EVALUATION OF THE ANTIDIABETIC EFFECT OF *BASELLA ALBA* LEAF SUPPLEMENT ON STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

BY

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DECLARATION

I declare that this research in this dissertation entitled EVALUATION OF THE ANTI DIABETIC EFFECT OF *BASELLA ALBA* LEAF SUPPLEMENT ON STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS has been carried out by me in the Department of Biochemistry. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this university or any other institution.

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "EVALUATION OF THE ANTI DIABETIC EFFECT OF *BASELLA ALBA* LEAF SUPPLEMENT ON STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS" by Israel Ocheje HARUNA meets the regulation governing the award of the degree of M.Sc Biochemistry of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this dissertation to my parents, Mr. and Mrs. Joseph Haruna and to my siblings, Enoch, Faith and Praise.

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ABSTRACT

The present study evaluated the antidiabetic effect of *Basella alba* leaf food supplement on streptozotocin-induced diabetic Wistar rats. Twenty five mixed sex Wistar rats weighing between 150-170g were grouped into five groups of five animals each namely; Group A (normal control) non–diabetic rats were fed with normal rat feed; Group B (diabetic control) diabetic rats were fed with normal rat feed; Group C (standard control) diabetic rats received 1mg/kg body weight of glibenclamide orally daily; Group D (treated group) diabetic rats were fed with 10% (w/w) of *Basella alba* leaf supplement; Group E (treated group) were fed with 20% (w/w) of *Basella alba* leaf supplement. At the end of 21 days feeding experiment there was a significant ($p<0.05$) higher blood glucose level in diabetic untreated group when compared with normal control and treated groups. Groups fed with *Basella alba* leaf had a significant ($p<0.05$) lowered blood glucose level. There was a significant ($p<0.05$) low serum insulin level in diabetic untreated group when compared with normal control and treated groups. Also, there was a significant ($p<0.05$) decrease in serum insulin level in the *Basella alba* leaf supplemented feed when compared to the normal control group. Among the *Basella alba* supplemented treated group there was no significant ($p>0.05$) difference. Histopathology of the pancreas of the diabetic group showed necrotic islets of the cells compared to the normal control. Group treated with 20% *Basella alba* leaf showed moderate necrosis, all other treated group showed no difference when compared with diabetic group. Histopathology of the liver of the diabetic group shows necrosis of hepatocytes and kupfer cell hyperplasia compared to the normal control. We concluded from the study that *Basella alba* leaves have hypoglycemic effect on diabetic rats, although the plant leaf did not reverse the damage effect on the histopathology of the liver and pancreas within the time of study.

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LIST OF ABBREVIATIONS

AGEs	Advanced glycation and products
ADA	American diabetes association
ATP	Adenosine triphosphate
Ca	Calcium
CVD	Cardiovascular disease
DAN	Diabetic autonomic neuropathy
DC	Diabetic control
DM	Diabetes mellitus
DME	Diabetic macular edema
DNA	Deoxyribonucleic acid
DPN	Diabetic peripheral neuropathy
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and agriculture organization of the united nation
Fe	Iron
GABA	Gamma amino butyric acid
GH	Glycogen hepatopathy
GLB	Glibenclamide
GI	Glycemic index
GL	Glycemic load
H & E	Haematoxylin and eosin stain
IDDM	Insulin dependent diabetes mellitus
IDF	International diabetic federation
IAR	Institute of Agricultural Research
ICH	Insulin control high

ICL	Insulin control low
MNU	Methyl-nitrosourea
MI	Myocardial infarction
NAD	Nicotinamide adenine dinucleotide
NAFLD	Non-alcoholic fatty liver disease
NIDDM	Non-insulin dependent diabetes mellitus
NC	Normal control
PDR	Proliferative diabetic retinopathy
PAS	Periodic acidic schiff
RIA	Radio-immunoassay
ROS	Reactive oxygen species
STZ	Streptozotocin
TMB	Tetramethylbenzidine
UAE	Urine albumin excretion
WHO	World health organization

CHAPTER ONE

1.1 INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies resulting from defects in insulin secretion, insulin action or both and the disease is characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism (Maritim *et al.,* 2003; Ann *et al.,* 2005). Broadly, it is classified into three categories: Type-1, insulin-dependent diabetes mellitus (IDDM) caused by lack of insulin secretion (WHO, 2013); Type-2, called non-insulin-dependent diabetes mellitus (NIDDM) caused by decreased sensitivity of target tissues to the metabolic effect of insulin (WHO, 2013) and others (genetic defects of beta-cell, insulin processing and functioning, exocrine and endocrine defects, drug induced aberrations and gestational diabetes) (Guyton and Hall, 2011).

In Nigeria, with about 150 million people (2006 census), an estimated 3.1 million People have full blown diabetes mellitus (Osibogun, 2012) and this is expected to rise due to the trend in food consumption (Pereira *et al.,* 2005 Krishnan *et al.,* 2010; Pan *et al.,* 2011;). Diabetes is known to have a multifactorial pathogenicity and therefore, demands a multi-modal therapeutic approach. Great efforts have been made in the understanding and management of diabetes but serious problems like diabetic neuropathy (Shaikh and Somani, 2010), diabetic retinopathy (Schwartz and Flynn Jr., 2007), diabetic nephropathy (Djordjevic, 2001), hepatopathy (Levinthal and Tavill, 1999), cardiovascular diseases (Stratmann and Tschoepe, 2009) and reproductive problems (Baccetti *et al.,* 2002) continue to confront diabetic patients. The recognition of the potential role for nutraceuticals and dietary supplements in helping to reduce health risks and improve health quality is on the increase (Singh *et al.,* 2012). Many drugs are available for use in

the treatment of diabetes, but their long-term use may cause adverse side effects and hence, the increased search for natural remedies for the effective treatment of diabetes exists (Nabeel *et al*., 2010).

The major source of drugs for the treatment and management of diabetes mellitus in many parts of the world especially developing countries has been plants (Dhasarathan and Theriappan, 2011). Phytochemicals such as alkaloids, terpenoids, glycosides, flavonoids have been found to be an essential component of plants that are used in the treatment and management of diabetes mellitus (Loew and Kaszkin, 2002). Among these antidiabetic plants *Basella alba* can be included, because it contains these substances possessed by the already discovered antidiabetic plants such *as Azadirachta indica*, *Ficus racemosa, Abelmoschus moschatus, Acacia Arabica. Basella alba* belongs to the family *Basellaceae* native to tropical Asia, probably originating from india and Indonesia and extremely heat tolerant areas (Grubben and Denton, 2004). It is a fast growing vegetable, grown throughout the tropics as a perennial plant and in warmer temperature regions as an annual crop. It is low in calories by volume and high in protein per calories (Duke and Ayensu, 1985).

1.2 Statement of research problem

Diabetes is a major concern all over the world due to its complication that results in severe cellular damage and mortality (Mohammed *et al*., 2007) and the number of diabetic patients are expected to rise due to the trend in food consumption (Pereira *et al.*, 2005; Krishnan *et al.,* 2010; Pan *et al.,* 2011). Diabetes mellitus has been managed using many of the synthetic drugs developed over time, some of the drugs being used presently are insulin, meglitinides, sulfonylureas, thiazolidinediones, biguanides, metformin and glibenclamide.

However, some of these therapies have limited efficacy and adverse side effects like severe hypoglycemia, fatigue and gastrointestinal tract upset, risk of liver disease, anemia and swelling of legs or ankles. They are also contraindicated in patients with kidney failure, pregnancy and ketotic patients (Triplitt and Reasner, 2011). A 2013 review of prescription drugs by Express script survey shows that diabetes medications are among the top 10 most expensive and research indicates that diabetes medication expenses are only going to increase, and predicts that spending will continue to grow between 10% and 13% every year through to 2016.

1.3 Justification

Functional food and neutraceutical have important role to play in both the primary and adjunctive treatment of diseases (Alissa and Fems, 2012). There has been increased interest in finding naturally occurring antioxidants for use in pharmaceutical application which can protect human body from free radicals and retards the progress of many diseases such as diabetes mellitus (Prior, 2003).

However, there have been no satisfactory anti-diabetic drugs in use for the management of this disease. And there is an emerging trend in research to support the biological activities of dietary products in the management of many diseases such as diabetes mellitus. The philosophy that food can be health promoting beyond its nutritional value is gaining acceptance within the public arena and among the scientific community as mounting research links diet or food supplements to disease prevention and treatment such as diabetes mellitus.

Basella alba contains phytochemicals such as alkaloids, terpenoids, glycosides, and flavonoids which have been found to be essential components of plants that are use in treatment of diabetes

mellitus. The adverse effects of oral anti hyperglycemic agents and the paucity of information on the anti-diabetic potential of *Basella alba* leaf, necessitate this research.

1.4 Aim and Objectives

1.4.1 Aim

This study aimed at evaluating the antidiabetic effect of *Basella alba* leaf supplement on Streptozotocin induced diabetic Wistar rats.

