COMPARATIVE EFFICACY OF ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACTS OF SOME PLANTS (CASHEW, GUAVA AND PAWPAW) AND THEIR COMPOSITES IN DIABETIC RATS.

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DECLARATION

I declare that the work in the thesis entitled "COMPARATIVE EFFICACY OF ANTI-DIABETIC AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACTS OF SOME PLANTS (CASHEW, GUAVA AND PAWPAW) AND THEIR COMPOSITE IN DIABETIC RATS" has been performed by me in the Department of Biochemistry under the supervision of Professor D. A. Ameh and Dr. E. Onyike. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Ayuba, Victoria	Signature	Date

CERTIFICATION

This thesis entitled "Comparative Efficacy of Anti-diabetic and Antioxidant Activities of Methanolic Extracts of some plants (Cashew, Guava and Pawpaw) and their composites in Diabetic rats" by Ayuba, Victoria meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The comparative efficacy of antidiabetic, anti-hyperlipidaemic and antioxidant activities of methanolic extracts of *Carica papaya*, *Psidium guajava*, *Anacardium occidentale* leaves and their composite extracts in streptozotocin induced diabetic rats was investigated. The investigation revealed that there was a significant ($P < 0.05$) decrease in the fasting blood glucose level in the composite treated group which is comparable with the glibenclamide treated group. The oral administration of the composite extract showed the highest hypolipidaemic effect, compared with the individual extracts. The highest reduction in alanine aminotransferase (ALT) and alkaline phosphatase (ALT) activities were also observed in the composite treated group. For the aspartate aminotransferase (AST) activity, *P. guajava* treated group showed the maximum decrease. Treatment with methanolic extracts of composite and leaf extracts of *C. papaya. P. guajava, A. occidentale* and glibenclamide brought about a significant increase in serum protein and albumin. Increase in urea level reduced significantly after administration of the composite extract, which had the lowest urea level, followed by *A. occidentale* leaves, *C. papaya and P. guajava* leaves treated groups. In this study, decreased activities of liver and kidney superoxide dismutase (SOD) and catalase (CAT) activities, as well as increased levels of malondialdehyde (MDA) were observed in diabetic control rats compared with the normal control rats. Based on the efficacy of the extracts in reducing the liver MDA levels, the order of effectiveness is as follows; A. *occidentale* > *P. guajava* > *C. papaya* > composite extracts. While for the kidneys, MDA levels is in the order; *A. occidentale* leaves ≥ *P. guajava* leaves ranks first, then composite ≥ *C. papaya* treated groups respectively. For the liver SOD and CAT activities, the composite showed the highest

increase, followed by *A. occidentale, C. papaya and P. guajava* leaves treated groups, which all had the same SOD activity and *A. occidentale* leaves extract treated group, *C. papaya* treated group, and *P. guajava* leaves treated group for catalase activity. A significant ($P < 0.05$) increase was observed in the kidney SOD activity after administration of the composite, followed by *P. guajava, C. papaya* and *A. occidentale* treated group. For the catalase activity, the composite and *C. papaya* showed the maximum activity followed by *A. occidentale* and lastly *P. guajava* treated groups. This study showed that treatment with the various methanolic leaves extract of *P. guajava, C. papaya* and *A. occidentale* and composite proved effective in hypoglycemic activity, hypolipidemic and antioxidant property. However, the composite extract had the highest activity and showed tissue- organ protective properties.

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LIST OF ABBREVIATIONS

ADA America diabetes association
ALP Alkaline phosphatase
ALT Alanine aminotransferase
AST Aspartate aminotransferase
ATP Adenosine triphosphate
BMI Body mass index
CAD Coronary artery disease
CAT Catalase
CVD Cerebrovascular disease
DKA Diabetic ketoacidosis
DM Diabetes mellitus
DN Diabetic nephropathy
DPP-IV Dipeptidyl peptidase –IV
DR Diabetic retinopathy
FDA Food and drug administration
FAO Food and agricultural organisation
GDM Gestational diabetes mellitus
GIP Gastric inhibitory polypeptide
GLP-1 Glucagon- like
GLUT 4 Glucose transporter 4
GSH Reduced glutathione
HDL-C High density lipoprotein cholesterol
HHNKC Hyperglycemic hyperosmolar nonketotic coma
HLA Human leucocytes antigene
IDDM Insulin-dependent diabetes mellitus
IGT Impaired glucose tolerance
IRSs Insulin receptor substrates
K^+ATP Potassium -adenine triphosphate
LD_{50} Lethal dose
LDL-C Low density lipoprotein cholesterol
MDA Malondialdehyde
NDFS National Diabetes Facts Sheets
NDS National Diabetes Statistics
NIDDM Non- insulin-dependent diabetes mellitus
PACAP Pituitary adenylate cyclase activating polypeptide
PAD Peripheral arterial disease
PCV Packed cell volume
PKB Protein kinase B
ROS Reactive oxygen species
SOD Superoxide dismutase
VIP Vasoactive intestinal peptide
VLDL-C Very low density lipoprotein cholesterol
WHO World health organization
t tonnes

cm	centimeters
s	Seconds

CHAPTER ONE

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder that presently affects 200 million people of the world's population (Wais *et al.,* 2012). It is projected to rise to over 366 million in the year 2030 (WHO, 2004). It is characterized by hyperglycaemia with alteration of carbohydrates, protein and fat metabolism, resulting from defects in insulin secretion or sensitivity of insulin to body cells or both (Hovens *et al.,* 2005). Such alterations result in elevated blood glucose concentration, which causes acute complications such as hyperglycemia and hypoglycemia and long-term complications in many organs leading to increase risk of atherosclerosis, coronary heart disease, renal failure, nerve damage, and blindness resulting in increasing disability (ADA, 2009). The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity (Upendra-Rao *et al.,* 2000). The International Diabetes Federation states that "every ten seconds, two people are diagnosed with diabetes somewhere in this world," and given the current trend, the number of diabetics world wide in 2025 will be more than the current populations of the United States, Canada and Australia combined (IDF, 2011). In developed countries, most patients of diabetes are over sixty, but in developing countries, diabetes is found to affect people in their prime. There is a considerable progress in the understanding and management of diabetes, however, the disease and its related complications continue to increase (Tiwari and Madhusudana, 2006). Increasing evidence indicates that the complications of diabetes are preventable by controlling the metabolic abnormalities. Lifestyle modifications of dietary habits and

physical activity appear to play an important role in delaying and preventing the risks associated with diabetes and its complications.

The search for natural products to cure diseases has received considerable attentions in which plants have been the most important source (Okwu, 2001). Herbal preparations form the basis for many therapeutic drugs and are the first line treatment for many of the world's population, being readily available, traditional and relatively inexpensive (Olaniyi, 1998; Okpara *et al*., 2007). Herbal medicinal products are assuming greater roles in the lives of the people across the world in the face of global upsurge of drug resistance, toxicity, adverse effects and increasing costs of synthetic products (Mbi and Bilikha, 1998). In Nigeria, several thousands of plant species have been claimed to possess medicinal properties and employed in the treatment of many ailments (Iweala and Oludare, 2011). Many of these indigenous medicinal plants are used as spices and food plants and for medicinal purposes (Nwaogu *et al*., 2007). Medicinal plants are believed to be an essential source of new chemical substances with potential therapeutic effects (Kuhn and Winston, 2000). Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional antidiabetic therapies (Grover, *et al.*, 2002; Katerere and Elof, 2005; Balde *et al*., 2006; Acharya and Shrivastava, 2008). Some of these medicinal plants having anti-diabetic properties are; *Anacardium occidentale*, *Carica papaya* and *Psidium guajava* leaves.

1.1 **STATEMENT OF RESEARCH PROBLEM**

Globally, the prevalence of diabetes mellitus is increasing and especially in adults which constitutes a global public health burden (Reetesh *et al.,* 2011**)**. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and is anticipated to cross 5.4% by the year 2025 (Patel *et al*., 2012). World Health Organisation projects that diabetes deaths will double between 2005 and 2030 (WHO 2011). According to a survey conducted in 2000 by the World Health Organization, more than 1.71 million Nigerians age 15 years and older are diabetics. Regardless of progress made in the management of diabetes using synthetic drugs, many traditional plants treatments are still used throughout the world. Management of diabetes without any side effect is still a challenge to the medical community. In modern medicine, no satisfactory effective therapy is available to cure diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance (Piedrola *et al.,* 2001), anorexia nervosa, brain atrophy and fatty liver (Yaryura-Tobias *et al.,* 2001) after chronic treatment.

1.2 **JUSTIFICATION**

Reports are available individually on the anti-diabetic activity of different extracts, of paw-paw (*Carica papaya*), guava (*Psidium guajava*) and cashew (*Anacardium occidentale*) plant extracts, but no information is available on the effect of composite, comprising the three plants. Hence, the need to work on these three plants and document the most potent for use and reference purpose.

1.3 **AIM AND OBJECTIVES**

The general aim of this research work is to compare the anti-diabetic and antioxidant efficacy of methanolic extracts of *Psidium guajava*, *Anacardium occidentale* and *Carica papaya* leaves and their composite in streptozotocin induced diabetic rats.

The specific objectives are:

1. To carryout quantitative phytochemical estimations of the plants extracts.
2. To determine the effect of the plant extracts on fasting blood glucose level in diabetic rats.
3. To evaluate the effect of the plant extracts on lipid profile of diabetic rats
4. To determine the effect of the plant extracts on the packed cell volume of the diabetic rats.
5. To assess the effect of the plant extracts on the liver and renal function indices.
6. To assess the effect of the plant extracts on the endogenous antioxidant enzymes.

CHAPTER TWO

LITERATURE REVIEW

2. 1. 0 **CLASSIFICATION AND DESCRIPTION OF THE PLANTS**

2.1.1 ***Pawpaw (Carica papaya* linn).**

Carica papaya belongs to the family of *Caricaceae*. Papaya is an herbaceous succulent plant that posses self supporting stems (Dick, 2003). It is a large perennial herb with a rapid growth rate. The plants are usually short-lived, but can produce fruit for more than 20 years (Ayoola and Adeyeye, 2010). The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female (Bruce and Peter, 2008). Hermaphrodite trees are the commercial standard, producing a pear shaped fruit. The plants are self pollinated (Jari, 2009).

Carica papaya belongs to a small family of only 4 genera and 27-30 species of which *C. papaya* is the most widely cultivated and best known species of the family. The plant was originally derived from the southern part of Mexico. However, it has naturalized in many tropical and subtropical countries (Randall, 2002). *C. papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular, *C. papaya* fruit circulates widely, and it is accepted as food. In Nigeria, pawpaw is one of the most popular, cheapest, economically important fruit tree grown and consumed for its nutritional content (Baiyewu and Amusa, 2005). *Papaya* is not a tree but an herbaceous succulent plant that possesses self supporting stems (Dick, 2003). The plants can produce fruit for

more than 20years (Ayoola and Adeyeye 2010). The plant may be male, hermaphrodite, or female (Bruce and Peter, 2008).

The top ten countries that produces *Carica papaya* plants world wide are; Brazil (25%), Nigeria (15%), India (12%), Mexico (11%), Indonesia (10%), Ethiopia (4%), Congo (4%), Peru (3%), Venezuela (3%), and lastly China (2%) (FAO, 2004).



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Figure 2.1 Pawpaw (*Carica papaya*) leaf.

2.1.2 **Cashew** (***Anacardium occidentale* linn*)*:**

The plant belongs to the family *Anacardiaceace*.The tree is small and evergreen, growing up to 15m high (Morton, 1987), with a short, often irregularly shaped trunk. The leaves are spirally arranged, leathery textured, elliptic to ovate, 4 to 22 cm long and 2 to 15 cm broad, with a smooth margin. The flowers are produced in a panicle or corymb up to 26 cm long, each flower small, pale green at first then turning reddish, with five slender, acute petals 7 to 15 mm long. The largest cashew tree in the world covers an area of about 7,500 square metres (81,000 sq ft) (Pillai and Santha 2008).

There are about 70 genera and 600 species, which are native mainly to the warmer regions of the world but also in some temperate regions. *Anacardium* contains 8 species, native to tropical America, of which the cashew is by far the most economically important. It is a multipurpose tree of the Amazon that grows up to 15m high. The cashew develops well between 70% and 85%, relative humidity. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground (Morton, 1987). There are 13 genera and 113 species native to southern Africa, an additional three genera and four species naturalised, and an additional 10 genera and 22 species that are cultivated in the region (Archer, 2000). The largest number of varieties of the genus *Anacardium* is found in northeastern Brazil. It is produced in around 32 countries of the world. The world production figure of cashew crop, published by the Food and Agriculture Organization (FAO), was around 3.1 million tonnes (t) per annum. The principal producing countries responsible for 83.9% of world production are Vietnam, India, Nigeria, Brazil, Indonesia and Tanzania. The major raw cashew producing countries with their production figures in 2006 (estimated by the FAO) are Vietnam

(941,600 (t)), Nigeria (636,000 (t)), India (573,000 (t)), Brazil (236,140 (t)) and Indonesia (122,000 (t)). Nigeria is the world's forth largest producer of raw cashew. The cashew tree produces many resources and products. During the past decade, the production of cashew nuts in Nigeria has increased almost six-folds from 30,000 (t) in 1990 to 176,000 (t) in 2000 (Olunloyo, 1996). As in the case of other developing countries, Nigeria has recognized the potential economic value of cashew and has made a concerted effort to improve production of the crop. During the last five to ten years, Nigeria has emerged as a largest producer of cashew nuts in Africa (Ogunsina and Lucas, 2008).



Figure 2.2 Cashew *(Anacardium occidentale)* leaves.

2.1.3 **Guava (*Psidium guajava* linn)**

Psidium guajava belongs to the family *Myrtaceae*. The leaves are tough and dark that are opposite, simple, elliptic to ovate and 5–15 centimetres (cm) (2.0–5.9 in) long. It is a low evergreen tree or shrub of 6 to 25 feet high, with wide spreading branches and square, downy twigs (Sanda *et al*., 2011). It is a common vegetation cover by roads and in waste places in Hawaii. Guava is a tropical and semitropical plant. It is well known in the islands for its edible fruit. The flowers are white, incurved petals, 2 or 3 in the leaf axils; they are fragrant, with four to six petals and yellow anthers.

Psidium guajava has about 133 genera and more than 3,800 species (Joseph and Priya 2011). It originated in the tropical South America. It grows wild in Bangladesh, India, Thailand, Brazil, Florida, and West Indies, California and also in several other countries (Pathak *et al*., 1993). It grows in all the tropical and subtropical areas of the world, and adapts to different climatic conditions but prefers dry climates. However, the plant is cultivated today from the west coast of Africa to the Pacific region, including India and China, with varieties originally introduced over the past 300 years from the United States. It is a genus of about 100 species of tropical shrubs and small trees. Thriving in all types of soils, the guava is widely distributed and the fruits enrich the diets of millions of people in the tropics of the world (El-Mahmood, 2009). Guava (P. guajava) and its cultivars are those most commonly traded internationally. Mature trees of most species are fairly cold-hardy and can survive as low as 5 °C for short periods of time, but younger plants will not survive. Generally, guava plant has spread widely throughout the tropics because it thrives in a variety of soils, propagates easily, and bears fruit relatively quickly (Lapik *et al.,* 2005; Jimenez-Escrig *et al.,* 2001;Lozoya *et al.,* 2002) . The guava

plant parts are used for the development of various industrial and pharmaceutical products. The tree is easily identified by its distinctive thin, smooth, copper colored bark that flakes off, showing a greenish layer beneath. It is the hardiest among tropical fruit trees and excels most of the other fruit crops in productivity and adaptability. Moreover, guava scores over other fruits in ascorbic acid, pectin and other mineral contents. Guava cultivars however display a greater diversity in tree size, bearing habit and yield, as well as in fruit size, shape, quality and ripening season. (Joseph *et al*., 2011). It plays a vital role in fulfilling the vitamin C deficiency among the people of the country, which is 2–5 times higher than the fresh orange. Guava is rich in antioxidants (exogenous antioxidants such as vitamin C and flavonoids specifically quercetin) compounds and contains a high level of ascorbic acid (Joseph and Priya, 2011).



Figure 2.3 Guava (Psidium guajava) leaves.

2.2 **MEDICINAL IMPORTANCE OF THE PLANTS.**

2.2.1 ***Guava* (*Psidium guajava* Linn.)**

This is an important plant used traditionally for medicinal purposes. It is a medicinal plant used in tropical and subtropical countries to treat many disorders. (Belemtougri *et al*., 2006). Guava, also known as the poor mans apple of the tropics, has a long history of traditional use, a good proportion of which have been validated by scientific research. In Ghana and in Nigeria the leaves are chewed to relieve toothache. (Burkill, 1997). It was reported that *P.guajava* leaf extract has a wide spectrum of biological activities such as anti-cough, antibacterial, haemostasis (Jaiarj *et al.*, 1999; 2000), antidiarrhoeal and narcotic properties (Lozoya *et al.*, 1990), and antioxidant properties (Qian and Nihorimbere, 2004). The decoctions made from the leaves and/or bark of *Psidium guajava* have been reported to be used by many tribes for diarrhea, dysentery, sore throats, vomiting, stomach aches, and vertigo throughout the tropical Amazon and India. The pharmacological actions and the medicinal uses of aqueous extracts of guava leaves in folk medicine include the treatment of different type of gastrointestinal disturbances such as vomiting, diarrhea (Ojewole *et al*., 2008), inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain. (Lutterodt, 1992; Aguilar *et al.*, 1994; Lozoya *et al.*, 1994). It is phytotherapic, used in folk medicine to treat respiratory disturbances and anti-inflammatory medicine (Abreu *et al.*, 2006). Some of the ethnomedicinal uses includes the crushing of the leaves and the application of the liquid extract on wounds, cuts, ulcers, boils, skin and soft tissue infectious site, rheumatic places (Bala, 2006) and the

chewing of the leaves to relieve oral ulcers, inflamed gums, throat, chest pains. Some *invitro* works have shown that the aqueous extracts of guava leaves are effective against a number of microbial strains: *Aeromonas hydrophila, Shigella* spp. and *Vibrio* spp. (Chulasiri *et al*., 1986), *Staphylococus aureus* and *β-streptococcus* group A (Jaiarj *et al.*, 1999), *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus subtilis* (Abdelrahim *et al.*, 2002). Anti-rotavirus activity has also been reported to exist in these extracts (Gonçalves *et al*., 2005). Many pharmacological studies have demonstrated the ability of the plant to exhibit antioxidant, hepato-protective, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, antidiabetic, anti-inflammatory and antinociceptive activities, supporting its traditional uses (Gutiérrez *et al.,* 2008; Abdul *et al.,* 2011). *Invitro* anti-diabetic activity of *Psidium guajava* leaves extracts has also been reported (Sabjan and Vinoji, 2012).

2.2.2 **Pawpaw (*Carica papaya L*inn)**

Several species of *Caricaceae* have been used as remedy against a variety of diseases (Mello *et al*., 2008). According to the reports of Okeniyi *et al*. (2007) *C. papaya* ***L.*** fruit and seeds have antihelminthic and antimoeboic activities. The high level of natural self-defense compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 1999). The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level (Seigler *et al*., 2002). In humans, following treatment with antibiotics, papaya juice helps to restore normal intestinal flora destroyed by the antibiotics (Nwinyi and Abikoye, 2010). Pawpaw peels serve as poultice for treatment of skin wounds that do not heal

quickly. Pawpaw fruit stimulates milk production in weaning mothers, prevents constipation, nausea, morning sickness, motion sickness, cataract formation, chronic obstructive pulmonary disease, diverticulosis and hypertension (Nwinyi and Abikoye, 2010). The seeds of pawpaw have been reported to cure cough when eaten raw in some parts of Nigeria (Gills, 1992). Pawpaw (*C. papaya L.)* improves the immune system and prevents illnesses such as recurrent ear infections, colds and flu (Nwinyi and Abikoye, 2010). *Carica papaya L*. leaf tea or extract has a reputation as a tumour-destroying agent. (Walter, 2008). The root of papaya is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases (Gills, 1992). The unriped fruit is used as a remedy for ulcer and impotence, (Kafaru, 1994). The tea prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2005). The yellow *Carica papaya* leaf is equally used as anti- anaemic agent while the brown leaf is used as a body cleanser (Atta, 1999). Previous studies has shown that papaya leaves contain carpaine which has high antioxidant content that may be helpful for the prevention of atherosclerosis, diabetic heart disease, heart attacks and strokes. Pawpaw (*C. papaya L.)* improves the immune system and prevents illnesses such as recurrent ear infections, colds and flu. It has exhibited inhibitory effects on gram-positive bacteria and gram-negative bacteria. These organisms include: *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterobacter cloacae* and *Proteus vulgaris* (Emeruwa, 1982). The hypoglycemic and hypolipidemic effects of the aqueous seed extract of *Carica papaya* was reported (Adeneye and Olagunju, 2009).

2.2.3 **Cashew (*Anacardium occidentale* L.)**

The leaves, stem and bark extracts of *Anacardium occidentale* are used widely for the treatment of diarrhea, dysentery and colonic pain (Bilcalho, 2001). It has also been reported to possess anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic properties (Akinpelu, 2001). The leaves are also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis, and syphilis-related skin disorders (Dare *et al.*, 2011). Several clinical studies have shown that anacardic acids a component of cashew, with highest concentration in the nutshells curb the darkening effect of aging by inhibiting tyrosinase activity, and that they are toxic to certain cancer cells (Kubo *et al*., 1994). Further studies have shown the anti-bacterial, antifungal and anti-tumor activities of cashew apple juice , a processed juice from the fruit of the cashew tree (Kubo *et al*., 1993a, b; Kozubek *et al*., 2001) as well as anti-oxidant effects (MeloCavalcante *et al*., 2003) and anti-mutagenic activity (Santos *et al*., 2002; Melocavalcante *et al*., 2003). Toyomizu *et al*. (2003) revealed a unique function of anacardic acid in that, for dietary conditions enhancing body fat deposition, that is consumption of a diet high in carbohydrates, dietary anacardic acid has the potential to decrease body fat deposition.

