# SOME PHARMACOLOGICAL PROPERTIES OF METHANOL EXTRACT OF *ANISOPUS MANNII* N.E.Br (ASCLEPIADACEAE) IN ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS

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**ZARIA, NIGERIA**

**June, 2011**

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**MSc/Pharm.Sci/49298/05-06**

**A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACOLOGY**

**DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,**

**FACULTY OF PHARMACEUTICAL SCIENCE,**

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**ZARIA, NIGERIA**

**JUNE, 2011**

## DECLARATION

I declare that the work in this thesis entitled "Some Pharmacological Properties of Methanol Extract of *Anisopus mannii* N.E.Br (Asclepiadaceae) in Alloxan-Induced Hyperglycaemic Wistar Rats" has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Prof. (Mrs.) H.O. Kwanashie and Prof. I. Abdu-Aguye.

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

Ibrahim Salahudeen MOHAMMED _____ _____

Name of student Signature Date

## CERTIFICATION

This thesis entitled "SOME PHARMACOLOGICAL PROPERTIES OF METHANOL EXTRACT OF *ANISOPUS MANNII* N.E.BR (ASCLEPIADACEAE) IN ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS" by Ibrahim Salahudeen MOHAMMED meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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## ACKNOWLEDGEMENT

I owe my gratitude to the Almighty God, by whose will, power and wisdom this thesis was successfully completed.

I wish to express my sincere gratitude and appreciation to my supervisors, Prof. (Mrs.) H.O. Kwanashie and Prof. I. Abdu-Aguye for their useful suggestions, advice and constructive criticisms throughout this work.

I would like to express my appreciation to those who showed concern, interest and gave moral, physical, spiritual and material resources, as well as time in seeing to the completion of this work, may God bless you all.

Finally, I thank my family for their tireless support, faith and love for me, which led to the completion of this work.

## DEDICATION

This work is dedicated to my mother, Hajiya Fatima Mohammed and my late father, Alh. Mohammed Abubakar.

## ABSTRACT

Diabetes mellitus is a metabolic disorder that affects carbohydrates, lipids and proteins resulting in sustained hyperglycaemia which could lead to serious organ(s) damage and death. The aim of this study was to investigate some of the pharmacological properties of the methanol extract of *Anisopus mannii*, a plant used in ethnomedical practice to manage diabetes mellitus. The specific objectives were to investigate acute toxicity test including $LD_{50}$, antihyperglycaemic properties and some other effects of the plant (if any) on, hematological indices, lipid profile, some liver enzymes (Alanine aminotransferase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP)) and preliminary phytochemical screening of the whole plant. The extract is relatively safe as the oral $LD_{50}$ was found to be 2,150 mg/kg. Alloxan (150 mg/kg) was used to induce hyperglycaemia in rats. Three graded doses of the extract (150, 300 and 600 mg/kg) were administered daily for 14 days and the antihyperglycaemic property was compared with normal saline as control, and the group administered standard hypoglycaemic drug, glibenclamide (10 mg/kg). The extract (300 – 600 mg/kg, *P.O*) produced significant ($P<0.05$ – $P<0.0005$) antihyperglycaemic properties when compared to control in alloxan-induced hyperglycaemic rats. Similarly, the extract (600 mg/kg, *P.O*) at 1 to 24h; and 300 mg/kg at 24h showed significant decrease ($P<0.05$ – $P< 0.0005$) in the blood glucose concentration in normoglycaemic rats. The extract also produced a significant decrease ($P<0.01$ – $P<0.0005$) in hemoglobin, packed cell volume and white blood cell (WBC) count in the alloxan-induced hyperglycaemic Wistar rats, although, lymphocytes and neutrophils were not significantly affected. The high density lipoproteins (HDL) was decreased ($P<0.05$), while low density lipoproteins (LDL) and total cholesterol (TC) were increased ($P<0.01$

and P<0.05) by 300 and 600 mg/kg of the extract respectively. The highest dose of the extract (600 mg/kg) showed a significant decrease (P<0.05 - P<0.0005), while 150 and 300 mg/kg did not produce any significant change in aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) levels in the alloxan-induced hyperglycaemic Wistar rats. Preliminary phytochemical screening of the crude methanol (whole plant) extract of *A. mannii* revealed the presence of saponins, alkaloids, tannins, steroids, terpenoids and flavonoids, while cardiac glycosides and cyanogenic glycoside were absent. The results of the study showed that, the methanol (whole plant) extract of *A. mannii* possesses dose dependent antihyperglycemic and hypoglycaemic properties in alloxan-induced hyperglycaemic and normoglycaemic Wistar rats respectively. The data may provide pharmacological basis for the use of the plant in the management of diabetes mellitus.

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## ABBREVIATIONS

| | |
|---|---|
| ALP | Alkaline Phosphatase |
| ALT | Alanine Aminotransaminase |
| AMI | Acute Myocardial Infarction |
| AP | Angina Pectoris |
| AST | Aspartate Aminotransferase |
| B | Basophils |
| CAD | Coronary Artery Disease |
| DKA | Diabetic ketoacidosis |
| DL | Decilitre |
| DM | Diabetes Mellitus |
| GDM | Gestational Diabetes Mellitus |
| Glb | Glibenclamide |
| Hb | Haemoglobin |
| HT | Hypertension |
| ICD | International Classification of Disease |
| IDDM | Insulin Dependent Diabetes Mellitus |
| IGT | Impaired Glucose Tolerance |
| IND | International Nomenclature of Disease |
| IP | Intraperitoneal |
| Kg | Kilogram |
| L | Lymphocytes |
| Mg | Milligram |
| Mmol | Millimol |
| MRDM | Malnutrition-Related Diabetes Mellitus |

| | |
|---|---|
| N | Neutrophils |
| N/Saline | Normal Saline |
| NIDDM | Non Insulin Dependent Diabetes Mellitus |
| NS | Not Significant |
| PCV | Packed Cell Volume |
| PO | Per Oral |
| WPRO | World Health Organization Regional Office for the Pacific |
| SEM | Standard Error of Mean |
| WBC | White Blood Cells |
| WHO | World Health Organization |

# CHAPTER ONE

# INTRODUCTION

## 1.1 HISTORICAL BACKGROUND

The use of medicines is as old as the existence of man because the need to find measures to fight diseases has always been as important to man's survival as his needs for food and shelter. In the past, all medications came directly from nature with main source being the plant (Sheriff, 2006).

The use of plants for medicinal purposes probably dates back as far as 300BC, during this period the early Greek naturalist, Philosopher, Theophrastus of Lesbos (283-372BC) described plants and classified them by use as herbs, shrubs and trees (Klein, 1979). However, since ancient times, man was able to know that, some fruits, stem, and leaves of many plants can be used to cure some diseases including treatment of wounds as done by our rural folks (Borodo, 2000). The same author further stated that the knowledge that plants can cure diseases is probably instinctive because, even animals seek out appropriate herbs when they are ill. The discovery of both the positive and negative values of plants have been studied and documented by many researchers.

Through the process of studying nature, man studied plants, some of which he found to be useful for food and cultivated same as food crops, while those he found harmful or poisonous were rejected. He found another group of plants to possess medicinal properties, used them as such and the special knowledge passed on to his progeny (Chame, 1987).

## 1.2 STATEMENT OF RESEARCH PROBLEM

Over 280 million people have diabetes worldwide (WHO, 2011).About 3.90 % of Nigerians live with diabetes mellitus (DM) (International Diabetes Federation, 2010).

More than 80% of all deaths occur in low-and middle- income countries (Diabetes Fact Sheets, 2011). Direct cost of diabetes mellitus in Nigeria is put at about N150 billion annually (Suleiman *et al.*, 2006).

### 1.2 .1 Significance of the Study

The need for continuous research into medicinal plants especially in the developing nations could not be overemphasized. The outcome of this research may explain the basis for the rational use of the plant or otherwise in the management of Diabetes. It was indicated by the WHO that over 30% of the world's plant species have at one time or another been used for one medicinal purpose or another (Akerele, 1990).

Traditional medicines have the potential to ameliorate the health needs of our country and Africa in general (WHO, 2008). The selection of antidiabetic plants for evaluation of their therapeutic activity would provide the scientific basis that will justify the claims or otherwise of using them. Zhang, (1996) stated that in 1977 (the year WHO's Traditional Medicine Program was established) the World Health Assembly urged governments "to give adequate importance to the utilization of their traditional systems of medicine, with appropriate regulations, as suited to their national health systems". The same author further stated that medicinal plants and herbs are of great importance to the health of individuals and communities hence between 35, 000 and 70, 000 species have at one time or another been used for medical purposes and international use of herbal medicines and natural products is steadily increasing (W/PRO, 1998). In 1993, the total sales of herbal

medicines in China amounted to more than 14 billion Yuan (US$ 2.4 billion), not including US$ 400 million worth of exports (WHO, 2008). The Malaysian Government estimates that sales of traditional medicine currently amount to US$ 60 million. In the United States and Canada, sales of herbal medicines showed a growth rate of 15% in 1990, while the national growth rates in western European countries ranged from 5% to 22%. In Japan, there was a 15-fold increase in herbal sales between 1979 and 1989, while sales of pharmaceutical products multiplied by only 2.6 (Zhang, 1996).Similarly, the same Author reported that, despite the existence of herbal medicines over many centuries, only a relatively small number of plant species, about 5000, have been studied for their possible medical applications. The provision of this scientific basis for the use of these plants is necessary in order to offer them a place in our health care system as alternative drug therapy.

Diabetes mellitus is a chronic disease that requires long term therapy, often with multiple drug administration (polypharmacy), the likelihood of side effects might hinder the use of synthetic drugs.The cost of long term treatment of diabetes mellitus in our country is a cause for concern and the need to integrate our resources (herbs) into our healthcare system will reduce the suffering of the patients. The choice of using these resources (plants) and their evaluation for therapeutic activity lies inthe hands of our scientists.

Little scientific research has been carried out on the plant, *Anisopus mannii.* This study was designed to explore the claim of *Anisopus manniia*s it is claimed in folklore to be useful in the treatment of diabetes mellitus (Personal communication, 2008).

## 1.3 DEFINITIONS

World Health Organization (WHO,2000) defined Traditional Medicine as the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses.

World Health Organization (WHO) estimated that 80% of more than 400 million inhabitants of the world rely mainly on traditional medicine for their primary health care needs (Farnsworth, 1998). There is an increasing use and popularity for traditional medicine throughout the world nowadays. In industrialized countries, upto 40% of all pharmaceuticals are derived from natural sources (Kayne, 2002).

Adaptation of traditional medicine is termed "Complementary" or "Alternative" Medicine (CAM). Complementary medicine is a diagnosis, treatment and/or prevention which complement mainstream medicine by contributing to a common whole, by satisfying a demand not met by orthodox or by diversifying the conceptual framework of medicine (Kayne, 2002). There are many reasons why CAM is necessary in a poor resource economy like ours, this is due to the fact that, orthodox medicine is becoming more and more expensive due to the cost of advanced technologies in producing new drugs and equipments. Severe toxicities and or adverse effects of conventional drugs push patients to look for a safer alternative therapy like homeopathy, osteopathy, acupuncture and herbal remedies. The need for continuous research into medicinal plants especially in the developing nations could not be over emphasized. It was indicated by the WHO that over 30% of the world's plant species have at one time or another been used for one medicinal purpose or another (Akerele, 1990).

## 1.4 MEDICINAL PLANTS

A medicinal plant is any plant or plant part used for its medicinal, flavoring, or fragrant properties (Andrews, 1982). Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. Europeans sometimes use the term phytomedicine, from phyto (Greek for "plant"), to describe herbal or botanical medicine.

Modern pharmaceuticals cannot cure every condition effectively and therefore, the need to include alternative therapy in form of medicinal herbs and other forms of alternative remedies could not be overemphasized. Today, more than 1,500 herbs are being marketed in stores, pharmacies, supermarkets and other health stores in the USA (Zang, 1996). Plants, a part from the protection they offer in our environment, serve as a source of medicine to the vast number of the world inhabitant. Huguma, (2008) reported that, many Nigerians rely on traditional herbs for their medication. Similarly, one-quarter of U.S. prescription drugs are derived from herbs (Pelletier, 2005). The pharmaceutical industry uses about 120 different compounds derived from plants in the drugs it manufactures, most of which (nearly three-quarters) arise from studying folk remedies (Pelletier, 2005). Examples of drugs from plants include quinine, from the bark of the South American cinchona tree, used to treat malaria; digitalis, a widely prescribed heart medication, derived from the foxglove plant; salicylic acid, the source of aspirin, from willow bark; and taxol, for treating ovarian cancer, from the yew tree. Others include the use of *Montanoa tomentosa* as a contraceptive, *Rauwolfiavomitoria* in treating lunatics and *Bridelia ferniginer* in managing dibetes mellitus (Aliyu, 2007).

