ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF MEDICINE

DEPARTMENT OF PHARMACOLOGY

EVALUATION OF HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC EFFECT OF AQUEOUS AND 80% METHANOL LEAVES EXTRACTS OF *THYMUS SCHIMPERI* (LAMIACEAE) *IN* MICE

Getu Melesie (B. Pharm)

November, 2016

Addis Ababa, Ethiopia

EVALUATION OF HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC EFFECT OF AQUEOUS AND 80% METHANOL LEAVES EXTRACTS OF *THYMUS SCHIMPERI* (LAMIACEAE) *IN* MICE

By: Getu Melesie (B. Pharm)

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL PHARMACOLOGY

Under the supervision of:

Prof. Teferra Abula (PhD), Department of Pharmacology, School of Medicine, Addis Ababa University, Ethiopia and

Frehiwot Teka (MSc), Directorate of Traditional and Modern Medicine Research, Ethiopian Public Health Institute, Addis Ababa, Ethiopia

November, 2016

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Mr. Getu Melesie entitled: *Hypoglycemic and antihyperglycemic effect of* aqueous and 80% methanol *leaves extracts of Thymus schimperi in mice* and submitted in partial fulfillment of the requirements for the degree of Master of Science in Medical Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved and signed by the examining committee:

prof. Yalemtsehay Mekonnen	_____	_____
External Examiner	Signature	Date
Prof. Eyasu Mekonnen	_____	_____
Internal Examiner	Signature	Date
Prof. Teferra Abula (PhD	_____	_____
Advisor	Signature	Date
Frehiwot Teka (MSc)	_____	_____
Co-Advisor	Signature	Date

_____

Chair of Department or Graduate Program Coordinator

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

Name: _____

Signature: _____

Place and date of submission: Addis Ababa, Ethiopia, December, 2016

Evaluation of hypoglycemic and antihyperglycemic effect of aqueous and 80% methanol leaves extracts of *thymus schimperi* (Lamiaceae) *in* mice

Getu Melesie

Addis Ababa University, 2016

ABSTRACT

Diabetes mellitus is one of the most common chronic diseases nearly in all countries and the burden continue to increase especially in developing countries. Current treatment interventions have been hampered by drawbacks like high costs, inaccessibility, and potential adverse effects. Hence it is necessary to evaluate medicinal plants for their pharmacological effects to support existing drugs in treating diabetes. The present study was undertaken to evaluate the hypoglycemic and antihyperglycemic activity of leaf extracts of *Thymus schimperi* in mice.

***Aim of the study**:* The present study was aimed to investigate the effects of the aqueous and hydroalcolic leaf extract of *T. schimperi* on blood glucose level.

Methods: The aqueous and 80% methanol extracts of *T. schimperi* leaves were prepared. Swiss albino mice of either sex weighing 20-30 grams were selected for the experiments. Normal mice were grouped into six groups (of each containing 6 mice) to carry out hypoglycemic effect of the extracts, whereas mice that were made diabetic were grouped into seven groups to study the antihyperglycemic effect of the extracts. Diabetes was induced by single intraperitonial injection of alloxan monohydrate (180mg/kg body weight). Each extract was tested for antihyperglycemic activity using glucose tolerance test in normal mice also. Then Blood glucose levels were measured. Preliminary phytochemical screening was done using common chemical test procedures and acute toxicity study was done as per OECD 425 guidelines. The results were analyzed using one way ANOVA at a 5% level of significance.

***Result**s:* The aqueous and hydroalcoholic extracts of *T. schimperi* leaves lack hypoglycemic effect on normoglycemic mice at dose of 250mg/kg and 500mg/kg. In oral glucose tolerance test, extracts of *T. schimperi* led to dose-dependent reductions in blood glucose levels. After diabetic mice were treated with extract of both solvent at dose of 250 and 500 mg/kg for 21 days,

there were significant decreases in fasting blood glucose when compared to those diabetic controls. The observed antidiabetic activity could be associated with the phytochemicals present in this plant extract. The extract of both solvent also prevented body weight loss of diabetic when compared to diabetic mice group. Results of preliminary phytochemical screening indicated that alkaloid, flavonoids, tannins, phenols, saponins and steroids were presented in leaves extracts. It was also observed that the extracts have shown no acute toxicity at a dose of 2 g/kg.

Conclusion: The aqueous and 80% methanol extracts of *T. schimperi* leaves have showed blood glucose level lowering effect in diabetic mice, but lack hypoglycemic effect in normoglycemic mice.

Key word: Hypoglycemic, Antihyperglycemic, *T. schimperi*, alloxan, in vivo.

ACKNOWLEDGEMENTS

First, I would like to present my special thanks and great appreciation to my advisor Prof. Teferra Abula for his unreserved, timely follow-ups, critical and constructive comments high encouragement which helped me to write this research paper. I have also a great honour for him for his great and invaluable contribution from the very beginning title selection to the completion of this study. It is also my pleasure to express my deepest gratitude to my co-advisor, Frehiwot Teka for her guidance, advice and helpful suggestions in problem solving that helped me to perform the laboratory activities and this thesis.

I would like to acknowledge the support of the management of Traditional and Modern Medicine Research Directorate, Ethiopian Public Health Institute who sponsored me to use their laboratory setup, laboratory animals, equipment, reagents and chemicals to perform each activities of the research work. I especially wish to thank, Mr. Samuel for his support in laboratory tasks.

My warmest gratitude goes to my sponsor Ambo University for the opportunity given to me to pursue this MSc program. I also thank Addis Ababa University for the financial support it provided me for conducting this study.

Last but not least, my gratitude goes to my family and friends who helped me in one way or the other to complete this study. I additionally wish to give my appreciation to Mr. Hailemeskel Meshesha and Ms. Fantu Assefa for their unfailing help during the laboratory activities and Ms. Yewubdar Haile for her dutiful and uninterrupted concern of the experimental animals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/ ACRONOMYS

DAG	Diacylglycerol
DNA	Deoxyribonucleic Acid
ER	Endoplasmic Reticulum
FBG	Fasting Blood Glucose
GD	Gestational Diabetes
Glc	Glucosamine
HDL	High Density Lipoproteins
IDDM	Insulin-dependent diabetes
IDF	International Diabetes Federation
LDL	Low Density Lipoproteins
MODY	Maturity Onset Diabetes in the Young
Nac-N-	Acetylglucosamine
NIDDM	Non-insulin-dependent diabetes,
OECD	Organization for Economic Co-operation and Development
OGTT	Oral Glucose Tolerance Test
PKC-	Protein kinase C
ROS	Reactive oxygen species
TM	Traditional Medicine
T1DM	Type 1 Diabetic Mellitus
T2DM	Type 2 Diabetic Mellitus
UDP	Uridine diphosphate
WHO	World Health Organization

1. INTRODUCTION

1.1. Background

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by persistent hyperglycemia due to alteration in carbohydrate, fat and protein metabolism (Shyam and Kadalmani , 2014), related with decrease in insulin secretion or insulin resistance. . Impairment of insulin secretion and defects in insulin action frequently coexist in the same individuals(Akter *et al*., 2013). The two most common forms of DM are type 1 DM (T1DM) previously known as insulin-dependent diabetes (IDDM) and type 2 DM (T2DM), previously known as non-insulin-dependent diabetes, NIDDM. However, there are other rare forms of diabetes that are directly inherited. These include gestational diabetes (GD), maturity onset diabetes in the young (MODY), and diabetes due to mutations in mitochondrial deoxyribonucleic acid (DNA) (Kumar *et al*., 2012). The predisposing factors associated with DM includes, lifestyle changes associated with urbanization, diet, obesity and physical inactivity are major determinant. Age, ethnicity, history of gestational diabetes and family history of type II diabetes are also the main determinants of diabetes prevalence (Colagiuri *et al*., 2006, Libman and Arslanian, 2007).

DM is one of the most important causes of death and disability in both developed and developing countries. According to the report by World Health Organization (WHO) 9% of adults in the world suffer from diabetes (Hosseini *et al*., 2015). DM has very serious effects on health. In addition to the consequences of abnormal metabolism of glucose, diabetes is associated with micro and macro-vascular complications, which are the major causes of morbidity and mortality in diabetic patient. This includes ketoacidosis of different grades, development of foot ulcer, cardiovascular, peripheral vascular, ocular, neurologic and renal abnormalities, which are responsible for morbidity, disability and premature death in young adults. Furthermore, the disease is associated with reproductive complications causing problems for both mothers and their children (Sunil and Kumar, 2014).

Early diagnosis is the best way for living with diabetes, the longer a person lives with undiagnosed and untreated diabetes, the worse their health outcomes are likely to be. For those

who are diagnosed with diabetes, a series of cost-effective interventions can improve their outcomes. These interventions include blood glucose control, through a combination of diet, physical activity and, if necessary, medication; control of blood pressure and lipids to reduce cardiovascular risk and other complications; and regular screening for damage to the eyes, kidneys and feet, to facilitate early treatment (WHO, 2016).

The currently available medicines to control hyperglycemia in DM management include: "insulins, insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, thiazolidinedione), agents that enhance incretin secretion and action (incretin analogues, incretin mimetics, dipeptidyl peptidase IV (DPP-IV) inhibitors), agents that decrease gastrointestinal glucose absorption (alpha glucosidase inhibitors, alpha amylase inhibitors, sodium-glucose co-transporter (SGLT-1) selective inhibitors), agents that promote renal glucose excretion (sodium-glucose co-transporter (SGLT-2) inhibitors) and others (amylin analogue, bile acid sequestrants, bromocriptine) (Flint and Arslanian, 2011; Verspohl, 2012).

Despite the wide range of therapeutic agents designed to fight hyperglycemia, the statistical projections are still alarming and the stability of communities is being threatened. Alternative strategies to the current pharmacological options of DM management are therefore urgently needed to manage this global health problem. The plant kingdom has become a target for the search of biologically active lead compounds for complementary/alternative management of DM. The effect of these plants may delay the development of diabetic complications and correct the metabolic abnormalities (Mardanyan *et al*., 2011 ; Osigwe *et al.*, 2015).

Traditional Medicine (TM) in Ethiopia has attracted very little attention in modern medical research and development, and less effort has been made to upgrade the role of TM practice and identify side effects that may be associated to the use of herbal medicines *(Abebe, 1996)* As a result, it has become imperative to conduct research on herbs to find out the effectiveness of drugs for the benefit of human and animals and discard the ineffective, toxic and worthless drug.

1.2. Epidemiology of Diabetic Mellitus

DM is found worldwide and becoming a serious threat to mankind. It is third killer of human beings after cancer, cardiovascular and cerebro-vascular diseases(Larbie *et al.*, 2014). It is estimated that 366 million people had DM in 2011 and by 2030 it will be increased to 552 million. The number of people with type 2 diabetes is increasing in all countries with 80% of people with DM living in low and middle income countries. DM caused 4.6 million deaths in 2011. It is estimated that 439 million people will be suffer from T2DM by 2030. The incidence of T2DM ranges from one geographic area to another, due to lifestyle and environmental risk factors(Deshmukh and Jain, 2015). The literature has shown that there is little available data on type 2 diabetes prevalence in Africa as a whole. Study done in Africa showed a dramatic increase in the prevalence DM of rural and urban areas and both gender equally. The majority of the weight in Africa appears to be T2DM, to be less than 10% of cases of DM are T1DM (Ashwini , 2015).