1.4.2 Specific Objectives of the Studies

The specific objectives include:

- To evaluate the effect of *Basella alba* leaf on blood glucose level in Streptozotocin-induced diabetic Wistar rats.
- To evaluate the effect of *Basella alba* leaf on the level of insulin in Streptozotocin-induced diabetic Wistar rats.
- To investigate the Histopathological effect of *Basella alba* leaf on the Pancreas and Liver of Streptozotocin-induced diabetic Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Plant: *Basella Alba*

2.1.1 Taxonomy of the plant

Kingdom: Plantea

Phylum: Magnoliopsida

Order: Caryophyllales

Family: Basellaceae

Genus: *Basella*

Species: *alba*

Basella alba is a tropical leafy – green vegetable. It is a succulent, branched, smooth, twinning herbaceous vine, several meters in length. The stems are purplish or green. Leaves are fleshy, ovate or heart-shaped, 5 to 12cms long, stalked, tapering to a pointed tip with a cordate base. Spikes are axillary, solitary, 5-29cm long. Fruit is fleshy, stalkless, ovoid, 5-6mm long, and purple when mature (Kumar, 2010). *Basella alba* is grown in tropical lowlands at elevations near 500-3000 meters. Although, it also grows in temperate regions, Sandy loam soil is most suitable for its growth but thrives in many soils.

2.1.2 Medicinal uses and importance of *Basella alba*

The leaves and stem are mainly used for medicinal purpose (kumar, 2010). Consumption of *Basella alba* increases the storage of vitamin A in men (Haskell *et al*., 2004). Leaves of *Basella alba* is used for the treatment of hypertension by Nigerians. The plant possesses antimicrobial (Oyewole and Kalejaiye, 2012), anti convulsant, analgesic (Anandarajagopal *et al.*, 2011), anti-inflammatory (Chaitanya, 2012), central nervous system depressant (Anandarajagopal *et al.*,

2011) and it is used for the treatment of anemia. Decoctions of leaves are used for its mild laxative effects. It is also an astringent and the cooked roots are used in the treatment of diarrhea. The leaf juice is a demulcent, used in cases of dysentery (Kumar, 2010). Thai traditional medicine uses it as topical application for irritant, bruise, ringworm and labouring. Stem and leaves are used as mild laxative, diuretic and antipyretic (Chou, 1997). The leaves and stem has been used as an anti cancer agent such as melanoma, leukemia and oral cancer (Premalatha and Rajgopal, 2005).

2.1.3 *Basella alba* as an Alternative Therapy in the Management of Diabetes

Basella alba commonly called amunu tutu in Yoruba has been used in folk medicine as a remedy for diabetes. *Basella alba* may be included among the plants that possess anti-diabetic potential because it contains most of the substances possessed by the already discovered anti diabetic plants. This agrees with the report of Bamidele et al (2014) that the aqueous extract of Basella alba leaves significantly reduce the blood glucose level in alloxan-induced diabetic albino rats. The flowers are used as an anti dote for poisons (Duke and Ayensu, 1985). The aqueous extract of the leaves has been reported to reduces anaemia and maintain good health (Bamidele *et al*., 2010)

2.1.4 Other uses of *Basella alba*

Basella alba is commonly grown for its young shoots which makes an excellent succulent, slightly mucilaginous vegetable, used as a pot herb in stews or soup; consumed boiled, fried in oil or sometimes as a green salad (Ramu *et al.,* 2011). Its fruits seem to have been earlier used for dyeing purposes in China. The red fruit juice can be used as ink, cosmetic and for colouring foods (Ramu *et al.,* 2011). The red forms are commonly planted as ornamentals.

2.1.5 Nutritional value of *Basella alba*

Shoots of *Basella alba* per 100 grams (g) edible portion, contain water (91g), protein (2.1g), fat (0.3g) carbohydrates (3.9g) and fibre (1.3g). The energy value is approximately 112KJ/100g. The vitamin and mineral content vary widely as follows: Vitamin A, 1686-8000IU. Vitamin C, 29-166mg; calcium (Ca), 16-177mg; and iron (Fe); 1.2-3.1milligrams per 100grams edible portion (FAO, 1988)

2.2 Plant Phytochemical Constituents

The medicinal value of medicinal plants lies in some chemical substances that produce definite physiological actions in human body (Edeoga *et al.*, 2005). Antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases (Edeoga *et al.,* 2005). These secondary metabolites of plants are flavonoids, alkaloids, tannins, saponins, anthraquinones, cardiac glycosides and cyanoenic glycosides.

2.2.1 Saponins

Saponins are amphipathic glycosides consisting of a polycyclic aglycone that is either a choline steroid attached via C3 and an ether bond to a sugar side chain. This class of phytochechemical have hypotensive and cardiac depressant properties (Oladeye, 2007). Saponins bind to cholesterol to form insoluble complexes; as a result humans do not suffer severe poisoning from saponins. Endogenous cholesterol excreted via the bile combined with dietary saponins; this prevents cholesterol reabsorption and results in a reduction of serum cholesterol (Cheeke, 1971). Saponins is therefore, useful in the treatment of hypercholesterolemia by interfering with intestinal absorption of cholesterol (Malinow *et al*., 1977)

2.2.2 Tannins

These are known for their astringent property, which are capable of tanning leather and can precipitate proteins and various other organic compounds including amino acids and alkaloids. They are found in almost every plant part: bark, wood, leaves, fruits and roots (Edeogo *et al.*, 2006). There are two groups of tannins i.e. hydrolysable and condensed tannins. Condensed tannins (proanthcyanides) are derived from flavonoids monomers. Hydrolysable tannins are based on gallic acid usually as multiple esters with D-glucose. Stimulation of phagocytic cells, host-mediated tumor activity, and anti infective actions observed in humans are said to be due to the presence of tannins in plants (Brantner *et al.*, 1996) an example of its molecular action is to complex with proteins through a non-specific forces such as hydrogen bond, hydrophobic effects and covalent bond formation (Edeoga and Gomina, 2000). Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesion; enzymes and cell envelopes transport proteins.

2.2.3 Flavonoids

Flavonoids are found in varying amounts in foods and medicinal plants. They exert potent antioxidant activity against the superoxide radical (Hertog *et al.*, 1993). Flavonoid is hydroxylated phenolic substances but occurs as a C3-C6 unit linked to an aromatic ring. They are synthesized by plants in response to microbial infection (Salisburg and Ross, 1992) they are effective antimicrobial substances against a wide array of microorganisms. Their activity is due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Lipophilic flavoid disrupt microbial membrane (Salisburg and Ross, 1992).

2.2.4 Alkaloids

Alkaloids are complex nitrogenous base in which the nitrogen is contained in a heterocyclic ring system. Alkaloids are widely used as therapeutic agent (Noble, 1990). Alkaloids are found in the form of salts with organic acids and are haemolytically active and are also toxic to microorganisms.

2.2.5 Cardiac glycosides

Cardiac glycosides are a class of triterpenoids compounds (Brian *et al.,* 1985) and their activity resides in the aglycone portion of their sugar attachment. They act directly on the smooth muscle of the vascular system. They exert a number of effects on neutral tissue and thus indirectly influence the mechanical and electrical activities of the heart and modify vascular resistance and capacitance (Oladeye, 2007).

2.3 Medicinal Plants in Traditional Medicine

Traditional medicine involves the use of herbs (medicinal plants) in the form of decoction, infusion, tisane, concoction with objectives of curing or preventing diseases (Kingsley, 2015). A decoction is a preparation of plant in cold, warm or boiled water. While concoction is a drink, soap preparation made from many plants and other ingredients. Infusion is a plant material on which boiling water is added. Tisane is an aqueous preparation of plant made by decoction or infusion. The use of medicinal plants is the most ancient approach to healing, practiced by traditional medicine practioners. A plant can be said to be medicinal when one or more of its parts contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (WHO, 2005).

World health organisation (WHO) has recommended that the ‘vegetable drug’ be applied to that part of a medicinal plant, such as leaf, bark etc used for therapeutic purposes. Herbal treatment is the conventional type of treatment (Akpata, 1979); it does not require incantation and other ceremonies such as sacrifice before the medicinal plant can act.

2.4 Medicinal Plants as Sources of Modern Drugs

Plants produce various organic substances with potential values in the treatment of diseases. It therefore become necessary to determine what these substances are, from what plants they are extracted, in order to eliminate the use of plant material which for whatever reason may be of no value. Research has enhanced the value of medicinal plants and through research medicinal plant has made a great contribution to modern medicine. This research consists of investigating all reputed therapeutic values ascribed to plants by herbalists and knowing the active component of the plant. (Sandbery and Bruhn, 1979).

A number of medicinal plants are in common use, but from ethnomedical considerations not all medicinal plants are safe for use. Some of the plants used as medicine are toxic as it has been revealed that some contain hepatotoxic and carcinogenic constituents (Sofowora, 1993). Despite their toxic effects, these plants are still very much in use in traditional medicine. This maybe because the dosage at which the constituent is utilized in herbal medicine is not sufficient for it to be toxic. Also, many of the plants used are in combination with other agents which may have attempting effect or counteract the toxic constituents (Sofowora, 1993).

Some medicinal plants are food items or of agricultural interest, for example, *Zea mays, Phaseolus vulgaris, Nicotiana tobacu , Pisium sativum,* etc. Some African medicinal plants have been thoroughly screened phytochemically and their active constituents found. Some of these

constituents have antimicrobial, anticonvulsant, and antihypertensive, sedative, tranquilizing and insecticidal properties. The anti sickling effect of *Zauthoxylium fagara* species have been investigated by (Isaac and Sofowora, 1971) while the *ocium gratissium* herb which produces a volatile oil has been shown to have both antibacterial (Nakaruma *et al.*, 1999) and antihelmintic properties (Pessoa *et al.*, 2002). Most medicinal plants are administered as crude extracts and the active principle of many are yet to be identified but many important orthodox drugs of the modern world have medicinal plants as their sources (Myers, 1982). Below are some example of African medicinal plants, their medicinal uses and active constituents:

Plants	**Therapeutic use**	**Essential ingredient**
Papaver somniferum	Analgesics, antipyretic	Morphine
Cephaetis pelacanna	Antiprotozoan	Emetine, quinine
Clavieps pupurea	Antimigraine	Ergotamine
Raawolfia serpentine	Cardiovascular	Reserpine
Catharanthus roseus	Antihypertensive	Raubasine

Source: Sofowora, 1993.

