STUDY OF THE EFFECTS OF *AZADIRACTA INDICA* SEED EXTRACT ON BLOOD GLUCOSE LEVELS OF NORMOGLYCEMIC AND STREPTOZOCIN-INDUCED DIABETIC WISTAR RATS.

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,AHMADU BELLO UNIVERSITY,ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN HUMAN PHYSIOLOGY.

DEPARTMENT OF HUMAN PHYSIOLOGY
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September, 2006

DECLARATION.

I ,TANKO,YUSUF, do hereby declare that this thesis has not been submitted in whatever form to any institution or organization other than Ahmadu Bello University,Zaria for the award of any degree. Results discussed in this thesis are work performed by me at the Department of Human Physiology and Veterinary Pathology , Ahmadu Bello University, Zaria, under the supervision of Professor M..A.OKASHA and Dr N.D.G. IBRAHIM. The information derived from the literature has been acknowledged in the text and list of references provided.

SIGN ---------------------------------------

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DEDICATION

This thesis is dedicated to my parents, Mal Tanko Musa Mamman, Malama Habiba Jummai, and my siblings, Adamu, Amina , Hafsat and Hadiza.

CERTIFICATION

This thesis titled "STUDY OF THE EFFECTS OF *AZADIRACTA INDICA* SEED EXTRACT ON BLOOD GLUCOSE LEVELS OF NORMOGLYCEMIC AND STREPTOZOCIN-INDUCED DIABETIC WISTAR RATS" by TANKO, YUSUF in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University ,Zaria, and meet the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

This study investigated the effects of *Azadiracta Indica* seed extract on blood glucose levels of streptozocin-induced diabetic and normoglycemic Wistar rats.

An acute toxicity studies of the extract *Azadiracta Indica* was done, the result of the acute toxicity study showed that the extract had an LD_{50} 3000mg/kg bodyweight via intraperitoneal route in Wistar rats.

A preliminary phytochemical screening was done which revealed the presence of flavonoids tannins, glycerin, cholesterol, steroids alkaloids saponin, triterpenoid and reducing sugar. After which the three doses (500mg/kg, 1000mg/kg and 1500mg/kg bodyweight) of the extract were administered to streptozocin-induced diabetic and normoglycemic Wistar rats. The effects of the extract on blood glucose levels of the animal were compared with untreated control and Biphasic Isophane Insulin treated groups.

In the streptozocin-induced diabetic groups, there was no significant change in the blood glucose levels after 1 hour of extract treatment in all the groups given different doses. After 3 hours there was a significant decrease ($P<0.05$) in the group when given 1000mg/kg bodyweight of the extract compared to the untreated control. After 5 and 7 hours there was a

significant decrease ($P<0.05$) in the groups given 500 and 1000mg/kg bodyweight of extract when compared to untreated control.

However after 1 and 3 hours there was no significant change in the blood glucose levels when compared to the insulin- treated group. After 5 and 7 hours there was only significant increase ($P<0.05$) in the group given 1500mg/kg bodyweight of the extract when compared to insulin- treated group .

In the normoglycemic groups there was no significant change in the blood glucose levels for all the groups that were given different doses, after 1 hour of extract administration compared to the control. After 3 hours there was a significant decrease in group given 500mg/kg of extract when compared to control group. However after 5 and 7 hours there was a significant decrease in the groups given 500 and 1000mg/kg bodyweight of extract when compared to the control group. In regard to group given 1500mg/kg bodyweight of the extract all the results revealed no significant change in the blood glucose levels when compared to control untreated. However after 1hour there was a significant increase ($P<0.05$) in the groups given 1000mg/kg of the extract when compared to the insulin-treated group. After 3 hours there was a significant increase in the group given 500 and 1000mg/kg bodyweight when compared to insulin group. However after 5 and 7 hours there was a highly significant increase ($P<0.001$) in all the groups given the three doses when compared to insulin-treated group .

The histopathological effects on the pancreas of the streptozocin-induced diabetic Wistar rats revealed focal areas of necrosis and degenerated pancreatic cells, while the livers revealed congested blood vessel of the hepatic cells. In the normoglycemic groups, there were no any observable microscopic lesion in the pancreas

These results show that the extract of *Azadiracta Indica* has a blood glucose lowering effects in both streptozocin-treated and normoglycemic Wistar rats. This may be due to insulin like action of the extract of *Azadiracta Indica* seed on the peripheral tissues as well as stimulation of the β-cell.

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LIST OF ABBREVIATIONS.

ATP-Adenosine triphosphate

ADP-Adenosine diphosphate

ANOVA- Analysis of Variances

CTL- Cytotoxic islet-antigen specific T lymphocyte

FFA- Free Fatty Acid

GAD-Glutamic acid decarboxylase

GDM-Gestational Diabetes Mellitus

GH- Growth hormone

GIP-Glucose-dependent Insulinotrophic polypeptide

GLP-Glucagon-like polypeptide.

GLUT-Glucose transporter

HDL-High Density lipoprotein

HLA-Human leucocyte antigen.

IDDM-Insulin Dependent Diabetic Mellitus

IRS-Insulin receptor substrate

LADA-Latent Autoimmune Diabetic in Adults

LDL-Low Density Lipoprotein

LD_{50}-Lethal Dose for 50% group

MODY-Maturity onset Diabetes of the Young

MHC-Major histocompatibility complex

NIDDM-Non Insulin Dependent Diabetic Mellitus

PKA-Protein Kinase

PPG-Postprandial Plasma Glucose.

PP-Pancreatic polypeptide

PTP-Protein-tyrosine phosphate

SGLT-Sodium Glucose Transporter

VDCC-Voltage Dependent Calcium Channel

VLDL-Very Low Density Lipoprotein.

WHO-World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 HISTORY OF DIABETES MELLITUS

Although diabetes has been known since antiquity, and treatments were known since the middle Ages, the elucidation of the pathogenes of diabetes occurred mainly in the 20th century (Patlak, 2000)**.** Until 1922 when insulin was discovered and made available, a clinical diagnosis of diabetes was an invariable death sentence, more or less quickly. Paul Langerhans identified pancreatic Islet in 1889,describing them as discrete island surrounded by exocrine tissue.

The discovery of the role of the pancreas in diabetes is generally credited to Joseph Von Mering and Oskar Minkowski, two European researchers who, in 1889, found that when they completely removed the pancreas of dogs, the dogs developed all the signs and symptoms of diabetes and died shortly afterward. In 1910,Sir Edward Albert Sharpey-Schafer of

Edinburgh in Scotland suggested diabetes were deficient in a single chemical that was normally produced by the pancreas, he proposed calling this substance insulin.

The endocrine role of the pancreas in metabolism, and indeed the existence of insulin, was not fully clarified until 1921,when Frederick Grant Banting and Charles Herbert Best repeated the work of Von Mering and Minkowski but went a step further and manage to show that they could reverse the induced diabetes in dog by giving them an extract from the pancreatic islet of langerhans of healthy dogs (Banting *et al.,*1922).They went on to isolate the hormone insulin from the bovine pancreas at the university of Toronto in Canada. This led to the availability of an effective treatment, insulin injections, and the first clinical patient was treated in 1922. For this ,Banting and his colleagues received the Nobel prize in physiology or medicine in 1923.The distinction between what is now known as type 1 and type 2 diabetes was made by Sir Harold Percival Himsworth in 1935,he published his finding in January 1936 in the lancet (Himsworth,1936).

The plant *AZADIRACTA INDICA* belongs to the family called maliaceace. It is known in English as the Neem tree in Hausa Dogonyaro. *Azadiracta indica*, commonly referred to in many centuries as the neem tree.

This broad leaves evergreen tree can reach height of 30 meters with trunk girth of 2.5 meters . Its deep root system is well adapted to retrieving water and nutrient from the soil profile, but this deep root system is very sensitive to water logging. The neem tree shrives in hot, dry climate where shade temperature often reach $50^{\circ C}$ and annual rainfall from 400 to 1200 millimeters (Mann, 1990). The tree can withstand shallow or acidic soil and it produces ellipsoidal fruits that are about two centimeters for each in length and are arranged in axillary clusters. The fruits contain kernels that have high concentration of active metabolites (National Research council,1992).There is evidence that no scientific correlation, that the tree grow in climate with lower rainfall produces kernels with higher content of metabolites (Schmutterer,1990).

National research council (1992) has released a report titled as "Neem tree for solving global problems". They considered neem tree to be one of the most promising of all plants that can yield as many as strange and varied products.

The neem tree is considered so important because of its medicinal properties. The active (ingredients) compounds can be extracted by many methods. Leaching with water is the oldest method and is still used by some firms selectively to extract the active compounds from *Azadiracta* *indica* .On the other hand, most companies are using non- polar solvents to obtain more varied mixture chemicals. Hexane, pentane, ethanol, methanol, ester and dichloromethane are used in the Extraction as well as mixture of these solvents with water (Lee *et al.,* 1988).

1.2 CONSTITUENTS OF NEEM EXTRACT

Mamoodin one (1) is the new limonoid which has been isolated from *Azadiracta indica* along with seven known tetranotripeniod: azadirone, epoxydirone ,nimbim, gedunin, azadiradione, deacetylumbin and 17 hydroazadiradione (Saddiqui, 1992).

A new protolimonoid, neheedin three (3), has been obtained from the neem fruit along with azadiractin. Azadiractin actually is a mixture of seven isomeric compounds and is labeled A to G with azadiractin A being present in the quality and E regarded as the effective insect growth (Verkert *et al.,* 1993).

1.3 NEEM EFFECTS

The mode of action of neem extract is not completely understood .It is quite possible that different chemicals or different ratios of chemicals found in neem tree have various effects. Azadiractin alone has several modes of action **(**Koul, 1991).

Hypoglycemic effect was observed with *Azadiracta Indica* leaves (Khosla *et al*., 2000).

1.4 STATEMENT OF THE RESEARCH PROBLEMS.

The growing interest in herbal medicine currently is global. in most circles, this is viewed as a welcome development. Many plants used as traditional medicine have been proved to be useful drugs.

Azadiracta Indica seed oil is claimed to have anti-diabetic effect by traditional healers (National Research Council, 1992**).** Since the plant extracts are used in treatment of different diseases, so it is essential to investigate their various effects on blood glucose and diabetics in general. However very little work has been carried out on this plant as an antidiabetic in Nigeria especially the seed extract.

The growing interest in herbal medicine currently witnessed globally has also affected African. There for In many African countries today, a place of prominence is being given to herbal medicine both for curative and nutritional purposes (Akah, 1994).

Many plants used in traditional medicine have been proven useful in drug development and modern medicine. According to Sofowora (1993) drugs developed from plants or plant parts can be classified into 3:

1. Drugs used in orthodox medicine today which are directly derived from traditional remedies e.g. digital from digoxin.
2. Drugs from plant origin used for treatment of different ailments in modern medicine.
3. Drugs which are synthetic derivatives of natural products.

Therefore a close scientific study of many such plants used locally in traditional medicine is of paramount importance.

1.5 AIMS AND OBJECTIVES.

1. To carry out acute toxicity study on *Azadiracta Indica* seed extract in this environment and to determine the lethal dose (LD_{50})
2. To carry out preliminary phytochemical analysis of the seed oil extract of *Azadiracta Indica*.
3. To determine the effect, if any of *Azadiracta Indica* on blood glucose levels of normoglycemic Wistar rats.

4. To determine the effect, if any of *Azadiracta Indica* on blood glucose levels of streptozocin-induced diabetes in Wistar rats.

5. Finally, to determine the toxic effects, if any, of the seed extract of *Azadiracta Indica* on the liver and pancreas of the streptozocin -induced diabetic and normoglycemic Wistar, rats through histopathological studies.

1.6 JUSTIFICATION

(a) Authentication of the claim will stimulate further researchers in drug development and general medicine.

(b) The study of the hypoglycemic effect of our indigenous *Azadiracta Indica* seed oil may provide us with bioactive compounds for the treatment of diabetes mellitus.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin (Guyton and Hall, 2000).

Diabetes is the major disorder of insulin production and metabolism. This is a clinical condition due to the inability of the body to utilize glucose even in the presence of high glucose level. This is described as starvation in the mist of plenty (Ganong, 2003).

During fasting, most of the glucose in the blood is supplied by the liver and is used by the brain, independently of insulin. After a meal, the rise in blood glucose level rapidly stimulates insulin secretion, which results within minutes in increased glucose transport, metabolism, and storage by muscle and adipocytes. In addition, insulin both inhibits glucagon secretion and lowers serum free-fatty-acid concentration, contributing to the sharp decline in hepatic glucose production. In healthy persons, post absorption level of plasma glucose stay within a narrow range about 60 to 100mg per deciliter (3.3 to 5.6 mM per litre) despite the intermittent ingestion of food. Insulin, the primary regulatory hormone that blunts postprandial hyperglycemia and maintain post absorptive euglycemia, its effect is counter balanced by several factors that provide a minimal level of glycemia in order to sustain the nutrition of the central nervous system. An uninterrupted flow of glucose into the blood is essential for normal metabolism in the brain (Mccal, 1993). Diabetes is an epidemic disorder that arises when insulin secretion from pancreatic cells fail to maintain blood glucose level in the normal range, especially when exacerbated by peripheral insulin resistance. The underlying pathophysiology of diabetes is diverse, but pancreatic β-cell failure is the common theme (Halban *et al.,* 2001). Type 2 diabetes is the most common form, which arises when pancreatic β-cell insulin secretion fail to compensate for peripheral insulin resistance (Defonzo, 1997).

Work over the past decade suggested that type 2 diabetes begin with skeletal muscle insulin resistance (Cline *et al.,* 1994). However peripheral insulin resistance might not be enough, as transgenic mice lacking muscle insulin receptor or patients with muscle insulin resistance owing to defective mRNA splicing do not develop diabetes (Brunning *et al.*, 1998, Savkur *et al.*, 2001).

Despite incontrovertible evidence of gastric Links for type 2 diabetes, the gene responsible have been difficult to identify, because diabetes is not a

Mendelian disorder (Burghes *et al.*, 2001). Consequently, linkage analysis with well defined population has made slow progress, although a possible role for the serine protease CAPN10 was recently revealed (Horikawa *et al.*, 2000, Sreenan *et al*., 2001).

2.2 BLOOD GLUCOSE HOMEOSTASIS

Paul Langerhans (1869) identified pancreatic Islet, describing them as discrete Islands or Islets surrounded by pancreatic exocrine tissues (Morrison, 1937) hence, the term "islet of langerhans".The islets make up approximately 2 to 3 percent of the total pancreatic volume. The Islets are more abundant in the body and tail than in the head of the pancreas. They consist of small cuboidal cells with a large vesicular nucleus arranged in cords separated by a fibro vascular stroma. The Islets have a portal circulation, with blood flowing from beta to alpha to delta cells as well as afferent fibers from the central nervous system which regulates secretion of hormones from these cells. The alpha, beta, delta, and pancreatic polypeptide (PP) cells secrete glucagon, insulin, somatostatin and pancreatic polypeptide, respectively. Alpha and beta cells are exquisitely sensitive to glucose, which stimulates the beta cell and inhibit the alpha cells, thereby maintaining blood glucose level within a narrow range. The principal action of insulin promotes the entry of glucose into the tissues and decreases hepatic gluconeogenesis. Whereas glucagon principally stimulate hepatic glycogenolysis when hypoglycemia threatens (Brunicardi *et al.,* 1996; Samols *et al.,* 1998).

At any moment, the change in concentration of glucose in any small volume element of blood plasma depends on the rate at which glucose is introduced into circulating blood, the rate at which it is distributed throughout the blood circulation, the rate at which it is apportioned on red blood cell and plasma and the rate at which it is removed from the circulation. The limiting event on rate of glucose removal from the circulation is its rate of entry into cells. The rate of glucose entry into cells may be limited by transmembrane transport or by glucose phosphorylation. All of these factors are changing throughout the day in relation to meals, physical activity, emotional state, and stage of development. Glucose in the gut is absorbed mainly in the duodenum, Jejunum and Ileum, by two routes; Transcellular and Paracellular. The Paracellular route is across the tight junction between enterocytes, into the lateral clefts between cells. The trancellular route is via Sodium glucose transporter (SGLT) and glucose transporter (GLUT-2). An increase in dietary carbohydrate increases glucose absorption from small intestinal segments via an unknown path that lead to an increase in number of SGLT in the brush-border membrane (Kavarsor and Diamond, 1983; Ferraris and Diamond, 1986).

It is inferred that SGLT-1 by successive exocytosis and endocytosis, cycles between the interior and the plasma membrane. Little is known yet about this cycle process (Ferraris and Diamond, 1997). High concentration of glucose in the gut lumen also increase paracellular glucose transport by an unknown mechanism, possible by a cascade of signal from brush-border SGLT, which may be the only available glucose sensor (Philpott *et al.*, 1992).

Increase in glucose transport exceeds the increased in number of GLUTs so that it was suggested that the intrinsic transport activity of GLUT was increased as well as the GLUT number. There is also a slow increase in basolateral GLUT number after 3 days in response to increase dietary carbohydrate (Cheeseman and Harley, 1991). Probably due to increase message and synthesis, combined with slow migration of enterocyte from the crypt. Paths for stimulating this GLUT response are unknown. Glucose absorption generates the familiar prandial blood glucose curve. The rapidity of rise in blood glucose concentration and the height of its maximum concentration depend on the rate at which the stomach empties its contents, because most glucose absorption occurs in the Jejunum and Ileum. The rate of gastric emptying is well controlled teleogically. It makes sense that hypoglycemia actually accelerates gastric emptying and hyperglycemia retards it. Two peptides decrease the rate of gastric emptying glucagons-like polypeptide -1 (GLP-1) and amylin (Young *et al.*, 1995). It is suggested that the effect of GLP -1 on gastric-emptying is mediated via vagal afferent (Imeryuz *et al.,* 1997). Amylin is a peptide hormone. Co-secreted with insulin from pancreatic β cells.

Hyperglycemia declines as glucose is transferred from interstitial fluid into cells emphasized that there are two components for this increase in glucose uptake, an insulin-dependent and an insulin-independent component. It is not simple to establish the relative contribution of the two components and there is some (debate) about the quantitative estimate. Increased glucose uptake has to occur with hyperglycemia, without intervention of hormones. Simply because the glucose concentration gradient from interstitial fluid to cytoplasm is greater in such condition resulting in increased facilitated diffusion via all GLUTs that are not saturated, unless hexokinase is limiting (Baron *et al.*, 1988; Best *et al.*, 1996).

There are two tissues (Brain and skeletal muscle) where glucose uptake is not increased in response to increase in glucose delivery alone. Brain does not respond to hyperglycemia because its GLUT-3 has so small a k_m

Value that it is saturated at euglycemia level. In situ, muscles of the forearm in humans do not increase glucose uptake when the local (but not systemic) forearm arterial blood glucose level is raised to a steady value of 10-15 mmol presumably because GLUT density in the basal state is so small (Andres and Zierler, 1958).

SGLT and GLUT-2 work in series in small intestinal enterocytes and in the renal proximal tubule. In both cases SGLT is on the brush-border luminal membrane, where it absorbs glucose from gut contents or from renal glomerular filtrate Absorbed glucose travels some how through cytoplasm to the basolateral membrane, through which it travels via GLUT-2 to interstitial fluid and the portal vein in the gut and to the renal vein in the kidney.

Arterial blood plasma glucose concentration is not tightly controlled. In normal healthy young adult, during the course of a day's activity, arterial blood glucose concentration may vary from a low to a while more then 3 to a high of 9m Mol (Somogyi, 1942). Whole blood values are some 10-15% lower than plasma values. Similarly, capillary values are 7% higher than venous in the fasting state and 8% higher after a glucose load. The high and low of blood glucose concentration are affected by dietary carbohydrate and fat, by blood plasma level of free fatty acid (FFA), by muscle exercise, by local hypoxia, and by families of hormones.

2.3 MECHANISM OF INSULIN SECRETION AND ACTIONS

Insulin is synthesized in the rough endoplasmic reticulum of the beta cell of the pancreas and in granules of the golgi apparatus as proinsulin. This is a precursor of insulin with a connecting peptide (C-peptide) between the α (21aa) and β (30aa) chains. The C-peptide is cleaved from proinsulin, stored in secretory granules, and eventually released into the bloodstream in amounts equimolor with those of insulin (Sterner *et al.,* 1967, Rubenstein *et al.,* 1969). C-peptide has an essential function in the Synthesis of insulin where it links the α and β chains in a manner that allows correct folding and interchain disulphide bond formation. When C-peptide is removed from proinsulin by proteolytic processing, the COOH -terminal part of insulin's β-chain becomes exposed and free to assume an appropriate conformation for effective interaction with the insulin receptor (Steiner *et al.,* 1995). After the discovery of the mode of insulin biosynthesis, several early studies addressed the question of possible physiological effect of C-peptide. Rat C-peptide was, however, found to diminish glucose -stimulated insulin release in rat both in vivo and in vitro (Dryburgh *et al.,* 1980, Wojcikowski *et al.,* 1983), whereas the corresponding findings in other animals were less conclusive (Dunbar *et al.,* 1976). C-peptide was also reported to inhibit arginine-stimulated glucagon release from the isolated perfused rat pancreas and to inhibit fat stimulated gastric inhibitory polypeptide secretion by intestinal cell (Dunbar *et al.*, 1980).

Despite these reported effects, it became generally accepted that C-peptide possesses little or no biological activity and has no other role than its participation in insulin synthesis, a role that is emphasized by its name "Connecting peptide". Later on, new data have been presented demonstrating specific binding of C-peptide to cell surface in a manner that suggest the presence of G protein coupled membrane receptor. C-peptide may thereby stimulate specific intracellular processes, influencing renal and nerve function in C-peptide deficient type 1 diabetic patients (Kitachi, 1977). Proteases cleave this peptide and the physiological active insulin is released by exocytosis. Insulin is transported in the plasma bound to β-globulin. Fasting plasma level of insulin is 0 -70μ unit /ml. Inactivation of insulin is usually done by enzyme glutathione insulin dehydrogenase found in the liver. There is thus splitting of the disulphide bridges between α and β chains, (cysteine amino acid at 7-7 and 20-19 position) of the chain respectively.

The first stimuli for insulin release from pancreatic β cell are a family of peptide, (incretins, release in their amino acid sequences), that include glucose-dependent insulinotropic polypeptide (GLP), glucose-like peptide1 (GLp-1) glucagons, vasoactive intestinal peptide, and pituitary adenylate cyclase -activating peptide. Of these, perhaps the most protein is GLP1 (Wang *et al.*, 1995). GLP was discovered in 1973 on the basis of its ability to inhibit acid secretion in denervated gastric pouches, but its insulinotropic properties were discovered soon after (Dupre et al., 1973). GLP is a peptide of 42 amino acids processed from a precursor of 153 amino acid (Takeda *et al.*, 1987).

The GLP receptor is a type II G protein -coupled receptor belonging to a super family of receptors the ligands of which are constituted by the members of the secretin -glucagons family of peptide (Mayo *et al.*, 2003). It is expressed in the pancreatic Islet and also in the gut, adipose tissue, heart, pituitary, and adrenal cortex. GLP is secreted from specific endocrine cells, the so-called K cells, which exhibit the highest density in the duodenum, but GLP producing cell may be found in the entire small intestinal mucosa (Mortensen *et al.*, 2003). Its secretion is stimulated by absorbable carbohydrate and by lipids. GLP secretion is therefore greatly increased in response to meal ingestion, resulting in 10-to-20 folds elevation of plasma concentration. Incretins are released in the circulation in response to food, primarily carbohydrate and long-chain fatty acids in the gut. With absorption of glucose and ensuring hyperglycemia, more insulin is released, beginning at 8mM blood glucose and increasing as blood glucose concentration increases up to a maximum. While it falls to a second peak, often obscured by fusion with the down limb of the first surge of insulin. Plasma insulin concentration then continues to fall to the upper range of normal basal and then falls slowly during the intermediate and late postprandial period. During the rise and peak portion of the blood insulin concentration curve, blood level of GH decreases, even to immeasurable small concentration. GH, glucagon, β adrenergic agonist, and cortisol are released into the circulation at some portion of the down limb of falling blood glucose concentration, reported to be 1.6 mmol below subject's basal level that is 3.3mmol or less (Defronze *et al.,* 1977).