2.3 **DIABETES MELLITUS**

Diabetes is a chronic disease that arises when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Insulin is a hormone made by the pancreas that enables cells to take in glucose from the blood and use it for energy. Failure to produce insulin, or of insulin to act properly, or both, leads to

raised glucose levels in the blood (IDF, 2011). The major clinical manifestation of the diabetic state is hyperglycemia. However, insulin deficiency and/or insulin resistance also are associated with abnormalities in lipid and protein metabolism, and with mineral and electrolyte disturbances. Diabetes is one of the oldest known diseases (Dallas and John 2011).Some of the common symptoms associated with Diabetes mellitus are: Polyuria (excessive urination), Polydipsia (excessive thirst) and Polyphagia (excessive appetite) (Cooke and Plotnick 2008). These symptoms are due to the high glucose level and inability of cells to make use of glucose as a source of energy.

2.4 **EPIDEMIOLOGY AND SIGNIFICANCE OF DIABETES MELLITUS**

The number of studies describing the epidemiology of diabetes over the last 20 years has been alarming. Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries (Erasto *et al.,* 2005). It is now recognized that it is the developing countries that presently face the greatest burden of diabetes. Diabetes is now one of the most common non-communicable diseases globally. It is one of the top five most significant diseases in the developed world, and the third 'killer' of mankind, after cancer and cardiovascular diseases, because of its high prevalence, morbidity and mortality (Li *et al.,* 2004). Diabetes mellitus and lesser forms of glucose intolerance, particularly impaired glucose tolerance can now be found in almost every population in the world and epidemiological evidence suggests that, without effective prevention and control programmes, diabetes will likely continue to increase globally (WHO, 1994). It is the most prevalent disease in the world affecting 25% of population and afflicts 150 million people and is set to rise to 300 million by 2025 (Vats *et al.*, 2000). This increase

is at an epidemic form and in near future the largest increases will take place in the regions dominated by developing economies. The prevalence of diabetes is reported to be higher in men than in women; however, there are more women than men with diabetes (Wild *et al.*, 2004). Population growth, urbanization, increasing prevalence of obesity and physical inactivity are thought to be the main factors responsible for the increasing prevalence of type 2 diabetes mellitus (Wild *et al.*, 2004). Diabetes causes about 5% of all deaths globally each year. 80% of diabetes deaths are now occurring in-low and middle-income countries (WHO, 2010). According to an estimate, one person is detected with diabetes every 5 s somewhere in the world, while someone dies of it every 10 s. According to the International Diabetes Federation, the overall cost assessment for the global prevention and treatment of diabetes will run up to US$490 billion by 2030. (Colagiuri, 2010). The greatest increase in prevalence is however expected to occur in Asia and Africa, where more patients will likely be found by 2030. According to the American Diabetes Association (ADA, 1997), Asia and Africa are regions with greatest potential where diabetics could rise to two or three fold above the present level. This is due to ageing population structure and the global increase in obesity as well as sedentary lifestyle (Vats *et al*., 2002). Epidemiologic evidences suggest that if effective preventive measures are not implemented, the global prevalence will continue to rise (Alberti and Zimmet 2007).

2.5 **CLASSIFICATION OF DIABETES MELLITUS**

Diabetes mellitus is classified on the basis of clinical presentation of the disorder and this can be grouped into three major types: type I diabetes, type II diabetes and gestational diabetes mellitus (Velho and Froguel, 2002).

2.5.1 **Type I Diabetes*:***

This is known as insulin-dependent diabetes mellitus (IDDM), is caused by immunological destruction of pancreatic beta (β) cells resulting in insulin deficiency (Notkins, 2002). This type of diabetes is further classified as auto-immune (immune mediated) diabetes (type 1A) or idiopathic diabetes with β -cell destruction (type 1B), (Alberti and Zimmet 1998). The majority of type 1 diabetes is of the immune-mediated nature, where β cell loss is a T-cell mediated auto-immune attack (Rother, 2007). Its pathogenesis involves environmental triggers that may activate auto-immune mechanisms in genetically susceptible individuals, leading to progressive loss of pancreatic islet β cells (Harrison and Honeyman 1999). A person's immune system attacks the insulin – producing β cells in the pancreas-hence type 1 diabetes is an auto-immune disease and is sometimes called "immune-mediated diabetes". Type 1 diabetes develops in individuals who are genetically susceptible. An exposure to some yet unknown triggering environmental factor or factors may be required. In type 1 diabetes, endogenous insulin is simply not available. Factors that can trigger an immune reaction include viruses, toxic chemicals (alloxan and streptozotocin) and certain drugs (diuretics, anti-hypertensive agents and thiazide). Genetic make – up may also be implicated, for type 1 diabetes; the disease may run in families. It was initially called juvenile onset diabetes because it

affects adolescents and young adults. While this form of diabetes occurs in children and adolescents, it can occur at any age (Anderson *et al.,* 2006). Younger individuals typically have a rapid rate of β cell destruction and present with ketoacidosis, while adults often maintain sufficient insulin secretion to prevent ketoacidosis for many years (Zimmet *et al*., 2001). Type I diabetes is not common, it accounts for less than 10% of all diabetes cases. People in this classification more frequently exhibit the classic symptoms, usually with ketones present in blood and urine. All type 1 diabetic patients require insulin therapy to maintain normoglycemia.

The pathogenesis of type 1 diabetes is summarized in figure 2.1

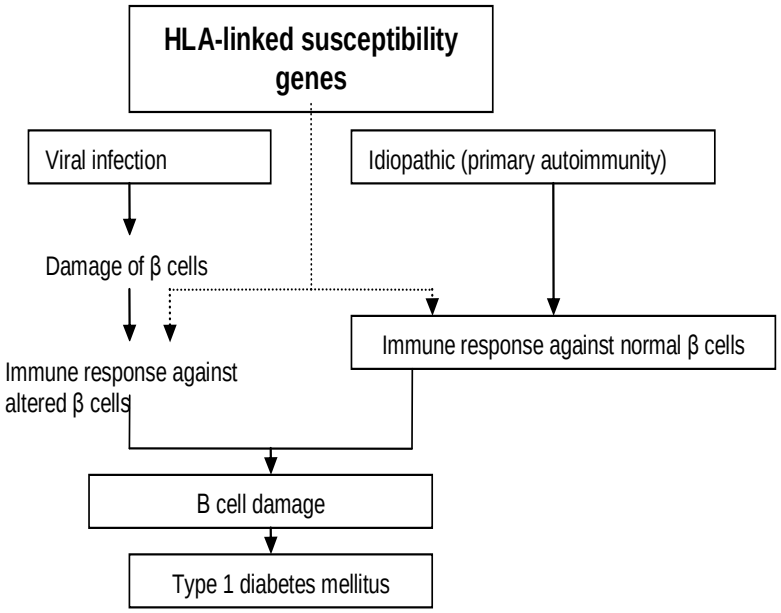


Figure 2.4 Pathogenesis of type 1 diabetes mellitus. (Kumar, *et al.*,1992)

2.5.2 **Type II Diabetes*:***

This type of diabetes develops gradually over a long period of time and is characterized by insufficient insulin or the inability of the body to utilize the insulin present. This is as a result of excessive hepatic glucose production, decreased insulin secretion from pancreatic β cells, and insulin resistance in peripheral tissue such as muscle adipose and liver (Ahmed, 2006; Joe *et al.,* 1999). The body produces excess, normal or slightly reduced insulin. However, the insulin is unable to adequately affect glucose entry into the cells and its subsequent breakdown into energy. In a person with type 2 diabetes the body also cannot properly utilize fats and protein. Because of its slow and gradual occurrence, it is mostly undetected until one or more of its long-term complications appear. During its early stage, insulin resistance is compensated for by hyperinsulinemia, thus preserving normal glucose tolerance. Deterioration into impaired glucose tolerance occurs where either insulin resistance increases or the insulin secretory responses decrease, or both (DeFronzo, 2004). Type II diabetes is increasing rapidly and is becoming a significant global health problem (Suriyaprom *et al.,* 2009). This is the most prevalent type of diabetes found in adults and the elderly. It is more common, accounting for 90–95% of all diabetic cases (Kurihara, 2008). Type II diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, hypertension, and race and/or ethnicity (African American, Hispanic or Native American, Asian American, Pacific Islander). Presently an alarming number of young people, some even in their teens are now being diagnosed with type 2 diabetes. Type 2 diabetes can lead to cardiovascular damage through a number of mechanisms, each of which in turn may accelerate or worsen the others (Ahmed *et al*., 2009). The

genetic causes of the common variety of type II diabetes is not well defined and, at present, no specific genes has been identified in the pathogenesis of this common metabolic disorder (DeFronzo, 1997 and van *et al*., 2001). This increasing trend in type 2 Diabetes Mellitus has become a serious medical concern worldwide that prompts every effort in exploring for new therapeutic agents to stem its progress. The pathogenesis of insulin resistance and type 2 diabetes is summarized in Figure 2.5

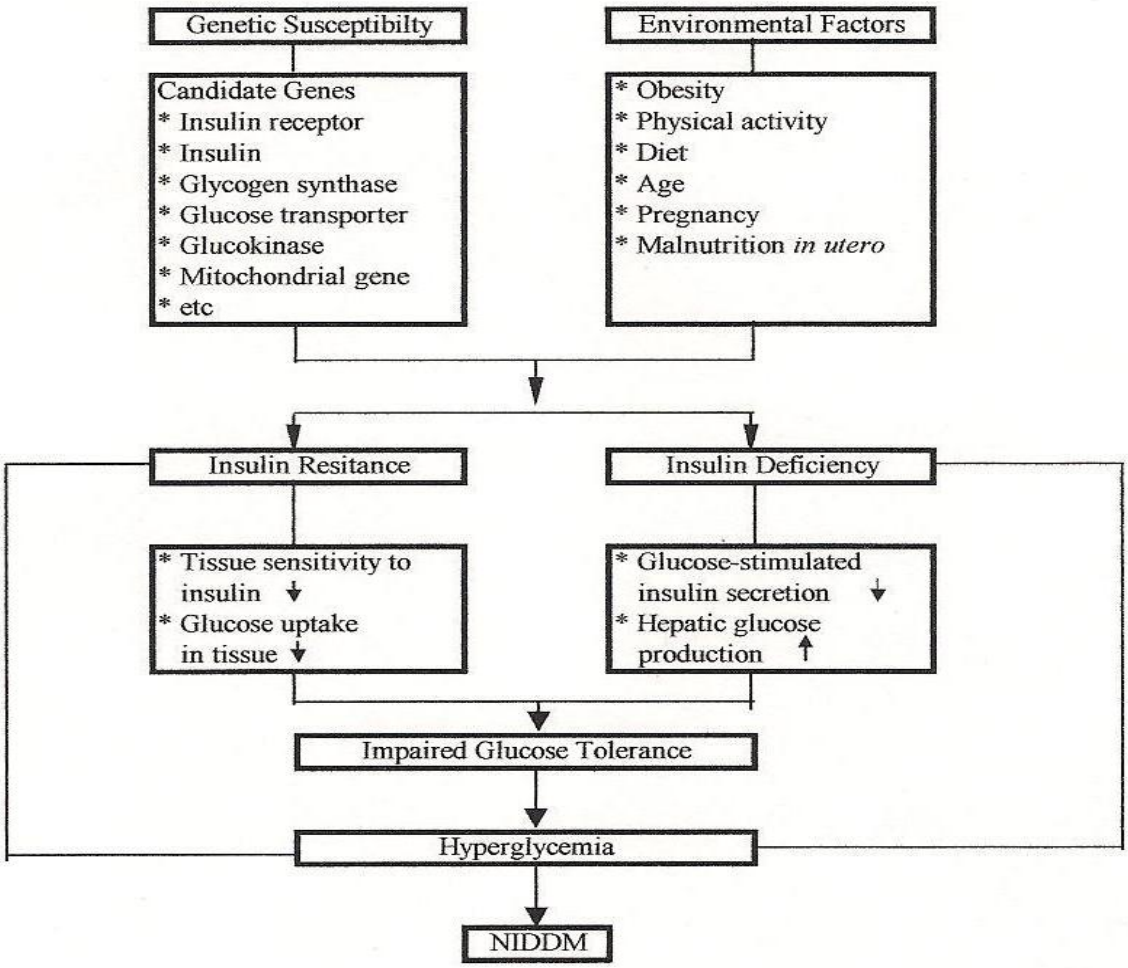


Figure 2. 2: Progressive pathogenesis of type 2 diabetes mellitus (DeFronzo, 2004)

Table 2.1 **Comparism of Type I and Type II Diabetes.**

Feature	Type I	Type II
Onset	Sudden	Gradual
Age of onset	Any age (mostly young)	Mostly in adults
Body habitus	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Concordance in Identical twins	50%	90%
Prevalence	Less prevalent	More prevalent

Source: (Agabegi. and Steven, 2008; Lambert, 2002)

2.5.3 **Gestational Diabetes Mellitus:**

(GDM) is defined as glucose intolerance, which is first recognized during pregnancy. It is the body's inability to effectively process carbohydrates, leading to increased glucose in the blood stream. Insulin resistance during pregnancy stems from a variety of factors, including alterations in growth hormone and cortisol secretion , human placental lactogen secretion (which is produced by the placenta and affects fatty acids and glucose metabolism, promotes lipolysis, and decreases glucose uptake), and insulinase secretion (which is produced by the placenta and facilitates metabolism of insulin). In addition, estrogen and progesterone also contribute to a disruption of the glucose insulin balance. Increased maternal adipose deposition, decreased exercise, and increased caloric intake also contribute to this state of relative glucose intolerance (Gilmartin *et al.,* 2008). It is a condition that can lead to pregnancy complications. In most women who develop GDM, the disorder has its onset in the third trimester of pregnancy. This type of diabetes occurs during pregnancy and disappears after delivery. As pregnancy advances insulin resistance and diabetogenic stress due to placental hormones necessitate compensatory increase in insulin secretion. When this compensation is inadequate gestational diabetes develops. Insulin resistance is one of many physiological changes occurring during pregnancy, and when insulin resistance is accompanied by pancreatic β -cell insufficiency, gestational diabetes may develop. Several lines of evidence suggest that gestational diabetes shares a common etiology with type 2 diabetes and support the hypothesis that gestational diabetes serves as a window to reveal a predisposition to type 2 diabetes. Pregnancy is an environmental stressor that may catalyze the progression to a diabetic state in genetically predisposed women (Robitaille and Grant 2008). Gestational diabetes complicates about

4% of all pregnancies (Engelgau, 1995) and almost half of these patients are prone to developing permanent diabetes later in life. Women diagnosed to have Gestational Diabetes Mellitus are at increased risk of future diabetes predominantly type 2 Diabetes Mellitus as are their children (Dornhorst and Rossi 1998). An estimated 15% to 50% will develop diabetes in the decades following the affected pregnancy. They need much more intensive antenatal care and close monitoring of blood sugar, blood pressure and weight (Jawad, 2006).

2.5.4 **Other specific Types of diabetes:**

These are also known as secondary diabetes. These diabetes are secondary to diseases in other organ-systems of the body. Diseases of the exocrine pancreas (e.g., Pancreatitis, pancreatic reaction, or cystic fibrosis) are less common causes of DM. There are a variety of uncommon and diverse types of diabetes, which are caused by infections, drugs, endocrinopathies; producing insulin counter regulatory hormones excessively (e.g.,Cushing's syndrome, acromegaly) may result in diabetes mellitus (ADA, 2001), pancreatic destruction, and genetic defects of β cell function and action. Genetic defects of the pancreatic ß cell or in insulin action pathways (Raffel *et al* 1997). These unrelated forms of diabetes are included in the "Other Specific Types" and classified separately. Such types of diabetes account for 1 to 5% of all diagnosed cases (NDS 2011).

2.6 **PATHOGENESIS OF DIABETES MELLITUS**

2.6.1. **Genetic factors**

Heredity is a major cause of diabetes. People who belong to family background having history of diabetes are 25% more prone to develop diabetes. If there is a close relative having the disease, then the vulnerability is high. The vulnerability is higher with close relatives and risk decreases as the relations become more and more distant. First degree relatives have a higher risk of developing Type 1 Diabetes than unrelated individuals from the general population (approximately 6% vs. <1%, respectively) (Dorman and Bunker, 2000). Family studies have revealed that first degree relatives of individuals with Type 2 Diabetes are about 3 times more likely to develop the disease than individuals without a positive family history of the disease (Flores, *et al.*, 2003; Hansen, 2003; Gloyn, 2003).

2.6.2. **Obesity**

Obesity is a prime precipitating factor for diabetes mellitus. It is estimated that three quarter of all Type II diabetes patients are obese. It is recognized as one primary independent cause of insulin resistance (Felber and Golay 2002). Insulin resistance in obesity and type II diabetes is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output (Reaven, 1995). These functional defects may result, partially, from impaired insulin signaling in all three target tissues and, in adipocytes, also from down regulation of the major insulin-responsive glucose transporter, GLUT4. The

molecular mechanisms that connect obesity with impaired glucose tolerance appear to become clarified (Abel *et al.,* 2001), making impaired glucose tolerance a modifiable risk factor. In people who are overweight, the sensitivity of the receptor cells to insulin decreases, which leads to glucose retention. Many epidemiologic studies shows that the risk for diabetes, and presumably insulin resistance, rises as body fat content increases from the very lean to the very obese, implying that the amount of body fat has an effect on insulin sensitivity across a broad range (Colditz *et al.*, 1990). Although this relationship is seen with measures of adiposity such as body mass index (BMI), which reflect general adiposity, it is critical to realize that all sites of adiposity are not equal in this regard. Intra-abdominal deposits of fat are much more strongly linked to insulin resistance, type II diabetes, and cardiovascular disease than are peripheral fat deposits (Kissebah and Krakower 1994). Obesity has been found to contribute to approximately 55% of cases of type II diabetes (Eberhart *et al.,* 2004). Chronic obesity leads to increased insulin resistance that can develop into type 2 diabetes, this is likely because adipose tissue (especially that in the abdomen around internal organs) is a source of several chemical signals, hormones and cytokines, to other tissues (Shoelson *et al*., 2006).

2.6.3. **Auto-immune disease**

The auto-immune process results in the circulation of antibodies that may either cause or be caused by ß-cell death. The body's response to the Type I diabetes is easier to control with treatment. The outcome is a lack of available insulin. While the onset is said to be sudden. Changes resulting in decreased insulin availability may have occurred over a

longer period of time. Insulin-dependent diabetes mellitus is an inherited defect of the body's immune system, resulting in destruction of the insulin-producing ß cells of the pancreas (Notkins, 2002).

2.6.4. **Drugs/Medications**

Certain drugs like; Glucocorticoids, Pentamidine, Niacin, and a-interferon may lead to diabetes mellitus (Pandit *et al.,* 1993). Thyroid hormone, β-adrenergic agonists also steroids, Dilantin, and others may elevate the blood sugar through a variety of mechanisms. Other drugs, such as alloxan, streptozotocin, and thiazide diuretics, are toxic to the ß cells of the pancreas and can cause diabetes.

2.6.5**. Viral Infection**

There are some suggestions that viruses can be a critical factor in some people and initiates diabetes by destroying the ß-cells of the pancreas (Weiss and Sumpio, 2006). A number of viruses have been associated with type 1 diabetes in humans, including infections such as enterovirus, rubella (McIntosh and Menser, 1992), mumps (Hyoty *et al*., 1993), rotavirus (Honeyman *et al.,* 2000), cytomegalovirus (CMV) (Pak *et al.,* 1988) and Coxsackie virus B.

2.6.6. **Lifestyle**

There are studies that have shown that lack of adequate daily physical activity increases the chances by 8% for a person to suffer from type II diabetes at some stage of life (Gorman *et a*l., 2004). The prevalence of type II diabetes is tightly associated with the

global adoption of a western lifestyle involving changes both in dietary habits and physical activity (Zimmet *et al.,* 2001). A number of lifestyle factors are known to be important to the development of diabetes mellitus type II including: obesity, physical activity, diet, stress, and urbanization. Excess body fat underlies 64% of cases of diabetes in men and 77% of cases in women (Peter *et al.,* 2005). A number of dietary factors such as sugar sweetened drinks (Malik *et al.,* 2010) and the type of fat in the diet appear to play a role. (Risérus *et al.,* 2009).

2.6.7. **Stress**

It has been suggested that emotional stress plays a role in the etiology of type II diabetes mellitus. A considerable number of depressed patients suffer from high levels of diabetes-specific emotional stress (Pouwer *et al*., 2005; Kokoszka *et al.,* 2009). Emotional stress was found to be associated with unhealthy lifestyle behaviors, i.e., inadequate eating behaviors in terms of quality and quantity of food, low exercise levels, smoking and alcohol abuse (Bonnet *et al.,* 2005; Rodbard *et al*., 2009). All these factors are well-known risk factors for the development of type II diabetes Chronic stress reactions and depression are often characterized by long term activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system which were found to be associated with the development of abdominal obesity, and this may explain why depression or chronic stress increases the risk of diabetes (Björntorp, 2001; Vogelzangs *et al.,* 2008). Stress, both physical and mental can send one's blood sugar out of control (Guzik *et al.,* 2002).

2.6.8. **Age**

Important factors contributing to the increasing prevalence of type 2 diabetes are obesity, physical inactivity, and an increase in the number of individuals older than 65 years (Wild *et al.,* 2004). Abrupt Symptoms such as weight loss and ketoacidosis usually start in childhood or adolescence in type I diabetes (DiMeglio *et al*., 2003) while in type II diabetes, is mostly discovered in adulthood, but an increasing number of children are being diagnosed with the disease (Kota *et al.,* 2012).

2.6.9. **Diet**

Diet is also a major factor responsible for causing diabetes**.** Sugar sweetened drinks (Malik *et al* 2010) and the type of fat in the diet play a role (Risérus *et al* 2009). The composition of dietary fat intake is linked to diabetes risk; decreasing consumption of saturated fats and trans- fatty acids while replacing them with unsaturated fats may decrease the risk (Riserus *et al* 2009, Mozaffarian *et al.,* 2009). Excess body fat underlies 64% of cases of diabetes in men and 77% of cases in women (Peter *et al.,* 2005). Sugar sweetened drinks appear to increase the risk of type II diabetes both through their role in obesity and potentially through a direct effect (Salmerón *et al.,* 2001).

