Nigeria (Obomanu *et al.*,2007). When put in contact with fishes, the extract caused high mortality rate of the fishes. According to

Obomanu *et al.* (2005), the plant possesses some antimicrobial activity and therefore finds its use by the locals for the treatment of abdominal pains and diarrhea. It also possesses larvicidal action against mosquito's larvae (Obomanu *et al.*, 2006). Phytochemical screening of the plant revealed the presence of alkaloids, tannins, saponins, glycosides and flavonoid (Obomanu *et al.*, 2005) all of which have been proven to posses some form of toxic effect on organisms. The aqueous extract of the leaves of *L. alopecuroides* on the haematological, biochemical, behavioral (Opercular and tail beat frequencies) and mortality of African catfishes of various sizes have been reported (Gabriel and Okey, 2009: Keremah, *et al.*, 2010). Leaves and stems of *L. allopecuroides* has ben reported to have a preservative effect on wood (*Bomabax buonopozense*) against termites attack. (Edori, *et al.*, 2016).

1.5 Botanical Description of *Lepidagathis alopecuroides* Plant

Lepidagathis alopecuroides (Vahl) is a decumbent slender branching herb, woody bellow, rooting at the lower nodes, with small pink or purplish flowers-1/3 inches long in rather dense almost bristly pseudo-spike; inflorescenes very variable in length and density. They are usually found on coaster region of west affrica. (Obomanu *et al.*, 2007).

1.5.1 Taxonomy of *Lepidagathis alopecuroides* Plant

Kingdom Plantae

Subkingdom Viridiplantae

Infrakingdom Streptophyta

Superkingdon Embryophata

Division Tracheophyta

Subdivision Spermatophytina

Class Magnoliopsida

Order Lamiales

Family Acantheceae

Genus Lepidagathis

Species *Lepidagathis alopecuriodes* (Burkill, H.M. 1985).

Fig.1 Digram Showing the Leaves of *Lepidagathis alopecuroides*

Source: http://www.westafricanplants.senckenberg.de/root/index.php?page_id=14&id=6109

1.5.2 Medicinal Uses of *Lepidagathis alopecuroides*

Lepidagathis alopecuroides (Vahl) like many other plants has been beneficial to the local users in the treatments of various diseases including abdominal pains, diarrhea (Obomanu, *et al.*, 2005). The whole plant has been screened for antimalaria activity (Claude and Al. 1994), The aqueous leaf crude extract is used locally for the treatment of persistent sores and administered orally in the local gin for serious stomach upset (Orlu and Ogugua, 2012). In Dominica, Caribs make an infusion of it which is given to calm frightened children (Uphof, 1968; Usher, 1974). These medicinal properties of the plant have been attributed to the important phytochemical constituents such as flavonoids, alkaloids, glycosides, terpenoids, tannins and saponin (Obomanu *et al.*, 2005).

1.5.3 Previous Scientific Investigation

Review of documented literatures showed that the plant possesses antimalaria, antidiarrhea, antimicrobial activity and have been effective in treatment of abdominal pains (Obomanu, *et al.*, 2005). Its larvicidal action against mosquito's larvae has also been reported (Obomanu *et al.*, 2006), Insecticidal activity has been reported (Edori, *et al.*, 2016). Its picicidal effect against hardy fish like mudskippers (Obomanu *et al.*, 2007), and other types of fishes like catfishes and tilapias in parts of Rivers and Cross River State has been documented (Ekanem *et al.*, 2003). Sublethal concentrations of the aqueous leaf extract have been reported to induce degenerative changes in the germinal epithelium of fresh water catfish *Clarias gariepinus* during spermatogenesis (Orlu and Gabbriel, 2011a). Post testicular effect vis-à-vis significant reduction in sperm quality, motility and hatchabilty (Orlu and Ogbalu, 2011) and the effect on some Liver biomarkers and biochemistry have also been reported (Orlu and Gabbriel, 2011b).

1.6 Phytochemicals

Phytochemicals are secondary metabolites produced by plants; they give plants its colours, flavour, smell and are part of a plant's natural defence system (Okwu, 2005). Phytochemicals are chemical compounds formed during the plant normal metabolic processes. They are made up of several classes of compounds which include: alkaloids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2005). These compounds have been linked to human health by contributing to protection against deteriorative diseases (Dandjesso *et al.*, 2012). Phytochemicals are present in varieties of plants and are utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Different mechanisms have been suggested for the action of phytochemicals. They may act as antioxidants, or modulate gene expression and signal transduction pathways (Dandjesso *et al.*, 2012). The medicinal values of these plants lie in their component phytochemicals, which produce the definite physiological actions on human body.

1.7 Glucose Homeostasis

Glucose is the main fuel for most tissues. Glucose metabolism is critical to normal physiological functioning. Glucose act both as a source of energy and as a source of starting material for nearly all types of biosynthetic reactions. Oxidative metabolism of glucose allows production of adenosine triphosphate (ATP), which is an energy source for many chemical reactions in the

body (Wolfe, 1998). Plasma glucose concentration is a reflection of the balance between glucose uptake in the gut, endogenous production, utilization and storage in the body (Woerle *et al*., 2003). Endogenous production of glucose is through gluconeogenesis and glycogenolysis, while tissue utilization is mainly mediated via glycolysis and to a lesser extent, the pentose phosphate pathway. Following ingestion of meal, a third of glucose is taken up by the brain tissue where it undergoes complete aerobic break down to water and carbon dioxide, 2 to 3 hours later; another third is used by the muscle where it is converted to glycogen or catabolized to lactate or water and carbon dioxide; and the rest is taken up by the liver where it is mainly converted to glycogen and stored (Morifuji *et al*., 2005). The liver, therefore, play an essential role in the maintenance of blood glucose. It maintains blood glucose levels by maintaining a balance between glucose uptake and its conversion to glycogen and the release of glucose into circulation via glycogenolysis and gluconeogenesis (Saadat *et al*., 2004). The role of other tissues such as the kidney in endogenous glucose production is minimal. Nevertheless, the contribution of kidney is significant during prolonged fasting.

1.7.1 Glucose Transport into Cells

The lipid nature of cell membranes offers a barrier against free glucose entry into cells. These are 2 major types of glucose transporters that overcome this membrane barrier. Sodium-glucose co-transporters are mainly located in the kidney and intestines; these actively transport glucose into cells against concentration gradient by transporting sodium. GLUT transporters are a family of glucose transporters that transport glucose into cells passively, down a concentration gradient. They include GLUT-2, located in the kidney, small intestines, liver and pancreatic tissues; GLUT-3, expressed in the neurons and placenta; GLUT-4 expressed in the skeletal, cardiac and adipose tissues. Transport of glucose via GLUT-4 into muscle and adipose is the rate-controlling step in insulin-mediated glucose disposal and this disposal is compromised in conditions of insulin-resistance (Herman and Kahn, 2006). GLUT-5 transporters are located in the small intestine, sperm, brain, kidney and muscle (Shepherd and Kahn, 1999).

1.7.2 Roles of Kidney in Glucose Metabolism

Kidney participates in glucose homeostasis by taking part in gluconeogenesis, glucose filtration, reabsorption and utilization. Kidneys have an important influence on maintaining body metabolic glucose homeostasis both in physiologic and pathologic conditions (Marsenic, 2009). Kidneys

not only take part in glycolysis and gluconeogenesis, but also perform essential functions of glucose filtration in glomeruli, and reabsorption in tubules. In these processes two types of transporters are involved. GLUT's (Glucose transporters), which are glucose specific protein carries and SGLT s (sodium-dependent glucose transporters), which require sodium ions for work (Hummel *et al.*, 2011 and Gerich, 2010). Every of these processes may be disturbed in diabetes (Flordellis *et al.*, 2005).

1.8 The Role of the Pancreas

The pancreas is a large gland that nestles under the stomach, plays an important part in glucose regulation and is unusual having both an exocrine and endocrine function. As an exocrine gland it produces several digestive enzymes that are secreted into the duodenum via the pancreatic duct (Pandol *et al.*, 2011). Over 90 percent of the pancreas is devoted to its exocrine and digestive function. As an endocrine gland, the pancreas secrets a variety of hormones that are concerned with the regulation of blood glucose, including insulin, glucagon, and somatostatin (Guthrie, 2002).

1.8.1 Synthesis and Release of Insulin

Insulin is produced by the beta cells of the pancreas; it is the principal hormone regulating glucose metabolism. Its presence enhances glucose uptake and metabolism by various tissue cells including skeletal muscle, white adipose tissue and the liver (Barros, *et al.*, 2006). During non-pathological states, glucose is the stimulus for insulin secretion in pancreatic beta cells. Glucose entry into the pancreatic cells is through GLUT-2 transporters (Herman and Kahn, 2006). Oxidation of glucose and the subsequent increase in ATP/ADP ratio in the cells triggers closure of ATP-sensitive potassium channels (Doyle and Egan, 2003). Inhibition of potassium efflux results in cell depolarization leading to influx of voltage dependent calcium ions that stimulate extrusion of insulin. Physiological insulin secretion consists of a basal and bolus component. During fasting, basal insulin secretion maintains glucose levels between 70 and 120 mg/dl, and bolus secretion keeps glycemic levels below 180 mg/dl after a meal (Fonseca, 2006).

1.4.2 Insulin Signaling Pathway

The insulin function involves signaling cascades, which are initiated by insulin binding to its receptor (IR) of target cells, stimulating autophosphorylation with activation of receptor tyrosine

kinases and subsequently stimulating the tyrosine phosphorylation of insulin receptor substrates (IRSs). This activates tyrosine kinase which further phosphorylates insulin receptor substrates (IRS) leading to a series of pathways that result into insulin signal transduction (Sale and Sale, 2008). Depending on the target tissue cell, the outcome of the signal transduction might be movement of GLUT 4; for instance, from intracellular pools toward the cell surface of skeletal muscle and white adipose tissues, or indeed, increasing levels and activities of glycogen synthase and glycogen phosphorylase in the liver (Barros *et al.*, 2006).

1.4.5. Insulin Regulatory and Counter-Regulatory Hormones

Cells are located in the islets of Langerhans in the pancreas. Other cell types present in islets include alpha and pancreatic polypeptide (PP) cells. Glucagon, the catecholamines epinephrine and norepinephrine , glucocorticoids, and growth hormone all act to raise plasma glucose levels. Their actions generally oppose those of insulin, and as a group, they are called counter regulatory hormones (Hussain *et al.*, 2000). Glucagon increases glycaemia by mediating hepatic glycogenolysis (Robertson and Harmon, 2006). Studies have shown that diabetics have basal or increased plasma glucogon concentrations. Furthermore, suppression of this hormone is associated with reduction of plasma glucose concentration (Cherrington *et al.*, 1977). Cells also secrete somatostatin which plays an inhibitory role in the secretion of several hormones including insulin (Robertson and Harmon, 2006).

Counter regulatory effects of epinephrine are due to its direct effects on target tissues and its effect on other participating hormones. For example epinephrine is released by the adrenal medulla in response to hypoglycemia (as well as other stress stimuli), and Norepinephrine is released from sympathetic neurons. Both catecholamines have significant roles in maintaining glucose levels in exercise (especially in supporting the massive increase in glucose use by the muscle), and in stressed-related conditions. Catecholamines stimulate glucagon release and inhibit insulin release, causing a decrease in insulin mediated glucose uptake as a result of glucose-6-phosphate-dependent inhibition of hexokinase (Raz *et al.*, 1991). In addition adrenaline compromises insulin-induced glycogen synthesis attributed to its inhibition of glycogen synthase (Raz *et al.*, 1991).

Glycogenolysis and inhibition of glycogen synthesis, by both epinephrine and glucagon, are mediated via increased generation of cAMP which activates kinase A. Protein kinase A

inactivates glycogen synthase and activates phosphorylase (Hodis, *et al.*, 2007). Glucocorticoids (primary cortisol in humans) are released from adrenal cortex in response to stress; one such stress is a decrease in plasma glucose. Glucocorticoids stimulate gluconeogenesis and glycogen stress is a decrease in plasma glucose. Glucocorticoids stimulate gluconeogenesis and glycogen synthesis in the liver, and reduce muscle and adipose tissue glucose uptake. They also acutely inhibit insulin release, and over longer term, insulin action; prolonged cortisol hypersecretion can result in darkness (Liu *et al.*, 2005). In addition, glucocorticoids enhance hepatic gluconeogenesis and impair effects of insulin to reduce glucose production in type II diabetes (Liu *et al.*, 2005). In states of intensive glycemic control resulting in specific condition. Adiponectin is an adipocyte derived peptide which improves insulin sensitivity via several mechanisms. It modulates fatty acid oxidation in the muscle (Mlinar *et al.*, 2007). These effects are mediated through stimulation of perixosome proliferator gamma receptors (PP AR-y) nuclear receptors and activation of AMP kinase (Mlinar *et al.*, 2007). Like adiponectin, leptin is an adipocyte derived –hormone that circulates in plasma in free and bound form. Acting through its receptors located in the hypothalamus, hippocampus, cortex, cerebellum, thalamus and choroids plexus, leptin decreases levels of circulating neuropeptide resulting in reduced appetite (Mantzoros, 1999).

Leptin also modulates glucose homeostasis (Levy and Stevens, 2001). In insulin deficient rats, administration of leptin restores glucose levels, enhances glucose metabolism in the post absorptive stages and improves hepatic insulin sensitivity during glucose clamp (Chinookoswong et al 1999). Leptin, therefore, acts agonistically to insulin. Moreover, leptin modulates release of thyroid hormones which play a homeostatic role in glucose metabolism (Wang *et al*, 2007). Thyroid hormones secreted by the thyroid glands also exert glucose homeostatic functions in synergy as well as antagonistic to insulin action. Thyroid hormones may increase glucose production via hepatic gluconeogenesis, to meet increased energy requirements by the body (Lenzen and Bailey, 1984). Thyroid hormones, however, enhance transcription of insulin sensitive glucose transporter- 4 (GLUT-4) (Chidakel, *et al.*, 2005). This allows increased uptake of glucose by tissue cells thereby offsetting initial increase in blood glucose (Lenzen and Bailey, 1984).

1.4.6. Insulin Resistance

Insulin resistance largely caused by central obesity is a key component in the pathogenesis of type II diabetes mellitus. It is defined as the inability of insulin to curtail hepatic glucose production and promote glucose disposal in the peripheral tissues (Guillermo and Luigi, 2013). Insulin resistance constitutes defective insulin receptor function, insulin receptor signal transduction, glucose transport and phosphorylation, glucose uptake and glycogen synthesis in adipose, skeletal and hepatic tissues (Panunti *et al*., 2004).

Adipose tissue secretes large quantities of tumour necrosis factor (TNF)-α. According to Mlinar *et al*., (2007). TNF α is the main factor that stimulates increased levels of circulating free fatty acids in circulation increased intraportal free fatty acids and impair insulin clearance. The mechanisms by which this is mediated are still unclear (Kahn and Flier, 2000). It is, however, suggested that accumulation of intramyocellular lipids in striated muscles suppresses glucose.

1.5. Diabetes Mellitus

Diabetes mellitus is a group of metabolic disorders with different underlying etiologies, each characterised by chronic hyperglycemia owing to over production and/or underutilization of glucose in the cellular system. It is the condition in which the body does not properly process food for use as energy. Most of the food is turned into glucose, or sugar, for our bodies to use for energy. The pancreas, an organ that lies near the stomach, makes a hormone called insulin to help glucose get into the cells of the bodies. Diabetes, occurs when our body either doesn't make enough insulin or can't use its own insulin as it should. This causes sugars to build up in the blood (ADA, 2012).

1.5.1. Signs and Symptoms of Diabetes Mellitus

The common signs and symptoms of diabetes include: frequent urination, excessive thirst, unexplained weight loss. extreme hunger, sudden vision changes, tingling or numbness in hands or feet. Feeling very tired much of the time. very dry skin, sores that are slow to heal and more infections than usual (Rother, 2007).

1.5.2. Types of Diabetes Mellitus

The major types of diabetes mellitus are Type I and 2, the former arising from inadequate production of insulin due to pancreatic β-cell dysfunction, and the latter arising from reduced sensitivity of insulin in target tissues and/or inadequate insulin secretion (Zhao, 2011).

1.5.3. Type 1 Diabetes Mellitus

Type 1 diabetes mellitus, previously called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, may account for 5 percent to 10 percent of all diagnosed cases of diabetes. Insulin is necessary for the body to be able to use glucose.Glucose is the basic fuel for the cells in the body, and insulin takes the sugar from the blood into the cells. Type 1 diabetes without any production of insulin is according to this understanding a severe problem for the body and will lead definitely to death (Kaufman *et al.*, 2012). Risk factors are less well defined for type 1 diabetes than for Type 2 diabetes. But autoimmune, genetic, and environmental factors are involved in the development of this type of diabetes. There are two major type 1 diabetes, namely, immune-mediated and Idiopathic diabetes. (Soumya and Srilatha, 2011).

1.5.4. Immune-Mediated Diabetes Mellitus

Immune-mediated type 1 diabetes mellitus, previously known as insulin dependent diabetes mellitus (IDDM). Constitutes 5 to 10 % of all diabetic cases (American Diabetes Association, 2004). The body's immune system can mistakenly destroy the insulin-production beta cells of the pancreas. The causes of autoimmune diabetes are poorly understood, but genetic and famil history play a role. viruses or other environmental factors are also believed to be a risk factor (Kukreja and Maclaren, 1999).

Risk factors for pathogenesis, of type 1 diabetes include genotype, diet and viral infections e.g. *rubella, coxsackie, mumps* (Wei, *et al.*, 2006). Coxsackie viral infections are a type of environmental factors that do not initiate, but enhance development of type 1 diabetes in genetically prone victims (Serreze, *et al.*, 2000). It is proposed that *coxsackie viral* infections probably provide a molecular mimic during replication that triggers a cross-reactive CD4+T-cell response against the candidate cell autoantigen (Serreze, *et al.*, 2000). Ultimately cell death occurs by apoptosis (Kurrer, *et al.*, 1997).

DISCOVERY MEDICINE

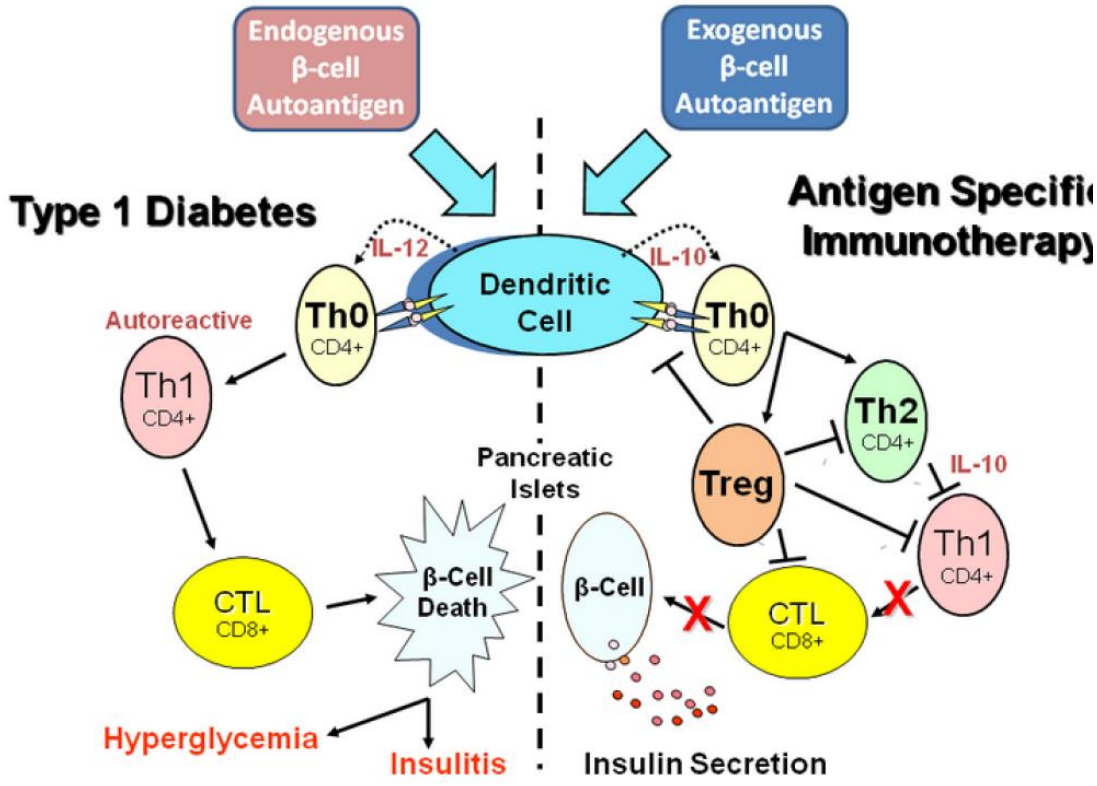

Figure 2: Auto-immune diabetes disorder mechanism, William *et al* (2011).

In addition, how birth weight is considered a risk factor for type I diabetes. Low birth weight is indicative of exposure of foetus to maternal viral infection and the interaction of immune cross-reactivity between virus and auto-antigens which may lead to destruction of pancreas cells (Wei *et al.*, 2006). Furthermore, low birth weight is commonly associated with a reduced beta cell mass which is a vital factor in the pathogenesis of diabetes. Worldwide, an estimated 4.9 million people have Type 1 diabetes (Gadsby, 2002).and approximately 35,100 children have Type 1 diabetes in Africa (Beran *et al.*, 2006).

1.5.5. Type II Diabetes Mellitus

Type II diabetes mellitus, formerly known as non-insulin dependent diabetes (NIDDM) is the most common type of diabetes mellitus accounting for 90 to 95 % of all diagnosed cases of diabetes (American Diabetes Association, 2004). Type II diabetes is a polygenic disease caused by diminished response of target cells to insulin action, and/or insufficient production of insulin. Inadequate insulin production often characterize later stages of Type II diabetes and is attributed to reduced cell mass or beta cell dysfunction (Jaime, 2013). Hepatic gluconeogenesis and renal gluconeogenesis significantly contribute to postprandial hyperglycaemia in Type II diabetes (Meyer *et al.*, 1998).

In recent times, interest has been generated into the possibility of involvement of the hypothalamus-pituitary- adrenal axis in the pathogenesis of type II diabetes (Koshiyama *et al.*, 2006). Risk factors for type 2 diabetes include genotype, age, previous history of gestational diabetes, behavioural factors including poor diet, and sedentary lifestyle. Genetic pre-disposition interacts with the environmental factors to influence disease prevalence (Zachary *et al.*, 2012). In industrialized nations the increase in prevalence and incidences of diabetes are due in part to the increase in the age of the populations. Obesity present 50 % and 70 % of type II diabetes men and women, respectively (Etie *et al.*, 2013). It leads to increased lipolysis which results in increased levels of circulating free fatty acids and triglycerides and their subsequent deposition in muscle bed (McGarry, 2011).

1.5.6. Gestational Diabetes Mellitus

Gestational diabetes, which occurs during pregnancy, is the third commonest type of diabetes. It is derangement in carbohydrate metabolism resulting to hyperglycemia with onset or first detection during pregnancy (ADA, 2016).The causative factors for gestational diabetes mellitus are unknown but autoimmune activities in the pancreatic b-cells of pregnant mothers are suggested (McEvoy, *et al.*, 2004). It has high prevalence in developed countries 3-19 %, and is low in Africa, at 0 to1 % (Seyoum *et al.*, 1999). The significance of gestational diabetes mellitus is that it poses 16 to 63 % risk factor to the mother for future development of type II diabetes within 5 to 16 years (Hyer and Shehata, 2005).