There are agents that act to prevent or reverse insulin- induced hypoglycemia, of these agents and the most important is glucagon, which stimulates endogenous glucose production. The incretin glucagons-like polypeptide (GLP-1) has multiple role, not only does it stimulate insulin release, but it also inhibits glucagon release, tending to delay hepatic glycogenolysis. GLP-1 is a product of the glucagon gene. The gene is expressed not only in the pancreatic cells but also in the L cell of the intestinal mucosa, perhaps the most abundant endocrine cell of the gut

(Mojsor *et al.,* 1986). The primary translation product proglucagen is cleaved, not to produce glucagons, as in the islet, but to release from its COOH-terminal part of the two glucagons-like peptides GLP-1 and GLP-2 (Orskor *et al.,* 1986) both showing 250% sequence homology with glucagon. The NH_2-terminal part of the precursor, which includes the glucagons sequence, is secreted as a single, rather large peptide designated glicentin (formerly called gut glucagons or GL1), which most likely is biologically inactive. Some of the glicentin moieties are cleaved further to release the peptide oxyntomodulin, which corresponds to the glucagon sequence plus the additional C00H-terminal octapeptide of glicentin. This peptide is insulinotropic and probably explains the insulinotropic activity of some preparation of “gut glucagons”. (Baldissera *et al.,* 1988).

However, as a circulating hormone in humans, its concentration is probably too low to significantly influence insulin secretion under physiological circumstances (Holst, 1983). Recently, GLP-1 attracted considerable interest because of its appetite-Inhibiting properties (Cohen *et al.,* 2003). GLP-1 secretion is stimulated by the presence of nutrient in the lumen of the gut (but additional neural or endocrine mechanisms may also operate), and the secretion of GLP-1 throughout the day is highly correlated to the release of insulin. GLP-1 insulinotropic activity, which is strictly glucose dependent, is exerted via interaction with a specific receptor located on the cell membrane of the β cells. The GLP receptor belongs to the same family as the GLP receptor (Mayo *et al.,* 2003). Binding of GLP-1 to the receptors causes activation, of adenylate cylase, via a stimulatory G protein, resulting in the formation of cAMP. There after, subsequent activation of protein kinase A and the cAMP-regulated guanine nucleotide exchange factor II (CAMP-GEFII, also known as EPac2) leads to a plethora of events, including altered ion channel activity, intercellular calcium handling, and enhanced exocytosis of insulin-containing granules (Holz, 2004).

Second, GLP-1 stimulates all steps of insulin biosynthesis as well as insulin gene transcription (Fehman and Habener, 1992), thereby providing continued and augmented supplies of insulin for secretion. Activation of pancreatic duodenal homeobox-1, a key regulation of Islet growth and insulin gene transcription, may be involved. In additions, GLP-1 up regulates the gene for cellular machinery involved in insulin secretion, such as the gluco-kinase and Glu T2 gene (Buteau *et al.,* 1999). Finally and most importantly, GLP-1 has been shown to have trophic effect on β cells (Egan *et al.,* 2003). Furthermore, GLP-1 stimulates β cell proliferation (Stoffers *et al.,* 2000) and enhances the differentiation of new β cell from progenitor cells in the pancreatic duct epithelium (Zhou *et al.,* 1990). Most recently, GLP-1 has been shown to be capable of inhibiting apoptosis of β-cell (Li *et al.,* 2003), where the normal number of β-cell is maintained in a balance between apoptosis and proliferation (Bonner-weir *et al.,* 2000). This observation is of considerable interest and also raises the possibility that GLP-1 could be used in conditions with increased β- cell apoptosis.

In the pancreas, the adenosine triphosphate (ATP),and ATP-sensitive potassium (K_{ATP}) channels are proposed to have a critical link in glucose-Induced insulin release from pancreatic β cells (Ashcroft and Riesman, 1990, Mister *et al.,* 1992). According to the paradigm, during the fed State, when glucose metabolism is increased, pancreatic K_{ATP} channel are inhibited by a high intracellular ATP to ADP concentration ratio. This depolarize the plasma membrane, which leads to Ca^{2+} entry through Voltage-dependent Ca^{2+} channel, (or VDCC), there by stimulating insulin secretion. A rise in circulating serum insulin, in turn, leads to increased glucose uptake in the periphery and a compensatory drop in blood glucose. Conversely, a falling of intracellular ATP to adenosine diphosphate (ADP) ratio during the fasting state is presumed to relieve inhibition of K_{ATP} channels, resulting in membrane hyperpolarization and cessation of insulin release (Mister et al., 1992).

After insulin binds to its receptor, the activated receptor tyrosine kinase catalyzes autophosphorylation of the receptor and subsequent phosphorylation of intracellular substrate on tyrosine residues. The major substrates of the insulin receptor (IR) kinases in most cells are IRS-1 and 2. When phosphorylated on tyrosine residue, those proteins serve as docking platforms for homology (SH)-2 dormin contain protein, such as Grb 2 or varret, of insulin biological effect (Virkamaki *et al.*, 1999). Another critical aspect of insulin action is the activation of the intracellular enzymes phospholinositide 3-kinase (P1- 3 kinase) (Shepherd *et al.,* 1998). P1-3-kinase is a heterodimer composed of a 110 KDa catalytic subunit and a regulating subunit. In insulin sensitive tissues most of P1-3-kinase activation is mediated by the regulatory subunit P85a and its shorter splice variant P50a and P55a. A large body of evidence obtained in cultured cell indicated that P1-3 kinase has a central role in the metabolic effect elicited by insulin, including glucose transport in muscle and fat, stimulation of glycogen synthesis and inhibition of hepatic glucose production (Shepherd *et al.*, 1998). Protein-tyrosine phosphates (PT-pases) act as physiological negative regulators of insulin signaling by diphosphorylating the IR and the IRS protein thereby limiting the insulin signal. Increased expression and activation of PTpases have been observed in muscle and fat from obsess and diabetic human and rodent and are believed to be involved in the pathogenesis of insulin resistance (Ahmed and Goldstein 1985; Ahmed *et al.*, 1997).

2.4 IN VIVO ROLE OF GLUCAGON IN GLUCOSE HOMEOSTASIS

Glucagon is a 29-amino acid peptide hormone processed from proglucagon. Proglucagon is expressed in various tissues (e.g. brain, pancreases, and intestine). And is proteolytically processed into multiply peptide hormone. In a tissue-specific fashion for example, proglucagon is processed into functional glucagons-like peptides-1 and 2 by subtillisin-like proprotein convertases (PCI-3) in intestinal L cells (Rouille *et al.*, 1997). Also, it is processed into functional glucagons by PC2 in the pancreatic cells (Rouille *et al.*, 1994, Furuta *et al.*, 2001). Glucagon act via a seven-Tran membrane G- protein-coupled receptor consisting of 4 and 5 amino acid (Jelinek *et al.*, 1993).

To date, glucagons – binding sites have been identified in multiple tissues, including liver, brain, pancreas, kidney, intestine and adipose tissue (Burcelin *et al.*, 1996). Glucagons are released into the blood stream when circulating glucose is low. The main physiological role of glucagons is to stimulate hepatic glucose output, thereby leading to increase in glycemia. This provides the major counter- regulatory mechanism for insulin in maintaining glucose homeostasis in vivo. There is ample evidence suggesting that glucagons play an important role in initiating and maintaining hyperglycemic conditions in diabetic animals and humans. Insulin and glucagons are the regulatory hormones for glucose homeostasis. The absolute level aids, even more so, the ratio of the two hormones are highly regulated in vivo, depending on nutritional status. It has been reported that the absolute level of glucagon or the ratio of glucagon to insulin are often elevated in various forms of diabetes in both animal and human subjects (Unger, 1985, Burcelin *et al.,* 1996). Diabetes is also one of the diseases associated with glucagonoma (glucagons-secreting tumor) derived from pancreatic Islet α-cells (Chaustain, 2001). Chronic hyperglucagonmia is correlated with and is at least partially responsible for increased hepatic glucose output and hyperglycemia in type 2 diabetes (Consoli, 1992).

Glucagon signals through its receptor on the surface. The binding of glucagons to the extracellular loops of the glucagons receptor result in conformational changes of the latter, leading to subsequent activation of the coupled G protein. At least two classes of G protein are known to be associated with and involved in the signal transduction of the glucagon receptor, namely; $G_{S\alpha}$ and G_q. The activation of $G_S\alpha$ leads to activation of adenylate cyclase, increase in intracellular cAMP level and subsequent activation of protein kinase A (PKA). The activation of G_q leads to the activation of a phospholipase C, production of inositol 1,4,5-triphosphate, and subsequent release of intracellular calcium (Christopher, 1995, Burcelin *et al.,* 1996). Glucagon and glucagon's receptor represent potential target for the treatment of diabetes (Zhang and Moller, 2000).

2.5 CLASSIFICATION OF DIABETES

2.5.1 TYPE 1 DIABETES MELLITUS

Type 1 diabetes is the most common diagnosed in children and adolescents, but can occur in adults as well. It is an autoimmune disorder, in which the body owns immune system attacks the beta cells in the Islet of Langerhans of the pancreas destroying them or damaging them sufficiently to reduce insulin production. The autoimmune attack may be triggered by reaction to an infection, for example by one of the viruses of the Coxsackie virus family. A subtype of Type 1(identify by the presence of antibodies against beta cells) develops slowly and so is often confused with Type 2. In addition, a small proportion of Type 1 cases has the hereditary maturity onset of the young (MODY) Formerly, Type 1 diabetes was called "childhood' or 'juvenile' or insulin-dependent diabetes. Each term is a misnomer, most especially in children and adolescent and insulin is used in some Type2 cases. Experimental replacement of beta cells (by transplant) is being investigated in several research programs and may become clinically available in future. (Weyer *et al.*, 2001; Homko et al., 2001).

2.5.2 TYPE 2 DIABETES MELLITUS

Type 2 diabetes is characterized by insulin resistance as body cells do not respond appropriately when insulin is present. This is a more complex problem than type 1, but is sometimes easier to treat, since insulin is still produced in many, especially in the initial years. Type 2 may go unnoticed for years in a patient before diagnosis, since the symptoms are typically milder (no ketoacidosis) and sporadic (Gerich, 2000;Porte,2001;Tripathy, 2000).

2.5.3 TYPE 3 DIABETES MELLITUS

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

2.5.4 TYPE 4 DIABETES MELLITUS

Type 4 or gestational diabetes mellitus appears in about 2-5% of all pregnancies. It is temporary and fully treatable, but if untreated it may cause problems with the pregnancy. It requires careful medical supervision during the pregnancy. In addition, about 20-50% of these women go on to develop type 2 diabetes (Porte, 2001).

2.6 DIAGNOSIS OF DIABETES MELLITUS

The diagnosis of type 1 diabetes is usually prompted by recent symptoms of excessive urination (polyuria) and excessive thirst (polydipsia), often accompanied by weight loss. Those symptoms typically worsen over days to weeks; about 25% of people with type one diabetes have developed diabetic ketoacidosis by the time it is recognized. The diagnosis of other types of diabetes is made in many ways. Genetic factors play an important role in the etiology of both type 1 and type 2 diabetes (Simpson, 1968; Rotter *et al.,* 1981; Abiru *et al.,* 2000; Ghosh *et al.,* 2000; Nolse *et al.*, 2000). The pathogenesis of type 2 diabetes has been an area of intense investigations. It is now clear that this is a heterogeneous condition both phenotypically and genotypically and that acquired reversible abnormalities/risk factors also plays an important role. Currently, type 2 can be viewed as developing in genetically susceptible individuals who because of impaired beta-cell function, are incapable or increasing their insulin release appropriately to compensate for reduced insulin sensitivity which is acquired through life for various reasons (e.g. Obesity, aging, physical inactivity, drug use or diet). Obesity has also been found to be a predisposing factor especially in type2 diabetes mellitus (Tafeit *et al.,* 2000). Environmental factors have also been incriminated in the causation of diabetes mellitus, especially cigarette smoking (Yoon *et al.,* 1987; Manson *et al.,* 2000; Sabbah *et al.,* 2000). In a comprehensive survey on the population of Western region of Saudi Arabia, a prevalence of diabetes of 4.95% and 4.3% was encountered in urban and rural areas (Fatani *et al.,* 1987) populations respectively. Some studies have shown some racial predisposition (Kahn and Wilhanson, 2000). Other investigators have shown that there is no difference with the socioeconomic status of individuals and development of diabetes mellitus (Robbins *et al.,* 2000).

2.7 CRITERIA FOR DIAGNOSIS

Diabetes mellitus is characterized by recurrent persistent hyperglycemia and is diagnosed by demonstrating any one of:

(a) Two fasting plasma glucose levels above 7mmol/l (125mg/dl). Plasma glucose above 11 mmol/l (200mg/dl) two hours after a 75 g glucose load. Elevated glucose bound to hemoglobin Hb1c,of 6.0 % or higher(2003 revised US standard). Besides, a screening and treatment-tracking test reflecting average blood glucose levels over the preceding 90 days(UKPDS,1998) .

2.8 RISK FACTORS IN DIABETES MELLITUS

Disorders of Insulin: This could either be disorders in the secretion (Gerich, 2000; Silva et al., 2000) or Insulin resistance from some receptor defects (Bilkhazi *et al.,* 2000; Walston *et al.,* 2000).

Autoimmune disorders: Autoimmune diabetes is caused by selective loss of insulin-producing pancreatic beta-cells.The main factors directly implicated in beta-cell death are autoreactive, cytotoxic Islet-antigenspecificT-Lymphocyte (CTL), and inflammatory cytokines (Pehuet-Figoni *et al.,* 2000).

Viral Infections: These have implicated especially in type 1 diabetes mellitus (Seewaldt *et al.,* 2000). Certain viruses have been associated with beta-cell destruction. Diabetes occurs in some patients with congenital rubella (Forrest *et al.,* 1971). In addition, Coxsackie B, cytomegalovirus and other viruses (e.g. adenovirus and mumps) have been implicated in inducing the disease (King *et al.,* 1983; Karjalainen *et al.,* 1988; Pak *et al.,* 1988).

Insulin like growth factor binding protein-1 is closely related to risk factors for diabetes and cardiovascular disease in people of European and Pakistani origin. It has potential use as a marker of (hepatic) insulin resistance in clinical intervention studies and further implicates the insulin like growth factor system in the development of macrovascular disease.

Heald *et al*. (2001) and Kalhan *et al.* (2001) reported altered lipid profile, Leptin, insulin and anthropometry in offspring of South Asian immigrants in the United States as a risk factor in diabetes mellitus. They found a significant correlation between plasma Leptin and insulin, triglycerides and body mass index (BMI) in the South Asian males. South Asian born in the United States show evidence for an altered metabolic profile in young adulthood. The relative contribution of this inheritance and nutritional practices early in life to this alterations remain unclear. Horn *et al*. (2001) studied correlates and predictors of adiposity among Mohawk children. They reported high rates of type 2 diabetes in native populations underlie the need for research on risk factors, including physical activity and obesity.

Falkenberg (2001) reported that the relative risk for type 2 diabetes increased with waist circumference. Making health care personnel and patients aware of overweight, obesity and abdominal obesity and the risk of associated diseases.

Defay *et al.* (2001) in a study of relationships between physical activity, obesity and diabetes mellitus in a French elderly population reported overall obesity in men and abdominal fat accumulation in women appeared strongly related to diabetes. Sport activity was negatively and independently associated with the prevalence of diabetes mellitus. Misra *et al.* (2001) studied the relationship between Plasma Leptin and anthropometric and metabolic covariates in lean and obese diabetic and hyperlipidaemic Asian Northern Indian subjects. They suggested that plasma Leptin concentration has a strong positive correlation with percent total body fat in Asian Northern Indian subjects. Among other components of metabolic syndrome, only abdominal obesity is weekly correlated to serum Leptin levels.

Pisunyer (2000) defined the criteria and classification of obesity. He reported that a waist circumference greater than 102 mm in men and 88 mm in women is a risk factor for insulin-resistant diabetes mellitus and cardiovascular disease. In another study for standard definitions of overweight and central adiposity for determining diabetes risk in Japanese Americans, it was reported that a waist circumference greater than or equal to the third percentile was associated with diabetes (McNeely *et al.,* 2001). They concluded that although central adiposity is an important risk factor, the guidelines for waist circumference are insensitive predictors of diabetes risk in this population.

Hultberg *et al.* (1996) reported that plasma/serum beta-hexosaminidase activity is known to be increased in chronic alcoholism, liver disorders, pregnancy and diabetes mellitus. They also showed that beta-hexosaminidase activity can be a risk factor in diabetes mellitus, hypertension and other vascular diseases. Chatila and West (1996) reported hepatomegally and abnormal liver tests as risk factors in patients with hepatocellular glycogen accumulation in adult diabetes mellitus. The typical biochemical findings were mildly to moderately elevated aminotransferases, with or without mild elevations of alkaline phosphatase.

It has been suggested that alterations in intracellular Ca^{2+} homeostasis may be responsible for development of diabetic cardiomyopathy (Lagadic *et al.,* 1996). They concluded that diabetes is associated with significant alterations in intercellular calcium homeostasis resulting primarily from dysfunction of sarcoplasmic reticulum.

2.9 PATHOPHYSIOLOGY AND COMPLICATIONS OF DIABETES MELLITUS

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and in absence of effective treatment, death. On presentation to hospital, the patient in diabetic ketoacidosis is typically dehydrated and hypokalemic.Urgent intravenous fluid resuscitation, potassium replacement and insulin replacement should be instituted (Tripathy *et al.,* 2000).

Symptoms sometimes, are not severe, or may be absent. Functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, define the etiological class.(Homko et al.,2001)

2.10 PATHOPHYSIOLOGY OF INSULIN DEPENDANT DIABETES MELLITUS (TYPE 1)

Type 1 indicated the processes of beta-cell destruction that may ultimately lead to diabetes mellitus in which “insulin is required for survival” to prevent the development of ketoacidosis, coma and death. An individual with a Type 1 process may be metabolically normal before the disease is clinically manifest, but the process of beta-cell destruction can be detected. Type 1 is usually characterized by the presence of anti-glutamic acid decarboxylase (GAD), islet cell or insulin antibodies. Some subjects with this clinical form of diabetes, particularly non-Caucasians are classified as “Type 1 idiopathic” Etiological classification may be possible in some circumstances and not in others. Thus, the etiological Type 1 process can be identified and sub-categorized if appropriate antibody determinations are performed. It is recognized that such measurements may be available only in certain centers at the present time. Where these measurements are performed, then the classification of individual patients should reflect this. Insulin-dependent diabetes mellitus (IDDM) occurs most frequently in persons of northern European decent. Among other racial groups as African American, Native Americans, and Asians, the disease is less common. The incidence ranges from a low level of 1 to 2 per 100,000 per year in Japan to a high level of more than 40 per 100,000 per year in parts of Finland (Green *et al.,* 1992). The differences in the incidence are largely explainable by the prevailing susceptibility of genes for IDDM in racially distinct populations, but diet and other environmental factors may also be important (Mclaren and

Atkinson, 1992). Evidence implying an environmental influence includes the increased incidence of IDD in some countries in the past three decades (Tuomilehto *et al.,* 1991; Drykoningen *et al.,* 1992). Also, it was reported that among patients with IDDM who are identical twins, the disease subsequently developed in only 33 percent of the twins of the index patients (Barnett et al., 1981) and that 90 percent of patients with newly diagnosed IDDM do not have an affected first-degree relative (LarPorte and Cruickshank, 1984).

Susceptibility to IDDM is inherited, and marked differences in risk have been found according to the familial relationship with the diabetic proband. The main gene associated with a predisposition to IDDM is the major histocompatibility complex (MHC) on chromosome 6, in the region associated with the genes for the highly polymorphic immune-system-recognition molecules known as Human leucocyte antigen (HLA). HLA class I molecules are dimeric proteins composed of a Tran membrane alpha chain and B_2-microglobulin that are present on the surface of all nucleated cells and platelets their function is to present antigenic peptides to CD_8 (cytotoxic or suppressor) T lymphocytes. The MHC also encodes three human leucocyte antigens (HLA) class II molecules: HLA-DP, DQ, and DR. HLA class II proteins are also dimeric, composed of an alpha and a beta chain, and expressed constitutively (e.g., dendritic cells and B lymphocytes) or inducible (e.g., macrophages and endothelial cells) on the surface of antigen-presenting cells. These molecules re-present antigenic peptides to CD_4 (helper or inducer) T lymphocytes. Both CD_4 and CD_8 cells have unique T-cell receptors for antigens on their surface, most often composed of an alpha and a beta chain, that have a high affinity for particular complexes of peptide antigens and HLA molecules. In addition to HLA class II genes, another gene for susceptibility to IDDM has been identified on the short arm of chromosome 11 near the genes for insulin and insulin-like growth factor II (Lucasson *et al.,* 1993).

Autoimmune diabetes mellitus previously encompassed by the terms insulin-dependent diabetes, Type 1 diabetes, or juvenile-onset diabetes, results from autoimmune-mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet *et al.,* 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey *et al.,* 1998). The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual beta-cell function, sufficient to prevent ketoacidosis, for many years (Zimmet, 1995). Individuals with this form of Type 1 diabetes often become dependent on insulin for survival eventually and are at risk for ketoacidosis (Willis *et al.,* 1996).

Markers of immune destruction, including islet cell autoantibodies, and/or autoantibodies to insulin, and autoantibodies to glutamic acid decarboxylase (GAD) are present in 85-90% of individuals with Type 1 diabetes mellitus when fasting diabetic hyperglycemia is initially detected (Verge *et al.,* 1996). The peak incidence of this form of Type 1 diabetes occurs in childhood and adolescence, but the onset may occur at any age, ranging from childhood to the ninth decade of life (Molbak *et al.,* 1994). There is a genetic predisposition to autoimmune destruction of beta cells, and it is also related to environmental factors that are still poorly defined. Although patients are usually not obese when they present with this type of diabetes, the presence of obesity is not due to autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, and Addison's disease (Betterle *et al.,* 1983).

Idiopathic diabetes mellitus are some forms of Type 1 diabetes which have no known etiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity (McLarty *et al.*, 1990). This form of diabetes is more common among individuals of African and Asian origin. In another form found in Africans an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis (Ahren and Corrigan, 1984).

The most striking histologic feature of the pancreas of a patient with long-standing IDDM is the near-total lack of insulin-secreting beta cells **(**Gepts, 1965). By contrast, islet cells secreting glucagons (alpha cells), somatostatin (delta cells), or pancreatic polypeptide (pancreatic-polypeptide cells) are preserved. Since beta cells constitute the majority (70 percent) of cells within normal islets, the islets of a patient with long-standing diabetes are abnormally small (Gepts, 1965). Aside from mild interstitial fibrosis and exocrine atrophy, there are no other obvious histologic abnormalities. Histologic studies suggested that 80 percent reduction in the volume of beta cells which is required to induce symptomatic IDDM (Foulis *et al.*, 1986). Histologic evidence of islet regeneration is uncommon, but it is found in the pancreas of some young patients with IDDM (Gepts and Lecompte, 1985).