2.6.10. **Environmental Factors**

A conclusion has been made that environmental factors are vital to the pathogenesis of diabetes mellitus (Gorman *et al.,* 2004). Cold climate may have a link with the onset of diabetes. Type 1 diabetes is higher in places with cold wheather, rather than areas that are

closer to the equator. It is remarkable that type 1 diabetes is diagnosed more often in winter time (Gorman *et al.*, 2004).

2.7 COMPLICATIONS OF DIABETES MELLITUS

Uncontrolled hyperglycemia in both type 1 and type II diabetes lead to the development of both acute and long term complications (Weiss and Sumpio, 2006).

2.7.1 **Short-term (acute) Complications**

Acute complications of diabetes mellitus include ketoacidocis in type I and nonketotic hyperosmolar coma in type II, hyperglycemia and hypoglycemia (WHO, 1999).

Ketoacidosis

Diabetic Ketoacidosis (DKA) develops when absolute insulin deficiency and excess contra-insulin hormones increase hepatic glucose production, decrease peripheral glucose utilization, and stimulate release of fatty acids from fat cells and production of ketones by the liver. These changes cause hyperglycemia, osmotic diuresis, volume depletion, and acidosis. An elevated level of ketone bodies in the blood reduces the blood's pH which results to Ketoacidosis (Laakso 1999). The annual incidence of DKA ranges from three to eight episodes per 1,000 persons with diabetes. It is much more common among persons with insulin-dependent diabetes mellitus (IDDM).

Hyperosmolar nonketotic coma

Hyperglycemic hyperosmolar nonketotic coma (HHNKC) is characterized by severe hyperglycemia, dehydration, and altered mental status -- in the absence of ketosis. In HHNKC, hyperglycemia causes glycosuria. Osmotic diuresis results in volume contraction and a reduction in both the glomerular filtration rate and glucose excretion. Worsening hyperglycemia causes further extracellular hypertonicity and intracellular dehydration. Central nervous system dysfunction in persons with HHNKC is probably due to hyperosmolarity. Most persons with HHNKC have a history of non insulin dependent diabetes. Persons, who are chronically ill, debilitated, have mild renal insufficiency and lack normal thirst mechanisms or access to water, are at risk of developing HHNKC (Maritim *et al.,* 2003).

Hypoglycemia

Severe hypoglycemia occurs more often in patients who are following an intensified insulin therapy protocol (with the target glucose level near the normal range), whose diet and activity changes widely, who have a long duration of diabetes, and/or who have autonomic neuropathy. Patients with a history of severe hypoglycemia are at increased risk for future episodes having symptoms of sympathetic activation of the autonomic nervous system (Gorman *et al.,* 2004). Often the cause is multifactorial, use of insulin, medication of oral hypoglycemic agents, a delay or decrease in food intake, vigorous physical activity may be the cause.

2.7.2 **Long-term (chronic) Complications**

Long term complications include cardiovascular diseases, hypertension, chronic renal failure (Nephropathy), retinal damage (Retinopathy), nerve damage (neuropathy), erectile dysfunction and macrovascular damage which may cause poor healing of wounds particularly of the feet and can lead to gangrene which may require amputation (WHO, 1999).

Diabetic Nephropathy

The principal indicator of diabetic nephropathy is proteinuria (Nathan, 1993). Diabetic Nephropathy is a microvascular complications and it is the commonest cause of end-stage renal failure. Diabetic nephropathy occurs when proteins deposit in the glomerulus (Clark and Lee, 1995). Thickening of the glomerular capillary basement membrane narrows the lumen of the capillaries impeding blood flow and subsequently reducing the filtering surface of the glomerulus (Koda-Kimble and Carlisle, 1995). Diabetes is the leading cause of kidney failure, accounting for 44 % of all new cases of kidney failure in 2008 (NDS, 2011). Diabetic nephropathy follows an outline of clinical course, starting with microalbuminuria through proteinuria, azotaemia and culminating in end-stage renal failure. Before the onset of overt proteinuria, there are various renal functional changes including renal hyperfiltration, hyperperfusion, and increasing capillary permeability to macromolecules. Basement-membrane thickening and mesangial expansion have been recognized as pathological hallmark of diabetes. It has been postulated that DN occurs as a result of the interplay of metabolic and hemodynamic factors in the renal microcirculation. The accumulation of advanced glycosylated end-products, the activation of isoform(s) of protein kinase C and the acceleration of the aldose reductase

pathway may explain how hyperglycemia damages tissue. Endothelial dysfunction is closely associated with the development of diabetic nephropathy, both in IDDM and in NIDDM (Raptis and Viberti 2001).

Diabetes Neuropathy

The development of neuropathy in affected patients has been shown in animal and *in vitro* models to be a result of hyperglycemia-induced metabolic abnormalities (Zochodone, 2008; Feldman *et al.,* 1999; Simmons and Feldman, 2002). One of the more commonly described mechanisms of action is the polyol pathway (Feldman *et al.,* 1999). In the development of neuropathy, the hyperglycemic state leads to an increase in action of the enzymes aldose reductase and sorbitol dehydrogenase. This results in the conversion of intracellular glucose to sorbitol and fructose. The accumulation of these sugar products results in a decrease in the synthesis of nerve cell myoinositol, required for normal neuron conduction (ADA, 2009). Diabetic neuropathy affects all parts of the nervous system but peripheral nerves are most commonly affected. It involves temporary or permanent damage to nerve tissue. Nerve tissue gets injured mainly due to decreased blood flow and rise in blood glucose levels. Approximately 50% of patients, suffering from diabetes from last 10-20 years, develop diabetic neuropathy. Nerve injury normally develops in stages. In earlier stages, tingling sensation or intermittent pain is noted particularly in the extremities such as feet. But in later stage, the pain is continuous and severe. At last, a painless neuropathy develops, which leads to loss of pain sensation in an area. This increases the chance of severe tissue injury because pain does not alerts the patients to injury. Therefore, the common symptoms of diabetic neuropathy are

Numbness (Holman and Turner, 1991), tingling, decreased sensation to a body part, diarrhoea, constipation, loss of bladder control, impotence, facial drooping, drooping eyelid, drooping mouth, vision changes, weakness, speech impairment etc. These symptoms usually develop gradually over years. People with diabetes are, in general, up to 30 times more likely to require a lower limb amputation compared with the general population (Calle-Pascual *et al*., 1997).

Atherosclerosis

Atherosclerotic complications include coronary artery disease (CAD), cerebrovascular disease (CVD), and peripheral arterial disease (PAD). Diabetes increases the risk of the development of CVD 2–4 times (Kanaya *et al.,* 2002).They account for more than 70% of deaths in persons with diabetes. Etiology of atherosclerosis is multifactorial and includes: Quantitative lipid abnormalities-primarily increases in VLDL-cholesterol (triglycerides) and decreases in HDL-cholesterol Qualitative lipid abnormalities-Increases in small, dense LDL-C as well as in glycated and oxidized LDL-C, Increased insulin resistance/hyperinsulinemia, Increased procoagulant and decreased anticoagulant factors, Hyperglycemia and other risk factors example is hypertension, abdominal obesity (Lawrence and Pamela, 2009)**.**

Retinopathy

Retinopathy is a major long-term complication of diabetes. Chronic hyperglycemia is thought to be the primary cause of diabetic retinopathy (NDFS, 2006). Diabetic retinopathy is a leading cause of blindness among working-age adult populations (Fong *et*

al., 2004). The incidence of blindness, for example, is 25 times higher in patients with diabetes than in the general population. The pathogenesis of DR is multifactorial but is primarily caused by the metabolic effects of chronic hyperglycemia, resulting in vascular changes and subsequent retinal injury and ischemia. Diabetic retinopathy is the most common cause of blindness in middle-aged subjects, accounting for at least 12,000 new cases in the United States each year (Frank , 2004). It affects about one in four people with diabetes. The development of retinopathy is strongly related to the length of time diabetes has been present and the degree of blood glucose control (Frank, 2004). There are diseased small blood vessels in the back of the eye which causes the leakage of protein and blood in the retina. Retinopathy-diabetics are also prone to cataract and Glaucoma.

2.8 **MECHANISM OF INSULIN RELEASE**

Insulin secretion by the ß-cells is in two phases; the first phase (fast requiring ATP and Ca^{2+}) and the second phase (slow requiring ATP) (Rorsman and Renstrom, 2003). It consist of an initial rapid phase of insulin secretion from the secretory granules in the β cell as a result of rising levels of plasma glucose concentrations, followed by a less intense but more sustained release of insulin (Bratanova-Tochkova *et al.*, 2002). Glucose entry into the β cell is sensed by glucokinase, which phosphorylates glucose to glucose-6-phosphate (G6P), generating ATP (De Lonlay and Saudubray 2000). Closure of K^+-ATP-dependent channels results in membrane depolarization and activation of voltage dependent calcium channels leading to an increase in intracellular calcium concentration; this triggers pulsatile insulin secretion (Soria *et al.*, 2004). Other mediators of insulin

release include activation of phospholipases and protein kinase C (e.g. by acetycholine) and by stimulation of adenylyl cyclase activity and activation of β cell protein kinase A, which potentiates insulin secretion. This latter mechanism may be activated by hormones, such as vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP), glucagon like polypeptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP). These factors appear to play a significant role in the second phase of glucose mediated insulin secretion, after refilling of secretory granules translocated from reserve pools (Bratanova-Tochkova *et al.,* 2002).

2.9 MECHANISM OF INSULIN ACTION

Insulin is an anabolic hormone, causing cells to store energy substrates at times of excess. Insulin triggers the uptake of glucose, fatty acids and amino acids into liver, adipose tissue and muscle and promotes the storage of these nutrients in the form of glycogen, lipids and protein respectively. Insulin's action is countered by the catabolic hormones glucagon, adrenalin, noradrenalin and growth hormone. The receptor for insulin is embedded in the plasma membrane and is composed of two α subunits and two β subunits linked by disulfide bonds (Kido *et al.,* 2001). The extracellular alpha chains have the insulin binding domains, while the beta chains penetrate through the plasma membrane. It functions as an enzyme that transfers phosphate groups from ATP to tyrosine residues on intracellular target proteins including insulin receptor substrates (IRSs) such as IRS-1 and -2, phosphatidylinositol (PI) 3-kinase, and protein kinase B (PKB) (Saltiel and Khan, 2001; Bader *et al*., 2004). These residues are recognised by phosphotyrosine-binding (PTB) domains of adaptor proteins such as members of the

insulin receptor substrate family (IRS) (Saltiel and Kahn 2001; Lizcano and Alessi 2002). Insulin is initiated with insulin binding to the alpha-subunit of the insulin receptor (IR) and activation of the intrinsic tyrosine kinase activity of the beta-subunit of the receptor (Saltiel and Kahn, 2001). Binding of insulin to the alpha subunits causes the beta subunits to phosphorylate themselves, thus activating the catalytic activity of the receptor.

2.10 OXIDATIVE STRESS AND DIABETES COMPLICATIONS

Oxidative stress which is associated with diabetes mellitus might play an important role in the initiation and progression of diabetic complications. Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications (Moussa, 2008). It is rather the detrimental effect of glucose toxicity due to chronic hyperglycaemia, which is mediated and complicated through oxidative stress (Tiwari and Rao, 2002). During diabetes, persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation. The increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by nonenzymic and enzymic catalase (CAT), reduced glutathione (GSH), and Superoxide dismutase (SOD) antioxidants, whose level critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes (Boguslaw, 2001). The pancreas has a relatively weak intrinsic defense system against oxidative stress (Tiedge *et al.,* 1997). Patients with diabetes have decreased antioxidant defenses with lower levels of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase (Ramakrishna and Jailkhani. 2008). Hyperglycaemia in diabetes

mellitus is responsible for the development of oxidative stress via glucose auto-oxidation and protein glycation (Pettevski *et al.,* 2003).

2.11 **MANAGEMENT OF DIABETES MELLITUS**

Treatment is predominantly directed at Microvascular and macrovascular complications (Roman and Harris 1997).

2.11.1 **Medication**

Currently, six classes of oral antidiabetic drugs are available: Biguanides, Sulfonylureas, Meglitinides, Thiazolidenediones, Dipeptidyl peptidase IV inhibitors, and α-glucosidase inhibitors (Nathan, 2007). These oral hypoglycaemic agents exert their glucose lowering effects through a variety of mechanisms.

Sulfonylureas

Sulfonylureas, including first generation (tolbutamide) and second generation (glyburide) sulfonylureas, enhance insulin secretion from the pancreatic ß-cells. Currently, all available sulfonylureas bind to specific receptors on β-cells, resulting in closure of potassium ATP channels. As a result, calcium channels open, leading to an increase in cytoplastic calcium that stimulates insulin release (Pilipson and Steiner 1995). A significant side effect is hypoglycaemia. Sulfonylurea therapy is also usually associated with weight gain due to hyperinsulinemia which results from anabolic effect of increased insulin concentration (Bailey and Day 2003).

Biguanides

Biguanides include the drug metformin, which was originally derived from a medicinal plant, *Galega officinalis*. This drug acts by causing the translocation of glucose transporters from the microsomal fraction to the plasma membrane of hepatic and muscle cells. It does not stimulate insulin release and does not, when given alone, cause hypoglycemia (Mahler, 1995). Metformin reduces plasma glucose via inhibition of hepatic glucose production and increase of muscle glucose uptake by decreasing peripheral insulin resistance. It also reduces plasma triglyceride and LDL-cholesterol levels. Side effects include weakness, fatigue, and shortness of breath, nausea, dizziness, lactic acidosis, and kidney toxicity (Matsuur *et al*, 2002).

Alpha-glucosidase Inhibitors

Alpha-glucosidase inhibitors include the drug acarbose and miglitol. Acarbose and miglitol competitively inhibit digestion of oligosaccharides to monosaccharides, so that glucose is slowly absorbed throughout the length of the small intestine, rather than rapidly in the proximal jejunum thereby delaying postprandial glucose absorption (van de Laar, 2008). The advantages includes; They specifically lower postprandial plasma glucose levels, their action is independent and additive to all other forms of pharmacologic therapy, they do not cause weight loss or gain, they are relatively nontoxic. (Lebovitz, 1998; Raptis and Dimitriadis 2001).This drug category decreases postprandial glucose levels by interfering with carbohydrate digestion and delaying gastrointestinal absorption of glucose. The major side effects of alpha-glucosidase inhibitors is their gastrointestinal side effects, which include abdominal discomfort,

flatulence, and, occasionally, diarrhea. (Chiasson *et al.*, 1994; Holman *et al.*, 1999). These side effects can be minimized by starting with very small doses with the evening meal and titrating upward very slowly.

Thiazolidenediones

Thiazolidenediones are represented by troglitazone, rosiglitazone and pioglitazone. These oral agents work by improving insulin sensitivity in muscle and, to a much lesser extent, in the liver. Thiazolidenediones are insulin-sensitizing drugs that improve whole-body insulin sensitivity through gene regulation (Krentz and Bailey 2005). These agents increase glucose uptake via glucose transporter-4 in skeletal muscle and reduce rates of gluconeogenesis in the liver. Reductions in plasma insulin concentration and lowering of circulating triglycerides are additional indirect mechanisms that may help improve whole-body insulin sensitivity. Thiazolidenediones have also been known to improve β-cell function and reduce insulin resistance; however, they are associated with weight gain and can cause peripheral edema (Del *et al* 2007). These drugs decrease plasma triglyceride levels, but such decrease may be associated with weight gain and an increase in LDL-cholesterol levels. Liver toxicity is a concern requiring monthly monitoring of liver function (Eurich *et al.*, 2007). Since troglitazone is more toxic to the liver than rosiglitazone and pioglitazone (having resulted in dozens of deaths from liver failure), in March 2000 the FDA asked the manufacturer of troglitazone to remove the product from the market.

Meglitinides

Meglitinides (Repaglinide) augment insulin secretion, its mechanism of action is that it binds to a different site on the KATP channel, and its binding is slightly slower and more prolonged than that of nateglinide (Guay, 1998; Damsbo *et al*., 1999). It greatly reduces postprandial hyperglycemia, but because its effects are more prolonged, it also greatly reduces fasting hyperglycemia (Jovanovic *et al*, 2000). But weight gain, gastrointestinal disturbances, and hypoglycemia are possible side effects (Eurich *et al.,* 2007).

Dipeptidyl Peptidase-IV Inhibitors

Dipeptidyl peptidase-IV (DPP-IV) inhibitors suppress the breakdown of a variety of bioactive peptides, including glucagon-like peptide-1, leading to an enhancement of their action (Hinnen *et al.*, 2006). DPP-IV inhibitors produce a significant effect on glucose tolerance and lasting improvement of glycosylated heamoglobin. Several agents are in different stages of clinical development. Sitagliptin was approved by the FDA in 2006 as an adjunct to diet and exercise in patients with Type 2 diabetes mellitus. A combination of sitagliptin and metformin was approved in 2007 and is also indicated as an adjunct to diet and exercise to improve glycemic control in adults with Type 2 diabetes mellitus, when treatment with both sitagliptin and metformin is appropriate. DPP-IV inhibitors are weight-neutral and well tolerated (Vilsboll and Knop 2009).

Insulin Therapy

All type 1 diabetic patients use exogenous insulin, as do many with type 2 diabetes. Insulin is taken via subcutaneous injection, most often with a syringe. There are a variety

of insulin preparations available; they vary in their onset, peak, and duration of activity and are classified as long-, intermediate-, short-, or rapid-acting (Bolli, 2001). Although beef and pork insulin species are still available, most individuals use human insulin preparations today. As ß cell function deteriorates during the course of type 2 diabetes, insulin secretogogues eventually become ineffective (Yki-Jarvinen *et al*., 2001). At this stage, insulin is used as adjunctive therapy inorder to achieve glycemic control. Weight gain and hypoglycemia are common side effects of insulin therapy. Vigorous insulin treatment may also carry an increased risk of atherogenesis.

Herbal Drugs

Ethno pharmacological surveys indicate that more than 1200 plants are used in traditional medical systems for their alleged hypoglycemic activity (Marles and Farnsworth, 1995; Dey *et al*., 2002; Grover *et al*., 2002). The hypoglycemic activity of a large number of these plants/plant products has been evaluated and confirmed in animal models (Gupta *et al*., 2005; Kesari *et al*., 2006) as well as in human beings (Jaouhari *et al*., 1999; Herrera-Arellano *et al*., 2004; Jayawardena *et al*., 2005). Numerous medicinal plants have been used for the management of diabetes mellitus in various traditional systems of medicine worldwide as they are a great source of biological constituents and many of them are known to be effective against diabetes. Medicinal plants with antihyperglycemia activities are being more desired, owing to lesser side-effects and low cost. (Khan *et al*., 2012). The reported mechanisms whereby herbal antidiabetic remedies reduce blood glucose levels are more or less similar to those of the synthetic oral hypoglycemic drugs and are classified into four categories according to their mode of action (Tanira, 1994;

Bastaki, 2005; Bnouham *et al*., 2006). Stimulation of insulin synthesis and/or secretion from pancreatic beta-cells, regeneration of damaged pancreatic ß cells, improvement of insulin sensitivity by improving glucose uptake by fat and muscle cells, acting like insulin, changing of the activity of some enzymes that are involved in glucose metabolism, reducing the rate of absorption of carbohydrates from the gut.

Physical activity

When moderate to mild exercise is performed post-prandially by patients with Type II Diabetes, glucose levels are reduced as well (Larsen *et al.,* 1997). Physical activity increases glucose uptake by muscle through an insulin-independent pathway (Goodyear and Kahn 1998). Exercise can increase glucose uptake by muscle up to two hours post-exercise, but insulin sensitivity may be increased by up to 16 hours for healthy and type 2 diabetes subjects (Borghouts and Keizer 2000). The mechanism of the beneficial effect of exercise on insulin sensitivity is, at least in part, mediated by an increase in the number of glucose transporters in skeletal muscle. How this translates into blood glucose levels depends on several factors, such as food consumption prior to exercise, exercise duration, exercise intensity, and whether Impaired Glucose Tolerance is present. Decreased intra-abdominal fat, an increase in insulin-sensitive glucose transporters (GLUT-4) in muscle, enhanced blood flow to insulin-sensitive tissues, and reduced free fatty acid levels appear to be the mechanisms by which exercise restores insulin sensitivity (Erisonn *et al*., 1997). Exercising five or six times per week enhances weight reduction (Boule *et al.,* 2001). People with diabetes who undertake regular physical activity have been shown to have substantially lower mortality rates over 12–14 years (Wei *et al.*, 2000; Hu *et al.*, 2001).

Diet therapy

Dietary modification and exercise can lower the risk of progression from impaired glucose tolerance to type II diabetes; and may also prevent the development of Impaired Glucose Tolerance in non-diabetic individuals at high risk. (Alberti, 1996). No single dietary approach is appropriate for all patients. Meal plans and diet modifications are generally individualized by a registered dietitian to meet patient needs and lifestyle. A typical conventional approach would recommend a diet composed of 60-65 % carbohydrate, 25-35 % fat, and 10-20 % protein, with limited or no alcohol consumption (Schlichtmann and Graber 1997).

Lifestyle

There are roles for patient education, dietetic support, sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure (Adler *et al.*, 2000).

2.12 **DEVELOPMENTS IN DISCOVERY OF ANTI-DIABETIC AGENTS FROM PLANTS.**

Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in developing countries, where many people do not have access to conventional antidiabetic therapies (Grover, *et al*., 2002; Katerere and Elof, 2005; Balde *et al.*, 2006; Acharya and Shrivastava, 2008). The hypoglycemic activity of a

large number of these plants/plant products has been evaluated and confirmed in animal models (Gupta *et al.,* 2005; Kesari *et al.,* 2006) as well as in human beings (Jaouhari *et al.,* 1999; Herrera-Arellano *et al*., 2004; Jayawardena *et al*., 2005). Some of the most studied and commonly used medicinal plants whose blood glucose lowering effects have been tested and confirmed in different parts of the world include: *Allium cepa, Allium sativum, Aloe vera, Cinnamomum tamala, Coccinia indica, Gymnema sylvestre , Momordica charantia , Murrayi koningii, Ocimum sanctum, Panax, Ginseng, Trigonella foenum-graecum , Pterocarpus marsupium* and *Syzigium cumini (*Bailey and Day, 1989; Grover *et al*., 2002; Bnouham *et al*., 2006).

