Evaluation of Anti-hyperglycemic effects of methanolic extract of *Cinnamomum zeylanicum* in laboratory animal models

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.

Submitted by

Amreen Ahmed

ID: 2014-1-70-058

Supervised by

Dr. JMA Hannan
Professor
Department of Pharmacy

East West University

Declaration by the candidate

I, **Amreen Ahmed**, hereby declare that the dissertation entitled **"Determination Anti diabetic Efficacy of barks of "*Cinnamomum zeylanicum*"** 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(Honors), genuine and authentic research 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.

_____

Amreen Ahmed

ID: 2014-1-70-058

Department of Pharmacy

East West University

Certificate by the Supervisor

This is to certify that the thesis entitled **"Determination Anti diabetic Efficacy of barks of "*Cinnamomum zeylanicum*"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Amreen Ahmed**(student ID: 2014-1-70-058) in 2017, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

_____

Dr. JMA Hannan

Professor and Supervisor

Department of Pharmacy

East West University, Dhaka.

Endorsement by the Chairperson

This is to certify that the thesis entitled **"Determination Anti diabetic Efficacy of barks of "*Cinnamomum zeylanicum*"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Amreen Ahmed** (student ID 2014-1-70-058) in 2017, under the supervision and guidance of **Dr. JMA Hannan,** Professor, Department of Pharmacy, East West University.

Dr. Chowdhury Faiz Hossain
Chairperson and Professor
Department of Pharmacy
East West University, Dhaka

ACKNOWLEDGEMENT

I am grateful to Almighty Allah for the good health and wellbeing that were necessary to complete this research work. I would like to express our deepest gratitude to our research supervisor **Dr. JMA Hannan,** Professor, East West University who had been always optimistic and full of passion and ideas. His generous advice, constant supervision, intense support, enthusiastic encouragements and reminders during the research work not only helped shape this study but also molded us into being a better researcher. His in-depth thinking, motivation, timely advice and encouragement have made it possible for us to complete this research.

I am also indebted to the Department of Pharmacy, East West University. We are very proud to be a part of this institute. This institute has given us opportunity to learn about our future goals, to learn how to show respect to the pharmacy profession.

I would like to show our gratitude to **Dr. Chowdhury Faiz Hossain**, Chairperson, Department of Pharmacy, East West University for giving us the courage that we needed most.

I would also like to be grateful to our wonderful lab officer, **Shofikul Islam** who taught us many techniques and methods and helped us complete the research work on time.my special thanks to my fellow researcher friends Iqbal Hossain Noyon (2014-1-70-010), Nazir Hossain Munna (2014-1-70-021), Towsif Azmain (2014-1-70-053) who helped me to conduct the research by being very co-operative.

Finally, I am immensely grateful to my beloved parents for their love and faith in me, especially for their unconditional love in life. I also would like to express my heartfelt love to our family for their continuous support for completion of B.PHRM Degree.

Thank You.

DEDICATION

This Research Paper Is Dedicated

To My Beloved **Parents**, Who Are My biggest inspiration…

Abstract

Our present studies were focused on the probable anti-diabetic activity of the plant ***"Cinnamomum zeylanicum*on"** in laboratory animals and the statistical significance of such effect. The plant extract was subjected to assessment of gastrointestinal motility and 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 the GI motility test, the extract showed laxative effect, which results the decreased absorption in small intestine. In Six Segment test, the amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 500mg/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 500mg/kg extract .The extract caused a significant, 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 ***"Cinnamomum zeylanicum*on"** claimed in Ayurveda medicine.

Keywords: Anti-Diabetic, ***"Cinnamomum zeylanicum*on",*** hypoglycemic, Glucose, Sucrose, Disaccharidase.

TABLE OF CONTENTS

CHAPTER 1. INTRODUCTION

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Overview

Diabetes is a disease caused by flawed carbohydrate metabolism and manifests itself by unusually large amounts of sugar in the blood and urine. (Ben-Jacob, T., 2011)

Diabetes mellitus describes a group of metabolic disorders characterised by increased blood glucose concentration. People living with diabetes have a higher risk of morbidity and mortality than the general population. The global prevalence of diabetes in adults has been increasing over recent decades. In 1964, it was estimated that 30 million people had diabetes. Less than 40 years later, the WHO estimated that there were 171 million people living with diabetes. The International Diabetes Federation (IDF) estimated the global prevalence to be 151 million in 2000, 194 million in 2003, 246 million in 2006, 285 million in 2009, 366 million in 2011, and 382 million in 2013. Each estimate was based on the latest data available. The IDF Atlas methodology was substantially updated in 2011 to incorporate an analytic hierarchy process that formalised the methods to prioritise the highest quality data from available sources. The dramatic increase in diabetes has occurred in all countries, and in rural as well as urban areas. Accurate global, regional, and country-level estimates and projections of diabetes prevalence are necessary for prevention and treatment strategies to be planned and monitored, and to assess progress towards reaching the targets set by the Global Action Plan for Non-Communicable Diseases and the Sustainable Development Goals. (IDF, D.A.G., 2015)

Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, referred to as microvascular disease. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases. This is referred to as macrovascular disease. Diabetes affects approximately 26 million people in the United States, while another 79 million have prediabetes. An estimated 7 million people in the United States have diabetes (WHO, 2004).

From an economic perspective, the total annual cost of diabetes in 2012 was estimated to be 245 billion dollars in the United States. This included 116 billion in direct medical costs (healthcare costs) for people with diabetes and another 69 billion in other costs due to

disability, premature death, or work loss. Medical expenses for people with diabetes are over two times higher than those for people who do not have diabetes. These numbers reflect only the population in the United States. (Guariguata, L., 2012)

Diabetes was the 7th leading cause of death in the United States listed on death certificates in 2007 (Seaman, 2014).

As of 2014, an estimated 387 million people have diabetes worldwide, (International Diabetes Federation, 2014) with type 2 diabetes making up about 90% of the cases. This is equal to 8.3% of the adult population, with equal rates in both women and men. In the years 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9 million deaths per year.The number of people with diabetes is expected to rise to 592 million by 2035. (Guariguata, L., et al, 2014.)

The global economic cost of diabetes in 2014 was estimated to be $612 billion USD. In the United States, diabetes cost $245 billion in 2012. (American Diabetes Association, 2014.)

1.2Diabetes Mellitus

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 macrovascular and microvascular system. Diabetic complications associated with macrovascular diseases are atherosclerotic macrovascular disease and ischemic coronary heart disease. Diabetic complications related to microvascular disease include retinopathy, nephropathy, neuropathy, and peripheral vascular diseases (Alberti, K.G.M.M. and Zimmet, P.F., 1998).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished

tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome.

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes. (American Diabetes Association, 2014)

1.3 Epidemiology of 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. 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 (Warram, J.H. and Krolewski, A.S., 2005).

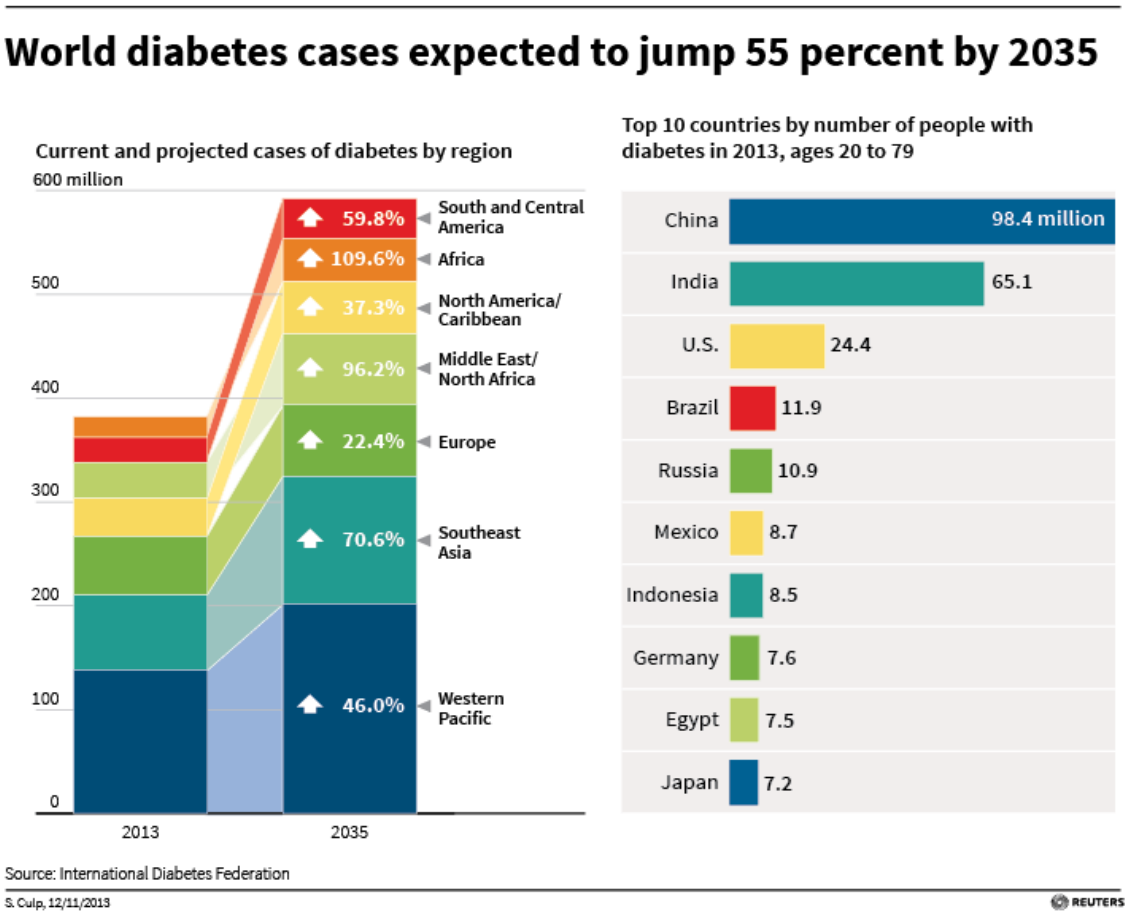


Figure 1.1: Epidemiology of Diabetes Mellitus

Bangladesh has a disproportionately high diabetes population with more than 7.1 million, 8.4% or 10 million according to research published in WHO bulletin in 2013, of the adult population affected by the disease. The number will be 13.6 million in 2040 (Rahman, M.S., *et al* 2015).

1.4 Classification of Diabetes Mellitus

It has been clearly established in recent years that diabetes mellitus is a genetically and clinically heterogeneous group of disorders that share glucose intolerance in common. The evidence in favor of this heterogeneity is overwhelming: (1) there are more than 30 distinct, mostly rare, disorders in which glucose intolerance is a feature; (2) ethnic variability in prevalence and clinical features; (3) genetic heterogeneity in diabetic animal models; (4)

clinical variability between thin, ketosis-prone, insulin-dependent diabetes and obese, nonketotic, insulin-resistant diabetes; (5) genetic and immunologic studies that show "juvenile" and "adult-onset" diabetes to be distinct entities; and (6) demonstration that a type of mild diabetes in young people, which is inherited in an autosomal dominant fashion, is clearly different from the classic acute-onset diabetes of juveniles. (National Diabetes Data Group, 1979.)

The World Health Organization (WHO) classifies diabetes into main groups: type 1 diabetes also called insulin-dependent diabetes mellitus or IDDM and type 2 diabetes also called non-insulin dependent diabetes mellitus or NIDDM. Maturity-Onset Diabetes of the Young (MODY) and gestational diabetes are less frequently occurring forms of diabetes (World Health Organization, 1999).

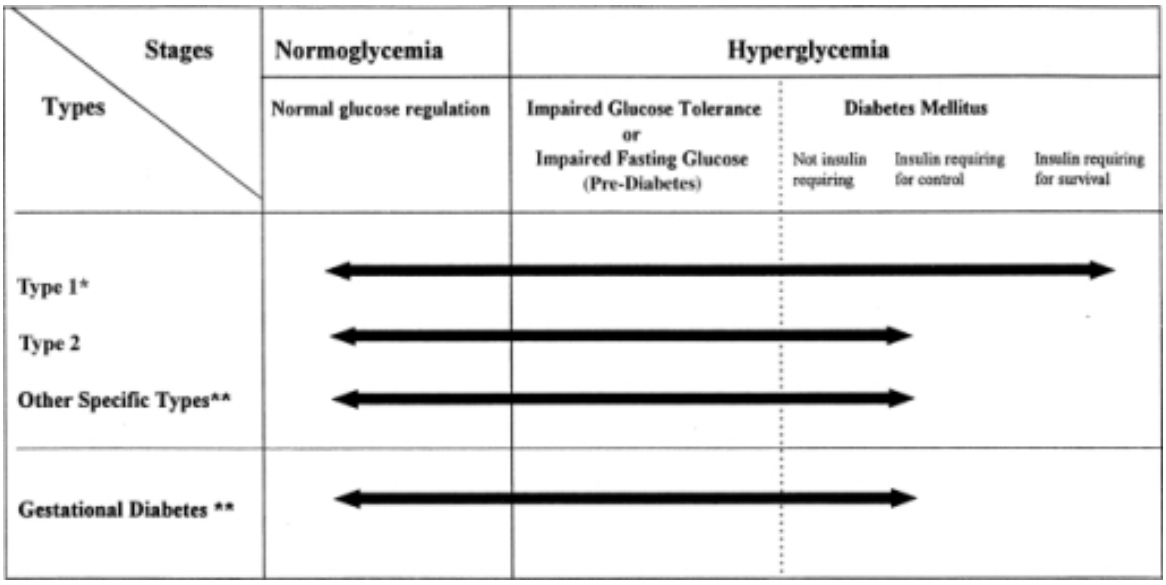


Figure: 1.2 Disorders of glycemia: etiologic types and stages.

*Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring continuous therapy (i.e., “honeymoon” remission); **in rare instances, patients in these categories (e.g., Vacor toxicity, type 1 diabetes presenting in pregnancy) may require insulin for survival. (American Diabetes Association, 2014)

1.4.1 Type 1 Diabetes Mellitus (beta-cell destruction, usually leading to absolute insulin deficiency)

Type 1 diabetes mellitus (DM) is a multisystem disease with both biochemical and anatomic consequences. It is a chronic disease of carbohydrate, fat, and protein metabolism caused by the lack of insulin, which results from the marked and progressive inability of the pancreasto secrete insulin because of autoimmune destruction of the beta cell. Type 1 DM can occurat any age. It is most common in juveniles but can also develop in adults, especially in those in their late 30s and early 40s.

1. **Immune-mediated diabetes.** This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes, type I diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the beta-cells of the pancreas. Markers of the immune destruction of the beta-cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to glutamic acid decarboxylase (GAD65), and autoantibodies to the tyrosine phosphatasesIA-2andIA-2B.One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective.

   In this form of diabetes, the rate of beta-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). 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; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life.

Autoimmune destruction of beta-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthe niagravis, and pernicious anemia.

2. **Idiopathicdiabetes.** Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only aminority of patients withtype1diabetesfallintothiscategory, of those who do, most are of African or Asianancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for beta-cell autoimmunity, and is not HLA associated. An absolute requirement for insulin replacement therapy in affected patients may come and go. (American Diabetes Association, 2014)

Common symptoms of type 1 diabetes include:

- Excessive thirst
- Increased urination (sometimes as often as every hour)
- Unusual weight loss
- Fatigue or tiredness
- Nausea, perhaps vomiting
- Blurred vision
- In women, frequent vaginal infections
- In men and women, yeast infections (thrush)
- Dry mouth
- Slow-healing sores or cuts
- Itching skin, especially in the groin or vaginal area.

Symptoms of type 1 diabetes can develop quickly, over weeks or sometimes days (Alberti, K.G.M.M. and Zimmet, P.F., 1998).

1.4.1.1 Pathophysiology of type 1 diabetes

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons in IDDM patients. Normally, hyperglycemia leads to reduced glucagons secretion, however, in patients with IDDM, glucagons secretion is not suppressed by hyperglycemia. The resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin deficiency. The most pronounced example of this metabolic disruption is that patients with IDDM rapidly develop diabetic ketoacidosis in the absence of insulin administration. Although insulin deficiency is the primary defect in IDDM, there is also a defect in the administration of insulin. There are multiple biochemical mechanisms that account for impairment of tissue's response to insulin. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Kutty, B.M. and Raju, T.R., 2010).

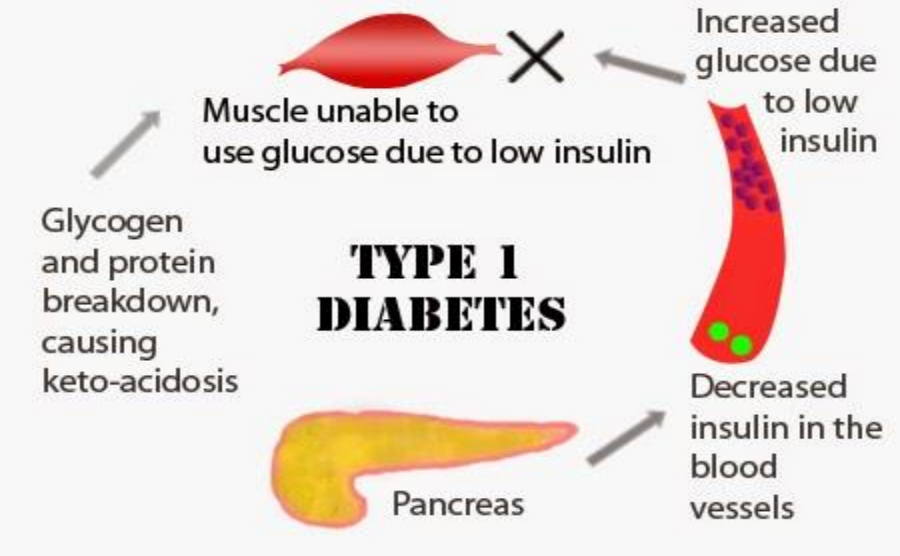


Figure1.3: Type 1 Diabetes (Alberti, 1998)

1.4.2 Type 2 Diabetes Mellitus (ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance)

Diabetes mellitus type 2 (formerly noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by hyperglycemia (high blood sugar) in the context of insulin resistance and relative lack of insulin. (Roy, S., *et al*, 2005).

Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity. As of 2010 there were approximately 285 million people diagnosed with the disease compared to around 30 million in 1985. (Smyth, S. and Heron, A., 2006) Long-term complications from high blood sugar can include heart disease, strokes, diabetic retinopathy where eyesight is affected, kidney failure which may require dialysis, and poor blood flow in the limbs leading to amputations. The acute complication of ketoacidosis, a feature of type 1 diabetes, is uncommon, (Evans, M.M., *et al*, 2016).

This form of diabetes, which accounts for 90–95% of those with diabetes, previously referred to as non-insulin dependent diabetes, type II diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of beta-cells does not occur, and patients do not have any of the other causes of diabetes listed above or below.

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of

developing macrovascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their beta-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined. (American Diabetes Association, 2014.)

Common symptoms of type 2 diabetes include:

- Increased thirst
- Increased hunger (especially after eating)
- Dry mouth
- Frequent urination
- Unexplained weight loss (even though you are eating and feel hungry)
- Fatigue (weak, tired feeling)
- Blurred vision
- Headaches
- Loss of consciousness (rare)

Other symptoms of type 2 diabetes may include:

- Slow-healing sores or cuts
- Itching of the skin (usually around the vaginal or groin area)
- Frequent yeast infections
- Recent weight gain or unexplained weight loss
- Velvety dark skin changes of the neck, armpit, and groin, called acanthosis nigricans

- Numbness and tingling of the hands and feet
- Decreased vision
- Impotency (Alberti, K.G.M.M. and Zimmet, P.F., 1998).

1.4.2.1 Pathophysiology of Type 2 Diabetes

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM. On the basis of oral glucose tolerance testing the essential elements of NIDDM can be divided into four distinct groups:

i) Those with normal glucose tolerance.

ii) Chemical diabetes (called impaired glucose tolerance).

iii) Diabetes with minimal fasting hyperglycemia (fasting plasma glucose less than

140mg/dl).

iv) Diabetes mellitus in association with overt fasting hyperglycemia (fasting plasma glucose greater than140mg/dl).The individuals with impaired glucose tolerance have hyperglycemia in spite of having highest levels of plasma insulin, indicating that they are resistant to theaction of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. Insulin resistance and insulin deficiency are common in the average NIDDM patients (Bushe, C. and Holt, R., 2004).