A number of islet-cell antigens have been associated with IDDM. Better characterization of these antigens could simplify immunoassays for the detection of IDDM-associated auto antibodies, improve our understanding of the role of cellular immune mechanisms in the disease, clarify whether antigen presentation is a function of susceptibility or resistance to IDDM, and lead to antigen-specific treatments to prevent IDDM. These antigens may be directly involved in the pathogenesis of IDDM, or they may merely serve as the targets of autoantibodies that are markers of immunity to beta cells. The component of the immune system that has the main role in the destruction of beta cells is unknown. CD_8 cells are the predominant type of lymphocyte in the inflamed islets of patients with newly diagnosed IDDM who died by diabetic ketoacidosis (Bottazzo *et al.,* 1985, Hanninen *et al.,* 1992). In non obese diabetic mice, however, CD_4 lymphocytes are also critical, because treatment with anti-CD_4 antibodies prevented IDDM in these mice (Wang *et al.,* 1987; Shizuru *et al.,* 1988). Furthermore, the elaboration of cytokines by cells within the inflamed islets may have an important pathogenic function. Interleukin-1 inhibits the secretion of insulin by beta cells and is cytotoxic at high concentrations (Mandrup-Poulsen *et al.,* 1986). Beta cells can produce interleukin-6, a cytokine that powerfully stimulates the immune response and may enhance insullitis and the destruction of beta cells (Campbell *et al.,* 1991). Interferon-α has consistently been detected in the pancreas of patients who have died because of diabetic ketoacidosis (Foulis *et al.,* 1987).

The production of interferon-α is induced by viruses, and it stimulates the expression of HLA molecules and is chemotaxic to other lymphocytes,(Steward *et al.,* 1993). Inflammatory responses often induce the production of superoxide radicals, and sites of tissue injury are characterized by high concentrations of nitric oxide. Both factors have been implicated as important mediators of beta-cell damage in IDDM. Since the enzymes that reduce injury by superoxide radicals (i.e. superoxide dismutases) are weakly expressed in beta cells (Asayama *et al.,* 1986; Nomikos et al., 1989). Beta cells may be especially prone to this form of injury. Beta cells also produce an abundant amount of nitric oxide in response to stimulation by cytokines (Corbett *et al.,* 1993). Therefore, the elaboration of cytokines by inflammatory cells within the inflamed islets may induce the expression of nitric oxide by beta cells, which in itself may be lethal to these cells.

2.11 PATHOPHYSIOLOGY OF NON-INSULIN-DEPENDENT DIABETES MELLITUS (TYPE 2)

It is a term used for individuals who have relative (rather than absolute) insulin deficiency. People with this type of diabetes frequently are resistant to the action of insulin (Lillioja *et al.,* 1993; DeFronzo *et al.,*1997). At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. This form of diabetes is frequently undiagnosed for many years because the hyperglycemia is often not severe enough to provoke noticeable symptoms of diabetes, Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Harris *et al.,* 1993; Mooy *et al.,*1995).There are probably several different mechanisms which result in this form of diabetes, and it is likely that the number of people in this category will decrease in the future as identification of specific pathogenetic processes and genetic defects permits better differentiation and a more definitive classification with movement into “Other types”. Although the specific etiologies of this form of diabetes are not known, by definition autoimmune destruction of the pancreas does not occur and patients do not have other known specific causes of diabetes. Insulin resistance is a diminished ability of insulin to exert its biologic action across a broad range of concentrations. Unless persons with insulin resistance secrete abnormally high amounts of insulin to compensate for this defect and properly to stimulate glucose transport in muscle and fat and suppress hepatic glucose production, plasma glucose concentrations will inevitably rise and diabetes will develop. In Western societies, insulin resistance is common and is associated with a variety of abnormalities, including obesity, hypertension, hyperlipidemia, and hyperuricemia, as well as a sedentary, lifestyle (Reaven, 1994). A number of molecular defects have been associated with insulin resistance. These include reduced expression of insulin receptors on the surface of insulin-responsive cells (Beck-Nielson, 1978; Defronzo *et al.*, 1979), and alterations in the signal-transduction pathways that become activated after insulin binds to its receptors (Golay *et al.*, 1988; Maegawa, *et al.*, 1991). Also, abnormalities in biologic pathways normally stimulated by insulin, including glucose transport (Garvey *et al*, 1988; Erickson *et al.*,1992) and glycogen synthesis (Groop *et al.*, 1993; Vestergaard et al.,1993).

Mutations in the insulin-receptors gene are responsible for insulin resistance in a limited number of persons (Taylor *et al.*, 1992) but the molecular basis of insulin resistance in the vast majority of persons with NIDDM remains unknown. Insulin resistance is clearly an important risk factor for NIDDM (Gulli *et al*, 1992; Martin *et al.*, 1992). However, although NIDDM is more common in people with insulin resistance than in those who are sensitive to insulin, the majority of people with insulin resistance do not become diabetic. Moreover, those who eventually do become diabetic may have insulin resistance for many years without having a substantial increase in their plasma glucose concentrations. The physiologic differences between persons with insulin resistance who become diabetic and those who do not are important for understanding the pathophysiology of NIDDM. The pancreatic beta cell is an obvious place to look for such differences, since the ability of the beta cell to produce more insulin to compensate for insulin resistance determines whether plasma glucose concentrations remain normal or increased. Insulin concentrations are increased in persons with insulin resistance who have normal glucose tolerance. Obesity is probably the commonest cause of insulin resistance. Basal and total 24-hours rates of insulin secretion are three to four times higher in obese subjects than in lean controls (Polonsky *et al.,* 1988).

The majority of patients with this form of diabetes are obese, and obesity itself causes or aggravates insulin resistance (Bogardus *et al.,* 1985; Campbell and Carlson, 1993). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Kissebah *et al.,* 1982).

Ketoacidosis is infrequent in this type of diabetes; when seen it usually arises in association with the stress of another illness such as infection (Banerji *et al.,* 1994; Umpierrez *et al.,*1995). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated. The high blood glucose levels in these diabetic patients would be expected to result in even higher insulin values and their beta-cell functions are normal (Polonsky *et al.,* 1996). However insulin secretion is insufficient to compensate for the insulin resistance. On the other hand, some individuals have essentially normal insulin action, but markedly impaired insulin secretion. Insulin sensitivity may be increased by weight reduction, increased physical activity, and/or pharmacological treatment of hyperglycemia but is not restored to normal (Simonson *et al.,* 1984; Wing *et al.,* 1994). The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity (Zimmet, 1991; Harris *et al.,* 1995). It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidaemia. Its frequency varies in different racial/ethnic subgroups (Zimmet, 1991). It is often associated with strong familial, likely genetic, predisposition (Knowler *et al.,* 1993). However, the genetics of this form of diabetes are complex and not clearly defined.

Some patients who present with a clinical picture consistent with Type 2 diabetes have autoantibodies similar to those found in Type 1 diabetes, and may masquerade as Type 2 diabetes if antibody determinations are not made. Patients who are non-obese or who have relatives with Type 1 diabetes and who are of Northern European origin may be suspected of having late onset Type 1 diabetes.

2.12 ACUTE COMPLICATIONS OF DIABETES MELLITUS

The principal biochemical manifestation is hyperglycemia. When the renal threshold for glucose reabsorption is exceeded, there is glycosuria and an osmotic diuresis and an accompanying loss of Na^+ and K^+ ions. An increase in serum osmolarity occurs, causing thirst and polydipsia. The loss of weight in spite of polyphagia results from failure to utilize glucose, and from a compensatory increase in catabolism of protein and fats. Glucagons, produced from the α-cells, which is relatively increased in diabetes, help to stimulate gluconeogenesis thus contributing to the hyperglycemia in diabetes. In insulin lack, there is an increased amount of acetyl-CoA formation which cannot be adequately catabolised because of the intracellular depletion of the carbohydrate component of the cycle. They are therefore preferentially converted to ketone bodies: Acetoacetic acid, Acetone, and β-hydroxybutyric acid. These ketone bodies dissociate to yield hydrogen ions, and produce metabolic acidosis. The metabolic acidosis stimulates the respiratory centre, resulting in hyperventilation (Kussmaul breathing) which reduces the plasma Hco_3^-. It also results in acidification of the urine; excretion of the acidic ions is being accompanied by an increased excretion of NH_4^+ and later of Na^+ and K^+ ions. This further aggravates the cationic loss which accompanies the glucose induced osmotic diuresis. The total body Na^+ and K^+ are thus depleted. The serum Na^+ ions levels decrease, but the serum K^+ ions increase. This level is maintained at the expense of the intracellular K^+ ions. This metabolic disturbance produces hyperosmolarity, hypovolumia, and electrolyte imbalance which seriously affect neuronal functions. This results in the clinical syndrome of diabetic keto-acidotic coma. This is commonly seen in type 1 diabetes mellitus **(**Sellers and Dean, 2000). Whereas in type 2 diabetes mellitus, a hyperosmolar non-ketonic coma occurs which is characterized by severe dehydration and profound hyperglycemia with minimal or no keto-acidosis (Liamis *et al.*, 2000).

Insulin deficiency is the basic defect in lipid handling among insulin-dependent diabetic subjects (Howard, 1987). The cause of the lipid alteration among non-insulin dependent diabetic subjects is differential insulin distribution (Reaven, 1987) which leads to increase in VLDL and triglyceride production through hepatic hyperinsulinemia. This is combined with decreased catabolism of triglyceride-rich lipoprotein due to relative peripheral insulin deficiency. There is a decrease in the fractional catabolic rate of apoprotein B in LDL (Briones *et al.*, 1984; Reaven, 1987). Inspite of this, the LDL concentration is not increased because of the alteration of lipid composition with increase in triglyceride-rich VLDL particles. The conversion to LDL, however, is decreased among NIDDM subjects (Goldberg, 1981; Kosther and Karadi, 1988).

2.13 LONG TERM COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus is a disease of metabolic dysregulation, most notably abnormal glucose metabolism, accompanied by characteristic long-term complications. The complications that are specific to diabetes include retinopathy, nephropathy, and neuropathy. Patients with all forms of diabetes of sufficient duration, including insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), are vulnerable to these complications, which cause serious morbidity and mortality.

The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joins, features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular diseases. If the diabetes is poorly controlled it can lead to diabetic complications. Diabetic complications are much more common in type 2 patients with approximately 50% suffering from 1 or more complications at the time of diabetes (Panzram, 1987).

These can be split into two main categories:

Microvascular complications

Macrovascular complications

2.13.1 MICROVASCULAR COMPLICATION

Diabetes affects the small blood vessels throughout the body. Damage of these vessels and the basement membrane causes impaired delivery of nutrients and hormones to the tissues, resulting in tissue damage. The sites most affected are the retina, renal glomerulus and the nerve sheath. The main complications are outlined below (Macauley *et al.,* 2000).

RETINOPATHY

This is a major cause of blindness in diabetic patients. Microangiopathy affecting the retina develops over a number of years. Vision is not affected by all retinopathies, however prompt treatment is necessary in controlling the condition and maintaining vision. Therefore it is important to advise patients to have eye examinations annually (Buchbinder *et al.,* 2000; Nwosu, 2000; Schmidt *et al.,* 2000).

NEPHROPATHY

This is a major cause of mortality in diabetics. Diabetic nephropathy is recognized by the detection of albuminuria from urine tests. Progress is evaluated by measuring albumin excretion (microalbuminuria = 30-300 mg/24hrs; macroalbuminuria >300 mg/24hrs) from a timed urine collection (Daily *et al.,* 2000; Steffes and Basgen, 2000). It is recommended that the patients are screened annually for proteinuria and heamaturia.

NEUROPATHY

Diabetic neuropathy affects the sensory nerve system causing pain (sharp, stabbing or burning particularly on the skins and soles of feet). Other symptoms of autonomic neuropathy include impotence in men, gastrointestinal dysfunction and lack of sweating in the feet (Li *et al.,* 2000; Sima *et al.,* 2000; Winkler *et al.,* 2000). In this case drug therapy is recommended according to the symptoms present .

2.13.2 MACROVASCULAR COMPLICATIONS

These affect the large vessels in the body and is more likely in type 2 diabetic patients. Risk factors for macrovascular complications are:-

Hyperglycemia, Obesity, Dyslipidaemia, Hypertension, Smoking and lack of exercise. Type 2 diabetic patients are in general more likely to suffer from coronary heart disease than their non-diabetic counterparts (Christensen *et al.*, 2000; Clark and Pierce, 2000; Toriyana *et al.*, 2000).

HYPERTENSION

This is an independent risk factor for cardiovascular, cerebral, renal and peripheral atherosclerotic vascular disease. The prevalence of hypertension increases with age, obesity and the duration of the diabetes. Management is by the way of anti-hypertensive e.g. ACE inhibitors.

DYSLIPIDAEMIA

Lipids and lipoproteins abnormalities are common in diabetes, particularly type 2 (Agbola-Abu *et al.*, 2000). It is very important to lower the levels of lipids in the body. This is done by drug therapy with drugs such as statins (Howard *et al.*, 1987; Schaper *et al.*, 2000).

DIABETIC FOOT

Peripheral neuropathy can cause symmetric sensory loss in the feet and legs, resulting in the loss of protective sensation in the feet. This combined with vascular impairment and infection can lead to development of the 'diabetic foot'. There is an increased risk of lesions or ulcers developing (Flynn, 2000; Lavey and Gazewood, 2000).

2.14 METABOLIC SYNDROME

Torgerson *et al.* (1997) reported elevated aminotransferases and decreased bilirubin as additional characteristics of the metabolic syndrome. They discovered that abnormal liver tests, as well as morphological changes in the liver, are frequent among obese patients. Other frequent disturbances are visceral fat accumulation, insulin resistance, non insulin-dependent diabetes mellitus (NIDDM), hypertriglyceridemia, and hypertension. These are set of aberrations known as metabolic syndrome. They examined associations between liver function tests, metabolic variables [insulin, glucose, and triglycerides], body composition and nutrition in both obese males and females of Swedish origin. In both genders aspartate amino-transferase and alanine amino transferase were, or tended to be positively correlated to fasting serum insulin, visceral adipose tissue [women] and alcohol intake. In women, the aminotransferase were also correlated with fasting blood glucose. In both genders alkaline phosphatase was, or tended to be positively associated with adipose tissue, insulin (women) and glucose. Bilirubin was negatively correlated to insulin and visceral adipose tissue in men and women. Additional multivariate analysis indicated that alcohol had less explanatory power than serum insulin for the examined liver tests, especially among women. These results suggested that pathological liver tests in the obese may represent an expression of metabolic syndrome. Insulin-induced hypoglycemia in the last trimester of diabetic pregnancy may cause embryopathic effects in rodents. There are no data in the humans to support a teratogenic effect (Person and Hansson, 1993).

The effects of alcohol intake and obesity on serum liver enzyme activity in obese men were studied by Fagerberg *et al.* (1993). They concluded that alcohol intake was an important determinant for elevated liver enzyme activity. The degree of obesity was a contributing factor for elevations of serum alanine aminotrasferase activity. They speculated that insulin may play a role in this relationship. O'Connor and his colleagues (1997) studied the etiological factor in pathogenesis of non alcoholic steatohepatitis. They found that clinical associations of probable relevance include gender (female), obesity, diabetes and hyperlipidemia. Liver biopsy confirmed the diagnosis. The association of steatosis with an inflammatory response being the sine qua non for the condition and "Creeping fibrosis" being a variable but possibly sinister feature.

Murakani *et al.* (1998) investigated the biological activity of a novel insulin sensitizer thiazolidinedione derivative and the molecular basis of this activity on the lipid metabolism in liver of obese rats. Their results suggested a protective effect against abnormal lipid metabolism in liver of obese rats.

Turnbull *et al.* (1997) studied the prevalence of hereditary haemochromatosis in a diabetic population. Of these patients, those at high risk were offered liver biopsy for histological assessment. Only one had hereditary haemochromatosis, but all had abnormal liver histology-largely steatosis but some with fibrosis. The study highlights the potential importance of diabetes as a cause of liver disease. Cooper (1997) reported that chylomicrons are formed in the intestine and transport dietary triglyceride to peripheral tissues and cholesterol to the liver. As a result, a new particle called a chylomicron remnant is formed. He reported also that in humans, delayed removal of chylomicron remnants has been documented in diabetes mellitus.

2.15 HEPATIC ABNORMALITIES AND CERTAIN DRUGS

The use of insulin and other drugs in management of diabetic patients have shown to cause some hepatic abnormalities (Twata *et al.,* 2000). Hepatic abnormalities are relatively frequent with the use of certain drugs like insulin, Amphotericin B especially in combination with potentially heptatotoxic agents. Surveillance of liver function should be included in the management of diabetic patients (Salerno *et al.,* 1993; Gill *et al.,* 1999). A study to evaluate the safety and efficacy of controlled-release niacin in patients with hyperlipoproteinemia By Gray *et al.* (1994) reported that niacin should be avoided in patients with hepatic dysfunction or a history of liver disease, patient with diabetes mellitus and patient who abuse alcohol. They also reported statistically, but not clinically meaningful does related increases in livers of liver enzymes and serum glucose.

Seifarth *et al.* (1999) reported that interferon-alpha therapy may lead to an augmented autoimmune reaction against islet cell antigens resulting in development of diabetes mellitus type 1, especially in the presence of other predisposing factors before interferon treatment. (Nishiyama *et al.,* 1999; Ichinose *et al.,* 1999).

2.16 SAFETY AND TOLERABILITY OF ANTIDIABETIC DRUGS

In a study to review the use of Troglitazone for the (non insulin-dependent) diabetes mellitus. Locker and Faulds (1999) reported an improved glycaemic control in patients with type 2 diabetes mellitus when used as monotherapy or in combination with other oral antidiabetic drugs or insulin. Its efficacy is similar to that of glibenclamide or metformin. They suggested that even though troglitazone is generally well tolerated, close monitoring of liver enzyme is required to minimize the rare occurrence of serious hepatic dysfunction. Repaglinide, an insulintrophic benzoic acid derivative, has been tested extensively in the preclinical and clinical setting for safety and tolerability. In an ascending dose, tolerability study on type 2 diabetic patients showed no clinical relevant changes in liver enzymes occurred during trial (Schatz, 1999).

The altered blood glucose, urea, cholesterol, triglycerides, liver glycogen and the activities of liver enzymes such as hexokinase, lactate dehydrogenase and fructose-1,6-diphosphatese, were reverted to normal levels in bisglycinate oxovanadium complex- treated diabetic rats, thereby suggesting for the insulin mimetic effect of bisglycinate oxovanadium in experimental diabetes. In comparative study of the efficacy and tolerability of policosanol and lovastatin in patients with hypercholesterolemia and non insulin-dependent diabetes mellitus. Crespo *et al.* (1999**)** reported that Lovastatin moderately but significantly ($p<0.05$) increased levels of aspartate aminotransferase, creatinine phosphokinase and alkaline phosphatase.

In another study on the insulin-dependent reduction in hepatic and splenic contents of interleukin -1 beta in experimental diabetes**.** Bitar *et al.* (1995) reported that insulin treatment restored the diabetes related decreases in liver and spleen interleukin-1 beta levels. Overall, the present data suggest that the abnormalities in hepatic and splenic interleukin -1 beta levels may contribute at least in part to immunodeficiency and increased susceptibility to infection in diabetes mellitus.

Little is known about the effects of islets grafts on the liver. De paepe *et al.* (1995) examined liver tissue of normal or streptozotocin diabetic rats at different intervals following intraportal injection of syngenic islets. They reported that implantation of islet grafts containing about 0.9 million beta cells, normalized overt diabetes within 14 days. Prevention of diabetes in the BB strain rats by early immunotherapy using Freund's adjuvant was reported by Sadelain *et al.* (1990). They suggested that it is possible to prevent diabetes by adjuvant treatment in early life without general immunosuppressant or sustained therapy.

Cam *et al.* (1993) demonstrated the insulin-like effects of oral vanadyl sulphate in the streptozocin diabetic rat, including the amelioration of hyperglycaemia and the prevention of diabetes elated cardiac and adipose dysfunction. They reported that prolonged treatment with vanadyl sulphate did not cause any apparent hepatic toxicity as assessed histologically. Diabetes induced morphological changes in the kidney were also prevented.

2.17 DIABETES MELLITUS AND FATTY LIVER

To test the hypothesis that fatty liver coexists with other metabolic abnormalities of insulin resistance syndrome and responds to their amelioration. Knobler *et al.* (1999) studied 48 consecutive patients with chronically elevated liver enzymes and clinical ultrasound and histologic findings revealed fatty infiltration of the liver. They found that fatty liver was strongly associated with many features of the insulin resistance syndrome.

Assy *et al.* (2000) found that serum aspartate transaminase values, hyperglycaemia and age, independently predict the presence of fatty infiltration, while hypertriglycerdemia and diabetes are the only risk factors that significantly increase the risk of fatty infiltration in hyperlipidemic patients.

Kral *et al.* (1993) reported that abdominal (truncal) fat distribution reflected by an elevated waist, to hip ratio predicts metabolic abnormalities such as diabetes and dyslipidemia as well as hypertension and strokes, all of which are associated with obesity. The pathogenesis is not known, although elevated splanchnic serum free fatty acid levels and reduced hepatic insulin clearance have been implicated.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS USED

3.1.0 ANIMALS:

Sixty three Wister rats of both sexes weighed between 120-150 grams were used for both acute toxicity studies as well as testing the effect of *Azadiracta indica* seed extract on blood glucose level of the animals. They were kept in the standard cages at 25^0C and 12 hour light/dark condition in the animal room of the Department of Human physiology, A.B.U., Zaria. They were fed on commercial feeds with tap water ad libitum. The animals were fasted of feeds for 12 hours before the commencement of each experiment, but were allowed water *ad libitum*. The experimental animals were classified into 4 main groups as follows:

Group A: Control group received normal saline only (5 rats)

Group B: Extract-treated group. It was sub classified into 3 groups, each group contained 5 rats:

Group B_1: Received 500mg/kg body weight extract i.p

Group B_2: Received 1000mg/kg body weight extract i.p

Group B_3: Received 1500mg/kg body weight extract i.p

Group C: Received biphasic isophane insulin 6 i.u /kg (5 rats)

Group D: Streptozocin (60mg/kg i.p)-treated group

(Streptozocin-induced diabetic group), which is sub

classified into 5 groups, each contained 5 rats:

Group D_1: Streptozocin-treated plus normal saline.

Group D_2: Streptozocin-treated plus biphasic isophane insulin (6

i.u/kg bodyweight i.p)

Group D_3: Streptozocin-treated plus 500mg/kg bodyweight

extract i.p

Group D_4: Streptozocin-treated plus 1000mg/kg bodyweight

extract i.p

Group D_5: Streptozocin-treated plus 1500mg/kg bodyweight

extract i.p.

3.1.1 PLANT MATERIAL

The samples of *azadiracta indica* seeds were collected from a village called kauran wali in kudan local government area of Kaduna State in the month of December 2005. It was taken to the herbarium section at Department of Biological Sciences, A.B.U., Zaria and was identified by Malam Musa and was given a batch voucher number

900151.

