Studies on the Effects of *Asteracantha longifolia* on Retardation of Glucose Absorption and Carbohydrate Digestion in Long-Evans Rats

A research paper is submitted to the Department of Pharmacy, East West University in conformity with the requirements for the degree of Bachelor of Pharmacy

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Declaration by the candidate

I, Tahmina Sultana Juthi hereby declare that the dissertation entitled **'Studies on the Effects of Asteracantha longifolia on Retardation of Glucose Absorption and Carbohydrate Digestion in Long-Evans Rats'** submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by us during the period 2017 of our research in the Department of Pharmacy, East West University, under the supervision and guidance of Dr. JMA Hannan, Professor, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

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Abstract

Our present studies were focused on the probable anti-diabetic activity of the plant *Asteracantha longifolia* in laboratory animals and the statistical significance of such effect. The plant extract was subjected to anti-diabetic study through assessing Disaccharidase activity and six segment method which was performed to assess the amount of sucrose remaining in the GIT at six different positions. In Six Segment test, the amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 100mg/kg extract at 30 minutes, 1hour, and 2hour. In Dissacharide activity the amount of unabsorbed sucrose in Pancreatic Enzymes are evaluated in control rats vs rats fed with 100mg/kg extract .The extract caused a significant ($p<0.05$), dose dependent inhibition of glucose absorption and showed hypoglycemic effects in Long-Evans rats weighing about 100-200 gm. The anti-diabetic effects were estimated by measuring the amount of glucose in the samples collected after the experiment. In conclusion, these observations provide evidence and possible mechanisms of action for the anti-diabetic properties of plant claimed *Asteracantha longifolia* in Ayurveda medicine.

Keywords: Anti-Diabetic, *"**Asteracantha longifolia**"* hypoglycemic, Glucose, Sucrose.

Chapter 1
INTRODUCTION

1. Introduction

Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria and they will become increasingly thirsty and hungry. Diabetes mellitus is a life-long disease affecting more than 150 million people all over the world and WHO has predicted the number will be doubled by the year 2025 (WHO 2002). Type 1 diabetes accounts for 5-10% of the diabetic population. Type 2 diabetes accounts for 90 - 95% of the people with diabetes and is more prevalent in adults (WHO 2002).

1.1 Diabetes Mellitus

Diabetes mellitus is a life-long disease affecting more than 150 million people all over the world and WHO has predicted the number will be doubled by the year 2025 (WHO 2002). Type 1 diabetes accounts for 5-10% of the diabetic population. Type 2 diabetes accounts for 90 - 95% of the people with diabetes and is more prevalent in adults (WHO 2002).

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association 2001). A consequence of the disease is adverse effects on both the macro vascular and micro vascular system. Diabetic complications associated with macro vascular diseases are atherosclerotic macro vascular disease and ischemic coronary heart disease. Diabetic complications related to micro vascular disease include retinopathy, nephropathy, neuropathy, and peripheral vascular diseases (Perring et al 1985, Clements & Bill 1986, WHO 2002).

1.1.1 Description of Diabetes Mellitus

Diabetes (diabetes mellitus) is classed as a metabolic disorder. Metabolism refers to the way our bodies use digested food for energy and growth. Most of what we eat is broken down into glucose. Glucose is a form of sugar in the blood. It is the principal source of fuel for our bodies. When our food is digested, the glucose makes its way into our bloodstream. Our cells use the glucose for energy and growth. However, glucose cannot enter our cells without insulin being present- insulin makes it possible for our cells to take in the glucose.

Insulin is a hormone that is produced by the pancreas. After eating, the pancreas automatically releases an adequate quantity of insulin to move the glucose present in our blood into the cells, as soon as glucose enters the cells blood-glucose levels drop.
A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated (hyperglycemia). This is because the body does not produce enough insulin, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the blood has plenty of glucose, the cells are not getting it for their essential energy and growth requirements. (American Diabetes Association 2002, WHO 2002).

1.1.2 Classification of Diabetes Mellitus

The World Health Organization (WHO) classifies diabetes into main groups: type1 diabetes also called insulin-dependent diabetes mellitus or IDDM and type2 diabetes also called non-insulin dependent diabetes mellitus or NIDDM (WHO 2002). Maturity-Onset Diabetes of the Young (MODY) and gestational diabetes are less frequently occurring forms of diabetes. It is also worth noting that the current classification of diabetes on the basis of age is becoming increasingly problematic because the age of individuals presenting type1 diabetes is getting older and there is an increase of type 2 diabetes in the young. In addition, increasing numbers of non-insulin dependent diabetic patients are becoming dependent on exogenous insulin administration.

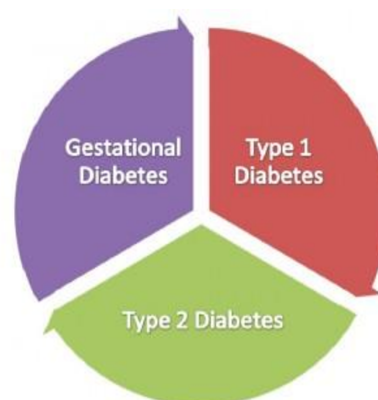

Figure 1.1 Classification of Diabetes Mellitus

1.1.2.1 Type 1 diabetes

Type1 diabetes, defined by an absolute requirement for administration of exogenous insulin, results from the autoimmune destruction of the insulin-secreting pancreatic β cells. Type 1 diabetes is a severe form associated with ketosis in the untreated state. It arises most commonly in juveniles but occasionally in non-obese adults and elderly. It is a catabolic disorder in which circulating insulin is virtually absent with elevated level of plasma glucagon. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis and reduce the elevated blood glucose level. It is thought to result from an infectious or toxic environmental-induced autoimmune disorder (Karam 1998). Autoimmunity has been proposed to be the main reason for β cell destruction associated with type1 diabetes (Eisenbarth 1986, Rossini et al 1993).

The pathogenesis of type 1 diabetes is initiated by activation of monocytes by unidentified factors from islet tissues. With the production of IL-1 by macrophages, nitric oxide and free radicals can be induced resulting in the abolishment of glucose-induced insulin secretion in pancreatic beta cells and ultimately beta cell death (Sandler et al 1989, Dunger et al 1996, Hoorens et al 2001, Suk et al 2001). The release of auto-antigens due to destruction of β cells could further trigger the activation of T lymphocytes and the production of islet cell antibodies leading to a self-perpetuating and self-limiting circuit of cytokine production (Nerup et al 1988). The secretion of tumor necrosis factor (TNF) by macrophages can also further enhance the effects of IL-1 on pancreatic β cells which is controlled by a gene in HLA regions (Nerup et al 1988). On the other hand, HLA-DR3, DR4, DR9, and HLA-DQ have been associated with susceptibility towards type 1 diabetes in various ethnic groups (Aparicio 1991, Baisch et al 1992, Ikegami et al 1992, Chuang et al 1995, Israel et al 1998). In addition, if one of a pair of identical twins has type 1 diabetes, the probability for the other to develop the condition is 20 - 30% (Abbas et al 1994, American Diabetes Association 2002). Therefore, genetic factors are considered to be quite important in type 1 diabetes (American Diabetes Association 2002, WHO 2002).

Although the role of viral infections in inducing type1 diabetes remains controversial, toxic chemicals with structural similarities to alloxan or streptozotocin, may contribute to pancreatic beta cell demise and destruction in animal model of type1 diabetes (Foulis et al 1997, Jorns et al 1997, Cheta 1998). This environmentally

mediated beta cell destruction could result in accidental release of self-antigens to the immune system leading to the triggering of islet-specific autoimmunity (Chowdhury et al 1999). The moderate to long-term symptomless phase of the disorder could readily be identified through circulating cytoplasmic auto-antibodies, such as islet cell cytoplasmic antibodies (ICA), including insulin autoantibodies (IAA) and glutamic acid decarboxylase (GAD) (Petersen et al 1994).

1.1.2.2 Type 2 diabetes

Type 2 or non-insulin-dependent diabetes mellitus is characterized (American Diabetes Association, 2001) by a relative insulin deficiency due to predominantly an insulin secretory defect with insulin resistance.

Type 2 diabetes represents a heterogeneous group of disorders comprising milder forms of diabetes that occur predominantly in adults but occasionally in adolescents. Circulating exogenous insulin is sufficient to prevent ketoacidosis but is often either subnormal or relatively inadequate because of tissue insensitivity (Rodger 1991). Obesity, which generally results in an impaired insulin action, is a common risk factor for this type of diabetes, and most patients with type 2 are obese. Genetic factors also underlie the disease (Karam 1998). It does not appear to have a strong autoimmune component with the observation that general autoantibody titres are low or absent. However, recent studies also suggested that reduction of β cell mass due to unbalanced islet neogenesis and apoptosis might play an important role in type2 diabetes apart from deficiency of insulin secretion (Zhu et al 1996, Butler et al 2003).

The clinical and other features of two major clinical classes – type 1 and type 2 are summarized in Table 1.1.

1.1.2.3 Gestational diabetes

Gestational diabetes develops in some women when they are pregnant. Most of the time, this type of diabetes goes away after the baby is born. However, if you've had gestational diabetes, you have a greater chance of developing type2 diabetes later in life. Sometimes diabetes diagnosed during pregnancy is actually type2 diabetes (Landon & Gabbe 1988).

1.1.2.4 Other types of diabetes

Diabetes caused by other identifiable etiologies such as: 1) Genetic defects of β cell function (e.g. MODY 1, 2, 3), 2) Genetic defects in insulin action, 3) Diseases of the

exocrine pancreas (e.g. cancer of the pancreas, cystic fibrosis, pancreatitis), 4) Endocrinopathies (eg Cushing's), 5) Drug or chemical induced (e.g. steroids), 6) Infection (e.g. rubella, Coxsackie, CMV), 7) Uncommon forms of immune-related diabetes, 8) Other genetic syndromes.

In 1985 fibro calculus pancreatic diabetes (FCPD) was grouped as a subtype of malnutrition related diabetes mellitus (MRDM) by the WHO study group on diabetes mellitus (WHO study Group on Diabetes Mellitus 1998). However, the ADA Expert Committee on diagnosis and classification of diabetes mellitus suggested it as secondary diabetes and termed it as fibro calculus pancreatopathy (American Diabetes Association 2001).

Table1.1

1.1.3 Differences between type 1 and type 2 diabetes

Type 1 diabetes	Type 2 diabetes
Symptoms usually start in childhood or young adulthood.	Usually the disease is discovered in adulthood, but an increasing number of children are being diagnosed with the disease.
Hypoglycemia is common	There are no episodes of low blood sugar level, unless the person is taking insulin or certain diabetes medicines.
It can’t be prevented	It can be prevented or delayed with a healthy lifestyle, including maintaining a healthy weight and exercising regularly.

(Leonid Barski, 2013)(Cold et al., 2017)

1.1.4 Sign and Symptoms

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger).

Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in type 2 diabetes. Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic derma dromes.

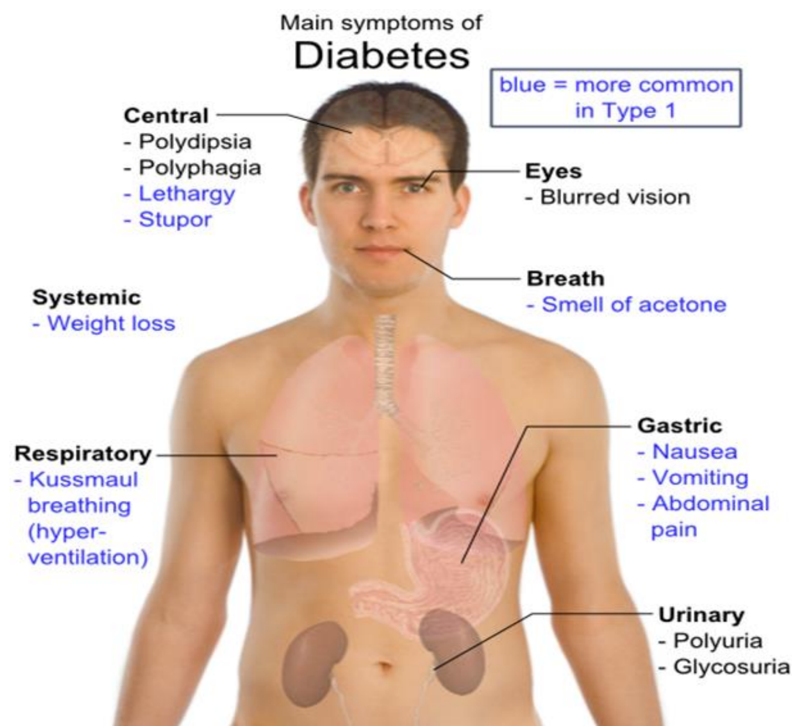

Figure1.2 Symptoms of diabetes - by Mikael Häggström

1.2 Biochemical abnormalities involved in the pathogenesis of diabetes

Diabetes mellitus is a heterogeneous group of metabolic disorders. The major metabolic lesions associated with diabetes mellitus include defective insulin secretion and insulin sensitivity by peripheral tissue targets.

1.2.1 Defective insulin secretion in diabetes mellitus

Defective insulin secretion is a feature of type 2 diabetes that results from inadequate compensatory increase of β cell mass and impaired glucose-dependent insulin release (Rutter 2001, Kahn and Porte 1990, Leahy 1990, Flatt et al 1992). The ability of

pancreatic β cells to synthesize, store, and release insulin in response to variations in circulating metabolite levels and intracellular glucose metabolism is regulated by changes in ATP/ADP ratios resulting in Ca2+ mobilization (Matschinsky et al 1996). Alterations of this sensing loop occur early in the pathogenesis of type 2 diabetes, but are initially compensated by an increase of β cell mass (Bonner-Weir 1994). In this respect, pancreatic β cells appear to differ from other terminally differentiated cell types by retaining their ability to proliferate, as demonstrated in both physiological conditions (growth, gestation) and disease states (obesity, insulin resistance) (Bonner-Weir, 2000). In addition to presumptive proliferation of existing β cells, there is evidence for β cell neogenesis from undifferentiated progenitors, apparently arising from the epithelial lining of pancreatic ducts (Bendayan 1987, Bertelli et al 2001, Bonner-Weir 2000, Bouwens & Pipeleers 1998).