Gestational hyperglycemia also exposes the foetus to risk of future diabetes, hypertension, and obesity (Maresh, 2005). In addition to the pre-natal morbidity, immediate dangers of gestational diabetes include pre-term birth, macrosomia, pre-eclampsia and caesarean section (Fan *et al.*, 2006). Excessive foetal exposure to glucose as a result of maternal hyperglycemia results in permanent reduction in levels of GLUT-1 and GLUT-4 transporters in the new born and adult offspring of a gestational diabetic mother (Boloker *et al.*, 2002). Down regulation offers these transporters an adaptive mechanism by which the foetus protects itself against the adverse effects hyperglycemia. The foetus adapts to a changed environment in the uterus that may enhance its chance of short-term survival but at the expense of a long-term capacity for normal growth and development resulting in the development of diabetes (Boloker *et al.*, 2002). Foetal exposure to excessive levels of glucocorticoids in diabetic pregnancy is one of several factors that subject them to growth retardation, hypertension and diabetes mellitus later in life (Fujisawa, *et al.*, 2005). Also demonstrated that increased plasma cortisol levels were strongly associated with hypertension, hyperglycemia, fasting, hypertriglyceridaemia and insulin resistance in adults.

It has been shown that diabetic pregnancy suppresses expression of 11 beta hydroxyl steroid dehydrogenase (11h-HSD) Type 2 in the placenta and foetal kidney (Fajisawa *et al.*, 2005). 11h-HSD is a high affinity NAD+-dependent enzyme that inactivates glucocorticoids. The role of glucocorticords in insulin resistance is well established. Glucocorticoids mediate lipolysis resulting in increased availability of free fatty acids which culminates in reduced glucose oxidation and uptake in peripheral tissues. Moreover, the presence of glucocorticoids also compromises translocation of GLUT-4 transporters to the cell membranes (Mlinar *et al.*, 2007). These are opposing views about influence of birth weight on future occurrence of chronic diseases including diabetes mellitus. One view asserts that gestational diabetes leads to low birth weight which is a risk factor for Type 1 diabetes (Boney *et al.*, 2005).

Other Types of Diabetes

Diabetes due to genetic defect of beta cells, also known as Maturity onset diabetes of the young (MODy), diabetes due to genetic defects in insulin action, diabetes due to diseases of the exocrine pancreas, diabetes due to endocrinopathies, and drug or chemical-induced diabetes (American Diabetes Association, 2004).

1.5. Diabetic Complications

Complications arising from hyperglycemia are both microvascular and macrovascular in nature. Micro vascular complications, affecting the smaller blood vessels frequently result from Type 1 diabetes while macro vascular complications affecting large blood vessels, originate from Type II (Girach and Vignati, 2006). The two are the leading cause of morbidity and death in people with diabetes.

1.6.1. Microvascullar Complications

Microvascular complications includes: (a) neuropathy, (b) retinopathy and (c) nephropathy.

1.6.2. Diabetic Neuropathy

Diabetes mellitus, a common metabolic disease with a rising global prevalence, associated with long-term complications of peripheral nervous system and the central nervous system (Chen, *et al.*, 2011). Diabetic neuropathy is a chronic micro-vascular complication affecting both somatic and autonomic peripheral nerves. It may be defined as the presence of symptoms or signs of peripheral nerve dysfunction in people with diabetes, after the exclusion of other causes of neuropathy. Neuropathy is the common complication of diabetes, and is due to high blood glucose, chemical changes that occur in the nerves (Heltianu and Guja, 2011). Generally it starts it starts in the nerves of feet as they are the longest nerves and nourished with longest blood vessels of the body. This condition is called diabetic foot or diabetic peripheral neuropathy or distal symmetric neuropathy. Diabetes can reduce the blood supply to the foot and gradually damages the nerves which carry sensation. Diabetic neuropathy can cause foot ulcers and foot infections as advanced complications in diabetic patients. signs and symptoms of diabetic neuropathy include, decrease or no sweating, numbness, or tingling, and some sort of burning sensation, weakness and loss of reflexes. Diabetic Polyneuropathy is a major complication of diabetes mellitus that frequently leads to foot ulceration (Viswanathan and Kumpatla, 2001).

1.6.3. Diabetic Nephropathy

Diabetic nephropathy is the most important cause of renal failure in developed countries (Amador, *et al.*, 2000). Patients with early renal damage manifest with micro-albuminuria which progresses into proteinuria (Leiter, 2005). In renal tissue advanced glycation end products can induce activities of transforming growth factor and raise expressions of various extracellular matrix mRNAs. These mediate hypertrophy of the glomeruli, thickening of basal membrane, and

expansion of the mesangial extracellular matrix. Such structural changes are associated with albuminuria and proteinuria (Stitt, 2003).

Blocking activities of advanced glycation end products by a phenyl hydrazine compound, amino guanidine, slows down mesangial expansion and proteinuria (Adler, 2003). In addition, hypertension associated with diabetes is one of the most important factors in the pathogenesis and progression of diabetes nephropathy (Bretzel, 1997). In turn, diabetic nephropathy is critical in the development of hypertension in diabetics (Parving *et al.*, 2008).Diabetic nephropathy results in progressive deterioration of glomerular function. The decline in renal function, however, can be attenuated by management of hypertension. Hyperglyceamia causes a redox imbalance. This is as a result of excessive reactive oxygen species (Marcheva *et al.*, 2010). Enzymes involved in cellular defence against excessive reactive oxygen species build up include catalase, glutathione peroxidase and superoxide dismutase. Increased incidences of cardiovascular complications and renal function deterioration are partly attributed to reduced excretion of electrolytes such as sodium (Dahar and Gutkowska, 2000). This was supported by other trials that showed that reduction of hypertension in addition of managing glycaemia to acceptable levels did reduce incidences of macrovascular complications (Bate and Jerums, 2003).

1.6.4. Diabetic Retinopathy

Diabetic retinopathy is the commonest cause of blindness in people afflicted with diabetes, aged between 20 and 74 years (American Acedemy of Ophthalmology, 2016). Retinopathy is characterized by increased vascular permeability by vascular closure mediated by the formation of new blood vessels neovascularization, on the retina and posterior surface of the vitreous. Diabetic retinopathy is a micro-vascular disease, characterized by damage to the blood vessels and retina of the eyes. This condition occurs in both Type 1 and Type 2 diabetics (da Silva *et al.*, 2010). It can be classified as non-proliferative diabetic retinopathy and proliferative diabetic retinopathy or diabetic macular edema (Abramoff, *et al.*, 2013). In diabetic retinopathy, the micro vessels supplying blood to the retina of eye is affected and can cause blindness. Retinopathy is related to high blood sugar level and obstructs the flow of oxygen to the cells of the retina (Zhang *et al.*, 2010). For the vision of eye, retina receives signals of light and sends them to the brain forming a three dimensional figure which is identified. Finally it is sent back to

the eyes by which one can recognize the things around (Muaka *et al.*, 2011). This working mechanism of passing light through the retina is hindered by the high glucose levels. The initial stage of this disease is known as Non- proliferate Diabetic retinopathy where as proliferative diabetic retinopathy is the advanced form of diabetic retinopathy in which new as well as weak blood vessels break and leak blood into vitreous of the eye causing floating spots in the eye (Chakrabarti *et al.*, 2012). Gradually, the swollen and scar nerve tissue of the retina is totally destroyed and leads to retinal detachment. The ground cause for blindness among diabetes is due to the retina detachment (Raman *et al.*, 2009).

1.6. 5. Macro- Vascular Complications

Macro-vascular complications are largely responsible for mortality in diabetes, with cardiovascular diseases accounting for up to 80 % morbidity (Akula *et al.*, 2003). Macro-vascular complications include (a) atherosclerosis, (b) reproductive problems, (c) stroke and (d) coronary artery diseases. (Chakrabarti *et al.*, 2012).

1.6.6. Atherosclerosis

Atherosclerosis is common in smokers and those with high blood pressure and abnormal fat levels in the blood. It is commonly fatty deposits in arteries or hardening of arteries. It accounts for virtually 80% of all deaths among diabetic patients (Soumya and Srilatha, 2011). Due to hyperglycemia, formation of advance glycation end products promote smooth muscle cell proliferation in blood vessels (Basta *et al.*, 2004). In coronary artery disease, advance glycation end products cause increased deposition of lipids in the cardiac cells, and compromise ventricular compliance (Sobel and Scheneider, 2005).

1.6.7. Reproductive Complications Caused by Diabetes Mellitus

Hyperglycaemia due to diabetes is a significant underlying cause of a number of sexual disorders associated with diabetes mellitus. About 90 % of diabetics manifest sexual problems characterized by impotence, infertility and low sexual drive (Amaral *et al.*, 2006). These are attributed to hyperglycemia-induced reactive oxygen species generation. Erectile dysfunction in diabetes is caused by lack of smooth muscle relaxation mediated by nitric oxide due to endothelial dysfunction and autonomic neuropathy (Price, 2006). Moreover, interaction of

reactive oxygen species generated as a consequence of diabetes mellitus, with DNA of spermatozoa, results in reduced motility and fertility of human sperm (Amaral *et al.*, 2006).

1.6.8. Pathogenesis of Diabetic Complications

Development and progression of diabetic complications are mediated by four major pathways which includes increased sorbitol (polyol) pathway flux, increased formation of advanced glycosylation end products, increased activities of protein kinase C, and increased hexosamine pathway flux (Geraldes and King, 2010).

Generation of reactive oxygen species have been implicated as the main pathogenic factor as well as the result, in these processes in endothelial cells, glucose enters into cells via plasma membrane transported by insulin-independent GLUT-1 transporter. Its subsequent accumulation in the cells causes generation of superoxide which is considered a key component in the initiation of all other pathways (Lapolla, 2005). Excessive formation of reactive oxygen species results in oxidative damage to DNA, proteins and lipids (Dandona *et al.*, 1996). The most affected organs appear to be those whose glucose uptake is independent of insulin influence including the nervous system, kidney, heart and small blood vessels (Ahmed, 2006).

1.7. Diagnosis and Clinical Manifestations

The diagnosis of diabetes depends on the demonstration of carbohydrate intolerance; the symptoms are characterized by elevated fasting or post- prandial blood glucose level.

Several assays are used in diagnosing diabetes, these includes; oral glucose tolerance assay (OGTA), fasting blood sugar assay (FBS), random blood sugar assay (RBS), urine assay and glycosylated haemoglobin assay (Yehuda *et al.*, 20011).

1.7.1. The Fasting Plasma Glucose Test

This test is done in the morning before eating. Normal fasting plasma glucose levels are less than 110 milligrams per deciliter (mg/dl). Fasting plasma glucose levels of more than 126 mg/dl on two or more tests on different days indicate diabetes.

1.7.2. Random Plasma Glucose Test

Differs from situation to situation and is done the same way as the fasting glucose test but without fasting. It shows different levels depending on the time difference of food-intake. Because of more and more problems of low-level glucose by diabetics in the morning, medical doctors change from tests after fasting to this king of testing.

1.7.3. Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test (or Impaired Glucose Tolerance Test, IGT), involves one fasting overnight (at least 8 but not more than 16 hours) and go to your doctor's office or the laboratory in the morning. First, your fasting plasma glucose is tested. After this test, you receive 75grams of glucose. Blood samples are taken up to four times at different time interval to measure the blood glucose (Saxena *et al.*, 2010). Other glucose level assay includes urine assay and glycosylated haemoglobin assay (Saxena, 2010).

1.8. Pharmacological Intervention

1.8.1. Insulin

Insulin is one of the major hypoglycemic agents presently used in diabetes mellitus management. It was discovered in 1921 and is the mainstay antidiabetic agent for the management of Type 1 diabetes and is also used late-stage Type II diabetes (Emilien, *et al.*, 1999). There are several types of insulin classified based on their duration of action. These are rapid (ultra short) acting (e.g. lispro, aspart), short-acting (e.g. regular insulin), intermediate-acting (e.g. lente) and long-acting (e.g. glargine, detemir) (Bethel, et al., 2005). Insulin is currently administered subcutaneously using multiple daily injections or external pump for continuous delivery (Hirsch, *et al.*, 2005). Other delivery routes include oral, inhaled, nasal, rectal, ocular, intravaginal and transdermal (William, 2011). Intensive insulin therapy to maintain optimal glucose levels in Type 1 diabetes can reduce incidences and exacerbation of micro-vascular complications by 50 to 70 % (Elizabeth *et al.*, 2014). Shortfalls of insulin therapy include ineffectiveness on oral application due to first pass hepatic metabolism, short shelf life, and severe hypoglycemia on over dosage, non-compliance to painful injections and weight gain (Shilpa *et al.*, 2012).

1.8.2. Oral Blood Glucose Lowering Agents

There are five classes of antidiabetic agents presently in use, including beguanides, sulphonylurea, thiazolidinediones, benzoic acid derivatives and α-glucosidase inhibitor. (Richard *et al*., 2012).

1.8.3. Sulphonylurea

Sulphonylureas act by stimulating insulin secretion. They bind beta cell receptors that are linked to ATP-dependent potassium gated channels. Interaction with these receptors inhibits opening of potassium channels. Potasium builds up in the cell causes cellular depolarization evoking opening of calcium chanels, and subsequently, influx of calcium ions which mediate exocytosis of secretory granules containing insulin (Simo and Hernandez, 2002). Sulphonylureas usage is restricted to Type II diabetes mellitus because it requires a functioning pancreatic beta cell mass. Examples of agents presently in use include Gilbenclamide, glipizide, tolbutamide and gliclazide Hypoglycemia is the main side effect of sulphonylureas (Porksen, 2006). The adverse effect of sulphonylurea class of antidiabetic agents is that they stimulate insulin release by the pancreatic beta cells independent of glucose loading thereby subjecting the beta cells to exhaustions. Furthermore they cause allergy and weight gain as a result of increased blood levels of insulin.

1.8.4. Biguanides

Biguanides were introduced for use between 1957 and 1960 (Simo and Hernandez, 2002). Biguanides (e.g. metformin) mediate anti-hyperglycemic effects by sensitizing target tissues cells to insulin action and blocking hepatic gluconeogenesis (Pari and Satheesh, 2006). Metformin (Dianben-is the principal drug in this class. The drug has been proved effective when used either in mono-therapy or in combination with other groups of drugs such as sulphonylureas or insulin (Chadwick *et al*., 2007). Its mechanisms of actions are still unclear, current studies, however, show that on a subcellular level, metformin mediates hypoglycemic effects by stimulating AMP-activated protein kinase (AMPK). Stimulation of AMPK results in fatty acid catabolism and enhanced muscle glucose uptake (Zhou *et al*., 2001). Therefore, usage of metformin prevents the occurrence of diabetic complications in addition to controlling glucose Levels (Julio, *et al*, 2010). Other examples of drugs in this class include fenformin and buformin (Kiho *et al.*, 2005).

1.8.5 Thiazolidinediones

Thiazolidinediones (TZDs) mediate their hypoglycemic effects by enhancing insulin sensitivity, and reducing gluconeogenesis by the liver (Emilian *et al*, 1999). This group of drugs targets the perixosome proliferator gamma receptors (pPAR-y) which are nuclear and expressed mainly in the adipose tissues. Other tissues expressing PP AR-y include liver, skeletal muscle, kidney, heart, large and small intestine and colon (Song *et al.*, 2004). Interaction TZDs with the receptors leads to modification of expression of major genes responsible for glucose metabolism (Hevener *et al*., 2001). When administered at high doses TZDs suppress hepatic gluconeogenesis (Flordelllis *et al.*, 2005). A major shortfall of thiazolidinediones is that they lead to increased plasma levels of cholesterol (Bailey, 2000).

1.8.6 Alpha-Glucosidase Inhibitors

Alpha-glucosidase inhibitors mediate their hypoglycemic effects by lowering glucose absorption into blood by suppressing digestion of polysaccharides and disaccharides into monosaccharides, thus decreasing post-prandial blood glucose (Fujisawa *et al*., 2005). This is primarily mediated by competitively and reversibly inhibiting the actions of alpha-glucosidase, a small intestine epithelial membrane bound enzyme which catalyses break down of polysaccharides into monosaccharides. Examples of α-glucosidase are acarbose and voglibose. The side effects include diarrhoea, flatulence abdominal bloating. These adverse effects are primarily due to malabsorption of glucose (Salvatore *et al.* 2013).

1.8.7 Non-Pharmacological Intervention

Weight loss as non-pharmacological intervention for diabetes is significant in that reduced adipose tissue results in lesser production of proinflammatory cytokine tumor necrosis factor (TNF-α) which is thought among others to mediate insulin resistance (Mlinar *et al*., 2007). Consumption of food with low glycemic index is beneficial in that it requires less insulin secretion and improves target tissues to insulin effects. Foods with low glycemic index allow slow absorption of glucose in the gut. Regular exercise reduces levels of circulating cholesterol. Furthermore, exercise is known to stimulate glucose uptake by the muscles and liver and its conversion to glycogen (Alan *et al.*, 2013).

1.9 Experimental Animal Models of Diabetes

Animal models of diabetes have allowed studies and provided understanding of pathogenesis and progression of diabetes mellitus in human. Furthermore, animal models have allowed the evaluation of various therapeutic interventions which may be potentially used in man. Uses of animal models have made possible studies of disease characteristics in humans by providing genetic and immunological modifications that are not possible in human (Bach, 1994).

1.10 Alloxan-Induced Diabetes

Alloxan, 2,4,5,6, *(IH 3H)* - pyrimidinetetrone, is probably the longest known and most potent diabetogenic agent (Piotrowski, 2003). Uptake of alloxan by the pancreatic cells is via GLUT-2 transporters, due similarly in molecular shape to glucose (Elsner *et al.*, 2006). Alloxan then undergoes reduction intracellulary in the presence of tripeptide thiol, glutathione, to yield dialuric acid whose re-oxidation, regenerates alloxan. Hence, a cyclic reaction (redox cycle) is created that ultimately results in reactive oxygen species generation (Elsner *et al.*, 2006). Alloxan toxicity, however, affects other organs including the liver, kidney, gonads and lungs. Alloxan causes mortality in 37 % of animals within a few days (Piotrowski, 2003).

1.10.1 Mechanism of Alloxan

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many animal species. This causes an insulin-dependent diabetes mellitus (called “alloxan diabetes”) in these animals, with characteristics similar to type diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT-2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cycle reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. One study suggests that alloxan does not cause diabetes in humans. Others found a significant difference in alloxan plasma levels in children with and without Type 1 (Lenzen, 2008).

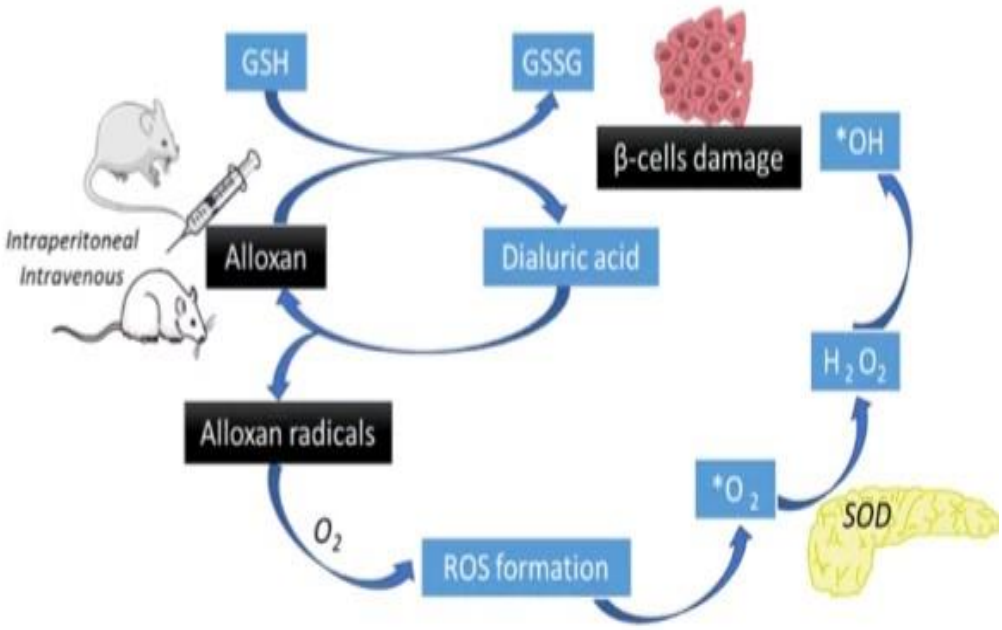

Figure 3: Mechanism of alloxan, Entaz *et al* (2017).

1.10. 2 Chemical Structures of Alloxan

The original preparation for alloxan was by oxidation of uric acid by nitric acid. In another method the monohydrate was prepared by oxidation of barbituric acid by chrominum trioxide. Alloxan is a strong oxidizing agent and it forms a hemiacetal with its reduced reaction product dialuric acid (in which a carbonyl group is reduced to a hydroxyl group) which is called alloxantin.

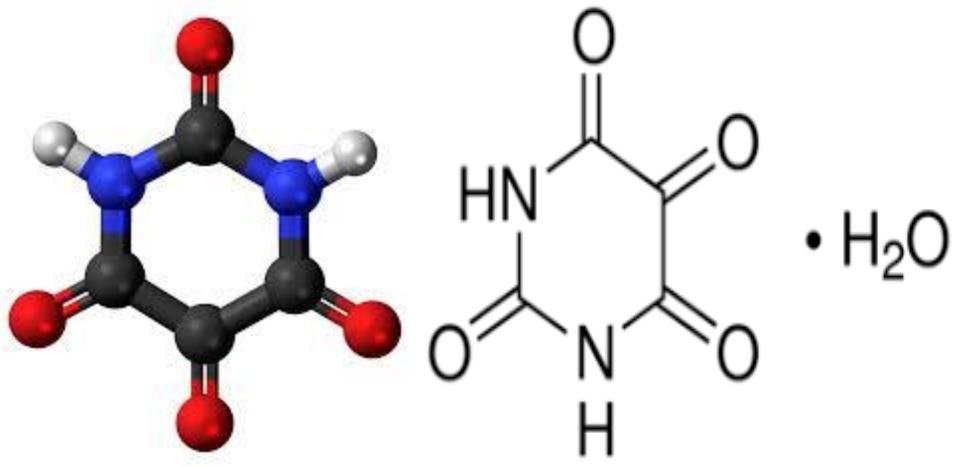

Figure 4: Alloxan monohydrate structure. Holmgren, A.V., Wenner, W. (1952), Alloxan monohydrate. org.synth. 32.6; Coll Vol. 4:23.

1.10.3 Streptozotocin (STZ)- Induced Diabetes

The streptozotocin-induced diabetic model is an extensively used animal model in studies of human diabetes mellitus. It is a well-described model and the toxicity of STZ is relatively low compared with other diabetogenic agents (Piotrowski, 2003). Streptozotocin *(*N-*[*meethylnitrocarbamoyl*]-D*-glucosamine*)* STZ, is an antibiotic synthesized by Streptomycetes achromogenes and is commonly used in induction of experimental diabetes. STZ selectively destroys β-cells by alkylation of DNA through its nitrosourea moiety (Vessal *et al.*, 2003). Other mechanisms of beta cell damage occur via reactive oxygen species and nitric oxide production. Pancreatic beta cells take up STZ via GLUT -2 transporters (Szkudelski, 2001).

1.11 Concepts Associated with Diabetes Mellitus

1.11.1 Free Radicals

A free radical is any molecule that contains one or more unpaired electrons. Free radicals are normal products of many metabolic pathways (Christian *et al.*, 2014). They interact with various tissue components; such interactions can cause both acute and chronic dysfunction, but can also provide essential control of redox regulated signaling pathways (Roberts *et al.*, 2010). It should also be clear that, while free radicals may initiate a series of damaging biochemical events, they are not necessarily directly responsible for the final tissue of dysfunction. Conversely, free

radicals may arise subsequent to some earlier events that are the proximate cause of tissue injury, but these radicals may have either no effect of amelioratin or aggravating the final damage.