Reports by Chakravart and Kalita (2011) showed in a review on 16 plants belonging to 12 families which show the efficiency of these medicinal plants in management of Diabetes mellitus; some of these plants are:

Crateva nurvula stem bark: Investigation was done to detect the antidiabetic activity of *C. nurvula* stem bark (family: *Capparidaceae*) extracts in alloxan-induced diabetic albino rats. A comparison was made between the actions of different extracts of C. *nurvula*. Petroleum ether extract and ethanolic extract showed significant antidiabetic activities (Sikarwar and Patil., 2010).

Annona squamosa leaf extract: An investigation was made to reveal the involvement of quercetin in the antidiabetic and antiperoxidative effects of *Annona squamosa* leaf extract (Panda and Kar, 2007).

Trichosanthes dioica (*Cucurbitaceae*): An investigation was made in rats with streptozotocin induced severe diabetes mellitus, aqueous extract of *T. dioica* fruits at a dose of 1000mg/kg body weight daily once for 28 days reduced the levels of fasting

blood glucose, postprandial glucose, aspartate amino transferase, alanine amino transferase, alkaline phosphatase (Rai *et al.,* 2008).

Barleria prionitis (*Acanthaceae):* Alcoholic extract of leaf and root of *Barleria prionitis* was tested for their antidiabetic activity (Dheer and Bhatnagar 2010). The study reveals that the alcoholic leaf extract of *B. prionitis* could be added in the list of herbal preparations beneficial in diabetes mellitus.

Capparis sepiaria (*Capparidaceae*): *Capparis sepiaria* ethanolic leaves extracts showed anti-hyperglycemic effect, hence the leaves may be prescribed as an adjunct to traditional formulation and drug treatment for controlling diabetes mellitus (Selvamani *et al*., 2008).

Cassia auriculata(*Caesalpiniaceae*) : the investigation describes the potent antidiabetic fraction from flowers of *Cassia auriculata* Linn (Surana *et al*., 2008).

Helicteres isora (*Sterculiaceae*): The investigations evaluated the antioxidant and antidiabetic activity of *Helicteres isora* (L.) fruits belonging to the family *Sterculiaceae* (Suthar *et al*., 2009).

Hemidesmus indicus (*Apocynaceae):* The Study was conducted to evaluate the antidiabetic activity of an aqueous extract of the roots of *Hemidesmus indicus*. *H. indicus* could be used as an antidiabetic and antioxidant agent for the prevention and treatment of diabetes mellitus (Mahalingam and Krishnan, 2008).

Argyreia speciosa (*Convulaceae)*: This plant is used traditionally in India as a hypoglycemic plant (Galani *et al.,* 2010).

Holostemma ada Kodien (*Asclepiadaceae*): Evaluation was done to study the antidiabetic activity of ethanolic extract of *Holostemma ada Kodien* Schults in normal,

glucose fed, alloxan-induced diabetic rats. The results justified the traditional use in the treatment of diabetes (Janapati, 2009).

Salacia fruticosa (*Celastraceae*): The Study was conducted to evaluate the anti hyperglycemic activity of methanolic extract of leaves of *Salacia fruticosa* in alloxan induced diabetic rats. Treatment with extract produced a significant dose dependent reduction in blood glucose level (Venkateshwarlul *et al.,* 2009).

Rosa canina (*Rosaceae*): The study was done to investigate the anti diabetic property of *Rosa canina*. The findings support the traditional usage of *R. canin*a fruits as a folk remedy in the treatment of diabetes in Turkey (Orhan *et al.,* 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 **MATERIALS**

Jenway 6405 UV/Vis Spectrophotometer, Sherwood Colorimeter 257, Grant JB Series Water Bath, Heraeus Labofuge 300 Centrifuge, Gallenkamp Incubator, Electronic weighing machine, dissecting set, curvettes, plain tubes, pipettes, water bath, dissect table and mortar and pestle and microheamatocrit reader, were used.

3.1.1 **Chemicals**

Assay kits for glucose, total cholesterol, triacylglycerols, high-density lipoprotein, AST, ALT, ALP, Total protein, Urea, Albumin, SOD, CAT and TBARS were bought from Randox laboratories Ltd. Ardmore, Co. Antrm UK. and reagents including Streptozotocin (STZ) were purchased from Sigma-Andrich Chemical Company St. Louis U.S.A. and all other chemicals used were of analytical grade.

3.1.2 **Plant materials**

Leaves of Pawpaw (*Carica papaya*) Cashew (*Anacardium occidentale*) and Guava (*Psidium guajava*) were obtained from Zaria metropolis in the month of January 2012. The leaves were identified and authenticated at the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria, where voucher specimen numbers (571, 184, and 3253) were deposited.

3.1.3 **Animals**

Eighty- seven male albino wistar rats of body weights between 150-200g were obtained from the Animal house, Department of Pharmacology and Therapeutics, A. B. U., Zaria. The animals were housed in well-ventilated cages and fed with commercial laboratory diet and water *ad libitum.*

3.2 **METHODS**

3.2.1 **Methanolic Extracts Preparation**

The plant materials were shade-dried and ground to powder. Extraction was performed at room temperature. Five hundred grams (500 g) of the dried and ground plant materials were soaked in 2 Litres of 70% methanol for about 72 hours stirring intermittently. The extracts were filtered and the filtrates were concentrated at 40^0C and kept in a dessicator for further use.

3.2.2 **Acute Toxicity Study**

This was carried out using the method described by Lorke (1983). In the initial phase, rats were divided into 3 groups of 3 rats each and were treated with 10mg, 100mg and 1000mg of the extract per kg body weight orally. They were observed for 24 hours for signs of toxicity, including death. In the final phase, 4 rats were divided into 4 groups of one rat each, and were treated with the extract based on the findings in the first phase. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

3.3 **PHYTOCHEMICAL ESTIMATIONS OF THE PLANTS**

3.3.1 **Determination of total polyphenolic contents.**

This was carried out spectrophotometrically according to the method described by Edeoga *et al.* (2005).

Procedure: Ten grams of each plant sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2h. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolics components for 15 min. Then 5 ml of the extract was pipetted into a 50 ml flask, 10 ml of distilled water, 2 ml of 5% ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added. The solution was made up to mark and left to react for 30 min for colour development. The mixture was then measured at 505 nm spectrophotometrically. This was carried out in triplicates.

3.3.2 **Determination of total flavonoids**

This was carried out using the methods developed by Bohm and Kocipaiabyazan (1994).

Procedure: Ten grams of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No. 42 (125 mm) filter paper. The filterate was later transferred into crucible and evaporated to dryness over a water bath and weighed to a constant weight. This test was carried out in triplicates.

3.3.3 **Determination of alkaloids**

This was carried out using the procedure described by Harbone (1973) as modified by Edeoga *et al.* (2005).

Procedure: Five grams of the powdered samples were weighed into 250 ml beaker. 100 ml of 10% acetic acid in ethanol was then added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the extract concentrated on a water bath to ¼ of the original volume. Thereafter, concentrated ammonium hydroxide was added drop wise until precipitation was completed. The solution was then allowed to settle and the precipitate collected, washed with diluted ammonium hydroxide and filtered. This was carried out in triplicates.

3.3.4 **Determination of saponin.**

This was carried out using the method described by Obadoni and Ochuko (2001).

Produre: Ten grams of the powdered sample for each plant extract was transferred into a conical flask, and 50 ml of 20% aqueous ethanol was added. This was heated over a hot water bath for 4h while stirring continuously at 50^{o}C.Thereafter, the mixture was filtered and residue re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90^{o}C. Then the concentrate was transferred into a 250 ml seperatory funnel. Diethyl ether (10 ml) was added to the funnel and the mixture shaken. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. There after, 30 ml of n-butanol was added. The combined n-butanol extract was washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was then heated on a water bath. After

evaporation, the samples were dried in a hot air oven at 70^{o}C to a constant weight. This was carried out in triplicates.

3.3.5 **Determination of tannin.**

This was determined by the method of Van-Burden and Robinson (1981).

Procedure: Exactly 500mg of the samples was weighed into a 50 ml plastic bottle. Distilled water (50 ml) was added and shaken for 1h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. Then 5 ml of the filterate was pipetted out into a test tube and mixed with 2 ml of 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance of the solution was measured at 420 nm within 10 min. This was carried out in triplicates.

IN VIVO STUDIES

3.4 **EXPERIMENTAL DESIGN**

Thirty-five rats were randomized into seven (7) groups, with five animals per group and treated for a period of 28 days.

Group I: Normal control fed with only normal commercial diet and water *ad libitum.*

Group II: Negative control induced with diabetes but not given any treatment.

Group III: Rats induced with diabetes and treated with 200 mg/kg b. w of methanolic extract of *Carica papaya* leaves orally.

Group IV: Rats induced with diabetes and treated with 200 mg/kg b. w of methanolic extract of *Anacardium occidentale* leaves orally.

Group V: Rats induced with diabetes treated with 200 mg/kg b. w of methanolic extract of *Psidium guajava* leaves orally.

Group VI: Rats induced with diabetes and treated with 102 mg/kg b. w of composite (1:1:1) of the methanolic extracts of the three (*Carica papaya, Anacardium occidentale* and *Psidium guajava*) plants orally.

Group VII: Rats induced with diabetes and treated with glibenclamide 2.5 mg/kg b. w orally.

3.5 **INDUCTION OF DIABETES**

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg b. w of streptozotocin, (dissolved in 0.1mol fresh cold citrate buffer, (pH 4.5)) into 24h fasted rats (Burcelin *et al.,* 1995). Glucose solution (5%) was administered after 6 hours of induction. After 3 days the blood sugar levels was determined with a glucometer (Acc-cheek Advantage Roche diagnostics GmbH, Germany) and the rats with fasting blood glucose level more than 200mg/dl (11.1mmol/L) were considered diabetic hence selected for experiment.

The extracts, composite and glibenclamide were administered on a daily basis to the experimental rats for 28 days by gastric intubation. The packed cell volume was determined weekly and the body weight was also taken weekly.

At the end of 28 days, the rats were anaesthetized using chloroform and sacrificed 24hrs after the last treatment. Blood samples were collected in specimen bottles, allowed to clot, centrifuged and serum was collected. The organs namely, liver and kidney were

collected, weighed and homogenized using 50 mM potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 3,000 x g for 15 minutes. The supernatant was decanted and used for the biochemical parameter analyses.

3.6 **BIOCHEMICAL ANALYSES**

3.6.1 **Fasting blood Glucose determination**

Fasting blood glucose levels was determined using the method described by Barham and Trinder (1972).

Principle: The glucose was determined after enzymatic oxidation in the presence of glucose oxidase. Hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4-aminophenazone which forms a red-violet quinoneimine dye as indicator.

Procedure: Exactly 1 ml of glucose working reagent containing glucose oxidase, peroxidase and 4- amino antipyrine as chromogen was pippetted into all the required test tubes. Then 20µL of the test samples was pippetted into the sample test tubes. Carefully, 20µL of standard reagent was pippetted into the standard test tube. They were all incubated at room temperature for 30 minutes and absorbance was read spectrophotometrically at 510nm.

3.6.2 **Determination of Packed Cell Volume (PCV).**

The determination of PCV was done weekly using microheamatocrit method (Schalm *et al*, 1975).

The weekly PCV was carried out using heparinized capillary tubes. Rats were bled by a slight cut on the tail tip using a razor blade, and the blood collected in heparinized capillary tubes and centrifuged at 6504 xg for 10min.

3.6.3 **Serum Lipid Profile Determination**

3.6.3.1 *Total Cholesterol Estimation.*

This was carried out using the method described by Stein (1987).

Principle:

The serum level of total cholesterol was quantified by spectrophotometric methods by the addition of enzyme present in reagent kit. A large portion of the cholesterol in blood is in the form of cholesteryl esters into cholesterol. Cholesterol is then oxidized by cholesterol oxidase to yield H_2O_2. The H_2O_2 produced interact with a sensitive cholesterol probe to produce resorufin, which can be detected spectrophotometrically. The value of total cholesterol present in serum was expressed in the unit of mg/dl.

Procedure: Exactly 1 ml of the cholestrol reagent was added to all the required test tubes. 10µL of the sample was added to the test sample test tube, 10µl of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 20min.The absorbance of the test sample and the standard was read at 505nm and the concentration of the sample was calculated using the formula;

Absorbance of test × Concentration of Standard

Absorbance of Standard.

3.6.3.2 *Determination of Serum Triacylglycerols*

Principle: The serum triacylglycerols level was determined by method of McGowan *et al*., (1983). Triglycerides are converted into quinoneimine dye. Quinoneimine dye is formed at a rate proportional to triglyceride concentration in the serum. It is detected and quantified spectrophotometrically. The value was expressed in the unit of mg/dl.

Procedure: Just 1 ml of the sample reagent was added to all the required test tubes. 10µL of the sample was added to the test sample test tube, 10µl of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 15min. The absorbance of the test sample and the standard was read at 500nm and the concentration of the sample was calculated using the formula;

Absorbance of test × Concentration of Standard

Absorbance of Standard.

3.6.3.3 *High Density Lipoprotein Cholesterol (HDL-c) Estimation*.

This was determined using assay kit as described by Wacnic and Albers (1978).The value was expressed in the unit of mg/dl.

Principle: High density lipoprotein (HDL) was separated from other lipoprotein fractions by treating the serum with phosphotungstic acid and magnesium chloride. HDL remained in solution while all other lipoproteins were precipitated.

Procedure: The proteins were precipitated using phosphotungstic acid, in the presence of magnesium and all other cholesterol in the solution. 1 ml the sample reagent was added to the required test tubes, 10µl of the sample was added to the test sample test tube, and 10µl of standard reagent was added to the standard test tube and none to blank. It was incubated at room temperature for 15min. The absorbance of the test sample and the standard were read at 500nm and the concentration of the sample was calculated using the formula;

$$\frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard}$$

3.6.3.4 *Determination of Serum Low Density Lipoprotein- Cholesterol* (*LDL-C*)

The serum level of (LDL-c) was measured according to the protocol of Friedewald *et al.*, (1972).The value was expressed in the unit of mg/dl. LDLC was calculated using the formula;

$$\text{LDLC} = \text{Total cholesterol} - \left(\frac{\text{Triglycerols}}{5} + \text{HDL-c}\right)$$

3.6.4 **Estimations of Indices of Liver Function.**

3.6.4.1 *Assessment of Aspartate Aminotransferase (AST)*

AST activities were determined by the method described by Amador and Wacker (1960).

Principle: In this reaction L-Aspartate and α-Ketoglutarate react in the presence of AST in the sample to yield oxaloacetate and L-glutamate. The oxaloacetate is reduced by malate dehydrogenase to yield L-malate with the oxidation of NADH to NAD. The

reaction is monitored by measurement of the decrease in the absorbance of NADH at 340nm. The rate of deduction in absorbance is proportional to AST activity in the sample.

Procedure: Exactly 1 ml of reagent was added to all the required test tubes. 500 µL of the sample was added to the test sample test tube and 50µl of the standard reagent was added to the standard test tube and none to the blank. It was incubated at room temperature for 20min, mixed immediately and first absorbance of test was read exactly at 1minute and thereafter at 30, 60, 90 and 120 secs at 340nm. The mean change in absorbance per minute was determined and the test results were calculated.

Serum AST activity (IU/L) = Change in A/min × F, where F= 3376

3.6.4.2 *Assessment of Alanine Aminotransferase (ALT)*

ALT activities were determined by method described by Amador and Wacker (1962).

Principle: In this reaction, L-Alanine and α-ketoglutarate react in the presence ALT in the sample to yield Pyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance at 340nm. The rate of reduction is proportional to ALT activity in the sample.

Procedure: To 1 ml of reagent added to all required test tubes, 500 µL of the sample was added to the test sample tube test and 50µl of the standard reagent was added to the standard test tube and none to the blank. It was incubated at room temperature for 20min, it was mixed immediately and first absorbance of test was read at exactly 1minute and thereafter at 30, 60, 90 and 120 secs at 340nm. The mean change in absorbance per minute was determined and test results were calculated.

Serum ALT activity (IU/L) = Change in A/min × F, where F= 3376

3.6.4.3 *Assessment of Alkaline Phosphatase (ALP) activities.*

Serum activities of alkaline phosphatase (ALP) were determined by the method described by Haussament, (1977).

Principle: This is based on measuring the concentration of phosphate hydrazone formed with p-nitro phenyl phosphate that absorbs maximally at 405 nm.

Procedure: Substrate solution (3 ml) was incubated at 37^0C for 15 min and then 0.5ml of the samples was added. The mixture was mixed thoroughly and immediately 0.05 ml of the mixture was removed and mixed with 9.5 ml of 0.085 N NaOH. This corresponded to zero time assay (blank). The remaining solution (substrate+enzyme) was incubated for 15min at 37^0C and then 0.5 ml was drawn and mixed with 9.5ml of 0.085N NaOH. Absorbance was measured at 405 nm against the reference blank. Specific activities were expressed as moles of p-nitrophenol formed per min per g tissue.

3.6.5 **Assessment of Indices of Renal Function**

3.6.5.1 *Determination of Serum Urea Concentration*

This was assessed using the method described by Fawcett and Scout (1960).

Principle: The Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia formed is then measured spectrophotometrically by Berthelot's reaction.

Procedure: To 2ml of water was added to all the required test tubes, 10µL of the sample was added to sample test tubes and 10µL of standard reagent was added to the standard test tube. 2 ml of uric acid colour reagent was added to all test tubes. It was incubated for

20min. The blank was used to zero the machine. The absorbance of the test sample and the standard were read at 500nm and the concentration of the test sample was calculated.

3.6.5.2 *Determination of Total Proteins*

This was carried out using Randox kits.

Principle: The protein assay is based on the reaction of protein with an alkaline copper tartarate solution and Folin reagent. Two steps led to colour development. First was the reaction between protein and copper at alkaline pH, and subsequently, the reduction of Folin reagent by the copper-treated protein. Colour formation was due to amino acids that reduced the Folin reagent, yielding reduced species that produced a characteristic blue colour with maximum absorbance at between 650 – 750nm.

Procedure: Exactly 1 ml of biuret reagent was added to all the required test tubes. 20µLof the sample was added to the test sample test tubes and 20µl of the standard reagent was added to the standard test tube. It was incubated for 10min under room temperature. The blank was used to zero the machine the absorbance was then read at 540nm. The concentration of the sample was calculated using the formula.

$$\frac{\text{Absorbance of test}}{\text{Absorbance of Standard.}} \times \text{Concentration of Standard}$$

3.6.5.3 *Determination of Albumin Level*

Principle: In an acidic medium, albumin binds with bromocresol green causing a shift in the absorption spectra of the yellow BCG dye. The blue green colour formed is directly proportional to the albumin present when measured at 630 nm.

Procedure: 1 ml of bromocresol green reagent was added to all the required test tubes. 20 µL of the sample was added to the test sample and 20µl of the standard reagent was added to the standard test tube. It was incubated for 10min under room temperature. The blank was used to zero the machine and the absorbance was read at 630nm.

3.6.6. **Assessment of Antioxidant Enzymes**

3.6.6.1 *Estimation of Superoxide Dismutase (SOD) Activity*

Superoxide Dismutase activity was measured using the method described by Martin *et al*., 1987.

Principle

Auto-oxidation of heamatoxylin (with increase in absorbance at 560 nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample was determined by measuring the amount of heamatin formed at 560 nm.

$0_2 + HTH_2 \xrightarrow{SOD} H_20_2 + HT$ (Abs 560nm)

HT = hematoxylin

Procedure

Exactly 920µL of phosphate buffer (0.05M, pH 7.8) was added to 40µL of the sample. A reagent test was prepared by replacing the sample with 40 µL of sample dilution buffer (0.85% NaCl). The mixtures were incubated for 2 min at 25^0C before 40 µL of hematoxylin was added. Following the addition of 40 µL of hematoxylin, absorbance of

the sample test and reagent test was read at 560 nm immediately and after 5 minutes against the sample blank which is distilled water.

SOD concentration in the sample was calculated thus;

$\text{Absorbance}_{\text{Reagent test}} (A_R) = \text{Absorbance}_{\text{Reagent test 2}} - \text{Absorbance}_{\text{Reagent test 1}}$

$\text{Absorbance}_{\text{sample test}} (A_s) = \text{Absorbance}_{\text{sample test 2}} - \text{Absorbance}_{\text{sample test 1}}$

$\% \text{ inhibition} = [1 - A_s / A_R] \times 100$

$\text{SOD } (\mu/\text{ml}) = [1 - A_s / A_R]_x \, 100 \times 1.2$

3.6.6.2 *Estimation of Catalase Activity*

Catalase activity was determined using the method described by Aebi and Bergmeyer (1983).

Principle

Catalase scavenges hydrogen peroxide (H_2O_2) converting it to water and molecular oxygen.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

The activity of catalase in the sample was determined by following the rate of decrease in absorbance at 240 nm.

Procedure

10µL (100-150 µg protein) tissue homogenate was added to 2.8 ml of 50 Mm potassium phosphate buffer (pH 7.0) in 3 ml cuvette. The reaction was initiated by adding 0.1 ml of

fresh 30 Mm H_2O_2 and the decomposition rate of H_20_2 was measured at 240 nm for 5 minutes on a spectrophotometer. A molar extinction coefficient of 0.041 mM^{-1} cm^{-1} was used to measure catalase activity which was expressed as n mole H_20_2 decreased/min/mg protein.

3.6.6.3 *Estimation of Thiobarbituric Acid Reactive Substance (TBARS)*

A Thiobarbituric acid reactive substance (TBARS) in the tissues was estimated using the method described by Fraga *et al* (1988).

Principle The formation of malondialdehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation.

At low pH of 2-3 and high temperature (100^0C), malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex, which absorbs maximally at 532 nm.