3.1.2 CHEMICALS USED

All chemicals and drugs used were of analytical grade. Streptozocin was obtained from Sigma Aldrich Company, A M schlengerbusch 2233106 Paderborn-Elsen Germany. The Biphasic Isophane Insulin AS Mixtard 30 HM Pen fill (Novo Nordisk AIS 2880 Bagsvaerd, Denmark. NAFDAC Reg.no 04-1601) were all brought commercially. ONE TOUCH BASIC meter (LIFESCAN,Inc 2001 Milpitas, CA 95035,USA) was used for the determination of the blood glucose levels of the animals. All the chemical used for the histopathological studies were obtained from the Department of Pathology and Microbiology, Faculty of Veterinary Medicine ,A.B.U. Zaria.

3.2 METHODS USED

3.2.0 PREPARATION OF THE PLANT EXTRACT

The neem seed extract was obtained from the seeds. The neem seeds were washed thoroughly with clean water and air dried. After dryness, the shell covering the neem seeds were peeled and the kernels were exposed. The seed kernels were crushed, pounded and transferred to the pharmacognocy laboratory for the extraction of the neem oil.

The extraction of the neem oil was carried out with the aid of a machine known as soxhlet extractor. The extraction of the neem oil was also made possible by the use of a chemical reagent known as petroleum ether maintained at a temperature range between 60^{0} C-80^{0} C. The ether helped to remove the oil from the pounded seed which was placed in a tumbler sack for easy extraction. The tumbler sack that contained the pounded seed was placed inside a glass jar, which was connected to the round bottom flask. Also, a condenser was placed above the glass jar and its function was to condense the vapourized petroleum ether The main extraction process of the oil from the seed kernel paste involved vapour heat application of petroleum ether that helped to melt the oil content of the seed kernel paste. Thus, removing the oil, and the vapour was converted back to liquid by the condenser. Hence, the condensed petroleum ether mixed with the extracted neem oil kept dropping back into the round bottom flask and the process repeated itself in a cyclic manner. At the end of the extraction, the content inside the round bottom flask was a mixture of petroleum ether and neem oil. Then the components were separated by the use of separating funnel because they were immiscible.

3.2.1 ACUTE TOXICITY STUDIES OF THE *AZADIRACTA INDICA* SEED EXTRACT ON WISTAR RATS

Investigation of the acute toxicity was the first step in toxicological investigation of any unknown substances. The index of the acute toxicity was LD_{50} according to the method of acute toxicity testing by (Lorke, 1983)**.**

The method involved two steps which was adapted in this study via the intraperitoneal route.

3.2.2 PRELIMINARY PHYTOCHEMICAL SCREENING

A preliminary phytochemical screening of the seed extract of *Azadiracta indica* seed was also done using standard methods of analysis (Trease and Evans,1989; Sofowora,1992).

3.2.3 EXPERIMENTAL INDUCTION OF DIABETES MELLITUS IN WISTAR RATS

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared streptozocin (STZ)(60mg/kg body weight) in 0.1 M citrate buffer(pH 4.5) in a volume of 1ml/kg. Three days after streptozocin treatment, rats showing a blood glucose level range of 150-230mg/dl were considered to be diabetic and were included in the study (Stanley *et al.*, 2001).

3.2.4 STUDIES ON THE EFFECTS OF SEED EXTRACT OF AZADIRACTA INDICA ON BLOOD GLUCOSE LEVELS OF WISTAR RATS

The research work was carried out on streptozocin-induced diabetic and normoglycemic Wistar rats. Before each experiment, the animals were fasted for twelve hours, but were allowed water *ad- libitum*.

3.2.5 DETERMINATION OF BLOOD GLUCOSE LEVELS

All blood samples were collected from the tail artery of the rats at intervals of 0,1,3,5, and 7 hours .The 0 hour is the fasting blood glucose levels. Determination of the blood glucose levels was done by the glucose-oxidase principle (Beach and Turner,1958) using the ONE TOUCH Basic (Lifescan,MilpitasCA) instrument and the results were expressed as mg/dl (Rheney and Kirk, 2000).

3.2.6 HISTOPATHOLOGICAL REPORT

One week after the conclusion of the studies on the effects of the seed extract of Azadiracta Indica on the blood glucose level of the streptozocin-induced diabetic and normoglycemic Wistar rats, all animals were sacrificed by euthanizing them with chloroform. Postmortem examination was done and the tissue samples of the pancreas and liver were collected from all the groups for histopathological studies. Histopathological slides, using haematoxylin and eosin stained paraffin embedded sections of the tissues, were prepared and photomicrographs were taken at the Department of Pathology and Microbiology, Faculty of Veterinary Medicine, A.B.U, Zaria.

3.2.7 STATISTICAL ANALYSIS

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of $p<0.05$ or $p<0.001$ were taken as significant (Duncan *et al.,* 1977).The streptozocin-induced diabetic groups and normoglycemic groups were analyzed separately for statistical significance.

CHAPTER FOUR

4.0 RESULTS

The various stages of this work are presented as follows:

Acute toxicity studies of the extract of *Azadiracta indica* seed via the intraperitoneal route to Wistar rats. The effects of the extract of Azadiracta Indica seed on blood glucose levels of Streptozocin -induced diabetic and normoglycemic Wistar rats as well as the Histopathological studies on pancreas and livers of the animals used.

4.1 PRELIMINARY PHYTOCHEMICAL SCREENING

Result of the preliminary phytochemical screening of *Azadiracta Indica* seed extract revealed the presence of tannin glycerin, cholesterol, steroids, alkaloids, triterpenoids, saponins, Flavonoids and reducing sugars.

4.2 ACUTE TOXICITY STUDIES

LD_{50} determination was conducted using the method of Lorke (1983). In the initial phase, male and female mice were divided into three groups of three mice each. They were treated with the *Azadiracta Indica* seed extract at doses of 250 mg/kg, 500 mg/kg and1000 mg/kg body weight intraperitoneally. Animals were observed for 24 hours. No mortality was recorded as shown in table (4.1). In the second phase of the toxicity study, the animals were in three groups with three mice in each group. They were treated with the *Azadiracta Indica* seed extract at doses of 1500 mg/kg, 2000 mg/kg, 2500 mg/kg and 3000mg/kg bodyweight intraperitoneally. Animals were observed for 24 hours and there was no mortality recorded, as shown in table (4.2). Sign of the toxicity were first noticed after 2-3 hours of extract administration. There were decreased locomotor activity and sensitivity to touch. Also there was decreased feed intake, tachypnoea and prostration after 8 hours of extract administration.

The LD_{50} was calculated as 3000 mg/kg by the log-probit using the method of Miller and Tainter (Lorke, 1983).

Table (4.1): The percentage mortality of the different doses of the extract of *Azadiracta indica* seed extract administered intraperitoneally to Wistar rats during the first phase of acute toxicity study.

Group	Dose(mg/kg)	Deaths	%Mortality.
1	250	0/3	0
2	500	0/3	0
3	1000	0/3	0

Table (4.2): The percentage mortality of the different doses of the extract of *Azadiracta Indica* seed administered intraperitoneally to Wistar rats during the second phase of acute toxicity study.

Group	Dose(mg/kg)	Deaths	%Mortality.
1	1500	0/3	0
2	2000	0/3	0
3	2500	0/3	0
4	3000	0/3	0

Table (4.3): Showing the blood glucose level (mg/dl) of control (Group A) and *Azadiracta indica* seed extract-treated normoglycemic rats(B_1,B_2 and B_3 groups).

Groups / Time (Hours)	GroupA (Control)	*Azadiracta indica* seed extract-treated normoglycemic rats		
		B_1 (500mg/kg)	B_2 (1000mg/kg)	B_3 (1500mg/kg)
0	47.0±2.50	49.4±6.32	56.0±4.82	48.8±3.91
1	49.8±2.70	45.4±6.32^{ns}	56.2±5.13^{ns}	46.2±5.87^{ns}
3	47.4±1.88	38.6±4.16^{a}	38.8±1.49^{ns}	45.0±2.16^{ns}
5	46.4±2.56	38.6±2.48^{a}	36.4±3.44^{a}	45.2±2.67^{ns}
7	49.2±3.20	33.6±2.88^{a}	34.2±2.88^{a}	42.0±2.91^{ns}

Values are mean ± SEM; n=5

Values are considered to be statistically significant compared to control: $^{a=}$ $P< 0.05$ while $^{ns=}$not significant

A study of the extract- treated groups (group B_1-B_3) as compared to the control group are shown in table (4.3) and figures (4.3a and 4.3b).As regards to the first group (group B_1),,that received 500mg/kgbody weight of the extract, there was no significant change in the blood glucose levels after 1 hour, when compared to control group. While after 3, 5 and 7 hours, the samples showed significant decrease in blood glucose levels as compared to the control group. In relation to the second group B_2 (received 1000mg/kg bodyweight of the extract), there was no significant change in blood glucose levels after 1 and 3 hours when compared with control group. While after 5 and 7 hours it showed a significant decrease in blood glucose levels. In regard to group B_3 (received 1500mg/kg bodyweight of the extract), all the results revealed no significant change in the blood glucose levels when compared to the control group. Thus, the effective dose appear to be 500mg/kg bodyweight of the extract.

Thus, it appears from the study that the extract had decreased the blood glucose levels in the normoglycemic rats especially the dose of 500mg/kg and 1000mg/kg bodyweight. The dose of 500mg/kg of the extract showed the best effect.

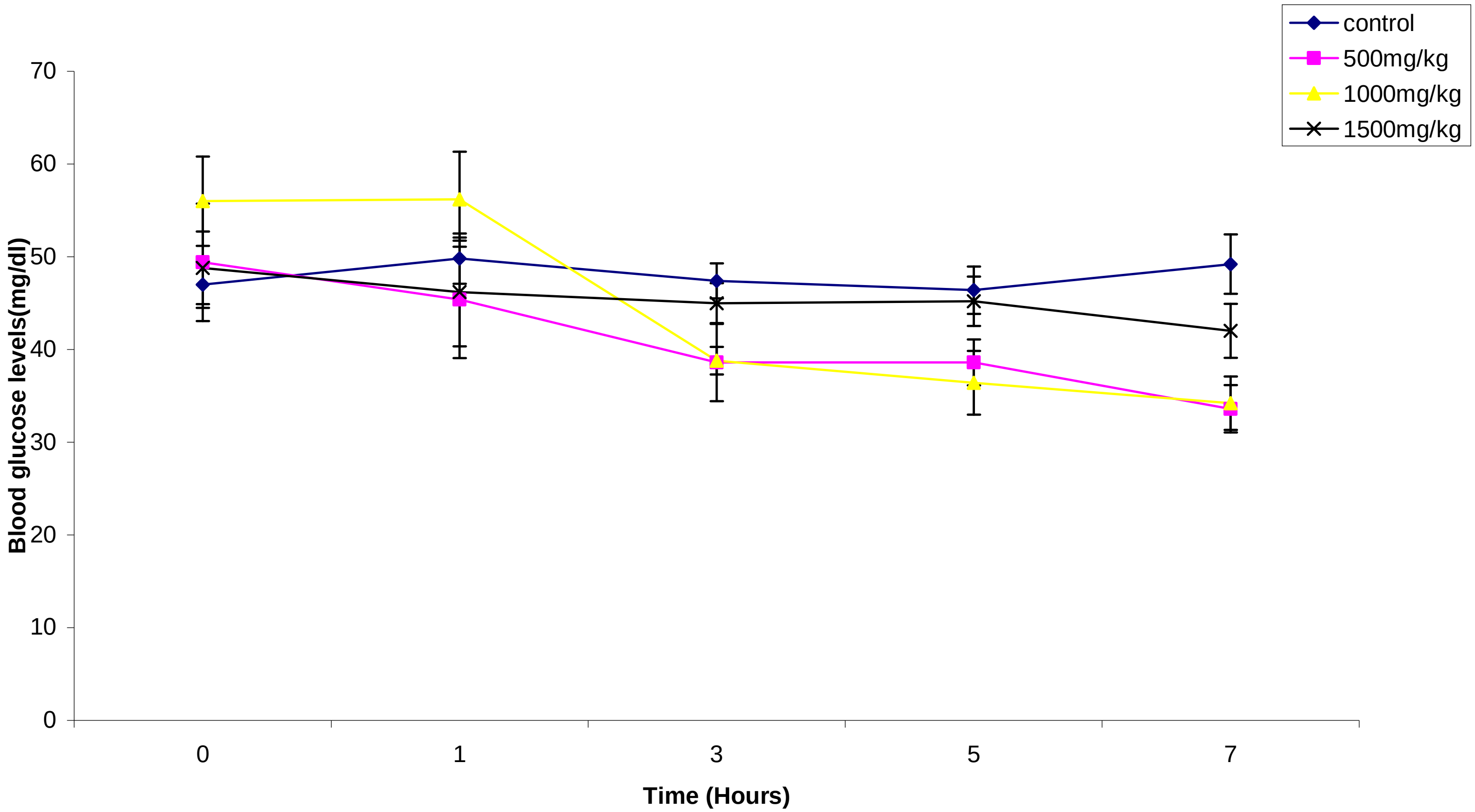

Fig 4.3a:Effect of *Azadiracta Indica* seed extract on blood glucose levels of normoglycemic Wistar rats compared to control.

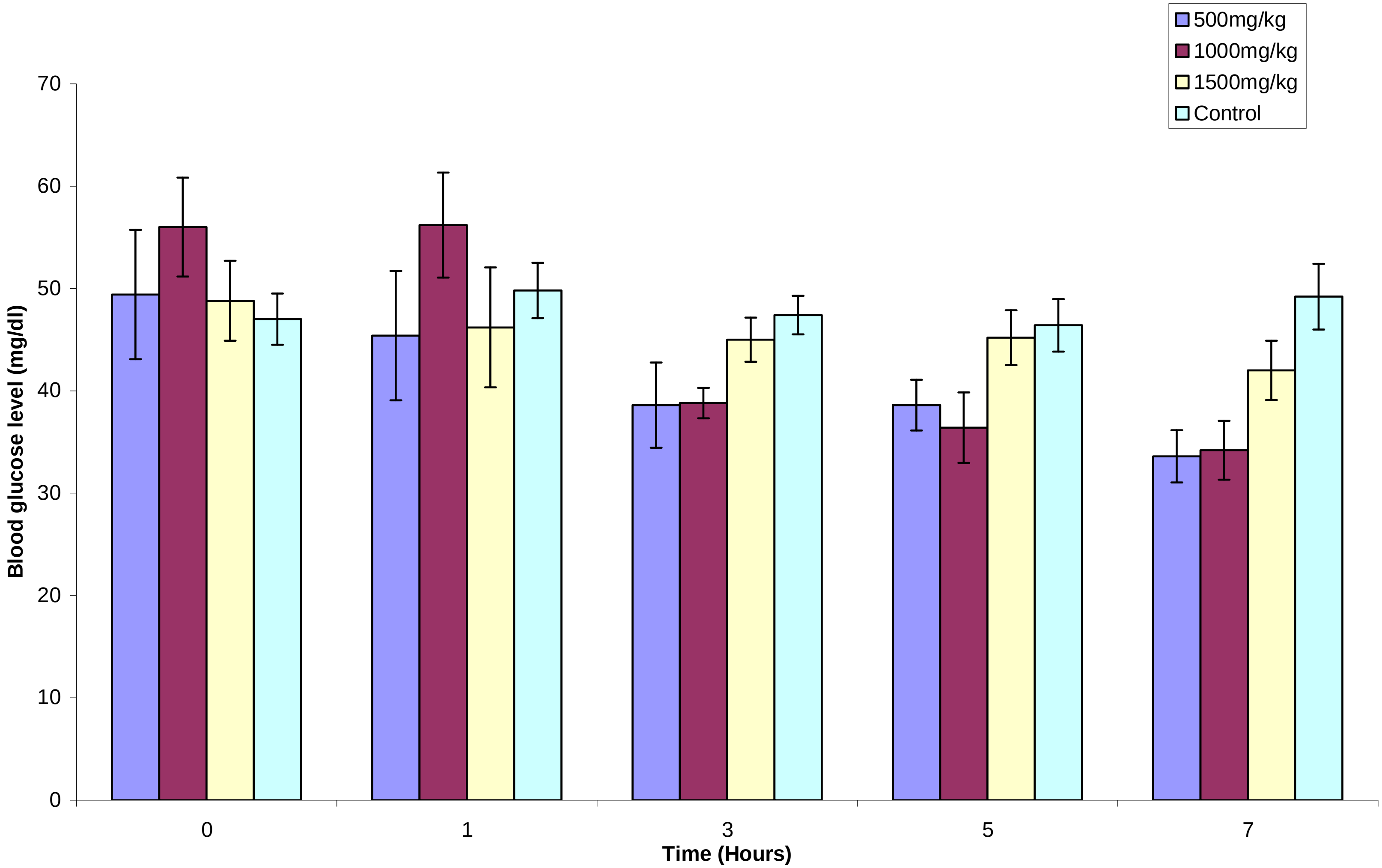

Fig. 4.3b: Effect of *Azadiracta Indca* seed extract on blood glucose levels of normoglycemic Wister rats compared to control

Table (4.4): Showing the blood glucose level (mg/dl) of biphasic insulin-treated group (Group C) and *Azadiracta indica* seed extract-treated normoglycemic rats(B_1 B_2 and B_3 groups).

Groups / Time (Hours)	Group C (Insulin-treated)	*Azadiracta indica* seed extract-treated normoglycemic rats		
		B_1 (500mg/kg)	B_2 (1000mg/kg)	B_3 (1500mg/kg)
0	44.2±0.86	49.4±6.32	56.0±4.82	48.8±3.91
1	30.2±2.26	45.4±6.32^{ns}	56.2±5.13^{a}	46.2±5.87^{ns}
3	28.6±3.56	38.6±4.16^{a}	38.8±1.49^{ns}	45.0±2.16^{a}
5	24.4±2.98	38.6±2.48^{b}	36.4±3.44^{b}	45.2±2.67^{b}
7	22.0±3.07	33.6±2.88^{b}	34.2±2.88^{b}	42.0±2.91^{b}

Values are mean ± SEM; n=5

Values are considered to be statistically significant compared to Isophane insulin treatment when $^{a=}$ P< 0.05 $^{b=}$P<0.001, while $^{ns=}$not significant.

A study of the effect of extract (500, 1000 and 1500mg/kgbody weight of the extract) on normoglycemic rats as compared to biphasic insulin (6 iu/kg) - treated group are shown in table (4.4) and figures (4.4a and 4.4b).The study showed that after 1 hour there was a significant increase in the blood glucose level in group B_2 when compared with biphasic isophane insulin group. After 3 hours, there was a significant increase in the blood glucose levels in groups (B_1 and B_3)while B_2 group showed no change as compared with the insulin- treated group. However after 5 and 7 hours there was a highly significant increase ($p<0.001$) in blood glucose levels in all the groups which were given 500, 1000, and 1500mg/kgbody weight of extract when compared to the biphasic isophane insulin- treated group. This indicates that biphasic insulin had a more reducing effect on blood glucose level of normoglycemic rats than the *Azadiracta indica* seed extract. So, the *Azadiracta indica* seed extract is more safe when used with normoglycemic rats.

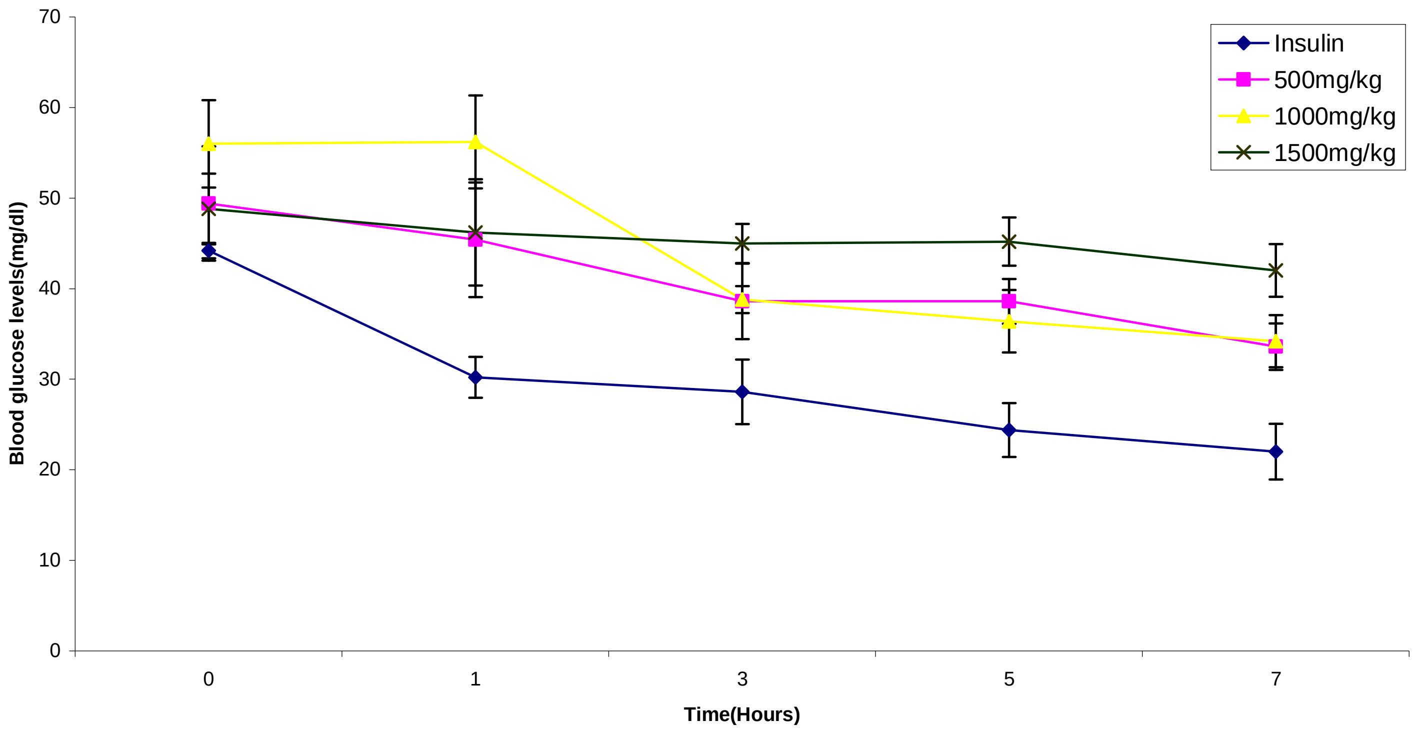

Fig 4.4a : Effect of Biphasic insulin and Azadiracta indica seed extract on blood glucose levels of normoglycemic Wistar ratss

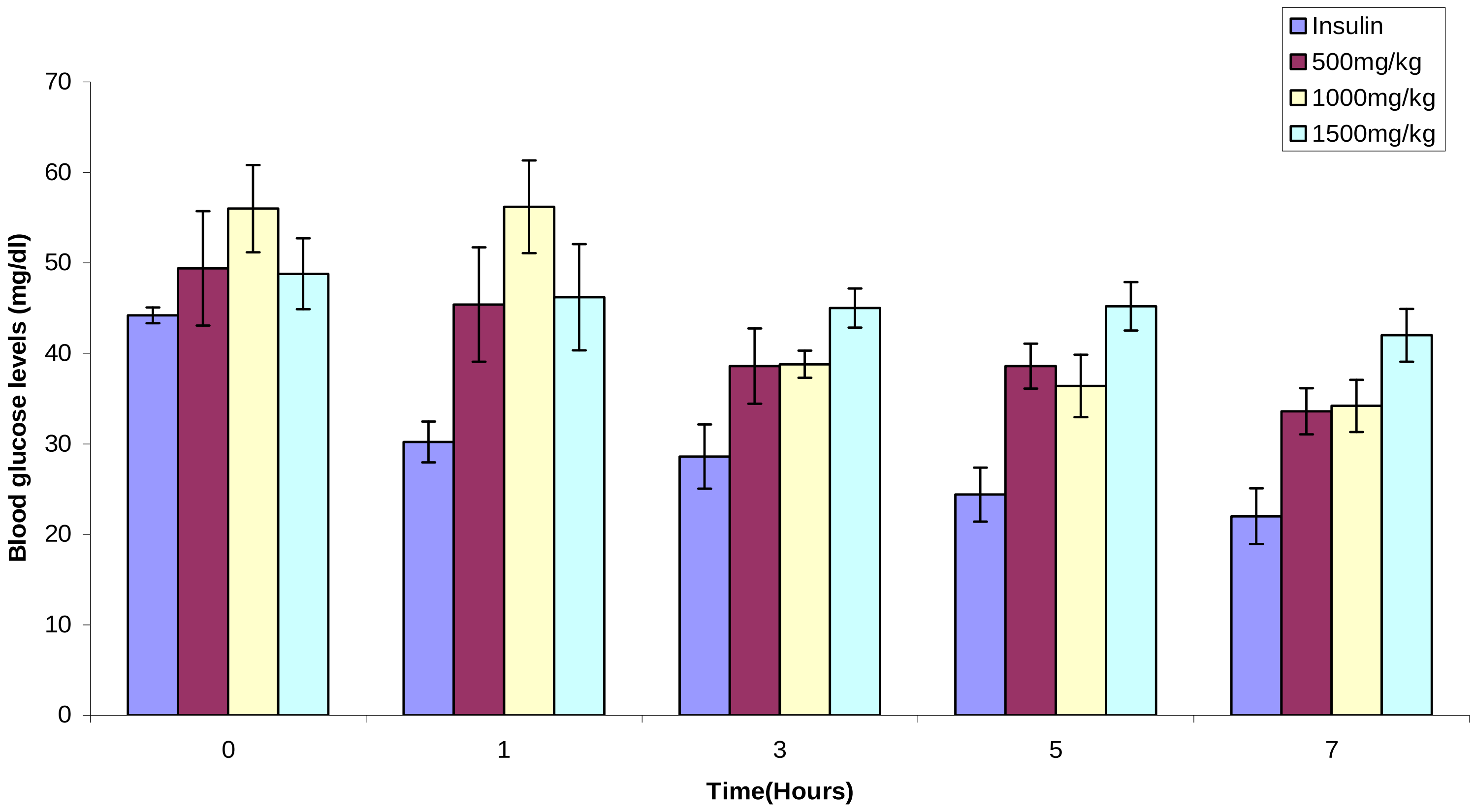

Fig 4.4b: Effect of Biphasic insulin and Azadiracta indica seed extract on blood glucose levels of normoglycemic Wistar rats.