International organizations such as Food and Agricultural Organization (FAO), World Health Organization (WHO) and United Nation Organization (UNO), have acknowledged the fact that, about 80% of world rural population rely on herbal medicine which is cheaper and more accessable than synthetic drugs (Arowolo, 1999).

In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60 percent of children with high fever resulting from malaria is the use of herbal medicine at home (WHO, 2003).

In San Francisco, London and South Africa, 75% of people living with HIV/AIDS use traditional medicine (WHO, 2003). Traditional medicine also has impact on infectious diseases. For example, the Chinese herbal remedy *Artemisia annua* is already in use against resistant malaria and has created a breakthrough in preventing almost one million deaths annually, most of them children, from severe fever (WHO, 2003). It is estimated that 25% of modern medicines are made from plants first used traditionally(WHO, 2003).Germany is the largest European medicinal plant importer and is also a major exporter of finished herbal products, accounting for at least 70% of the European market (Kayne, 2002).

Recently there has been a growing interest in hypoglycaemic agentsfrom natural products, especially those derived from plants because plant sources are usually considered tobe less toxic, with fewer side effects than synthetic sources.Currently over 200 pure phytochemicals are known to havehypoglycaemic properties (Jung *et al.*, 2004).

### 1.4.1 Traditional Medicine

Zhang, (1996) stated that ‘A progress report on traditional medicine and modem health care presented to the World Health Assembly in 1991 emphasized five major areas of concern: national program development; health systems and operational research; clinical and scientific investigations; education and training; and exchange of information’. The Program's future directions were to focus on three main activities: national policies, medicinal plantsand acupuncture. Then in 1994 at the World Health Assembly governments called on the Director-General "to consider the contribution WHO might make to promoting respect for, and maintenance of, indigenous knowledge, traditions and remedies, in particular, their pharmacopoeia". The cornerstones of traditional medicine practices are herbs which are cheap and available; their use depends on ancestral information (Marin-Bettolo, 1980).

### 1.4.2 Problems of HerbalMedicine

There is the need to state that, despite the advantages of folkloric medicine, it has its own shortcomings. Problems ranging from inadequate regulations, safety concerns, quality control, inaccurate labeling to potential interactions with food and other drugs as well as poor diagnosis (WHO, 2008). The problems are worthy of investigations to establish the reasons behind their success or failure.

## 1.5 CLASSIFICATION OF PLANTS CONSTITUENTS

Plants constituents can be classified into two main groups; the primary metabolite which the plant uses such as carbohydrate (polysaccharides, disaccharides and monosaccharide), fats and proteins and these are of pharmaceutical importance too and the secondary

metabolites such as flavonoids glycosides, sequesterpines, steroids, tannins and terpenoids, which have pharmacological activities.

The chemical constituents can be concentrated in any part of the plant like the leaves, roots, bark etc. and are often responsible for the pharmacological properties. The trend now is that phytochemists exploit medicinal plants and isolate bioactive compounds from which different analogs are synthesized with the aim of obtaining agents with better actions or even different biological properties. Plant's active constituents, thus, serve as templates for future drug developments (Aliyu, 2007).

## 1.6 FAMILY OF ASCLEPIADACEAE

The Asclepiadaceae are mostly herbs and shrubs with white sap comprising about 250 genera and 2,000 species, many of which are lianous and some of which are cactus-like succulents with reduced leaves. The leaves are simple and nearly always opposite or whorled; minute stipules are present. The flowers are bisexual, nearly always actinomorphic, and usually include an elaborate crown or corona of nectariferous appendages between the corolla and sexual parts. The calyx consists of 5 distinct or basally connate sepals. The inner perianth is a 5-lobed sympetalous corolla. The androecium and gynoecium are nearly always adnate into a gynostegium with five highly modified stamens and a massive, 5-lobed stigma. The anthers usually produce paired sacs of pollen called pollinia that are transferred as a unit during pollination. The gynoecium consists of a single compound pistil of two nearly distinct carpels that are separate at the level of the ovaries and styles and are united only by a single massive stigma. The ovaries are distinct, nearly always superior, and each has a single locule with numerous marginal ovules. The fruit is a

follicle. Seeds usually have a tuft of hairs at one end. Examples include: *Asclepias speciosa*, *Calotropis gigantean*, *Calotropis procera*,

### 1.6.1 *Anisopus mannii* (*A. mannii*)

*A. mannii* (Asclepiadaceae) is a glabrous twinning shrub with leaves petiole, elliptic, ovate and shortly cuspidate at apex upto 15 cm or more and 12 cm broad. The stem twines to a height of 3.7 – 4.6 m (Hutchinson and Dalziel, 1963).It belongs to the family Asclepiadaceae and a genus of *Anisopus* with specific epithet called *mannii* (Aluka, 2009). The family consists of 130 genera and 2,000 species (Evans, 2004); they are found often in the tropics and subtropical regions.*A. mannii* is a herb that grows in many parts of Kano state, Northern Nigeria and is popularly known as *Kashe Zaki, Kar zaki* or *Yado* in *Hausa* language. The whole plant (leaves, stems and roots) is being mixed together in a dried powdered form by traditional herbalists to manage patients with diabetes in the local areas (Personal communication, 2008). The dried powdered plant has been used by many traditional medical practitioners in the management of diabetes mellitus with reported success by some patients.

Very little information exists on the proximate composition, mineral elements and anti nutritional contents of the plant.

Recent studies showed the presence of crude fiber (89.64), moisture (8.41), soluble carbohydrate (7.94), fats (8.67), crude protein (8.4), soluble oxalates (0.34), tannins (10.55) and free cyanides (6.5) in the plant. It contains other minerals such as potassium, calcium, iron, zinc, copper and manganese (Aliyu *et al.*, 2009). These authors also

demonstrated that*in vitro*studies showed that, thecrude methanol extract and solvent fractions of *A. mannii* have strong antioxidant activities.

The plant has been shown to have antimicrobial properties and can inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonellagallinarium,Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Sani *et al*., 2009).

*A. mannii* was also shown to possess some analgesic and anti-inflammatory properties (Musa *et al*., 2009).In ethno medicine, the decoction of the whole plant is use to treat diabetes, hypertension, diarrhea and painful conditions like hemorrhoids. Some other members of the family include: *Anosopus batesii*, *Marsdenia rhynchogyna*, *Anisopus bicoranotus*, *Masdenia rostrifera*, *Anisopus restriferus* (Aluka, 2009).Very little information is known on its antihyperglycaemic and hypoglycaemic activity amongst other properties of the plant.

## 1.7 THEORETICAL FRAMEWORK

More and more plants are being investigated using modern scientific approaches and tools. These approaches involve the use of scientific models which simulate or mimic human disease conditions including neuropathology. However, experimental models for pharmacological screening and evaluation should be chosen based on their simplicity, rapidity and reproducibility. Animal models have been used extensively in diabetes research. 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). Similarly, the same authors stated that, today, animal experimentation is contentious and subject to legal and ethical restrictions that vary

throughout the world. The animal models chosen in this study have been found to satisfy this condition in various published research works.

### 1.7.1 Models for the Studies

Typically, models are animal preparations that mimic a human condition including diabetes mellitus. A number of models have been developed to screen drugs for beneficial effects in hyperglycaemic disorders. One of the most straightforward ways of studying the effects of hyperglycaemia in an animal is to remove the pancreas, either partially or totally, i.e, pancreatectomised rats and mice are the most commonly used. One of the guiding principles of animal research is to use the 'lowest' possible animal andnowadays, permission would not be granted to remove the pancreas of a dog unless a similar experiment could not be performed in a rodent. However, a major criticism of using rodents is that, they may not adequately reflect the human situation and occasionally, justification is sought to use larger animals such as cats, dogs, pigs and primates(ReesandAlcolado,2005).

## 1.8 AIM AND OBJECTIVES OF THE STUDY

### 1.8. 1 Aim of the Study

The aim of the study was to ascertain some of the pharmacological properties of *Anisopusmannii*, which is used in ethnomedical practice to manage hyperglycaemia in diabetes mellitus.

### 1.8.2 Specific Objectives

The specific objectives of this study were:

1. To investigate acute toxicity studies on the whole plant extract (including $LD_{50}$ determination).
2. To investigate the effect of the crude methanol(whole plant) extract of *A. mannii* on blood glucose concentration of:

    i. Alloxan-induced hyperglycaemic Wistar rats after 24h.

    ii. Alloxan-induced hyperglycaemia in Wistar rats within 14 days.

    iii. Normoglycaemic Wistar rats.

3. To investigate the effect of the crude methanol extract of *A. mannii*(if any) on the Alloxan-induced hyperglycaemic Wistar rats after 14 days (as in 2 above) on the following:

    i. Change in Hematological indices (Hemoglobin (Hb), Packed Cell Volume (PCV), Lymphocytes (L), Neutrophils (N) and White Blood CellsCount (WBC)).

    ii. Change in Lipid Profile (Low Density Lipoproteins (LDL), Triglycerides (TG), Total Cholesterol (TC), High Density Cholesterol (HDL) and Very Low Density Lipoproteins (VLDL)).

    iii. Liver Function Test (Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP)).

4. To carry out preliminary phytochemical screening of the crude methanol (whole plant) extract of *A. mannii.*

## 1.9 RESEARCH HYPOTHESIS

The crude methanol(wholeplant) extract of *A. mannii*, contain some pharmacologically active constituents that may be responsible for the beneficial effects claimed in the management of diabetes mellitus by traditional herbalists.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 OVERVIEW OF DIABETES MELLITUS

Diabetes mellitus (DM) is simply described as a disease in which the pancreas produces little or no insulin, a hormone that helps the body tissues absorbs glucose (Christoper *et al*., 2008). Diabetes mellitus is a clinical syndrome characterized by hyperglycaemia due to absolute or relative deficiency of insulin (Frier *et al.*, 1999). The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO, 1999). The effects of DM include long-term damage, dysfunction and failure of various organs. DM may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss (Cooke, 2008). In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made (WHO, 1990). The long-term effects of DM include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, charcot joints and features of autonomic dysfunction, including sexual dysfunction (Williams, 2009). People with diabetes are at increase risk of cardiovascular,

peripheral vascular and cerebrovascular disease (WHO, 1999). Several pathogenetic processes are involved in the development of diabetes mellitus. These include processes which destroy the beta (ß) cells of the pancreas with consequent insulin deficiency and others that result in resistance. This disease is the 7th leading course of death in the world (Ene *et al.*, 2007).

### 2.1.1 Classifications of Diabetes Mellitus

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (WHO, 1980) and, in modified form, in 1985 (Diabetes, 1979). The 1980 and 1985 classifications of diabetes mellitus and allied categories of glucose intolerance included clinical classes and two statistical risk classes. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, Insulin Dependent Diabetes Mellitus (IDDM) or Type 1, and None Insulin Dependent Diabetes Mellitus (NIDDM) or Type 2. In the 1985 Study Groupreports the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition-related Diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports other classes of Diabetes included Other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM) (WHO, 1999). These were reflected in the subsequent International Nomenclature of Diseases (IND) in 1991, and the tenth revision of the International Classification of Diseases (ICD-10) in 1992. The 1985 classification was widely accepted and is used internationally. It represented a compromise between clinical and aetiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or aetiology was unknown. The recommended classification includes both staging of diabetes mellitusbased on clinical

descriptive criteria and a complementary aetiological classification (Expert Committee, 1997).

The classification encompasses both clinical stages and aetiological types of diabetes mellitus and other categories of hyperglycaemia, as suggested by Kuzuya and Matsuda, (1997).

The clinical staging reflects that diabetes, regardless of its aetiology, progresses through several clinical stages during its natural history(WHO, 1999). Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, diabetes mellitus can be categorized by stage according to the clinical characteristics, even in the absence of information concerning the underlying aetiology (WHO, 1999). The classification by aetiological type results from improved understanding of the causes of diabetes mellitus.