The factors inducing β cell proliferation under normal or pathological conditions are largely unknown, although some evidence exists about the involvement of fibroblast growth factors (FGFs) (Hart et al 2000), hematopoietic growth factors (HGFs) (Garcia-Ocana et al 2000), and placental lactogen (Vasavada 2000). Moreover, signaling by receptor tyrosine kinases has been implicated as a regulatory mechanism in both β cell proliferation (Rhodes 2000, Withers 1999, Hugl et al 1998) and insulin release (Hart et al 2000, Khan et al 2001, Leibiger et al 1998). In particular, insulin/insulin-like growth factor (IGF) signaling through insulin receptor substrate (IRS) and phosphoinositide 3-kinase (PI 3-kinase) appears to regulate several aspects of β cell function. Thus, ablation of the insulin/IGF receptor substrate IRS-2 impairs β cell proliferation (Withers 1998, Kubota 2000), whereas ablation of p70s6k1, an Akt substrate, is associated with a decrease in β cell size (Pende 2000).

The mutations of insulin receptor (IR) (Kulkarni 1999a) or IRS-1 (Kulkarni 1999b) impair insulin synthesis and secretion mediated by PI 3-kinase–dependent pathways (Aspinwall 2000, Kulkarni 1999b). The signals regulating β cell proliferation and insulin secretion diverge downstream of PI 3-kinase and this strongly suggests that Akt is not the sole effector of PI 3-kinase. Nevertheless, the role of growth factor signaling through PI 3-kinase as related to insulin secretion remains poorly understood. It has been demonstrated that mice lacking IRS-1 develop defective insulin secretion, whereas mice lacking IRS-2 develop impaired β cell proliferation (Accili 2001). Recently it has been shown that β cells lacking IGF1R exhibit a

profound decrease of insulin secretion in response to both glucose and arginine (Xuan et al 2002).

1.2.2 Defective insulin action in diabetes mellitus

Insulin-mediated glucose utilization and metabolism is the final result of the activation of a complex cascade of events involved in the insulin signaling process (Khan 1993). Alteration of one or more of these events can result in impaired insulin action. Three main steps are involved in the generation of insulin resistance:

1) Insulin binding to the cell membrane receptor,

2) Insulin receptor phosphorylation, and

3) Intracellular insulin signaling.

The insulin receptor is consisting of two alpha-subunits in the extracellular domain and two beta-subunits with main intracellular domain. Upon insulin binding of the beta-subunits, the intrinsic kinase activity in the alpha-subunits is activated leading to phosphorylation of the adjacent beta-subunit. The auto phosphorylation of the insulin receptor allows the activation of insulin receptor substrate (IRS-1, -2, -3, -4) protein family. These proteins exert an important regulatory action on other mediators like phospho-inositol-3-kinase (PI3-kinase). The contribution of IRS-1 and IRS–2 to insulin resistance has been recently demonstrated with knock-out genetic experiments. These studies proved that IRS-2 can play a vicariate role in absence of IRS-1, while IRS-2 knock-out results in impaired insulin action (Mauvais-Jarvis et al 2002).

Activation of PI3-kinase catalysis the formation of PI-3,4,5-phosphate allowing the activation of PKB/AKT and phosphatidylinositol-3,4,5-phosphate kinase-1 (PDK-1). The phosphorylation of PKB/AKT regulates the kinase cascade involved in the insulin signal transduction responsible for GLUT-4 translocation from the intracellular membrane compartment to the cell membrane allowing active trans membrane glucose transport and phosphorylation, activation of the glycolytic flux, as well as glycogen and protein synthesis (Khan & Pessin 2002).

Several of the steps of insulin signaling cascade involved in the generation and propagation of the insulin signal can contribute to the molecular defect of insulin action. A reduced expression and a phosphorylation of the elements involved in the first steps of insulin signaling (IRS, PI3-kinase, PKB) have been found in tissue of type 2 diabetic patients. The role of specific defects of these proteins has been established by knock-out animal models (Mauvais-Jarvis et al 2002). For instance,

IRS-1, IRS-2 and GLUT-4 knock-out mice have been shown to develop insulin resistance and glucose intolerance. β-cell insulin receptor knock-out (βIRKO) mice lose acute insulin response to glucose and develop glucose intolerance. Human pancreatic islets carrying the Gly972βArg IRS-1 polymorphism have impaired insulin action (Marchetti et al 2002).

The principle defect in type 2 is the loss of insulin sensitivity in peripheral tissue such as muscle and liver resulting in impairment of glucose uptake and utilization by these tissues. Together with excessive glucose production by the liver, these defects lead to widespread disruption of nutrient homeostasis (DeFronzo 1988). Insulin resistance appears to be the primary metabolic defect with relative (but not absolute) insulin deficiency being the factor determining conversion to diabetes (Stern 1988). Once diabetes is established, the abnormalities of insulin secretion and insulin resistance worsens, hand in hand, in direct relationship to the degree of fasting hyperglycemia (Zimmet et al 1978, Kolterman et al 1981). In type 2 diabetes, gross insulin resistance combined with hyperinsulinaemia results in a state of relative (not absolute) insulin deficiency (Campbell et al 1988). Postreceptor defects are currently believed to be primarily related to insulin resistance in human diabetes (Olefsky & Kolterman 1981, Becker & Roth 1990, Kahn & Folli 1993, Kahn 1994).

1.3 Complications of diabetes mellitus

Diabetes is a complex heterogeneous disease where multiple levels of abnormalities are present in various tissues. Defects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. The major long-term complications of diabetes mellitus are macro vascular and micro vascular diseases such as nephropathy, retinopathy and neuropathy (Donnelly et al 2000).

1.3.1 Macro vascular complications

Diabetes is a very complex metabolic disorder, which produces vascular complications including cardiovascular disease, which is the major cause of death (Laakso & Lehto 1997). The macro vascular complications lead to coronary heart disease, hypertension and other peripheral vascular diseases (Wei et al 1998). Atherosclerotic macro vascular disease accounts for more than 80% of the mortality in the diabetic population (Escalante et al 1998) and ischemic coronary heart disease contributes about 60% to the mortality in the adult diabetic population (Barrett-

Connor & Orchard 1985). The major risk factors for coronary artery disease are elevated levels of low density lipoprotein (LDL) and decrease in high density lipoprotein (HDL) which have been found in many diabetic patients. However, to what extend these changes in lipid metabolism in diabetes are a reflection of metabolic abnormalities is unknown (Montague 1983). Other factors that account for the increased prevalence of macro vascular complications include hyperglycemia, hypertension and obesity (Escalanate et al 1998). The aetiological role of hyperglycemia in the pathogenesis of macro vascular diseases is not clear.

Type 2 diabetes and macro vascular disease may share several of the recognized risk factors, but hyperinsulinaemia and insulin resistance (due to obesity) may be important. Epidemiological studies have found a significant association between insulin concentration and subsequent development of ischaemic heart disease (Jarrett 1988), which may be due to insulin resistance as seen in type 2 diabetes (Ducimetiere et al 1980). Some studies have revealed that hyperinsulinaemia may be predictive for the development of coronary heart disease in diabetic patients (Pyorala). Individuals with insulin resistance often have elevated serum concentrations of triglyceride with low concentrations of high density lipoprotein cholesterol (HDL-cholesterol) and this dyslipidemia contributes to their increased risk of atherosclerotic cardiovascular disease (Krentz 1996, Erickson et al 1989, Reaven 1988, Piodor 2000, Haffner & Stern 1989).

Platelet hyperaggregability is found in both type 1 and type 2 diabetes, which may precede the development of vascular disease (Winocour 1989). Non-enzymatic glycosylated collagen in vessel walls in diabetes can significantly enhance the platelet adhesion and aggregation. Oxidative damage by free radicals has been implicated also in the development of vascular disease in patients with diabetes. Increased free radical damage may play an important role in pathogenesis of platelet hyperaggreegation in diabetes mellitus (Reaven & Greenfield 1993).

1.3.2 Micro vascular complications

The long-term effects of diabetes mellitus include progressive development of retinopathy with potential blindness, nephropathy that may lead to renal failure, and neuropathy with risk of foot ulcers, amputation, charcot joints and autonomic dysfunction including sexual dysfunction (Alberti & Zimmet 1999). The level of

hyperglycemia is clearly a risk factor for micro vascular complications in diabetic patient (Wei et al 1998).

Diabetic retinopathy is the most common cause of blindness in about 86% of people with type 1 diabetes and in 33% of type 2 diabetic patients (Klein et al 1984). Diabetes retinopathy is a progressive disorder classified according to the presence of various clinical abnormalities. Despite the growing concern about this disease, its natural history and etiopathogenesis are still not completely understood. However several risk factors have been identified (Klein et al 1985, Moss et al 1994) which may play an important role to the development of retinopathy (Kohner 1993). The prevalence of the disease increases with the duration of diabetes. In general, significant visual impairment is usually caused by proliferative retinopathy in type 1 and maculopathy in type 2 diabetes. Diabetes maculopathy is the most common cause of visual loss in type 2 diabetes and may be exudative, edematous or schaemic. If untreated, proliferative retinopathy and maculopathy will have an appalling prognosis for the patient's eyesight. Emphasis must therefore be placed on the primary prevention of retinopathy.

Diabetic nephropathy (defined clinically as the presence of micro albuminuria or overt nephropathy in patients with diabetes who lack indicators of other renal diseases) is the most common cause of renal failure in the Western World (Canadian Organ Replacement Registry 2001). Diabetic nephropathy is characterized by proteinuria, decreased glomerular filtration rate (GFR) and increase blood pressure. Longitudinal and cross sectional studies have shown that hypertension, poor metabolic control, smoking and general factors are generally accepted risk factors in the development of diabetic nephropathy (Friedman 1996, Quinn et al 1996, Earle et al 1997). The major pathological features are thickening of the basement membrane, messingial enlargement and glomerular sclerosis due to schaemia, which relates to glomerular filtration rate and albuminuria (Deckert et al 1991). As the disease progresses albuminuria increases, glomerular filtration rate declines and blood pressure rises progressively with eventual development of end stage nephropathy (Watkins 1998).

Several studies, including the Diabetes Control and Complications Trial (DCCT), have established that better metabolic control, as reflected by lower HbA_{1c} values, reduces the incidence of diabetic nephropathy (DCCT 1983). However the threshold of metabolic control, below which patient might be protected from this complication

remains controversial (Jacobson et al 1988, Danne et al 1996, DCCT 1996). It has been shown that hypertensive treatment greatly slows the decline in renal function and improves survival in patients with this disease (Santiago 1986).

Diabetic neuropathy constitutes a diverse group of conditions and is one of the major health problems among patients with type 1 and type 2 diabetes. This disease is characterized by diffuse or focal damage to peripheral somatic or autonomic nerve fibers resulting from diabetes mellitus (Wiengrad & Greene 1977). The common most form is a diffuse polyneuropathy, which damages distal peripheral nerves (mostly of the feet), together with the autonomic nervous system. The disease progress as the duration of diabetes lengthens and is often associated with other long-term diabetic complications (Watkins 1988). The pathogenesis of this complication is still not fully understood. However animal studies have shown the link with a wide range of metabolic abnormalities such as disturbances in the nerve conduction velocity, resistance of nerve impulse conduction, schaemia and altered nerve structure (Greene et al 1990).

Various observations indicate that hyperglycemia, by altering flux through the polyol pathway and decreasing myo-inositol levels within the cell, plays an important role in the development of peripheral neuropathy (Terkildsen & Chrietensen 1968, Graf et al 1979, Stevens et al 1998). Glycation of neuroproteins and ischemia were also thought to contribute to the degenerative neuropathic changes. Autonomic neuropathy can affect both the parasympathetic and sympathetic nervous system through cholinergic and adrenergic mechanisms (Santiago 1986).

1.4 Physiology of insulin secretion and action

Insulin is the most potent anabolic hormone promoting the synthesis and storage of carbohydrates, lipids and proteins, and inhibiting their degradation and release back into the circulation. Insulin regulates glucose homeostasis by inhibiting gluconeogenesis and the breakdown of glycogen in the liver and by stimulating glucose uptake, utilization and storage in insulin-sensitive tissues, such as adipose tissue, skeletal muscle and cardiac muscle (Pessin & Saltiel 2000). In muscle and liver, insulin increases glycogen synthesis (Champe & Harvey 1994).

1.4.1 Mechanism of insulin secretion

Insulin secretion occurs by the process of exocytosis in which the granule membrane fuses with the cell membrane, the membranes are disrupted at the point of fusion, and insulin crystals are discharged to the extracellular space. The process of exocytosis is the rate-limiting step for the physiologic insulin secretion. In this mechanism, cytoplasmic free calcium concentration and two second messenger systems, the cyclic-AMP and phosphoinositide systems are critically important for controlling the secretory steps and for setting the sensitivity of the release sites to the prevailing free calcium level (Daniel & Gerald 1997). The levels of the second messengers are tightly regulated by various secretagogues, such as glucose, other nutrients, hormones, and neurotrasmitters (McClenaghan & Flatt 1999b, Rutter 2001). Such stimulators can be further divided into two categories including initiators and potentiates. The fuel hypothesis has been proposed and is the generally accepted model of glucose induced insulin secretion (Trus et al 1981, Ashcroft & Ashcroft 1992). It is based on the following observations. Firstly, glucose induced insulin secretion is tightly related to glucose utilization and oxidation and blocking glucose phosphorylation or glycolysis abolishes insulin secretion (Sweet et al 1996).

