# THE EFFECTS OF *CORIANDRUM SATIVUM* SEED EXTRACTS ON HYPERGLYCEMIA, LIPID PROFILE AND RENAL FUNCTION IN STREPTOZOTOCIN INDUCED TYPE - 2 DIABETIC SWISS ALBINO MICE

ENDRIS YIBRU (BSc)

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY

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# THE EFFECT OF *CORIANDRUM SATIVUM* SEED EXTRACTS ON HYPERGLYCEMIA, LIPID PROFILE AND RENAL FUNCTION IN STREPTOZOTOCIN INDUCED DIABETIC TYPE- 2 SWISS ALBINO MICE

A thesis submitted to the School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry

Endris Yibru

**Advisor:**

Daniel Seifu (PhD)
Head of Department of Biochemistry
School of Medicine, Addis Ababa University

**Co-Advisors:**

Menakath Menon (PhD)
Department of Biochemistry
School of Medicine, Addis Ababa University

Yididya Belayineh (PhD)
Department of Biochemistry
School of Medicine, Addis Ababa University

Addis Ababa, Ethiopia

May, 2014

Addis Ababa University

School of Graduate studies

This is to certify that the thesis prepared by **Endris Yibru Hanurry** entitled: "***Effect of Coriandrum sativum Seed Extracts on Hyperglycemia, Lipid Profile and Renal Function in Streptozotocin Induced Type- 2 Diabetic Swiss Albino Mice***" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry complies with regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner:

Dr. Solomon Genete (PhD) Signature: _____ Date: _____

Advisors:

Dr. Daniel Seifu (Advisor) Signature: _____ Date: _____

Dr. Menakath Menon (Co-advisor) Signature: _____ Date: _____

Dr. Yididya Belayneh (Co-advisor) Signature: _____ Date: _____

_____

Chair of Department or Graduate Program Coordinator

# ABSTRACT

**Background:** Convectional drug treatment for diabetes mellitus carries risks that lead to many adverse effects such weight loss, hypoglycemia and many others. Ethiopia is rich in natural resources and medicinal plants useful in the treatment of diabetes.

**Aim of the study:** To investigate the effect of *Coriandrum sativum* seed extracts on hyperglycemia, lipid and renal profile in streptozotocin induced diabetic mice.

**Methods:** Thirty six male Swiss albino mice were kept in six different groups for 21 days. Group I served as normal controls; Group II served as diabetic control; Groups III, IV and V were given 300 mg/kg, 400 mg/kg and 500mg/kg of *Coriandrum sativum* seed extracts (70% ethanol), respectively; and Group VI received 5mg/kg glibenclamide drug. The effect of extracts on hyperglycemia, lipid profile 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 *Coriandrum sativum* extract concentration as compared to the diabetic group. It also reduces total cholesterol, triglyceride, low density lipoprotein, urea and creatinine, and improves high density lipoprotein level and total protein in treated diabetic mice.

**Conclusion:** Reduction in the fasting blood glucose, total cholesterol, triglyceride level, low density lipoprotein, urea, creatinine, and improvement in the high density lipoprotein and total protein by *Coriandrum sativum* extract indicates that it has anti-hyperglycemic, anti-hyperlipidemia and renal failure restoration effect in streptozotocin induced diabetic mice.



**Keywords**: *Diabetes mellitus, Coriandrum sativum, Hyperglycemia, hyperlipidemia, Renal function*

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## 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 |
| TG | Triglyceride |
| TNF-α | Tumor Necrosis Factor –α |
| VLDL | Very Low Density Lipoprotein |
| WHO | World Health Organization |

# 1. INTRODUCTION

Diabetes mellitus (DM) is defined as a group of metabolic diseases manifest by hyperglycemia which results from defects in insulin production and/or insulin action. Untreated chronic hyperglycemia can lead to long-term complications including microvascular and macro-vascular problems that cause disturbances of carbohydrate, fat and protein metabolism, and it covers a wide range of heterogeneous diseases (Sherita and Tamar, 2012; Edwin *et al.*, 2008).

Diabetes mellitus could be categorized into several classes but the major types are type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus. Both T1DM and T2DM lead to hyperglycemia, excessive urine production, compensatory thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism (Shukla *et al.*, 2011).

Type -2 diabetes mellitus is caused by the failure of insulin secretion or action. The impairment of insulin actions is known as insulin resistance, presented as a suppression or retard in metabolic responses of the muscle, liver and adipose tissue to insulin action. This failure is located at the signaling pathways held after insulin binding to its specific receptor. Chronic insulin resistance leads to hyperglycemia which mainly is involved in the etiology of development of diabetic complications (Sasso *et al.*, 2012).

Currently, T2DM is managed by a combination of diet, exercise and conventional therapy. Drugs for diabetes normally includes biguanides, thiazolidinediones, sulfonylureas, D-phenylalanine derivatives, etc (Ahren, 2013). Some of these convectional or synthetic drugs can cause side-effects including hematological, gastrointestinal reactions, hypoglycemic coma, and disturbances in liver and kidney metabolisms. In addition, these drugs are not ideal for use during pregnancy (Gomathi *et al.*, 2013). Due to these several side effects of conventional drugs, there is a growing tendency toward finding medications with less subsidiary effects, and as a result therapeutic herbs are taking lots of attention. Plant remedies are frequently considered to be less toxic and free from side-effects than synthetic drugs, and the World Health

Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes (Mohammadi *et al.*, 2010). WHO has listed 21000 herbs which are used as medicines all over the world and this magnifies the importance of herbs in curing diseases (Zahmatkesh and Khodashenas, 2013). According to previous studies, some herbs are effective in lowering blood glucose by decreasing sugar absorption in intestine, increasing glucose consumption in body, creating glycogen in liver, enhancing phosphorylation of glucose receptors, and increasing insulin sensitivity (Gomathi *et al.*, 2013).

Medicinal plants play an important role in the management of T2DM, especially in developing countries where resources are meager. The treatment of T2DM relies heavily on dietary measures, which includes the use of traditional plant therapies (Menakshi and Bimba, 2013). Some of the reports in ethno-botany suggested that about 800 medicinal plants possess anti-diabetic potential and the bioactive compounds such as glycosides, alkaloids, terpenoids and flavonoids (phenols) are effective medications both in preclinical and clinical studies (Auddy *et al.*, 2003). These bioactive compounds of remedial plant lower blood glucose in experimental animal models, and, currently, there is considerable interest in exploring these plant extracts for compounds that might also be useful in the clinic or might have novel effects such as stimulation of β-cell proliferation (Harvey, 2010). There are many reports of plants showing pancreatic islet regeneration and increase insulin secretion in diabetic conditions such as extracts of *Nigella sativa, Evertiamia microphylla* and many others (Kazuo and Itaru, 2006). Generally, hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver.

*Coriandrum sativum* is an annual herb originating from the Mediterranean and cultivated all over the world including Ethiopia (Momin *et al.*, 2012). Phytochemical constituents of *Coriandrum sativum* seeds have been studied and analysis had revealed presence of polyphenols (rutin, caffeic acid derivatives, ferulic acid, galic acid, and chlorogenic acid), flavonoids (quercetin and isoquercetin) and β- carotenoids (Ullagaddi and Bondada, 2011).

It is among such most commonly used spices in cooking and baked food, possessing the nutritional as well as medicinal properties. In Ethiopian traditional medicine, *Coriandrum sativum* has been considered for a number of medical complications such as loss of appetite, dyspeptic complaints, convulsion, insomnia, vomiting and carminative. It is also used in the preparation of many household medicines to cure bed cold, nausea, seasonal fever and stomach disorders (Reddy *et al.*, 2012).

*Coriandrum sativum* has been reported to exhibit several pharmacological effects such as anti-mutagenic (Cortes *et al*., 2004), anthelmintic (Eguale *et al*., 2007), sedative-hypnotic (Emamghoreishi and Heidari, 2006), anti-feeding (Catherine *et al*., 2006), anti-parasite (Matasyoh *et al*., 2009), anticancer (Chithra and Leelamma, 2000) and post-coital anti-fertility activities (Asgarpanah and Kasemivash, 2012). Interestingly, *Coriandrum sativum* also possessed heavy metal detoxification properties such as lead-detoxifying potential (Kansal *et al.*, 2011). The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical clues as well as a good source of dietary supplement to existing therapies. Therefore, the main aim of this study is to investigate the effects of *Coriandrum sativum* seed extracts on hyperglycemia, lipid profile and renal function in STZ induced diabetic Swiss albino mice.

## 1.1 LITERATURE REVIEW

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, 2012). It is also associated with an enhanced risk for developing premature atherosclerosis 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 Anand, 2010).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Insulin resistance is characterized by decreased tissue sensitivity to insulin and marked compensatory hyperinsulinemia. Initially, plasma glucose levels are maintained in the normal range. The first glucose abnormality is detected by a rise in the postprandial glucose levels because of reduced first-phase insulin secretion. With time, further decline in β- cell function leads to elevation of the fasting glucose levels. Eventually, diabetes occurs with more insulin secretory loss (Rajbharan *et al.*, 2008).

The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is impaired action of insulin on target tissues (Sherita and Tamar, 2012; Edwin *et al*., 2008). In diabetes, there is a decline in β-cell secretory capacity. Impaired 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.

### 1.1.1. 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 1.1 (Abel *et al.*, 2012).

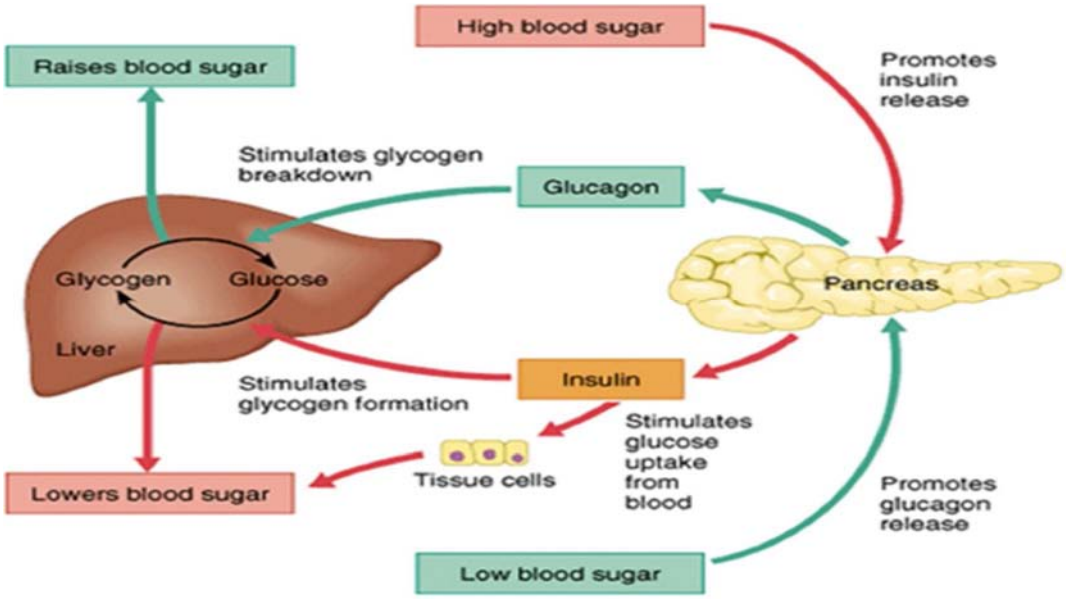



Figure 1.1: The role of the pancreas, liver and other tissue cells in glucose homeostasis: *When glucose goes too high, the pancreas releases insulin into the blood stream and insulin stimulates the liver to convert the blood glucose into glycogen for storage. If the blood sugar goes too low, the pancreas 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 $\beta$-cells which results in increased ATP production in mitochondria (Sharma, 2011). The increase in intracellular ATP closes the ATP-sensitive $K^+$ channels ($K_{ATP}$), decreasing the hyperpolarizing outward $K^+$ flux. This results in depolarization of the plasma membrane and influx of extracellular $Ca^{2+}$ through the voltage-gated $Ca^{2+}$ channels. Figure 1.2 illustrates the activation of protein motors and kinases by the increasing intracellular $Ca^{2+}$, which then mediate exocytosis of insulin-containing vesicles which lead to increased insulin and decreased fasting blood glucose levels (Bays *et al.*, 2004).

