TOXICOLOGICAL AND ANTI-HYPERGLYCAEMIC PROPERTIES OF AQUEOUS LEAF EXTRACT OF *PERSEA AMERICANA* MILL (LAURECEAE) IN NORMOGLYCAEMIC AND ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS

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DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
FACULTY OF PHARMACEUTICAL SCIENCES
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DECEMBER, 2014

DECLARATION

I declare that the work in this thesis entitled "Toxicological and Anti-Hyperglycaemic Properties of Aqueous Leaf Extract of *Persea americana* Mill (Laureceae) in Normoglycaemic and Alloxan-Induced HyperglycaemicWistar Rats" has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been presented for another degree or diploma at this or any other institution.

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "TOXICOLOGICAL AND ANTI-HYPERGLYCAEMIC PROPERTIES OF AQUEOUS LEAF EXTRACT OF *PERSEA AMERICANA* MILL (LAURECEAE) IN NORMOGLYCAEMIC AND ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS" by Stephen Chiadikaobi OKAFOR meets the regulations governing the award of the degree of Master of Science in Pharmacology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABBREVIATIONS, DEFINITIONS, GLOSSARY AND SYMBOLS

ABU - Ahmadu Bello University

ABUTH - Ahmadu Bello University Teaching Hospital

ADA - American Diabetes Association

AEPAL - Aqueous Extract of *Perseaamericana*

ALB - Albumin

ALP - Alkaline phosphatase

ALT - Alanine transaminase

ANOVA - Analysis of Variance

AST - Aspartate transaminase

ATP - Adenosine triphosphate

BGC - Blood Glucose Concentration

BGL - Blood Glucose Level

BMI – Basal Metabolic Index

CDA - Canadian Diabetes Association

CDC - Centre for Disease Control and Prevention

CHOL - Cholesterol

CETP - Cholesterol ester transfer protein

CHD - Coronary heart disease

DAG - Diacyl Glycerol

DKA - Diabetic ketoacidosis

DM - Diabetes mellitus

DNA - Deoxyribose nucleic acid

DPP-IV - Dipeptidyl peptidase-IV

EDTA - Ethylene diaminetetraacetic acid

FBS/G - Fasting blood sugar/glucose

FBGC/L - Fasting blood glucose concentration/level

FDA - Food and Drug Administration

fL - Femtolitre

g - Grams

g/day - gram per day

g/L - gram per Litre

GAD65 - Glutamic acid decarboxylase

GDIP - Glucose Dependent Insulinotrophic Polypeptide

GDM - Gestational Diabetes Mellitus

GHb - Glycohaemoglobin

GIP - Gastric Inhibitory Peptide

GIT - Gastrointestinal tract

GLP - Good Laboratory Practice

GLP-1 - Glucagon like peptide-1

Hb - Haemoglobin

HbA1C- Haemoglobin A1C or GlycatedHaemoglobin

H & E - Haematoxylin and Eosin

HDL - High density lipoprotein

HFD - High fat diet

HMG CoA 3-hydroxyl-3-methyl glutaryl Coenzyme A

HNS - Hiperosmolar Non-ketotic State

IAAs - Insulin autoantibodies

ICAs - Islet cell antibodies

ICF - Intra Cellular Fluid

IDDM - Insulin dependent diabetes mellitus

IGT - Impaired glucose tolerance

IDL - Intermediate density lipoprotein

i.p - Intraperitoneal

IU/L - International Unit per Litre

LD_{50} - Lethal dose in 50% of population

LDL - Low density lipoprotein

MAOIs - Monoamine oxidase inhibitors

MCH - Mean Corpuscular Haemoglobin

MCHC - Mean Corpuscular Haemoglobin Concentration

MCV - Mean Corpuscular Volume

mg/dL - Milligram per deciliter

mg/kg - Milligram per kilogram

ml/day - milliliter per day

mmol - millimoles

mmol/L - millimoles per Litre

mRNA - Messenger Ribonucleic Acid

n - Number of animals in a group

NIDDM - Non-insulin dependent diabetes mellitus

NIH - National Institute of Health

NIHCE - National Institute of Health and Care Excellence

NIHCD National Institute of health Consensus Development

OECD - Organization for Economic Cooperation and Development

P< - P less than

P> - P greater than

PA - *Persea americana*

PCV - Packed Cell Volume

Pg - Picogram

pH - Potential of hydrogen

PIG - Phosphatidyl Inositol Glycan

Plt - Platelate

p.o. - Per Oral

PPAR- Peroxisome proliferators activated receptor

PPG - Post Prandial Glucose

PPRE - Peroxisome proliferators Responsive Elements

PVD - Peripheral Vascular Disease

RBC - Red Blood Cell

ROW - Relative Organ Weight

RXR - Retinoid X receptor

SEMDSA - Society for Endocrinology, Metabolism and Diabetes of South Africa

SEM - Standard Error of Mean

SPSS - Statistical Package for Social Sciences

SU - Sulphonyl Urea

SUR - Sulfonylurea receptors

T1DM - Type 1 diabetes mellitus

T2DM - Type 2 diabetes mellitus

TBIL - Total bilirubin

TC - Total cholesterol

TCAs - Tricyclic anti-depressant

TMP - Traditional Medicine Practitioners

TP -	Total Protein
TRIG -	Triglyceride
TZDs -	Thiazolidinediones
WBC -	White Blood Cell
WHO -	World Health Organisation
B-cells -	Beta cells
± -	Plus or Minus
* -	Asterisk
µL	microlitre
µmol/L -	micromole per Litre
% -	Percentage

ABSTRACT

Diabetes mellitus has become a global health burden leading to an increase in the search for herbal anti-hyperglycaemic agents as alternative to synthetic ones. *Persea americana* popularly known as avocado pear have been known for its nutritional and medicinal properties. The leaves of this plant are used in Eastern Nigeria especially in Anambra state for the management of Diabetes Mellitus. This study was aimed at evaluating the toxicological and anti-hyperglycaemic properties of aqueous leaf extract of *P. americana* Mill. (laureceae) in normoglycaemic and alloxan-induced hyperglycaemic Wistar rats. Preliminary phytochemical screening, acute toxicity and sub-chronic toxicity and anti-hyperglycaemic studies of the extract were carried out. Preliminary phytochemical screening revealed the presence of saponins, steroids, flavonoids, tannins, alkaloids, cardiac glycosides, triterpenoids and carbohydrates whereas anthraquinones and cyanogenetic glycosides were absent. The oral median lethal dose (LD_{50}) of the extract in rats was estimated to be greater than 5000 mg/kg. In the sub-chronic toxicity study, the extract did not produce significant changes on feed and water intake, body weights and the relative organ weights of treated rats when compared with control. *P. americana* leaf extract significantly ($P<0.05$) reduced the blood glucose level in treated normoglycaemic rats compared to normoglycaemic control. The extract also significantly ($P<0.05$) decreased only the white blood cell (WBC) count among the haematological parameters assessed. The extract significantly ($P<0.05$) increased AST, urea, creatinine and ALT, while ALP showed no significant difference when compared with the control. There were no histological changes found in the heart and spleen tissues of the treated rats. However, dose dependent pathological changes were observed in the liver, kidney and lung tissues of the treated rats compared with control. Anti-hyperglycaemic study showed that the extract significantly ($P<0.05$)

reduced feed and water intake and significantly ($P<0.05$) decreased the blood glucose concentration. The body weights of the treated animals increased significantly ($P<0.05$) when compared to hyperglycaemic control. There were significant ($P<0.05$) decrease observed in the levels of AST, ALT, ALP, urea and creatinine while total protein and albumin were increased significantly ($P<0.05$) in treated animals compared to untreated hyperglycaemic control. No significant changes were observed in all the haematological parameters of the treated rats when compared to hyperglycaemic control. The extract also caused significant ($P<0.05$) decrease in TC, TG, and LDL while HDL was increased significantly ($P<0.05$) in the treated rats compared to non-treated hyperglycaemic control. Histopathological study showed a degenerative effect on the pancreatic tissue of the hyperglycaemic control for 28 days but revealed restorative (protective) effect of the extract on pancreatic islet cells of the hyperglycaemic treated rats. The findings from these studies showed that the aqueous leaf extract of *P. americana* is relatively safe on acute administration but revealed possible toxic effects following sub-chronic daily oral administration in rats at the doses used. The extract also showed blood glucose lowering activity (both in normogycaemic and hyperglycaemic rats) alongside anti-hyperlipidaemic activity with reversal of most alterations observed in hyperglycaemic control. Therefore, the aqueous extract of *P. americana* leaf has anti-hyperglycaemic property but toxic on sub-chronic administration and should be used with caution.

CHAPTER ONE

INTRODUCTION

1.1 Preamble

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system. The disease occurs worldwide and its incidence is increasing rapidly in most parts of the world (Kumar *et al*., 2008). It is a chronic medical condition. DM is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves (Grundy *et al.*, 1999; Barnett and O' Gara, 2003). Hyperglycaemia in diabetes mellitus can result from an absolute deficiency in insulin secretion (type 1 DM), insulin action (type 2 DM) or both (Ahmed and Goldstein, 2006; Andrade-Cetto *et al*., 2007). If not controlled, hyperglycaemia can lead to the development of microvascular disorders such as nephropathy, retinopathy and macrovascular complications including cardiomyopathy, neuropathy, atherosclerosis and myocardial infarction (WHO, 2012). A number of pathogenic processes are involved in the development of diabetes ranging from an autoimmune destruction of the β cells of the pancreas, with consequent insulin deficiency, to abnormalities that result in resistance to insulin action (Xu *et al.,* 2008). Obesity and sedentary life- style are known to be the major risk factors of type 2 DM.

At least 346 (about 5.6% of the population) million people worldwide suffer from diabetes (WHO, 2011). DM affects all races around the world, but is more prevalent in the more developed countries and type 2 diabetes accounts for almost 90% of all cases of diabetes in adults worldwide (Ramachandran *et al.,* 2001). The mortality rate of

diabetes varies sharply with the prosperity of the country (WHO, 2011). In 2011, the disease caused more than 3.5 million deaths in middle-income countries, of which more than 1 million were in China and just less than a million were in India. Approximately 1.2 adults die of a diabetes-associated illness per 1,000 cases in 2011 in low- and middle-income countries which are more than double the mortality rate of high-income countries (WHO, 2011). Mortality rates are much lower in high-income countries with the greater healthcare resources, but those tolls are still high: approximately 180,000 people died in the United States in 2011 (WHO, 2011). Diabetes afflicts only about 4.5% of adults in sub- Saharan Africa but prevalence is predicted to double over the next 20 years, the fastest rise of any region in the world (WHO, 2011).

Toxicology is the science that deals with the study of the adverse effects caused by chemicals or physical agents in living organisms under specific conditions of exposure. Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of a test item and to estimate safety margins (James *et al.*, 2000). The relationship between the dose of a toxicant and the response produced follows a predictable pattern. As the dose of a toxicant increases, the response also increases, which may be either in terms of the proportion of the population responding or in terms of the severity of the graded responses (Ballantyne *et al.*, 1993). Results obtained from toxicity studies can be different for the same dose, depending on the route of exposure to the toxicant (Everhard *et al.*, 1976). A toxicant may be inhaled, ingested, injected, or absorbed through the skin.

Herbal medicine practice has been the main form of treatment especially among indigenous populations around the world particularly in developing countries, it has been estimated that 80% of people worldwide rely on herbal medicines for some aspect

of their primary healthcare (WHO, 2002). Although many plants and plant-based materials have been in use for several years for the ethnotherapy of DM, the use of herbs and natural drug products from various plant sources is of great interest in the management of DM (Subramonium *et al*., 1996). Many Nigerians rely on traditional herbs for their medication. Plants from different botanical sources have been used by many Traditional Medical Practitioners (TMPs) in Nigeria for the treatment of numerous diseases that are locally endemic including DM (Builders *et al.,* 2012). *Persea americana* represents one such plants.

1.2 Statement of Research Problem

DM is a disease of significant public health importance due to the global rising estimates of prevalence morbidity and mortality as well as the adverse health and socioeconomic burden especially in developing countries including Nigeria (Akaogu, 2009). In Nigeria, it afflicts about 3 million people or about 3 percent of the population (WHO, 2011). There are many drugs that are used to control DM but due to lack of accessibility, adverse effects, cost, etc., many people especially in rural areas have resorted to herbal remedies in treating diabetes (Jarald *et al.,* 2008).

It is important to state that, despite the advantages of folkloric medicine, it has its own shortcomings; Problems ranging from inadequate regulations, safety concerns, quality control, dose, efficacy and inaccurate labeling to potential interactions with food and other drugs as well as poor diagnosis (WHO, 2005). Traditional Medicine Practitioners (TMPs) make claims of successful treatments of diseases using a variety of herbal medicines usually with little or no supporting evidence (Chindo, *et al.,* 2012). These

claims and herbal medicine shortcomings listed above are worthy of investigations to establish the reasons behind their success or failure.

1.3 Justification

Herbal medicines have the potential to meet the demands of the health needs of our country and Africa in general (WHO, 2002). Alternative strategies to the current modern pharmacotherapy of DM are urgently needed because of the inability of existing therapies to control all pathological aspects and the resultant complications of the disorder as well as enormous cost and poor availability of the modern therapies for many rural populations in developing countries (Jarald, 2008). Also, with increasing incidence of DM in rural population throughout the world and due to adverse effects of synthetic medicine, there is a clear and urgent need for the development of indigenous, relatively inexpensive plant derived ethno-medicines or purified drugs as effective alternative remedies (Jarald, *et al.,* 2008).

The increasing use of herbs calls for a proper scientific evaluation of herbal medicines with important emphasis on known toxicological and pharmacological paradigms which is imperative in order to determine their safety and efficacy (Salawu *et al.,* 2009; Chindo, *et al.,* 2012). Thus, the selection of anti-diabetic plants for evaluation of their safety and therapeutic activity would provide the scientific basis that will justify the claims of using them as alternative to orthodox anti-diabetes drugs (WHO, 2002).

This study was prompted by the claims of some TMPs in the south-eastern (precisely Anambra state) Nigeria, that maceration, decoctions and infusions of *P. americana* leaf are effective remedies for the management and/or control of DM. Hence, the outcome

of this research may form the basis for the use of this plant or otherwise in the management of DM in alternative medicine.

1.4 Aim and Objectives

1.4.1 Aim

To evaluate the toxicological and anti-hyperglycaemic properties of the aqueous extract of *P. americana* leaf (AEPAL) in normoglycaemic and alloxan-induced hyperglycaemic rats.

1.4.2 Specific Objectives

1. To validate the phytochemical constituents of AEPAL.
2. To determine the toxicity profile of AEPAL in normoglycaemic Wistar rats.
3. To screen the extract for hypoglycaemic/anti-hyperglycaemic activity in normoglycaemic and alloxan-induced hyperglycaemic Wistar.
4. To determine the effect of AEPAL on biochemical and haematological parameters in normoglycaemic and alloxan-induced hyperglycaemic Wistar rats.
5. To determine the effect of AEPAL on body and organ weights as well as on some metabolic parameters such as feed and water intake in normoglycaemic and alloxan-induced hyperglycaemic Wistar rats.
6. To determine the effect of AEPAL on tissue morphology (hispathology) of some essential organs of normoglycaemic and alloxan-induced hyperglycaemic Wistar rats.

1.5 Research Hypothesis

The crude aqueous extract of *P. americana* leaf possesses anti-hyperglycaemic properties and is safe for use as an anti-diabetic ethno medicine.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Diabetes Mellitus

The term DM has been defined and/or described in several ways. DM is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (WHO, 2012). It is also described as a metabolic disorder with heterogeneous aetiologies which is characterized by chronic hyperglyceamia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (ADA, 2012; SEMDSA, 2012). Insulin is a hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar, is a common feature of uncontrolled diabetes and over time leads to serious damage to many of the body's structures, especially the nerves and blood vessels (WHO, 2012). Currently DM is not regarded as a single disease in the classic sense of the word since it has no distinct and definable pathogenesis, aetiology, invariable clinical features, specific laboratory tests and definitive and curative therapy. Rather it is regarded as a syndrome comprising a variety of metabolic disorders that have in common a persistently elevated blood glucose level and normally associated with glycosuria as well as certain definite pathological changes (Gyang, 2009). It is a chronic medical condition, meaning that although it can be managed, it lasts a lifetime.

Several pathological processes are involved in the development of DM. These include processes which destroy the beta cells of the pancreas with consequent insulin

deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (SEMDSA, 2012). Diabetes is commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, prothrombic factors and microvascular problems involving eyes, kidney and peripheral nerves (Grundy *et al.*, 1999; Barnett and O'Gara, 2003). DM is associated with profound alteration in the serum lipid and lipoprotein profile with an increased risk in coronary disease. Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein composition. Increased levels of triglyceride-rich lipoproteins have been reported in diabetic patients and this atherogenic profile becomes more apparent (Hanachi *et al.,* 2009).

The term diabetes, without qualification, usually refers to DM. The rare disease diabetes insipidus (DI) has similar symptoms as diabetes mellitus, but without disturbances in the sugar metabolism (insipidus meaning "without taste" in Latin).

2.2 Epidemiology and Statistics of Diabetes Mellitus

The prevalence of diabetes mellitus worldwide has increased dramatically during the past few decades, and it is expected to increase even more in the future. Currently, 347 million people worldwide have diabetes which is about double the number (171 million) estimated in 2000 (WHO, 2012). In 2004, an estimated 3.4 million people died from consequences of high blood sugar and more than 80% of diabetes deaths occur in low- and middle-income countries (WHO, 2012). WHO in 2008 projects that diabetes death will increase by two thirds between 2008 and 2030. The greatest increase in

prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030. The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet (Wild *et al*., 2004).

In Africa, about 7.2 million people are diabetic while in Nigeria 1.7 million people were estimated to be diabetic in the year 2000 with a projection of over 4.8 million diabetics by 2030 (WHO, 2012).Over the past 30 years, the prevalence of diabetes in Nigeria has been increasing steadily (Ogungbe and Madehin, 2011). According to the 2004 estimates, the Diabetes Association of Nigeria (DAN) puts the diabetics' population in Nigeria at about 10 million and about half of this number is in Lagos State because of its very cosmopolitan nature (Ogbera *et al*., 2005).

On the whole, results from a properly standardized epidemiologic study worldwide will have an important bearing on many aspects of DM including aetiology, natural history, treatment and most importantly prevalence of the disease (Gyang, 2009).

2.3 Classification of Diabetes Mellitus

Diabetes is classified into four (4) general categories namely: type 1 DM, type 2 DM, type 3 DM and type 4 (Gestational) DM.

2.3.1 Type 1 diabetes mellitus

The term "type 1 diabetes" has replaced several former terms, including childhood-onset diabetes, juvenile diabetes, and insulin-dependent diabetes mellitus (IDDM) (ADA, 2012). It is the most common diagnosed in children (juvenile onset) and

adolescents, but can occur in adults as well and it accounts for 5-10% of the diabetic population. It is an autoimmune disorder, in which the body owns immune system attacks the beta cells in the Islets of Langerhans of the pancreas destroying them or damaging them sufficiently to reduce insulin production. Such diabetics are prone to ketoacidosis and often manifest the polytriad of diabetic symptoms: polyphagia, polyuria and polydipsia (Ukwe, 2006).

2.3.2 Type 2 diabetes mellitus

Likewise, the term "type 2 diabetes" has replaced several former terms, including maturity or adult-onset diabetes, obesity-related diabetes, and non-insulin-dependent diabetes mellitus (NIDDM) (ADA, 2005). It is characterized by relative insulin deficiency or insulin resistance as body cells do not respond appropriately when insulin is present. This is a more complex problem than type 1, but is sometimes easier to treat, since insulin is still produced, especially in the initial years. Type 2 may go unnoticed for years in a patient before diagnosis, since symptoms are typically milder (no ketoacidosis) and sporadic hyperglycaemia and some of the classical manifestation may not be present. Majority of the patients with type 2 DM are usually obese or overweight (Gerich, 2000; Tripathy, 2000; Porte, 2001).

2.3.3 Type 3 diabetes mellitus

All other specific forms of DM, accounting for up to 5% of all diagnosed cases are termed type 3; Type 3A caused by a genetic defect in beta cells, type 3B genetically related to insulin resistance, type 3C is related to disease of the pancreas, type 3D caused by hormonal defects and type 3E is caused by chemicals or drugs (Tripathy *et al.*, 2000).

2.3.4 Type 4 or gestational diabetes mellitus (GDM)

Gestational DM is a diabetes or any degree of glucose intolerance that is diagnosed during pregnancy. It appears in about 2-5% of all pregnancy. It is temporary and completely treatable, but if untreated it may cause problems to the pregnancy and degenerates to type 2 DM. It requires careful medical supervision during the pregnancy. In addition, about 20-50% of these women go on to develop type 2 DM (Porte, 2001; Bupa, 2008).

2.4 Causes of Diabetes Mellitus

The causes of diabetes mellitus are unclear; however, there seem to be both hereditary (genetic factors passed on in families) and environmental factors involved. Factors that could lead to the development of DM are listed below:

2.4.1 Genetic factors

The good evidence of genetic and environmental interactions in the aetiology of NIDDM (Matsutani *et al*., 1990) has created renewed interest in the concept of the so called thrifty genotype which was originally proposed by Neel (1962). The proposal states that diabetes represents a thrifty genotype rendered detrimental by progress. This raises important questions about the evolutionary significance of the diabetes genotype especially in high prevalence populations such as American Indians, Polynesians and Australian Aborigines where under primitive circumstances, diabetes was hitherto unknown (Mueckler, 1990).

Type 1 DM is likely to be inherited amidst the other possible links and cause. First degree relatives have a higher risk of developing Type 1 diabetes than unrelated

individuals from the general population (Sinha *et al*., 2002). Data suggest that genetic factors are involved with the development of the disease. At present, there is evidence that more than 20 regions of the genome may be involved in genetic susceptibility to Type 1 diabetes. However, none of the candidates identified have a greater influence on type 1 diabetes risk than that conferred by genes in the HLA region of chromosome 6. This region contains several hundred genes known to be involved in four immune responses. Those most strongly associated with the disease are the HLA class II genes (i.e., HLA-DR, DQ, and DP). The HLA class II genes, also referred to as IDDM1, contribute approximately 40-50% of the heritable risk for T1D (Lawrence *et al.,* 2008).

Studies have revealed that first degree relatives of individuals with Type 2 diabetes are about 3 times more likely to develop the disease than individuals without a positive family history of the disease (Gorman *et al*., 2004). Thus, it is clear that Type 2 diabetes has a strong genetic component. One approach that is used to identify disease susceptibility genes is based on the identification of candidate genes (Stumvoll *et al*., 2000). To date, more than 50 candidate genes for Type 2 diabetes have been studied in various populations worldwide (Stumvoll *et al*., 2000).

2.4.2 Obesity

The WHO Expert Committee on DM concluded that the most powerful risk factor for NIDDM is obesity (WHO Expert Committee, 1982 as cited in Petersen *et al*., 1993). Its occurrence correlates with the incidence and prevalence of DM in most population. Although there are other factors involved in the pathogenesis of DM (especially NIDDM), obesity appears to be a constant feature particularly in groups with high diabetes prevalence (Petersen *et al*., 1993).

Many recent studies (Lawrence *et al*., 2008), have shown a direct link between obesity and the diagnosis of Type 2 diabetes or NIDDM. Type 2 is increasingly being diagnosed in overweight children and adolescents. In this type of diabetes, the body produces insulin, but interference from the complications of obesity doesn't allow the body to use it as it should. The body of a type 2 diabetic, due to an overproduction of insulin, can no longer produce the correct amount to maintain healthy blood sugar levels and begins to develop a resistance to insulin. Ninety percent of diabetics are diagnosed with type 2 diabetes. Usually, this diagnosis comes after 40 years of age, but the condition is now increasingly found in children. This increase in obesity has been directly linked to the rise in Type 2 diabetes in both children and adults (Gorman *et al*., 2004).

2.4.3 Lifestyle (diets and exercise)

Sedentary lifestyle and excessive body weight may be two very important causes of diabetes. After all, diabetes along with cardiovascular disease is the most characteristic condition in developed countries. Sedentary life and diet high in saturated fats play a key role in development of both diseases (Fuller *et al*., 1980).

2.4.3.1 Diet

It has been thought that dietary factors e.g. composition of diet, (independent of obesity) may have a role in precipitating diabetes in genetically susceptible individuals (White and Campbell, 1992). This submission presumes that excessive consumption of carbohydrate based foods may exhaust the carbohydrate metabolizing mechanisms of the body. Today, the place of diet as a causative factor in DM has remained both conflicting and controversial.

2.4.3.2 Exercise

In current studies it has been difficult to isolate exercise and study it as a single factor in relation to the prevalence and incidence of DM. However, evidence exists to suggest that exercise has a strong protective effect against the development of DM (Bantle, *et al.*, 2006). People with sedentary lifestyle are more prone to diabetes, when compared to those who exercise thrice a week. Studies have shown that lack of adequate daily physical activity increases the chances by 8% for a person to suffer from Type 2 diabetes at some stage of life for people with a genetic predisposition the same percentage reaches 20%. Muscles along with brain are prominent glucose consumers in our body. Lack of fitness makes our muscular system to decline in terms of size and functionality. This situation reduces a muscle's ability to use glucose properly and can be a potential cause of insulin resistance (Ono, 2008).

2.4.4 Environmental factors

In numerous cases, people do not develop diabetes, despite their genetic background. Specialists have come to the conclusion that environmental factors are very important in diabetes etiology. Cold climate might be related to the onset of the disease. For instance, the occurrence of type 1 diabetes is higher in places with cold weather, rather than other areas being closer to equator and remarkable that type 1diabetes is diagnosed more often in winter time (Gorman *et al.*, 2004).

2.4.5 Age

The prevalence and incidence of NIDDM increases with age, and this increase is typical for most populations studied (Benneth, 1979; Zimmet, 1982 as cited in Gyang, 2009). As at today, it is still very unclear why there is a significant deterioration of glucose

tolerance with age (Gyang, 2009). The generally held view however is that the increase with age in the prevalence of DM may be part of the aging process where there is loss of the effectiveness of the body to regulate (metabolize) carbohydrates (Gyang, 2009).

2.4.6 Autoimmunity

The autoimmune process results in the circulation of antibodies that may either cause or be caused by beta-cell death. If it is found that the antibodies cause beta-cell destruction (the body fighting what it now considers foreign to itself), the body's response to the Type 1 diabetes is much less severe. Until then, the outcome is a lack of available insulin. While the onset is said to be sudden, changes resulting in decreased insulin availability may have occurred over a longer period of time. In short, insulin-dependent diabetes mellitus is an inherited defect of the body's immune system, resulting in destruction of the insulin-producing beta cells of the pancreas (Fuller *et al*., 1980).

Some researchers have given evidence that Type 1 diabetes may be of autoimmune nature. In young people with inherited susceptibility to diabetes, scientists traced certain types of auto-antibodies which may be related to the disease. Overtime, the majority of these people developed Type 1 diabetes. Auto-antibodies are heavy-chained proteins produced in the body. The causes of the production and action of such proteins are unknown. Normally antibodies are produce by the immune system to attack and destroy harmful invaders such as viruses, bacteria or toxins and not our own body (Fuller *et al*., 1980).