In addition, non-metabolizable sugars, such as 3-O-methylglucose, galactose, and fructose characteristically do not induce insulin secretion whereas metabolizable nutrients such as the amino acid, leucine are potent stimulators of insulin secretion (McClenaghan et al 1996b, McClenaghan et al 1996c, Lindskog et al 1998). As such, fuel metabolism plays a fundamental role in the initiation of insulin secretion. In contrast, the potent insulinotropic actions of other agents, including incretin hormones, require the presence of fuel secretagogues to mediate their actions and are referred to as potentiates of insulin secretion. The potentiation of insulin secretion by these agents is usually mediated by second messengers, such as cAMP, via binding and regulation of specific G protein-coupled receptor pathways.

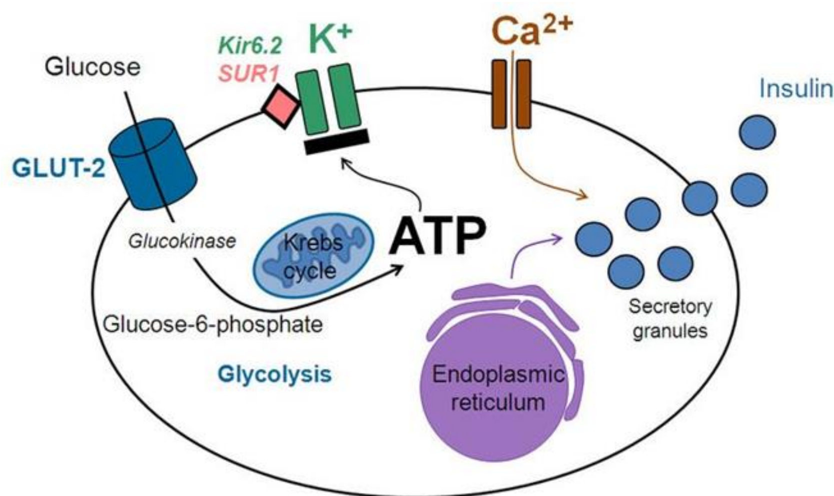

Figure 1.3 Mechanism of insulin secretion

1.4.1.1 ATP-sensitive K+ channels (KATP channels) – membrane depolarization – voltage dependent calcium channel (VDCC) pathway

Glucose is the main stimulator of insulin secretion and utilizes this pathway. Glucose (>5 mM) is transported into pancreatic beta cells (via GLUT2) and metabolized through glycolysis and Krebs cycle inside the mitochondria (Katagiri et al 1994). This process leads to the elevation of the intracellular ATP. The increase of intracellular ATP, results in the increase of ATP/ADP ratio, causes closure of KATP channels and inhibits the efflux of potassium ions (Deeney et al 2000). Under basal glucose levels (0 – 3 mM), the membrane potential of pancreatic beta cells is about –60 to –70 mV (Ashcroft et al 1992). However, with membrane depolarization via the closure of KATP channels, the resting cell membrane will be depolarized (raising to 0 mV from –70 mV) and results in the opening of the voltage-dependent calcium channels (VDCC). The intracellular Ca2+ concentration is increased by the influx of calcium via VDCC. Finally, the mobilization of secretory granules will be triggered and insulin will be discharged by exocytosis (Rotig et al 1996, Rutter 2001).

Activation of certain key components of this pathway can trigger secretion. Firstly, amino acids, such as leucine, and keto acids, can generate intracellular ATP via metabolism resulting in a rise of the ATP/ADP ratio (Meglasson et al 1986). In this way these agents stimulate insulin secretion utilizing essentially the same pathway as glucose. In addition, the oral hypoglycemic agents, such as the sulphonylureas, tolbutamide and glibenclamide, can trigger insulin secretion by closure of KATP

channels as a consequence of binding to the sulphonylurea binding subunit (SUR1) (Ashcroft et al 1992). Moreover, membrane depolarization agents, such as KCl and arginine, have been shown to increase intracellular calcium via opening VDCCs (Herchulz et al 1984, Hermans et al 1987). On the other hand, alanine depolarizes the cell membrane by co-transportation with Na+ which depolarizes the cells and thereby increases intracellular calcium via activation of VDCCs (Yada 1994).

1.4.1.2 KATP channel independent pathway (amplification pathway)

Glucose can stimulate insulin secretion in pancreatic beta cells under conditions where KATP channels are fully opened by KCl and diazoxide (Henquin 2000). Interestingly, a significantly reduced first phase but maintained second phase of glucose induced insulin secretion was observed in SUR knockout mice (Seghers et al 2000), These observations suggest that glucose stimulated insulin secretion is not only via KATP channel–VDCC pathway but also by KATP channel-independent pathways.

1.4.1.3 Potentiation of insulin secretion via regulation of second messengers

(i) <u>cAMP – Protein kinase A pathway:</u>

Cyclic AMP augments glucose-induced insulin secretion through a number of mechanisms including increased opening of voltage-sensitive Ca2+ channels (Kanno et al 1998), calcium-induced Ca2+-release (Kang & Holz 2003), activation of ryanodine receptors in the ER (Islam et al 1998, Holz et al 19990, stimulation of beta cell lipolysis (Yaney et al 2001) and direct effects on exocytosis (Harndahl et al 2002, Hedskov 1980, Wollheim & Sharp 1981, Weidenkeller & Sharp 1983, Supattapone et al 1988, Sculptoreanu et al 1993). Most actions of cyclic AMP in the beta cell seem to be mediated through protein kinase A (PKA)-catalysed phosphorylation events but direct effects of the cyclic nucleotide on exocytosis are partly PKA-independent (Renstrom et al 1997). PKA-independent effects on exocytosis can be mediated by the cyclic AMP-binding protein cAMP-GEFII, interacting with Rim2, a target of the small G-protein Rab3 (Kashima et al 2001).

Furthermore, incretin hormones, such as glucagons-like peptide 1 (GLP-1) and gastric–inhibitory-polypeptide (GIP), can enhance glucose-induced insulin secretion by binding to their own specific stimulatory G protein coupled receptors, thereby increasing intracellular cAMP by activation of adenylate cyclase (Hedeskov 1980,

Wolheim & Sharp 1981). An increase in intracellular cAMP by activation of adenylate cyclase with forskolin has been shown to enhance glucose induced biphasic insulin secretion (Weidenkeller et al 1983). Although it has been accepted that cAMP regulate insulin exocytosis due to protein phosphorylation; nonetheless, cAMP-dependent pathways still remained to be fully characterized.

Cyclic AMP is hydrolysed to its biologically inactive 5′ derivative by cyclic nucleotide phosphodiesterases (PDE1-PDE11) enzymes. Selective inhibition of phosphodiesterases (PDEs) augments insulin secretion by increasing cyclic AMP. Thus PDEs offer a target for developing drugs for the treatment of type 2 diabetes mellitus (Pyne & Furman 2003). IBMX, an inhibitor of cyclic AMP phosphodiesterase, has been shown to augment glucose-induced insulin secretion via increased levels of intracellular cAMP (Sharp 1979). Several selective PDE3 inhibitors (Org 9935, siguazodan, SK&F 94120, ICI118233) augmented glucose-induced insulin secretion from rat and human islets (Shafiee-Nick et al 1995). Org 9935 and siguazodan augmented insulin secretion in the insulin-secreting cell line BRIN-BD11 (Ahmad et al 2000). A novel piperazine hypoglycaemic agent was shown to inhibit PDE3 and PDE4 in islets and augmented insulin secretion (Leibowitz et al 1995).

(ii) Phospholipase C- protein kinase C pathway:

Phospholipase C (PLC) is a key component of activation of the calcium-calmodulin and protein kinase C system (Niwa et al 1998). This activation is via hydrolysis of PtdInsP2 into InsP3 and diacylglycerol (DAG). As a result, IP3 increases intracellular calcium via mobilazation of intracellular calcium stores in ER or microsomes (McClenaghan & Flatt 1999b). Elevation of intracellular calcium is directly associated with insulin exocytosis and along with DAG activates PKC which has been suggested to contribute in KATP channel independent pathways for insulin release (McClenaghan & Flatt 1999b). Neurotransmitters, such as acetylcholine, and the gastrointestinal hormone, cholecystokinin-8 (CCK-8), enhance glucose induced insulin secretion by activation of the PLC-PKC pathway following binding to specific muscarinic and CCK-8 receptors, respectively (Karlsson & Ahren 1991, Tang et al 1995). Direct activation of PKC with the phorbol ester, phorbol 12-myristate 13 acetate (PMA) stimulates insulin secretion (Wolf et al 1989). However, down-

regulation of PKC activity by chronic culture with phorbol esters has little effect on glucose-stimulated insulin secretion (Hii et al 1988).

1.4.2 Mechanism of insulin action

Insulin binds to specific, high-affinity receptors in the cell membrane of most tissues, including liver, muscle, and adipose. This is the first step in a cascade of reactions ultimately leading to a diverse array of biologic actions (Champe & Harvey 1994).

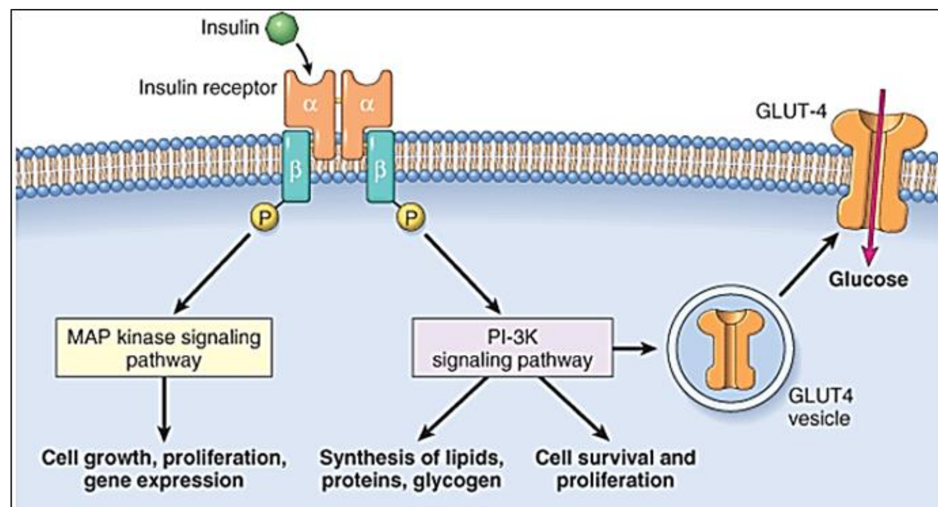

Figure 1.4 Mechanism of insulin action

1.4.2.1 Insulin receptor

The insulin receptor is synthesized as a single polypeptide that is glycosylated and cleaved into α and β subunits, which are then assembled into a tetramer linked by disulfide bonds. A hydrophobic domain in each β subunit spans the plasma membrane. The extracellular α subunit contains the insulin-binding site. The cytosolic domain of the β subunit is a tyrosine kinase, which is activated by insulin (Champe & Harvey 1994).

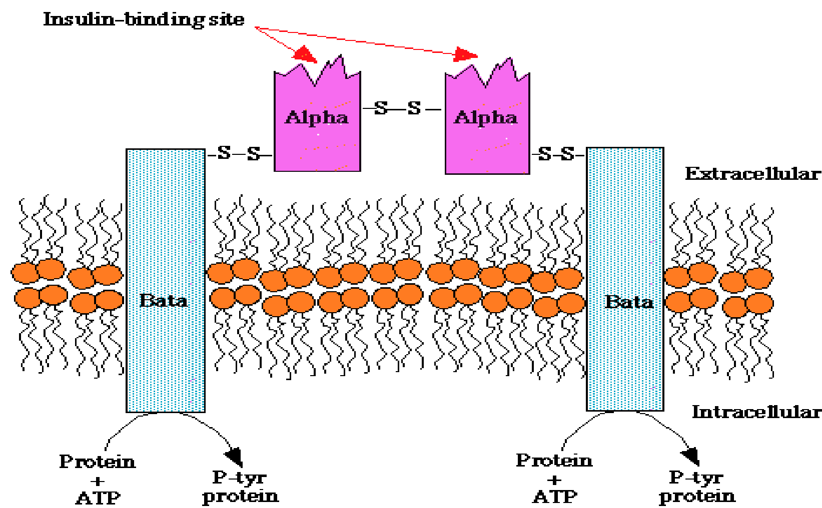

Figure 1.5 Insulin receptor

1.4.2.2 Insulin receptor substrates

The insulin receptor belongs to a subfamily of tyrosine kinases that includes the insulin-like growth factor (IGF)-I receptor and the insulin receptor-related receptor (IRR). These receptors are tetrameric proteins consisting of two α- and two β-glycoprotein subunits (Saltiel & Kahn 2001). Primary substrates of the insulin receptor include the four proteins, insulin receptor substrate (IRS)-1, -2, -3 and -4. The participation of IRS proteins in mediating intracellular signals from the insulin receptor is well documented (Cheatham 2000).

1.4.2.3 Signal transduction

The binding of insulin to the α subunits of the insulin receptor induces conformational changes that are transduced to the β subunits, promoting a rapid autophosphorylation of specific tyrosine residue of each β subunit (Champe & Harvey 1994).

The signaling mechanism involved in the various biologic responses to insulin remain somewhat elusive, but recent progress has shed light on a few pathways that are critical for its regulation of glucose and lipid metabolism (Pessin & Saltiel 2000). The action of insulin is characterized by a diverse variety of effects, including changes in vesicle trafficking, stimulation of protein kinases and phosphatases, promotion of cellular growth and differentiation, and activation, or in some cases, repression of transcription. The diverse mechanisms involve multiple signaling pathways that diverge at or near the receptor (Christian et al 2001). It has also been documented that both phosphoinositide (PI) 3-kinase-independent and -dependent signaling pathways

are a necessary component of insulin-stimulated GLUT4 translocation (Christian et al 2001). Insulin-stimulated activation of PI 3-kinase is a crucial step linking signaling of GLUT4 translocation (Cheatham & Kahn 1995).