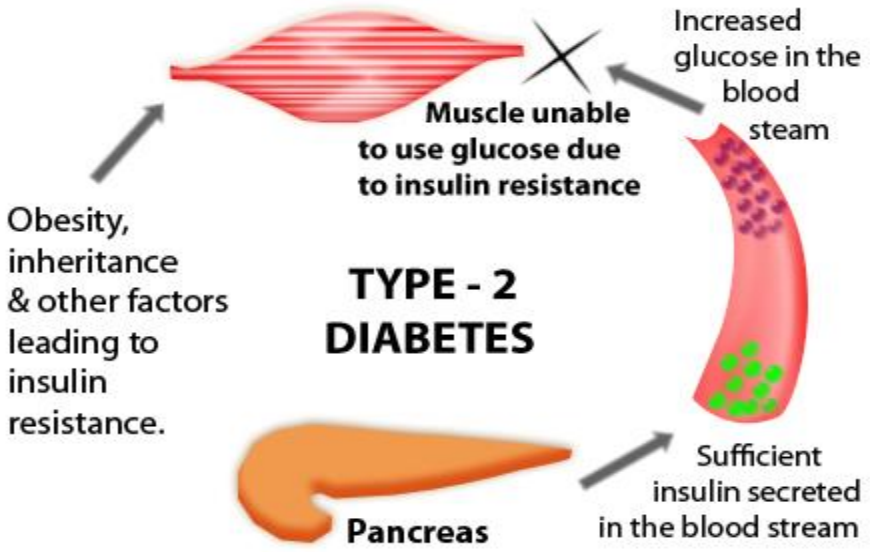


Figure1.4: Type 2 Diabetes (Alberti, 1998)

1.4.2.2 The risk factors for type 2

- Age and ethnicity: The older people are at higher risk, especially over 40 (for white people), and over 25 (for black, South Asian and some minority groups). It has been found in the UK that black people and people of South Asian origin have five times the risk of developing Type 2 compared to white people.
- Diabetes in the family: If a relative has/had diabetes risk might be greater. The risk increases if the relative is a close one.
- Bodyweight (and inactivity combined with bodyweight): Four-fifths of people who have Type 2 became so because they were overweight. The more overweight a person is the higher his/her risk will be. The highest risk is for a person who is overweight and physically inactive.
- Cardiovascular problems and stroke: A person who has had a stroke runs a higher risk of developing Type 2. This is also the case for people who suffer from hypertension (high blood pressure), or have had a heart attack. Any diagnosis of a problem with circulation indicates a higher risk of developing Type 2.
- Gestational Diabetes: A woman who became temporarily diabetic during pregnancy - gestational diabetes - runs a higher risk of developing Type 2 later on. Women who give birth to a higher weight baby may run a higher risk, too.

- Impaired fasting glycaemia (IFG) - Impaired glucose tolerance (IGT): A person who has been diagnosed as having impaired fasting glycaemia or impaired glucose tolerance and does not have diabetes runs a significantly higher risk of eventually developing Type 2. People with IFG or IGT have higher than normal levels of glucose in their blood.
- Severe mental health problems: It has been found that people with severe mental health problems are more likely to develop Type 2. (Baliunas, D.O., *et al*, 2009)

1.4.3 Difference 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

Table 1.1: Difference between type 1 and type 2 diabetes (Alberti, 1998)

1.5 Other specific types of diabetes

Genetic defects of the beta-cell. Several forms of diabetes are associated with monogenetic defects in -cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes

have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1.A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the-cell. Thus, glucokinase serves as the "glucose sensor" for the -cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. The less common forms result from mutations in other transcription factors, including HNF-4, HNF-1, insulin promoter factor (IPF)-1, and NeuroD1. Point mutations in mitochondrial DNA have been found to be associated with diabetes mellitus and deafness The most common mutation occurs at position3243inthetRNAleucinegene,leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome); however, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion. Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families, and such traits are inherited in an autosomal dominant pattern. The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism. (Kota, S.K., *et al,* 2012).

Genetic defects in insulin action. There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson-Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia.

Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin resistant lipo atrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the post receptor signal transduction pathways (Kahn, S.E., 2003).

Diseases of the exocrine pancreas. Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adreno carcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in -cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage -cells and impair insulin secretion. Fibro calculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy.

Endocrinopathies. Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved. Somatostatinoma- and aldosteronoma-induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion. Hyperglycemia generally resolves after successful removal of the tumor.

Drug- or chemical-induced diabetes. Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of -cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic -cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients receiving interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency. The

list shown in Table 1 is not all-inclusive, but reflects the more commonly recognized drug-, hormone-, or toxin-induced forms of diabetes.

Infections. Certain viruses have been associated with cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackie virus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.

Uncommon forms of immune-mediated diabetes. In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD autoantibodies, and approximately one-third will develop diabetes. Anti–insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues. However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti–insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. As in other states of extreme insulin resistance, patients with anti–insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance.

Other genetic syndromes sometimes associated with diabetes. Many genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down's syndrome, Klinefelter's syndrome, and Turner's syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin-deficient diabetes and the absence of -cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness. (American Diabetes Association, 2014)

1.6 Gestational diabetes

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of various degrees that is first detected during pregnancy. GDM is detected through the screening of pregnant women for clinical risk factors and testing for abnormal glucose tolerance that is usually,

but not invariably, mild and asymptomatic. GDM appears to result from the same broad spectrum of physiological and genetic abnormalities that characterize diabetes outside of pregnancy. Indeed, women with GDM are at high risk for having or developing diabetes when they are not pregnant. Thus, GDM provides a unique opportunity to study the early pathogenesis of diabetes and to develop interventions to prevent the disease (Thomas, 2005).

GDM complicates about 4% of all pregnancies in the U.S., resulting in about 135,000casesannually.Theprevalence may range from 1 to 14% of pregnancies, depending on the population studied. GDM represents nearly 90% of all pregnancies complicated by diabetes. Deterioration of glucose tolerance occurs normally during pregnancy, particularly in the 3rd trimester.(American Diabetes Association, 2014.)

Table 1.2: Etiologic classification of diabetes mellitus (American Diabetes Association, 2014.)

I. Type 1 diabetes (beta-cell destruction, usually leading to absolute insulin deficiency) A. Immune mediated B. Idiopathic
II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III. Other specific types
A. Genetic defects of beta-cell function 1. Chromosome 12, HNF-1 (MODY3) 2. Chromosome 7, glucokinase (MODY2) 3. Chromosome 20, HNF-4 (MODY1) 4. Chromosome 13, insulin promoter factor-1 (IPF-1; MODY4) 5. Chromosome 17, HNF-1 (MODY5) 6. Chromosome 2, NeuroD1 (MODY6) 7. Mitochondrial DNA 8. Others
B. Genetic defects in insulin action 1. Type A insulin resistance

2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipoatrophic diabetes
5. Others

C. Diseases of the exocrine pancreas

1. Pancreatitis
2. Trauma/pancreatectomy
3. Neoplasia
4. Cystic fibrosis
5. Hemochromatosis
6. Fibrocalculouspancreatopathy
7. Others

D. Endocrinopathies

1. Acromegaly
2. Cushing's syndrome
3. Glucagonoma
4. Pheochromocytoma
5. Hyperthyroidism
6. Somatostatinoma
7. Aldosteronoma
8. Others

E. Drug- or chemical-induced

1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. -adrenergic agonists
8. Thiazides
9. Dilantin

10. Alpha Interferon 11. Others
F. Infections 1. Congenital rubella 2. Cytomegalovirus 3. Others
G. Uncommon forms of immune-mediated diabetes 1. "Stiff-man" syndrome 2. Anti–insulin receptor antibodies 3. Others
H. Other genetic syndromes sometimes associated with diabetes 1. Down's syndrome 2. Klinefelter's syndrome 3. Turner's syndrome 4. Wolfram's syndrome 5. Friedreich's ataxia 6. Huntington's chorea 7. Laurence-Moon-Biedl syndrome 8. Myotonic dystrophy 9. Porphyria 10. Prader-Willi syndrome 11. Others
IV. Gestational diabetes mellitus (GDM)

1.7 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:

1. Insulin Glucose Challenge Test – This should be done with a 2-hour glucose challenge, 75 grams measuring fasting, 1- and 2-hour blood sugar AND insulin. The blood sugar

should be less than 80 fasting and never rise above 110 or 120 after one to two hours. Insulin should be less than 5 fasting and should never rise above 30 after one to two hours.

2. Hemoglobin A1C Test – This is an important measure of glycated hemoglobin, which can be an early indicator of sugar problems. It measures sugars and proteins combining into glycated proteins called AGEs (advanced glycation end products), like the crust on bread, or the crispy top on creme brule. These create inflammation and oxidative stress throughout the body, and promote heart disease and dementia and accelerating aging. The hemoglobin A1C should ideally be less than 5.5. Anything over 6 is considered diabetes.

Figure 1.5: Hemoglobin A1C Test

3. Lipid Profiles – An HDL or good cholesterol level under 60 and triglycerides over 100 may be due to insulin resistance. An HDL under 40 and a triglyceride level over 150 usually means diabetes.

4. NMR Lipid Profile – This test is slightly different from the one above as it identifies the size of your cholesterol particles. With insulin resistance or Type 2 diabetes, one may develop small LDL and HDL cholesterol particles. They are much more dangerous than larger particles and lead to increased risk of atherosclerosis or heart disease.

5. High Sensitivity C- Reactive Protein Test – This is a measure of inflammation, one of the classic conditions that is both the cause and result of insulin resistance and diabetes. It should be less than 1, and is often associated with diabetes. In fact, anyone with a high C reactive protein has a 1,700 percent increased risk of getting diabetes.

6. Homocysteine Test – Homocysteine levels are often abnormal in people with diabetes. The test is a measure of folic acid deficiency. It should be between 6 and 8.

7. Fibrinogen Test – This measures your risk of clotting, which can cause heart attacks and strokes. It is also a sign of inflammation and is associated with insulin resistance and diabetes. It should be less than 300.

8. Check Ferritin Levels – These are often elevated in people with diabetes. It is a nonspecific marker of inflammation associated with the disease. It also can mean an overload of iron in the body. It should be less than 150.

9. Uric Acid Test – Your level should be less than 6. Higher levels indicate problems with insulin resistance. This can lead to gout, which is related to insulin resistance and Type 2diabetes.

10. Liver Function Tests – Elevated liver function can result from insulin resistance. This is the major cause of fatty liver and elevated liver function in this country. This is entirely due to sugar and carbohydrates in our diet that cause fatty liver, liver damage, and even cirrhosis. (Genuth S et al)

1.7.1 Diagnostic Criteria for Diabetes Mellitus

The criteria for the diagnosis of diabetes are shown in table. Three ways to diagnose diabetes are possible, and each, in the absence of unequivocal hyperglycemia, must be confirmed, on a subsequent day, by any one of the three methods given in table. The use of the hemoglobin A1c (A1C) for the diagnosis of diabetes is not recommended at this time.

Criteria for the diagnosis of diabetes mellitus

1. Symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
Or
2. FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.
Or

3. 2-h postload glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

- In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine clinical use (American Diabetes Association, 2014).

1.8 Causes

Body digests the food which is eaten to produce sugar (glucose) that enters your bloodstream. In response to pancreas (large gland behind stomach) produces insulin. Insulin is a hormone that helps glucose move from your bloodstream into body's cells, where it's used as energy. During pregnancy, the placenta, which connects baby to blood supply, produces high levels of various other hormones. Almost all of them impair the action of insulin in cells, raising blood sugar. Modest elevation of blood sugar after meals is normal during pregnancy. As baby grows, the placenta produces more and more insulin-blocking hormones. In gestational diabetes, the placental hormones provoke a rise in blood sugar to a level that can affect the growth and welfare of baby. Gestational diabetes usually develops during the last half of pregnancy sometimes as early as the 20th week, but generally not until later (Notkins, A.L., 1979).

1.9 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.

1.9.1 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 Ca^{2+} mobilization (Matschinsky, F.M., 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 (Juang, J.H., *et al*, 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). 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 (Lipsett, M., *et al,* 2006).

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), hematopoietic growth factors (HGFs) (Garcia-Ocaña, A., *et al,* 2000), and placental lactogen. Moreover, signaling by receptor tyrosine kinases has been implicated as a regulatory mechanism in both β cell proliferation and insulin release (Leibiger, I.B., *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, whereas ablation of $p70^{s6k1}$, an Akt substrate, is associated with a decrease in β cell size.

The mutations of insulin receptor (IR) or IRS-1 impair insulin synthesis and secretion mediated by PI 3-kinase–dependent pathways. 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. 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, S., *et al,* 2002).

1.9.2 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. 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 alpha-subunits, the intrinsic kinase activity in the beta-subunits is activated leading to phosphorylation of the adjacent 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.

Activation of PI3-kinase catalyses 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 transmembrane glucose transport and phosphorylation, activation of the glycolytic flux, as well as glycogen and protein synthesis (Shang, W., *et al*, 2008).

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. For instance, IRS-1, IRS-2 and GLUT-4 knock-out mice have been shown to develop insulin resistance and glucose intolerance. Beta-cell insulin receptor knock-out mice lose acute insulin response to glucose and develop glucose intolerance. Human pancreatic islets carrying the $Gly^{972} \rightarrow Arg$ IRS-1 polymorphism have impaired insulin action.

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. 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 (Olefsky, J.M. and Kolterman, O.G., 1981). In type 2 diabetes, gross insulin resistance combined with hyperinsulinaemia results in a state of relative (not absolute) insulin deficiency. Postreceptor defects are currently believed to be primarily related to insulin resistance in human diabetes (Kolterman, O.G.,*et al*, 1981).

1.10 Signs 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 dermadromes. (Lustman, P.J., *et al*, 1989)

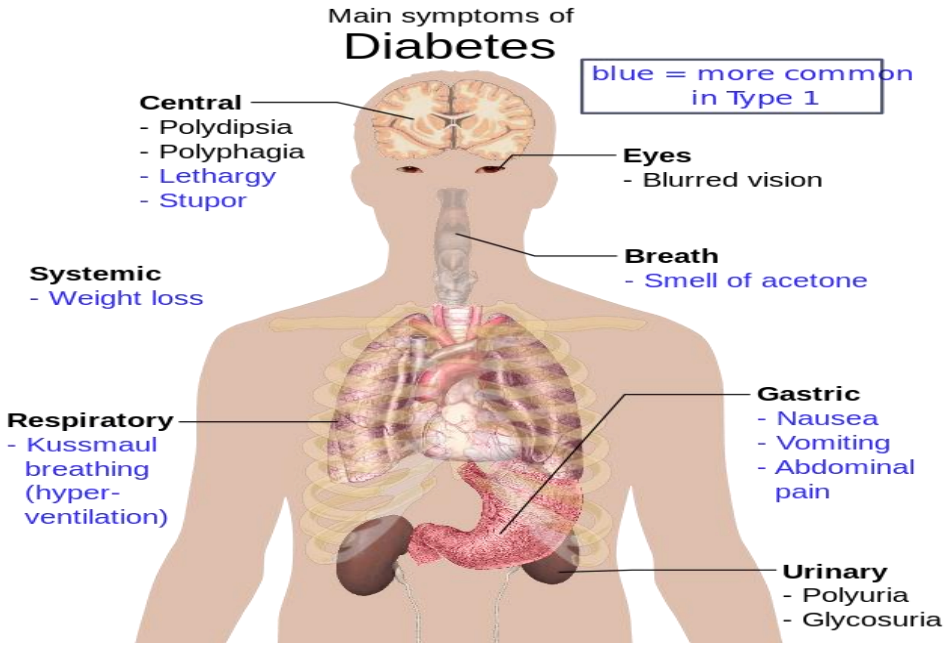


Figure 1.6: Overview of the most Significant Symptoms of Diabetes Mellitus. (Jackson et al, 1991)

1.11 Diabetic Emergencies

Low blood sugar is common in persons with type 1 and type 2-diabetes. Most cases are mild and are not considered medical emergencies. Effects can range from feelings of unease, sweating, trembling, and increased appetite in mild cases to more serious issues such as confusion, changes in behavior, seizures, unconsciousness, and (rarely) permanent brain damage or death in severe cases. Mild cases are self-treated by eating or drinking something high in sugar. Severe cases can lead to unconsciousness and must be treated with intravenous glucose or injections with glucagon.

People (usually with type 1 diabetes) may also experience episodes of diabetic ketoacidosis, a metabolic disturbance characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness.A rare but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 diabetes and is mainly the result of dehydration.

1.12 Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5 fold greater rate of decline in cognitive function (Amos, A.F., *et al*, 1997).

1.13 Prevention

There is no known preventive measure for type 1 diabetes. Type 2 diabetes can often be prevented by a person being a normal body weight, physical exercise, and following a healthful diet. Dietary changes known to be effective in helping to prevent diabetes include a diet rich in whole grains and fiber, and choosing good fats, such as polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat

and other sources of saturated fat can also help in the prevention of diabetes. Active smoking is also associated with an increased risk of diabetes, so smoking cessation can be an important preventive measure as well. Although the genes you inherit may influence the development of type 2 diabetes, they take a back seat to behavioral and lifestyle factors. Data from the Nurses' Health Study suggest that 90 percent of type 2 diabetes in women can be attributed to five such factors: excess weight, lack of exercise, a less-than-healthy diet, smoking, and abstaining from alcohol (Ratner, R.E. *et al*, 2006).

1.13(a) Simple Steps to Lowering the Risk

Making a few lifestyle changes can dramatically lower the chances of developing type 2diabetes. The same changes can also lower the chances of developing heart disease and some cancers.

1.13(b) Control Your Weight

Excess weight is the single most important cause of type 2 diabetes. Being overweight increases the chances of developing type 2 diabetes seven fold. Being obese makes you 20 to 40 times more likely to develop diabetes than someone with a healthy weight.

1.13(c) Dietary Changes

Four dietary changes can have a big impact on the risk of type 2 diabetes-

- Choose whole grains and whole grain products over highly processed carbohydrates. Whole grains don't contain a magical nutrient that fights diabetes and improves health. It's the entire package—elements intact and working together—that's important. The bran and fiber in whole grains make it more difficult for digestive enzymes to break down the starches into glucose. This leads to lower, slower increases in blood sugar and insulin, and a lower glycemic index. As a result, they stress the body's insulin-making machinery less, and so may help prevent type 2-diabetes.
- Skip the sugary drinks, and choose water, coffee, or tea instead. Several studies show that children and adults who drink soda or other sugar-sweetened beverages are more likely to gain weight than those who don't, and that switching from these to water or unsweetened beverages can reduce weight. Even so, however, weight

gain caused by sugary drinks may not completely explain the increased diabetes risk. There is mounting evidence that sugary drinks contribute to chronic inflammation, high triglycerides, decreased good (HDL) cholesterol, and increased insulin resistance, all of which are risk factors for diabetes. Water is an excellent choice. Coffee and tea are also good calorie-free substitutes for sugared beverages (as long as you don't load them up with sugar and cream). And there's convincing evidence that coffee may help protect against diabetes.

- Choose good fats instead of bad fats. The types of fats in your diet can also affect the development of diabetes. Good fats, such as the polyunsaturated fats found in liquid vegetable oils, nuts, and seeds can help ward off type 2 diabetes.
- Eating polyunsaturated fats from fish—also known as ―long chain omega or marine omega fats does not protect against diabetes, even though there is much evidence that these marine omega fats help prevent heart disease. If you already have diabetes, eating fish can help protect you against a heart attack or dying from heart disease.
- Limit red meat and avoid processed meat; choose nuts, whole grains, poultry, or fish instead. It may be that the high iron content of red meat diminishes insulin's effectiveness or damages the cells that produce insulin; the high levels of sodium and nitrites (preservatives) in processed red meats may also be to blame. Red and processed meats are a hallmark of the unhealthful Western dietary pattern, which seems to trigger diabetes in people who are already at genetic risk (Ratner, R.E., 2006).

1.13(d) Quit Smoking

Add type 2 diabetes to the long list of health problems linked with smoking. Smokers are roughly 50 percent more likely to develop diabetes than nonsmokers, and heavy smokers have an even higher risk (Wei, M., *et al*, 1996).

1.13(e) Alcohol Now and Then May Help

A growing body of evidence links moderate alcohol consumption with reduced risk of heart disease. The same may be true for type 2 diabetes. Moderate amounts of alcohol—up to a drink a day for women, up to two drinks a day for men—increases the efficiency of insulin

at getting glucose inside cells. And some studies indicate that moderate alcohol consumption decreases the risk of type 2 diabetes. If you already drink alcohol, the key is to keep your consumption in the moderate range, as higher amounts of alcohol could increase diabetes risk (Koppes, L.L., *et al*, 2005).

1.14 Management

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing low blood sugar. This can usually be accomplished with a healthful diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes). Learning about the disease and actively participating in the treatment is vital for people with diabetes, since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels. The goal of treatment is an HbA1C level of 6.5%, but should not be lower than that, and may be set higher.

Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise. Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal, however (Colberg, S.R., *et al*, 2010).

1.14.1 Lifestyle

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and sensible exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure.

1.14.2 Insulin

The discovery of insulin by Banting, Best and co-workers in 1922 dramatically improved the prospects of individuals with diabetes mellitus. As type 1 is characterized by insulin

insufficiency caused by partial or total destruction of insulin releasing pancreatic beta cells (Mordes, J.P., *et al*, 2004), 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 (Marteau, T.M., *et al*, 1987). 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 (Dörzbach, E. and Müller, R., 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). Insulin can be broadly classified as having short, medium, or long duration of action, however their effects vary considerably from one patient to another and in the same patient from time to time.

Examples of rapid acting insulins include

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

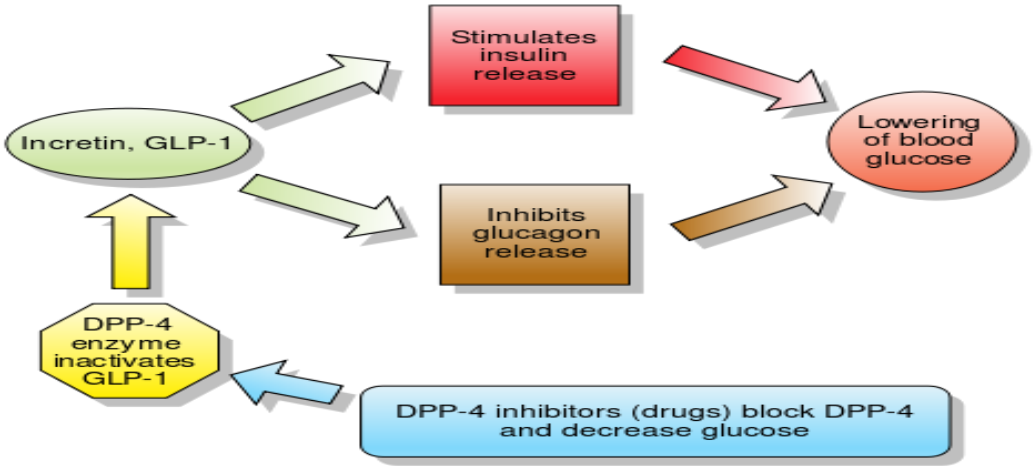


Figure 1.7: Overview of Insulin Secretion.