Table (4.5): Showing the blood glucose level (mg/dl) of streptozocin-diabetic (D_1) group and streptozocin-diabetic groups plus *Azadiracta indica* seed extract (D_3,D_4 and D_5 groups).

Groups / Time (Hours)	Streptozocin-Diabetic group (D_1)	Streptozocin-diabetic plus *Azadiracta indica* seed extract rats		
		D_3 (500mg/kg)	D_4 (1000mg/kg)	D_5 (1500mg/kg)
0	189.4±7.60	188.0±14.9	186.0±7.01	190.2±10.4
1	192.4±7.22	169.2±9.48^{ns}	148.4±23.3^{ns}	193.6±12.5^{ns}
3	192.6±9.16	162.2±16.9^{ns}	132.0±20.3^{a}	185.2±9.17^{ns}
5	191.8±8.77	134.0±16.7^{a}	134.0±18.9^{a}	174.8±8.15^{ns}
7	191.6±7.74	127.6±14.5^{a}	148.0±7.51^{ns}	163.0±11.5^{ns}

Values are mean ± SEM; n=5

Values are considered to be statistically significant compared to streptozocin-diabetic group when :$^{a=}$ P< 0.05, while $^{ns=}$not significant

Results of the extract-treated groups (group D_3-D_5) as compared to the streptozocin-diabetic group are shown in table (4.5) and figures (4.5a and 4.5 b). As regards the group D_3 (received 500mg/kg body weight of the extract) there was no significant change in the blood glucose levels after 1 and 3 hours. In the meantime, it showed marked decrease in blood glucose level after 5 and 7 hours, where the decrease was time- dependent.

In relation to the group D_4 (received 1000mg/kg body weight of the extract) there was no significant change in the blood glucose levels after 1and 7 hours when compared with streptozocin-treated group (group D_1), while after 3 and 5 hours, there was significant decrease ($p<0.05$) in blood glucose levels . In regard to group D_5 (received 1500mg/kg bodyweight of the extract) all the results revealed no significant change when compared with streptozocin-treated group (group D_1). Thus it appears from analysis of the results that the effective dose in the reduction of blood glucose levels of the streptozocin-diabetic rats was 500mg/kg body weight of the extract.

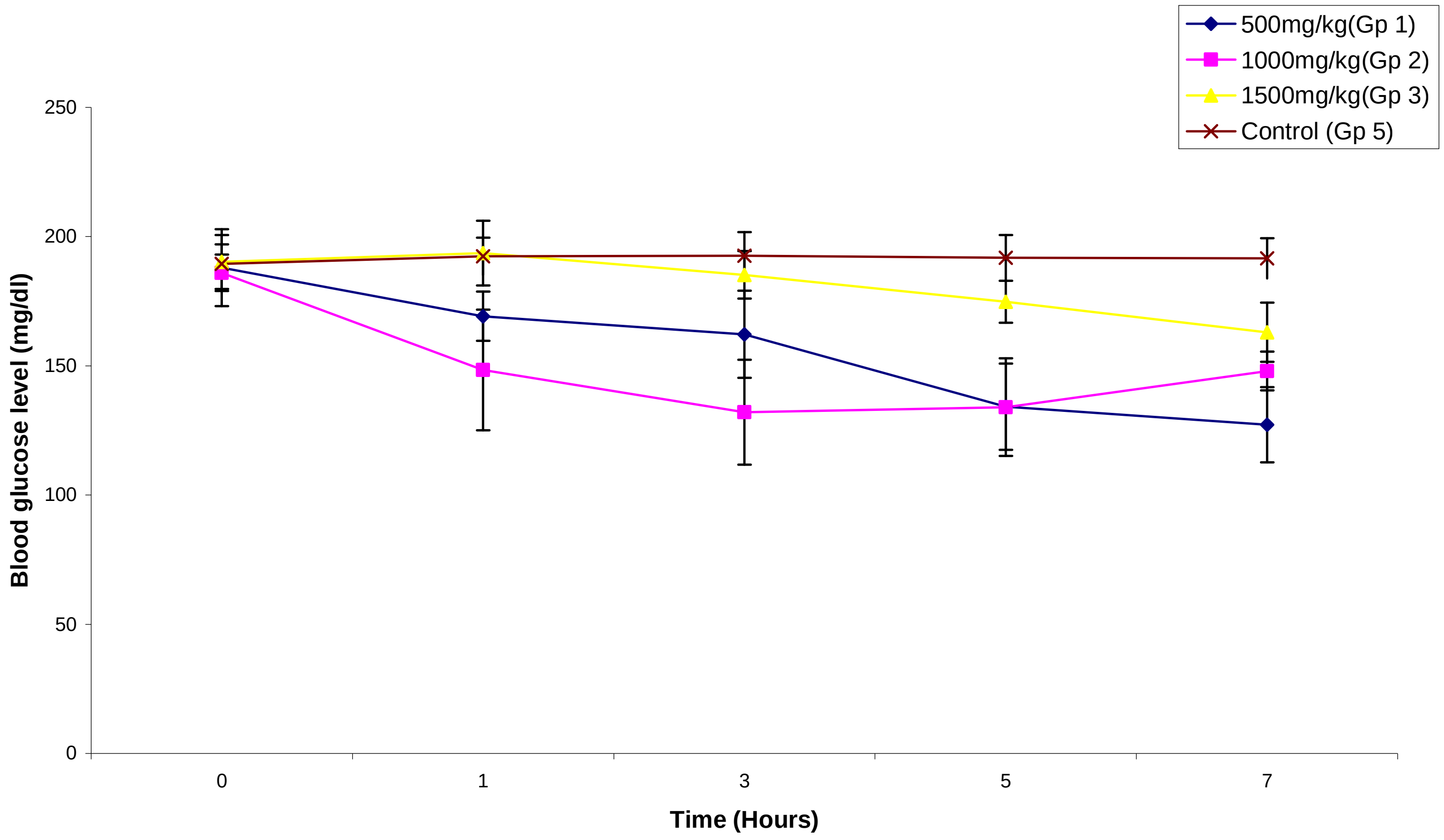

Fig 4.5a: Effect of Azadireacta Indica seed on blood glucose level of streptozocin-induced diabetic in Wistar rats compared to untreated diabetic control

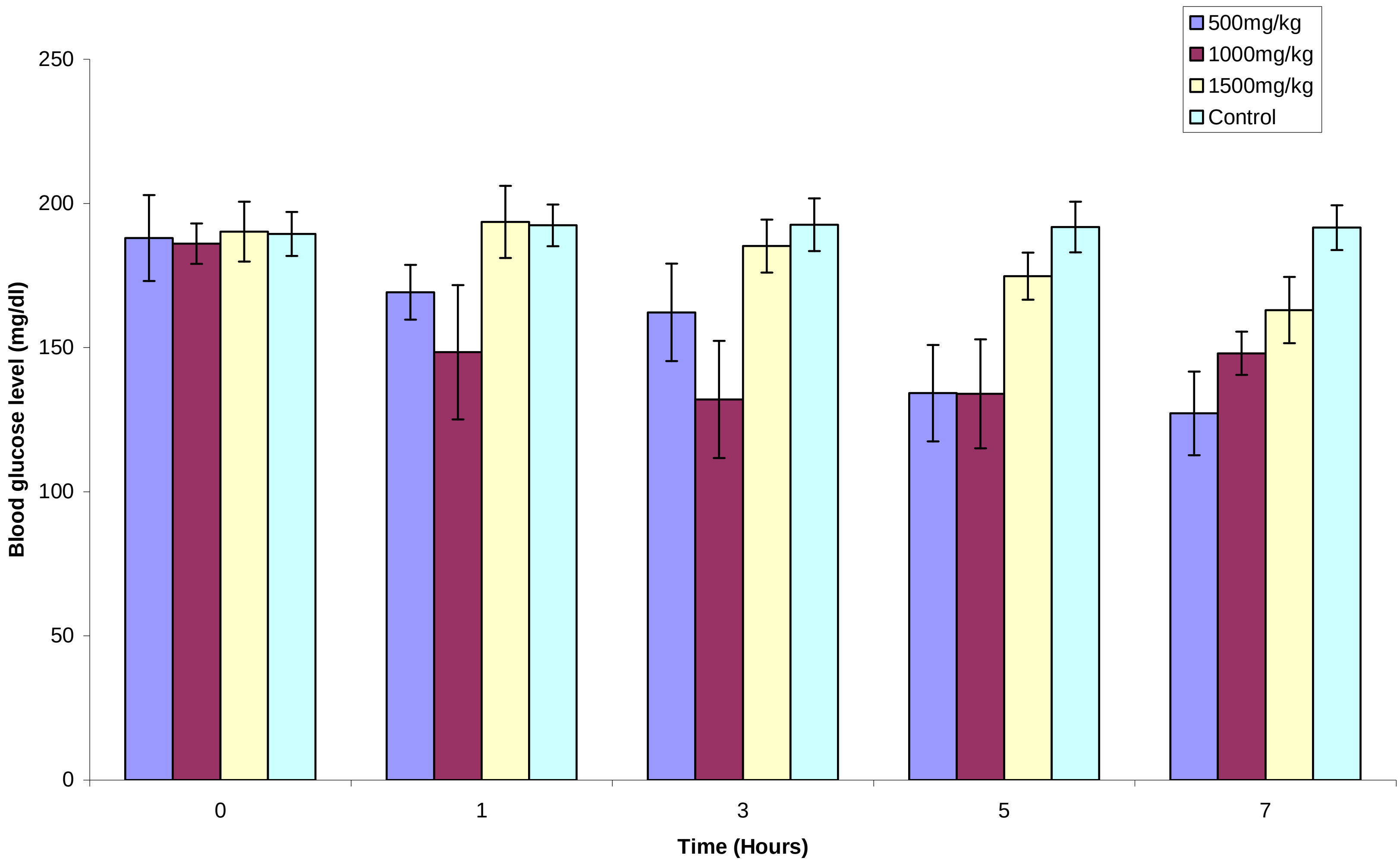

Fig. 4.5b: Effect of *Azadiracta Indica* seed extract on blood glucose level of Streptozocin-induced diabetic Wistar rats compared t un treated diabetic control

Table (4.6): Showing the blood glucose levels(mg/dl) of insulin-treated diabetic(D_2)group and *Azadiracta indica* seed extract-treated diabetic (D_3,D_4 and D_5 groups) .

Groups / Time (Hours)	Insulin-treated (D_2)	*Azadiracta indica* seed extract-treated diabetic rats		
		D_3 (500mg/kg)	D_4 (1000mg/kg)	D_5 (1500mg/kg)
0	192.6±11.9	188.0±14.9	186.0±7.01	190.2±10.4
1	165.6±11.5	169.2±9.48^{ns}	148.4±23.3^{ns}	193.6±12.5^{ns}
3	140.0±9.35	162.2±16.9^{ns}	132.0±20.3^{ns}	185.2±9.17^{ns}
5	112.2±6.23	134.0±16.7^{ns}	134.0±18.9^{ns}	174.8±8.15^{a}
7	99.6±5.80	127.6±14.5^{ns}	148.0±7.51^{a}	163.0±11.5^{a}

Values are mean ± SEM; n=5

Values are considered to be statistically significant when compared to Biphasic Isophane Insulin treatment at $^{a=}$ P< 0.05, $^{ns=}$not significant

Results of the effect of extract (500, 1000 and 1500mg/kg body weight of the extract) on diabetic rats compared to biphasic insulin (6.i u/kg) -

treated group are shown in table (4.6) and figures (46a and 4.6 b).The study showed that there was no significant change in the blood glucose levels after 1 and 3 hours in all groups (D_3, D_4 and D_5) as compared with the insulin-treated group. In relation to the group D_4 after 7 hours revealed a significant increase ($P<0.05$) in blood glucose level as compared to the insulin -treated group D_2. In regard to group D_5 (1500mg/kg bodyweight of the extract) after 5 and 7 hours revealed a significant increase glucose levels as compared to the insulin-treated group D_2. However no significant change in blood glucose levels in group D_3 (500mg/kg bodyweight of the extract) after 5 and 7 hours of extract when compared to biphasic insulin treated group (D_2).

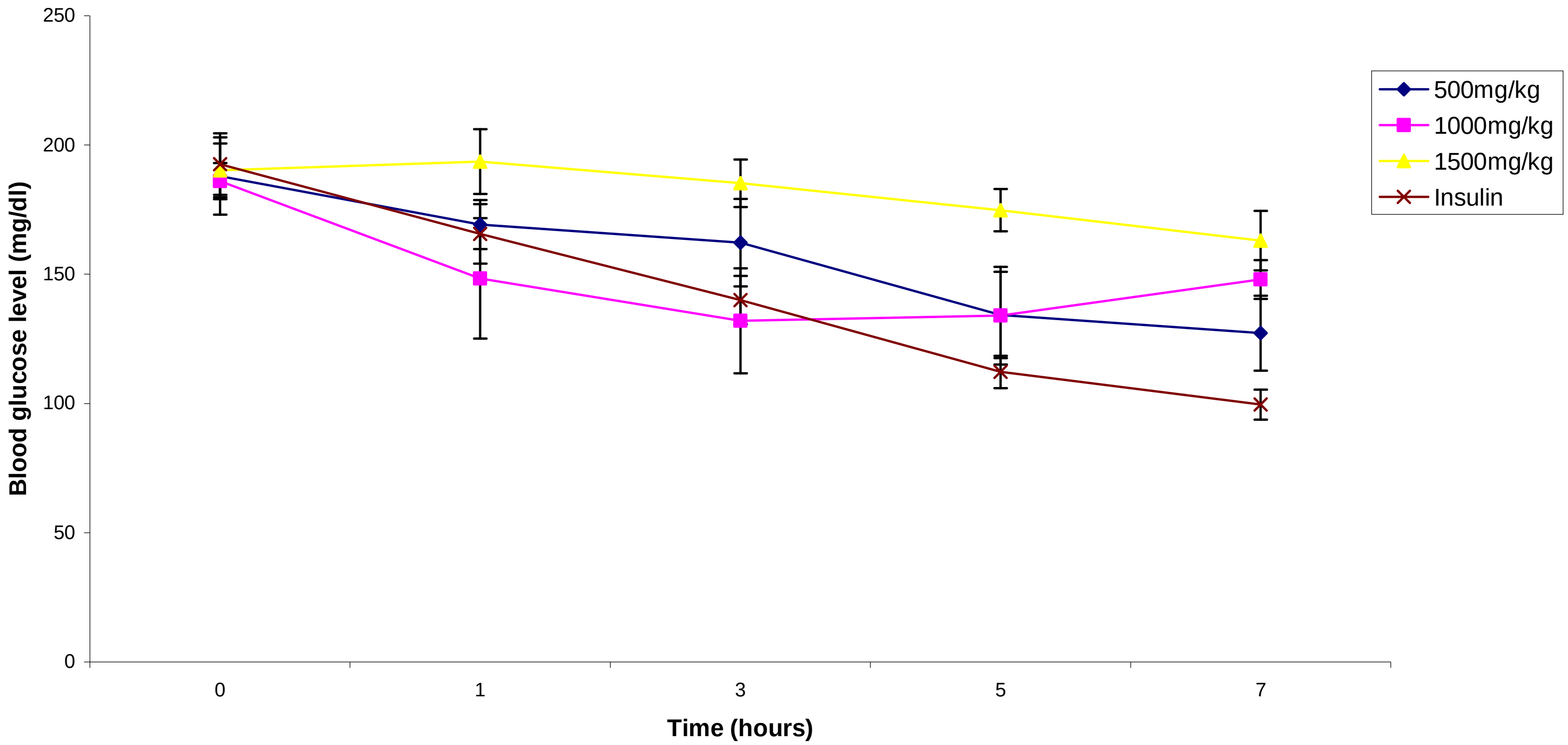

Fig. 4.6a: Effect of Azadiracta Indica seed extract on blood glucose level of Streptozocin-induced diabetic Wistar rats compared to Biphasic Isophane Insulin treatment

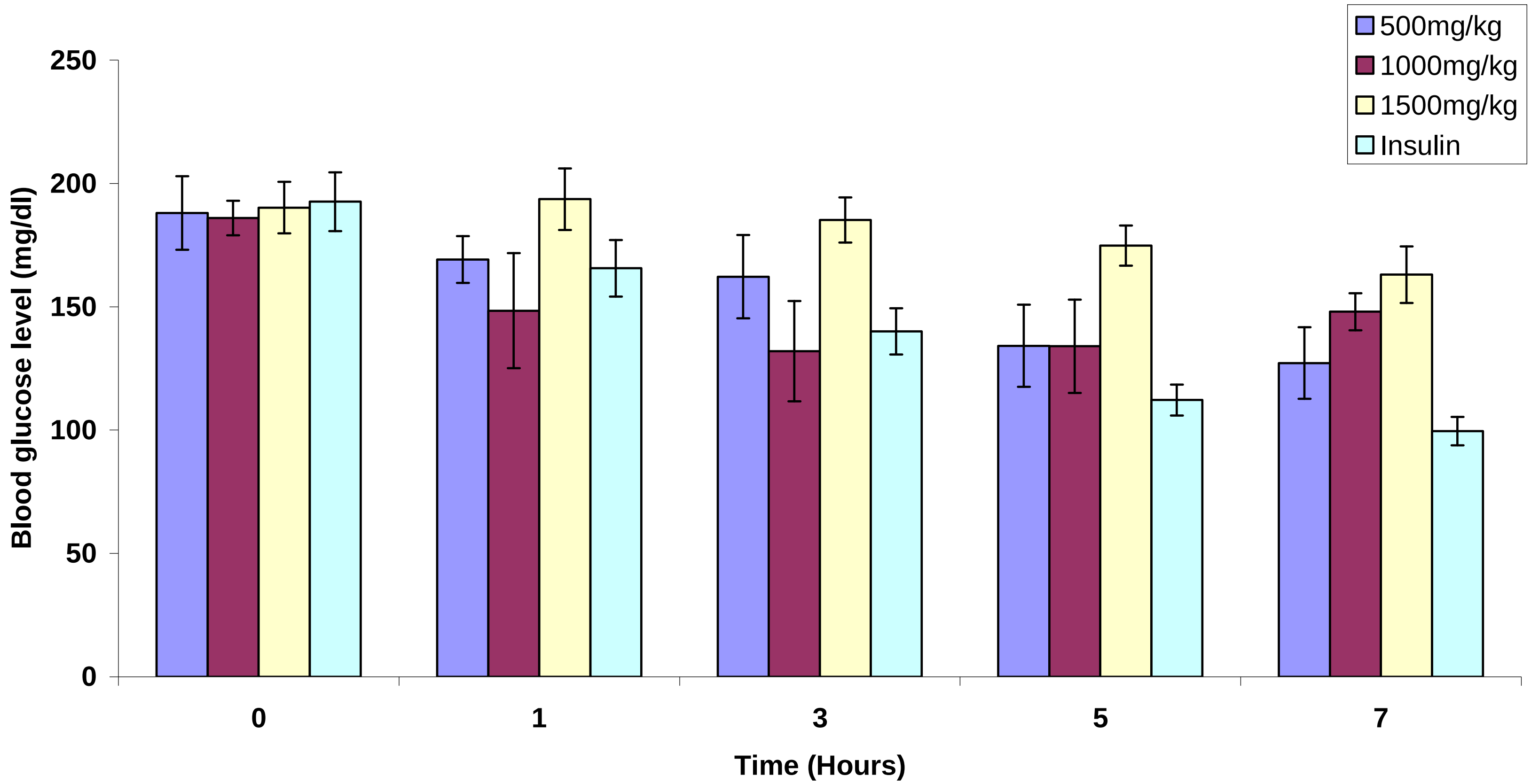

Fig. 4.6b: Effect of Azadiracta Indica seed extract on blood glucose level of Streptozocin-induced diabetic Wistar rats compared to Biphasic Isophane Treatment

4.7 HISTOPATHOLOGICAL EFFECT OF AZADIRACTA INDICA SEED EXTRACT ON NORMOGLYCEMIC WISTAR RATS.

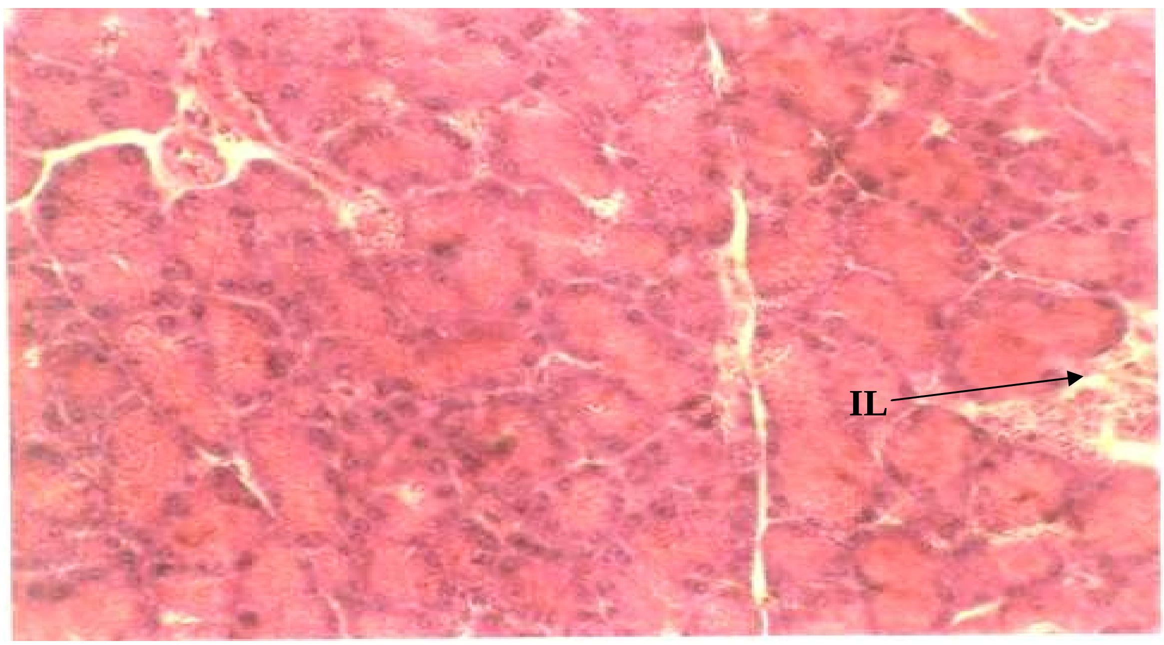

Plate 4.3a: Photomicrograph of a section of Pancreas of rats from Normoglycemic group treated with normal saline (Group A) Intraperitoneally administered. Note: there was no observable microscopic Lesion. The islet of Langerhans is present (I.L) H & E Stain x 400.