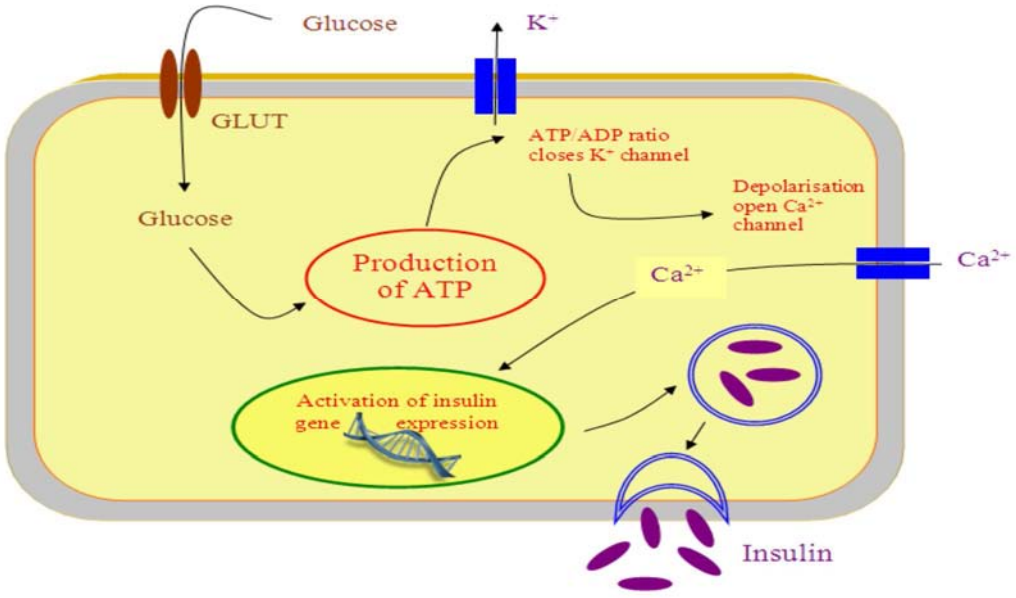



Figure 1.2: $K_{ATP}$ channel pathway of glucose sensing in the β-cell: *The increase of the ATP concentration closes the $K^+$ channel, leading to a depolarisation which in consequence opens the $Ca^{2+}$ channel. $Ca^{2+}$ activates the insulin gene expression via the Calcium Responsive Element Binding 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, 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 1.3, 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).

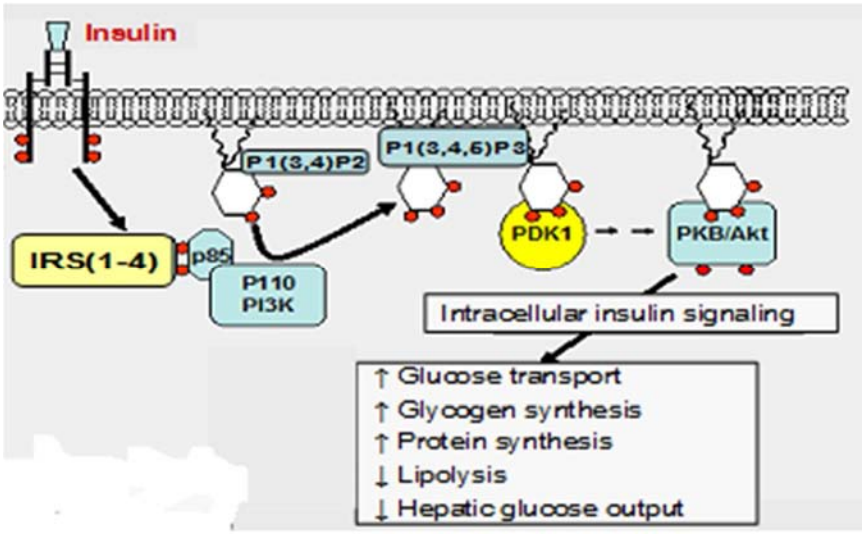



Figure 1.3: Metabolic actions of insulin: *IRS- Insulin Receptor Substrates, PKB-Protein kinase B, PI3K - PI 3-kinase, PI(3,4)P3-phosphatidylinositol-3,4- bisphosphate, PI(3,4,5)P3 – phosphatidylinositol (3,4,5)P3, PDK1– phosphatidyl- inositol dependent kinase -1 (Saltiel and Kahn, 2001).*

### 1.1.2 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".

#### 1.1.2.1 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).

#### 1.1.2.2 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, 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, 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, 2012).

#### 1.1.2.3 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% of GDM affected women develop T2DM later in life (Shukla *et al*., 2011).

#### 1.1.2.4 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.

### 1.1.3 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 as shown below in figure 1.4 (IDF, 2012). 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 *et al.*, 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).

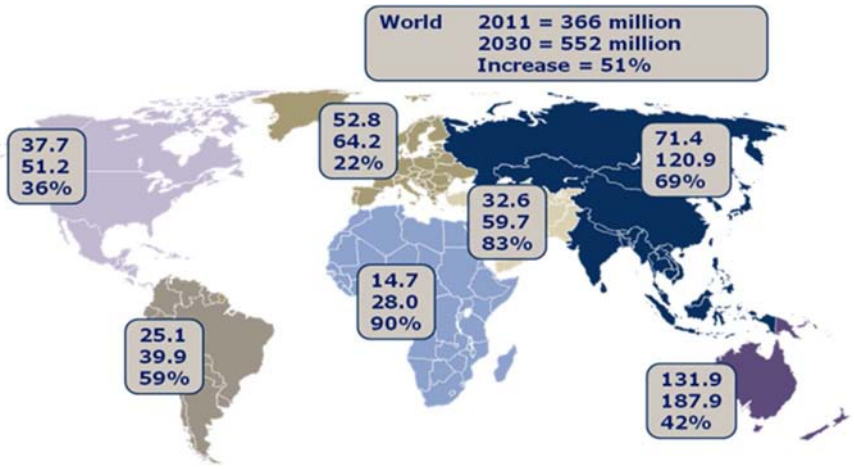



Figure 1.4: The Diabetes Epidemic: Global Projections, 2010–2030 (IDF, 2011)

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 Africa. Type 2 Diabetes Mellitus accounts for over 90% of diabetes cases in Sub-Saharan Africa (Levitt, 2008). The expected growth for sub- Saharan Africa is 98%, from 12.1 million in 2010 to 23.9 million in 2030 (Sicree and Zimmet, 2009). Similarly, the prevalence of DM in Ethiopia was 2.5 % in the year 2000 which is estimated to rise to 3.5 % by 2030 (Shaw *et al.*, 2010).

According to IDF (2012), 4.6 million deaths are directly attributable to diabetes, constituting 6.8% of the total global (all-age and all-cause) mortality in each year (Philip *et al.*, 2013). It has been projected that diabetes-related deaths will increase by 50% in the next 10 years if no urgent action is taken (Lal *et al.*, 2012). In Ethiopia, the number of deaths attributed to diabetes reached over 21,000 in 2007. This estimate has increased to about 25,000 in 2011(Tilahun and Yibeltal, 2012).

### 1.1.4 Diagnostic Tests for Type 2 Diabetes Mellitus

Diabetes can be diagnosed by the presence of four classic signs that include polyuria, polyphagia, polydipsia, and foremost hyperglycemia (Vasudev and Jann, 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 |

### 1.1.5 Pathophysiology of Type 2 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 1.5). 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 (Harrera *et al.*, 2004).

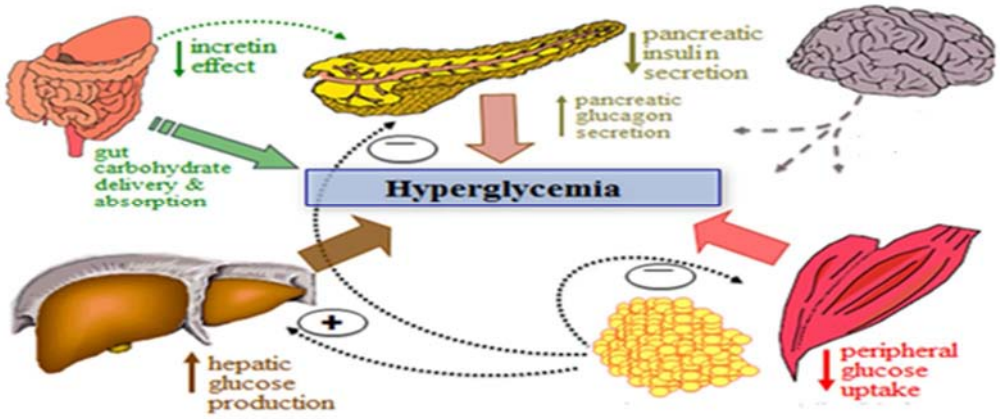



Fig 1.5: Pathophysiology of type two diabetes mellitus (Inzucchi *et al.*, 2012**)** (⬆) (increase), (⬇)-indicates decrease, (+) indicates activation and (-) indicates inhibition

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 impaired 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, 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 β cells), causing insulin resistance and impaired insulin secretion (Inzucchi *et al.*, 2012).

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

### 1.1.6 Molecular basis of Type -2 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-stimulated 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, 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, 2013).

The increased levels of non-esterified fatty acid, glycerol, leptin, resistin, 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 *et al.*, 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). Overexpression of PPARα in mice leads to enhanced β-oxidation of FAs and reduced glucose oxidation and accumulation of triglycerides (Abel *et al*., 2012).

### 1.1.7 Complication of Type 2 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-ketotic hyperosmolar coma, and diabetic coma. In case of chronic complication, chronic elevation of fasting blood glucose level leads to damage to blood vessels (Edwin *et al*., 2008). This chronically elevated blood glucose levels lead to increase production of mitochondrial reactive oxygen species (ROS), which activate a number of metabolic pathways whose end products contribute to the development of long term complication of diabetes (Weiss and Sumpio, 2006). These metabolic pathways are activated by hyperglycemia-induced ROS that includes the polyol pathway, formation of AGE, hexosamine pathway and the protein kinase C (PKC) pathway as shown the next figure (Figure 1.6) (Forbes and Cooper, 2013).

In diabetes, the subsequent problems are grouped under "micro-vascular complication" due to damage to small blood vessels and "macro-vascular complication" due to damage of the arteries (ADA, 2011). 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).

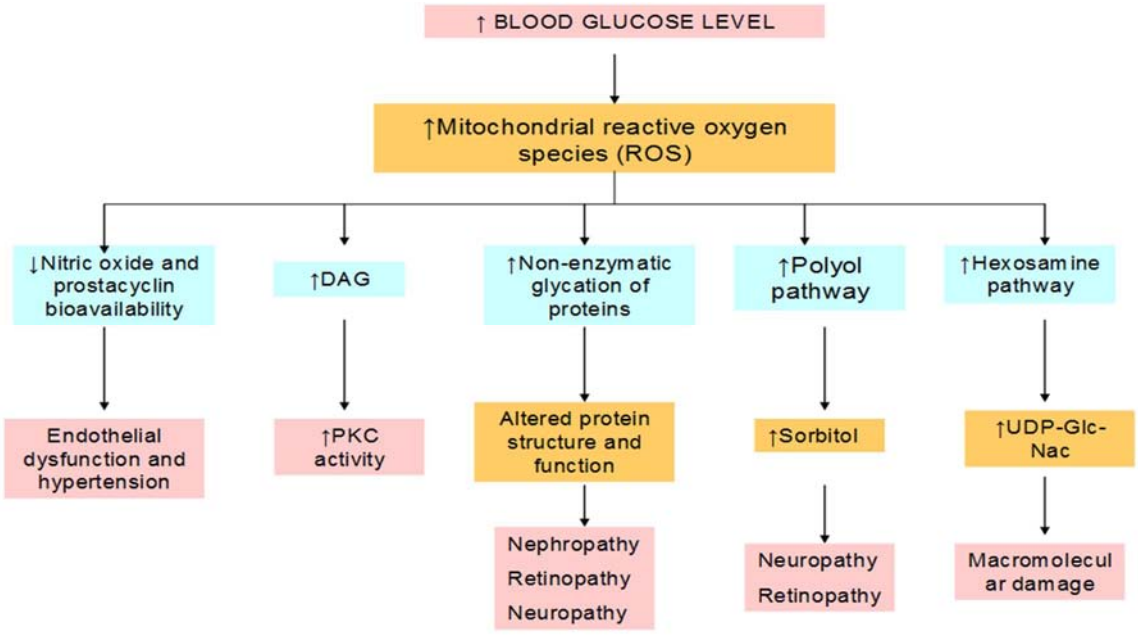



Figure 1.6: Metabolic pathways activated by chronically elevated blood glucose levels and long term complications of diabetes mellitus, *DAG- Diacylglycerol, PKC- protein Kinase C, Glc - Glucosamine, UDP- Uridine diphosphate, Nac-N-Acetylglucosamine (Weiss and Sumpio, 2006)*

### 1.1.7.1 Dyslipidemia and Type 2 Diabetes Mellitus

Dyslipidemia is common in T2DM, as both insulin deficiency and resistance affect enzymes and pathways of lipid metabolism. Atherogenic dyslipidemia describes the combination of raised TG level and low concentrations of HDL together with elevated apolipoprotein B (ApoB). Small dense LDL and small HDL particles are independently atherogenic, and which is commonly observed in both T2DM and other metabolic syndrome. Thus, Dyslipidemia is characterized by hypercholesterolemia, hypertriglyceridemia, and low HDL cholesterol, and is also influenced by glycemic control (Michael, 2008). Otamere and his colleagues also found that duration of diabetes was associated with higher incidence of dyslipidemia. Similarly, insulin resistance has a negative effect on lipid production, increasing VLDL, LDL, and TG the bloodstream and decreasing HDL (Otamere *et al*., 2011).