Procedure

To 0.5 ml of tissue homogenate, 0.5 ml saline and 1.0 ml of 10% TCA were added and 0.25 ml of 0.1 M TBA was then added to the mixture. The mixture was incubated for 1 hour at 95^0C, cooled and centrifuged at 6504 xg for 20 min. The absorbance of the pink colour produced was read at 535 nm.

3.7 **DATA ANALYSIS**

The results were expressed as mean ± standard error of mean (SEM) except where otherwise stated. The data was analyzed by the analysis of variance (ANOVA). The difference between the various animal groups was compared using the Duncan Multiple Range Test. P value less than 0.05 was taken as significant ($P< 0.05$).

CHAPTER FOUR

RESULTS

4.1 **PERCENTAGE YIELD**

The Percentage yield following the methanolic extraction of leaves extract of Pawpaw, Guava and Cashew is shown in table 4.1. The percentage yield (w/w) of Pawpaw leaves was highest, followed by Cashew leaves and lastly Guava leaves.

4.2 **ACUTE TOXICITY TEST**

The mean lethal dose testing showed that there was no record of death after the oral administration of methanolic extracts of *C. papaya, P. guajava, A. occidentale* and Composite up to a dose of 5000mg/kg body weight.

4.3 **PHYTOCHEMICAL ANALYSIS**

The phytochemical constituents of the methanolic extracts of *Carica papaya, Psidium guajava,* and *Anacardium occidentale* leaves are shown in table 4.2. Flavonoids concentration was highest in *C. papaya* leaves, and was significantly higher than what was obtained in *P. guajava* and *A. occidentale* leaves ($p < 0.05$). Alkaloids, tannins, saponins and total polyphenolic concentration were however higher in *P. guajava* leaves and was significantly higher than what was obtained in *C. papaya* and *A. occidentale* leaves.

Table 4.1 **Percentage yield of methanolic extract of the leaves of Pawpaw, Guava and Cashew**

Leaves	% yield
Pawpaw	27.35
Guava	16.35
Cashew	21.14

Table 4.2 **Phytochemical Constituents of Methanolic Leaves Extract (mg/g) of pawpaw, guava and cashew**

Leaves	Flavonoids	Alkaloids	Phenols	Saponins	Tannins
Pawpaw	37.55±2.29[b]	48.17±1.89[b]	7.83±0.35[a]	25.55±2.36[a]	3.85±0.76[a]
Guava	32.73±1.25[a]	16.98±5.07[a]	11.95±0.81[b]	36.89±1.06[b]	5.76±0.45[b]
Cashew	29.36±1.05[a]	24.08±2.02[a]	6.21±0.54[a]	27.03+1.48[a]	3.44±0.39[a]

Values are means ±SEM of three replicates for each group. Values with different superscript (a, b,) on the same column are statistically different (P< 0.05).

IN VIVO STUDYING

4.4 **BODY WEIGHT**

Figure 4.1 shows the effect of oral administration of methanolic leaves extracts of *C. papaya, P. guajava, A. occidentale,* the composite and Glibenclamide on body weight of diabetic and non-diabetic rats for 28 days. The diabetic control group showed a sharp decrease in body weight when compared with the normal control group. The individual extracts and the composite treated group showed a relative improvement in body weight when compared to the diabetic control group.

4.5 **PACKED CELL VOLUME (PCV)**

Figure 4.2 shows the effect of oral administration of methanolic leaves extract of *C. papaya, P. guajava, A. occidentale*, their composite and Glibenclamide (Standard drug) on packed cell volume of diabetic and normal control rats for 28 days is shown in figure 4.2 .A significant ($P< 0.05$) decrease in the level of PCV in diabetic control group was observed when compared with the normal control group. After treatment with the individual extracts and their composite, there was improvement in the PCV compared to the normal control group.

4.6 **ASSESSMENT OF FASTING GLUCOSE LEVEL.**

Table 4.3 shows the effect of daily oral treatment of methanolic leaves extracts of *C. papaya, P. guajava, A. occidentale* , Composites and Glibenclamide (standard drug) on serum blood glucose after 28 days of treatment (after induction with streptozotocin). The blood glucose levels of diabetic control rats increased significantly ($P < 0.05$). In the treated diabetic group, a significant decrease in blood glucose levels compared with diabetic control group was observed. There is also a significant decrease in the composite treated group which is comparable with the glibenclamide treated group.

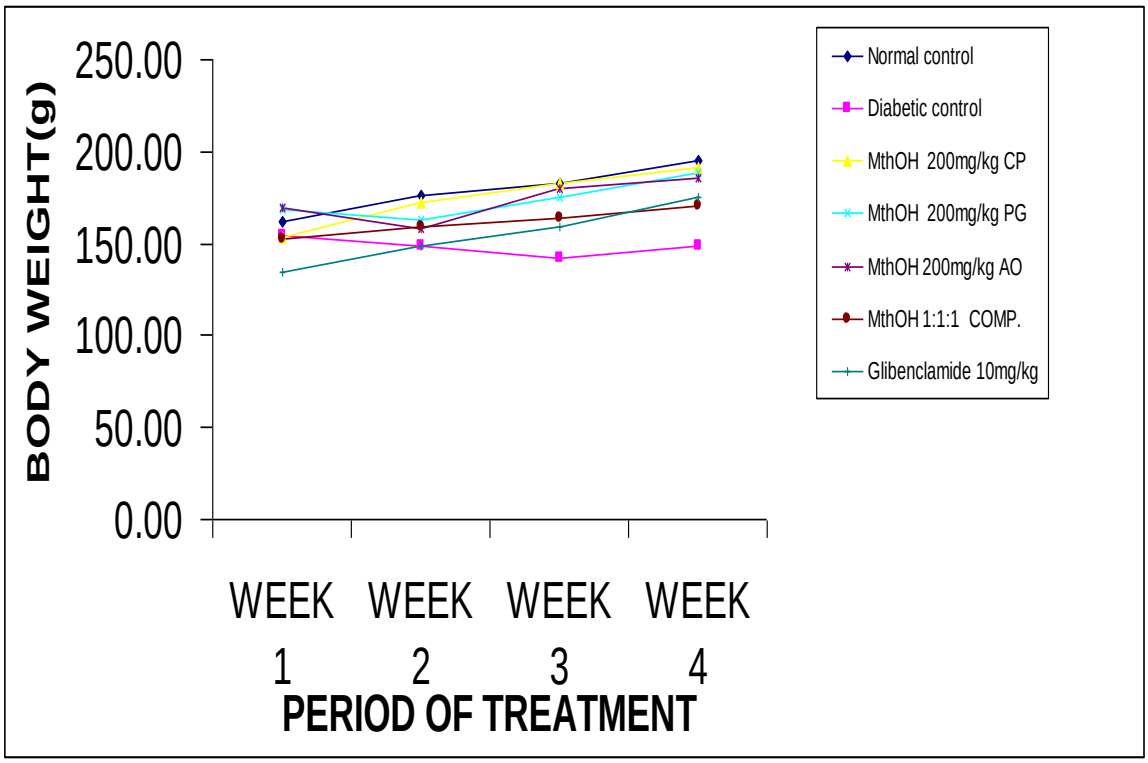


Figure 4.1: Effect of Daily Oral Administration of Methanolic Leaf Extracts of *C. papaya, P.guajava, A. occidentale* and Composite on Body Weight of Normal and STZ-induced Albino rats.

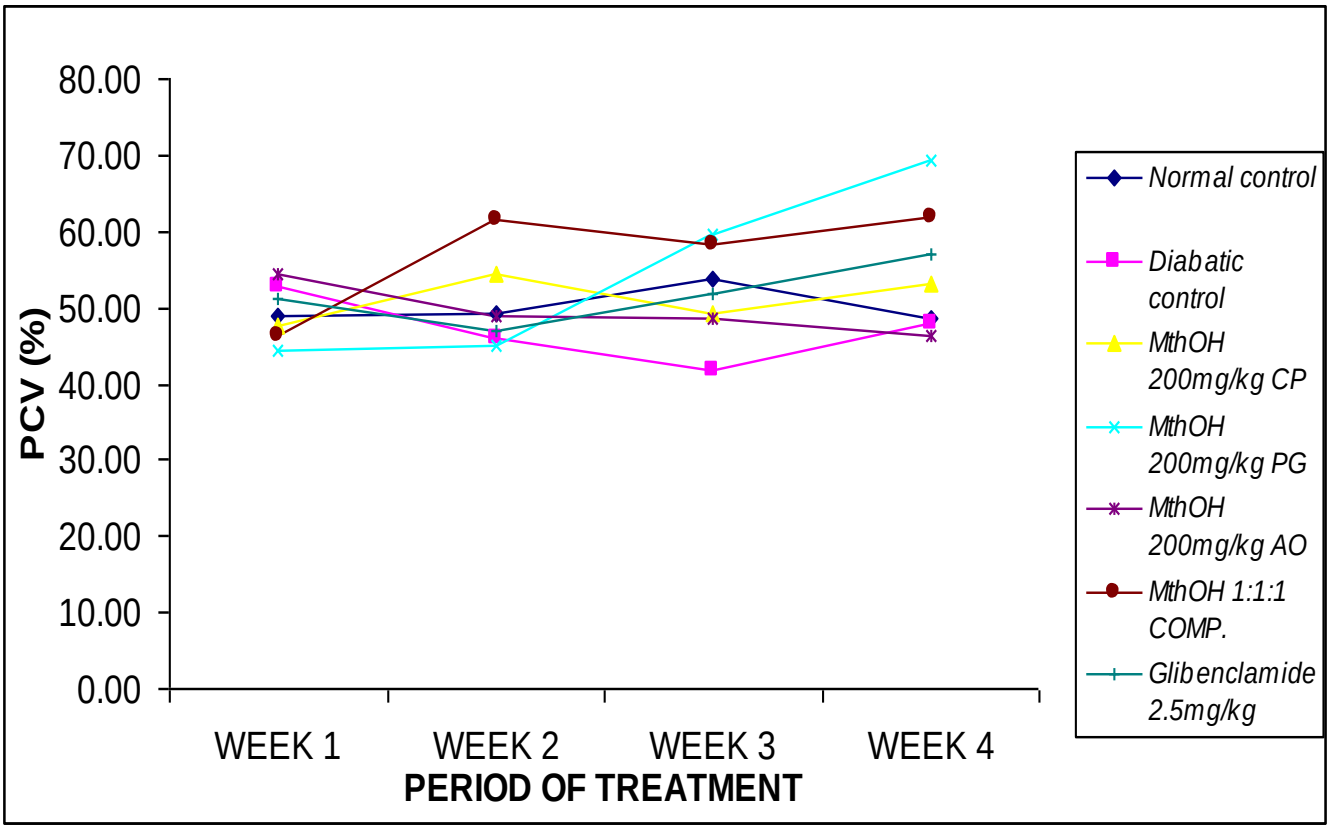


Figure 4.2: Effect of Daily Oral Doses of Methanolic Leaf Extracts of *C. papaya, P.guajava, A. occidentale* and Composite on Serum PCV Levels of Normal and STZ-induced Albino rats.

Table 4.3: **Effects of methanolic leaves extract (200mg/kg bw) of *Carica papaya, Psidium guajava, Anacardium occidentale,* Composite (102mg/kg bw) and Glibenclamide (2.5mg/kg bw) on fasting blood glucose levels in experimental rats after 28 days of treatment.**

Treatments	**Fasting Blood Glucose Level (mg/dl)**
NC	77. 33 ±3.71[a]
DC	268.33±16.41[c]
D + Mth *C. papaya*	125.67±2.96[b]
D +Mth *P. guajava*	121.33±5.78[b]
D + Mth *A. occidentale*	121.67±4.41[b]
D + Mth Composite	98. 00±4.16[a]
D + Glibenclamide	97.33±3.71[a]

Values are means ±SEM of five replicates for each group. Values with different superscript (a, b, c, d) on the same column are statistically different (P< 0.05).
NC: normal control, DC: diabetic control, D + Mth CP: Diabetic+ methanolic extracts of *Carica papaya*, D +Mth PG: Diabetic+ methanolic extracts of *Psidium guajava*, D +Mth AO: Diabetic + methanolic extracts of *Anacardium occidentale*, D+Mth Composite: Diabetic + Methanolic extracts of composite, D + Glibenclamide (Standard drug).

4.7 **ASSESSMENT OF INDICES OF LIVER FUNCTION.**

Table 4.4 shows the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities of diabetic and treated rats following the oral administration of methanolic leaves extracts of *C. papaya, P. guajava, A. occidentale,* the composite and Glibenclamide (standard drug) for 28 days. A significant ($P < 0.05$) increase in serum AST, ALT and ALP was observed in the diabetic control group compared with the normal control group. Group treated with the extracts and composite showed significant reduction in AST, ALT and ALP levels. The reduction in levels of AST, ALT and ALP following the administration of the extracts are statistically comparable with the composite and the standard drug (Glibenclamide). The reduction in level of ALT in the individual extracts is statistically similar to that of the composite and the standard drug (Glibenclamide). The decrease in ALT and ALP levels in the drug treated group is statistically comparable with the treated groups.

4.8 **ASSESSMENT OF INDICES OF RENAL FUNCTIONS.**

Table 4.5 shows the serum urea, total protein and albumin levels of diabetic and non-diabetic rats following the oral administration of methanolic leaves extracts of C. *papaya, P. guajava, A. occidentale,* Composite and Glibenclamide (standard drug) for 28days. The serum level of urea significantly ($P < 0.05$) increased in diabetic control group compared with the normal control group. Diabetic groups treated with the extracts, composite and standard drug showed significant decreases in urea levels. The reduction in level of urea in the extracts treated group is statistically comparable with the composite treated group. Significant ($P < 0.05$) decrease in total protein and albumin were observed

in diabetic control group when compared with groups treated with extracts, composite and standard drug. Diabetic rats treated with the extracts, composite and standard drug led to a significant increase in serum total protein and albumin levels. The increase in total protein and albumin in the composite and standard drug treated groups is statistically comparable with the extracts treated groups. A significant increase in Albumin levels was observed in the composite treated group followed by *A. occidentale leaves, P. guajava and C. papaya* leaves treated groups. This was also observed in the total protein levels, but after the composite comes *guajava, A. occidentale leaves and C. papaya* leaves treated groups.

4.4 **Effects of methanolic leaves extract (200mg/kg bw) of *Carica papaya, Psidium guajava, Anacardium occidentale,* Composite (102mg/kg bw) and Glibenclamide (2.5mg/kg bw) in serum Aspartate Transferase, Alanine Transferase and Alkaline Phosphatase in experimental rats after 28 days of treatment.**

Treatments	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
NC	19.33 ± 0.67^{a}	36.67 ± 1.20^{a}	57.67 ± 4.33^{a}
DC	26.67 ± 1.33^{b}	51.00 ± 1.73^{d}	84.33 ± 4.91^{b}
D +Mth C. papaya	22.00 ± 1.15^{a}	41.67 ± 1.20^{bc}	65.67 ± 1.76^{a}
D +Mth P. guajava	21.00 ± 1.00^{a}	43.67 ± 1.20^{c}	60.67 ± 6.36^{a}
D + Mth A. occidentale	21.33 ± 1.33^{a}	40.33 ± 1.45^{abc}	60.33 ± 5.78^{a}
D + Mth Composite	22.67 ± 1.45^{a}	37.67 ± 0.88^{ab}	56.33 ± 6.33^{a}
D + Glibenclamide	22.67 ± 1.45^{a}	38.00 ± 2.31^{ab}	70.67 ± 2.33^{ab}

Values are means ±SEM of five replicates for each group. Values with different superscript (a, b, c, d) on the same column are statistically different ($P< 0.05$).

NC: normal control, DC: diabetic control, D + Mth CP: Diabetic+ methanolic extracts of *Carica papaya*, D +Mth PG: Diabetic+ methanolic extracts of *Psidium guajava*, D +Mth AO: Diabetic + methanolic extracts of *Anacardium occidentale*, D +Mth Composite: Diabetic + Methanolic extracts of composite, D + Glibenclamide (Standard drug).

Table 4.5: Effects of methanolic leaves extract (200mg/kg bw) of *Carica papaya, Psidium guajava, Anacardium occidentale,* Composite (102mg/kg bw) and Glibenclamide (2.5mg/kg bw) on Urea, Total protein and Albumin levels in experimental rats after 28 days of treatment.

Treatments	Urea (g/dl)	TP(g/dl)	Albumin (g/dl)
NC	28.67±3.17^{a}	69.33±3.48^{ab}	39.67±1.45^{c}
DC	48.00± 1.53^{c}	64.33±2.03^{a}	26.00±1.15^{a}
D + Mth *C. papaya*	38.67±0.88^{b}	67.33±2.40^{ab}	32.00±1.00^{b}
D +Mth *P. guajava*	39.00±2.08^{b}	66.33±0.88^{ab}	39.33±2.40^{c}
D +Mth *A. occidentale*	37.00±2.08^{b}	66.33±1.15^{ab}	40.00±2.52^{c}
D + Mth Composite	33.00±1.53^{ab}	73.66±3.18^{b}	42.00±3.05^{c}
D + Glibenclamide	30.67±0.67^{a}	73.66±1.45^{b}	41.00±0.58^{c}

Values are means ±SEM of five replicates for each group. Values with different superscript (a, b, c, d) on the same column are statistically different (P< 0.05).

NC: normal control, DC: diabetic control, D + Mth CP: Diabetic+ methanolic extracts of *Carica papaya*, D +Mth PG: Diabetic+ methanolic extracts of *Psidium guajava*, D +Mth AO: Diabetic + methanolic extracts of *Anacardium occidentale*, D +Mth Composite: Diabetic + Methanolic extracts of composite, D + Glibenclamide (Standard drug).

4.9 **ASSESSMENT OF LIPID PROFILE.**

The effect of oral administration of doses of methanolic leaves extract of *C. papaya, P. guajava, A. occidentale*, Composite and glibenclamide on serum HDL-cholesterol, LDL-cholesterol, and Triacylglycerols on diabetic and normal rats for 28 days is presented in Table 4.6. The result showed a significant ($P < 0.05$) increases in the level of total cholesterol, triacylglycerols, and LDL-cholesterol in diabetic control group when compared with normal control group. There was a significant ($P < 0.05$) decrease in the level of HDL-cholesterol in diabetic control group compared with that of normal control rats, extracts, Composite and glibenclamide treated groups. Treatment with Composite and extracts of *C. papaya, P. guajava and A. occidentale* leaves caused significant ($P < 0.05$) decreases in LDL-cholesterol, Total cholesterol and Triglycerides as well as a significant increase in HDL-cholesterol when compared with the diabetic control group. The effects are comparable to the effects of glibenclamide.

4.10 **DETERMINATION OF LIVER AND KIDNEY ANTIOXIDANT ACTIVITIES.**

Effects of methanolic leaves extract of *C. papaya, P. guajava, A. occidentale*, composite and Glibenclamide on the activity of hepatic SOD, catalase and MDA in experimental and normal rats is shown in Table 4.7 following the oral administration of the leaves extracts for 28 days. There was a significant ($P < 0.05$) increase in Malondialdehyde (MDA) in the liver of diabetic control group when compared with the treated groups. There was a significant reduction in MDA levels in the liver following treatment with the leaves extracts, composite and Glibenclamide treated groups. In table 4.8, composite

group was statistically similar with the leaves extracts groups. The glibenclamide group has the highest reduction which was statistically comparable with other treated groups. There was observed significant ($P< 0.05$) reduction in the activity of catalase in the diabetic control group compared with the leaves extracts, composite and glibenclamide treated groups. Treatment with different leaves extracts, Composite and standard drug caused the increase in catalase activity in the liver of diabetic rats. Maximum increase of 47.00 U/ml was observed in the Composite group which is statistically similar with the 44.67, 41.00 and 45.33 U/ml respectively observed for groups treated with methanolic leaves extract of *C. papaya, P. guajava and A. occidentale*. These increase in catalase caused by the Composite and extracts is statistically comparable with 49.67 U/ml increase observed in the Glibenclamide group. Also, there was a significant ($P< 0.05$) reduction in the activity of Superoxide dismutase (SOD) in the liver of the diabetic control group when compared with the composite, extracts and Glibenclamide treated group. Treatment with the composite, different leaves extract of *C. papaya P. guajava A. occidentale* and Glibenclamide (standard drug) caused an increase in the SOD activity in the liver of diabetic rats. The level of increase in the SOD activity in the composite and the three extracts statistically differ.

The level of MDA, SOD and Catalase in the kidney of experimental and normal rats is shown in Table 4.8 above following the oral administration of methanolic leaves extracts of *C. papaya P. guajava A. occidentale,* composite and Glibenclamide for 28 days. There was a significant ($P< 0.05$) increase in Malondialdehyde in the kidney of diabetic control rats when compared with the treated groups. Treatment with composite, different leaves

extracts and Glibenclamide led to a significant reduction in the MDA level in the kidney of diabetic rats. There was a significant increase of the kidney MDA activity in the composite group which was statistically comparable to the groups treated with the different leaves extracts. For the catalase activity a significant ($P< 0.05$) increase was observed in the diabetic control group when compared with the treated group. A significant increase was observed in rats from the Glibenclamide group compared with the rats from the treated group. Also, there was a significant ($P< 0.05$) decrease in the activity of Superoxide dismutase (SOD) in the kidney of the diabetic control group when compared with extracts of *C. papaya, P. guajava, A. occidentale,* Composite and glibenclamide treated group. A significant ($P< 0.05$) increase was observed in the Composite treated group compared with the different extracts treated group which was also comparable to the Glibenclamide treated group.