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)

1.14.3 Anti-Diabetes Medications

Conventional therapies

Diet and exercise are the first step of therapy for type 2 diabetes; if these do not keep blood sugar at goal levels, then antihyperglycemic agents are added. Drug therapy for type 2 diabetes aims to control blood sugar levels both in the basal (fasting) state and postprandially; rational combinations of agents with different mechanisms of action can be used.

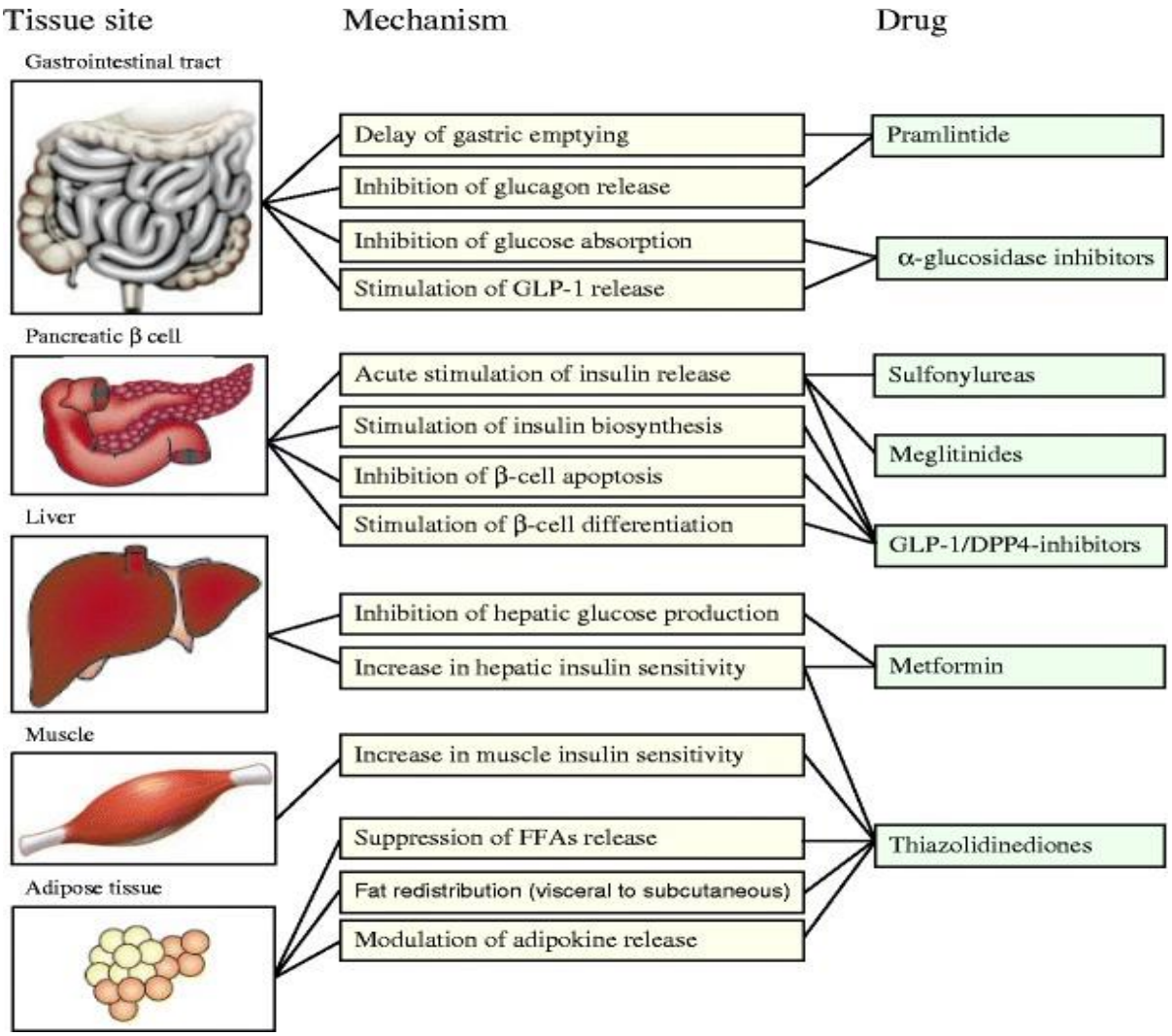


Figure 1.8: Pharmacological treatment of hyperglycemia according to site of action. Adapted from (Stumvoll, M., *et al*, 2005).

Four major classes of antihyperglycemic agents can be used, either as monotherapy or, more appropriately, in combination with one another:

- Insulin secretogogues
- Insulin sensitizers
- Insulin
- α-glucosidase inhibitors

Insulin secretogogues correct hyperglycemia by stimulating insulin secretion—but only if the patient still has enough functioning β-cells. They close ATP-sensitive potassium

channels in the β-cells of the pancreas, increasing insulin production; slow-acting and rapid-acting agents are available. The major side effects of insulin secretogogues (and insulin replacement) are hypoglycemia and weight gain.

Sulfonylureas

Sulfonylureas are insulin secretagogues, enhancing insulin secretion by binding to a unique receptor on pancreatic β-cells and having their greatest effect on fasting hyperglycemia. In this group we can include the second-generation agents gliclazide, glyburide, and glimepiride, as well as the first-generation agents acetohexamide, chlorpropamide, tolazamide, and tolbutamide.

When used as monotherapy, sulfonylureas generally result in HbA1C improvements of a magnitude similar to metformin (1.5%). Sulfonylureas are commonly associated with hypoglycemia and weight gain (∼2 kg). Based on long experience, efficacy, and low cost, sulfonylureas are recommended by the ADA-EASD as options for second-step pharmacotherapy in patients whose HbA1C remains elevated on metformin (Nathan, D.M., *et al*, 2009).

Meglitinides

The recently introduced class of meglitinides consists of nateglinide, which binds to the same site of sulphonylurea receptor 1 as do the sulfonylurea derivatives, and repaglinide, which binds to a nearby site of the receptor, both leading to insulin release. They stimulate rapid, short-lived, insulin secretion.

They lower postprandial glucose levels, although fasting hyperglycemia is also improved. Meglitinides are more specific that sulfonylureas and are associated with lower risk of hypoglycemia but clinical experience remains limited. These agents cannot further stimulate insulin release in patients on maximal doses of sulfonylurea derivatives. These drugs can be used in patients with decreased renal function and for individuals with varying daily meal patterns (Hasslacher, C., 2003).

Metformin

Metformin is an insulin sensitizer well accepted as a first-line agent for treatment of type 2 diabetes (Grant, P.J., 2003). It is a biguanide derivative that exerts an antihyperglycemic effect with minimal risk of hypoglycemia.

Metformin lowers blood glucose concentration and improves insulin sensitivity by reducing hepatic gluconeogenesis and enhancing insulin-stimulated peripheral glucose uptake. In addition, metformin reduces insulin resistance in muscle tissue and the liver, decreasing postprandial hyperglycemia and inhibits adipose tissue lipolysis thereby reducing circulating levels of FFAs (Kirpichnikov, D., *et al*, 2002). Metformin may also suppress inflammation independently of action on glucose, insulin and FFAs. When used as monotherapy, metformin typically reduces HbA1C by about 1.5%. Metformin also improves the lipid profile and lowers blood pressure and plasminogen activator inhibitor-1 levels in both patients and animals with impaired glucose tolerance and type 2 diabetes. In overweight type 2 diabetic patients, metformin use is associated with decreases in macrovascular morbidity and mortality, effects that appear to be independent of the improvement in glycemic control (Dandona, P.,*et al*, 2003).

Thiazolidinediones

Thiazolidinediones (pioglitazone, rosiglitazone) With the introduction of this new class of drug in 1997, the world has watched the peroxisome proliferator activated receptor (PPAR)-γ agonists with anticipation. The net effect of these drugs results from stimulation of a nuclear PPAR-γ that regulates the transcription of genes culminating in an increase in insulin sensitivity.

Thiazolidinediones (TZDs) mediate their function through binding to the PPAR-γ receptor that is expressed predominantly in adipocytes. It is expressed to a lesser extent in muscle and liver tissue. Binding of the PPAR receptor in turn mediates binding to the retinoic-X receptor (RXR-receptor). This heterodimer then binds to a nuclear response element which then switches on gene transcription.

Many of the genes that are activated play a central role in carbohydrate and lipid metabolism. TZDs, like metformin, require the presence of insulin to mediate a blood

glucose-lowering effect. Interestingly, the thiazolidinediones also suppress the expression of TNF-α by adipocytes (Xiang, A.H.,*et al*, 2006).

α-glucosidase inhibitors

The α-glucosidase inhibitors, acarbose, miglitol and voglibose, slow digestion of oligosaccharides, thereby providing an alternative to reduce postprandial glucose levels. The α-glucosidase inhibitors do not cause weight gain, can reduce postprandial hyperinsulinemia, and have been shown to lower plasma triglyceride levels in some studies. They must be dosed multiple times per day and are associated with frequent gastrointestinal side effects (Nathan, D.M., *et al*, 2009).

They generally have less potent glucose-lowering effects than other oral anti-diabetics. In the STOP-NIDDM (Study to Prevent Non-Insulin Dependent Diabetes Mellitus) trial, acarbose reduced the incidence of new cases of type 2 diabetes in high-risk subjects with impaired glucose tolerance. Acarbose therapy is also associated with a reduction in incidence of cardiovascular events although some controversy is present (Van De Laar, F.A. and Lucassen, P.L., 2005).

Novel antidiabetic agents

The currently available therapies used for type 2 diabetes do not significantly improve β-cell function. In addition, the current approach does not address defects in hormonal secretion thought to play key roles in the pathophysiology of type 2 diabetes.

New emerging therapies for type 2 diabetes have become available in some countries in recent years. As a result of their recent availability, long-term studies are lacking and full safety profiles of these compounds are largely unknown, even though they are being used in large numbers of patients.

Incretins (exendin-4, liraglutide, vildagliptin, sitagliptin)

The small intestine secretes glucagon-like peptide-1 (GLP-1) as well as glucose-dependent insulinotropic polypeptide (GIP, previously called gastric inhibitory peptide) in response to food intake. These hormones stimulate insulin secretion, insulin gene expression and pancreatic betacell growth. Furthermore, they mediate the incretin effect which augments

insulin secretion following oral administration of glucose. The GLP-1 molecule is subject to rapid degradation by the DPP-IV (dipeptidyl peptidase) enzyme.

Amylin analogues (Pramlintide)

Human amylin is a 37-amino acid glucoregulatory peptide that is co-secreted with insulin by the pancreatic beta-cells. Pramlintide, a synthetic analogue, exerts its effect by slowing down gastric emptying and increasing satiety. Post-prandially, it decreases glucose levels and reduces the reintroduction of glucose in the circulation.

Pramlintide is administered as a subcutaneous injection immediately before a meal.

Insulin

Several different insulin analogs are available for type-1 and advanced type-2 diabetic patients. The injected insulin types differ in their onset and duration. Insulin therapy often has two components, an intermediate acting or long-acting insulin given at bedtime, and a rapid-acting insulin given before meals (Crasto, W., *et al*, 2009).

Insulin administration is the most effective means of restoring glycemic control; because there is no maximum dose, any HbA1C level can be reduced to the target range if insulin is dosed adequately. However, insulin has a number of limitations. It is typically administered by subcutaneous injection, often requiring multiple injections per day. Insulin carries a tangible risk of hypoglycemia, and regular self-monitoring of blood glucose is usually required. It is typically associated with weight gain that significantly increases in patients on intensive insulin therapy with adverse cardiovascular consequences. The ADA-EASD guidelines recommend addition of insulin as a second-step option for patients who are not adequately controlled on metformin monotherapy or as a third-step option for patients who still do not reach the HbA1C target goal on oral combination therapy. Insulin is also the treatment of choice for patients with severely uncontrolled or symptomatic type 2 diabetes (Nathan, D.M., *et al*, 2009).

Table 1.3: Comparison of glucose-lowering agents

Class	**Examples**	**Primary mode of action**	**Route of glycemic control**	**Adverse effects**
α-Glucosidase inhibitors	Acarbose, miglitol	Inhibit enzyme central to digestion of carbohydrates	Postprandial glucose	Diarrhea, abdominal pain, flatulence, ↑transaminases
Biguanides	Metformin	↓Hepatic glucose production, ↑muscle sensitivity to insulin	Fasting glucose, insulin sensitivity	Diarrhea, nausea, lactic acidosis
DPP-4 inhibitors	Sitagliptin	Inhibition of DPP-4 results in ↑GLP-1	Postprandial glucose	Upper respiratory infection, nasopharyngitis, headache
Meglitinides	Nateglinide, repaglinide	β-cell secretagogue	Postprandial glucose	Hypoglycemia

Sulfonylureas	Glimepiride, glipizide, glyburide	β-cell secretagogue	Fasting and postprandial glucose	Hypoglycemia, weight gain
Thiazolidinediones	Pioglitazone, rosiglitazone	Enhanced peripheral insulin sensitivity, improved hepatic insulin sensitivity	Insulin sensitivity, postprandial and fasting glucose	Fluid retention, weight gain, heart failure
Amylin analogues	Pramlintide	↓Glucagon secretion, gastric emptying, and food intake	Postprandial glucose	Nausea, hypoglycemia
Incretin mimetics	Exenatide	↓Glucagon secretion, gastric emptying, and food intake;	Postprandial glucose	Nausea, diarrhea, hypoglycemia, pancreatitis

		↑insulin secretion		
Insulin	—	—	Fasting and postprandial glucose	Hypoglycemia, weight gain

↓ decreased; ↑ increased; *DPP-4* dipeptidyl peptidase 4; *GLP-1* glucagon-like peptide-1

These drugs offer a range of different mechanisms of action that complement established therapies. Several of the novel drugs are based on the incretin hormone, GLP-1. GLP-1 controls glucose levels through various mechanisms including glucose-mediated insulin secretion, suppression of inappropriate glucagon release, slowing gastric emptying and increasing satiety. The natural hormone is rapidly degraded by the enzyme dipeptidyl peptidase 4 (DPP-4), and the two approaches to new agents are inhibitors of DPP-4 activity and development of GLP-1 analogues resistant to degradation. An important property of these agents is their neutral or beneficial effect on bodyweight (Sena, C.M., *et al*, 2013).

1.15 Medicinal Plants

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo pharmaceutical semi-synthesis. When a plant is designated as ‘medicinal’, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes.

1.16 Utilization of Medicinal Plants in Various Cultures

1.16.1 Greek period

Greek civilization was an epoch of science and philosophy. The Greeks have made worthy contribution in pharmaceutical sciences, especially in phytopharmaceuticals. Aristotle has described 500 crude drugs used in the cure of different pathological conditions. (JA., 1908) Hippocrates (460-337 BC) is considered as the father of allopathic medicine. He formulated the first scientific medical paradigm of treatment. He proposed that a large number of pathological conditions were due to disturbance in the normal physiology of human systems. The treatment was, therefore, based on the causes of the diseases to normalize the imbalance body systems. (Sykiotis et al, 2006). He has pointed out nearly 400 samples of medicinal substances from plant origin. Theophrastus (370-287 BC), a student of Aristotle, (J., 1978) has also mentioned 500 crude drugs in his book. Another important name is that of Claudius Galen Pergamum (modern-day Bergama, Turkey: 129-199). He prepared vegetable drugs using different extraction techniques called Galenicals and introduced the concept of pharmaceutical formulation to formulate stable and therapeutically effective drugs. (Newman et al, 2000). He wrote some 300 books on plants.

1.16.2 Traditional Chinese Medicine

Traditional Chinese Medicine represents one of the oldest systems of treatment. Traditional Chinese medicine is unique in theories, treatment, and therapies. This effective system of medicine has tremendous importance in the history of medicine and has now received global recognition due to its evidence basis approach (Patwardhan et al, 2005) (Buerki and Higby, 2007) This system is nearly free of external influence. Fu His (2953 BC) is considered as the pioneer of this system of medicine. The prescription of traditional Chinese medicine addresses those exogenous factors that are considered to be engaged in the pathology. Later, emperors Shen Nung and Hong Ti developed this system more significantly. Chinese pharmacopoeia Pen Tsao contained large numbers of remedies for various medical problems. Crown of written Chinese medicine goes to Shen Nong Ben Cao Jin (22-250 AD). CaoYuan Fang (550-630) wrote a book titled Zhu Bing Yuan Ji Lun, which described the etiology and symptoms of various diseases. This book is considered as a standard reference book for Chinese medical students. Wang Tao (702-772) has had

an important contribution to traditional Chinese medicine. His published work Waitai Miyao described approximately 600 prescriptions. The foundation of his diagnostic philosophy was tongue. During different pathological conditions, the color and status of tongue changes. (Kopp et al, 2003) A great Chinese physician and naturalist, Li Shizen, has written a more inclusive pharmacopoeia Ben Ca Gang Mu, which was published in 1596. It has 1894 prescriptions and is still in use as reference and guide for research and schooling in China and several other communities. Importantly, traditional Chinese medicine was traditional knowledge that passed through generations, but only in the 1950s was it formatted in the form of academic educational training. (Xu and Yang, 2009)

1.16.3 Traditional Indian Medicine

Traditional Indian Medicine or ayurveda (known as the mother of all therapies) is considered as the oldest health care system on earth. The descriptions of the system are available in ancient literatures such as Rig-Veda and Atharva-Veda, approximately 5000 years BC. (Hsu and Barrett, 2008) (Mukherjee and Wahile, 2006). Ayurveda is a Sanskrit word that literary means knowledge of life. It is a natural healing system consisting of a mixture of physiologic and holistic medicine. Ayurveda defines man as a matrix of 7 basic tissues that works in harmony while disease is the outcome of imbalance in these components of the body. (Routh and Bhowmik, 1999)

1.16.4 Arabic Period

The Arabs made enormous progress in the field of science and medicine after the fall of the Roman Empire. Scholars from the Islamic world translated books from Greece and Rome. Arab physicians introduced the concept of diet control and exercise along with medications. (Azaizeh et al, 2003). Arabs are actually the pioneers in the start of basic pharmacy practices. This includes the foundation of drug stores, the job description of physicians as diagnosticians of disease, and pharmacists being deputed for drug extraction and formulation. Due to this demarcation, the development in each field has started. As a result of this, Jaber Bin Hayan, a Muslim chemist, extracted and isolated various chemicals like alcohols, nitric acids, sulfuric acids, and so on. (Azaizeh et al, 2006). The religion of Islam has set a new breadth to the science of medicine in Arabia. Islam has specified means for a hygienic life style. (HM., 1978) These principles are primarily focused on Al Quran

and Sunnnah and are titled as Tibb al-Nabi. (Qureshi and Ghufran, 2007). Ali Ibn Rabban Al Tabri (782-855 AD) was a renowned Muslim scientist. His book Firdous Al Hikmat, 11 consists of 7 parts in which one is specially focused on drugs and poisons. Abu Ali Al Hussan Ibn Sina (Avicenna, 980-932 AD) is the creator of the Greco-Arabic school of medicine.His book Canon was considered as a textbook on medicine in Europe, which describes more than 1000 drugs. His other book, Kitab Ash-Shifa, is considered as a scientific encyclopedia. Apart from the therapeutic and healing characteristics, the Arabs also described the toxic aspects of various plants. Abu Musa Jabir ben Hayyan has written a very comprehensive book on different plant poisons and antidotes: The Book on Poisons and Antidotes. (Saad et al, 2006)

1.16.5 South American medicine

The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca and tobacco was common in these countries in the 14th and 15th centuries.
(Sofowora, 1982).

1.17 Plants as a Basis of Some Important Drugs

Higher plants have been used as a source of drugs by mankind for several thousand years. In fact, ancient man was totally dependent on green plants for his day-to-day needs of medicaments. With the development of modern medicine, synthetic drugs and antibiotics, the importance of plants as raw material for drugs decreased considerably. However, plants were used as a basis of some of the most important drugs, even in the modern system of medicine. With the advancement of synthetic organic chemistry most of the active constituents of plants used in medicine were synthesized. At one time it was thought that ultimately all the plant drugs would be obtained from synthetic sources. However, in spite of phenomenal progress in the development of new drugs from synthetic sources and the appearance of antibiotics as major therapeutic agents, plants continue to provide basic raw materials for some of the most important drugs. Although data are not available for all countries, a study carried out in the United States by Farnsworth and his colleagues between 1958 and 1980 indicated that although the number of prescriptions issued by

community pharmacies in the United States increased considerably, the percentage of prescriptions containing one or more plant products 12 remained constant at a figure of 25%. It has been found that in highly developed countries like the United States more than 100 chemical constituents of definite structure derived from 41 species of plants were used in modern medicine. It has also been estimated that in addition to these active constituents, more than 96 crude extracts were also used in the United States. (Faried et al, 2000).

1.18 Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

Synergic Medicine:

The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

Support of Official Medicine:

In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

Preventive Medicine:

It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment. (Bassam and Rasool, 2012).