According to the International Diabetes Federation (IDF), in 2013 approximately 50% of all people with diabetes reside in three countries: China (98.4 million), India (65.1 million) and the USA (24.4 million). Africa accounts a 22 million (5.1%) people with diabetes which is likely to increase by 70% in 2035. The ranges of prevalence between countries reflect the rapid socioeconomic and demographic transitions faced by communities throughout the Region. The highest prevalence of diabetes in the Africa Region is on the island of Reunion (15.4%), followed by Seychelles (12.1%), Gabon (10.7%) and Zimbabwe (9.7%). Some of Africa's most populous countries have the highest numbers of people with diabetes, including: Nigeria (3.9 million), South Africa (2.6 million), Ethiopia (1.9 million), and the United Republic of Tanzania (1.7 million) (Aseffa *et al.*, 2014).

Ethiopia is one of the top five countries with the highest number of people affected by diabetes mellitus in sub-Saharan Africa. The prevalence of diabetes mellitus is considerably higher among urban compared to the rural populations. Diabetes is largely undiagnosed and untreated in many populations in Ethiopia (Abebe *et al.*, 2014).

1.3. Classification of Diabetes Mellitus

Diabetes can be classified based on the etiology and clinical symptoms into the following general categories: T1DM (due to b-cell destruction, usually leading to absolute insulin deficiency), T2DM (due to a progressive insulin secretory defect on the background of insulin resistance, GD (diabetes diagnosed in the second or third trimester of pregnancy), Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes(such as neonatal diabetes and MODY), diseases of the exocrine pancreas (such as cystic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of HIV/AIDS or after organ transplantation) (American Diabetes Association, 2015).

1.3.1. Type 1 Diabetic mellitus

T1DM was previously called IDDM or juvenile-onset diabetes. Although disease onset can occur at any age, the peak age for diagnosis is in the mid-teens. T1DM develops when the cells that produce the hormone insulin, known as the beta cells, in the pancreas are destroyed. This destruction is initiated or mediated by the body's immune system and limits or completely eliminates the production and secretion of insulin, the hormone that is required to lower blood glucose levels. In adults, T1DM accounts for approximately 5 -10 % of all diagnosed cases of diabetes. There is no known way to prevent T1DM. The patients with T1DM rely on insulin medication for survival. Autoimmune, genetic and environmental factors are the major risk factors for T1DM (Control and Prevention, 2014).

1.3.2. Type 2 Diabetic mellitus

It was previously called NIDDM or adult-onset diabetes. In adults, T2DM accounts for about 90% to 95% of all diagnosed cases of diabetes. T2DM causes are usually multifactorial, because more than one diabetes cause is involved. It is primarily due to lifestyle factors, obesity, living a sedentary lifestyle, bad diet and genetics(Ijaola *et al*., 2014). NIDDM is multifunctional disease which is characterized by hyperglycemia and lipoprotein abnormalities. These traits are hypothesized to damage cell membrane, which results in excess generation of reactive oxygen species (ROS). NIDDM has also associated with an increased risk for developing premature

atherosclerosis due to an increased in triglycerides and low density lipoproteins (LDL), and decrease in high density lipoproteins (HDL) (Singh and Singh, 2010).

T2DM involves at least two primary pathogenic mechanisms: (I) a progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and inadequate suppression of glucagon secretion and (II) peripheral insulin resistance resulting in a decrease in the metabolic responses to insulin. It is widely recognized that both insulin secretion and insulin resistance are important elements in the pathogenesis of T2DM. The resulting insulin deficiency disrupts the regulation of glucose production in the liver and is a clue element in the pathogenesis of glucose intolerance (American Diabetes Association., 2010; Boada and Martínez-Moreno., 2013; Weyer *et al.*, 1999).

1.3.3. Gestational Diabetes mellitus

GD refers to the onset or initial recognition of glucose intolerance during pregnancy, usually in the second or third trimester. It occurs in about 4% of all pregnancies. Patients with GD have a 30% to 50% chance of developing DM. During pregnancy, the placenta and placental hormones create an insulin resistance that is most pronounced in the last trimester (Bastaki , 2005; Tripathi and Verma, 2014). It is temporary and fully treatable, but if untreated, may cause problems with the pregnancy such as high birth weight, fetal malformation and congenital heart disease. Fetal and neonatal risks associated with GD include congenital anomalies such as cardiac, central nervous system and skeletal malformations. In severe cases perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment (Mohammed *et a*l., 2013).

1.3.4. Other Types

In this type, there are causes of hyperglycemia, include genetic defects of the pancreatic ß cell or in insulin action pathways (insulin receptor mutations). Certain drugs like thiazides diuretics diazoxide, growth hormone and with some protease inhibitors (e.g. saquinavir), glucocorticoids, niacin, and a-interferon may also lead to DM (Bastaki, 2005).

1.4. Clinical Features Of Diabetic Mellitus

1.4.1. Pathophysiology of type 2 Diabetic mellitus

Various complex mechanisms with multifactorial pathways ultimately lead to DM. Although much is known about the mechanisms involved, there are still questions which need to be answered. The severity of the disease and the occurence of complications may vary from one individual to another. The interplay of genes, lifestyle, environment and oxidative stress may determine the course of the illness(Sakthiswary *et al*., 2014).

T2DM is characterized by insulin insensitivity as a result of insulin resistance, declining insulin production, and eventual pancreatic beta-cell failure. This leads to a decrease in glucose transport into the liver, muscle cells and fat cells. There is an increase in the breakdown of fat with hyperglycemia(Deshmukh and Jain , 2015). At a cellular level dysfunction of the mitochondria and endoplasmic reticulum (ER) were discovered to be partially responsible for the cell dysfunction and insulin resistance. ER stress can trigger mitochondrial dysfunction with subsequent apoptosis signaling via Ca2+- and reactive oxygen species dependent mechanisms (Sakthiswary *et al*., 2014).

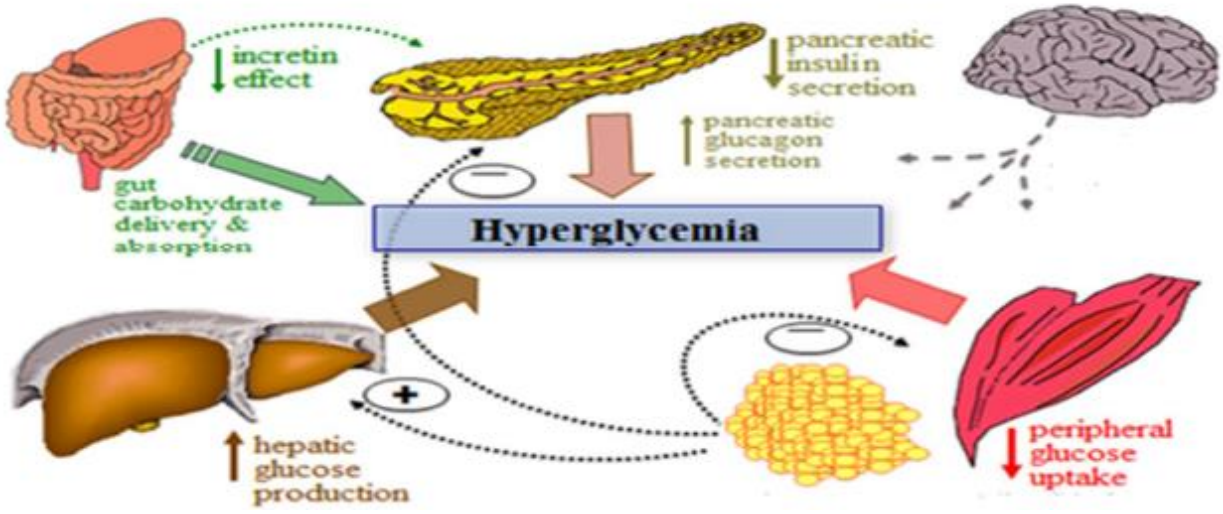


Figure 1: Pathophysiology of type two diabetes mellitus.

(Inzucchi et al., 2012) (↑) (Increase), (↓)-indicates decrease, (+) indicates activation and (-) indicates inhibition

ROS are constantly produced in the human body. Multiple studies have shown that the T2DM is accompanied by increased oxidative damage to all biomolecules in body. Diabetes produces disturbances of lipid profiles. An increased ROS has been observed in diabetic patients as indicated by high free radical production. There are several potential resources of free radical production in DM including autoxidation of plasma glucose, activation of leucocytes, and increased transition metal bioavailability (Rohilla and Ali, 2012; El-Desouki *et al*, *2015*).

1.4.2. Complications due to diabetes mellitus

Untreated diabetes of types all can cause many complications in many parts of the body. The general categories of chronic diabetic complications include macrovascular disease and microvascular disease (retinopathy, nephropathy and neuropathy). Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar. Serious long-term complications include heart disease, kidney failure and damage to the eyes (Sunil and Kumar, 2014; WHO, 2016).

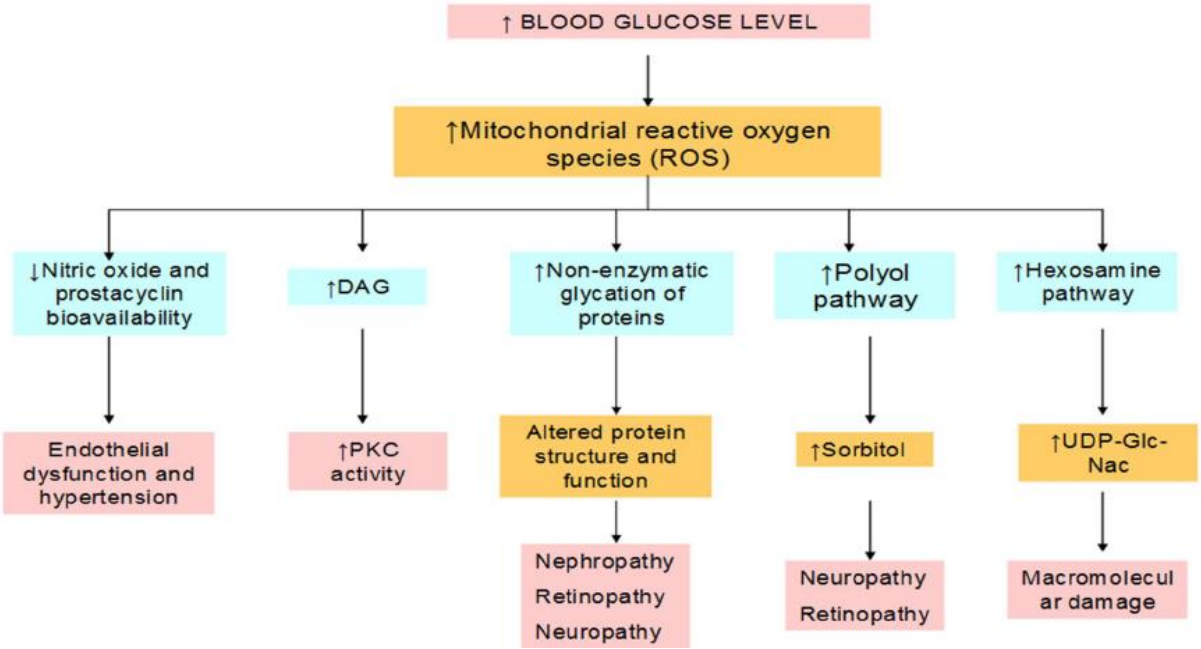


Figure 2: Metabolic pathways activated by chronically elevated blood glucose levels and long term complication of DM

DAG-Diacylblycerol, PKC- Protein kinase C, Glc-Glucosamine, UDP-Uridine diphosphate, Nac-N-Acetylglucosamine (Weiss and Sumpio, 2006).