1.11.2 Reactive Oxygen Species

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. Reactive Oxygen Species (ROS) have long been known to be a component of the killing response of immune cells to microbial invasion (Kops *et al* 2002). The production of oxygen based radicals is the bane to all aerobic species. ROS have a role in cell signaling, including; apoptosis; gene expression; and the activation of cell signaling cascades (Hancock *et al.*, 2001). The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including; superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide (Sunil, 2014).

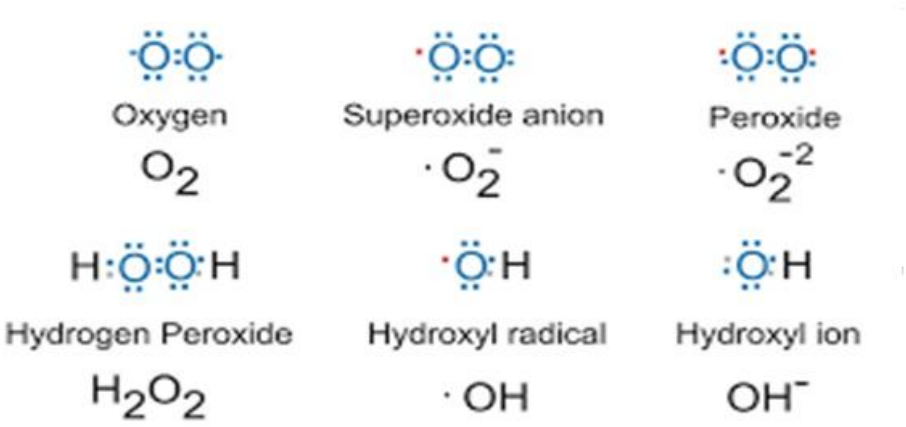

Figure 5: Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. (Sunil, 2014).

All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage, ROS are generated by a number of pathways (Sunil, 2014).

1.11.3 The Role of ROS in Metabolic Syndrome (e.g diabetes).

The role of ROS in the pathogenesis of the metabolic syndrome, as well as both Type 1 and Type 2 diabetes, has been studied extensively (Rochette *et al.*, 2014). Both an enhanced generation of ROS and a dysfunctional cellular antioxidant response appear to be factors. For example, elevated blood glucose and saturated fatty acid levels are linked to an enhanced production of superoxide, hydrogen peroxide through stimulated mitochondrial metabolism, as well as through activation of NADPH oxidases (Rochette *et al.*, 2014). In addition, the hyperglycemia associated with poorly controlled diabetes can induce oxidative stress and may play an important role in diabetic complications, especially β-cell damage (Ogugua, 2000).

1.11.4 Oxidative Stress

Reactive oxygen species percentage increases during infections, exercise, stress and exposure to pollutants. UV ionizing radiation increases the degree of oxidative stress in the internal biological system (Manda *et al.*, 2009).

Oxidative stress is a term used to refer to the shift towards the pro-oxidants in the antioxidants balance that can occur as a result of an increase in oxidative metabolism (Manda, *et al* 2009). ROS reactions with biomolecules such as lipid, protein and DNA, produce different types of secondary radicals like lipid radicals, sugar and base derived radical, amino acid radicals depending upon the nature of the ROS (Niki, *et al.*, 2005). These radicals in the presence of oxygen are converted to peroxyl radicals. Peroxyl radicals are critical in biosystems, as they often induce chain reactions. These reactions exert oxidative stress on the cells, tissues and organs of the body. The biological implications of such reactions depends on several factors like site of generation, nature of the substrate, activation of repair mechanisms, redox status among many others (Goldstein *et al.*, 1993). Oxidative stress is increased in diabetes mellitus owing to an increase in the production of oxygen free radicals, such as superoxide ($O_2\cdot$), hydrogen peroxide (H_20_2) and hydroxide ($OH^{\cdot -}$) radicals which overwhelm the natural antioxidant defence mechanisms (Soliman, 2008).

1.11.5 Enzymes Scavenging ROS

Mammalian enzymatic antioxidant defense systems include the SODs, a family of metalloenzymes that convert two superoxide anions to triplet oxygen and H_2O_2. The enzymatic rate of dismutation is approximately 10,000 times greater than the spontaneous disproportionation (Freidovich, 1983). The major mammalian forms of SOD are the cytosolic Cu/Zn enzyme, the mitochondrial Mn enzyme and extracellular Cu/Zn containing SOD (Ogunlana and Ogunlana, 2008). Although molecularly different, all catalyze the same reaction. Multiple enzymes exist to deal with H_2O_2. Notably, these include heme peroxidases, such as catalase, that catalyzes the dismutation of H_2O_2 bound at the active heme site (catalase compound 1) in order to react very rapidly with a second molecule of H_2O_2. Other peroxidases, such as the five selenium-dependent glutathione peroxidases also reduce H_2O_2, as well as organic hydrogen peroxides, by transferring reducing equivalent s from GSH, producing water and GSSG (Brigelius and Maiorino, 2013).

$$2O_2 + 2H \longrightarrow H_2O_2 + O_2, \quad 2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

1.12 Lipid Peroxidation

Lipid peroxidation is described generally as a process under which oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) that involve hydrogen abstraction from a carbon, with oxygen insertion resulting in lipid peroxyl radicals and hydrogen peroxides (Yoshida and Niki, 2004). One of the consequences of uncontrolled oxidative stress is cells, tissues, and organs injury caused by oxidative damage. It has long been recognized that high levels of free radicals or reactive oxygen species (ROS) can inflict direct damage to lipids, this produce highly reactive species, disruption of the intracellular membranes and cellular damage (Ahmadian *et al.*, 2013). The primary sources of endogenous ROS production are the the mitochondria,plasma membrane, endoplasmic recticulum, and perixisomes. (Moldovan and Moldovan 2004). Different exogenous stimuli, such as the ionizing radiation, ultraviolet rays, tobacco smoke, pathogen infections, environmental toxins, and exposure to herbicide/insecticides, are sources of in vivo ROS production (Belles *et al.*, 2010). The two most prevalent ROS that can affect profoundly the lipids are mainly hydroxyl radical (HO.) and hydroperoxyl (HO_2^-). Lipid peroxidation causes a

decrease in membrane fluidity and in the barrier functions of the membranes. The many products of lipid peroxidation such as hydroperoxides or their aldehyde derivatives inhibit protein synthesis, blood macrophage actions and alter chemotactic signals and enzyme activity *(*Fischer *et al.,* 2008).

Glycolipids, phosopholipids (PLs), and cholesterol (Ch) are also well-known targets of damaging and potentially lethal peroidative modification. Lipids also can be oxidized by enzymes like lipoxygenases, cyclooxygenases, and cythochrome P_{450}. The lipid peroxidation of polyunsaturated fatty acids may be enzymatic and non-enzymatic. Enzymatic lipid peroxidation is catalyzed by the lipoxygenases family, a family of lipid peroxidation enzymes that oxygenates free and esterified PUFA generating as a consequence, peroxy radicals. Non enzymatic lipid perioxidation and formation of lipid peroxides are initiated by the presence of molecular oxygen and is facilitated by Fe^{2+} ions (Repetto *et al.,* 2010).

Many aldehydes are produced during the peroxidative decomposition of unsaturated fatty acids. Compared with free radicals, aldehydes are highly stable and diffuse out from the cell and attack targets far from the site of their production. About 32 aldehydes were identified as products of lipid peroxidation) saturated aldehydes (propanal, butanal, hexanal, octanal, being the decanal the most important); b) 2,3-trans-unsaturated-aldehydes (hexenal, octenal, nonenal, decenal and undecenal); c) a series of 4-hydroxylated, 2,3-trans-unsaturated aldehydes: 4-hydroxyundecenal, being 4-hydroxinonenal (HNE) the most important quantitatively. Malondialdehyde (MDA) was considered for a long time as the most important lipid peroxidation metabolite. However, MDA is practically not toxic (Ossani *et al.,* 2007). Living organisms have evolved different molecules that speed up termination by catching free radicals and therefore, protecting the cell membrane. One important such antioxidant is vitamin E. Other antioxidants made within the body include the enzymes superoxide dismutase, catalase, and peroxidase. If not terminated fast enough, there will be damage to the cell membranes, which consists mainly of lipids (Seiler *et al.,* 2008). By-products of lipid peroxidation such as conjugated diseases and malondialdehyde (MDA) are increased in the circulation of diabetes mellitus patients. MDA is generated as a relatively stable end product from the oxidative has been causatively implicated in the aging process, atherosclerosis, Alzheimer's disease and cancer (Niki *et al.,* 2005). Serum MDA has been used as a biomarker of lipid peroxidation and has served as an indicator of free radical damage.

Malondialdehyde is a highly reactive three carbon dialdehyde that occurs naturally and exists primarily in an enol form. It is a toxic compound that reacts with DNA to form covalently-bonded adducts with deoxyadenosine and deoxyguanosine, an event that can cause a mutagenic transformation within DNA. Additionally, malondialdehyde can interact with several functional groups on proteins and lipoproteins, altering their chemical behaviour and possibly contributing to carcinogenesis and mutagenesis (Ogugua and Ikejiaku, 2005). Due to its highly reactive nature, malondialdehyde also functions as an electrophile that can cause toxic stress within the cell and is, therefore, a potent market for measuring the overall level of oxidative stress within an organism (Tangvarasittichai *et al.* 2009).

1.13 Antioxidants

Antioxidants are man-made or natural substances that play a very important role in the body defense system against ROS. It may prevent or delay some types of cell damage, antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well-being (Anuj *et al,* 2016). Antioxidants are found in many foods, including fruits and vegetable. The addition of synthetic antioxidants to oils and/or foods is one of the most efficient ways to prevent lipid oxidation. However, the safety of synthetic additives has been questioned stimulating the evaluation of naturally occurring compounds with antioxidative properties (Aziz *et al*., 2012). Although there is no assurance of the safety of natural antioxidants, there is some comfort knowing that such antioxidants are purified of natural products that have been consumed for generations. Phenolic compounds in plants are recognized as important compounds in conferring stability against oxidation (Sunil, 2014). Natural antioxidant phenolics can be classified into a lipophilic group, tocopherols, and a hydrophilic group, including simple phenolics, phenolic acids, anthocyanin, flavonoids and tannins. Even though chemists have elucidated the structures of thousands of phenolic, there are still many compounds that have not yet been fully characterized and they are referred as phenolic extracts (Sunil, 2014).

To protect the cells, organ and systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system (Vertuani, *et al.*, 2004). It involves a variety of compounds, both endogenous and exogenous in origin, that function interactively and synergistically to naturalize free radicals. These components include;

antioxidant enzymes e.g., superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions (Sathiya *et al.,* 2015). Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids and other low molecular weight compounds such as glutathione and lipoic acid also defends the body against reactive oxygen species. (Waggiallah and Alzohairy, 2011).

1.13.1 Superoxide Dismutase

Superoxide dismutase (SOD) is an enzyme with a generalized presence in the body which catalyzes the dismutation of superoxide. It is a prime antioxidant enzyme found in two forms. One, complexed with zinc and copper, is localized in the cytosol, while the other, bound with manganese, is found in the mitochondrial matrix (Hancock *et al., 2001*). Both forms of this metalloenzyme catalyze the inactivation of destructive superoxide ion by converting them to hydrogen peroxide which is then transformed to water and oxygen by the enzyme catalase (Soliman, 2008). Reactive forms of oxygen such as superoxide, leak from respiratory enzymes and wreak havoc on the cell. This superoxide can then cause mutations in DNA or attack enzymes that make amino acids and other essential molecules (Vertuani *et al.,* 2004). To combat this potential danger, most cells make superoxide dismutase, an enzyme that detoxifies superoxide (Sies, 1997). The reduction of ferricytochrome c to ferrocytochrome c has been used in a number of situations to assess the rate of superoxide formation (McCord *et al.,* 1968).

Fe^{3}+cytc +O_2 ⟶ Fe^{2}+cyt c +O_2

1.13.2 Catalase

Catalase is an antioxidant enzyme found in aerobically active cells that catalyzes the conversion of the ROS hydrogen peroxide to molecular oxygen and two molecules of water, represented by the equation below. $H_2O_2 \longrightarrow O_2 + 2H_2O$. It nullifies the effect of hydrogen peroxide that is present intracellularly (*Nelson et al.,* 2006). The precise amount of catalase present in the cytoplasm cannot be assessed because most of it is lost during tissue manipulation (Aly and Shahin, 2010). Catalases, since they must fight against reactive molecules, are also unusually stable enzymes and perform its rapid destruction of hydrogen peroxide in two steps. First, a molecule of hydrogen peroxide binds and is broken apart. One oxygen atom is extracted and

attached to the iron atom, and the rest is released as harmless water. Then, a second hydrogen peroxide molecule binds. It is also broken apart and the pieces are combined with the iron-bound oxygen atom, releasing water and oxygen gas (Switala and Loewen, 2002).

1.13.3 Reduced Glutathione/Glutathione Peroxidase

Glutathione (GSH) can be used to "detoxify" reactive oxygen species such as hydrogen perioxide H_20_2, a process in which glutathione is oxidized to the dimer glutathione disulfide in a reaction catalyzed by glutathione peroxidase (GPx). Reduced glutathione in turn is regenerated from glutathione disulfide by glutathione reductase (GR) in a reaction requiring NADPH as a cofactor (Ukeda *et al.,* 1997). Glutathione peroxidase protects the erythrocyte against peroxides that are generated intracellularly or exogenously (Waggiallah and Alzohairy, 2011). Glutathione reductase plays an important role in protecting hemoglobin, red cell enzymes, and biological cell membranes against oxidative damage by increasing the level of reduced glutathione in the process of aerobic glycolysis. Deficiency of the enzymes may result in mild to moderately severe hemolytic anemia upon exposure to certain drugs or chemicals (Okezie, 1996).

1.14 Cholesterol

Cholesterol is a waxy substance that is present in the blood plasma and in all animal tissues. Chemically cholesterol is an organic compound belonging to the steroid family; its molecular formula is $C_{27}H_{46}O$ (Abell *et al.,* 1952). Cholesterol is essential to life; it is a primary component of the membrane that surrounds each cell, and it is the starting material or an intermediate compound from which the body synthesizes bile acids, steroid hormones, and vitamin D (Olson, 1998). Cholesterol circulates in the blood stream and is synthesized by the liver and several other organs. Human beings also ingest considerable amounts of cholesterol in the course of a normal diet. A compensatory system regulates the amount of cholesterol synthesized by the liver, with the increased dietary intake of cholesterol, resulting in the liver's decreased synthesis of the compound (Olson, 1998).

Cholesterol is insoluble in the blood; it must be attached to certain protein complexes called lipoproteins in order to be transported through the bloodstream. Low-density lipoproteins (LDL) transport cholesterol from its site of synthesis in the liver to the various tissues and body cells, where it is separated from the lipoprotein and is used by the cell. Cholesterol attached to LDL is

primarily that which builds up in atherosclerotic deposits in the blood vessels hence LDL are termed 'bad' cholesterol. When cholesterol levels get too high (that is a level greater than 240 mg/dL), the excess cholesterol is deposited on the inside walls of arteries in the form of plague. A build up of plague eventually narrows the arteries, a condition known as arteriosclerosis, which can lead to coronary artery disease and/or a heart attack (Olson, 1998). High-density lipoproteins (HDLs) may possibly transport excess or unused cholesterol from the tissues back to the liver, where it is broken down to bile acids and is then excreted thereby serving to retard or reduce atherosclerotic buildup, thus, it is termed 'good' cholesterol (Lewis and Rader, 2005).

1.14.1. Implications of Increased Level TAG, HDL and LDL Cholesterol In Diabtes

The cluster of lipid abnormalities associated with Type 2 diabetes is defined by a high concentration of TG and small dense LDL and a low concentration of HDL cholesterol. Plasma LDL cholesterol levels are generally normal. Insulin resistance is believed to contribute to this atherogenic dyslipidaemia by increasing the hepatic secretion of VLDL and other apolipoprotein (apo) B-containing lipoprotein particles, as a result of increased free fatty acid flux to the liver (Krauss and Siri, 2004). This may also be the result of a diminished suppressive effect of insulin on apoB secretion, either at the level of the regulation of apoB degradation, or inhibition of microsomal TG transfer protein. TGs are transferred from VLDL to HDL, creating TG-rich HDL particles, which are hydrolyzed by hepatic lipase and rapidly cleared from plasma (Hopkins and Barter, 1986). A similar cholesterol ester protein-mediated transfer of TGs from VLDL to LDL contributes to the formation of small dense LDL particles (Berneis, and Krauss, 2002). Other mechanisms, including impaired clearance of lipid and lipoproteins may also be involved.

1.15 The Liver

The liver has an important role in carbohydrate metabolism since it is responsible for the balance of blood glucose levels by means of glycogengenesis and glycogenolysis (Pieardi *et al.*, 2006). In the presence of hepatic disease, the metabolic homeostasis of glucose is impaired as a result of disorders such as insulin resistance, glucose intolerance and diabetes (Nielsen, *et al.*, 2005). Liver is a self-regenerating organ that plays important roles in the body. It functions not only in metabolism and removal of exogenous toxins and therapeutic agents responsible for metabolic derangement but also in the biochemical regulation of fats, carbohydrates, amino acids, protein,

blood coagulation and immunomodulation function (Ram and Goel, 1999). Due to its ability to regenerate even a moderate cell injury is not reflected by measurable change in its metabolic function. However, damage caused by lipid peroxidation on the membrane of the hepatocytes allows the leakage of some cytosolic enzymes of the liver into the blood stream (Plaa and Hewitt, 1982).

Serum enzyme markers involved in hepatic disorder when the integrity of the membrane of the hepatocytes is compromised, certain enzymes located in the cytosol are released into the blood. Their estimation in the serum is useful quantitative marker for the evaluation of liver damage (Ram and Goel, 1999). Glutamate dehydrogenase activity is not found in normal serum but moderate elevation is found in most cases of acute hepatitis indicating cellular damage. Another demonstrable type of membrane damage involves injury to lysosomes which leads to the release of acid ribonuclease and acid phosphatases, and other liver enzymes such as alanine aminotranferase, aspartate aminotranferase and alkaline phosphatase, into the blood stream. These enzymes are elevated to distinguish and assess the extent and type of hepatocellular injury (Ram and Goel, 1999).

1.15.1 Alanine Aminotransferase (ALT)

Systematic name L-alanine 2-oxoglutarate aminotransferase (EC 2.6.1.2).also pyruvate aminotransferase, glutamate pyruvate aminotransferase; formerly known as alanine transminase (Mussoa *et al.*, 2009). ALT is an intracellular cytoplasmic enzyme. Alanine aminotransferase (ALT) is an enzyme that catalyzes the transfer of amino groups to form the hepatic metabolite oxalocetate. It is composed of 496 amino acid, which are encoded by a gene located in the long arm of chromosome (Kim *et al.,* 2008). ALT is found abundantly in the cytosol of the hepatocyte. ALT activity in the liver is about 3000 times that of serum activity. Although it is generally thought to be specific to the liver, it is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells. High level (> 50) indicate damage to liver cells as a result of infection (hepatitis, infectious mononucleosis), or toxic levels of drugs (e.g acetaminophen (Tylenol) or chemicals (e.g chloroform) or alcohol (Kim *et al.,* 2008). Excessive alcohol consumption plus heavy use of acetaminophen is particularly dangerous. ALT levels may be modestly elevated with liver tumors or cirrhosis of the liver. ALT catalyzes the transfer

of amino groups from alanine (forming pyruvate) to 2-oxoglutarate (forming glutamate). It is key enzyme in gluconeogenesis (Mussoa *et al.*, 2009).

Alanine + 2-oxoglutarate ⟶ (ALT) ⟶ pyruvate + glutamate.

Pyruvate + NADH + H+ ⟶ lactate + NAD+ ALT is measured in plasma or serum.

1.15.2 Aspartate Aminotransferase (AST)

Aspartate aminotransferase (AST) also known as (serum glutamic oxaloacetic transaminase (SGOT) is an enzyme found in red blood cells and in liver, heart, muscle, pancreas and kidney. Damage to these tissues releases AST into the blood stream. Very high levels suggest recent liver damage. More moderate elevation can indicate damage to liver or other tissues. It normal range is from 17-59 Units per liter (Kunde *et al.*, 2005). In acute hepatocellular injury, serum AST levels usually rise immediately, reaching a higher level than ALT initially, due to the higher activity of AST in hepatocytes and its release with liver injury. The use of many medications has been associated with elevated AST levels (Green and Flamm, 2002).

1.15.3 Alkaline Phosphatase (ALP)

The normal values of alkaline phosphates are 0-130 I.U./L. It is a metalloenzyme linked to the cell membrane (Wallach and Analized, 2001). It is distributed in the liver, placenta and bone. Bone-specific alkaline phosphatase is in fact an isoenzyme involved in bone formation processes and the main enzymatic mediator in the removal of inorganic pyrophosphate, an inhibitor of bone mineralization (Lazar *et al.*, 2016). Abnormally high levels suggest liver damage or obstruction of the bile ducts from gallstones or cancer. Bone disease may also cause elevated ALP (Green and Flamm, 2002). Cholestatsis-inducing drugs (aminosalicylic acid, amitriptyline, anabolizing steroids, androgens, azathiopine, carbamazepine, carbazone, chlorothiazide, chlorpropamide, clavulanic acid, dapsone), drugs that induce hepatocullular damage and many other drugs may cause increases of alkaline phosphatase, which in general are transient, but indicate hepatotoxicity in certain cases.

1.15.4 Bilirubin

Bilirubin (BR) is a yellow-brown pigment that is produce when the liver breaks down hemoglobin from old red blood cells. It is secreted into bile and then enters the intestine where it is eventually excreted in faeces, accounting for the characteristic brown colour of stool (Valaskova and Muchova, 2016). There are two forms of bilirubin; indirect (unconjugated) bilirubin, which is poorly soluble in water and direct (conjugated) bilirubin, which is water soluble. The sum of the two is "total bilirubin. High blood levels of bilirubin can be caused by liver damage from hepatitis or from blockage of bile ducts or abnormally rapid destruction of red blood cells (hemolytic anemia) (Van de Steege *et al.,* 2012).

The physiological concentrations of BR in the organism are relatively low but its antioxidant effects might be increased by bilirubin-biliverdin redox cycle, in which is BR regenerated by biliverdin reductase (Valaskova and Muchova, 2016). BR was found to be a free radical scavenger by donating a hydrogen atom attached to the C-10 Bridge of the tetrapyrrole molecule to form carbon-centered radical and an inhibitor of superoxide production by mechanism employing the inhibition of NADPH oxidase (Kwak *et al.,* 1991). BR is able to protect fatty acids, phospholipids and proteins against peroxidation and neuronal cultures from the oxidative stress (Stocker and Ames, 1987). The normal ranges are Total bilirubin 0.3 – 1.0 miligrams per deciliter (mg/dL). Direct bilirubin 0.1-0.3 mg/dL, Indirect bilirubin (total bilirubin level minus direct bilirubin level) 0.2-0.7 mg/dL.

1.16 Kidney

When blood sugar is high, it can put too much stress on kidneys causing serious damage to the blood vessels, leading to kidney disease. In rare, severe cases, this can lead to kidney failure and the need for a kidney transplant. Diabetic kidney disease takes many years to develop. Overall, kidney damage rarely occurs in the first 10 years of diabetes and usually 15 to 25 years will pass before kidney failure occurs. The kidneys excrete metabolic waste products and regulate the serum concentration of a variety of substances (Anupriya *et al.,* 2011). At some stage during the course of renal disease, the following routinely measured substances often become abnormal and the extent of the abnormality generally depends on the severity of the disease. Serum creatinine and urea concentrations change inversely with changes in glomerular filtration rate (GFR) and

are therefore useful in knowing the degree of renal dysfunction (Schutte *et al.*, 1981). Changes in serum creatinine concentration more reliably reflect changes in GFR than do changes in serum urea concentrations and blood concentrations depend almost solely upon GFR. Urea formation is influenced by a number of factors such as liver function, protein intake and rate of protein catabolism (Griffin *et al.*, 2008). Urea and creatinine are the parameters to diagnose kidney function.