2.5 Diabetes

Diabetes mellitus is a metabolic disorder of multiple etiologies resulting from defects in insulin secretion, insulin action or both and the disease is characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism (Maritim *et al.*, 2003).

People with diabetes cannot properly process glucose, a sugar the body uses for energy. As a result, glucose stays in the blood, causing blood glucose to rise. At the same time, the cells of the body become starved of glucose. Broadly, diabetes is classified into three categories: Insulin – dependent diabetes mellitus (IDDM) or type 1 or childhood onset diabetes. In type 1 diabetes, the pancreas cannot make the insulin needed to process glucose. Natural therapies cannot cure type 1 diabetes but they may help by making the body more receptive to insulin supplied by injection. It is particularly critical for people with Type 1 diabetes to work carefully with the doctor prescribing insulin before contemplating the use of any herbs, supplements or dietary changes. Any change that makes the body more receptive to insulin would require critical changes in insulin dosage that must be determined by the treating physician.

Non-insulin-dependent diabetes mellitus (NIDDM) also called type 2 or adult onset diabetes. In type 2 diabetes the pancreas often makes enough insulin, but the body has trouble using the insulin. Type 2 diabetes frequently responds well to natural therapies.

And others which could be due to genetic defects of beta-cell, insulin processing and functioning, exocrine and endocrine defects, drug induced aberration and gestational diabetes. People with diabetes have a high risk for heart disease and atherosclerosis'. In addition, those with diabetes have a higher mortality rate if they have high homocysteine levels (Hoogeveen *et al.*, 2000). Eating carbohydrate containing food whether high in sugar or high in starch (such as

bread, potatoes, processed breakfast cereals and rice) temporarily raise blood sugar and insulin levels (Wolever and Brand Miller, 1995)

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates fat and protein. This results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems (Noor *et al.,* 2008). Management of diabetics without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicines for diabetes as well. Although herbal medicines have long been used effectively in treating disease in Asian communities and throughout the world the mechanism of most of the herbs has not been defined/elucidated. Many traditional plants treatment for diabetes are also used, but most of the evidence for their beneficial effects is anecdotal (Bailey and Day, 1989). Traditional anti-diabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines/present day drugs for many rural populations in many developing countries. Medicinal plants like *Trigonella foenum, Allium sativum, Gymnema slyvestre and Zyzigium cumini* have been studied (Grover *et al.,* 2002) for treatment of diabetes mellitus. However, detailed studies on the efficacy, mechanism of action and safety of plant extract are needed. Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world (Burke *et al.,* 2003). Diabetes is one of the leading causes of death in humans and animals. In animals it occurs most frequently in the dog with an incidence of approximately 0.2%. World health organisation (WHO, 1980) has recommended the evaluation of the effect of plants in conditions

where there are no safe modern drugs. The ethno botanical information reports state that about 800 plants may possess anti-diabetic potential (Aguilaza *et al*., 1998).

Recently, the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research. (Daisy and Eliza, 2007; Noor *et al*., 2008). Various parts of herbs have been used for medicinal purposes including the treatment of diabetes mellitus.

2.6 Complications of Diabetes

Hyperglycaemia is the initiating cause of diabetic tissue damage and the process is modified by both genetic determinants of individual susceptibility and by independent accelerating factors such as hypertension and hyperlipidaemia (Brownlee, 2005).

2.6.1 Neuropathy

Neuropathy (disease or abnormality of the nervous system) is a microvascular complication of diabetes mellitus which results in considerable morbidity and a decreased quality of life (Van Acker *et al.,* 2009; Mijnhout *et al.,* 2010). It is characterized by a slowly progressive, length-dependent loss of sensation that correlates with duration of diabetes and glycaemic control (Kern *et al.,* 2009). Neuropathy is the most common complication of diabetes mellitus and occurs in 60% of the patients and affects their quality of life (Shaikh and Somani, 2010). Diabetic neuropathy causes foot ulceration, may lead to amputation and chronic pain with reduced quality of life and the most common among the diabetic neuropathies are diabetic peripheral neuropathy (DPN) and diabetic autonomic neuropathy (DAN). Shaikh and Somani (2010) reported that many abnormalities that are found in diabetic patients with neuropathy, including hyperalgesia (extreme sensitivity to pain), allodynia (pain that results from a non-injurious stimulus to the

skin), slow nerve conduction velocity and progressive sensory and sensory motor deficit are seen in diabetic rodents.

2.6.2 Retinopathy

Diabetic retinopathy (disorder of retinal blood vessels) is often the cause of new cases of blindness among adults aged 20–74 years and the duration of diabetes is probably the strongest predictor for development and progression of retinopathy (Fong *et al.*, 2004). Diabetic retinopathy is duration-dependent which develops in stages and it is often not detected in the first few years of diabetes, but increases to 50% by 10 years and to 90% by 25 years of diabetes (Kowluru and Chan, 2007). It is regarded as a disease of the retinal microvasculature and has been divided into an early, non-proliferative (or background) stage, and a later, proliferative stage (Kern, 2007). The two most vital visual complications of diabetic retinopathy are diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) (Schwartz and Flynn Jr., 2007). Retinopathy is characterized by a spectrum of retinal lesions and abnormalities that show vascular damage and death or dysfunction of the neural retina (Kern *et al*, 2009). At all stages of retinopathy, macular edema, characterized by retinal thickening from leaky blood vessels, can develop (Fong *et al.*, 2004). In this diabetic complication, the microvasculature of the retina is damaged, the blood vessels swell and seep out fluid and if not prevented, new vessels start to grow, which eventually lead to the detachment of the retina (Frank, 2004; Aylward, 2005; Kowluru and Chan, 2007).

2.6.3 Nephropathy

Diabetic nephropathy (diabetic kidney disease) is a major microvascular complication (Anjaneyulu and Chopra, 2004). It is categorized into stages: microalbuminuria, the presence of

small amounts of albumin in the urine (UAE > 20 μg/min and ≤ 199 μg/min) and macroalbuminuria, the presence of high amounts of albumin in the urine (UAE ≥ 200 μg/min) (Gross *et al.,* 2005). Diabetic nephropathy occurs in ≈ 30% of people with type 1 diabetes and 25-40% of people with type 2 diabetes often, irrespective of glycaemic control (Hall, 2006). The presence of microalbuminuria is considered to be a manifestation of renal and generalized endothelial injury and strongly predicts progressive diabetic nephropathy and cardiovascular risk and hence, microalbuminuria appearance is used as an important indicator of effective treatment intervention (Hall, 2006). It is the chief cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality (Gross *et al.,* 2005). Pathophysiological changes associated with diabetic nephropathy include renal and glomerular hypertrophy, mesangial cell hypertrophy and matrix accretion, glomerular basal membrane thickening and functional alterations in glomerular filtration barriers (Djordjevic, 2001).

2.6.4 Hepatopathy

Diabetic hepatopathy (disease of the liver) causes lesions to develop in the liver. Diabetic patients have a high prevalence of liver disease and it is an important cause of death in type 2 diabetes (Abolfathi *et al.*, 2011). Increased occurrence of liver disease arises in both type 1 and type 2 diabetic patients, resulting in an increased prevalence of hepatic complications (Albright and Bell, 2003). Liver disease such as abnormal liver enzymes, non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and acute liver failure are seen in patients with type 2 diabetes (Abolfathi *et al.*, 2011). There has been a reported increase in the incidence of cirrhosis and cholelithiasis (presence of stones in the gall bladder) in diabetes mellitus and conversely, at least 80% of patients with cirrhosis have glucose intolerance (Levinthal and

Tavill, 1999). Excess hepatic glycogen and fat accumulation are also reported to be seen in diabetic complications (Levinthal and Tavill, 1999). In poorly controlled diabetes, glycogen hepatopathy (GH) has been characterized as a pathologic overloading of hepatocytes with glycogen which leads to clinical signs and symptoms such as abdominal discomfort, tender hepatomegaly and elevated transaminases (Fridell *et al.,* 2007). It is also the rare cause of elevated serum transaminases, mostly confined to type 1 diabetics (Van den Brand *et al.,* 2009).