1.19 Classification of medicinal plants

Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification (Joy et al. 1998)

Table 1.4: Classification of medicinal plants

Based on part used	1. Whole plant: diffusa, Phyllanthus neruri 2. Root: Dasamula 3. Stem:Tinospora cordifolia, Acorus calamus 4. Bark: Saraca asoca 5. Leaf: Indigofera tinctoria, Lawsonia inermis, Aloe vera 6. Flower:Biophytum sensityvum, Mimusops elenji 7. Fruit: Solanum species 8. Seed: Datura stramonium
Based on habitant	1. Tropical: Andrographis paniculata 2. Sub-tropical: Mentha arvensis 3. Temperate: Atropa belladonna
Based on therapeutic Value	1. Antimalarial: Cinchona officinalis, Artemisia annua 2. Anticancer: Catharanthus roseus, Taxus baccata 3. Antiulcer: Azadirachta indica, Glycyrrhiza glabra 4. Antidiabetic: Catharanthus roseus, Momordica charantia 5. Anticholesterol: Allium sativum 6. Antiinflammatory: Curcuma domestica, Desmodium gangeticum 7. Antiviral: Acacia catechu 8. Antibacterial: Plumbago indica 9. Antifungal: Allium sativum 10. Antiprotozoal: Ailanthus sp., Cephaelis ipecacuanha 11. Antidiarrhoeal: Psidium gujava, Curcuma domestica 12. Hypotensive: Coleus forskohlii, Alium sativum 13. Tranquilizing: Rauvolfia serpentina 14. Anaesthetic: Erythroxylum coca 15. Spasmolytic: Atropa belladona, Hyoscyamus niger 16. Diuretic: Phyllanthus niruri, Centella asiatica 17. Astringent: Piper betle, Abrus precatorius 18. Anthelmentic: Quisqualis indica, Punica granatum

	19. Cardiotonic: Digitalis sp., Thevetia sp. 20. Antiallergic: Nandina domestica, Scutellaria baicalensis 21. Hepatoprotective: Silybum marianum, Andrographis paniculata

1.20 Use of Medicinal Plants to Treat Diabetes

Popularity of herbal medicine in Bangladesh is high. There are many people, who have chosen to take alternative medicine over allopathic medicine as ailment. There are lots of people who still believes in natural treatment. That is why, the market share by herbal medicine is high. The market value for Bangladeshi medicinal plants are approximately Tk. 3,300 million at trade prices. The yearly turnover for the Ayurvedic sector is around Tk. 1,000 million, Unani around Tk. 1,800 million and homeopathy around Tk. 500 million.

Plants have formed the basis for the treatment of diseases in traditional medicine systems for thousands of years, and continue to play a major role in the primary health care of about 80% of the world's inhabitants (Farnsworth, N.R.,*et al,* 1985). It is estimated that 66-80% of medicines used in developing countries are based on plants. Many of the currently available drugs have been derived directly or indirectly from plants. Within developed countries 25% of medicinal therapies contain active priciples derived from plants (Bailey, C.J. and Day, C., 1989). Besides providing active raw materials, plants can offer molecules that serve as templates for the development of new drugs.

World ethnobotanical information about medicinal plants reports that almost 800 plants are used in the control of diabetes mellitus. Over the last two decades, several comprehensive reviews (Bever, B.O. and Zahnd, G.R., 1979) (Ivorra, M.D., *et al,* 1989) have been written on the evidence that higher plants are of use in the treatment of diabetes, providing discussions of the botany, phytochemistry, pharmacology, and in some cases, toxicology, of the botanical agents. Literally hundreds of extracts of higher plants used in folk medicine for diabetes (or active principles derived from these plants) have been screened for their biologic activity in both in vitro and in vivo assays. The most extensive review (Marles, R.J. and Farnsworth, N.R., 1995). evaluated available data on more than 1000 species of

plants reported to have been used to treat diabetes and/or been investigated for antidiabetic activity, and indicated that approximate 80% of the traditional plants used for the treatment of diabetes demonstrated some antidiabetic activity. In many instances the chemical constituent in the plant responsible for the biological activity has been isolated and identified, and information is also available concerning the mechanism of action. Galega officinalis (goat's rue), used in Europe as a treatment for diabetes since medieval times, yields a hypoglycemic principle rich in guanidine (Bailey 1985). Further derivatives of this principle have given rise to biguanides and the present anti-diabetic agent metformin (Sterne, J., 1969).

Prior to the discovery of insulin in 1992 and the later development of oral hypoglycemic agents, the major form of treatment of diabetes mellitus involved dietary manipulation and the use of plant therapies. The recommended use of plants dates back to the Ebers papyrus of around 1550 BC. More than 400 plants world-wide have been documented for the treatment of diabetes and the majority await proper scientific and medical evaluation. Most of these traditional medicines are prepared from herbs, spices and plants, which do not form part of the normal diet (Bailey, C.J. and Day, C., 1989). However, several common components of the diet are traditionally recommended for regular consumption, and some are additionally taken as infusions, decoctions or alcoholic extracts. The World Health Organization has recommended accordingly that traditional plant treatments for diabetes warrant further evaluation (World Health Organization, 1999)

With few exceptions, traditional plant treatments for diabetes have not claimed to be alternatives to insulin therapy in type 1. Isolated reports have described plant-derived materials that exert an insulin-like effect in type 1 diabetes. However these reports have not been independently evaluated, and there is no evidence that they could provide a long-term botanical substitute for insulin. A small number of review articles exist which document hypoglycemic agents derived from plant treatments for diabetes (Alam, M.B., 2017). Many traditional plant treatments owe their folklore reputation, at least in part, to the presence of polysaccharides, which achieve beneficial effects through reduction of gastrointestinal processing and post-prandial hyperglycemia. However for the majority of traditional plant treatments the active principles present together with their mode of action

have yet to be realized (Swanston-Flatt, S.K., *et al*, 1991.). Hypoglycemic compounds from plants that help directly combat insulin resistance and/or promote endogenous insulin release are realistic possibilities.

1.21 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 (Jouad, H., *et al,* 2001).

1.22 Medicinal Plants with reported Antidiabetic Effect on experimental models

Plant(Family)	Part of Plant Used	Material	Result
Annona Sqamosa (Annonaceae)	Fruit peel	Alcohol, ether, ethyl acetate	Significant increase body weight and diminished blood glucose level
Piper longum (Piperaceae)	Root	Aqueous and ethanolic extract	Streptozotocin HbAlc level were well regulated near to normal
Calamus erectus (Arecaceae)	Fruit	Methanolic extract	Reduction of blood glucose level
Tamarandusindica Linn	Seeds	Aqueous extract	effective in type II diabetic rat model
MomordicaCharantia (Cucurbitaceae)	Plant	Alcoholic extract	lower the blood sugar level
dactyliferalinn (Arecaceae)	dried dates	Aqueous extract	reduction in blood glucose level
Zizyphusnummularia (Rhamnaceae)	Leaves	Aqueous and 12% ethanolic extract	reduction in blood glucose level and body weight maintained
SwertiaChirata (Gentianaceae)	Whole plant	Aqueous and 12% ethanolic extracts	Significant antidiabetic activity

Tamarandusindica Linn (Caesalpiniaceae)	Fruit pulp	Ethanolic extracts	Antidiabetic effect
ParmeliaPerlata. Ach (Permeliaceae)	Leaves	Aqueous extract	Reduced the fasting blood glucose and HbA1C level
Gomphrenagobosa (Amaranthaceae)	whole plant	Methanolic n-Hexane, chloroform, Carbon tetrachloride and aqueous extracts	Lower the blood glucose level
Psidiumguvajava (Myrtaceae)	Leaves	Ethanolic extract	reduction in blood glucose level

Table 1.5: Medicinal Plants with reported Antidiabetic Effect

(Babu, P.A., *et al,* 2006.)

1.23 Cinnamomum zeylanicum

Cinnamon (Cinnamomum zeylanicum, and Cinnamon cassia), the eternal tree of tropical medicine, belongs to the Lauraceae family. Cinnamon is one of the most important spices used daily by people all over the world. Cinnamon primarily contains vital oils and other derivatives, such as cinnamaldehyde, cinnamic acid, and cinnamate. In addition to being an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering compound, cinnamon has also been reported to have activities against neurological disorders, such as Parkinson's and Alzheimer's diseases. This review illustrates the pharmacological prospective of cinnamon and its use in daily life (Rao, P.V. and Gan, S.H., 2014).

1.23.1 General Description

The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Overall, approximately 250 species have been identified among the cinnamon genus, with trees being scattered all over the world. Cinnamon is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products. The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde (Cin), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon. A study on Cinnamomumosmophloeum (C. osmophloeum) indicated that the essential oil from cinnamon leaves contains a high level of Cin. Consequently, C. osmophloeum is also used as an alternative spice for C. cassia. One of the major constituents of essential oil extracted from C. zeylanicum named (E)-cinnamaldehyde has an antityrosinase activity, while cinnamaldehyde is the principal compound responsible for this activity. Cinnamon bark contains procyanidins and catechins. The components of procyanidins include both procyanidin A-type and B-type linkages. These procyanidins extracted from cinnamon and berries also possess antioxidant activities (Rao, P.V. and Gan, S.H., 2014).

Figure 1.9: Cinnamon Tree

1.23.2 Varieties of Cinnamon

In North America, the most common spice labeled as 'cinnamon' is actually cassia, a similar spice also known as Chinese cinnamon. It is harvested from the bark of the *Cinnamomumaromaticum* tree. *Cinnamomumaromaticum* is an evergreen tree native to southern Bangladesh, China, India, Uganda and Vietnam. Cassia is cheaper to produce and has a bolder, less subtle flavor than true cinnamon, so it is sometimes referred to as 'bastard cinnamon.'

The spice more correctly known as cinnamon is harvested from the inner bark of the *Cinnamomumverum* tree. It is also known as 'Ceylon cinnamon,' a reference to its native country of Sri Lanka (which was formerly known as Ceylon). Today, Ceylon cinnamon is commonly grown in Sri Lanka, India (particularly in the southern state of Kerala), Bangladesh, Brazil, Vietnam, and Madagascar, amongst other countries.

Ceylon cinnamon has a delicate, nuanced flavor that works well in sweet and savory foods and drinks. It has a paler color than cassia and is comprised of many thin layers of bark rather than a single coiled strip of bark. True cinnamon is soft enough to be ground in a (clean) coffee grinder.

Other varieties of cinnamon include *Cinnamomumaromaticum* (a close relative of Ceylon cinnamon, Saigon cinnamon (also known as Vietnamese cinnamon or *Cinnamon loureiroi*), camphor laurel (*Cinnamon camphora*), malabathrum (*Cinnamon tamala*) and Indonesian cinnamon (*Cinnamon burmannii*).

1.23.3 Chemical Constituents

Cinnamon consists of a variety of resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and numerous essential oils (Table 1). The spicy taste and fragrance are due to the presence of cinnamaldehyde and occur due to the absorption of oxygen. As cinnamon ages, it darkens in color, improving the resinous compounds. Various physiochemical properties of cinnamon presented on Table 2. The presence of a wide range of essential oils, such as trans-cinnamaldehyde, cinnamyl acetate, eugenol, L-borneol, caryophyllene oxide, b-caryophyllene, L-bornyl acetate, E-nerolidol, α-cubebene,

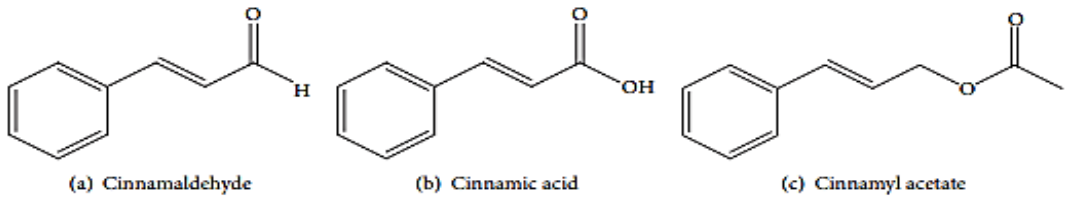
α-terpineol, terpinolene, and α-thujene, has been reported. The chemical structures of some important constituents of cinnamon are shown in Figures 1, 2, 3, 4, and 5.

Table 1.6: Chemical constituents of different parts of cinnamon (Vangalapati et al., 2012).

Part of the plant	Compound
Leaves	Cinnamaldehyde: 1.00 to 5.00% Eugenol: 70.00 to 95.00%
Bark	Cinnamaldehyde: 65.00 to 80.00% Eugenol: 5.00 to 10.00%
Root bark	Camphor: 60.00%
Fruit	trans-Cinnamyl acetate (42.00 to 54.00%) andcaryophyllene(9.00 to 14.00%)
C. zeylanicum buds	Terpene hydrocarbons: 78.00% alpha-Bergamotene: 27.38% alpha-Copaene: 23.05% Oxygenated terpenoids: 9.00%
C. zeylanicum flowers	(E)-Cinnamyl acetate: 41.98% trans-alpha-Bergamotene: 7.97% Caryophyllene oxide: 7.20%

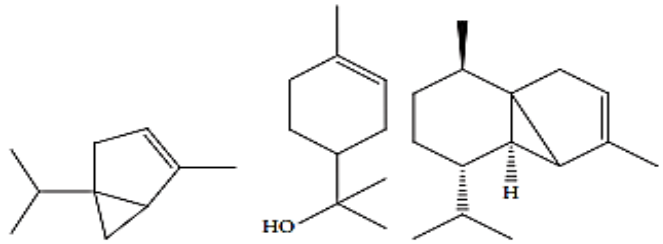
Table 1.7: Physicochemical properties of cinnamon (Sangal. 2011).

Parameter	Leaf oil	Bark oil
Specific gravity (20°C)	1.030–1.050	1.010–1.030
Optical rotation (°) (20°C)	1°9'–0°40'	Slightly laevorotatory
Refractive index (20∘ C)	1.529–1.537	1.573–1.591
Aldehyde content	4%	65–76%
Eugenol content	77.3–90.5%	4–10%
Solubility characteristics	Soluble in 1.5 volumes of 70% alcohol	Soluble in 2.0–3.0 volumes of 70% alcohol



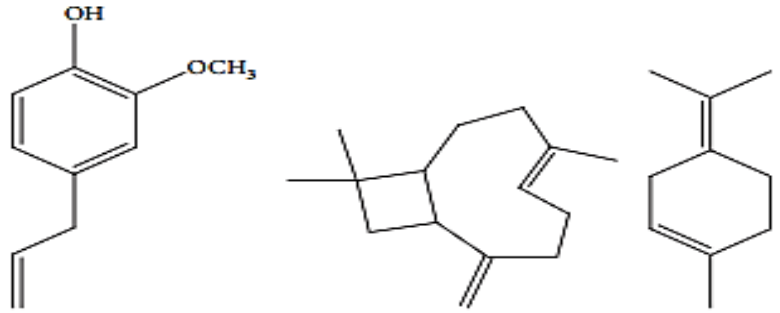
(a) Cinnamaldehyde (b) Cinnamic acid (c) Cinnamyl acetate

FIGURE 1: Cinnamyl group-containing compounds.



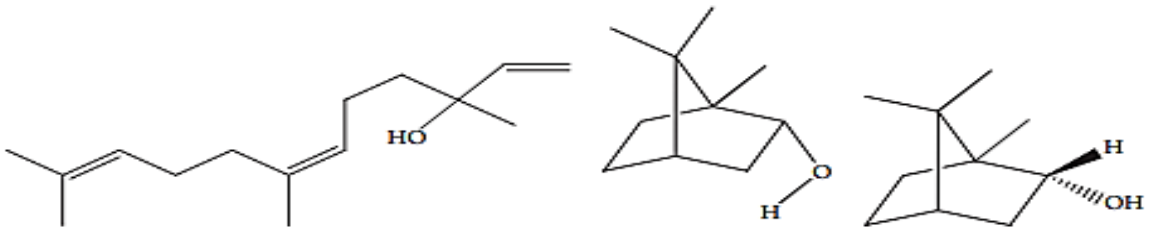
(a) α-Thujene (b) α-Terpineol (c) α-Cubebene

FIGURE 2: Endocyclic double bond-containing compounds.



(a) Eugenol (b) β-Caryophyllene (c) Terpinolene

FIGURE 3: Unconjugated exocyclic double bond-containing compounds.



(a) E-Nerolidol (b) L-Borneol (c) Borneol

FIGURE 4: Hydroxy-substituted aliphatic compounds.

Figure 1.10: Chemical constituents of Cinnamon

1.23.4 Classification of Acacia

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Super division	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Magnoliidae
Order	Laurales
Family	Lauraceae – Laurel family
Genus	*Cinnamomum* Schaeff. – cinnamon
Species	*Cinnamomum zeylanicum* Garcin ex Blume

Figure1.11: Cinnamon Bark

1.23.5 Medicinal Uses and Pharmacological Effects

- **Antioxidant activity**

Antioxidant compounds present in foodstuffs play a vital role in human life, acting as health-protecting agents. In addition to this role, antioxidants are one of the key additives used in fats and oils. Even in the food processing industry, Chemical constituents of different parts of cinnamon (Vangalapati et al., 2012). Part of the plant Compound Leaves

Cinnamaldehyde: 1.00 to 5.00% Eugenol: 70.00 to 95.00% Bark Cinnamaldehyde: 65.00 to 80.00% Eugenol: 5.00 to 10.00% Root bark Camphor: 60.00% Fruit trans-Cinnamyl acetate (42.00 to 54.00%) and caryophyllene (9.00 to 14.00%) C. zeylanicum buds Terpene hydrocarbons: 78.00% alpha-Bergamotene: 27.38% alpha-Copaene: 23.05% Oxygenated terpenoids: 9.00% C. zeylanicum flowers (E)-Cinnamyl acetate: 41.98% trans-alpha-Bergamotene: 7.97% Caryophyllene oxide: 7.20% antioxidants have been used to delay or prevent food spoilage. Spices and medicinal plants have received rapid consideration as sources of beneficial antioxidants against various diseases (Mancini-Filho, J., *et al*, 1998). Antioxidants have been considered the most important drivers in the progress and existence of humans, as they respond to free radicals and damage in metabolic diseases and age-related syndromes of humans and other animals [53, 54]. Mancini-Filho et al. reported various extracts of cinnamon, such as ether, aqueous, and methanolic extracts that have shown considerable antioxidant activities (Halliwell, B., 2011).

Anti-Inflammatory Activities

Several studies on medicinal plants and their components have indicated the anti-inflammatory activities of cinnamon. Various studies reported the anti-inflammatory activity of cinnamon and its essential oils. To date, there are several flavonoid compounds (e.g., gossypin, gnaphalin, hesperidin, hibifolin, hypolaetin, oroxindin, and quercetin) that have been isolated and have antiinflammatory activities. A recent study reported that 2-hydroxy cinnamaldehyde isolated from C. cassia bark exhibited an inhibitory effect on the production of nitric oxide by inhibiting the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), indicating that this substance can potentially be used as an anti-inflammatory agent. The ethanolic extract of C. cassia showed significant anti-inflammatory effects by reducing the activation of Src/spleen-tyrosinekinase- (Src/Syk-) mediated NF-κB. Various compounds contained in C. ramulus showed anti-inflammatory effects by suppressing the expression of inducible nitric oxide synthesis (iNOS), cyclooxygenase-2 (COX-2), and nitric oxide (NO) production in the central nervous system (CNS). By this mechanism, C. ramulus could be a potential source for the therapeutic treatment or prevention of inflammation-mediated neurodegenerative diseases. Furthermore, the aqueous extract of cinnamon decreases the lipopolysaccharide-induced tumor necrosis factor-α levels in the serum (Hong, J.W., *et al*, 2012).

Neurological Disorders

Cinnamophilin is a novel thromboxane A2 receptor antagonist isolated from C. philippinensis. A study reported that cinnamophilin confers protection against ischemic damage in rat brains when administered at 80 mg/kg at different time intervals (2, 4, and 6 h) after insult. The effects were found to have a considerable effect (by 34–43%) on abridged brain infarction and further enhance neurobehavioral outcomes. Cinnamophilin also dramatically condenses the oxygen glucose deprivation-induced neuronal damage in organotypic hippocampal slices in experimental rats. A substance called procyanidin type-A trimer (trimer 1) isolated from cinnamon's water-soluble extract showed that trimer 1 may reduce cell swelling by controlling the movement of intracellular calcium [Ca2+]. Trimer 1 also considerably alleviates the oxygen glucose deprivation-induced diminishing effects on glutamate uptake. The protective effects of trimer 1 in attenuating the diminution in glutamate uptake are possibly arbitrated via their effects on the mitochondria. Parkinson's disease (PD) is the second major widespread neurodegenerative disorder after Alzheimer's disease, with a prevalence of 2% in people 65 years and older. PD protein 7 (PARK7) is an autosomal recessive form of early-onset parkinsonism caused by alterations in the DJ1 gene. Cinnamon and its metabolite sodium benzoate also upregulate the neurotropic factors BDNF (brain-derived neurotropic factors) as well as neurotrophin-3 (NT-3) in the mouse central nervous system [99]. PARK7 is one of the main neuroprotective proteins that protects cells from damage and from the further detrimental effects of oxidative stress; therefore, this protein may be an effective molecule that can be incorporated into the therapeutic intervention of Parkinson's disease. A natural compound isolated from cinnamon extract (CEppt) significantly reduces the formation of toxic β-amyloid polypeptide (Aβ) oligomers and prevents its toxicity on neuronal pheochromocytoma (PC12) cells. The study indicated that CEppt resolved the reduced permanence, fully improved deficiencies in locomotion, and totally eradicated the tetrameric species of Aβ in the brain of the fly model of Alzheimer's disease, leading to a noticeable reduction in the 56 kDa Aβ oligomers, reducing plaques and improving the cognitive performance of transgenic mice models. Another study reported that the aqueous extract of C. zeylanicum can reduce tau aggregation and filament formation, two of the main features of Alzheimer's disease. The extract can also encourage the complete

fragmentation of recombinant tau filaments and cause the considerable modification of the morphology of paired helical filaments from Alzheimer's disease brain, indicating the potential of cinnamon in the treatment of Alzheimer's disease (Peterson, D.W., *et al*, 2009).