The Pathophysiology of diabetic lipid profile is an outcome of elevated free fatty acid (FFA) release from insulin-resistant fat cells (Samatha *et al*., 2012). The excess FFA are then converted to TG in the liver that in turn stimulates VLDL cholesterol and apolipoprotein B synthesis; and then reduced the activity of lipoprotein lipase (LPL) (Byambaa *et al*., 2010).

Specifically, there is an interconversion between the HDL-transported cholesterol ester and the VLDL-transported triglyceride mediated by cholesteryl ester transfer protein (CETP) (Shiju and Pragasam, 2012). This exchange results in triglyceride-rich HDL particles that are subsequently hydrolyzed by hepatic lipase or LPL. LPL hydrolyzes triglycerides from the core of HDL, resulting in production of smaller, denser particles. The apolipoprotein A-I (apoA-I) released from this enzymatic hydrolysis is then filtered through the renal glomeruli and broken down (Bays *et al*., 2004) as shown below in figure 1.7.

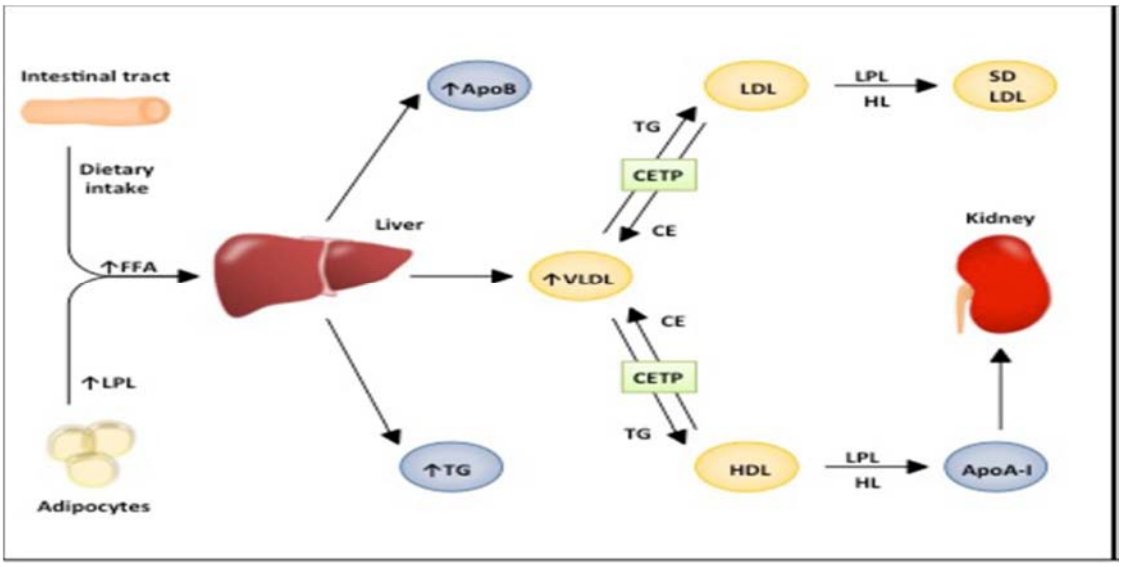



Figure 1.7: Pathophysiology of diabetic dyslipidemia: Abbreviations: *ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FFA free fatty acid; HL, hepatic lipase; LPL, lipoprotein lipase; SD LDL, small dense LDL; TG, triglyceride (Elboudwarej et al., 2011)*

Cholesterol ester transfer protein (CETP) is responsible for the exchange of LDL-transported cholesteryl ester and the VLDL-transported triglyceride, with the resulting triglyceride-rich LDL undergoing hydrolysis by hepatic lipase or LPL to become lipid-depleted small dense LDL particles. These small dense LDL particles are more atherogenic and more vulnerable to oxidation when glycated (Puddu *et al.*, 2013). Small dense LDL particles are relatively more common in diabetes, and have a high susceptibility to oxidation.

Oxidative modification of LDL results in rapid uptake by macrophages, which leads to foam cell formation and promotion of the expression of intercellular adhesion molecule (Byambaa *et al.*, 2010). Furthermore, LDL oxidation seems to be affected by an acute increase in glycaemia. Apolipoprotein B in LDL can be modified by advanced glycosylation end products, reducing the binding affinity of the LDL-modified particles to their hepatic receptors and subsequently leading to increased oxidation of these particles. In addition, glycated and oxidized LDL particles induce the release of foam cell cytokines (TNF-α, interleukin-1), which play important roles in the atherosclerotic process (Hunt *et al*., 2011). Thus, oxidative modification of LDL may contribute to the higher risk of cardiovascular disease among diabetic patients, and elevated levels of TG level may contribute to the rapid LDL oxidation in T2DM.

Insulin resistance also contributes to functional changes in the enzymes involved in HDL metabolism. Cholesterol esterification within the lipoprotein particles via lecithin-cholesterol acyltransferase (LCAT) is only mildly increased relative to the increase in CETP activity; this discrepancy in enzymatic activity levels leads to lower HDL levels because of the greater efflux of cholesterol ester from HDL (Byambaa *et al*., 2010).

#### 1.1.7.2 Kidney Dysfunction and Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus is the single most common cause of end-stage renal disease worldwide (Stenvinkel, 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, 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, 2012). Kidneys affected by diabetic nephropathy have no longer work efficiency, and trace amounts of protein appear in the urine (Isra'a, 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, 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 by various 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 creatine is converted to creatinine every day. Creatine 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 creatine. 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 used

as 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, 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).

### 1.1.8 Current Type 2 Diabetes Mellitus Therapy and Associated Problems

Since both micro- and macro-vascular complications contribute in the increasing morbidity and mortality of patients with T2DM, novel anti-diabetic therapies are intensively studied with respect to their possible beneficial effects on the long-term complications (Sharma, 2011). According to the road map for T2DM research that was recently commissioned by the European Research Council (2012), the status of current T2DM treatment can be summarized as follows: 'Today, there is no cure for this disease; while new drugs and a holistic approach to treatment have certainly improved the prognosis for individuals with diabetes and their quality of life, the menace, morbidity and increased mortality from micro- and macro-vascular complications remain' (Campbell *et al*., 2012).

Similarly, the latest statement of the American Diabetes Association's Standards of Medical Care in Diabetes in 2011 states that: 'T2DM is a chronic illness that requires continuing medical care and on-going patient self-management education and support to prevent acute complications and to reduce the risk of long-term complications. T2DM care is complex and requires that many issues, beyond glycemic control, be addressed' (Campbell *et al.*, 2012).

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, 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 peptide 1 (GLP-1) agonists (incretin mimetics), increase insulin secretion with the incretin drugs also normalizing glucagon secretion (Moore, 2007) as shown in figure 1.8.

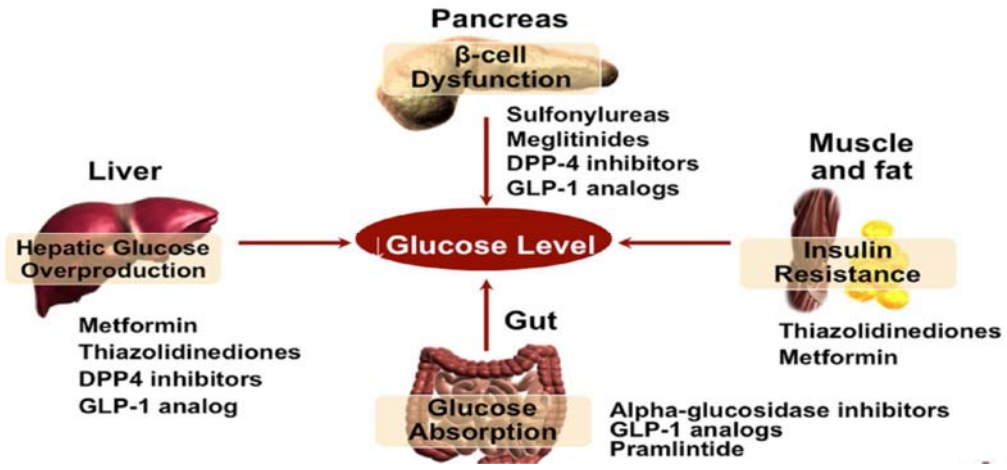



Figure 1.8: Sites of action and mechanism of antihyperglycemic drugs in treatment of hyperglycemia and T2DM: abbreviation; *DPP-4 – dipeptidyl peptidase-4 and GLP1, glucagon-like peptide-1* (Molitch, 2013)

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 1.9 (Sharma, 2011).

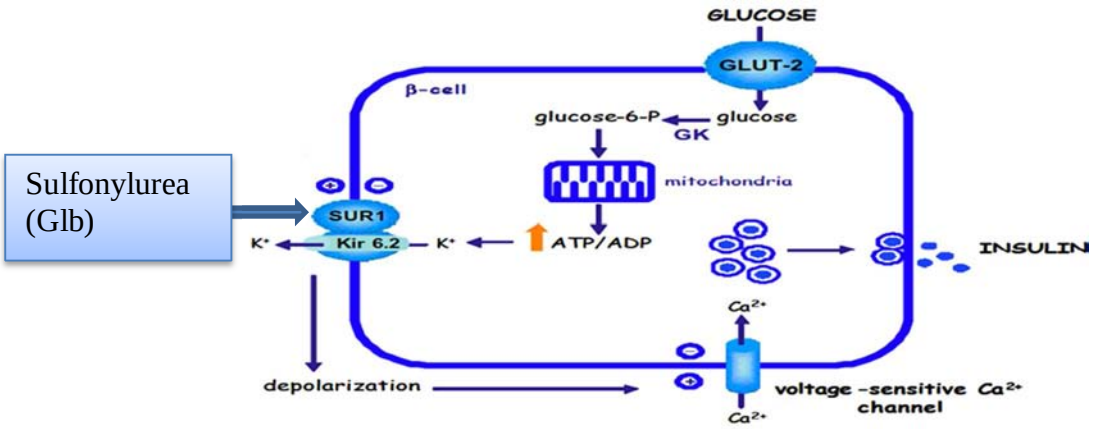



Figure 1.9: Mechanism of action of sulfonylureas on ATP-sensitive K+ channels

*Sulfonylureas (glibenclamide) promote the closure of $K_{ATP}$ channels leading to membrane depolarization, opening of $Ca^{++}$ channels and initiation of insulin secretion mediated via the binding to a sulfonylurea receptor-1 (SUR1) together with protein Kir6.2., Glb-glibenclamide, GK-glucokinase,GLUT2-Glucose transport-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 extrapancreatic 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.

## 1.2 Medicinal Plant

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 also to restore health (Zahmatkesh and Khodashenas, 2013).

Medicinal plants and traditional medicine play an important role in the health care system of most developing countries. Ethiopia has 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 Ethiopia (Reta, 2013). Some study showed that nearly 80% of human population and 90% of livestock in Ethiopia rely on traditional medicine (Yeweyenhareg and Fikre, 2005).

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic, anti-hyperlipidemia 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, anti-dyslipidemia 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).

Many traditional plant treatments for diabetes are used throughout the world where resources are meager, but most of the evidence for their beneficial effects is unreliable. In addition, there is strong desire to use herbs or plants for treatment, due to less side effects, easier consumption or availability (Joan *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 in

β cells 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, 2012).

