EFFECT OF CINNAMON CASSIA EXTRACTS ON HYPERGLYCEMIA AND RENAL FUNCTION IN STREPTOZOTOCIN INDUCED DIABETIC MICE

A thesis

By

AmAR NATH CHAUDHARY

rEGISTRATION nO.: 1505017

Session: 2015-2016

Semester: Jan-June, 2017

MASTER OF SCIENCE (MS)

IN

PHYSIOLOGY

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR -5200, BANGLADESH

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Submitted to the Department of Physiology & Pharmacology Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh in partial fulfillment of the requirements For the degree of

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JUNE, 2017

DEDICATED

TO MY

BELOVED PARENTS

ACKNOW LEDGEMENTS

At first of all praises are due to the almighty of God, the invincible, ubiquitous &omniscient who empowers the author to complete the thesis. Without His grace and wish, I will not be here where I am now and obviously this work cannot be accomplished successfully.

The author express the deepest sense of appreciation, heartiest gratitude and indebtedness to his reverend, beloved supervisor Dr. Rakibul Islam, Associate professor and Chairman, Department of Physiology & Pharmacology, Faculty of veterinary and Animal science, Hajee Mohammad Danesh science and Technology university, Dinajpur, Bangladesh for his sincere interest, Scholastic supervision, continuous, encouragement, constructive suggestion and all round help throughout the period of the research work and preparation of thesis.

The author also like to express to his Co-supervisor Dr. Dinesh kumar singh, Professor ,Department of pathology, Institute Of Agriculture & Animal science(IAAS), Tribhuban university, Nepal by his constructive criticism, guidance and countless fruitful discussions, he used to bring me back to the track, recharge and stimulate my enthusiasm for pressing on further. Undoubtedly, his innovative comments greatly improved the content of this thesis.

The Author humbly desire to express his profound respect and sincere appreciation to his respected & beloved Dr. Fahima Binthe Ajij, Associate professor ,Dr. Mahammudul Hasan, Assistant Professor and Dr. Mst. Misrat Masuma Parvez, lecturer, Department of Physiology & Pharmacology, HSTU, Dinajpur for their unreserved help in reviewing this paper, provision of valuable comments as well as for providing me with tremendous amount of information regarding my study area.

I would like to thank my senior brother Dr. Belal Hossain and my junior brother Dr. Rakesh Yadav lecturer Department of pharmacology IAAS. TU, Nepal, for cooperating in research process for giving a chance to advance my knowledge.

Next, I would like to thank all institutions, staff member and Lab staff and my junior brother of HSTU, Amit, Mukesh, Jitu, Suman who helped me in many ways during research Period.

Last but not least, I am greatly indebted to my Parents, my wife, all my sweet brother and sister ,my daughter and son and also my friends for their constant support, encouragement and prayers during my study.

The Author

ABSTRACT

Background: Diabetes mellitus (DM) is defined as a group of metabolic diseases manifested by hyperglycemia which results from defects in insulin production and/or insulin action. The present study was conducted to demonstrate the diabetogenic effect of Streptozotocin in Mice. Conventional drug treatment for diabetes mellitus carries risks that lead to many adverse effects such weight loss, hypoglycemia and many others. Asian countries including India, Bangladesh and Nepal are rich in natural resources and medicinal plants useful in the treatment of diabetes. To investigate the antidiabetic or anti-hyperglycemic effect and renal profile restoration effect of the Cinnamon cassia extract on Streptozotocin induced diabetes in experimental mice. The extract of cinnamon cassia was tested for its efficacy in Streptozotocin at a dose 100 mg/ kg of body weight induced diabetic mice.

Aim of the study: To investigate the effect of Cinnamon cassia extracts on hyperglycemia, and renal profile in **Streptozotocin** induced diabetic mice.

Methods: Thirty six male Swiss albino mice were kept in six different groups and each group have six male Swiss albino mice for 21days. Group T_0 served as normal controls; Group T_1 Streptozotocin induced 100 mg/ kg of body weight served as positive control mice,; Groups T_2, T_3 and T_4 mice were also Streptozotocin induced 100 mg/ kg of body weight and mice treated with 300 mg/kg, 400 mg/kg and 500mg/kg of *Cinnamon* extracts (70% ethanol), respectively; and Group T_5 were induced mice Streptozotocin 100 mg/ kg and treated with 5mg/kg Glibenclamide drug. The effect of extracts on hyperglycemia, and renal function were tested by chemistry analyzer. Results were analyzed using one way ANOVA at a 5% level of significance.

Results: The fasting blood glucose level was significantly ($p<0.05$) reduced at 400mg/kg and 500mg/kg of *Cinnamon* extract concentration as compared to the diabetic group. It also reduces urea and creatinine in induced diabetic mice.

Conclusion: Reduction in the fasting blood glucose, urea and creatinine by *Cinnamon* extract indicates that it has anti-hyperglycemic, and renal failure restoration effect in **Streptozotocin** induced diabetic mice.

Keywords: *Diabetes mellitus, Cinnamon cassia extracts, Hyperglycemia, Renal function*

LIST OF ACRONYMS

ADA	: American Diabetes Association
AGE	: Advance Glycation End
Akt	: Tyrosine/Threonine Kinase Activity
ATP	: Adenosine Triphosphate
CETP	: Cholesterol Ester Transfer Protein
CVD	: Cardiovascular Disease
FBG	: Fasting Blood Glucose
FFA	: Free Fatty Acid
GLUT2	: Glucose Transport-2
HDL	: High Density Lipoprotein
HGP	: Hepatic Glucose Production
IDF	: International Diabetes Federation
IRS-1	: Insulin Receptor Substrate–1
JNK	: c-Jun N-terminal Kinase
LCAT	: Lectin Cholesterol Acyltransferase
LDL	: Low Density Lipoprotein
LPL	: Lipoprotein Lipase
NEFA	: Non-Esterified Fatty Acid
PKC	: Protein Kinase C
PPAR	: Peroxime Proliferator Activated Receptor
STZ	: Streptozotocin
TC	: Total Cholesterol
WHO	: World Health Organization

CONTENTS

		SUBJECTS	PAGE NO.
Chapter I		**INTRODUCTION**	**01-02**
Chapter II		**REVIEW OF LITERATURE**	**03-27**
	2.1	Introduction of Diabetes	03
	2.2	Overviews on Glucose Metabolism and Insulin Signaling	03-06
	2.3	Classification of Diabetes Mellitus	07-08
	2.3.1	Type 1 Diabetes Mellitus	07
	2.3.2	Type 2 Diabetes Mellitus	07-8
	2.3.3	Gestational Diabetes Mellitus	08
	2.3.4	Other Specific Types of Diabetes Mellitus	08
	2.4	Prevalence of Diabetes Mellitus	08-09
	2.5	The Clinical Features of Diabetes Mellitus	09-10
	2.6	Diagnostic Tests for Type 2 Diabetes Mellitus	11
	2.7	Pathophysiology of Type 2 Diabetes Mellitus	11-13
	2.8	Molecular basis of Type -2 Diabetes Mellitus	13-14
	2.9	Complication of Type 2 Diabetes Mellitus	14-15
	2.10	Kidney Dysfunction and Type 2 Diabetes Mellitus	15-17
	2.11	The Treatments of Diabetes Mellitus	17
	2.12	Current Type 2 Diabetes Mellitus Therapy and Associated Problems	17-20
	2.13	Medicinal Plant for Diabetes	20-25
	2.13.1	The Cinnamon	21
	2.13.1.1	General Description of Cinnamon	21-23
	2.13.1.2	The Types of Cinnamon	23
	2.13.2	The Traditional Uses of Cinnamon	24
	2.13.3	Safety and Precautions	24
	2.13.4	Some Plants Effect on Hyperglycemia	25
	2.14	Streptozotocin	26-27
	2.14.1	The Role of Streptozotocin in Type 2 Diabetes	26-27

CONTENTS (Continue)

		SUBJECTS	PAGE NO.
Chapter III		**MATERIALS AND METHODS**	**28-36**
	3.1.	Experimental Animals and Study Protocol	28
	3.2	Place of Study	28
	3.3	Experimental Layout	29
	3.4	Design of Experiment	30-31
	3.5	Instruments, Reagents and Drugs	31-32
	3.6	Preparation and Alcoholic Extraction of Cinnamon bark	32-33
		3.6.1 Yield of Cinnamon Extracts	32
		3.6.2 Acute Toxicity Test of Cinnamon bark extracts	33
	3.7	Induction of Experimental Type 2 Diabetes Mellitus	33
	3.8	Biochemical Test Assay	33-35
		3.8.1 Fasting Blood Glucose Level	34
		3.8.2 Serum Creatinine	34
		3.8.3 Serum Urea	35
	3.9	Recording of Different Parameters	35-36
		3.9.1 Recording of body weight	35-36
		3.9.2 Recording of blood glucose	36
	3.10	Statistical Analysis	36
Chapter IV		**RESULTS**	**37-42**
	4.1	The Effects of Cinnamon extracts on Body Weight	37
	4.2	The Effect of Cinnamon extract on Fasting Blood Glucose Level	38-39
	4.3	The Effects of Cinnamon extract on Serum Creatinine	40
	4.4	The Effects of Cinnamon extract on Serum Urea	41
	4.5	The Effect of Cinnamon extract on Acute Toxicity Test	42
Chapter V	**DISSCUSSION**		**43-45**

Chapter VI	**CONCLUSION AND RECOMMENDATION**	**46**
	REFERENCES	**47-56**

LIST OF TABLES

Table No	Topic	Page No
1.1	The standard values of diagnostic tests in type-2 diabetes mellitus	11
4.1	Effects of Cinnamon extracts on Body Weight	37
4.2	Effect of Cinnamon extract on Fasting Blood Glucose Level	39
4.3	Effects of Cinnamon extract on Serum Creatinine	40
4.4	Effects of Cinnamon extract on Serum Urea	41

LIST OF FIGURES

Figure No	Topic	Page No
2.1	The role of the pancreas, liver and other tissue cells in glucose homeostasis	04
2.2	KATP channel pathway of glucose sensing in the β-cell	05
2.3	Metabolic actions of insulin	06
2.4	Pathophysiology of type 2 diabetes mellitus	12
2.5	Metabolic pathways activated by chronically elevated blood glucose levels and long term complications of diabetes mellitus	15
2.6	Sites of action and mechanism of antihyperglycemic drugs in treatment of hyperglycemia and T2DM	18
2.7	Mechanism of action of sulfonylureas on ATP-sensitive K+ channels	19
2.8	Cinnamon Leaf and Cinnamon Bark	22
2.9	The mechanism of STZ induced toxic events in B cells of Pancreas	27
2.10	Layout of the experiment	29
4.1	The effects of Cinnamon extract on body weight in STZ induced diabetic mice	37
4.2	The effects of Cinnamon extract on fasting blood glucose in diabetic mice on 1st, 7th, 14th and 21st days	38
4.3	The effects of Cinnamon extract on Creatinine in STZ induced diabetic mice	40
4.4	The effects of Cinnamon extract on urea in STZ induced diabetic mice	41

1. INTRODUCTION

Diabetes mellitus is the commonest endocrine disorder, which arise from many environmental and genetic factors often acting together causing an absolute or relative insulin deficiency, leading to hyperglycemia in the blood and urine, sometimes accompanied by thirst and graduate loss of weight (Jahadar, 1993., Murrag *et al.,* 1996).The vast majority of diabetic patients are classified into Type I diabetes is characterized by an absolute deficiency of insulinType II diabetes is characterized by insulin resistance involving muscle, liver and adipocytes (Curtis *et al.,* 2005).

The synthetic drugs for diabetes treatment may produce serious side effects such as cardiovascular disorder, hypoglycemia, coma and damage of the kidney and liver that can endanger the life of diabetic patient. Therefore, in recent years, herb researches has been increased all over the world and a greater importance is given to therapeutic use of drugs isolated from medicinal herbs for their activities, safety and commercial factors (Katin and Schechter *et al.,* 1991).

In diabetes mellitus, the major contribution was made in the field of hypoglycemic action of various plant products and drug interaction to hypoglycemic agents. Thus, in some countries the use of medicinal plants as anti-diabetic remedies is a common practice. These plants were found to possess active constituents called hypoglycemia agents. Several updated researches about experimental and clinical investigation of plant used for control of diabetes mellitus have been published. They include many nutrients and nutraceuticals, of which cinnamon extract, that enhance insulin sensitivity and/ or reduce circulating insulin concentrations (Babu and Prince *et al.,* 2004).

Cinnamon is widely used in Ayurveda medicine to treat diabetes in India, Cinnamon extract has strong antioxidant activity due to the presence (cinnamaldehyde, eugenol, weitherthin, cinnamic acid and pinene, terpenoids, Mucilage, Diterpenes, Proanthocyanidins, Mannitol, Gum and coumarins and is beneficial in preventing and controlling the glucose intolerance and diabetes by activating insulin, glucose transport and improving glucose metabolism. It also lowered blood level fat and bad cholesterol which are, also, partly controlled by insulin (Preuss *et al.,* 2006).

Cinnamon plants (Family: Lauraceae) is a common food additive for its flavor and aromatic properties and it contains low carbohydrate, fat, a lot of fiber and other constituents present in the

plant which could act synergistically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes. Cassia cinnamon (Cinnamoum cassia) is one of the popular species of cinnamon, which is widely distributed in Asia especially in India and China, Bangladesh, Nepal. (Jayprakasha *et al.,* 2003).

In spite of using inner bark of cinnamon as a food additive in cooking or to treat digestive system and urinary problem, fight bad breath, stave off common cold and promotion of wound healing for many years (Archer,1998), but recently it has become increasing popular for its beneficial role in glucose metabolism (Preuss *et al.,*2006). Some study were mentioned that the active compounds of cinnamon (such as cinnamaldehyde, eugenol and other compounds) possess wide ranges of pharmacological effects that seems to be highly bioactive against diabetes by its effect on insulin secretion and stimulate glucose uptake by hepatocytes and adipocytes (Qin, *et al.,* 2003).

It was hoped that this study has been important in giving direction for finding new application on hyperglycemia, and renal dysfunction of *cinnamon extract* that could be used for future treatment of T2DM. The general objective of this study was to see that the effect of cinnamon on blood glucose level in Streptozotocin induced mice with the following specific objectives:

I. To determine the effect of cinnamon on blood glucose level in Streptozotocin induced mice.
II. To know the effect of cinnamon on serum urea, creatinine in Streptozotocin induced mice.