The new classification contains stages which reflect the various degrees of hyperglycaemia in individual subjects with any of the disease processes which may lead to diabetes mellitus. All subjects with diabetes mellitus can be categorized according to clinical stage, and this is achievable in all circumstances (WHO, 1999). The stage of glycaemia may change over time depending on the extent of the underlying disease processes. The disease process may be present but may not have progressed far enough to cause hyperglycaemia. The aetiological classification reflects the fact that the defect or process which may lead to diabetes may be identifiable at any stage in the development of Diabetes - even at the stage of normoglycaemia (WHO, 1999). Thus, the presence of islet cell antibodies in a normoglycaemic individual makes it likely that, that person has the Type 1 autoimmune

process (WHO, 1999). The same disease processes can cause impaired fasting glycaemia and/or impaired glucose tolerance without fulfilling the criteria for the diagnosis of diabetes mellitus. In some individuals with Diabetes, adequate glycaemic control can be achieved with weight reduction, exercise and/or oral agents. These individuals, therefore, do not require insulin and may even revert to Impaired Glucose Tolerance (IGT) or normoglycaemia. Other individuals require insulin for adequate glycaemic control but can survive without it. These individuals, by definition, have some residual insulin secretion. Individuals with extensive beta-cell destruction, and therefore no residual insulin secretion, require insulin for survival (WHO, 1999). The severity of the metabolic abnormality can regress (e. g. with weight reduction), progress (with weight gain) or stay the same. It is recommended that, the terms "Insulin-Dependent Diabetes Mellitus" and "Non-Insulin-Dependent Diabetes Mellitus" and their acronyms "IDDM" and "NIDDM" no longer be used. These terms have been confusing and frequently resulted in patients being classified based on treatment rather than on pathogenesis. The term type I and Type 2 should be used instead(National Diabetes data,(1979) and American Diabetes Association, (2001)).

Type I encompasses all cases that result due to pancreatic ß -islet cell destruction which is prone to ketoacidosis (American Diabetes Association, (2001)). Type I is also attributed to autoimmune process as well as those cases prone to ketoacidosis witch neither aetiology nor a pathogenesis is known while Type 2 refers to the most common form seen due to a defect in insulin secretion or insulin resistance and Gestational Diabetes Mellitus(GDM) and “Other specific types” of diabetes mellitus (WHO, 1999).

Diabetes can cause many complications. Acute complications (hypoglycemia, ketoacidosis or nonketotic hyperosmolar coma) may occur if the disease is not adequately controlled.

Serious long-term complications include cardiovascular disease (doubled risk), chronic renal failure, retinal damage (which can lead to blindness), nerve damage (of several kinds), and micro vascular damage, which may cause impotence and poor healing (American Diabetes Association, 2001). Poor healing of wounds, particularly of the feet, can lead to gangrene, which may require amputation. Adequate treatment of diabetes, as well as increased emphasis on blood pressure control and lifestyle factors (such as not smoking and keeping a healthy body weight), may improve the risk profile of most aforementioned complications (American Diabetes Association, 2001). In the developed world, diabetes is the most significant cause of adult blindness in the non-elderly, the leading cause of non-traumatic amputation in adults, and diabetic nephropathy is the main illness requiring renal dialysis (Nathan*et al.,* 2005).

### 2.1.2 Epidemiology

In 2006, according to the World Health Organization, at least 171 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will double.Global prevalence is 6.4%; Over 280 million people have diabetes worldwide (WHO, 2011).I57 million Indians will be affected by DM by the year 2025 (Thakkar & Patel, 2010). WHO in 2004 projects that, death will double between 2005 and 2030. More than 80% of all deaths occur in low and middle-income countries (Diabetes fact Sheet, 2011). Direct cost of diabetes mellitus in Nigeria is put at about N150 billion (Suleiman *et al.*, 2006).

Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will likely be found by 2030(Wild*et*

*al.*,2004). The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a "Western-style" diet. This has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation, some of it most compellingly presented.(Wild*et al.*,2004).

Diabetes is in the top 10, and perhaps the top 5, of the most significant diseases in the developed world, and is gaining significance there and elsewhere(Wild*et al.*,2004).

### 2.1.3 Pathophysiology

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 diabetes mellitus (Christopher *et al*., 2008 and American Diabetes Association, 2001).

Much of the carbohydrate in food is converted within a few hours to the monosaccharide glucose, the principal carbohydrate found in blood and used by the body as fuel. Some carbohydrates are not so converted. Notable examples include fruit sugar (fructose), usable as cellular fuel but it is not converted to glucose, and which therefore does not participate in the insulin/glucose metabolic regulatory mechanism. Additionally, the carbohydrate cellulose (though it is actually many glucose molecules in long chains) is not converted to glucose, as humans and many animals have no digestive pathway capable of breaking up cellulose.

Insulin is released into the blood by beta cells (β-cells), found in the Islets of Langerhans in the pancreas, in response to rising levels of blood glucose after eating. Insulin is used by

about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose toglycogenfor internal storage in liver and muscle cells. Lowered glucose levels result both in the reduced release of insulin from the beta cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon which acts in an opposite manner to insulin. Glucose thus recovered by the liver re-enters the bloodstream; muscle cells lack the necessary export mechanism.

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

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not 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.

### 2.1.4 Diagnosis

The diagnosis of type1 diabetes and many cases of type 2, is usually prompted by recent-onset symptoms of excessive urination (polyuria) and excessive thirst (Polydipsia), and often accompanied by weight loss (American Diabetes Association, 2001). These

symptoms typically worsen over days to weeks; about a quarter of people with new type 1 diabetes have developed some degree of diabetic ketoacidosis by the time the diabetes is recognized(American Diabetes Association, 2001). The diagnosis of other types of diabetes is usually made in other ways. These include ordinary health screening; detection of hyperglycaemia during other medical investigations; and secondary symptoms such as vision changes or unexplainable fatigue. Diabetes is often detected when a person suffers a problem that is frequently caused by diabetes, such as aheart attack, stroke, neuropathy, poor wound healing or a foot ulcer, certain eye problems, certain fungal infections, or delivering a baby with macrosomia or hypoglycaemia(CookeandPlotnick, 2008). Diabetes mellitus is characterized by recurrent or persistent hyperglycaemia, and is diagnosed by demonstrating any one of the following (WHO, 1999):Fasting plasma glucose concentration at or above 126 mg/dL (7.0 mmol/l), Plasma glucose at or above 200 mg/dL (11.1 mmol/l), two hours after a 75 g oral glucose load as in a glucose tolerance testandrandom plasma glucose at or above 200 mg/dL (11.1mmol/l).

A positive result, in the absence of clinical symptoms of diabetes, should be confirmed by another of the above-listed methods on a different day. Most physicians prefer to measure a fasting glucose concentration because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete. According to the current definition, two fasting glucose measurements above 126 mg/dL (7.0 mmol/l) are considered diagnostic for diabetes mellitus (Saydah*et al*.**,** 2001).

*Diabetic Complication*:Diabetic complications can be classified as Acute or Chronic:

*Acute complications:* Diabetic ketoacidosis (DKA) – Type 1, Nonketotic hyperosmolar coma – Type 2 and Hypoglycemia.

DKA-is a potentially life-threatening deficiencyof insulin (hypoinsulinism), resulting in severe hyperglycaemia and requiring prompt diagnosis and treatment. Because insulin is unavailable to allow glucose to enterthe cell, dangerously high levels of glucose build up inthe blood (hyperglycaemia). The body, needing energy,begins to break down fat for energy. As fats are brokendown, ketones are produced by the liver. As more andmore fat is used for energy, higher levels of ketonesaccumulate in the blood. This increase in ketones disruptsthe acid–base balance within the body, leading toDKA (Christopher, *et al*., 2008).

Hyperosmolar (nonketotic hyperglycaemic) coma. This occurs mainly in elderly type 2 cases. It appears to be precipitated by same factors as in ketoacidosis(Nathan *et al*., 2005). There is uncontrolled glycosuria producing severe diuresis and thus leads to dehydration and haemoconcentration over several days. Plasma osmolarlity is high and leads to coma and death if not treated. Treatment of precipitating factors and associated illness is vital (Nathan *et al*., 2005).

*Chronic Complications*: This can further be sub classified into, microvascular and macrovascular chronic complications.

Microvascular disease –This occurs due to damage to small blood vessels which can cause diabetic retinopathy, neuropathy &nephropathy.Diabetic nephropathy is the leading cause of DM- related morbidity and mortality. The pathogenesis of diabetic nephropathy is related to chronic hyperglycaemia and hemodynamic alterations in renal microcirculation and structural changes in glomerulus (Cryer, 2001).

Macrovascular disease– this occurs due to damage to the arteries through atherosclerosis This can cause Hypertension (HT), Coronary artery Disease (CAD), Angina Pectoris(AP), Acute Myocardial Infections(AMI), Pulmonary Vascular Disease and Cardiovascular Accident (Ischemic).

### 2.1.5 Goals of Therapy

The goals of therapy are to achieve the following:

a. Glycaemic Control: FBS 70 – 126 mg/dL
b. Glycosylated hemoglobin $HbA_{1c}$ less than 7%
c. BP Control: Less than 130/80 mmHg
d. Cholesterol control: Total cholesterol less than 200 mg/ dL (American Diabetes, (2006) and Qaseem*et al*. (2007)).

## 2.2 NONPHARMACOLOGICAL MANAGEMENT OF DIABETES MELLITUS

Type 2 diabetes is usually first treated by changes in physical activity (usually increase), diet (generally decrease carbohydrate intake, especially glucose generating carbohydrates), and through weight loss. These can restore insulin sensitivity, even when the weight loss is modest, for example, around 5 kg, most especially when it is in abdominal fat deposits,(Bantle*et al*., (2006) and Ono, (2008)).

## 2.3 DRUG MANAGEMENT OF DIABETES MELLITUS

Diabetes Mellitus treatment is based on four fundamental factors: patient education concerning the disease; physical exercise; diet and hypoglycaemic agents. The most currently used agents in diabetes mellitus control are sulphonylurea drugs, biguanides, thiazolidinediones derivatives, and insulin. Although these drugs have been extensively used because of their beneficial effects in the control of hyperglycaemia, these agents have not been able to establish adequate control of diabetes mellitus, being unable to suppress the associated chronic and acute complications. In addition to specific adverse effects for each one of these medicines, administration and dosage problems are also found (Hernandez-Galicia *et al.*, 2002). These reasons coupled with inadequate economic resources force some patient to empirically use traditional herbal medicine in the management of diabetes mellitus.

Apart from diet and exercise, drugs are the next line of managing diabetes mellitus. The drugs are many and classified as follows:

**1. Biguanides**:-These are very effective agents mainly for the treatment of Type 2 DM. The agents under this class work primarily by reducing liver release of blood glucose from glycogen stores as well as provoking some increase in cellular uptake of glucose in body tissues. These agents have a drawback of twice daily dosing, contraindications and gastro intestinal complaints. Examples include metformin which is contraindicated in renal and hepatic insufficiency (Eurich *et al.,* 2007).

**2. Sulfonylureas:-**Sulfonylureas enhance insulin secretion by blocking ATPsensitivepotassium channels in the cell membranes of pancreaticThis action results in membrane depolarization,allowing an influx of calcium to cause the translocation ofsecretory granules of insulin to the cell surface, and enhancesinsulin secretion. The extent of insulin secretion depends onthe blood glucose level.More insulin is released in response tohigher blood glucose levels, whereas the additional insulinsecretion from sulfonylureas is less at near-normal glucose

levels.Insulin is then transported through the portal vein to theliver, suppressing hepatic glucose production (Inzucchi,2002).Examples include tolbutamide, glibenclamide, chlorpropamide, etc. They cause mild hypoglycemia and minimal weight gain. They are contraindicated in renal and hepatic insufficiency. Another limitation of sulfonylurea therapy is the inability of these products to stimulate insulin release from β-cells at extremely high glucose levels, a phenomenon called glucose toxicity (Christopher *et al*., 2008).

**3.Thiazolidinediones (TZDs)**:- These agents increase tissue insulin response. TZDs also increase the synthesis of certain proteins involved in fat and glucose metabolism, which reduces levels of certain types of lipids, and circulating free fatty acids. TZDs generally decrease triglycerides and increase high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Although the increase in LDL-C may be more focused on the larger LDL particles, which may be less atherogenic, the clinical significance of this is currently unknown. Nonetheless, rosiglitazone, a certain glitazone, was suspended from allowed use by medical authorities in Europe, as it has been linked to an increased risk of heart attack and stroke (NHS, 2010). They however, have the drawback of weight gain, fluid retention, slow onset and expensive. Periodic liver function test should be performed durin TZD theraphy (Christopher *et al*., 2008). Examples includerosiglitazone, pioglitazone, and troglitazone.

**4.α-Glucosidase inhibitors**:-These agents interfere with absorption of some nutrients. Examples include acarbose and miglitol. They cause GI- complaints and are expensive. Acarbose does not cause hypoglycemia, because it does not cause the release of insulin from pancreas nor does it increase insulin action in peripheral tissues.Another member of this class is miglitol (Christopher *et al*., 2008).

**5. Meglitinides**:-these agents and their analogs stimulate insulin release quickly; they can be taken with food, unlike the sulfonylurea. Examples include nateglinide and repaglinide. Meglitinides help the pancreas produce insulin and are often called "short-acting secretagogues." They act on the same potassium channels as sulfonylureas, but at a different binding site (Rendell, 2004). By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, hence enhancing insulin secretion (Healthvalue, (2007) and Rendell, (2004)).