Table 4.6: Effects of methanolic leaves extract (200mg/kg bw) of *Carica papaya, Psidium guajava, Anacardium occidentale*, Composite (102mg/kg bw) and Glibenclamide (2.5mg/kg bw) on serum Total cholesterol, Triglycerides, High Density Cholesterol Lipoprotein, and Low-Density cholesterol Lipoprotein levels in experimental rats after 28 days of treatment

Treatments	TC(mg/dl)	TG(mg/dl)	HDL (mg/dl)	LDL (mg/dl)
NC	86.67±6.67^{a}	93.33±6.67^{ab}	43.33±3.33^{b}	24.67±4.67^{a}
DC	160.00±11.55^{b}	193.33±12.02^{a}	23.33±3.33^{a}	104.67±7.69^{c}
D + Mth *C. papaya*	123.33±8.82^{b}	123.33±6.67^{c}	33.33±3.33^{ab}	65.33±12.72^{b}
D + Mth *P. guajava*	126.67±6.67^{b}	116.67±6.67^{bc}	33.33±6.67^{ab}	70.00±6.11^{b}
D+ Mth *A. occidentale*	120.00±5.77^{b}	96.67±6.67^{ab}	36.67±3.33^{ab}	64.00±9.87^{b}
D+ Mth Composite	103.33±3.33^{a}	93.33±6.67^{ab}	36.67±3.33^{ab}	48.00±2.00^{ab}
D+ Glibenclamide	103.33±8.82^{a}	86.67±8.81^{a}	40.00±5.78^{b}	46.00±15.01^{ab}

Values are means ±SEM of five replicates for each group. Values with different superscript (a, b, c, d) on the same column are statistically different ($P < 0.05$).

NC: normal control, DC: diabetic control, D + Mth CP: Diabetic+ methanolic extracts of *Carica papaya*, D +Mth PG: Diabetic+ methanolic extracts of *Psidium guajava*, D +Mth AO: Diabetic + methanolic extracts of *Anacardium occidentale*, D +Mth Composite: Diabetic + Methanolic extracts of composite, D + Glibenclamide (Standard drug).

Table 4.7: Effects of methanolic leaves extract (200mg/kg bw) of *Carica papaya, Psidium guajava, Anacardium occidentale,* Composite (102mg/kg bw) and Glibenclamide (2.5mg/kg bw) on, Superoxide Dismutase, Catalase and Malondialdehyde activity in liver of experimental rats after 28 days of treatment.

Treatments	SOD(U/ml)	CAT (U/ml)	MDA(nmol/mgprotein)
NC	1.70 ± 0.100^{bc}	48.67 ± 4.48^{b}	1.63 ± 0.09^{a}
DC	0.97 ± 0.203^{a}	32.00 ± 0.58^{a}	2.47 ± 0.22^{b}
D + Mth *C. papaya*	1.60 ± 0.100^{b}	44.67 ± 2.73^{b}	1.77 ± 0.13^{a}
D + Mth *P. guajava*	1.60 ± 0.116^{b}	41.00 ± 2.31^{b}	1.70 ± 0.10^{a}
D +Mth *A. occidentale*	1.60 ± 0.578^{b}	45.33 ± 2.40^{b}	1.63 ± 0.88^{a}
D + Mth Composite	1.70 ± 0.58^{bc}	47.00 ± 2.65^{b}	1.83 ± 0.16^{a}
D + Glibenclamide	2.00 ± 0.58^{c}	49.67 ± 2.73^{b}	1.63 ± 0.15^{a}

Values are means ±SEM of five replicates for each group. Values with different superscript (a, b, c, d) on the same column are statistically different ($P< 0.05$).

NC: normal control, DC: diabetic control, D + Mth CP: Diabetic+ methanolic extracts of *Carica papaya*, D +Mth PG: Diabetic+ methanolic extracts of *Psidium guajava*, D +Mth AO: Diabetic + methanolic extracts of *Anacardium occidentale*, D +Mth Composite: Diabetic + Methanolic extracts of composite, D + Glibenclamide (Standard drug).

Table 4.8 Effects of methanolic leaves extract (200mg/kg bw) of *Carica papaya, Psidium guajava, Anacardium occidentale,* Composite (102mg/kg bw) and Glibenclamide (2.5mg/kg bw) on SOD, Catalase and MDA activity in kidney of experimental rats after 28 days of treatment.

Treatments	SOD (U/ml)	CAT (U/ml)	MDA(nmol/mgprotein)
NC	1.53 ± 0.88^{b}	42.00 ± 1.55^{b}	1.67 ± 0.33^{a}
DC	0.93 ± 0.15^{a}	32.67 ± 0.67^{a}	2.50 ± 0.46^{b}
D + Mth *C. papaya*	1.63 ± 0.88^{b}	48.67 ± 2.40^{bc}	1.87 ± 0.12^{a}
D + Mth *P. guajava*	1.67 ± 0.88^{b}	42.00 ± 2.31^{b}	1.60 ± 0.17^{a}
D + Mth *A. occidentale*	1.63 ± 0.88^{b}	46.33 ± 3.18^{bc}	1.60 ± 0.20^{a}
D + Mth Composite	1.83 ± 0.67^{bc}	47.33 ± 1.76^{bc}	1.87 ± 0.32^{a}
D + Glibenclamide	2.03 ± 0.12^{c}	50.67 ± 2.73^{c}	1.73 ± 0.36^{a}

Values are means ±SEM of five replicates for each group. Values with different superscript (a, b, c, d) on the same column are statistically different ($P< 0.05$).
NC: normal control, DC: diabetic control, D + Mth CP: Diabetic+ methanolic extracts of *Carica papaya*, D +Mth PG: Diabetic+ methanolic extracts of *Psidium guajava*, D +Mth AO: Diabetic + methanolic extracts of *Anacardium occidentale*, D +Mth Composite: Diabetic + Methanolic extracts of composite, D + Glibenclamide (Standard drug).

CHAPTER FIVE

DISCUSSION

The median acute toxicity value (LD_{50}) of the methanolic leaves extracts and composite of the plants were determined and no death was recorded up to the dose of 5000 mg/kg bw. The effective dosage of the various leaves extracts of *Carica papaya, Psidium guajava, and Anacardium occidentale* leaves and composite were 200mg/kg body weight. The composite was prepared in 1:1:1 (w/ w) ratio which is in conformity with that of Kumar *et al.,* (2010).

Phytochemical Analysis of the plant extracts revealed the presence of phenols, tannins, flavonoids, saponins, and alkaloids, which are known to exhibit pharmacological activities. The therapeutic potentials of plants have been linked with their antioxidant potentials (Akinmalodun *et al*., 2007; Eleazu *et al*., 2011). Flavonoids are potent water soluble antioxidants and free radical scavengers that prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis (Okwu, 2004). Most plants containing glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., are also frequently implicated as having antidiabetic effect (Grover *et al.,* 2002). Umeh *et al.* (2005) reported that many of the phytoconstituents with antibacterial properties in plants are preferably concentrated in leaves. The accumulation of these compounds in plant cells have attracted the attention of scientists worldwide and have been the subject of intense investigations. Tannins also react with proteins to produce the typical tanning effect which is important in the treatment of inflamed or ulcerated tissues, burns, wounds, pneumonia and dysentery (Mota *et al.,* 1985). Plant

parts that contain tannins are astringent in nature and have important roles as stable and potent antioxidants (Dharmanda, 2003). Phenols, saponins and flavonoids found in the leaves of guava have been observed to exert anti-bacterial properties (Marjorie, 1999; Ogbonnia *et al*., 2008). Much of guava's therapeutic activity is attributed to tannins, phenols and flavonoids, in particular, quercetin (Morton, 1987; Scalbert, 1991). Flavonoids have also been found to play a very important role in protection against oxidative stress (Said *et al*., 2008). As shown in Table 4.2, *Carica papaya* has the highest flavonoids concentration, followed by *Psidium guajava* and then *Anacardium occidentale*. For alkaloids concentration, *Psidium guajava* had the highest concentration, followed by *Carica papaya*, then *Anacardium occidentale*. For tannins, *Psidium guajava* had the highest concentration followed by *Carica papaya* and then *Anacardium occidentale*. Similarly, for saponins concentration, *Psidium guajava* had the highest concentration followed by *Anacardium occidentale* then *Carica papaya*. Finally, for phenols concentration, *Psidium guajava* has the highest concentration, followed by *Carica papaya* and then *Anacardium occidentale*. Thus the high alkaloid and flavonoids contents of these plants may explain their therapeutic use in herbal medicine**.**

Streptozotocin (STZ) selectively destroys the insulin secreting ß-cells, leaving less active cells and resulting in severe hyperglycemia (Kamtchouning *et al.,* 1998). STZ-induced diabetes is characterized by severe loss in body weight and this reduction is due to loss or degeneration of structural proteins, as the structural proteins are known as a major contributor to body weight (Chen and Ianuzzo, 1982). Significant weight loss was observed in the diabetic group which was improved significantly by the composite extract (1:1:1) treated group than the other treated groups. This may be due to the efficacy of the

plant ingredients for this purpose and it may be due to herb-herb interaction. This result is in agreement with the report of Kumari and Devi (1993).

In severe diabetic state, rupture of red blood cells within the blood stream may occur which brings about a reduction in packed cell volume. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin (Kumar *et al.,* 2005). The total hemoglobin level is decreased in diabetic rats which brought about a decrease in packed cell volume as observed in the diabetic control group (fig 4.1). This is in accordance with report by Umar *et al*., (2012). The increase in the packed cell volume of the treated group after administration of the various extracts and glibenclamide may be due to the decrease in the level of blood glucose after receiving treatment.

A significant ($P< 0.05$) increase in fasting blood glucose (FBS) levels was observed in the diabetic control group compared to the normal control. The oral administration of the composite treated group showed the maximum reduction in the diabetic rats compared to the groups treated with extracts of *C. papaya, P. guajava, and A. occidentale*. The reduction on FBS caused by the composite is statistically comparable with the reduction in the group treated with the Glibenclamide (standard drug) (Table4.3). Though all the three extracts and composite proved effective in this study, the composite extract had the maximum capacity to restore fasting blood glucose back to near normal levels (Table 4.3). The hypoglycemic activity of the extracts and composite may be due to its protective action against STZ-mediated damage to the pancreatic ß cells and also possibly

through regeneration of damaged ß cells or increased insulin release or secretion. (Subramoniam *et al.,* 2012). Some of these phytochemicals (saponins, flavonoids, tannins and alkaloids) are believed to be responsible for the blood glucose lowering effects of these plant materials (Galvez *et al*., 1992; Ojewole 2003). It may also be that the extracts regulate the absorption of glucose in the gastrointestinal tracts thereby reducing the postprandial glucose or regulate the metabolism of glucose in the liver by reducing hepatic glucose production (James *et al.*, 2009).

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease (Subramanian *et al*., 2012). Abnormalities in lipid profile are common complications in diabetes mellitus and it is found in about 40% of diabetics (Stanley *et al.,* 1998).This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin (Kumar *et al*., 2010). In this study, increased levels of total cholesterol, triacylglycerols and reduced level of high density lipoprotein along with altered composition of low density lipoprotein were observed in the diabetic control rats as also reported by Howard *et al* (2000). But after treatment with the different extracts, composite and glibenclamide, it reversed to near normal. The oral administration of the Composite caused the highest hypolipidemic effect, compared with the individual extracts, followed by *A. occidentale leaves, C. papaya and P. guajava* leaves (Table 4.6). This lipid lowering action following treatment may be due to an increase in insulin secretion that led to a decrease in the synthesis of cholesterol and fatty acid by inhibiting

lipolysis as a result of proper stabilization of the glucose level and increase in insulin level. This is in conformity with the report by Subramanian *et al.,* (2012)**.**

Kidney disease is one of the most common and severe complications of diabetes (Tierney *et al., 2002*). One of the functions of the kidney is to remove the metabolic waste such as urea, creatinine, etc, thereby helping to maintain body homeostasis.The blood urea level increased significantly in diabetic control group (Table 4.8). This result is as reported by Aurell and Bjorck (1992). Treatment with methanolic extracts of composite and the leaves extracts of *C. papaya. guajava, A. occidentale* and glibenclamide brought about a significant increase in serum protein and albumin and a decrease in urea levels of diabetic rats (Table 4.5). Increase in urea level was reversed by administration of the composite treated group which had the lowest urea level, followed by *A. occidentale leaves, C. papaya and P. guajava* leaves treated groups. Increase in serum urea levels in the diabetic rats may be as a result of renal dysfunction which results in elevated urea levels. Decrease of serum protein level in diabetic control rats may be due to impaired protein turnover and muscle wasting in diabetic condition. The uncontrolled diabetes is associated with severe muscle wasting (Castaneda, 2002). A significant increase in Albumin levels was observed in the composite treated group. This may be due to inhibition of proteolytic activity due to enhance insulin secretion and proper utilization of blood glucose. Similar effect was reported by (Sivajothi *et al,* 2007). The order of effectiveness in restoring serum total protein was composite extracts≥ glibenclamide> *P. guajava*> *A. occidentale*> *C. papaya* leaves and for serum albumin was composite> glibenclamide> *A. occidentale*> *P. guajava*> *C. papaya* leaves. These

findings suggest that the composite of the plants may enhance the function of the kidneys to remove these waste products from the blood as indicated by the reduction in serum urea level and confer a protective effect on the kidneys of diabetic rats. It is also possible to suggest that this extracts might improve the functional integrity of the kidney cells.

This study also showed elevated levels of AST, ALT and ALP in STZ- induced diabetic rats (Table4.4). These observations may be due to STZ mediated liver damages which may cause leakage of these enzymes from the liver cytosol into the blood (Jasmine and Daisy 2007). The liver, kidney and heart release ALT and ALP and an elevation in their plasma concentrations indicates liver and heart damage (Ogbonnia *et al.,* 2008). As shown in table 4.4, there was a significant increase in the serum AST, ALT and ALP levels in diabetic control group when compared with other leaves extracts treated diabetic groups. However, the highest reduction in ALT and ALP levels was observed in the composite treated group, followed by *A. occidentale, C. papaya and P. guajava* treated group for ALT levels and *A. occidentale, P. guajava and C. papaya* treated group for ALP levels. For the AST levels, *P. guajava* treated group showed the maximum decrease followed by *A. occidentale, C. papaya* and composite treated groups. This suggests that the composite have a curative effect, by the reduction in ALT and ALP levels.

In diabetes, oxidative stress seems to be caused by increased production of Reactive Oxygen Species (ROS), a sharp reduction in antioxidant defenses and altered cellular redox status (West, 2000). It is well established that free radicals derived from oxygen have been implicated in the pathophysiology of diabetes mellitus and other diseases

(Kakkar *et al*., 1998). Boguslaw (2001) reported that the level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complications in diabetes.

In this study, decreased level of liver and kidney SOD, and CAT, as well as increased levels of MDA were observed in diabetic control rats compared with the normal control rats (Table 4.8 and 4.9). There is sharp reduction of *in vivo* antioxidant enzymes levels in various tissues as reported in diabetic condition (Ahmed, 2005). The increase in MDA levels seen in diabetic control group was also reported by Subramoniam *et al.,* (2012). This may be due to a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Wilson *et al.,* 2001).

There is a significant difference in the liver MDA levels in the diabetic control group compared to the normal control group. The MDA levels for the different extracts and composite treated group is statistically similar. Based on the efficacy of the extracts in reducing the liver MDA levels, the *A. occidentale* leaves extract treated group comes first, then the *P. guajava* leaves treated group, the *C. papaya* treated group, and lastly the composite treated group. While for the kidney MDA levels, is in the order; *A. occidentale* leaves extract treated group ≥ *P. guajava* leaves treated group ranks first then ; composite ≥ *C. papaya* treated groups respectively.

For the liver SOD and CAT activities, the glibenclamide treated group showed the highest increase, followed by the composite, *A. occidentale, C. papaya and P. guajava* leaves treated groups which all had the same SOD activities and *A. occidentale* leaves extract treated group, *C. papaya* treated group, and *P. guajava* leaves treated group for

catalase activity. A significant increase was observed in the kidney SOD levels after administration of the composite, followed by *P. guajava, C. papaya* and *A. occidentale* leaves treated group and for the catalase activity the composite and *C. papaya* had the maximum activity followed by *A. occidentale* and lastly *P. guajava* leaves treated groups. The increase in SOD and CAT activity in this study shows that the composite of the methanolic leaves extract contain high free radical scavenging activity hence a potent antioxidant property due to the presence of tannin and phenolic compounds (Subramoniam *et al* ., 2012).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 **SUMMARY**

In the traditional system of plant medicine it is usual to use plant formulation and combined extract of plant as a drug of choice rather than individual ones (Kumar, 2010), to get the benefit of synergism and to find suitable antidiabetic and antioxidant combination therapy (Srivastava *et al.*,2012). This is in order to reduce the several side effects associated with conventional drugs, whether used singly or as combination therapy.

The investigation revealed that there was a significant ($P < 0.05$) decrease in the fasting blood glucose level in the composite treated group which is comparable with the glibenclamide treated group. The oral administration of the Composite showed the highest hypolipidemic effect, compared with the individual extracts, followed by *A. occidentale leaves, C. papaya* and *P. guajava* leaves. The highest reduction in ALT and ALP activities were observed in the composite treated group, and *A. occidentale,* for ALP activities. For the AST levels, *P. guajava* treated group showed the maximum decrease. Treatment with methanolic extracts of composite and the leaves extracts of *C. papaya. guajava, A. occidentale* and glibenclamide brought about a significant increase in serum protein and albumin. Increase in urea level reduced significantly after administration of the composite extract. In this study, decreased level of liver and kidney SOD, and CAT, as well as increased levels of MDA were observed in diabetic control rats compared with the normal control rats. The liver MDA levels for the different extracts and composite

treated group is statistically similar. While for the kidney MDA levels, they are also statistically similar. For the liver SOD and CAT activities, the glibenclamide treated group showed the highest increase, followed the composite treated group. A significant ($P < 0.05$) increase was also observed in the kidney SOD and CAT activities for the glibenclamide treated group followed by the composite treated group.

6.2 **CONCLUSIONS**

The results of this study indicated that all the extracts of *C. papaya, P. guajava and A. occidentale* possess hypoglycemic, hypolipidemic and antioxidant activities.Composite formulated from the extracts also possess hypoglycemic, hypolipidemic and antioxidant activities and in most cases better than the individual extracts. Therefore it may be useful in controlling diabetes and its related complications.

Despite the use of advanced oral hypoglycemic agents for the management of diabetes, use of herbal remedies is gaining higher importance because these oral hypoglycemic agents have drawbacks and limitations (Wais *et al.*, 2012). The increasing interest in the use of herbal medicine demands information on the efficacy, toxicity and also risk assessment on various plant concoctions used in management of diseases. Numerous medicinal plants have been reported to be effective in treating diabetes, yet plenty of research is still needed to be done. Polyherbal formulations (Singh *et al*., 2005) have proved more useful and beneficial in the management of various ailments including those that seem to defy conventional medication (Ebong *et al*., 2008).

6.3 **RECOMMENDATIONS**

1. There is need for further study on sub- acute toxicity study and efficacy of the composite extract, by using different doses of the composite extract on normal rats.

2. There is need to determine the insulin levels in order to know the mechanism of anti-diabetic activity of the extracts.

3. There is also need to check the effects of the leaves extract on the activities of some selected diabetic related carbohydrate metabolizing enzymes.

4. There is need to assess the effect of the leaves and composite extract on endogenous antioxidant such as glutathione.

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Appendices

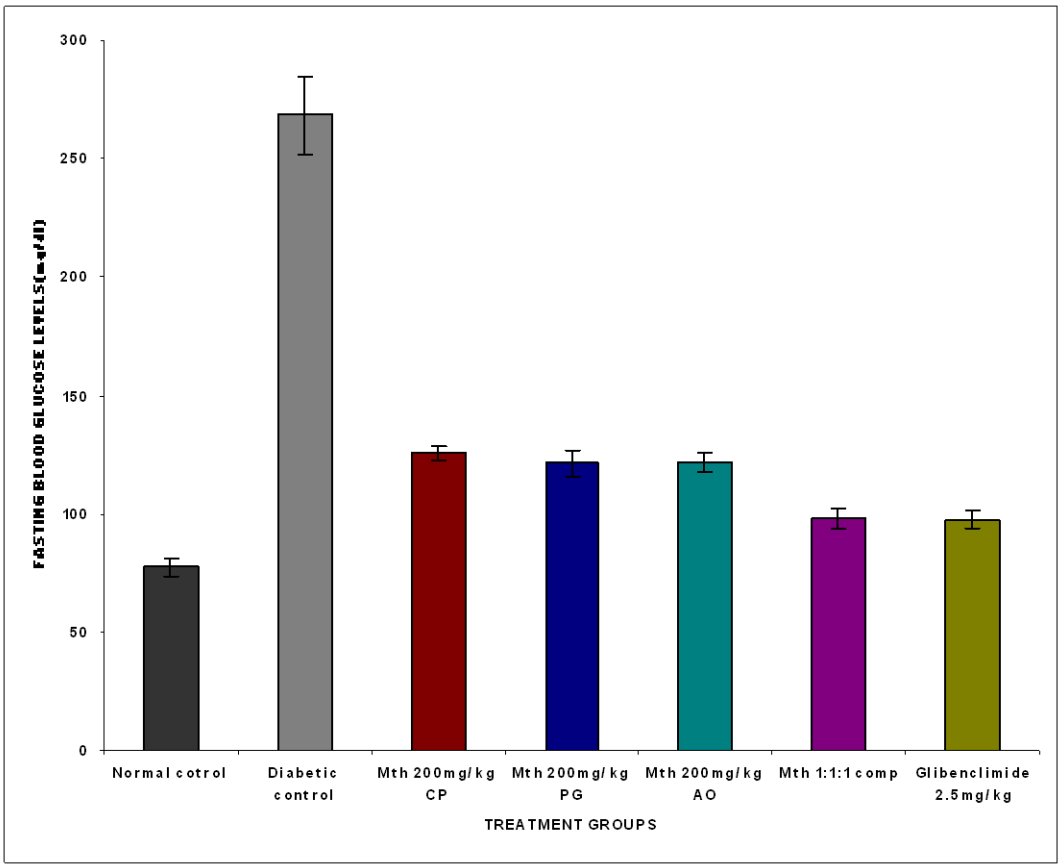


Figure I: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum fasting glucose Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group
Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$.