2.4.7 Drugs

Several common medications can impair the body's use of insulin, causing a condition known as secondary diabetes (Bupa, 2008). These medications include treatments for high blood pressure (furosemide, clonidine, and thiazide diuretics), drugs with hormonal activity (oral contraceptives, thyroid hormone, progestins, and glucocorticorids), and the anti-inflammation drug indomethacin. Several drugs that are used to treat mood disorders (such as anxiety and depression) can also impair glucose absorption. These drugs include haloperidol, lithium carbonate, phenothiazines, tricyclic antidepressants, and adrenergic agonists. Other medications that can cause diabetes symptoms include isoniazid, nicotinic acid, cimetidine, and heparin. Drugs such as steroids, Dilantin, and others may elevate the blood sugar through a variety of mechanisms. Certain other drugs, such as alloxan, streptozotocin, and thiazide diuretics, are toxic to the beta cells of the pancreas and can cause diabetes. Certain syndromes (for example, Prader-Willi, Down's, Progeria, and Turner's) may result in a hyperglycemic state; if this state is prolonged, the result can be permanent diabetes (Bupa, 2008). Other drugs, including theophylline, aspirin and nalidixic acid can cause transient hyperglycaemia in overdosage, but only streptozotocin, alloxan and the rodenticide Vacor are likely to cause permanent diabetes (Bupa, 2008).

2.4.8 Infections

Viral infections might be another causative factor for DM. There are some suggestions that viruses can be a critical factor in some people and help diabetes to appear by destroying the β-cells of the pancreas. Of course, the virus is not the primary cause, since the affected person has to be prone to diabetes, because of genetic factors. The

virus perhaps works as a trigger for the onset of the disease (Weiss and Sumpio, 2006). Examples of such viruses are Cytomegalovirus Coxsackievirus B, etc.

2.4.9 Stress

Studies have given evidence that prolonged stressful periods of time or of bad psychological condition may cause diabetes. Stress, both physical and mental, can send your blood sugar out of control (Guzik *et al*., 2002). It is hard to dispute that most of us live life at breakneck speed. It is the nature of a fast-paced society, where numerous family, social, and work obligations can easily overpower your precious time and resources. But for people with diabetes, both physical and emotional stress can take a greater toll on health (Gordon and Rifkind, 1989).

During stress hormones like epinephrine (A substance produced by the medulla (inside) of the adrenal gland, cortisol (the primary stress hormone) becomes active and their major function amongst others is to raise blood sugar level which helps to boost energy when needed. Emotional and physical trauma keeps these hormones working as they are designed to aid when one is stressed. Logicallythe hormones want to give energy to fight the cause of such stress, but, the adverse effect becomes eminent. Individuals who have inherited the predisposition of Type 1 diabetes mellitus seem to require additional factors such as virus infection and stress. People who are not diabetic have compensatory mechanisms to keep blood sugar from swinging out of control (Ihekwaba and Ojule, 2001).

A variety of severe stress states have been associated with carbohydrate intolerance and/or symptomatic glycosuria. Pregnancy with its increased oestrogen and chorionic somatotropin concentrations, produce resistance to the action of insulin, and this is one

example of stress-related carbohydrate intolerance. Other examples of stress-related carbohydrate intolerance include injury and chronic/severe illnesses especially those associated with secretion of regulatory hormones such as glucagon, glucocorticoid, catecholamines and growth hormone (Aguwa and Omole, 1996). The exact mechanism by which stress influences the course of diabetes is not entirely understood.

2.5 Pathophysiology of Diabetes Mellitus

The pathophysiology of the disease involves two hormones: insulin and glucagon, which are respectively secreted by the beta and alpha cells of the islets of Langerhans of the pancreas. These two hormones work together to maintain a homeostatic mechanism for blood glucose levels (Christopher *et al*., 2008).Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of DM (ADA, 2001).

Higher insulin levels increase some anabolic ("building up") processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and vice versa. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat burning metabolic phase) (Rang *et al.,* 2007).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not have its usual effect so that glucose will not be absorbed properly by

those body cells that require it nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (Ukwe, 2006).

When the glucose concentration in the blood is raised beyond its renal threshold (about 10 mmol/L, although this may be altered in certain conditions, such as pregnancy), reabsorption of glucose in the proximal renal tubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst (polydipsia) (Rang *et al.,* 2007).

2.6 Chemistry and Mechanism of Insulin Synthesis, Secretion, Action and Degradation

Like other peptides hormones, insulin is synthesized as a precursor (preproinsulin) in the rough endoplasmic reticulum. Preproinsulin is transported to the Golgi apparatus, where it undergoes proteolytic cleavage first to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide. Insulin and C-peptide are stored in granules in B cells, and are normally cosecreted by exocytosis in equimolar amounts together with smaller and variable amounts of proinsulin (Rang *et al.,* 2007).

The main factor controlling the synthesis and secretion of insulin is the blood glucose concentration (Fig. 2.1). Beta cells respond both to the absolute glucose concentration and to the rate of change of blood glucose (Rang *et al.,* 2007). Insulin is released from

pancreatic beta cells at a low basal rate and at a much higher stimulated rate in response to a variety of stimuli, especially glucose. Other stimulants such as other sugars (e.g., mannose), certain amino acids (e.g. leucine, arginine), hormones such as glucagon-like polypeptide-1 (GIP-1), glucose-dependent insolinotropic polypeptide (GIP), glucagon, cholecystokinin, and vagal activity are recognized. Inhibitory signals include somatostin, leptin and chronically elevated glucose and fatty acid levels (Martha and Nolte, 2009).

One mechanism of stimulated insulin release is diagrammed in the figure below. Hyperglycaemia results in increased intracellular ATP levels, which close the ATP-dependent potassium channels. Decreased outward potassium efflux results in depolarization of the beta cell and opening of voltage-gated calcium channels. The resulting increased intracellular calcium triggers secretion of the hormone. The insulin secretagogue drug group (sulfonylureas, meglitinides and D-phenylalanine) exploits parts of this mechanism (Martha and Nolte, 2009).

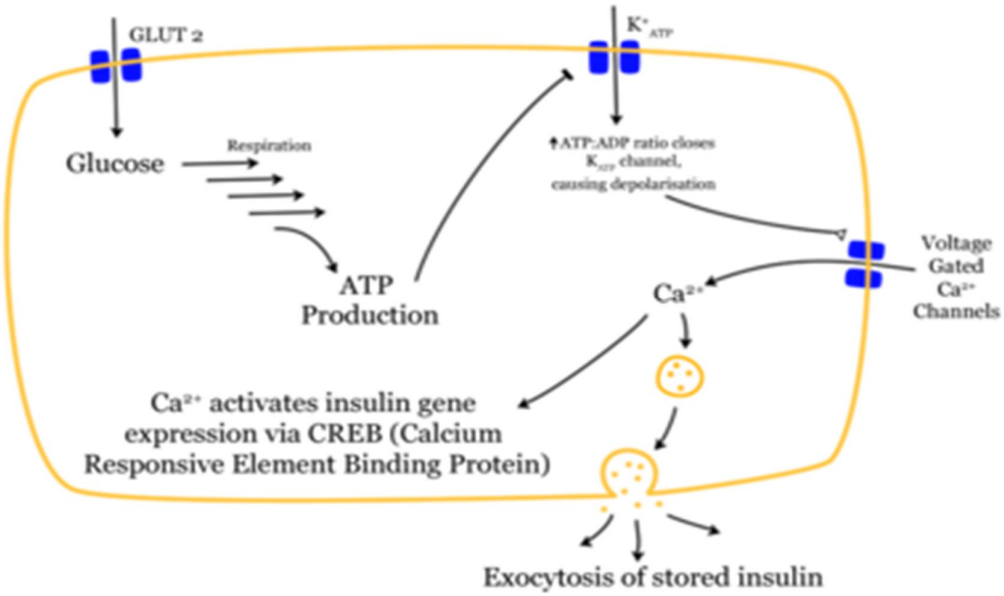


Figure 2.1: Mechanism of insulin release.

Source: (Seidell, 2000).

Insulin is the hormone controlling intermediary metabolism, having actions on liver, muscle and fat. It is an anabolic hormone: its overall effect is to conserve fuel by facilitating the uptake and storage of glucose, amino acids fats after a meal. Acutely, it reduces blood sugar. Consequently, a fall in plasma insulin increases blood glucose. Insulin receptors are found in different cells of the body, including cells in which insulin does not increase glucose uptake (Rang *et al.,* 2007).

When insulin binds to its receptor, they aggregate in patches and are taken into the cell by receptor mediated endocytosis. Eventually, the insulin-receptor complexes enter lysosomes, where the receptors are broken down or recycled (Williams *et al*., 1998). The number or the affinity, or both, of insulin receptors is affected by insulin and other hormones, exercise, food, and other factors. Exposure to increased amounts of insulin decreases receptor concentration (down regulation), and exposure to decreased insulin levels increases the number of receptors (Hellman *et al*., 2007).

The liver and the kidney are the two main organs that remove insulin from the circulation. The liver normally clears the blood of approximately 60% of the insulin released from the pancreas by virtue of its location as the terminal site of portal vein blood flow, with the kidney removing 35-40% of the endogenous hormone. However, in insulin-treated diabetics receiving subcutaneous insulin injections, this ratio is reversed, with as much as 60% of exogenous insulin being cleared by the kidney and the liver removing no more than 30-40%. The half-life of circulating insulin is 3-5 minutes (Martha and Nolte, 2009).

2.7 Signs and Symptoms of Diabetes Mellitus

Symptoms of diabetes can develop suddenly (over days or weeks) in previously healthy children or adolescents, or can develop gradually (over several years) in overweight adults over the age of 40. The classic symptoms include fatique (feeling tired and sick), frequent urination (polyuria), excessive thirst (polydipsia), excessive hunger (polyphagia) and weight loss (Rowland, 2001).

Ketoacidosis, a condition due to starvation or uncontrolled diabetes, is common in Type I diabetes. Ketones are acid compounds (e.g. acetoacetic acid, acetone and β-hydroxybutyric acid) which are formed in the blood when the body breaks down fats and proteins. Symptoms include abdominal pain, vomiting, rapid breathing, extreme tiredness, and drowsiness. Patients with ketoacidosis will also have a sweet breath odour. Left untreated, this condition can lead to coma and death (Rowland, 2001). With

Type II diabetes, the condition may not become evident until the patient presents for medical treatment for some other condition. A patient may have; heart disease, infections of the gums, infections of the urinary tract, blurred vision, numbness/tingling in the feet and legs, slow-healing of wounds and women may experience genital itching.

2.8 Clinical Features of Diabetes Mellitus

Diabetes as a disorder may cause widespread damage over the years, requiring the victim to cope with visual loss, deteriorating peripheral and autonomic nervous function, as well as renal insufficiency, coronary artery disease, cerebrovascular disorders, and peripheral vascular abnormalities. These are all in addition to nervous, physical, emotional and socio-economic impact the disorder as a whole can have on the victim (WHO, 2012). Insulin lack impairs protein synthesis (hence increased weight loss) and promotes protein breakdown especially in muscles. Plasma amino acid concentration increases and in the liver, deamination becomes the main cause of gluconeogenesis. The amine (NH2) - containing amino acids become converted to urea and excreted in urine, giving rise to a negative nitrogen balance (Ebadi, 1996). On the whole increased protein breakdown or catabolism leads to wasting of the body mass and therefore loss of body weight. Protein depletion, and the unexplained reduced activity of phagocytic cells, predisposes the victim to infections. The hyperglycaemic state presents glucose-rich body fluids which are good culture media for microbial growths (Peel *et al*., 2007) resulting in clinical infections and poor wound healing. In addition, increased protein breakdown and tissue hypoxia due to peripheral circulatory failure, enhances loss of potassium from intra-cellular fluid (ICF) to extra-cellular fluid (ECF) and its subsequent loss via the kidneys.

2.9 Diagnosis of Diabetes Mellitus

The importance of neuropathy, microangiopathy and large vessel disease to the overall diabetic syndrome notwithstanding, they are rarely useful parameters in the diagnosis of the disorder. This is because the diagnosis has come to be based entirely on the hyperglycaemic status of the patient. This situation results from the fact that there is a lack of quantitative methods of evaluating the importance and relative contribution of neuropathy, microangiopathy and large vessel pathology in the overall disease process of diabetes. Furthermore, abnormal carbohydrate tolerance is also a dominant feature of many other conditions besides DM (WHO, 2012).

A definitive diagnosis of diabetes is usually based on any one of the following criteria:

1. Fasting plasma glucose level ≥ 7.0 mmol/L (126 mg/dL).
2. Plasma glucose ≥ 11.1 mmol/L (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test.
3. Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/L (200 mg/dL).
4. Glycated hemoglobin (Hb A1C) ≥ 6.5%.

Of the major tests, fasting plasma glucose is the most common one used to aid in proper diagnosis. The physician performs this after the patient fasts for at least eight hours, usually in the morning before a patient has breakfast. In this test, the patient ingests a 75g glucose load dissolved in water and the doctor checks his blood glucose levels after two hours (ADA, 2012). A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above-listed methods on

a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (SEMDSA, 2012). According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/L) are considered diagnostic for diabetes mellitus.

People with fasting glucose levels from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease (WHO, 2012).

Measurements of this hemoglobin in glycated haemoglobin test, also called glycosylated haemoglobin, glycoheamoglobin, haemoglobin AIc or haemoglobin Al, will help you evaluate the stable linkage of glucose to minor haemoglobin components. It is believed that this link is a function of the tightness of glycemic control. Because glycated hemoglobin levels are highly correlated to adverse clinical outcomes like retinopathy, this test is commonly used to monitor glycemic control in patients with diagnosed diabetes (SEMDSA, 2012). In most cases, this test is not used in the initial diagnosis because there is currently no agreement on standardization. So, a variety of measurement methods and normal ranges are utilized. The major advantage to using glycated hemoglobin is that the specimen can be collected regardless of when the patient last ate (WHO, 2012).

2.10 Diabetes Complications

In recent years, important advances have been made in understanding the pathogenesis of diabetes. Scientists are learning more about how diabetes affects the cardiovascular, renal, nervous, and ophthalmological systems. Diabetic complications can be classified as follows;

2.10.1 Acute (short term) complications

Diabetic ketoacidosis (DKA) is an acute, dangerous complication and is always a medical emergency. DKA is potentially life threatening deficiency of insulin (hypoinsulinism), resulting in severe hyperglycaemia and requiring prompt diagnosis and treatment. Because insulin is unavailable to allow glucose to enter the cells, a dangerous high level of glucose builds up in the blood (hyperglycaemia). Low insulin levels cause the liver to turn to fat for fuel (ketosis); this is normal when periodic, but can become a serious problem if sustained. The body, needing energy, begins to break down fat for energy. As fats are broken down, ketones are produced by the liver. Ketone bodies (e.g. acetone, acetoacetic acid, etc.) are intermediate substrates in that metabolic sequence (Sellers and Dean, 2000). As more and more fat is used for energy, higher levels of ketones accumulate in the blood. This increase in ketones disrupts the acid-base balance within the body, i.e. Elevated levels of ketone bodies in the blood decrease the blood's PH, leading to DKA (Christopher *et al*., 2008).

Hyperosmolar non-ketotic state (HNS) or hyperosmolar (non-ketotic hyperglycaemic) coma is another acute problem associated with diabetes mellitus. This occurs mainly in elderly type 2 cases. It appears to be precipitated by the same factors as in ketoacidosis.

There is uncontrolled glycosuria producing severe diuresis and thus leads to dehydration and haemoconcentration over several days. Electrolyte imbalances are also common Plasma osmolarity is high and leads to coma and death if not treated. Treatment of precipitating factors and associated illness is vital (Liamis *et al.,* 2000).

2.10.2 Chronic (long term) complications

This can further be sub classified into; microvascular and macrovascular chronic complications.

2.10.2.1 Microvascular Complications

Occur due to damage to small blood vessels and the basement membranes, causes impaired delivery of nutrients and hormones to the tissues, resulting in tissue damage. The sites most affected are the retina, renal glomerulus and the nerve sheath. The main complications are diabetic neuropathy, diabetic retinopathy and Nephropathy (Macaulay *et al*., 2000; Camici and Crea, 2007).

Diabetic neuropathy**:** Of all of the long-term complications of diabetes, diabetic neuropathy is the most common and least understood. Fifty percent of all patients with diabetes will experience diabetic neuropathy when they have had the disorder for 25 years or more. Some patients with type 2 diabetes may develop signs of diabetic neuropathy soon after they are diagnosed. Diabetic neuropathy leads to visual, gastrointestinal, sexual, and peripheral vascular disturbances (Dubby and Campbell, 2004). The pathogenesis of diabetic neuropathy is multifactoral with metabolic and vascular involvement. Microangiopathy of the vasa nervorum (i.e, small arteries located in the outer and middle coats of the larger arteries and veins) develop and impair

normal blood flow. Diminished nerve blood flow, in turn, reduces nerve perfusion and oxygenation, which ultimately results in damage to axons and the myelin sheath (Porte, 2001).

Diabetic retinopathy: Diabetic retinopathy is the leading cause of blindness in people younger than 75 years of age in the United States. The risk of retinopathy is related directly to the degree and control or lack of control of hyperglycemia (Huber, 2008). It is present in almost all people who have had diabetes for 20 years or more. The 20-year prevalence of retinopathy is approximately 80% in people who require insulin and approximately 20% in those who do not require insulin (Ejaz *et al*., 2008).

Diabetic Nephropathy**:** Diabetic nephropathy is characterized by albuminuria, hypertension, and progressive loss of renal function. It is a microvascular complication that is the most common single cause of end-stage renal disease if not treated adequately. The earliest clinical indicator of nephropathy is microalbuminuria, which is low but abnormal levels of albumin in the urine (that is, ≥30 mg per day or 20 µg per minute) (He *et al.,* 2004).

2.10.2.2 Macrovascular Complications

These occur due to damage to the large vessels in the body and are more likely in type 2 diabetes. These include; peripheral vascular disease (PVD) and diabetic foot problems.

Peripheral vascular disease**:** Atherosclerosis is common in patients with diabetes, and it causes increased incidences of microangiopathy and macroangiopathy. Macroangiopathy causes an accelerated form of atherosclerosis that affects important

vessel sites, including the coronary, carotid, aortic, iliac, femoral, popliteal, tibial, and peroneal vessels (Boyle *et al.,* 2007). Patients with diabetes are approximately four times more likely to have PVD than patients without diabetes. Many patients with PVD have few symptoms, and others have significant clinical manifestations. Patients with diabetes who also have PVD have an increased incidence of calf vessel involvement (e.g, peroneal and tibial arteries). Patients with diabetes also have greater difficulty developing collateral blood supply in the lower extremities, although the reason for this is not clear. Symptoms include claudication or leg pain on rest, loss of foot hair, dependent rubor and coolness of the feet, and diminished or absent peripheral pulses (Libby, 2011).

Diabetic foot problems**:** Both PVD and diabetic neuropathy are the indirect cause of many diabetic foot problems. Approximately 20% of all hospital admissions of patients with diabetes are for diabetic foot conditions (eg, ulceration, infection, foot deformities, Charcot neuroarthropathy, osteoarthritis, amputation) (Sheetz and King, 2002). Diabetic foot infections may be minor and non-limb threatening or major and limb threatening. Minor infections do not involve bones or joints and usually are caused by staphylococcus aureus. Major and limb-threatening infections usually are polymicrobial and may involve aerobic gram positive cocci, gram negative bacilli, anaerobes, and enterococci. Approximately one-third to two-thirds of patients with diabetes who have foot infections will develop osteomyelitis. These patients are at high risk for amputation (Skyler *et al.,* 1996).

2.11 Metabolic Syndrome

Torgerson *et al.,* (1997) reported elevated aminotransferases and decreased bilirubin as additional characteristics of the metabolic syndrome. They discovered that abnormal liver tests, as well as morphological changes in the liver, are frequent among obese patients. Other frequent disturbances are visceral fat accumulation, insulin resistant, hyperglycaemia and hypertension. These are set of aberrations known as metabolic syndrome.

2.12 Management of Diabetes Mellitus

DM is incurable and so treatment should be directed towards certain objectives. In type 1 cases, objectives should be including the maintenance of optimal physical health, including normal growth and participation in all activities appropriate to the age group. Relief of symptoms of hyperglycaemia and glycosuria as well as prevention of acute complications of hypoglycaemia, ketoacidosis and hyperosmolar coma are some of the attainable goals of therapy (Ukwe, 2006).

The long term goals of diabetic management are to prevent the acute complications of the disorder (e.g. ketoacidosis, hyper-osmolar coma etc) and to control or prevent the "concomitants" of DM including retinopathy, nephropathy, microangiopathy and neuropathy. The maintenance of normoglycaemia in association with ideal body weight is considered fundamental to maintenance of efficient intermediary metabolism in DM (ADA, 2001). The major modalities in diabetic management are dietary control with appropriate physical activity and insulin or oral hypoglycaemic therapy in addition to adequate patient education. On the whole, the general consensus today is that treatment of overt DM should always be directed towards a control of the measured metabolic

abnormalities so as to normalize fat, protein and daily carbohydrate metabolism (Zarich, 2003).

Basically management of DM is divided into two, namely: Non-pharmacological management and Pharmacological management (drug treatment).

2.12.1 Non-pharmacological management of diabetes mellitus

Diet and exercise play important role in control of blood glucose and overall management of DM (Nelson *et al.*, 2002).

2.12.1.1 Diet

In all diabetics, the intake of nutrients should be matched to that with which the individual's supply of insulin, wheather endogenous or exogenous, can cope. The objectives are to maintain an ideal body weight and to avoid large swings in blood glucose concentration. In some obese mild diabetics, weight reduction through dieting is often associated with improvement in glucose tolerance to the extent that no additional therapy is required, at least in the early stages of the disease (Nelson *et al.*, 2002).

2.12.1.2 Exercise

Exercise has a positive role to play in contributing to one's well-being both physically and mentally. It increases glucose utilization. Diabetics should participate in some form of regular exercise as it will enhance stable blood glucose (Goodpaster *et al.*, 2003). In addition to normalization of blood glucose, exercise decreases elevated plasma cholesterol, and thus reduce the development of atherosclerosis (NCEP, 2003).

2.12.2 Pharmacological/drug management of diabetes mellitus

Insulin and oral hypoglycaemic agents are used for the pharmacological management of DM.

2.12.2.1 Insulin therapy

Insulin is used in all type 1 patients who do not have the capability of making insulin and about one third of type 2 may require insulin to normalize blood glucose, especially during times of severe emotional or physical stress. Insulin is necessary in the treatment of diabetic ketoacidosis and in the treatment of hyperosmolar, hyperglycaemic non-ketotic syndrome (Martha and Nolte, 2009). Patients with gestational diabetes may need insulin treatment if diet alone does not adequately control blood glucose levels and some patients without DM who are receiving parenteral nutrition, may require insulin to maintain normal glucose levels during periods of insulin resistantce. The dose of insulin must be adjusted to the individual needs of the patient so that adequate control is achieved with avoidance of ketoacidosis and without the production of hypoglycaemia (Ukwe, 2006).

Insulin mechanism of action: Significant advances have been made in elucidating the molecular aspect of the action of insulin. Insulin binds to a specific receptor on the surface of its target cells. The receptor is a glycoprotein complex (molecular weight 350,000) consisting of two alpha- and two beta-subunits linked by sulphide bridges. The alpha-subunits are entirely extra cellular and carry the insulin-binding site. The beta-subunits are transmembrane proteins, and when insulin binds to the receptor these beta-subunits manifest tyrosine kinase activity, acting upon themselves

(autophosphorylation). This enhances the action of the kinase on other target proteins (Martha and Nolte, 2009). Certain second messengers like phosphatidyl inositol glycan (PIG) and Diacyl glycerol (DAG) which are generated through activation of a specific phopholipase C are also believed to mediate the action of insulin on metabolic enzymes (Taylor and lnsel, 1990).

Insulin preparations: All insulin preparations vary with respect to onset of action, peak of action and duration of action. At present, there are five different types of insulins with reference to species source; Beef (bovine) insulin, purified pork (porcine), beef-pork combination insulin, human insulin converted from pork (semi-synthetic) and human insulin from recombinant DNA technology. Genetic technology has assisted in the synthesis of human insulin for use in spiral cases. There is always a 5% contamination with such substances as insulin polymers, pro-insulin, insulin intermediates and insulin products of degradation in most insulin preparation (Ukwe, 2006).

Human insulin is considered the insulin of choice for most patients with DM. The less similar insulin is to endogenous human insulin, the greater the chances for immunological response. Beef insulin differs from human insulin by three amino acids whereas only a single amino acid distinguishes pork and human insulin. Beef insulin is more antigenic than pork insulin in humans (Ukwe, 2006).

Based on the onset and duration of action, insulin preparation can be classified into (Rang *et al.,* 2007):

i. Regular or short or rapidly acting preparations

ii. Semi-lente or intermediate acting preparations and

iii. Ultra-lente or long acting preparations.

Complications of insulin therapy:

A. Hypoglycaemia**:** This is the most common complication or undesirable effect of insulin therapy. They commonly result from inadequate carbohydrate consumption, unusual physical exertion, and too large a dose of insulin (Martha and Nolte, 2009). Episodes of hypoglycaemia is characterized by hunger, cold sweats and palpitation, nervousness, weakness, headache, inability to concentrate, disturbances in speech and vision. Severe signs are loss of consciousness, convulsions and possible death. In the later stage brain damage may occur. Treatment is usually by appropriate administration of glucose. Glucagon is used in an unconscious patient and will usually restore consciousness within 15 minutes to permit ingestion of sugar (Rang *et al.,* 2007).

B. Immunopathology**:** At least five molecular classes of insulin antibodies may be produced in diabetics during the course of insulin therapy: IgA, IgD, IgE, IgG and IgM. There are two major types of immune disorders in patients:

Insulin allergy: Insulin allergy, immediate type hypersensitivity, is a rare condition in which local or systemic urticaria results from histamine release from tissue mast cells sensitized by anti-insulin IgE antibodies. In severe cases, anaphylaxis results (Martha and Nolte, 2009). Shallow injections result in intradermal insulin delivery and pain, reddened lesions which may scar or form abscess, local allergic reactions due to cutaneous hypersensitivity which may occur early in insulin therapy (but generalized allergic responses are extremely rare) Because sensitivity is usually to non-insulin

contaminants, the human and analog insulins have markedly reduced the incidence of insulin allergy, especially local reactions (Martha and Nolte, 2009).