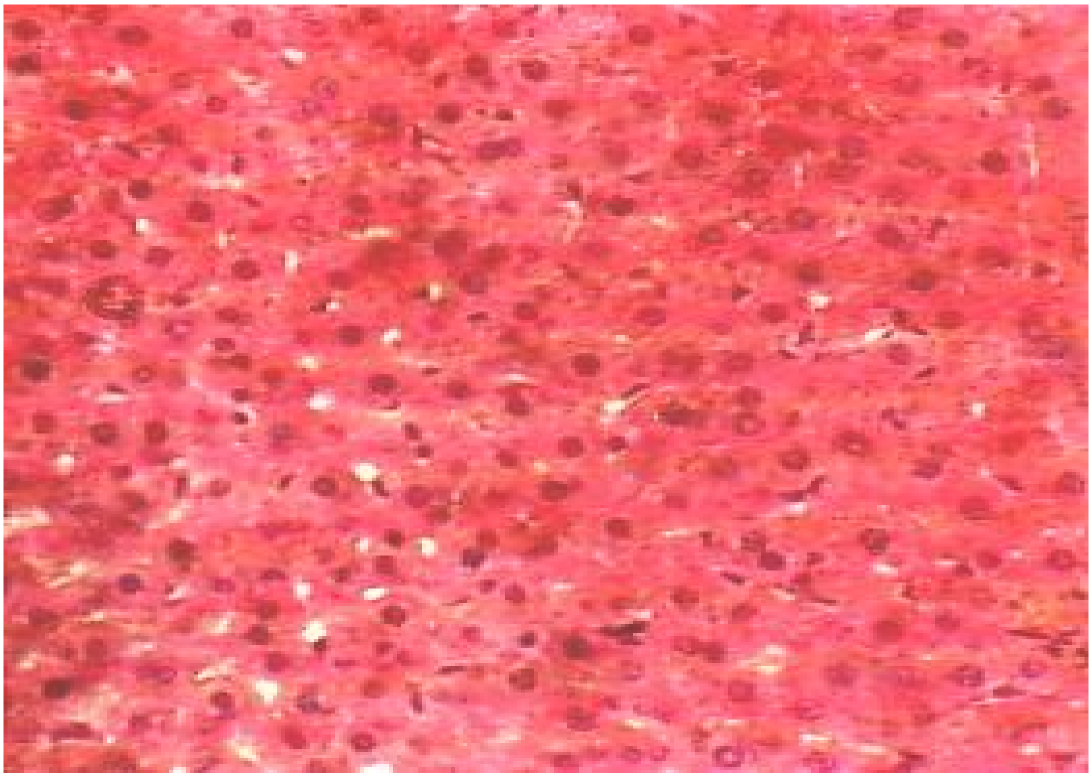

Plate 4.3b: Photomicrograph of a section of liver of rats from Normoglycemic group treated with normal saline (Group A) intraperitoneally administered. Note: there was no observable microscopic lesion H & E Stain x 400.

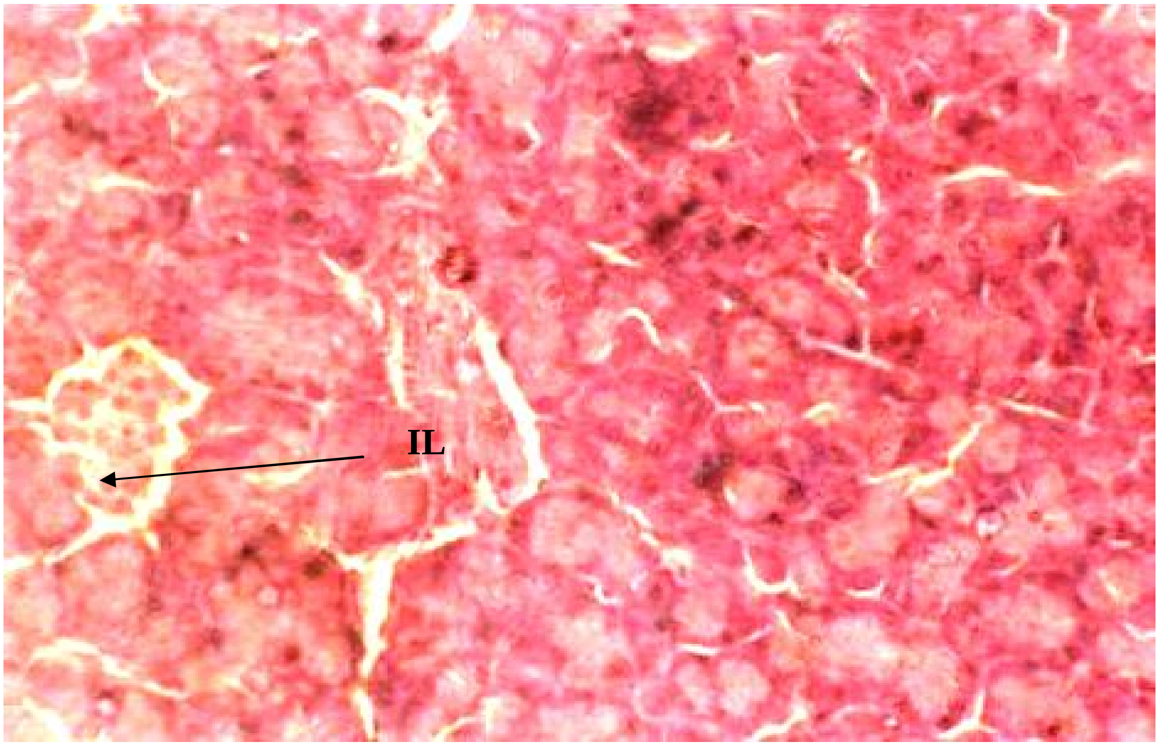

Plate 4.4a: Photomicrograph of a section of Pancreas of rats from Normoglycemic group treated with 500mg/kg body weight (Group B_1) of seed extract of *Azadiracta Indica* intraperitoneally administered .Note: there was no observable microscopic lesion. The islet of Langerhans is present (IL) H & E Stain x 400.

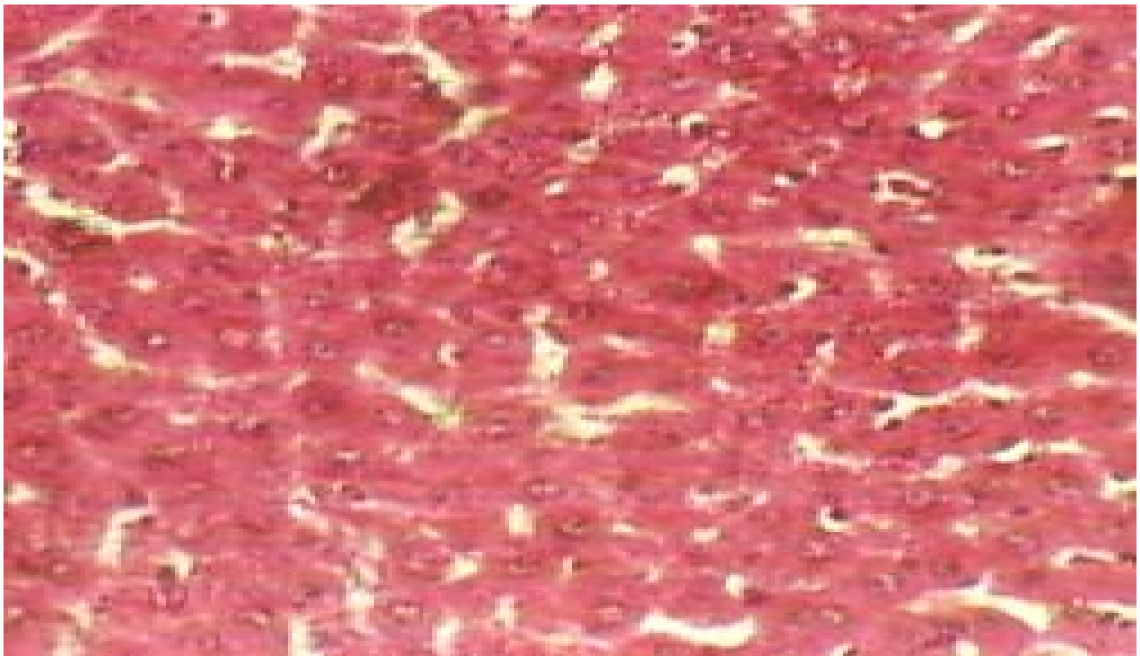

Plate 4.4b: Photomicrograph of a section of liver of rats from Normoglycemic group treated with 500mg/kg body weight (Group B_1) of seed extract of *Azadiracta Indica* Intraperitoneally administerd. Note: there was no observable microscopic lesion. H & E Stain x 400.

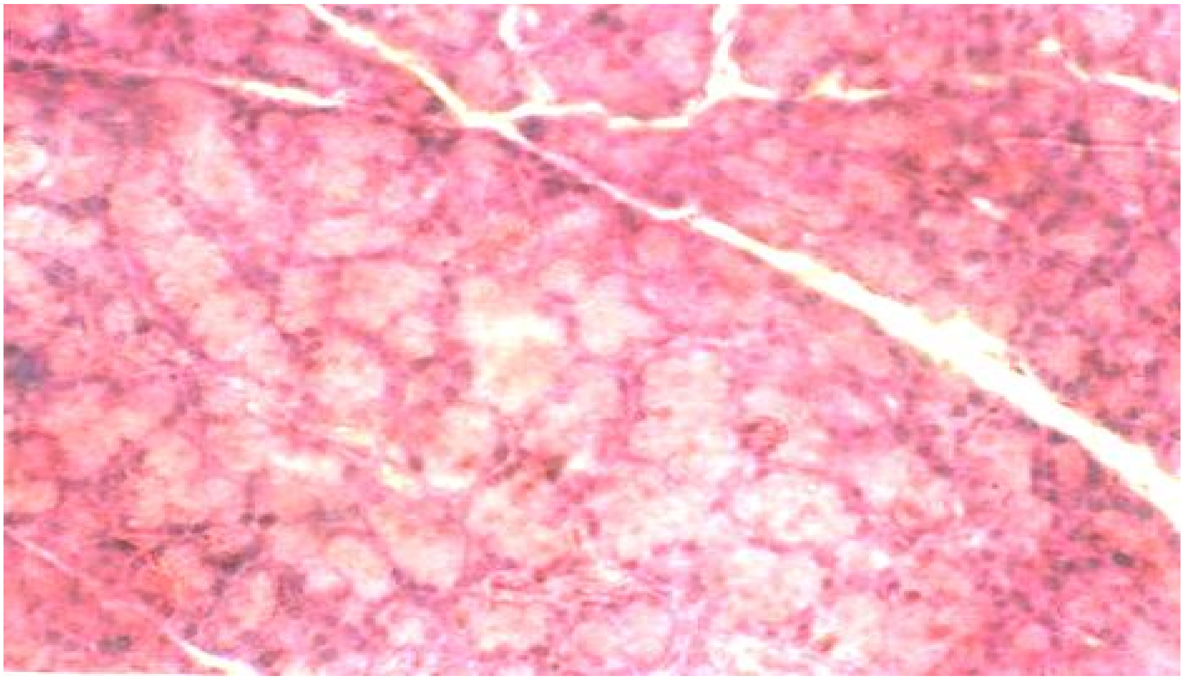

Plate 4.5a: Photomicrograph of a section of Pancreas of rats from Normoglycemic group treated with 1000mg/kg body weight (Group B_2) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: there was no observable microscopic lesion. H & E Stain x 400.

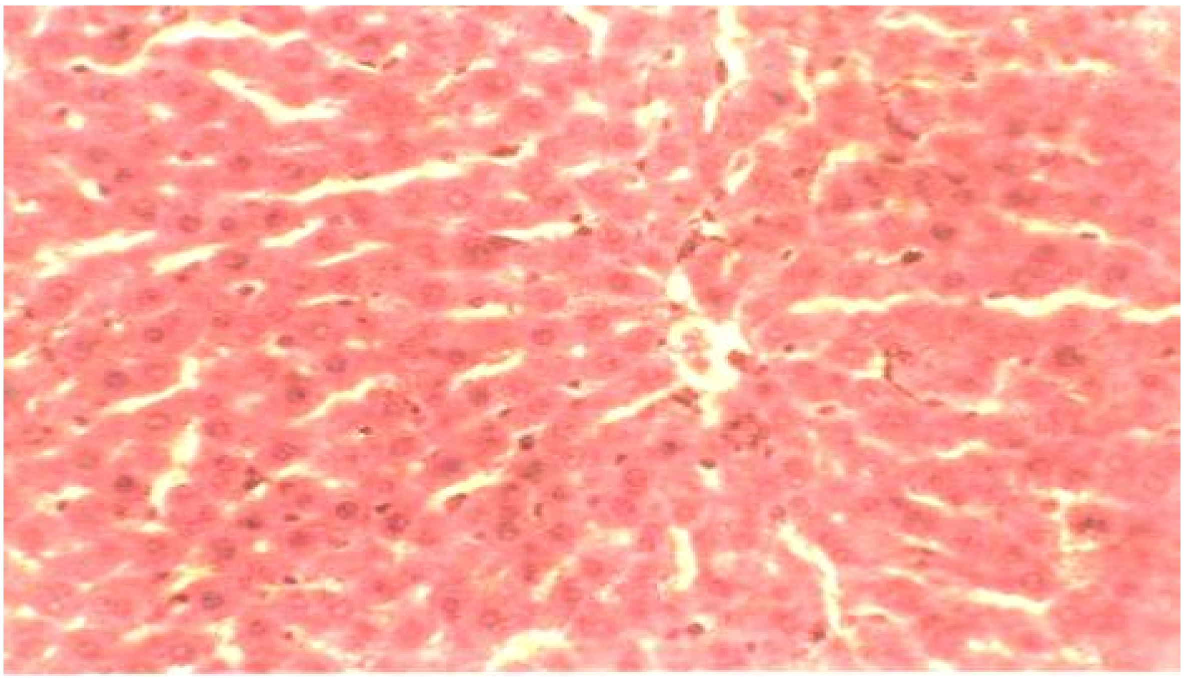

Plate 4.5b: Photomicrograph of a section of liver of rats from Normoglycemic group treated with 1000mg/kg body weight (Group B_2) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: there was no observable microscopic lesion. H & E Stain x 400.

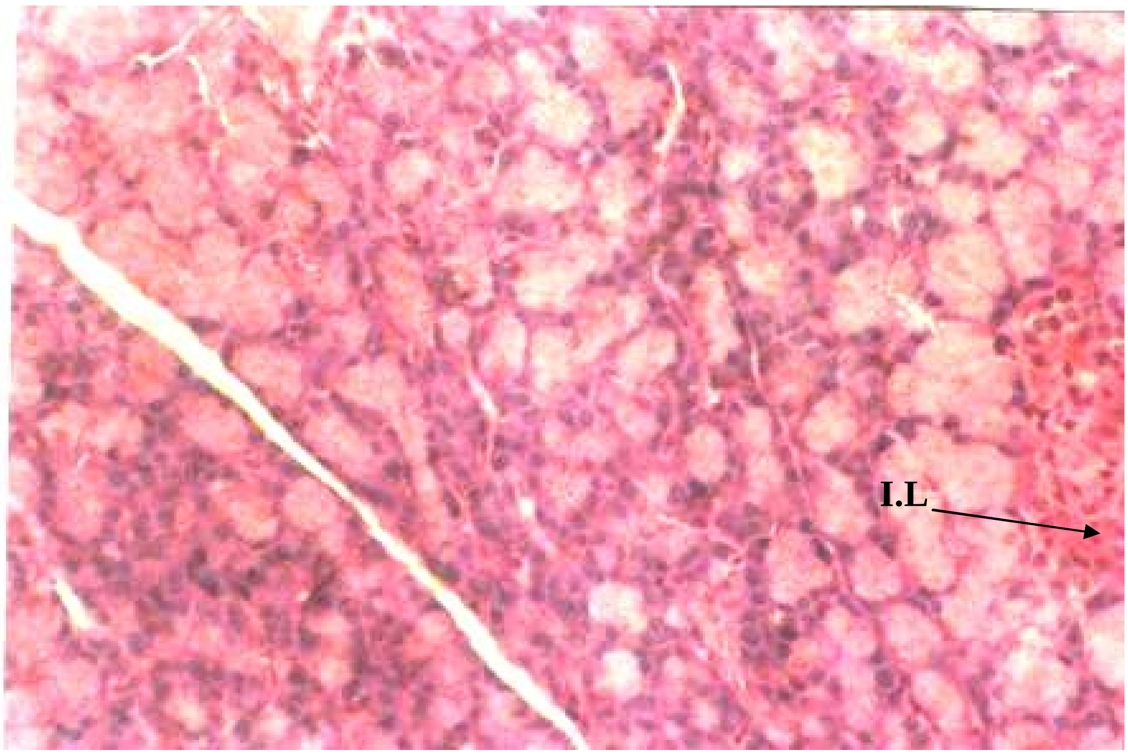

Plate 4.6a: Photomicrograph of a section of Pancreas of rats from Normoglycemic group treated with 1500mg/kg body weight (Group B_3) of seed extract of *Azadiracta Indica* seed intraperitoneally administered. Note: there was no observable microscopic lesion. The islet of Langerhans is present (IL)H & E Stain x 400.

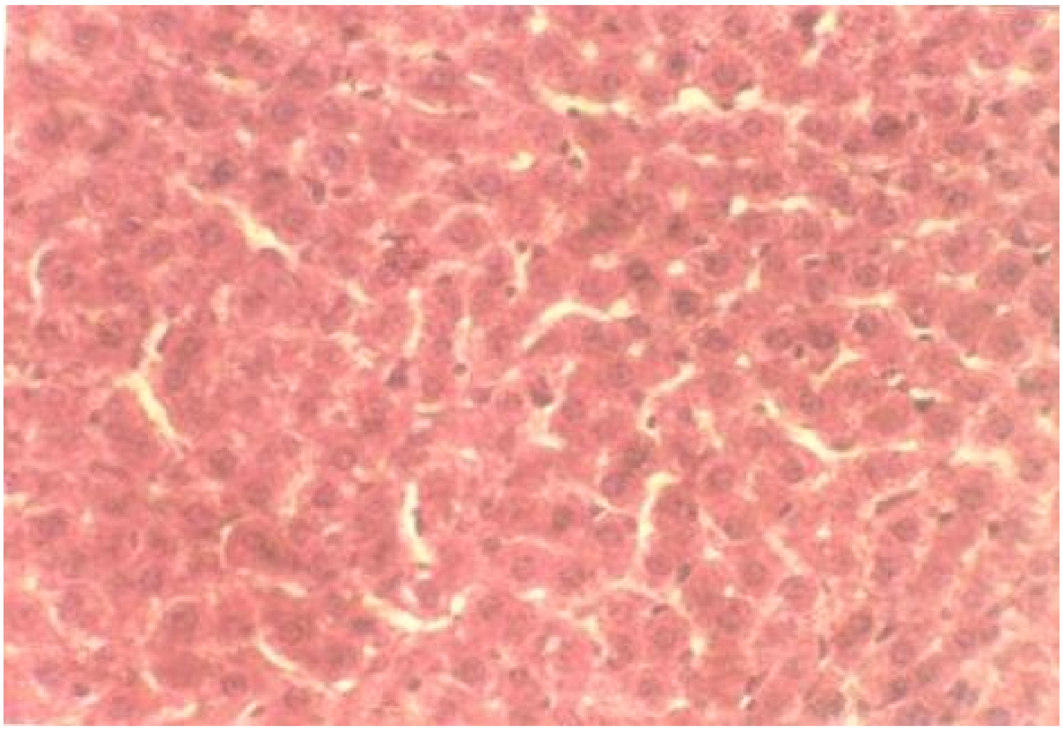

Plate 4.6b: Photomicrograph of a section of liver of rats from Normoglycemic group treated with 1500mg/kg body weight (Group B_3) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: there was no observable microscopic lesion. H & E Stain x 400.

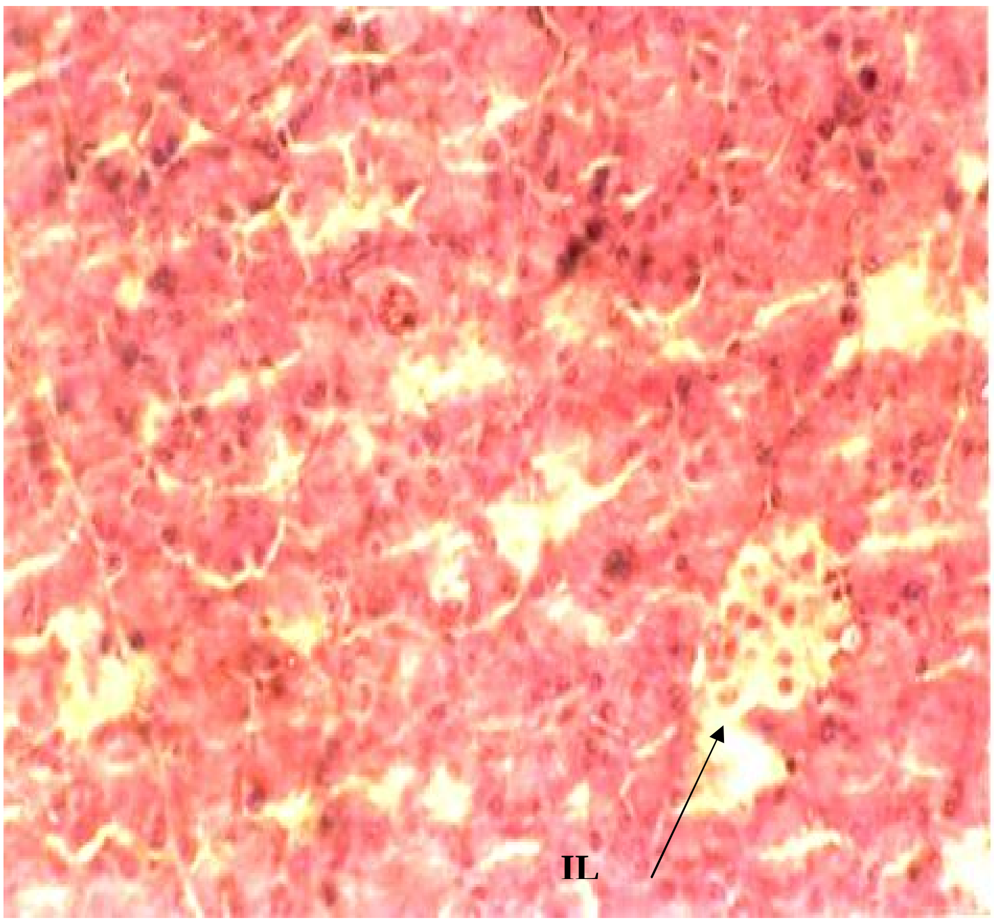

Plate 4.7a: Photomicrograph of a section of Pancreas of rats from Normoglycemic group treated with Isophane Insulin 6 i.u/kg body weight (Group C)of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: there was no observable microscopic lesions. The islet of Langerhans is present (IL). H & E Stain x 400.