Antidiabetic Activity

A substance from cinnamon has been isolated and coined as "insulin-potentiating factor" (IPF), while the antidiabetic effects of cinnamon bark have been shown in streptozotocin-induced diabetic rats. Several studies have also revealed that cinnamon extracts lower not only blood glucose but also cholesterol levels. A study comparing the insulin-potentiating effects of many spices revealed that the aqueous extract of cinnamon was 20-fold higher than the other spices. Methylhydroxychalcone polymer (MHCP) is the purified polymer of hydroxychalcone with the ability to stimulate glucose oxidation. Anderson et al. isolated and characterized the polyphenol type-A polymers from cinnamon and found that these substances act as insulin-like molecules. Following this characterization, a new compound from hydroxycinnamic acid derivatives named naphthalenemethyl ester, which has blood glucose-lowering effects, has been identified, further confirming cinnamon's antidiabetic effects. Several polyphenols have been isolated from cinnamon. These polyphenols include rutin (90.0672%), catechin (1.9%), quercetin (0.172%), kaempferol (0.016%), and isorhamnetin (0.103%). Cao et al. (2007) demonstrated that the aqueous extract of cinnamon containing polyphenols purified by high performance liquid chromatography (HPLC) showed insulin-like activity (H. Cao, *et al*, 2007).

The aqueous extract of cinnamon markedly decreased the absorption of alanine in the rat intestine. Alanine plays a vital role in gluconeogenesis, is altered back to pyruvate in the liver, and is utilized as a substrate for gluconeogenesis. However, another study conducted on diabetic postmenopausal women supplemented with cinnamon showed poor glycemic control, even though cinnamon is generally believed to be useful for diabetes. However, it is plausible that differences in the dose of cinnamon used, as well as baseline glucose and lipid levels, have led to these variations. In a recent study, suitable doses of cinnamon (5, 10, and 20 mg/kg) of the linalool chemotype were found to help with glycemic control in diabetics due to enhanced insulin secretion. It is plausible that the amelioration of oxidative

stress and the proinflammatory environment in the pancreas may confer protection to pancreatic β cells, which should be further investigated.

Antimicrobial Activity

To date, several antimicrobial activities of cinnamon and its oils have been reported in various studies. For example, Matan et al. reported the effects of cinnamon oils on different bacterial (Pediococcushalophilus and Staphylococcus aureus), fungal (Aspergillus flavus, Mucorplumbeus, Penicilliumroqueforti, and Eurotium sp.), and yeast species (Candida lipolytica, Pichia membranaefaciens, Debaryomyceshansenii, and Zygosaccharomycesrouxii), indicating that cinnamon is a natural antimicrobial agent. Goni et al. described the antibacterial activity of a com- ˜ bination of cinnamon and clove oils against Gram-positive organisms (Listeria monocytogenes, Enterococcus faecalis, Staphylococcus aureus, and Bacillus cereus), as well as against Gram-negative bacteria (Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa, and Yersinia enterocolitica). A study from Hili et al. indicated that cinnamon oils have potential action against various bacteria (Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli) and yeast (Torulopsisutilis, Schizosaccharomycespombe, Candida albicans, and Saccharomyces cerevisiae) [18]. A recent study reported the activity of the aqueous extract of cinnamon and other plants against oral microflora. Overall, the essential oil from cinnamon is more potent than other tested plant extracts, such as Azadirachtaindica and Syzygiumaromaticum (Goni, P., *et al*, 2009).

Anticancer Activity

The aqueous extract and the fraction of cinnamon (procyanidins) from HPLC inhibit vascular endothelial growth factor subtype 2 (VEGFR2) kinase activity, thereby inhibiting the angiogenesis involved in cancer. The results of the study revealed that cinnamon could potentially be used in cancer prevention. Cinnamaldehydes have been synthesized and tested as inhibitors against angiogenesis. Jeong et al. reported that CB403, a chemical that can be synthesized from 2-hydroxycinnamaldehyde derived from cinnamaldehyde, can inhibit tumor growth. Overall, the antitumor and growthinhibitory properties of CB403 in animal-based studies as well as in cell culture-based studies indicate the potential of cinnamon to be used as an anticancer agent. Cabello et al. reported that cinnamic aldehyde inhibits the activity of NF-κB and the production of tumor necrosis factor alpha (TNFα-)

induced interleukin-8 (IL-8) in A375 cells. This inhibition provides additional support to the existing unrecognized role of cinnamic acid as a potential anticancer agent. Fang and others reported the anticancer effect of trans-cinnamaldehyde from C. osmophloeum, finding that trans-cinnamaldehyde showed potential effects in restraining tumor cell growth and in enhancing tumor cell apoptosis. A preliminary study on cinnamon and cardamom against azoxymethane- (AOM-) induced colon cancer in Swiss albino mice has been conducted. (Bhattacharjee, S., Rana, T. and Sengupta, A., 2007).Treatments with the aqueous extracts of cinnamon and cardamom augment the activities of the detoxifying and antioxidant enzyme glutathione-stransferase (GST) with a concomitant reduction in lipid peroxidation levels in animals with colon cancer compared to controls. The essential oils extracted from C. cassia inhibit alpha melanocyte-stimulating hormone's induced melanin production, thereby suppressing oxidative stress in murine B16 melanoma cells (Cabello, C.M., *et al,* 2009).

Cardiovascular Diseases

One of the active components isolated from C. cassia named 2-methoxycinnamaldehyde (2-MCA) decreases the expression of vascular cell adhesion molecule-1 (VCAM1) in TNFα-activated endothelial cells, suggesting that ischemia/reperfusion (I/R) injury is ameliorated due to the induction of hemeoxygenase- (HO-) 1. A recent study reported the potential effects of two compounds, cinnamic aldehyde and cinnamic acid, isolated from C. cassia against myocardial ischemia, indicating that cinnamon also has the potential to be used to treat cardiovascular diseases. Several studies have reported the protective effects of cinnamaldehyde on the cardiovascular system. Cinnamophilin is one of the important lignans isolated from C. philippinensis and has been confirmed to have thromboxane A2 (TXA2) receptor blocking activity in rats as well as in guinea pigs (Song, F., Li, H., Sun, J. and Wang, S., 2013).

Cinnamophilin acts as a potential thromboxane synthase inhibitor and TXA2 receptor antagonist and may be helpful when incorporated in the treatment of diseases involving TXA2 disorders, such as platelet aggregation and cancers. Cinnamophilin mainly inhibits thromboxane receptor-mediated vascular smooth muscle cell proliferation and may have the potential for use in the prevention of vascular diseases and atherosclerosis [128]. Cinnamaldehyde produces hypotensive effects, which are possibly mainly due to

peripheral vasodilatation in anesthetized dogs and guinea pigs. The vasodilatation induced by cinnamaldehyde in dogs lasted and remained over the recovery period of the fall in blood pressure to the baseline [130]. A recent study showed that cinnamaldehyde expands rat vascular smooth muscle in an endotheliumindependent manner. The ability of cinnamaldehyde in vasodilatory function may be because it impedes both Ca2+ influx and Ca2+ release. Cinnamaldehyde averts the progress of hypertension in types 1 and 2 diabetes by abridging vascular contractility, in addition to its insulinotropic effect in insulin deficiency (El-Bassossy, H.M., *et al*, 2011).

Cholesterol- and Lipid-Lowering Effects

The administration of cinnamon to mice positively affected the lipid profile, whereby the high density lipoprotein (HDL) cholesterol levels decreased, and plasma triglycerides were reduced [27]. Another study by [133] found a reduction in the total cholesterol, triglycerides, and low-density lipoproteins in rats administered Cinnamomum cassia powder (15%) for 35 days. Additionally, cinnamon oils reduced the cholesterol levels in broiler chickens. A study by Khan et al. reported that the administration of cinnamon at 1, 3, and 6 g doses per day caused a reduction in serum glucose,triglyceride, total cholesterol, and LDL cholesterol levels in humans (Khan, A., Safdar, M., Khan, M.M.A., Khattak, K.N. and Anderson, R.A., 2003. Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes care*, *26*(12), pp.3215-3218.

Advanced Glycation End Products (AGEs)

Different types of phenolic and flavonoid compounds have been isolated from cinnamon. Epicatechin, catechin, and procyanidin B2, which are the phenolic compounds isolated from cinnamon, showed noteworthy and potentially inhibitory activities on the formation of AGEs. These antiglycation activities of the phenolic compounds not only are attributed to their antioxidant activities but also are associated with the entrapping capabilities of reactive carbonyl species, such as methylglyoxal (MGO), an intermediate reactive carbonyl of AGE formation. The inhibition of AGE formation by trapping the reactive carbonyl species could be a logical therapeutic approach to treat diabetes and its complications.

Cinnamon has been used as a spice in daily life without any side effects. Several reports have dealt with the numerous properties of cinnamon in the forms of bark, essential oils, bark powder, phenolic compounds, flavonoids, and isolated components. Each of these

properties plays a key role in the advancement of human health. The antioxidant and antimicrobial activities may occur through the direct action on oxidants or microbes, whereas the anti-inflammatory, anticancer, and antidiabetic activities occur indirectly via receptor-mediated mechanisms. The significant health benefits of numerous types of cinnamon have been explored. Further investigations are necessary to provide additional clinical evidence for the traditional uses of this spice against cancer and inflammatory, cardioprotective, and neurological disorders. (Rao, P.V. and Gan, S.H., 2014.)

CHAPTER 2

OBJECTIVE OF THE STUDY

2.1 Research Objective

The objective of this research work has therefore focused on the following point:

- To evaluate the anti-hyperglycemic effect of the methanolic extract of the barks of plant *Cinnamon* in long evans rats.

- To determine the anti-diabetic efficacy of the plant *Cinnamon.*

CHAPTER 3

LITERATURE REVIEW

3.1 Literature Review on *Cinnamon*

We have studied on stem, bark and leaf of this plant. Here is some literature review on different parts of this plant.

3.1.1 Diverse Mechanisms of Antidiabetic Effects of the Different Procyanidin Oligomer Types of Two Different Cinnamon Species on db/db Mice (Chen, L., *et al*, 2012)

The procyanidin oligomers are thought to be responsible for the antidiabetic activity of cinnamon. To investigate the hypoglycemic effects of different procyanidin oligomer types, the procyanidin oligomer-rich extracts were prepared from two different cinnamon species. Using high-performance liquid chromatography with purified procyanidin oligomers as reference compounds, we found that the *Cinnamomum cassia* extract (CC-E) and *Cinnamomum tamala* extract (CT-E) were rich in B- and A-type procyanidin oligomers, respectively. Both CC-E and CT-E exhibited antidiabetic effects. Moreover, histopathological studies of the pancreas, liver, and adipose tissue showed that CC-E promoted lipid accumulation in the adipose tissue and liver, whereas CT-E mainly improved the insulin concentration in the blood and pancreas.

3.1.2 In vivo and in vitro antidiabetic effects of aqueous cinnamon extract and cinnamon polyphenol-enhanced food matrix (Cheng, D.M., *et al*, 2012)

Cinnamon has a long history of medicinal use and continues to be valued for its therapeutic potential for improving metabolic disorders such as type 2 diabetes. In this study, a phytochemically-enhanced functional food ingredient that captures water soluble polyphenols from aqueous cinnamon extract (CE) onto a protein rich matrix was developed. CE and cinnamon polyphenol-enriched defatted soy flour (CDSF) were effective in acutely lowering fasting blood glucose levels in diet induced obese hyperglycemic mice at 300 and 600 mg/kg, respectively. To determine mechanisms of action, rat hepatoma cells were treated with CE and eluates of CDSF at a range of 1–25 μg/ml. CE and eluates of CDSF demonstrated dose-dependent inhibition of hepatic glucose production with significant levels of inhibition at 25 μg/ml. Furthermore, CE decreased the gene expression of two major regulators of hepatic gluconeogenesis,

phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. The hypoglycemic and insulin-like effects of CE and CDSF may help to ameliorate type 2 diabetes conditions.

3.1.3 Antidiabetic effects of cinnamon oil in diabetic KK-Ay mice (Ping, H., *et al*, 2010)

Abstract

The hypoglycemic effect of cinnamon oil (CO) in a type 2 diabetic animal model (KK-Ay mice) was studied. The main component of CO was cinnamaldehyde, and other nineteen components were also determined. CO was administrated at doses of 25, 50 and 100 mg/kg for 35 days. It was found that fasting blood glucose concentration was significantly decreased ($P < 0.05$) with the 100 mg/kg group ($P < 0.01$) the most efficient compared with the diabetic control group. In addition, there was significant decrease in plasma C-peptide, serum triglyceride, total cholesterol and blood urea nitrogen levels while serum high density lipoprotein (HDL)-cholesterol levels were significantly increased after 35 days. Meanwhile, glucose tolerance was improved, and the immunoreactive of pancreatic islets β-cells was promoted. These results suggest that CO had a regulative role in blood glucose level and lipids, and improved the function of pancreatic islets. Cinnamon oil may be useful in the treatment of type 2 diabetes mellitus.

3.1.4 Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats (Shihabudeen, *et al*, 2011)

α-glucosidase inhibitors regulate postprandial hyperglycemia (PPHG) by impeding the rate of carbohydrate digestion in the small intestine and thereby hampering the diet associated acute glucose excursion. PPHG is a major risk factor for diabetic vascular complications leading to disabilities and mortality in diabetics. *Cinnamomum zeylanicum*, a spice, has been used in traditional medicine for treating diabetes. In this study we have evaluated the α-glucosidase inhibitory potential of cinnamon extract to control postprandial blood glucose level in maltose, sucrose loaded STZ induced diabetic rats.

3.1.5 Effects of cinnamon extract in diabetic rat models in comparison with oral hypoglycemic drugs (Fahim, A., 2001)

Tolbutamide and Acarbose showed better anti diabetic effect in comparison with cinnamon extract treated groups when used individually. This effect was enhanced when cinnamon was used in combination with either tolbutamide or acarbose.

3.1.6 Effectiveness of Cinnamon for Lowering Hemoglobin A1C in Patients with Type 2 Diabetes: A Randomized, Controlled Trial (Crawford, P., 2009)

Multiple trials in the past have shown conflicting results of whether cinnamon lowers glucose or hemoglobin A1C (HbA1C). The purpose of this study was to determine whether cinnamon lowers HbA1C in patients with type 2 diabetes. I performed a randomized, controlled trial to evaluate whether daily cinnamon plus usual care versus usual care alone lowers HbA1c.

Cinnamon lowered HbA1C 0.83% (95% CI, 0.46–1.20) compared with usual care alone lowering HbA1C 0.37% (95% CI, 0.15–0.59).

3.1.7 Role of cinnamon as beneficial antidiabetic food adjunct: a review (Sangal, A., 2011)

Diabetes mellitus (DM) is the most common of the endocrine disorders. It is an important human ailment, afflicting many, from various walks of life in different countries. Insulin is the ideal treatment for diabetes in the conditions where blood glucose levels cannot be controlled. The introduction of insulin and oral hypoglycemic agents has revolutionized the management of diabetes. Despite advances in drug management of diabetes, the adverse drug effects have made scientists to look towards hypoglycemic agents of plant origin, especially in the developing countries. Cinnamon is one such spice, which has the potential to attenuate the development of diabetes and its complication. It also does not have much troublesome side effects. The present paper reviews the outstanding ability of cinnamon, to tackle diabetes by boosting insulin function. It also points to the areas of future research, to further control many of the pathological mechanisms that cause diabetes.

3.1.8 Effects of a cinnamon extract on plasma glucose, HbA1c, and serum lipids in diabetes mellitus type 2 (Mang, B., *et al*, 2006)

According to previous studies, cinnamon may have a positive effect on the glycaemic control and the lipid profile in patients with diabetes mellitus type 2. The aim of this trial was to determine whether an aqueous cinnamon purified extract improves glycated haemoglobin A1c (HbA_{1c}), fasting plasma glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triacylglycerol concentrations in patients with type 2 diabetes.

3.1.9 Cinnamon Use in Type 2 Diabetes: An Updated Systematic Review and Meta-Analysis (Allen, R.W., *et al*, 2013)

Cinnamon has been studied in randomized controlled trials (RCTs) for its glycemic-lowering effects, but studies have been small and show conflicting results. A prior meta-analysis did not show significant results, but several RCTs have been published since then. We conducted an updated systematic review and meta-analysis of RCTs evaluating cinnamon's effect on glycemia and lipid levels.

3.1.10 Effect of Cinnamon on Glucose and Lipid Levels in Non–Insulin-Dependent Type 2 Diabetes (Blevins, S.M., *et al*, 2007)

Interest in cinnamon as a potentially useful treatment for type 2 diabetes began with the discovery almost 20 years ago of cinnamon's insulin-sensitizing properties (1). Numerous in vitro and in vivo studies have elucidated cinnamon's effect on insulin signal transduction (2–6). A study in diabetic mice showed that cinnamon lowered blood glucose, total cholesterol, and triglyceride levels while raising HDL cholesterol levels (7).

It report the first U.S. study examining the effects of cinnamon on glucose and lipid levels in subjects with type 2 diabetes.

3.1.11 Cinnamon Supplementation Does Not Improve Glycemic Control in Postmenopausal Type 2 Diabetes Patients (Vanschoonbeek, K., *et al*, 2006)

In vitro and in vivo animal studies have reported strong insulin-like or insulin-potentiating effects after cinnamon administration. Recently, a human intervention study showed that cinnamon supplementation (1 g/d) strongly reduced fasting blood glucose concentration

(30%) and improved the blood lipid profile in patients with type **k**2 diabetes. The objective of this study was to investigate the effects of cinnamon supplementation on insulin sensitivity and/or glucose tolerance and blood lipid profile in patients with type 2 diabetes. They conclude that cinnamon supplementation (1.5 g/d) does not improve whole-body insulin sensitivity or oral glucose tolerance and does not modulate blood lipid profile in postmenopausal patients with type 2 diabetes. More research on the proposed health benefits of cinnamon supplementation is warranted before health claims should be made.

3.1.12 Cinnamon: Potential Role in the Prevention of Insulin Resistance, Metabolic Syndrome, and Type 2 Diabetes (Qin, B., *et al*, 2010)

Metabolic syndrome is associated with insulin resistance, elevated glucose and lipids, inflammation, decreased antioxidant activity, increased weight gain, and increased glycation of proteins. Cinnamon has been shown to improve all of these variables in *in vitro*, animal, and/or human studies. In addition, cinnamon has been shown to alleviate factors associated with Alzheimer's disease by blocking and reversing tau formation *in vitro* and in ischemic stroke by blocking cell swelling. In summary, components of cinnamon may be important in the alleviation and prevention of the signs and symptoms of metabolic syndrome, type 2 diabetes, and cardiovascular and related diseases.

3.1.13 Cinnamon Supplementation in Patients with Type 2 Diabetes Mellitus (Pham, A.Q., *et al*, 2007)

Diabetes mellitus is the sixth leading cause of death in the United States, and most patients with the disease have type 2 diabetes. The effectiveness of cinnamon supplementation in patients with type 2 diabetes has received a great deal of media attention after a study was published in 2003. Although the efficacy of cinnamon in patients with diabetes has not been established, many patients seek other therapies and supplement their prescribed pharmacologic therapy with cinnamon. Here, two of the studies reported modest improvements in lowering blood glucose levels with cinnamon supplementation in small patient samples. One trial showed no significant difference between cinnamon and placebo in lowering blood glucose levels. Overall, cinnamon was well tolerated. These data suggest that cinnamon has a possible modest effect in lowering plasma glucose levels in patients with poorly controlled type 2 diabetes. However, clinicians are strongly urged to refrain

from recommending cinnamon supplementation in place of the proven standard of care, which includes lifestyle modifications, oral antidiabetic agents, and insulin therapy.

3.1.14 The effect of cinnamon cassia powder in type 2 diabetes mellitus (Suppapitiporn, S. and Kanpaksi, N., 2006)

Type 2 diabetes is a chronic metabolic disorder and the incidence of cardiovascular is increased two- to fourfold in its complications. Cinnamon is expected to have some degree of anti-diabetic efficacy without troublesome side effects. The objective of the present study was to investigate the anti-diabetic effect of cinnamon cassia powder in type 2 diabetic patients. The cinnamon cassia powder 1.5 g/d did not have any significant difference in reducing fasting plasma glucose, HbA1c and serum lipid profile in type 2 diabetes patients who had mean fasting plasma glucose 154.40 +/- 24.72 mg/dl.

3.1.15 From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark (Dugoua, J.J., *et al*, 2007)

Common (Cinnamomum verum, C. zeylanicum) and cassia (C. aromaticum) cinnamon have a long history of use as spices and flavouring agents. A number of pharmacological and clinical effects have been observed with their use. The objective of this study was to systematically review the scientific literature for preclinical and clinical evidence of safety, efficacy, and pharmacological activity of common and cassia cinnamon. One pharmacological study on antioxidant activity and 7 clinical studies on various medical conditions were reported in the scientific literature. Two of 3 randomized clinical trials on type 2 diabetes provided strong scientific evidence that cassia cinnamon demonstrates a therapeutic effect in reducing fasting blood glucose by 10.3%–29%; the third clinical trial did not observe this effect. Cassia cinnamon, however, did not have an effect at lowering glycosylated hemoglobin (HbA1c). One randomized clinical trial reported that cassia cinnamon lowered total cholesterol, low-density lipoprotein cholesterol, and triglycerides; the other 2 trials, however, did not observe this effect.