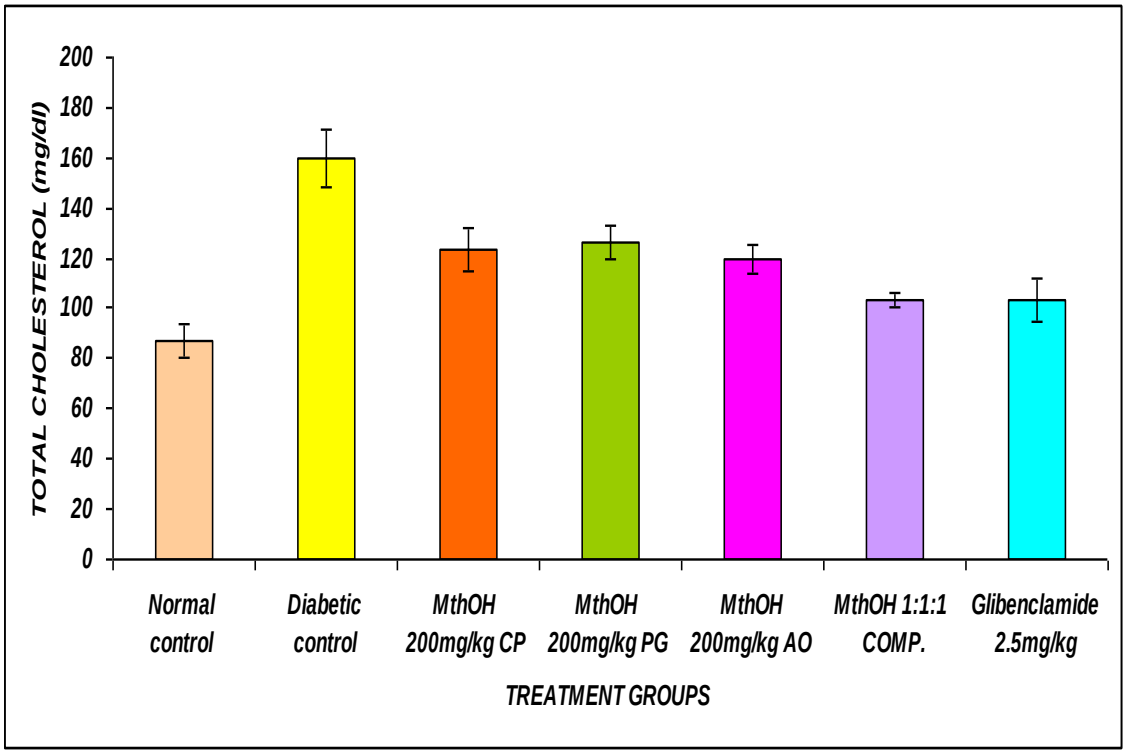


Figure II: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum Levels Total cholesterol of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

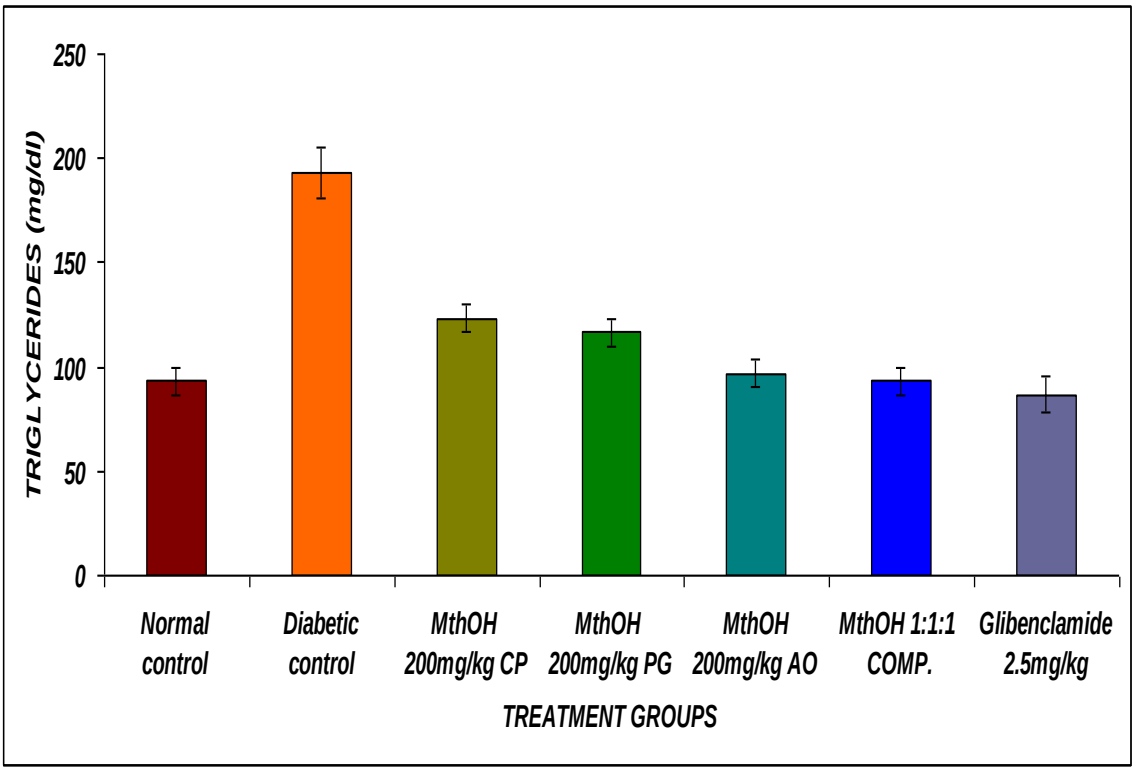


Figure III: Effect of daily oral doses of methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum Triglycerols Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

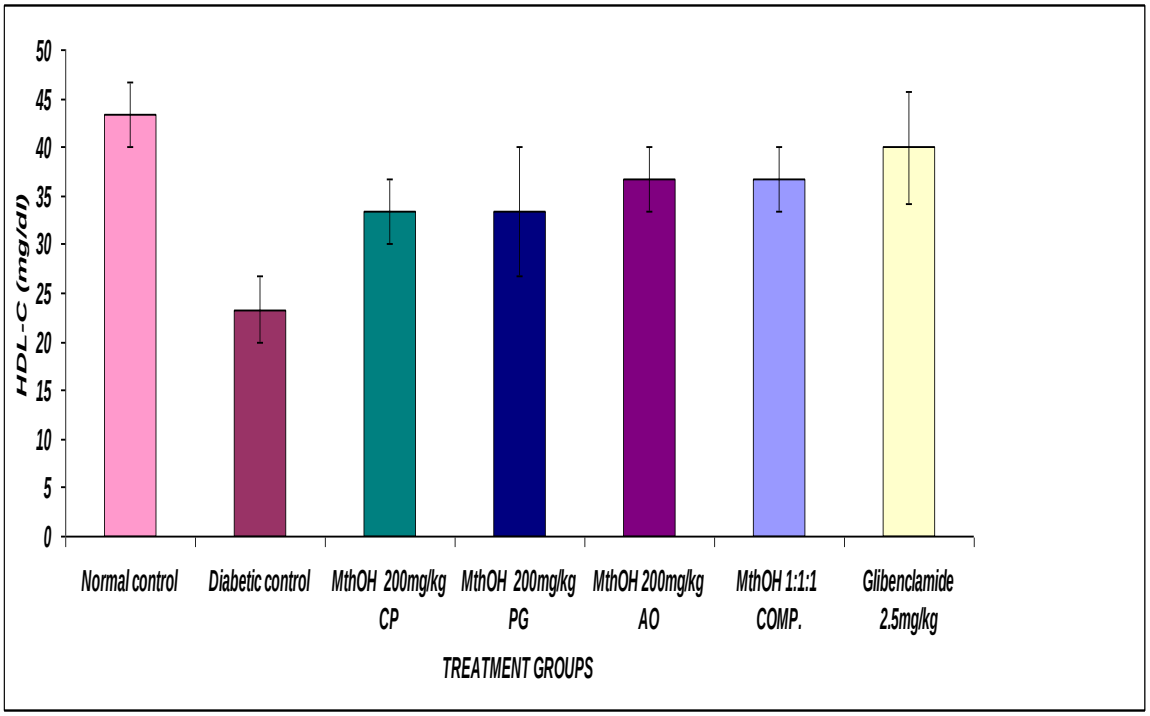


Figure IV: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum HDL Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

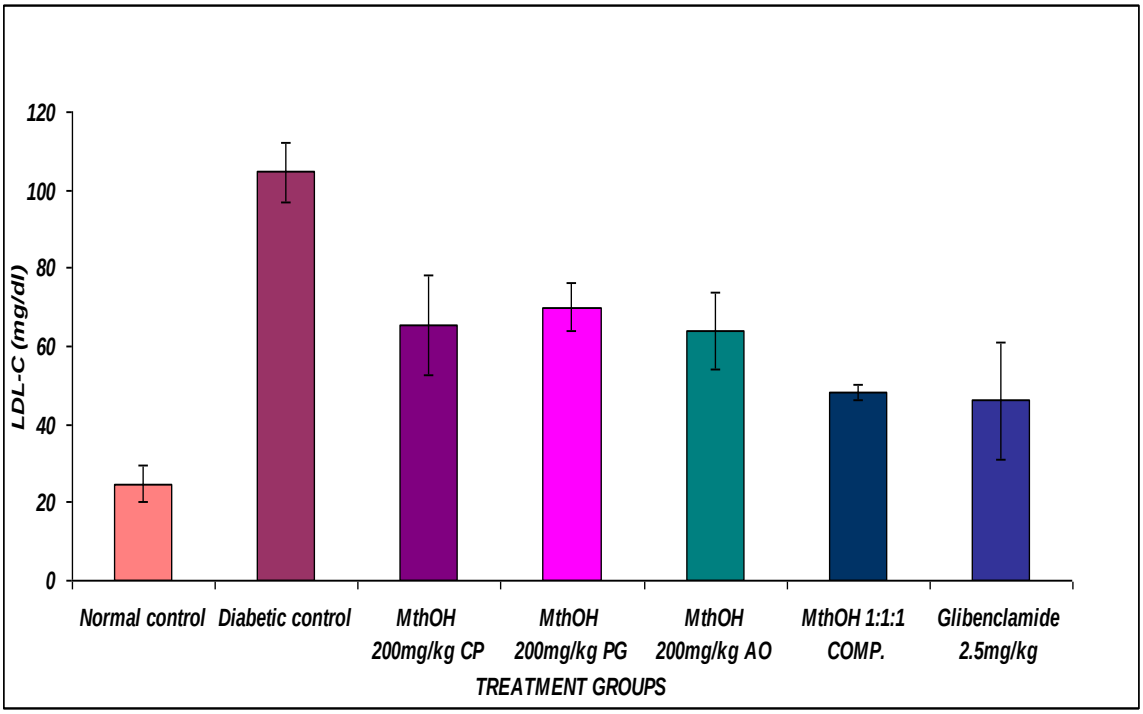


Figure V: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum LDL Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant

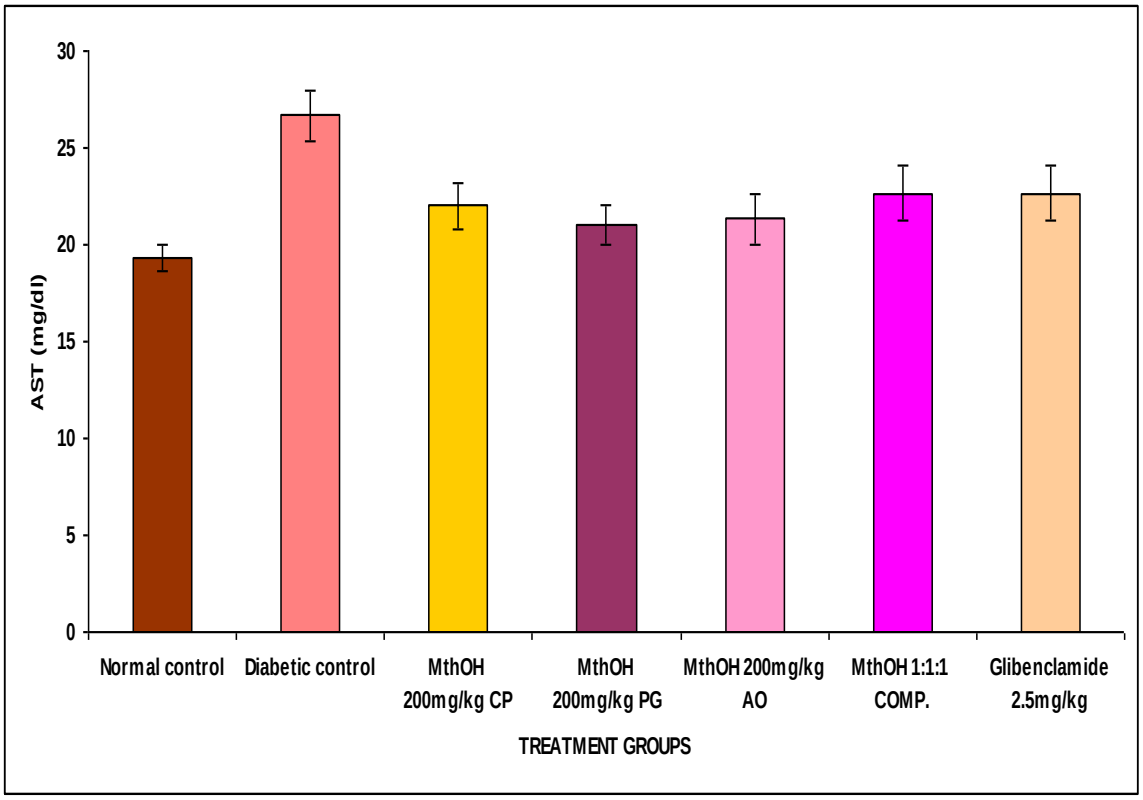


Figure VI: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum AST Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant

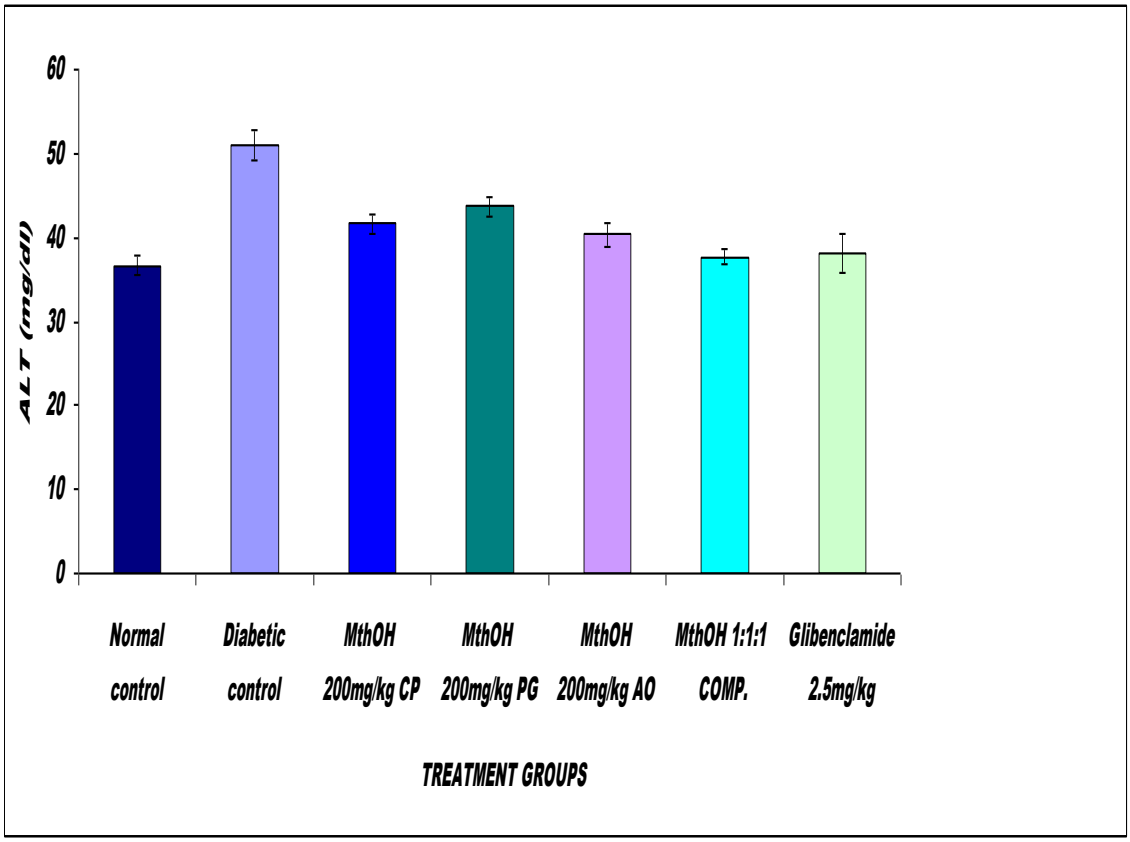


Figure VII: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum ALT Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

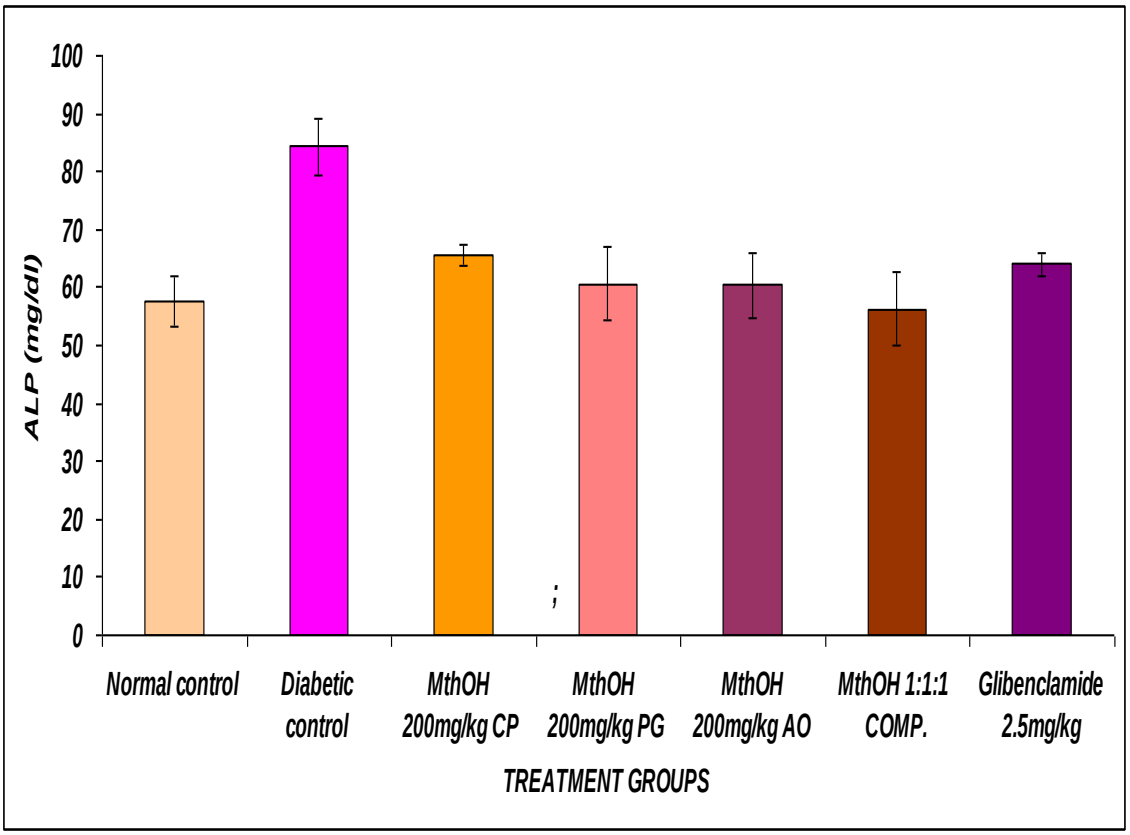


Figure VIII: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum ALP Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

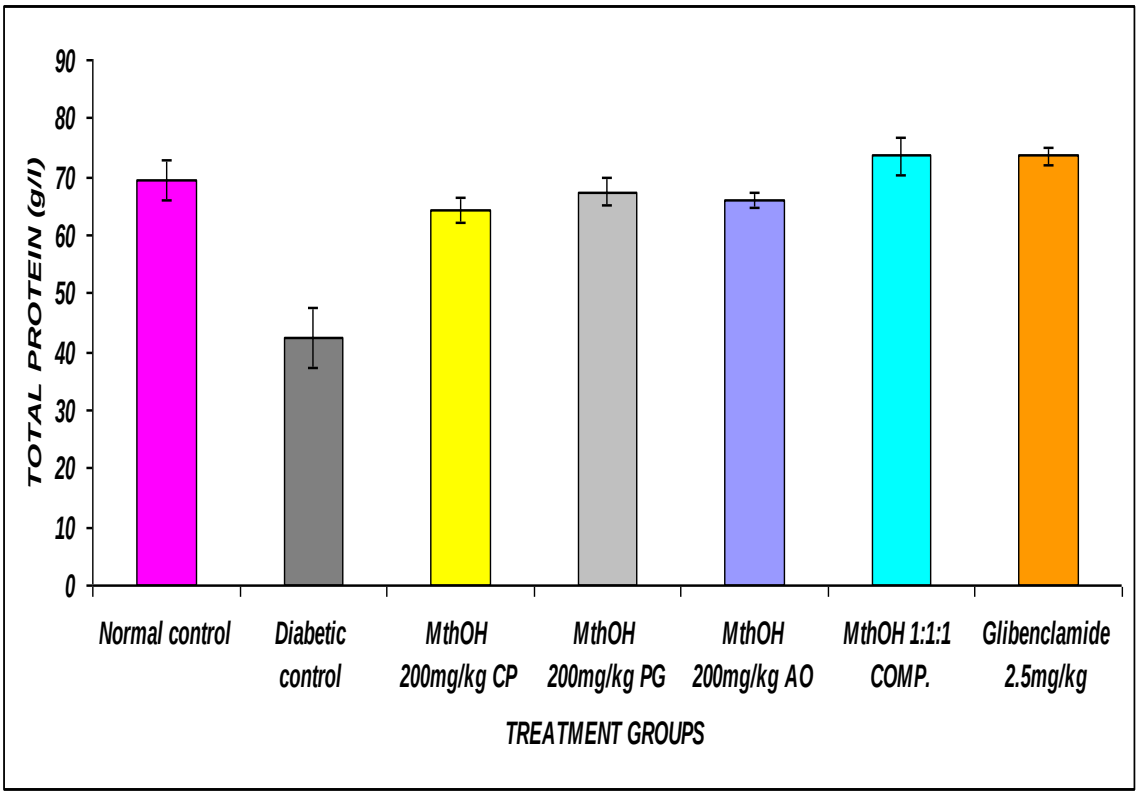


Figure IX: Effect of daily oral doses of methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum Total Protein Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