## 1.3 *Coriandrum sativum*

The genus *Coriandrum* includes the cultivated plant *Coriandrum Sativum* and the wild species *Coriandrum tordylium*. *Coriandrum sativum*, belongs to the family Apiaceae, is an annually grown herb which is extensively cultivated in India, Russia, Central Europe, Asia and Middle East, Ethiopia, China, Bangladesh and other countries (Leena *et al*., 2011). *Coriandrum sativum* has different names in various languages as mentioned in table 1.2.

Table 1.2: Common names of the *Coriandrum sativum*

| Language | Name | Language | Name |
|---|---|---|---|
| Amharic | Dimbilal | Greece | Koriannon, Korion |
| Arabic | Kuzbara | Hindi | Dhania, Dhanya |
| Chinese | Yuan Sul | Persian | Geshnes |
| English | Coriander | Tigrigna | Tsagha, Zagda |
| Oromifa | Debo, Shucar | Turkish | Kisnis |

*Coriandrum sativum* has a very strong aroma that can be felt from meters away from the growing spot. It is a glabrous, aromatic, herbaceous annual plant, small sized tree growing throughout Ethiopia. It has thin, spindle-shaped roots, erect stalk, and small,

pinkish-white flowers. The plant flowers yields round seeds and these seeds are almost ovate globular and there are many longitudinal ridges on the surface. The whole plant and especially the unripe seed, is characterized by a strong disagreeable odor. The color of dried seed is usually brown, but may be green, straw-colored or off white as shown below in Figure 1.10.

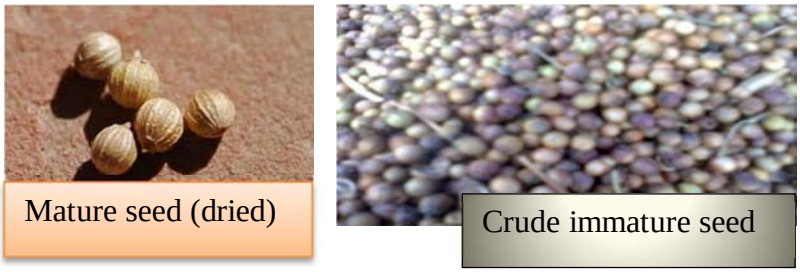



Figure 1.10: *Coriandrum sativum* plant seeds (Picture by Endris)

*Coriandrum sativum* is used as culinary spices and recognized as one of the most important medicinal plant all over the world. *Coriandrum sativum* was used in traditional Greek medicine by Hippocrates (460–377 B.C.). *Coriandrum sativum* is named in an Egyptian papyrus dating from 1550 BC that lists medicinal plants. In Ethiopia, it has traditionally been used for its anti-inflammatory properties. In addition, *Coriandrum sativum* is a commonly used domestic remedy. The raw seed is chewed to stimulate the flow of gastric juices and to cure foul breath and will sweeten the breath after garlic has been eaten (Ullagaddi and Bondada, 2011).

### 1.3.1 Phytochemistry of *Coriandrum sativum* Seed

The chemical composition of *Coriandrum sativum* seeds contains various components like water, crude fiber, fat, starch, minerals, crude protein and essential oils (Momin *et al*., 2012).

Table 1.3: The chemical composition of *Coriandrum sativum* seeds (Momin *et al*., 2012)

| Component Content | Percentage (%) | Component Content | Percentage (%) |
|---|---|---|---|
| Water | 11.37 | Pentosans | 10.29 |
| Crude Protein | 11.49 | Sugar | 1.92 |
| Fat | 19.15 | Minerals | 4.98 |
| Crude Fibre | 28.43 | Essential Oil | 0.84 |
| Starch | 10.53 | | |

Seeds of *Coriandrum sativum* are the most widely used components of the plant with the most important constituents being the essential oil and the fatty oil. This essential oil includes Lenoleic acid, Palmitic acid, Oleic acid and Stearic acid (Sahib *et al.*, 2012). However, the composition of the essential oil appears to be dependent on biological and geographical variability. Bhuiyan *et al*. carried out a comprehensive analysis on the chemical composition of seed essential oil of *Coriandrum sativum* from Bangladesh using gas chromatography mass spectroscopy (GC-MS) (Bhuiyan *et al*., 2009). Fifty-three compounds were identified, with linalool being the major one (37.7%), followed by geranyl acetate (17.6%) and g-terpinene (14.4%). Other compounds present are b-pinene (1.82%), m-cymene (1.27%), citronellal (1.96%), citronellol (1.31%), citral (1.36%), geraniol (1.87%), citronellyl acetate (1.36%), a-cedrene (3.87%), a-farnesence (1.22%) and b-sesquiphell-andrene (1.56%). Anwar et al. (2011) investigated the physico-chemical properties of *Coriandrum sativum* seed essential oil from Pakistan. The oil analyzed by GC-flame ionization detector and further authenticated by GC-MS mainly contained linalool with contribution of 69.60%, where as other important constituents were identified to be g-terpinene (4.17%), a-pinene (1.63%), anethol (1.15%) and p-cymene (1.12%) (Anwar *et al*., 2011).

Apart from environmental and genetic factors, other parameters such as, states of maturity and methods of extraction from seeds affect the composition of the essential oil. Msaada and colleagues studied the changes on essential oil composition of *Coriandrum sativum* seeds during three stages of maturity. The main components of immature seeds were geranyl acetate (46.27%), linalool (10.96%), nerol (1.53%) and neral (1.42%).

Seeds in the middle stage contained mainly linalool (76.33%), cis-dihydocarone (3.21%) and geranyl acetate (2.85%). Predominant component of mature seeds include linalool (87.54%) and cis-dihydocarone (2.36%) (Msaada *et al*., 2007).

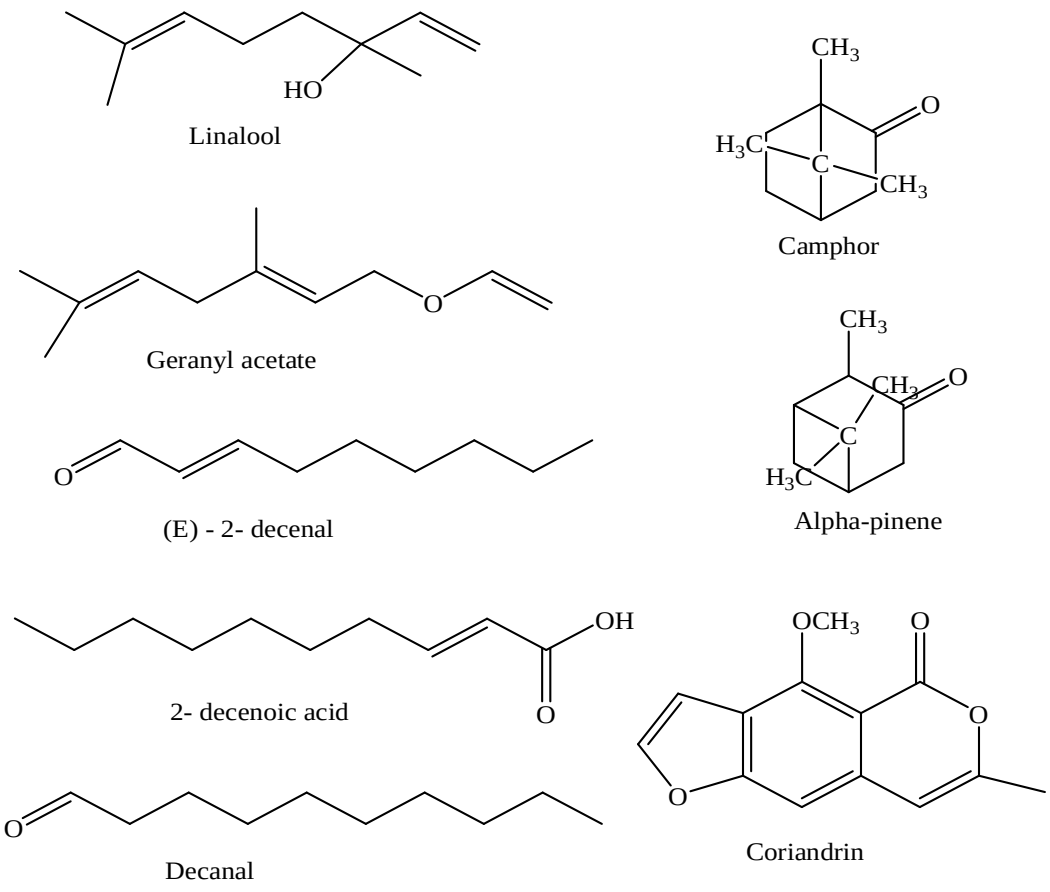



Figure 1.11: Structures of selected phytochemicals from *Coriandrum sativum*

Generally, the most commonly known phytochemicals from *Coriandrum sativum* are volatile components, flavonoids, isocoumarins, fatty acids, sterols, coriandrones, coumarins, catechins, and polyphenolic compounds (Sriti *et al*., 2009).

### 1.3.2 Common Applications and Uses of *Coriandrum sativum* Seeds

Many spices are used as a source of vitamins and minerals and to treat human disorders. The traditional health benefits of *Coriandrum sativum* include treatment of swellings, diarrhea, Mouth ulcers, anemia, digestion, menstrual disorders, small pox, eye care,

conjunctivitis, skin disorders, etc (Asgarpanah and Kazeminash, 2012). The seeds have medical uses and traditionally applied for curing digesting disorders, pain in joints and rheumatism, Stomachic, spasmolytic, carminative, diarrhea and dyspepsia. *Coriandrum sativum* are also used in aromatherapy as 'Appetite stimulant' (Reddy *et al*., 2012).

Moreover, it has been verified by many other researchers that the essential oils from *Coriandrum sativum* possess antibacterial, anti-cancerous, anti-mutagenic and antihemolytic activities (Ullagaddi and Bondada, 2011; Rajeshwari *et al*., 2012). Recent research studies (though still on animals) have confirmed all these medicinal effects.

## 1.4 The Role of Streptozotocin in Type 2 Diabetes Mellitus

The most common chemicals to induce diabetes in the animal model are alloxan 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 β cells 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 with glucose (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 reactive oxygen 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 1.12.

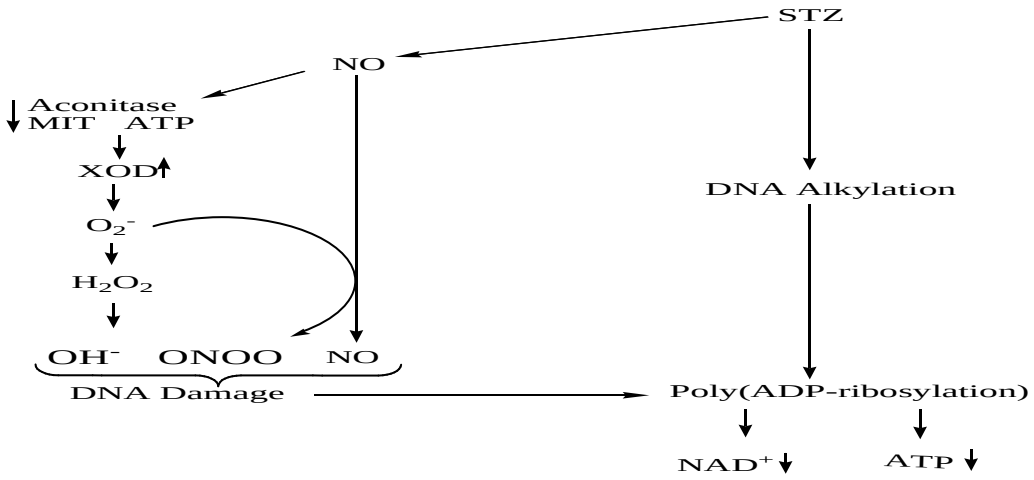



Figure 1.12: The mechanism of STZ induced toxic events in B cells of Pancreas:
abbreviation: *MIT –mitochondria; XOD – xanthine oxidase*

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, 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).

## 1.5. Hypotheses

It was hoped that this study has been important in giving direction for finding new application on hyperglycemia, dyslipidemia and renal dysfunction of *Coriandrum sativum* seed that could be used for future treatment of T2DM. To achieve these, the study was conducted with the following objectives.

# 2. OBJECTIVES

## 2.1 General Objective

The main goal of this study is to investigate the effect of *Coriandrum sativum* seed extracts on hyperglycemia, lipid profile and renal function in STZ induced type 2 diabetic Swiss Albino mice.