CHAPTER 4

MATERIALS & METHODS

4.1 Plant Material

Plant sample of ***Cinnamomum zeylanicum*on** were used for the experiment. They were processed in the laboratory.

4.1.1 Collection of Plant

The Plant sample ***Cinnamomum zeylanicum*on** collected and washed with water several times.

4.1.2 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.

4.1.3 Extraction (Methanol extraction)

500 gm of powered material was taken in a clean, flat bottomed glass container and soaked in 5000 litre of methanol, 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 (methanol 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 gummy concentrate was designated as the crude ethanol extract. It was then dried in the freeze drier and preserved at +4°C for two weeks.

4.1.4 Extraction Procedure

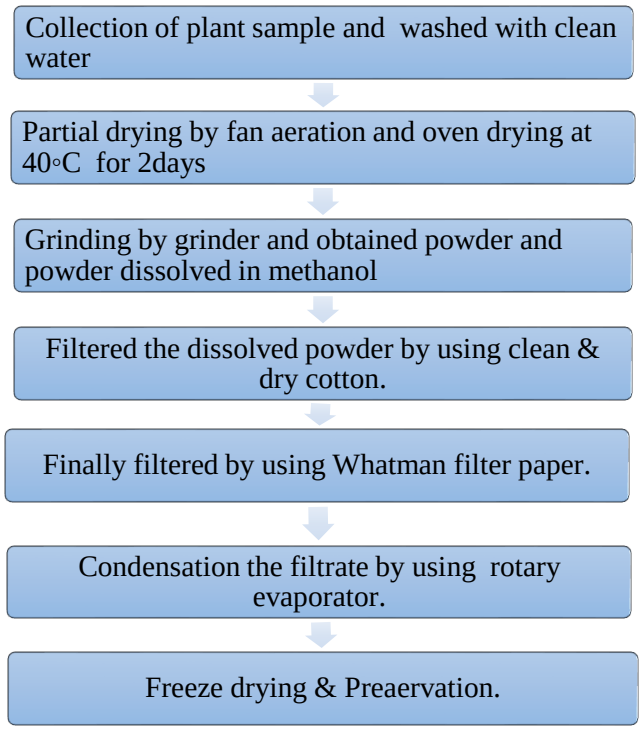


Figure 4.1: General Plant Extraction Procedure

4.2 Experimental animals

Mice and 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)

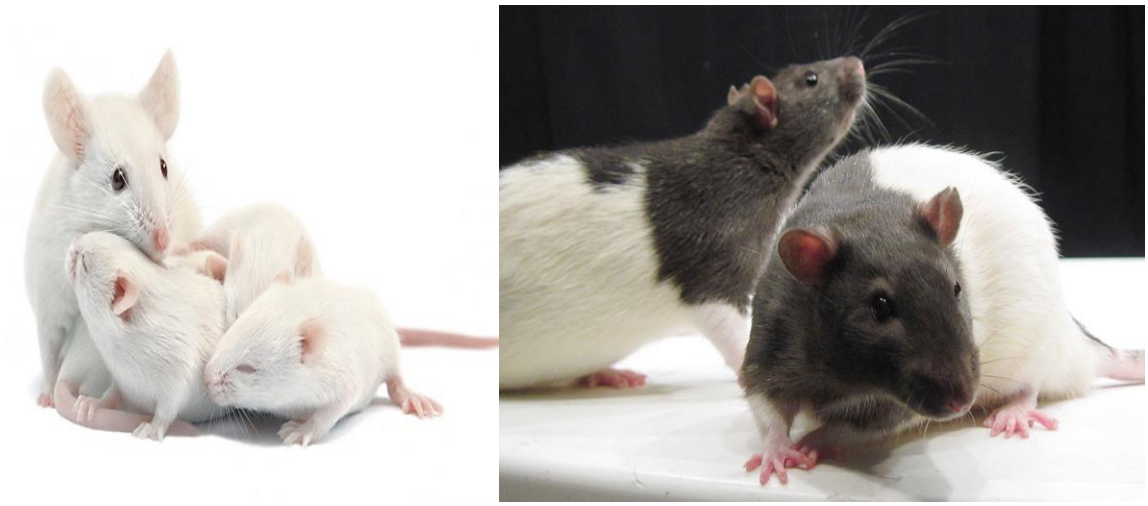


Figure 4.2: Long Evans rats and Mice

4.2.1Biomedical 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 4.3: Rat Used in Research

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.

4.3 Assessment of the Effect of Plant Materials on Gastrointestinal Motility:

One of the pivotal tasks of gastrointestinal tract is its ability to organize coordinated transport of luminal content which is perfectly adjusted to the digestive needs of the body. To achieve this gastrointestinal tract exhibits a wide repertoire of motor patterns that are based on spatiotemporal coordination of muscle activity. The gastrointestinal tract is able to monitor caloric density; osmolarity and pH of the luminal content and reacts with the initiation of the appropriate motility pattern.

The fascinating variety of motility patterns is best appreciated by imaging gut motility and transit of luminal content by Video fluoroscopy.

Motility disorders in the gut are major causes and concomitant phenomena of various functional, structural and inflammatory bowel diseases; one of the most prominent examples is irritable bowel disease (IBS).

4.3.1 Procedure:

1. For this experiment, 12 hours fasted mince is taken from the animal house.
2. Distilled water is administered to one mouse and marked it with marker as control mouse.
3. Plan extract is administered to another mouse and marked it as test mouse.

4. Barium sulphate milk is prepared by dissolving 10% (W/V) barium sulphate milk in 0.5% (W/V) sodium carboxymethyl cellulose (Na- CMC) suspension.
5. $BaSo_4$ milk is administered to all mice after 1 hour of administration of test drug.
6. Mice are sacrificed after 15 minutes of administration of the $BaSo_4$ milk.
7. The distance travelled by $BaSo_4$ milk is determined by scale.

4.3.2 Steps in Gastrointestinal Motility:

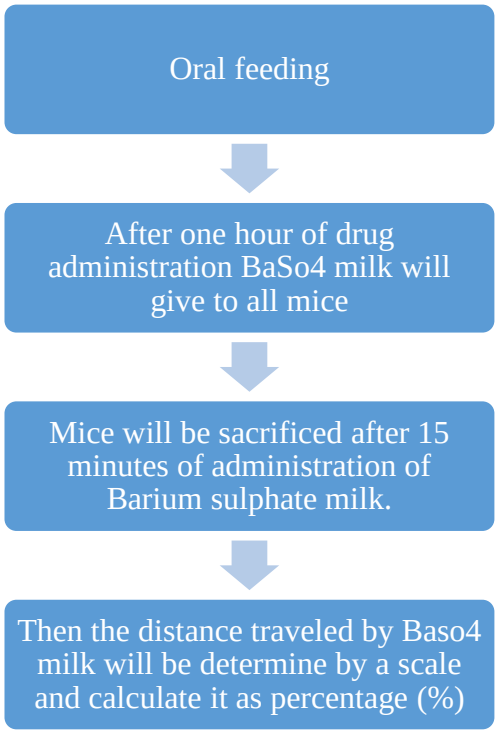


Figure 4.4: steps in gastrointestinal motility

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

4.4.1 Chemicals and reagents

Normal saline, 2N H2SO4, 1N NaOH, Sucrose (2.5g/Kg body weight of rat in 5ml deionized water)

Drug: 500mg/Kg body weight of rat

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

4.4.2 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 H2SO4 (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.

Figure 4.5: Six segments of Gastrointestinal tract

The gastrointestinal tract was excised and divided into 6 segments. They are –

1. Stomach,

2. Upper 20 cm of small intestine,

3. Middle part of small intestine,

4. Lower 20 cm of small intestine,

5. Cecum and

6. Large intestine.

4.4.3 Steps of the experiment

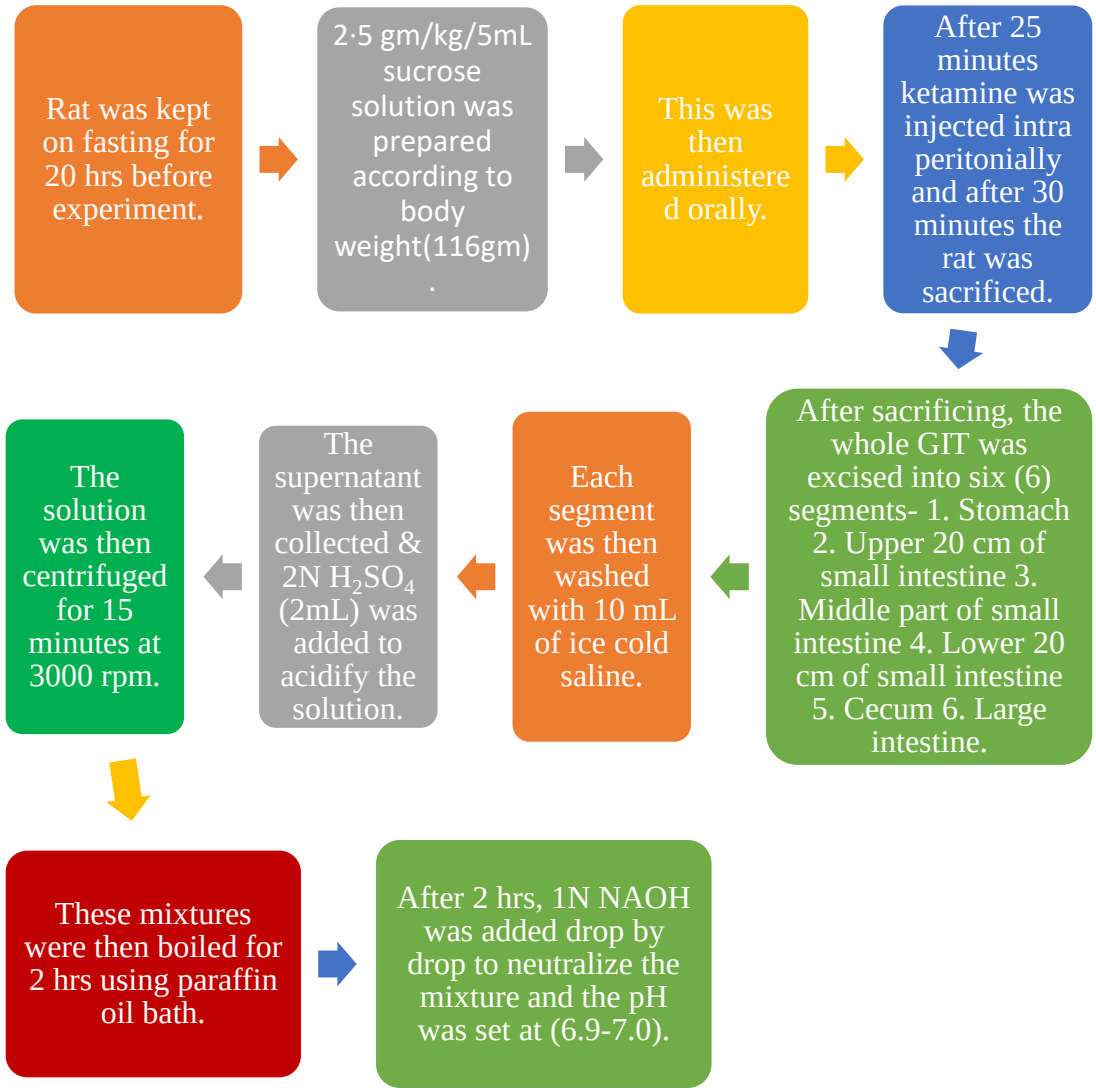


Figure 4.6: Flowchart of the experiment

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

4.5.1 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.

4.5.2 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 HeidolphDiax 600 homogenizer.

4.5.3 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.

4.5.4 Steps of the experiment

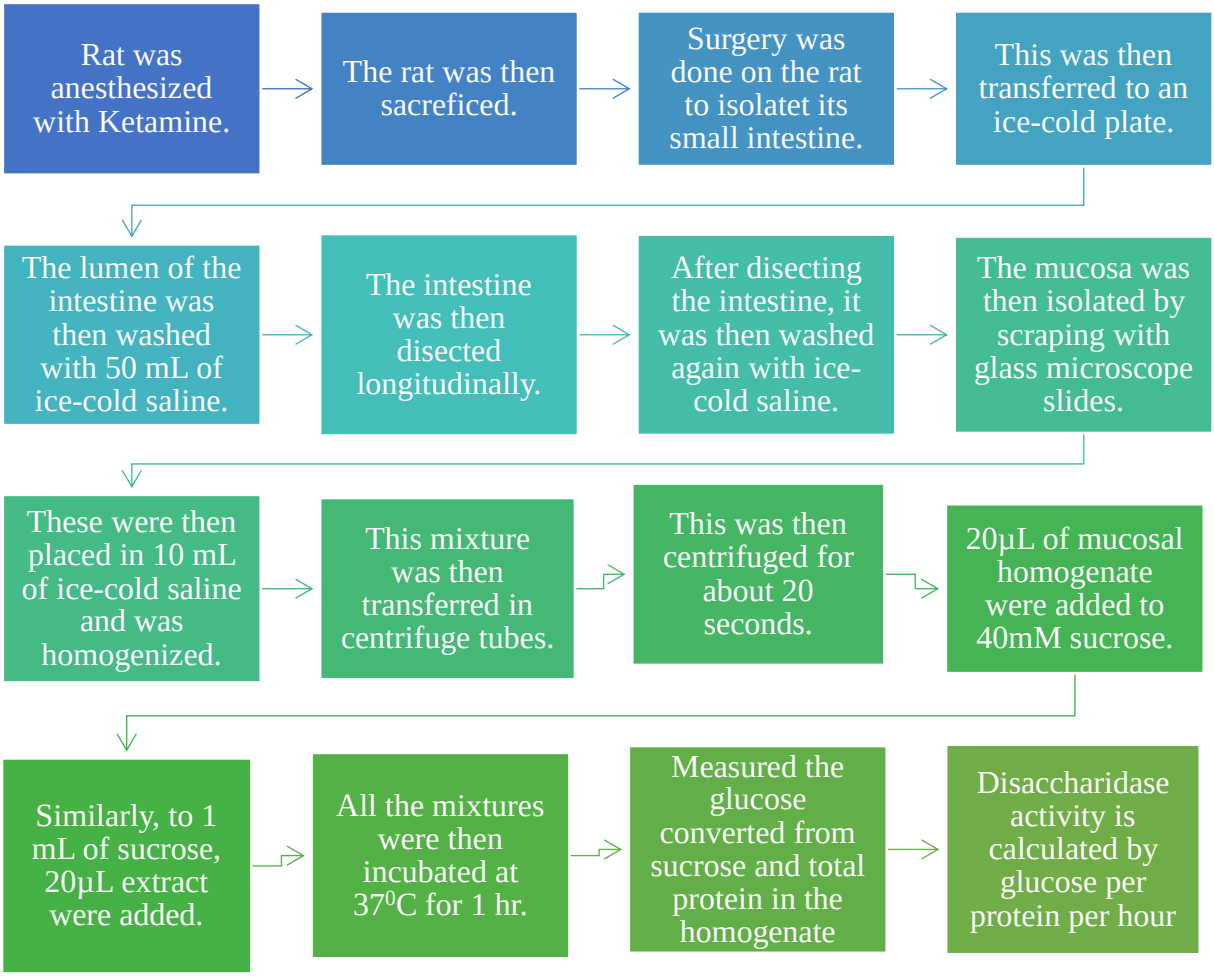


Figure 4.7: Flowchart of the experiment

CHAPTER 5

RESULT

5.1 Effect of *Cinnamomum zeylanicum* on GI Motility

Cinnamomum zeylanicum extract showed highly significant effect ($p<0.001$) in GI motility. It showed laxative effect. The absorption in the small intestine was decreased on the ingestion of extract.

Table 5.1: GI Motility Test

Group	% GI Motility ±SEM
Control	53.161±2.29
Cinnamon	70.130±1.98***
Standard (Bisacodyl)	84.157±0.587

*p<0.5

**p<0.01

***p<0.001

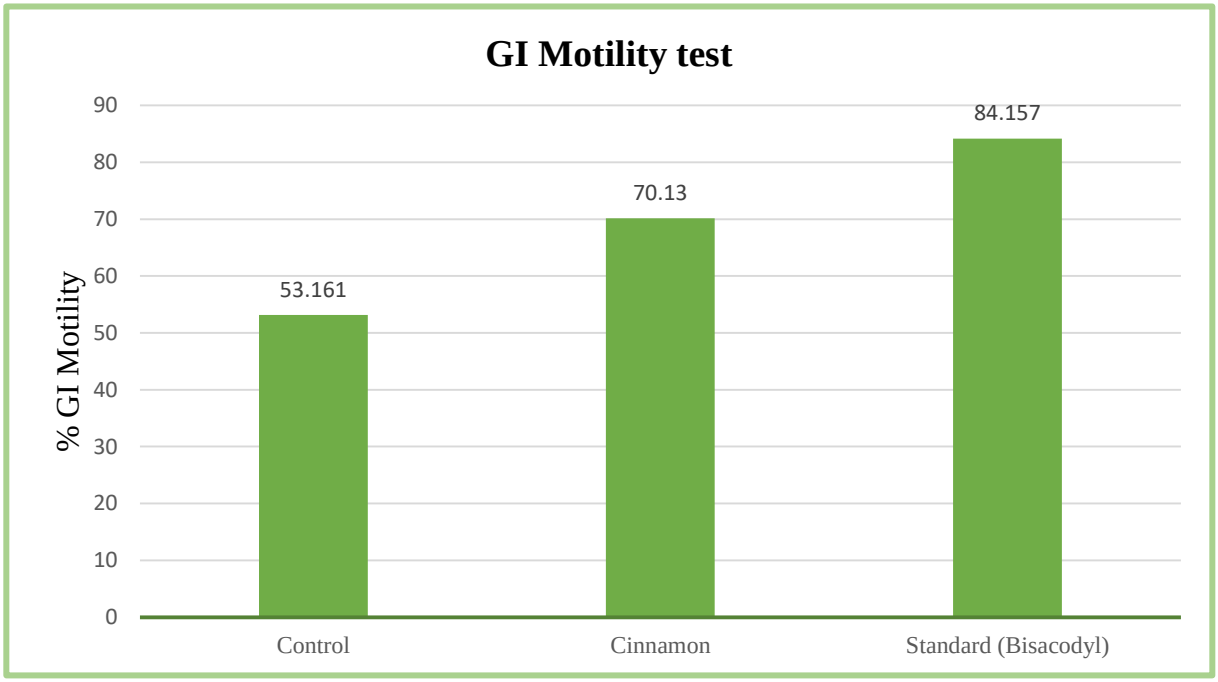


Figure 5.1: Effect of Cinnamomum zeylanicum on GI motility

5.2 Effect of bark *Cinnamomum Zeylanicum* on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Upon oral administration of sucrose along with ***Cinnamomum Zeylanicum*** (500mg/Kg), significant amount of unabsorbed sucrose was remained in the stomach, upper, middle, and lower intestine at 30 min and 1h. This amount of residual sucrose remained significant in caecum and large intestine till 4h (*p<0.5, **p<0.01, ***p<0.001; Table 1 - Table 6, Figure 5.2).