2.6.5 Cardiovascular diseases

Cardiovascular disease (CVD) is a class of diseases which affect the heart and/or blood vessels and it is frequently linked with any disease that affects the cardiovascular system such as atherosclerosis (Oguntibeju *et al.,* 2009). Diabetic patients have an increased risk of cardiovascular disease (Desouza *et al.,* 2010). There is an increase in the correlation of type 2 diabetes and the death rate from cardiovascular disease which is two-fold to eight-fold higher in diabetics than people without diabetes (Grundy *et al.,* 2002; Lago *et al.,* 2007). The leading cause of death in diabetes mellitus (DM) is cardiovascular disease and it has been implicated in more than 80% of the cases of the diabetes disease (Selvaraju *et al.,* 2012). About 80% of all patients with CVD may have diabetes or impaired glucose tolerance (Giugliano *et al.,* 2009). Diabetes mellitus is a major cause of cardiovascular morbidity and mortality in developed countries and atherothrombosis (a condition in which a thrombus originates in an atheromatous blood vessel) is the cause of most deaths among diabetic patients (Stratmann and Tschoepe, 2009). Atherothrombosis comes up as a result of atherosclerosis progression with clinical manifestations such as sudden cardiac death, myocardial infarction (MI), ischaemic stroke, and peripheral arterial ischaemia (Stratmann and Tschoepe, 2009). In diabetic patients, the interaction of auto-antibodies with AGEs is capable of forming AGE-immune complexes which

may play a role in atherogenesis (Turk *et al.*, 2001). Atherogenesis involves endothelial dysfunction, activation and injury, inflammation, and smooth muscle cell migration and proliferation (Mehta *et al*., 2006). One mechanism by which diabetes promotes atherosclerosis is through abnormal lipid metabolism (Dokken, 2008). Insulin deficiency and insulin resistance promote dyslipidaemia with an increased oxidation, glycosylation, and triglyceride enrichment of lipoproteins (Dokken, 2008).

2.6.6 Reproductive damage

Erectile dysfunction is a common complication of diabetes (Agostini *et al.,* 2006). Diabetes has been linked with reproductive impairment in both men and women (Baccetti *et al.,* 2002). The occurrence of sexual dysfunction in diabetic men approaches 50% while diabetic women seem to be slightly lower (Amaral *et al.,* 2008). This deleterious effect on male reproductive function is possibly through an increased production of reactive oxygen species and imbalance between antioxidants and oxidants (Amaral *et al.,* 2006). Thakur and Dixit (2008) also reported that oxidative stress is increased in diabetes resulting in impaired sexual dysfunction and impotence in the modern world. Diabetes causes damage to nerves throughout the body which includes the penis (Agostini *et al.,* 2006). In diabetic men, poor semen quality which includes decreased sperm motility and concentration, abnormal morphology, increased seminal plasma abnormalities as well as decreased serum testosterone due to impaired Leydig cell function have been reported (Amaral *et al.,* 2008). The sexual problems in diabetic women include decreased sexual arousal with slow and/or inadequate lubrication and sexual desire (Enzlin *et al.,* 2002) and good glycaemic control would be essential to restore a normal sexual activity in diabetic women (Bultirini *et al.,* 2004). Streptozotocin caused testicular dysfunction and degeneration under situations of experimentally induced diabetes in animals (Shrilatha and Muralidhara, 2007) and

alloxan-induced diabetes in male rats was reported to reduce semen parameters and impair distinct phases of spermatogenesis (Arikawe *et al*., 2007).

2.7 Glycemic Index

The blood sugar-raising effect of a food is called its "glycemic index" and it depends on how rapidly its carbohydrate is absorbed. Many starchy foods are said to have a glycemic index similar to sucrose (Wolever and Brand Miller, 1995). Consumption of large amount of foods with high glycemic indices, have been reported to have increase the risk of type 2 diabetes (Salmeran *et al.,* 1997[a]; Salmeran *et al.,* 1997[b]). But consumption of diet high in carbohydrate rich foods with low glycemic indices is associated with a low risk of type 2 diabetes (Feskens *et al.,* 1995)

Beans, peas, fruits and oats, have low glycemic indices despite their high carbohydrate content, due mostly to the health-promoting effects of soluble fiber. Diabetes disrupts the mechanism by which the body controls blood sugar. Health professionals have recommended sugar restriction to people with diabetes (Colagiur *et al.,* 1989). The American diabetes association (ADA) guideline do not prohibit the use of moderate amounts of sugar, as long as the goals of normalizing blood levels of glucose, triglycerides and cholesterol are being achieved. It is mostly recommended that people with diabetes should cut intake of sugar from snacks and processed foods and replace the food with high fiber. Other authorities also recommend lowering the glycemic index of the diet to improve the control of diabetes. High fiber diet has been shown to work better in controlling diabetes than the diet recommended by the ADA and may control blood sugar levels as well as oral anti-diabetic drugs (Chandalia *et al*., 2000).

Glycation end products tend to accumulate as a result of disorders of sugar metabolism such as diabetes, it is generally believed that a diet low in refined carbohydrates is a logical choice for those with diabetes. Diets naturally high in vitamin C have been shown to result in reduced protein glycation (Boeing *et al.,* 2000). Though this would not stop all glycation end products from forming and accumulating, it may reduce their formation and possibly delay the onset of some of the complications associated with diabetes. Reducing weight, increasing consumption of fiber, exercise, and reducing the intake of saturated fats also reduce the risk of developing diabetes, even for those with impaired glucose tolerance (Tuomilehto, 2001). Consumption of whole grains is also associated with reduced risk of developing diabetes (Montonen *et al.,* 2003). This could be due to the natural B vitamins. Similarly, nut consumption has been found to be inversely associated with the risk of type 2 diabetes mellitus (Jiang *et al.,* 2002). It has been reported that wheat, soy and perhaps other foods can provoke pancreatic beta cell destruction through immunological mechanism in some people (Werbach, 1999). In type 1 diabetes mellitus, hyperglycemia results in the intracellular accumulation of the sugar alcohol sorbitol by the action of aldose reductase on glucose (Cunningham *et al.,* 1994). Based on this, sorbitol and similar sugar alcohols should probably be avoided. It has been proposed that insulin dependent diabetes may be started by an immunization from environmental proteins such as a virus with molecular mimicry with the islet cell enzyme glutamic acid dehydrogenase, initiating the process (Maclaren, 1992). In the islets, GABA (gamma amino butyric acid) inhibits glucagon secretion. In susceptible individuals the anti enzyme glutamic acid dehydrogenase immune response may be sufficient to initiate islet cell auto immunity and damage. Auto antibodies to insulin and islet cell cytoplasmic glycolipid antigens resulting to the damage of beta cells. Type 1 diabetes mellitus occurs only after destruction of the bulk of the insulin secreting beta cells. The entire

process usually takes years to complete but is faster in young children than adults. Eating frequently small meals can also be helpful in keeping blood glucose levels in more desired ranges (Werbach, 1999). Low intensity exercise, such as walking, has been found to significantly reduce blood-glucose level in type 2 diabetes mellitus (Fritz and Rosenquist 2001). Caloric restriction and exercise are among the best ways to reduce insulin resistance. Additionally, "high carbohydrate, high fibre diets increase insulin sensitivity and decrease insulin requirements" while caffeine may decrease it. Thus caffeine itself is not advised for diabetes (Keijzers *et al.*, 2002)

2.7.1 Glycemic factors

Nearly all calories are converted by the body into glucose (blood sugar). If the amount of glucose converted is excessive, this will trigger the body's need to secret insulin. But all caloric foods are not equal in their conversion to glucose; some foods take longer time to be converted. Those foods that take longer time to convert have lower glycemic effect, whereas those that convert quickly (in a short time) are known to have a higher glycemic effect. Those foods with a low glycemic effect do not make the body to secret insulin because they provide glucose at a rate that is consumed rather than accumulated. Food like fruits, vegetables, whole grains are preferable for diabetes as well as for basically everyone else.

Foods behave differently because of the particle size, for example, whole oats behave differently from instant oats. This realization that different food raised glucose levels differently resulted in a method of evaluating foods known as glycemic index (GI). The GI measures the degree to which foods trigger a rise in blood sugar levels. Foods with low GI provide smaller, more sustained elevations and provide a near steady supply of glucose and energy. Foods with high

GI, prompt rapid blood sugar spikes, followed by equally dramatic plummets. The concept of GI has been expanded into something more practical by Jenkins *et al.,* (1981); the Glycemic Load (GL). Glycemic load takes into account quality and quantity. It is determined by both the GI of a food, plus the amount available and net carbohydrates (fibre excluded) in a standard serving. The GL has revealed a few surprises. Some foods with a high GI actually have minimal effects on blood sugar levels when eaten in normal quantities: others with low GI are potentially problematic. For example, a large carrot and a cup of spaghetti have similar GIs. Yet carrot contains only 5grams of available carbs (it is mostly H_20) while the spaghetti contains 38grams, giving them GLs of 2 and 16 respectively (Whitake, 2005).