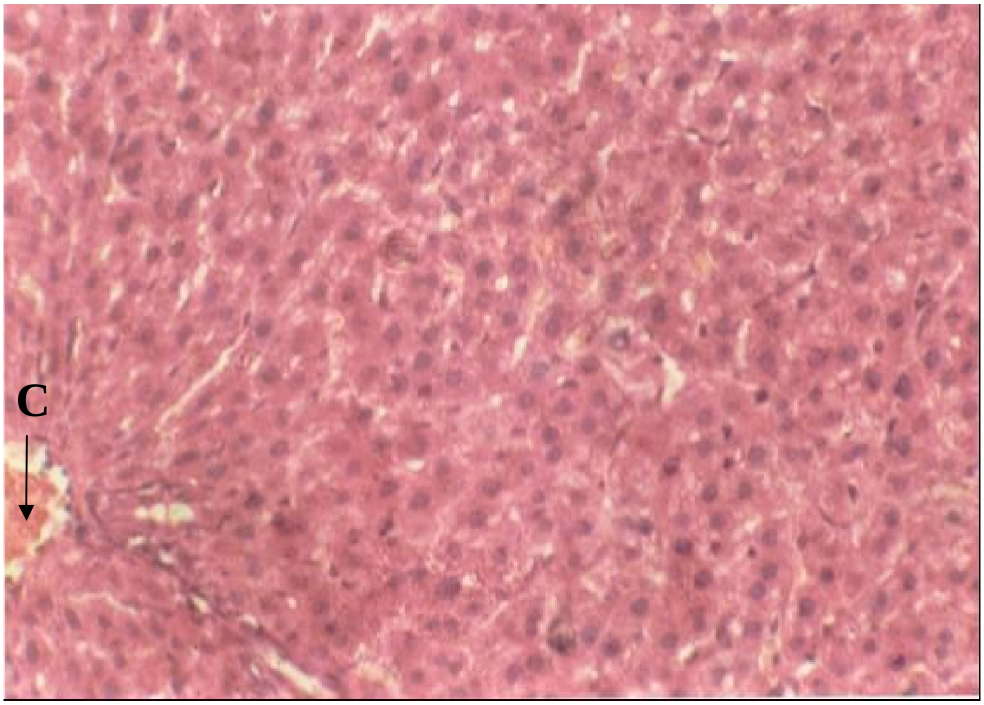

Plate 4.7b: Photomicrograph of a section of liver of rats from Normoglycemic group treated with Isophane Insulin 6 i.u/kg body weight (Group C intraperitoneally administered. Note: congested blood vessels(C) hepatic cells H & E Stain x 400.

4.8 HISTOPATHOLOGICAL EFFECT OF *AZADIRACTA INDICA* SEED EXTRACT ON STREPTOZOTOCIN- INDUCED DIABETIC WISTAR RATS

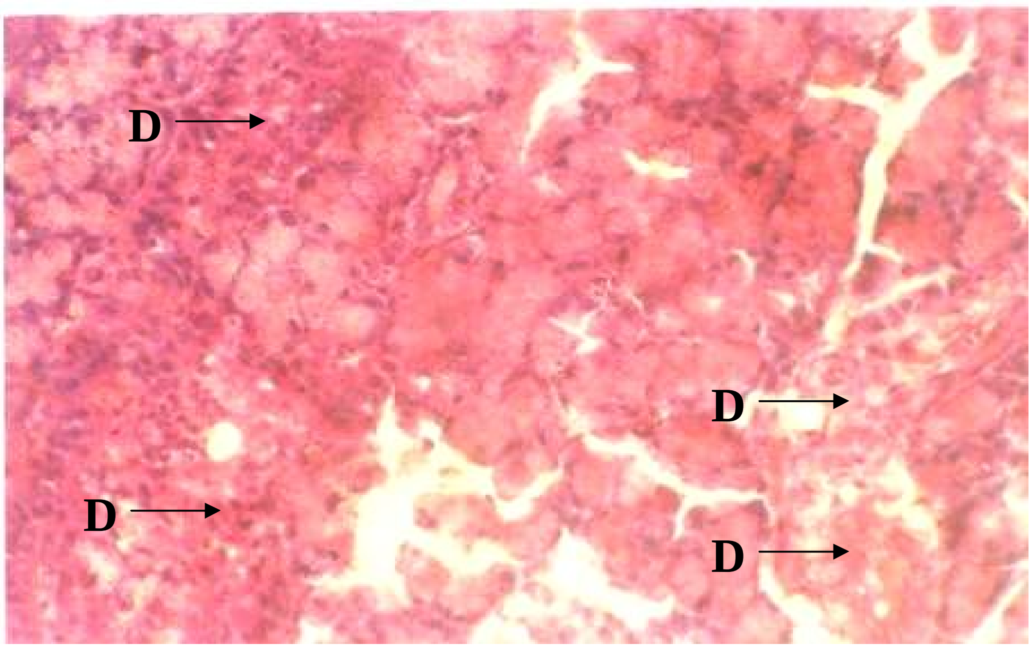

Plate 4.8a: Photomicrograph of a section of Pancreas of rats from streptozocin-induced diabetic group treated with normal saline (Group D_1) intraperitoneally administered. Note: areas of degenerated pancreatic cells(D) H & E Stain x 400.

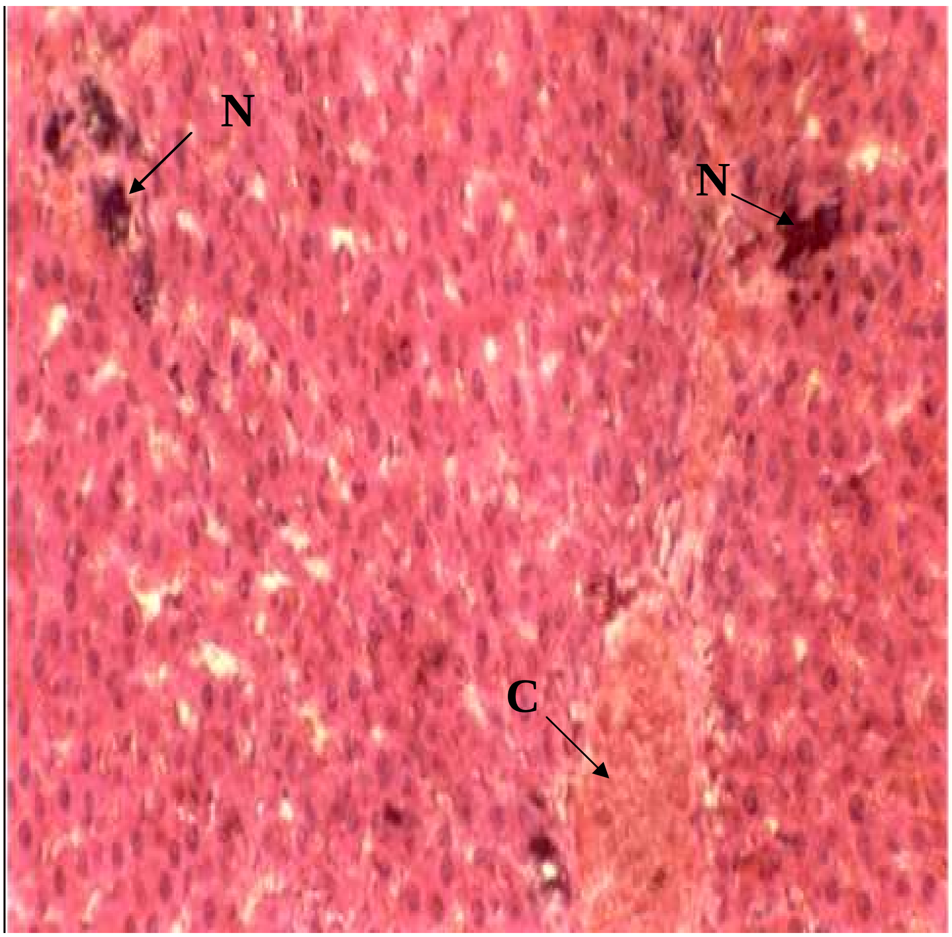

Plate 4.8b: Photomicrograph of a section of liver of rats from streptozocin-induced diabetic group treated with Normal saline (Group D_1) intraperitoneally administered. Note: focal areas of Necrosis (N) of the hepatic cells and Congested (C) blood vessels H & E Stain x 400.

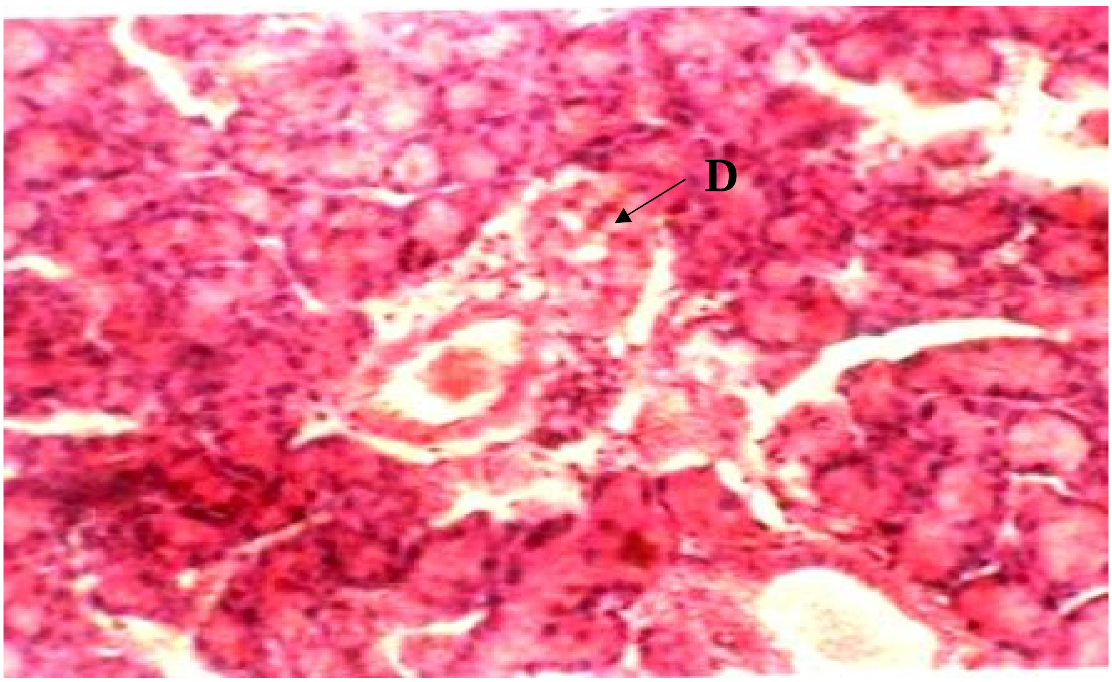

Plate 4.9 a: Photomicrograph of a section of Pancreas of rats from streptozocin-induced diabetic group treated with Isophane Insulin 6 i.u/kg body weight (Group D_2) intraperitoneally administered.Note: few areas of degenerated (D) pancreatic cells H & E Stain x 400.

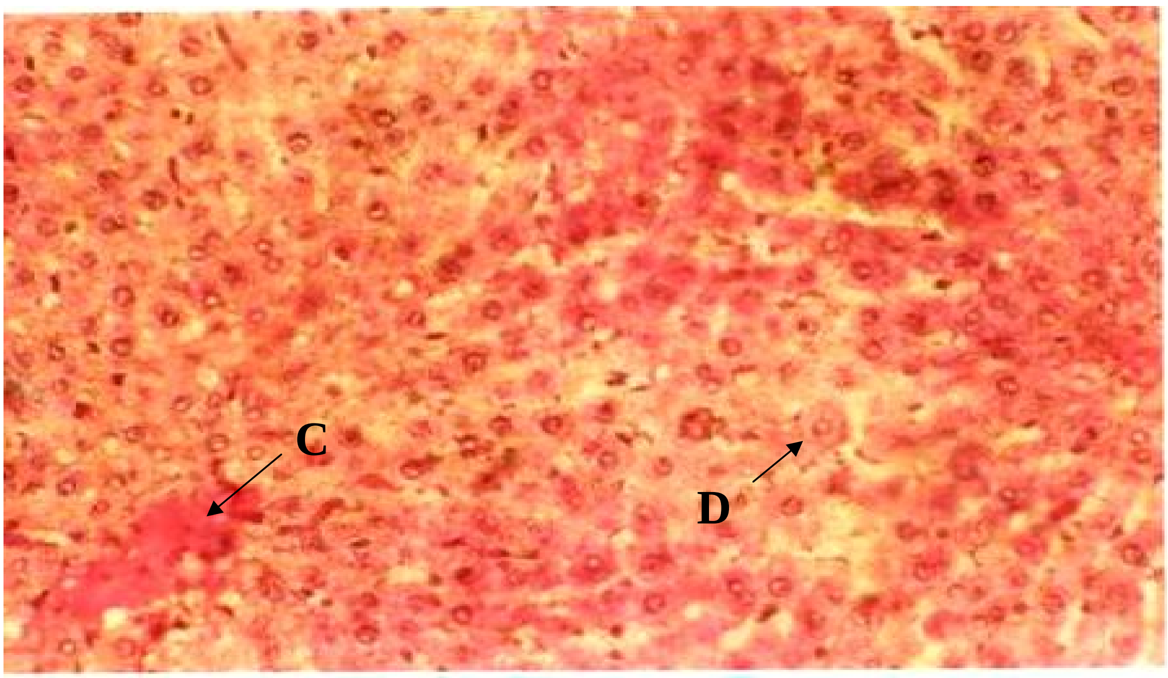

Plate 4.9: Photomicrograph of a section of liver of rats from streptozocin-induced diabetic group treated with biphasic insulin 6 i.u/kg bodyweight(Group D_2) intraperitoneally administered. Note fatty degenerated (D) hepatic cells and Congested (C)Blood vessels H & E stain x 400.

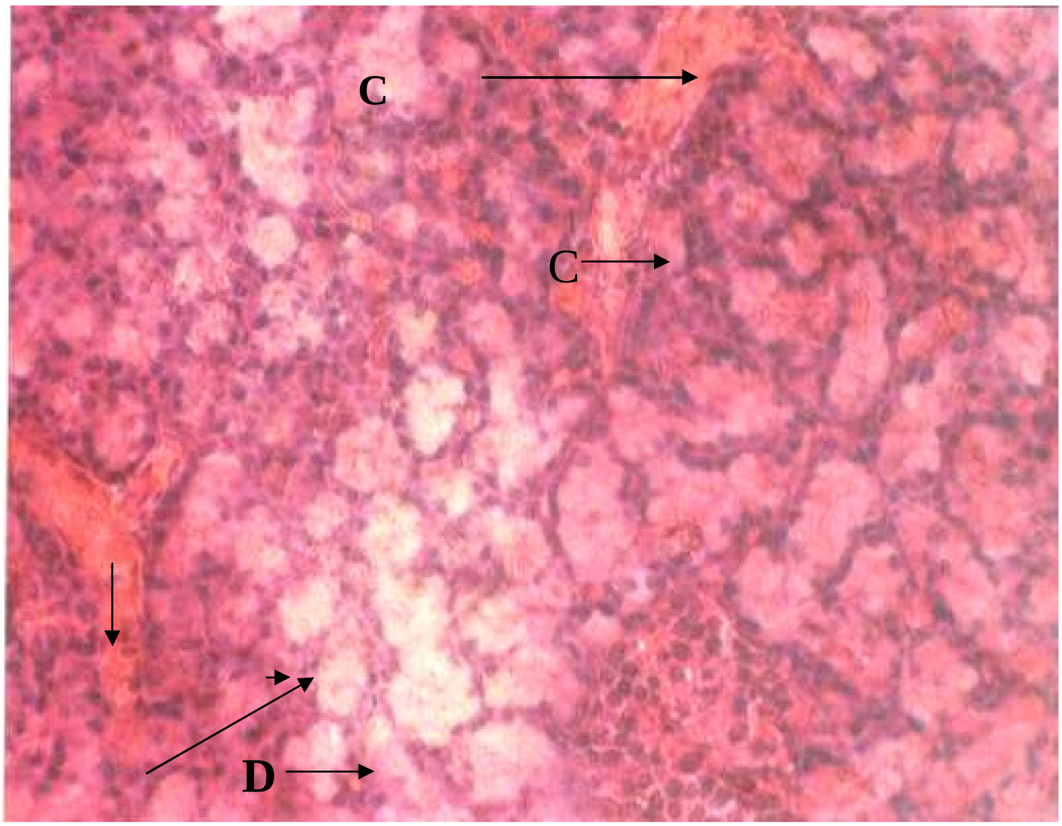

Plate 4.10a: Photomicrograph of a section of pancreas of rats from streptozocin-induced diabetic group treated with 500mg/kg body weight (Group D_3) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: Congested (C) blood vessel and focal areas of degenerated (D) pancreatic cells H & E Stain x 400.

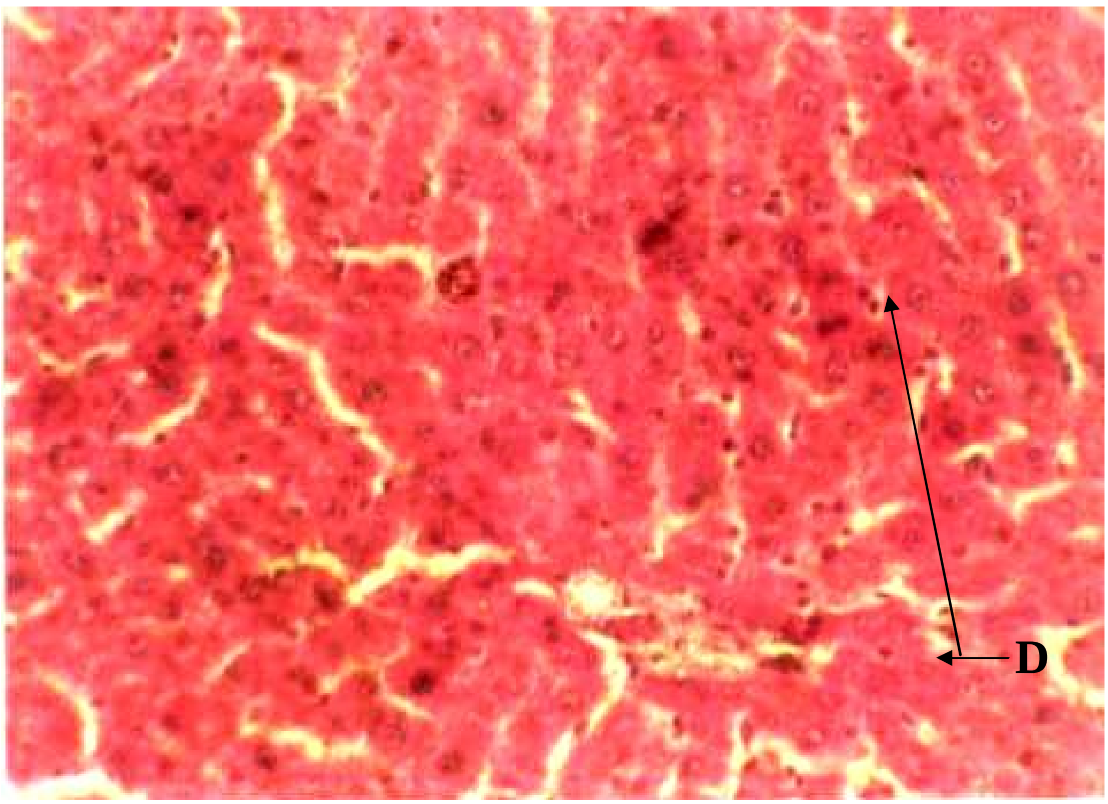

Plate 4.10b. Photomicrograph of a section of liver of rats from streptozocin – induced diabetic group treated with 500mg/kg body weight (Group D_3) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: focal areas of degenerated (D) hepatic cells H & E Stain x 400.

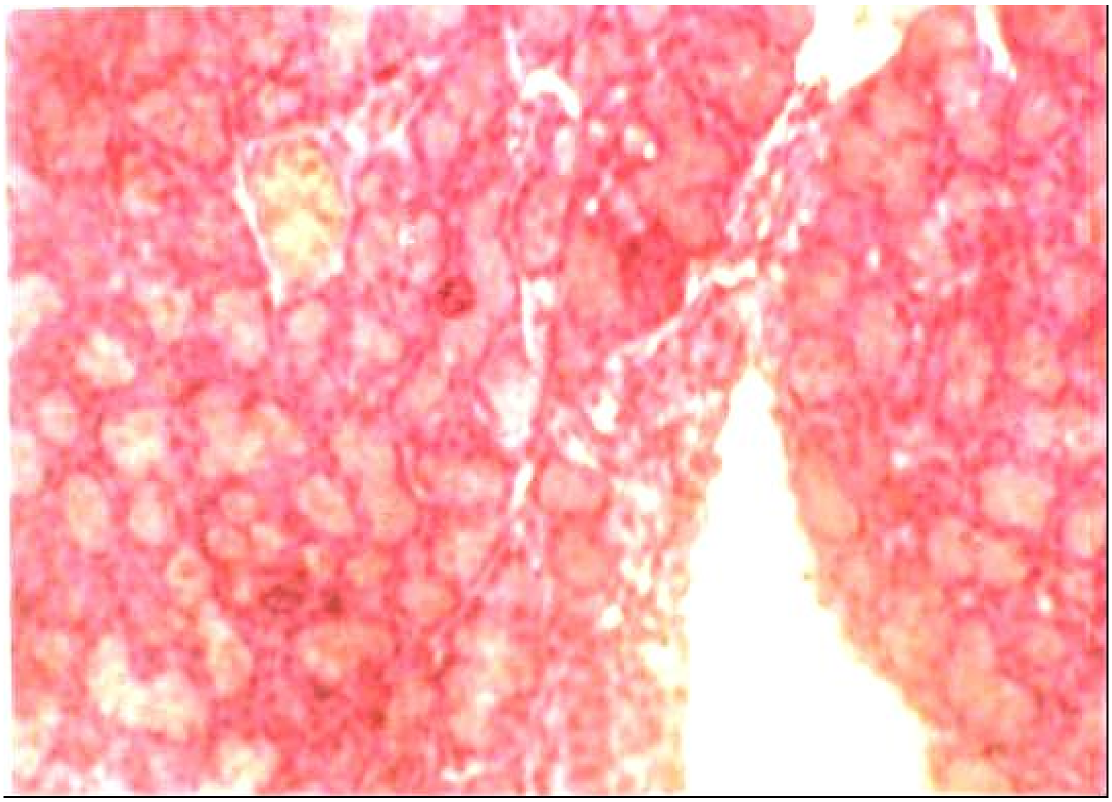

Plate 4.11a: Photomicrograph of a section of Pancreas of rats from streptozocin – induced diabetic group treated with 1000mg/kg body weight (Group D_4) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: few areas of degenerated (D) pancreatic cells H & E Stain x 400.