1.16.1 Creatinine

Creatinine is synthesized in the liver and after being released, it is taken up in a proportion of over 90 % of muscle, where phosphorylation occurs. In this form, it plays an important role in storing muscle energy (Winklem and Parma, 2000). In case when this muscle energy is required for metabolic needs, phosphocreatine is broken down to creatine. The reduction of protein intake diminishes the level of creatinine through the absence of the amino acids arginine and glycine, which are creatine precursors. The amount of creatine converted to creatinine is maintained at a constant level, which is directly related to the muscle tissue mass of the body. The normal creatinine values in rats are 0.0-0.9 mL/dL (Wilfred and Ulrich, 2005). Glomerular filtration of creatinine, however, is only one of the variables that determine its concentration in serum. Alterations in renal handling and metabolism of creatinine and methodological interferences in its measurement may have a profound impact on the serum concentration of creatinine. Renal disease, serum creatinine values do not increase significantly until renal function has been considerably impaired. If the filtration in the kidney is deficient creatinine blood level will rise, this means that the creatinine levels in blood and urine may be used to calculate the creatinine clearance, which correlates with the glomerular filtration rate (Winklem and Parma, 2000). Determination of creatinine clearance ratios, however, may more sensitively indicate renal impairment and thus can be useful for following the effect of a course of treatment in cases of actue glomerulonephritis, to identify a primarily glomerular dysfunction and to assess overall renal function (Crowe and Hatch, 1977). Reactions involved in the metabolism of creatine and creatinine at various sites in the body are summarized as follows:

Transamidinase

Arginine + glycine ==========ornithine + guanidoacetic acid

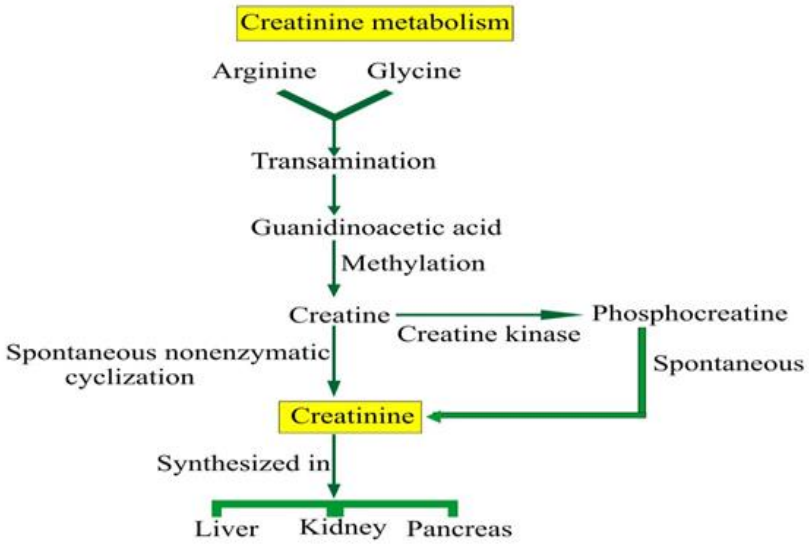

Figure 6. Creatinine metabolism. http://wwwlabpedia.net/test/52.

1.16.2 Urea

Urea is the principal nitrogenous waste product of metabolism and is generated from protein breakdown. It is eliminated from the body almost exclusively by the kidneys as urine (Witting, 2006). Urea is a small organic molecule comprising two amino (NH_2) groups and a linked carbamyl (C-O) group:

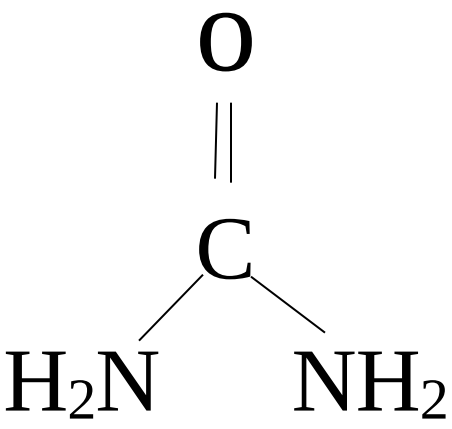

Figure 7: Structure of urea. Watford, (2003).

It is the principal nitrogenous end-product of protein and amino acid catabolism. Proteins are first degraded to constituent amino acids, which are in turn degraded with production of ammonia (NH_3), which is toxic. In a series of five enzymatically controlled reactions, known collectively as the "urea cycle", toxic ammonia resulting from protein breakdown is converted to non-toxic urea (Watford, 2003). Normal range: Serum/plasma urea 2.5-7.8 mmol/L. serum/plasma urea concentration reflects the balance between urea production in the liver and urea elimination by the kidneys, so increased plasma/serum urea can be caused by increased urea production, decreased urea elimination, or a combination of the two. This may be caused due to advanced renal disease and associated marked reduction in glomerular filtration rate (GFR). Gastro-intestinal haemorrhage is associated with increased protein intake (blood in the gut is effectively a high-protein meal) and thereby increased urea production and consequent increased plasma/serum urea (Mehta, 2008). Drugs that induced a catabolic state with increased protein breakdown and consequent increased urea production can cause plasma/serum urea to rise slightly.

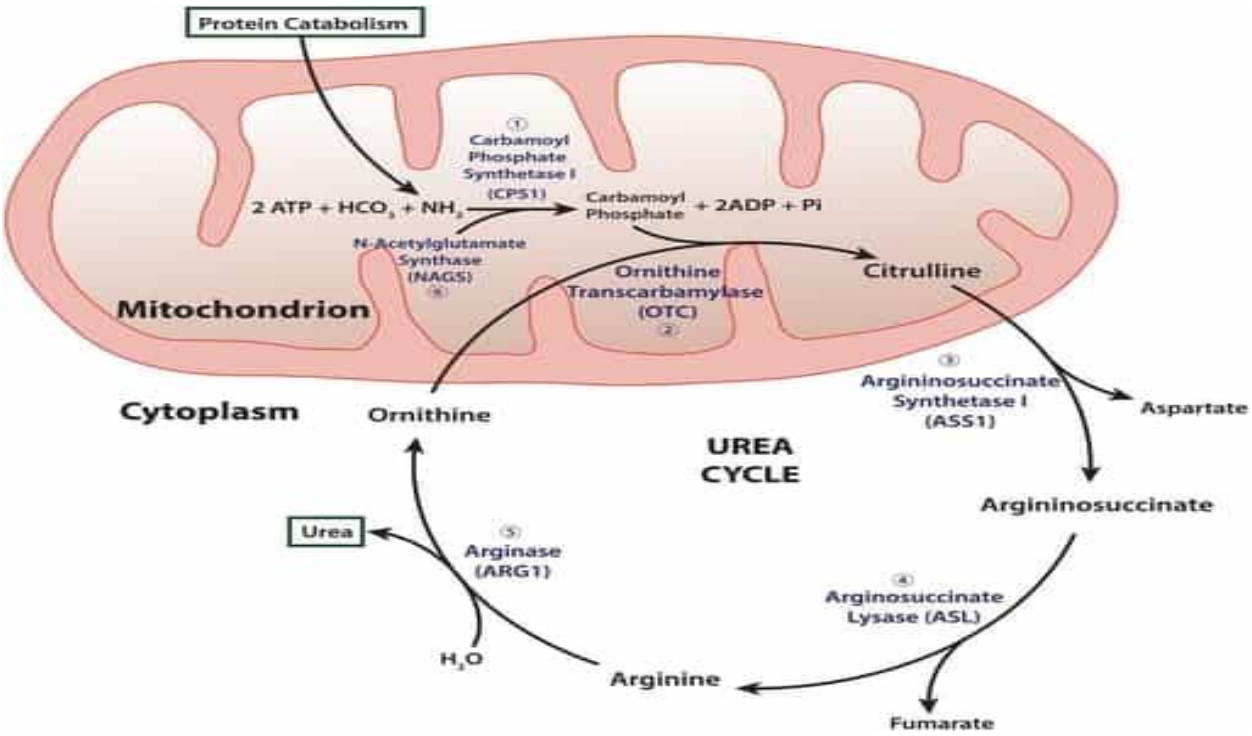

Figure 8: Urea cycle Watford, (2003).

1.17 Haematology

Hematology is the study of blood in health and disease. It includes problems with the red blood cells, white blood cells, platelets, blood vessels, bone marrow, lymph nodes, spleen, and the protein involved in bleeding and clotting (hemostasis and thrombosis). The mechanisms responsible for regulating steady-state hematopoisis and the capacity to modulate blood cell production in response to stresses such as anemia or infection consist of a series of progenitor cells in the bone marrow and a complex array of regulatory factors (Alam *et al.,* 2015). Hematological indices are important indicators for the evaluation of variations in size, number, and maturity of different blood cells. They are important for the assessment and management of patients with DM (Alam *et al.,* 2015). Hemoglobin is life giving substance of every red blood cell, the oxygen carrying component of the red cells, major organ of the human body depend on oxygenation for growth and function (Norman, 2009). In healthy adult individuals the red blood cells constitute approx. 40-48 %, whereas newborns may have hematocrits of up of 60 % (Soldin *et al.,* 1995). A low hematocrit reflects a low number of circulating red blood cells and is an indicator of a decrease in the oxygen-carrying capacity or of over hydration. Examples of conditions causing a low hematocrit (anemia) include, kidney disease, bleeding, also bone-

marrow failure (Linne and Ringsrud, 1999). A high hemaocrit may reflect an absolute increase in the number of erythrocytes, or a decrease in plasma volume, in conditions such as, severe dehydration, excessive red blood cell production, an inherited iron metabolism disorder (Linne and Ringsrud, 1999).

High platelet activity enhances vascular complications in Diabetes Mellitus patients. Mean platelet volume (MPV) is a marker showing platelet function and activation (Mortensen, et al., 2010). Altered platelet morphology and function can be reflected as a factor for risk of microvacular and macrovascular diseases. These abnormalities have been shown to markedly increase blood viscosity that unfavourably affects the microcirculation, leading to microangiopathy (Parker *et al.,* 2016). Higher White blood Cell (WBC) count, is one of the major components of inflammatory process, contributes to atherosclerotic progression and CVD (Zuberi *et al.,* 2008).

1.18 Histology

Histopathology refers to microscopic examination of tissue in order to study the manifestation of disease. Histopathological examination starts with surgery, biopsy or autopsy. Fixation stabilizes the tissue and thereby prevents decay. The most common fixation is done using formalin (10 % formaldehyde in water). The medical diagnosis is formulated as pathology report describing the histological findings and the opinion of the pathologist.

1.19 Rationale of the Study

In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural product with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. There are numerous traditional medicinal plants reported to have antihyperglycemic properties, however, many of these plants are less effective in lowering glucose level in severe diabetes, thus the need for this study.

1.20 Aim of the Study

This study is aimed at investigating the antidiabetic activity of n- hexane fraction of *Lepidagathis alopecuroides* leaves in alloxan-induced diabetic rats.

1.20.1 Specific Objectives of the Research

To determine qualitatively and quantitatively the phytochemical constituents of the ethanol leaf extract of *Lepidagathis alopecuroides*.

To determine the acute toxicity (LD_{50}) of the ethanol extract of leaf of *Lepidagathis alopecuroides.*

To determine the body weight of the rats before induction with alloxan and after treatment with *Lepidagathis alopecuroides* leaf fraction.

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on blood glucose level of alloxan induced diabetic rats.

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on insulin secretion capacity of the pancreas in alloxan-induced diabetic rats.

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on the activities of serum liver marker enzymes (AST, ALT and ALP) in alloxan-induced-diabetic rats.

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on serum lipid peroxidation parameters (MDA).

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on serum kidney function parameters (Urea and Creatinine).

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on the activities of serum endogenous antioxidants (SOD, CAT and GSH) in alloxan-induced diabetic rats

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on lipid profile (LDH, TAG, HDL and Total Cholesterol)

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on haematological parameters (RBC, WBC, HB and PCV).

To determine the effect of *Lepidagathis alopecuroides* leaf fraction on the histology of the pancreas.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemical and Reagents

The chemicals used for this study were of analytical grade and products of Sigma Aldrich (USA), British Drug House (BDH, England) and Andalucia (Spain). They include ethanol (BDH, England), hydrochloric acid (BDH, England), Dragendorff's reagent (Andalucia), Mayer's reagent (Andalucia), Fehlings solutions (A and B) (Sigma Aldrich), Phosphate buffer (BDH, England), Distilled water, Ferric chloride solution (Sigma Aldrich), Trichloroacetic acid solution (Sigma Aldrich), Ammonium thiocyanate solution (BDH, England), Iron II Chloride (Sigma Aldrich), Griess reagent (Andalucia), Sulphanamide, Methylethemediamine dihydrochloride (Sigma Aldrich), Methanol (BDH, England), Alkaline Picrate solution (Sigma Aldrich), Phosphate buffer (BDH, England), Potassium ferricyanide solution (BDH, England), ethylacetate, ammonium solution, Chloroform (BDH, England), alkaline copper reagent (BDH, England), sodium carbonate (BDH, England), ferric chloride (BDH, England), formaldehyde (Andalucia), Concentrated hydrogen tetraoxosulphate IV acid (BDH, England), calcium chloride, oxalic acid, 2,6-dichlorophenol indophenol (Sigma Aldrich), petroleum ether (BDH, England), acetic anhydride (BDH, England), aqueous bovine albumin (Andalucia), alcoholic potassium (Andalucia), sodium carbonate (Sigma Aldrich), folinciocalteau reagent (Sigma Aldrich), sodium hydroxide (BDH, England),. glibenclimide. (Germany).

2.1.2 Equipment and Instruments

Rotary evaporator, (Model 349/2, Corning Ltd, England), Centrifuge (Gallenkamp, Germany) Spectrophotometer (Jeol 400 MHz, Strathclyde Scotland University), glass column chromatography (Prex, England), vacuum pump, milling machine, filter paper, digital photo colorimeter, Olympus microscope, water bath (Chikpas Instrument, Enugu), chemical balance (Gallenkamp, England). Micro-pipettes (perfect, USA), microscope slides, capillary tube.Refrigerator (Haier thermocool, England), microscope (XSZ-107BN, India), counting chamber (MC Qiujing, China).

2.1.3 Animals

Twenty four (24) adult male rats were used for the studies (80 - 150 g) and eighteen (18) adult male Swiss albino mice (20 – 30 g) obtained from the animal holding unit of the Department o Zoology and Environment Biology, University of Nigeria, Nsukka were used for the acute toxicity (LD_{50}) study. The animals were housed under standard conditions (25 $\pm$ 2 ^{o}C and 12h light/ dark cycle). The rats and mice were fed two times in a day with standard pellets (Grand Cereals Ltd. Enugu Nigeria) and had unrestricted access to clean drinking water. The guide for the care and use of laboratory animals procedures were followed in this study (Indian Council o Medical research, 2001).

2.1.4 Plant Material

The leaves of *Lepidagathis alopecuroides* used for this study were collected from Abanwan community in Erei, Biasel local government area of Cross River State Nigeria. The leaves were identified and authenticated by Mr. Alfred Ozioko of the Bioresource Development and Conservation Program (BDCP), Research Centre Nsukka.

2.2 Methods

2.2.1 Experimental Design

The study was carried out in stages as follows:

2.2.2 Animal Grouping

Twenty four (24) Wistar albino male rats weighing 80 – 150 g were used for the study. They were acclimatized for fourteen (14) days with free access to feed and water. After acclimatization, they were evenly distributed into six (6) groups of four rats each. The route of administration of extracts was via oral route with the aid of an oral intubation tube. The groups and doses administered are summarized below.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan induced diabetic rat + 5 mg/kg body weight of Glibenclamide

Group 4: Alloxan induced diabetic rat + 100 mg/kg body weight of n-hexaneleaf fraction

Group 5: Alloxan induced diabetic rat + 200 mg/kg body weight of n-hexane leaf fraction

Group 6: Alloxan induced diabetic rat + 400 mg/kg body weight of n-hexane leaf fraction

2.2.3 Preparation of Plant Material

The fresh leaves of *Lepidagathis alopecuroides* were harvested, washed and dried to constant weight at room temperature (29 ^{O}C – 35 ^{O}C) for three weeks. The dried leaves were pulverized into coarse form with a Crestor high speed milling machine.

2.2.4 Extraction of Plant Materials

The ethanol extraction of pulverized leaves of *Lepidagathis alopecuroides* was cried out. A weighted quantity (634.91 g) of the pulverized leaves of *Lepidagathis alopecuroides* was soaked and macerated in 95% ethanol for 72hr, after which it was filtered with a mesh, followed by a Whatman filter paper. The clear filtrate was concentrated using rotary evaporator. In a 500ml separating funnel, 20g of the crude extract was dissolved in 200ml of 20% ethanol after which it was partitioned with 200ml of n-hexane to obtain n-hexane fraction. The n-hexane fraction was further concentrated using an oven at 45 $^{\circ}$C. To the samples were stored in the refrigerator for subsequent studies.

2.2.5 Qualitative Phytochemical Analysis of the Leaves of *Lepidagathis alopecuroides*

The phytochemical analysis of the leaves of *Lepidagathis alopecuroides* were carried out according to the method of Sofowora (1993) and Trease and Evans (2002) to identify its active constituents.

2.2.5.1 Tannins

The sample (0.5 g) was stirred with 10ml of distilled water and then filtered. Few drops of 1 % ferric chloride solution were added to 2ml of the filtrate. The occurrence of a blue-black, green or blue-green precipitate indicated the presence of tannis (Trease and Evans, 2002)

2.2.5.2 Flavonoids

The ssample (0.5 g) was dissolved in water and filtered. To 2ml of the filtrate, few drops 10% ferric chloride solution was added to produce a green-blue or violet colouration. A change in colour from green-blue or violet on addition of dilute ferric chloride was an indication of the presence of flavonoids (Trease and Evans, 2002).

2.2.5.3 Alkaloids

The sample (1 g) was stirred with 5ml of 1 % aqueous HCL on water bath and then filtered. The filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second 1 ml of Mayer's reagent was added and appearance of buff-coloured precipitate was in indication for the presence of alkaloids (Sofowora, 1993).

2.2.5.4 Saponins

The sample (1 g) was boiled with 5ml of distilled water and filtered. To the filtered, 3 ml of distilled water was added and shaken vigorously for 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of Saponin (Trease and Evans, 2002).

2.2.5.5 Determination of Phenols

The sample (1 g) was dissolved in water and filtered, to this; 2 ml of the 10 % aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

2.2.5.6 Determination of Terpenoids

The sample (0.5 g) was dissolved in ethanol. A 1 ml of acetic anhydride was added followed by the addition of conc, H_2SO_4. A change in colour from pink to violet showed the presence of terpenoid (Sofowora, 1993).

2.2.5.7 Determination of Steroids

To 0.2 g of the sample, 2ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac portion of cardiac glycoside (Sofowora, 1993).

2.2.5.8 Determination of Glycosides

The sample (0.5 g) was dissolved in glacial acetic acid. 1 ml of the filtrate was pipetted into the test tube and 10ml water was added. This was boiled for 30 minutes and 2 ml of dilute ammonia was added. Then 0.4 ml Fehling's solution A and B was added and boiled again for 5 minutes and a colour change to brick red showed the presence of glycoside (Trease and Evans, 2002).

2.2.6 Quantitative Phytochemical Analysis of *Lepidagathis alopecuroides*

2.2.6.1 Determination of Tannins

Tannins content of the leaves of *Lepidagathis alopecuroides* was determined by Folin Denis colometric method of Nwaokonkwo (2009). Each sample (g) was macerated with 20 ml of distilled water and filtered. The filtrate (5 ml) was pipette into a test tube. To this added 0.3 ml of 0.1N ferric chloride in 0.1 N HCL and 0.3 ml of 0.0008M potassium ferricyanide were added. This was mixed well. Measurement of absorbance was at 720 nm.

$$\text{Tannin} = \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$$

2.2.6.2 Determination of Flavonoids

This was determined according to the method of El-Olemyl (1994). A quantity, 1 g of the extract was maccrated with 20 ml ethylacctate and filtrated. The filtrate (5 ml) was pipette inside the test tube. The dilute ammonia (5 ml) was added and we collect the upper layer. Measurement of absorbance at 490 nm.

$$\text{Flavoniod} = \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$$

2.2.6.3 Determination of Alkaloids

This was determined according to the method of El-Olemyi (1994). A portion (1 g) of the extract was maccrated with 20 ml of ethanol 20 % sulphuric acid (1:1) and filtrate. Each sample (1 ml) of the filtrate and 5 ml of 60 % H_2SO_4 mixed and allowed to stand for 3 hr. measurement of absorbance was at 490 nm.

$$\text{Alkaloid} = \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$$

2.2.6.4 Determination of Saponins

This was determined according to the method of El-Olemyi (1994). A quantity, 1 g of the extract was maccrated with 20 ml of petroleum ether and decanted into a beaker and was washed again with 10 ml of petroleum ether. The combined filtrate was evaporated to dryness. The residue was dissolve with 6ml of ethanol.into a test tube transfer 2 ml of the solution and adds 2ml of chromogen solution. Stand for 30 minutes. Measurement of absorbance was at 550 nm.

$$\text{Saponin} = \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$$

2.2.6.5 Determination of Phenols

This was determined according to the method of El-Olemyi (1994). A quantity, 1 g of the extract was macerated with 20 ml of 80% ethanol and filtered.To each sample (5 ml) of the filtrate was added inside test tube. The folincicalteus reagent (0.5 ml) was added and after 30 minutes i added 2 ml of 20 % sodium carbonate. the absorbance was read at 650 nm

$$\text{Phenols} = \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$$

2.2.6.6 Determination of Trepenoids

This was determined according to the method of El-Olemyi (1994). A quantity, 0.5 g of the extract was macerated with 20 ml of ethanol and filter. 2.5ml of filtrate was pipetted into a test tube . 1 ml of 5 % phosphomolybdic acid solution was added together with 1 ml of conc. H_2SO_4 . allowed to stand for 30 minutes and the absorbance read at 700 nm

Trepenoids $= \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$

2.2.6.7 Determination of Steroids

This was determined according to the method of El-Olemyl (1994). A quantity, 2 g of the extract was macerated with 20 ml of ethanol and filter. Each sample (2 ml) of filtrate and 2 ml ethanol was pipette inside the test tube. After that, 2 ml of colour reagent was added in sample test tube and blank test tube, and stand for 30 minutes. Measure the absorbance at 550 nm.

Steroids $= \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$

2.2.6.8 Determination of Glycosides

This was determined according to the method of El-Olemyl (1994) A quantity, 1 g of the extract was macerated with 20 ml of distilled water and filtered. We added 2.5 ml of 1 % lead acetate and we filtered then we also added 2.5ml of chloroform and shook vigorously. We collected the lower layer and evaporate to dryness. The residue was dissolve with 3 ml of glacial acetic acid and we add 0.1ml of 5 % ferric chloride. The concentrated H_2SO_4 (0.25 ml) was added and kept in the dark for 2 hours. the absorbance was measured at 530 nm.

Glycoside $= \frac{Abs-blk}{Slope} x \frac{volume\ of\ solvent}{wt\ of\ sample}$

2.2.7 Acute Toxicity Studies

The acute toxicity studies of the crude ethanol extract were estimated in mice using the method of Lorke (1983). The tests involved two phases. The first phase was determination of the toxic rang. The mice were placed in three groups of three (3) mice each and were given 10,100 and 1000 mg/kg body weight of the extract solubilized in normal saline. The treated mice were observed for 24 h for number of deaths. The pattern in the first phase determined the doses used for the second phase. In the second phase, the mice received 1600, 2900 and 5000 mg/kg body weight of the extract. The treated animals were observed for 24 h for lethality or signs of acute intoxication.