2.8 Beneficial Food Nutrient

Many nutrients have been found to be of benefit to people with diabetes, B vitamins are involved in the production of energy as well as other metabolic processes, B complex vitamins, potassium and trace minerals are often recommended for diabetics, particularly for the prevention and reversal of oxidative dysoxynative insulin. Omega–3 fatty acids are required for optimal function of the body. They are linked to improved well being and a lower risk of many serious diseases (Ruxton *et al.,* 2004). Phycocyannins and carotenoids are antioxidant with powerful anti inflammatory activity (Ku *et al.,* 2013). Calcium and vitamins D has been link to lower the risk of type 2 diabetes in people with pre- diabetes (Mitrie, 2013) as calcium and vitamin D may have direct effect on the pancreatic beta cells to enhance insulin secretion for people who don't have diabetes, eating a balanced potassium rich diet may help prevent it (Ranee *et al*., 2012)

Vitamin E is known to improve glucose control and prevent blood vessels and nerve from free radical damage accelerated by diabetes (Patrick *et al*., 2005) Magnesium also has been shown to

decrease insulin resistance helping to keep blood sugar levels in check (Adala hruby *et al*., 2013). Vanadium (vanadyl sulphate) mimics insulin in the body and helps maintain normal blood sugar levels (Boden *et al*., 1993)

2.9 Use of Streptozotocin (Stz) as a Diabetogen

Streptozotocin (STZ) originally identified in the late 1950`s as an antibiotic is a naturally occurring nitrosourea product of bacterium *Streptomyces achromogenes* and shows broad spectrum antibacterial properties (Vavra *et al.,* 1959; Sharma, 2010). Usually, the intraperitoneal injection of a single dose (60mg/kg body weight) of it exerts direct toxicity on cells resulting in necrosis within 48-72 hours and causes a permanent hyperglycemia. STZ was later discovered to be particularly toxic to pancreatic β-cells that secrete insulin and has since been used extensively to create animal models of type I diabetes (Mansford and Opie, 1968; Pathak *et al.,* 2008). It induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models (Weir *et al.*, 1981). STZ selectively destroys the insulin producing β-cells by inducing necrosis and hence, it is diabetogenic (Sharma, 2010). Its action on β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations (Szkudelski, 2001). The glucose moiety in the structure of STZ enables it to be transported through GLUT 2 (Elsner *et al.,* 2000) and thus, insulin-producing cells that do not express this glucose transporter are resistant to STZ (Lenzen *et al.*, 2007). STZ is able to produce nitric oxide (NO), a bioregulatory and cytotoxic molecule and it has been indicated that direct NO-generation may be a mechanism of STZ toxicity in diabetogenesis (Kwon *et al.,* 1994). Wada and Yagihashi (2004) also reported that nucleic acid alkylation or excessive nitric oxide (NO) generation has been proposed to contribute to STZ-induced beta-cell damage. The production of NO by STZ could damage genomic DNA and may cause beta-cell dysfunction by inhibiting mitochondrial enzymes (Wada

and Yagihashi, 2004). The DNA damage caused by alkylation that is mediated by STZ is being repaired by an excision repair process and requires the activation of the NAD dependent enzyme poly (ADP-ribose) synthetase (Wilson and Leiter, 1990; Sharma, 2010). This process leads to depletion of cellular NAD and ATP and the increased ATP dephosphorylation provides substrate for xanthine oxidase which leads to generation of superoxide radicals and consequently leads to the formation of hydrogen peroxide and hydroxyl radicals (Szkudelski, 2001).

The link between STZ and a cytosolic protein post-translational modification through O-glycosylation with N-acetylglucosamine (O-GlcNAc) has recently been proposed to be a mechanism of STZ toxicity effects and it is referred to as O-GlcNAc-dependent model of STZ toxicity (Pathak *et al.,* 2008). Streptozotocin is proposed to induce apoptosis by inhibiting O-GlcNAcase, the enzyme that, together with O-GlcNAc transferase, is responsible for the reversible intracellular O-GlcNAc post-translational modification (Pathak *et al.,* 2008). *O*-GlcNAc-selective *N*-acetyl-b-d-glucosaminidase (OGlcNAcase) removes *O*-GlcNAc from protein and is the final enzyme in the pathway of O-glycosylation in the β-cells (Konrad *et al.,* 2001). Streptozotocin elevated *O*-GlcNAc levels in pancreatic islets and contributed to the destruction of β-cells (Liu *et al.,* 2000). Evidence has also been shown that protein modification may be specifically important in the β-cells because *O*-GlcNAc transferase (OGT) is very much enriched in β-cells than any other cell (Liu *et al.,* 2000).

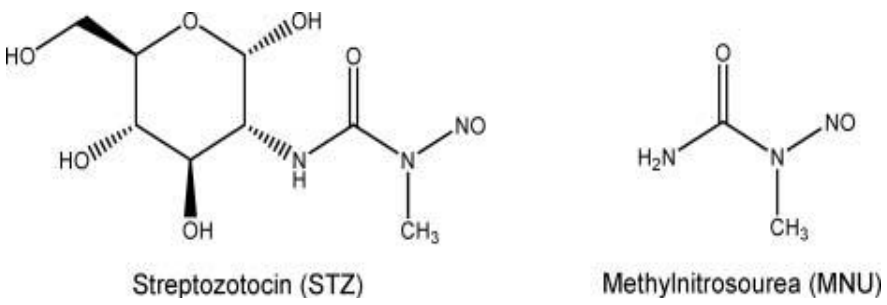

Figure 2.1: Chemical formula of streptozotocin (STZ) and Methyl-nitrosourea (MNU)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical, Reagents and Equipment

The Glibenclamide used for the study was manufactured by Jiangxi Xier Kantai Pharmaceutical Co., Ltd, China and obtained from a pharmacy within Kaduna Metropolis. The Streptozotocin used was manufactured by shanghai Rengoung Pharmaceuticals. Other materials used included: Spectrophotometer (V-16 model, United Kingdom); Centrifuge Hettich (Universal 32, Made in Germany); Automated Analyzer (Selectra XL-model Netherland); Glucometer and Strips (Accu-chek® Advantage, Roche USA); Sample bottles (EDTA and plain tubes); Laboratory Mortar and pestle; Insulin Elisa kits.

3.1.2 Plant materials

Fresh leaves of *Basella alba* were procured from their natural habitat in Ikoro-Ekiti South West Nigeria in October, 2014. The plant specimen was identified and authenticated at the herbarium in the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria. The fresh leaves were removed, washed with clean water and dried under shade. The dried leaves were then grounded and stored in air tight container until used.

3.1.3 Animals

Twenty five (25) Wistar albino rats weighing between 150-170g each was purchased for this research work from Faculty of Veterinary Medicine Ahmadu Bello University, Zaria. The animals were kept in clean cages and kept at the animal house, Department of Human

Physiology, Faculty of Medicine Ahmadu Bello University, Zaria. The animals were fed on growers' marsh (vital feed) *ad libitum*. The animals were allowed to acclimatize under standard laboratory conditions before the commencement of the experiment.

3.2 Methods

3.2.1 Preparation of food supplement

The grounded leaves of Basella alba was mixed with the animal feed in various proportions i.e. 10% (w/w) and 20% (w/w) which was used in feeding the diabetic Wistar rats.

3.2.2 Induction and confirmation of diabetes mellitus

Diabetes mellitus was induced by single intra-peritoneal injection of 60 mg/kg body weight dose of Streptozocin (STZ) dissolved in fresh 0.1M cold citrate buffer of pH 4.5 into rats that were fasted over night. Three days after STZ injection, blood was taken from tail artery of the rats. Animals having blood glucose levels equal to or greater than 250mg/dl were considered diabetic and included in the study. The diabetic animals were randomly divided into different groups.

3.2.3 Animal grouping and experimental design

Animals with blood glucose levels equal to or greater than 250 mg/dL were selected and divided into five (5) groups of five animals each as follows:

Group A (Normal control): Non diabetic rats were fed with normal feed.

Group B (Diabetic control): Diabetic rats were fed with normal feed.

Group C (Standard control): Diabetic rats received 2mg/kg body weight of glibenclamide orally daily.

Group D: Diabetes rats were fed with 10% (w/w) *Basella alba* leaf supplement.

Group E: Diabetic rats were fed with 20% (w/w) *Basella alba* leaf supplement

3.2.4 Determination of blood glucose level

Blood glucose level was determined by collection of blood sample from the tail artery of the rats at interval of 0 Week (72 hours after STZ administration), 1st Week, 2nd Week, and 3rd Week of the treatment period respectively by glucose-oxidase principle (Beach and Turner, 1958) using digital glucometer (Accu-chek Advantage) and was expressed in the unit of mg/dL (Rheney and Kirk, 2000).

3.2.5 Collection of samples for analysis

After the treatment period all animals were anaesthetized using chloroform and thereafter sacrificed. Blood samples (4ml) were drawn from the heart of each animal from all groups by cardiac puncture. The blood was allowed to clot and serum collected for insulin estimation. Pancreas was equally dissected out for histological assessment.

3.3 Determination of Serum Insulin Level

The estimation of serum insulin levels was done by radio-immunoassay (RIA) using Mercodia Ultrasensitive Rat Insulin ELISA kits (10-1251-01). Briefly, all reagents and samples were brought to room temperature before use. The required amount of enzyme conjugates 1X and wash buffer solution was prepared. The samples, insulin control solutions, and calibrators were also prepared as well as sufficient micro plate wells to accommodate calibrators and samples in duplicate. A recommended plate plan which includes: Cal 0-5: calibrator solutions (standards); insulin control low (ICL), Insulin control high (ICH) and sample (S) were made. Twenty five

micro litre each of Calibrators was pipette into appropriate wells and one hundred micro litre of enzyme conjugate 1X solution added into each well and incubated on a plate shaker (700-900 rpm) for two hours at room temperature (18-25°C). Each well was washed six times with wash buffer 1X solution and reaction volume discarded by inverting the micro plate over a sink. Three hundred and fifty micro litre of wash solution was added into each well and the wash solution discarded and taps firmly several times against absorbent paper to remove excess liquid. This was repeated five times and to avoid prolonged soaking during washing procedure two hundred micro litre Substrate TMB was added into each well. This was incubated for fifteen minutes at room temperature (18-25°C) and fifty micro litre stop solution was added to each well. The plate was placed on the shaker for approximately five seconds to ensure mixing. The absorbance was measured at 450 nm within thirty minutes.