Immune insulin resistance: A low titer of circulating IgG anti-insulin antibodies that neutralize the action of insulin to a negligible extent develops in most insulin-treated patients. Insulin resistance occurs when a patient requires extremely large doses – often more than 200 units daily (Lahtela *et al.,* 1997). The most common cause of this phenomenon is obesity. The patient that has developed insulin resistance clinically requires increasing doses of insulin for adequate glycaemic control. It may be acute (develops rapidly and of short duration) and this is caused by such factors as infections, trauma, surgery, emotional stress, corticosteroids and other hyperglycaemic or anti-insulin hormones which are usually produced, in response to the formation of ketone bodies and free fatty acids both of which have been shown to inhibit glucose entry in brain and skeletal muscle cells (Tripathi, 1999). Insulin resistance may on the other hand be chronic where the patient who has been on insulin for a long time gradually needs increasing amounts for adequate glycaemic control. This type of resistance is common with bovine and porcine insulin. Development of chronic insulin resistance is an indication for switching over to a more purified newer preparation. After instituting purer preparations, insulin requirement gradually decreases over weeks or months and most of the patients eventually stabilize at about 60 I.U/day (Tripathi, 1999).

Lipodystrophy at injection site: Shallow injections result in intradermal insulin delivery and pain, reddened lesions which may scar or form abscess (Le Roith *et al.,* 2000). Local allergic reactions due to cutaneous hypersensitivity which may occur early in insulin therapy (but generalized allergic responses are extremely rare) and

lipodystrophies including lipoatrophy (loss of fat around the site of insulin injection) and lipohypertrophy (development of fibrofatty masses due to over-use of a single injection site). However, this may be corrected by avoiding the specific injection site or by liposuction (Altan-Gepner *et al*., 1996).

C. Rebound hyperglycaemia (smogyi effect): Can follow excessive administration of insulin. This results from the release of the insulin-opposing or counter-regulatory hormones in response to insulin-induced hyperglycaemia. This can cause hyperglycaemia before breakfasts following an unrecognized hypoglycaemic attack during sleep in the early hours of the morning. It is important to recorgnise this possibility to avoid the mistake of increasing (rather than reducing) the dose of insulin in this situation (Rang, *et al.,* 2007).

D. Weight gain: Patients that do not comply strictly to their diet regulations and are therefore predisposed to weight gain may show progressive weight gain especially where the insulin dose is increased inappropriately since insulin tend to induce a feeling of hunger (Gaster and Hirsh, 1998).

2.12.2.2 Oral hypoglycaemic agents

Anti-diabetic medications treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, liraglutide and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral anti-hyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors (Ukwe, 2006).

Clinically used oral hypoglycaemics can be classified as follows:

Insulin secretagogue (sulfonylureas (SUs))**:** They are grouped as;

First-generation agents: tolbutamide, acetohexamide, tolazamide and chlorpropamide.

Second-generation agents: glipizide**,** glyburide or glibenclamide**,** glimepiride**,** gliclazide**,** glycopyramide and gliquidone.

SUs were the first widely used oral anti-hyperglycaemic medications. As a group, the SUs were developed as a result of the finding that a sulfonamide derivative used for the treatment of typhoid fever resulted in a marked lowering of blood glucose. They are insulin secretagogues. The "second-generation" drugs are now more commonly used. They are more effective than first-generation drugs and have fewer side-effects. All may cause weight gain. Typical reductions in glycated hemoglobin (A1C) values for second-generation sulfonylureas are 1.0–2.0% (Jonsson *et al.,* 1994).

*Mechanism of Action***:**

Stimulation of insulin release from beta-cells of the pancreas: The drug molecules bind to a specific receptor -sulphonylurea receptor on the pancreatic beta-cell membrane causing depolarization by reducing conductance of ATP sensitive K channels. This results in the opening of voltage-gated calcium channel causing Ca2+ influx and degranulation with the release of pre-formed insulin. Calcium channel blockers can inhibit this action of sulphonylureas in-vitro (Jonsson *et al.,* 1994).

Reduction of serum glucagon levels: It has been recently established that chronic administration of sulphonylureas in Type 2 patients reduce plasma level of glucagon (Mycek *et al.,*2000) and increase somatostatin release and possibly concomitant

decrease in hepatic degradation of insulin (Tripathi, 1999). The mechanism for suppressive effect on glucagon levels is unclear but may involve indirect inhibition due to enhanced release of both insulin and somatostatin, which inhibit alpha cell secretion (Mycek *et al.,* 2000).

Potentiation of binding of insulin to target tissues (and insulin receptors): SUs increase the binding affinity of insulin to insulin receptors as well as increase the numbers of insulin receptors in responsive tissues such as liver and skeletal muscles (Mycek *et al*., 2000). The ultimate effect is enhanced effectiveness of insulin especially in Type 2 patients (Jonsson *et al.,* 1994).

Pharmacokinetics: All sulphonylureas are well absorbed orally and most reach peak concentrations within 2-5 hours. They are more than 90% plasma protein bound and are implicated in interactions with other drugs. They have low volumes of distribution (0.2-0.4 L/kg). Some are primarily metabolized to produce active metabolites (tolazamide and glyburide), while others are mainly excreted unchanged in urine. They should therefore be used with caution in patients with hepatic and/or renal insufficiency. SUs cross the placenta and stimulate fetal beta cells to release insulin; as a result, their use is contra-indicated in pregnancy, and gestational diabetes is managed with diet supplemented if necessary with insulin (Karam, 1997).

General side effects of SUs: The SUs as a group are usually well tolerated. Hypoglyceamia is the main side effect associated with these drugs. It can be severe and its incidence is related to the potency and duration of action. The highest incidence occurs with chlorpropamide and glyburide, and the lowest with tolbutamide. Such

hypoglyceamia can be prolonged, and this can be serious in elderly patients and in patients with impaired renal function (Aguwa and Omole, 1996). About 3% of patients experience gastrointestinal disturbances which include nausea, vomtting, constipation or diarrhea. Allergic skin reactions may appear as erythema, urticaria, pruritus and photosensitization. Haemopoietic effects though very rare have been reported and may be severe. These include leukopenia, thrombocytopenia, pancytopenia and rarely aggranulocytosis. Cholestatic jaundice occurs more often with chlorpropamide. Other adverse effects such as muscular weakness, paresthesia, headache, tinnitus and mental confusion may occur and are sometimes confused with the normal progression of DM (Aguwa and Omole, 1996).

Contraindications: SUs are contraindicated in Type 1 DM, liver or kidney disease, sulfa allergy; they should be avoided in those with increased risk of hypoglycaemia such as the elderly, debilitated or malnourished patients, and those with adrenal or pituitary insufficiency. Where a SU needs to be used in patients, at increased risk of hypoglycaemia, a short-acting drug such as tolbutamide, gliquidone, or gliclazide may be preferred; these three SUs, being principally inactivated in the liver, are perhaps particularly suitable in renal impairment, although careful monitoring of blood-glucose concentration is essential (Ogungbe and Madehin, 2011).

Drug interactions: Drugs which may increase hypoglycaemic effects of SUs include: analgesics like azapropazone, phenylbutazone and the salicylates, cimetidine, Ace inhibitors (mainly captopril and enalapril), sulfonamides, coumarin anticoagulants like warfarin, beta-blockers, alcohol, allopurinol, azole antifungals, floroquinolones, heparin, MAOIs, tetracycline and TCAs. Drugs which may decrease hypoglyceamic

effects of SUs are: thiazides, hydantoins, oral contraceptives, corticosteroids, rifamycins, thyroid hormones and chlorpromazine (Ogungbe and Madehin, 2011).

Clinical uses/advantages: SUs require functional B cells, so they are useful in the early stages of type 2 DM. They can be combined with metformin or with thiazolidinediones.Used in lean patients with high blood glucose (Rang *et al*, 2009).

Non-sulfonylurea secretagogues (meglitinides): Examples are; repaglinide and nateglinide. Nateglinide is structurally derived from D-phenylalanine. Although these drugs are not SUs, they have similar pharmacological actions. Typical reductions in glycated hemoglobin (A1C) values are 0.5–1.0%.

Mechanism of action: Megliinides help the pancreas produce insulin and are often called "short-acting secretagogues". They also act on the same potassium channels as sulfonylureas, but at a different binding site. They interact with the ATP-sensitive K^+ channel and increase insulin secretion from beta cell of pancrease. They act like SUs, by blocking the SU receptor on K_{ATP} channels in pancreatic beta cells (Rang *et al*, 2009).

Pharmacokinetics: They have fast onset of action, short half-lives (1hr), short duration of action (4hr), they are metabolized in the liver and excreted with bile. So these agents are taken immediately before meal (Rang *et al*, 2009; Ogungbe and Madehin, 2011)).

Side effects: Meglitinides generally have mild side effects which are well tolerated. A potential advantage is that they may cause less weight gain and hypoglyceamia than conventional SUs (Rang *et al*, 2009).

Contraindications: They should be used with caution in hepatic and renal insufficiency (Ogungbe and Madehin, 2011).

Clinical advantage: Effective in reducing postprandial glucose rise in type 2 diabetic patients whose condition is inadequately controlled with diet and exercise (Rang *et al*, 2009).

Biguanides**:** Examples include:

1. Metformin is the only drug of this class presently in use and may be the best choice for patients who also have heart failure (Martha and Nolte, 2009).
2. Phenformin was used from 1960s through 1980s, but was withdrawn due to lactic acidosis risk.
3. Buformin also was withdrawn due to lactic acidosis risk.

They differ significantly from the sulphonylureas in that they do not cause hypoglycaemia in normoglycaemic (non-diabetic) individuals and do not stimulate pancreatic beta-cells to produce insulin. Among common diabetic drugs, metformin is the only widely used oral drug that does not cause weight gain (Mycek *et al.,* 2000). Metformin is usually the first-line medication used for treatment of type 2 diabetes. In general, it is prescribed at initial diagnosis in conjunction with exercise and weight loss, as opposed to in the past, where it was prescribed after diet and exercise had failed.

There is an immediate release as well as an extended-release formulation, typically reserved for patients experiencing GI side-effects. It is also available in combination with other oral diabetic medications (Ukwe, 2006). Typical reduction in glycated hemoglobin (A1C) values for metformin is 1.5–2.0%.

Mechanism of action: The mechanism of action of this group of drugs remains elusive. The biguanides do not act through insulin release as they are effective even in the absence of functioning pancreatic beta cells. Their main action is probably to increase glucose uptake across the cell membrane in the skeletal muscle. However, currently proposed mechanisms of action include (Ukwe, 2006):

i. Direct stimulation of glycolysis in peripheral tissues, with increased glucose removal from blood.
ii. Reduced hepatic gluconeogenesis
iii. Slowing of glucose absorption from the GIT
iv. Reduction of plasma glucagon levels
v. Increased insulin binding to insulin receptors

Metformin is claimed to have additional metabolic actions in that it reduces plasma concentrations of low-density and very low-density lipoproteins (LDL and VLDL, respectively), effects that could be useful in reducing atheroma (Ukwe, 2006).

Pharmacokinetics: Metformin has a half-life of 1.5-3 hours, is not bound to plasma proteins, is not metabolized, and is excreted by the kidneys as the active compound. As a consequence of metformin's blockade of gluconeogenesis, the drug may impair the hepatic metabolism of lactic acid. In patients with renal insufficiency, biguanides

accumulate and thereby increase the risk of lactic acidosis, which appears to be a dose-related complication (Martha and Nolte, 2009).

Side effects: The commonest unwanted effects of metformin are dose-related GI disturbances (e.g. bitter taste, anorexia, diarrhoea, nausea, vomitting), which are usually but not always transient. Lactic acidosis complicates biguanides therapy in diabetic patients with renal insufficiency. This occurs most often with phenformin. Metformin does not carry a high risk of lactic acidosis. Long-time use of metformin may interfere with absorption of vitamin B12 (Rang *et al*, 2009).

Clinical advantage/use: No hypoglycaemia and no weight gain. Metformin is used to treat patients with type 2 DM. it does not stimulate appetite, rather it causes anorexia and is consequently useful in the majority of type 2 patients who are obese and fail treatment with diet alone. It can be combined with SUs glitazones or with insulin to decrease its dosage in certain patients (Mycek *et al.,* 2000).

Contraindications: Metformin is contraindicated in patients with renal/liver diseases, any form of acidosis, congestive heart failure, in pregnancy and use of contrast radiography (should be stopped 48hrs before) (Ogungbe and Madelin, 2011).

Thiazolidinediones (glitazones): Drugs in this group are new entrants in drug therapy of DM. Examples are: rosiglitazone (the European Medicines Agency recommended in September 2010 that it be suspended from the EU market due to elevated cardiovascular risks), pioglitazone, Ciglitazone, englitazone and troglitazone (used in 1990s, withdrawn due to hepatitis and liver damage risk) (Rang *et al.,* 2009). The

thiazolidinediones (TZDs) also known as glitazones were developed following the chance observation that a clofibrate analogue, ciglitazone, which was being screened for effects on lipids, unexpectedly lowered blood glucose (Rang *et al*., 2009). Typical reductions in glycated hemoglobin (A1C) values are 1.5–2.0%.

Mechanism of action: TZDs, bind to a nuclear receptor called the peroxisome proliferator-activated receptor-y (PPARγ), a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. It is complexed with retinoid X receptor (RXR). These PPARs act on peroxysome proliferator responsive elements (PPRE) (Mycek *et al.,* 2000). The PPREs influence insulin-sensitive genes, which enhance production of mRNAs of insulin-dependent enzymes. The final result is better use of glucose by the cells. PPARy occurs mainly in adipose tissue, but also in muscle and liver. It causes differentiation of adipocytes (this contributes to the unwanted effect of weight gain), increases lipogenesis and enhances uptake of fatty acids and glucose. It also promotes amiloride-sensitive sodium ion reabsorption in renal collecting ducts, explaining the adverse effect of fluid retention. TZDs are exogenous agonists, which cause the PPARy-RXR complex to bind to DNA, promoting transcription of several genes with products that are important in insulin signaling. These include lipoprotein lipase, fatty acid transporter protein, and adipocyte fatty acid binding protein, Glut-4, phosphoenolpyruvate carboxykinase, malic enzyme and others (Rang *et al*., 2009).

Pharmacokinetics: Both rosiglitazone and pioglitazone are rapidly and nearly completely absorbed, with time to peak plasm concentration of less than 2 hours. Both are highly (□99%) bound to plasma proteins, both are subject to hepatic metabolism

and both have a short (□7 hours) elimination half-life for the parent drug, but substantially longer (up to 150 hours for rosiglitazone, up to 24 hours for pioglitazone) for the metabolites. Rosiglitazone is metabolized by CYP2C8 to weakly active metabolites, pioglitazone mainly by a CYP2C isozyme and CYP3A4 to active metabolites. The metabolites of rosiglitazone are eliminated mainly in urine, and those of pioglitazone mainly in bile (Rang *et al.,* 2009).

Side effects: The serious hepatotoxicity of ciglitazone and troglitazone was not encountered during clinical trials of rosiglitazone or pioglitazone, and reports of liver dysfunction since their general release have been rare. Regular blood tests of liver function are currently recommended. The commonest unwanted effects of rosiglitazone and pioglitazone are weight gain and fluid retention. Fluid retention is a substantial concern, because it can precipitate or worsen heart failure, which contraindicates their use. Symptoms of uncertain course, including headache, fatique and GI disturbances, have also been reported (Rang *et al*., 2009).

Contraindications: TZDs are contraindicated in patients with liver disease or congestive heart failure, in pregnant or breast feeding women and in children. It is theoretically possible that these drugs could cause ovulation to resume in women who are anovulatory because of insulin resistance (e.g. with polycystic ovary syndrome) (Ogungbe and Madehin, 2011).

Drug interactions: TZDs are additive with other oral hypoglycaemic drugs. In Europe, both rosiglitazone and pioglitazone are contraindicated for use with insulin because of

concern that these combinations increase the risk of heart failure, although in the USA TZDs are widely used in combination with insulin (Rang *et al*., 2009).

Clinical uses: Because insulin resistance is one important component of the pathogenesis of type 2 DM, and has been implicated in the excess cardiovascular mortality that accompanies the common 'metabolic syndrome' (visceral obesity, hypertension, dyslipidemia, insulin resistance, etc), there is a good rationale for glitazones in type 2 DM. This probably explains their wide spread adoption into clinical practice, especially in the USA. There is, however, as yet no evidence that this optimism is justified in terms of improved clinical outcomes. Clinical trial evidence to date is from short-term studies and supports their use in combination with metformin or with a SU in patients whose condition is inadequately controlled on one of these drugs and are unsuited to addition of the other. It is hoped that evidence to support wider and more useful applications (e.g. as monotherapy or as triple therapy with both metformin and a SU) will soon be forthcoming. Potential clinical uses unrelated to diabetes, including fatty liver and atheromatous disease, are under investigation (Gale, 2001).

Alpha glucosidase inhibitors**:** Examples include; miglitol, acarbose and voglibose.

Alpha-glucosidase inhibitors are "diabetes pills" but not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or sensitivity (Reabasa-Lhoret and Chiasson 1998). These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity. These agents are effective by themselves only in the earliest stages

of impaired glucose tolerance, but can be helpful in combination with other agents in type 2 diabetes (Rosak *et al.,* 1995).

Acarbose is relatively new and was approved by the FDA in the USA in September 1995 as monotherapy in patients with type 2 DM whose previous treatments with diet and exercise regimens has failed. It was also approved as an adjunct treatment for patients with type 2 DM who are taking oral SUs without adequate blood glucose control (Willms and Ruge, 1999). Acarbose is a water soluble pseudotetrasaccharide that resembles dietary oligosaccharides derived from starch digestion. Selected clinical trials show that acarbose is effective in reducing haemoglobin A1C (Hb A1C) and post prandial glucose (PPG) (Kageyama, *et al.,* 1997). Typical reductions in glycated hemoglobin (A1C) values are 0.5–1.0%.

Mechanism of action: One effective way to treat overweight diabetics is to administer drugs that inhibit the enzymes involved in the breakdown of carbohydrates in the intestines. Acarbose is a sham sugar (a complex oligosaccharide) that binds 1000 times more avidly than natural carbohydrates to GIT disaccharidases such as alpha-glucosidase which is situated in the brush border of the intestine. This result in poor absorption of dietary monosaccharides (glucose and other hexoses) and concomitant decrease in postprandial blood glucose level (Bischoff, 1995). The inhibitory effects of acarbose vary, depending on the enzyme on which it is acting. Inhibition is greatest with glucoamylase, followed by sucrose, maltase, and isomaltase. Acarbose has little to no effect on lactose, so lactose absorption is not affected. This competitive inhibition is reversible, so the digestive process is slowed down but not permanently blocked. The result is a reduced rate of glucose production and absorption, and a more even

distribution of glucose absorption throughout the small and large intestine. The delayed absorption decreases the sharp, postprandial blood glucose peak that normally occurs after meals (Bischoff, 1995).

Pharmacokinetics: following oral administration of acarbose, very little drug (1-2%) is systematically absorbed. Approximately 51% of the drug is excreted unchanged in the feaces. The remainder of the drug is degraded either by digestive enzymes in the GIT and/or by the natural of the gut. Systematically absorbed acarbose is excreted renally (Bischoff, 1995).

Side effects: the main adverse effects of acarbose are flatulence, abdominal pain and diarrhea, which occur quite frequently (Ukwe, 2006).

Clinical advantage: used in pre-diabetic and obese petients (Ogungbe and Madehin, 2011).

2.12.2.3 Newer hypoglycaemic agents (peptide analogues)

Injectable incretin mimetics: Incretins are insulin secretagogues. The two main candidate molecules that fulfill criteria for being an incretin are glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (glucose-dependent insulinotropic peptide, GIP). Both GLP-1 and GIP are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4) (Abraham, *et al.,* 2002; Demuth *et al.,* 2005).

Glucagon-like peptide (GLP) agonists bind to a membrane GLP receptor. As a consequence, insulin release from the pancreatic beta cells is increased. Endogenous

GLP has a half-life of only a few minutes, thus an analogue of GLP would not be practical (Rocca *et al.,* 1999). Examples include:

Exenatide: (also Exendin-4, marketed as Byetta) is the first GLP-1 agonist approved for the treatment of type 2 diabetes. Exenatide is not an analogue of GLP but rather a GLP agonist (Chee *et al.,* 2009). Exenatide has only 53% homology with GLP, which increases its resistance to degradation by DPP-4 and extends its half-life. In clinical studies, exanatide therapy was shown to have multiple actions such as potentiation of glucose mediated insulin secretion, suppression of postprandial glucagon release through as yet unknown mechanisms, slowed gastric emptying, and central loss of appetite. The increased insulin secretion is speculated to be due in part to an increase beta-cell mass. It is not known whether the increased beta-cell apoptosis, increased beta-cell formation, or both (Martha and Nolte, 2009). Exanatide is absorbed equally from injection sites, reaching a peak concentration in about 2 hours with duration of action of up to 10 hours. It undergoes glomerular filtration. Exanatide is injected subcutaneously within 60 minutes before meal. The major adverse effects are nausea (about 44% of users) and vomiting and diarrhea. Weight loss is reported in some users, presumably because of the nausea and anorectic effects. A serious and, in some cases, fatal adverse effect of exanatide is necrotizing and hemorrhagic pancreatitis (Martha and Nolte, 2009). Typical reductions in A1C values are 0.5–1.0%.

Liraglutide: a once-daily human analogue (97% homology), has been developed by Novo Nordisk under the brand name Victoza. The product was approved by the European Medicines Agency (EMEA) on July 3, 2009, and by the U.S. Food and Drug Administration (FDA) on January 25, 2010.

Taspoglutide: is presently in Phase III clinical trials with Hoffman-La Roche. These agents may also cause a decrease in gastric motility, responsible for the common side-effect of nausea, and is probably the mechanism by which weight loss occurs.

Dipeptidyl peptidase-4 (dpp-4) inhibitors: GLP-1 analogs resulted in weight loss and had more gastrointestinal side-effects, while in general DPP-4 inhibitors were weight-neutral and increased risk for infection and headache, but both classes appear to present an alternative to other antidiabetic drugs. However, weight gain and/or hypoglycaemia have been observed when DPP-4 inhibitors were used with sulfonylureas; effect on long-term health and morbidity rates are still unknown (Rang, *et al.,* 2007). Examples are; Sitagliptin (Januvia) FDA approved Oct 2006, vildagliptin (Galvus) EU Approved 2008, saxagliptin (Onglyza) FDA Approved July 2009, linagliptin (Tradjenta) FDA Approved May 2, 2011 and alloglíptin.

DPP-4 inhibitors lowered hemoglobin A1C values by 0.74%, comparable to other antidiabetic drugs (Martha and Nolte, 2009). DPP-4 inhibitors increase blood concentrations of the incretin GLP-1 and GIP by inhibiting their degradation by dipeptidyl peptidase-4, the enzyme that degrades incretin and other GLP-1-like molecules. This ultimately decreases postprandial glucose excursions by increasing glucose-mediated insulin secretion and decreasing glucagon levels. Sitagliptin has an oral bioavailability of over 85% and a half-life of approximately 12 hours; the dosage is 100 mg orally once daily. Common adverse effects include nasopharyngitis, upper respiratory infections, and headaches. Rarely, severe allergic reactions have been reported. Dosage should be reduced in patients with renal impairment. Sitagliptin can

be given as monotherapy or combined with metformin or TZDs (Martha and Nolte, 2009).

Injectable amylin analogues: Amylin agonist analogues slow gastric emptying and suppress glucagon release via unknown mechanisms, and have central nervous system-mediated anorectic effects. They have all the incretins actions except stimulation of insulin secretion. Until 2007, pramlintide was the only clinically available synthetic amylin analogue approved for use in persons with type 1 and type 2 DM. Like insulin, it is administered by subcutaneous injection and in addition to insulin in those who are unable to achieve their target postprandial blood sugars. It is rapidly absorbed after subcutaneous administration; levels peak within 20 minutes, and the duration of action is not more than 150 minutes. Pramlintide is renally metabolized and excreted.The most frequent and severe adverse effects of pramlintide is hypoglycaemia and GI symptoms including nausea, vomiting, and anorexia. Nausea occurs mostly at the beginning of treatment and gradually reduces (Martha and Nolte, 2009). Typical reductions in A1C values are 0.5–1.0%.

2.12.2.4 Potential new antidiabetic drugs

Several agents are currently being studied, including α_2-adrenoceptor antagonists and inhibitors of fatty acid oxidation. Lipolysis in fat cells is controlled by adrenoceptors of the β_3 subtypes. The possibility of using selective β_3 agonists, currently in development, in the treatment of obese patients with type 2 diabetes is being investigated. There is interest in inhibitors of protein kinase C, for example ruboxistaurin (LY333531), an inhibitor specific for the β isoform of PKC, because of evidence implicating activation

of this pathway in the development of vascular diabetic complications (Rang *et al.,* 2007).

2.12.3 Islet cell and pancreas transplant

Islet cell transplants have received increased attention with the success of the Edmonton Protocol□, which used a steroid-free immunosuppressive regime as well as other techniques (Shapiro, 2000). All patients achieved insulin independence after one year in contrast to a previous rate of 8%. Many issues remain regarding islet cell transplantation, including availability of transplant material and assessment of long term outcomes (Oberholzer, 2003; Robertson, 2004).

Pancreas transplantation on the other hand is the only available treatment for type 1 DM that induces an insulin-dependent normoglycaemic state (Gruessner and Sutherland, 2002). Because it requires immunosuppression, it has been mostly used in uraemic diabetic recipients of kidney transplant with high success rate, particularly when performed simultaneously. As of October 2002, 14,000 pancreas transplantations had been performed in the United States (Sutherland *et al*., 2004). The 1 year graft survival rates (defined as total freedom from insulin therapy, normal fasting glucose concentrations, and normal or only very slightly elevated Aic values) are more than 80% (Spellman, 2007). Pancreas transplantation improves the quality of life, and there is evidence that the developments of microvascular or macrovascular abnormalities are prevented or slowed (Meiver-Levy and Ferber, 2004). Furthermore, pancreatic transplantation prolongs life. Moreover, pancreatic transplantation is considered a viable option for diabetic patients with end stage renal disease who must undergo kidney transplant. However, pancreatic transplantation alone in a patient with diabetes

mellitus remains controversial because the disadvantages of exogenous insulin therapy are replaced with risks of the transplantation procedure itself and the complications of immunosuppressive medications (Robertson, 2004).

2.13 Need and Scope for Alternative Remedies

Regardless of the type of diabetes, patients are required to control their blood glucose level with medications and/or by adhering to an exercise program and a dietary plan (Alam and Mahpara, 2003). Insulin therapy by injection is given to those with type 1 DM and also to some patients with type 2 DM when oral hypoglycaemic drugs fail to lower blood glucose.These treatment agents have their setbacks, ranging from developing of resistance and adverse effects to lack of responsiveness in large number of patients population (Dey *et al.,* 2002). Sulfonylureas lose effectiveness for 44% population within six years. Also, these treatments are associated with side effects or even toxic effects (e.g., thiazolidinedione (troglitazone) may cause liver toxicity; sulfonylureas might worsen heart disease, lower the glucose level below the normal range and increase the body weight gain; bloating, flatulence, diarrhea and abdominal discomfort and pain are the major complaints with glucosidase inhibitors) (Defronzo, 1999; Micheal *et al.,* 2005). Moreover, none of these glucose-lowering agents adequately controls the hyperlipidaemia that is frequently met with the disease (Derek, 2001).