2.2.8 Induction of Diabetes and Blood Glucose Determination

Reaction Principle:

The baseline blood glucose levels were determined and recorded before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in ice cold normal saline at a dose of 140 mg/kg body weight and the route of administration was intraperitoneal. After two (2) days, rats with blood glucose levels greater than 200 mg/dl were considered diabetic and used for the investigation (Frode and Medeiros, 2008). The treatment lasted for fourteen (14) days in which blood glucose levels and body weights of the rats were determined on day 0, 7. and day 14. Accu-Chek blood glucose monitoring meter and test strips were used for the assay. This method is based on the reaction of glucose and oxygen in the presence of glucose oxidase (GOD) to yield gluconic acid and hydrogen peroxide. The hydrogen peroxide formed subsequently reacts under catalysis of peroxidase (POD), with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. In other words, it oxidizes the dyes in a reaction mediated by peroxidase producing a blue coloured product. The intensity of the colour which is proportional to the glucose concentration in the sample was read from the Accu-Chek glucometer.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + 4\text{–aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

2.2.8.1 Treatment of the Alloxan-induced Diabetes with Plant Extract

The N-hexane fraction of *Lepidagathis alopecuroides* was dissolved in normal saline. The dissolved fraction was administered orally to group 4 – 6 once daily for 14 consecutive days. The dissolve standard drug, glibenclimide was administered orally to group 3 once daily for 14 days. Blood was collected by ocular puncture for biological analysis, after which the animals were sacrificed.

2.2.9 Determination of Insulin Concentration

Principle

The essential reagent required for an immunoenzymometric assay include high affinity and specificity antibodies [enzyme and immobilized] with different and distinct epitope recognition, in excess, and native antigen, in this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal insulin antibody.

Test Procedure

1. Format the microplate wells for each serum reference, control and the specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 ºC

2. Pipette 0.05 ml of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.1 ml of insulin-enzyme reagent to all wells.

4. Swirl the microplate gently for 30 seconds to mix and cover.

5. Incubate for 120 minutes at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper

7. Add 350 of wash buffer tap and blot or aspirate. Repeat two (2) additional times for a total of three (3) washes. Decant the wash and repeat two (2) additional times.

8. Add 0.100 ml of working substrate solution to all wells [do not shake the plate after substrate addition].

9. Incubate at room temperature for fifteen (15) minutes.

10. Add 0.05 ml of stop solution to each well and gently mix for 20 seconds.

11. Read the absorbance in each well at 450 nm in a microplate reader and the results within thirty (30) minutes of adding the stop solution.Sacks and Tietz. (1994).

2.2.10 Liver Enzyme Markers:

2.2.10.1 Assay of Aspartate Aminotransferase (AST) activity

The activity of aspartate aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit used. Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazine with 2, 4-dinitrophenylhydrazine.

Kit Reagents

S/N	Content	Initial Concentration of Reagents
R1	Phosphate Buffer	100mmol/1, pH 7.4
	L- Aspartate	100mmol/1
	Beta – oxoglutarate	2mm0l/l
R2	2, 4-dinitrophenylhydrazine	2mm0l/l

Procedure

The serum samples (0.1ml) pipette into the samples test tubes only and 0.1 ml of distilled water was pipette into the blank test tube. The, 0.5 ml of Reagent one (R_1) containing phosphate buffer, L –aspartate and beta – oxoglutarate was pipette into both the blank and serum sample test tubes respectively. The entire reaction medium was well mixed and incubated for 30 minutes in a water bath at 37 ^{O}C. immediately after incubation, 0.5ml of Reagent two (R_2) containing 2,4-dinitrophenylhydrazine was added to the blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25 ^{O}C. finally, 5.0 ml of 0.4 N sodium hydroxide solutions was added to both the blank and serum sample test tubes respectively and mixed thoroughly. The absorbance was read at a wavelength of 54 nm after 5 minutes.

$$\frac{AST = Abs\ of\ sample - abs\ of - blk}{Slope}$$

2.2.10.2 Assay of Alanine Aminotransferase (ALT) activity

The activity of alanine aminotransferase were assayed by the method of Reitman and Frankel (1975) as outlined in Randox Kit.

Kit Reagents

S/N	Content	Initial Concentration of Reagents
R1	Phosphate Buffer	100mmol/1, pH 7.4
	L- alanine	100mmol/1
	Beta – oxoglutarate	2mm0l/l
R2	2, 4-dinitrophenylhydrazine	2mm0l/l

Procedure

The serum samples (0.1ml) was pipetted into the samples test tubes only and 0.1 ml of distilled water was pipetted into the blank test tube. 500μl of the ALT substrate buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate (R_1) were added. The entire reaction media were well mixed and incubated for 30minutes in a water bath at 37 ^{O}C and pH 7.4. Immediately after incubation, 0.5 ml of Reagent two (R_2) containing 2,4- dinitrophenylhydrazine was added to the blank and sample test tubes. These were thoroughly mixed and allowed to stand for exactly 20 minutes at 25 ^{O}C. Finally, 5.0 ml of sodium hydroxide solutions was added to both the blank and serum sample test tubes respectively and mixed thoroughly. The absorbance was read at a wavelength of 546 nm after 5 minutes.

$$ALT = \frac{Abs\ of\ sample - abs\ of - blk}{Slope}$$

2.2.10.3 Assay of Alkaline Phosphatase Activity

Principle

The activity of alkaline phosphatase was assayed by the method of Bason *et al.*, (1996), as outlined in Randox kit, used. The alkaline phosphatase act upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure

Into three test tubes labeled test, serum, standard and blank were added 50μl distilled water respectively. Then, 0.05ml (50μl) of alkaline phosphatase substrate into labeled test tubes for 3 minutes mix gently and incubates for exactly 10 minutes at 37 ^{O}C. After that, alkaline phosphatase colour developer (2.5 ml) was added at timed intervals and mix well. Measure the absorbance at 630 nm

Calculation.

$$\text{Alkaline phosphatase} = \frac{Absorbance\ of\ sample\ \ x\ value\ of\ standard}{Absorbance\ of\ standard}$$

2.2.11 Estimation of the Extent of Lipid Peroxidation (Malondialdehyde)

Principle

Lipid peroxidation was estimated by measuring spectrophotometerically the level of the lipid peroxidation product, malondialdehhyde (MDA) as described by Wallin *et al.*, (1993). Lipid degradation occurs forming such products as malondialdehyde (from fatty acids with two or more double bonds), ethane and pentane (from the n-terminal carbons of 3 and 6 fatty acids. respectively malondialdehyde reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution absorbs maximally at 532 nm.

$$MDA + 2TBA \longrightarrow MDA{:}\ TBA\ adduct - H_2O$$

Procedure

Into three test tubes labeled test, serum and blank were added with 10μl of distilled water respectively. Then, 0.5ml of 25 % TCA (trichloroacetic acid) and 0.5 ml of 1% TBA (thiobarbituric acid) in 0.3 % NaOH were added also. The mixture was boiled for 40 minutes in a water bath and cooled in cold water. Then, 0.1 ml of 20 % sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbances were taken at wavelengths of 532 nm and 600 nm against a blank.

MDA = $\frac{abs\,1-abs\,2}{0.00693}$

2.2.12. Kidney Function Markers

2.2.12.1 Determination of Urea Concentration

The concentration of serum urea was determined using the method of Tietz, (1983) as outlined in Randox kit, UK

Urea + H_2O $\longrightarrow$ $2NH_3 + CO_3$

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

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction

Procedure

In three test tubes labeled serum. Standard and blank were added 10 μl serum, standard and distilled water each. Then, 10 μl of sodium nitroprusside and urease R_1 were added to each of tubes. The tubes were mixed and incubated at 37 ^{O}C for 10 minutes. Then, reagent R_2 (2.50 ml) was added to each of the test tube. After that, 2.50 ml of R_3 was also added. Mix immediately and incubated at 37 ^{O}C for 15 minutes and Measurement the absorbance at 546 nm.

Urea concentration (mg/l) = $\frac{Absorbance\ of\ sample\ \ x\ standard\ conc}{Absorbance\ of\ standard}$

2.2.12.2 Determination of Creatinine Concentration

The concentration of serum Creatinine was determined using the method of Tietz (1983) as outlined in Randox kitg, UK.

Principle

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

Procedure

Into three test tubes labeled test, serum, standard and blank were added 50 μl of serum, 50 μl of standard and 50 μl distilled water respectively. Then, 500 μl of the working reagents were pipetted into each of the three tubes and mixed. the absorbance was read at 510 nm.

$$\text{Creatinine (mg/l)} = \frac{Absorbance\ of\ sample\ x\ standard\ conc}{Absorbance\ of\ standard}$$

2.2.13 Lipid Profile

2.2.13.1 Determination of Serum Cholesterol (CHOL) Concentration

This was done according to the method of Roeschlau *et al.,* (1974).

Principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{cholesterol}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{Cholesterol -3-} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{Aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4H_2O$$

Procedure

I added (10 μl) of distilled water into test tube as blank, 10 μl of the standard reagent was added into a separate test tube and 10 μl of the sample was added to thesame test tube. Then, 1000 μl of reagent R1 was equally added and mixed. Then, incubated for 10 minute at 37^{O}C. the absorbance of all the cuvettes was read and recorded at 546 nm.

Cholesterol conc. (mg/dl) $= \frac{change\ in\ Abs\ sample}{Chnage\ in\ Abs\ standard}$x conc. of standard

2.2.13.2 Estimation of the Concentration of Serum Triacylglycerol Concentration

This was done according to the method of Tiez, (1976)

Principle

Triglycerides + H_2O $\xrightarrow{\text{lipases}}$ glycerol + Fatty acid

Glycerol + APT $\xrightarrow{GK}$ glycerol -3-phosphate + ADP

Glycerol -3-phosphate + O_2 $\xrightarrow{GPO}$ dihydroxyacetone + phosphate + H_2O_2

H_2O_2 + 4-aminophenazone + 4-chorophenol $\xrightarrow{POD}$ quinoneimine + HCI + $4H_2O$

Procedure

The 5 μl of the distilled water was added into test tube blank, 5 μl of the standard reagent was added into another test tube and 5 μl of the sample was added into another test tube. Then, 500 μl reagent R1 was added into each test tube and mixed. This was incubated for 5 minute at 37 ^{O}C.

TAG (mmol/1) $= \frac{change\ in\ Abs\ sample}{Chnage\ in\ Abs\ standard}$x conc. of standard

2.2.13.3 Determination of Serum High Density Lipoproteins Concentration

High density lipoprotein (HDL) - cholesterol concentration was determined by the method of Alber *et al.,* (1978) using Randox kit. High density lipoproteins (DL) was determined using biosystem commercial kit method was based on the principle that very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the sample precipitated with phosphotungstate and

magnesium ions. The supernatant contains high density lipoproteins (HDL). The high density lipoproteins (HDL) were spectrophotometrically measured.

Procedure

The 1000 μl of the serum and standard was pipetted into the centrifuge tube which was immediately accompanied with the addition of 500 μl of the diluted precipitation reagent (R1) to the centrifuge tube. The content were mixed and allowed to stand for 10 minutes at 25 ^{O}C, then, centrifuged for 15 minutes at 3500 rpm. The cholesterol concentration of the supernatant was determined after centrifugation. Into three test tube labeled test, sample supernatant, standard and blank were added 50 μl of sample supernatant, 50 μl of standard and 50 μl distilled water respectively. Then, 500 μl each CHOL reagent solution was added the test tubes and mixed incubated for 10min at 25 ^{O}C. the absorbance was read at 500 nm after 60 mins.

HDL (mg/d1) = $\frac{change\ in\ Abs\ sample}{Chnage\ in\ Abs\ standard}$x conc. of standard

2.2.13.4 Determination of Serum Low Density Lipoproteins Concentration

LDL-Cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (pvs) in the presence of polyethyleneglycol monomethyl ether.

LDL= Cholesterol - VLDL - HDL

2.2.14 Determination of Antioxidant Concentrations/ Activities

2.2.14.1 Assay of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was assayed by the method of Arthur and Boyne (1985) as contained in the Randox Kit used. The method employed xanthine and xanthine oxidase to generate superoxide radicals which reacted with 2 (-4-iodophenyl) -3(-4nitrophenol), -5-phenltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activities were measured by the degree of inhibition of this reaction. One unit of SOD is that which caused a 50 % inhibition of the rate of reduction of INT under the conditions of the assay.

The requirements were the following:

R1a (Mixed substrate)

R1b (Buffer)

R2 (Xanthine Oxidase)

Standard

Procedure

Into three test tubes labeled test, serum, standard and blank were added 15 μl of serum, 15 μl of standard 15 μl distilled water respectively. Then, R1 (500 μl) was added and mixed well. Xanthine oxidase (75 μl) was added, mixed again and the initial absorbance taken after 30 sec. The final absorbance was taken after 3 minutes at wavelength 505 nm and units of SOD per gramme haemoglobin were extrapolated from a standard curve.

Superoxide dismutase (SOD) = $\frac{Abs\ of\ sample - abs\ of - blk}{slope}$

2.2.14.2 Assay of Catalase Activity

Principle

The activity of catalase was assayed by the method of Sinha (1972). Dichromate in acetic acid was reduced to chromic acetate, when heated in presence of hydrogen peroxide with the formation of per chromic acid as an unstable intermediate. The chromic acetate formed was measured at 570nm. Catalase was allowed to split and H_2O_2 was determined by measuring chromic acetate colormetrically. The requirements are the following:

Phosphate buffer, 0.01, pH_7

Hydrogen peroxide, 0.2 M

Potassium dichromate, 5 %

Dichromate acetic acid reagent: Potassium dichromate and glacial acetic acid were mixed in the ratio 1:3

Standard hydrogen peroxide 0.2 M

Procedure

The distilled water (0.9 ml) and 0.1ml of plasma in a test tube were added 2 ml of H_2O_2 and 2 ml phosphate buffer. The reaction was initiated by adding 2 ml of dichromate acetic acid reagent to 1 ml portion of this mixture. Absorbance of the reaction was taken in 30 seconds intervals for 2 mins. The activity of catalase was expressed as U/ml of plasma (U – micromoles of H_2O_2 utilized/second).

Catalase activity (U/mg) = 0.23 x log Abs 1

Abs 2

0.00693

Where Abs 1 is absorbance at t = 0 seconds

Abs 2 is absorbance at t =30 seconds

2.2.14.3 Determination of Glutathione Concentration

Principle

This was determined according to the method of King and Wootton (1959). Blood sample (0.1 ml) was mixed with (0.9 ml) of distilled water in a beaker; 0.02 ml of sodium sulphate was also added, shaken and allowed to stand for 2 min at room temperature. This was filled by the addition of 0.02 ml of lithium sulphate 20 %, 0.2 ml of 20 % Na_2CO_3 and 0.2ml of phosphor- 18-tungstic acid were added to the beaker, it was shaken and allowed to stand for 4min while observing for maximum colour development. Then, 2.5 ml of 20 % sodium sulphate was also added and the absorbance was read at 680 nm within 10 min. a blank, 0.1ml of H_2O was used as blank and glutathione concentration was calculated from a standard cysteine curve.

Glutathione concentration $= \frac{Abs\ of\ sample - abs\ of - blk}{Slope}$

2.2.15 Determination of Haematological Parameters

After two weeks of treatment with the extract, blood samples were obtained through ocular puncture of the rats for the determination of the blood parameters. Red blood cells (RBC) and platelets using the method of Dacie and Lewis (2000).

2.2.15.1 Determination of White Blood Cells Counts

The white blood cell (WBC) count was determined following the method described by Ochei and Kolatkar (2008). The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leucocyte. The blood specimen was diluted 1:20 in a WBC pipette with the diluting fluid and the cells were counted under low power, microscope by using a counting chamber. The number of cells in undiluted blood was reported as the number of white cells/cu.nm of whole blood.

Procedure

Whole blood (20 μ1) was added to 380μ1 of diluting fluid (acetic acid, with gentian violet) and mixed. The counting chamber was charged with the well mixed diluted blood (after discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 minutes. The four corners of the chamber were visualized under a low power (10X) objective microscope and the cells were counted in all the four marked corner squares.

Total WBC (mm^3) = $\frac{N}{0.1}$ x $\frac{20}{A}$

N = numbers of cells counted

0.1= depth of the chamber

A = area counted

20 = dilution factor

2.2.15.2 Determination of the Packed Cell Volume

Packed cell volume (PCV) was estimated as described by Ochei and Kolhatkar (2008). Blood sample was taken with a heparinized capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the micro-haematocrit centrifuge and spun at 10,000 rpm for 5 minutes. Spunned tubes were placed into a specially designed scale and the PCV was read as a percentage

$$\text{PVC\%} = \frac{Packed\ RBC\ column}{Total\ blood\ volume\ height}\ x\ 100$$

2.2.15.3 Determination of Haemoglobin Concentration

Haemoglobin (HB) concentration was determined using cynamethaglobin technique as outlined by Ochei and Kohatkar (2008).

Principle

Drabkin's solution which contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate was mixed with the haemoglobin. The ferricyanide forms methaemoglobin which is converted to cyanmethaemoglobin by the cyanide. The cynmethaemogloin produces a colour which is measured colorimetrically.

Procedure

Whole blood (20 μl) was added to 4 ml of Drabkin's solution in a test tube in a 1:25 dilution. This was well mixed, allowed to stand for 10 minutes at room temperature and the absorbance was read colorimetrically at 540 nm with Drabkin's solution as a blank.

$$\text{Haemoglobin (HB)} = \frac{reading\ of\ test}{reading\ of\ standard}\ \text{x}\ \frac{conc.standard}{4}$$

2.2.16 Histological Examination

The histological examination of the tissues of the kidney and liver of wistar albino rats was done using the method of Drury *et al.,* (1967).

2.2.16.1 Tissue Preparation

Sections of the pancreas were collected for histopathological examination at the end of the study period. The samples were fixed in 10 % phosphate buffered formalin for a minimum of 48 hours prior to tissue preparation. The tissues were subsequently trimmed, dehydrated in 4 ascending grades of alcohol (70 %, 80 %, 90 % and 100 %), cleared in 3 grades of xylene and embedded in molten wax. After embedding, the tissue-containing wax blocks were cut into 5µm thick sections with a rotary microtome, floated in water bathe at 60˚C, placed on clean grease-free glass slides and placed on slide warmer set at 60˚C overnight. The 5µm thick sectioned tissues on glass slides were subsequently cleared in 3 grades of xylene and rehydrated in 3 descending grades of alcohol (90 %, 80 % and 70 %). The sections were then stained with Mayer's hematoxylin for 5 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

2.2.16.2 Slide Examination

The prepared slides were examined with a Motic™ compound light microscope using x 4, x10 and x 40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x 400 magnifications.

2.2.17 Statistical Analysis

The data obtained were analyzed using Statistical Package of Social Sciences (SPSS) version 23.0 and the results expressed as mean $\pm$ standard error of mean. Significant differences of the result were established by one-way and two-way ANOVA and the acceptance level of significance was $p < 0.05$ for all the results.

CHAPTER THREE

RESULTS

3.1. Percentage Yield of Ethanol Extract of *Lepidagathis alopecuroides* Leaves

Table 1 shows the percentage yield of the ethanol extract of the sample.

The results showed that 634.91 g dried leaves samples of *Lepidagathis alopecuroides* after extraction with ethanol gave 47. 62 g.

3.2. Qualitative Phytochemical Analysis of Ethanol Extract of *Lepidagathis alopecuroides* Leaves

The phytochemical screening of the ethanol extract showed high presence of bioactive compounds such as alkaloids, terpenoids, flavonoids and saponins which were found to be abundantly present. Phenol and tannin were moderately present, while glycosides were slightly present in the leaf extract.

3.3 Quantitative Phytochemical Screening of Ethanol Extract of *Lepidagathis alopecuroides* Leaves

The result of the quantitative phytochemical is presented in Table 3. The result shows the concentration in mg/100g of the various bioactive compounds detected during the qualitative analysis with flavonoid, alkaloid terpenoid and saponin having the highest concentration followed by tannin and phenol, and glycoside which has the least concentration.

Table 1: Yield of ethanol extract of *Lepidagathis alopecuroides* leaves

Ground leaves (g)	Weight of extract (g)	% yield of extract
634.91	47.62	7.50

Table 2: Qualitative phytochemical composition of ethanol extract of *Lepidagathis alopecuroides* leaves.

Phytochemical constituents	Present/absent
Alkaloids	+++
Flaonoids	+++
Glycosides	+
Terpenoids	+++
Total Phenols	++
Tannins	++
Saponins	+++

Key: + slightly present, ++ moderately present, +++ highly present

Table 3: Quantitative phytochemical constituents of ethanol extracts of *Lepidagathis alopecuroides* leaves

Phytochemicals	Quantity Present (mg/100g)
Flavonoids	290.40±0.18
Terpenoids	250.60±0.12
Alkanoids	216.34±0.19
Tannins	180.00±0.20
Glycosides	3.071±0.53
Phenols	196.37±0.14
Saponins	215.00.±0.14

Results are expressed in Mean±STD.(n=3)

3.4 Acute Toxicity and Lethality (LD_{50}) of the N-hexane Leaf Fraction of *Lepidagathis alopecuroides*

Oral administration of the fraction of up to 5000 mg/kg caused no death in mice. Therefore, the oral LD_{50} of the n-hexane fraction in mice was > 5000 mg/kg. Although, there was weakness and decreased activity/movement following the administration of a high dose (5000 mg/kg) of the extract in the phase two (2).

Table 4: Outcome of phase I and II acute toxicity test of n-hexane fraction of *Lepidagathis alopecuroides* leaves.

phase	Groups of mice	Doses of the n-hexane fraction (mg/kg)	Mortality rate
Phase 1	1	10	0/3
	2	100	0/3
	3	1000	0/3
Phase 2	1	1600	0/3
	2	2900	0/3
	3	5000	0/3

n=3

3.5 Weights of Animal Before and After Administration of Treatments.

The effect of the fraction on body weight of diabetic rats changes in two weeks are shown in Figure 9. It was observed that in untreated diabetic rats, there were weight losses, relative to day 0, ie. before the start of the treatment. But after treatment, the treatment groups (group 4, 5 and 6) and standard control group (group 3), showed significant ($p < 0.05$) increases in weights of the animals when compared to the positive control (Untreated group 2). Also the positive control (untreated group), when compared to normal group (group 1), showed a significant decrease, this maybe as a result of the alteration effect of the alloxan on the group 2 animals which was not given any treatment. In the various experimental groups it was observed that there was an increase in the weights of the animals at week 0 when compared with the weight of the animals at week two of the experimental study, which may be as a result of feed intake of the experimental rats.