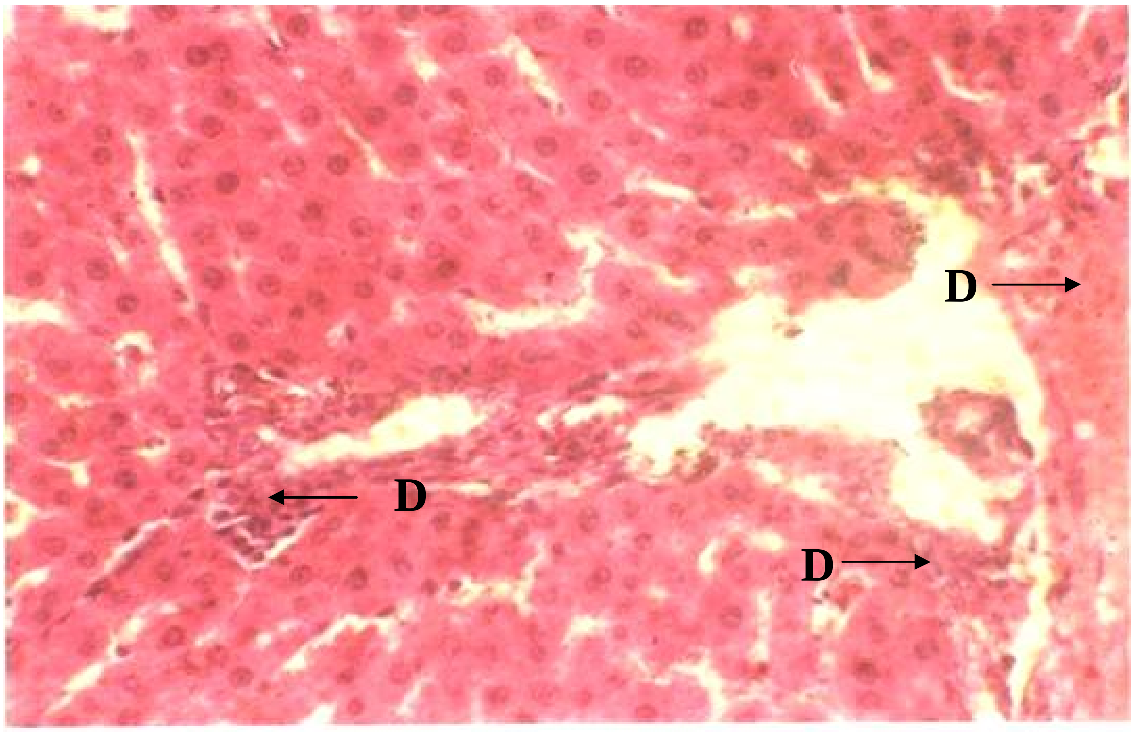

Plate 4.11b: Photomicrograph of a section of liver of rats from streptozocin- induced diabetic group treated with 1000mg/kg body weight (Group D_4) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: focal areas of degenerated (D) hepatic cells. H & E Stain x 400.

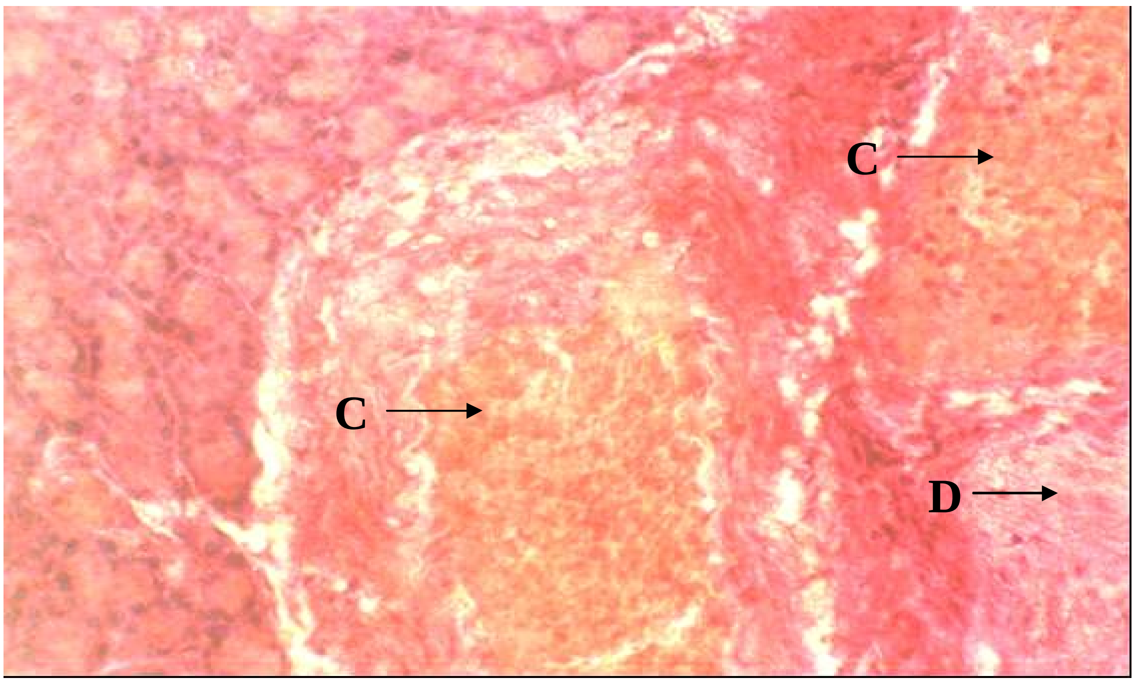

Plate 4.12a: Photomicrograph of a section of Pancreas of rats from streptozocin-induced diabetic group treated with 1500mg/kg body weight (Group D_5) of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: congested blood vessel (C) and degenerated (D) of the pancreatic cells H & E Stain x 400.

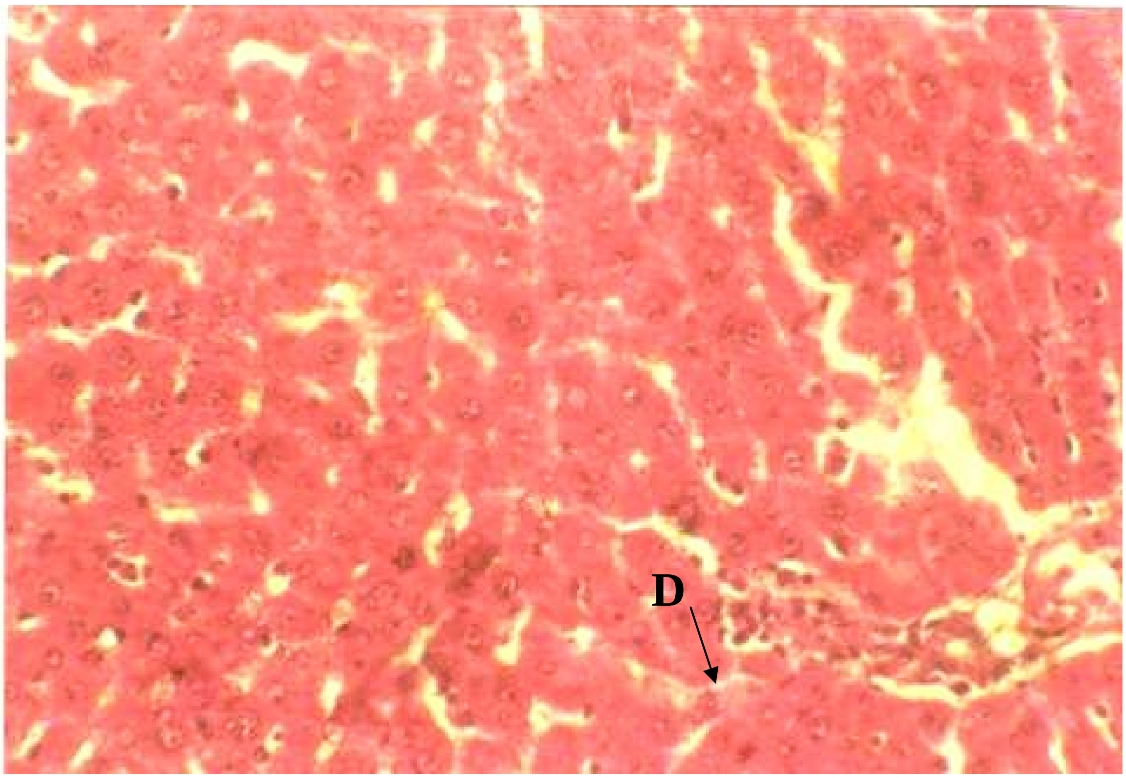

Plate 4.12b: Photomicrograph of a section of liver of rats from streptozotocin-induced diabetic group treated with 1500mg/kg body weight (GroupD_5)of seed extract of *Azadiracta Indica* intraperitoneally administered. Note: degenerated of hepatic cells (D) H & E Stain x 400.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSIONS

Although the use of herbal remedies for treatment of diabetes mellitus has greatly declined in Europe and many other western countries. Following introduction of insulin and synthetic oral hypoglycemic agents in the 1920s and 1950s respectively (Swanston et al., 1989 and 1990) approximately 80% of the people in rural African communities still rely on the use of plant remedies to control and/or manage diabetes mellitus. Administration of various plant extracts for the reduction of blood sugar levels of diabetics comprises an important aspect of the indigenous medicinal systems of many countries (Jayaweera, 1982). Most of the plants prescribed for diabetes mellitus are not edible (Atta-ur-rahma, Zaman 1989). In Africa, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus to date, however, only a few of such African medicinal plants have been scientific evaluated. Despite the fact that the World Health Organization has recommended that medical and scientific examination of such plants should be undertaken (WHO Expert Committee on Diabetes Mellitus, 1980).

Since streptozocin (STZ) is known to destroy insulin producing pancreatic β-cells, the STZ- treated rats, therefore, appear to represent a good laboratory model for IDDM, experimental diabetes state, with residual or remnant insulin production by the pancreatic β cells. The diabetic state of STZ- treated diabetic rats is therefore, not the same as that obtained by total pancreatectomy, as daily administration of insulin is not required for the survival in STZ-treated diabetic animals.

Azadiracta indica seed contain many chemical compounds, including tannins, alkaloids, flavonoids, glycerin, steroids, cholesterol and terpenoids. A number of investigations have shown that flavonoids and a host of other secondary plant metabolites including arginine and glutamic acid, possess hypoglycemic effect in various animal models **(**Akah and Okafor, 1992; Marles and Farnsworth, 1995; Ross, 1999 and 2001; Ojowole, 2002). Although the hypoglycemic effect of terpenoids appear to involve stimulation of pancreatic B-cells and subsequent secretion of preformed insulin. The exact chemical constituents of the plant extract that is/are specifically responsible for the hypoglycemic effect up till now are not fully studied. However, if the hypothesis of Marles and Farnsworth (1995) which stipulates that plants which contain terpenoids possess hypoglycemic activities in diabetic and normal mammals. It would seem reasonable to assume that, in part at least the hypoglycemic effect of *Azadiracta indica* seed may probably due to the treenails present in the plant. One or more of the other chemical constituents of the plant especially flavonoids. Nevertheless, the exact mechanism of the hypoglycemic action of the extract remains largely speculative.

5.1.0 EFFECT OF AZADIRACTA INDICA ON BLOOD GLUCOSE LEVELS OF NORMOGLYCEMIC WISTAR RATS

The normoglycemic extract-treated groups (groups B_1-B_3) were compared to the control group As regards the first group, B_1 (received 500mg/kg of the extract) there was no significant change in the blood glucose level after 1 hour, when compared to control group. While after 3, 5 and 7 hours there was significant decrease ($p<0.05$) in blood glucose levels as compared to the control group. In relation to the second group B_2 (received 1000mg/kgbody weight of extract) there was no significant change in blood glucose levels after 1 and 3 hours when compared with control group. While after 5 and 7 hours it showed a significant decrease ($p<0.05$) in blood glucose levels. With regard to group B_3 (received 1500mg/kg bodyweight of the extract), all the results revealed no significant change in the blood glucose levels when compared to the control group. Thus, the effective dose appears to be that of 500mg/kg of extract.

Also on normoglycemic rats as compared to biphasic insulin (6 i.u/kg) - treated group. After 1 hour there was a significant increase ($p<0.05$) in the blood glucose level in group B_2 when compared with biphasic isophane insulin treated group. After 3 hours there was a significant increase in the blood glucose level in groups (B_1 and B_3) as compared with the insulin-treated group. However after 5 and 7 hours there was a significant increase in blood glucose levels in all groups which were given 500 ,1000 and 1500mg/kg bodyweight of extract when compared to the biphasic isophane insulin- treated group. Thus, we can conclude that *Azadiracta indica* seed extract is more safe because its power to reduce the normal blood glucose level is less than that of insulin. So, it is more safer for diabetics to use the extract in its smallest dose to protect them against the severe hypoglycemic effect which is produced by insulin.

Our results are in agreement with the result of Khosla and his colleagues (2000). They reported in their study that *Azadiracta indica* leaves extract produced hypoglycemic activity in normal and alloxan-diabetic rats. Also our results are consistent with the study of Gupta *et al.* (2004) who reported a chronic administration of 2gm/kg of *Azadiracta indica* seed extract administered orally for 28 days produced hypoglycemic activity in normal and streptozocin-diabetic rats.

5.1.1 EFFECT *OF AZADIRACTA INDICA* SEED ON BLOOD GLUCOSE LEVEL OF STREPTOZOCIN-INDUCED DIABETIC WISTAR RATS.

The diabetic groups after receiving *Azadiracta indica* seed extract in different doses (groups D_3-D_5) were compared to the diabetic non insulin-treated control group (D_1). As regards the group D_3 (received 500mg/kg body weight of the extract) there was no significant change in the blood glucose levels after 1 and 3 hours, while after 5 and 7 hours, there was a marked decrease in blood glucose levels. In relation to the group D_4 (received 1000mg/kg bodyweight of the extract) there was no significant change in the blood glucose levels after 1 hour and 7 hours when compared with diabetic control group, while after 3 and 5 hours it showed significant decrease in blood glucose levels as compared with group D_1 but still the blood glucose levels are higher than normal values. In regard to group D_5 (received 1500mg/kg of extract) all the results revealed no significant change when compared to the diabetic control group (group D_1). Thus the effective dose appears to be 500mg/kg bodyweight of the extract. Also, when the blood glucose levels of the same previous groups (D_3, D_4 and D_5) are compared to that of the streptozocin-diabetic rats (group D_2) received isophane insulin. There was no significant change in the blood glucose levels after 1 and 3 hours in all groups (D_3, D_4 and D_5) as compared with the diabetic insulin -treated group. In relation to the group D_4 after 7 hours revealed a significant increase in blood glucose level as compared to the diabetic insulin -treated group D_2. In regard to group D_5 (1500mg/kg bodyweight of the extract) after 5 and 7 hours revealed a significant increase in blood glucose levels as compared to the diabetic insulin-treated group D_2. As regards group D_3 received 500mg/kg bodyweight of the extract after been diabetic, there was no significant change in the blood glucose levels of all samples when compared to the diabetic biphasic insulin- treated group (D_2).

Thus it appears from our study that the dose of 500mg/kg bodyweight of *Azadiracta indica* seed extract when administered to streptozocin-diabetic group (D_3) rats, produced an effect similar to that of insulin in reducing the blood glucose levels of diabetic rats. As regard to the other two dose of the extract (1000 and 1500mg/kg bodyweight), they showed a slight reduction in the blood glucose levels which had no significance indication when compared to insulin treated diabetic group. Hence, it appears that the best result is obtained by the dose of 500mg/kg bodyweight of the extract.

Our results are in agreement with the results of Khosla and his colleagues (2000). They reported in their study that *Azadiracta indica* leaves extract produced hypoglycemic activity in normal and alloxan-diabetic rats. Also our results are consistent with the study of (Gupta *et al.*, 2004) who reported that chronic administration of 2gm/kg bodyweight of *Azadiracta indica* seed extract administered orally for 28 days produced hypoglycemic activity in normal and streptozocin-diabetic rats.

5.1.2 HISTOPATHOLOGICAL EFFECTS OF *AZADIRACTA INDICA* SEED EXTRACT ON NORMOGLYCEMIC WISTAR RATS.

Histopathological examination of the livers and pancreas of rats from group A (control group) reported no observable microscopic lesions. In regards to groups B_1, B_2 and B_3 (normoglycemic rats received 500,1000 and 1500mg/kg bodyweight of the extract respectively),no observable microscopic lesion were detected in such groups.

In relation to group “C” (normoglycemic received insulin 6 iu/kg), in the pancreas there was no observable microscopic lesion. In the same time the livers showed congested blood vessels of the hepatic cells.

5.1.3. HISTOPATHOLOGIAL EFFECTS OF *AZADIRACTA INDICA* SEED EXTRACT ON STREPTOZOCIN – INDUCED DIABETIC WISTAR RATS.

The pancreas of the rats in group D_1 (received streptozocin and normal saline) had focal areas of degenerated pancreatic cells (D) involved the cells of the islet of langerhans. The livers of that rats had focal areas of degenerated hepatic cells (D) and congested blood vessels (C).

As regards the pancreas of the rats in group D_2 (received streptozocin and Biphasic insulin 6.iu/kg bodyweight)) had few focal areas of degenerated pancreatic cells (D) while the livers of the same rats had fatty degenerated hepatic cells (D).

In relation to the pancreas of the rats in group D_3 (received streptozocin and 500mg/kg bodyweight of the extract), they had congested blood vessels and focal areas of degenerated pancreatic cells (D). The livers of the same rats had focal areas of degenerated hepatic cells and congested blood vessels (C).

In regard to the pancreas of the rats in group D_4 (received streptozocin and 1000mg/kg bodyweight of the extract), they showed had focal areas of few degenerated pancreatic cells (D). The liver of the same rats had focal areas of degenerated hepatic cells (D).

Finally, pancreas of rats in group D_5 (received streptozocin and 1500mg/kg bodyweight of extract) revealed congested blood vessels (C) and degenerated . pancreatic cells(D) and their livers had few areas of degenerated hepatic cells (D). The extract did not produce remarkable improvement in the pancreas and livers of the extract-treated, may be because of the acute used.

5.2 CONCLUSIONS

The acute toxicity study of the extract *Azadiracta Indica* seed as determined by the intraperitoneal route of an LD_{50} was 3000mg/kg bodyweight .As chronic toxicity study was not carried out in this research work to determine whether the histopathological effect could be reversible, the therapeutic use of the extract should be monitored cautiously.

The research work has demonstrated that the hypoglycemic effects of the extract of *Azadiracta Indica seed* when administered intraperitoneally in both streptozocin-induced diabetic and normoglycemic Wistar rats had an effective dose of 500mg/kg of the extract. However at 1500mg/kg it was not effective.

5.3 RECOMMENDATIONS

This research work demonstrated the hypoglycemic effects of *Azadiracta Indica* seed extract intraperitoneally. It may be used as a cheap alternative to currently used acting insulin preparations for diabetic patients. For actualization of this goal I recommend the following,

(a) Further research work to be carried out on animal models of type 2 diabetes using this extract in our environment to determine its effects.

(b) Chronic toxicity study should be done to determine the reversibility of the effects of the extract on various organs

(c) The isolated active components can further be subjected to research with animal models of diabetes mellitus.

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Appendix A : Effect of *Azadiracta Indica* seed extract on blood glucose levels of normoglycemic Wistar rats

Group A: Normal saline administered intraperitoneally.

Rats	**Time(Hours) Blood glucose levels (mg/dl)**				
	0	1	3	5	7
1	44	46	44	42	45
2	51	53	50	55	53
3	55	59	52	45	60
4	43	46	49	41	45
5	42	45	42	49	43

Group B_1 :500mg/kg extract of *Azadiracta indica seed* administered intraperitoneally.

Rat	**Time(Hours) Blood glucose levels (mg/dl)**				
	0	1	3	5	7
1	37	33	31	38	27
2	49	40	37	35	37
3	34	35	29	32	29
4	61	52	45	42	34
5	66	67	51	46	41

Group B_2 :1000mg/kg extract of *Azadiracta indica seed* administered intraperitoneally.

Rats	Time(Hours) Blood glucose levels (mg/dl)				
	0	1	3	5	7
1	43	58	33	28	26
2	66	59	41	43	31
3	46	73	40	32	32
4	60	47	39	33	42
5	65	44	41	46	39

Group B_3 :1500mg/kg extract of *Azadiracta indica seed* administered intraperitoneally..

Rats	Time(Hours) Blood glucose levels (mg/dl)				
	0	1	3	5	7
1	45	48	40	40	36
2	48	43	45	46	40
3	64	68	53	55	53
4	45	39	43	44	42
5	42	34	44	41	39

Group C : Biphasic Isophane insulin(6.iu/kg) administered

Rats	**Time(Hours) Blood glucose levels (mg/dl)**				
	0	1	3	5	7
1	42	30	28	28	24
2	45	20	38	31	31
3	47	29	27	21	19
4	44	38	28	24	16
5	43	34	22	18	20

Appendix B : Effect of *Azadiracta Indica* seed extract on blood glucose level of streptozotocin–induced diabetes Wistar rats

Group D_1 :Normal saline administered intraperitoneally.

Rats	**Time(Hours)**	**Blood glucose levels (mg/dl)**			
	0	1	3	5	7
1	200	204	208	193	205
2	162	169	163	163	165
3	185	183	180	185	189
4	195	198	201	203	190
5	205	208	211	215	209

Group D_2 : Biphasic Isophane Insulin (6 i.u /kg) administered intraperitoneally.

Time(Hours)		**Blood glucose levels (mg/dl)**			
	0	1	3	5	7
1	180	145	115	101	92
2	165	134	138	108	117
3	210	189	144	112	90
4	178	168	131	104	110
5	230	192	172	136	89

Group D_3 : 500mg/kg extract of Azadiracta indica seed administered intraperitoneally

Rats	**Time(Hours) Blood glucose levels (mg/dl)**				
	0	1	3	5	7
1	200	180	160	160	240
2	192	140	155	182	177
3	221	153	120	173	144
4	159	188	105	113	106
5	135	162	153	100	88

Group D_4 : 1000mg/kg extract of *Azadiracta indica* seed administered intraperitoneally.

Rats	**Time(Hours) Blood glucose levels (mg/dl)**				
	0	1	3	5	7
1	165	129	160	145	106
2	183	115	155	97	100
3	179	92	120	83	105
4	203	215	105	180	141
5	200	191	153	165	122

Group D_5 :1500mg/kg extract of *Azadiracta indica* seed administered intraperitoneally.

Rats	**Time(Hours)**	**Blood glucose levels (mg/dl)**			
	0	1	3	5	7
1	180	182	175	145	168
2	158	152	158	97	129
3	190	199	183	83	145
4	220	225	210	180	181
5	203	210	200	165	192