**6. Incretin mimetics**:-which increase insulin output from the beta cells among other effects. This includes the Glucagon-like peptide (GLP) analog Exenatide.

A Synthetic form of exendin-4, is an Incretin mimetic which increases insulin secretion, suppresses glucagon secretion, and slows gastric emptying. It is given by subcutaneous injection for the treatment of Type 2 diabetes in combination with metformin or a sulphonylurea, or both, in patients who have not achieved adequate glycaemic control with these drugs alone or in combination. They have to be administered parenterally and are expensive. Other side effects include nausea, vomiting and diarrhea (Christopher *et al*., 2008).

**7. Dipeptidyl peptidase-4 (DPP-4) inhibitors**:- increase Incretin levels (sitagliptin) by decreasing their deactivation rates.Dipeptidyl peptidase-4 (DPP-4) inhibitors increase blood concentration of the incretin GLP-1 by inhibiting its degradation by dipeptidyl peptidase-4. Adverse effects, including nasopharyngitis (the common cold), headache, nausea, hypersensitivity and skin reactions, have been observed in clinical studies(Christopher et al., 2008). Other possible adverse effects, including hypersensitivity reactions and pancreatitis, have been reported (NPS, 2010). DPP-4 inhibitors lowered hemoglobin A1C values by 0.74%, comparable to other antidiabetic drugs (Amori *et al.*, 2007). Examples of members include:vildagliptin, sitagliptin, saxagliptin and linagliptin.

**8. Amylin agonist analog:-**which slows gastric emptying and suppresses glucagon e.g. Pramlintide.The most frequent and severe adverse effect of pramlintide is nausea, which occurs mostly at the beginning of treatment and gradually reduces(Christopher *et al*., 2008).They have to be administered parenterally and are expensive**.**

**9.Fast acting scretagogues:-**e.g. Glinides. They have dosage flexibility and have fast-on and fast - off effects. They are expensive and have to be administered three times a day.

**10 Insulins*:-***play a key role in the regulation of carbohydrate, fat, and protein metabolism. It is a polypeptide hormone of complex structure. There are differences in the amino-acid sequence of animal insulin and the human insulin analogs. Insulin may be extracted from pork pancreas and purified by crystallization; it may also be extracted from beef pancreas, but beef insulins are now rarely used. Human sequence insulin may be produced semi-synthetically by enzymatic modification of porcine Insulin or biosynthetically by recombinant DNA technology using bacteria or yeast**.**Insulin is almost needed by all children with diabetes and those with types 1 and 2 especially those that cannot be controlled with other medication (BNF-56, 2008).

Insulin preparations can be divided into 3 types:

a. Those of short duration which have a relatively rapid onset of action, namely: soluble, lispro and aspart**.**
b. Those with an intermediate action, e.g. Isophane insulin and insulin zinc suspension.
c. Those whose actions are slower in onset and lasts for long periods, e.g. insulinzinc suspension.

The duration of action of a particular type of insulin varies considerably from one patient to another, and needs to be assessed individually. Generally most common side effects of insulins are hypoglycemia, lipodystrophy and allergic reactions.

### 2.3.1 Drug Interactions in Diabetics

While more than one drug is being administered the response may consist of effectgreater than anticipated or a decrease in effectiveness of one or both drugs or unanticipated response. According to the likely effectiveness, importance of drug interactions, in a diabetic, these are sub grouped as follows:

**A. In Alteration of glucose homeostasis**

1. Anti-inflammatory Drugs and Diabetes:-Salicylates and phenylbutazone displace tolbutamide and chlorpropamide from the plasma proteins (White and Campbell, 200).This increase the hypoglycaemic effects of sulfonylureas.

2**.** Antigout Drugs:-Allopurinol competes with chlorpropamide for renal tubular secretion thus increasing the half life of chlorpropamide and its hypoglycaemic effect. Sulfinpyrazone and Probenacid also potentiate sulphonylurea effects (Kritz *et al*., 1983).

3**.** Antibiotics:-Long acting sulphonomide, chloramphenicoland clarithrommycin potentiate the hypoglycaemic effects of sulphonylureas (Robert and Amy, 2002).

4**.**Anticoagulants:-Dicumoral group of drugs compete for same liver enzymes as sulphonylurea, this prolongs their half life, e.g. chlorpropamide from 40 to 90 hours-so effectiveness is potentiated, and risk of hypoglycaemia is increased.

5. Lipid lowering agents:-competes, with chlorpropamide for renal secretion and as a result half life of chlorpropamide is increased.

6. Anabolic steroids: - Anabolic steroids affect protein binding, inhibit metabolism of sulphonylureas, and enhance their hypoglycaemic effect. These agents have been employed in improving Insulin sensitivity in Insulin resistant patients.

7. Marijuana:-Marijuana may impair glucose tolerance further (Podolsky *et al*., 1971)

8. Monoamine oxidase inhibitors (MAO) and Diabetes

MAO inhibitors enhance the hypoglycaemic effect of insulin and sulphonylureas andas well MAO inhibitors inhibit the adrenergic response to hypoglycemia.

9. Diuretics:-Thiazide diuretics aggravate hyperglycaemia. The mechanism of action being due toinduced hypokalemia or inhibition of insulin secretion. Continued use may induce diabetes in a susceptible or potential diabetic (Martindale, 2008).In combination with chlorpropamide it may accentuate hyponatraemia, the mechanism being different for each; Thiazide diuretics reduce the free water clearance while Chlorpropamide enhances Anti Diuretic Hormone(ADH) effect on renal tubules. Other diuretic agents such as acetazolamide, ethacrynic acid and furosemide may also impair glucose tolerance.

10. Oral contraceptives:-Oestrogens are antagonistic to insulin and known to induce abnormal glucosetolerance. These agents also decrease peripheral tissue sensitivity to insulin. Lowdose estrogens are devoid of this effect. This is, however, more likely event inindividuals who are obese, have strong family history of diabetes or previously known for a poor obstetrical history (Berenson *et al*., 2011).

**B.In Induction of Syndromes that offer Diagnostic Egnimes**

1. Chlorpropamide interacts with alcohol and leads to antbuse like reaction-flushingsensation, headache, nausea, vomiting. This may as well be associated with cerebella ataxia, scanning speech.

2. Ethanol ingestion concomitantly with biguanides (phenformin) markedly contributes to lactic acidosis (Julie, 2010).

## 2.4 MEDICINAL PLANTS USED IN THE MANAGEMENT OF DIABETES MELLITUS

A great number of medicinal plants used in the control of the DM have been reported (Aliyu, 2007). However, although these plants may represent alternatives to developing new oral hypoglycaemic agents, appropriate ethnobotanical information about them is scarce, obscure and ambiguous**.**Many herbal supplements have been used for the treatment of Diabetes, but not all of them have scientific evidence to support their effectiveness. Bitter melon (*Mormodica charantia*), Fenugreek and Soy beans have been studied as possible treatment in patients with diabetes, but the results of these were inconclusive or showed these products to be ineffective (Ene *et al*., 2007). In another survey carried out by Gbolade, (2008), the following plants have also shown to have antidiabetic properties: *Vernonia amygdalina*, *Bidens pilosa*, *Carica papaya*, *Citrus aurantiifolia*, *Ocimumgratissimum*, *Momordica charantia*and *Morinda lucida* .

## 2.5 ANIMAL MODELS

Model for hyperglycaemic studies are many and the following are some of them, including those adopted for this study.

### 2.5.1 Animal Model of Hyperglycaemia

One of the most straightforward ways of studying the effects of hyperglycaemia in an animal is to remove the pancreas, either partially or totally (Rees and Alcolado, 2005). The species of animal used is determined by several factors. The smaller the animal, the more manageable and cheaperthe experiment becomes(Rees and Alcolado, 2005).

*Animal models of Type 1 Diabetes Mellitus:*Type 1 diabetes mellitus in humans is characterized by a specific destruction of the pancreatic β cells commonly associated with immune-mediated damage. Although the damage may occur silently over many years, at clinical presentation there is little surviving β cell mass and the disorder progresses to absolute insulinopaenia. High doses of β cell toxins like streptozotocin and alloxan induce insulin deficiency and Type 1 diabetes mellitus with ketosis. However, doses calculated to cause a partial destruction of β cell mass can be used to produce a mild insulin deficient state of Type 2 diabetes mellitus, without a tendency to cause ketosis (Porter *et al*., 1989) The dosage is difficult to judge to create stable Type 2 diabetes mellitus without either gradual recovery or deterioration into Type 1 diabetes mellitus. Streptozotocin is preferred because it has more specific ß cell cytotoxicity, but the sensitivity of this agent varies with species, strain, sex and nutritional state and there are batch differences in activity (Okamato, 1981). A single large dose of streptozotocin can produce diabetes in rodents, probably as a result of direct toxic effects. Alternatively, multiple small doses of streptozotocin are used (e.g. 40 mg/kg on five consecutive days). In susceptible rodents this induces insulinopenic diabetes in which immune destruction plays a role, as in human Type 1 diabetes (Rees and Alcolado, 2005).

*Animal model of type 2 diabetes:*-There are basically two main classes of animal model of Type 2 diabetes:

Genetic and spontaneous models: Spontaneous animal models of Type 2 diabetes mellitus are highly heterogeneous. At one end of the spectrum there is a mild hyperglycaemia associated with obesity and hyperinsulinemia. At the other extreme, animal models of Type 2 diabetes mellitus can develop a severe form of diabetes mellitus with extensive ß cell degeneration, occasionally resulting in ketosis and the requirement of exogenous insulinto sustain life (Arulmozhi *et al*., 2004).Experimental Type 2 diabetes mellitus can be induced by: 1. Chemical destruction or surgical removal of part of the ß cell mass, 2. Lesioning the ventromedial hypothalamus. 3. Feeding with high-fat and high-sugar diets 4. Malnutrition in utero. 5. High doses of counter-regulating hormones, particularly glucocorticoids and 6. Prolonged cell exposure to hyperinsulinemia (Arulmozhi *et al*., 2004). Chemical destruction is often employed to achieve the partial destruction of the pancreatic ß cells (Pont *et al*., 1979). Some of these agents capable of inducing diabetes include the following:

*Irreversible beta cytotoxic agents*:alloxan, streptozotocin, Diphenyl thiocarbazine, Oxine-9- hydroxyquinoloneand vacor.

*Reversible beta cytotoxic agents:* 6- aminonicotinamide, l-asparginase, azide cyanide,cyproheptadine, phenytoin, thiazides and malonates,

*Other agents*: Tacrolimus, anti-insulin antibodies, somatostatins, catecholamines,glucocorticoids, glucagon and β-receptor antagonists (Flier, 1992).

*Streptozotocin (Streptozocin)*:- is a broad-spectrum antibiotic, which causes beta islet cell damage by free radical generation(Szkudelski, 2001). It induces diabetes in almost all species of animals excluding rabbits and guinea pigs. Streptozocin is a synthetic nitrosoureido glucopyranose derivative isolated from fermentations of *Streptomyces achromogenes* that is classically an anti tumour, antibiotic and chemically is related to other nitrosoureas used in cancer chemotherapy(Brentjens, (2001) and Etuk, (2010) ).

Streptozocin prevents DNA synthesis in mammalian and bacterial cells (Etuk, 2010).In the bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA(Rerup, 1970 and Reusser, 1971). The biochemical mechanism results in mammalian cell death (Etuk, 2010). Streptozocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connection to the DNA or inhibiting many of the enzymes involved in DNA synthesis ((Holemans and van Assche, 2003). The Induction of hyperglycaemia with streptozocin takes some time, and experiment carried out at suitable time intervals after administration of the agent will give additional information into mechanism of action of the tested compound (Szkudelski, (2001) and Etuk, 2010)). Etuk, (2010), reported that, during earlier part of this induction period, hypoglycaemic action of plant extract may be due to stimulation of residual activity of beta cells; however, after induction of the diabetic state, hypoglycemic activity will be due to some other mechanism, since in this model of hyperglycemia insulin activity is thought to be negligible. Single doseof streptozocin in sterile citrate buffer (e.g. pH 4.5, 0.1M) may be used: mice 150 mg/kg; rats 80 mg/kg, administered intraperitoneally (Williamson *et, al.,* (1996) and Etuk, 2010)). Hyperglycaemia develops gradually and may be assessed after few days, usually

four days for mice and seven days for rats. Usually a serum glucose level of about 180-500 mg/dL indicates the induction of hyperglycaemia. Sometimes diabetic animals are maintained on insulin if the experiments are not to commence immediately to prevent the animals' death (Williamson *et, al.,* (1996) and Etuk, 2010).