## 2.2 Specific Objectives

- To determine the hyperglycemic effect the extract in experimental mice
- To evaluate lipid profile of treated mice and compare it among the groups
- To assess atherosclerotic and cardioprotective index in each group
- To assess and compare serum urea, creatinine and total protein among the groups

# 3. MATERIALS AND METHODS

## 3.1 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, erlenmeyer flasks (400 mL), 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, water bath, Rota vapor, 902 automated chemistry analyzer and A 25 BioSystems Chemistry analyzer.

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

**Drugs:** Glibenclamide (Sanofi Winthrop industrie, France) was purchased from a local drug store. Streptozotocin was also purchased from sigma Aldrich Company, India.

## 3.2. Place of Study

The experiment was conducted in Addis Ababa University, college of health science, department of Biochemistry, and Ethiopian Health and Nutrition Research Institute, Ethiopia.

## 3.3 Preparation and Alcoholic Extraction of *Coriandrum sativum* Seed

*Coriandrum sativum* seeds were purchased from the local market, Merkato, Addis Ababa, Ethiopia. It was identified and authenticated by taxonomist in national herbarium, Addis Ababa University, Ethiopia, and deposited in the Department of Plant Biology and Biodiversity Management (Voucher number; 086790/2013). The seeds were washed carefully with distilled water to remove any extraneous materials and then grounded to a coarse powder using electric grinder. Three hundred gram of dried and grounded seeds was extracted with ethanol (70%) in a soxhlet apparatus for 48 hr at 60 $^{\circ}$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.

### 3.4 Experimental Animals and Study Protocol

Laboratory male Swiss albino mice (26-34g), 8th week age, were obtained from the department of Biomedical science, Arat killo campus, Addis Ababa University, Ethiopia. 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 Biochemistry Research and Ethics Review Committee (DRERC 07/2013). The animals were allowed to acclimatize in the laboratory environment for a week before the commencement of the experiment. The animals were housed in polypropylene plastic cages and maintained under standard laboratory conditions of temperature of 22 ± 3°C and 12 hour light/dark cycle. The mice were fed a standard commercial pellet diet and water *ad libitum* throughout the experimental period.

### 3.5 Acute Toxicity Test of *Coriandrum sativum* Seed extracts

Acute oral toxicity study was conducted according to Organization for Economic Co-operation and Development guideline 423, and six male mice were orally administered a single concentration of 2000mg/kg body weight of *Coriandrum sativum* seed 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.

### 3.6 Design of Experiment

The mice were divided into six groups comprising of six mice in each group as follows.

- Group I Normal control and were given only 0.5ml saline daily
- Group II STZ induced diabetic mice that served as Diabetic Control and were given 0.5ml saline only.

- Group III STZ induced diabetic mice treated with 300 mg/kg of *Coriandrum sativum* seed extracts
- Group IV STZ induced diabetic mice treated with 400 mg/kg of *Coriandrum sativum* seed extracts
- Group V STZ induced diabetic mice treated with 500 mg/kg of *Coriandrum sativum* seed extracts
- Group VI STZ induced diabetic mice treated with 5mg/kg of body weight of glibenclamide (Glb) (Tamiru *et al*., 2012)

Concentration selections were based on the safe doses of extract in oral acute toxicity studies carried out earlier in this study. *Coriandrum sativum* seed extracts and glibenclamide were administered orally for 21 days.

## 3.7 Induction of Experimental Type 2 Diabetes Mellitus

Diabetic were induced to fasting mice by a single intraperitoneal injection of freshly prepared STZ at a concentration of 120 mg/kg body weight in 0.1 M citrate buffer (pH 4.5) 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.

## 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 direct cardiac puncture. 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 $^{\circ}$C until further analyses of various biochemical parameters were determined. TC, TG, HDL, total protein, urea and creatinine were estimated with chemistry analyzer. LDL level was calculated using Friedwald equation (Burtis *et al.*, 2008).

### 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 0, $8^{th}$, $15^{th}$ and $21^{st}$ days. The method involves two coupled reactions (Mukherjee, 1988).

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

With a second reaction of;

$$\text{Glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6PD}} \text{6-phosphategluconate} + \text{NADPH} + \text{H}^+$$

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

### 3.8.2 Triglyceride Level

Triglyceride (TG) level is estimated by the enzymatic colorimetric method (Werner *et al*., 1981). TG is measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol and free fatty acids by the enzyme lipase. The glycerol formed phosphorylated to glycerol-3-phosphate by glycerol kinase. The glycerol-3-phophate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Then, Peroxidase catalyzes the redox-coupled reactions of $H_2O_2$ with 4-aminoantipyrine (4-AAP), producing a bright purple color. The absorbance is measured at 540 nm. The reaction sequence is as follows:

$$\text{Triglycerides} + 3\text{H}_2\text{O} \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{dihydroxyacetone phosphate} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 2\text{H}_2\text{O}$$

For this study, the specimen or sample was serum of the mice and, the reagents are standard and ready for use on automated analyzer. Enzymatic assay was done at 540 nm wavelength, 1cm optical path, 37 $^{o}$C temperature and measurement done against reagent blank.

**Procedure:** Samples, standard and blank were preincubated for 5 minutes at 37 °C. Reagent blank (1000 µL) and samples (10 µL) or standard (10 µL) were put into cuvette and mixed gently by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and blank was measured at 540 nm. Finally, the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank were calculated.

$$\text{TG level concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times C_{standard}$$

### 3.8.3 Total Cholesterol

Cholesterol is determined by enzymatic colorimetric method (Allain *et al*., 1974). Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-ene-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminophenazone and phenol in the presence of peroxidase to yield a chromogen. The absorbance is measured at 400 nm.

The reaction sequence is as follow:

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{Cholesteryl Ester Hydrolase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{Peroxide}} \text{Quinoneimine} + 4H_2O$$

For this study, the specimen or sample was serum of the mice and, the reagents are standard and ready for use on automated analyzer. Enzymatic assay was adjusted at 400

nm in wavelength, 1 cm in optical path, 37 $^{o}$C in temperature and measurement done against reagent blank.

**Procedure:** Samples, standard and reagent blank were preincubated for 5 minutes at 37 °C. Samples (10 µL) or standard (10 µL) and reagent blank (1000 µL) were pipetted into cuvette and mixed thoroughly by inversion. The cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of sample, standard and the reagent blank were measured at 400 nm within 60 minutes. Finally the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank were calculated.

$$\text{TC concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times C_{standard}$$

### 3.8.4 High Density Lipoprotein

The VLDL and LDL from serum are precipitated by phosphotungstate in the presence of magnesium ions. After removal by centrifugation, the clear supernatant containing high density lipoproteins (HDL)-fraction and their cholesterol content was determined enzymatically.

The reactions are as follows:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol ester hydrolase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-ene-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

For this study, the specimen or sample was serum of the mice and, the reagents are standard and ready for use on automated analyzer. Enzymatic assay was done at 593 nm wavelength, 1 cm optical path, 37 °C temperature and measurement done against reagent blank.

**Procedure for precipitation:** 100 µL of reagent and 10µL of samples were pipetted into centrifuge tube, mixed well, allowed to stand for 5 minutes at 37 °C and centrifuged at 3000 rpm for 20 minutes. The supernatant (sample) was collected for HDL test.

**Procedure for determination of HDL**: Reagent blank, samples and calibrator were preincubated for 5 minutes at 37 °C. Reagent blank (10 µL distilled water and 750 µL enzymes) and samples (10 µL samples and 750 µL enzymes) or calibrator (10 µL calibrator and 750 µL enzymes) 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 of sample, standard and the reagent blank were measured at 593 nm after 5 minutes. Finally the absorbance of the samples (ΔA sample) and the calibrator (ΔA standard) against the reagent blank were calculated.

$$\text{HDL concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{calibrator}} \times C_{caliibrator}$$

### 3.8.5 Low Density Lipoprotein

Indirect method, TC, TG and HDL are measured and LDL cholesterol is calculated from the primary measurements by use of the empirical Freidewald Formula equation:

$$\text{Concentration of LDL-c (mk/dl)} = [\text{Total cholesterol}] - \frac{[\text{Triglyceride}]}{5} - [\text{HDL}]$$

### 3.8.6 Estimation of Atherosclerotic and Cardioprotective Index

**Atherosclerotic index (AI)** is a marker of atherosclerosis that has a direct correlation with the risk of cardiovascular disease. It is calculated by the formula (Li *et al*., 2009):

$$\text{AI} = \frac{\text{TC - HDL}}{\text{HDL}}$$

**Cardioprotective index (CI)** is a superior measure of the risk of cardiovascular disease. It is calculated as the ratio of HDL-cholesterol to total cholesterol (Stevens *et al*., 2004).

### 3.8.7 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 colorimetrically through the increase of the absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

$$\text{Creatinine} + \underset{\text{(Yellow)}}{\text{Picric acid}} \xrightarrow[\text{(alkaline medium)}]{\text{NaOH}} \underset{\text{(Orange)}}{\text{Creatinine picrate}}$$

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, 37 $^{o}$C 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 ($A_1$) and after 90 seconds ($A_2$) of the sample or standard addition.

$$\text{Concentration of Creatinine (mg/dL)} = \frac{(A_2 - A_1)_{\text{Sample}}}{(A_2 - A_1)_{\text{Standard}}} \times \text{Con. of Standard(mg/dL)}$$

### 3.8.8 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).

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} NH_4^+ + HCO_3^-$$

$$\text{alpha-ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutarate} + H_2O + NAD^+$$

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, 37 $^{o}$C temperature and measurement done against air (decreasing absorbance).

**Procedure:** Working reagent, samples and standards were pre-incubated at 37 $^{\circ}$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 (A1) and exactly 90 seconds later (A2) of the sample and standard addition. Finally, after 30 and 90 seconds absorbance, the difference was calculated.

$$\text{Concentration of Urea (mg/dL)} = \frac{(A_1 - A_2)_{\text{Sample}}}{(A_1 - A_2)_{\text{Standard}}} \times \text{Concentration of Standard(mg/dL)}$$

### 3.8.9 Serum Total Protein (Biuret method)

In alkaline solution, peptide bonds bind with $Cu^{2+}$ ions to form a blue violet colored complex. This complex is formed between the $Cu^{2+}$ ion, the carbonyl oxygen and amide hydrogen atoms. Each $Cu^{2+}$ ion is combined to six peptide bonds. The intensity of the color is proportional to the reacting number of peptide bonds, and therefore to the amount of protein present in the medium, in which the absorbance is measured at 546nm (Bradford, 1976). The reaction is described as follows:

$$Cu^{2+} + \text{Protein} \xleftrightarrow[37\ ^{o}C]{} \text{Cu-Protein Complex (Blue violet color)}$$

For this study, the sample was mice serum, and the single reagent was ready for use. The assay was done at 546nm wavelength, 1cm optical path, 37 $^{o}$C temperature and measurement done against the blank.

**Procedure:** Samples (1000µL reagent and 10µL sample) or calibrator (1000 µL reagent and 10µL calibrator) and blank reagent (1000 µL reagent and 20 µL distilled water) were put into cuvettes and mixed at 37 °C by inversion. Then, the cuvettes were inserted into the cell holder and stopwatch was started to count. The absorbance of both the sample and the reagent blank were measured at 546 nm after 10 minutes. Finally, the absorbance of the samples (ΔA sample) and the calibrator (ΔA calibrator) were calculated against the blank reagent.

$$\text{Concentration of total protein(mg/dL)} = \frac{A_{sample} - A_{blank}}{A_{calibrator} - A_{blank}} \times \text{Concentration of Calibrator(mg/dL)}$$

### 3.9 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 by Tukey test at a 5% level of significance.

# 4. RESULTS

## 4.1 Yield of *Coriandrum sativum* Seed Extracts

The percentage yield of ethanollic (70%) extract of the dried *Coriandrum sativum* seeds was found to be 5.7% (w/w).