In pregnancy, poorly controlled diabetes increases the risk of fetal death and other complications (El-Desouki *et al*, 2015). Oxidative stress has a great role in the development of complications of DM. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase; glutathione peroxidase, glutathione levels, and lipid peroxidation hyperglycemia in diabetes have been reported. There are many potential mechanisms whereby excess glucose metabolites traveling along these pathways might promote the development of complications of DM (Gupta *et al*., 2015).

1.4.3. Diagnosis of Diabetes Mellitus

Main diagnostic criteria of diabetes are elevated blood glucose level and presence or absence of symptoms such as polyuria, polydipsia and fatigueness, burring vision and weight loss, in association with glycosuria and ketonuria. The diagnosis of diabetes based on plasma glucose level may require continued observation with fasting or 2 hour post prandial blood glucose levels and an oral glucose tolerance test (OGTT) (Hemalatha *et al*, 2012). The WHO and the IDF have recommended If any one of the criteria listed in table1 is met, confirmation by repeat testing on a subsequent day is necessary to establish the diagnosis.

Table 1: Criteria for the diagnosis of diabetes.

Test	**Results**	**Interpretation**
HbA1c	6.5% or higher	Diabetes
	5.7–6.4%	Impaired glucose tolerance
	Lower than 5.7%	Normal
Random plasma glucose	200 mg/dL or higher	Diabetes
	140–199 mg/dL	Impaired glucose tolerance
	Lower than 140 mg/dL	Normal
Fasting plasma glucose	126 mg/dL or higher	Diabetes
	100–125 mg/dL	Impaired glucose tolerance
	Lower than 100 mg/dL	Normal

(Madhu and Srivastava, 2015; American Diabetic association, 2016)

1.5. The Role of Alloxan in Type 2 Diabetes Mellitus

The most common chemicals to induce diabetes in the animal model are alloxan and streptozotocin. Alloxan is the most commonly used chemical for induction of diabetes mellitus. It is a well- known diabetogenic agent. Alloxan is a toxic glucose analogue (Sharma *et al*., 2010) urea derivative, which causes selective necrosis of the pancreatic islet β-cells. It is used experimentally to induce T2DM in animals such as rabbits, rats, mice and dogs. The experimental dose of the drug needs careful consideration in order to avoid excessive pancreatic tissue damage (Matheka *et al*., 2012).

Using alloxan, it is possible to produce different grades of severity of the disease by varying the dose of alloxan used: these may be classified by measuring fasting blood sugar level. Moderate diabetic animals are recommended for use in testing drugs for use in T2DM. For mice and rats a single dose of alloxan, 140 –180 mg/kg is administered intraperitoneally (Etuk, 2010) to induce T2DM. Thus alloxan induced DM served as a pathological for testing a substance with supposed antioxidant activities *in vivo.*

1.6. Management and control of DM

Primary prevention is the main aim at preventing diabetes from occurring in susceptible individuals or in general population. Regular physical activity, dietary and lifestyle modifications are an important component of the prevention and management of T2DM. The common strategy for treatment focused mainly on regulating and decreasing blood sugar to fall within the normal level. The main mechanisms involved in decrease blood sugar are stimulating pancreatic β-cells; inhibiting other hormones elevating blood sugar; increasing the affinity, and sensitivity of insulin receptor. On the other hand, lowering glycogen release; enhancing glucose utilization within many tissues and organs; clearing free radicals, fighting lipid peroxidation, correction of the lipid and protein metabolic disorders and improving human blood circulation are also involved(Singab *et al*., 2014). Another aims of DM therapies are to prevent long-term diabetic complications by eliminating various risk factors to increase longevity (Bastaki, 2005).

Pharmacological therapy is aimed at maintaining the glycemic and reducing the long term complications of Diabetes. The two broad categories of pharmacological approach to DM are insulin therapy and oral hypoglycaemic agents. Oral antidiabetic agents include sulphonylurea, biguanides, alpha glucosidase inhibitors and thiazolidenediones(Madhu and Srivastava, 2015).

1.6.1. Insulin

Insulin is a hormone produced by beta cells of the islets of Langerhans in the pancreas of animals, humans' and synthetically. It has an AB heterodimeric structure with one intrachain and two interchain disulfide bridges (Mohammed *et al*., 2013). Insulin is the mainstay of treatment for patients with T1DM and it is also important in T2DM when blood glucose levels cannot be controlled by diet, weight loss, exercise, and oral medications (Sheeja *et al*., 2010). Insulin therapy is aimed to mimic nature, which is remarkably successful both in limiting postprandial hyperglycemia and preventing hypoglycemia between meals. Insulin therapy is no free from complications and adverse effects. Weight gain and hypoglycemia are the major adverse effect of insulin (Deshmukh and Jain , 2015).

Various substitutions on the insulin molecule and other modifications have led to multiple types of insulin. These are characterized and administered based on their pharmacodynamics and pharmacokinetic characteristics like onset, peak, and duration of action (Mohammed *et al*., 2013). On the basis of their action profile, insulins can be divided into short, intermediate and long acting. Short acting insulin is used to control blood glucose level after meals, since it has a quick onset (30 minutes), peaks 2-3 hours after injection and has a short duration of action. Intermediate acting insulins consist of regular insulin modified by adding zinc or basic protein (NPH). These are used primarily to provide basal insulin replacement. These insulins have duration of action of 12-14 hours and usually need to be given twice in a day to provide cover for the entire 24 hours. Human long-acting insulin has duration of action of approximately 18-20 hours, but its absorption is highly variable (Bhatia and Aggarwal , 2007; Sheeja et al., 2010)

1.6.2. Oral antidiabetic agents

Four categories of oral antidiabetic agents are available namely; insulin secretagogues, biguanides, thiazolidinediones and alpha-glucosidase inhibitors and also additional recently introduced into market and some others which are under clinical trial at different stages.

A. Insulin secretagogues: sulfonylureas

Sulfonylureas are one of the most widely used classes of oral hypoglycemic agents. Sulfonylureas act by increasing insulin release from the pancreas beta cells by binding to the high-affinity plasma membrane receptor coupled to a beta cell inward rectifier-type Adenosine tri-phosphate (ATP-dependent K+ channel). The binding of a sulfonylurea inhibits the efflux of potassium ions through the channel and results in depolarizes the plasma membrane, leading to an opening of voltage-gated calcium channels. Calcium influx and a corresponding increase in intracellular calcium levels, causes release of insulin from the beta-cell. Depolarization, in turn, opens a voltage-gated calcium channel that results in a calcium influx and the release of insulin (Topi, 2014).

Sulfonylurea drugs are conventionally divided into first and second generation agents, which differ primarily in their potency. First-generation agents (chlorpropamide, tolazamide, and tolbutamide) and second-generation agents (glibenclamide glimepiride, glipizide, glyburide). The first generation agents have longer half-lives, increased incidence of hypoglycemia, and more drug interactions. The second generation agents have quicker onsets of action, shorter half-lives, and lower incidence of hypoglycemia(Oderda *et al*., 2013). These drugs are still a popular choice for first-line therapy in a T2DM patient who has failed on non-pharmacological measures and is non-obese. They can be used in combination with other classes of antidiabetic drugs except other secretagogues (including the meglitinides). They can also be used in combination with longer-acting insulin as part of the day time sulphonylurea- night-time-insulin regimen (Bösenberg and G. van Zyl D., 2008).

Glibenclamide is a potent anti-diabetic and it improves glucose control by acting both on insulin secretion and insulin action. The drug inhibits ATP sensitive K+ channels in pancreatic beta cells. 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 (Girani *et al*., 2016**).** Glibenclamide has also role on insulin action at the level of different organ/tissues and it has action at the liver, skeletal muscle, heart muscle and smooth muscle sites. In liver the drug has a positive action on glycogen deposition with direct action on the synthesis of glucose transport 2 proteins (Moore, 2007).

B. Insulin secretagogues: Meglitinides

Repaglinide and nateglinide are non-sulfonylurea secretagogues which act on the ATP dependent K-channel in the pancreatic beta cells thereby stimulating the release of insulin from the beta cells, similar to sulfonylurea, though the binding site is different. Meglitinides have a rapid onset and a short duration of action (4-6 hrs.) and thus lower risk of hypoglycemia. Repaglinide is mainly metabolized in the liver with very minimal amounts excreted via the kidneys and thus dose adjustment is not necessary in patients with renal insufficiency except those with end-stage renal disease (Olokoba *et al.*, 2012).

C. Biguanides

Metformin is one of the most commonly used of biguanides, which is used in overweight and obese patients. Even though the molecular mechanisms of action have not as yet been clearly established it is thought that it suppresses hepatic glucose production, primarily by decreasing gluconeogenesis, as a lesser effect, it increase glucose uptake by skeletal muscles, increases insulin sensitivity, enhances glucose uptake by phosphorylation GLUT-enhancer factor, increases fatty acid oxidation, and decreases the absorption of glucose from the gastrointestinal tract(Olokoba *et al*., 2012)

D. Thiazolidinediones

Thiazolidinediones is a recently introduced class of oral antidiabetic drug that enhances target tissue insulin sensitivity. Pioglitazone and Rosiglitazone are the two approved thiazolidinediones for T2DM. They act by binding to the peroxisome proliferative insulin activated receptors enhancing Sensitizing effects of insulin at liver, muscle as well as fat tissues also by inhibiting glucose formation by liver (Cheng and Fantus, 2005; Ibrahim, 2010).