Etuk, (2010) reported that streptozocin is the most commonly used drug for induction of hyperglycaemia in rats. However, there are some disadvantages to its use in chronic experiments, especially spontaneous recovery from high blood glucose levels by the development of functioning insulinoma and high incidence of kidney and liver tumours(Steiner et al., 1970). These problems are due strongly to oncogenic action of streptozocin. Non insulin dependent diabetes mellitus was induced by a single intraperitoneal injection of streptozocin (60 mg/kg) and nicotinamide (120 mg/kg) to rats.Injection of a single high dose of streptozocin (200 mg/kg body weight) induced rapid and permanent hyperglycaemia in mice. In contrast, the injection of the same total dose divided into multiple "subdiabetogenic" doses (40 mg/kg per day for five consecutive days) caused the development of delayed but progressive hyperglycaemia only in the thymus competent mice(Paikand Fleischer, (1980) and Etuk, (2010)).

*Alloxan*:-Alloxan is another most commonly used chemical for induction of diabetes mellitus in laboratory animals though not in humans. It is a well-known diabetogenic agent widely used to induce type 2 diabetes in animal(Vianna *et al.,*(2004), Antia*et al.*, (2005), and Etuk, (2010)). Alloxan is a toxic glucose analogue–which selectively destroys insulin-producing ß cells in the pancreas of rodents and many animal species (Etuk, 2010). Similarly the same author reported that, alloxan preferentially accumulates in the ß cells through up-take via GLUT2 glucose

transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The action of reactive oxygen species with a massive increase in cytosolic calcium causes rapid destruction of ß cells (Szkudelski, 2001).

Alloxan is a urea derivative which causes selective necrosis of the pancreatic isletß-cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs. With this agent, it is possible to produce different grades of severity of the disease by varying the dose of alloxan used(Huralikuppi, (1991), McNeill, (1990) and Etuk, (2010)).These may be classified by measuring fasting blood sugar (FBS) levels: e.g. in rabbits moderate diabetes has been defined as an FBS level of 180-250 mg/dL, and severe diabetes as FBS level above 250 mg/dL(Huralukuppi, 19991). The severe diabetes produced by alloxan results in blood sugar levels equivalent to a total pancreatectomy, hence sulphonylureas such as tolbutamide, which act mainly by stimulating insulin release from ß-cell, show only a small hypoglycaemic effect in their instance(Etuk, 2010). Therefore a test plant extract producing a significant hypoglycemia (in a severely alloxan diabetic animal) must be operating through a different mechanism (Williamson *et al*., (1996) and Etuk, (2010)). Moderate diabetic animals are recommended for use in testing drugs for uses in Non insulin dependent diabetes mellitus. For all animals a single dose of alloxan, 140- 180 mg/kg (usually 150 mg/kg) is administered as a 5% v/v in distilled water injected intravenously into the marginal ear vein of rabbit or intraperitoneally in case of mice and rats(Szkudelski, 2001). A rest period of seven days for rabbits, 12 days for rats and mice is allowed during which the animals have free access to food and water. Alloxan and its reduction product dialuric acid establish a Redox cycle with the formation of superoxide radicals. Theseradicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by Fenton reaction.

The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells (Szkudelski, 2001).

Thus alloxan-induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activities *in vivo*. One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in beta cells exposed to alloxan (Takasu *et al*., 1991). The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan (Crouch *et al*., (1981), Bartosikova *etal., (*2003) and Etuk, 2010)).

The simplistic argument often made against the use of Alloxan to induce type 2 diabetes mellitus is that, alloxan administration produces beta cells damage and thus leading to type 1 rather than type 2 diabetes mellitus. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content (McNeill, 1990). This perhaps explains why drug at a low or medium dose, does not produce absolute but insufficient insulin deficiency in experimental animals. Therefore the experimental dose of the drug must be carefully selected in order to avoid excessive pancreatic tissue damage. The most frequently used intravenous dose of alloxan in rats is 65 mg/kg, but when it is administered intraperitoneally or subcutaneously its effective dose must be higher (Antia *et al.*, 2005).

*Insulin antibodies induced hyperglycaemia*:-A transient diabetic syndrome can be induced by injecting gunea pigs with anti insulin serum. Diabetes persists as long as antibodies are capable of reacting with insulin remaining in the circulation.

*Hormone induced hyperglycaemia*:-Dexamethasone is a steroid possessing immunosupressive action, which caused an autoimmune reaction in the islets and produces type 1 diabetes(Gill *et al.,* 1994 and Barbera *et al*., 2001).

*Adrenaline induced acute hyperglycaemia*:-Adrenaline is a counter regulatory hormone to insulin. It increases the rate of glyconeolysis and the glucose levels in blood causing hyperglycaemia.

*Dithizone induced hyperglycaemia*:-Organic agents react with zinc in islets of langerhans causing destruction of islet cells and causes diabetes. Compounds such as dithizone, EDTA, 8-hydroxy quinoline are used to induce spontaneous type 2 diabetes in experimental animals. Dithizone at 40-100 mg/kg (*i.v*) produces type 2 diabetes in mice, cats, rabbits and golden hamsters(Awai *et al.,* 1979).

*Ferric nitrilotriacetate inducedhyperglycaemia*:- This chemical is rarely used. Rat and rabbits parenterally treated with a large daily dose of ferric nitrilotriacetate manifested diabetic symptoms such as hyperglycaemia, glycosuria, ketonaemia and ketonuria after approximately 60 days of treatment. The blood insulin response to oral glucose loading was poor (Awai *et al.,* 1979).

## 2.6 HYPERGLYCAEMIA AND HYPOGLYCAEMIC EVENTS

The origin of the term Glycaemia isGreek:*hyper*-, meaning excessive; -*glyc*-, meaning sweet; and -*emia*, meaning "of the blood".

Hyperglycaemia, or high blood sugar, is a condition in which an excessive amount of glucosecirculates in the blood plasma. This is generally a glucose concentration higher than 10mmol/l (180 mg/dL), but symptoms may not start to become noticeable until even

higher values such as 15-20 mmol/l (270-360 mg/dL). However, chronic levels exceeding 7mmol/l (125 mg/dL) can produce organ damage (WHO, 1999).

Temporary hyperglycaemia is often benign and asymptomatic. Blood glucose levels can rise well above normal for significant periods without producing any permanent effects or symptoms. However, chronic hyperglycaemia at levels more than slightly above normal can produce a very wide variety of serious complications over a period of years, including kidney damage, neurological damage, cardiovascular damage, damage to the retina etc (American Diabetes Association,2000).

In diabetes mellitus (by far the most common cause of chronic hyperglycaemia), treatment aims at maintaining blood glucose at a level as close to normal as possible, in order to avoid these serious long-term complications.

Acute hyperglycaemia involving glucose levels that are extremely high is a medical emergency and can rapidly produce serious complications (such as fluid loss through osmotic diuresis). It is most often seen in persons who have uncontrolled insulin-dependent diabetes (Gennari and Kassirre, 1974).

### 2.6.1 Signs and Symptoms of Hyperglycaemia

The following symptoms may be associated with acute or chronic hyperglycaemia, with the first three composing the classic hyperglycaemic triad:

- Polyphagia - frequent hunger, especially pronounced hunger
- Polydipsia - frequent thirst, especially excessive thirst
- Polyuria - frequent urination

- Blurred vision
- Fatigue (sleepiness).
- Weight loss
- Poor wound healing (cuts, scrapes, etc.)
- Dry mouth
- Dry or itchy skin
- Tingling in feet or heels
- Erectile dysfunction
- Recurrent infections external ear infections (swimmer's ear)
- Cardiac arrhythmia
- Stupor
- Coma (Kitabchi*et al.*, 2006).

Frequent hunger without other symptoms can also indicate that blood sugar levels are too low. This may occur when people who have diabetes take too much oral hypoglycaemic medication or insulin for the amount of food they eat. The resulting drop in blood sugar level to below the normal range prompts a hunger response. This hunger is not usually as pronounced as in Type I diabetes, especially the juvenile onset form, but it makes the prescription of oral hypoglycaemic medication difficult to manage.

Polydipsia and polyuria occur when blood glucose levels rise high enough to result in excretion of excess glucose via the kidneys (glycosuria), producing osmotic diuresis (Gennari and Kassirre, 1974).

### 2.6.2 Symptoms of Diabetic Ketoacidosis

- Ketoacidosis
- Kussmaul hyperventilation: deep, rapid breathing
- Confusion or a decreased level of consciousness
- Dehydration due toglycosuria and osmotic diuresis
- Acute hunger and/or thirst
- 'Fruity' smelling breath odor
- Impairment of cognitive function, along with increased sadness and anxiety(Somerfield *et al.,* 2004).

### 2.6.3 Complication of Hyperglycaemia

Hyperglycaemia can be a serious problem if untreated in time. If hyperglycaemia is not treated, a condition called ketoacidosis could occur. Ketoacidosis develops when the body does not have enough insulin. Without insulin, the body is not able to utilize the glucose for fuel, so the body starts to breaks down fats for energy(Somerfield*et al.,* 2004).

When the body breaks down fats, produces a waste product called ketones. The body cannot tolerate large quantity of ketones and will try to remove it through the urine. Unfortunately, the body is not able to remove all the ketones and thus they build up in the blood stream, which can lead to ketoacidosis (Somerfield *et al.*, 2004).

Ketoacidosis is life-threatening condition which needs immediate treatment. Symptoms include: Shortness of breath, breath that smells fruity, nausea, vomiting and very dry mouth.

*Treatment of hyperglycaemia*:-Treatment of hyperglycaemia requires elimination of the underlying cause, e.g., treatment of diabetes when diabetes is the cause. Acute hyperglycaemia can be treated by direct administration of Insulin in most cases. SevereHyperglycaemiacan be treated with oral hypoglycaemic therapy and lifestyle modification (Walls *et al.*, 2009).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 PLANT MATERIAL

The whole plant, *A. mannii* was collected from Burunburun, Tudun wada, Local Government Area of Kano State, Nigeria. The plant was identified and authenticated by Malam Musa and Umar Galla of the Herbarium Section in the Department of Biological Sciences, A.B.U., Zaria, Nigeria. A voucher specimen(217) was deposited at the herbarium for future reference. The whole plant was air dried under shade until a constant weight was obtained. It was then crushed into coarse powder with a pestle and mortar. About 200g of the powered plant was extracted with 1.5L 70%v/v methanol for 72hours using soxhlet extraction apparatus. The solvent was evaporated to give an average yield of 9.55 % w/w (19.1g). The extract was stored in a desiccator and only prepared freshly for each study (Appendix 2).

## 3.2 ANIMALS

### 3.2.1 Rats

Wistar rats of both sexes (weighing 200-220g) obtained fromAnimal House, Department of Pharmacology and Therapeutics, A.B.U, Zaria were used for this study. The animals were maintained in a well ventilated room, fed on Excel feeds (Feed Masters, Ilorin) and water *ad libitum*.They were kept in clean cages under 12/12 hours normal light/dark cycle and allowed to acclimatize to the laboratory environment for a period of 12 days before the commencement of the experiment.

## 3.3 DRUGS AND CHEMICALS

The following drugs were used:Distilled water, alloxan (Sigma Chemical Co. USA),Chips of magnesium metal (BDH Ltd Poole, England),chloroform (Sigma Chemical Co.USA),concentrated hydrochloric acid (BDH Ltd Poole, England),5% dextrose (Unique Pharmaceuticals),Dragendorff reagent (BDH Ltd Poole, England),ferric chloride (BDH Ltd Poole, England), glibenclamide (Hovid ),Meyer reagent (BDH Ltd Poole, England),methanol,Sulphuric acid (BDH Ltd Poole, England),Wagner reagent (BDH Ltd Poole, England).

## 3.4 EQUIPMENTS AND OTHER MATERIALS

Photoelectric colorimeter model AE IID (Erma Inc. Japan), haematology analyzer (Sysmex USA), haematocrit centrifuge, spectrophotometer, glucometre (Accu-check®) made by Roche, test strips (Accu-check® Advantage II), weighing balance, clock, cotton wool, scissors, syringes (1cc, 2cc, 5cc and 10cc), spatulas, Animal cages,pestle and mortar.