Recently there has been a growing interest in hypoglycaemic agents from natural products, especially those derived from plants because plant sources are usually considered to be less toxic, with fewer side effects than synthetic sources (Farswan *et al.,* 2009). Currently, over 200 pure phytochemicals are known to have hypoglycaemic properties (Li *et al*., 2004). There has been increasing demand for the use of plant

products with anti-diabetic activity due to low cost, easy availability and lesser side effects. Therefore, plant materials are continuously being scrutinized and explored for their effect as hypoglycaemic agents. Plants used in traditional medicine to treat DM represent a valuable alternative for the control of this disease (Marles and Farnsworth, 1995).

2.14 Herbal Remedies

Most herbs and plant derivatives have been employed even as foods and medicines over the years and are still being used. As per ancient literature, more than 800 plants are reported to have antidiabetic properties. (Eddouks and Maghrani, 2004 as cited in Jarald *et al.,* 2008). DM whether type 1 or 2 has been managed over the centuries in traditional communities before the advent of orthodox medicine. Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycaemic activity (Kesari *et al*. 2007). Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine (Jarald *et al*., 2008).

Plants-based products have been popular all over the world for the centuries. A great number of medicinal plants used in the control of DM have been reported. Some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complications of the disease. Some herbs have also been proven to help in the regeneration of β-cells and in overcoming resistance. In addition to maintaining normal blood sugar level, some herbs are also reported to possess antioxidant activity and cholesterol-lowering action. The hypoglycaemic effect of some herbal extracts have been confirmed in human and animal models of type 2 diabetes and conventional drugs have been derived from the active molecules of these medicinal plants (Jarald *et al*.,

2008). Metformin, a less toxic biguanides and potent oral glucose-lowering agent, was developed from *Galega officianalis* and used to treat diabetes (Daniel and Norman, 2001).

2.15 Anti-diabetic Plants in Traditional Medicines

The NAPRALERT database lists over 1200 species of plants representing 725 genera in 183 families extending from the marine algae and fungi with anti-diabetic activity. Over half of these have been used ethnopharmacologically in traditional medicine as anti-diabetics, and some 50% of these traditional remedies have been studied experimentally (Marles and Farnsworth, 1995).

In Africa, the following plants have been reported to be used in the treatment of diabetes: *Pileostigma thonningii, Xylopia aethiopica, Combretum micratum, Ficus capensis, Cassia sieberiana, Nauclea pobeguinii, Ocimum sanctum, Anacardium occidentale, Jatropha curcas, Nauclea latifolia, Allium sativum, Citrus medica, Moringa oleifera, Persea Americana, Tamarindus indica, Afzeria Africana, Carica papaya, Garcinia kola, Psidium guajava,* among others (Balde *et al.,* 2006).

In Nigeria, different ethnic groups use different plants for treatment of diabetes (Gbolade, 2009). Most of the herbal remedies in DM are multi-component in nature. Some of the mono-component herbal remedies used in management of diabetes among various ethnic groups and which are more frequently cited include; *Morinda lucida, Allium cepa, Allium sativum, Ceiba pentandra, Xanthosoma sagittifolia, Musa sapientum, Persea americana, Congronema latifolium, Vernonia amygdalina, Carica papaya, Citrus aurantiifolia, Bidens pilosa, Momordica charantia, Ocimum*

gratissimum, Afromomum meleguat and *Aloe vera* (Aiyeloja and Bello, 2006; Okoli *et al*., 2007; Gbolade, 2009; Lawal *et al*., 2010).

2.15.1 *Persea americana* Mill. (laureceae)

2.15.1.1 Description

P. americana is a medium to large tree, 9-20 m in height. Avocado tree is distributed geographically. The avocado is classified as an evergreen, although some varieties lose their leaves for a short time before flowering. The tree canopy ranges from low, dense, and symmetrical to upright and asymmetrical. Leaves are 7-41 cm in length and variable in shape (elliptic, oval, and lanceolate). They are often pubescent and reddish when young, becoming smooth, leathery, and dark green when mature. Flowers are yellowish green and 1-1.3 cm in diameter. The many-flowered inflorescences are borne in a pseudoterminal position. The central axis of the inflorescence terminates in a shoot. The fruit is a berry, consisting of a single large seed, surrounded by a buttery pulp. It contains 3-30% oil (Florida varieties range from 3% to 15%). The skin is variable in thickness and texture. Fruit color at maturity is green, black, purple, or reddish, depending on the variety. Fruit shape ranges from spherical to pyriform and weighs up to 2.3 kg (Cobley and Steele, 1976; Wolstenholme and Whiley, 1999). An average avocado tree produces about 120 avocados annually. *P. americana* originated from Mexico and Central America, but was introduced to the West African sub-region over a century ago. It is now cultivated for commercial purposes (Storey, *et al.,* 1987). The plant grows well in the forest and savanna areas of Nigeria and is known by most ethnic groups.

2.15.1.2 Taxonomy (scientific classification)

Kingdom:	Plantae
Phylum/Division:	Magnoliophyta
Subphylum:	Magnoliophytina
Class:	Magnoliopsida
Subclass:	Magnoliidae
Order:	Laurales
Family:	Lauraceae
Genus:	Persea
Species:	*americana*
Authority:	Miller

2.15.1.3 Names of Persea americana

Among the international names include: "avocado", "avocado pear", "alligator pear" or "butter fruit" (Common name, English), *"Poire d'alligator"*, *"avocat"* "*zabelbok*" or "*zaboka"* (French), *"avocadobirne"* or *"alligatorbirne"* (German), *"aguacate"* or *"pagua"* (Spanish), *"awokado"* (Thai), *"you li"* (Chinese), *"bo"* or *"lê dâù"* (Vietnamese) *"adpukat"* or *"avokad"* (Indonesian), *"bata"* (Pidgin English) *"mparachichi", "mpea"* or *"mwembe mafuta"* (Swahili).

The local names amongst some ethnic groups in Nigeria include: *"ube bekee"* or *"ube oyibo"* (Igbo, South-east), *"pia"* or *"piya"* (Hausa, North), *"igba* or *apoka"* (Yoruba, South-west), *"uber"* (Ndokwa, Delta State), *"eben mbakara"* (Ibiobio, Akwa Ibom State) and *"orumwa"* and *"olumuebo"* (Bini and Esan, Edo State) (Aiyeloja and Bello, 2006; Okoli *et al*., 2007; Gyang, 2009; Lawal *et al*., 2010).



Plate I: *Persea americana* tree



Plate II: *Persea americana* leaves

Source: Pictures taken from the plants's natural habitat in Samaru, Zaria

2.15.1.4 Phytochemical constituents of Persea americana

From the phytochemical screening earlier conducted on *PA,* it has been reported that the plant parts (leaf, stem bark and root bark) contain pharmacologically active compounds including but not limited to alkaloids, saponins, flavonoids, steroids, carbohydrates, fatty acids, flavonols, essential oils, tannins, phlobatannins, flavones, terpenoids, cardiac glycosides and polysaccharides (Anita *et al*., 2005; Gyang, 2009; Owolabi *et al.,* 2010; Ayinde *et al*., 2011). According to Owolabi *et al*., (2010), Isolation of the bioactive phytoconstituents of the leaves of *PA* (lauraceae), yielded; isorhamnetin, luteolin, rutin, quercetin and apigenin. Isohamnetin has been fully characterized. These compounds were evaluated for their ability to scavenge free radical using $DPPH^+$ and H_2O_2 systems. Persin (fatty acid derivative), isolated from avocado leaves has been shown to possess antifungal properties. The avocado and other fruits have been reported to slow liver damage caused by galactosamine in rat. Researchers have also isolated compounds from avocado, which showed marked inhibitory activities towards superoxide and nitric oxide generation in cell culture systems (Muanya, 2007).

2.15.1.5 Ethnomedical uses of Persea americana

Reported ethno medical uses of the plant include (Muanya, 2007; Oluwole *et al.,* 2011):

Fruit - food, wound healing, etc.

Seed - hypertension, diabetes, stomach ache, bronchitis, etc.

Leaf - diabetes, hypertension, cough, ulcer, diarrhea, as an emmenagogue, etc.

Stem bark - asthma, cough, fever, diabetes, kidney and liver problems, etc.

Root bark - for skin blemishes, convulsion and epilepsy, wound, as an abortifacient, for female disorders among others.

2.15.1.6 Research reports/scientifically validated ethnomedical uses of Persea americana

Previous studies have shown that *PA* possesses a catalogue of pharmacological activities, including but not limited to:

The leaf extract:

Anti-ulcer properties (Ukwe and Nwafor, 2004; Oluwole *et al.,* 2011)

Hypotensive activity (Adeboye *et al*., 1999)

Hepatoprotective effect (Ekor *et al*., 2006)

Analgesic and anti-inflammatory properties (Adeyemi *et al.,* 2002)

Anti convulsant property (Ojewole and Amabeoku, 2006)

Vasorelaxant property (Owolabi *et al.,* 2005)

Anti-hyperlipidaemic effect (Kolawale *et al.,* 2012)

The seed extract:

Anti-microbial activity (Idris *et al.,* 2009)

Hypoglycaemic effect (Edem, 2009; Edem *et al.,* 2009)

Hypotensive property (Anaka *et al.,* 2009)

Anti-hypertensive and anti-hyperlipidaemic properties (Imafidon and Amaechina, 2010)

Hypolipidaemic and hypocholesterolic effect (Nwaoguikpe and Braide, 2011)

The stem barks extract:

Hypoglycaemic property (Gyang, 2009)

2.16 Theoretical Framework

A large number of drugs including herbs and minerals with suspected anti-diabetic activity have been successfully tested in the laboratory. These approaches involve the use of scientific models which simulate or mimic human disease conditions. Animal models have been used extensively in diabetes. For example, early studies used Pancreatectomised dogs to confirm the central role of the pancreas in glucose homeostasis, culminating in the discovery and purification of insulin (Rees and Alcolado, 2005). Presently, experimental models for pharmacological screening and evaluation are chosen based on their simplicity, rapidity and reproducibility. Today, animal experimentation is contentious and subject to legal and ethical restrictions that vary throughout the world. Thus, the animal model chosen for this study have been found to satisfy this condition in various published research works.

2.16.1 Models for alloxan-induced diabetes

Models are typically animal preparations that mimic a human condition including DM. A number of models have been developed to screen drugs for beneficial effects in diabetic disorders. Hyperglycaemia and glycosuria after administration of alloxan has been described in several species, such as in dogs, rabbits and rats (Baily and Baily 1943; Brunschwig *et al.*1943; Dunn and McLetchie, 1943; Goldner and Gomori, 1944). Dosage and treatment regimen have been elaborated for the most frequently used species. In most species, a triphasic time course is observed: an initial rise of glucose is followed by a decrease, probably due to depletion of insulin from islet cells, again followed by a sustained increase of blood glucose (Lenzen, 1989).

Alloxan is a cyclic urea compound which induces permanent diabetes. It is a highly reactive molecule which produces free radical damage to beta islet cells and causes cell death. Alloxan at the doses of 150mg/kg in rats produces hyperglycaemia (Lenzen and Panten, 1988a).

Thus, alloxan-induced hyperglycaemic rat models will be used for the anti-hyperglycaemic study while normoglycaemic rats will used for the toxicity profile study.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

3.1.1 Plant material

Fresh leaves of *P. americana* were collected from Samaru, Zaria and was identified and authenticated on 16 July, 2012 by a taxonomist, Mallam U.S Gallah of the Herbarium Unit, Department of Biological Sciences A.B.U., Zaria. The voucher specimen number is 992.

3.1.2 Experimental animals

Young adult Wistar rats of both sexes (separated) weighing between 150 – 200 g were obtained from the Animal House, Department of Pharmacology and Therapeutics, A.B.U., Zaria. The animals were maintained on standard pelleted laboratory animal feed (Vital feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions and weighed prior to commencement of each experiment. All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NRC, 1985).

3.1.3 Drugs/chemicals, reagents and solvents

Alloxan monohydrate (Sigma-Aldrich Co. LLC St. Louis, USA).

Metformin 500 mg tabs (Glucophage) (Merke Sante s.a.s, France).

Chloroform (Sigma-Aldrich Co. LLC St. Louis, USA).

Formalin (Sigma-Aldrich Co. LLC St. Louis, USA).

ELITech reagent kits, (Vital Scientific, Netherlands).

3.1.4 Equipment and other materials

Glucometer and strips (Accu-check® Advantage, Roche Germany).

Haematocrit centrifuge (Denley BS400 centrifuge, England).

Automated haematology analyzer (Sysmex model 2X-12N, USA).

Automated biochemistry analyzer (Selectra XL, Vital Scientific, Netherlands).

Steam bath (Rajat Scientific Instrument, India).

Electronic weighing machine (Precision Electronic Instrument Company, India).

Glass and ceramic wares.

Cotton wool, Hand gloves, Nose masks.

Dissecting set, cannula, syringes and needles.

Cages and feeders.

Spatula, pestle and mortar.

3.1 Methods

3.2.1 Plant preparation

The fresh leaves of *P. americana* were collected and cleaned with water to remove dirt/contaminants and air dried under the shade until a constant weight was obtained. The dried leaves were pulverized into a coarse powder using pestle and mortar. The powdered sample was stored in an air tight container until needed.

3.2.2 Extraction

To 500 g of powdered leaf material, 2 litres portion of cold distilled water was added and then stirred in a conical flask until evenly mixed. The mixture was left to stand for twenty four (24) hours with occasional shaking and stirring. After the set time, the mixture was filtered using muslin cloth followed by Whatman filter paper (No 1). The resultant filtrate was concentrated on a steam bath maintained at 50^{o}C until all the moisture evaporates and a constant weight is obtained. The weight of the crude extract was taken and the percentage yield was calculated. The extract was stored in a sample bottle, labeled and kept in a desiccator until required. The extract was prepared fresh on each day of the experiment by reconstituting in distilled water. The extract will henceforth be referred to as AEPAL (aqueous extract of *P. americana* leaf).

3.2.3 Phytochemical screening

Preliminary phytochemical screening of AEPAL was carried out to identify the constituents, using standard methods as described by Trease and Evans (1989) and Sofowora (1993).

3.2.3.1 Test for alkaloids

One gram of extract was stirred in 10 ml distilled water and then filtered. The filtrate was divided into 3 portions and the following reagents were used to test for the presence of alkaloids.

Mayer's Test: The filtrate was treated with few drops of Mayer's reagent (Potassium Mercuric Iodide).The formation of a yellow coloured precipitate indicated the presence of alkaloids.

Wagner's Test: The filtrate was treated with few drops of Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: The filtrate was treated with few drops of Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of reddish precipitate indicated the presence of alkaloids.

3.2.3.2 Test for flavonoids

Shinoda's Test: The extract was dissolved in 2 ml of distilled water and warmed in a test tube. 4 drops of concentrated hydrochloric acid were then added to 1ml of the solution followed by addition of some magnesium ribbons. Immediate appearance of orange colour denoted flavones, red crimson colour denoted flavonols, and pink magenta colour denoted flavonones.

Sodium hydroxide test: The other 1ml solution was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids.

3.2.3.3 Test for tannins

Lead acetate test: To 1 ml of extract dissolved in distilled water, 2 drops of lead subacetate solution was added. A coloured precipitate indicated the presence of tannins.

Bromine water test: 1 ml of extract solution was treated with 3 drops of bromine water. Non-formation of coloured precipitate indicated the presence of hydrolysable tannins.

3.2.3.4 Test for saponins

Frothing Test: 0.5g of extract was dissolved in 5ml distilled water and shaken in a test tube for 5 mins. The occurrence of a honeycombs froth, which lasts for about 45 minutes, indicated the presence of saponins.

Hemolysis Test: 0.5g of extract was dissolved in 5ml distilled water. 2 drops of fresh whole blood was added to the solution. Immediate hemolysis of blood indicated the presence of saponins.

3.2.3.5 Test for phytosterols / triterpenoids

One gram of extract was dissolved in distilled water and then treated with chloroform. The liquids were separated using separating funnel. The chloroform portion was collected and then divided into 2 portions and was used for:

Salkowski's Test: A few drops of Conc. Sulphuric acid was added to the filtrate by the wall of the test tube. The appearance of golden yellow colour indicated the presence of triterpenes.

Libermann Burchard's test: A few drops of acetic anhydride were added to the filtrate in a test tube, followed by the addition of Conc. Sulphuric acid by the wall of the test tube. The formation of brown ring at the junction indicated the presence of phytosterols.

3.2.3.6 Test for phenolic compounds

Ferric chloride test: 1g of extract was dissolved in distilled water. Few drops of a solution of ferric chloride were added. The appearance of blue - black colour indicated the presence of tannins.

3.2.3.7 Test for of anthraquinones

Borntrager's Test: One gram of extract was dissolved in distilled water and fractionated with petroleum ether. The petroleum ether fraction was concentrated and shaken with 5mls of 25% ammonia solution. A cherry-red colour of the alkaline solution indicated anthraquinones in an oxidized form.

3.2.3.8 Test for cardiac glycosides

Keller- Kiliani's Test: 1 g of extract was dissolved in 20mls of distilled, 1ml of strong lead sub-acetate solution was added to precipitate pigments, which were filtered off. The filtrate obtained was shaken with equal volume of chloroform and allowed to separate into two layers in a separating funnel. The chloroform layer was removed and evaporated to dryness over a water bath. The residue was dissolved in 3ml of ferric chloride in glacial acetic acid, and then transferred to a dry test tube. Few drops of concentrated sulphuric acid were added by the wall of the test tube. On standing, a brown colour at the interphase (due to deoxysugars) and a pale green colour in the upper layer (due to steroidal nucleus) is a preliminary test for Cardiac Glycosides.

3.2.3.9 Test for carbohydrates

One gram of extract was dissolved in 5mls of distilled water and filtered. The filtrate was divided into 2 portions and was used to test for the presence of carbohydrates using the following reagents:

Molisch's Test: The filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. The formation of the violet ring at the junction indicated the presence of carbohydrates.

Fehling's Test: The filtrate was hydrolysed with dil. HCl, and then neutralized with alkali and warmed with Fehling's A and B solutions. The formation of red precipitate indicated the presence of reducing sugars.

3.2.4 Route of administration

AEPAL, distilled water, normal saline and metformin were administered orally (*p.o.*) throughout the study while alloxan was administered intraperitoneally (*i.p.*) for induction of hyperglycaemia.

3.2.5 Acute toxicity study

Acute toxicity was determined using the method described by Lorke (1983) and was used to determine the oral median lethal dose (LD_{50}). The study was carried out in two phases. In Phase 1; nine (9) rats were randomly divided into three groups of three rats per group. The animals in the three groups were administered (via a cannula) with single graded doses (10, 100 and 1000 mg/kg body weight respectively) of the extract per oral. In phase 2; based on the results of the phase 1 study, three (3) rats were each administered orally single graded doses 1600, 2,900 and 5,000 mg/kg body weight of

the extract. In each of the two phases of the study, the rats were kept under the same conditions and observed for signs of toxicity and mortalityfor 24 hours. The LD_{50} was then calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses which 0 and 100% survival rates were recorded (figure 3.1).

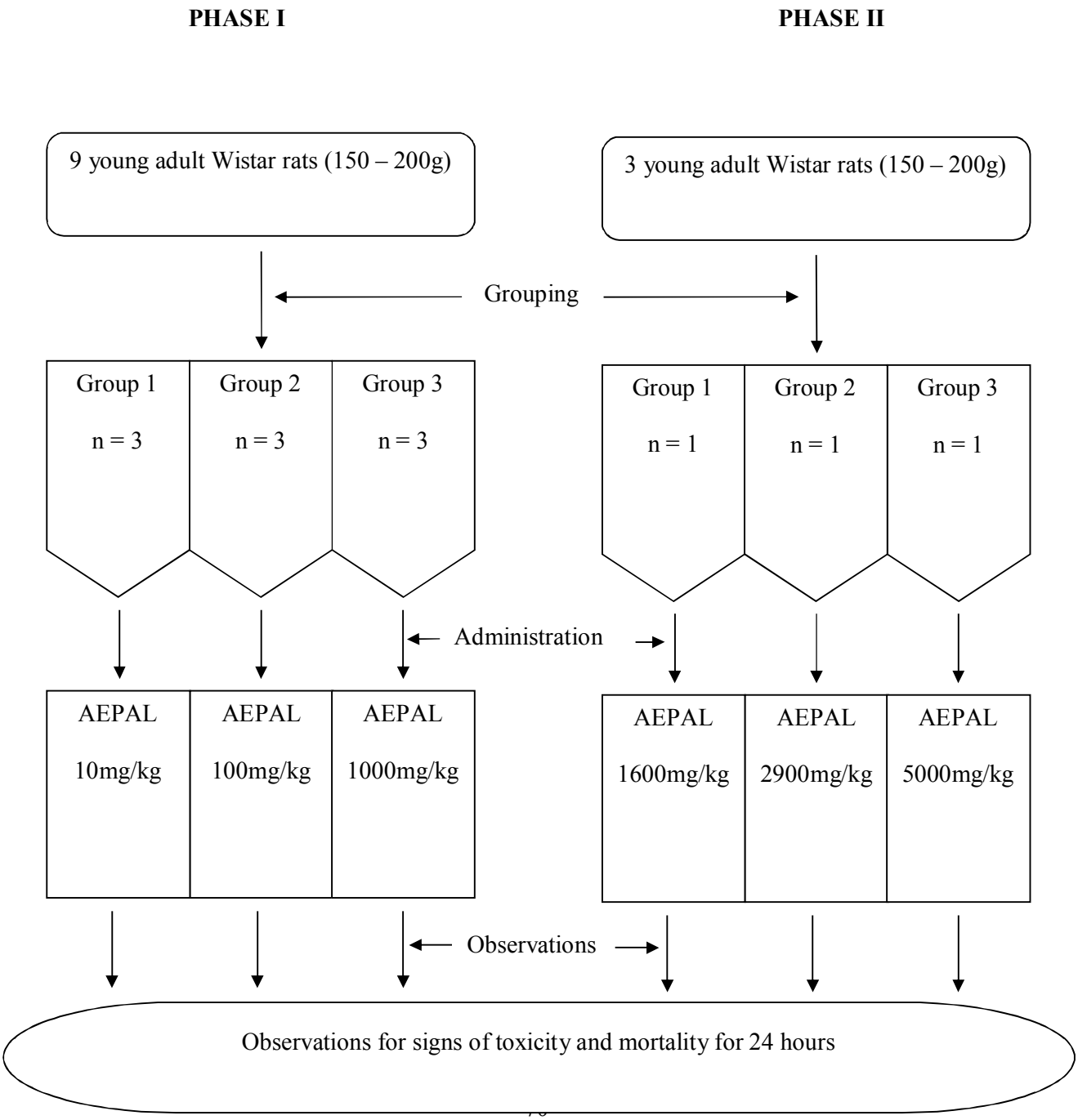


Figure 3.1: Experimental Design for Acute Toxicity Study

3.2.6 Sub-chronic toxicity study

Sub-chronic toxicity study was carried out in accordance with WHO (1992) and OECD 407 (1995) guidelines as shown in Figure 3.3. Twenty four rats of both sexes deprived of food for 24 hours, were randomly divided into four test groups of six animals (n=6) each. Group A served as control and were administered with distilled water the vehicle for reconstitution of the extract. Animals in groups B, C and D were administered graded doses (250, 500 and 1000 mg/kg body weight) of AEPAL respectively daily for 28 days *p.o*. All the animals had free access to feed and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality.

3.2.6.1 Feed and water intake

The weight of feed and volume of water consumed by rats in each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 hours.

3.2.6.2 Weekly body weight change

The body weight of each rat was measured once during the acclimatization period, once before commencement of dosing (Day 0) and once every 7 days (Day 7, Day 14, Day 21 and Day 28) during the dosing period.

3.2.6.3 Relative organ weight

At the end of the treatment (28 days), animals were fasted, weighed and humanely sacrificed on day 29 with an overdose of chloroform. The essential organs including the liver, kidneys, spleen, heart and lungs were surgically dissected out and weighed in grams (absolute organ weight). The relative organ weight (ROW) was then calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

3.2.6.4 Biochemical analysis

The fasting blood glucose concentration (FBGC) was measured weekly using a digital glucometer and strips by taken blood from the tail vein. The results were expressed in mg/dl. Blood samples were collected from the heart of each animal by cardiac puncture after sacrifice on 29th day. The blood samples were dispensed into plain bottles, allowed to clot and centrifuged. The sera was separated and used for evaluation of biochemical parameters, which include alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) levels, as well as serum urea and creatinine using automated Biochemistry Analyzer.

3.2.6.5 Haematological analysis

Blood samples were also collected from the heart of each animal by cardiac puncture into K^+ EDTA bottles for analysis of blood indices including white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), platelet (PLT), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) using automated Haematology Aanalyzer.

3.2.5.6 Histopathological studies

Following the sacrifice of the animals after the treatment period, post mortem gross examination was done and essential organs including liver, kidneys, spleen, heart and lungs were dissected out and washed in ice cold saline immediately. The organs were fixed in 10% formalin fixative solution for at least 48 hours. After fixation, the organs were processed routinely, and the tissues embedded in paraffin wax. Histological sections were cut at 5 – 6 µm and stained with haematoxylin and eosin (H and E) stain as described by Strate *et al.,* (2005). Detailed microscopic examination was carried out by a Consultant Histopathologist. The slides were viewed at magnification of ×250 and photomicrographs of the tissues taken.