3.4 Histological Preparation of Pancreas and Liver Tissues

At the end of four weeks of supplement and treatment, all animals from each group were euthanized, pancreas and liver tissues dissected out and fixed immediately in 10% formaldehyde fixative solution for histological studies. The tissues were processed in Shandon Southern Automatic tissue processor and sections of 5µm thick were made using Leitz base sledge microtome.

3.5 Staining of the Sections

3.5.1 Haematoxylin and Eosin stain

Briefly, the sections were taken to water after which the section was placed in haematoxylin for 5minutes. The sections were then washed in tap water and were “blued” in Scolt’s tap water. The sections were then washed in tap water again and were differentiated with 1% acid alcohol for a few seconds. The sections were washed in tap water then placed in Eosin for 1minute. The

sections were washed in water after which it was dehydrated, cleared and cover slip. The slides were viewed at the magnification of ×250 and photomicrographs taken

3.6 Statistical Analysis

Data obtained from each group was expressed as mean ± SD. The data was statistically analyzed using ANOVA followed by Duncan's a multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software. The values of $p < 0.05$ were considered as significant.

CHAPTER FOUR

RESULTS

4.1 Effect of *Basella alba* Leaf Supplementation on Fasting Blood Glucose Level in Streptozotocin – induced Diabetic Wistar Rats

The effect of *Basella alba* leaf supplementation on fasting blood glucose level in Streptozotozin induced diabetic Wistar rat is shown in Figure 4.1. The result shows that 72hours at the start of the experiment, there was a significantly ($p<0.05$) higher level of blood glucose of rats in diabetic control group when compared to the normal control group, also the blood glucose level of rats in all the treated groups increased significantly ($p<0.05$) when compared to the normal control but was not significantly ($p>0.05$) different from the diabetic control group.

On day 7, 14 and 21 rats in the diabetic control groups shows a significantly ($p<0.05$) higher blood glucose level when compared to rats in the normal control and treatment groups. Also on day 7, 14 and 21, 10% (w/w) and 20% (w/w) *Basella alba* leaf supplemented feed was able to lower the blood glucose levels significantly ($p<0.05$) in the treatment groups compared to the diabetic control group. However, the blood glucose level was significantly ($p<0.05$) high when compared to normal control group glibenclamide treated group.

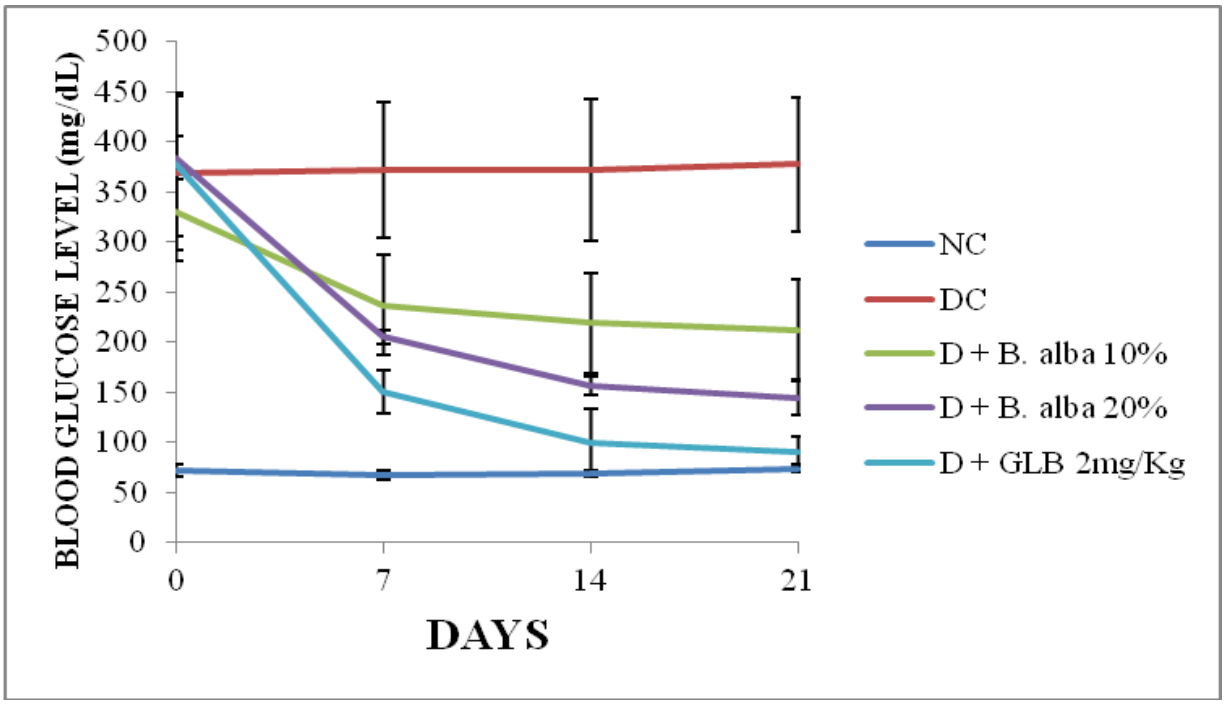

Figure 4.1 Effect of *Basella alba* Leaf on Fasting Blood Level Glucose in Streptozotozin-induced Diabetic Wistar rats.

NC = (Normal control): Non-diabetic rats, DC = Diabetic rats, D + *B. alba* (10%) = Diabetic rats fed with 10% (w/w) of *Basella alba* leaf supplement, D + *B. alba* (20%) = Diabetic rats fed with 20% (w/w) of *Basella alba* leaf supplement, D + GLB (2mg/kg body weight) = Diabetic rats treated with 2mg/kg body weight of glibenclamide orally daily.

4.2 Effect of *Basella alba* Leaf Supplementation on Serum Insulin Level in Streptozotocin – induced Diabetic Wistar Rats

The effect of *Basella alba* leaf on serum insulin level in Streptozotocin induced diabetic Wistar rats is shown in Figure 4.2. At day 21, results obtained show that the serum insulin levels decreased significantly ($p<0.05$) in the diabetic control rats when compared to the normal control group and the treated groups. There was a significant ($p<0.05$) decrease in the serum insulin levels in the groups treated with 10% w/w and 20% w/w *Basella alba* leaf supplemented feed when compared to the normal control group and glibenclamide treated group. Between the *Basella alba* supplemented treated group there was no significant ($p>0.05$) difference.

4.3 Effect of *Basella alba* Leaf Supplementation on the Histopathology of the Pancreas of Streptozotocin induced Diabetic Wistar Rats

The effect of *Basella alba* leaf supplementation on the histopathology of the pancreas of streptozoticin induced diabetic Wistar rats are shown on plate I. The pancreatic section of rats in the normal control group shows normal pancreatic structure (normal islet of langerhans and normal acini tissues) when compared to rats in the diabetic control and treatment groups. The rats in the diabetic control group shows necrotic islet. The pancreatic section of rats treated with glibenclamide shows slight distortion and necrosis of islet cells while the pancreatic section of rats supplemented with 10% *Basella alba* shows vacoulation and necrosis of islet cells also rats supplemented with 20% *Basella alba* leaf shows necrosis of islet cells.

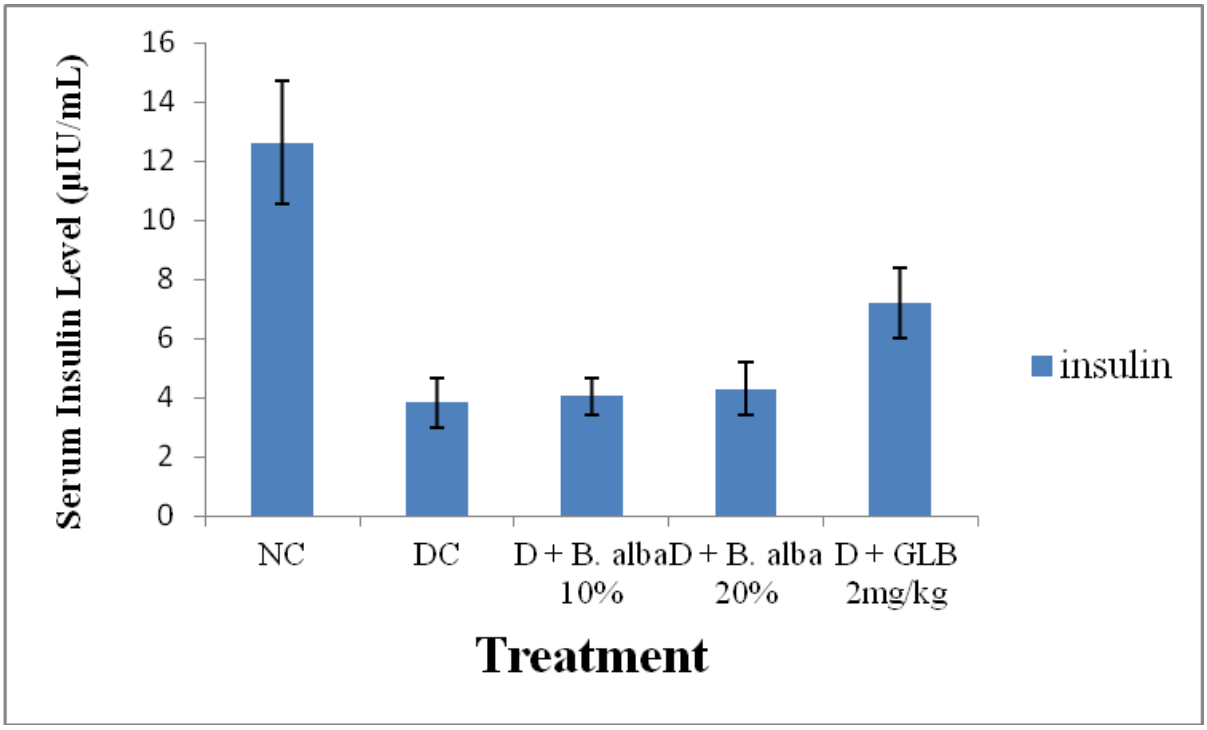

Figure 4.2: Effect of *Basella alba* on Serum Insulin Level in Streptozotocin-induced Diabetic Wistar rats

Each bar represent mean of five animals

NC = (Normal control): Non-diabetic rats, DC = Diabetic rats, D + *B. alba* (10%)= Diabetic rats fed with 10% (w/w) of *Basella alba* leaf supplement, D + *B. alba* (20%) = Diabetic rats fed with 20% (w/w) of *Basella alba* leaf supplement, D + GLB (2mg/kg body weight) = Diabetic rats treated with 2mg/kg body weight of glibenclamide orally daily.