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

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

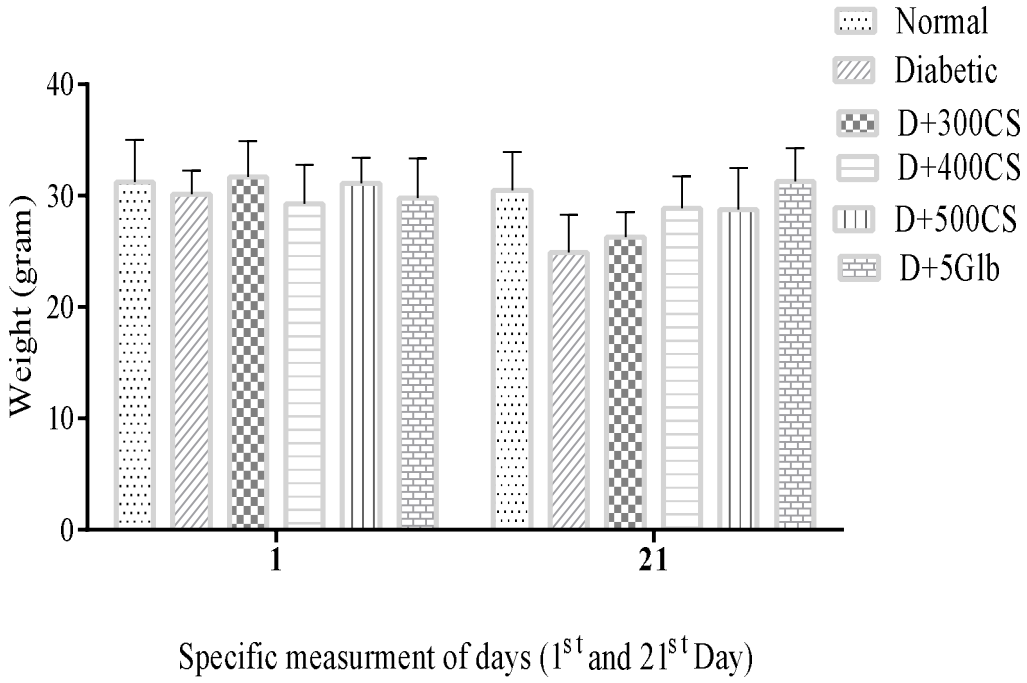
The extract was dark-brown jelly and solidified when stored in a deep freezer and turn to semisolid state on re-exposure to room temperature.

## 4.2 The Effect of *Coriandrum sativum* Seed extracts on Acute Toxicity Test

In acute oral toxicity test, *Coriandrum seed* extracts 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, diarrhea, seizures, and coma which received *Coriandrum sativum* seed extracts. Thus, the *Coriandrum sativum* seed extracts, 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.

## 4.3 The Effects of *Coriandrum sativum* Seed 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 *Coriandrum sativum* seed extracts treated diabetic mice as shown below in figure 4.1.



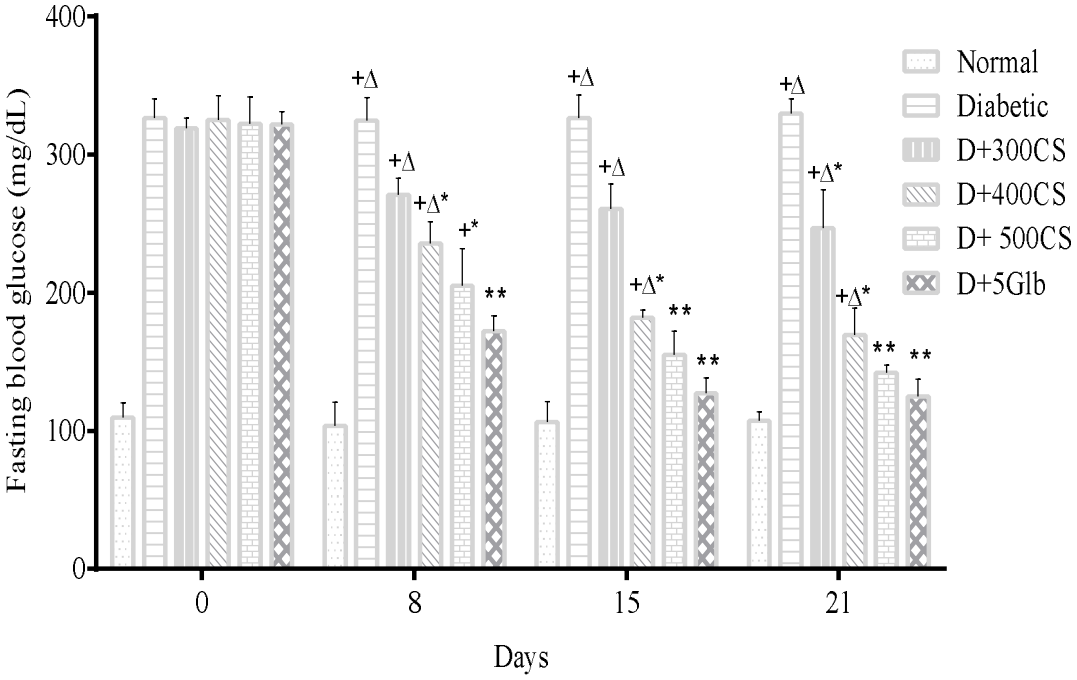

*The results are expressed as mean ± SD (n =6). D-Diabetic, CS: Coriandrum sativum, Glb: Glibenclamide*

Figure 4.1: The effects of *Coriandrum sativum* seed extracts on body weight in STZ induced diabetic mice

### 4.4 The Effect of *Coriandrum sativum* Seed extracts on Fasting Blood Glucose Level

The anti-hyperglycemic effects of graded concentration of *Coriandrum sativum* seed extracts on the FBG levels of STZ induced diabetic mice were presented in figure 4.2 as shown 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 *Coriandrum sativum* seed extracts, the FBG levels were significantly ($p<0.01$) decreased on $8^{th}$, $15^{th}$ and $21^{st}$ days. Similarly, treatment with glibenclamide, which has been used as standard anti-diabetic reference drug to compare the beneficial effects of *Coriandrum sativum* seed extracts, also led to a significant ($p<0.001$) reduction in FBG levels on $8^{th}$, $15^{th}$ and $21^{st}$ days.

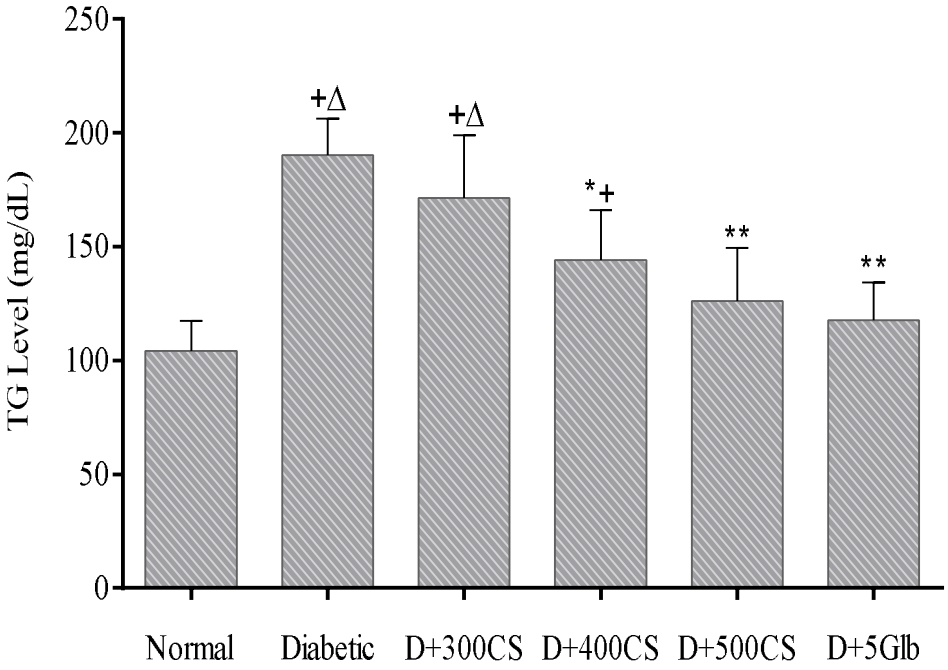



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with Glb (glibenclamide) treated group, D-Diabetic,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

Figure 4.2: The effects of *Coriandrum sativum* seed extracts on fasting blood glucose in diabetic mice on 0, 8th, 15th and 21st days.

## 4.5 The Effects of *Coriandrum sativum* Seed Extracts on Serum Triglyceride

Figure 4.3 illustrates the effect of *Coriandrum sativum* seed extracts on serum TG level in normal and diabetic mice. There were significant (P<0.01) rises in serum TG in diabetic control as compared to normal control. However, administration of *Coriandrum sativum* seed extracts at 300mg/kg, 400mg/kg and 500mg/kg concentration reduced serum TG level as compared to diabetic mice on 21th day. And, treatment with 5mg/kg of glibenclamide also led to a significant (p<0.001) reduction in TG level after 21th days.

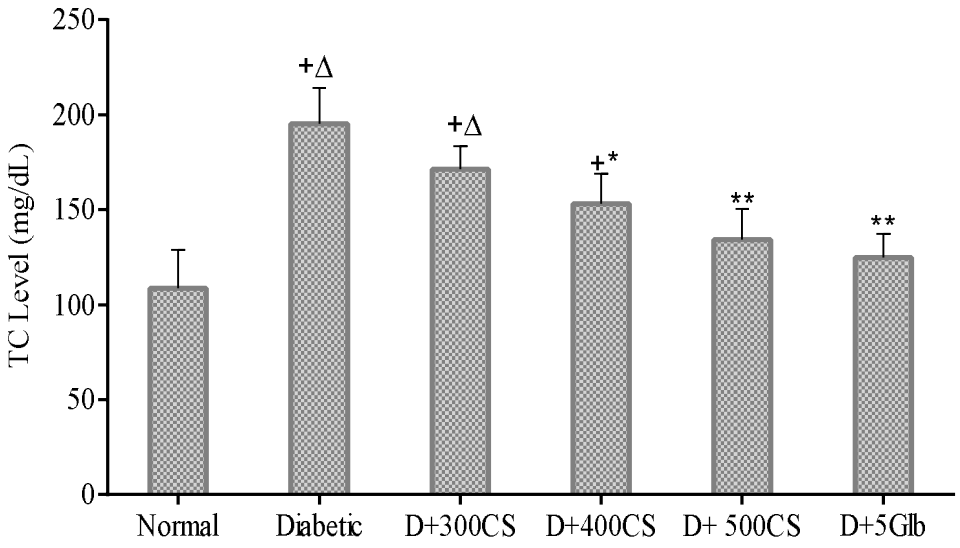



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, +- significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.3: The effects of *Coriandrum sativum* seed extracts on TG in STZ induced diabetic mice.

## 4.6 The Effects of *Coriandrum sativum* Seed extracts on Total Cholesterol

Figure 4.4 illustrates the effect of *Coriandrum sativum* seed extracts on serum TC in normal and diabetic mice. There were a significant ($p<0.001$) rise in serum TC in diabetic group as compared to normal control. Also, administration of *Coriandrum sativum* seed extract at 300mg/kg, 400mg/kg and 500mg/kg concentration reduced serum TC as compared to diabetic mice on $21^{th}$ days treatment. And, treatment with glibenclamide also led a significant ($p<0.001$) reduction of TC.

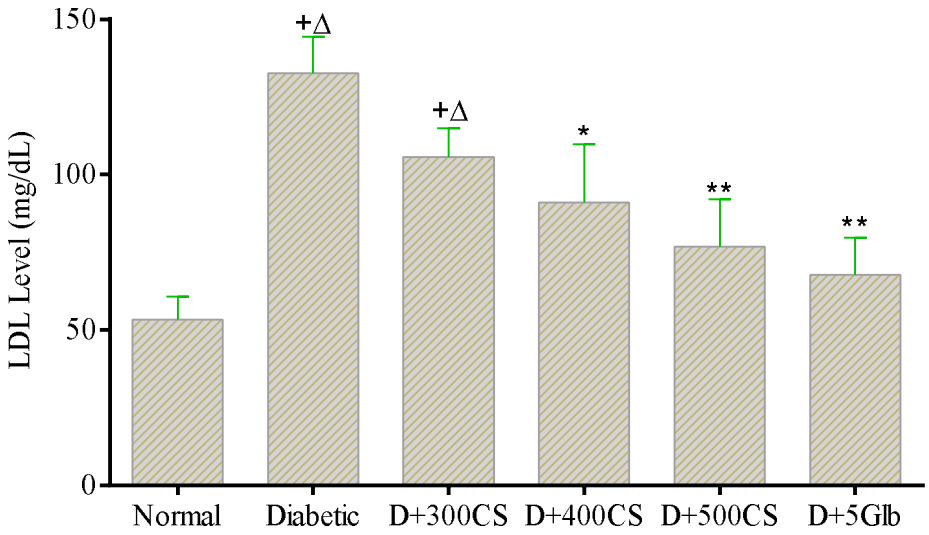



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, +- significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.4: The effects of *Coriandrum sativum* seed extracts on TC in STZ induced diabetic mice.