Table 5.2: Sucrose Absorption Test

Table: 1 (sucrose content in Stomach)					
Groups	30 min	60 min		120 min	240 min
	Sucrose(mg)	**Sucrose(mg)**		**Sucrose(mg)**	**Sucrose(mg)**
Control	54.13±1.7	34.04±3.94		8.50±0.60	1.32±0.32
Extract	64.23±0.93*	52.37±1.56*		18.50±2.30	3.55±0.11*

Table: 2 (sucrose content in Upper 20cm of intestine)				
Groups	30 min	60 min	120 min	240 min
	Sucrose(mg)	**Sucrose(mg)**	**Sucrose(mg)**	**Sucrose(mg)**
Control	14.69±0.89	11.68±0.66	4.56±1.08	0.95±0.15
Extract	19.06±1.62*	18.31±0.15*	7.30±0.06*	1.67±0.06*

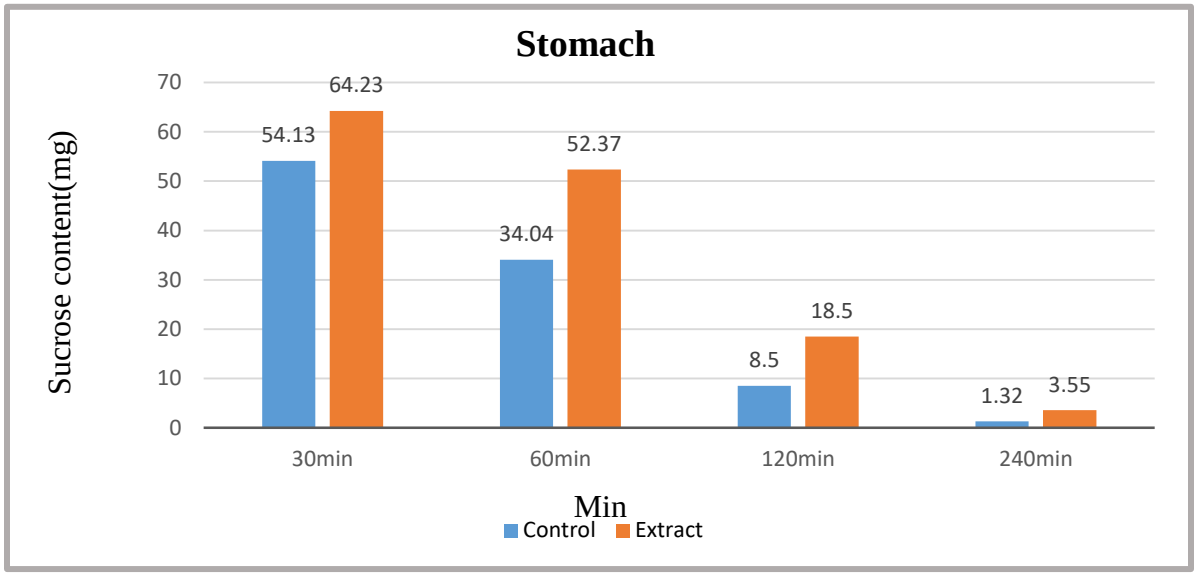
Table: 3 (sucrose content in middle 20cm of intestine)				
Groups	30 min	60 min	120 min	240 min
	Sucrose(mg)	**Sucrose(mg)**	**Sucrose(mg)**	**Sucrose(mg)**
Control	20.17±1.95	17.48±0.72	7.99±0.05	1.26±0.08
Extract	32.49±1.70*	31.74±0.55*	11.30±0.03*	1.97±0.06*

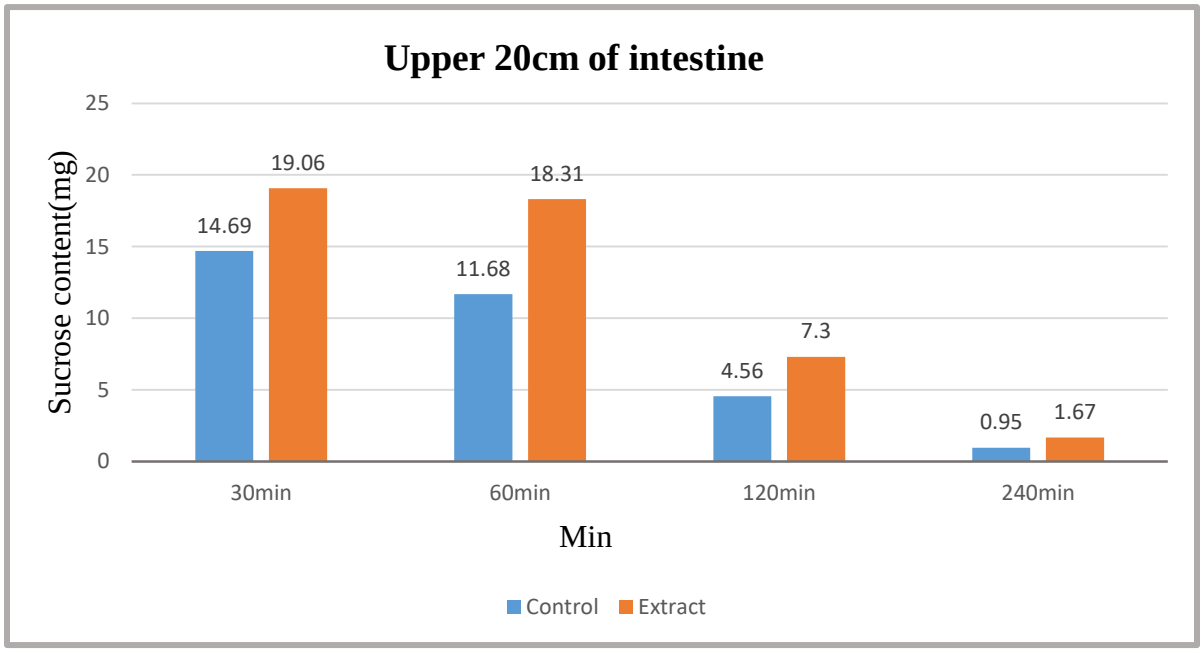
Table: 4 (sucrose content in Lower 20cm of intestine)				
Groups	30 min	60 min	120 min	240 min
	Sucrose(mg)	**Sucrose(mg)**	**Sucrose(mg)**	**Sucrose(mg)**
Control	5.57±0.7	3.24±0.73	1.26±0.56	0.98±0.02
Extract	5.97±0.16	7.32±0.48*	6.17±0.26*	1.53±0.04*

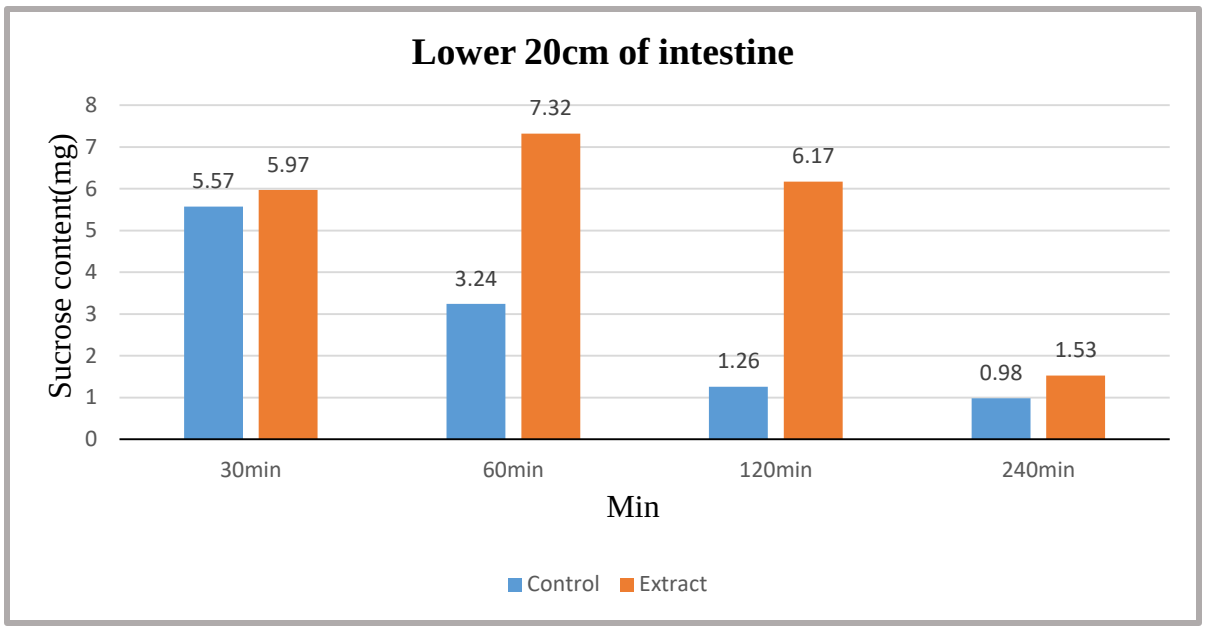
Table: 5 (sucrose content in Caecum)				
Groups	30 min	60 min	120 min	240 min
	Sucrose(mg)	**Sucrose(mg)**	**Sucrose(mg)**	**Sucrose(mg)**
Control	2.7±0.4	2.01±0.0	1.76±0.04	0.74±0.08
Extract	5.50±0.03**	6.81±0.65*	5.60±0.08**	1.54±0.06*

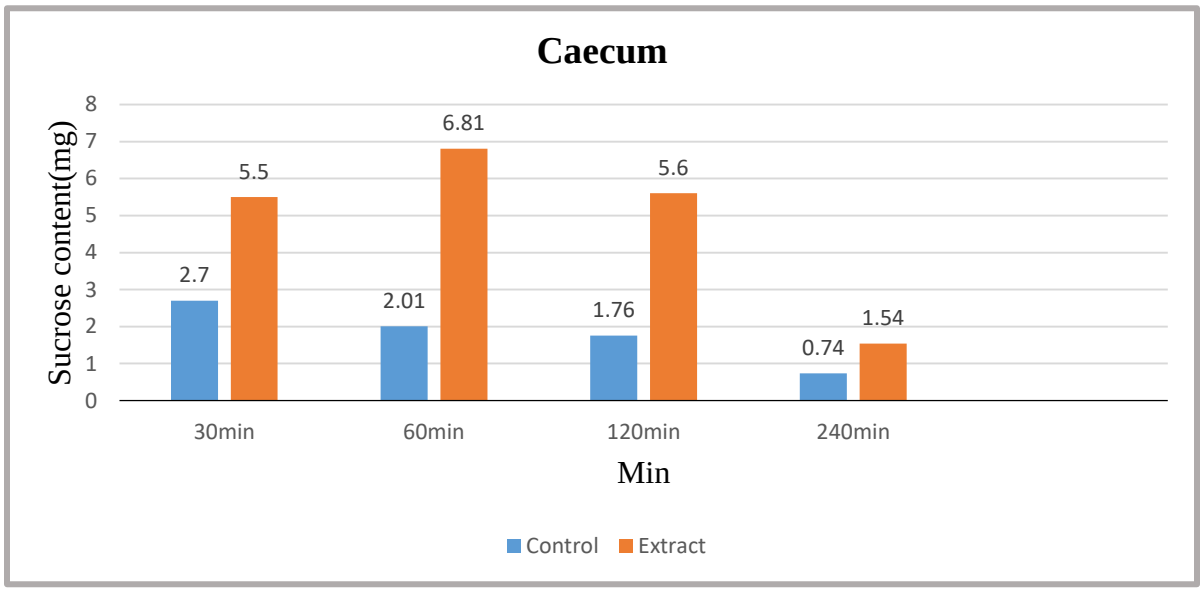
Table:6 (sucrose content in Large intestine)				
Groups	30 min	60 min	120 min	240 min
	Sucrose(mg)	**Sucrose(mg)**	**Sucrose(mg)**	**Sucrose(mg)**

Control	1.32±0.22	0.94±0.06	0.96±0.15	0.48±0.01
Extract	5.39±0.20*	5.70±0.25**	5.40±0.07***	1.02±0.03*






Lower 20cm of intestine
Sucrose content(mg)
8
7
6
5
4
3
2
1
0
5.57
5.97
3.24
7.32
1.26
6.17
0.98
1.53
30min
60min
120min
240min
Min
Control
Extract


Caecum
Sucrose content(mg)
8
7
6
5
4
3
2
1
0
2.7
5.5
2.01
6.81
1.76
5.6
0.74
1.54
30min
60min
120min
240min
Min
Control
Extract

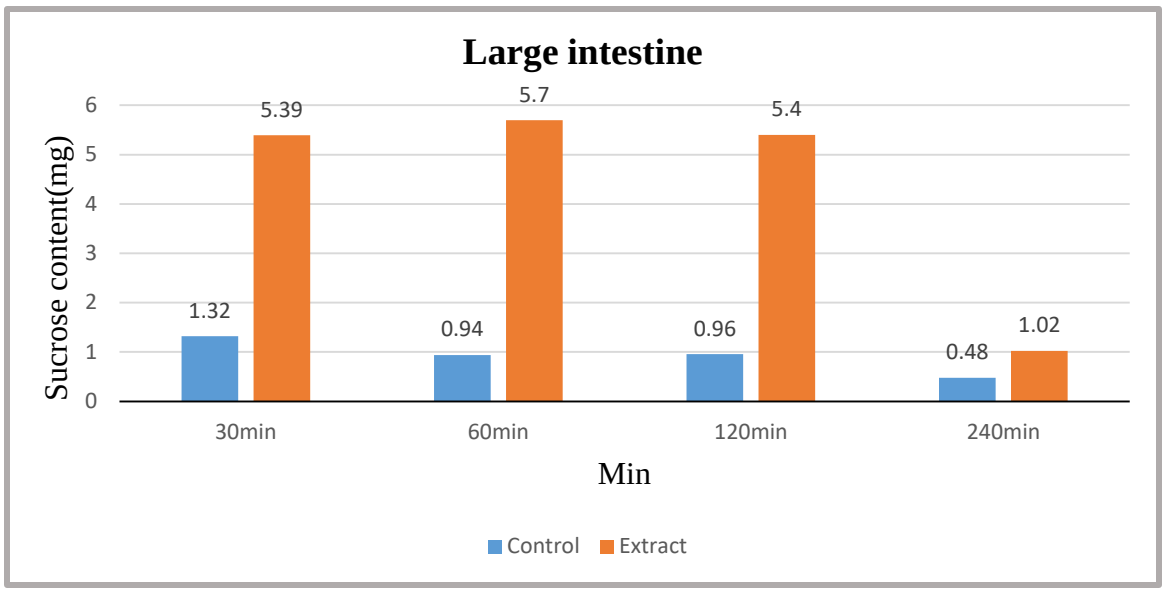


Figure 5.2: Effect of Cinnamomum zeylanicum on Unabsorbed Sucrose Content in the Gastrointestinal Tract

Effects of ethanol extract of Cinnamomum zeylanicum on gastrointestinal sucrose content after oral sucrose loading in normal rats: Rats were fasted for 20 h before the oral administration of a sucrose solution (2·5 g/kg body weight) with (treated group) or without (control group) ethanol extract of Cinnamomum zeylanicum (500mg/kg body weight). Values are means and standard deviations represented by vertical bars. This is derived from repeated-measures ANOVA and adjusted using Bonferroni correction.

5.3 Effect of *Cinnamomum zeylanicum* on Intestinal Disaccharidase Enzyme Activity

Cinnamomum zeylanicum extract showed highly significant ($p<0.001$) inhibition of disaccharidase enzyme activity.

Table 5.3: Disaccharidase activity test of Cinnamomum zeylanicum

Disacharidase Activity Test	
Group	**Disaccharidase Activity ±SEM (µmol/mg/h)**
Control	1.545±0.026
Cinnamon	1.047±0.031***
Standard (Acarbose)	1.065±0.02

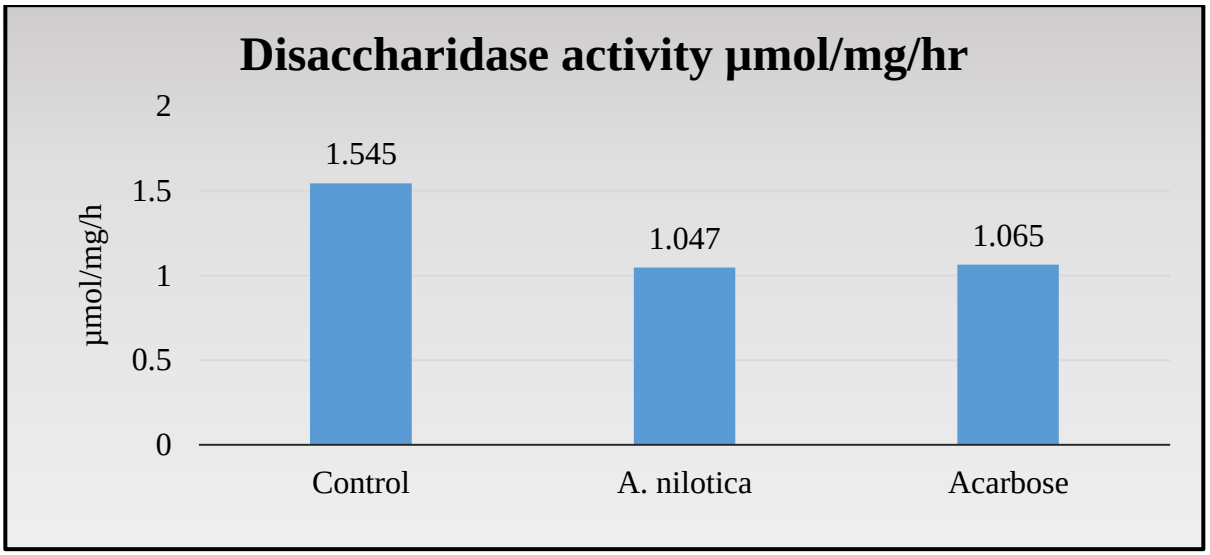


Figure 5.3: Effects of methanol extract of *Cinnamomum zeylanicum* on intestinal disaccharidase activity

Effects of ethanol extract of *Cinnamomum zeylanicum* on intestinal disaccharidase activity in normal rats: Rats were fasted for 20 h before the oral administration of ethanol extract of *Cinnamomum zeylanicum* (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 ($p<0.001$) disaccharidase enzyme activity (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

5.4 Discussion

Diabetes and its complications is becoming the third leading cause of death after cancer and cardiovascular diseases. Many serious side effects of insulin therapy and oral hypoglycaemic drugs necessitate the search for newer effective and safer class of compounds to overcome diabetic problems. In recent years, herbal products have started to gain importance as a source of antidiabetic medicines. It has been estimated that more than 1000 plant species are used as folk medicine for treating diabetes though most lack scientific evidence. Our study is directed to evaluate the anti-diabetic property of a methanolic extract of barks of ***Cinnamomum zeylanicum*** on normal rats. Additionally, unpublished, preliminary screening data, of this plant, showed highly promising hypoglycemic activity. Oral treatment with the defatted methanolic leaf extract showed hypoglycemic activity in normal rats. However, the tissue level mechanism of action of ***Cinnamomum zeylanicum*** antidiabetic property is yet to be investigated. According to established studies, the initiator of diabetic tissue damage is the hyperglycaemic states. The cells which are damaged by hyperglycemia cannot maintain a constant internal level of glucose which ultimately results in altered cellular mechanism and long-term changes in cellular macromolecular content. Postprandial glucose spike causes perturbation in endothelial cell function, and increased blood coagulation. An increase in the products of glycosylation is another result of hyperglycaemic states, which significantly influences the development of diabetic induced vascular disease. Thus, management of hyperglycaemic states in diabetes patients is the most important method of diabetes control. Commonly used diabetic drugs follow the basic mechanism of enhancing insulin secretion or enhancing sensitivity to insulin, improving peripheral glucose utilization, inhibiting glucose absorption and intestinal disacharidase enzymes. Through our studies on ***Cinnamomum zeylanicum***, after using several techniques, we are trying to prove any of the above mentioned mechanism that this plant follows.

Cinnamomum zeylanicum showed highly significant effect in GI motility which means it has laxative effect, which results the decreased absorption in small intestine.

Six Segment test showed significantly higher amount of sucrose in stomach, upper, middle and lower intestine in ***Cinnamomum zeylanicum*** administered groups. The latter three part of GI are most important for absorption of nutrients including sugar. Disaccharides in its own form does not get absorbed due to lack to sucrose carriers, as carriers monosaccharaides only are present in the GI tract. Therefore, it is imperative that disaccharides get converted to monosaccharaides first for absorption. Higher sucrose content in the GI Tract clearly reflects a reduced sucrose digestion throughout the GI Tract. This in turn, is shown by a significantly higher concentration of sucrose reaching the large intestine and caecum, which eventually remains unabsorbed and egested with faeces. In the intestinal disaccharidase activity assay, ***Cinnamomum zeylanicum*** was shown to have reduced the catabolism of sucrose and starch respectively. Since complex carbohydrates and disaccharides have first to be broken down into simpler monosaccharaides, it follows that any inhibition of this catabolic process would retard sugar absorption, which would in turn, be shown as a lower glycemic peak.

Dietary fibers of plant ingredients or powders can often provide a barrier to diffusion caused due to its high viscosity and ability to bind to glucose. Because, dietary fibers are capable of significantly reducing the transit time in GI Tract of ingested food. Reduced transit time is responsible for lesser time available for di-and polysaccharides in the meal to be digested and absorbed.

So, our results can be fully attributed to the significant increase amount of unabsorbed sucrose was remained in 6 different parts of intestine and decrease in disaccharide enzyme activity which validates anti-hyperglycemic activity of ***Cinnamomum zeylanicum***.

Extract of ***Cinnamomum zeylanicum*** showed significant effect on inhibiting disaccharidase enzyme activity, which does not allow the breakdown of disaccharide to monosaccharide.

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

CHAPTER 6

CONCLUSION

6.1 Conclusion

Our studies confirm the previous findings showing anti-hyperglycemic action of ***Cinnamomum zeylanicum.*** Additionally, we have elucidated that ***Cinnamomum zeylanicum*** has highly significant capabilities on GI motility and inhibiting absorption of glucose by inhibition of intestinal disaccharidase enzyme. Therefore, its traditional use, as mentioned above is justified and calls for further research, to optimize its anti-diabetic activity.

CHAPTER 7

REFERENCES

7.1 References:

- Ahmed, I., Furlong, K., Flood, J., Treat, V.P. and Goldstein, B.J., 2007. Dual PPAR α/γ agonists: promises and pitfalls in type 2 diabetes. *American journal of therapeutics*, *14*(1), pp.49-62.
- Alam, M.B., 2017. *Studies on the Mechanism of Anti-Hyperglycemic Effects of Acacia Nilotica* (Doctoral dissertation, East West University)
- Alberti, K.G.M.M. and Zimmet, P.F., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine*, *15*(7), pp.539-553.
- Alberti, K.G.M.M. and Zimmet, P.F., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabetic medicine*, *15*(7), pp.539-553.
- Allen, R.W., Schwartzman, E., Baker, W.L., Coleman, C.I. and Phung, O.J., 2013. Cinnamon use in type 2 diabetes: an updated systematic review and meta-analysis. *The Annals of Family Medicine*, *11*(5), pp.452-459.
- American College of Sports Medicine and the American Diabetes Association: joint position statement. Exercise and type 2 diabetes. *Medicine and science in sports and exercise*, *42*(12), pp.2282-2303.
- American Diabetes Association (ADA) 2001 Report of the expert committee on the diagnosis and classification of diabetes mellitus, *Diabetes Care* **24** S5-S20.
- American Diabetes Association, 2014. Diagnosis and classification of diabetes mellitus. *Diabetes care*, *37*(Supplement 1), pp.S81-S90.
- Amos, A.F., McCarty, D.J. and Zimmet, P., 1997. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetic medicine*, *14*(S5).