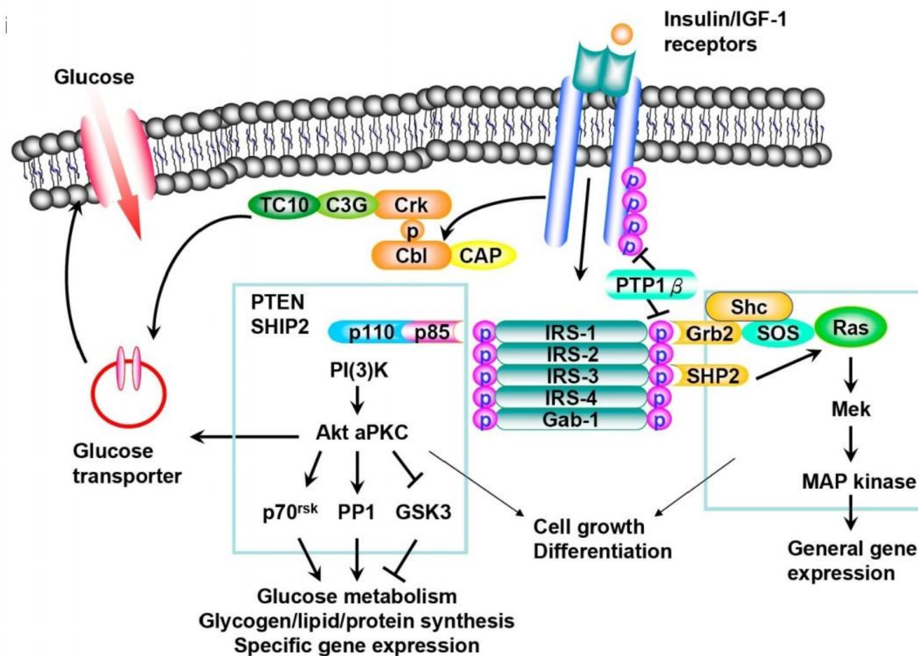

Figure 1.6 Signal transduction in insulin action.

1.4.3 Effects of insulin on glucose uptake

Insulin stimulates glucose uptake in muscle and adipose tissue by translocating intracellular glucose transporter protein-4 (GLUT4) units to the plasma membrane. Basal glucose uptake is mediated primarily by GLUT1 and GLUT3. Any increase in the plasma glucose levels will enhance glucose uptake into peripheral tissues by these transporters (Kruszynska 2003).

1.4.3.1 Glucose transport and GLUT4

Glucose, being hydrophilic, cannot diffuse across the cell membrane. Entry of glucose into tissues from the bloodstream is by a family of facilitative GLUTs, which catalyze (in an energy-independent process) the transport of glucose down its concentration gradient. Seven functional GLUT isoforms (GLUT14 and GLUT8-10) have so far been identified; GLUT5 is a fructose transporter (Kruszynska 2003). However GLUT4 is the only major insulin regulator glucose transporter and its expression is limited to insulin-responsive tissues, namely adipose tissue, skeletal muscle and cardiac muscle. Unlike most of the other GLUTs, which are primarily localized to the

cell surface membrane, GLUT4 sequestered in specialized vesicles are predominantly located in the cytosol under basal conditions.

Insulin stimulates glucose transport in muscle and adipocytes primarily by causing the translocation of vesicles containing GLUT4 to the plasma membrane. They function as pores allowing glucose entry (Kruszynska 2003). This process is reversible when circulating insulin levels fall, GLUT4 proteins are removed from the plasma membrane by endocytosis and are recycled back to their vesicular storage compartment. In the long-term, insulin plays a role in maintaining normal levels of the GLUT4 protein in muscle and fat (Kruszynska 2003). However, the exact mechanisms of these processes are unknown. The docking and fusion of the GLUT4 vesicle at the plasma membrane may be subjected to regulation by insulin (Saltiel & Kahn 2001). Furthermore, the GLUT4 compartment is enriched in v-SNARE protein VAMP2 (Christian et al 2001). Again the plasma membrane target for the GLUT4 vesicle is the t-SNARE, syntaxin 4 (Syn4) (Christian et al 2001). The v-SNARE protein VAMP2 physically interacts with its t-SNARE counterpart in the plasma membrane during GLUT4 vesicles docking and fusion (Saltiel & Kahn 2001). Several lines of evidence have suggested that insulin specifically stimulates the translocation of the GLUT4 from VAMP2-containing compartments (Pessin & Saltiel 2000).

The intravenous administration of insulin thus causes an immediate decrease in the concentration of blood glucose (Champe & Harvey 1994). The β-cells specialization for regulating blood glucose levels in the normal range (roughly 90 mg/dl or 5 mM).

1.5 Diagnosis of Diabetes

Doctors can determine whether a patient has a normal metabolism, prediabetes or diabetes in one of three different ways - there are three possible tests:

- ❖ The A1C test
  - at least 6.5% means diabetes
  - between 5.7% and 5.99% means prediabetes
  - less than 5.7% means normal
- ❖ The FPG (fasting plasma glucose) test
  - at least 126 mg/dl means diabetes
  - between 100 mg/dl and 125.99 mg/dl means prediabetes
  - less than 100 mg/dl means normal

An abnormal reading following the FPG means the patient has impaired fasting glucose (IFG)

- ❖ The OGTT (oral glucose tolerance test)
- at least 200 mg/dl means diabetes
- between 140 and 199.9 mg/dl means prediabetes
- less than 140 mg/dl means normal

An abnormal reading following the OGTT means the patient has impaired glucose tolerance (IGT)

1.6 Current therapies for diabetes mellitus

Since diabetes conditions encompass a multiplicity of endocrine and metabolic disturbance, it is necessary to consider a wide range of pharmacological approaches to manage these. These may be required individually or in combinations to treat different features of the disease process. Ideal treatments will target the fundamental causes of insulin resistance, defective cell function, and loss of cell mass, and reinstate near-normal glucose homeostasis (Bailey & Flatt 1995).

Currently glycaemic control is achieved by dietary manipulation, oral hypoglycamics agents (for example sulphonylurase or biguanides) or insulin injections. Approximately 75% of diabetic patients in UK achieve glycaemic control without exogenous insulin treatment (Campbell 1990).

1.6.1 Diet

The regulation of food intake is central to the treatment of diabetes mellitus and various dietary regimes have been considered to assist in the control of hyperglycemia. The control of diet should be the first treatment offered to type 2 patients before drugs are considered. The main goal of nutritional management is to correct obesity as weight loss will improve glucose control (Savage et al 1979, Knowler et al 1991, Ohneda et al 1995), lower blood pressure and lipid concentration, all of which may help in preventing or diminishing long term complications (Henry & Griver 1998). Various dietary regimes have been considered to assist in the control of hyperglycemia. However, in most cases the dietary recommendations for type 2 diabetic patients are identical to those for the general population (British Diabetic Association 1981). Calorie restriction in the overweight and obese, with the emphasis on low-fat, high-carbohydrate and high-fibre is recommended (Simpson et al 1979b)

1.6.2 Insulin

The discovery of insulin by Banting, Best and co-workers in 1922 dramatically improved the prospects of individuals with diabetes mellitus. As type1 is characterized by insulin insufficiency caused by partial or total destruction of insulin releasing pancreatic β cells (Eisenbarth 1986, Rossini et al 1993), patients with this condition required exogenous insulin replacement for treatment. The last decade has seen increasing refinement of exogenous insulin delivery in type 1 diabetes. In an attempt to reinstate normoglycemia, efforts have been made to match exogenous insulin delivery with the 24 h glucose profile. These have led to the introduction of continuous subcutaneous insulin infusion (CSII) and practice of multiple (4/d) subcutaneous insulin injections (Schiffrin & Belmonte 1982). Although intensive insulin regimes have unquestionably improved the control of diabetes they have not consistently achieved normoglycemia in clinical practice. In certain cases of type 2, exogenous insulin is required to achieve glycemic control.

A number of insulin preparations have been developed since its discovery based on the duration of action. Although various procedures were attempted to prolong the duration of insulin action (Dorzbach and Muller 1971), the two forms endured; the production of neutral protamine hagedorn (NPH) insulin, where absorption is retarded by protamine and development of the lente series by the use of zinc-insulin complexes (Galloway & Chance 1994, Skyler 1998). In general, there are three types of insulin, characterized by the rate which they are metabolized by the body. They are rapid acting insulins, intermediate acting insulins and long acting insulins. (P. Sonksen ,J. Sonksen,2000)

Examples of rapid acting insulin's include

- Regular insulin (Humulin R, Novolin R)
- Insulin lispro (Humalog)
- Insulin aspart (Novolog)
- Insulin glulisine (Apidra)
- Prompt insulin zinc (Semilente, Slightly slower acting)

Examples of intermediate acting insulins include

- Isophane insulin, neutral protamine Hagedorn (NPH) (Humulin N, Novolin N)
- Insulin zinc (Lente)

Examples of long acting insulins include

- Extended insulin zinc insulin (Ultralente)
- Insulin glargine (Lantus)
- Insulin detemir (Levemir)

1.6.2.1 Structure of Insulin

Insulin is composed of two peptide chains referred to as the A chain and B chain. A and B chains are linked together by two disulfide bonds, and an additional disulfide is formed within the A chain. In most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids.

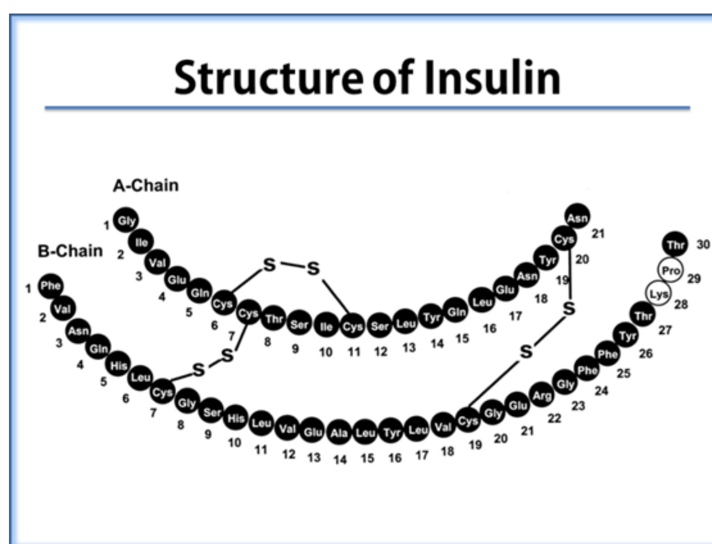

Figure1.7 Structure of Insulin

1.6.3 Anti-diabetic drugs

Those patients who fail to achieve glycemic control through dietary intervention measures require oral hypoglycemic agents. Approximately 50% of type 2 patients in the UK are treated with oral hypoglycemic agents (Campbell 1990). Although there are new oral hypoglycemic agents on the horizon, the choice at the present is primarily between sulphonylureas and biguanide (metformin).

Sulphonylureas, developed after initial observations of sulphonamide in patients with typhoid fever (Janbon et al 1942), have been the foundation of antidiabetic therapy for many years. The various sulphonylureas differ in potency, pharmacokinetic properties and side effects (Ferner & Chaplin 1987, Lebovitz 1990). The sulphonyurea drugs

have direct and immediate stimulating effects on the cell (Pfiefer et al 1984, Gorus et al 1988, Panten 1989, Henquin 1990) mediated via the inhibition of KATP channels in the β cell (Henquin 1988, Henquin 1990). The potentiation of the stimulatory effect of the amino acids alanine and leucine by sulphonylureas through enhanced β cell recognition has been documented (Fajans 1967). Some authors claim an extrapancreatic action for sulphonylureas on the insulin receptor (Beck-Neilson et al 1984) and at the post-receptor level (Mandarino & Gerich 1984) which require the presence of endogenous insulin. Recently promotion of insulin exocytosis was demonstrated and was shown to be partly independent of KATP channels and dependent on protein kinase C (Eliasson 1996).

Repaglinide has recently been introduced in the US. The reports of trials in patients with type 2 diabetes have demonstrated that it promptly increase insulin concentrations and reduce postprandial hyperglycemia without causing interprandial glucose concentration to fall below the normal range (Graul & Castener 1996).

Metformin, the major biguanide in clinical use, was used before the characteristic insulin resistance was discovered. In contrast to sulphonylurea drug, metformin enhances the extra pancreatic actions of insulin in insulin resistance and hyperglycemic status but has no effect on glycaemia of type 1 diabetic individuals. Metformin does not change insulin-receptor binding (Bailey 1988) or alter phosphorylation and kinase activity of insulin receptors after insulin-mediated glucose uptake in vitro with metformin indicating a post-receptor site of action (Jacobs et al 1986). In addition to insulin-mediated glucose disposal, metformin and related biguanides decrease hepatic glucose output and increase glucose utilization by the small intestine. Some of these effects are independent of insulin but in patients devoid of insulin these drugs are ineffective. The glucose-lowering efficacy of sulphonylureas and metformin in type 2 diabetes are reviewed elsewhere (Bailey & Nattrass 1988, Bailey & Day 1989, Henquin 1990, Lebovitz 1990, Bailey 1991).

Table 1.2 Drugs used as Anti-diabetic agent

Antidiabetic agent	Recommended dosage and/or administration
Insulin	400 IU per vial - 40 IU per day (mean value)
Gliclazide (Diamicron)	80 mg/tablet - 1 to 4 tablets per day
Glibenclamide (Daonil) or Glyburide (Micronase, Glynase, Diabeta)	5 mg/tablet - 1 to 3 tablets per day (Glibenclamide); 1.25 to 6 mg/tablet - 1 to 2 tablets per day (Glyburide)
Glipizide (Glucotrol, Glibenese)	5 mg/tablet - 1 to 4 tablets per day
Glimepiride (Amaryl, Amarel)	1 to 4 mg/tablet - 6 mg per day maximum
Chlorpropamide (Diabinese)	250 mg/tablet - 125 to 1000 mg per day per day
Tolbutamide	500 mg/tablet - 1 to 4 tablets per day
Repaglinide (Prandin)	0.5 to 16 mg per day

Troglitazone, rosiglitazone and pioglitazone (thiazolidinediones derivative), are more recently discovered antidiabetic drugs that improve action of insulin through different cellular mechanisms (Cusi & DeFronzo 1998, Saleh et al 1999).