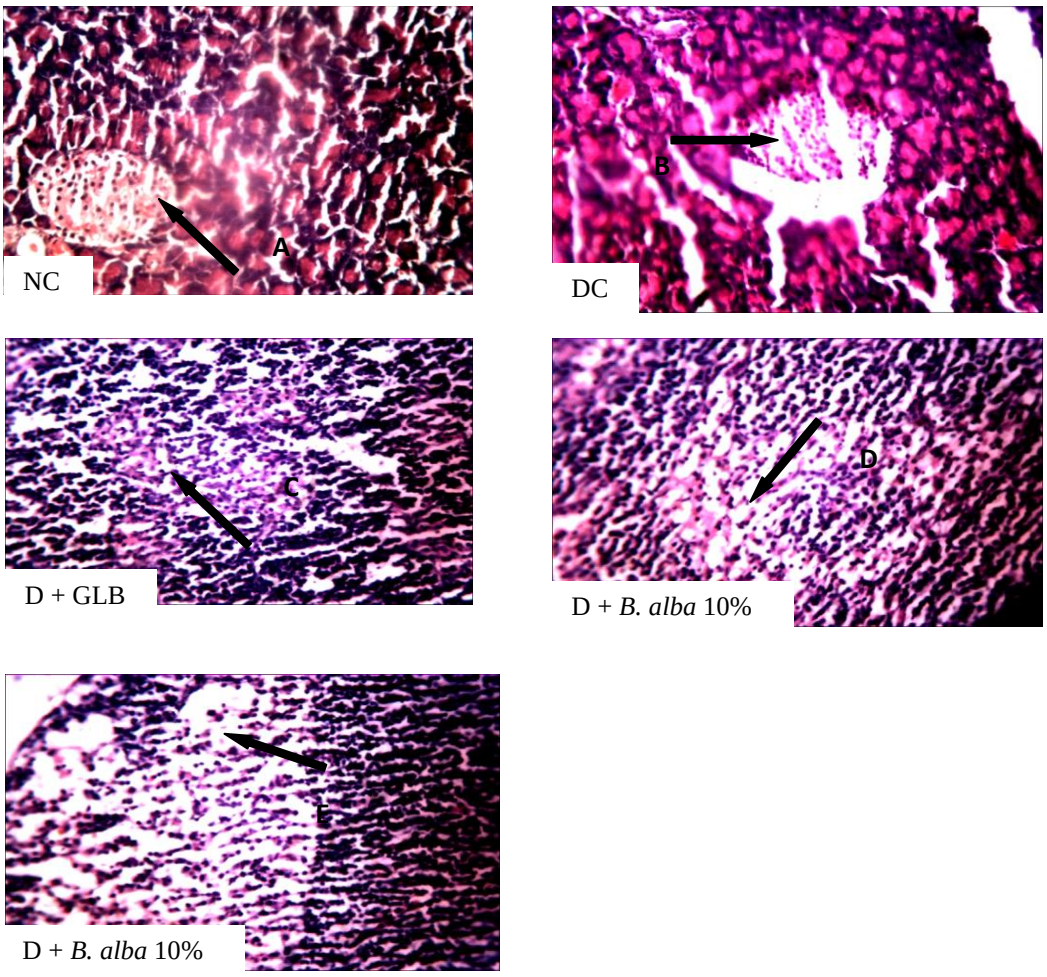

Plate 4.1: **Photomicrograph of Pancreas Sections showing the effect of *Basella alba* leaf Supplementation on the Histopathology of the Pancreas of Streptozotocin induced Diabetic Wistar rats (H&E stain). Magnification X250.**

NC = (Normal control): Non-diabetic rats, DC = Diabetic rats, D + *B. alba* (10%) = Diabetic rats fed with 10% (w/w) of *Basella alba* leaf supplement, D + *B. alba* (20%) = Diabetic rats fed with 20% (w/w) of *Basella alba* leaf supplement, D + GLB (2mg/kg body weight) = Diabetic rats treated with 2mg/kg body weight of glibenclamide orally daily.

(A=Normal Islet; B=Necrotic Islet; C=Slight distortion and necrosis; D=Vacoulation and necrosis of islet; E=Necrosis of islet cell)

4.4 Effect of *Basella alba* Leaf Supplementation on the Histopathology of the Liver of Streptozotocin induced Diabetic Wistar Rats

The effect of *Basella alba* leaf supplementation on the histopathology of the liver of streptozotocin induced diabetic Wistar rats is shown on plate II. The liver section of rats in the normal control group shows normal hepatocytes. The rats in the diabetic control group shows necrosis of hepatocytes. The liver section of rats treated with glibenclamide shows slight hepatocellular necrosis while the liver sections of rats supplemented with 10% (w/w) *Basella alba* leaf shows slight vacuolation and vascular congestion of the cells, also rats supplemented with 20% (w/w) *Basella alba* leaf shows slight periportal necrosis and kupfer cell hyperplasia.

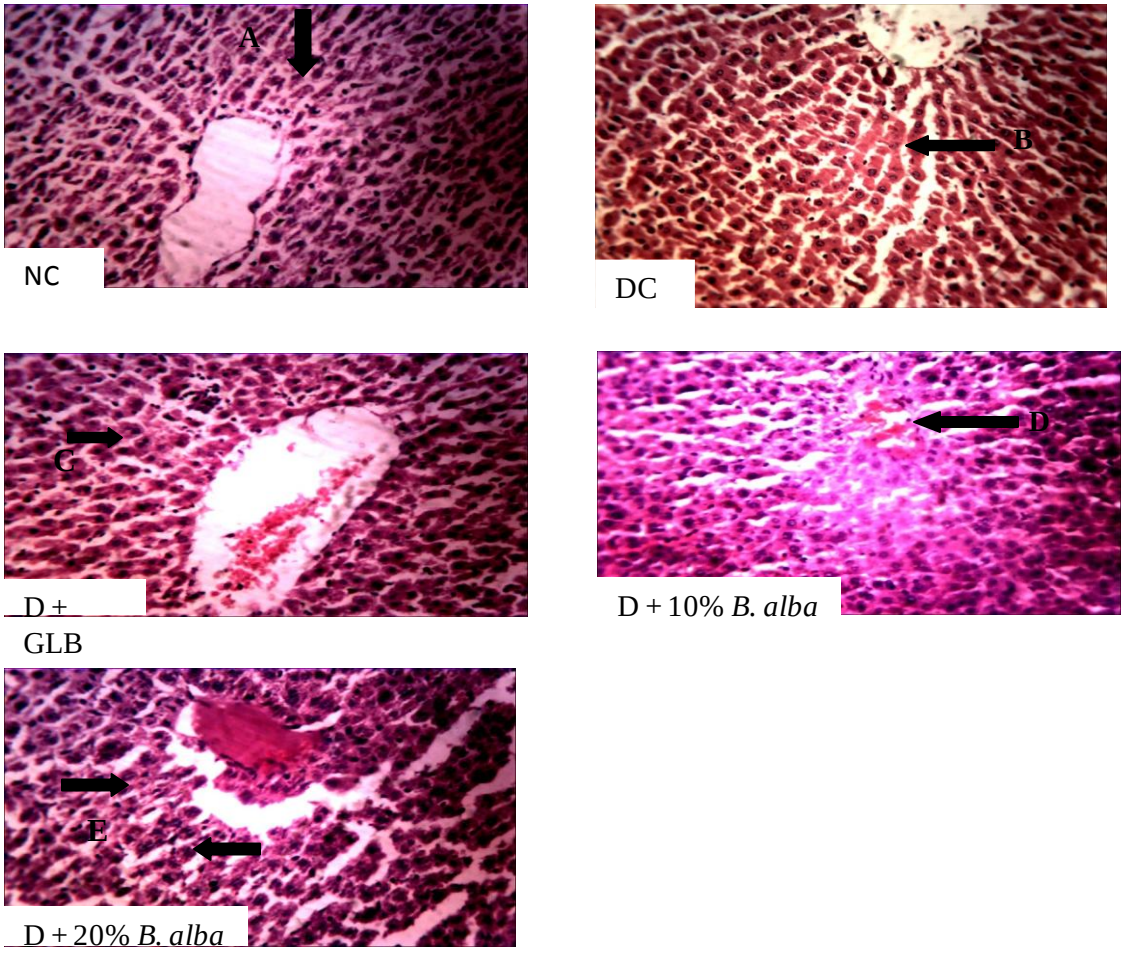

Plate 4.2: Photomicrograph of Liver Sections Showing the Effect of *Basella alba* Leaf Supplementation on the Histopathology of the Liver of Streptozotocin induced Diabetic Wistar rats (H&E stain). Magnification X250

NC = (Normal control): Non-diabetic rats, DC = Diabetic rats, D + *B. alba* (10%) = Diabetic rats fed with 10% (w/w) of *Basella alba* leaf supplement, D + *B. alba* (20%) = Diabetic rats fed with 20% (w/w) of *Basella alba* leaf supplement, D + GLB (2mg/kg body weight) = Diabetic rats treated with 2mg/kg body weight of glibenclamide orally daily.