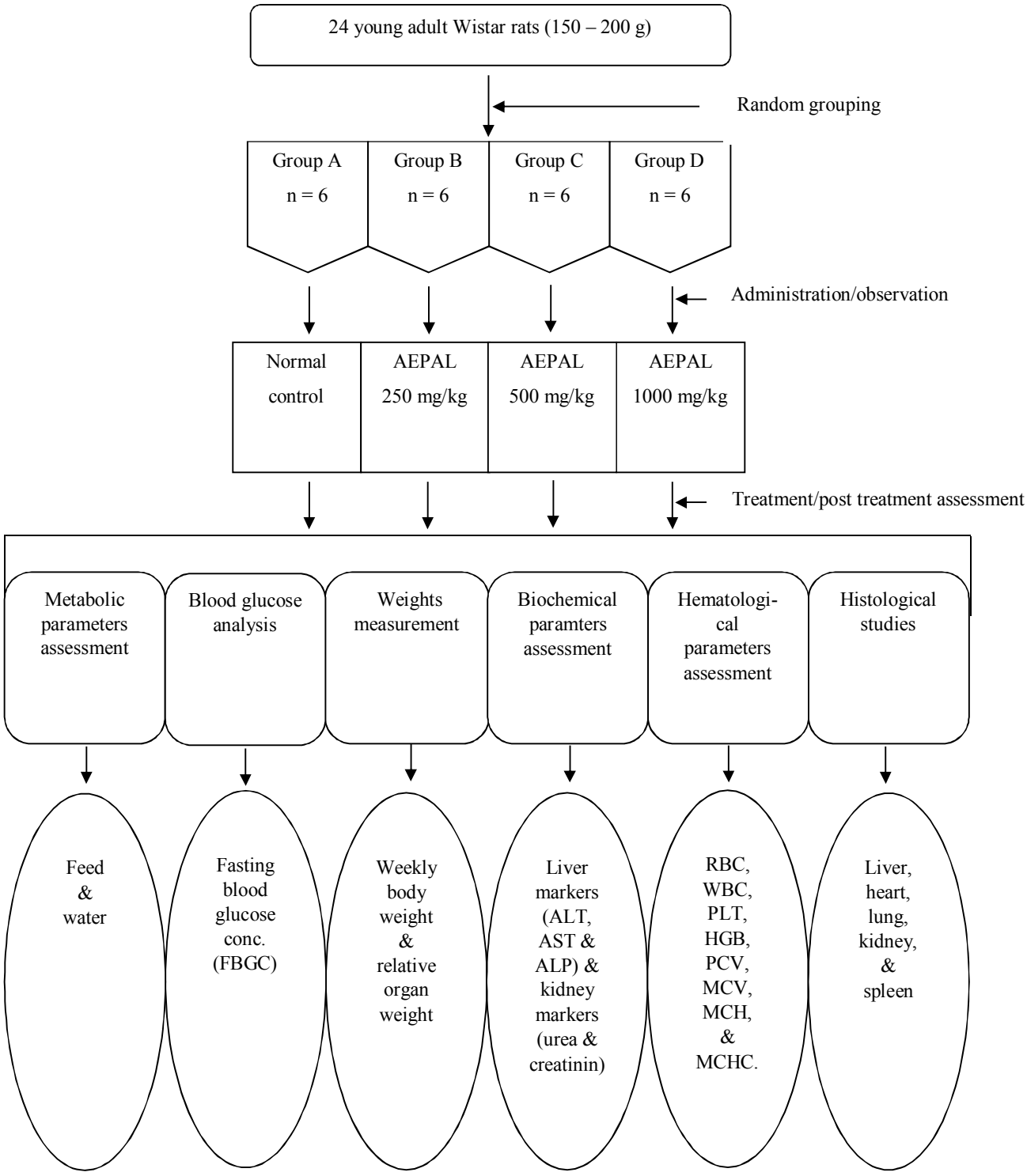


Figure 3.2: Experimental Design for Sub-Chronic Toxicity Study

3.2.7 Anti-hyperglycaemic study

3.2.7.1 Induction of hyperglycemia

Hyperglycemia was induced using alloxan according to the method described by Dunn and McLetchie (1943). Thirty (30) animals were fasted for 16 hours with free access to water prior to the induction of hyperglycemia which was carried out by a single *i.p*. injection of freshly prepared alloxan monohydrate (150 mg/kg body weight) dissolved in 0.9% cold normal saline solution. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 hours. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani *et al*., 2002). After 72 hours of alloxan administration, blood was collected from the tail vein of the rats and the blood glucose concentration were measured with digital glucometer and strips (Accu-Chek® Advantage, Roche Diagnostic, Germany). Rats having fasting blood glucose concentration (FBGC) ≥200 mg/dl (11.1mmol/L) were considered hyperglycaemic and selected for the study. The animals were then allowed to stabilize with consistent hyperglycaemia for another 3 days before commencement of the experiment in order to avoid reversal effect (Dhandapani *et al*., 2002).

3.2.7.2 Grouping and treatment

In this study, the method of Vivek *et al.,* (2010) was adopted. The duration of the study was twenty eight (28) days. A total of thirty six (36) rats were randomly selected and grouped into six groups of six (n=6) each as follows:

Group 1: Normal rats, were administerd distilled water (*p.o*) and served as normoglycaemic control.

Group 2: Alloxan-induced hyperglycaemic rats, were administered normal saline (*p.o*) and served as hyperglycaemic control.

Group 3: Alloxan-induced hyperglycaemic rats, were administered AEPAL 125 mg/kg body weight *p.o.*

Group 4: Alloxan-induced hyperglycaemic rats, were administered AEPAL 250 mg/kg body weight *p.o.*

Group 5: Alloxan-induced hyperglycaemic rats, were administered AEPAL 500 mg/kg body weight *p.o.*

Group 6: Alloxan-induced hyperglycaemic rats which were administered reference standard drug, metformin 250 mg/kg body weight *p.o.* and served as positive control.

3.2.7.3 Blood samples collection and preparation

At the end of the treatment (after 28 days), similar procedure for blood samples collection in the sub-chronic study above was used. Blood samples were drawn from the heart of each sacrificed animal from all groups by cardiac puncture. Pair of blood samples were collected into heparinized and non-heparinized bottles. The heparinized blood samples were used for haematological evaluation while the non-heparinized blood were allowed to coagulate, centrifuged and the sera separated were analyzed for biochemical parameters.

3.2.7.4 Determination of effect of AEPAL on fasting blood glucose concentration (FBGC) of alloxan-induced hyperglycaemic rats

FBGC were determined and the results were expressed in the unit of mg/dl (Rheney and Kirk, 2000). All blood samples for FBGC determination were collected from the tail vein of the rats on days 0, 7, 14, 21 and 28.

3.2.7.5 Assessment of effect of AEPAL on feed and water intake in alloxan-induced hyperglycaemic rats

The weight of feed and volume of water consumed by rats in each group were measured in same way as described in sub-chronic toxicity study above.

3.2.7.6 Determination of effect of AEPAL on body weights of alloxan-induced hyperglycaemic rats

The body weight of each rats were assessed using the same procedure as in the sub-chronic toxicity study above.

3.2.7.7 Evaluation of effect of AEPAL on serum liver enzymes and proteins in alloxan-induced hyperglycaemic rats

ALT, AST, ALP, Total protein (TP) and albumin (Alb) were determined using the same method and equipment as described in sub-chronic study above.

3.2.7.8 Evaluation of effect of AEPAL on serum kidney function parameters in alloxan-induced hyperglycaemic rats

Serum urea and creatinine were measured as above.

3.2.7.9 Evaluation of effect of AEPAL on serum lipid profile in alloxan-induced hyperglycaemic rats

Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) were analyzed using the same biochemical method and equipment.

3.2.7.10 Evaluation of effect of AEPAL on haematological parameters of alloxan-induced hyperglycaemic rats

This was carried out using the same procedures and equipment, and the same parameters were assessed as described in sub-chronic toxicity study above.

3.2.7.11 Histopathology of the pancreas

The pancreas was surgically dissected out from each animal after the sacrifice. The same procedures for tissue preparation and examination as described in sub-chronic toxicity study was also employed here.

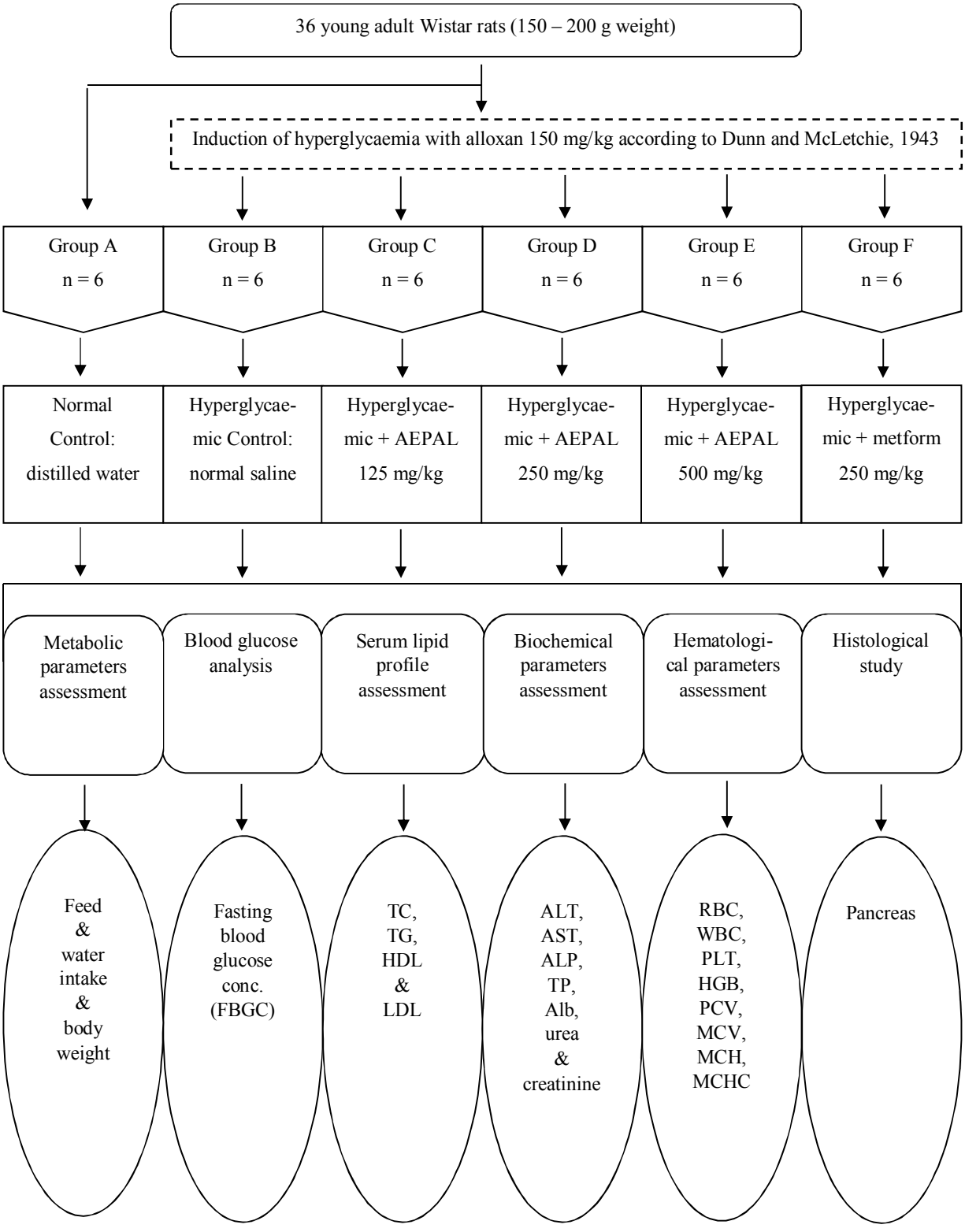


Figure 3.3: Experimental Design for Anti-Hyperglycaemic Study

3.2.8 Statistical analysis and data presentation

The data obtained from these studies were analyzed with Statistical Package for Social Science (SPSS) software, version 20 (IBM, USA), using one-way analysis of variance (ANOVA) with Dunnet (for sub-chronic toxicity study) and Turkey's (for anti-hyperglycaemic study) multiple comparison post hoc test which compared the level of significance between the control and test groups. All data were expressed as mean ± SEM and $P \leq 0.05$ were considered as significant (Duncan *et al*., 1997). The data were presented as tables, graphs and charts as appropriate.

CHAPTER FOUR

RESULTS

4.1 Aqueous Extraction of *P. americana* Leaf

The crude extract (AEPAL) obtained from *P. americana*leaf was a non-sticky dark brown solid residue with a mild sweet smell. The extract was readily and completely soluble in water. A weight of 49.66g with a yield of 9.93% $^{w}/_{w}$ was obtained.

4.2 Phytochemical Screening

Preliminary phytochemical screening of AEPAL showed the presence of saponins, steroids, flavonoids, tannins, alkaloids, cardiac glycosides, triterpenoids and carbohydrates while anthraquinones and cyanogenetic glycosides were absent (Table 4.1).

Table 4.1: Phytochemical constituents of aqueous extract of *P. americana* leaf

Phytochemical constituents		
Constituents tested	**Test method**	**Result**
Alkaloids	Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
	Picric acid test	+
	Tannic acid test	+
Anthraquinones	Borntrager's test	_
Carbohydrates	Molisch's test	+
	Fehling's test	+
Cardiac glycosides	Keller-Keliani test	+
	Kadde's test	+
	Salkowski's test	+
Cyanogenetic glycosides	Ferric chloride test	_
	Fehling's test	_
Flavonoids	Shinoda's test	+
	Sodium hydroxide test	+
Saponins	Frothing test	+
Steroids	Liebermann-Burchard's test	+
Triterpenoids	Salkowski's test	+
Tannins	Ferric chloride test	+
	Lead sub-acetate test	+

(-) = Absent, (+) = Present

4.3. Acute Toxicity Study

No signs of toxicity or mortality were recorded at all doses of AEPAL in both phases of the experiment during the acute (24 hours) observation period following the single dose oral administration in rats (Table 4.2). The absence of death at doses up to 5,000mg/kg showed that the LD_{50} of the extract was greater than 5,000 mg/kg body weight *p.o*.

Table 4.2: Observations in phase I and II of the acute toxicity study following single oral administration of aqueous extract of *P. americana* leaf (AEPAL) in adult Wistar rats

Acute toxicity study phase I and II

PHASE	Treatment	Dose (mg/kg)	Signs of toxicity	No of deaths
PHASE I				
	AEPAL	10	None	0/3
	AEPAL	100	None	0/3
	AEPAL	1000	None	0/3
PHASE II				
	AEPAL	1600	None	0/1
	AEPAL	2900	None	0/1
	AEPAL	5000	None	0/1

AEPAL – aqueous extract of *P. americana* leaf.
Phase I: n = 3; Phase II: n = 1

4.4 Sub-Chronic Toxicity Study

4.4.1 Effect of aqueous extract of *P. americana* leaf (AEPAL) on feed intake

AEPAL has no significant (P>0.05) effect on the feed intake of all the treated animals compared to the control (Table 4.3).

Table 4.3 Effect of aqueous extract of *P. americana* leaf (AEPAL) on feed intake in rats following daily oral administration for 28 days

	Feed intake (g/day)			
Treatment/dose	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	107.86 ± 4.12	116.00 ± 4.20	122.43 ± 2.92	124.29 ± 3.18
AEPAL 250 mg/kg	105.57 ± 3.36	112.00 ± 4.79	120.86 ± 5.15	126.57 ± 3.80
AEPAL 500 mg/kg	111.86 ± 3.84	118.71 ± 4.79	123.00 ± 5.08	123.71 ± 4.20
AEPAL 1000 mg/kg	108.00 ± 4.32	115.86 ± 3.16	121.14 ± 2.11	119.86 ± 4.51

AEPAL – aqueous extract of *P. americana* leaf.

n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; no statistically significant difference (P>0.05) between control and test groups.

4.4.2 Effect of aqueous extract of *P. americana* leaf (AEPAL) on water intake

There were no significant (P>0.05) difference observed in the water intake of all the treated groups compared to the control (Table 4.4).

Table 4.4 Effect of aqueous extract of *P. americana* leaf (AEPAL) on water intake in rats following daily oral administration for 28 days

	Water intake (ml/day)			
Treatment/dose	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	134.86 ± 3.60	141.57 ± 4.23	140.43 ± 4.65	142.14 ± 4.54
AEPAL 250 mg/kg	136.86 ± 5.45	143.00 ± 5.48	144.29 ± 5.37	141.71 ± 3.98
AEPAL 500 mg/kg	130.29 ± 3.29	137.29 ± 3.62	142.71 ± 5.71	146.29 ± 5.06
AEPAL 1000 mg/kg	132.57 ± 3.88	138.43 ± 5.14	137.86 ± 5.07	138.71 ± 4.02

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; no statistically significant difference (P>0.05) between control and test groups.

4.4.3 Effect of aqueous extract of *P. americana* leaf (AEPAL) on body weights

In spite of the decrease in body weight observed on week 3 and week 4 in 1000 mg extract/kg treated group, there were no significant (P>0.05) decrease in body weight changes of all the treated rats compared to control (Table 4.5).

Table 4.5 Effect of aqueous extract of *P. americana* leaf (AEPAL) on body weights of rats following daily oral administration for 28 days

	Weekly body weights (g)				
Treatment/dose	**Initial**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	195.33±4.65	201.50±3.91	208.00±3.63	214.17±3.65	218.83±3.67
AEPAL 250 mg/kg	200.00±5.05	206.17±4.87	211.50±4.40	217.33±4.04	219.33±4.00
AEPAL 500 mg/kg	197.17±4.32	202.33±3.99	209.00±3.83	212.83±3.74	214.8±3.68
AEPAL 1000 mg/kg	202.50±6.57	205.50±6.53	212.50±6.41	212.00±5.68	210.83±5.89

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; no statistically significant difference (P>0.05) between control and test groups.

+4.4.4 Effect of aqueous extract of *P. americana* leaf (AEPAL) on relative organ weights (ROW)

Also, there were no significant (P>0.05) increase in relative organ weights of all the treated rats compared to control, despite the increase in liver weights observed in rats administered 500 and 1000 mg/kg AEPAL and slight decrease in the kidneys, lungs, heart and spleen weights observed in the rats administered 250, 500 and 1000 mg/kg AEPAL daily *p..o.* (Figure 4.1).

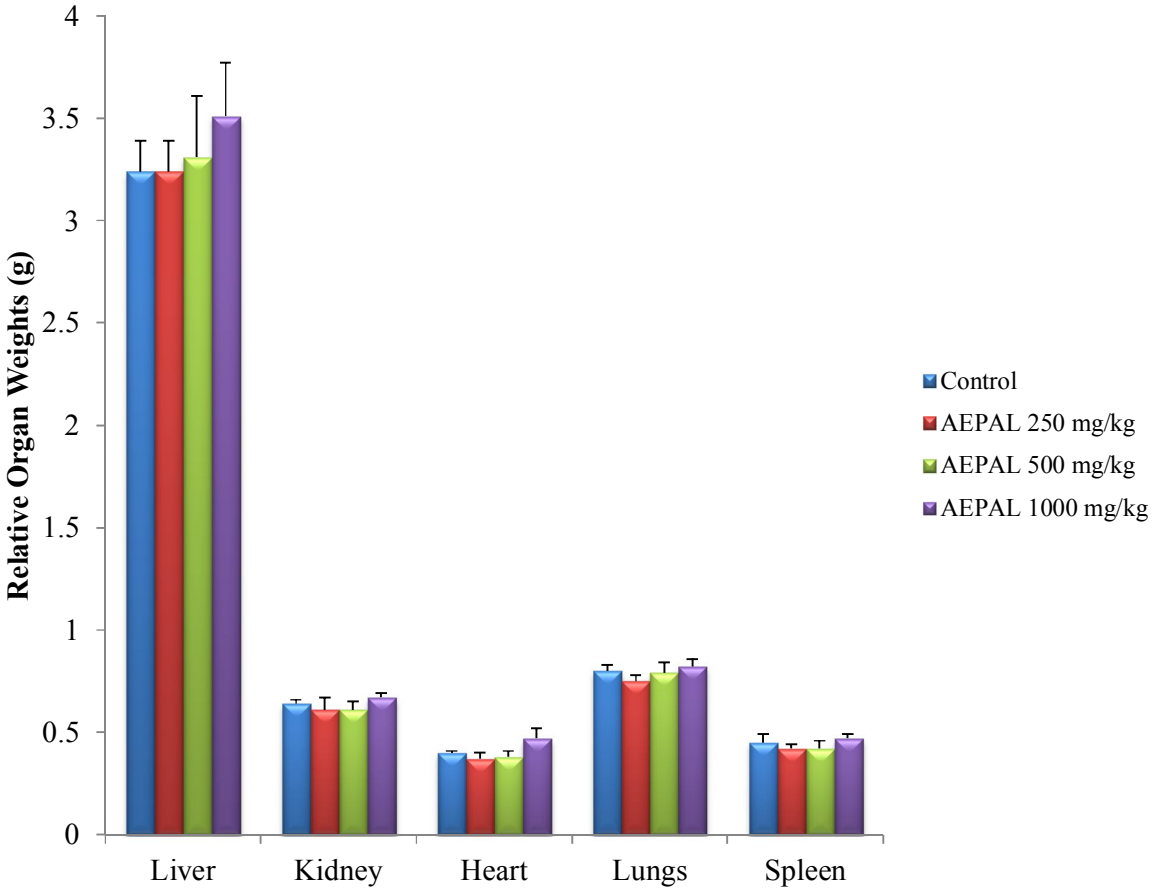


Figure 4.1: Effect of aqueous extract of *P. americana* leaf (AEPAL) on relative organ weights (ROW) of rats following daily oral administration for 28 days

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; no statistically significant difference (P>0.05) between control and test groups.

4.4.5 Effect of aqueous extract of *P. americana* on fasting blood glucose concentration (FBGC) of normoglycaemic rats

Significant (P<0.05) reduction in blood glucose levels were observed in all the treated groups on days 7, 14 and 28 compared to control. Higher significant (P<0.01) reduction was also observed in all the treated groups on days 14 and 21. While the highest significant (P<0.001) reduction was observed only in the group that was treated with the highest dose (1000 mg/kg body weight) of extract on day 21. Thus, the reduction was dose dependent (Figure 4.2).

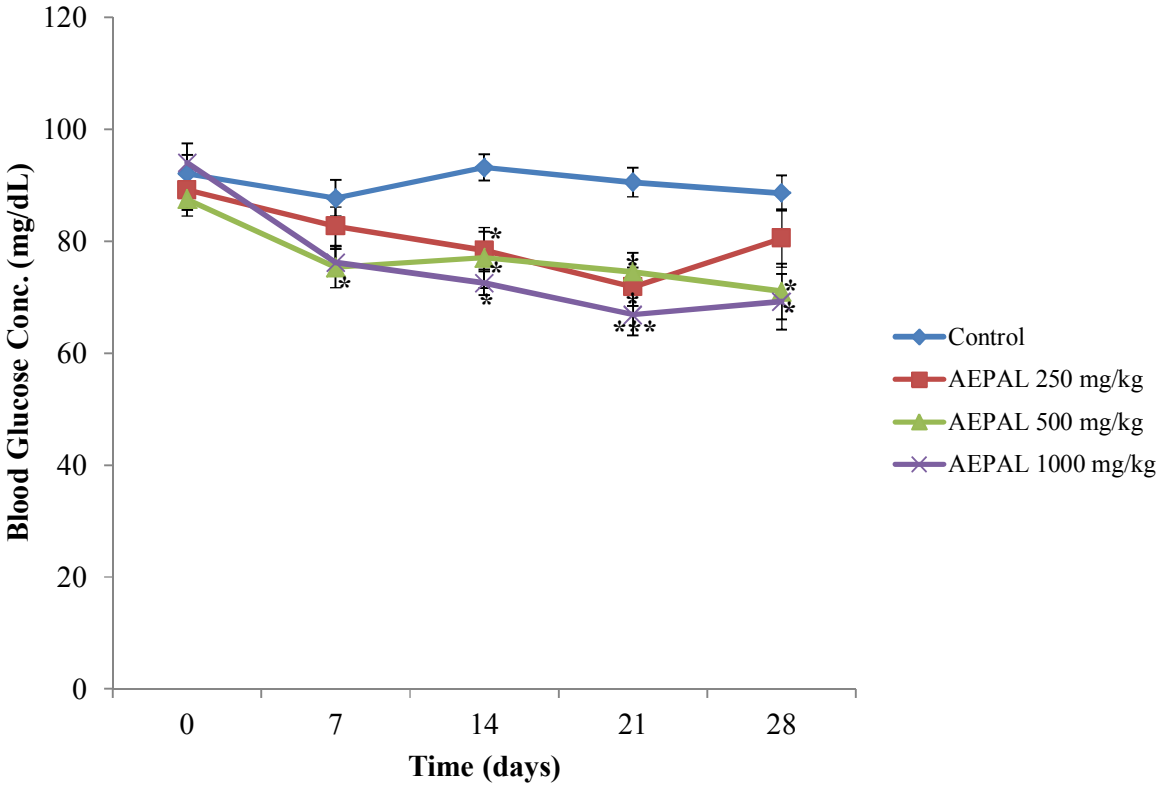


Figure 4.2: Effect of aqueous extract of *P. americana* leaf (AEPAL) on weekly fasting blood glucose concentration (FBGC) of normoglycaemic rats following daily oral administration for 28 days

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; *P<0.05, **P<0.01, ***P<0.001, statistically significant compared to control.

4.4.6 Effect of aqoueus extract of *P. americana* leaf (AEPAL) on serum biochemical parameters

There were significant (P<0.01) increase observed in the serum levels of AST, urea and creatinine in 1000 mg/kg group. ALT showed significant (P<0.05 and P<0.01) increase in 500 and 1000 mg/kg group respectively, while ALP showed no significant (P<0.01) increase/decrease in all the treated groups compared to control (Table 4.6).

Table 4.6: Effect of aqueous extract of *P. americana*leaf (AEPAL) on serum biochemical parameters of rats following daily oral administration for 28 days

	Serum biochemical parameters				
Treatment/dose (mg/kg)	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**	**Urea (mmol/L)**	**Creat (µmol/L)**
Control	31.17 ± 3.34	37.33 ± 3.25	64.50 ± 6.21	2.48 ± 0.25	45.33 ± 2.26
AEPAL 250	40.67 ± 2.85	43.00 ±3.52	61.33 ± 5.35	1.93 ± 0.12	52.67 ± 1.75
AEPAL 500	34.83 ± 3.80	56.00 ± 4.37*	72.83 ± 3.16	2.33 ± 0.13	49.50 ± 1.59
AEPAL 1000	51.50 ± 3.64**	62.50 ± 5.71**	81.50 ± 5.98	3.47 ± 0.15**	55.83 ± 2.73**

AEPAL – aqueous extract of *P. americana* leaf, AST – aspartate transaminase, ALT – alanine transaminase, ALP – alkaline phosphatase, Creat – creatinine.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; *P<0.05, **P<0.01, statistically significant compared to control.

4.4.7 Effect of aqueous extract of *P. americana* leaf (AEPAL) on haematological parameters

In table 4.7, the extract caused a significant ($P<0.05$) and highly significant ($P<0.01$) decrease in WBC count in 500 and 1000 mg extract/kg treated groups respectively. There were no significant ($P>0.05$) changes observed for all the treated groups in RBC, HGB, PCV, MCV, MCH, PLT and MCHC compared to control.