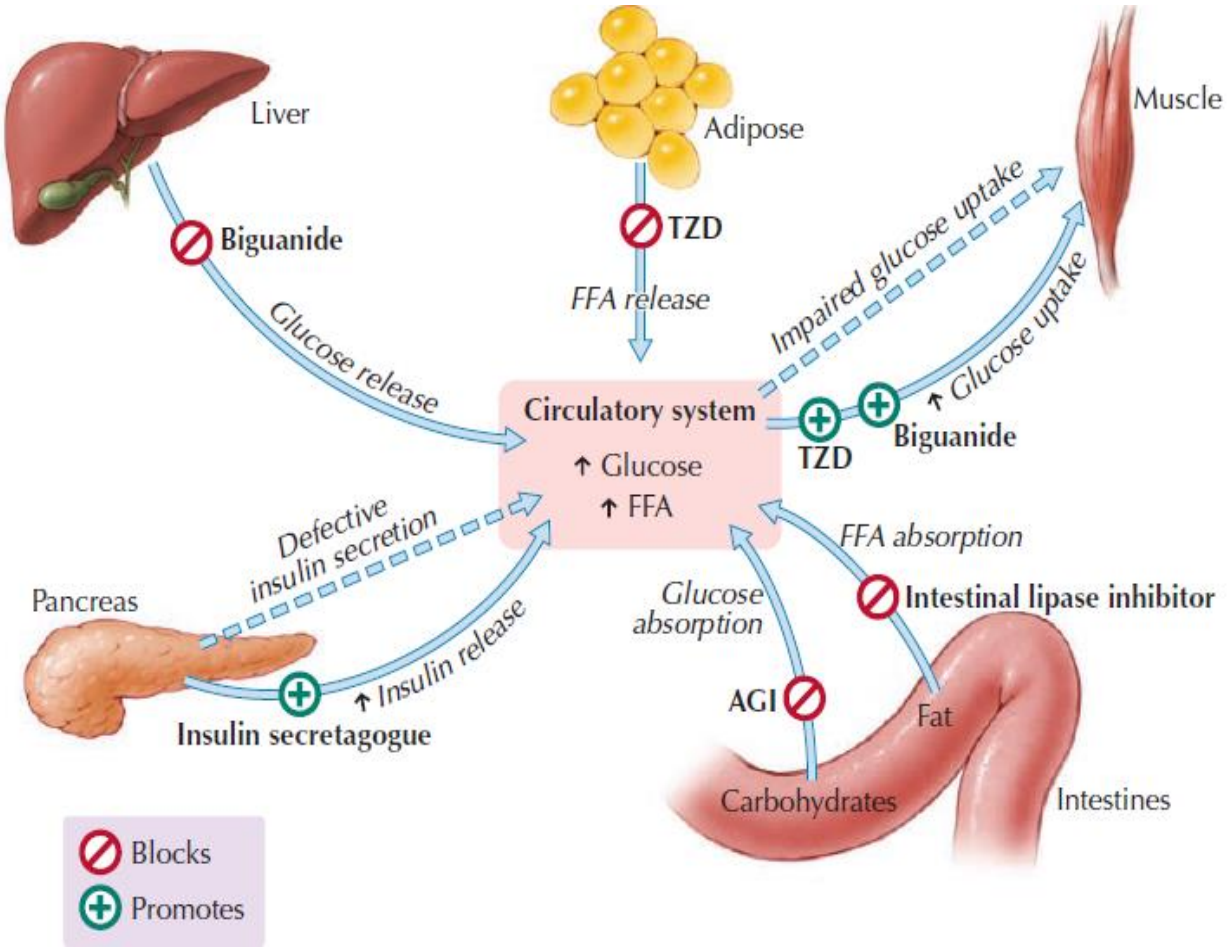
The pharmacokinetics of these drugs indicates that both rosiglitazone and pioglitazone are rapidly absorbed after a meal, reaching peak concentrations within 1-2hrs. Both drugs undergo hepatic metabolism, with rosiglitazone excreted mainly in urine and pioglitazone in bile. Although rosiglitazone and pioglitazone are metabolized by cytochrome p450, no major drug interactions have been reported (Bösenberg and G. van Zyl D., 2008). Adverse effects associated with this class are hepatotoxicity (Ibrahim, 2010).

E. Alpha-glucosidase inhibitors

Acarbose and miglitol are the two agents available in this class. Alpha-glucosidase inhibitors act by inhibiting the enzymes, pancreatic alpha-amylase and alpha-glucosidase, found in the brush border cells that line the small intestine. These agents slow down the digestion of starch in the small intestines, so that glucose from starchy meal enters the blood stream more slowly, and can be matched more effectively by an impaired insulin response or insensitivity. It is very effective in the treatment of T2DM (Mohammed *et al*., 2013).

F. Dipeptidyl-Peptidase IV Inhibitor

Dipeptidyl-peptidase (DPP) IV inhibitors inhibit dipeptidyl peptidase-4 (DPP-4), a ubiquitous enzyme that rapidly inactivates both Glucagon-Like Peptide 1(GLP-1) and Glucose-dependent Insulinotropic Peptide (GIP), increase active levels of these hormones and, this process leads to an increase in the release of insulin and a decrease in the levels of glucagon in the blood (Rajput, 2009) in doing so, improves islet function and glycemic control in T2DM. Four already approved DPP-4 inhibitors are: Sitagliptin, vildagliptin, saxagliptin and alogliptin. Although they differ in terms of their chemistry, they are all small molecules which are orally available. They are different in terms of their absorption, distribution, metabolism and elimination, as well as in their potency and duration of action, but their efficacy, both in terms of inhibiting plasma DPP-4 activity and as antidiabetic agents, appears to be similar. They improve glycemic control, reducing both fasting and postprandial glucose levels, without weight gain and with an apparently benign adverse event profile (Deacon, 2011).



.

Figure 3: Major target organs and actions of orally administered antihyperglycemic agents in type 2 diabetes mellitus.

TZD = thiazolidinedione; FFA = free fatty acid; AGI = alpha-glucosidase inhibitor (Cheng and Fantus, 2005).

The treatment of diabetes with synthetic drugs is generally not preferred because of these drugs are not adequately effective in glycemic controlling of most patients, with genetic polymorphism of drug metabolizing enzymes, its high cost and side effects such as severe hypoglycemia, lactic acidosis, peripheral edema and abdominal discomfort). For this reason, it is necessary to develop alternative medicine (García Mesa, 2014). Therefore searching for new antidiabetic more effective agents with fewer side effects is a scientific challenge.

1.7. Use of Medicinal Plants

Natural products play a key role in the discovery and development of new drugs. About 25% of prescribed medicines are plant derivatives and about 80% of the world's population relies on herbal medicines as a folk medicine and alternative remedies to cure different ailments and diseases (Qamar and Qureshi, 2013). Interest in traditional medicine can be explained by the fact that it is a fundamental part of the culture of the people who use it and also due to the economic challenge: on one side, the modern drugs are not accessible to the poor and on the other side, the richness and diversity of the fauna and flora of Africa are an inexhaustible source of therapies of ailments (Mahomoodally, 2013). During the last two decades, traditional systems of medicine and medicinal plant research have become topics of global interest and importance. In many developing countries of the world, large number of peoples still relies heavily on traditional healers and medicinal plants to meet their daily primary health-care needs. Indeed, traditional medicine systems that use medicinal plants are part of the national health care system in several countries, including China, Gambia, India, Ethiopia, Zambia, Cameroon, Ghana, Congo, and Nigeria, among other (Saganuwan, 2009).

In Ethiopia, about 80% of populations relied on traditional medicine due to the cultural acceptability, relatively low cost of traditional medicine and limited access to modern health facilities. In addition to this, the government sturdily supports and encourages traditional medicine through its policies despite sustainable use of traditional medicine and their integration with modern medical practice has been limited (Kassaye *et al*., 2006).

1.7.1. Role of Medicinal Plants in Diabetic Mellitus

Traditional Medicine derived from plants play a major role in the management of DM. WHO has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less side effects and considered to be excellent candidates for oral therapy (Rupeshkumar *et al*., 2014). Recent scientific investigation has confirmed the efficacy of many antidiabetic plant preparations. As an alternative approach, medicinal herbs with antihyperglycemic activities are increasingly sought by diabetic patients and health care

professionals. Commonly used herbs, less likely to have the side effects of conventional approaches for T2DM, have been exploited (Piero *et al*., 2012).

Ethnobotanical and ethno pharmacological surveys report that more than 1200 plants are being used in many ethnic societies around the world in traditional medicine for their alleged hypoglycemic activity (Fraser *et al.*, 2007) and *T. schimperi* is one of such plants (Enyew *et al*., 2014). The seeds of *Trigonella foenum-graecum* (family: Fabaceae), *Syzygium guineense* (family: Myrtaceae) and *Glinus lotoides* (family: Aizoceae), the leaves of *Rubus apetalous* (family: Rosaceae), *Moringa stenopetala(*Moringaceae), *Rubus pinnatus* (family: Rosaceae), *Thymus schimperi* (family: Lamiaceae), *Ajuga integrifolia* (family: Lamiaceae) and *Otostegia integrifolia* (family: Lamiaceae), the roots of *Rumex abyssinicus* (family: Polygonaceae) and *Verbena officinalis* (family: Verbenaceae) are some of the medicinal plants used by Ethiopian folkloric medicine for the treatment of diabetes (Abate, 1998).

The main active constituents derived from medicinal plants which have antidiabetic activity include alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycan, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. These affect various metabolic cascades, which directly or indirectly affect the level of blood glucose in the human body (Prabhakar and Doble, 2011).

Plants may act on blood glucose through different mechanisms including adrenomimeticism, pancreatic beta cell potassium channel blocking, secondary messenger stimulation; providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the beta-cells; Inhibition of β-galactocidase and α-glucocidase; preventing oxidative stress that is possibly involved in pancreatic β-cell dysfunction found in diabetes (Jarald *et al.*, 2008); Stimulation of glycogenesis, glycolysis and citric acid cycle and hexose monophosphate shunt; inhibition of gluconeogenesis and glycogenolysis (Khanavi *et al.*, 2012), facilitating insulin's activity, acting as potential insulin-like substances, inhibiting insulinase activity and increasing the quality and/or quantity of the β- cells in the pancreas by enhancing regeneration of these cells. The fiber of plants may also interfere with carbohydrate absorption; thus affecting blood glucose(Hashemnia *et al*, 2012).

1.7.2. *Thymus schimperi*

The genus *Thymus (*Lamiaceae) is one of the eight most important genera as regards number of species within the Lamiaceae family. This family has about 220 genera and more than 350 specie (Morales, 1997*)*. The genus *Thymus* (Lamiaceae) is widely distributed in temperate zones. Among the various species, *T.schimperi is* indigenous to Ethiopia locally known as "*Tosign*". *T. schimperi* is a common spicy flavoring agent used since ancient times. The leaves are opposite, grayish-green, entire, linear or elliptic, up to 15 mm long. The flowers are small, pale-purple or white, arranged in terminal inflorescences that may be dense or loose. Flowers appear since the beginning of summer until the end of autumn. *T. Schimperi is* growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes *(*Demsew, 1993*)*.

In Ethiopian *T. Schimperi* has many medicinal applications. The leaves of *Thymus* are used in Ethiopia as spices to flavor a wide range of food products as well as medicines *(*Ermias *et al.*, 1998). The essential oil known as thyme oil is used in the food flavoring and preservatives, perfumery and pharmaceutical industries. Analysis of the essential oils from different species indicated the presence of different components mainly the high phenolic monoterpenes such as carvacrol, thymol and α- terpineol and solvent extracts contain many phenolic acids such as rosmarinic, ferulic, caffeic, chlorogenic and p-coumaric acids and also different flavonoids. The essential oils exhibited strong antioxidant (Dessalegn *et al*, 2015).