2. LITERATURE REVIEW

2.1 Introduction of Diabetes

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs especially the eyes, kidneys, nerves, heart and blood vessels (ADA, 2011). It is also associated with an enhanced risk for developing prematureatherosclerosis as evident by an increase in the concentration of serum triglyceride level (TG), increase in low density lipoprotein (LDL) and decrease in high density lipoprotein (HDL) (Yamini and Anande *et al.,* 2010).

Diabetes mellitus is a metabolic disorder characterized by resistance to the action of insulin, insufficient insulin secretion or both which is associated with abnormalities in carbohydrate, fat and protein metabolism, result in chronic complications including micro vascular, macro vascular, and neuropathy disorders (Curtis *et al.,* 2005).

The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and will be 4.4% in 2030. The total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million in 2030. (Wild, *et al*., 2004).

In diabetic patients, the body loses insulin producing capacity as a result of pancreatic β-cell apoptosis or insulin insensitivity. The cytokines, lipo-toxicity and gluco-toxicity are three major stimuli for β-cell apoptosis. (Hui, *et al*., 2004).

Evidence is increasing that control of hyperglycaemia, hypertension and dyslipedemia may postpone the development of diabetic complications in type 2 diabetes mellitus. (Kim, *et al.,* 2006).

2.2 Overviews on Glucose Metabolism and Insulin Signaling

Diabetes mellitus is actually a group of diseases characterized by high fasting blood glucose levels. Glucose is a simple sugar that provides energy to all of the cells. The cells take glucose from the blood and break it down for energy. Glucose gets absorbed from the intestines and distributed by the bloodstream to keep a constant supply of sugar, by maintaining a constant glucose concentration in the blood (Neeland *et al.,* 2012). To maintain a constant blood-glucose level, the two antagonistic hormones (insulin and glucagon) are produced in the pancreas.

Insulin is an important signaling molecule required by almost all of the cells, but its major targets are liver, fat and muscle cells. For these cells, insulin has the following function: stimulates liver and muscle cells to store glucose in glycogen, stimulates fat cells to form fats from fatty acids and glycerol, activate liver and muscle cells to make proteins from amino acids, and inhibits the liver and kidney cells from making glucose from intermediate compounds of metabolic pathways (gluconeogenesis) as described below in figure 2.1 (Abel *et al.,* 2012).

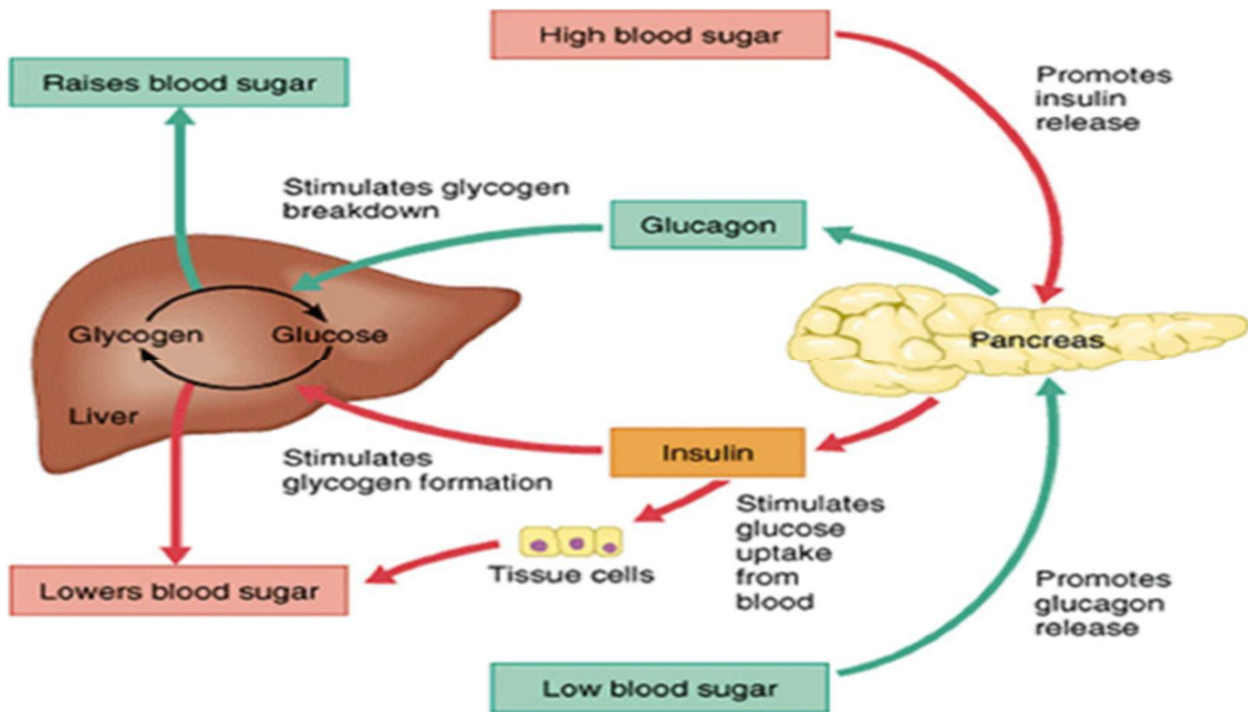


Figure 2.1: *The role of the pancreas, liver and other tissue cells in glucose homeostasis: When glucose goes too high, the pancreas releases insulin into the blood stream and insulin stimulates the liver to convert the blood glucose into glycogen for storage. If the blood sugar goes too low, the pancreas release glucagon which causes the liver to turn stored glycogen back into glucose and release it into the blood, Green arrow-express the role of glucagon and red arrow-express the role of insulin to balance glucose (Evan and Bruce, 2006).*

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states *via* glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted.

In conditions of high fasting blood glucose level, oxidative metabolism is increased in pancreatic *β*-cells which results in increased ATP production in mitochondria (Sharma, 2011). The increase in intracellular ATP closes the ATPsensitive K+ channels (*K*ATP), decreasing the hyperpolarizing outward K+ flux. This results in depolarization of the plasma membrane and influx of extracellular Ca2+ through the voltage-gated Ca2+ channels. Figure 2.2 illustrates the activation of protein motors and kinases by the increasing intracellular Ca2+, which then mediate exocytosis of insulin-containing vesicles which lead to increased insulin and decreased fasting blood glucose levels (Bays *et al.,* 2004).

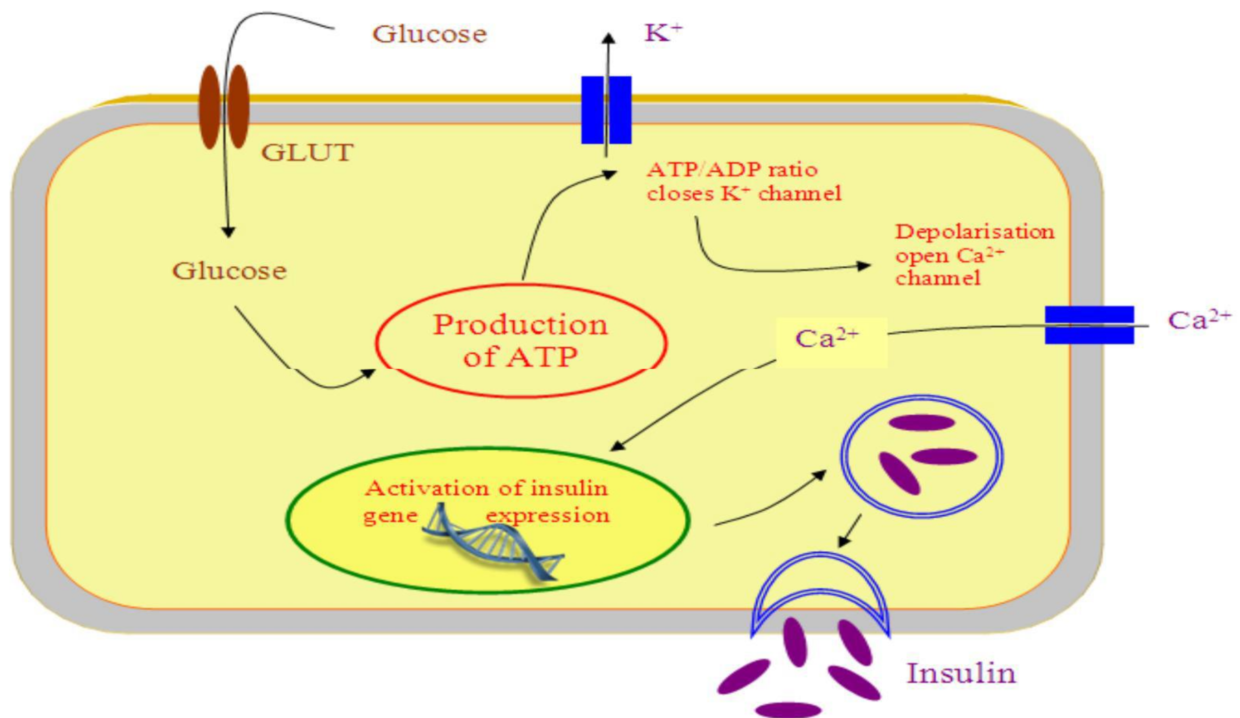


Figure 2.2: KATP channel pathway of glucose sensing in the β-cell: *The increase of the ATP concentration closes the K+ channel, leading to a depolarization which in consequence opens the Ca2+ channel. Ca2+ activates the insulin gene expression via the Calcium Responsive Element Binding Protein (CREB) through exocytosis; the produced insulin is set free in the blood. Abbreviations: ATP- Adenosine. Triphosphate, ADP-adenosine Diphosphate, GLUT-Glucose transporter (Wiltgen and Tilz et al., 2012)*

Metabolic actions of insulin result from its interaction with the insulin receptor (IR) found in all insulin responsive target cells like liver, muscle and adipose tissue (Hu *et al.*, 2013). As shown in Figure 2.3, insulin binds to the α-subunit of IR and activates the intrinsic tyrosine kinase activity (Akt) of the β-subunit of the receptor. Activated IR results in the subsequent phosphorylation of intracellular substrates including insulin receptor substrates (IRSs) such as IRS-1 and -2, phosphatidylinositol (PI) 3-kinase, and protein kinase B (PKB). Normal insulin action leads to increased glycogen synthesis, glucose transport, and lipogenesis, and decreased gluconeogenesis, glycogenolysis, and lipolysis (Postic*et al.,* 2004).

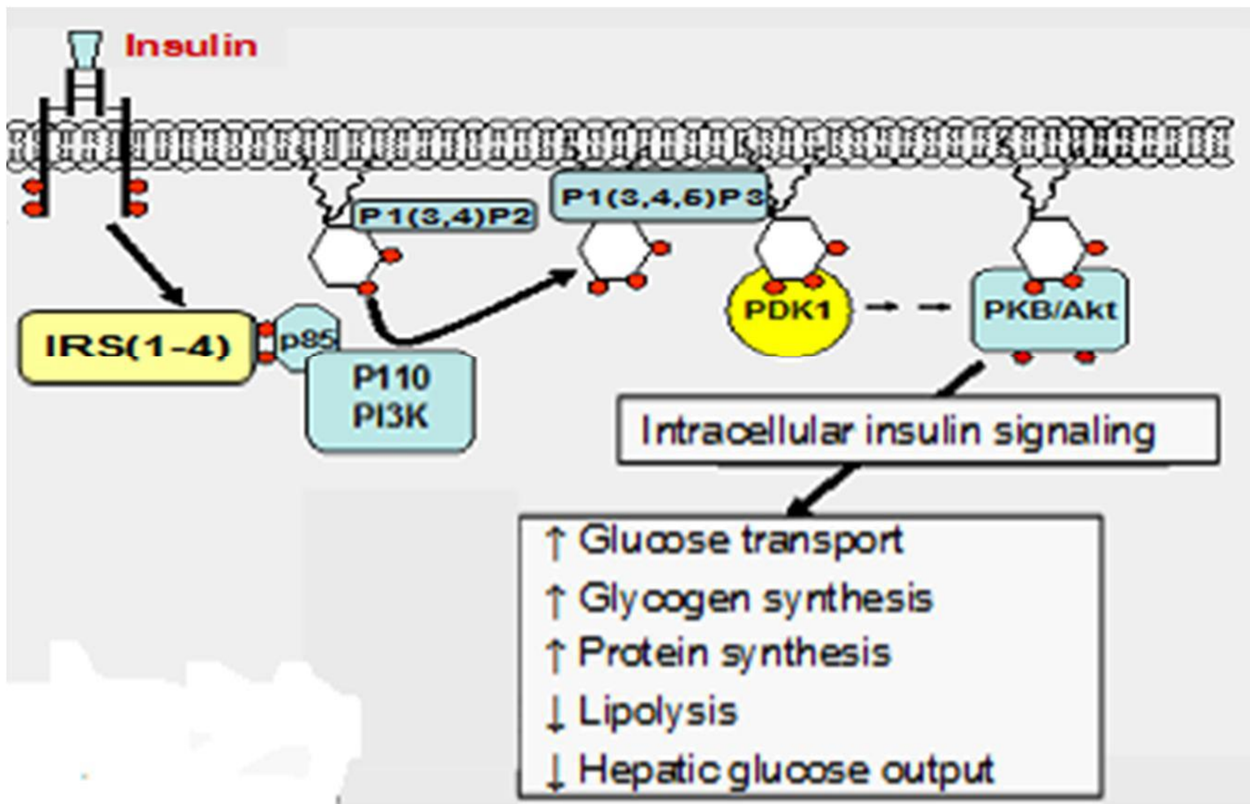


Figure 2.3: Metabolic actions of insulin: *IRS- Insulin Receptor Substrates*, *PKB-Protein kinase B*, *PI3K - PI 3-kinase, PI(3,4)P3-phosphatidylinositol-3,4- bisphosphate, PI(3,4,5)P3 phosphatidylinositol (3,4,5)P3, PDK1– phosphatidyl- inositol dependent kinase -1 (Saltiel and Kahn, 2001).*

2.3 Classification of Diabetes Mellitus

Diabetes mellitus (DM) is classified into four broad categories: type 1 DM, type 2 DM, gestational DM and "other specific types".