Acarbose, an β-glucosidase enzyme inhibitor, is a new class of antidiabetic drug that reduces postprandial peak of glucose level, by inhibiting the breakdown of oligosaccharides and disaccharides in the proximal half of the small intestine so that they must be digested throughout the length of the small intestine (Puls 1996, Puls 1980, Caspary 1978). The existing classes of antidiabetic drugs with modes of action are summarized in Table 1.2.

There are also many other promising agents, such as gluconeogenesis inhibitors, amylin, glucagon-like-peptide 1 (GLP-1) and analogues (Druker 2001), gastric inhibitory polypeptide (GIP) and analogues (Gault et al 2003, Meier et al 2002), DPP IV inhibitors (Scharpe & De-Meester 2001), and insulin mimic agents (Bailey & Flatt 1995), considered as potential drugs for the future treatment of diabetes.

Drug List – Oral Antidiabetics

Insulin secretagogues		Biguanides	Thiazolidinediones	α-glucosidase inhibitors
Sulfonylureas	Meglitinides			
Tolbutamide* Chloroprapamide* Glyburide** Glipizide** Glimepiride** (amaryl)	Repaglinide	Metformin	Rosiglitazone	Acarbose Miglitol
* 1st generation sulfonylureas, ** 2nd generation sulfonylureas				

(GLIP ih zyd), (glye-MEP-ir-ide), (met FOR min), (thy-a-zoll-i-deen-dye-ones)

Figure 1.8 Oral Anti-diabetics

1.7 Medicinal plant as anti-diabetic

Among the most common chronic diseases in the world, Diabetes mellitus (DM) is an extremely studied and widely manifested multi-factorial disease which deliberately requires multi-modal therapeutic strategies. It has an age-old history of being recognized and even symptomized in various cultures of the world majorly as glycosuria (sweet urine). Hence, the treatment strategies for DM have been in the process of development and documentation since a long time in traditional medicine systems. Back then the nature of drug used to be mostly unorganized and crude. The major difference now in the modern era is that the treatment strategies basically concentrate on identifying, isolating, modifying or searching alternatives of the lead compounds and exact active principles which attribute to the desired therapeutic nature of the plant. The aim of this paper is to acknowledge the various treatment methods available for Diabetes mellitus and to review the Traditional Indian herbs and plants which are most efficiently, safely and widely accepted medicament for DM and source of future lead compounds and family-wise segregation of these plants. This review is in total compliance with the strong and effective traditional medicinal systems of India.

Diabetes mellitus is a global metabolic epidemic affecting essential biochemical activities in almost every age group. Diabetes mellitus is not a single disease but rather a group of metabolic disorders. Hyperglycemia in diabetes results from defect in insulin secretion and or insulin action. Conventionally insulin dependent diabetes mellitus is treated with exogenous insulin, and non-insulin dependent diabetes mellitus is treated with synthetic oral hypoglycemic agents like sulphonyl urea's and

biguanides. Synthetic oral drugs produce adverse health effects. Different medicinal systems are using the active plant constituent which discovered as natural hypoglycemic medicine came from virtue of traditional knowledge. Herbal drugs are considered free from side effects than synthetic one.

A large diversity of animal models has been developed to better understand the pathogenesis of diabetes mellitus and new drugs introduced in market to treat this disease. This review also studied the animal model used in testing of drug. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. The active principal present in medicinal plants have been reported to possess pancreatic beta cells regenerating, insulin releasing and fighting the problem of insulin resistance. The ethanobotonical information reports about 800 plants that may possess antidiabetic potential and more than 1200 species of plants have been screened for activity on the basis of ethanopharmacology.

In India indigenous remedies have been used for treatment of diabetes since the time of charaka and sushruta. The World Health Organization has recommended the evaluation of traditional plant treatments for the diabetes. Diabetes mellitus can be induced by pharmacological, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals (Ayurhelp.com, 2017).

1.7.1 Traditional herbal anti-diabetics

It is now internationally accepted and acknowledged that traditional medicines systems of India and other ancient origins report, advocate and justify the significance of floral biodiversity as an effective and reliable treatment strategy of hyperglycemia and related malfunctions.

Several disadvantages associated with insulin and synthetic drugs and their failure to divert the course of diabetic complications have opened up tremendous horizons for searching possibilities in complementary and alternative medicine (CAM) for diabetes as well as many other chronic diseases. Plants, herbs and their derivatives owing to their wide spectrum of active principles representing numerous chemical compounds hold promising potentials for their consistent usages in the treatment of Diabetes. According to WHO, 21,000 plants around the globe have been reported for medicinal uses. India is posted to have an enormous medicinal flora of some 25,000 species, out of these 150 species are commercially exploited for medicinal extractions or drug

formulation. There are about 800 plants species reported having the probability of possessing antidiabetic potentials in the ethnobotanical surveys. The antidiabetic effects of the plants are attributed to the wide range of chemicals and secondary metabolites. Reports have essayed approximately 200 pure compounds from plant sources to show blood glucose lowering effect. These compounds range vividly in chemical nature like alkaloids, carbohydrates, glycosides, flavonoids, steroids, terpenoid, triterpenoid, peptides and amino acids, lipids, phenolics, glycopeptides, and iridoids. (Ayurhelp.com, 2017)

1.8 *Asteracantha longifolia*

Asteracantha longifolia is a source of the ayurvedic drug, 'Kokilaaksha' and the Unani drug, Talmakhana. The seeds are acrid, bitter, aphrodisiac, tonic, sedative, used for diseases of the blood. The plant is known to possess antitumor, hypoglycemic, aphrodisiac, antibacterial, free radical scavenging and lipid peroxidation and hematopoietic activity. It contains lupeol, stigmasterol, butelin, fatty acids, and alkaloids. The present review article is focused on phytochemical, pharmacological and other important aspects of Talmakhana. (Chauhan and Dixit, 2017)

It is a spiny, stout, annual herb, common in water logged places. Leaves subsessile, oblong-lanceloate or linear lanceolate, spines yellowish brown, 2-3 cm long, Flower yellowish brown, fruit two celled, linear oblong, compressed about 8 cm long, pointed, 4-8 seeded. Seed ovate, flat or compressed, 0.2-0.25 cm long and 0.1-0.15 cm wide, hairy but appearing smooth; when soaked in water immediately get coated with mucilage, light brown: taste slightly bitter and odor not distinct (The Ayurvedic Pharmacopeia of India, 1999).

Figure 1.9 *Asteracantha longifolia*

1.8.1 Traditional uses

The urinary infections,edema and gout. It is classified in ayurvedic system as seethaveeryam, mathuravipaka and used for the treatment of diabetes, dysentery etc (Nadkarni, 1978; Chopra et al., 1986). Whole plant, roots, seeds, and ashes of the plant are extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain,

Figure 1.10 Leaf powder of *Asteracantha longifolia*

1.8.2 Quantitative standards

Foreign matter: Not more than 2.0 percent. Ash: Not more than 8.5 percent. Acid-insoluble ash: Not more than 3.5 percent. Ethanol soluble extractive: Not more than 13.0 percent. Water soluble extractive: Not more than 17.0 percent (Gupta et al., 2006).

1.8.3 Scientific Classification

Kingdom: Plantae

Division: Angiospermae

Class: Equisetopsida C. Agardh

Order: Personales

Family: Acanthaceae

Genus: *Asteracantha*

Species: *Asteracantha longifolia*

1.8.4 Chemical constituents

The plant gave lupeol, stigmasterol and hydrocarbons, the seed gave sterols and the flowers have apigenin glucuronide.

Seeds of this plant are very good aphrodisiac. Ayurveda Acharya's recommend this herb in male infertility. The seeds effectively increase sperm count and sperm motility. Hence the conditions like low sperm count can be improved by use of this herb. The seeds of Kokilaksha are best herbal ayurvedic remedy for Erectile Dysfunction. The erectile dysfunction capsule Rejuzoa from Moolika Ayurveda contains this herb.

Researches have shown that this plant has hypoglycemic activity and improves blood sugar level in diabetic patients. Hence Rejuzoa Capsules which contain kokilaksha are recommended in Vajikarana Therapy for diabetic Patients. It has dual benefits i.e. it controls blood sugar level and also helps to rectify erectile dysfunction.

This plant has very good diuretic properties. It increases urine output. Due to this property Kokilaksha is used in conditions like urinary calculi and cystitis. (Chauhan and Dixit, 2017)

1.8.5 Phytochemical studies

The phytochemical investigation of the *A. longifolia* as carried out so far contains various compounds with varying structural patterns. *A. longifolia* seed oil pale yellow in color about 23% contain about 72% of linoleic, 10% of oleic, 12% of stearic, and 6% of palmitic and myristic acids. Mineral elements Mn, Mg, Zn, Ca, Fe, Ni, Cr, Na, K and Al were found in the *A. longifolia* determined by using Flame photometer, Atomic Absorption Spectrometer and Inductively Coupled Plasma. Plant also contains

minerals Fe, Cu, Co. Root contain stimagsterol. Aerial parts of *A. longifolia* have been reported to contain lupeol, stigmasterol and butelin while the seeds of the plant are reported to contain mainly fatty acids. Petroleum ether extract of *A. longifolia* root found lupeol and lupenone. Misra et al (2001) isolated the two aliphatic esters (25-oxo-hentriacontanyl acetate, and methyl 8-n-hexyltetracosanoate, and betulin from the aerial parts of *A. longifolia*. The HPTLC estimation of lupeol and sitosterol in various part like root, leaves, seeds and stems was reported in solvent system toluene:ethylacetate:methanol 15:3:1.5 (% v/v). The whole plant contains lupeol, stigmasterol, an isoflavone glycoside, an alkaloid and small quantities of uncharacterized bases. From the seeds isolation of asterol I, II, III, and IV, asteracanthine and asteracanthicine have been reported (Basu & Rakhit, 1957a, 1957b). Flowers contain apigenin 7-*O*-glucuronide. Also, amino acids histidine, lysine and phenyl-alanine have been detected in the seeds. From the plant collected from Saharanpur, lupeol, betulin and stigmasterol isolated; betulin was found to be absent in aerial parts and stigmasterol in roots.

1.8.6 Anti-diabetic (hypoglycemic) effects

Muthulingam investigated the effect of leaf extract of *Asteracantha longifolia* on diabetic rats. It appears that *Asteracantha longifolia* increased insulin secretion which brought glucose back to normal levels. The antidiabetic effect of leaf extracts of *Asteracantha longifolia* may be due to increased release of insulin from the existing beta-cells of pancreas similar to that observed after glibenclamide (antidiabetic drug) administration.

1.8.7 Liver damage protection effects of *Asteracantha Longifolia* extract

Many animal studies are suggesting that seed as well as root of *Asteracantha longifolia* extract may possess liver damage protection effects.

1.8.8 Pharmacognostic studies

Plants having spines, having perennial root stocks; bluish-purple 2-lipped flower; leaves - sessile, multi-chambered thick-walled sclerotic cell, among the epidermal cells of midrib, large flat bunch-shaped calcium carbonate crystals and needle-shaped calcium oxalate crystals in the epidermal cells of lamina and in the cortical cells of

midrib respectively; stomata-caryophyllaceous; stomatal index on upper surface: 23.46±47); lower surface: 27.44±52); palisade ratio: 10.23±19) and vein islet number: 25.8±69).

1.8.9 Tissue culture

High Plant regeneration frequency in *A. longifolia* was achieved from leaf explant implanted on MS basal medium supplemented with NAA (0.5 mg/L) + BA (2.0 mg/L) through intervening callus phase. Protein and total soluble sugar contents were maximum during organogenesis and multiple shoot induction phase compared with non-organogenic callus and root induction phase. Esterase and catalase activities were maximum during organogenic differentiation, while activities were minimum at non-differentiated callus stages. Peroxidase activities were higher during rhizogenesis whereas acid phosphatase activities were high during organogenesis and declined during rhizogenesis (Panigrahi et al., 2007).

1.8.10 Medicinal activity

The medicinal activities are given below-

1.8.10.1 Hematopoietic activity

Petroleum ether extract of root from A. longifolia increases WBC count significantly (Mazumdar et al., 1996). Ethanolic extract (100 and 200 mg/kg) of the aerial parts of H. spinosa significantly increased the hemoglobin, hematocrit, RBC and total WBC, as compared with vehicle treated control rat. In anemic male albino rats, the extract significantly increased hemoglobin, hematocrit and RBC count (Gomes et al., 2001). Petroleum ether and chloroform extract of leaves show haematopoietic activity as it significantly increases erythrocyte count, leukocyte count, and hemoglobin count (Pawar et al 2006).

1.8.10.2 Hypoglycemic activity

Ethanolic extract (Al Eth) of aerial parts of A. longifolia (100 and 250 mg/kg body weight) when administered to rats for three weeks showed significant reduction in blood glucose level. There is also decrease in thiobarbituric acid reactive substances (TBARS) and hydro peroxide in both liver and kidney. The treatment with Al Eth significantly increased the glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) in the drug-treated group, which

is comparable to the control group. Al Eth treated rats also showed decreased lipid peroxidation that is associated with increased activity of superoxide dismutase (SOD) and catalase (Vijayakumar et al., 2006). Fernando et al. (1991) investigated the effects of hot water extracts of A. longifolia whole plant material on the glucose tolerance of normal human subjects and maturity onset diabetic patients. Administration of aqueous extract of A. longifolia to rats prior to glucose loading showed hypoglycemic action as it was significant increase in the glycogen content of liver and muscle and a significant increase in triacylglycerol content of adipose tissue in comparison with control rats. However, the plant extract had no effect on the gluconeogenic capacity of the kidney or intestinal glucose absorption (Fernando et al., 1998, Fernando et al. 1989).

1.8.10.3 Antioxidant Activity

Ethanolic extract of leaves contain phenolic and flavonoid shows promising antioxidant activity (Sawadogo et al., 2006). Aqueous extract of leaves of A. longifolia shows potent antioxidant activity in various in vitro model (Dasgupta & De, 2007).