## 3.5 TOXICOLOGICAL STUDIES

### 3.5.1 Acute Toxicity Studies in Rats ($LD_{50}$)

$LD_{50}$ determinations were conducted using the method of Lorke, (1983).The method was divided into two phases. In the initial phase, 3 groups of three rats each were treated with the methanol extract of the plant at doses of 10,100 and 1000 mg/kg body weight orally and observed for signs of toxicity and death for 24 hours. In the second phase, 3 groups of one rat each were orally administered with three more specific doses of the extract 1600, 2900 and 5000 mg/kg. The $LD_{50}$ value was determined by calculating the geometric mean

of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1). The final $LD_{50}$ was calculated as follows:

$LD_{50} = \sqrt{XxY}$

Where x = maximum dose that killed

y = minimum dose that did not killed

## 3.6 PHARMACOLOGICAL STUDIES

### 3.6.1 Alloxan-Induced Hyperglycaemia

Alloxan- induced hyperglycaemia was conducted by employing the method of Okokon*et al*., (2006). Twenty five (25) overnight fasted rats were grouped into 5 and administered alloxan monohydrate 150 mg/kg intraperitoneally.Blood samples, by cutting the tail tip arteries,were withdrawn at the time of injection to determine the initial fasting blood glucose concentration (FBGC). Food and water (containing 5% dextrose) were immediately returned to them. The dextrose water was allowed to stay with the rats for only 24h in order to protect the massive hypoglycemia that will be seen as a result of excessive depletion of pancreatic beta cells containing insulin. The rats were observed over a 72h period for signs of hyperglycemia. The Fasting Blood Sugar (FBS) was taken and compared to the control.

*Effect of acute single oral administration of A. mannii on the blood glucose concentration of hyperglycaemic Wistar rats:*This test was conducted employing the method described by Okokon *et al*., (2006). After 72h, the rats in 3.6.1 were fasted overnight, i.e. food was withdrawn overnight

but water was allowed and the tail artery was cut to squeeze blood for use with Accu-check® glucometre (Trinder, 1969). Those that were found to be hyperglycaemic were selected for the studies. A FBS of 200-500 mg/dL range were considered suitable for this study.

The rats were then grouped into 5 groups each containing 5 rats with proportional FBS and weight. The 1st group represents the control group and was given normal saline at 10 ml/kg body weight orally; the 2nd, 3rd, and 4th groups received150, 300and 600 mg/kg body weight orally of the methanol (whole plant) extract of *A. mannii* respectively. The 5th group was given Glibenclamide (10 mg/kg)*P.O*. Theextract of *A. mannii* was administered to the rats immediately as soon as the blood was taken; food and water was returned to the rats. The blood was then withdrawn for testing at interval of 0h, 1h, 2h, 4h, 8h,16h and 24h for the first day using the Accu-check® glucometre and the Advantage II® Test strips.

### 3.6.2 Subacute Toxicity Tests

Thesubacute toxicity studies were carried out using another group of 25 hyperglycaemic rats. The rats were administered the various concentrations of the extract as above (group 1 N/Saline 10 ml/kg, 2nd, 3rd, and 4th groups received 150300, 600 mg/kg respectively and 5th group Glibenclamide (10 mg/kg) once daily for 14 days). The blood samples were withdrawn from the tail arteries and tested for change in blood glucose concentration on interval of 1st, 3rd, 6th,9th,12thand 14th days. The determination of blood glucose concentration was done by the glucose-oxidase principles (Beach and Turner, 1958) using Accu-check® glucometre basic instrument. On the 14th day, the rats were sacrificed; blood samples were collected through cardiac puncture and tested for lipid profile, liver function and changes in the blood indices.

### 3.6.3 Effect of Acute Single Administration of Crude Methanol (whole plant) of *A. mannii*on the Blood Glucose Concentration of NormoglycaemicWistar Rats

The effect of the crude extract of *A. mannii* in normoglycaemic Wistar rats of both sexes was investigated. The rats were grouped into 5 rats per group of fives. Thegroups were administered N/Saline 10 ml/kg, 150, 300, 600 mg/kg and glibenclamide (10 mg/kg) *P.O* respectively. The determination of blood glucose concentration was done by the glucose-oxidase principles (Beach and Turner, 1958) using Accu-check® glucometre basic instrument.

### 3.6.4 Determination of Effect of *A. mannii* on Some Blood Indices

The collected blood sample above (3.6.2 ) was investigated to determine any change in blood indices like hemoglobin (Hb), packed cell Volume (PCV), lymphocytes(L), neutrophils (N) after 14 days of administering the extract using hematology analyzer (Sysmex USA).

### 3.6.5 Determination of the Effect of *A. mannii* on Lipid Profile

The blood samples collected in (3.6.2) were also analyzed using the photoelectric colorimeter model AE IID (Erma Inc. Japan) to determine any change in the lipid profile.

### 3.6.6 Determination of Effect of *A. mannii*on Liver Function

The blood collected above was also analyzed for any effect on Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Aspartate transaminase (AST) using the Photoelectric colorimeter model AE IID (Erma Inc. Japan).

### 3.7 PRELIMINARY PHYTOCHEMICAL SCREENING OF A. MANNII

The preliminary phytochemical screening of *A. manni*was carried out on the whole plant extract.The tests were as follows:

#### 3.7.1 Test for Carbohydrates

*Molisch test:*About 0.5g of the extract was dissolved in water in a test tube. Three drops of 1% α- naphthol in 80% ethanol was added followed by 5 drops of concentrated sulphuric acid without mixing. Appearance of purple ring at the interface as a result of the reaction between α- naphthol and furfural and 5- hydroxymethylfurfural aldehydes produced by dehydration of saccharides would indicate the presence of carbohydrates (Silva *et al*., 1998).

#### 3.7.2 Test for Flavonoids

*Shinoda reduction test* (*Cyanidin test*): 3 ml of the alcoholic solution of the extract was evaporated to dryness. The residue wasdissolved in 2 ml of 50% methanol with heat. Four drops of concentrated Hydrochloric acid was added followed by some chips of magnesium metal. Immediate appearance of orange color denotes flavones, red – crimson color denotes flavonols and pink – magenta color denotes flavonones (Mahrain *et al*., 1980).

#### 3.7.3 Test for Cardiac Glycosides

*Keller Kiliani test*: 1 ml of the extract was diluted with 20 ml ofwater and 1 ml of strong lead sub acetate solution was added to precipitate pigments, which were filtered off. The

filtrate obtained was shaken with an equal volume of chloroform and allow separating into two layers in a small separating funnel. The chloroform layer was removed and evaporated to dryness on a water bath. The residue was dissolved in 3 ml of ferric chloride in glacial acetic acid, left for 1minute and then transferred into a dry test tube. Six drops of concentrated sulphuric acid was added by the walls of the test tube. On standing, a brown colour at the interface (due to deoxy sugars) and a pale green colour at the upper layer (due to the steroidal nucleus) is a preliminary indication for cardenolides (Brain and Turner, 1975).

### 3.7.4 Test for Cyanogenic Glycosides

*Guinard test*: 0.5g of the powdered drug was mixed with 0.1 ml of water in a test tube. A prepared damp sodium picrate paper was suspended at the mouth of the test-tube by means of a cork. The tube was placed in a water bath and allowed for an hour. A brick – red colour on the picrate paper indicated the presence of cyanogenic glycoside (Silva *et al*., 1998).

### 3.7.5 Test for Saponins

*Froth test*:2 ml of the aqueous methanol extract was diluted with twice its volume of water and shaken in a test tube for 15minutes. The occurrence of foam column, of at least 1cm in height persisting for a minimum of 15minutes would indicate the presence of saponins (Silva*etal*., 1998).

### 3.7.6 Test for Tannins

*Ferric Chloride test*:1 ml of the extract was diluted with 2 ml ofwater. Dilute solution of ferric chloride (3 drops) was then added. The occurrence of black-blue or black-green would show the presence of tannins (Evans, 1996).

*Lead Sub acetate test*: 1 ml of the aqueous methanol extract was diluted with 2 ml water. The dilute solution wasmixed with lead sub acetate solution. Appearance of a light brown precipitate indicates the presence of tannins (Evans, 1996).

### 3.7.7 Test for Steroids and Terpenoids

*Liebermann-Burchard's test*:5 mg of the powdered plant material was extracted with 5 ml of methanol and then filtered.The filtrate was evaporated to dryness on water bath. The residue was shaken with chloroform and filtered into a clean and dry test tube. 2 ml of acetic acid anhydride was added to the filtrate and shaken. 1 ml of concentrated sulphuric acid was added carefully down the side of the tube to form a lower layer. A brownish-red or violet ring at the zone of contact of the two liquids and the upper layer turning green denote the presence of sterols and terpenes (Cuilei, 1990).

### 3.7.8 Test for Alkaloids

1 ml of the extract of *A. mannii* was treated with strong ammonia to make alkaline (pH 8) and then extracted twice with 10 ml portions of chloroform. The chloroform extract were combined and concentrated in vacuum to about 5 ml. The concentrate was then tested with the following reagents; Dragendorff's, Meyer, picric acid and Wagner. The presence of precipitate in most or all of the reagent tests indicates the presence of alkaloids (Evans, 1989), that is

- Light brown to brownish precipitate - Wagner's reagent

- White or creamy white precipitate - Mayer's reagent

- Orange or orange-yellow precipitate -Dragendoff's reagent

- Yellowish precipitate - picric acid reagent.

### 3.7.9 Test for Anthraquinones and Their Derivatives

2 g of the plant material was extracted with 10 ml of benzene and filtered. 5 g of 10%ammonia solution was added to the filtrate and shaken. The presence of a pink, red or violet colour in the ammoniacal (lower layer) phase indicates the presence of free hydroxyl anthraquinone. For the anthraquinone glycosides, 2 g of the powdered plant material was boiled with 10 ml of dilute sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml benzene. The benzene layer was then separated and to it, 3 ml of 10% ammonia solution was added and the mixture shaken. Appearance of a pink, red or violet colouration in the lower layer indicates the presence of anthraquinones glycosides (Sofowora, 1993).

## 3.8 STATISTICAL ANALYSIS

The results were analyzed and expressed as mean ± standard error of mean, and student's t-test were used to determine level of significance of all results obtained (Appendix 3). Results were considered statistically significant at $P< 0.05$.

# CHAPTER FOUR

# RESULTS

## 4.1 ACUTE TOXICITY STUDIES

In the first phase of the experiment all the rats in the group treated with 10, 100 and 1000mg/kg extract did not die. In the second phase, rats treated with 1600 mg/kg survived and 2,900 and 5,000 mg/kg extract died. The calculated median lethal dose ($LD_{50}$) for methanol extract (whole plant) of *A. mannii* was 2,154 mg/kg (Table4.1).

**Table 4.1: Median lethal Dose ($LD_{50}$) values of the crude Methanol (whole plant) extract of *A. mannii* in Wistar rats**

| Dose mg/kg(*po*) | No. of death/used | Mortality % |
|---|---|---|
| 10 | 0/3 | 0 |
| 100 | 0/3 | 0 |
| 1,000 | 0/3 | 0 |
| 1,600 | 0/1 | 0 |
| 2,900 | 1/1 | 100 |
| 5,000 | 1/1 | 100 |

$LD_{50} = \sqrt{X} \times Y$

Where X= maximum dose that killed and Y= Minimum dose that did not kill

$LD_{50}$ = Geometric mean of 2900 x 1600 mg/kg $=\sqrt{2900} \times 1600$mg/kg = 2,154 mg/kg (approximately 2,150 mg/kg).

## 4.2 EFFECT OF ACUTE ADMINISTRATION OF CRUDE METHANOL (WHOLE PLANT) EXTRACT OF *A. MANNII*ON THE BLOOD GLUCOSE CONCENTRATION OFALLOXAN-INDUCED HYPERGLYCAEMICWISTAR RATS

The effect of acute single oral administration of methanol (whole plant) extract of *A. mannii* on Alloxan-induced hyperglycaemic rats showed that, the 150 mg/kg did not show any significant change in the blood glucose concentration of the hyperglycaemic rats compared to control (at 0 to 8 hour). However, at 16 h, it showed a significant reduction ($P<0.0005$) in blood glucose concentration. The extract at 300 mg/kg showed significant decrease ($P<0.05$ - $P< .0005$) in blood glucose concentration between 1 to 24 hour when compared to the control. Similarly, the 600 mg/kg showed a significant decrease ($P<0.01$ - 0.0005) in the blood glucose concentration from 4 to 24 h when compared to control (Table 4.2).

## 4.3 EFFECT OF 14 DAYS SINGLE ADMINISTRATION OF CRUDE METHANOL (WHOLE PLANT) EXTRACT OF *A. MANNII* ON BLOOD GLUCOSECONCENTRATIONOFALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS

The effect of the extract at various doses administered for 14 days to hyperglycaemic rats showed that, the extract at 150 mg/kg(starting on the 3$^{rd}$ day) decreased significantly($P<0.05$ ) the blood glucose concentration of the hyperglycaemic rats while 300 and 600 mg/kg showed significant hypoglycaemic activity($P<0.05$ - $P<0.0005$) when compared to control. The 600 mg/kg on days 9 and 12 showed comparable response ($P<0.0005$) to Glibenclamide 10 mg/kg in reducing the blood concentration of the hyperglycaemic rats (Table 4.3).