T. schimperi is recommended for a myriad of indication based upon its antimicrobial, antitussive, and spasmolytic and antioxidant activity (Teshale *et al.*, 2013*).* The dried leaves of *T. schimperi* are also used in traditional medicine for the treatment of headache, gonorrhea, inflammation, spasm, thrombosis, urinary retention, mental illness, eye disease, toothache, stomachache, earache, liver disease, gonorrhea, leprosy, lung TB, acne and ascaris *(*Demsew, 1993; Haile K, 2013)

Some of pharmacological studies on extracts of different parts of *T. schimperi* done includes, the antioxidant activity and preservative effect (Gebrehana and Shimelis, 2013), antimicrobial (Nasir *et al*., 2015), diuretic and antihypertensive activity of leaf extracts (Haji *et al.*, 2016) and antioxidant and α-amylase inhibition activities (Dessalegn *et al.*, 2015). Scientific reports on the

phytochemical constituents of different plants of the plant revealed the presence of, tannins, phenols (Haji *et al.*, 2016) phenols and flavonoids (Dessalegn *et al.*, 2015).

In Oromia region, North Shoa Zone, *T.* schimperi leaves has been used in folk medicine for the management of diabetes mellitus (Enyew *et al.*, 2014) and based on a claim the present study is initiated to assess the hypoglycemic and antidiabetic activity of crude leaf extract of *T. schimperi* in alloxan diabetic mice (acute study and sub-acute).

1.8. Significance of the study

The finding of this study could scientifically validate its claimed use by the traditional practitioners. Beside, this work will be helpful in devising experiments aimed at validating the effective use of *T.schimperi*. Results against tested diseases provided an avenue for further studies to validate the use of the plant against the disease. Finally, results of the current work will also save as a template for further research on the use of *T.schimperi.*

1.9. Statement of Problem

DM is one of the most common endocrine-metabolic disorders worldwide. It affects millions of people and has an incidence that is increasing at a striking rate. Diabetes is considered as an alarming global health problem due to the high rate of morbidity and mortality Moreover; uncontrolled diabetes leads too many chronic complications such as blindness, heart failure, and renal failure (Kabbaoui *et al.*, 2016).

DM is a growing problem worldwide entailing enormous financial burden and medical care policy issues (Joshi *et al.*, 2015). In 2002, the estimated cost of treating diabetes and its complications, both in terms of direct (medical) and indirect (disability, work loss, death, etc.) costs, was estimated at $132 billion. The direct medical costs of diabetes more than doubled in five years from $44 billion in 1997 to $92 billion in 2002. Treatment costs for people with diabetes are more than double those for people without diabetes, mainly because of the high costs associated with complications (NDEP, 2007).

Even though no population based prevalence study exists, Ethiopia is one of the countries with the highest number of people affected by diabetes mellitus in sub-Saharan Africa. Ethiopia has

an alarming problem with the prevalence diabetes trending upwards. According to International Diabetes Federation (IDF) estimation, 3.5 % of Ethiopians had diabetes in 2011 and by 2030 there will be almost 2 million diabetics in Ethiopia (Abebe *et al.*, 2014).

Due to lack of organized health care systems in developing countries, people with chronic diseases like diabetes are among the worst sufferers in their communities today. Hence, majority of the populations still have limited access or no access, especially those in remote areas, to modern medicines. Instead they use traditional medicines for a range of diabetic complications. Different species of medicinal plants are used in the treatment of diabetes mellitus (Kumar *et al,* 2015).

The treatment of diabetes mellitus is considered as the main global problem and successful treatment has yet to be discovered. The available treatments for DM have their own drawbacks ranging from development of resistance and adverse effects to lack of responsiveness in a large segment of patient population Eventhough insulin therapy and oral hypoglycemic agents are the first line treatment for diabetes mellitus they have some side effect and fail to significantly alter the course of diabetic complication (Hemalatha *et al.*, 2012). Due to the heterogeneity of T2DM, current therapies are limited and unable to modify the natural history of this disorder (Hawthorne *et al.*, 2006). The limitation of currently available oral anti-diabetic agents either in terms of efficacy/safety coupled with the emergence of the disease into global epidemic have encouraged alternative therapy that can manage diabetes more efficiently and safely. Therefore it is necessary to explore new antidiabetic therapies. Recently, there has been a resurgent interest in the herbal treatment of diabetes. The growing public interest and awareness of natural medicines have led the pharmaceutical industry and academic researchers to pay more attention to medicinal plants (Erasto *et al*., 2005).

1.10. Hypotheses

Although *T.schimperi* is widely used in many communities for treatment of various diseases, as mentioned in the general introduction section, there is no scientific evidence that justifies the claimed uses for DM. This work was therefore undertaken to investigate the biological activities of the plant against DM and to establish the ethno-botanical use of *T. schimperi*. To achieve these, the study was conducted with the following objectives.

2. OBJECTIVES

2.1. General Objective

This study is intended to evaluate the effect of crude aqueous and 80% methanol extract of *T.schimperi* on blood glucose level in laboratory mice.

2.2. Specific objectives

- To evaluate hypoglycemic effect of aqueous and methanol crude extracts of *T.schimperi* leaves on Normoglycemic mice.
- To investigate the effect of the crude extracts of the *T. schimperi* on OGTT.
- To evaluate antihyperglycemic effect of aqueous and methanol crude extracts of *T.schimperi* leaves on alloxan-induced mice.
- To determine the effect of *T.schimperi* leaves on body weight.
- To detect the presence/absence of some secondary metabolites in the extracts of the study plants using chemical tests.
- To determine acute toxicity of the crude extract of *T. schimperi*.

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals and Instruments

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

Instruments: Whatman filter paper (Number 1) (Maidstone-, UK), rotary evaporator (Buchii model R-200, Switzerland), lyophilizer (Operan, Korea vacuum limited, Korea)test tube, volumetric flask (5 L), beakers (800 mL), Erlenmeyer flasks (400 mL), measuring cylinder (1000 mL), spatula, magnetic stirrer, test tubes, semi-automatic pipettes, gavages (oral feeding syringe), Syringe (1 mL, 3 mL), desiccators, heater, refrigerator, sensitive digital weighing balance(Mettler Toledo, Switzerland), deep freezer, one touch basic glucometer (Prodigy Autocode blood glucose monitoring system, Taiwan), strip (Prodigy blood glucose test strip, Taiwan) and mice cages.

Reagents and drugs: Methanol absolute (Blulux, India, Purchased from ZAF pharmaceuticals Pvt. Ltd. Co.), 5% and 30% of glucose solution, Tweens 80% (BDH Laboratory Supplies Poole, BH151TD lot ZA2088516, England),, sulfuric acid (Park scientific Ltd, Lot 8114/10, UK), Acetic anhydride (Techno Pharmchem, India), Ferric chloride(Hopkin and Williams Ltd, England), potassium iodide BP (Evans medical Ltd, England), Wagner's reagent, Lead acetate, Acetic anhydride, chloroform, normal saline (IV infusion BP Medsol pharmaceuticals) were all obtained from Ethiopian public health institute. Glibenclamide was purchased from a local drug store and Alloxan (Sigma Chemical Company) obtained from school of medicine department of pharmacology.

3.1.2. Collections and preparation of plant materials

Fresh leaves of *T. schimperi* were collected from Sululta, one of the woredas in the Oromia Region of Ethiopia located at 910'59.988"N and 3845'0.000"E and about 18 kilometers north of the capital city, Addis Ababa, Ethiopia in December 2015. After collection, the plant materials were identified and authenticated by a taxonomist at Ethiopian Public Health Institute (EPHI) and deposited with voucher specimen HH-001 in the herbarium for future reference. Fresh leaves

were cleaned from extraneous materials, dried under shade at room temperature, and grinded by manual crusher to obtain fine powder particles.

Figure 4: Leaf of *Thymus Schimperi* Collected From Suluta in December 2015.

3.1.3. Experimental animals and Study Protocol

Swiss albino mice weighing 20-30g, 6weeks-9weeks, of both sexes were obtained from EPHI, Addis Ababa. 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 Medical pharmacology Research and Ethics Review Committee. After randomization in to various groups and before initiation of experiment, the mice were acclimatized to animal house conditions for a period of 7 days before experimentation to minimize effects of environment-induced physiological, cardiovascular, immune central nervous and endocrine systems changes due to stress associated with transportation (Obernier and Baldwin, 2006). Mice were housed in groups of six in polypropylene cages, lined soft wood shavings as bedding (renewed every 24 h), with natural night-daytime exposure and at room temperature. Before and during the experiment, the mice were allowed free access to standard mice pellets, made from ground animal food and tap water regularly. All the animal experiments were conducted at EPHI.

3.2. Methods

3.2.1. Preparation of crude extract

Six hundred gram powders of *T. schimperi* leaves macerated with 1000 ml of 80% methanol for 72 hrs in an Erlenmeyer conical flask with frequent agitation using mini orbital shaker adjusting at 170 revolutions per minute for 90 minute at room temperature. First extract was filtered using folded gauze and nylon clothe. Then, the extract was filtered through Watmann filter paper No.1 using pressurized suction filtration system and the marc was remacerated twice using the same volume of methanol to exhaustively extract the plant material. Then filtrates from each extraction were combined and methanol was removed from the extract by evaporation under vacuum using rota vapor at 40^{0}c. Then the filtrates were frozen overnight using deep freezer and set water free by using Lyophilizer. The yield obtained then kept in refrigerator at 2-8 degree centigrade and fresh stock solution was prepared for the experiment whenever required.

The aqueous extract was prepared by maceration of the powdered leaves (400g) by using distilled water for 72 hrs. in an Erlenmeyer conical flask with frequent agitation using mini orbital shaker adjusting at 170 revolutions per minute for 90 minute at room temperature. First extract was filtered using folded gauze and nylon clothe. Then, the extract was filtered through Watmann filter paper No.1 using pressurized suction filtration system and the marc was remacerated twice using the same volume of methanol to exhaustively extract the plant material. Then filtrates from each extraction were combined. Then the filtrates were frozen overnight using deep freezer and set water free by using Lyophilizer and the lyophilized product was collected and kept in desiccators until used for the experiment.

3.2.2. Grouping and dosing

The animals were randomly assigned into different groups of six animals per group to perform hypoglycemic and antihyperglycemic activity test. Dose selection was based on, acute toxicity study and previous study of the crude aqueous extract, (Haji H *et al*, 2016) as well as pilot experiments.

3.2.3. Administration of Extracts to Normoglycemic Mice

Mice were divided into six groups (four test groups and two control groups), each group comprising a minimum of six mice. The mice were starved overnight prior to performing the experiment. The positive control group received glibenclamide, while the negative control group received distilled water, and the test groups were administered aqueous extracts of 250mg/kg and 500mg/kg; and ethanol extract of 250mg/kg and 500mg/kg of *T. schimperi were* administered orally by using gavage. The effects of the plant extract were compared with the control groups. Blood sample from the control and test animals was collected after at 0, 1, 3, 4 and 6 hrs following glibenclamide, extracts and vehicle administration. Blood glucose level was measured using glucometer on blood drawn from the tail of the mice.