1.8.10.4 Aphrodisiac Activity

The ethanolic extract of seeds shows androgenic as well as improvement of sexual behavior of rat in dose dependent manner, it also improve the histoarchitecture of testis and increase the concentration of sperm count in epididymis and also increase testosterone level (Chauhan et al., 2009, 2010).

1.8.10.5 Liver protecting activity

The aqueous extract of whole plant and root of A. longifolia possesses hepatoprotective and antioxidative properties against CCl_4- and paracetamol-induced hepatotoxicities (Hewawasam et al., 2003, Usha et al., 2007). Petroleum ether extract of A. longifolia affects liver and kidney functions and metabolism and hematological parameters in high doses (40 and 80 mg/kg) whereas low weekly dose (20 mg/kg) and low and moderate daily/therapeutic dose (2 and 4 mg/kg) does not exhibit any appreciable toxic action (Mazumdar et al., 1996). Methanolic extracts of the seeds show hepatoprotective activity against paracetamol and thioacetamideintoxification in rats (Singh &Handa, 1999). Ahmed et al. (2001) studied seeds against chemically

induced hepatocarcinogensis in Wistar rats. Methanol extract of seed showing antitumor promoting potential inhibit hepatocarcinogenesis in Wistar rats, increase GPx and CAT, ODC. Shivashangari et al. (2004) studied the protective efficacy of A. longifolia on acetaminophen-induced liver damage in rats. Shanmugasundaram & Venkataraman (2006) studied the aqueous extract of the roots for hepatoprotective in CCl4-induced liver toxicity in rats and in vitro antioxidant activity using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. Shailajan et al. (2005) showed the whole plant slurry of A. longifolia was hepatoprotective activity against CCl4 induced liver dysfunction in rats. Later they also reported that the slurry, aqueous extract and ethanolic extract of whole plant powder showed hepatoprotective effect against galactosamine induced hepatotoxicity.

1.8.10.6 Miscellaneous activity

Petroleum ether extract of root potentiated the sedative-hypnotic action of chlorpromazine, diazepam, pentobarbitone, chlordiazepoxide and protected against strychine-induced convulsions. Preliminary study shows it possess diuretic activity. Aqueous extract of root and leaves cure patient suffering from dropsy .Ethanolic extract of whole plant showed diuretic effects in rats.

1.8.11 Non medicinal use

Talmakhana is used in the preparation of fuchka in Bangladesh. It is a popular snack in Bangladesh.

Chapter 2

MATERIALS & METHODS

2.1 Plant Material

Plant sample of ***Asteracantha longifolia*** were used for the experiment. They were processed in the laboratory.

2.1.1 Collection of plant

The Plant sample ***Asteracantha longifolia*** was collected and washed with water several times.

2.1.2 Drying and grinding

The collected plant sample were washed with water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2 days. The fully dried leaves was then grinded to a powdered form and stored in there refrigerator at +4°C for a few days.

2.1.3 Extraction (Ethanol extraction)

300 gm of powered material was taken in a clean, flat bottomed glass container and soaked in 800 ml of 80% ethanol, sealed and kept for a period of 2 days with occasional shaking and stirring. It was then filtered first by cotton material and twice through whatman filter paper to obtain a finer filtrate. The filtrate (Ethanol extract) obtained was evaporated by Rotary evaporator (Eyela n 1000, Tokyo Rikaki Kai Co. Ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65◦C temperature. The separated filtrate was found to be a precipitate of dark green color and the gum my concentrate was designated as the crude ethanol extract. It was then dried in the freeze drier and preserved at +4°C for two weeks.

Figure 2.1 Filtration of the sample

Figure 2.2 Evaporation of the sample

2.1.4 Extraction Procedure

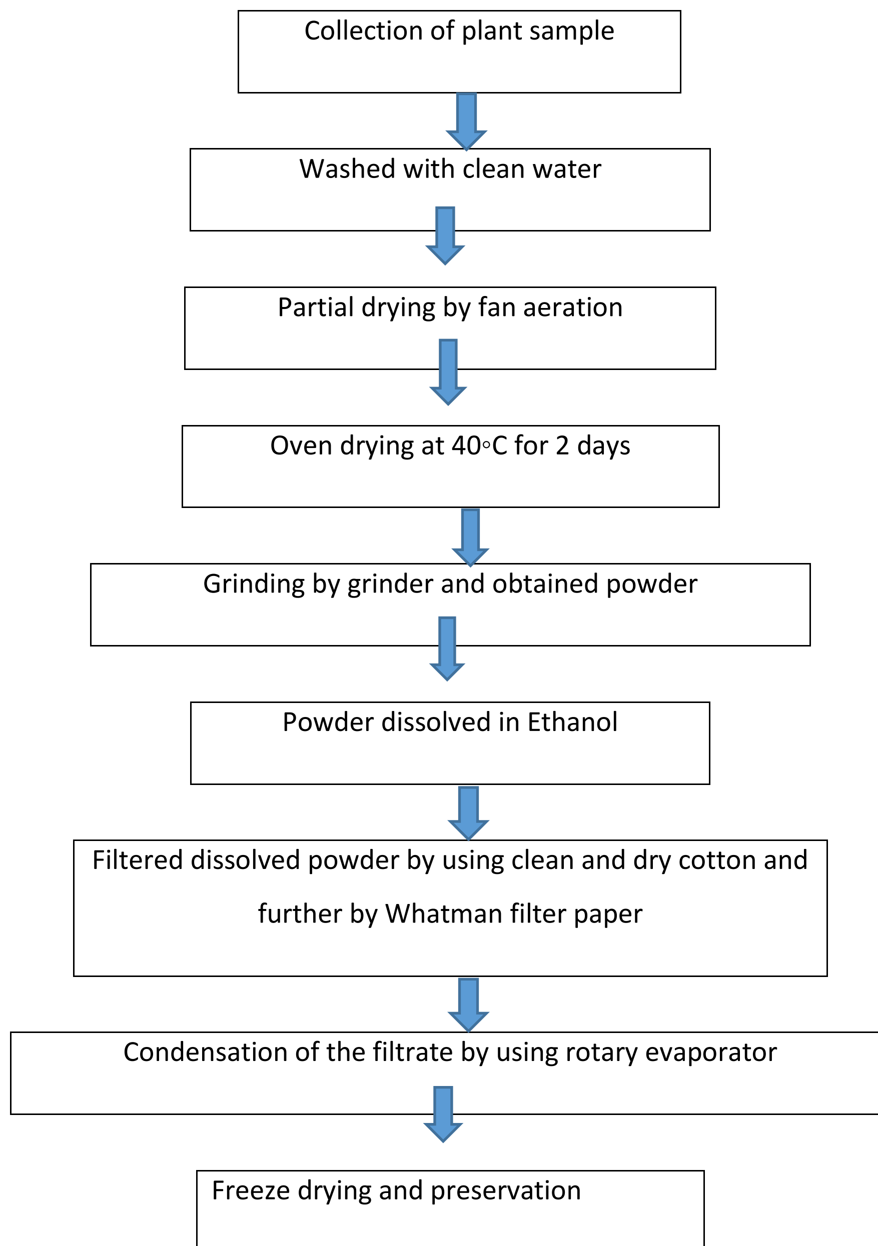

Figure 2.3 General Plant Extraction Procedure

2.2 Experimental animals

Long Evans rats (male and female), weighing 80-200g of either sex are bred in ICDDR, B and grown in the animal house of the Department of Pharmacy, East West University. All the animals acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2°C, and 12 hours light dark cycle). The animals were fed with standard diet from ICDDR, B and had free access to filtered water (M.K. Sharif et al, 2011).

Figure 2.4 Experimental animals

2.2.1 Biomedical research

Rats have a prevalence within biomedical research second only to humans and they share 90% of the genome with humans. Almost all disease-linked human genes we currently know of have equivalent genes within the rat genome, making them a suitable research tool.

Figure 2.5 Rat handling during procedure.

Rats were the first mammalian species specifically domesticated to be used in the laboratory.

Records dating back to the 1850s show these animals were derived from those bred by rat fanciers who collected them for their unique coat colors and behavioral characteristics.

The success of the rat in research today has been linked to the Wistar Institute in America and their development of the Wistar albino strain. There are currently 117 albino strains of the laboratory rat, all of which can be traced genetically back to the one rat, likely to have arisen as a mutation from a hooded (piebald) rat strain. Since their development as a laboratory species, rats have been used to answer a wide range of basic science questions ranging from physiology,immunology, pharmacology, toxicology, nutrition, behavior and learning.

2.3 Screening for the possible inhibition of carbohydrate absorption by plant material

2.3.1 Chemicals and reagents

Normal saline, 2N H_2SO_4, 1N NaOH, Sucrose (2.5g/Kg body weight of rat in 5ml deionized water)

Drug: 100mg/Kg body weight of rat

Kits: Glucose kit was used for the determination of Glucose.

2.3.2 Procedure

Rats were fasted for 20hours before experiment. Sucrose (2.5g/Kg/5ml, average 443 mg) with or without extract (effective dose of hypoglycemic effect). Each segment was washed out with ice-cold saline (10ml), acidified with H_2SO_4 (2ml) and centrifuged at 3000rpm for 10minutes. The supernatant thus obtained was boiled for 2hours to hydrolyze the Sucrose and then neutralized with NaOH (approximately 2.5ml). The blood glucose level and the amount of Glucose liberated from residual Sucrose in the gastrointestinal tract were measured by Glucose Oxidase (GOD-PAD) Method. Then the gastrointestinal sucrose content was calculated from the amount of liberated glucose.

2.3.3 Steps of the experiment

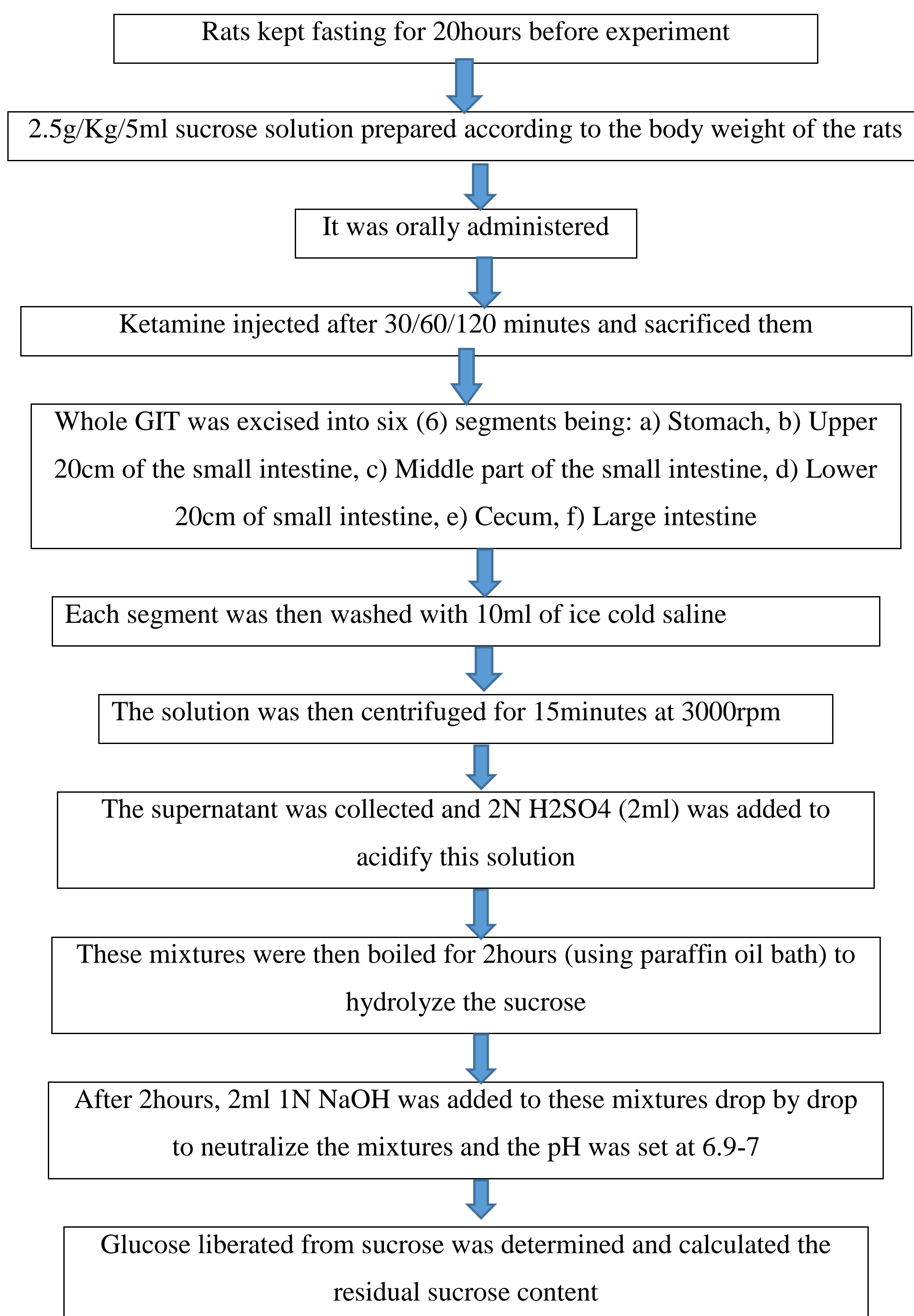

Figure 2.6 Flowchart of the experiment

2.4 Assessment of the effect of plant materials on intestinal disaccharidase activity

2.4.1 Assessment of conditions

All rats were fasted overnight (12hours) before being tested but still allowed free access to distilled water. Extract is administered orally to experiment group and water to control group.

2.4.2 Mucosa/Tissue Collection

After one hour of drug administration, rats are anesthetized with pentobarbital-Na/ether, the entire length of the small intestine (from pylorus to ileocaecal junction) is carefully removed from the pylorus to the ileocaecal junction. The lumen of the intestine is washed out with 50ml of ice cold saline. Intestine is then placed on ice-cold glass plates over ice and cut longitudinally. The mucosa is isolated bt scrapping with glass microscope slides and homogenized with 10ml of saline for 20seconds at medium speed in a Heidolph Diax 600 homogenizer.