Table 4.7: Effect of aqueous extract of *P. americana* leaf (AEPAL) on haematological parameters of rats following daily oral administration for 28 days

	Haematological parameters of animals							
Treatment/dose (mg/kg)	**WBC ($\times 10^3/\mu l$)**	**RBC ($\times 10^6/\mu l$)**	**HGB (g/dl)**	**PCV (%)**	**MCV (fl)**	**MCH (pg)**	**PLT ($\times 10^3/\mu l$)**	**MCHC (g/dl)**
Control	7.7 ± 0.63	7.55 ± 0.17	13.70 ± 0.21	49.83 ± 0.98	65.92 ± 1.18	18.13 ± 0.30	739.83 ± 28.13	27.48 ± 0.37
AEPAL 250	6.13 ± 0.41	7.37 ± 0.19	13.92 ± 0.13	50.00 ± 0.52	69.03 ± 1.75	19.15 ± 0.31	735.33 ± 49.49	27.23 ± 0.54
AEPAL 500	5.72 ± 0.67*	7.72 ± 0.22	14.27 ± 0.31	51.83 ± 1.25	67.10 ± 1.42	18.48 ± 0.22	600.83 ± 50.47	25.90 ± 1.63
AEPAL 1000	4.78 ± 0.32**	7.65 ± 0.56	14.00 ± 0.22	50.50 ± 0.72	66.50 ± 1.76	18.60 ± 0.41	601.67 ± 26.63	27.88 ± 0.46

AEPAL – aqueous extract of *P. americana* leaf, WBC – white blood cell, RBC – red blood cell, HGB – haemoglobin, PCV – packed cell volume, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, PLT – platelets, MCHC – mean corpuscular haemoglobin concentration.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; *P<0.05, **P<0.01, statistically significant compared to control

4.4.8 Histopathological studies

No structural changes were identified by histological studies in the liver, kidney, heart, lungs and spleen of the control group. Also, there were no pathological changes in the heart and spleen tissues of rats in all the treated groups. However, there were dose dependent pathological changes (hepatocellular degeneration and necrosis, periportal mononuclear cell infiltrations and vacuolar degeneration and sinusoidal congestion) in the liver tissue. Also hemorrhagic interstitium with lymphocytocis, glomerular and tubular congestion, atrophy, degeneration and necrosis were observed in the kidney tissue of rats in all the treated groups. While changes (lymphocytes hyperplasia, alveoli congestion and atrophy) in the lungs tissue were identified only in rats treated with 500 and 1000 mg extract/kg b.w. (plate III-VII).

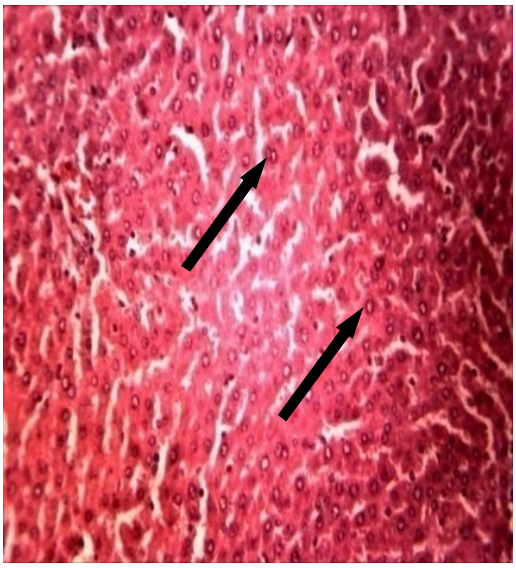


Plate IIIa: Control

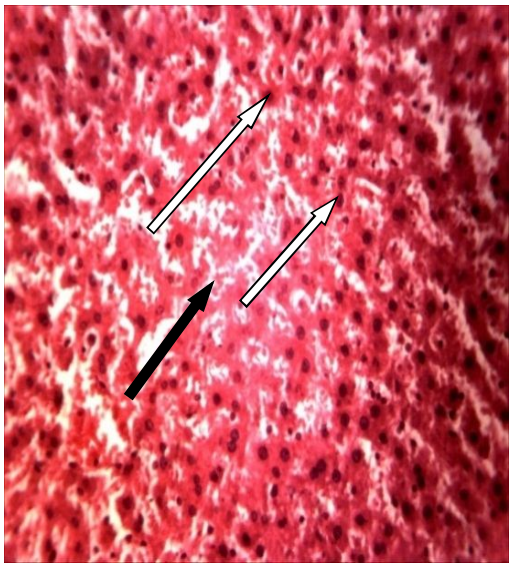


Plate IIIb: 250 mg/kg AEPAL

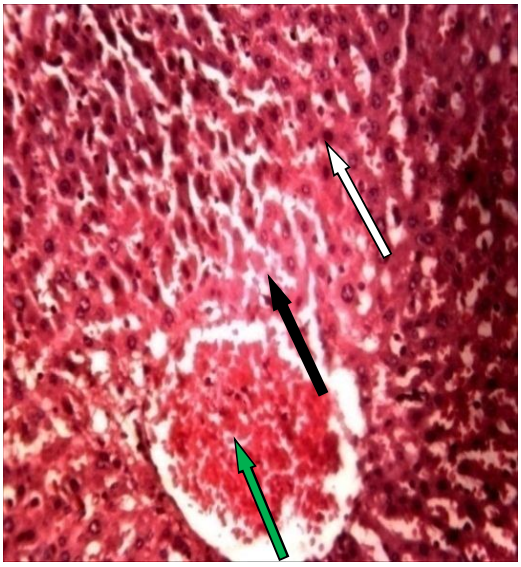


Plate IIIc: 500 mg/kg AEPAL

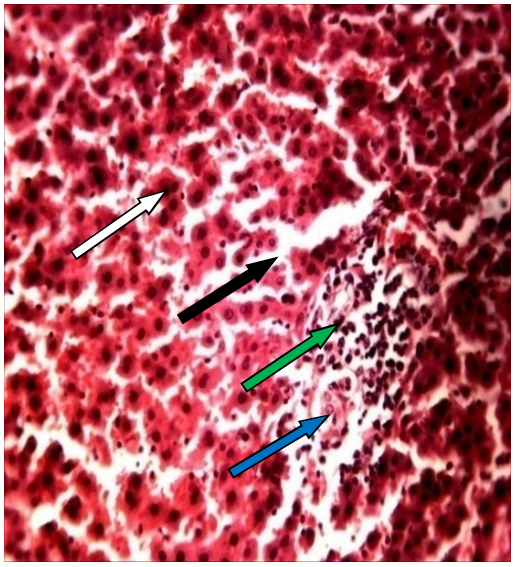


Plate IIId: 1000 mg/kg AEPAL

Plate III: Photomicrographs of sections of liver tissues of control and treated rats (H & E stained, ×250)

IIIa: Section showing normal histological appearance of liver with normal hepathocyte (black arrow)

IIIb: Section showing slight hepatocellular degeneration (white arrows) and periportal mononuclear cell infiltrations and vacuolar degeneration (black arrows).

IIIc: Section showing moderate diffuse vacuolar and hepatocellular degeneration (white and black arrow) andmononuclear cell infiltrations and venous congestion (green arrow).

IIId: Section showing intense lymphocyte hyperplasia (green arrow), sinusoidal congestion (black arrow), vacoular degeneration (blue arrow) and hepathocellular necrosis (white arrow) .

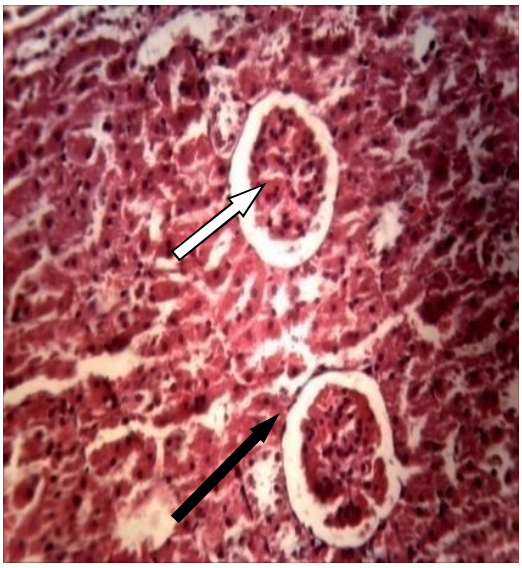


Plate IVa: Control

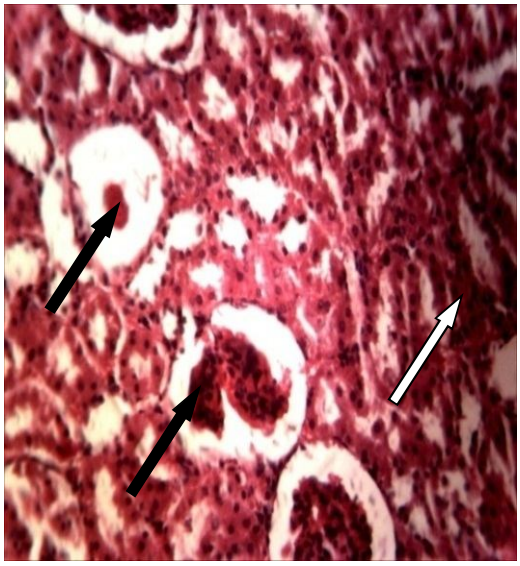


Plate IVb: 250 mg/kg AEPAL



Plate IVc: 500 mg/kg AEPAL

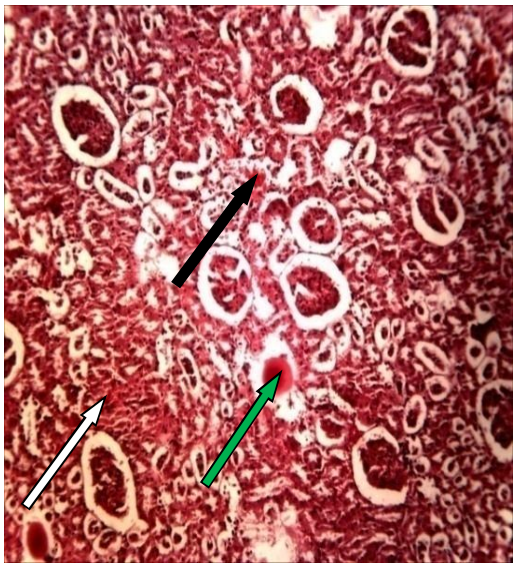


Plate IVd: 1000 mg/kg AEPAL

Plate IV: Photomicrographs of sections of kidney tissues of control and treated rats (H & E stained, ×250)

IVa: Section showing normal histological appearance of kidney with normal glomerulus (white arrow) and normal renal tubule (black arrow).

IVb: Section showing moderate focal glomerular atrophy (black arrow) and slight tubular epithelial vacuolar degeneration (white arrow).

IVc: Section showing glomerular congestion and degeneration, (black arrow), hemorrhagic interstitium with lymphocytocis (white arrow) and diffuse tubular degeneration and necrosis (green arrow).

IVd: Section showing intense glomerular atrophy and necrosis (green arrow), interstitial hemorrhage and lymphocytosis (black arrow) and mononuclear cell infiltration, tubular degeneration and necrosis (white arrow).

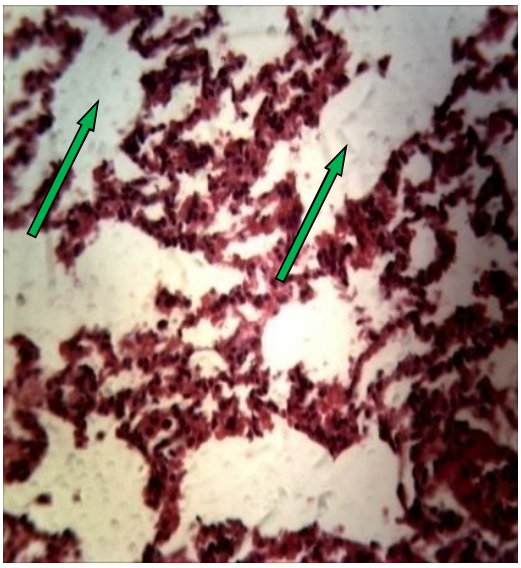


Plate Va: Control

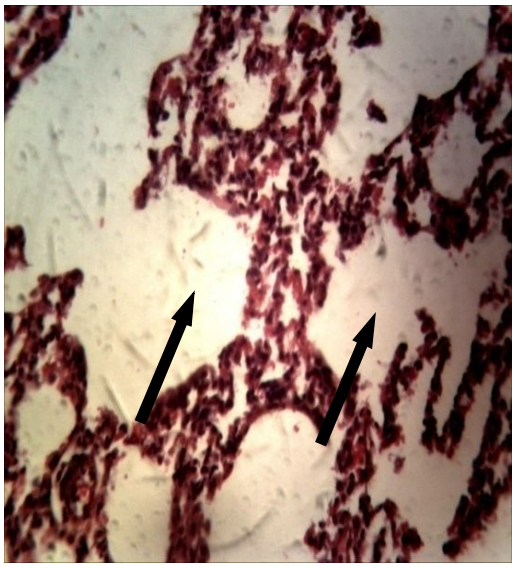


Plate Vb: 250 mg/kg AEPAL

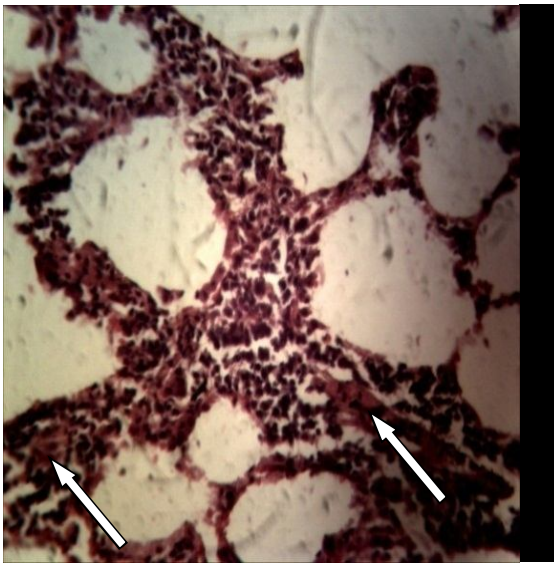


Plate Vc: 500 mg/kg AEPAL

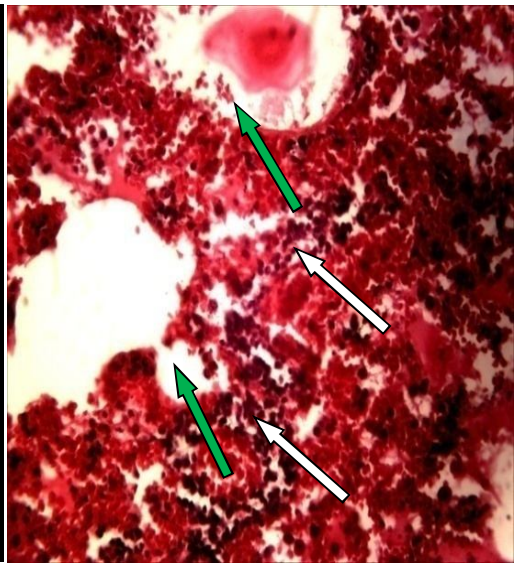


Plate Vd: 1000 mg/kg AEPAL

Plate V: Photomicrographs of sections of lung tissues of control and treated rats (H & E stained, ×250)

Va: Section showing normal histological appearance of lung with normal alveoli and respiratory ducts (green arrows)

Vb: Section showing normal architure with normal alveoli and respiratory ducts (black arrows)

Vc: Section showing moderate lymphocytes hyperplasia (white arrows)

Vd: Section showing alveoli congestion and atrophy (green arrows) and intense lymphocytes hyperplasia and emphysematous changes (white arrows).

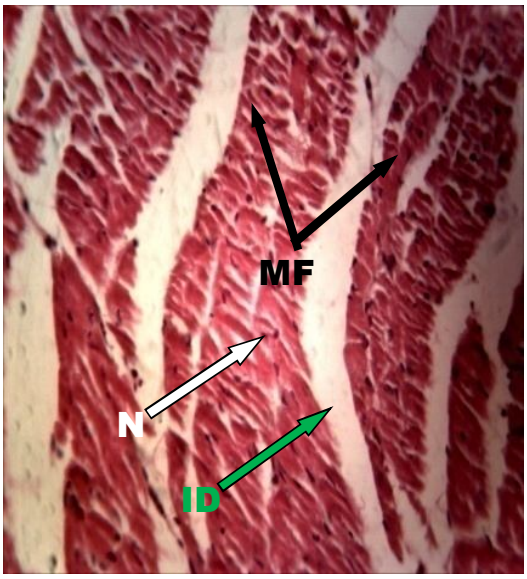


Plate VIa: Control

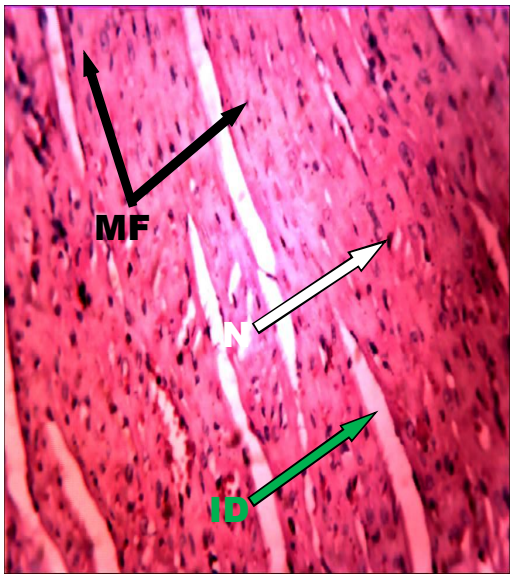


Plate VIb: 250 mg/kg AEPAL

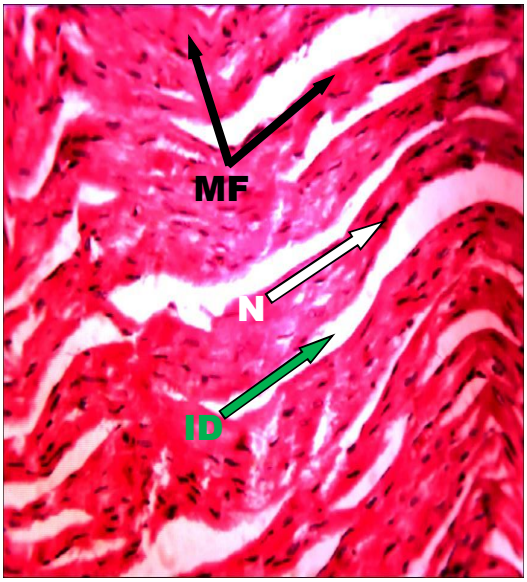


Plate VIc: 500 mg/kg AEPAL



Plate VId: 1000 mg/kg AEPAL

Plate VI: Photomicrographs of sections of heart tissues of control and treated rats with normal features (H & E stained, ×250)

N – nucleous; MF – muscle fibre; ID – intercalated disc



Plate VIIa: Control

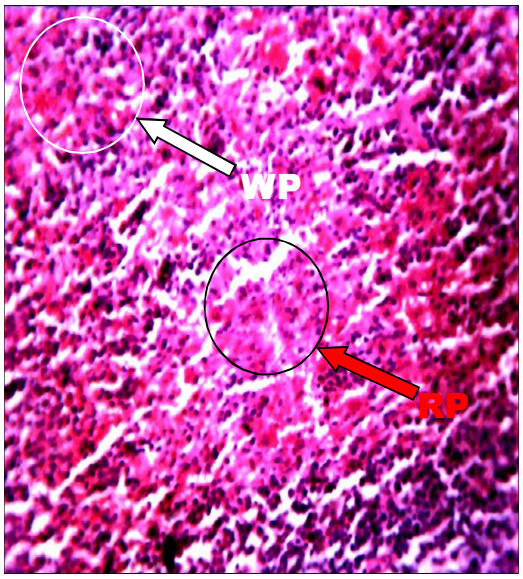


Plate VIIb: 250 mg/kg AEPAL

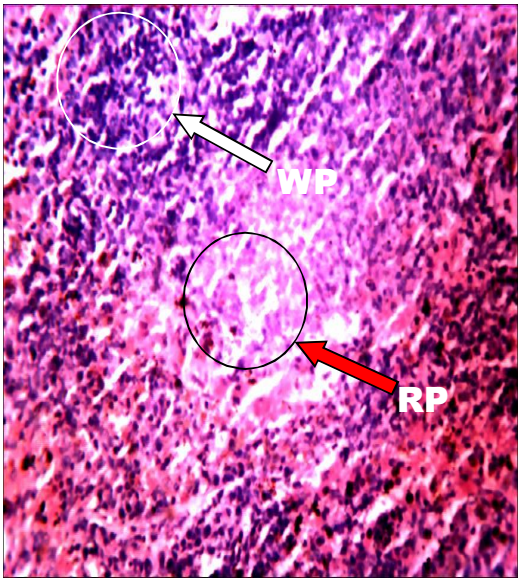


Plate VIIc: 500 mg/kg AEPAL

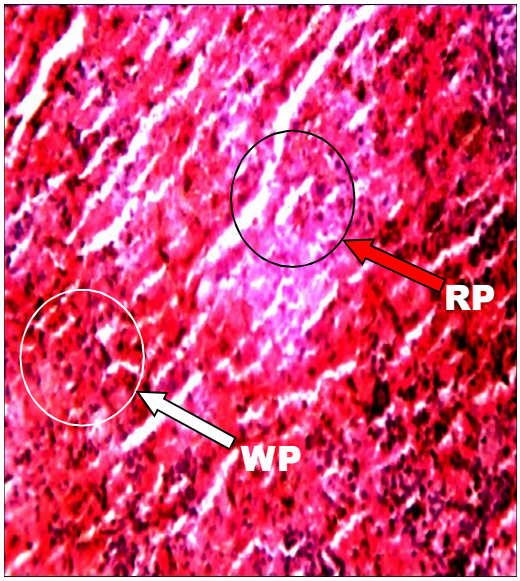


Plate VIId: 1000 mg/kg AEPAL

Plate VII: Photomicrographs of sections of spleen tissues of control and treated rats with normal features (H & E stained, ×250)

RP – red pulp; WP – white pulp

4.5 Anti-hyperglycaemic Study

The doses used for this study were reduced to half (i.e. 125, 250 and 500 mg extract/kg b.w) the doses used in the sub-chronic toxicity study representing 2.5, 5 and 10% respectively of the LD_{50}. This reduction was as a result of the toxicity observed with the high doses (250, 500 and 1000 mg/kg) used in the sub-chronic toxicity study.

4.5.1 Effect of extract on feed intake in alloxan-induced hyperglycaemic rats

An increase in feed and water intake was observed in hyperglycaemic control rats compared to normal control rats. Within each group, a decrease with time was recorded in both feed and water intake. The extract elicited a significant ($P<0.05$) reduction in feed consumption in week 1 only in the reference standard drug (metformin 250 mg/kg) group and in week 2 in 250 and 500 mg/kg groups as well as in metformin 250 mg/kg group compared to hyperglycaemic control. A higher significant ($P<0.01$) reduction was elicited by the extract in week 3 and 4 only in 125 mg/kg group while the highest significant ($P<0.001$) decrease in feed intake was produced in week 3 and 4 in 250 and 500 mg extract/kg group as well as in metformin 250 mg/kg group compared to hyperglycaemic control (Figure 4.3).

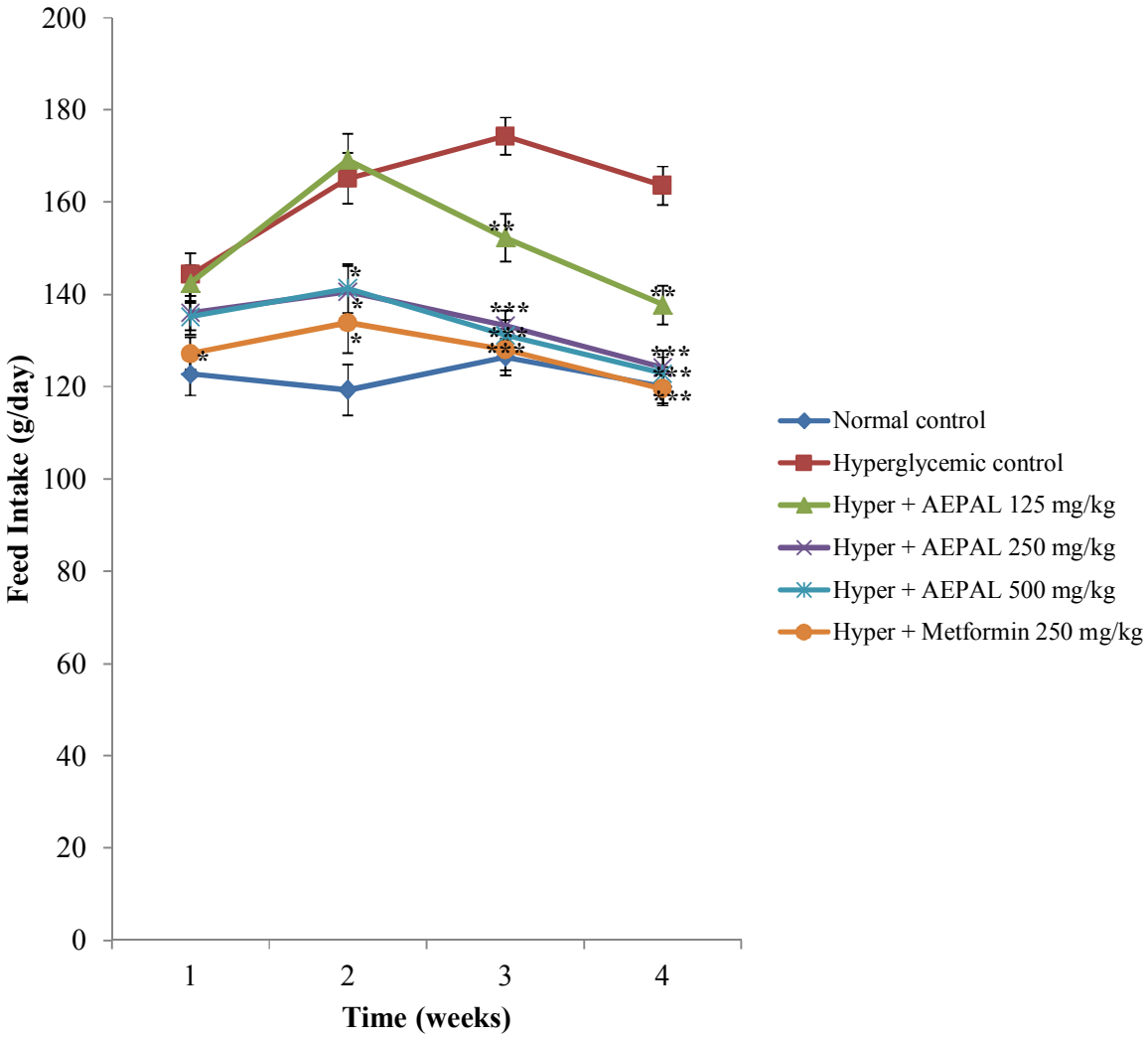


Figure 4.3: Effect of aqueous extract of *P. americana* leaf (AEPAL) on feed intake in alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.05, **P<0.01**,** ***P<0.001, statistically significant compared to hyperglycaemic control.

4.5.2 Effect of extract on water intake in alloxan-induced hyperglycaemic rats

A highly significant (P<0.001) reduction in water intake was observed in standard drug (metformin 250 mg/kg) group and in all the treated groups in week 1 - 4 except in 250 mg extract/kg treated group which showed a significant (P<0.01) decrease only in week 1 compared to hyperglycaemic control (Figure 4.4).