3.2.4. Oral Glucose Tolerance Test (OGTT)

OGTT were carried out as per the procedure previously described by (Lanjhiyana, 2011). Fasted mice were grouped into seven groups of six mice each. Then group 1 received normal saline, 10 ml/kg and served as normal control Group 2 received 2g/kg of glucose (negative control); Group 3 received glibenclamide, 0.66 mg/kg and served as positive control. Groups 4 and 5 received aqueous leaf extracts of *T.schimperi* at doses of 250 and 500 mg/kg body weight, 6 and 7 given 80% methanol leaf extracts at doses of 250 and 500 mg/kg body weight. All substances were orally administered. Thereafter, following 30 min post extract administration all the animals were fed with glucose (2 g/kg) except normal control group. Blood samples were collected from tail vein prior to dosing and 30, 60 and 120 minutes after administration of glucose in order to evaluate their blood glucose level. The blood glucose level was analyzed using glucose-oxidase-peroxide reactive strips.

3.2.5. Induction of Experimental Diabetes

Swiss albino mice of both sex were fasted overnight and their weight and fasting blood glucose level was recorded. Alloxan was first weighed individually for each animal according to their weight & then solubilized with 0.9% w/v normal saline just prior to injection Mice were then made diabetic by a single intraperitoneal injection of 180 mg/kg body weight of alloxan monohydrate solution to overnight fasted mice. Food and water were presented to the animals 30

minutes after drug administration (Nagappa *et al*., 2003). Besides, to prevent hypoglycemic shock and mortalities during hypoglycemic phase, oral solution of 10% glucose in tap water was provided via water bottle for next 24 hrs. Seven days after alloxan injection, plasma blood glucose level of each animal was determined and animals with a fasting blood glucose range above 200 mg/dl (Gidado *et al*., 2005) were included in the study. The blood samples were collected from the tail of the mice.

3.2.6. Administration of extracts to Alloxan-induced diabetic mice

Alloxan-induced diabetic mice were administered orally 250mg/kg and 500mg/kg of the hydroalcoholic and aqueous extracts of *T.schimperi* for test groups; 0.66mg/kg glibenclamide for positive control group; and distilled water (10ml/kg) for negative control group. There was also normal control group that received distilled water (10ml/kg) orally. Extract, standard drug and water were administered once daily for 21 days. Blood glucose level was measured using glucometer blood drawn from the tail of the mice. In the acute experiments, for blood glucose measurement, blood samples were collected from the tail vein at 0, 2 and 4 h post gavage of the plant extract. Blood glucose was measured using with glucometer. In the chronic experiments, blood glucose was measured at weekly intervals till end of the study (3 weeks). The animal fasting glucose levels were estimated on day 1, 7, 14 and 21. Percent reduction of blood glucose level was calculated using the following formula:

$(G0 - G21)/G0$, Where G0 is blood glucose level at day 0; G21 is blood glucose level at day 21.

3.2.7. Determination of Body Weight

Body weight of all the treated groups and control group of mice were recorded before treatment (on day 0) and during treatment period i.e. 7th, 14^{th} and 21^{st} day. A properly calibrated and standardized electronic balance was used for taking body weight of the mice and expressed as gram (g).

3.2.8. Phytochemical Analysis

Preliminary phytochemical qualitative analysis was carried out on the extract of aqueous and methanol using standard phytochemical reagents and procedures as described by (Trease and Evans, 1983; Harborne, 1988; Yadav. and Agarwala M., 2011) to detect the either presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, and tannins.

A. Test for alkaloids

Ten mg of each of the crude extracts of both solvent were dissolved in 1ml of distil water. With this solution three drops of Wagner's reagent(solution prepared by dissolving 2 gm of Iodine in a solution of 6 gm of potassium iodide in 100ml distill water) was added. The presence of alkaloids was confirmed by the formation of reddish brown colored solution.

B. Detection for Phenols

Ferric Chloride Test: Ten mg of each of the extracts was dissolved in 1ml of water. 0.5 ml of 5% ferric chloride solution was added to it and development of deep blue or black colour was taken as an indicator for the presence of phenols.

C. Detection for Tannins

Ferric Chloride Test: About 0.5 g of the dried powdered was dissolved in 1ml of distil water was treated with 3-4 drops of 5% ferric chloride and formation of brownish green or a blue black colouration indicates the presence of tannins.

*Lead acetate test***:** Ten mg of each of the extracts was dissolved in 1ml of distil water. Then 0.5 ml 0f 1% lead acetate was added to it. Formation of yellowish precipitate was observed for the presence of tannins.

D. Detection for Flavonoids (*Lead acetate Test)*

About 0.3gm of each of the crude extracts was dissolved in 2ml of distil water To this 3 drops of 10% lead acetate was added and formation of yellow precipitate was observed for the presence of flavonoids.

E. Detection for Saponins (*Foam test*)

About 0.5mg of each of the crude extract were taken and dissolved in 2ml of distilled water and shaken vigorously for 15 min. The formation of foam to length 1cm it indicates the presence of saponins.

F. Test for steroids (*Liebermann-Burchard test).*

About one half gram (0.5 g) of each of the crude extracts was dissolved in 0.5mL dichloromethane to produce a dilute solution. To this solution 0.5 mL of acetic anhydride was added, followed by three drops of concentrated sulphuric acid. Formation of a blue-green colouration indicated the presence of steroids.

3.2.9. **Acute toxicity test**

Acute oral toxicity of the extract of *T. schimperi* leaves was evaluated in female Swiss albino mice (25-30 g), as per OECD guideline (Organization for Economic Co-operation and Development, Guideline-425, adopted on 3rd October, 2008). The mice were fasted overnight and the weight of each mouse was recorded just before use. Animals were divided randomly into a control and two treatment groups, each group consisting of five female mice. Control group received only the vehicle and each treatment group were administered orally a single dose of 2000 mg/kg body weight of *T.schimperi* leaves of 80% methanol and aqueous extract of maceration methods. Mice were closely observed for the initial 4 h after the administrations, and then once daily during the following days. The behavioral changes closely observed for were: hyperactivity, ataxia, tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma (Shah, 1997; Bürger *et al*., 2005), and then they were observed daily for 14 days for any change in general behavior, other physical activities and/or possible mortality of the mice.

3.2.10. Ethical considerations

The experiment was carried out in accordance with ethical principles and the proposal was approved by Addis Ababa University, college of health sciences, faculty of medicine, Department of pharmacology.

3.2.11. Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). Differences between means of all parameters were carried out by using analysis of variance (ANOVA). Subsequently, the Tukey post-hoc tests with multiple comparisons were used to determine the source of significant differences. $P < 0.05$ was considered to be statistically significant (*). SPSS Version 20 Software was used for statistical analysis. Both Extract treated groups were compared to positive control, negative control and standard control.

4. RESULTS AND DISCUSSION

4.1. Results

The present study was conducted to evaluate the hypoglycemic and anti-hyperglycemic activity of methanol 80% and aqueous extract of *T. schimperi* leaf in alloxan induced diabetes mice and the results were presented as follows.

4.1.1. Percent yield of plant extracts

In the preparation of crude aqueous extract from the dried leave of *T. schimperi* a yield 10.7% was obtained. In the case of crude 80% methanol extract preparation, a yield of 13.2% of *T. schimperi* was obtained.

4.1.2. Hypoglycemic Effect of *T. Schimperi* Crude Extract on Normoglycemic Mice

The effect of treatment with *T.schimperi* crude extract on the blood glucose level in normal fasted mice is shown in Figure 5. In normoglycemic mice, aqueous and 80% methanol leaves extract at two doses i.e. 250 and 500mg/kg orally did not significantly reduce the plasma glucose in normal mice. However, the mice treated with Glibenclamide showed a significant reduction in blood glucose levels when compared with untreated group. The lowest dose taken i.e. 250mg/kg body weight of aqueous extract showed suppressing glucose blood glucose by 26.71% after 6 hrs of extract administrations, while the dose of 500mg/kg body weight was observed to exert glucose lowering effect after 6 hrs. of extract administration by 27.97 %. The doses of 250 and 500 mg/kg body weight of 80 % methanol leaves extract exerted a more prolonged effect on blood glucose with a reduction of 34.29 % and 39.46 %, respectively after 6hr of extract administrations.

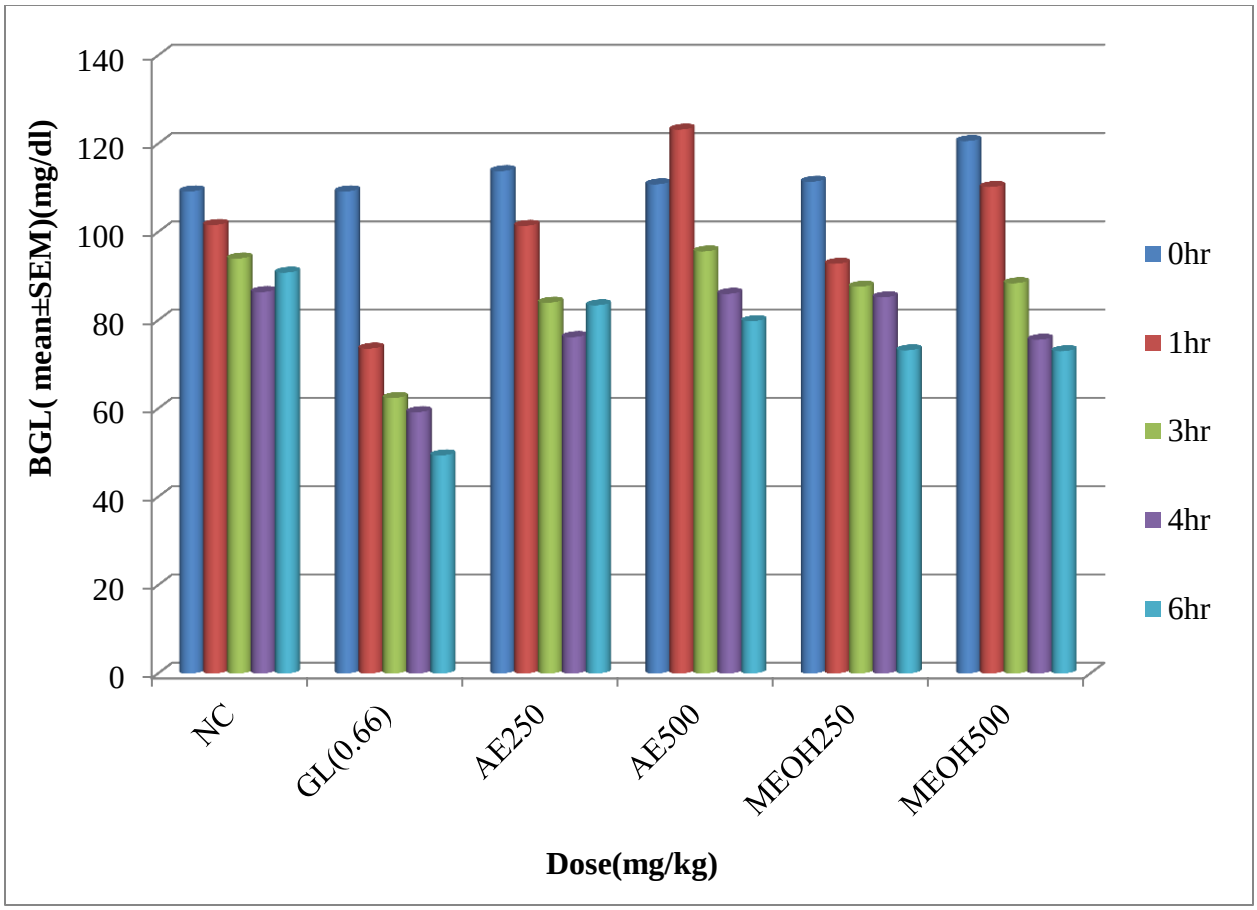


Figure 5: Hypoglycemic Effect of *T. Schimperi* crude extract of aqueous and 80% methanol on normal mice (non-diabetic)

Key: GL: Glibenclamide; AE250: aqueous extract 250mg/kg; AE500: aqueous extract 500mg/kg; MEOH250: methanol extracts 250mg/kg; MEOH500: methanol extracts 500mg/kg.