(A= Normal hepatocytes; **B**= Necrosis of hepatocyte and kupfer cell hyperplasia; **C**= Hepatocellular necrosis; **D**= Vascular congestion vacoulation; **D**= Periportal necrosis and kupfer cell hyperplasia)

CHAPTER FIVE

5.0 DISCUSSION

Experimental animal models have been suggested to be one of the best ways to understand the pathophysiology of any disease (Chatzigeorgiou *et al.,* 2009; Ali *et al.,* 2011). In this study, the intra-peritoneal administration of streptozotocin (STZ) effectively induced diabetes mellitus in rats which was confirmed by elevated levels of fasting blood glucose, three days after STZ injection. This agrees with the reports of Mohammed *et al*. (2008), and Krishna *et al*. (2012).

Results obtained in our present study also indicate that the serum insulin levels decreased significantly ($p<0.05$) in the diabetic untreated animals following Streptozotocin (STZ) treatment when compared to normal control rats. This finding has been substantiated by other researchers (Mallick *et al.,* 2006; Daisy *et al.,* 2012). Streptozotocin has been reported to induce insulin dependent diabetes mellitus in animal models (Bedoya *et al.,* 1996). Intracellular action of STZ results in changes of DNA in pancreatic β-cells comprising its fragmentation (Morgan *et al*. 1994). This results to impaired glucose oxidation (Bedoya *et al*., 1996) and decreases insulin biosynthesis and secretion (Nukatsuka *et al*., 1990a, 1990b).

Basella alba leaf had a more pronounce effect at a high dose than when administered at a low dose. This agrees with the report of Bamidele *et al* (2014), that aqueous leaf extract of *Basella alba* leaves significantly reduced blood glucose level in alloxan induced diabetic Wistar rats. The possible mechanism involved in the anti hyperglycaemic action of *Basella alba* leaves may be due to reduction of nitric oxide (NO) production of reactive oxygen species (ROS) (Kim *et al.,* 2007) due to the presence of anti oxidants presence in the plant (Olajire and Azeez, 2011) which

are known to scavenge the free radicals produced by oxidative damage in the disease state (Bamidele *et al.,* 2010; Nirmala *et al.,* 2011) or decrease blood glucose via suppression of STZ-induced peroxidation and apoptosis in beta cells by increasing glycogen content in liver and muscle; and activating glucokinase, hexpkinase and phosphofructokinase (Rathi *et al.,* 2002). Other reasons which may account for the reduction in fasting blood glucose may possibly include: inhibition of p53/p21 expression and inhibition of cleavage of caspases and poly (ADP-ribose) polymerase (PARP) (Xiang *et al.,* 2008), inhibition of aldose reductase, or due to bulk laxative effect and also slowing of carbohydrate absorption from the gastrointestinal tracts.

However, the results of our present finding in this study revealed that *Basella alba* leaf administration to diabetic rats did not produce any significant ($p>0.05$) increase on serum insulin level when compared with diabetic control group. Based on our findings, it may be suggested that insulin secretion may not be part of the observed anti hyperglycaemic property of *Basella alba* leaf. Unlike glibenclamide which produce a significant elevated serum insulin level when compared with the diabetic control group. Glibenclamide have been reported to stimulate insulin secretion from pancreatic β-cells and also reduces hepatic glucose production resulting in reduced blood glucose level (Erejuwa *et al.,* 2011).

Oxidative stress induced by reactive oxygen species (ROS) which are generated due to hyperglycaemia has been implicated in the onset and progression of diabete mellitus and its related complications (Jay *et al.,* 2006; Wei *et al.,* 2009; Giacco and Brownlee, 2010). Hyperglycemic in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increase the levels of free radicals (Sharma *et al.,* 2010; Tsuruta *et al.,* 2010). *Basella alba* leaves have been reported to have a good free radical scavenging capacity (Duke and Ayensu, 1985).

Disorganization of the endocrine and exocrine cells and a decrease in the langerhans cells with damaged and necrotic pancreatic acinic was observed in STZ-induced diabetic untreated rats. This observation agrees with the report of other investigator (Kondeti *et al.,* 2010; Baquer *et al.,* 2011; Comaoglu *et al.,* 2011). It has earlier been proposed that plant extracts can be effective in cell regeneration and restoration of islet size, even producing cell hyperplasia (Pepato *et al.,* 2004; Kondeti *et al.,* 2010; Comaoglu *et al.,* 2011) and this could be responsible for the slight but insignificant ($p>0.05$) increase in serum insulin level observed in the *Basella alba* leaves supplemented group when compared with the diabetic control group. In addition, it has been reported using pancreatic islets cell line that plant play a role in stimulating insulin secreation in β-cell (Pepato *et al.,* 2004; Shen *et al.,* 2010; Gaamoussi *et al.,* 2010; Kondeti *et al.,* 2010).

The necrosis of the hepatocytes observed in the STZ- induced diabetic untreated rats is in agreement with the reports of other investigators (Halmilton 1987; Herman et al., 1999). Papaccio et al (2000) found that STZ interfers with cellular metabolic mechanism. The liver mitochondrial dysfunction in diabetes is related to the oxidative stress enhanced in diabetic animals (Kucharska et al., 2012; Bukker et al 2000). However, glibenclamide prevent severe liver damage caused by hyperglycemia (Sokolovska *et al.,* 2012). The effect of glibenclamide in the liver tissue is probably due to nonalcoholic steatohepatitis (Ketoh et al., 2001; Wiggins and Gibbons 1992; Broadway and Saggerson, 1995). The administration of *Basella alba* leaf did not prevent alteration in liver pathology. Enhanced blood sugar yields to imbalance of oxidation-reduction reactions in hepatocytes, so that hyperglycaemia through increasing AGES facilitates free radicals production via disturbance in ROS production. Hence, it reveals that, diabetic hepatic injuries results from several agents and is not controllable only via inhibition of

hyperglyceamia (Hanaa and Seham, 2012). But the restoration of the hepatocytes to the normal architecture could be time dependant.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The summary made at the end of this study is as follows:

i. Administration of *Basella alba* leaf to diabetic animals produced a significantly decreased blood glucose level.

ii. Administration of various doses of *Basella alba* leaf to diabetic rats did not produce any significant increase in serum insulin level. Conversely, glibenclamide produced a significantly elevated serum insulin level.

iii. The histopathology of the pancreas of diabetic rats fed with *Basella alba* leaf indicated necrosis of the islet cells. Also liver sections of diabetic rat fed with *Basella alba* leaf showed slight periportal necrosis and kupfer cell hyperplasia.

6.2 Conclusion

We concluded from the study that *Basella alba* leaves have hypoglycemic effect on diabetic rats, although the plant leaf did not reverse the damage effect on the histopathology of the liver and pancreatic cells.

6.3 Recommendations

Based on the findings of this study, it is recommended that:

i. *Basella alba* leave can be included in the diet of hyperglycemic and diabetic patients

ii. Further studies varying the proportion of *Basella alba* leaves supplementation and longer duration of treatment should be carried out.

iii. There is need to elucidate the mechanism of action of the *Basella alba* leaf

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APPENDICES

APPENDIX A: BLOOD GLUCOSE LEVEL

Table 1: Effect of *Basella alba* Leaf on Fasting Blood Glucose in Streptozotozin-induced Diabetic Wistar rats.

	Day0	Day7	Day14	Day21
NC	71.60±6.11[a]	67.60±5.13[a]	69.40±3.21[a]	73.80±3.77[a]
DC	368.80±77.37[b]	372.20±68.38[d]	372.20±70.19[d]	377.60±67.55[d]
D+ B. alba (10%)	330.60±21.59[b]	237.20±6.83[c]	219.40±8.88[c]	212.80±17.17[c]
D+ B. alba (20%)	384.40±72.10[b]	205.40±21.36[c]	156.40±33.89[b]	144.40±14.08[b]
D+Glibenclamide	377.60±43.46[b]	150.80±36.70[b]	100.40±15.13[a]	91.20±6.50[a]

- Values are the mean of five replicates ± (SD)

APPENDIX B: BLOOD SERUM INSULIN LEVEL

Table 2: Effect of *Basella alba* leaf on Serum Insulin Level in Streptozotocin - induced Diabetic Wistar rats

NC	DC	D+ B. alba (10%)	D+ B. alba (20%)	D+ Glb (2mg/Kg)
12.62 ± 2.10^{d}	3.82 ± 0.82^{a}	4.04 ± 0.91^{a}	4.30 ± 1.20^{a}	7.18 ± 0.64^{c}

- Values are the mean of five replicates ± (SD)