2.4.3 Enzyme activities

Disaccharidase activity is assessed using the Dahlqvist method with modifications. Twenty (20) µl of mucosal homogenate were added in duplicate to 40 mM sucrose and incubated at 37◦C for 60minutes. The glucose converted from sucrose and total protein (using Lowry's methods) in the homogenate are measured. Disaccharidase activity will be calculated by glucose concentration converted from sucrose as µmol-mg glucose/protein/h.

2.4.4 Steps of the experiment:

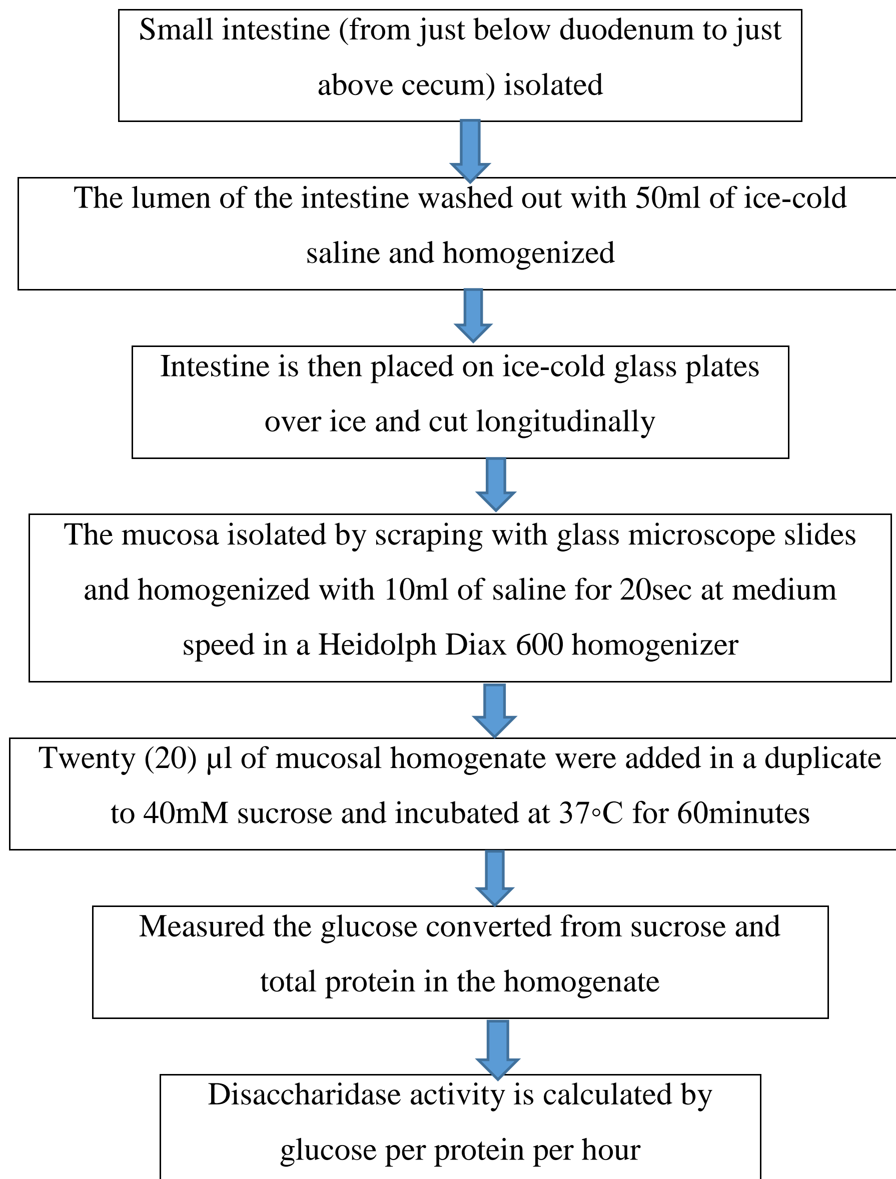

Figure 2.7 Flowchart of the experiment

Chapter 3

RESULTS

3.1 Effect of A. longifolia on unabsorbed sucrose content (mg) in the intestinal tract after sucrose load in six segment test.

Table 3.1.1 (sucrose content in Stomach)								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	58.3	8.5	35.3	5.9	9.1	1.4	1.1	0.3
Asteracantha longifolia	53.8	9.5	40.2	7.3	11.1	2.1	1.4	0.4

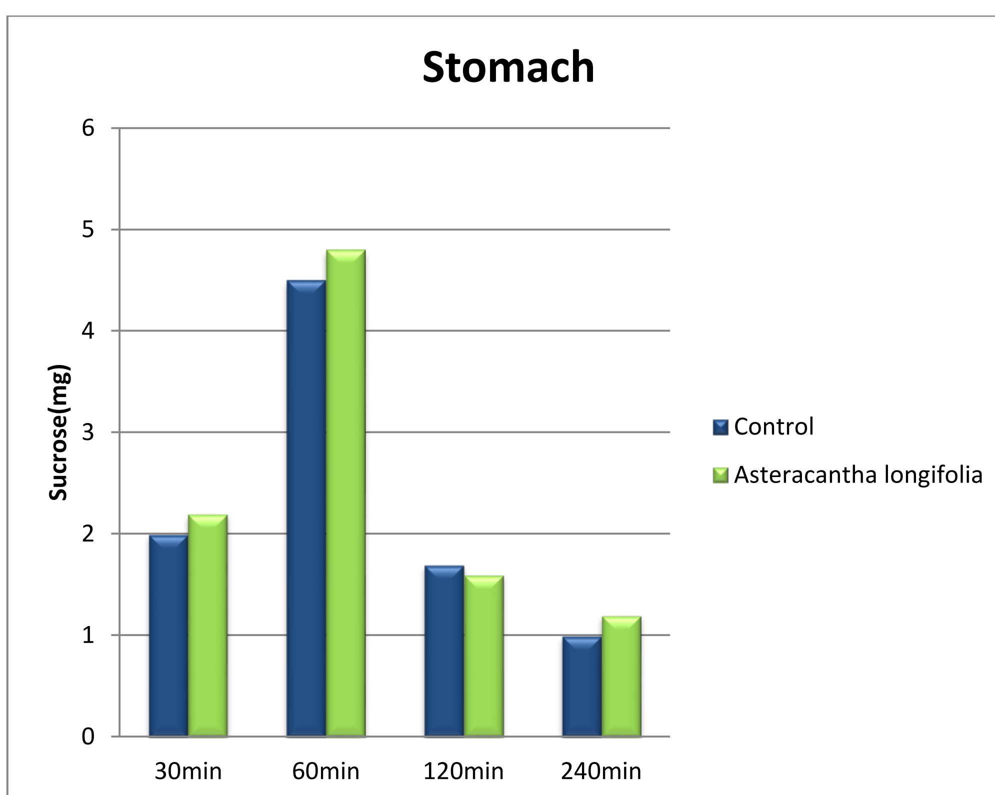

Figure 3.1 Sucrose in Stomach.

Table 3.1.2 (sucrose content in Upper 20cm of intestine)								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	13.9	2.3	10.2	1.8	4.9	1.1	1.7	0.4
Asteracantha longifolia	14.2	2.1	10.3	1.9	5.6	0.9	2.1	0.6

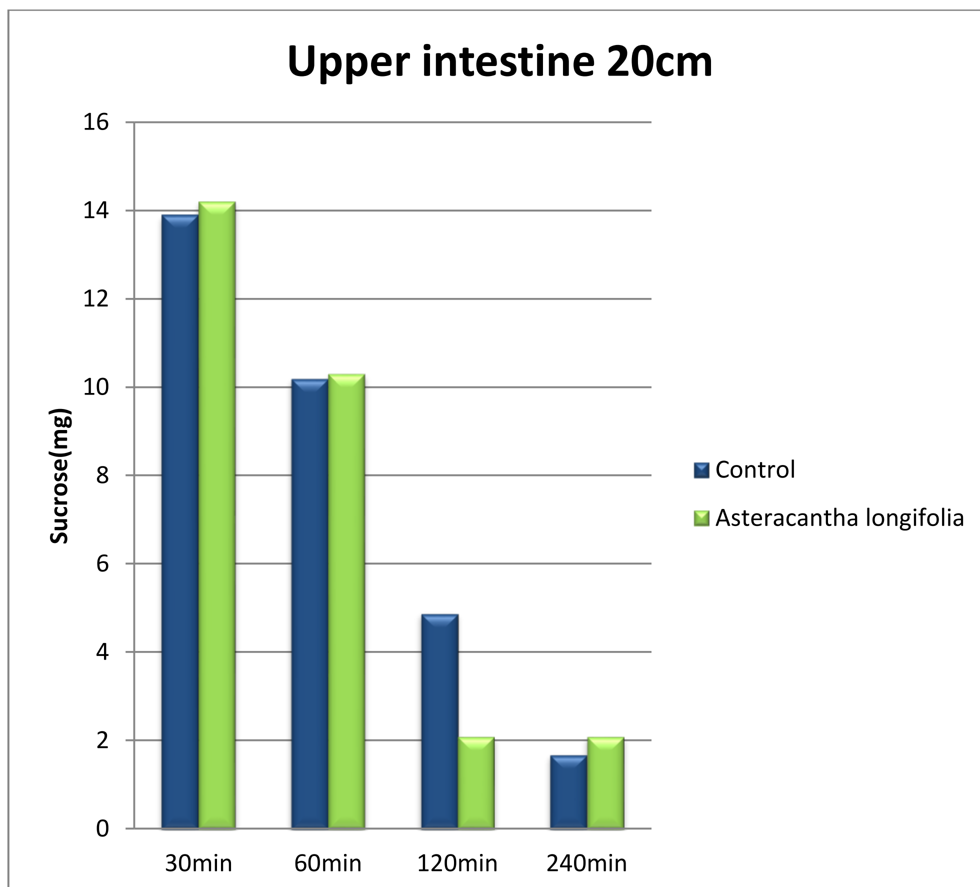

Figure 3.2 Sucrose in Upper intestine (20cm).

Table 3.1.3 (sucrose content in middle of intestine)								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	20	4.3	16	4.2	7.5	1.8	1.3	0.3
Asteracantha longifolia	21.3	6.6	4.5	1.1	6.8	0.9	1.5	0.5

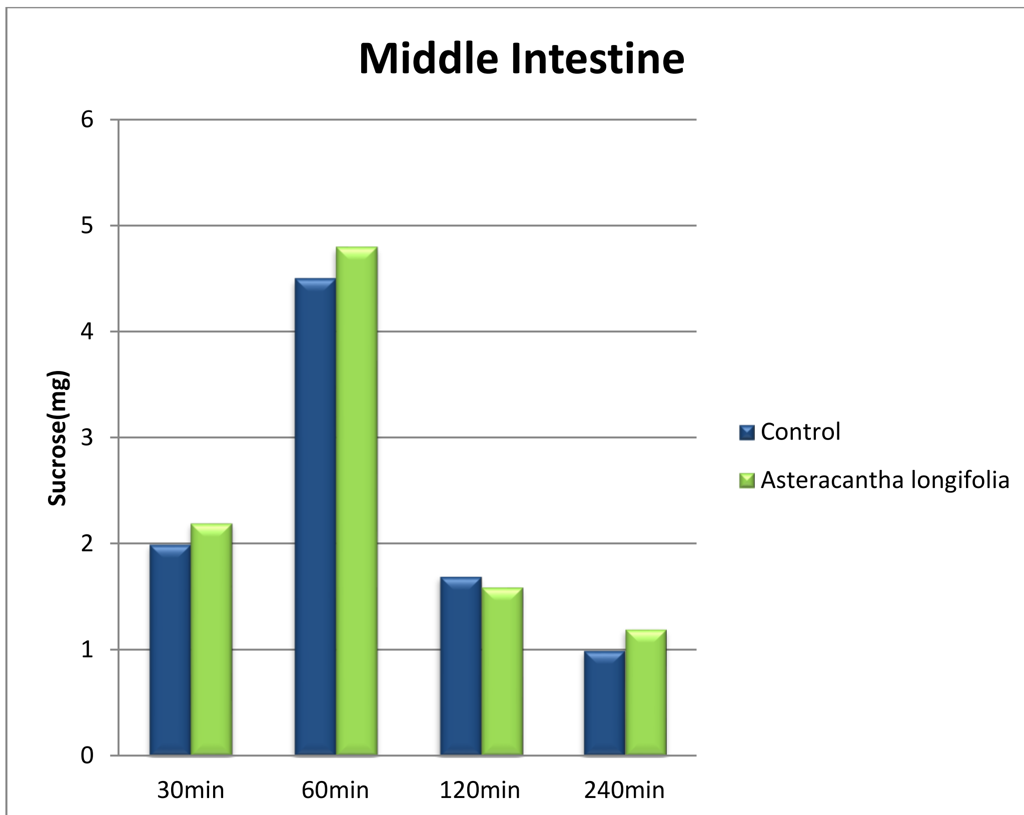

Figure 3.3 Sucrose in Middle intestine.