2.3.1 Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin-secreting β cells (Sachin *et al.*, 2009). Type 1 Diabetes Mellitus is characterized by loss of the insulin-producing β cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. A trigger-either an illness or stress causes the immune system to attack and destroy the β cells of the pancreas (Amreen *et al.*, 2012). The treatment for T1DM is to take insulin injections every day to survive. This form of DM is also called Insulin Dependent DM. Type 1 Diabetes Mellitus develops suddenly in childhood or adolescence (Shukla *et al.,* 2011).

2.3.2 Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) refers to a non-autoimmune form of diabetes characterized by peripheral insulin resistance and impaired insulin secretion (Amy and Irinn *et al.,* 2009). This type of DM occurred when the pancreas produces insulin, but the cells are unable to use it efficiently; this effect is called ‘insulin resistance’. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor.

However, the specific defects are not known. People living with T2DM are more vulnerable to various forms of both short- and long-term complications, which often lead to their premature death (Olokoba and Obateru *et al.,* 2012).

Type 2 Diabetes Mellitus is also called Non-Insulin Dependent DM (NIDD). Type 2 Diabetes Mellitus is far more common than T1DM and approximately 90% of all DM cases are T2DM. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of T1DM. However, the genetics of this form of diabetes are complex and not clearly defined. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (Shukla *et al.,* 2011).

It occurs more frequently in women with prior gestational DM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups (Olokoba and Obateru *et al.,* 2012).

2.3.3 Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM), resembles T2DM in several respects, involve a combination of relatively inadequate insulin secretion and action during pregnancy. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery.

Gestational Diabetes Mellitus is fully treatable but requires careful medical supervision throughout the pregnancy. It can complicate pregnancy leading to prenatal morbidity and mortality, so clinical detection is important (Amreen *et al.,* 2012). About 20%-50% ofGDM affected women develop T2DM later in life (Shukla *et al.,* 2011).

2.3.4 Other Specific Types of Diabetes Mellitus

Prediabetes indicates a condition that occurs when a person's fasting blood glucose levels are higher than normal but not high enough for a diagnosis of T2DM. Many people destined to develop T2DM, and spend many years in a state of prediabetes. Maturity onset DM of youth is due to impaired insulin secretion minimal or no insulin resistance, so hyperglycemia is noticed at an early stage. Genetic inability to convert proinsulin to insulin causes mild hyperglycemia (Shukla *et al.,* 2011).

Latent autoimmune DM of adults (LADA) is a condition in which T1DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having T2DM, based on age rather than etiology.

2.4 Prevalence of Diabetes Mellitus

Diabetes mellitus is a heterogeneous disorder with varying prevalence among different ethnic groups. It affects large number of people around the world. It is estimated that 366 million people had DM in 2011, and this number is expected to reach 552 million by 2030. According to the IDF report in 2011, China, India, and USA have 90.0, 61.3, and 23.7 million peoples living with diabetes that may be increase up to 129.7, 101.2, and 29.3 million people, respectively, in

2030 (Hu *etal.*, 2011). The IDF recently reported that the number of people with T2DM will escalate from 285 million in 2010 to 438 million by 2030, with more than 70% of cases already from developing countries (Shaw *et al.,* 2010).

In another report, WHO also states that the highest increases in diabetes prevalence have occurred in low- and middle-income countries of Africa, Asia, and South America (IDF, 2012). It was once believed that DM is uncommon in the developing world, but has now emerged as an important public health problem in Asia. Type 2 Diabetes Mellitus accounts for over 90% of diabetes cases in Asia (Levitt, 2008). The expected growth for South is 98%, from 12.1 million in 2010 to 23.9 million in 2030 (Sicree and Zimm *et al.,* 2009). Similarly, the prevalence of DM in Nepal was 2.5 % in the year 2000 which is estimated to rise to 3.5 % by 2030 (Shaw *et al.,* 2010).

2.5 Clinical Features of Diabetes Mellitus

The classical symptoms of type I diabetes are thirst, polyuria, nocturia and rapid weight loss. These symptoms are often absent in patients with type II diabetes. (Frei *et al.,* 1990., Murrag *et al.,* 1996).

Other symptoms that appear in diabetic patients include intense hunger, slow heal, being tired, very dry skin, headache sudden changes in vision, rapid breathing, high blood pressure and the appearance of ketone bodies in the blood and urine as well as an increase in blood acidosis (Brown *et al.,* 1982).

These symptoms are appearing in acute form where as in chronic form associated with more complication and metabolize-disturbances (Mcmillaa *et al.,* 1978).

The Biochemical Changes: The principle biochemical manifestation is hyperglycemia, most of metabolic changes of diabetes mellitus are the consequences of insulin deficiency. In the absence of insulin affects the metabolism of carbohydrate, fat and protein which caused a significant disturbance of water and electrolyte homeostasis. Also, the glucose is not rapidly taken up by adipose tissue and muscle, the inability to clear the blood glucose is atypical characteristic of diabetes. If blood glucose level exceeds (200 mg/dl) and renal function is normal, glucose urea will be present (Ditzel and Slandl *et al*; 1975).

High urinary glucose concentration produces osmotic diuresis and therefore polyuria which with the increased plasma osmolarity due to hyperglycemia causes thirst and polydipsia (Zilva *et al.,* 1988). A prolonged osmotic diuresis may lead to excessive electrolyte loss (water, Na+, K+, calcium and other inorganic constituents) and to fall in circulation blood volume (Whitby *et al.,* 1988). Blood glucose levels are maintained by hepatic glucose production from gluconeogenesis. Besides, glycolysis extra hepatic tissue utilizes glucose and corresponds an amount of glucose provided by liver. Thus, keeping glucose homeostasis in balance (Ammone *et al.,*1996).

Therefore, the maintenance of glucose homeostasis depends on glucose ingestion and glucose up take, insulin secretion, hormonal counter-regulation and hepatic glucose efflux (gluconeogenesis and glycogenolysis). These processes are simultaneously ongoing and are coordinated physiologically to avoid hypo-and hyper- glycemic states. (Ammone *et al.,*1996). Normally, insulin stimulates the transport of amino acid into muscle cells and their synthesis to protein. If there is a lack in insulin, this synthesis is impaired. There is an accelerated rate of amino acid catabolism to CO_2 and H_2O via citric acid cycle and marked increase in the rate of conversion of amino acid to glucose in the liver (gluconeogenesis) (King, 2004).

Normal fatty acids are catabolized to acetyl CoA which may be synthesized into new fatty acid, converted into ketone or completely catabolized because of the intracellular depletion of the carbohydrate components of the cycle (Anderson *et al*;2003). They are, therefore, converted into ketone bodies: two acetyl–CoA moieties are formed and converted into acetoacetic acid, and β-hydroxy butyrate. Acetoacetic continually undergoes spontaneous decarboxylation to yield acetone (Harpers *et al.,* 1993).

Acetoacetic acid and 3-hydroxy butyric acid production give rise to metabolic acidosis, as the liver cannot metabolize. In general, then completely metabolized the increase an amount of these ketone bodies that are being formed. The acidosis is partly compensated by hyperventilation with reduction in PCO_2. Urinary excretion of ketone bodies results in the loss of sodium and state of acidosis occurs (Murrag *et al.,* 1996). In diabetes patient, the level of insulin is too low when it's compared with glycogen relative to the needs of the patient. Therefore, glycolysis is inhibited and gluconeogenesis is stimulated. Further, glycogen break down is promoted, hyperglycemia is state is exaggerated and the elevated blood glucose spills in the urine. (Ganong *et al*.,1997).

2.6 Diagnostic Tests for Diabetes Mellitus

Diabetes can be diagnosed by the presence of four classic signs that include polyuria, polyphagia, polydipsia, and foremost hyperglycemia (Vasudev and Jann *et al.,* 2011). Type 2 diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating one of the following tests:

1. Fasting blood glucose test (most common) - fasting blood glucose levels are checked after fasting for between 12 and 14 hours
2. Random blood glucose test - blood glucose levels are checked at various times during the day. Blood glucose levels tend to stay constant in a person who does not have diabetes.
3. Oral glucose tolerance test - a high-glucose drink is given. Blood samples are checked at regular intervals for two hours.
4. Glycohemoglobin HbA1c **-** measures how much glucose is stuck to red blood cells. It also shows how well diabetes has been controlled in the last 2 to 3 months and whether diabetes medicine needs to be changed. HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes mellitus, diagnosed using glucose tests (WHO, 2011)

Table 1.1: The standard values of diagnostic tests in type-2 diabetes mellitus

Test to diagnosis	Normal (mg/dL)	Pre-Diabetes (mg/dL)	Diabetes (mg/dL)
Fasting blood sugar	70-99	100-125	≥126
Random blood sugar	70-139	140-199	≥200
2 - hour glucose tolerance test	70-139	140-199	≥200

2.7 Pathophysiology of Diabetes Mellitus

The etiology of T2DM is complex and comprises a variety of different dysfunctions involving multiple organs and tissue types. In previous understanding, the pathophysiology of T2DM largely focused on β cell dysfunction and insulin resistance in skeletal muscle and liver, and that understanding has expanded in recent years to include defects in the adipose tissue, pancreatic α cells, gastrointestinal tract, brain, heart and kidney (Lorenzo *et al.,* 2010). Indeed, it is now

apparent that T2DM is a multisystem disease with multiple metabolic abnormalities that contribute in varying degrees to the development and maintenance of hyperglycemia (Figure 2.4). Chronic hyperglycemia is a primary factor in the pathophysiology of T2DM because of its contribution to the development of insulin resistance and β cell dysfunction, both of which, and in turn, aggravate hyperglycemia (Herrera *et al.,* 2004).

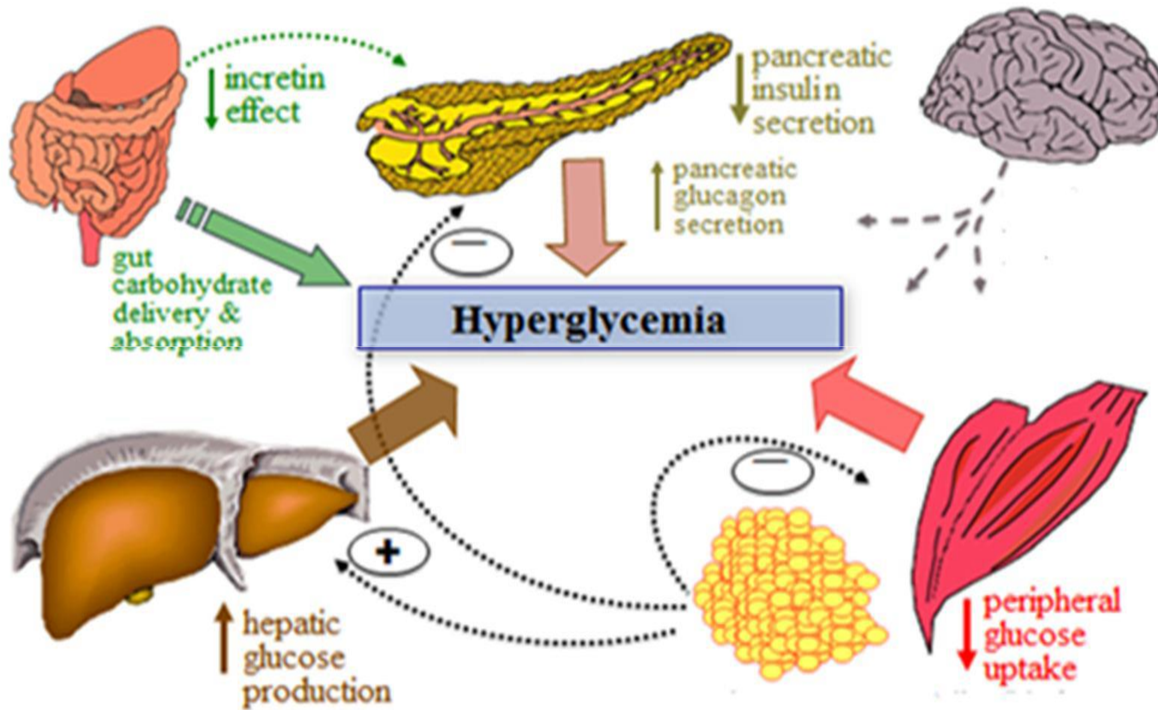


Fig 2.4: Pathophysiology of type 2 diabetes mellitus (Inzucchi *et al.,* 2012) (↑) (increase), (↓) indicates decrease, (+) indicates activation and (-) indicates inhibition.

The term insulin resistance refers to impairment in insulin action in insulin-target tissues such as skeletal muscle, liver, and adipocytes (fat cells). Insulin is the major anabolic hormone in the body and it is the major regulator of glucose metabolism. It stimulates glucose uptake and metabolism in skeletal muscle, suppresses hepatic glucose production (HGP), and restrains lipolysis in adipocytes (Neeland *et al.,* 2012). In the presence of insulin resistance, all of these insulin actions are markedly impaired, leading to impair insulin-mediated muscle glucose uptake and increased rates of HGP and lipolysis (Sachin *et al.,* 2009).

In addition to hepatic insulin resistance, multiple other factors contribute to accelerated rate of HGP, including

1) Increased circulating glucagon levels and enhanced hepatic sensitivity to glucagon;
2) Increased circulation of gluconeogenic precursors such as lactate, alanine, and glycerol; and
3) Increased free fatty acid (FFA) oxidation (Ismail, 2012).

Chronic elevation of plasma FFA concentration has been shown to cause severe insulin resistance in skeletal muscle and liver and may impair insulin secretion (Shiju and Pragasam *et al.*, 2012). Moreover, enlarged adipocytes have diminished capacity to store fat, and when the maximal fat storage capacity of the adipocytes is exceeded, excess lipid spills over to lean tissue (e.g., skeletal muscle, liver, and βcells), causing insulin resistance and impaired insulin secretion (Inzucchi *et al.,* 2012).

Lastly, dysfunctional fat cells produce excessive amounts of insulin resistance–inducing inflammatory adipocytokines (e.g., TNfα and interleukins), and fail to produce insulin sensitizing adipokines such as adiponectin (Bays *et al.,* 2004).

2.8 Molecular basis of Diabetes Mellitus

Indeed, T2DM is enriched with high levels of glucose, advanced glycation end-products (AGEs), proinflammatory cytokines, free fatty acids, and other lipid intermediates. These factors are toxic for β-cells and might activate several stress response pathways including oxidative and endoplasmic reticulum stress, mitochondrial dysfunction, apoptosis, and necrosis (Puddu *et al.,* 2013).