## 4.7 The Effects of *Coriandrum sativum* Seed extracts on serum LDL

Figure 4.5 describes the effect of *Coriandrum sativum* seed extracts on serum LDL in normal and diabetic mice. There were a significant ($p<0.001$) rise in serum LDL in diabetic group as compared to normal control. Administration of *Coriandrum sativum* extract at 300mg/kg, 400mg/kg and 500mg/kg concentration reduced serum, LDL levels as compared to diabetic mice on 21$^{th}$ days treatment. And, treatment with glibenclamide also led to a significant ($p<0.001$) reduction in LDL.

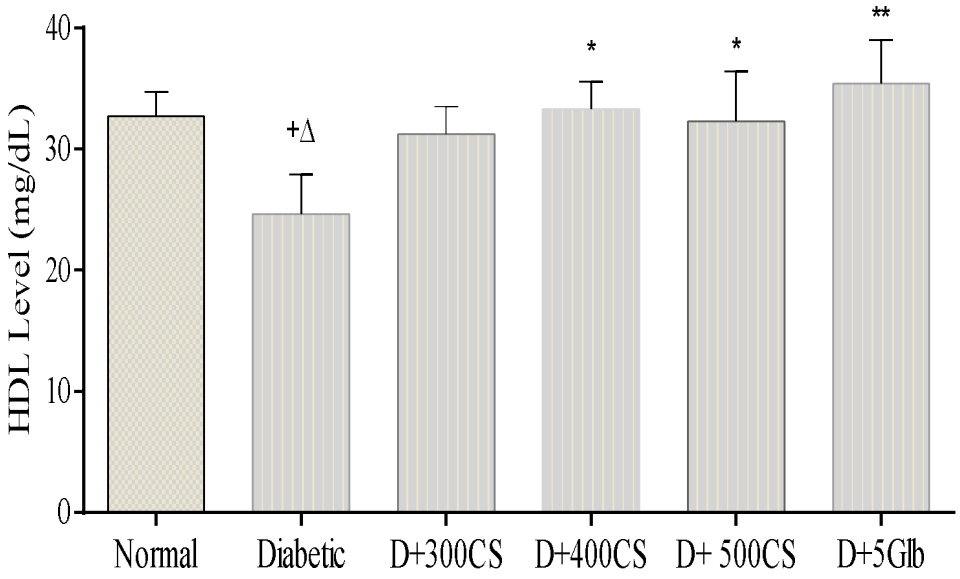



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, +- significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.5: The effects of *Coriandrum sativum* seed extracts on LDL in STZ induced diabetic mice.

### 4.8 The Effects of *Coriandrum sativum* Seed extracts on HDL

Figure 4.6 illustrates the effect of *Coriandrum sativum* seed extracts on serum HDL in normal and diabetic mice. There were a significant (P<0.01) decrease in serum HDL in diabetic group as compared to normal control. Also, administration of *Coriandrum sativum* seed extracts at 300mg/kg, 400mg/kg and 500mg/kg concentration increased the HDL levels as compared to diabetic mice on 21$^{th}$ days treatment. And, treatment with glibenclamide also led to a significant (p<0.001) increase in HDL.

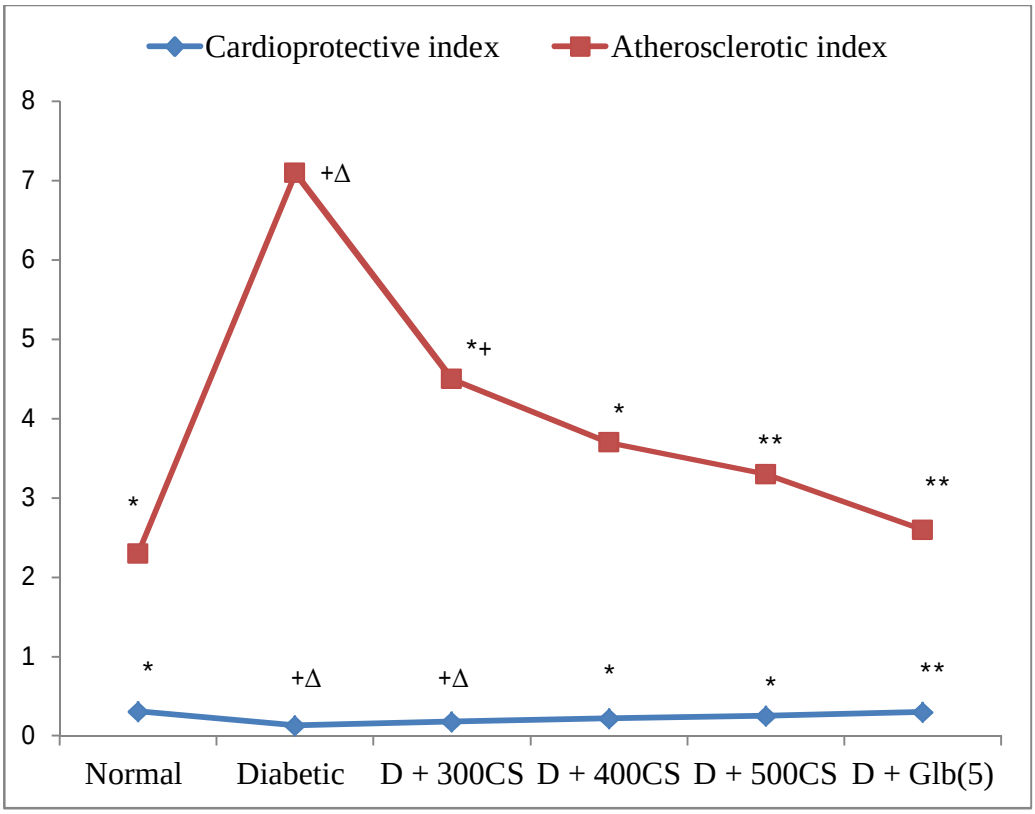



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.6: The effects of *Coriandrum sativum* seed extracts on HDL in STZ induced diabetic mice.

## 4.9 The Effect *Coriandrum sativum* Seed Extracts on Atherosclerotic and Cardioprotective Index

Figure 4.7 illustrates the effect of *Coriandrum sativum* seed extracts on atherosclerotic and cardioprotective index in normal and diabetic mice. There were significant ($p<0.001$) rises in atherosclerotic and decrease cardioprotective index on diabetic group as compared to normal control. However, administration of *Coriandrum sativum* seed extract at 300mg/kg, 400mg/kg and 500mg/kg concentration reduced atherosclerotic and increased cardioprotective index as compared to diabetic mice. And, treatment with glibenclamide also led to a significant ($p<0.001$) reduction of atherosclerotic index and rise cardioprotective index.

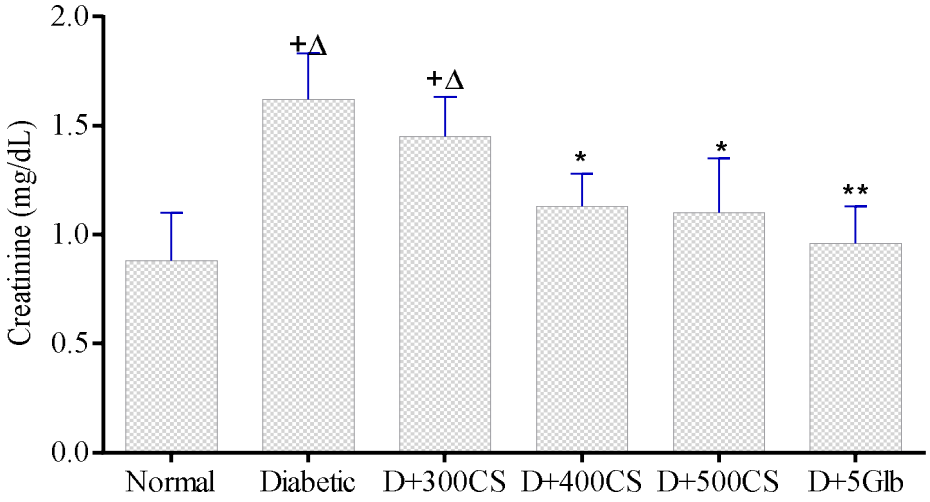



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, +- significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.7: The effects of *Coriandrum sativum* extract on atherosclerotic and cardioprotective index in diabetic mice.

## 4.10 The Effects of *Coriandrum sativum* Seed Extracts on Serum Creatinine

Figure 4.8 describes the effect of *Coriandrum sativum* seed extracts 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 *Coriandrum sativum* seed extracts at all concentrations and 5mg/kg glibenclamide in treated diabetic mice as compared to diabetic mice.

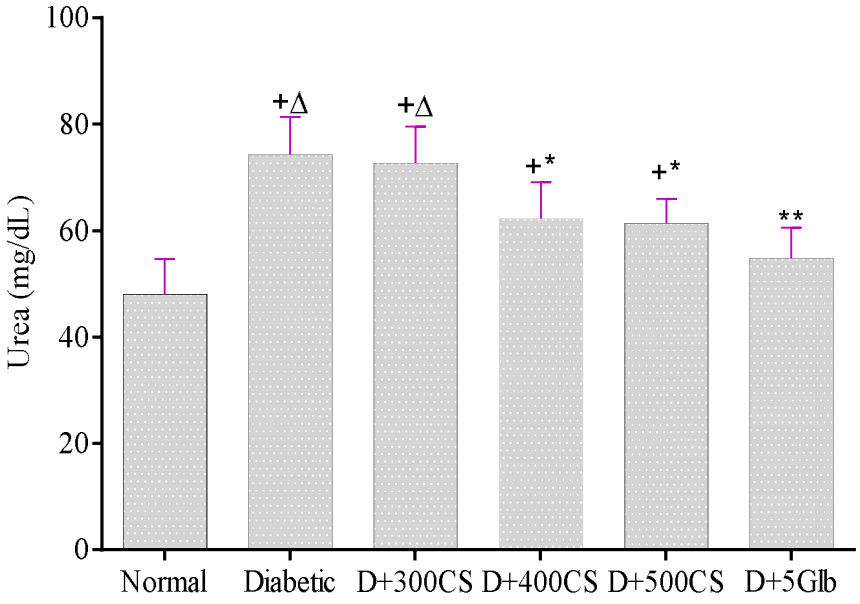



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, + - significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.8: The effects of *Coriandrum sativum* seed extracts on Creatinine in STZ induced diabetic mice.

## 4.11 The Effects of *Coriandrum sativum* Seed Extracts on Serum Urea

Figure 4.9 describes the effect of *Coriandrum sativum* seed extracts 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 were reduced after the administration of *Coriandrum sativum* seed extracts at all concentrations and 5mg/kg glibenclamide in treated diabetic groups as compared to diabetic group after treatment for three weeks.

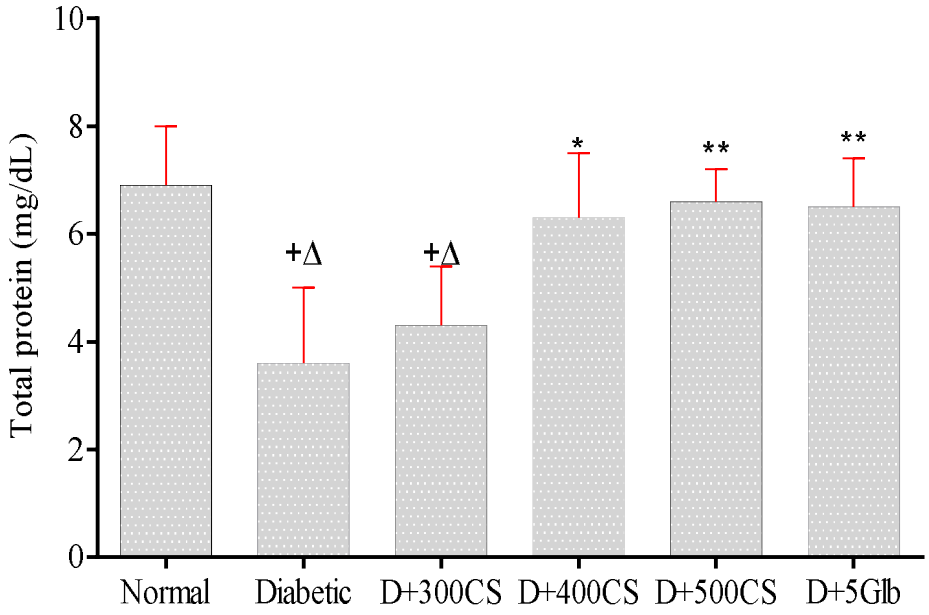



*The results are expressed as mean ± SD (n =6). * – significant at p<0.05 compared with diabetic control, ** - significant at p<0.001 compared with diabetic control, +- significant at p<0.05 compared with normal control, Δ - significant at p<0.05 compared with glibenclamide treated group, D-Diabetic,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

Figure 4.9: The effects of *Coriandrum sativum* seed extracts on urea in STZ induced diabetic mice.