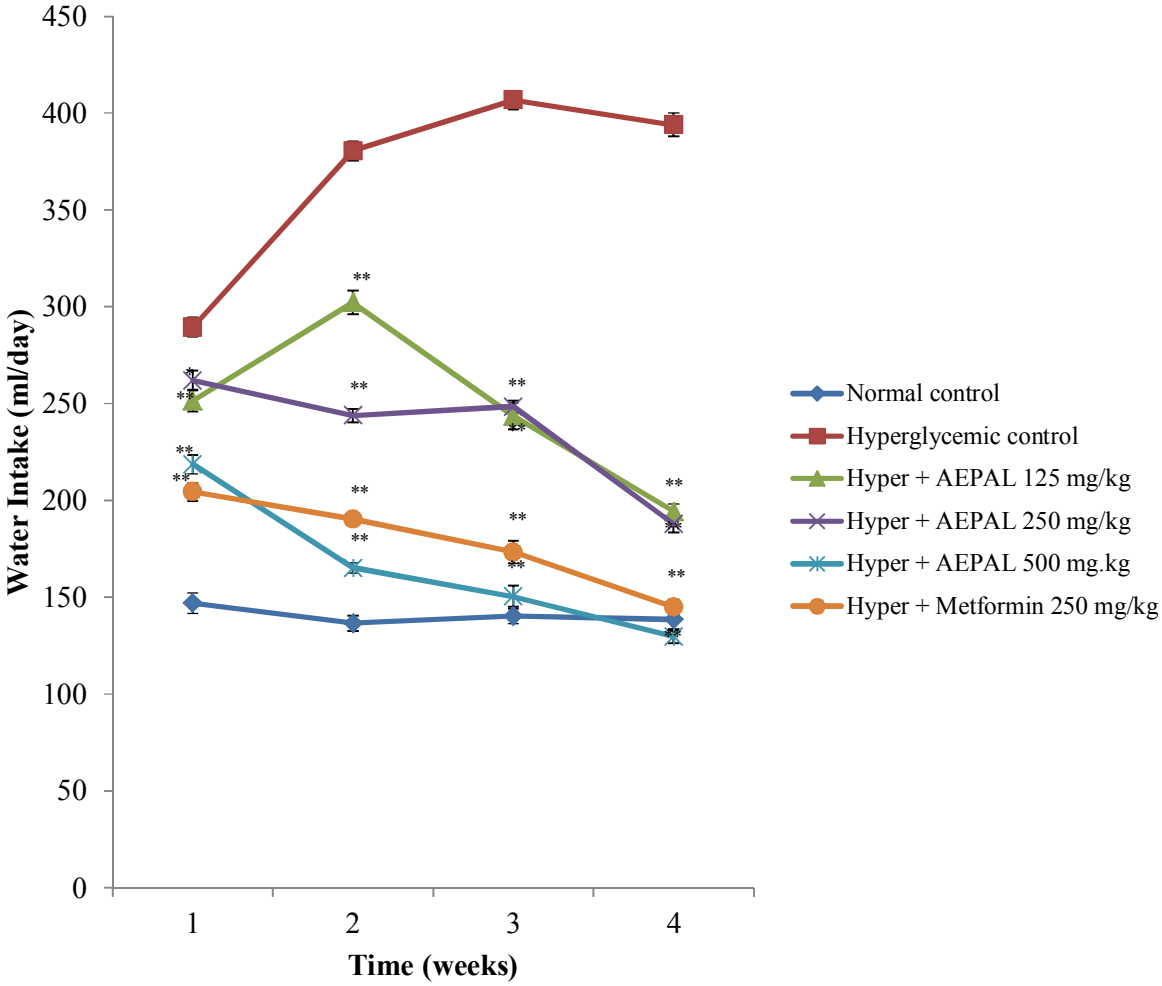


Figure 4.4: Effect of aquoeos extract of *P. americana* leaf (AEPAL) on water intake in alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.01, **P<0.001 statistically significant compared to hyperglycaemic control.

4.5.3 Effect of extract on body weights of alloxan-induced hyperglycaemic rats

The normal control showed a progressive increase in body weight with time while the reverse is the case in hyperglycaemic control (non-treated) which showed a decrease in body weight throughout the 28 days of treatment. The body weight reduction mediated by alloxan-induced hyperglycaemia was significantly ($P<0.05$) reversed in hyperglycaemic rats treated with 250 mg AEPAL/kg on day 28 and standard drug (metformin 250 mg/kg) on day 21. A highly significant ($P<0.01$) weight gain was observed in hyperglycaemic rats treated with 500 mg/kg of extract and metformin 250 mg/kg on the last day of treatment compared to hyperglycaemic control (Figure 4.5).

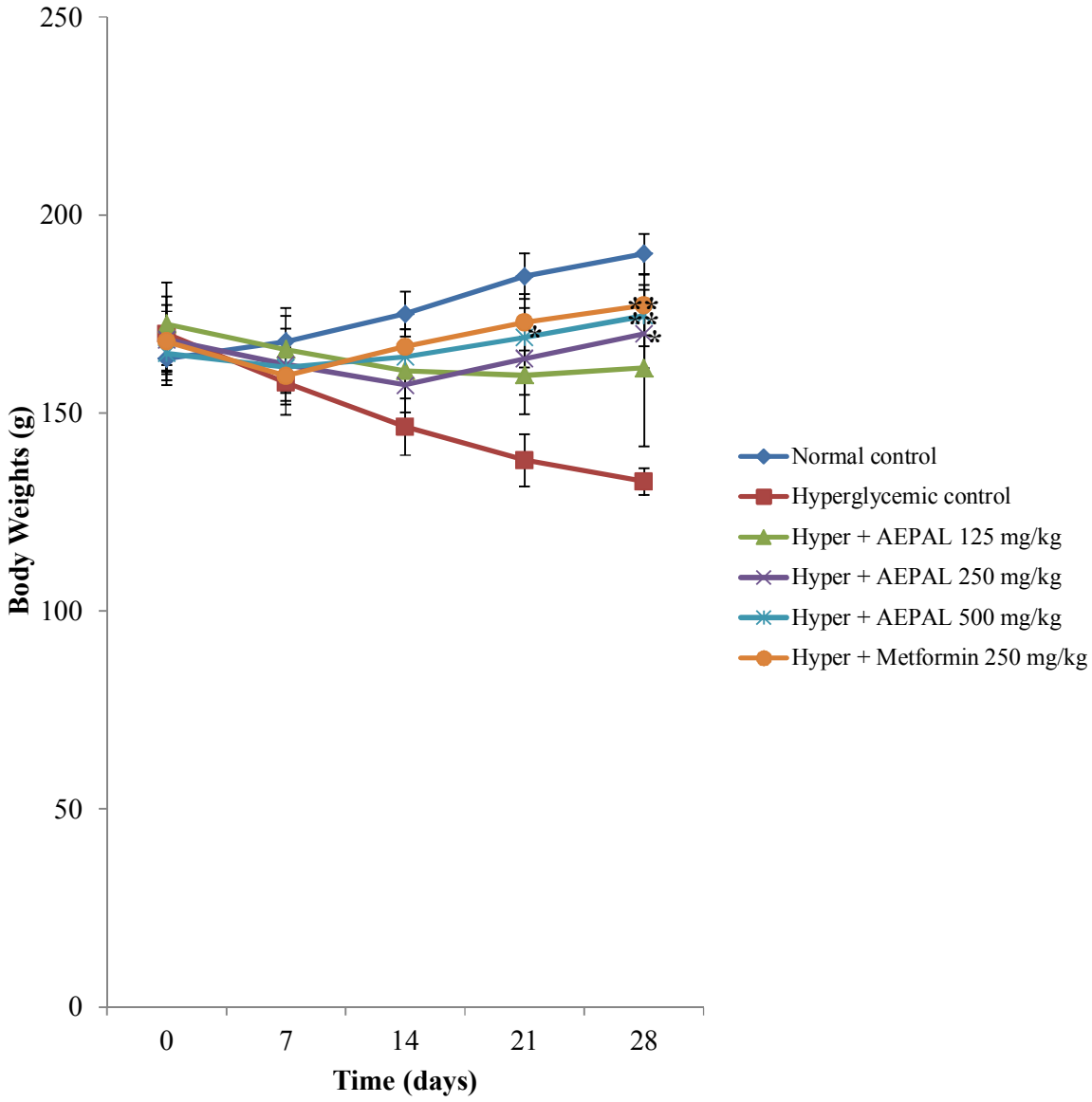


Figure 4.5: Effect of aquoeos extract of *P. americana* leaf (AEPAL) on body weights of alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; $^{*}P<0.05$, $^{**}P<0.01$, statistically significant compared to hyperglycaemic control.

4.5.4 Effect of extract on fasting blood glucose concentration (FBGC) of alloxan-induced hyperglycaemic rats

There was a marked rise in fasting blood glucose levels observed in hyperglycaemic control compared to normal control. All the doses (125, 250 and 500 mg/kg) of AEPAL including metformin 250 mg/kg exhibited a highly significant (P<0.001) anti-hyperglycaemic activity from day 7 – 28 of the treatment period (Figure 4.6).

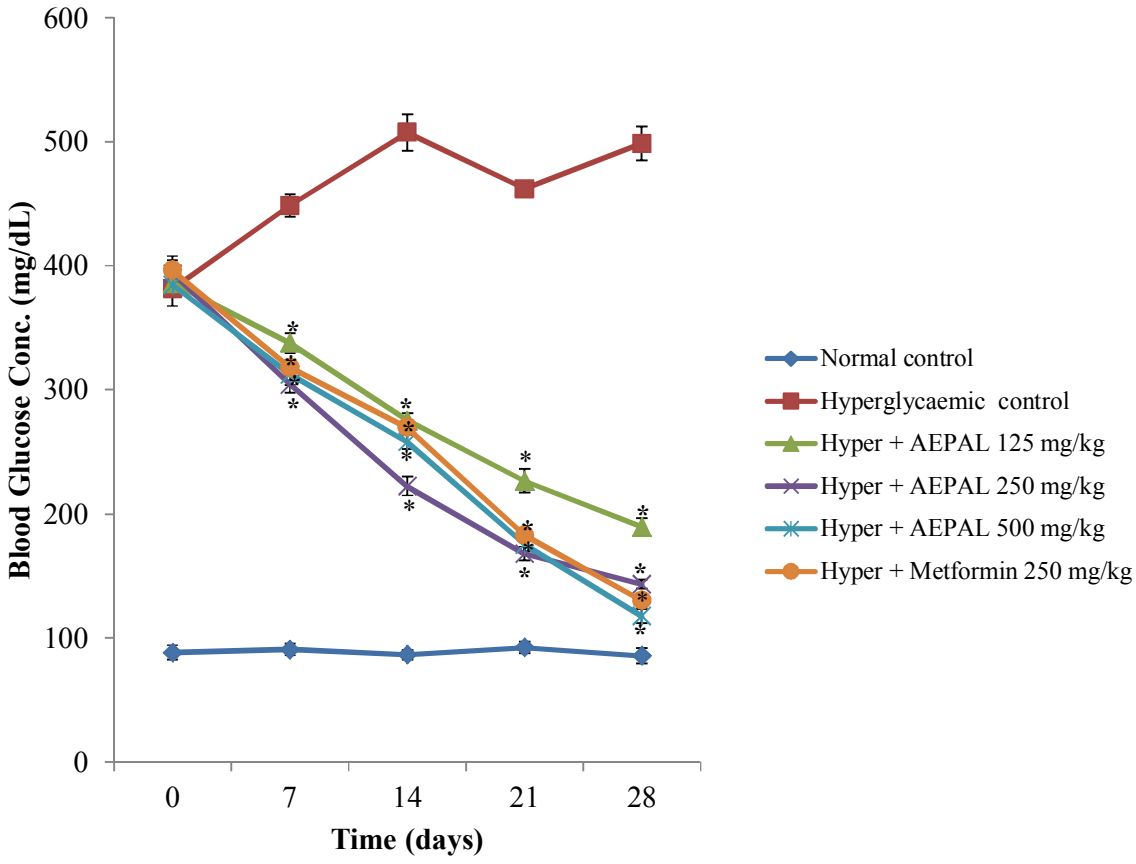


Figure 4.6: Effect of aquoeos extract of *P. americana* leaf (AEPAL) on fasting blood glucose concentrations in alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.001, statistically significant compared to hyperglycaemic control.

4.5.5 Effect of extract on serum biochemical parameters in alloxan-induced hyperglycaemic rats

AST and creatinine levels were decreased significantly ($P<0.05$) by 125 and 250 mg/kg AEPAL respectively. A highly ($P<0.01$) reduction in AST, urea, TP and ALB were observed in 125, 250 and 500 mg extract/kg respectively while ALT and ALP were both decreased at the same significant ($P<0.01$) level by 500mg/kg extract and reference standard, metformin 250 mg/kg compared to hyperglycaemic control. The highest significant ($P<0.001$) decrease in serum biochemical parameters were observed as follows; AST and urea in 500 mg/kg extract and metformin, TP in metformin only and creatinine in 250 and 500 mg extract/kg as well as in metformin compared to hyperglycaemic control (Table 4.8).

Table 4.8: Effect of aqoueos extract of *P. americana* leaf (AEPAL) on biochemical parameters in alloxan-induced hyperglycaemic rats following daily oral administration for 28 days

	Serum biochemical parameters						
Treatment/dose (mg/kg)	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**	**TP (g/L)**	**ALB (g/L)**	**Urea (mmol/L)**	**Creat (µmol/L)**
Normal control	40.83 ± 4.67	47.67 ± 5.11	62.83 ± 7.94	65.00 ± 6.55	37.17 ± 6.68	2.18 ± 0.17	49.50 ± 1.48
Hyperglycaemic control	78.67 ± 5.46	90.50 ± 11.42	102.17 ± 8.28	28.00 ± 3.90	19.17 ± 4.31	5.05 ± 0.20	68.50 ± 2.57
Hyperglycaemic + AEPAL 125	54.33 ± 3.94**	71.67 ± 5.65	93.50 ± 5.97	32.33 ± 3.46	23.83 ± 2.97	4.62 ± 0.28	60.50 ± 1.34*
Hyperglycaemic + AEPAL 250	57.50 ± 3.30*	64.67 ± 7.62	77.33 ± 7.50	42.00 ± 4.76	30.50 ± 2.57	3.72 ± 0.26**	45.67 ± 1.20***
Hyperglycaemic + AEPAL 500	43.67 ± 6.49***	46.50 ± 4.69**	61.50 ± 6.20**	57.00 ± 7.40**	46.50 ± 5.06**	2.95 ± 0.17***	43.67 ± 2.03***
Hyperglycaemic + Metformin 250	39.83 ± 1.35***	52.83 ± 5.53**	58.68 ± 8.02**	65.33 ± 1.63***	38.67 ± 4.76	2.63 ± 0.17***	47.67 ± 1.84***

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.05, **P<0.01, ***P<0.001, statistically significant compared to hyperglycaemic control

4.5.6 Effect of extract on lipid profile in alloxan-induced hyperglycaemic rats

In figure 4.7, hyperglycaemic control showed significant hyperlipidaemia compared to normal control. Treatment with AEPAL reversed the hyperglycaemic-induced hyperlipidaemia in a dose related fashion. HDL was significantly ($P<0.05$) increased by 125 mg extract/kg where as TG and LDL were highly significantly ($P<0.01$) decreased by the same extract dose. Rats treated with 250 and 500 mg/kg AEPAL and 250 mg/kg metformin showed a highest significant ($P<0.001$) reversal in all the lipid profile compared to hyperglycaemic control.

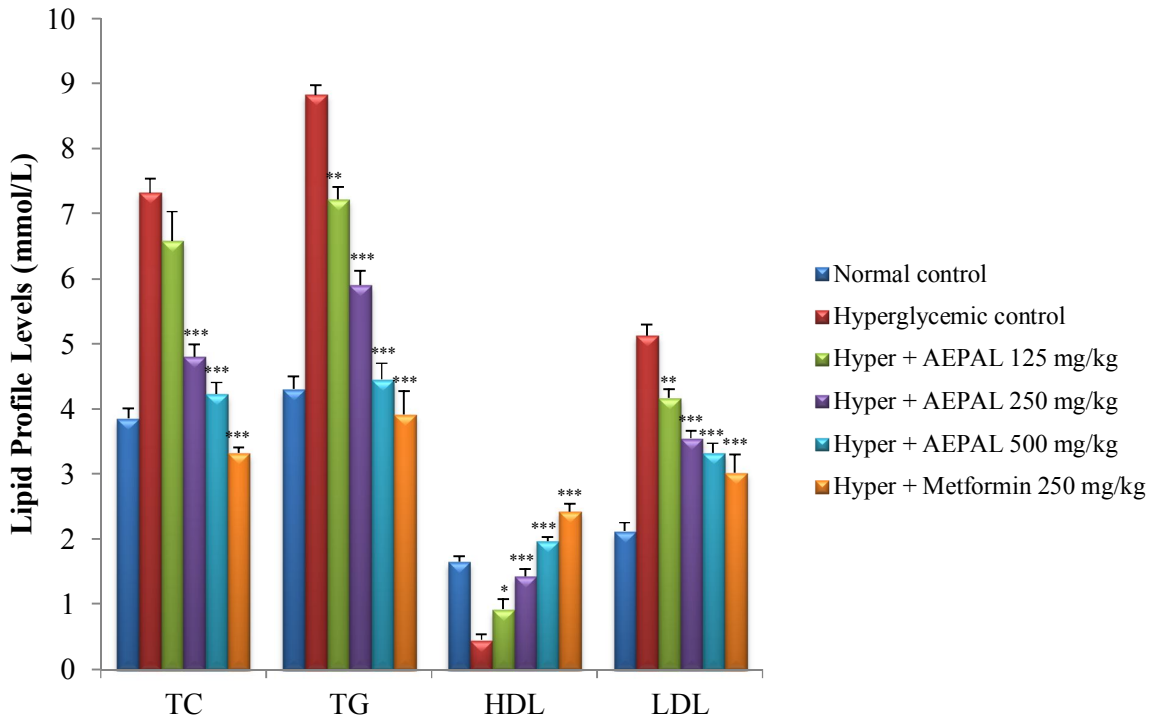


Figure 4.7: Effect of aqoueos extract of *P. americana* leaf (AEPAL) on lipid profile levels in alloxan-induced hyperglycaemic rats following daily oral administration for 28 days

AEPAL – aqueous extract of *P. americana* leaf, TC – total cholesterol, TG – triglyceride, HDL – high density lipoprotein, LDL – low density lipoprotein.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.05, **P<0.01, ***P<0.001 statistically significant compared to hyperglycaemic control.

4.5.7 Effect of extract on haematological parameters of alloxan-induced hyperglycaemic rats

There were insignificant (P>0.05) variations observed in all the haematological indices of the treated groups compared to hyperglycaemic control (Table 4.9). **Table 4.9:** Effect of aqueous extract of *P. americana* leaf (AEPAL) on haematological parameters of alloxan-induced hyperglycaemic rats following 28 days oral administration

	Haematological parameters							
Treatment/dose (mg/kg)	**WBC ($\times10^3$/µl)**	**RBC ($\times10^6$/µl)**	**HGB (g/dl)**	**PCV (%)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**PLT ($\times10^3$/µl)**
Normal control	11.93 ± 1.86	7.22 ± 0.31	13.70 ± 0.43	45.67 ± 1.59	63.27 ± 1.28	19.08 ± 0.43	30.13 ± 0.33	649.67 ± 36.22
Hyperglcaemic control	10.87 ± 1.59	6.95 ± 0.30	13.05 ± 0.57	42.50 ± 1.38	61.25 ± 1.02	18.80 ± 0.50	30.68 ± 0.51	473.69 ± 45.05
Hyperglycaemic + AEPAL 125	10.75 ± 1.82	7.28 ± 0.20	13.55 ± 0.43	44.50 ± 1.52	61.23 ± 1.25	18.67 ± 0.42	30.47 ± 0.50	515.50 ± 31.86
Hyperglycaemic + AEPAL 250	12.25 ± 1.39	7.85 ± 0.30	14.48 ± 0.64	47.17 ± 2.17	59.73 ± 0.70	18.43 ± 0.36	30.88 ± 0.50	586.50 ± 67.44
Hyperglycaemic + AEPAL 500	13.45 ± 0.77	7.90 ± 0.14	14.30 ± 0.23	46.83 ± 0.83	58.98 ± 0.68	18.17 ± 0.38	30.58 ± 0.42	605.67 ± 34.01

Hyperglycaemic + Metformin 250	10.75 ± 1.49	7.47 ± 0.18	13.55 ± 0.44	44.17 ± 1.42	59.17 ± 0.89	18.17 ± 0.33	30.75 ± 0.48	663.17 ± 55.90

AEPAL – aqueous extract of *P. americana* leaf, WBC – white blood cell, RBC – red blood cell, HGB – haemoglobin, PCV – packed cell volume, MCV – mean corpuscular volume, MCH – mean corpuscular haemoglobin, PLT – platelets, MCHC – mean corpuscular haemoglobin concentration.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; P>0.05, no statistically significant difference between hyperglycaemic control and treated group.

4.5.8 Histopathology of Pancreas

Multiple sections of pancreas of rats from each group were taken and studied for any histological changes. The cellular integrity and architecture were intact in the normal control group which showed normal appearance of pancreatic lobules and cells. The islets were normal in size, shape and number (plate VIIIa). Pancreatic sections of hyperglycaemic control rats showed that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets. Also nuclear changes, disappearance of nucleus, relative reduction of size and number of islets (atrophy) especially around the central vessel and severe reduction of beta cells were clearly seen (Plate VIIIb). Study of pancreas of treated hyperglcaemic groups (125, 250 and 500 mg/kg AEPAL) showed a progressive dose dependent regeneration features. There were increased sizes of islets and hyperchromic nucleus, relative granulated and normal beta cells from lowest to the highest dose of extract compared to hyperglycaemic control (plate VIIIc – VIIIe). But the metformin treated group showed a less regeneration of pancreatic beta cells (plate VIIIf).

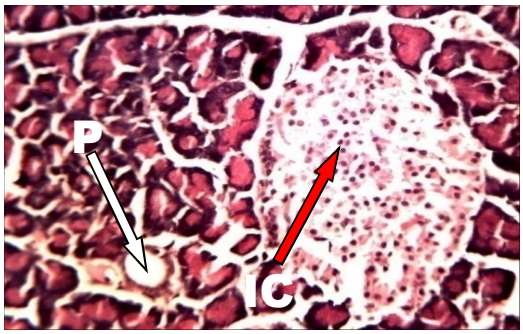


Plate VIIIa: Normal control

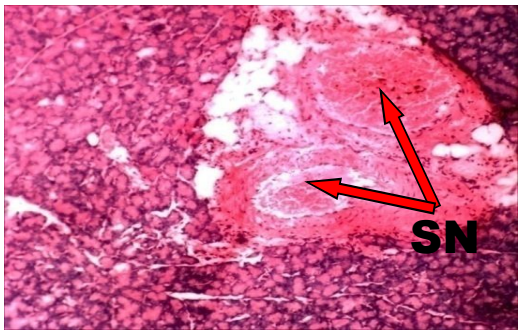


Plate VIIIb: Hyperglycaemic control



Plate VIIIc: Hyper + 125 mg/kg AEPAL

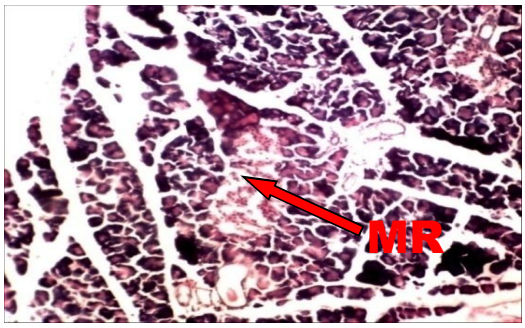


Plate VIIId: Hyper + 250 mg/kg AEPAL



Plate VIIIe: Hyper + 500 mg/kg AEPAL

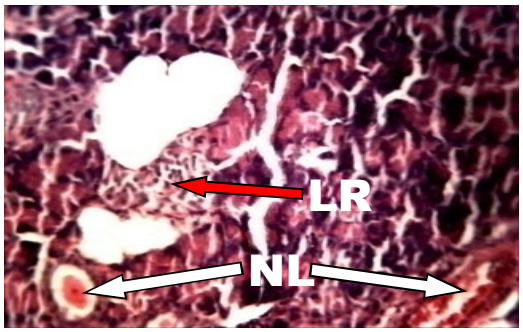


Plate VIIIf: Hyper + 250 mg/kg metformin

Plate VIII: Photomicrographs of sections of pancreas of normal control, hyperglycaemic control and alloxan-induced hyperglycaemic treated rats (H & E stained, ×250)

Plate VIIIa: IC – Intact Islet cells, PL – normal pancreatic lobule
Plate VIIIb: SN – Severe necrosis and atrophy of Islet cells
Plate VIIIc: SR – Slight regeneration of Islet cells
Plate VIIId: MR – Moderate regeneration of Islet cells
Plate VIIIe: HR – High regeneration and almost intact islet cells
Plate VIIIf: LR – Low regeneration of islet cells, NL – Necrosis, lesion, distortion and atrophy still present.

CHAPTER FIVE

DISCUSSION

Herbal medicines have received greater attention as an alternative to orthodox pharmaceutical products for reasons of cost, side effects, availability and general cultural/traditional awareness (Kripa *et al.,* 2011). These herbal preparations are believed to be harmless and their utilization is often based on long-term clinical experience (Chindo *et al.,* 2012). Several medicinal plants have been scientifically evaluated and found to possess hypoglycaemic activity (Gyang *et al.,* 2001), thus the basis for their continue use in herbal medicine practice for the treatment of diabetes mellitus (DM). *Persea americana* is one of those plants used as food and traditional medicine in some rural communities in Nigeria (Oluwole *et al*., 2011). The leaf preparations are mostly used in the south-eastern Nigeria for the management of diabetes. With the upsurge in the use of herbal remedies in the last two decades, there is need for a thorough scientific evaluation of these medicinal plants. Hence to validate the claimed pharmacological properties and investigate the possible toxicity, preclinical toxicity and anti-hyperglycaemic studies were carried out on the aqueous extract of *Persea americana* leaf (AEPAL) in rat models.

The yield (9.93% $^{w}/_{w}$) of AEPAL is similar to that reported by Gyang (2009) which are 12.25 and 10.41% $^{w}/_{w}$ from hot water and chloroform stem bark extract respectively as well as Idris *et al*., (2009), 11.34% $^{w}/_{w}$ from methanol seed extract. But differs from the yields reported by Ayinde *et al*., (2011) which are 4.78, 4.47 and 3.67% $^{w}/_{w}$ respectively from methanol extract of leaf, stem and root bark of *P. americana*

respectively. These differences might be due to the various parts of *P. americana,* extraction solvents and procedures/duration used.