Since oxidative mitochondrial metabolism is required for normal glucose-stimulate insulin secretion from pancreatic β-cells, subtle defects in mitochondrial function result in insulin secretion and β-cell dysfunction (Barlow et al, 2011; Carrera and Martínez *et al.,* 2013).

The Endoplasmic reticulum stress pathway which is active in adipose tissue and liver has a molecular link between obesity, decreased insulin sensitivity and T2DM. This endoplasmic reticulum stress in obese individuals leads to suppression of insulin receptor signaling by

increased activation of c-Jun N-terminal kinase (JNK) and phosphorylation of IRS-1 on serine residues (Grover and Luthra *et al*., 2013).

The increased levels of non-esterified fatty acid, glycerol, leptin, resist in tumor necrosis factor-α (TNF-α), and cytokines in the blood plasma lead to the increased level of insulin resistance and reduced insulin sensitivity while adiponectin improves resistance (Bays *etal.*, 2004). Some common gene variants are also reported to be associated with T2DM such as PPAR i.e. key regulators of FA metabolism. Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear hormone receptors that function as transcription factors regulating the expression of a number of genes involving lipid metabolism and insulin resistance (Hu *et al.,* 2013). Over expressionof PPARα in mice leads to enhancedβ-oxidation of FAs and reduced glucose oxidation and accumulation of triglycerides (Abel *et al.,* 2012).

2.9 Complication of Diabetes Mellitus

Effective management of glycemic and lipid plays a vital role in diabetes mellitus. The complications are far less common and less severe if the blood sugar levels have been well-controlled. According to Edwin and his colleague's, acute complications include diabetic ketoacidosis, non-ketosis, hyperosmolar coma, and diabetic coma. In case of chronic complication, chronic elevation of fasting blood glucose level leads to damage toblood vessels (Edwin *et al.,* 2008).

Micro-vascular complication can leads to retinopathy, neuropathy and nephropathy. Macro-vascular complication can also leads to cardiovascular disease, mainly by accelerating atherosclerosis disorders. These disorders include:

(1) Coronary artery disease,

(2) Stroke (mainly ischemic type),

(3) Peripheral vascular disease,

Which contributes to intermittent claudication (exertion-related foot pain) as well as diabetic foot (Edwin *et al.,* 2008).

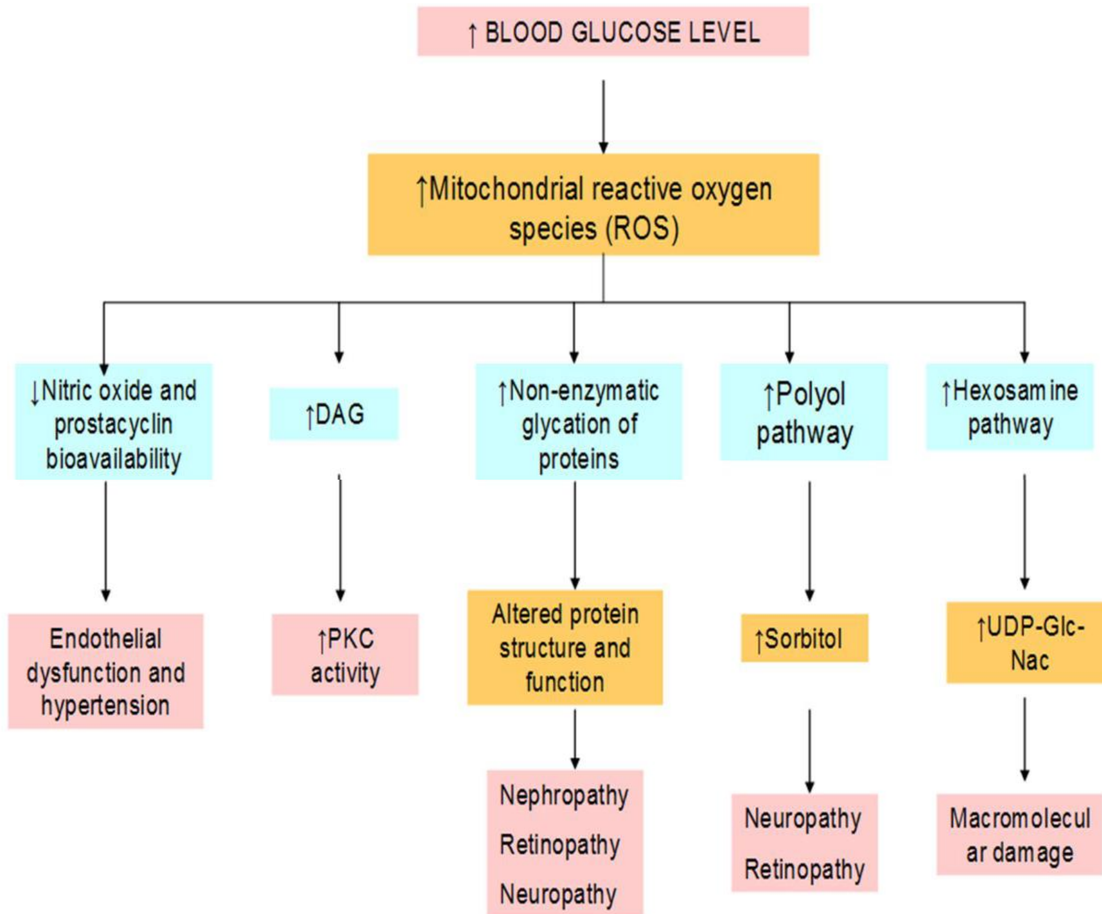


Figure 2.5: Metabolic pathways activated by chronically elevated blood glucose levels and long term complications of diabetes mellitus, *DAG- Diacylglycerol*, *PKCproteinKinase C, Glc - Glucosamine, UDP Uridine diphosphate, Nac-NAcetylglucosamine(Weiss and Sumpio, 2006)*

2.10 Kidney Dysfunction and Diabetes Mellitus

Type 2 Diabetes Mellitus is the single most common cause of end-stage renal disease worldwide (Stenvink *et al*., 2010). The main function of the kidneys is to remove waste from the blood and return the cleaned blood back to the body. In kidney dysfunction, the kidneys have no a capacity

to remove wastes and maintain the level of fluid and salts in the body. This end stage renal disease in diabetics is described as diabetic nephropathy.

Risk factors for development of diabetic nephropathy are glucose level, activity of insulin in plasma and glycosylation end products (Stojimirovic and Vlatkovic *et al*., 2008). It is believed that uncontrolled high blood sugar leads to the development of kidney damage and can cause renal end- stage disease (Amreen *et al.*, 2012). Over time, the high levels of sugar in the blood damage the millions of tiny filtering units of nephron within each kidney. The mechanisms involved in the pathogenesis of diabetic nephropathy are multiple and complex (George, 2011) but, it affects the kidney in stages (Volker and Scott *et al*., 2012). Kidneys affected by diabetic nephropathy have no longer work efficiency, and trace amounts of protein appear in the urine (Isra'a *et al.,* 2010).

The hyperglycemic state itself is a strong risk factor for diabetic kidney disease and causes the proliferation of mesangial cells and their matrix, as well as the thickening of the basement membrane (Volker and Scott*et al*; 2012). In recent years, many discoveries elucidated the mechanisms by which hyperglycemia affect the renal glomerular and tubule-interstitial cells and suggested that podocyte injury have a crucial role in the pathogenesis of diabetic nephropathy. Molecular pathogenesis of diabetic podocyte injury is likely multifactorial involving a number of interrelated signaling pathways that have yet to be well understood. Sustained hyperglycemia affects the glomerular cells byvarious mechanisms that lead to altered structure and function in the glomerulus (Rodica *et al.,* 2012).

There are many biomarkers from blood and urine for detections of kidney damage in T2DM such as creatinine, urea and proteinuria (Morteza *et al.,* 2012). These parameters are used for diagnostics, clinical outcomes and efficiency of therapy.

Approximately 2% of the body's creatinine is converted to creatinine every day. Creatinine is a naturally occurring nitrogenous organic acid that helps to supply energy to muscle cells. Creatinine is the metabolic waste product resulting from the breakdown of creatinine. It is transported through the bloodstream to the kidneys. Creatinine is eliminated by glomerular filtration through the kidneys and excreted in urine without tubular reabsorption. In renal dysfunction, the ability of the kidneys to filter creatinine is diminished leading to a rise in serum

creatinine. Therefore, serum creatinine level is usedas an indicator of renal function (Sasso *et al.,* 2012). The high level of creatinine in serum is caused by break down of skeletal muscle cells. Skeletal muscle is a major target tissue of insulin, and a lower volume of skeletal muscle would mean fewer target sites for insulin which causes increase in insulin resistance, and then leads to the development of T2DM (Arora *et al.,* 2010).

Similarly, urea is formed in the liver from ammonia released by deamination of amino acids. Over 75% of non- protein nitrogen is excreted as urea mainly by the kidneys; small amounts are lost through the skin and gastrointestinal tract. In kidney disease, urea accumulates in the body and is not excreted normally (Vrhovac *et al.,* 2008).

2.11 Treatments of Diabetes Mellitus

Diabetes mellitus is a chronic condition that can be controlled with lifestyle adjustment and medical treatment (Perryll *et al.,* 1996).

The treatment of diabetes is divided into two pathways:

A- The Pharmacologic Therapy which involve.

1. Insulin therapy
2. Drug therapy
3. Plants and natural material therapy

B- The Non-pharmacological Therapy.

1. Diet therapy
2. Activity modification

2.12 Current Type 2 Diabetes Mellitus Therapy and Associated Problems

The first-line treatment for T2DM is diet, weight control and physical activity. The combination of lifestyle modification, appropriate exercise and conventional therapies are recommended for the management of T2DM through improvements of metabolic risk factors such as blood pressure, blood glucose, plasma lipids, and oxidative stress markers (Molitch *et al.,* 2013).

Moreover, current convectional drugs available for T2DM include sulfonylureas and related compounds, biguanides, thiazolidenediones, α-glucosidase inhibitors of insulin.

Most oral anti-diabetic treatments target insulin resistance or β-cell dysfunction as their primary mechanisms of action. Sulfonylureas drugs acting via the incretin system, dipeptidyl peptidase-4 (DPP-4) inhibitors (incretin enhancers) and glucagon-like peptide1 (GLP-1) agonists (incretin mimetics), increase insulin secretion with the incretin drugs also normalizing glucagon secretion (Moore, 2007) as shown in figure 2.6

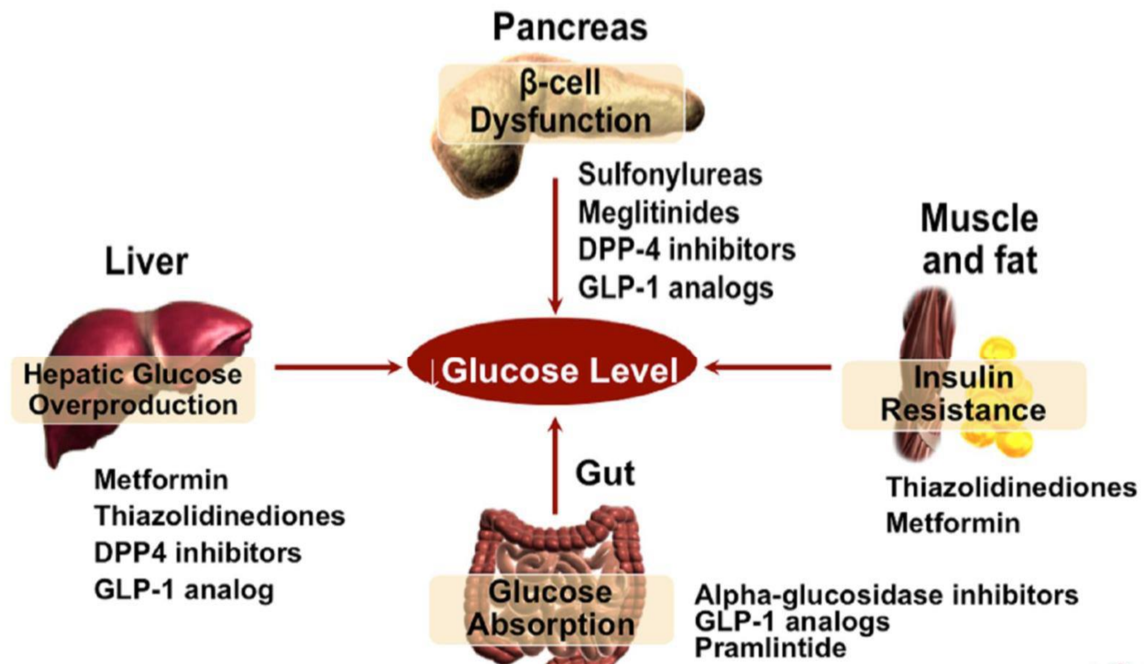


Figure 2.6: Sites of action and mechanism of antihyperglycemic drugs in treatment of hyperglycemia and T2DM: abbreviation; *DPP-4 dipeptidyl peptidase-4 andGLP1, glucagon-like peptide-1* (Molitch, 2013)

Glibenclamide is a potent anti-diabetic and second-generation of oral sulfonylurea drug that improves glucose control by acting both on insulin secretion and insulin action (Bodhankar *et al.,* 2009). Currently, it is available for treating hyperglycemia in T2DM.

The mechanism of action of Glibenclamide seems to be initiated by the linkage of drug molecules with receptor in the β-cell surface and subsequent reduction of conductance of the ATP-sensitive K+ channels. This inhibition causes cell membrane depolarization, opening of

voltage-dependent calcium channels, thus triggering an increase in intracellular calcium into the β cell which stimulates insulin release as shown below in figure 2.7 (Sharma *et al.,* 2011).