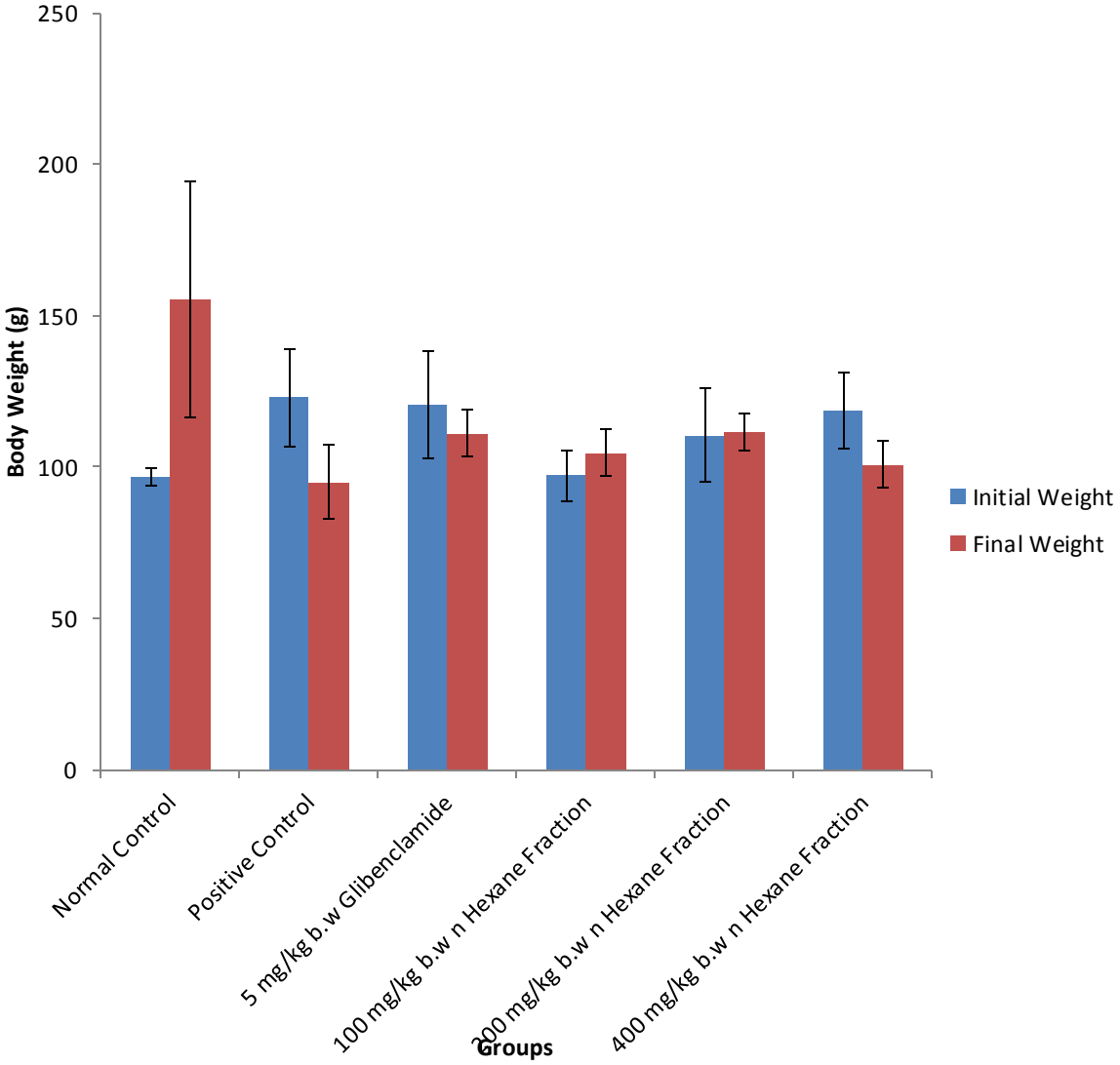

Figure 9: Weight of animals before and after administration of treatments.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan induced diabetic rats + 5mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.6 Effect of n-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Blood Glucose Level of Diabetic Rats

As shown in figure 10, there were significant ($p < 0.05$) increases in blood glucose levels of all diabetic rats on day 0 when compared to their initial glucose level. After post treatment with graded doses of *Lepidagathis alopecuroides* fractions (100, 200 and 400 mg/kg) on day 7, blood glucose level decreased when compared with the positive control significantly ($p < 0.05$) at tested doses of 100 and 400 mg/kg. The standard control similarly, showed a significant ($p < 0.05$) fall in blood glucose level. On day 14, *Lepidagathis alopecuroides* fractions significantly ($p < 0.05$) reduced the blood glucose level relative o the positive control. Pronounced reduction was observed at the tested dose of 200 mg/kg, almost similar to the effect of the reference drug. The decreases in the sugar level exerted by the plant sample were no significant ($p > 0.05$) when compared to the standard drug.

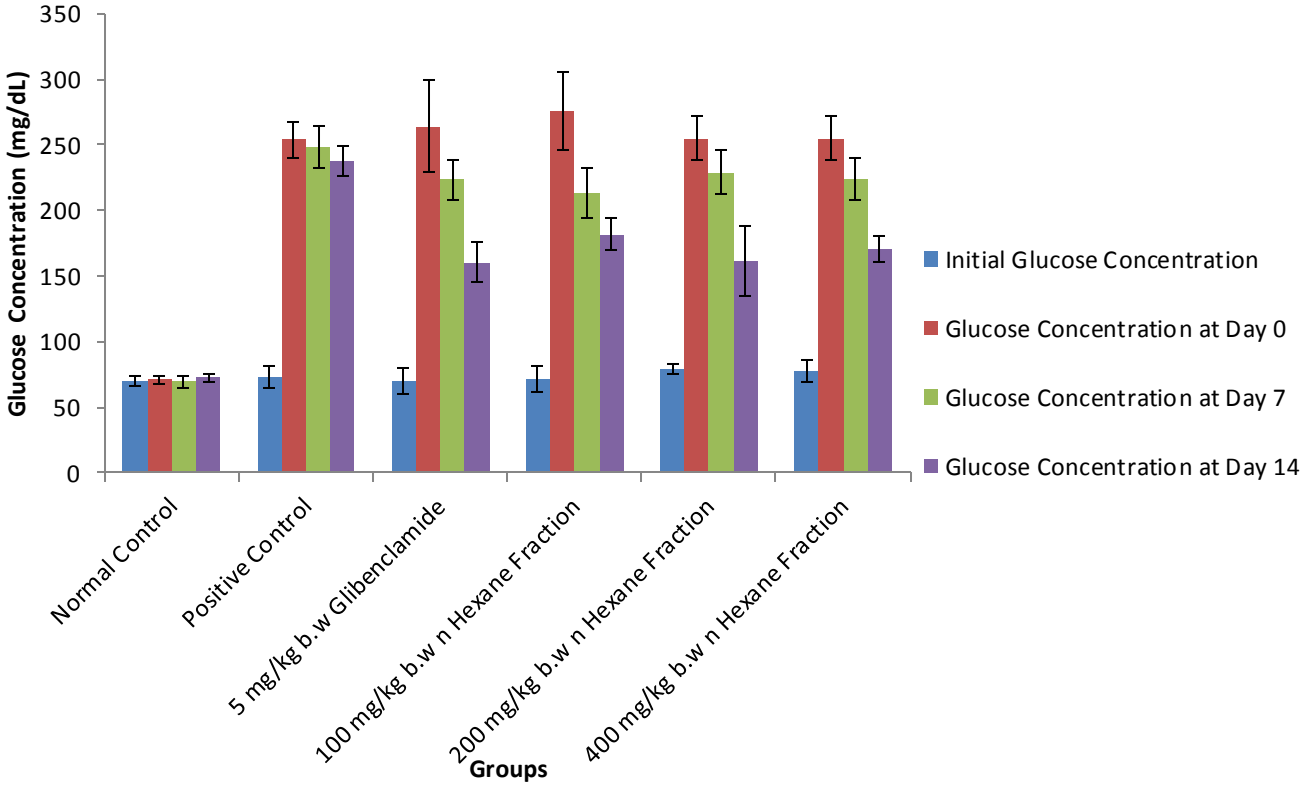

Figure 10: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on blood glucose levels of diabetic rats.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan induced diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.7: Effect of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Insulin Concentration of Diabetic Rats

Induction with alloxan significantly ($p < 0.05$) decrease insulin concentration in the untreated diabetic rats, when compared to normal control. (Figure11). Administration of leaf fraction of *Lepidagathis alopecuroides* produced significant increase in insulin concentration in the various treatment groups relative to the positive control. Similar effect was observed in the group treated with the standard drug.

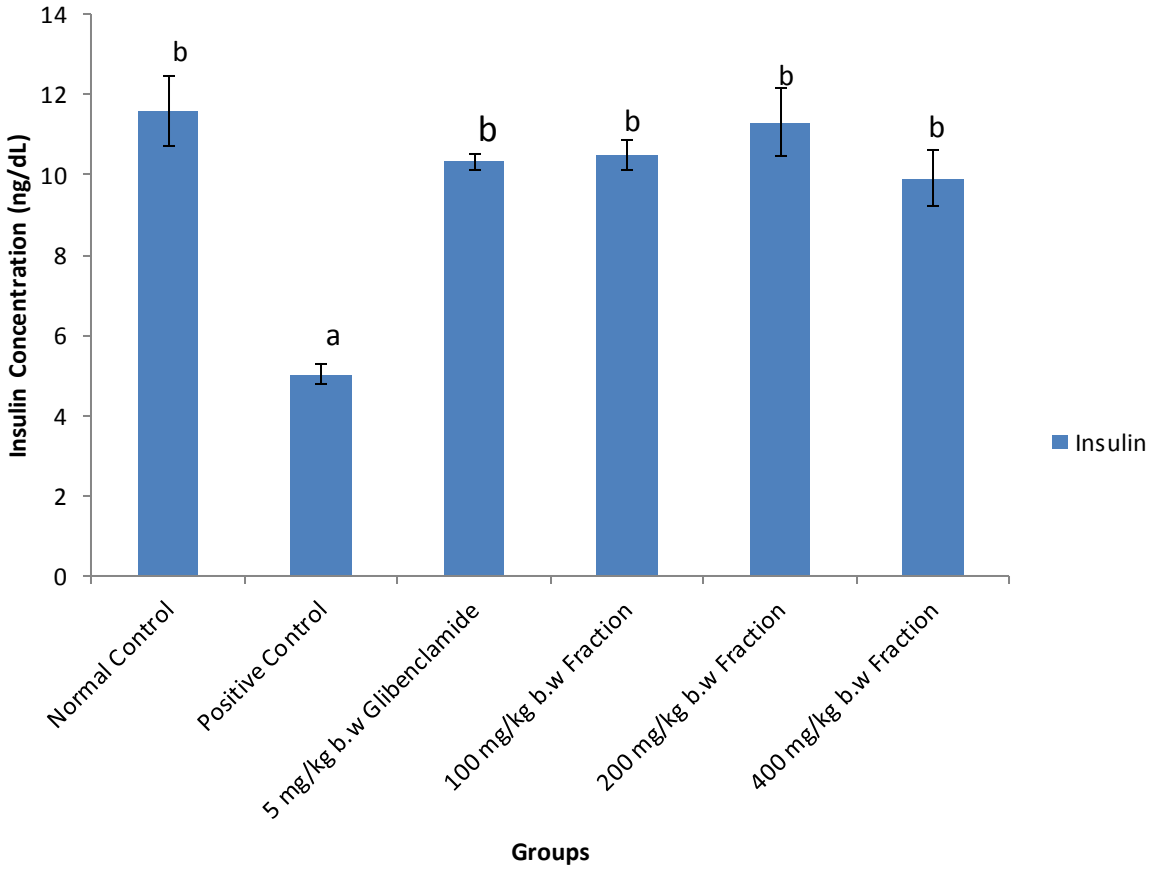

Figure 11: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on insulin levels of diabetic rats.

Results are presented as Mean±STD. Bars with different letters as superscripts are considered significant at $p < 0.05$.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan induced diabetic rats + 5mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100mg/kg body weight of n-hexane leaves fraction.

Group 5: Alloxan induced diabetic rats + 200mg/kg body weight n-hexane leaves fraction.

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.8 Effect of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Liver Function Enzymes of Diabetic Rats

As shown in Table 5, induction with alloxan produced significant ($p < 0.05$) increases in the concentration of ALT, AST and ALP in the untreated groups when compared to the normal control. Relative to the untreated groups, post treatment with graded doses of *Lepidagathis alopecuroide*s fraction as well as the standard drug (glibenclamide) caused decreases in the activities of these enzymes evident by the decrease in ALT, AST and ALP concentration. This effect was observed to be significant ($p < 0.05$) at tested doses of 200 and 400mg/kg. Similar significant ($p < 0.05$) effect was also observed in the standard control group.

Table 5: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on liver function enzymes of diabetic rats.

Groups	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Normal Control	17.50±1.29^{b}	30.00±3.27^{a}	19.00±0.82^{a}
Positive control	26.25±3.30^{c}	46.25±1.71^{c}	26.00±0.82^{d}
5 mg/kg b.w Glibenclamide	11.25±2.87^{a}	35.25±3.77^{b}	20.25±3.69^{ab}
100 mg/kg b.w Fraction	22.25±0.96^{bc}	42.75±2.87^{c}	24.25±0.96^{cd}
200 mg/kg b.w Fraction	18.00±7.26^{b}	37.25±5.74^{b}	20.75±1.89^{ab}
400 mg/kg b.w Fraction	19.25±0.96^{b}	37.50±1.91^{b}	22.00±0.82^{bc}

n=4

Results are presented as Mean±STD. Mean values on the vertical or horizontal axis with same superscripts are not significantly different at ($p > 0.05$).

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan inducxed diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.9 Effect of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Kidney Function Markers

Increases in urea and creatinine concentration were observed in the untreated diabetic groups relative to the normal control. Post treatment with *Lepidagathis alopecuroides* leaf fraction caused a decrease in the concentration of urea and creatinine when compared to the untreated diabetic groups. These decreases were only observed to be significant ($p < 0.05$) at tested mid dose of 200 mg/kg.

Table 6: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on renal function parameters of diabetic rats.

Groups	UREA (mg/dL)	CREATININE (mg/dL)
Normal Control	57.75±4.03[a]	2.04±0.09[ab]
Positive control	72.00±4.97[b]	2.16±0.01[c]
5 mg/kg b.w Glibenclamide	60. 00±5.89[a]	2.03±0.10[a]
100 mg/kg b.w Fraction	69.50±3.32[b]	2.15±0.03[bc]
200 mg/kg b.w Fraction	61.50±2.08[a]	2.08±0.07[abc]
400 mg/kg b.w Fraction	69.25±4.50[b]	2.13±0.04[abc]

n=4

Results are presented as Mean±STD. Mean values on the same row or column with same letters as superscripts are not significantly different at $p > 0.05$.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan inducxed diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.10 Effect of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Lipid Peroxidation Marker: Malondialdehyde

The effect of *Lepidagathis alopecuroides* leaf fraction on malondialdehyde (MDA) showed a significant ($p < 0.05$) increase in the positive control when compared to normal control. Standard control (Group 3), and the treatment groups that received 200 and 400 mg/kg of leaf fraction, showed significant ($p < 0.05$) decreased MDA concentration when compared to positive control (diabetic untreated groups). Similarly the significant ($p > 0.05$) reduction observed in the the group that received 200mg/kg of leaf fraction was comparable to that of the normal control.

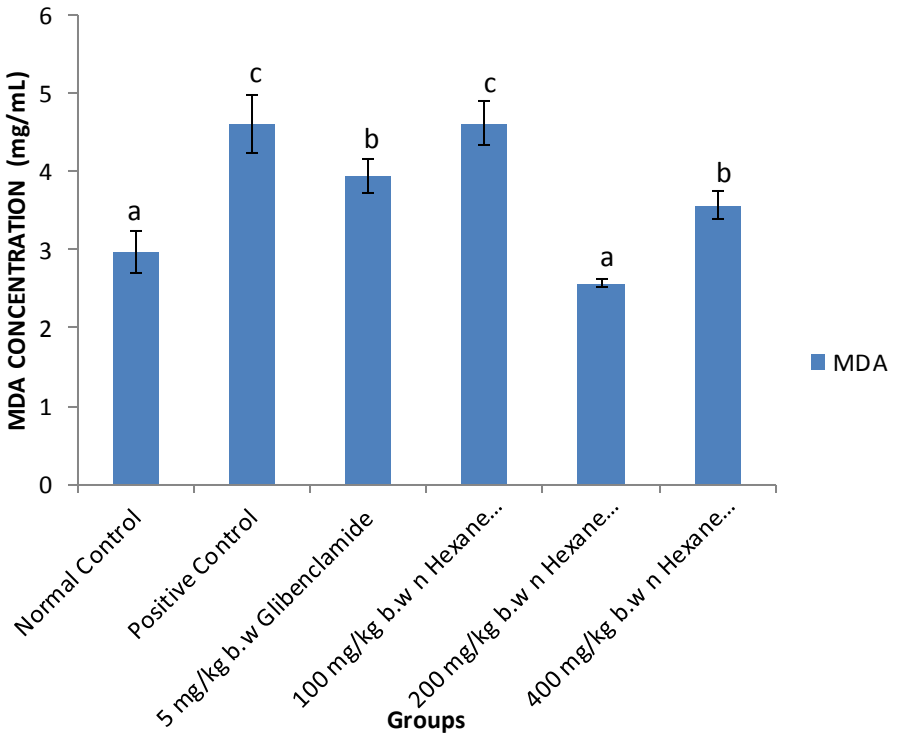

Figure 12: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on lipid peroxidation marker; malondialdehyde

Results are presented as Mean±STD. Mean values with different letters as superscripts across column are considered significant at $p < 0.05$.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan inducxed diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.11 Effect of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Antioxidant Activities in Diabetic Rats

There was a significant ($p < 0.05$) reduction in antioxidant activities of CAT, SOD and GSH in the untreated groups when compared to normal control. There was also an increase in the antioxidant enzyme activities in the diabetic groups post treated with graded doses of *Lepidagathis alopecuroides* leaf fraction when compared to the positive control, although not significant ($p > 0.05$) when compared to standard control. However, a significant ($p < 0.05$) increase in GSH was observed at a dose of 200 and 400 mg/kg.

Table 7: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on antioxidant activities in diabetic rats.

Groups	CAT (U/mg)	SOD (U/mg)	GSH (mg/dL)
Normal Control	4.77±0.34^{c}	11.40±0.09^{ab}	5.73±0.24^{c}
Positive Control	2.44±0.20^{a}	11.29±0.09^{a}	3.57±0.07^{a}
5 mg/kg b.w Glibenclamide	3.58±0.16^{b}	11.44±0.04^{b}	5.72±0.32^{c}
100 mg/kg b.w Fraction	2.91±0.08^{ab}	11.31±0.14^{ab}	3.70±0.28^{a}
200 mg/kg b.w Fraction	3.18±1.05^{ab}	11.36±0.07^{ab}	4.73±0.51^{b}
400 mg/kg b.w Fraction	3.03±0.38^{ab}	11.37±0.07^{ab}	5.15±0.34^{b}

n=4

Results are presented as Mean±STD. Mean values with different letters as superscripts across column are considered significant at $p < 0.05$.while values with same superscript at ($p>0.05$) are not significantly different.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan inducxed diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.12 Effects of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Lipid Profile.

The animal in the diabetic untreated groups showed significant ($p < 0.05$) increases in LDL, TAG and Total Cholesterol, with a significant ($p < 0.05$) decrease in HDL concentration when compared to the normal control. The reverse is the case in the test groups that received varying dose of *Lepidagathis alopecuroides* leaf fraction when compared to diabetic untreated rats, decreases in LDL, TAG and T-Cholesterol and an increase in HDL concentration. Similar effect was observed in the group post treated with the reference drug.

Table 8: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on lipid profile of diabetic rats.

Groups	LDL (mmol/l)	TAG (mmol/l)	HDL (mmol/l)	T-CHOL (mmol/l)
Normal Control	1.38±0.11^{a}	1.08±0.02^{a}	2.27±0.13^{d}	4.40±0.20^{a}
Positive Control	2.27±0.13^{d}	1.49±0.36^{c}	1.38±0.11^{a}	4.90±0.24^{b}
5 mg/kg b.w Glibenclamide	2.09±0.08^{cd}	1.20±0.00^{ab}	2.24±0.19^{cd}	4.40±0.16^{a}
100 mg/kg b.w Fraction	2.24±0.19^{cd}	1.39±0.17^{bc}	2.05±0.09^{c}	4.85±0.25^{b}
200 mg/kg b.w Fraction	2.05±0.09^{c}	1.15±0.07^{ab}	2.09±0.08^{cd}	4.35±0.39^{a}
400 mg/kg b.w Fraction	1.60±0.13^{b}	1.23±0.14^{ab}	1.60±0.13^{b}	4.85±0.13^{b}

n=4

Results are presented as Mean±STD. Mean values with different letters as superscripts across column are considered significant at $p < 0.05$. while values with same superscript at ($p>0.05$) are not significantly different.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan inducxed diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.13 Effect of N-hexane Leaf Fraction of *Lepidagathis alopecuroides* on Haematological Indices of Diabetic Rats

Results on Haematological parameters shows a significance ($p < 0.05$) decrease in the untreated groups across the heamatological parameters (WBC, RBC, HB, and PCV). The result of WBC shows significant ($p < 0.05$) decrease in positive control when compared to the normal control. Standard control (Group 3) and the treatment groups that received 100 and 400 mg/kg showed a significant ($p < 0.05$) increase in WBC concentration when compared to positive control. The reference drug was also comparable to the normal control. The result of RBC shows a significant ($p < 0.05$) decrease in the diabetic untreated group when compared to normal control. However, both standard control and the group that received graded dose of 100 mg/kg b.w leaf fraction significantly ($p > 0.05$) increased in RBC count when compared to positive control. More so, the increases observed in groups that received 200 and 400 mg/kg b.w were not significant ($p>0.05$) when compared to positive control. Result of haemoglobin shows a significant ($p < 0.05$) decrease in diabetic untreated group when compared to normal control significant ($p < 0.05$) elevation was observed in both standard control and in the groups that were given graded dose of 100 and 400 mg/kg when compared to positive control. In the same vein these increased observed was comparable to the normal control. The result of PCV on the other hand shows a significant ($p < 0.05$) decrease in positive control when compared to normal control.. There is no significant ($p > 0.05$) difference between group 2 and 4. Group 3, 5 and 6 also show no significant ($p > 0.05$) difference when compared to positive control. Similarly, comparing group 3 to normal control shows no significant ($p > 0.05$) difference.

Table 9: Effect of n-hexane leaf fraction of *Lepidagathis alopecuroides* on haematological indices of diabetic rats

Groups	WBC ($X10^9$/L)	RBC ($X10^{12}$/L)	Hb (g/dL)	PCV (%)
Normal Control	76.00±3.27^{c}	4.50±0.08^{bc}	10.24±0.74^{b}	40.25±1.89^{c}
Positive Control	62.75±1.89^{a}	4.20±0.22^{a}	8.15±0.46^{a}	33.25±2.75^{a}
5 mg/kg b.w Glibenclamide	73.25±0.96^{bc}	4.55±0.10^{c}	9.81±1.39^{b}	38.75±2.87^{bc}
100 mg/kg b.w Fraction	70.00±4.32^{b}	4.50±0.14^{bc}	9.75±0.48^{b}	33.25±2.75^{a}
200 mg/kg b.w Fraction	64.00±1.63^{a}	4.25±0.25^{ab}	9.05±0.40^{ab}	36.50±3.42^{ab}
400 mg/kg b.w Fraction	70.00±3.27^{b}	4.40±0.16^{ab}	10.05±0.54^{b}	34.00±4.24^{ab}

n=4

Results are presented as Mean±STD. Mean values with different letters as superscripts across column are considered significant at $p < 0.05$. while values with same superscript at ($p>0.05$) are not significantly different.

Group 1: Normal control (Normal rats)

Group 2: Positive control (Diabetic not treated rats)

Group 3: Alloxan inducxed diabetic rats + 5 mg/kg body weight of Gilbenclamide.

Group 4: Alloxan induced diabetic rats + 100 mg/kg body weight of n-hexane leaves fraction

Group 5: Alloxan induced diabetic rats + 200 mg/kg body weight n-hexane leaves fraction

Group 6: Alloxan induced diabetic rats + 400 mg/kg body weight of ethanol leaves extract.

3.14 Histological Examination of the Pancreas of Rat

3.14.1 Histological Examination of the Pancreas of Rat in Group 1 (normal control) not Induced, not Treated.

Sections of the pancreas presented in plate 1 showed the normal histomorphology of the endocrine pancreas. Normal pancreatic islets (black arrow) were observed, randomly embedded in-between the acinar cells of the exocrine pancreas. H&E x 400.