**Table 4.2: Effect of acute single oral administration of crude methanol (whole plant) extract of *A. mannii*on the blood glucose concentration of hyperglycaemicWistar rats**

| | Mean blood glucose concentration ( mg/dL) |
|---|---|

| Treatment | Dose (mg/kg) | 0h | 1h | 4h | 8h | 16h | 24h |
|---|---|---|---|---|---|---|---|
| N/Saline | 10 | 306.40 ±26.46 | 467.40 ±49.40 | 552.80 ±30.57 | 560.20 ±39.3 | 568.0 ±20.12 | 592.25 ±7.78 |
| *A. mannii* | 150 | 332.00 ±30.34 | 393.40 ± 7.44$^{NS}$ | 452.40 ±47.11$^{NS}$ | 523.00 ±27$^{NS}$ | 343.00 ±21.3$^{d}$ | 520.20 ±50.05$^{NS}$ |
| *A. mannii* | 300 | 331.60 ±15.43 | 352.20 ±13.07$^{a}$ | 373.80 ±8.29 $^{d}$ | 402.40 ±9.7$^{e}$ | 321.40 ± 12.21$^{d}$ | 328.80 ±16.10$^{d}$ |
| *A. mannii* | 600 | 402.00 ±4.91 | 410.40 ±3.55$^{NS}$ | 395.80 ±2.59$^{c}$ | 394.60 ±2.40$^{c}$ | 289.40 ±4.73$^{d}$ | 368.20 ±12.50$^{d}$ |
| Glibenclamide | 10 | 323.80 ±1.15 | 309.60 ±2.50$^{b}$ | 176.20 ±0.73$^{d}$ | 165.6 ±1.40$^{d}$ | 149.20 ±2.34$^{d}$ | 139.00 ±0.89$^{d}$ |

n=5, NS = Not Significant, a=P<0.05, b= P<0.01, c = P<0.001, d= P<0.0005,

e= P<0.00025

**Table 4.3: Effect of 14 days administration of crude methanol (whole plant) extract of *A. mannii* on blood glucose concentration of alloxan-induced hyperglycaemic Wistar rats**

| Treatment | Dose (mg/kg) | Mean Blood glucose concentration ( mg/dL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Day 1 | Day 3 | Day 6 | Day 9 | Day 12 | Day 14 |
| N/ Saline | 10 | 308.60 ±4.34 | 484.90± 4.47 | 497.80 ±3.89 | 510.65 ±1.72 | 532.00 ±1.38 | 543.20 ±3.43 |
| *A. mannii* | 150 | 323.40 ±2.46 | 321.80 ±4.63$^{d}$ | 313.00 ±3.97$^{d}$ | 304.60 ±5.58$^{d}$ | 304..20 ±3.95$^{d}$ | 300.00 ±2.96$^{a}$ |
| *A. mannii* | 300 | 381.60 ±6.67 | 349.20± 2.03$^{c}$ | 312.20 ±5.03$^{c}$ | 315.60 ±2.77$^{d}$ | 303.60 ±3.08$^{d}$ | 277.60 ±3.54$^{b}$ |
| *A. mannii* | 600 | 306.60 ±3.47 | 292.40± 3.83$^{c}$ | 278.00 ±3.54$^{a}$ | 246.20 ±2.96b$^{d}$ | 208.80 ±4.06$^{b}$ | 202.40 ±2.93$^{d}$ |
| Glibenclamide | 10 | 323.60 ±1.86 | 307.20± 1.46$^{b}$ | 176.80 ±1.71$^{d}$ | 153.00 ±1.82$^{d}$ | 140.06 ±1.96$^{d}$ | 135.80 ±1.66b$^{d}$ |

n= 5, a = P<0.05, b= P<0.02, c= P<0.005, d= P<0.0005

## 4.4 EFFECT OF ACUTE ADMINISTRATION OF CRUDE METHANOL EXTRACT OF *A. MANNII* ON NORMOGLYCAEMIC WISTAR RATS

The extract at 150 mg/kg did not show any significant effect on the blood glucose concentration of the rats when compared to control, whereas, at 300 mg/kg, it showed significant ($P<0.0005$) hypoglycaemic effect at 24 h. The extractat 600 mg/kg also showed significant ($P<0.05$-$P<0.0005$) hypoglycaemic effect within 1 to 24h. Glibenclamide (10 mg/kg) showed significant ($P<0.0005$) hypoglycaemic effect in the blood glucose concentration of normoglycaemic rats which is comparable to the 600 mg/kg at the $16^{th}$ and $24^{th}$ hour (Table 4.4).

**Table4.4: Effect of single oral administration of crude methanol (whole plant) extract of *A. mannii* on normoglycaemic Wistar rats**

| Treatment | Dose (mg/kg) | Mean blood glucose concentration (mg/dL) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0h | 1h | 4h | 8h | 16h | 24h |
| N/Saline | | 71.50 ±0.96 | 74.50 ± 1.23 | 73.00 ± 1.09 | 69.00 ± 0.90 | 71.00 ± 1.02 | 70.00 ± 0.87 |
| *A. mannii* | 150 | 76.00 ± 0.98 | 74.00 ± 1.02$^{NS}$ | 72.00 ± 0.87$^{NS}$ | 68.00 ± 0.79$^{NS}$ | 69.00 ± 1.03$^{NS}$ | 70.00 ± 0.65$^{NS}$ |
| *A. mannii* | 300 | 79.00 ± 1.09 | 76.00 ± 1.02$^{NS}$ | 74.00 ± 0.62$^{NS}$ | 75.00 ± 0.78$^{NS}$ | 70.00 ± 0.98$^{NS}$ | 78.00 ± 0.89$^{d}$ |
| *A. mannii* | 600 | 79.00 ±0.50 | 71.00 ± 0.83$^{a}$ | 68.00 ± 0.65$^{b}$ | 64.00 ± 0.46$^{c}$ | 54.00 ± 0.98$^{d}$ | 59.00 ± 0.63$^{d}$ |
| Glibenclamide | 10 | 78.00 ± 0.56 | 68.00 ± 0.23$^{d}$ | 56.00 ± 1.02$^{d}$ | 53.00 ± 0.23$^{d}$ | 47.00 .± 0.78$^{d}$ | 49.00 ±0.09$^{d}$ |

n = 5,NS= Not significant, a = P<0.05, b= P<0.005, c = P<0.001, d=P<0.0005

## 4.5 EFFECT OF 14 DAYS ADMINISTRATION OF CRUDE METHANOL EXTRACT OF*A. MANNII* ON SOME HAEMATOLOGICAL INDICES OF ALLOXAN-INDUCED HYPERGLYCAEMIC WISTAR RATS

The crude extract of *A. mannii* (whole plant) after oral administration for 14 days at 150 mg/kg did not show any significant effect on Hb, WBC, PCV, N, and L, when compared to the control. However, at doses of 300 and 600 mg/kg it showed significant ($P<0.01$ and $P<0.005$) reduction in haemoglobin concentration, respectively.These doses also showed significant reduction in PCV($P<0.0005$) and WBC count ($P<0.005$). All the three doses, 150, 300 and 600 mg/kg did not have any significant effect on lymphocyte and neutrophil counts. Macrophage, eosinophils and basophils were not detected in all the groups (Table 4.5).

## 4.6 EFFECT OF CRUDE METHANOL (WHOLE PLANT) EXTRACT OF *A. MANNII* ON LIPID PROFILE OF ALLOXAN–INDUCED HYPERGLYCAEMIC WISTAR RATS

The crude methanol (whole plant) extract of *A. mannii* at 150 mg/kg did not show any significant effect on HDL, LDL, TC, VLDL and triglycerides after 14 days subacute administration, while 300 and 600 mg/kg of the extract reduced significantly ($P<0.005$ and $P<0.05$) the HDL respectively. The extract at 300 and 600mg/k significantly ($P<0.01$and $P<0.005$)increased total cholesterol

while triglyceride and VLDL, were not significantly affected by all the doses used including glibenclamide 10 mg/kg (Table 4.6)

**Table 4.5: Effect of 14 day subacute administration of crude methanol(whole plant) extract of *A. mannii* on some hematological indices of Alloxan- induced hyperglycaemic Wistar rats**

| Treatment | Dose (mg/kg) | Hb (g/dL) | PCV ( % ) | WBC ($x10^9$/L ) | N ( %) | L ( %) | B (%) | E (%) | M (%) |
|---|---|---|---|---|---|---|---|---|---|
| N/Saline | | 14.98 ±0.14 | 44.75 ±0.65 | 8.35 ±0.46 | 53.00 $\pm6.35^{Ns}$ | 49.50 ±8.85 | 0 | 0 | 0 |
| *A. mannii* | 150 | 15.00 $\pm0.20^{NS}$ | 45.00 $\pm0.43^{NS}$ | 7.00 $\pm0.26^{NS}$ | 52.00 $\pm3.67^{NS}$ | 45.00 $\pm3.28^{NS}$ | 0 | 0 | 0 |
| *A. mannii* | 300 | 10.00 $\pm1.69^{a}$ | 30.00 $\pm2.04^{c}$ | 5.00 $\pm0.36^{a}$ | 52.00 $\pm1.71^{NS}$ | 48.00 $\pm1.63^{NS}$ | 0 | 0 | 0 |
| *A. mannii* | 600 | 10.00 $\pm0.82^{b}$ | 30.00 $\pm2.04^{c}$ | 5.75 $\pm0.48^{b}$ | 52.00 $\pm5.79^{NS}$ | 45.25 $\pm2.50^{NS}$ | 0 | 0 | 0 |
| Glibenclamide | 10 | 10.00 $\pm0.82^{b}$ | 30.00 $\pm0.60^{c}$ | 5.75 $\pm0.48^{b}$ | 52.00 $\pm5.76^{NS}$ | 45.20 $\pm2.5^{NS}$ | 0 | 0 | 0 |

n = 5, NS = Not significant,a= P<0.01, b= P<0.005, c=P<0.0005

PCV = Packed Cell Volume, WBC= White Blood cell Count, Hb = Hemoglobin,

N = Neutrophils, L= Lymphocytes, B= Basophils, E = Eosinophils, M= Macrophage

| Treatment | Dose (mg/kg) | Total Cholesterol | HDL | Triglycerides | VLDL | LDL |
|---|---|---|---|---|---|---|
| N/Saline | | 3.58 | 1.77 | 1.12 | 0.51 | 1.30 |
| | | ±0.1 | ±0.10 | ±0.05 | ±0.07 | ±0.06 |

**Table 4.6:Effect of crude methanol (whole plant) extract of *A. mannii* onlipid profile of alloxan–induced hyperglycaemic Wistar rats**

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. mannii* | 150 | 3.58 ±0.10$^{NS}$ | 1.46 ±0.18$^{NS}$ | 1.36 ±0.22$^{NS}$ | 0.57 ±0.05$^{NS}$ | 1.46 ±0.08$^{NS}$ |
| *A. mannii* | 300 | 3.23 ±0.11$^{b}$ | 0.90 ±0.05$^{c}$ | 1.12 ±0.05$^{NS}$ | 0.50 ±0.05$^{NS}$ | 1.79 ±0.05$^{b}$ |
| *A. mannii* | 600 | 3.88 ±0.11$^{a}$ | 1.41 ±0.08$^{a}$ | 1.55 ±0.12$^{NS}$ | 0.70 ±0.11$^{NS}$ | 1.76 ±0.12$^{b}$ |
| Glibenclamide | 10 | 3.60 ±0.09$^{NS}$ | 1.75 ±0.07$^{NS}$ | 1.10 ±0.13$^{NS}$ | 0.52 ±0.06$^{NS}$ | 1.31 ±0.08$^{NS}$ |

n=5, NS=Not significant, a=P<0.05, b =P<0.01, c = P<0.005

High Density Lipoproteins (HDL), Very Low Density Lipoproteins (VLDL) and

Low Density Lipoproteins (LDL)

## 4.7 EFFECT OF CRUDE METHANOL (WHOLE PLANT) EXTRACT OF *A. MANNII* ON SOME LIVER FUNCTION PARAMETERS AFTER SUBACUTE ADMINISTRATION

Subacute administration of 150 and 300 mg/kg did not exert any significantly affect on the levels of AST, ALT and ALP when compared to control. The extract of *A. mannii* at 600 mg/kg significantly decreased the levels of AST ($P<0.005$), ALP ($P<0.0005$) and significantly increased ($P<0.05$) the level of ALT (Table 4.7).