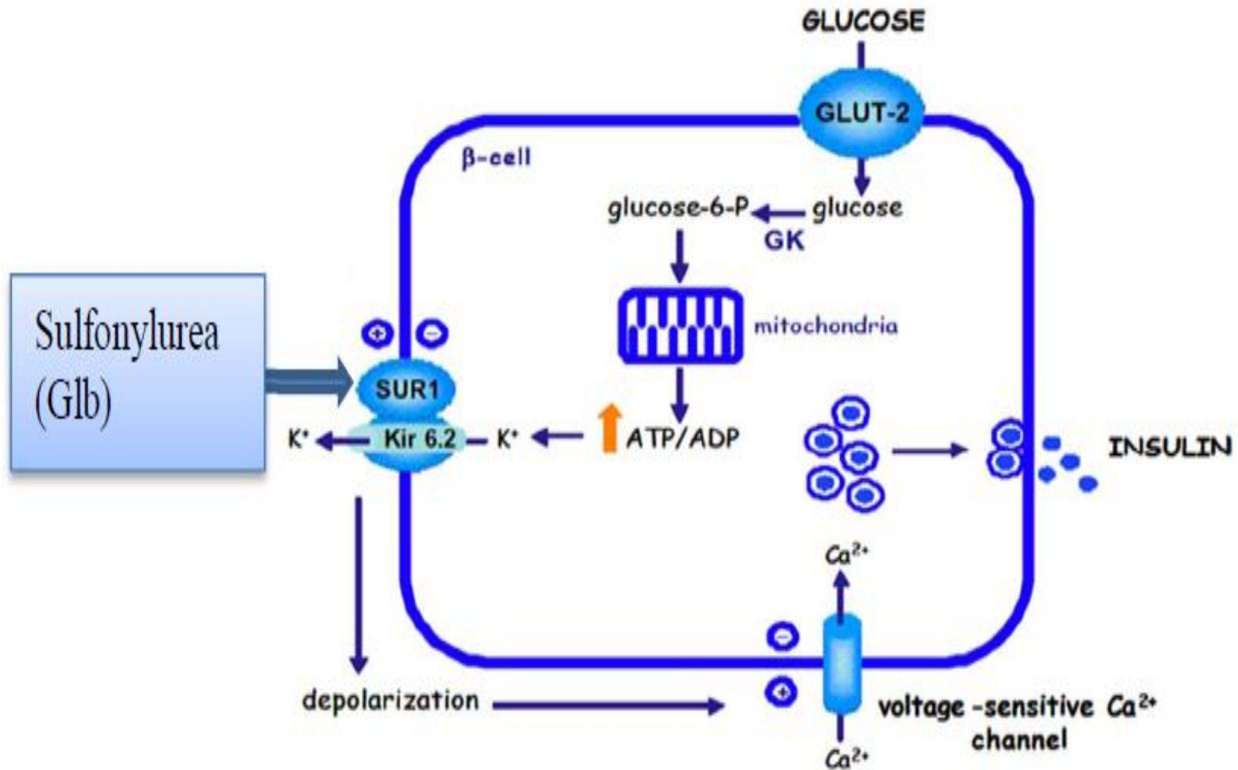


Figure 2.7: Mechanism of action of sulfonylureas on ATP-sensitive K+ channels *Sulfonylureas (Glibenclamide) promote the closure of KATP channels leading tomembrane depolarization, opening of Ca++ channels and initiation of insulin secretion mediated via the binding to a sulfonylurea receptor-1 (SUR1) togetherwith protein Kir 6.2., Glb-glibenclamide, GK-glucokinase, GLUT2- Glucosetransport-2, ATP-adenosine triphosphate, ADP-adenosine diphosphate.* (Rodríguez, 2004)

In recent years increasing evidence has suggested a possible role of Glibenclamide on insulin action at the level of different organ/tissues. Based on the same mechanism, there are also extra pancreatic action of the drug at the liver, skeletal muscle, heart muscle and smooth muscle sites. In liver, additional studies have shown that the drug has a positive action on glycogen deposition with direct action on the synthesis of GLUT-2 rather than GLUT-4 proteins and at the glycogen phosphorylase level. In a similar set of experiments Glibenclamide has been shown to increase

the fructose -2, 6-biphosphate levels, reducing the rate of glucose formation from a mixture of labeled lactate/pyruvate. The effect of the drug was mediated through an increased synthesis of GLUT1 with no effect on GLUT3 and GLUT4 in skeletal muscle cells (Moore, 2007).

Due to several different side effects of these convectional medications, there is a growing tendency toward finding medications with less subsidiary effects and as a result therapeutic herbs are taking lots of attention.

2.13 Medicinal Plant for Diabetes

The use of medicinal plants is as old as human civilization. Throughout history, humans have found that some plants and herbs can not only enhance the flavor of foods but alsoto restore health (Zahmatkesh and Khodashenas *et al.,* 2013).

Medicinal plants and traditional medicine play an important role in the health care system of most developing countries. Asian countries including India, Bangladesh and Nepal have glorious tradition of health care system based on plants, which dates back to many years. About 887 species used for medicinal purposes, constituting over 10% of the vascular species; exist in Asia (Reta *et al.,* 2013).Some study showed that nearly 80% of human population and 90% of livestock inAsia rely on traditional medicine (Yeweyenhareg and Fikre *et al.,* 2005).

The dietary antioxidants are the substances which are present in foods that significantly decrease the adverse effect of reactive oxygen species (Ros), reactive nitrogen species (RNs) or both on normal physiological function in humans (Dragland et al., 2003).

Commonly used herbs and medicinal herbs may contain novel antioxidants which have specific action perhaps at different levels of organisms biology, e.g. garlic onion and the desert plant Aloe vera which is exposed to high levels of solar radiation, are all known to have antioxidant and properties. These and other plants probably may also have other compounds which may positively help cell and tissues to repair damages caused by oxidation processes. Commonly used spices, ginger, and tumeric are also effective in preventing oxidation (Brighthope, 1994).

There is some early evidence that the vascular disease associated with diabetes and of course the cardiovascular mortality and morbidity may be reduced with better antioxidant natriture (Brighthope, 1994).

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic, and renal dysfunction remedies. Despite the presence of known anti-diabetic medicine in the pharmaceutical market, diabetes and related complications continued to be a major medical problem (Campbell *et al.,* 2012). Anti-hyperglycemic, and anti-renal failure effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes (Amreen *et al.,* 2012).

Traditional medicinal plants may act on blood glucose through different mechanism, some of them may have insulin-like substances, some may inhibit insulinase activity, others may cause increase inβcells in pancreas by activating regeneration of these cells. The fiber of these plants may also interfere with carbohydrate absorption; thereby affecting blood glucose (Jelodar *et al*., 2007).

Traditional medicinal plant practitioners of the study area reported that leaves were the dominant plant part used to prepare medications (31.9%), followed by seeds (19%), roots (15.3%), bulb (5.52%), shoot tip (4.29%), stem and stem bark (3.68%), fruits (1.84%), latex of stem, rhizome, flowers, gum of stem and whole plant (1.23%) and others (8.6%). The administration of remedy preparations was mainly through oral (74.8%), dermal (20.3%), nasal (3.7%) and optical (1.2%) (Reta *et al.,*2012).

2.13.1 Cinnamon

2.13.1.1 General Description of Cinnamon

Cinnamon is one of the species of Lauraceae family. It is also known as sweet wood, cassia and Guizhi. However, the name of cinnamon encompasses many varieties, including Cinnamoum cassia and Cinnamoum zeylanicum (Anderson *et al.,* 2003).

The origin of cultivated cinnamon is India, Burma and Sir Lanka (Ceylon) recently it is now cultivated in many tropical countries including Mexico, China and Brazil. This plant has been used in Ayurveda (India traditional medicine) and other medicinal traditions in South Asia and Americans (Linares *et al.,* 1994).

Fig.2.8: Cinnamon Leaf and Cinnamon Bark

The part of this important used in medicinally are the outer bark inner bark, leaves and essential oil (Stuart *et al.,* 2005). The peel bark is characterized by the tan to light brown color, easily break down, pungent taste, aromatic odor. The peels bark the important constituent of volatile oil percent 4% which contains important compounds such as cinnamaldehyde. In fact, most pharmacological activity is contributed to it the other compound is eugenol (Anderson *et al.,* 2003).

Broadhurst *et al.,* (2000) re-evaluated the extract of cinnamon on insulin function in the insulin-dependent utilization of glucose using a rat epiddymal adipocyte assay. Cinnamon was the most bioactive product. The glucose oxidation enhancing bioactivity was lost from cinnamon by poly-vinylpyrrolidone (PVP) treatment. Indicating that the active phytochemicals were likely to be phenolic in nature, they concluded that the extract of cinnamon had improved the glucose and insulin metabolism.

However, the cinnamon might have brought some biochemical /physiological changes in the sites of resistance to insulin, transfer of glucose through cell membrane, enzyme system of carbohydrate metabolism and receptor sites. The biochemical and physiological changes in the sites of resistance to insulin or other parameter are true, then, a permanent cure for diabetes mellitus is present in cinnamon therapy (Begum *et al.,* 1991).

Anderson (2006) shows that the active components of cinnamon are found in the water-soluble portion of cinnamon and are not present in cinnamon oil which is largely a fat-soluble. In addition to ground cinnamon which are consumed directly (Anderson *et al.,* 2004). They identified cinnamon's bioactive compound poly phenol type-A polymer which is a water-soluble.

The cinnamon is an ancient herbal medicine mentioned in Chinese text as long 4.000 years ago, cinnamon was used in ancient Egypt for embalming- in ancient times. It is added to food to prevent spoiling. And it is one of the oldest remedies prescribed for everything from diarrhea, chills to influenza and parasitic worms (Anderson *et al.,* 2003).

2.13.1.2 Types of Cinnamon

There are two types of the cinnamon

1- **Cinnamon cassia:**

This tree is usually grown in china and Burma besides, it is grown commercially in many sub-tropical countries. It is characterized by it is long shiny leaves, small pale green flowers, tan to reddish color of peel bark pungent taste, aromatic odor also loose thin and peeling bark. The flavor of the bark is not as subtle as true cinnamon, but strong and pungent and often used as a substitute (Garland *et al*., 1979).

2- **Cinnamon zeylanicum**:

Which is another type of family Lauraceae. It is origin belongs to Sri Lanka, but it is cultivated in the West Indies and in many eastern countries. This type is characterized by its tree smaller than cassia with similar tough shiny leaves, small yellowish-white flowers and a dark blue berry. It has aromatic odor, pungent taste, reddish-brown to variation of brown color, the bark harvested from the young shoots and the palest bark being of the finest quality. The quills are thinner and more fragile than those cassia. This type is richer with aromatic oil than other types (Garland *et al.,* 1979).

Chemical Composition of Cinnamon: according to (Jayaprakasha and Rao *et al.,* 2000)

Volatile oil that involves:

A) (Cinnamaldehyde, eugenol, weitherthin, cinnamic acid and pinene).
B) The various terpenoids found in the volatile oil are believed to account for cinnamon's medicational effectsMucilage, Diterpenes, Proanthocyanidins, Mannitol, Gum and coumarins. Further it contains low carbohydrate, fat and a lot of fiber (Anderson *et al.,* 2003; Stuart, 2005).

2.13.2 Traditional Uses of Cinnamon

According to (Jayaprakasha and Rao 2002)

- To treat upset stomach and diarrhea.
- Cinnamon bark may possess a potentiating effect on insulin and can be useful in the treatment of type II diabetes as well as lowering triglyceride levels and serum cholesterol.
- Treatment of bronchitis, coughs and other respiratory ailments.
- To treat loss of appetite and dyspepsia.
- Treatment of hypertension.
- As an in Vigo rating tonic.
- Externally as a poultice to treat the minor bacterial and fungal infection of the skin.
- The essential oil is employed in aroma therapy as a rub to promote blood circulation
- Cinnamon constituents possess antioxidant and may prove beneficial against free radicals damage to cell membranes.
- Cinnamon oil has been proved to be particularly effective against some species of toxicogenic fungi as well as against respiratory tract pathogenesis including species belonging to the genera aspergillus, candida, Cryptococcus and Histoplasma.

2.13.3 Safety and Precautions

According to (Jayaprakasha and Rao *et al.,* 2002)

- Cinnamon bark should not be used during pregnancy and lactation or small children in medicinal doses, only as aspice or food condiment.
- Cinnamon constituents may be irritating to the oral mucous membranes.
- Contact dermatitis in susceptible individuals has been reported after using cinnamon containing ointments.
- Do not employ tea in large amounts for patients with ulcers.
- Some people may be hypersensitive to the essential oil used topically to treat skin infections or as arubefacient in aroma therapy.
- Cinnamon essential oil should not be ingested due to it is potential toxicity especially for children and adolescents.
- Undiluted essential oil should not be applied topically.

2.13.4 Some Plants Effect on Hyperglycemia

In recent years, focus on herbs research has increased all over the world. In diabetes mellitus, the major contributions were in the field of hypoglycemic action of various plant products and drug interaction of hypoglycemia agents.

The role of diet in the prevention and treatment of diabetes and cardiovascular disease is clear. However, the plants that are used as antidiabetes are also rich with vitamins, minerals and fiber which are necessary for the adequate nutrition of the diabetes patients. But these are characterized by a low carbohydrate and fat content and by providing some proteins (Roman-Ramos *et al.,* 1995).

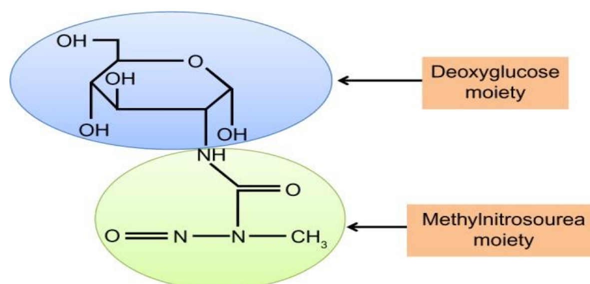
Botanical products can improve glucose metabolism and overall condition of patients suffering from diabetes not only by hypoglycemic effect, but also by improving renal function lipid metabolism, antioxidant status and capillary function (Broadhurst *et al.,* 1997).

These plants were found to possess active constituents like glycosides, saponins, alkaloids, oils, flavonoids, tannin, polysaccharides and terpenes (Jahadar *et al.,*1993). A number of medicinal/ culinary herbs have been reported to yield hypoglycemic effects in subject with diabetes such as: Eugenia jambolana, Green tea, Momordica charantia, Gymnema sylvester, Piper nigrum, Vinca rosea and Azadirachta indica, these plants are effective for both prevention and treatment of diabetes mellitus (Sharma *et al.,* 2006).