- Azaizeh H, Fulder S, Khalil K, Said O., 2003. Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region.. *Fitoterapia.*, Volume 74, pp. 98-108.
- Azaizeh H, Saad B, Khalil K, Said O. , 2006. The state of the art of traditional Arab herbal medicine in the eastern region of the Mediterranean: a review.. *Evid Based Complement Alternat Med.* , Volume 3, pp. 229-235.
- Babu, P.A., Suneetha, G., Boddepalli, R., Lakshmi, V.V., Rani, T.S., RamBabu, Y. and Srinivas, K., 2006. A database of 389 medicinal plants for diabetes. *Bioinformation*, *1*(4), p.130.
- Bailey, C.J. and Day, C., 1989. Traditional plant medicines as treatments for diabetes. *Diabetes care*, *12*(8), pp.553-564
- Bailey, C.J. and Day, C., 1989. Traditional plant medicines as treatments for diabetes. *Diabetes care*, *12*(8), pp.553-564.
- Baliunas, D.O., Taylor, B.J., Irving, H., Roerecke, M., Patra, J., Mohapatra, S. and Rehm, J., 2009. Alcohol as a risk factor for type 2 diabetes. *Diabetes care*, *32*(11), pp.2123-2132.
- Ben-Jacob, T., 2011. Type 1 and Type 2 Diabetes: Causes, Treatments, and Manifestations in Several Different Ethnicities. *Undergraduate Research Journal for the Human Sciences*, *10*(1).
- Bever, B.O. and Zahnd, G.R., 1979. Plants with oral hypoglycaemic action. *Quarterly Journal of Crude Drug Research*, *17*(3-4), pp.139-196.,
- Bhattacharjee, S., Rana, T. and Sengupta, A., 2007. Inhibition of lipid peroxidation and enhancement of GST activity by cardamom and cinnamon during chemically induced colon carcinogenesis in Swiss albino mice. *Asian Pac J Cancer Prev*, *8*(4), pp.578-582
- Blevins, S.M., Leyva, M.J., Brown, J., Wright, J., Scofield, R.H. and Aston, C.E., 2007. Effect of cinnamon on glucose and lipid levels in Non–insulin-dependent type 2 diabetes. *Diabetes care*, *30*(9), pp.2236-2237.
- Buerki RA, Higby GJ., 2007. Encyclopedia of Pharm Technology: Dosage Forms and Basic Preparations: History.. *PharmaceuTech, Inc,* Volume 3, p. 948.

- Bushe, C. and Holt, R., 2004. Prevalence of diabetes and impaired glucose tolerance in patients with schizophrenia. *The British Journal of Psychiatry*, *184*(47), pp.s67-s71.
- Cabello, C.M., Bair, W.B., Lamore, S.D., Ley, S., Bause, A.S., Azimian, S. and Wondrak, G.T., 2009. The cinnamon-derived Michael acceptor cinnamic aldehyde impairs melanoma cell proliferation, invasiveness, and tumor growth. *Free Radical Biology and Medicine*, *46*(2), pp.220-231.
- Chen, L., Sun, P., Wang, T., Chen, K., Jia, Q., Wang, H. and Li, Y., 2012. Diverse mechanisms of antidiabetic effects of the different procyanidin oligomer types of two different cinnamon species on db/db mice. *Journal of agricultural and food chemistry*, *60*(36), pp.9144-9150.
- Cheng, D.M., Kuhn, P., Poulev, A., Rojo, L.E., Lila, M.A. and Raskin, I., 2012. In vivo and in vitro antidiabetic effects of aqueous cinnamon extract and cinnamon polyphenol-enhanced food matrix. *Food chemistry*, *135*(4), pp.2994-3002.
- Colberg, S.R., Albright, A.L., Blissmer, B.J., Braun, B., Chasan-Taber, L., Fernhall, B., Regensteiner, J.G., Rubin, R.R. and Sigal, R.J., 2010. Exercise and type 2 diabetes:
- Crasto, W., Jarvis, J., Khunti, K. and Davies, M.J., 2009. New insulins and new insulin regimens: a review of their role in improving glycaemic control in patients with diabetes. *Postgraduate Medical Journal*, *85*(1003), pp.257-267.
- Crawford, P., 2009. Effectiveness of cinnamon for lowering hemoglobin A1C in patients with type 2 diabetes: a randomized, controlled trial. *The Journal of the American Board of Family Medicine*, *22*(5), pp.507-512.
- Dandona, P., Aljada, A., Chaudhuri, A. and Bandyopadhyay, A., 2003. The potential influence of inflammation and insulin resistance on the pathogenesis and treatment of atherosclerosis-related complications in type 2 diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *88*(6), pp.2422-2429.

- Dörzbach, E. and Müller, R., 1971. Die Insulintherapie: Die Insulinpräparate. In *Pathophysiologie und Klinik/Pathophysiology and Clinical Considerations* (pp. 1087-1111). Springer Berlin Heidelberg.
- Dugoua, J.J., Seely, D., Perri, D., Cooley, K., Forelli, T., Mills, E. and Koren, G., 2007. From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark This article is one of a selection of papers published in this special issue (part 1 of 2) on the Safety and Efficacy of Natural Health Products. *Canadian journal of physiology and pharmacology*, *85*(9), pp.837-847
- El-Bassossy, H.M., Fahmy, A. and Badawy, D., 2011. Cinnamaldehyde protects from the hypertension associated with diabetes. *Food and Chemical Toxicology*, *49*(11), pp.3007-3012.
- Evans, M.M., Miller, A., Favuzza, A., Stombaugh, N., Harrison, G. and Shaffer, A., 2016. Diabetic ketoacidosis--a case study describing standards of care. *MedSurg Nursing*, *25*(3), pp.S8-S8.
- Fahim, A., 2001, EFFECTS OF CINNAMON EXTRACT IN DIABETIC RAT MODELS IN COMPARISON WITH ORAL HYPOGLYCEMIC DRUGS.
- Garcia-Ocaña, A., Takane, K.K., Syed, M.A., Philbrick, W.M., Vasavada, R.C. and Stewart, A.F., 2000. Hepatocyte growth factor overexpression in the islet of transgenic mice increases beta cell proliferation, enhances islet mass, and induces mild hypoglycemia. *Journal of Biological Chemistry*, *275*(2), pp.1226-1232.
- Goni, P., López, P., Sánchez, C., Gómez-Lus, R., Becerril, R. and Nerín, C., 2009. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food chemistry*, *116*(4), pp.982-989.
- Grant, P.J., 2003. Beneficial effects of metformin on haemostasis and vascular function in man. *Diabetes & metabolism*, *29*(4), pp.6S44-6S52.
- Guariguata, L., 2012. By the numbers: new estimates from the IDF Diabetes Atlas Update for 2012. *Diabetes research and clinical practice*, *98*(3), pp.524-525.
- Guariguata, L., Whiting, D.R., Hambleton, I., Beagley, J., Linnenkamp, U. and Shaw, J.E., 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice*, *103*(2), pp.137-149.

- H. Cao, M. M. Polansky, and R. A. Anderson, “Cinnamon extract and polyphenols affect the expression of tristetraprolin, insulin receptor, and glucose transporter 4 in mouse 3T3-L1 adipocytes,” *Archives of Biochemistry and Biophysics*, vol. 459, no. 2, pp. 214–222, 2007
- Halliwell, B., 2011. Free radicals and antioxidants–quo vadis?. *Trends in pharmacological sciences*, *32*(3), pp.125-130.
- Hasslacher, C., 2003. Safety and efficacy of repaglinide in type 2 diabetic patients with and without impaired renal function. *Diabetes care*, *26*(3), pp.886-891.
- HM., S., 1978. Traditional Medicine in the Service of Health..
- Hong, J.W., Yang, G.E., Kim, Y.B., Eom, S.H., Lew, J.H. and Kang, H., 2012. Anti-inflammatory activity of cinnamon water extract in vivo and in vitro LPS-induced models. *BMC complementary and alternative medicine*, *12*(1), p.237.
- Hsu E, Barrett RL, 2008. Traditional Asian medical systems. *International Encyclopedia of Public Health*, pp. 349-357.
- IDF, D.A.G., 2015. Update of mortality attributable to diabetes for the IDF Diabetes Atlas: Estimates for the year 2013. *Diabetes research and clinical practice*, *109*(3), p.461
- Ivorra, M.D., Paya, M. and Villar, A., 1989. A review of natural products and plants as potential antidiabetic drugs. *Journal of ethnopharmacology*, *27*(3), pp.243-275.,
- J., S., 1978. Theophrastus on herbals and herbal remedies.. *J Hist Biol.* , Volume 11, pp. 353-385.
- JA., C., 1908. Avicenna and Arabian medicine.. *Bull Johns Hopkins Hosp.* , Volume 19, pp. 157-160.
- Juang, J.H., Bonner-Weir, S., Wu, Y.J. and Weir, G.C., 1994. Beneficial influence of glycemic control upon the growth and function of transplanted islets. *Diabetes*, *43*(11), pp.1334-1339.

- Kahn, S.E., 2003. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*, *46*(1), pp.3-19.
- Kirpichnikov, D., McFarlane, S.I. and Sowers, J.R., 2002. Metformin: an update. *Annals of internal medicine*, *137*(1), pp.25-33.
- Kolterman, O.G., Gray, R.S., Griffin, J., Burstein, P., Insel, J., Scarlett, J.A. and Olefsky, J.M., 1981. Receptor and postreceptor defects contribute to the insulin resistance in noninsulin-dependent diabetes mellitus. *Journal of Clinical Investigation*, *68*(4), p.957.
- Kopp J, Wang GY, Horch RE, Pallua N, Ge SD, 2003. Ancient traditional Chinese medicine in burn treatment: a historical review.. *Burns,* Volume 29, pp. 473-478.
- Koppes, L.L., Dekker, J.M., Hendriks, H.F., Bouter, L.M. and Heine, R.J., 2005. Moderate alcohol consumption lowers the risk of type 2 diabetes. *Diabetes care*, *28*(3), pp.719-725.
- Kota, S.K., Meher, L.K., Jammula, S., Kota, S.K. and Modi, K.D., 2012. Genetics of type 2 diabetes mellitus and other specific types of diabetes; its role in treatment modalities. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *6*(1), pp.54-58.
- Kutty, B.M. and Raju, T.R., 2010. New vistas in treating diabetes-Insight into a holistic approach.
- Leibiger, I.B., Leibiger, B., Moede, T. and Berggren, P.O., 1998. Exocytosis of insulin promotes insulin gene transcription via the insulin receptor/PI-3 kinase/p70 s6 kinase and CaM kinase pathways. *Molecular cell*, *1*(6), pp.933-938.
- Lipsett, M., Aikin, R., Castellarin, M., Hanley, S., Jamal, A.M., Laganiere, S. and Rosenberg, L., 2006. Islet neogenesis: a potential therapeutic tool in type 1 diabetes. *The international journal of biochemistry & cell biology*, *38*(5), pp.715-720.

- Lustman, P.J., Clouse, R.E. and Carney, R.M., 1989. Depression and the reporting of diabetes symptoms. *The International Journal of Psychiatry in Medicine*, *18*(4), pp.295-303.
- Mancini-Filho, J., Van-Koiij, A., Mancini, D.A., Cozzolino, F.F. and Torres, R.P., 1998. Antioxidant activity of cinnamon (Cinnamomum Zeylanicum, Breyne) extracts. *Bollettino chimico farmaceutico*, *137*(11), pp.443-447.
- Mang, B., Wolters, M., Schmitt, B., Kelb, K., Lichtinghagen, R., Stichtenoth, D.O. and Hahn, A., 2006. Effects of a cinnamon extract on plasma glucose, HbA1c, and serum lipids in diabetes mellitus type 2. *European journal of clinical investigation*, *36*(5), pp.340-344.
- Marles, R.J. and Farnsworth, N.R., 1995. Antidiabetic plants and their active constituents. *Phytomedicine*, *2*(2), pp.137-189
- Marteau, T.M., Bloch, S. and Baum, J.D., 1987. Family life and diabetic control. *Journal of Child Psychology and Psychiatry*, *28*(6), pp.823-833
- Matschinsky, F.M., 1996. A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes*, *45*(2), pp.223-241.
- Mordes, J.P., Bortell, R., Blankenhorn, E.P., Rossini, A.A. and Greiner, D.L., 2004. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. *Ilar Journal*, *45*(3), pp.278-291
- Mukherjee PK, Wahile A. , 2006. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *J Ethnopharmacol*, Volume 103, pp. 25-35.
- Nathan, D.M., Buse, J.B., Davidson, M.B., Ferrannini, E., Holman, R.R., Sherwin, R. and Zinman, B., 2009. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. *Diabetes care*, *32*(1), pp.193-203.

- Nathan, D.M., Buse, J.B., Davidson, M.B., Ferrannini, E., Holman, R.R., Sherwin, R. and Zinman, B., 2009. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. *Diabetes care*, *32*(1), pp.193-203.

- National Diabetes Data Group, 1979. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*, *28*(12), pp.1039-1057.
- Newman D, Cragg G, Snader K. , 2000. The influence of natural products upon drug discovery. *Nat Prod Rep*, Volume 17, pp. 215-234.
- Notkins, A.L., 1979. The causes of diabetes. *Scientific American*, *241*(5), pp.62-73.
- Olefsky, J.M. and Kolterman, O.G., 1981. Mechanisms of insulin resistance in obesity and noninsulin-dependent (type II) diabetes. *The American journal of medicine*, *70*(1), pp.151-168.
- Patwardhan B, Warude D, Pushpangadan P, Bhatt N. , 2005. Ayurveda and traditional Chinese medicine: a comparative overview.. *Evid Based Complement Alternat Med.* , Volume 2, pp. 465-473.
- Peterson, D.W., George, R.C., Scaramozzino, F., LaPointe, N.E., Anderson, R.A., Graves, D.J. and Lew, J., 2009. Cinnamon extract inhibits tau aggregation associated with Alzheimer's disease in vitro. *Journal of Alzheimer's Disease*, *17*(3), pp.585-597.
- Pham, A.Q., Kourlas, H. and Pham, D.Q., 2007. Cinnamon supplementation in patients with type 2 diabetes mellitus. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *27*(4), pp.595-599.
- Ping, H., Zhang, G. and Ren, G., 2010. Antidiabetic effects of cinnamon oil in diabetic KK-Ay mice. *Food and chemical toxicology*, *48*(8), pp.2344-2349.
- Qin, B., Panickar, K.S. and Anderson, R.A., 2010. Cinnamon: potential role in the prevention of insulin resistance, metabolic syndrome, and type 2 diabetes. *Journal of diabetes science and technology*, *4*(3), pp.685-693.
- Qureshi RA, Ghufran MA., 2007. Indigenous knowledge of selected medicinal wild plants of district Attock. *Pak J Bot*, Volume 39, pp. 2291-2299.

- Rahman, M.S., Akter, S., Abe, S.K., Islam, M.R., Mondal, M.N.I., Rahman, J.S. and Rahman, M.M., 2015. Awareness, treatment, and control of diabetes in Bangladesh: a nationwide population-based study. *PloS one*, *10*(2), p.e0118365.
- Rao, P.V. and Gan, S.H., 2014. Cinnamon: a multifaceted medicinal plant. *Evidence-Based Complementary and Alternative Medicine*, *2014*.
- Ratner, R.E. and Prevention Program Research Group, D., 2006. An update on the diabetes prevention program. *Endocrine Practice*, *12*(Supplement 1), pp.20-24.
- Routh HB, Bhowmik KR., 1999. Traditional Indian medicine in dermatology.. *Clin Dermatol,* Volume 17, pp. 41-47.
- Roy, S., Sehgal, R., Padhy, B.M. and Kumar, V.L., 2005. Antioxidant and protective effect of latex of Calotropis procera against alloxan-induced diabetes in rats. *Journal of Ethnopharmacology*, *102*(3), pp.470-473.
- Saad B, Azaizeh H, Abu-Hijleh G, Said O, 2006. Safety of traditional Arab herbal medicine.. *Evid Based Complement Alternat Med.,* Volume 3, pp. 433-439.
- Sangal, A., 2011. Role of cinnamon as beneficial antidiabetic food adjunct: a review. *Advances in Applied Science Research*, *2*(4), pp.440-450.
- Sena, C.M., Bento, C.F., Pereira, P., Marques, F. and Seiça, R., 2013. Diabetes mellitus: new challenges and innovative therapies. In *New Strategies to Advance Pre/Diabetes Care: Integrative Approach by PPPM* (pp. 29-87). Springer Netherlands.

- Sena, C.M., Bento, C.F., Pereira, P., Marques, F. and Seiça, R., 2013. Diabetes mellitus: new challenges and innovative therapies. In *New Strategies to Advance Pre/Diabetes Care: Integrative Approach by PPPM* (pp. 29-87). Springer Netherlands.

- Shang, W., Yang, Y., Zhou, L., Jiang, B., Jin, H. and Chen, M., 2008. Ginsenoside Rb1 stimulates glucose uptake through insulin-like signaling pathway in 3T3-L1 adipocytes. *Journal of Endocrinology*, *198*(3), pp.561-569.

- Shihabudeen, H.M.S., Priscilla, D.H. and Thirumurugan, K., 2011. Cinnamon extract inhibits α-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. *Nutrition & metabolism*, *8*(1), p.46.
- Smyth, S. and Heron, A., 2006. Diabetes and obesity: the twin epidemics. *Nature medicine*, *12*(1), pp.75-80.
- Sofowora, A., 1982. Medicinal plants and traditional medicine in Africa. *Popline*, Volume 018476, p. 256.
- Song, F., Li, H., Sun, J. and Wang, S., 2013. Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats. *Journal of ethnopharmacology*, *150*(1), pp.125-130.
- Sterne, J., 1969. Pharmacology and mode of action of hypoglycaemic guanidine derivatives. *Oral hypoglycaemic agents*, pp.193-245
- Stumvoll, M., Goldstein, B.J. and van Haeften, T.W., 2005. Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet*, *365*(9467), pp.1333-1346.
- Suppapitiporn, S. and Kanpaksi, N., 2006. The effect of cinnamon cassia powder in type 2 diabetes mellitus. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet*, *89*, pp.S200-5.
- Swanston-Flatt, S.K., Flatt, P.R., Day, C. and Bailey, C.J., 1991. Traditional dietary adjuncts for the treatment of diabetes mellitus. *Proceedings of the Nutrition Society*, *50*(3), pp.641-651.
- Sykiotis G, Kalliolias G, Papavassiliou A. , 2006. Hippocrates and genomic medicine.. *Arch Med Res.,* Volume 37, pp. 181-183.
- Van De Laar, F.A. and Lucassen, P.L., 2005. α-Glucosidase inhibitors for patients with type 2 diabetes. *Diabetes care*, *28*(7), pp.1841-1841.
- Van De Laar, F.A. and Lucassen, P.L., 2005. α-Glucosidase inhibitors for patients with type 2 diabetes. *Diabetes care*, *28*(7), pp.1841-1841.
- Vanschoonbeek, K., Thomassen, B.J., Senden, J.M., Wodzig, W.K. and van Loon, L.J., 2006. Cinnamon supplementation does not improve glycemic control in

postmenopausal type 2 diabetes patients. *The Journal of nutrition*, *136*(4), pp.977-980.

- Warram, J.H. and Krolewski, A.S., 2005. Epidemiology of diabetes mellitus. *Joslin's Diabetes Mellitus. 14th ed. Kahn CR, King GL, Moses AC, Weir GC, Jacobson AM, Smith RJ, Eds. Philadelphia, Pa., Lippincott, Williams & Wilkins*, pp.341-354.
- Wei, M., Mitchell, B.D., Haffner, S.M. and Stem, M.P., 1996. Effects of cigarette smoking, diabetes, high cholesterol, and hypertension on all-cause mortality and cardiovascular disease mortality in Mexican Americans: the San Antonio Heart Study. *American journal of epidemiology*, *144*(11), pp.1058-1065.
- WHO, E.C., 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet (London, England)*, *363*(9403), p.157.
- World Health Organization, 1999. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus.
- Xiang, A.H., Peters, R.K., Kjos, S.L., Marroquin, A., Goico, J., Ochoa, C., Kawakubo, M. and Buchanan, T.A., 2006. Effect of pioglitazone on pancreatic β-cell function and diabetes risk in Hispanic women with prior gestational diabetes. *Diabetes*, *55*(2), pp.517-522.

- Xiang, A.H., Peters, R.K., Kjos, S.L., Marroquin, A., Goico, J., Ochoa, C., Kawakubo, M. and Buchanan, T.A., 2006. Effect of pioglitazone on pancreatic β-cell function and diabetes risk in Hispanic women with prior gestational diabetes. *Diabetes*, *55*(2), pp.517-522.

- Xu J, Yang Y. , 2009. Traditional Chinese medicine in the Chinese health care system.. *Health policy* , Volume 90, pp. 133-139.

- Xu J, Yang Y. , 2009. Traditional Chinese medicine in the Chinese health care system.. *Health policy* , Volume 90, pp. 133-139.

- Xuan, S., Kitamura, T., Nakae, J., Politi, K., Kido, Y., Fisher, P.E., Morroni, M., Cinti, S., White, M.F., Herrera, P.L. and Accili, D., 2002. Defective insulin secretion in pancreatic β cells lacking type 1 IGF receptor. *The Journal of clinical investigation*, *110*(7), p.1011.