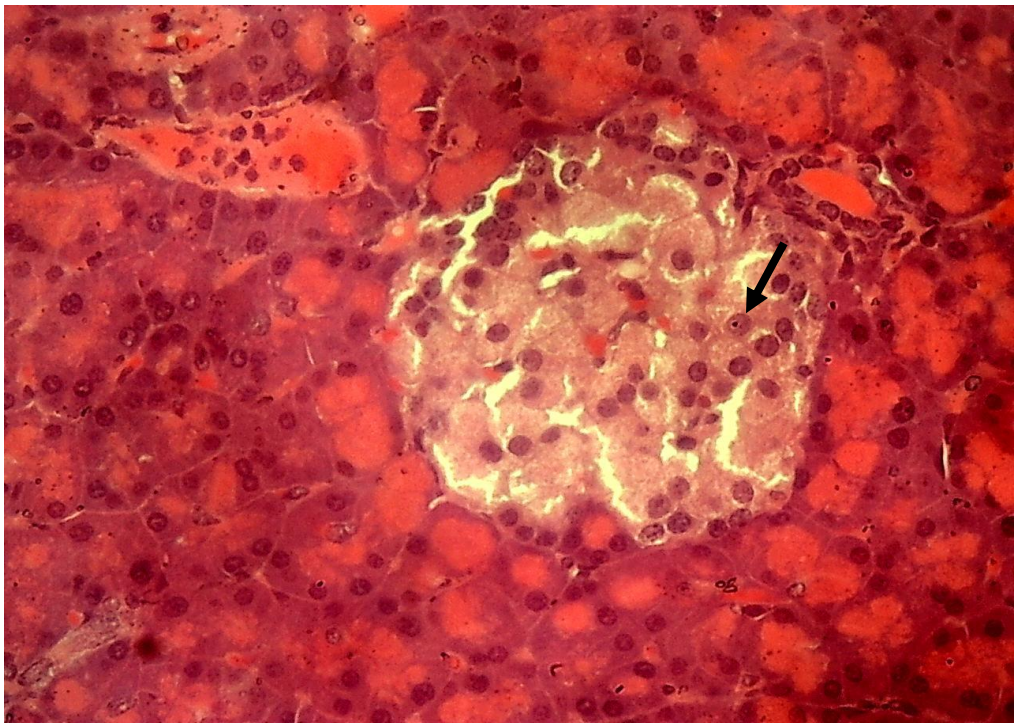

Plate 1: Photomicrograph of normal histomorphology of the endocrine pancreas, of group 1 (normal control) showing normal pancreatic islets.

3.14.2 Histological Examination of the Pancreas of Rat in Group 2 (positive control) Induced with 140 mg/kg b.w Alloxan but not Treated.

Sections of the pancreas presented in plate 2 showed a marked decrease in the number and sizes of the pancreatic islets. The islet cells showed marked vacuolar degeneration and necrosis. The degenerate cells were markedly swollen with foamy cytoplasm (white arrow), admixed with eosinophilc necrotic debris (black arrow). H&E x 400.

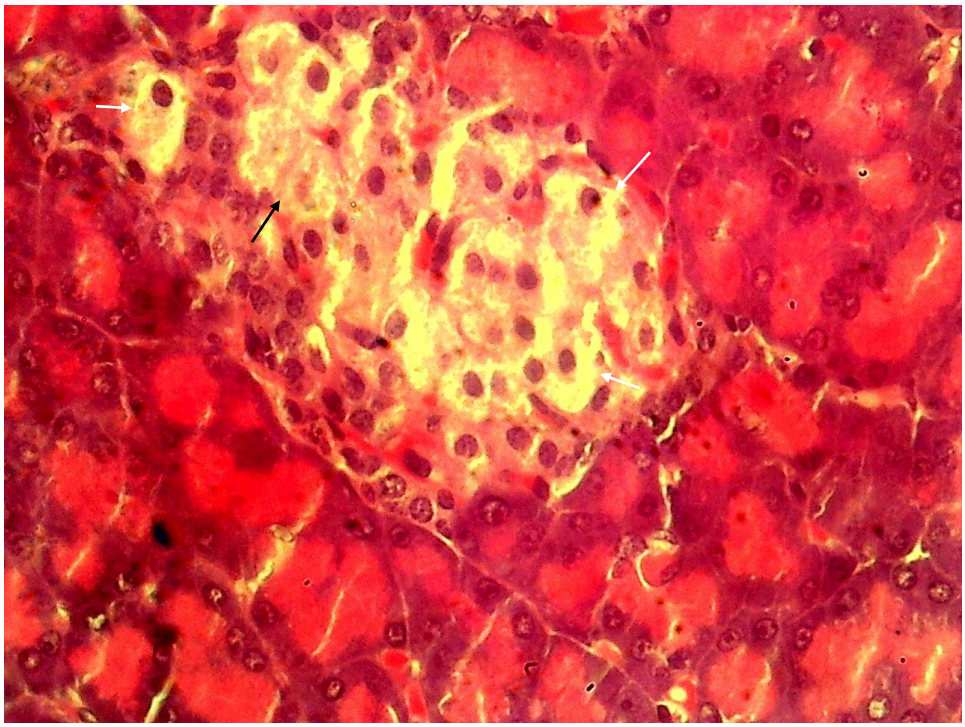

Plate 2: Photomicrograph of group 2 (positive control) showing a marked decrease in the number and sizes of the pancreatic islets, with marked vacuolar degeneration and necrosis of normal histomorphology of the endocrine pancreas, admixed with eosinophilc necrotic debris.

3.14.3 Histological Examination of the Pancreas of Rats in Group 3 (standard control) Induced and Treated with 5 mg/kg b.w Glibenclamide

Sections of the pancreas presented in plate 3 showed a reduction in the number and size of the pancreatic islets (black arrow). The islets that were present were mostly small and inconspicuous. H&E x 400.

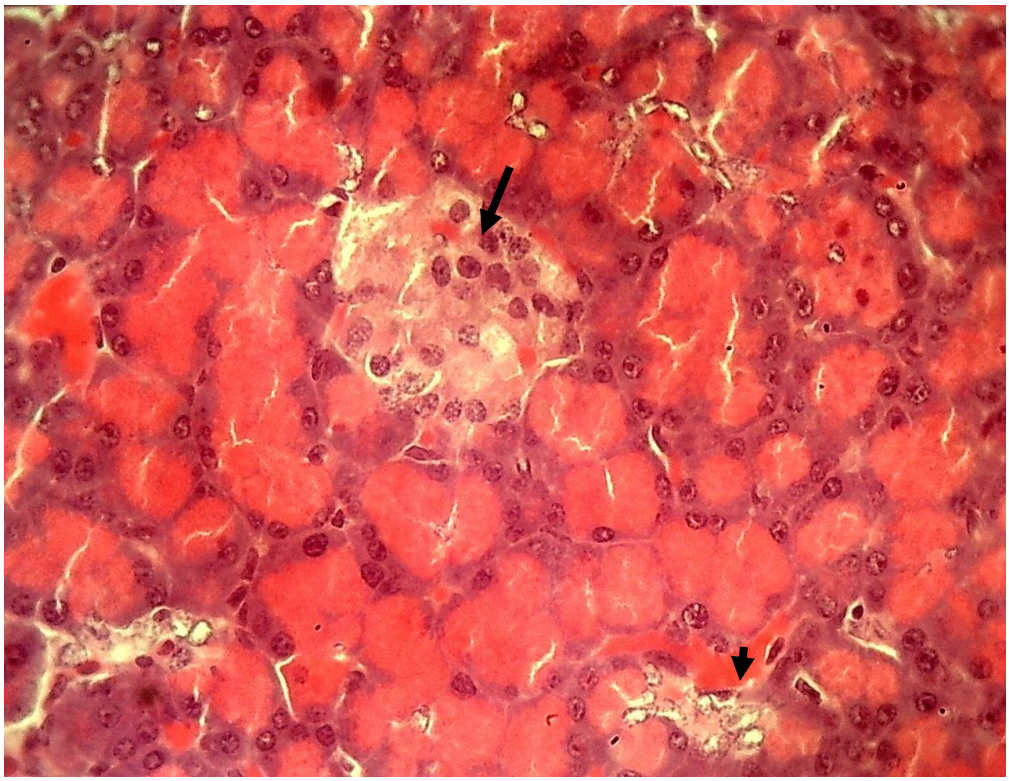

Plate 3: Photomicrograph of group 3 (standard control) showing a reduction in the number and sizes of the pancreatic islets.

3.14.4 Histological Examination of the Pancreas of Rats in Group 4 Induced and Treated with 100 mg/kg b.w N-hexane Fraction

Sections of the pancreas presented in plate 4 showed a reduction in the number and size of the pancreatic islets (black arrow). The islets that were present were mostly small and inconspicuous. Some of the islets are also necrotic with fibrosis (white arrow). H&E x 400.

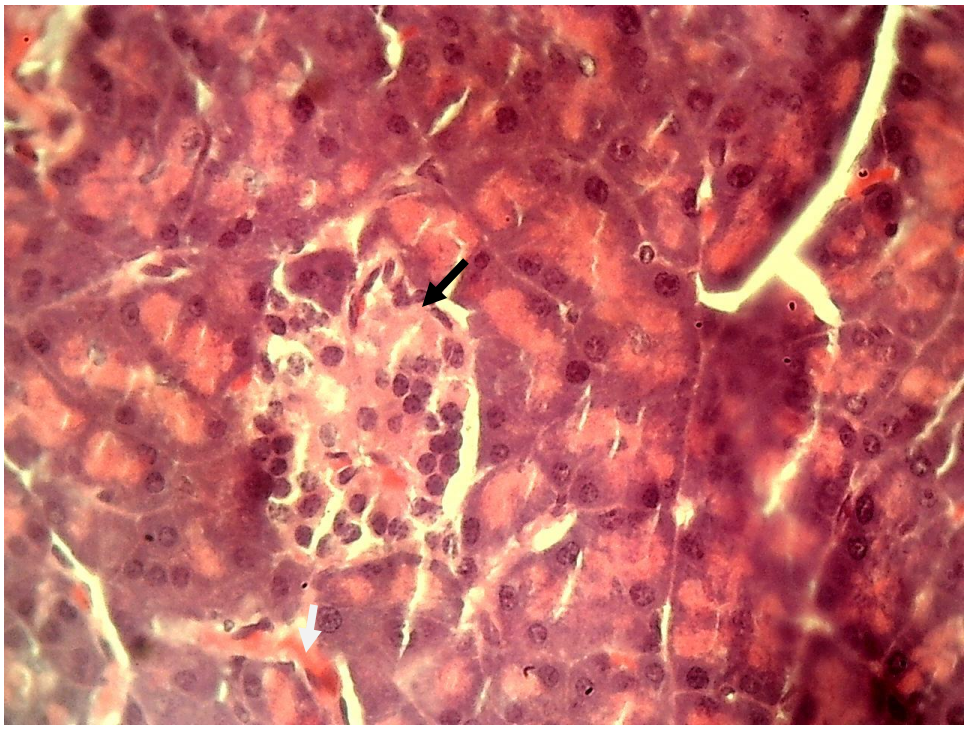

Plate 4: Photomicrograph of group 4 (100mg/kg fraction) showing a reduction in the number and sizes of the pancreatic islets. Some of the islets are also necrotic with fibrosis.

3.14.5 Histological Examination of the Pancreas of Rats in Group 5 Induced and Treated with 200 mg/kg b.w N-hexane Fraction

Sections of the pancreas presented in plate 5 showed a marked proliferation of the α-cells (black arrow) in the pancreatic islets. The α-cells are small cells with scant cytoplasm and heterochromatic nuclei, located at the borders of the pancreatic islet. The slide shows a less abundant β-cell located at the centre of the pancreatic islets (white arrow). H&E x 400.

Note: Recent studies have associated proliferation of the α-cells and other cells of the islet with β-cell regeneration via process of trans-differentiation (Sangan and Tosh, 2010; Courney *et al.*, 2011).

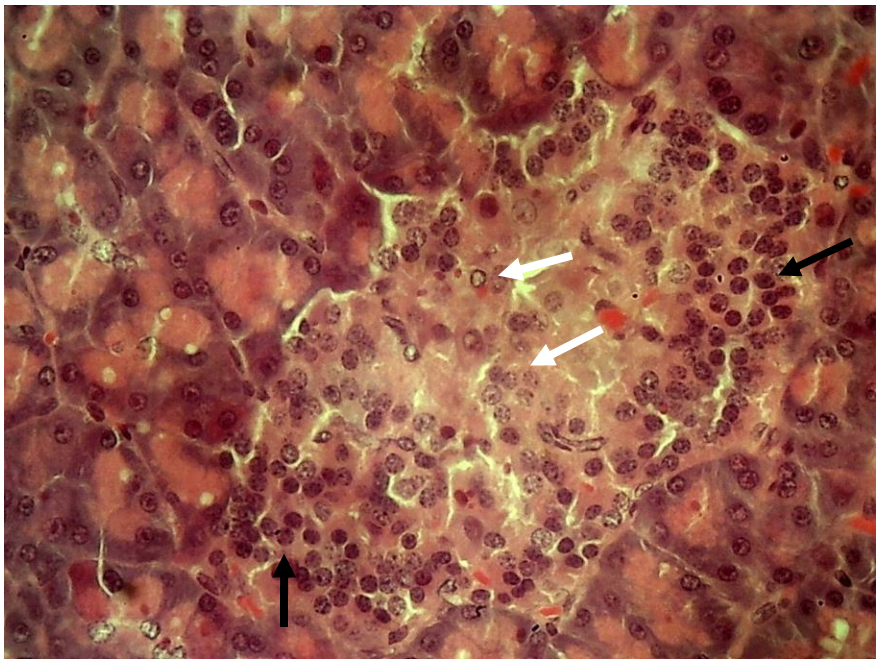

Plate 5: Photomicrograph of the pancreas of group 5 (200 mg/kg fraction) rat showing a marked proliferation of the α-cells in the pancreatic islets with less abundant β-cell located at the centre of the pancreatic islets.

3.14.6 Histological Examination of the Pancreas of Rats in Group 6 Induced and Treated with 400 mg/kg b.w N-hexane Fraction

Sections of the pancreas presented in plate 6 showed normal histomorphology of the endocrine pancreas. Normal sized pancreatic islet cells with seemingly proliferation of the α-cells were observed (black arrow). However, multifocal areas of necrosis of the acinar cells of the pancreas were observed (white arrow). H&E x 400.

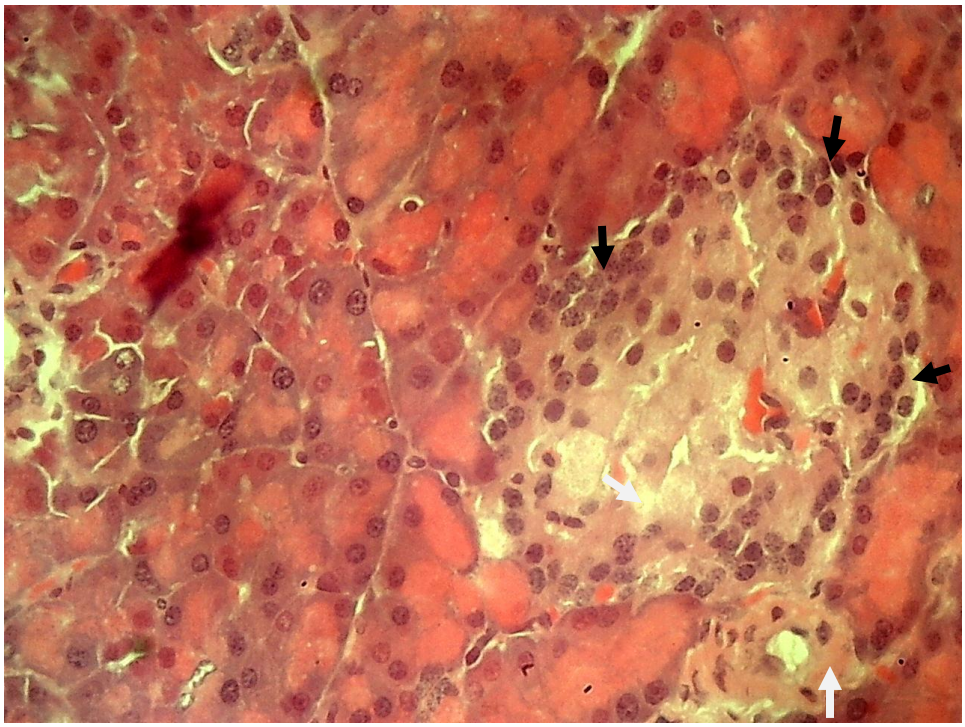

Plate 6: Photomicrograph of the pancreas of group 6 (400 mg/kg fraction) rats showing Normal sized pancreatic islet cells with seemingly proliferation of the α-cells. However, multifocal areas of necrosis of the acinar cells of the pancreas were observed.

CHAPTER FOUR

4.1. DISCUSSION

The need for bio-prospecting and development of ethnomedicinal plants as hypoglycemic agents is imperative especially now that most diabetic patients in Nigeria find it increasingly difficult to manage hyperglycemic conditions due to high cost of synthetic antidiabetic drugs with their attendant side effects. There are numerous traditional plants reported to have anti-hyperglycemic properties. However, many of these are less effective in lowering blood glucose levels in severe diabetes thus the need for this study.

The phytochemical screening of the ethanol extract showed that the presence of bioactive compounds such as alkaloids, terpenoids, flavonoids and saponins were found to be abundantly present. Phenol and tannin were moderately present, while glycosides were slightly present in the leaf extract. Quantitatively, the ethanol extract constitutes respectively in (mg/100g) showed flavonoids (290.40±0.18), terpenoids (250.60±0.12), alkanloids (116.34±0.19), tannins (180.00±0.20), glycosides (3.71±0.53), Phenols (196.37±0.14), saponins (215.00±0.14).

The presence of these biologically active compounds suggests that these plants could exert some biological activities when taken by animals (Okafor, 1983). Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms (Trease and Evans, 1989). Many studies have implicated that alkaloids and flavonoids such as (quercetin, kaempferol and caffeoyl glucoside) have all been demonstrated to manage hyperglycemia in animal models (Shimzu *et al.,* 2001; Ezekwe *et al.,* 2014).

Flavonoids have aroused considerable interest, recently because of their potential benefits on human health; they are potential anti-diabetic agents because they exert multiple actions that are both hypoglycemic (insulinomimetic action) and anti-hyperglycemic (insulin secretagogue) (Cazarolli *et al.,* 2008). Flavonoids have also been reported to have anti-viral, anti- platelet, anti-tumor and anti-inflammatory effects (Middleton and Kandaswami, 1992). Their anti-inflammatory activities are due to inhibitory effect involved in the production of chemical mediators of inflammation and arachidonic acid breakdown (Oweyele *et al.,* 2005). Generally flavonoids were found to regenerate the damaged beta cells of pancreas (Tiwari and Rao, 2002).

`Saponins have been shown to possess beneficial properties by lowering cholesterol level, anti-diabetic and anti-carcinogenic properties (Trease and Evans, 1989). Saponins also have hypotensive and cardiac depressant properties (Olaleye, 2007). Alkaloids are the active components of many anesthetics, sedatives, stimulants, relaxants, and tranquilizers. The primary use of alkaloids, however, is in medicine, because they can act quickly on specific areas of the nervous system (Almeri, 1998).

The acute lethality (LD_{50}) of the extract indicated a very high safety profile with no morality recorded by the test mice up to a dose of 5000 mg/kg body weight.

The body weight loss represents one of the most common signs of diabetes. Despite the increased appetite, insulin deficiency reduces all anabolic processes and accelerates catabolic processes, contributing further to body weight loss, as seen in the result of group 2 (positive control) which is already occurring by glycosuria and polyuria (ADA, 2014). There was a significant ($p < 0.05$) increase in body weight of both standard control and in group 4, 5 and 6 that received 100, 200 and 400 mg/kg graded doses of leaf fraction respectively when compared to positive control which may be as a result of the effect of the standard drug (glibenclamide) and the leaf fraction. This is in line with the report of Yisa *et al.* (2010), but contradicted the findings that there was significant decrease at high dose as observed in 500 mg/kg body weight, which reveals a wasting effect of the extract which is likely to be due to the presence of saponin in the plant as reported by Yisa *et al.* (2010).

The result in this study showed that the n-hexane leaf fraction of *Lepidagathis alopecuroides* significantly reduced the hyperglycemia in alloxan-induced diabetic rats. This could be attributed to the anti-hyperglycemic properties of flavonoids, which act by improving the body's sensitivity to insulin (Lean *et al.*, 1999). According to Kambouche *et al.* (2009), the Saponin possesses an antihyperglycemic effect. Thus, the antihyperglycemic effect of the leaf fraction of *Lepidagathis alopecuroides* in rats is likely due to the individual or synergistic activity of flavonoids , saponin and other active components of the plant therefore, it can be adduced that the presence of such phytochemicals in the *L. alopecuroides* leaf fraction might account for its hypoglycemic activity. (N'Diaye *et al.*, 2008).

The cells of the liver, which are known as hepatocytes carry out many biochemical activities. Some of the uses include excretion of bile, carbohydrate metabolism, protein metabolism, synthesis of blood clotting factors, storage of iron and some vitamins, detoxification and lipid metabolism. It is therefore, very obvious that any disease condition and adverse physiological conditions, which affect the hepatocytes will cause concerted and tremendous metabolic derangement. It is therefore very pertinent to ascertain the effect of any ingestible food or drug on the serum activity of the liver enzymes so as ensure the hepatoprotectiveness of such food and drug. This can be achieved through liver function tests which include assay of aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) (Ochei and Kolhatkar, 2008). The ALT, AST and ALP increased in activities in the alloxan intoxicated rats (positive control). The increased levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver (Drotman and Lawhorn, 1978). In this study, administration of different doses of n-hexane leaf fraction of *Lepidagathis alopecuroides* to respective groups of diabetic rats suppressed the elevated serum levels of these enzyme towards the respective normal control. This clearly indicates that on continued treatment, the plant fraction may possibly stabilize the plasma membrane as well as help in healing of the hepatic tissue damage as reported by Effrong and Akpan, (2015). Reduction in serum liver markers enzymes activities recorded in treatment groups is suggestive of cellular membrane/ hepatocellular membrane protective effects of the fraction. The ALP functions as a biochemical marker enzyme for maintaining membrane integrity.Increase in its plasma activity indicates lipid peroxidation of cell membrane which occurs during diabetes mellitus (Akanji *et al.,* 1993). The hepatocellular protection evidence in the present study might be due to the presence of flavonoids in the plant extract. Flavonoids have been reported to possess antioxidant activity (Middleton, 1996) and thus, are capable of protecting cell membranes from peroxidative actions of free radicals. According to Steffen and Menzel (2009), significant increase in the serum activities of liver enzymes is an indication of hepatic injury, since there was a significant reduction in the serum concentration of these enzymes when compared with the untreated group showed that *L. alopecuroides* is safe to be used as antidiabetic drug.

Kidneys are the major excretory organs and renal function tests are devised to detect possible renal damage. Increased serum levels of urea and creatinine are among the most sensitive indicator of kidney injury. Hyperglycemia is known to induce these elevations. Alarcon *et al.,*

(2002) reported that alloxan-induced hyperglycemia resulted in increased levels of serum urea and creatinine. The high serum urea in diabetic control rats could be attributed to the stimulation of gluconeogenesis as alternative glucose source as a result of insulin deficiency (Abdulazeez *et al.*, 2013). In this study, urea and creatinine levels were significantly ($p < 0.05$) higher in diabetic control rats indicating renal impairment. However, administration of *Lepidagathis alopecuroides* n-hexane leaf fraction for 14 days significantly lowered these indices. The stabilization of these parameters indicates improvement in renal function which could be attributed to the anti-hyperglycemc effect of the fraction and thus increased insulin effect causing a decline in proteolysis. Similar observation has been reported (Shah, and Khan, 2014).