Cinnamon cassia, called Chinese cassia or Chinese cinnamon, is an evergreen tree originating in southern China, and widely cultivated there and elsewhere in southern and eastern Asia (India, Bangladesh, Nepal, Indonesia, Malaysia, Taiwan, Thailand, and Vietnam). It is one of several species of Cinnamon used primarily for their aromatic bark, which is used as a spice. In the United States, Chinese cassia is the most common type of cinnamon used. The buds are also used as a spice, especially in India, and were once used by the ancient Romans.The tree grows to 10–15 m tall, with greyish bark and hard, elongated leaves that are 10–15 cm long and have a decidedly reddish color when young. (Jayaprakasha and Rao 2000).

2.14 Streptozotocin

Streptozotocin or streptozocin (INN, USP) (STZ) is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas in mammals.



Chemical structure of Streptozotocin

2.14.1 Role of Streptozotocin in Type 2 Diabetes Mellitus

The most common chemicals to induce diabetes in the animal model are Alloxane and Streptozotocin (STZ). Streptozotocin is a glucosamine–nitrosourea compound that has a capacity of producing mild to severe types of diabetes according to the dosages used when it is given to animals by either single intravenous or intraperitoneal injection. (Sachin *et al.,* 2009).

Streptozotocin damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia (Katherine and Laura, 2009). The selectivity for βcells is associated with preferential accumulation of the chemical in β cells after entry through the GLUT-2 glucose transporter receptor due to similar chemical structural withglucose (Mahmoud, 2009).

Since STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, it was proposed that this molecule contributes to STZ-induced DNA damage (Kamal *et al.,* 2011). Streptozotocin was also found to generate reactiveoxygen species, which also contribute to DNA fragmentation and induce other deleterious changes in the cells. The formation of superoxide anions in turn causes an increase in the activity of xanthine oxidase. NO and reactive oxygen species can act separately or form the highly toxic peroxynitrate (ONOO) as shown below in Figure 2.9.

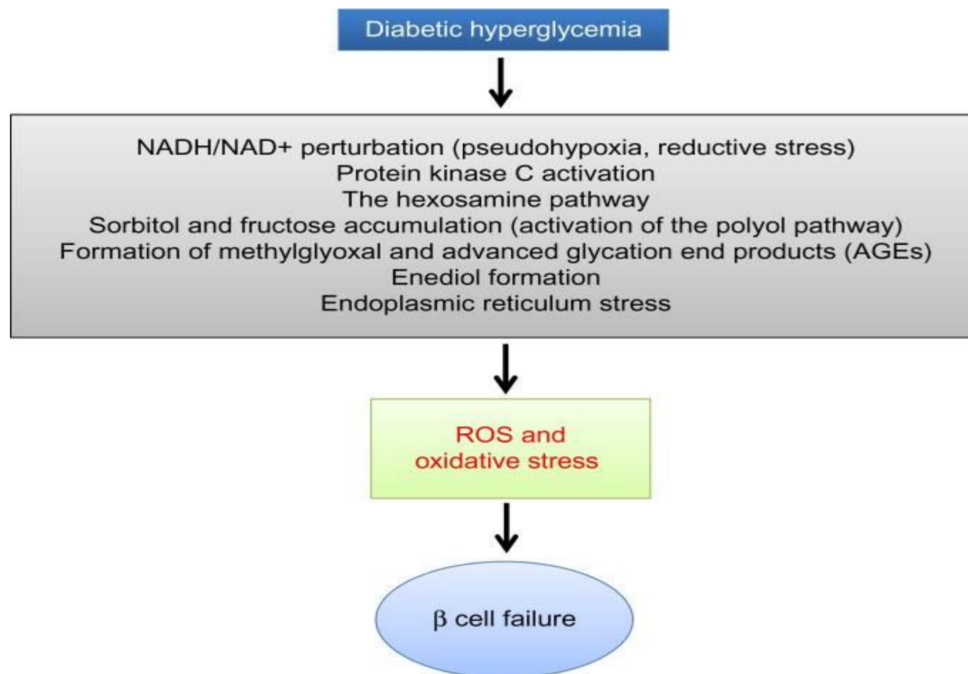


Figure 2.9: The mechanism of STZ induced toxic events in B cells of Pancreas: Streptozotocin induced DNA damage activates poly ADP- ribosylation. This process leads to depletion of cellular NAD+, then further reduction of the ATP content and subsequent inhibition of insulin synthesis and secretion (Lenzen *et al*., 2008).

According to Graham, serum glucose level was increased significantly, while insulin level decreased in STZ induced nude mice. The diabetogenic agent Streptozotocin selectively destroys β-cells in the pancreas. This results an inhibition of insulin synthesis and elevation of blood glucose level, firstly due to reduction in entry of glucose to peripheral tissues, muscle and adipose tissue; secondly due to increased glycogen breakdown and increased gluconeogenesis and hepatic glucose production (Graham *et al.,* 2011).

3. MATERIALS AND METHODS

3.1 Experimental Animals and Study Protocol

Laboratory male Swiss albino mice (36-40) gm, 8th week age, were obtained from the department of Pharmacology, Institute of Agriculture and Animal science (IAAS), Tribhuban University, Nepal. All experimental animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by Committee for Purpose and Control of Supervision of Experiments on Animals, and approved by Department of Pharmacology. The animals were allowed to acclimatize in the laboratory environment for a week before the commencement of the experiment. The mice were housed in a standard plastic cage measuring 30×13×15 cm at temperature $(25\pm2)^0$C and 12/12 light/dark cycle under controlled environment and sawdust substrate was changed weekly. The mice were fed a standard commercial pellet diet at a dose of (120-150) gm/kg recommended or advised by Nimbus feed ltd. and water *ad libitum* throughout the experimental period.

3.2 Place of Study

The experiment was conducted in Tribhuban University, Institute of Agriculture & Animal science (IAAS), Department of Pharmacology, Nepal. At first the Shed with plastic cage was cleaned by using detergent and clean water. The debris or dust were expelled or flushed by using hose pipe connected with tape and disinfected the room and cage phenolic disinfectant (Phenol). For ensuring the proper ventilation and light, settled the electric fan and fluorescent bulb.

3.3 Experimental Layout

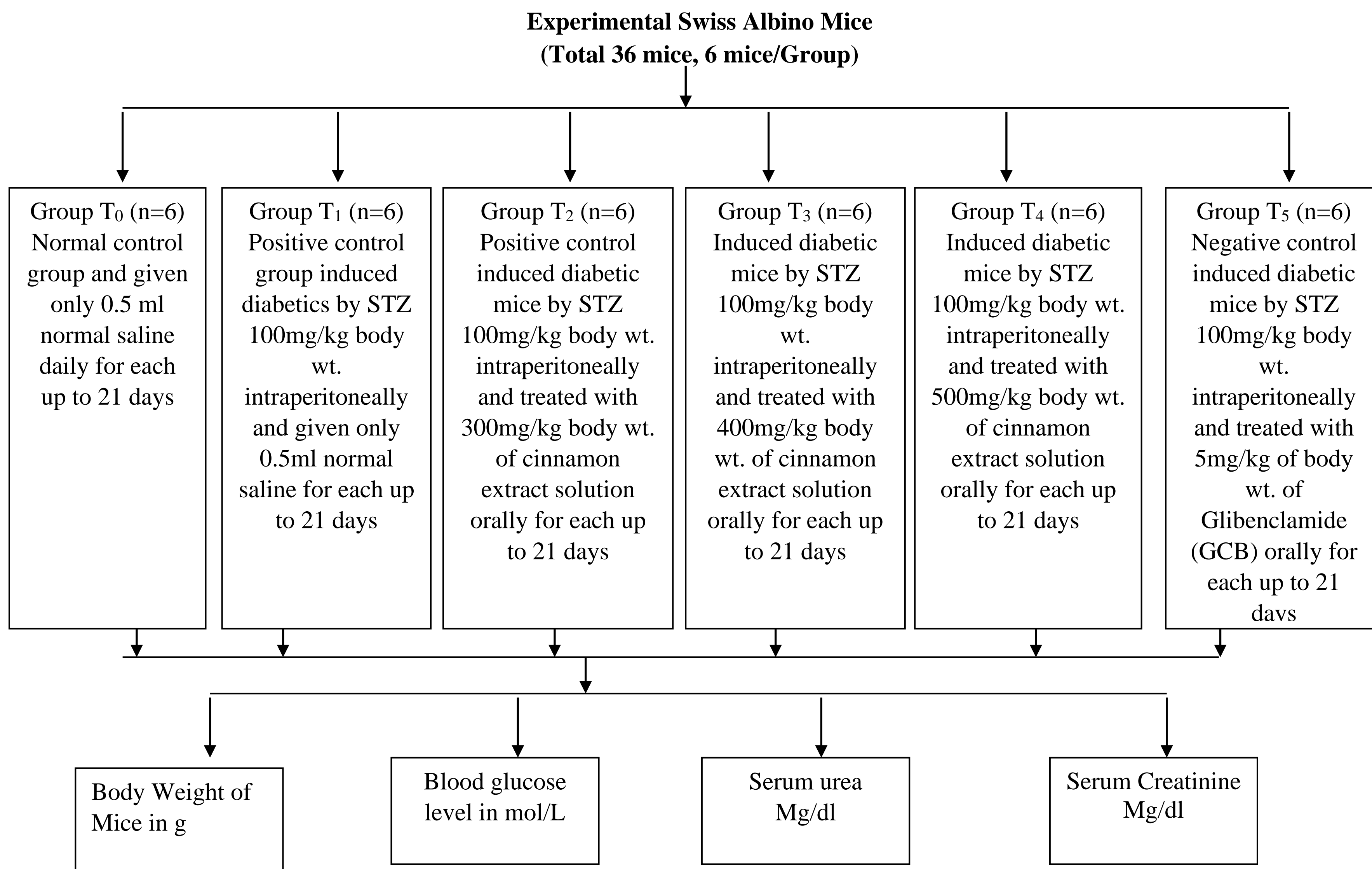


Fig 2.10: Layout of the experiment

3.4 Design of Experiment

36 male Swiss albino mice were used to carry out this investigation, and the mice were divided into six groups comprising of six mice in each group designed and maintained as follows:

Group T_0: The mice were fed normal diet and water supplied *ad libitum* and their body weight and blood glucose were recorded after acclimatization. This group served as a Normal control group and were given only 0.5ml saline daily. Body weights and glucose level were measured at the time when that of other groups were measured.

Group T_1: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then STZ induced by intraperitonealinjection at a dose rate 100mg/kg body weight to each of the group mice. The mice were fed normal diet and supplied *ad libitum* water from day 1 to 21. On 14th day blood glucose level and the body weight were again measured to ensure hyperglycemic condition or diabetic condition that served as Positive Control and were given 0.5ml saline only. Then all the mice of this group were kept for 14 days without any treatment, during that period on day 1, 7, 14 & 21 the body weight and blood glucose level were measured.

Group T_2: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then STZ induced by intraperitoneal injection at a dose rate 100mg/kg body weight to each of the groupmice. The mice were fed normal diet and supplied *ad libitum* water from day 1 to 21 day. The blood glucose level and the body weight were again measured on 14th day andSTZ induced to mice by injection to ensure diabetic condition. After that extraction of cinnamon was given for 21 days. During treatment of cinnamon extract body weight and blood glucose level were recorded on day 1 and 7, 14 & 21 days. This group served as treatment group to find the effect of treatment with 300 mg/kg of *Cinnamon bark* extracts

Group T_3: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then STZ induced by intraperitonealinjection at a dose rate 100mg/kg body weight to each of the groupmice. The mice were fed normal diet and supplied *ad libitum* water from day 1 to 21 day. The blood glucose level and the body weight were again measured on 14th day and STZ induced to mice by injection to ensure diabetic condition. After that extraction of cinnamon was given for 21 days. During treatment of cinnamon extract body weight and blood

glucose level were recorded on day 1 and 7, 14 & 21 days. This group served as treatment group to find the effect of treatment with 400 mg/kg of *Cinnamon bark* extracts

Group T_4: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then STZ induced by intraperitonealinjection at a dose rate 100mg/kg body weight to each of the groupmice. The mice were fed normal diet and supplied *ad libitum* water from day 1 to 21 day. The blood glucose level and the body weight were again measured on 14th day and STZ induced to mice by injection to ensure diabetic condition. After that extraction of cinnamon was given for 21 days. During treatment of cinnamon extract body weight and blood glucose level were recorded on day 1 and 7, 14 & 21 days. This group served as treatment group to find the effect of treatment with 500 mg/kg of *Cinnamon bark* extracts

Group T_5: After acclimatization, body weight and blood glucose level were measured after 18 hours of starvation, then STZ induced by intraperitonealinjection at a dose rate 100mg/kg body weight to each of the groupmice. The mice were fed normal diet and supplied *ad libitum* water from day 1 to 21 day. The blood glucose level and the body weight were again measured on 14th day and STZ induced to mice by injection to ensure diabetic condition. After that extraction of cinnamon was given for 21 days. During treatment of Glibenclamide (GIb) 5mg/kg body weight and blood glucose level were recorded on day 1 and 7, 14 & 21 days (Tamiru *et al.,* 2012). This group served as negative control group.

Concentration selections were based on the safe doses of extract in oral acute toxicity studies carried out earlier in this study Cinnamon bark extract extracts and Glibenclamide were administered orally for 21 days

3.5 Instruments, Reagents and Drugs

The following instruments, reagents and drugs were used for this study.

Instruments:

Whatman filter paper No.1, test tube, gel tube, volumetric flask 5 L, beakers 400 mL, funnels, measuring cylinder 1000 mL, glass rod, spatula, magnetic stirrer, semi-automatic pipettes of 10, 200 and 1000 µL, gavage (oral feeding syringe), Syringe 1 mL, 3 mL, desiccator, heater,

refrigerator, digital electronic balance, pH meter, one touch basic glucometer, strip,(Johnson & Johnson company, India).

Reagents: Ethanol, citric acid, sodium hydroxide, tri-sodium citrate, 5% glucose solution, diethyl ether.

Drugs:

- ✓ Streptozotocin was also purchased from Sisco Research Laboratories Pvt. Ltd. (SRL) - India
- ✓ Glibenclamide (Maan Medex private limited, India) was purchased from a local drug store in Patna of India.