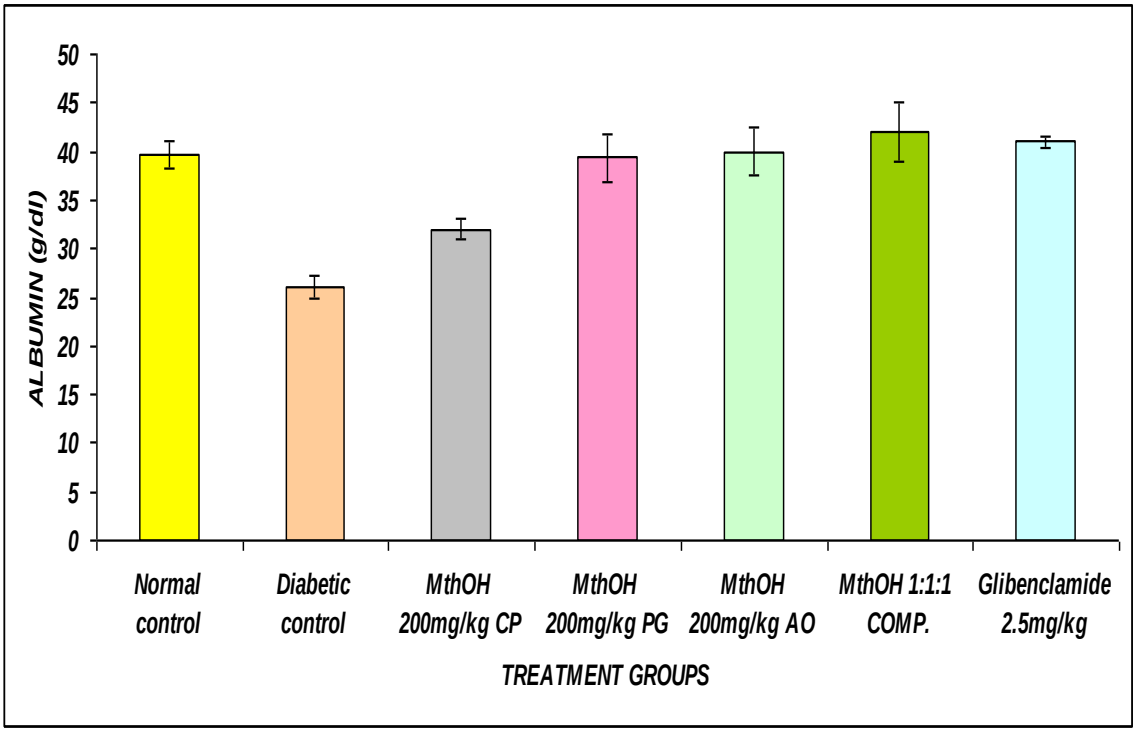


Figure X: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum Albumin Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

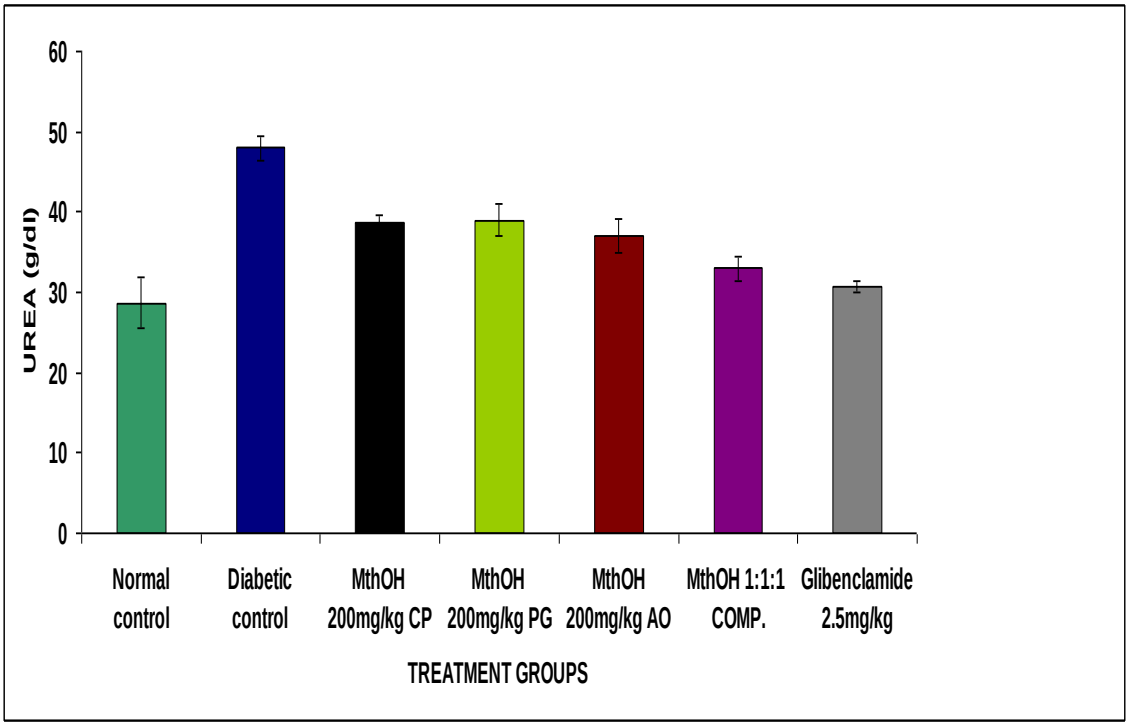


Figure XI: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Serum Urea Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

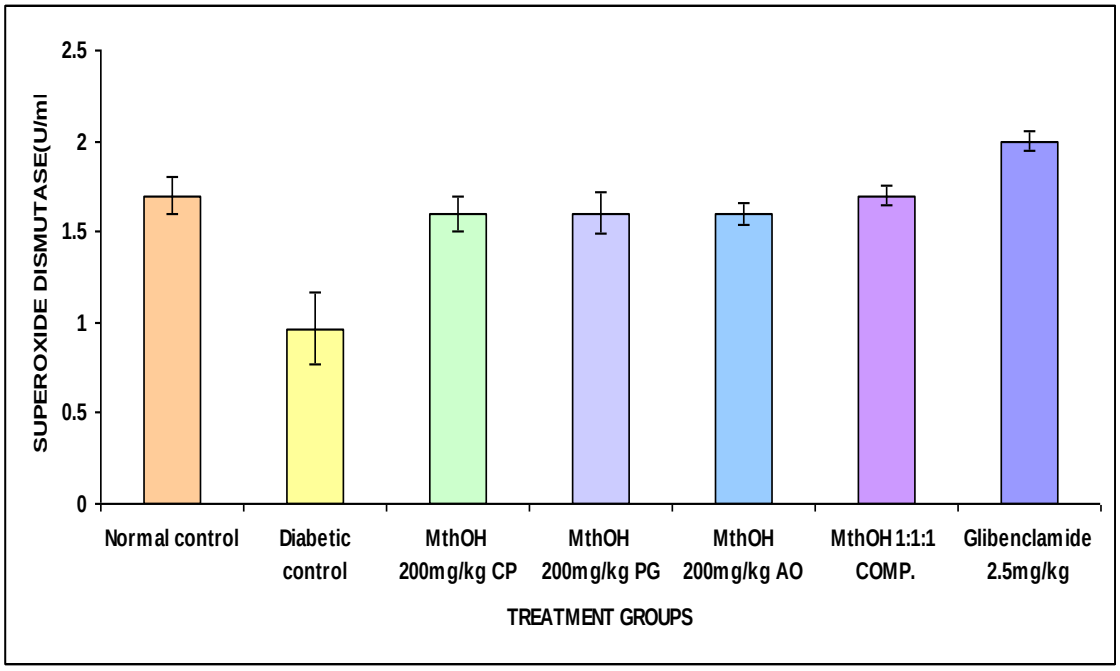


Figure XII: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Liver SOD Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

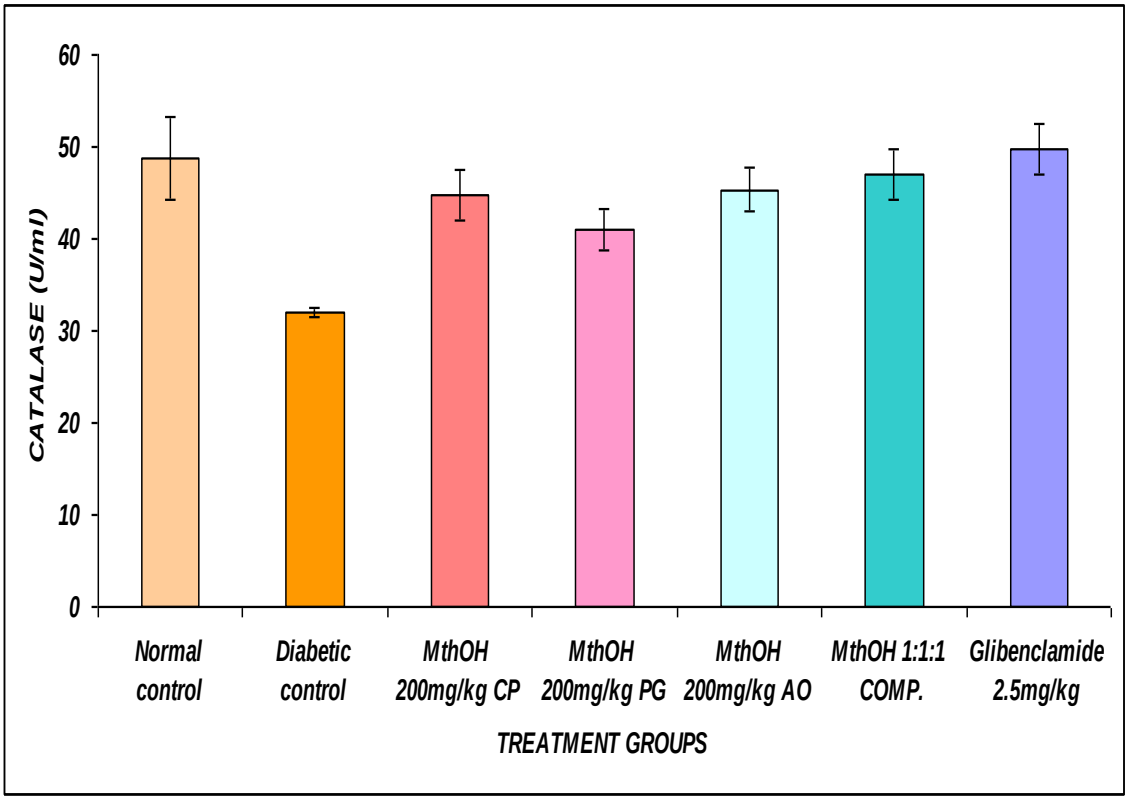


Figure XIII: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Liver Catalase Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

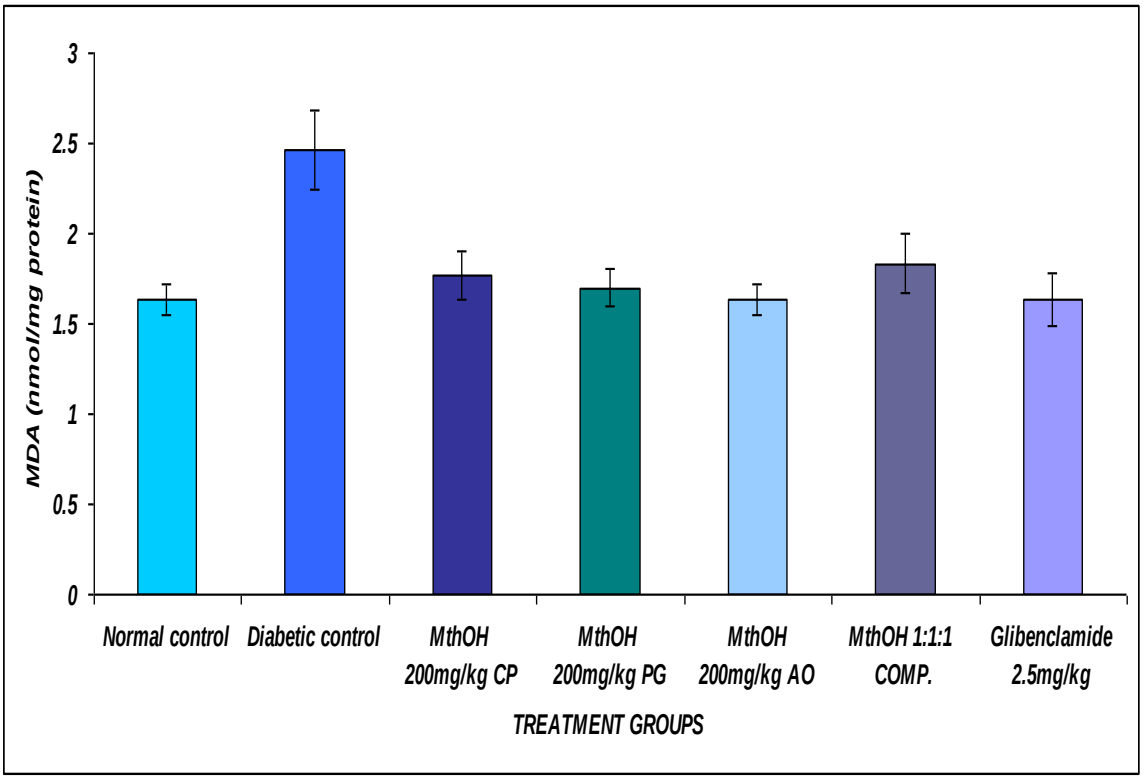


Figure XIV: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Liver MDA Levels of Normal and STZ-induced Albino rats

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

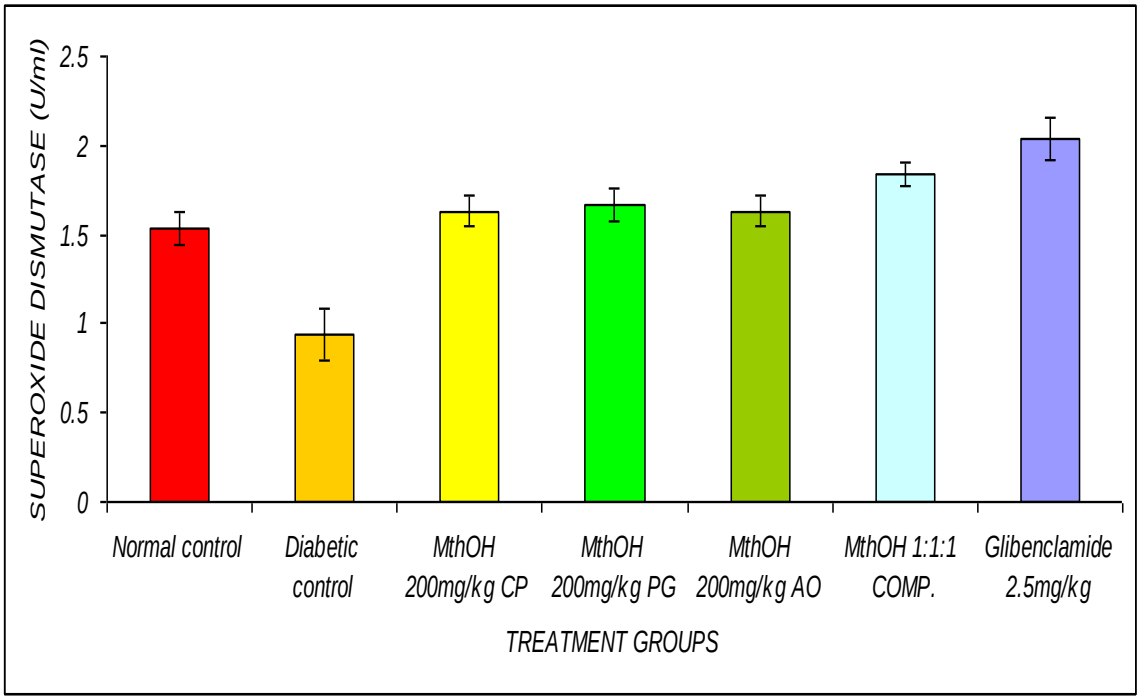


Figure XV: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Kidney SOD Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

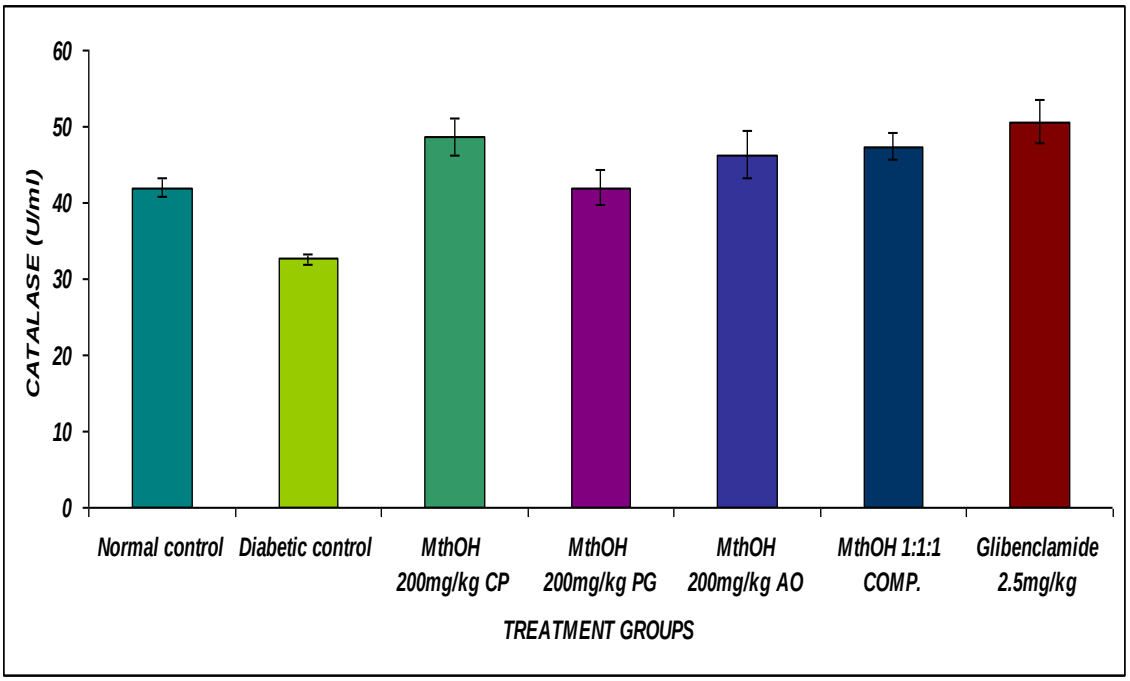


Figure XVI: Effect of daily oral doses of methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Kidney Catalase Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.

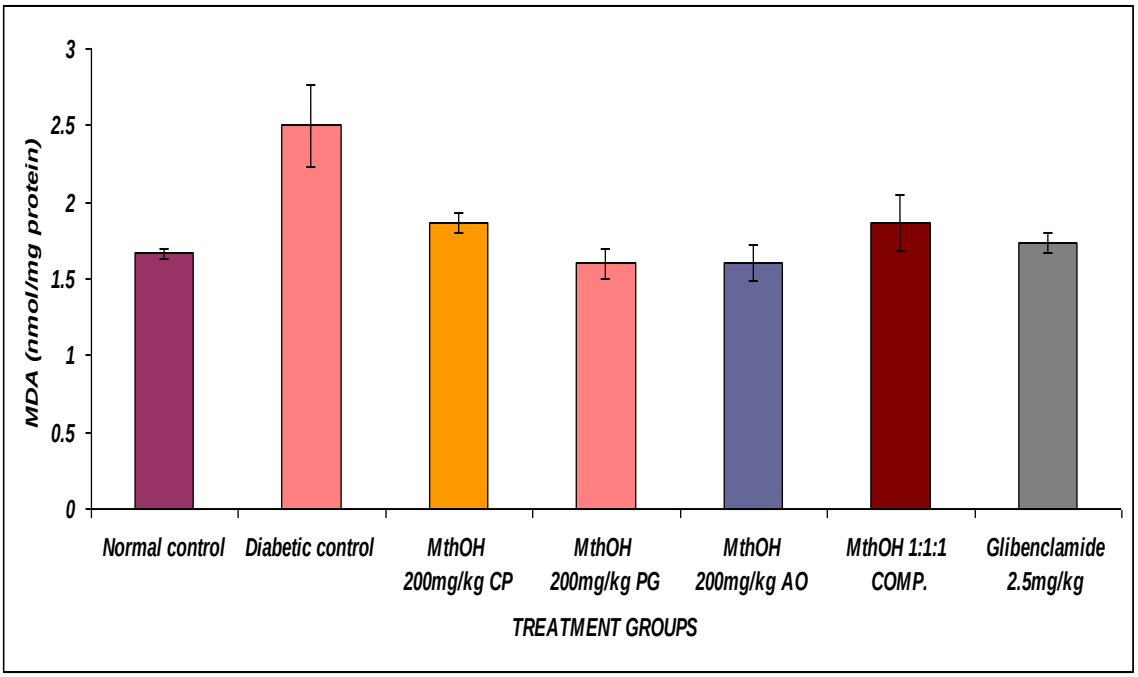


Figure XVII: Effect of daily oral doses of Methanolic leaf extracts of *C. papaya, P. guajava, A. occidentale and Composite* on Kidney MDA Levels of Normal and STZ-induced Albino rats.

Values are presented as mean ± SEM (Bar represents mean ± SEM n=5) for each group. Values are statistically significant compared to control group at a $p < 0.05$, b $p < 0.01$ while ns=not significant.