4.1.3. Effect crude extract of *T. Schimperi* on OGTT in Normal Mice

For OGTT, the blood samples were analyzed for glucose content at 0, 30, 60, 90 and 120 minutes, respectively. The effect of both extracts on OGTT mice is shown in table 2. Both aqueous and 80% methanol extracts caused a significant raise of blood glucose at 30 minute time point of the test. After that, the blood glucose level of all extracts treated mice decreased. The 80% methanol Extract (dose 500mg/kg) showed a higher reduction of blood sugar level of normal mice compared to aqueous extract treated and negative control (2g/kg of body weight of glucose) treated groups. The overall pattern of Glucose tolerance was more improved in extract treated mice than in those untreated.

Table 2: Effect of crude extract of aqueous and 80% methanol of *T. Schimperi* on OGTT in Normal Mice

Group	**Treatment (dose mg/kg)**	**Blood Glucose Level (mg/dl)**				
		0min	**30 min**	**60 min**	**90 min**	**120 min**
I	NS (10ml/kg)	102.8±2.5	103.4±2.5*	100.2±2.2*	97.0±2.4**	94.4 ± 1.7**
II	Glucose (2gm)	94.4 ±4.8	217.8±10.3	149.2 ± 5.4	128.2 ± 5.1	126.8 ± 3.6
III	GL(0.66)+GLU	99.8 ±4.5	181.2±10.5	106.2±9.2*	80.2 ± 4.6*	67.8 ± 4.2**
IV	AE 250 +GLU	95.8 ±5.2	258.0 ± 5.4	183.6±12.5	131.2 ±3.6	115.2 ± 3.2
V	AE 500+GLU	93.6 ±2.9	185.6±16.2	137.2± 6.2	125.4 ±5.0	105.6±1.3**
VI	ME250 +GLU	94.8 ±3.8	228.8 ± 8.9	138.2±10.5	114.4 ± 4.3	106.2 ± 4.4*
VII	ME500 +GLU	95.4 ±1.7	238.6±19.1	135.4±4.12	103.0±4.8*	84.6 ± 4.7**

Key: NS: normal saline, GL: Glibenclamide, AE: aqueous extract, MEOH: Methanol extract.
Data are expressed as Mean ± Standard Error of Mean (SEM); n=6
* Significant values at P<0.05 compared to group II
** Significant values at P<0.01 compared to group II

4.1.4. Effects of Crude Extract of *T. Schimperi* on Diabetic Mice After Acute Treatment

The effect on blood glucose level in alloxan induced mice after oral administration of the aqueous and methanol 80% extract of *T. schimperi* at varying doses showed a reduction in blood glucose levels in a dose- and time dependent manner (Table 3). Treatments wit *T. schimperi* aqueous extracts of 250 and 500 mg/kg showed reduction of 22.65% and 33.15% in plasma glucose levels, respectively after 4hr of extract administration. In case of 80% methanol extraction at 250 and 500 mg/kg showed reduction of 30.06% and 38.35% in plasma glucose levels, respectively after 4hr of extract administration. The 500 mg/kg of 80% methanol extract showed a significant ($p < 0.05$) decrease in the blood sugar level while both aqueous extract (250 and 500 mg/kg) and other dose of 80 % methanol extract (250mg/kg) showed no significant effect ($p>0.05$) when compared to diabetic control group at 4hr after extracts administration.

Glibenclamide (0,66mg/kg) produced a significant reduction of 48.21% in the plasma glucose level 4hr of drug administration.

Table 3: Effects of aqueous and 80% methanol crude extract of *T. schimperi* on diabetic mice after acute treatment.

Group	Treatment (mg/kg)	Blood Glucose Level (mg/dl)			
		0hr	2hr	4hr	% of Reduction from 0h to 4 hr.
I	NC	104.2 ± 3.056	101.00 ± 2.70	96.20 ± 3.48	7.67
II	NS (10ml/kg)	345.6 ± 13.47	363.40 ±10.75	375.6 ± 10.56	-8.68
III	GL (0.66)	329.8 ± 36.7	197.00 ± 26.34*	170.80 ± 25.16**	48.21
IV	AE 250	319.6 ± 59.59	278.00 ± 60.57	247.20 ± 60.38	22.65
V	AE 500	330.00 ±35.06	250.60 ± 26.69	220.60 ± 34.13	33.15
VI	MeOH 250	328.6 ±55. 26	272.60 ± 53.89	230.00 ± 55.13	30.06
VII	MeOH 500	331.60 ±35.73	252.40 ± 31.96	204. 40 ± 26.99*	38.35

Key: NC: Normal control, GL: Glibenclamide; AE250: aqueous extract 250mg/kg; AE500: aqueous extract 500mg/kg; ME250: methanol extracts 250mg/kg; ME500: methanol extracts 500mg/kg
* Significant values at P<0.05 compared to group II.

4.1.7. Effect of extract of *T. Schimperi* on BGL in Diabetic Mice after Prolonged Treatment

A marked increase in fasting blood glucose level was recorded in alloxan-induced diabetic rats when compared with the normal control (group I). Statistical analysis by One-way ANOVA revealed that there was significant difference among diabetic control and the group received the standard drug (Table 4). Post hoc test revealed that aqueous (500 mg/kg) and 80% methanol extract (500 mg/kg) showed significant reduce in the plasma sugar level compared to diabetic control (P< 0.05) at 14 day. Furthermore, the similar trend was observed at 21 days like at 14 days, however, there was significant decrease in sugar level in 80 % of methanol extract (500mg/kg) (P <0.001) and also significant decrease in BGL for aqueous 250mg/kg and 80%

methanol extract at dose 250mg/kg($P< 0.05$)compared to diabetic control. Glibenclamide induces a significant reduction ($P<0.001$) in blood glucose levels when we used in treating alloxan-induced diabetic mice at 21 day. However, Glibenclamide and *T. Schimperi* treatments failed to bring the blood glucose levels to normal values as in the non-diabetic control mice (group I).

Table 4: Effects of aqueous and 80%methanol crude extracts of *T. Schimperi* on the blood glucose level in alloxan-induced diabetic mice after prolonged treatment

Group	Treatment (mg/kg of body weight)	Blood Glucose Level (mg/dl) (Mean ± SEM)			
		day 0	Day 7	day14	day21
I	Normal control	97.5 ± 3.57**	98. 8 ± 2.5**	102.0 ± 2.9**	99.3 ± 4.1**
II	NS (10ml/kg)	324.8 ± 19.6	340.3 ± 20.9	339.5 ± 21.7	328.8 ± 23.1
III	GL(0.66)	270.5 ± 32.4	218.0 ± 36.7*	195.3 ± 30.8**	145.8 ± 7.7**
IV	AE 250	269.0 ± 45.0	254.8 ± 41.5	238.0± 39.2	226.8 ± 39.2*
V	AE 500	265.3 ± 13.5	247.5± 8.2	227.3± 10.5*	209.5 ± 8.3*
VI	MEOH 250	274.3 ± 28.7	258.3 ± 30.7	230.0± 28.5	206.8 ± 29.8*
VII	MEOH 500	270.3± 13.2	239.0 ± 12.9	215.8 ± 13.1*	195.5 ± 13.1**

Key: Statistical significant test for comparison was done by ANOVA, followed by Tukey. NS: normal saline, GL: Glibenclamide, AE: aqueous extract, MEOH: Methanol extract.
Data are expressed as Mean ± Standard Error of Mean (SEM); n=6
* Significant values at $P<0.05$ compared to group II
** Significant values at $P<0.01$ compared to group II

4.1.5. Effect of *T. Schimperi* on Body Weight in Alloxan Induced Diabetic Mice

At the end of experimental study, body weights of mice in normal control group were increased as compared to their initial body weights whereas in the diabetic control group significant decrease in the body weight was observed, when their final body weights were compared with their initial body weights. Alloxan induced diabetic mice produced significant loss in body weight as compared to normal mice during the study as shown above in figure 6. Aqueous and methanol extract of two doses (250mg/kg and 500mg/kg body weight) showed improvement in

body weight compared to diabetic control group, but it was still less than normal control group. Similarly the body weights of group of alloxan induced Diabetes+ Glibenclamide were also increased significantly.

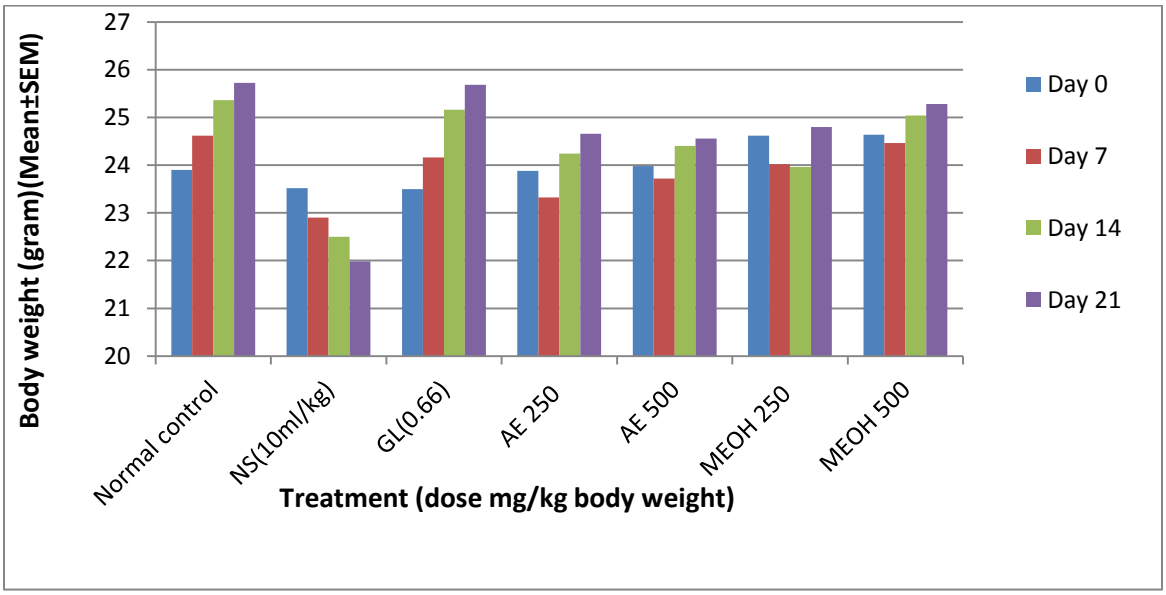


Figure 6: Effects of aqueous and 80% methanol *T.schimperi* leaves extracts on body weight in alloxan induced diabetic mice

Key: GL: Glibenclamide; AE250: aqueous extract 250mg/kg; AE500: aqueous extract 500mg/kg; MEOH250: methanol extracts 250mg/kg; MEOH500: methanol extracts 500mg/kg, NC: Normal control; DC: Diabetic control; NS: 0.9 % Normal saline.