3.6 Preparation and Alcoholic Extract of *Cinnamon* bark

Cinnamon bark was purchased from the local market, Bhairwaha, Rupendehi Nepal. It was identified and authenticated by taxonomist in Tribhuban University, Nepal, The bark were washed carefully with distilled water to remove any extraneous materials and then grounded to coarse powder using electric grinder. Three hundred gram of dried and grounded bark was extracted with ethanol 70% in a soxhlet apparatus for 48 hr. at 60°C. After extraction, the solvent was evaporated to dryness at (40° - 45°C) by using a rotary evaporator and the extract left behind was stored at 4°C

3.6.1Yield of Cinnamon Extracts

The percentage yield of ethanolic 70% extract of the dried *Cinnamon extract* was found to be 5.7% (w/w).

$$\% \text{ Yield} = \frac{\text{Actual mass obtained from experment (gm)}}{\text{Theoretical Mass (gm)}} \text{ x } 100\%$$

$$\% \text{ Yield} = \frac{17.2\text{gm}}{300\text{gm}} \text{ x } 100\% = 5.7\%$$

The extract was dark-brown jelly and solidified when stored in a deep freezer and turn to semisolid state on re-exposure to room temperature.

3.6.2 Acute Toxicity Test of *Cinnamon bark* extracts

Acute oral toxicity study was conducted according to Organization for Economic Cooperation and Development guideline 423, and six male mice were orally administered a single concentration of 2000mg/kg body weight of *cinnamon bark* extracts. Mortality and toxicity signs such as coma, anxiety, polyuria and other behavioral changes were observed and recorded after 1, 3 and 6 hours of administration of the extract for three days.

3.7 Induction of Experimental Diabetes

Diabetic were induced to fasting mice by a single intraperitoneal injection of freshly prepared STZ at a concentration of 100 mg/kg body weight in 0.1 M citrate buffer (pH4.5) in a volume of 20 ml/kg body weight (Sachin *et al.,* 2009). After one week of Streptozotocin induction, fasting blood glucose levels were estimated and mice with blood glucose 200 mg/dl were considered as diabetic, and used for the experiments.Streptozotocin can selectively destroy the pancreatic β-cells with rapid and irreversible necrosis and can be used to generate a chronic model of hyperglycemia with diabetes complications.

Protocol:

- ✓ Administer an intraperitoneal injection of Streptozotocin (100mg/kg body weight).
- ✓ Monitor glucose level for onset of hyperglycemia.

3.8 Biochemical Test Assay

At the end of the experimental period, all groups of animals were euthanized by anesthetizing with diethyl ether and then blood was collected via lateral Tail vein.After the blood was coagulated at room temperature for 30 minutes, it was centrifuged for 10 minutes at 3000 rpm. Serum samples were stored in deep freezer at -20°C until further analyses of various biochemical parameters were determined. Urea and creatinine were estimated with chemistry analyzer.

3.8.1 Fasting Blood Glucose Level

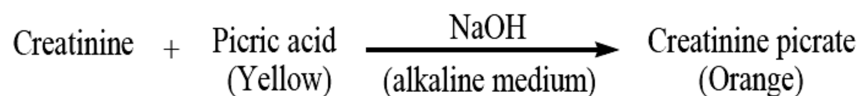
Blood sample was collected from the tail vein of the mice, and fasting blood glucose was estimated with One Touch Basic Glucometer after 6 hour fasting on 1st, 7th, 14th and 21stdays. The method involves two coupled reactions (Mukherjee, 1988).

With a second reaction of;

The increase in absorbance of NADPH at 340 nm was measured and directly proportional to concentration of glucose.

3.8.2 Serum Creatinine

Colorimetric estimation of serum creatinine is done by using the alkaline picrate method via Jaffe's Method (Peake and Whiting, 2006). Creatinine in an alkaline medium forms a colored complex with picric acid. The formation rate of the complex measured calorimetrically through the increase of the absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.



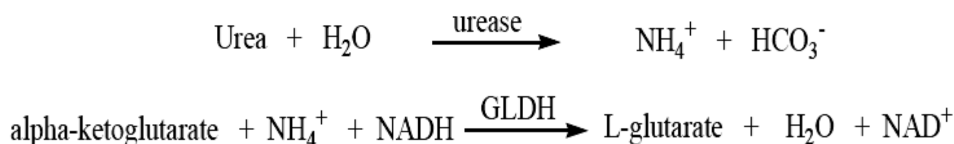
For this study; the sample was serum of the mice and, the reagents are standard and ready for use on automated analyzer. This enzymatic Assay was done at 400nm wavelength, 1cm optical path, room temperature and measurement done against air (increasing absorbance).

Procedure:

Working reagent, sample and standard were pre-incubated at 37 °C. The spectrophotometer was adjusted to zero absorbance with air. Working reagent (10µl) and sample (10µl) or standard (10µl) were pipetted into cuvette and mixed gently. The cuvettes were put into the cell holder and stopwatch started to count. The absorbance was recorded at 400 nm after 30 seconds (A1) and after 90 seconds (A2) of the sample or standard addition.

3.8.3 Serum Urea

Urea is split into ammonia and carbon dioxide in the presence of water and urease. Then, ammonia combines with 2-oxoluglutarete and NADH by the enzyme glutamate dehydrogenase (GLDH) to yield glutamate and NAD+. The reaction is monitored kinetically at 340 nm by the rate of the decrease in the absorbance resulting from the oxidation of NADH to NAD+, proportional to the concentration of urea present in the sample (Fawcett and Scott, 1960).



For this study, the sample was serum of mice and, the Reagents are standard and ready for use on automated analyzer. This enzymatic Assay was done at 340 nm wavelength, 1 cm optical path, room temperature and measurement done against air (decreasing absorbance).

Procedure:

Working reagent, samples and standards were pre-incubated at 37°C. The spectrophotometer was adjusted to zero absorbance with air. Samples (10µL) or standard (10µL) and working reagents (10 µL) were pipetted into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance was measured at 340 nm exactly after 30 seconds (A_1) and exactly 90 seconds later (A_2) of the sample and standard addition. Finally, after 30 and 90 seconds absorbance, the difference was calculated.

3.9 Recording of Different Parameters

3.9.1 Recording of body weight

Determination of body weight

Body weight was taken on day1 and 21st day of treatment. (During treatment)

Materials required

- Leather gloves
- Electric balance

Procedure:

Body weight of all groups was recorded before treatment on Day 1 and21st day by the help of electric balance.

3.9.2 Recording of blood glucose

Collection of blood

Material required: Leather gloves, pinching needles, ethanol, cotton, One Touch Ultra® 2, one touch basic glucometer, strip, (Johnson & Johnson Company, India).

Procedure:

For time to time blood glucose level determined, the blood samples were collected from the tail vein of each mice of group as a drop. The drop was then immediately placed on the strip of OneTouch Ultra® 2 active monitor to find the glucose level quickly. The values were expressed in m mol/L.

Determination of blood glucose level:

Blood sample were collected from tail vein at a day 1st, 7th , 14thand 21st (during treatment for estimation of blood glucose levels. estimation of blood glucose level was performed by OneTouch Ultra® 2 active monitor blood glucose system(Strip method)

Material required:

- OneTouch Ultra® 2 active monitor
- One touch basic glucometer, strip, (Johnson & Johnson Company, India).

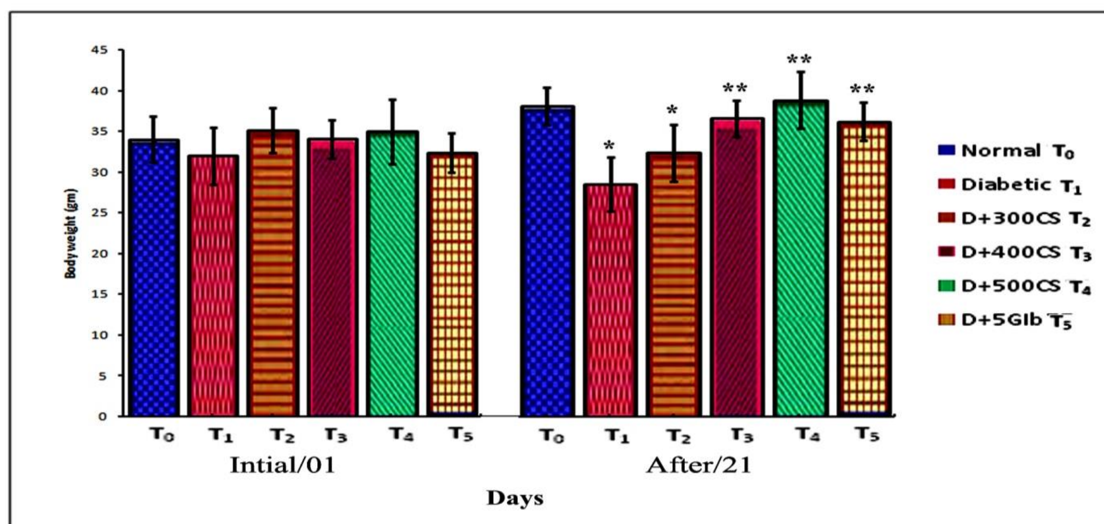
3.10 Statistical Analysis

The results of various biochemical parameters were expressed as mean ± SEM. Data analysis of the Statistics were done using SPSS version 20 and Microsoft Excel. Statistical difference between means analysis was done using analysis of variance (ANOVA) followed at a 5% level of significance.

4. RESULTS

4.1 Effects of Cinnamon extracts on Body Weight

The body weights were found to drop in diabetic mice as compared with normal control group. However, there were slight increases of the body weights in all concentrations of *Cinnamon extract* treated diabetic mice as shown below in figure 4.1.

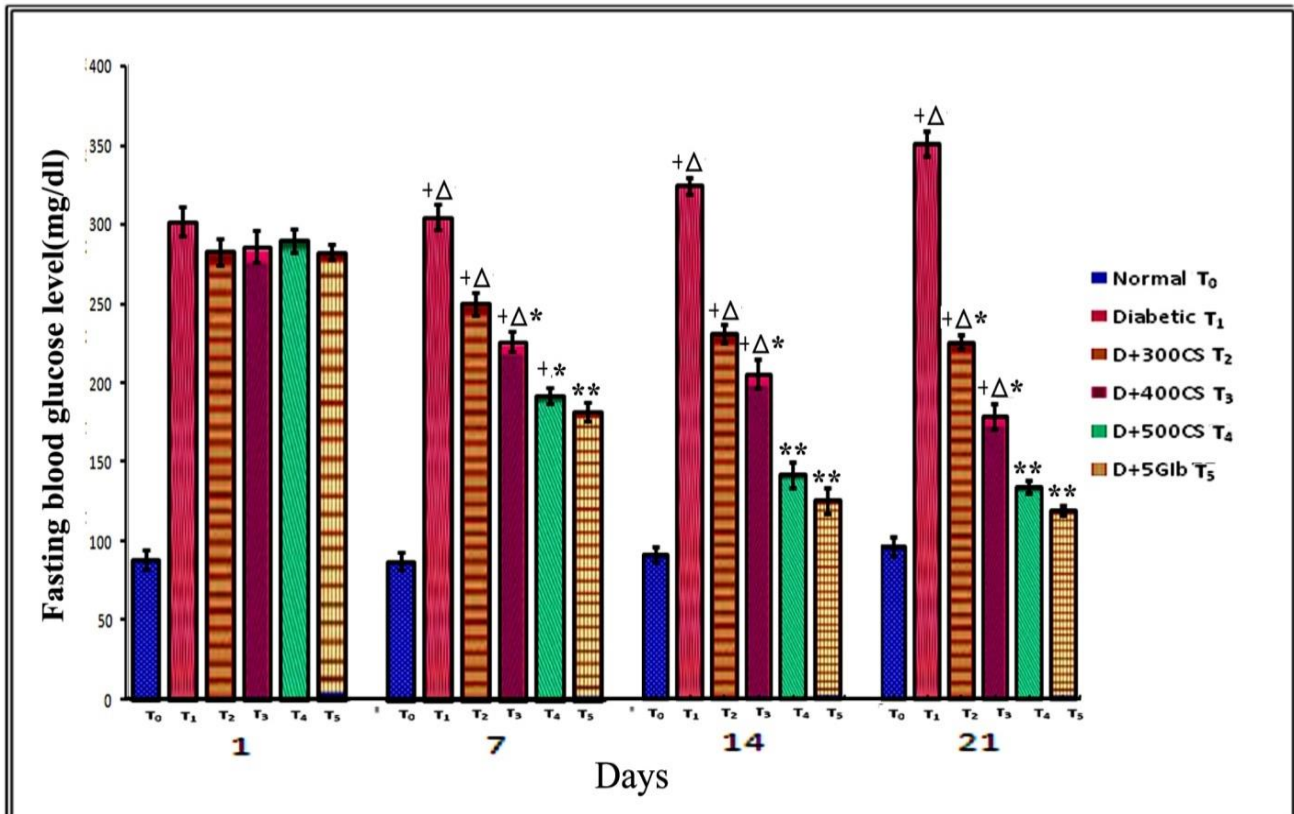


Experimental Groups of Mice	**Body weight (g)**		
	Initial (01 days)	After (21 days)	Level of Significant
Normal T_0	34.03±2.86	38.34±2.29	
Diabetic T_1	31.99±3.51	28.7±3.32	*
D+300CS T_2	32.07±2.75	35.5±3.52	*
D+400CS T_3	34.05±2.35	36.81±2.24	**
D+500CS T_4	34.97±3.96	39.07±3.53	**
D+5GIb T_5	32.42±2.41	36.43±2.35	**

Figure 4.1: Effects of *cinnamon extract* on body weight in STZ induced diabetic mice. *The each value is a mean ±SD for six mice is group. Values are statistically significant at *-$p<0.05$ diabetic control compared with normal control, **-$p<0.001$ treated group compared with diabetic control. D-Diabetic, CS - cinnamon extract solution, Glb -Glibenclamide.*

4.2 Effect of *Cinnamon extract* on Fasting Blood Glucose Level

The anti-hyperglycemic effects of graded concentration of *Cinnamon extract* on the FBG levels of STZ induced diabetic mice were presented in figure 4.2 asshown below. The FBG levels were significantly ($p<0.001$) increased as compared to normal control group throughout the study period. This increase of blood glucose was almost three-fold higher even after three weeks compared to normal control mice. However, treatments of diabetic mice with *Cinnamon* extracts, the FBG levels were significantly ($p<0.01$) decreased on 7th, 14th and 21st days. Similarly, treatment with Glibenclamide, which has been used as standard anti-diabetic reference drug to compare the beneficial effects of *Cinnamon extract*, also led to a significant ($p<0.001$) reduction in FBG levels on 7th, 14th and 21st days.