Various studies have shown that diabetes is associated with increased formation of free radicals and suppression of antioxidant enzyme system. Our data shows decreases in SOD and CAT activities in serum and an increase in MDA concentration in the untreated rats when compared to the normal group. The administrations of *Lepidagathis alopecuroides* leaf fraction to respective groups improved endogenous antioxidant system, and suppressed the generation of free radicals evident by the decreases in MDA concentration in the treated groups. The antioxidant effect of *Lepidagathis alopecuroides* fraction could be explained by two mechanisms. Firstly, the *Lepidagathis alopecuroides* leaf fraction possibly prevented protein glycosylation and peroxidation by scavenging free radicals, thereby inhibiting their damaging effects. In addition, *Lepidagathis allopecuroides* leaf fraction possibly induced protein synthesis of the antioxidant enzymes. Based on previous studies, it has been reported that polyphenolic compounds increased enzyme expression of SOD and GPx in transcriptional level (Vina *et al.*, 2006 and Ben *et al.*, 2017). Furthermore, GSH is a major non-protein in living organisms which play a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences (Uday *et al.*, 1999). Decline in GSH content in the serum of diabetic induced rats, and its subsequent return towards near normal in leaf fraction treated groups reveals the antioxidant effect of *Lepidagathis alopecuroides* leaves. The possible mechanism underlying the antioxidant properties of most drugs include the prevention of GSH depletion and destruction of free radicals (Valenzuela *et al.*, 1985). These two factors may possibly be attributed to the antioxidant properties of *Lepidagathis alopecuroides*. This is in line with the report by Shajeela *et al.*, (2012).

The total serum lipids (cholesterol, LDL-chol and TAG) levels in treated animals were statistically lower when compared with the positive control and the increase in HDL-Chol concentrations in the treated rats could account for the use for the plant in traditional medicine for the treatment of diabetes and hypertension. The results of this study clearly indicate that the administration of n-hexane leaf fraction of *Lepidagathis alopecuroides* produces hypolipidaemic effects and may prevent cardiovascular diseases. Studies have shown that increase in the risk factor of cardiovascular disease correlate with increase in serum TC, LDL-chol, atherogenic index level and a decrease in HDL-Chol concentrations. A similar decrease in serum total lipids was also reported by Mattar and Helal, (2001); Longe *et al.,* (2005). The level of serum triacylglyceride in the standard control (group 3) and in groups that received 200 and 400 mg/kg leaf fraction were not significantly different from normal control. The decrease in lipid profile, therefore, may be due to a comparable decrease in free fatty acids (Harper *et al.,* 1993). Such a decrease is expected in the case of accumulation of fat in adipocytes, both by synthesis and decreased liberation of fatty acids. It may also be due to a number of other mechanisms including the inhibition of rate limiting enzyme of cholesterol biosynthesis, HMG COA reductase into bile acids and inhibition of cholesterol absorption from the intestine due to formation of complexes with compounds such as glycosides and saponins (Gamoussi *et al.,* 2010).

Alteration in the various haematological parameters and the immune system during the course of diabetes has been reported (Mansi and Lehham, 2008). Also toxicological studies revealed that ingestion of medicinal plants or drugs can alter the normal haematological values (Ajabonna, *et al.,* 1999). Therefore, haematological parameters could be an important tool in the assessment of deleterious effect of drugs as well as medicinal plants and their extracts (Yakubu, *et al.,* 2007). The RBC count in the fraction treated group 4 was significantly ($p < 0.05$) higher than the positive control group. Similar report of slight increase in RBC and its indices in *N. laevis* leaf-supplemented normal rats has been made and suggested that the increase may be due to stimulatory effect of the extract on the production of haematopoietic regulatory elements such as erythropoietin and colony-stimulating factors by the stromal cells and macrophages in the bone marrow. Phytoconstituents such as flavonoids present in the leaf of *N. laevis* may be responsible for these effects (Usman and Osuji, 2007). In the present study, some haematological parameters were analysed and the result showed that white blood cell (WBC), packed cell volume (PCV), and haemoglobin (Hb) concentration values were decreased in diabetic rats and treatment with n-

hexane leaf fraction of *L.alopecuroides* increased the WBC, PCV, and Hb concentration values in diabetic rats. This is in line with previous work where occurrence of anaemia in diabetes has been documented (Merlin *et al.,* 2005).

Insulin is produced by the beta cells of the pancreas; it is the principal hormone regulating glucose metabolism. Its presence enhances glucose uptake and metabolism by various tissue cells including skeletal muscle, white adipose tissue and the liver (Barros, *et al.,* 2006). During non-pathological states, glucose is the stimulus for insulin secretion in pancreatic beta cells. Glucose enters into the cells through GLUT-2 transporters (Herman and Kahn, 2006). Oxidation of glucose and the subsequent increase in ATP/ADP ration in the cells triggers closure of ATP-sensitive potassium channels (Doyle and Egan, 2003). Inhibition of potassium efflux results in cell depolarization leading to influx of voltage dependent calcium ions that stimulate extrusion of insulin.

The result on insulin level showed a significant ($p < 0.05$) decrease in the group 2 (positive control) when compared to normal control. In contrast, there is no significant ($p > 0.05$) increase in the treatment groups when compared to normal control. Both standard control (group 3) and groups that received 100, 200 and 400 mg/kg graded doses of n-hexane leaf fraction increased significantly ($p < 0.05$) when compared to positive control. The result further implies that the leaf fraction of *Lepidagathis alopecuroides* has both protective and stimulative effect on the pancreas. this can be as a result of the phytoconstituent such as flavonoids, alkaloids, tannin and phenol present in the plant as reported by (Gupta and De, 2012; Mamun *et al.*, 2014).

The result of the histopathology of group 2 (positive control) presented showed a marked decrease in the number and sizes of the pancreatic islets. The islet cells showed marked vacuolar degeneration and necrosis, swollen with foamy cytoplasm and eosinophilc necrotic debris when compared to group 1(normal control) which showed the normal histomorphology of the endocrine pancreas, with normal pancreatic islets that are randomly embedded in-between the acinar cells of the exocrine pancreas. There is also a marked decrease in the number and sizes of the pancreatic islets in group 3 and 4. However, group 5 and 6 showed a marked proliferation of the α-cells in the pancreatic islets, with less abundant β-cells located at the centre of the pancreatic islets. Recent studies have associated proliferation of the α-cells of the islet with β-cell regeneration via process of trans-differentiation. (Sangan and Tosh, 2010) Several lines of

evidence has also indicated an important role of α cells as direct progenitors of β-cells both in the embryonic development of the islets and in the regeneration of islets in the adult pancreas (Sangan and Tosh, 2010; Courney *et al*., 2011). The ability of *Lepidagathis alopecuroides* fraction to induce the proliferation of β-cells supports other results in this work and also serve as an indication that *Lepidagathis alopecuroides* could serve as a potent anti-diabetic drug.

4.2. Conclusion

The results of this study showed that the n-hexane fraction of *Lepidagathis alopecuroides* significantly reduced hyperglycemia in alloxan-induced diabetic rats and also restored some altered haematological indices as well as some biochemical parameters in alloxan-induced diabetic rats. The investigation possibly authenticates the use of *Lepidagathis alopecuroides* leaf fraction in folkloric treatment of diabetes and its associated complication.

4.3. Suggestion for Further Studies

1. Further studies should be carried out to analyse the bioactive components of the n-hexane leaf fraction of *Lepidagathis alopecuroides* and the molecular weight of the respective components via GC-MS analysis.

2 Structural elucidation of the bioactive component responsible for the antihyperglycemic and antihyperlipidaemic properties of the fraction should be carried out.

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APPENDICES

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
INSULIN	Normal Control	4	11.6000	.87696	.43848	10.2046	12.9954	10.44	12.56
	Positive Control	4	6.0050	2.81243	1.40622	1.5298	10.4802	3.37	8.75
	5mg/kg bw of glibenclamide	4	10.3250	.21237	.10618	9.9871	10.6629	10.07	10.59
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	11.3125	.84389	.42195	9.9697	12.6553	10.61	12.50
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	10.5025	.35743	.17872	9.9337	11.0713	10.04	10.91
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	9.9125	.68665	.34333	8.8199	11.0051	9.35	10.88
	Total	24	9.9429	2.21125	.45137	9.0092	10.8766	3.37	12.56
ALP	Normal Control	4	17.5000	1.29099	.64550	15.4457	19.5543	16.00	19.00
	Positive Control	4	26.2500	3.30404	1.65202	20.9925	31.5075	24.00	31.00

	5mg/kg bw of glibenclamide	4	11.2500	2.87228	1.43614	6.6796	15.8204	8.00	15.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	22.2500	.95743	.47871	20.7265	23.7735	21.00	23.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	18.0000	7.25718	3.62859	6.4522	29.5478	11.00	28.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	19.2500	.95743	.47871	17.7265	20.7735	18.00	20.00
	Total	24	19.0833	5.64082	1.15143	16.7014	21.4652	8.00	31.00
GSH	Normal Control	4	5.7250	.23587	.11793	5.3497	6.1003	5.55	6.06
	Positive Control	4	3.5725	.06652	.03326	3.4667	3.6783	3.48	3.63
	5mg/kg bw of glibenclamide	4	5.7200	.32259	.16130	5.2067	6.2333	5.43	6.17
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	3.7000	.28178	.14089	3.2516	4.1484	3.36	4.05
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	4.7250	.50954	.25477	3.9142	5.5358	4.30	5.44

	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	5.1500	.33951	.16975	4.6098	5.6902	4.70	5.52
	Total	24	4.7654	.93284	.19041	4.3715	5.1593	3.36	6.17
CAT	Normal Control	4	4.7725	.34345	.17173	4.2260	5.3190	4.34	5.18
	Positive Control	4	2.4350	.19672	.09836	2.1220	2.7480	2.15	2.58
	5mg/kg bw of glibenclamide	4	3.5750	.15546	.07773	3.3276	3.8224	3.37	3.74
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	2.9125	.07588	.03794	2.7918	3.0332	2.85	3.02
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	3.1825	1.04513	.52256	1.5195	4.8455	2.39	4.66
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	3.0250	.37616	.18808	2.4264	3.6236	2.53	3.44
	Total	24	3.3171	.86429	.17642	2.9521	3.6820	2.15	5.18
SOD	Normal Control	4	11.4025	.08808	.04404	11.2623	11.5427	11.28	11.49
	Positive Control	4	11.2900	.09092	.04546	11.1453	11.4347	11.19	11.41
	5mg/kg bw of glibenclamide	4	11.4425	.04113	.02056	11.3771	11.5079	11.39	11.49

	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	11.3100	.14445	.07223	11.0801	11.5399	11.15	11.50
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	11.3600	.07528	.03764	11.2402	11.4798	11.27	11.45
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	11.3675	.06850	.03425	11.2585	11.4765	11.27	11.42
	Total	24	11.3621	.09578	.01955	11.3216	11.4025	11.15	11.50
MDA	Normal Control	4	2.9750	.27489	.13745	2.5376	3.4124	2.60	3.24
	Positive Control	4	4.6225	.04787	.02394	4.5463	4.6987	4.58	4.69
	5mg/kg bw of glibenclamide	4	3.5700	.28083	.14042	3.1231	4.0169	3.26	3.94
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	4.4800	.36851	.18426	3.8936	5.0664	4.01	4.91
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	3.5700	.18019	.09009	3.2833	3.8567	3.36	3.80
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	3.9525	.22217	.11108	3.5990	4.3060	3.64	4.13

	Total	24	3.8617	.61947	.12645	3.6001	4.1232	2.60	4.91
LDL	Normal Control	4	1.3800	.11314	.05657	1.2000	1.5600	1.30	1.54
	Positive Control	4	2.2650	.13304	.06652	2.0533	2.4767	2.08	2.38
	5mg/kg bw of glibenclamide	4	2.0850	.08226	.04113	1.9541	2.2159	1.98	2.18
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	2.2425	.18733	.09366	1.9444	2.5406	1.98	2.40
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	2.0500	.09416	.04708	1.9002	2.1998	1.93	2.16
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	1.6000	.13064	.06532	1.3921	1.8079	1.44	1.76
	Total	24	1.9371	.35707	.07289	1.7863	2.0879	1.30	2.40
TAG	Normal Control	4	1.0825	.02363	.01181	1.0449	1.1201	1.05	1.10
	Positive Control	4	1.4875	.35650	.17825	.9202	2.0548	1.01	1.87
	5mg/kg bw of glibenclamide	4	1.1950	.09678	.04839	1.0410	1.3490	1.06	1.27
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	1.3900	.16872	.08436	1.1215	1.6585	1.20	1.61

	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	1.1525	.06602	.03301	1.0475	1.2575	1.08	1.24
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	1.2300	.13736	.06868	1.0114	1.4486	1.04	1.36
	Total	24	1.2563	.21194	.04326	1.1668	1.3457	1.01	1.87
HDL	Normal Control	4	2.2650	.13304	.06652	2.0533	2.4767	2.08	2.38
	Positive Control	4	1.3800	.11314	.05657	1.2000	1.5600	1.30	1.54
	5mg/kg bw of glibenclamide	4	2.2425	.18733	.09366	1.9444	2.5406	1.98	2.40
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	2.0500	.09416	.04708	1.9002	2.1998	1.93	2.16
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	2.0850	.08226	.04113	1.9541	2.2159	1.98	2.18
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	1.6000	.13064	.06532	1.3921	1.8079	1.44	1.76
	Total	24	1.9371	.35707	.07289	1.7863	2.0879	1.30	2.40
T_CHOL	Normal Control	4	4.4000	.20000	.10000	4.0818	4.7182	4.10	4.50
	Positive Control	4	4.9000	.24495	.12247	4.5102	5.2898	4.60	5.20

	5mg/kg bw of glibenclamide	4	4.4000	.16330	.08165	4.1402	4.6598	4.20	4.60
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	4.8500	.25166	.12583	4.4496	5.2504	4.60	5.20
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	4.3500	.38730	.19365	3.7337	4.9663	4.00	4.90
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	4.8500	.12910	.06455	4.6446	5.0554	4.70	5.00
	Total	24	4.6250	.32870	.06710	4.4862	4.7638	4.00	5.20
WBC	Normal Control	4	76.0000	3.26599	1.63299	70.8031	81.1969	72.00	80.00
	Positive Control	4	62.7500	1.89297	.94648	59.7379	65.7621	60.00	64.00
	5mg/kg bw of glibenclamide	4	73.2500	.95743	.47871	71.7265	74.7735	72.00	74.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	70.0000	4.32049	2.16025	63.1251	76.8749	64.00	74.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	64.0000	1.63299	.81650	61.4015	66.5985	62.00	66.00

	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	70.0000	3.26599	1.63299	64.8031	75.1969	66.00	74.00
	Total	24	69.3333	5.40263	1.10281	67.0520	71.6147	60.00	80.00
RBC	Normal Control	4	4.5000	.08165	.04082	4.3701	4.6299	4.40	4.60
	Positive Control	4	4.2000	.21602	.10801	3.8563	4.5437	4.00	4.50
	5mg/kg bw of glibenclamide	4	4.5500	.10000	.05000	4.3909	4.7091	4.50	4.70
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	4.5000	.14142	.07071	4.2750	4.7250	4.40	4.70
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	4.2500	.25166	.12583	3.8496	4.6504	4.00	4.60
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	4.4000	.16330	.08165	4.1402	4.6598	4.20	4.60
	Total	24	4.4000	.20216	.04127	4.3146	4.4854	4.00	4.70
Hb	Normal Control	4	10.2375	.73894	.36947	9.0617	11.4133	9.50	11.06
	Positive Control	4	8.1525	.45544	.22772	7.4278	8.8772	7.52	8.54
	5mg/kg bw of glibenclamide	4	9.8100	1.38530	.69265	7.6057	12.0143	8.82	11.86

	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	9.7525	.47759	.23879	8.9925	10.5125	9.28	10.27
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	9.0525	.40186	.20093	8.4131	9.6919	8.59	9.57
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	10.0475	.53680	.26840	9.1933	10.9017	9.43	10.59
	Total	24	9.5088	.98084	.20021	9.0946	9.9229	7.52	11.86
PCV	Normal Control	4	40.2500	1.89297	.94648	37.2379	43.2621	39.00	43.00
	Positive Control	4	33.2500	2.75379	1.37689	28.8681	37.6319	30.00	36.00
	5mg/kg bw of glibenclamide	4	38.7500	2.87228	1.43614	34.1796	43.3204	35.00	42.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	33.2500	2.75379	1.37689	28.8681	37.6319	30.00	36.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	36.5000	3.41565	1.70783	31.0649	41.9351	32.00	40.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	34.0000	4.24264	2.12132	27.2490	40.7510	29.00	38.00

	Total	24	36.0000	3.90095	.79628	34.3528	37.6472	29.00	43.00
UREA	Normal Control	4	57.7500	4.03113	2.01556	51.3356	64.1644	52.00	61.00
	Positive Control	4	72.0000	4.96655	2.48328	64.0971	79.9029	68.00	79.00
	5mg/kg bw of glibenclamide	4	60.0000	5.88784	2.94392	50.6311	69.3689	52.00	66.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	69.5000	3.31662	1.65831	64.2225	74.7775	66.00	74.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	61.5000	2.08167	1.04083	58.1876	64.8124	59.00	64.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	69.2500	4.50000	2.25000	62.0895	76.4105	64.00	75.00
	Total	24	65.0000	6.73085	1.37393	62.1578	67.8422	52.00	79.00
CREATININE	Normal Control	4	2.0425	.09465	.04732	1.8919	2.1931	1.92	2.15
	Positive Control	4	2.1625	.01258	.00629	2.1425	2.1825	2.15	2.18
	5mg/kg bw of glibenclamide	4	2.0325	.10145	.05072	1.8711	2.1939	1.89	2.11
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	2.1450	.03109	.01555	2.0955	2.1945	2.12	2.19

	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	2.0800	.07071	.03536	1.9675	2.1925	1.98	2.13
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	2.1325	.03862	.01931	2.0710	2.1940	2.08	2.17
	Total	24	2.0992	.07846	.01602	2.0660	2.1323	1.89	2.19
AST	Normal Control	4	30.0000	3.26599	1.63299	24.8031	35.1969	26.00	34.00
	Positive Control	4	46.2500	1.70783	.85391	43.5325	48.9675	44.00	48.00
	5mg/kg bw of glibenclamide	4	35.2500	3.77492	1.88746	29.2433	41.2567	32.00	39.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	42.7500	2.87228	1.43614	38.1796	47.3204	39.00	46.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	37.2500	5.73730	2.86865	28.1207	46.3793	30.00	44.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	37.5000	1.91485	.95743	34.4530	40.5470	35.00	39.00
	Total	24	38.1667	6.14793	1.25494	35.5706	40.7627	26.00	48.00

ALT	Normal Control	4	19.0000	.81650	.40825	17.7008	20.2992	18.00	20.00
	Positive Control	4	26.0000	.81650	.40825	24.7008	27.2992	25.00	27.00
	5mg/kg bw of glibenclamide	4	20.7500	1.89297	.94648	17.7379	23.7621	18.00	22.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	24.2500	.95743	.47871	22.7265	25.7735	23.00	25.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	20.2500	3.68556	1.84278	14.3855	26.1145	16.00	25.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	22.0000	.81650	.40825	20.7008	23.2992	21.00	23.00
	Total	24	22.0417	2.94115	.60036	20.7997	23.2836	16.00	27.00
Initial_Glucose_Level	Normal Control	4	70.2500	3.77492	1.88746	64.2433	76.2567	66.00	75.00
	Positive Control	4	83.2500	18.28251	9.14125	54.1585	112.3415	58.00	99.00
	5mg/kg bw of glibenclamide	4	69.5000	9.88264	4.94132	53.7745	85.2255	60.00	79.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	71.5000	9.67815	4.83908	56.0999	86.9001	61.00	82.00

	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	79.5000	23.78375	11.89187	41.6547	117.3453	58.00	113.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	76.7500	28.42974	14.21487	31.5119	121.9881	59.00	119.00
	Total	24	75.1250	16.63760	3.39614	68.0996	82.1504	58.00	119.00
Glucose_level_Day_0	Normal Control	4	70.7500	3.09570	1.54785	65.8241	75.6759	68.00	75.00
	Positive Control	4	253.7500	4.27200	2.13600	246.9523	260.5477	251.00	260.00
	5mg/kg bw of glibenclamide	4	263.7500	35.07492	17.53746	207.9380	319.5620	241.00	316.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	276.2500	29.94857	14.97428	228.5951	323.9049	247.00	316.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	255.0000	7.11805	3.55903	243.6736	266.3264	247.00	261.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	255.2500	7.41058	3.70529	243.4581	267.0419	247.00	262.00
	Total	24	229.1250	74.78218	15.26485	197.5473	260.7027	68.00	316.00

Glucose_level_Day_7	Normal Control	4	69.7500	4.57347	2.28674	62.4726	77.0274	64.00	75.00
	Positive Control	4	248.2500	16.58061	8.29031	221.8665	274.6335	232.00	264.00
	5mg/kg bw of glibenclamide	4	223.5000	5.06623	2.53311	215.4385	231.5615	216.00	227.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	213.7500	19.10279	9.55140	183.3532	244.1468	187.00	228.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	229.2500	17.48094	8.74047	201.4339	257.0661	216.00	255.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	223.7500	5.73730	2.86865	214.6207	232.8793	216.00	229.00
	Total	24	201.3750	62.15186	12.68669	175.1306	227.6194	64.00	264.00
Glucose_level_Day_14	Normal Control	4	68.5000	3.10913	1.55456	63.5527	73.4473	64.00	71.00
	Positive Control	4	238.2500	11.55783	5.77891	219.8589	256.6411	226.00	252.00
	5mg/kg bw of glibenclamide	4	160.5000	5.44671	2.72336	151.8331	169.1669	156.00	168.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	181.7500	11.84272	5.92136	162.9056	200.5944	167.00	196.00

	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	162.0000	26.69582	13.34791	119.5210	204.4790	122.00	177.00
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	170.2500	9.77667	4.88834	154.6931	185.8069	161.00	181.00
	Total	24	163.5417	52.49180	10.71484	141.3763	185.7070	64.00	252.00
Initial_Body_Weight	Normal Control	4	96.8400	2.77359	1.38679	92.4266	101.2534	95.40	101.00
	Positive Control	4	122.8650	6.30008	3.15004	112.8402	132.8898	113.74	128.00
	5mg/kg bw of glibenclamide	4	120.6000	17.60227	8.80114	92.5909	148.6091	96.40	138.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	97.1250	8.46341	4.23170	83.6578	110.5922	85.61	106.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	110.3700	15.43323	7.71661	85.8123	134.9277	93.23	130.44
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	118.6500	12.52325	6.26162	98.7227	138.5773	104.98	129.39
	Total	24	111.0750	15.05207	3.07249	104.7191	117.4309	85.61	138.00

Final_Body_Weight	Normal Control	4	155.5125	39.08062	19.54031	93.3265	217.6985	100.47	187.24
	Positive Control	4	95.0400	12.09010	6.04505	75.8020	114.2780	78.30	106.60
	5mg/kg bw of glibenclamide	4	111.0025	7.76077	3.88038	98.6534	123.3516	101.00	118.00
	100 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	104.7825	7.49633	3.74816	92.8542	116.7108	94.83	113.00
	200 mg/kg bw of n-HexaneFfraction of L. alopecuroides	4	111.5775	6.28210	3.14105	101.5813	121.5737	103.81	118.69
	400 mg/kg bw of n-Hexane Fraction of L. alopecuroides	4	100.6950	7.81725	3.90862	88.2560	113.1340	93.69	110.00
	Total	24	113.1017	25.61458	5.22855	102.2856	123.9178	78.30	187.24