Table 3.1.4 (sucrose content in lower 20 of intestine)								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	2	0.5	4.5	1.1	1.7	04	1	0.3
Asteracantha longifolia	2.2	0.6	4.8	0.9	1.6	0.3	1.2	0.2

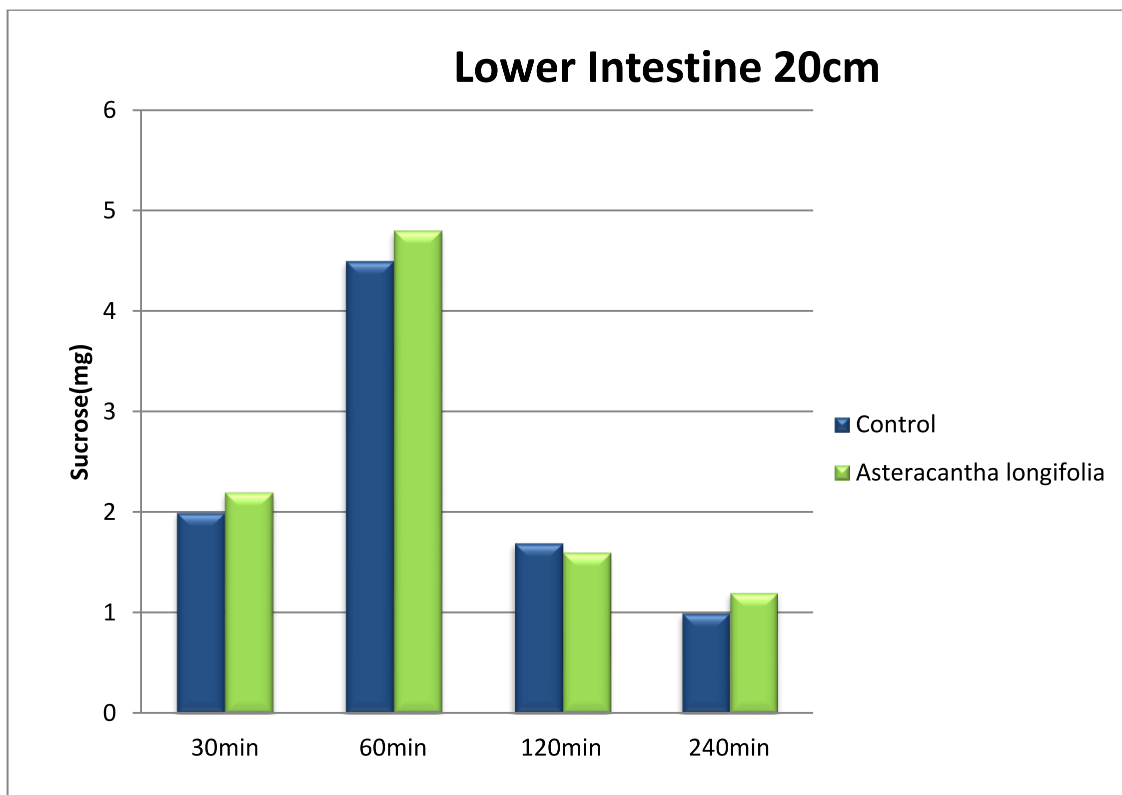

Figure 3.4 Sucrose in Lower intestine (20cm).

Table 3.1.5 (sucrose content in caecum of intestine)								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	1.6	0.5	1.7	0.3	1.6	0.4	1.8	0.5
Asteracantha longifolia	2.1	0.5	2.2	0.4	1.9	0.4	1.6	0.3

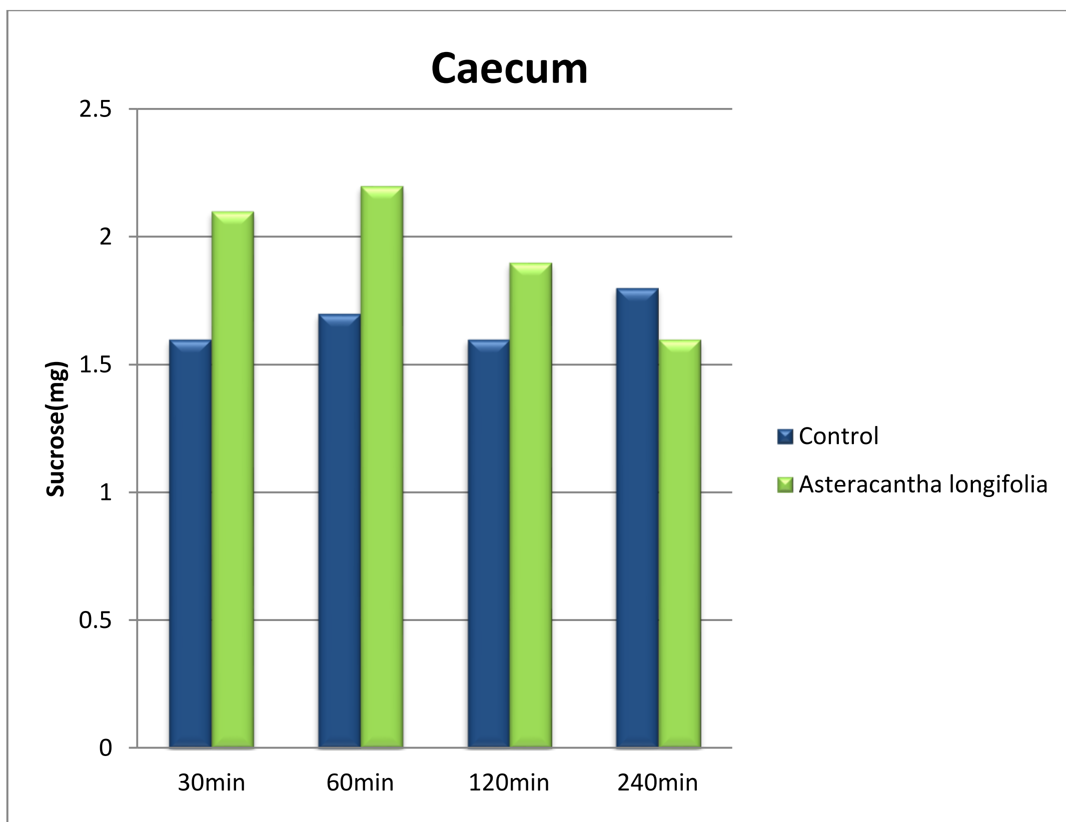

Figure 3.5 Sucrose in Caecum.

Table 3.1.6 (sucrose content in large intestine)								
Groups	30 min		60 min		120 min		240 min	
	Sucrose(mg)	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**	**Sucrose(mg)**	**SD**
Control	1	0.3	0.9	0.3	1.2	0.2	1.1	0.2
Asteracantha longifolia	0.9	0.3	1.0	0.3	1.1	0.2	1.3	0.4

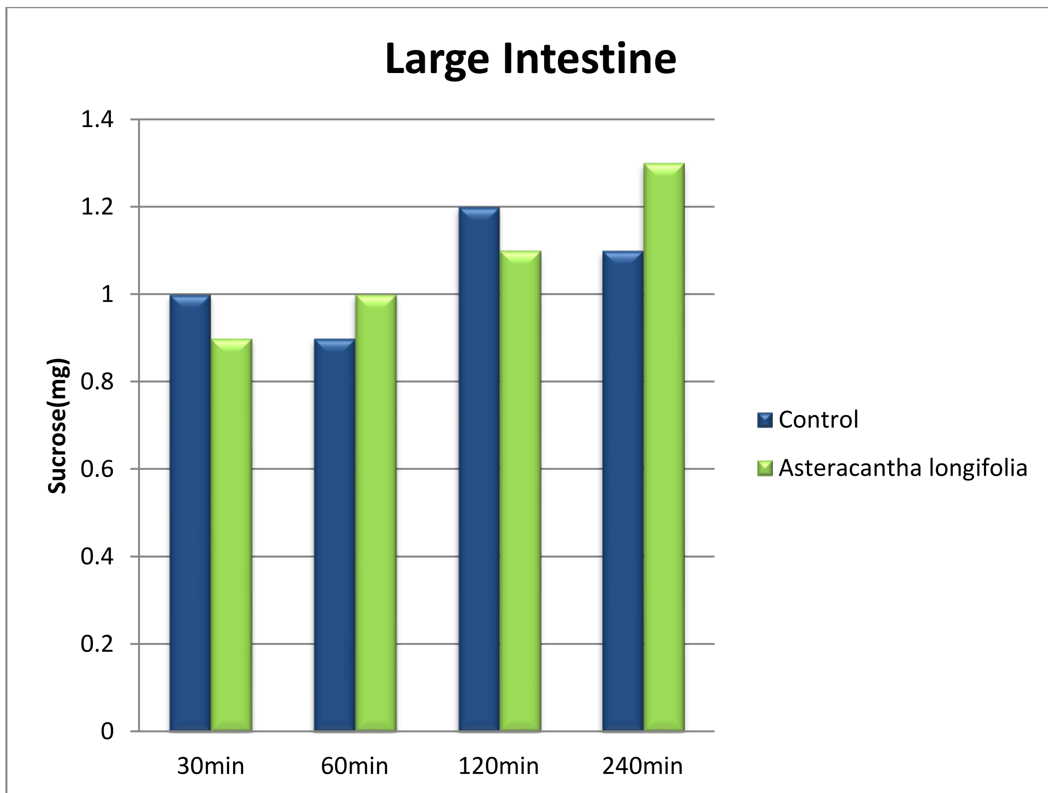

Figure 3.6 Sucrose in Large intestine

Data are presented as Mean±SEM; n=4. Data values are significantly different from the corresponding values of the CONTROL group at $p<0.05$

3.2 Effect of *A. longifolia* on Intestinal Disaccharidase Enzyme Activity

Asteracantha longifolia extract showed significant inhibition (p<0.05) ofdisaccharide activity.

Table 3.2.1 Effect of A. longifolia on Intestinal Disaccharidase Enzyme Activity

Groups	Disaccharidase activity (µmol/mg/h)	SEM
Control	1.6	0.2
A. longifolia	1.06	0.1
Acarbose	1.09	0.1

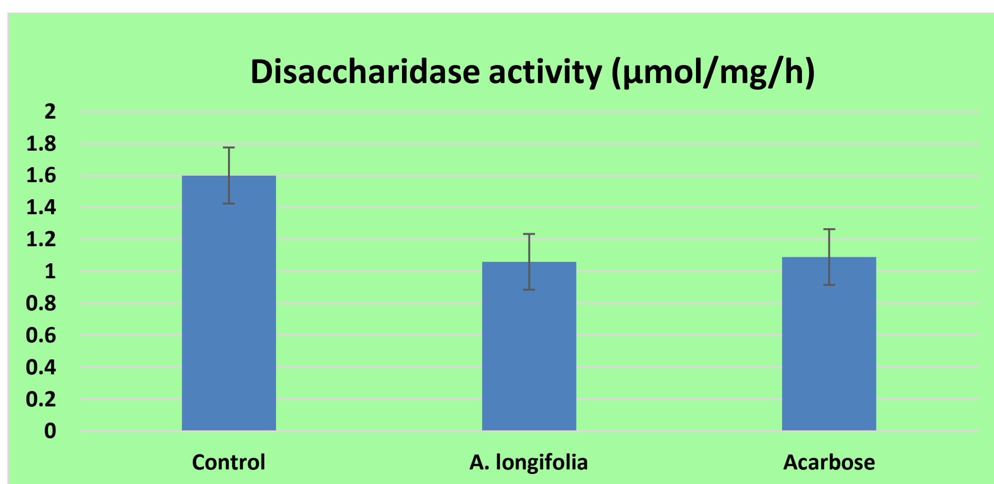

Figure 3.7 Effects of A. longifolia on intestinal disaccharides activity.

Effects of ethanol extract of A. longifolia on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of A. longifolia (100mg/kg body weight) or water (control). Enzyme activity was determined at 60min. Acarbose (200 mg/Kg) was used as reference control for disaccharidase activity test. Values are means and standard deviations represented by vertical bars (n=12). It significantly decreased (p<0.05) disaccharidase enzyme activity (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

Chapter 4

DISSCUSSION & CONCLUSION

4.1 Discussion of *Asteracantha longifolia*

The prevalence of diabetes is rising relentlessly around the world. Current estimates suggest that, globally, the number of persons with diabetes will rise from 151 million in the year 2000, to 221 million by the year 2010, and to 300 million by 2025 (Amos et al 1997, King et al 1998).

This rise is predicted to occur in virtually every nation, with the greatest increases expected in developing countries.

Nature has been a source of medicinal treatments for thousands of years, and plants-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries (King et al 1998). Biguanides developed from a prototypic plant molecule is an excellent example of anti-diabetic drug development from plants. Thus, it is prudent in the current context to look for new and if possible more efficacious hits from the vast reserves of phytotherapy. Many herbal medicines have been recommended for the treatment of diabetes. On the other hand, as indicated by Marles & Farnsworth (1995), not all of the plants reported to be useful are entirely safe, and they emphasize the need for carefully planned scientific research to identify those hypoglycemic plants with true therapeutic efficacy and safety.

Renewed attention in alternative medicines and natural therapies has led to a revived interest in the use of traditional plants for the treatment of diabetes. In this regard the screening of plant materials for hypoglycemic properties is important as it might provide a new lead as anti-diabetic agent. *Asteracantha longifolia* has been using as an antidiabetic agent for a long time. Efficacy of this plant in the treatment of diabetes has been studied in details. In the present study, this plant was selected to explore the mechanism of action in Long Evans rat.

In previous studies it has been found that Annona squamosal helped in total control of diabetes. In the present study we explored the extra pancreatic action of the plant in Long Evans rats.

In six segment method, the sucrose extract solution was administered to the model rat, water and sucrose was administered to the control. Then after 30 minutes, 60 minutes, 180 minutes and 360 minutes the rats were sacrificed to observe the amount of sucrose remaining in the gastrointestinal tract. From the result we can deduce that the

extract of the leaf of *Asteracantha longifolia* was capable to cause a decrease in the amount of unabsorbed sucrose from the gastrointestinal tract.

The results obtained from both six-segment method and Intestinal Disaccharidase Enzyme Activity test significantly demonstrates, more conclusively, that the ethanol extract of *Asteracantha longifolia* can be effective in diabetic treatment.

4.2 conclusions of *Asteracantha longifolia*

The rapidly increasing prevalence of diabetes mellitus throughout the world will continue to challenge the existing therapies and encourage new approaches to counter DM.

The present study has evaluated potential antidiabetic activity of *Asteracantha longifolia,* traditionally used in the treatment of DM. The experiment carried out showed positive hypoglycemic effects of the plant. Hopefully this will provide as a lead to carry out further investigation to assess whether or not Asteracantha longifolia extracts may be used commercially.

Chapter 5

REFFERENCES

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