Experimental Groups of Mice	Fasting Blood glucose level (mg/dl)						
	1st day	7th day	Level of Significant	14th day	Level of Significant	21st day	Level of Significant
Normal T_0	89.38±6.23	92.61±5.94		96.25±5.79		98.28±5.85	
Diabetic T_1	304.49±9.21	319.75±8.80	Δ+	342.33±5.45	Δ+	357.92±8.35	Δ+
D+300CS T_2	285.4±8.56	262.69±7.51	Δ+	243.92±6.28	Δ+	229.81±4.62	Δ+*
D+400CS T_3	288.66±10.27	237.52±6.74	Δ+*	216.77±9.81	Δ+*	182±8.45	Δ+*
D+500CS T_4	292.53±7.62	201.93±5.29	Δ+	149.56±8.41	**	136.84±4.21	**
D+5GIb T_5	285.24±4.96	191.34±5.96	**	132.25±8.51	**	121.53±3.29	**

The results are expressed as mean ± SD (n =6).

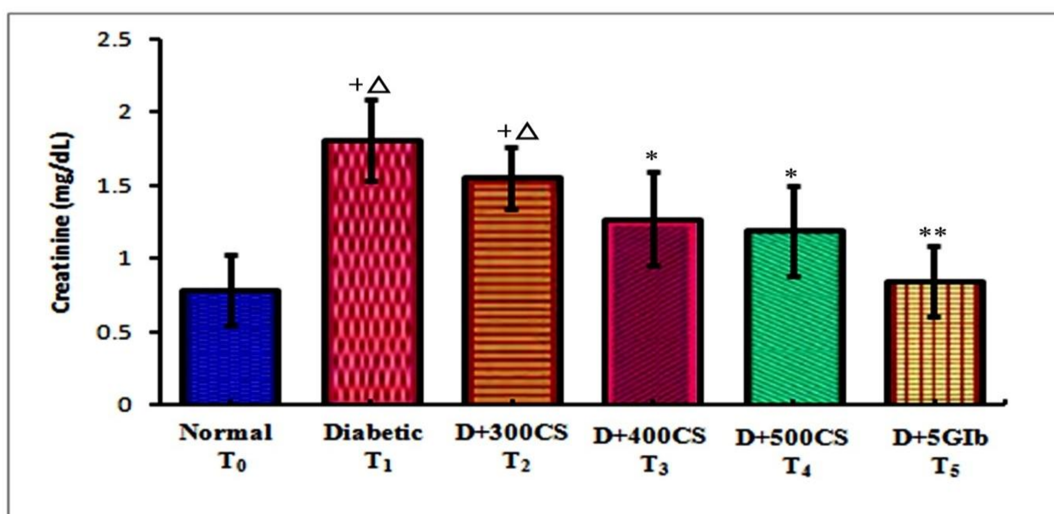
** – significant at $p<0.05$ compared with diabetic control,*

*** - significant at $p<0.001$ compared with diabetic control, + - significant at $p<0.05$ compared with normal control, Δ -significant at $p<0.05$ compared with (Glb) Glibenclamide treated group, D-Diabetic, CS- Cinnamon extract solution*

Figure 4.2 Effects of *Cinnamon extract* on fasting blood glucose in STZ induced diabetic mice on 1st, 7th, 14th and 21st days.

4.3 Effects of Cinnamon extract on Serum Creatinine

Figure 4.3 describes the effect of *Cinnamon extract* on serum creatinine in normal and diabetic mice. There were significant ($P<0.01$) increases in serum creatinine in diabetic group as compared to normal control. However, serum creatinine was reduced after the administration of *Cinnamon extract* at all concentrations and 5mg/kg Glibenclamide in treated diabetic mice as compared to diabetic mice.



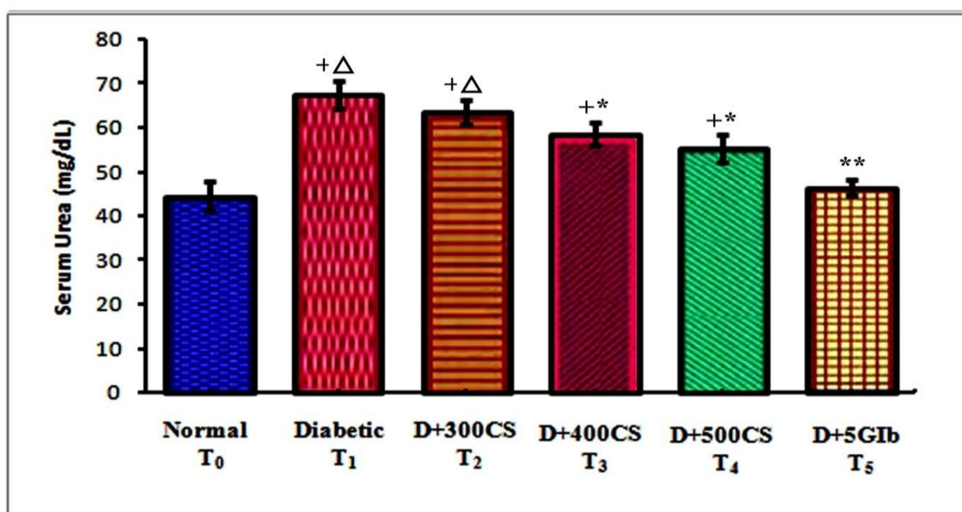
Experimental Groups of Mice	Serum Creatinine (mg/dl)	Level of Significant
Normal T_0	0.78±0.24	
Diabetic T_1	1.81±0.28	+Δ
D+300CS T_2	1.55±0.21	+Δ
D+400CS T_3	1.27±0.32	*
D+500CS T_4	1.19±0.31	*
D+5GIb T_5	0.84±0.24	**

*The results are expressed as mean ± SD (n =6). * – significant at p<0.05compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ -significant at p<0.05 compared with Glibenclamide treated group, D-Diabetic, CS - Cinnamon extract.*

Figure 4.3: The effects of *Cinnamon extract* on Creatinine in STZ induced diabetic mice.

4.4 Effects of Cinnamon extract on Serum Urea

Figure 4.4 describes the effect of *Cinnamon extract* on serum urea in normal and diabetic mice. There were significant ($p<0.05$) increases in serum urea in diabetic group as compared to normal control. However, serum urea was reduced after the administration of *Cinnamon extract* at all concentrations and 5mg/kg Glibenclamide in treated diabetic groups as compared to diabetic group after treatment for three weeks.



Experimental Groups of Mice	Serum urea (mg/dl)	Level of Significant
Normal T_0	44.49±3.48	
Diabetic T_1	67.52±3.14	+Δ
D+300CS T_2	63.48±2.69	+Δ
D+400CS T_3	58.54±2.53	+*
D+500CS T_4	55.36±3.13	+*
D+5 GIb T_5	46.44±1.85	**

*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with Glibenclamide treated group, D-Diabetic, CS - Cinnamon extract*

Figure 4.4: Effects of *Cinnamon extract* on serum urea in STZ induced diabetic mice.

4.5 Effect of Cinnamon extracton Acute Toxicity Test

In acute oral toxicity test, Cinnamon extract revealed no mortality at the 2000 mg/ kg body weight concentration in mice. The mice did not also show any toxic effects like changes in behavioral activities such as anxiety, polyuria, diarrhoea, seizures, and coma which received *cinnamon extract*. Thus, the *Cinnamon extract*, 2000 mg/Kg body weight of mice were found be a good safety margin indicator. Therefore, one-fifth (20%) of the safe doses were taken by the researcher for the experiments

5. DISCUSSION

Diabetes mellitus is now described as a disorder of multiple etiologies with abnormalities in carbohydrate, lipid as well as protein metabolism. Abnormalities in glucose metabolism and renal function are important risk factors for diabetes, cardiovascular and many other diseases.

STZ induced animal model has been described as a useful experimental model to study the effect of anti-diabetic agent such as Glibenclamide against T2DM. STZ is known to induce diabetes, hyperinsulinemia, or hyperglycemia by damaging the pancreatic β cells (Graham *et al.,* 2011).

Management of T2DM is indeed a tough task with the convectional medicines as they may cause many side-effects. Thus, as an alternative, there is an immense interest in medicinal plants for finding a cure to reduce the risk of T2DM. Scientists have started looking into the herbal extracts to observe their effective and protective role in the diabetic animal models. The present work demonstrates a significant role of cinnamon extracts in normalizing body weight, reducing blood glucose levels and restoring renal function in STZ induced diabetic mice.

Slight body weight Loss was observed in STZ-induced diabetic mice and almost normalized by treatment with extract of Cinnamon bark. In diabetic mice, this slight loss of weight may be due to tissue protein break down and muscle wasting via unavailability of carbohydrate as an energy source and catabolism of fats (Gougean *et al.,* 2008).

However, 5mg/kg of Glibenclamide treated mice gained weight (25.7%) incomparison with the diabetic group after 21 days treatment.

This study result revealed that Cinnamon extract treated groups did not show important body weight gain; this could support that Cinnamon to be an important for treatment of diabetes mellitus over convectional drugs (Glibenclamide) which are mostly known to cause body weight gain in diabetes mellitus treatment (Prabhakar and Doble*, et al,* 2011).

The protective effect of the extract on body weight loss may be due to its ability to reduce hyperglycemia. Here, the bioactive compounds of Cinnamon may help in suppressing the free radicals generated via due to hyperglycemia, and control over muscle wasting resulted from glycemic control in treated diabetic mice, and ultimately lead to normalize the level of body weight (Auddy *et al.,* 2003).

The increase in fasting blood glucose concentration is an important characteristic feature of T2DM. In this study, there were elevations in FBG level in diabetic treated group. However, the extract of Cinnamon reduced FBG level in diabetic mice. The FBG level was decreased by 16.4% and 21.12% at 300mg/kg extract concentration on 7th and 14th days respectively, and decreased significantly ($p<0.01$) by 25.13% on 21^{st}day as compared to diabetic group. On the other hand, administration of 400mg/kg of the extract significantly ($p<0.01$) reduced the FBG level by 27.04%, 44.97% and 48.59% on 7th, 14th and 21st days respectively as compared to diabetic group. Administration of 500mg/kg of the extract reduced the FBG significantly ($p<0.001$) by 36.49%, 53.06% and 57.0% on 7th, 14th and 21^{st}t days respectively as compared to diabetic group.

Similarly, Glibenclamide (5mg/kg) led to reduction of FBG level by 46.03%, 61.30% and 62.12% on 8th, 15th and 21st days respectively as compared to diabetic group. These results of reduction in FBG are in agreement with pervious work on effect of Cinnamon extract on insulin release from pancreatic β cells in Streptozotocin-induced diabetic rats (Eidi *et al.,* 2009).

Hence, when the concentrations of Cinnamon extracts increased, the FBG level was shown to have been decreased. The glycemic control was nearly similar between Glibenclamide and Cinnamon extracts treatment. Thus, the increment of the Cinnamon extracts concentration may further provide a similar result as Glibenclamide drug.

The present findings indicate the hypoglycemic and/or potential ant hyperglycemic effect of the extract. There were many possible explanations for this finding. The ant hyperglycemic effect of Cinnamon extracts may be due to restoration of insulin response via the presence of ant hyperglycemic, “insulin-releasing” and “insulinlike” activity in Cinnamon bark (Gray and Flatt, 1999). It was also suggested that the anti-hyperglycemic effects of the Cinnamon extracts could be caused by high level of fiber which interfere carbohydrate absorption, increased peripheral uptake of glucose, improved sensitivity of insulin receptor, and regenerative effect of Cinnamon on pancreatic tissue (Byambaa *et al.,* 2010).

Type 2 diabetes mellitus also causes renal damage due to abnormal glucose regulation including elevated glucose and glycosylated protein tissue level, hemodynamics changes within the kidney and oxidative stress. Both negative balance of nitrogen and lowered protein synthesis leads to

increased level of serum urea and creatinine that indicates progressive renal damage in diabetic mice (Musabayane *et a*l; 2012).

Nevertheless, the Cinnamon extracts reduced both serum urea and creatinine in diabetic treated mice. The level of serum urea and creatinine were decreased insignificantly (P>0.05) by 2.02% and 10.49% at 300mg/kg extract concentration respectively as compared to diabetic group. The level of serum urea and creatinine were decreased significantly (P<0.01) by 16.17% and 30.25% at 400mg/kg extract respectively as compared to diabetic group. The level of serum urea and creatinine were decreased significantly (P<0.01) by 17.25% and 32.72% at 500mg/kg extract respectively as compared to diabetic group. Similarly, Glibenclamide (5mg/kg) also significantly (P<0.01) reduced the serum urea and creatinine by 26.14% and 40.74% on the diabetic treated mice as compared to diabetic group respectively.

These reductions of serum urea and creatinine may show the beneficial effects of the Cinnamon extracts on the kidney function of diabetic mice. Thus, this renephroprotective function could be mediated via antioxidant and/or free radical scavenging activities as they possess high concentration of flavonoids and alkaloids (Udayakumar *et al.,* 2009).

6. CONCLUSION AND RECOMMENDATION

In this study, hydro-ethanolic extracts of Cinnamon extract showed a reduction on fasting blood glucose level in STZ induced diabetic mice. This could be due to ‘’insulin like’’ and insulin releasing activities of the extract. Thus, it may be concluded that Cinnamon extracts has potent hypoglycemic effect and provided better glycemic control in STZ induced diabetic mice. Cinnamon extracts also showed a decrease of serum urea and creatinine which indicates restoring properties on the function of kidney of diabetic mice. Generally, from the above findings, it is possible to conclude that extract of Cinnamon has anti-hyperglycemic, and restoring the function of kidney in STZ induced diabetic Swiss albino mice.Hence, it could be conclude that 500mg/kg concentration of Cinnamon extract have a better anti-diabetic capacity, and almost equipotent with Glibenclamide drug. Cinnamon extract solution possesses antihyperglycemic and antioxidant effects in diabetic animals.

It is evidenced that the extract of cinnamon exhibit that the hypoglycemic activity on STZ induced diabetic mice. Further, research study can be done to investigate the effect of cinnamon extract on lipid profile and can also see histopathology effect on pancreas, liver and kidney to make the study more comprehensive.

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