## 4.12 The Effects of *Coriandrum sativum* Seed Extracts on Serum Total Protein

Figure 4.10 describes the effect of *Coriandrum sativum* seed extracts on serum total protein in normal and diabetic mice. There were significant ($p<0.001$) decreases in total protein on diabetic group as compared to normal control. On the other hand, the levels of serum total protein were increased after the administration of *Coriandrum sativum* seed extracts and glibenclamide on treated diabetic mice as compared to diabetic control mice.




*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- Coriandrum sativum*

Figure 4.10: The effects of *Coriandrum sativum* seed extracts on total protein in STZ induced diabetic mice.

# 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, lipid profile, and renal function are important risk factors for diabetes, cardiovascular and many other diseases. The increased vascular risk and coronary events associated with T2DM is the most likely to the multifactorial, and dyslipidemia and atherogenic failure has emerged. 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). STZ enters the β cell *via* GLUT2 transporter and causes alkylation of DNA, thereby induces the activation of poly ADP ribosylation. Poly ADP-ribosylation leads to depletion of cellular $NAD^+$ and ATP (BalkisBudin *et al*., 2009).

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 *Coriandrum sativum* seed extracts in normalizing body weight, reducing blood glucose levels, decreasing cholesterol levels, cardioprotective effect 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 *Coriandrum sativum* seed. 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 (Gouggean *et al*., 2008). However, 5mg/kg of glibenclamide treated mice gained weight (25.7%) in comparison with the diabetic group after 21 days treatment.

This study result revealed that *Coriandrum sativum* seed treated groups did not show important body weight gain; this could support that *Coriandrum sativum* seed 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, 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 *Coriandrum sativum* seed 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 *Coriandrum sativum* seed reduced FBG level in diabetic mice. The FBG level was decreased by 16.4% and 21.12% at 300mg/kg extract concentration on $8^{th}$ and $15^{th}$ 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 $8^{th}$, $15^{th}$ and $21^{st}$ 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 $8^{th}$, $15^{th}$ and $21^{st}$ 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 $8^{th}$, $15^{th}$ and $21^{st}$ days respectively as compared to diabetic group. These results of reduction in FBG are in agreement with pervious work on effect of *Coriandrum sativum* extract on insulin release from pancreatic β cells in streptozotocin-induced diabetic rats (Eidi *et al*., 2009).

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

The present findings indicate the hypoglycemic and/or potential antihyperglycemic effect of the extract. There were many possible explanations for this finding. The anti-hyperglycemic effect of *Coriandrum sativum* seed extracts may be due to restoration of insulin response *via* the presence of antihyperglycemic, "insulin-releasing" and "insulin-like" activity in *Coriandrum sativum* (Gray and Flatt, 1999). It was also suggested that the anti-hyperglycemic effects of the *Coriandrum sativum* seed 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 *Coriandrum sativum* seed extracts on pancreatic tissue (Byambaa *et al*., 2010).

In addition to its anti-hyperglycemic effect, *Coriandrum sativum* seed extracts were also able to alter the levels of lipid metabolites including TC, TG, HDL, and LDL cholesterol levels in diabetic mice suggesting a remarkable anti-hyperlipidemic effect. The levels of serum lipids are usually raised in T2DM and such an elevation represents a risk factor for coronary heart disease (Sahib *et al*., 2012).

In this study, there were significant ($P<0.01$) increase in the TC, TG and the LDL levels on diabetic mice. The elevated TG level in diabetic mice might be due to the consequence of increased synthesis of triglyceride rich lipoprotein particles (VLDL) in liver and diminished catabolism. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Byambaa *et al*., 2010). The increased levels of LDL and VLDL in the STZ diabetic mice might also be due to over production of LDL and VLDL by the liver in turn by the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Samatha *et al*., 2012). Recent researches have also reported that the elevated TG level-rich lipoproteins could be a consequence of the reduction of LPL activity due to its glycation (Puddu *et al*., 2013).

The elevated levels of TC, TG and LDL were reduced in *Coriandrum sativum* seed extracts treated diabetic mice. Administration of 300mg/kg *Coriandrum sativum* extract were reduced by 12.29 % in TC, 9.88 % in TG, 20.23 % in LDL, and increased by 26.34 % in HDL as compared to diabetic group. Administration of 400mg/kg *Coriandrum*

*sativum* seed extracts were also reduced significantly ($p<0.01$) by 21.52% in TC, 24.23% in TG and 31.32% in LDL, and increased by 34.82% in HDL as compared to diabetic group. Administration of 500mg/kg *Coriandrum sativum* seed extracts were reduced significantly ($p<0.001$) by 31.2% in TC, 33.68% in TG and 42.04% in LDL, and increased by 30.77% in HDL as compared to diabetic group. After glibenclamide (5mg/kg) treatment of diabetic mice, there were reduction by 35.06 % in TC, 38.10 % in TG and 48.98 % in LDL, increased the HDL by 43.32% as compared to diabetic control group. These results are in line with Ramadan *et al*, who stated that *Coriandrum sativum* seed oil improves plasma lipid profile in rats fed a diet containing cholesterol (Ramadan *et al*., 2008). Thus, these results suggested that *Coriandrum sativum* seed extracts would be helpful to the prevention of diabetic complications through improving dyslipidemia and/or hypercholesterolemia.

Nevertheless, the maximum reduction in TC, TG and LDL was associated with the concentration of 500mg/kg among the *Coriandrum sativum* treated groups, and it was comparable with mice treated by 5mg/kg concentration of glibenclamide.

The reductions of TG, TC and LDL level by *Coriandrum sativum* seed extracts might be due to the involvement of polyphenolic part of the extract in preventing the formation of AGEs in diabetic mice (Jia *et al*., 2009; Krishnan *et al.*, 2007). *Coriandrum sativum* fiber may delay the absorption of glucose and fatty acids from the upper small intestine, thus providing less substrate for synthesis of triglycerides (Jelodar *et al*., 2007).

Both HDL and plasma LCAT are believed to be involved in the transport of cholesterol from extra hepatic tissues to the liver for its excretion (Shiju and Pragasam, 2012). The higher levels of cholesterol associated with HDL and the increase in the activity of plasma LCAT on administration of *Coriandrum sativum* may result in a higher amount of cholesterol being removed from extra hepatic tissues which may contribute to the anti-hypercholesterolemia observed in these animals. Hence, the lowering in cholesterol levels of serum and tissues by the administration of this extract would seem to be mediated through its increased rate of degradation to bile acids and neutral sterols.

The phenotype of dyslipidemia in Ethiopian population is high in TG level concentration, low in HDL and high in LDL cholesterol (Siraj *et al.*, 2006). In this study, similar results were obtained in animal models of STZ induced diabetic mice. Although, no comparison with human population is attempted in the present work, induced insulin resistance can be described as a potent stimulus for T2DM in mice. Therefore, it is suggested that an intervention with *Coriandrum sativum* seed extracts in patients with T2DM requires supra-physiological dosing as well as confirmation with immune regulatory treatment.

Furthermore, there were an elevation of atherosclerotic index and reduction of cardioprotective index in diabetic mice. These high levels of atherosclerotic index and lower level of cardioprotective index are usually found in T2DM and such an elevation represents a risk factor for coronary heart disease (Sahib *et al*., 2012). The increased TG level in T2DM is also accompanied by pro-atherosclerotic functional change in HDL and LDL particles (Mulugeta *et al*., 2012). Accumulation of LDL within the arterial wall appears to play a crucial role in the initiation and progression of atherosclerotic plaque (Hunt *et al*., 2011). However, *Coriandrum sativum* seed extracts were able to improve the levels of atherosclerotic index and cardioprotective index in diabetic mice suggesting a remarkable cardioprotective effect. These findings indicate that the extracts might be beneficial to diabetic mice with atherosclerosis, since a decrease of LDL and elevated HDL were obtained and associated with a reduced risk on of the development of atherosclerosis in T2DM (Ramadan *et al*., 2008).

Administration of the *Coriandrum sativum* seed extract decreased fasting blood glucose, which has a direct and independent relationship with cardiovascular disease, reduced lipid and lipoprotein and decreased the calculated atherosclerotic index and increased cardioprotective index. It may be deduced that the *Coriandrum sativum* seed extracts have an overall cardiovascular protective effect and reduce atherosclerotic risk in STZ induced diabetic mice.

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, 2012).

In this study, the level of serum urea and creatinine were raised in STZ induced diabetic mice. The increased urea and creatinine production in diabetic might be due to accelerated catabolism of both liver and plasma proteins (Hassan *et al.*, 2009). Nevertheless, the *Coriandrum sativum* seed 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 *Coriandrum sativum* seed 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; Jayaprakasam *et al*., 2005).

In this study, it was found that serum total protein was decreased significantly ($p<0.01$) in STZ induced diabetic group. Protein metabolism is impaired in T2DM as a result of insulin resistance that led to a defect in amino acid metabolism and suppression of protein synthesis. Insulin resistance of protein metabolism could be impaired as one of the causes of protein malnutrition (Gougeon *et al.*, 2008). However, it was improved in the *Coriandrum sativum* seed extracts treated diabetic groups. Total protein was raised by 20.95% at 300mg/kg extract concentration as compared to diabetic group. On the other hand, there were significant ($P<0.01$) increase by 74.56% and 83.79% of serum total protein after a 400mg/kg and 500mg/kg *Coriandrum sativum* extract treatment

respectively as compared to diabetic group. Similarly, there were also significant ($P<0.01$) improvements of total protein (81.56%) in treated diabetic mice with glibenclamide (5mg/kg) as compared to diabetic group. Here, *Coriandrum sativum* seed extracts might be attributed to an improvement in glycemic control and insulin secretion that in turn leads to increase protein synthesis or decrease in protein degradation.

# 6. CONCLUSION AND RECOMMENDATION

## 6.1 Conclusion

In this study, hydro-ethanollic extracts of *Coriandrum sativum* seed 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 *Coriandrum sativum* seed extracts has potent hypoglycemic effect and provided better glycemic control in STZ induced diabetic mice.

Similarly, the seed extract also caused a decrease in TC, TG and LDL, and an increase in HDL for diabetic mice implying that it has anti-hyperlipidemia effect on STZ induced diabetic mice.

*Coriandrum sativum* seed extracts also showed a decrease of serum urea and creatinine which indicates restoring properties on the function of kidney diabetic mice.

Generally, from the above findings, it is possible to conclude that extract of *Coriandrum sativum* seed has anti-hyperglycemic, anti-dyslipidemia effect and restoring the function of kidney in STZ induced diabetic Swiss albino mice. Thus, *Coriandrum sativum* seed extract can be helpful in preventing future damages caused by T2DM and its complications, such as cardiovascular diseases, which is among the main causes of death.

There were also a gradual improvement of hyperglycemia, dyslipidemia and renal failure within the graded concentration. Hence, it could be conclude that 500mg/kg concentration of *Coriandrum sativum* seed extract have a better anti-diabetic capacity, and almost equipotent with glibenclamide drug.

## 6.2 Recommendation

- The present observation provided evidence that extract of *Coriandrum sativum* seed exhibited hypoglycemic activity on STZ induced diabetic mice. This effect may be due to the presence of flavonoids, terpens, tripenes, alkaloids, polyphenols and other constituents present in the plant which could act synergistically or independently in enhancing the activity of glycolytic and gluconeogenic enzymes. Thus, it is essential that comprehensive chemical and pharmacological investigation should be carried out to isolate and characterize a specific bioactive compound and appropriate elucidation of its mechanism of action.

- Generally, *Coriandrum sativum* seed extracts provided better glycemic control and improved dyslipidemia in STZ induced diabetic mice. Other studies are needed to assess the potential negative effect of dyslipidemia in vascular disorder and endothelial function. It should also be mentioned that this study described all short-term (three weeks) effects with restricted concentration. Thus, it needs further comprehensive work to assess and to conform long term outcomes resulting from higher concentration of *Coriandrum sativum* seed extracts.

- Further, it is proposed that researches on histopathology of pancreas, liver and kidney should be undertaken to make the study more comprehensive.

- The use of *this* seed extracts could be a potential strategy to aid in the prevention of T2DM and its complications. Although the results found using this extract are promising, further studies are essential to evaluate its effects on human beings.

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