## 4.8 PRELIMINARY PHYTOCHEMICAL SCREENING OF *A. MANNII*

The preliminary phytochemical screening of the crude methanol (whole plant) extract of *A. mannii* revealed the presence of alkaloids, carbohydrates, flavonoids,saponins, tannins and terpenoids, while cardiac glycosides, cyanogenic glycosides and anthraquinones were absent(Table 4.8).

**Table4.7: Effect of crude methanol (whole plant) extract of *A. mannii* on liver function parameters after 14days administration**

| Treatment | Dose (mg/kg) | AST IU/L | ALT IU/L | ALP IU/L |
|---|---|---|---|---|
| N/Saline | | 80.50 ±2.50 | 48.50 ±3.90 | 238.50 ±12.76 |
| *A. mannii* | 150 | 75.00 ±2.31$^{NS}$ | 43.75 ±2.95$^{NS}$ | 235.00 ±11.98$^{NS}$ |
| *A. mannii* | 300 | 78.30 ±2.46$^{NS}$ | 45.80 ±0.31$^{NS}$ | 231.00 ±14.01$^{NS}$ |
| *A. mannii* | 600 | 67.75 ±2.25$^{b}$ | 58.75 ±1.13$^{a}$ | 123.00 ±14.43$^{c}$ |
| Glibenclamide | 10 | 76.00 ± 0.91$^{NS}$ | 47.75 ±3.30$^{NS}$ | 239.00 ±1.63$^{NS}$ |

n =5, NS = Not Significant, a= P<0.05, b= P<0.005, c=P<0.0005.

AST= Aspartate Transaminase, ALT =Alanine Aminotransferase,

ALP= Alkaline Phosphatase.

**Table 4.8:Prelimnary phytochemical constituents in the crude methanol (whole plant) extract of *A. mannii***

| Constituents | Remark |
|---|---|
| Tannins | + |
| Saponins | + |
| Flavonoids | + |
| Alkaloids | + |
| Cardiac glycosides | - |
| Cyanogenic glycosides | - |
| Steroid/Terpenoids | + |
| Carbohydrates | + |
| Anthraquinones | - |

+ Present

- Absent

# CHAPTER FIVE

## DISCUSSION

The oral median lethal dose ($LD_{50}$) of the extract was calculated to be 2,150mg/kg. This according to the classification of Matsumma, (1975) and Lorke, (1983)is slightly toxic.

Hyperglycemia was induced using alloxan in Wistar rats. The observed hyperglycemia in the rats could be as result of rapid destruction of beta cells of the pancreas there by causing reduction in insulin which eventually leads to increase in bloodglucose concentration as reported in the literature (Antia *et al.,* 2005; Etuk, 2010).

The data of the bloodglucose concentration ofhyperglycaemic rats treated with methanol (whole plant) extract of *A. mannii* showed a significant decrease in blood glucose concentration at higher doses of 300 and 600 mg/kg as compared to 150 mg/kg. This may suggest a dose dependent response (Okokon *et al*., 2006). The 300 and 600 mg/kg significantly decreased the blood glucose concentration within the first hour and fourth hour respectively. The reference drug, glibenclamide (10mg/kg) also starts its action within the first hour as compared to the 600 mg/kg.Both Glibenclamide (10mg/kg) and the 600 mg/kg showed significant decrease in blood glucose at the16$^{th}$ hour.

The comparable onset of action at 300 mg/kg with the oral hypoglycaemic agent, glibenclamide might suggest similar pharmacokinetic profile withthe extract. Sulfonylureas are effectively absorbed from the gastrointestinal tract, although food and hyperglycemia can reduce the absorption(Goodman and Gilman, 2008). The delay (4h) in reducing the blood

glucoseconcentration (600mg/kg group) could be as a result of the severe hyperglycaemic condition ( > 400 mg/dL) a condition called glucose toxicity when compared to the 300 mg/kg and glibenclamide 10 mg/kg groups(< 400 mg/kg)and thus, the effect is not seen immediately (Etuk, 2010; McNeil, 1999).Sulphonylurea compounds arepotent in mild alloxan-induced diabetes and inactive in intense alloxan diabetes where nearly all beta cells have been destroyed (Yallow *et al.*,(1960) and Christopher *et al.,* (2008)).

The antihyperglycaemic activity seen in 300 and 600 mg/kg last upto 24 h and is comparable with the reference drug,glibenclamide (10 mg/kg). This may suggest too that, the higher doses (300 and 600mg/kg) stays in the system bound to proteins for them to be effective upto 24h. Glibenclamide is also largely (90–99%) bound to protein, especially albumin (Goodman and Gilman, 2008). This means there is the possibility of the extract to be taken once or twice daily to achieve its effect.

Similarly, all the doses used in this study showed significant and consistent antihyperglycaemic effects throughout the period of 14 days in the hyperglycaemic rats.The comparable pattern of hypoglycaemic activity of the extract with that of the reference drug, glibenclamide,may mean a possible similarity in their mechanism of action. The extract could possibly be acting through increase in therelease of insulin from the remaining beta cells or increase in peripheral glucose utilization by the cells or both (Erah *et al*., 1996). The fact that the rats were able to survive upto 14 days, may mean there is little insulin in the β cells or a regeneration of the β cells as reported by Sheeja and Augusti, (1993); Kumud and Mathew, (1995) and Subramoniam *et al*.,(1996).

Theextract at 150 mg/kg did not show any significant change in blood glucose concentration of normoglycaemic Wistar rats and the result is comparable to control. However, the extract at 300

mg/kg showed significant decrease in blood glucose concentrationat 24 h while, at 600 mg/kg it caused significant decrease in blood glucose concentration in normoglycaemic rats which compares with the reference drug, glibenclamide (10 mg/kg) throughout the 24h period. The decrease in blood glucose concentration of normoglycaemic rats showed the possibility of the extract causing hypoglycemia at higher doses (300 and 600 mg/kg). Hypoglycemia is a common side effect amongst some oral hypoglycaemic agents such as glibenclamide and this may mean the extract should be used with caution and blood glucose monitoring should be considered in patients taking the extract.

The extract atboth 300 and 600 mg/kg significantly decreased the concentration of hemoglobin (Hb), WBC and PCV,while at 150 mg/kg there was no significant change, in Hb, WBC, Neutrophils (N) and Lymphocytes (L) when compared to control. However, the lowering effect on hemoglobin in the 600 mg/kg is comparable to that of glibenclamide(10 mg/kg).The changes in some haematological parameters observed with higher doses, may be due to alteration in blood homeostasis by the extract and therefore may have toxic potential. Some oral hypoglycaemic agents are known to cause anaemia as their side effects (Atanu, *et al*., 2011). This may suggest that, the extract at higher doses has the potential to cause anemia(Okokon*et al*., (2006) and Olukunle and Abatan, (2008)). The reduction in WBC counts observed at 600mg/kg maycompromiseimmunity and predispose individuals to opportunistic and supra infections inspite of the antihyperglycemic properties (Adepoju and Adebanjo, 2011), asWBC contributes to the host defense mechanism (Lloyd, *et al*., 1999).Similarly, the reduction in PCV observed in this study could be due to the toxic effect ofalloxan as reported by Martins *et al.,* (2010) or may be due to the effect of the extract on blood haemostasis as reported by Okokon *et al*., (2006).

The extract in this study showed a dose dependent activity on the lipid profile of the hyperglycemic rats.The increase in LDL and TC observed with higher doses of the extract may be as a result of the hyperglycaemic condition which is associated with an increased risk of thrombotic, atherosclerotic and cardiovascular disease. Hyperglycaemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins , with about 70 -80% of deaths in diabetic patients being due to vascular disease.(Chattopadhyay and Bandyopadhyay, 2005). Dyslipidemia is a frequent complication of DM and is characterized by low levels of high-density lipoprotein-cholesterol (HDL-C) and high levels of low density lipoprotein. Hyperlipidemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and phospholipids; and changes in lipoprotein composition (Betteridge, 1997). The extract showed significant increase in TC and LDL and a decrease in HDL which may suggest that, higher doses of the extract may cause elevated levels of lipoproteins which may add to the macrovascular effect of the disease.

ALT is found in liver cells in high concentration (Romito and Kahrilas, 2010). Alanine

aminotransferase (ALT) is an enzyme produced by the liver that acts as a catalyst in the transamination reaction necessary for amino acid production.When liver damage occurs,ALT levels increase, which makes ALT testing, a valuable test for monitoring liver function. In this study, oral administration of the extract at 150 and 300 mg/kgfor14 days did not show any significant change in ALT, ALP and AST levels. However, at

600 mg/kg there was significant (P<0.005) increase in ALT, a significant decrease in AST (P<0.005)and ALP (P<0.0005). The high levels of ALT alone may indicateliver damage as reported in the literature (Schiele *et al*., 1998; Romito and Kahrilas, 2010 and Adepoju and Adebanjo, 2011). Lower levels of AST may mean that the extract do not have significant effect on other organs where AST is found. AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys(Romito and Kahrilas, 2010). Alkaline phosphatase (ALP) is a hydrolaseenzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids(Romito and Kahrilas, 2010).Some diseases may lead to reduced levels of alkaline phosphatase such as hypophosphatasia, an autosomal recessive disease, severe anemia, malnutrition etc, (Schiele *et al.*, 1998). The low levels of ALP observed in this study may be due to anaemia reported (Okokon *et al*., (2006) and Olukunle and Abatan, (2008)). The fact that the extract can lower the ALP levels it may also affect some drug biotransformation due to the importance of ALP in removing phosphate groups from many molecules in the body (Romito and Kahrilas, 2010).

Some of the chemical constituents found present in plants are known to have various pharmacological activities in man and animals(Couper, 1987). Medicinal plants are known to possess various phytochemical agents, which are responsible for their pharmacological activities and the side effects associated with their use (Aliyu, 2007).The phytochemical constituents (alkaloids, carbohydrates, flavonoids, saponins, tannins and steroids/terpenoids) found in this plant, *A. manni*ihave been shown to have therapeutic effect and may be responsible for the antihyperglycaemic/hypoglycaemic effects observed in this study. Flavonoids and tannins for examples have been reported to have analgesic

and anti-inflammatory effects (Ahmadiani *etal.*, 1998; Ahmadiani *etal.*, 2000).Similar studies were reported by Andrade-Cetto and Wiedenfeld, (2001), where, flavones, isoorientin and 3-caffeoylquinic acid, were isolated from *Cecropia obtusifolia* and were found to be responsible for its hypoglycemic action. Tanko *etal.*,(2011)reported that the antidiabetic actions of*Indigofera*. *pulchra*may be due to flavonoids and tannins.

# CHAPTER SIX

## CONCLUSION AND RECOMMENDATIONS

### 6.1 CONCLUSION

The acute toxicity studies on *A. mannii*showed an estimated $LD_{50}$ of 2,150 mg/kg body weight orally in rats, which makes it relatively safe to use. The crude methanol (whole plant) extracts of *A. mannii* showed significant dose dependant antihyperglycaemic and hypoglycaemic properties in alloxan-induced hyperglycaemic Wistar rats. It may therefore be concluded, that the use of the plant *A. mannii* in traditional medicine to manage hyperglycaemia in diabetic patients is justifiable. However, subacute (14 days) administration of the crude methanol extract of *A. mannii* showed a dose dependant toxic potential on some haematological and biochemical parameters which should be monitored.

### 6.2 Recommendations

It is recommended that, more research be carried out to determine:

1. The active constituents and possibly the pharmacologically active compound(s) responsible for the observed hypoglycaemic activity.
2. Subchronic and chronic toxicity studies to be carried out on the crude extract to determine long term toxicity of the extract.

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## APPENDICES

### APPENDIX I

**Calculation of Percentage Yield of the Methanol Extract of *A. mannii***

Weight of the Crude drug to be extracted = 200g

Weight of the crude extract obtained = 19.1 g

Percentage yield =19.1/200 x100 = 9.55%

# APPENDIX II

## Statistical Formulae

1. **Mean**

$$mean\left(\bar{x}\right) = \sum \frac{x}{n}$$

Where; x = sample

∑x = sum of x

n = number of x

2. **Standard deviation**

Standard deviation (s) = $\sqrt{\frac{\sum x^2 - \left(\frac{\sum x}{n}\right)^2}{n-1}} = \sqrt{\frac{\sum\left(x-\bar{x}\right)^2}{n-1}}$

3. **S** = $\sqrt{Variance}$

4. **Standard error of mean(SEM**)

Standard error of mean (SEM) = $\frac{s}{\sqrt{n}}$

5. **T-test**.

$t_{test}$ = $\frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{n} + \frac{S_2^2}{n}_2}}$

Where; $\bar{x}_1 - \bar{x}_2$ = difference between sample means

$S_1$ and $S_2$ = standard deviations

$n_1$and $n_2$ = number of the samples

$n_1 + n_2 - 2$ = degree of freedom.