The activities of plant extracts in effecting any therapeutic or biological changes in ailing or diseased animals or living tissues are direct functions of the chemical constituents inherently present in them after extraction (Ayinde and Agbakwuru 2010). The results of the preliminary phytochemical screening revealed the presence of numerous medically useful phytoconstituents or biologically active compounds which could serve as potential source of drugs in herbal medicine. In this study, the phytochemicals detected include alkaloids, cardiac glycosides, flavonoids, saponins, tannins, and polysaccharides. This screening result concurs with the work of Nwaoguikpe and Braide, (2011) who reported the quantitative values of phytochemicals from aqueous seed extract of *P. americana* as follows; 'that the seed is rich in saponins (51.0 ± 0.0 %), tannins 21.66 ± 0.0%), flavonoids (21.00 ±0.0%) and alkaloids 9.43 ± 0.2%)'. Also, this study agreed with the reports of Anita, (2005), Idris *et al.,* (2009) and Leite *et al.,* (2009), and similar with the works of Gyang, (2009) and Ayindele *et al.,* (2011). The two later studies showed the absence of flavonoids in the hot water extract of stem bark of *P. americana*and alkaloids in the methanol extract of leaf, stem and root bark of *P. americana* respectively. These slight variations could be possibly due to regional, harvesting season, plant species and parts, differences in phytoconstituents and probably the extraction solvents used.

The acute toxicity of *P. americana* has been investigated to determine the median lethal dose (LD_{50}) as well as any adverse effect that may arise as a result of a short time animal exposure to the extract within 24 h period. *P. americna* has been used by TMPs

without report of any mortality due to toxicity (Kripa, *et al.,* 2011). There was no death recorded at oral treatment of up to 5000 mg/kg b.w. of AEPAL which agreed with the work of Ozolua *et al*., (2009). The result thus suggests that the extract of *P. americana* is relatively safe on acute administration at the doses used, since the LD_{50} was greater than 5000 mg extract /kg b.w. this relative safety observed may have been responsible for its widespread use in different ethno-therapeutic interventions.

There were no significant changes in feed and water intake. Although rats administered 1000 mg/kg extract showed slight decrease feed intake. The determination of these parameters is important in the study of safety of a therapeutic product, as proper intake of nutrients and water are essential to the physiological status of the animals and to the accomplishment of proper response to the drug tested (Teo *et al.,* 2002; Feres *et al.,* 2006).

Body and organ weight changes serve as an indicator of adverse effects (Raza *et al.,* 2002). Organ-to-body weight ratios are indices which are often used in toxicological evaluations (Michael *et al*., 2007). The results in this study show that these indices were not significantly altered by sub-acute treatment. This observation agrees with that reported in the work (acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea americana* Mill (lauraceae) in rats) of Ozolua *et al.,* (2009). However, this lends no credence to the presence of injuries to the liver, kidney and lungs with exception to the heart and spleen. Thus, it is possible that the injuries elicited by AEPAL on some of the essential organs may have not gotten to the stage of causing growth retardation in the treated animals. In this study, the effect of AEPAL on normoglycaemic fasting blood glucose concentration in the course of the 28 days sub-

chronic study was evaluated. It was observed that AEPAL caused a significant reduction in blood glucose level in all the doses used, with the highest dose (1000 mg/kg extract) eliciting the lowest reduction on day 21. This suggests that extract of *P. americana*may cause hypoglycaemia at higher doses since in this study, decrease in fasting blood glucose level increases with dose. This result is similar to the one reported by Edem, (2009) in which the ethanolic seed extract of *PA* caused decrease in blood glucose level in normoglycaemic rats but did not cause hypoglycaemia as observed with AEPAL in this study.

Elevated levels of liver enzymes are often diagnostic of underlying cellular injuries (Wittekind, 1995; Karthikeyan *et al*., 2006). From the serum biochemistry result of this study, AEPAL appear safe at low and moderate doses of 250 and 500 mg/kg, but toxic changes (increased AST and ALT) were elicited in the liver of rats treated with the 1000 mg/kg dose of the extract at the end of 28 days study. This is evident in the observed histopathological changes in the liver tissue. The absence of significant increase in the activity of the ALP might have been due to the prolong administration of the extract, since liver enzymes activity normally increases at the earlier phase of hepatic injury and the liver is capable of repairing itself. This suggest that possible cholestasis may have not occurred at the dose levels tested since a rise in plasma ALP level is usually a characteristic finding in cholestatic liver disease (Kaneko, 1989). The observed moderate increase in urea and creatinine at highest dose treated group indicates deleterious effect on kidney which is absent at low doses probably due to regeneration on the earlier assault on the organ. Thus, the above findings did not concur with that of Ozolua *et al.,* (2009).

The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological status in man and animals (Adeneye *et al.,* 2006). This study has shown that sub-acute treatment with AEPAL caused changes only in WBC counts which showed decrease in 500 and 1000 mg/kg extract treated groups. Decrease in haematological parameters has been associated with anaemia (Onyenyili *et al.,* 1998). Haematological changes such as anaemia are often accompaniments of bone marrow toxicity (Flanagan and Dunk, 2008). Thus, this normocytic type of aneamia observed in this study might have been due to the chronic or prolonged administration of high doses of AEPAL which may have caused bone marrow toxicity.

Histological examination of essential organs is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD, 1995). The examination of the liver indicated tissue damage. The lesions may have occurred as a result of metabolism of the extract by the liver which serves as the primary organ of biotransformation (Baggot, 1984) and also agree with the theory of target organ toxicity (Heywood, 1981). The glomerulus in the kidney is the site of removal of several chemicals and it may be injured by any toxic, metabolic and immunologic mechanism (Himri *et al*., 2004). The toxic irritant substances brought to the kidney by circulating blood cause degenerative changes in the kidney tissues according to Varely (1987). The lung tissue also showed lesions with higher doses (500 and 1000 mg/kg) of AEPAL. In all, there were dose dependent pathological changes in the liver, kidney and lung tissues (excluding heart and spleen tissues) elicited by AEPAL following sub-acute treatment. These changes may be due to any one or more of the phytochemical constituents present in the extract. Furthermore, the phytochemical screening of the aqueous extract of *P. americana* indicated presence of appreciable amount of tannins. Studies

conducted by Yamasaki *et al.,* (2002) and Bajaj (1988), showed that a large intake of tannins may cause kidney and liver damage.From the findings of the sub-chronic toxicity study above, in which some toxic effects where observed at the high doses used especially in 1000 mg/kg extract, the doses for this anti-hyperglycaemic study were reduced to half the doses used in sub-chronic toxicity study above, that is, 125, 250 and 500 mg/kg representing 2.5, 5 and 10% of the LD_{50}.

In the anti-hyperglycaemic study, AEPAL significantly reversed the excess feed and water intake and body weight loss in hypeglycaemic treated rats. These reversal effects of decrease in feed and water intake and increase in body weight compared to hyperglyglycaemic non-treated rats were evident towards the end of the treatment. Those observations may be due to reversal of hyperglycaemia and the effect of the plant extract on lipolysis, thus countering the basic symptoms of DM viz; polyphagia, polydipsia, and weight loss.

The extract had a remarkable effect on the plasma glucose levels. AEPAL significantly reduced blood glucose levels of treated rats ($P<0.001$) compared to hyperglycaemic control, an effect which is dose dependent. The plant extract at the doses tested decreased the plasma glucose concentration more than the standard reference drug, metformin. The anti-hyperglycaemic activity of AEPAL appear to be related to a number of investigations which have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids possess hypoglycemic properties in various experimental animal models (Akah and Okafor, 1992; Marles and Farnsworth, 1995; Grover *et al.,* 2002; Pulok *et al.,* 2006). This hypothesis stipulates that plant which contain terpenoid and/or flavonoids posses

hypoglycemic activities in diabetic and normal mammal. One or more of the other chemical constituents of the plant especially flavonoid may have played a role in the anti-hyperglycemic action of the plant extract (Valcheva- Kuzmanova *et al.*, 2007; Goji *et al.*, 2009; Aragao *et al.,* 2010). Thus, the decrease in plasma glucose level observed in this study is in line with the works of Anita *et al.,* (2005), Edem (2009) and Edem *et al.,* (2009) which lends support to the folkloric use of *P. americana* leaf preparations as an anti-diabetic agent. Metformin used as a control drug in this study, exert its action mainly by reduction of hepatic glucose output, largely by inhibiting hepatic gluconoegenesis. It also slows intestinal absorption of glucose and improves peripheral glucose uptake and utilization (Richard and Pamela, 2009). Unlike sulfonylurea, metformin does not promote insulin secretion from the pancreatic β-cells; hence its action is mainly extra pancreatic (Richard and Pamela, 2009). The decrease in blood glucose concentration exhibited by AEPAL is greater than that elicited by metformin in this study.

Furthermore, the anti-hyperglycaemic study showed elevated serum liver enzymes (AST, ALT and ALP) in hyperglycemic control compared treated groups and normal control. These increases may be due to the leakage of these enzymes from the liver cytosol into the blood stream and/or change in the permeability of liver cell membranes (Ogbonnia *et al.,* 2008). ALT is predominantly found in the liver and an increased plasma concentration is an indicator of liver damage while AST is not only located in the liver but also in the heart and kidney and elevation in their plasma concentrations indicate liver and possible heart damage and high level of ALP is a sign of possible liver cholestasis (Ogbonnia *et al.,* 2008). There were significant reduction in the level of AST, ALT and ALP in extract and metformin treated hyperglycaemic groups.

Regarding the disturbance of protein metabolism in a diabetic state, the level of serum protein and albumin were significantly reduced in hyperglycaemic control compared to treated groups and normal control. A number of factors that might likely be responsible for this reduction in the serum total protein include increased rate of amino acids conversion to glucose (Chaung *et al.,* 2007), decreased amino acids uptake (Garber *et al.,* 1980), and increased conversion rate of glucogenic amino acids to CO_2 and H_2O (Ahmed, 2005). Other studies have also revealed that total protein decrease might be as a result of a decrease in the amount and availability of mRNA, a loss of transitional factor, reduction of ribosomal protein synthesis as a result of insulin deficiency (Ahmed *et al.,* 2006) and decreased defensive mechanism (Mahboob *et al.,* 2005). The improvement in the total protein and albumin content observed in the extract and metformin treated groups could be a as a result of increased protein synthesis, increase hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzes amino acids transamination. Good correlation between protein synthesis and insulin level has been recorded by Nahla *et al.,* (2006). Thus, this indicates the protective effect of AEPAL on liver and improvement in liver function efficiency.

Renal disease is one of the most common and severe complications of diabetes (Tierney *et al.,* 2002). The blood urea and creatinine levels increased significantly in hyperglycaemic control group. Increase in serum urea and creatinine concentrations, which are considered as markers of kidney dysfunction (Gross *et al.,* 2005), were reversed in hyperglycaemic treated rats. An increase in serum urea and creatinine levels may indicate diminished ability of the kidneys to filter these waste products from the

blood and excrete them in the urine (Gross *et al.,* 2005) . Based on these findings, AEPAL may have enhanced the ability of the kidney to remove these waste products from the blood as indicated by the reduction in serum urea and creatinine levels and thus, confer a protective effect on the kidney of hyperglycaemic treated rats.

Hyperglycemia and hyperlipidemia as well as oxidative stress generally coexist in diabetes subjects (Mironova *et al*., 2000; Beckman *et al*., 2002). Dyslipidemia which includes not only quantitative but also qualitative abnormalities of lipoproteins plays a significant role in the proatherogenesis of vascular complications in diabetes (Sobngwi *et al*., 2001; Rotimi *et al*., 2011). Hyperlipidemias are consequences of diabetes (Odetola *et al*., 2006; Iweala and Oludare, 2011). The present study showed an increase in the concentration of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in hyperglycaemic (non-treated) control. Diabetic-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to under utilization of glucose (Krishnakumar *et al*., 2000; Nimenibo-uadia, 2003). The lack of insulin and elevations of the counter-regulatory hormones lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah *et al*., 2006). The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride (Suryawanshi *et al*., 2006). The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Claudia *et al*., 2006). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Claudia *et al*., 2006). In this study, the administration of graded doses of AEPAL significantly reduced serum levels of total cholesterol, triglyceride, low-density

lipoprotein and increased serum levels of high-density lipoprotein (good cholesterol) in alloxan-induced hyperglycaemic treated rats. Preliminary phytochemical screening of the extract revealed the presence of saponin among other polyphenolic compounds. This may be responsible for the lipid-lowering effect of *P. americana* on plasma lipid. Saponins are known anti-nutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its faecal excretion (Nimenibo-uadia, 2003). Increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol, hence saponins have been reported to have hypocholesterolic effect (James *et al*., 2010). Kumarappen et *al.*, (2007) reported that administration of polyphenolic compounds to alloxan-induced diabetic rats reduced hyperlipidemia, and attributed this to a reduction in the activity of hepatic HMG-CoA reductase, which is the first committed enzymatic step of cholesterol synthesis. This lowers elevated LDL cholesterol levels, resulting in a substantial reduction in coronary events and deaths from congestive heart disease (CHD) that occurs in diabetics (Richard and Pamela, 2009). Thus, the observed hypolipidemic effect of AEPAL may be linked to the synergistic actions of phytochemicals like saponins and polyphenolic compounds contained in the plant extract. It is reported that the derangement of glucose, fat and protein metabolism in the diabetic state, results into the development of hyperlipidemia (Brown and Goldstein, 1983; Austin and Hokanson, 1994). About 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol aetheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease (Kwiterovich, 2000). Therefore, the observed increase in the serum HDL-C level on

administration of various doses of the extract in alloxan-induced hyperglycaemic rats, indicates that the extract have HDL-C boosting effect. More so, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fat (Momo *et al*., 2006). This implies that the plant extract may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics (Sharma *et al*., 2003). The study also revealed that administration of the extract at various doses significantly lowered the serum LDL-C in alloxan-induced hyperglycaemic treated rats. Studies have shown that chronic insulin deficiency as observed in alloxan-induced hyperglycaemia in experimental animals is associated with diminished levels of LDL-C receptors. This results to an increase in LDL particles and consequently increases serum level of LDL-C (Suryawanshi *et al*., 2006). In this study, all doses of the plant extract used produced a significant beneficial effect on serum lipid profile in alloxan-induced hyperglycaemic treated rats. The significantly lowered cholesterol level may have contributed to the observed significant high serum high-density lipoprotein cholesterol (HDL-c) in the animals. Thus, this result corresponds with the works of Brai *et* al., (2007), Nwaoguikpe and Braide, (2011) and Kolawale *et al.,* (2012).

In this study, the blood parameters assessed was to ascertain if AEPAL has any ameliorative effect on the haemopoeitic system in a diabetic state. Interestingly, AEPAL indeed corrected the hyperglycaemic-induced alterations in the blood indices. Though, there was no significant increase in WBC, RBC, HGB, PLT and PCV which were decreased in hyperglycaemic control group. It has been suggested that the anaemia occurrence in DM is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia (Twaij and Al-Badr, 1988).

Oxidation of these glycosylated membrane proteins and hyperglyceamia in DM cause an increase in the production of lipid peroxides causing the hemolysis of RBC.

Pancreas is the primary organ involved in regulating the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted (Edem, 2009 cited in Sharma *et al.,* 2010). In this study, the pancreatic ß-cells were destroyed. Alloxan is one of the usual substances used for the induction of DM. Alloxan has a destructive effect on the beta cells of the pancreas (Prince and Menon, 2000; Jelodar *et al*, 2007). Histopathological study of hyperglycaemic untreated rats showed degeneration of pancreatic islet cells, which was due to alloxan used in this study. This probably gave rise to insulin deficiency. Insulin deficiency (diabetes mellitus) causes excessive elevation of blood glucose and underutilization leading to hyperglycemia (Standl *et al*, 2003). The histopathological study of hyperglycaemic treated groups indicated increased number of beta cells, which may be a sign of regeneration. Signs of regeneration of ß-cells, potentiation of insulin secretion from surviving ß-cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of some plant extracts (Shanmugasundaram *et al*, 1990; Abdel-Monein *et al*, 1997; Jelodar *et al,* 2007; Yadav *et al*, 2008). AEPAL may have some chemical components that exert regenerative effects on ß-cells, stimulate these cells to produce more insulin (pancreatotrophic action) or may have some insulin-like substances. Induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreas (Adewole and Ojewole, 2007). The restorative effect of AEPAL on the islet cells of hyperglycaemic treated rats followed a dose dependent manner. This improvement observed in the pancreas of the hyperglycaemic treated rats tallies with the work of Edem (2009) and Edem *et al.,* 2009.

Taken together, it is possible to suggest that AEPAL might directly improve the structural and functional integrities of cells of the blood, liver, kidney, pancreas and other organs in a hyperglycaemic state.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The results from these studies have shown that *P. americana* has a wide range of phytochemical constituents which could be responsible for its various pharmacological activities. The acute toxicity study revealed that the extract is relatively safe orally since the LD_{50} was greater than 5000 mg/kg. However the continuous oral daily dosing (sub-chronic toxicity study) for 28 days showed a varying degree of effects by the extract ranging from having no effects on the feed and water intake as well as body weight and organ weight changes to eliciting alterations in the normal levels of liver enzymes and kidney function markers and haematological parameters. The extract also showed deleterious effects on the structural morphology of some organs including liver, kidney and lungs. These long term toxic effects were mostly observed with higher doses (500 and 1000 mg extract/kg b.w.) treated rats. Meanwhile the reduced doses (125 – 500 mg/kg extract b.w.) used in the anti-hyperglycaemic study showed remarkable beneficial effects. AEPAL reversed the observed symptoms of diabetes such as polyphagia, polydipsia and weight loss. It also corrected all the metabolic dysfuntions observed in the hyperglycaemic untreated rats in the course of this study. These include hyperglycaemia (evident from destroyed β-cells of the pancreas), increased serum liver and kidney function markers, decreased serum total protein and albumin, dyslipidaemia (decreased serum HDL and increased LDL, triglyceride and total cholesterol) and altered haematological parameters.

6.2 Conclusion

From the results of these studies, we conclude that oral administration of *P. americana*leaf extract has relative acute safety but toxic at doses used on long term administration. Also, the extract has hypoglycaemic activity alongside desirable effects such as anti-hyperlipidaemic activity. This finding (blood glucose lowering effect) has justified the use of *P. americana*traditionally for management of DM. However, it can be suggested that caution should be taken in the consumption of high doses of *P. americana* leaf extract especially in liver and kidney disease condition owing to the hepathotoxicity and nephrotoxicity recorded in this study.

6.3 Recommendations

Based on the findings of this work, we therefore recommend the followings;

i. Further studies are needed to quantify, isolate, purify and characterize the specific active compound(s) responsible for the toxicity observed as well as the hypoglycaemic and anti-hyperlipidaemic activities of the leaf extract of *P. americana* plant.

ii. Also, further studies should be carried out in order to ascertain the toxic effect (safety profile) of the plant leaf at lower doses on long term treatment.

iii. There is need to investigate the actual mechanisms/mode of actions for the anti-hyperglycaemic as well as anti-hyperlipidaemic properties of the extract.

iv. Lastly, assessment of insulin level should be carried out in the experimental animals, as this may give more insight into the mechanism of anti-hyperglycaemic activity exhibited by the plant extract.

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APPENDICES

APPENDIX I

Effect of aqueous extract of *P. americana* leaf (AEPAL) on relative organ weights (ROW) of rats following daily oral administration for 28 days

	Relative organ weights (g)						
Treatment/dose	**Liver**	**Kidney**	**Heart**	**Lungs**	**Spleen**	**Testes**	**Ovaries**
Control	3.24±0.15	0.64±0.02	0.40±0.01	0.08±0.03	0.45±0.04	1.27±0.06	0.20±0.01
AEPAL 250 mg/kg	3.24±0.15	0.61±0.06	0.37±0.03	0.75±0.03	0.42±0.02	1.29±0.01	0.21±0.02
AEPAL 500 mg/kg	3.31±0.30	0.61±0.04	0.38±0.03	0.79±0.05	0.42±0.04	1.35±0.02	0.23±0.02
AEPAL 1000 mg/kg	3.51±0.26	0.67±0.02	0.47±0.05	0.82±0.04	0.47±0.02	1.40±0.03	0.24±0.03

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; no statistically significant difference (P>0.05) between control and test groups.

APPENDIX II

Effect of aqueous extract of *P. americana* leaf (AEPAL) on weekly fasting blood glucose concentration (FBGC) of normoglycaemic rats following daily oral administration for 28 days

	Weekly fasting blood glucose concentration (mg/dL)				
Treatment/dose	**Basal value**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	92.00±3.45	87.67±3.24	93.17±2.36	90.50±2.57	88.6±3.15
AEPAL 250 mg/kg	89.17±3.60	82.67±3.42	78.33±3.38*	71.83±3.42**	80.50±5.18
AEPAL 500 mg/kg	87.50±3.06	75.33±3.68*	77.00±5.40*	74.50±3.41**	71.00±5.01*
AEPAL 1000 mg/kg	94.00±3.48	76.17±2.34	72.50±2.11**	66.83±3.65***	69.17±4.98*

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM; data analyzed using one-way ANOVA followed by Dunnet multiple comparison post hoc test; *P<0.05, **P<0.01, ***P<0.001, statistically significant compared to control.

APPENDIX III

Effect of aquoeos extract of *P. americana* leaf on feed intake in alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

Food consumption in g per 24 hours

Treatment/dose	Week 1	Week 2	Week 3	Week 4
Normal control	122.71 ± 4.63	119.29 ± 5.52	126.43 ± 3.98	120.00 ± 4.12
Hyperglycaemic control	144.29 ± 3.66	165.14 ± 5.75	174.29 ± 4.68	163.57 ± 6.98
Hyperglycaemic + AEPAL 125 mg/kg	142.43 ± 3.93	169.14 ± 5.63	152.29 ± 5.22**	137.71 ± 4.23**
Hyperglycaemic + AEPAL 250 mg/kg	136.00 ± 4.72	140.57 ± 5.63*	133.29 ± 3.25***	124.14 ± 3.62***
Hyperglycaemic + AEPAL 500 mg/kg	135.14 ± 2.97	141.29 ± 5.30*	131.14 ± 3.24***	122.86 ± 3.54***
Hyperglycaemic + Metformin 250 mg/kg	127.14 ± 3.45*	133.86 ± 6.52*	128.00 ± 4.50***	119.57 ± 3.21***

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Tukey's post hoc test; *P<0.05, **P<0.01, ***P<0.001 statistically significant compared to hyperglycaemic control.

APPENDIX IV

Effect of aquoeos extract of *P. americana* leaf on water intake in alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

	Water intake in ml per 24 hours			
Treatment/dose	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Normal control	147.00 ± 5.18	136.57 ± 3.89	140.29 ± 3.82	138.57 ± 4.87
Hyperglycaemic control	289.43 ± 4.99	380.57 ± 5.10	406.71 ± 4.69	394.00 ± 6.02
Hyperglycaemic + AEPAL 125 mg/kg	251.57 ± 5.56**	302.29 ± 6.08**	243.57 ± 6.74**	194.43 ± 3.84**
Hyperglycaemic + AEPAL 250 mg/kg	262.00 ± 5.06*	244.00 ± 3.44**	248.43 ± 3.14**	187.86 ± 4.19**
Hyperglycaemic + AEPAL 500 mg/kg	218.57 4.74**	165.14 ± 2.69**	150.43 ± 5.39**	129.71 ± 3.12**
Hyperglycaemic + Metformin 250 mg/kg	204.43 ± 4.79**	190.43 ± 3.19**	173.29 ± 5.86**	145.00 ± 3.86**

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Tukey's post hoc test; **P<0.01, ***P<0.001 statistically significant compared to hyperglycaemic control.

APPENDIX V

Effect of aquoeos extract of *P. americana* leaf on body weights of alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

	Weekly body weights (g)				
Treatment/dose	**Initial**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal control	163.67±6.62	168.00±6.45	175.00±5.72	184.50±5.74	190.17±5.04
Hyperglycaemic control	170.00±9.33	157.67 ±8.09	146.50±7.14	138.00±6.63	132.67±3.37
Hyperglycaemic + AEPAL 125 mg/kg	172.50±10.47	166.00±10.50	160.67±10.48	159.50±9.84	161.33±9.78
Hyperglycaemic + AEPAL 250 mg/kg	168.50±8.68	162.17±9.12	157.00±9.33	163.67±9.03	170.00±8.66*
Hyperglycaemic + AEPAL 500 mg/kg	165.00±6.83	161.50±6.44	164.17±6.92	169.00±7.47	174.50±7.69**
Hyperglycaemic + Metformin 250 mg/kg	168.00±7.67	159.33±7.17	166.67±7.75	172.83±7.14*	177.17±7.60**

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; $^{*}P<0.05$, $^{**}P<0.01$, statistically significant compared to hyperglycaemic control.

APPENDIX VI

Effect of aquoeos extract of *P. americana* leaf on fasting blood glucose concentrations in alloxan-induced hyperglycaemic rats following daily oral treatment for 28 days

Fasting blood glucose concentration (mg/dl)

Treatment/dose	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	88.17±5.83	90.67±4.79	86.33±4.10	92.33±4.65	85.50±6.17
Hyperglycaemic control	381.33±13.73	448.50±9.24	507.50±14.60	462.00±15.13	498.50±13.61
Hyperglycaemic + AEPAL 125 mg/kg	386.00±10.62	337.67±8.09*	275.33±5.89*	226.67±9.55*	189.50±6.94*
Hyperglycaemic + AEPAL 250 mg/kg	393.67±10.94	304.50±6.65*	222.33±7.40*	168.00±5.73*	143.17±3.70*
Hyperglycaemic + AEPAL 500 mg/kg	384.50±6.25	312.67±8.72*	258.00±5.69*	175.50±5.64*	117.50±5.54*

Hyperglycaemic + Metformin 250 mg/kg	396.33±11.36	318.00±4.40*	269.83±5.74*	182.67±3.27*	130.17±4.97*

AEPAL – aqueous extract of *P. americana* leaf.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.001, statistically significant compared to hyperglycaemic control.

APPENDIX VII

Effect of aqoueos extract of *P. americana* leaf on lipid profile levels in alloxan-induced hyperglycaemic rats following daily oral administration for 28 days

	Lipid profile			
Treatment/dose	**TC (mmol/L)**	**TG (mmol/L)**	**HDL (mmol/L)**	**LDL (mmol/L)**
Normal control	3.85 ± 0.16	4.30 ± 0.20	1.65 ± 0.08	2.12 ± 0.13
Hyperglycaemic control	7.32 ± 0.22	8.83 ± 0.14	0.45 ± 0.08	5.13 ± 0.16
Hyperglycaemic + AEPAL 125 mg/kg	6.58 ± 0.45	7.22 ± 0.19**	0.92 ± 0.16*	4.17 ± 0.13**
Hyperglycaemic + AEPAL 250 mg/kg	4.80 ± 0.19***	5.90 ± 0.23***	1.43 ± 0.11***	3.55 ± 0.12***
Hyperglycaemic + AEPAL 500 mg/kg	4.23 ± 0.18***	4.45 ± 0.25***	1.97 ± 0.06***	3.32 ± 0.15***

Hyperglycaemic + Metformin 250 mg/kg	3.32 ± 0.09***	3.92 ± 0.36***	2.42 ± 0.12***	3.02 ± 0.28***

AEPAL – aqueous extract of *P. americana* leaf, TC – total cholesterol, TG – triglyceride, HDL – high density lipoprotein, LDL – low density lipoprotein.
n = 6; values expressed as mean ± SEM.; data analysed using one-way ANOVA followed by Turkey's post hoc test; *P<0.05, **P<0.01, ***P<0.001 statistically significant compared to hyperglycaemic control.