4.1.6. Preliminary phytochemical screening

Preliminary phytochemical analysis revealed the presence of steroids, alkaloids, flavonoids, saponins and tannins in *T. schimperi leaves*. Phytochemical constituents of both solvent extracts of *T. schimperi* are given in table 6.

Table 5: Results of qualitative phytochemical Analysis of aqueous and 80% methanol crude extract of *T. Schimperi* leaves

S. No	Constituent	Aqueous extract	80% Methanol extract
1	Flavonoids	++	+ + +
2	Phenol	+ + +	+ + +
3	Tannins	+ + +	+ + +
4	Saponins	±	+ + +
5	Alkaloids	±	+ + +
6	Steroids	++	+ + +

Key: **+++:** Very strong positive; **++:** Strong positive; ±: Trace

4.1.7. Acute Toxicity Study

The present study conducted as per the OECD guidelines 425 revealed that aqueous and methanol extracts of *T. schimperi* did not produce any morbidity throughout the study period of 14 days at dose of 2g/kg body weight. There were no any behavioral, neurological, autonomic or physical changes such as alertness, motor activity, restlessness, convulsions, coma, diarrhea, lacrimation and appearance of the animals and also there were no changes in eyes, respiratory circulation, sleep, etc. Besides, the extract did not cause mortality in the animals at a dose of 2 mg/kg during the observation time. The result showed that in single dose; the plant extracts of both solvents had no adverse effect, indicating that the LD_{50} could be greater than 2g/kg per body weight in mice.

4.2. Discussion

The use of *T.schimperi* in diabetics has been reported in the literature along with several other traditional claims. Hence, it was thought that investigations of these medicinal properties should be scientifically authenticated to validate the traditional claims. In the present study, in order to establish the scientific basis for the utility of *T. schimperi in* the treatment of diabetes, evaluation of the hypoglycemic and antihyperglycemic effects of the crude aqueous and 80% methanol leaves extracts were performed in normoglycemic and alloxan-induced diabetic mice, respectively.

Alloxan is a specific toxin that destroys the pancreatic β-cells, provoking a state of primary deficiency in insulin without affecting other types of islets and is used in the laboratory to induce both T1DM and T2DM in animals. The diabetic effect of alloxan is due to an excess in the production of ROS. This excess leads to toxicity in pancreatic cells, which, in turn, reduces the synthesis and release of insulin while concurrently affecting other organs, such as liver. Increased lipid peroxidation products and decreased plasma or tissue concentrations of superoxide dismutase, catalase, and glutathione have been well documented in the literature on alloxan induced diabetes (Aloulou *et al*., 2012).

In the preparation of crude aqueous extract from the dried leave of *T. schimperi* a yield 10.7% was obtained. In the case of crude 80% methanol extract preparation, a yield of 13.2% of *T. schimperi* was obtained. Other researchers also reported that the percentage yield of crude extract of 80% methanol extract was 12% which is similar to the present finding Tsegaye *(2015)*.

Both the aqueous and the methanolic extracts of *T.schimperi* leaves didn't show any significant hypoglycemic effect after the mice were administered with (250 and 500mg/kg doses) of the extracts. Therefore, this study indicates that single dose 250 and 500mg/kg of both the aqueous and the 80% methanolic extracts of *T.schimperi* leave have no hypoglycemic effect on normoglycemic mice. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and these compounds are active in mild alloxan diabetes. From the results, glibenclamide produce reduction in blood glucose levels, which

validated its activity as a hypoglycemic agent and the type of diabetic induced by alloxan monohydrate, was mild type of hyperglycemic in our study also.

OGTT is a measures the body's ability to utilize glucose and it seen as the "gold standard" in diagnosing diabetes mellitus Sucharitha and Estari (2013). The effect of extracts on glucose tolerance test in normal mice is shown in table 2. At 30 min after glucose administration, the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. Statistical analysis by One-way ANOVA showed that there was no significant difference among the groups at 0 min. Similarly, statistical analysis at 30 min showed that there was significant difference among the groups. Further, statistical analysis at 60 min showed that there was significant difference among the groups at 60 min. Post hoc test revealed that glibenclamide (0.66 mg/kg) showed significant ($P<0.05$) attenuation in the plasma sugar level compared to glucose loaded control group at 60 min.. Furthermore, Post hoc test revealed that normal control, glibenclamide and 80% methanol extract at dose of 500 mg/kg) showed significant ($P<0.05$) decrease in the plasma sugar level compared to glucose loaded control group at 90 min. Similar trend was observed at 120 min, i.e., there is significant reduction of blood glucose level by all treatment group except those group treated with aqueous extract of dose 250mg/kg, when compared to glucose loaded control group.

In general, in OGTT the crude extract showed significant reduction in blood glucose level from 90 min onwards. This suggests that the extract is endowed with the ability to enhance regulatory mechanisms, indicating a potential advantage of the extract in minimizing hyperglycemia related complications of diabetes.

The increase in fasting blood glucose concentration is an important characteristic feature of DM. In this study, there were elevations in fasting blood glucose (FBG) level in diabetic group. However, the extract of *T.schimperi* leaves reduced FBG level in diabetic mice. The aqueous and the 80% methanol extract of *T. schimperi leaves* (dose: 250mg/kg and 500mg/kg body weight) were administered daily for three weeks to alloxan-induced diabetic mice. The significant reduction of the blood glucose levels of the mice were observed gradually from the second week end to the last week of treatment. Therefore, the present study revealed that both the aqueous and

the methanolic extracts of *T.schimperi* leaf have a significant antihyperglycemic effect on alloxan-induced diabetic mice in dose and time dependent manner.

The present findings indicate the potential antihyperglycemic effect of the extract. There were many possible explanations for this finding. Blood glucose levels are maintained within a narrow range by homeostatic mechanisms Widyawati (2015). The inhibition of pancreatic α-amylase activity in the human digestive tract represents one of the therapeutic approaches commonly used for the control and prevention of postprandial hyperglycemia in T2DM patients through reducing the uptake of glucose released by this enzyme from starch Aloulou *(*2012). Complex starches, oligosacharides, and disaccharides must be broken down into their individual monosaccharides before being absorbed in the duodenum and the upper jejunum; because, only monosaccharides can be transported out of the intestinal lumen and into the bloodstream. This digestion is facilitated by enteric enzymes like pancreatic alpha amylase that are attached to the brush border of the intestinal cells. Inhibition of this enzyme involved in the delaying of postprandial hyperglycemia (Widyawati *et al*., 2015). In a study conducted by Dessalegn (2015) *T. schimperi* demonstrated the α-amylase inhibitory effect *in vitro*. So, this property may have partly contributed to the antihyperglycemic activity of *T.schimperi* leaf extract, which support our study.

It has been suggested that enhanced production of free radicals and oxidative stress are central events to the development of diabetic complications. Use of antioxidants reduces oxidative stress and alleviates diabetic complications (Hasani-Ranjbar *et al.,* 2008). Alloxan produce hyperglycemia by selective cytotoxic effect on pancreatic beta cells, via disruption of the cell membrane integrity. The pancreatic beta - cells are known to be involved in the synthesis, storage, and release of insulin, the peptide hormone regulating carbohydrate, protein, and lipid metabolism. One of the intracellular phenomenons for its cytotoxicity is through generation of free radicals as reviewed by Ijaola (2014). It is equally possible for *T. schimperi* to have regenerated remnants of the already alloxan - destroyed cells. *T. schimperi* probably prevented the destruction of b cells of islets in the pancreas. This is an interesting finding and suggests that it may have antioxidant or free radical scavenger properties in preventing these changes. Antioxidant/ free radical scavenging property of *T. schimperi* leaves have been reported earlier by Gebrehana and Shimelis (2013). The antioxidant activity of the plant might also contribute

towards the anti-diabetic effect of the extract by providing protection against the cytotoxic effect of free radicals generated by the alloxan or diabetes itself. Thus *T. schimperi* may have a role in prevention of diabetes.

Secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-malarial, anti-cholinergic, anti-leprosy activities as reported by Yadav (2014). Preliminary phytochemical screening of the extract of *T. schimperi* demonstrated the presence of potent phytochemicals like saponins, flavonoids, alkaloids, tannins, and phenolic compounds, even though 80% methanolic leaf extracts of *T. schimperi* show strong content of the phytochemical constituents than the aqueous leaf extracts. Oliver (1980) and Moha (2013). reported that these secondary metabolites are known to be bioactive antidiabetic principles

Flavonoids have been shown to have a significant antidiabetic activity and are also known for their ability to regenerate pancreatic beta cells, increasing the peripheral utilization of glucose and inhibiting the glucose transporter activity from intestine (Jadhav and Puchchakayala, 2012). Steroidal compounds have also been reported to decrease blood glucose via inhibition of intestinal glucose uptake and increased hepatic glucose deposition.

A review on the mode of action of flavonoids (Brahmachari, 2011) discussed about the various effects of the drug candidates in regulating diabetic syndromes. It has been demonstrated that flavonoids compounds act against diabetes mellitus either through their capacity to avoid glucose absorption (inhibition of α-glucosidase activity in the intestine), or to improve glucose tolerance. Moreover, it has also been demonstrated that flavonoids can act *per se* as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. Also in other study flavonoids, especially quercetins have been reported to possess antidiabetic activity. Vessal and co-workers *(*2003) reported that quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in drug-induced diabetic.

Researchers have found that the antihyperglycemic effect of plant extract may be due to the presence of tannin. Oxidative stress or excessive production of reactive oxygen species is being implicated in diabetes. Tannins are excellent free radical scavengers, this property arising mainly from the presence of well-known antioxidants (Borgohain *et al.*, 2012). Hence this activity will be another reason for the glucose lowering effect of *T. schimperi*

Reduction of blood glucose level action of saponins is through, restoration of insulin response, improvement in insulin signaling, increase plasma insulin levels and induction of insulin release from the pancreas, inhibition of disaccharides activity, activation of glycogen synthesis, inhibition of gluconeogenesis, inhibition of α- glucosidase activity and inhibition of mRNA expression of glycogen phosphorylase and glucose 6 phosphatase (Lavle *et al*., 2016). Phenolic constituents of plants have been reported to have a significant blood glucose lowering effect (Gaikwad *et al*, 2014). In another study by (Ramu and Vijayakumar, 2016) 7-methoxycoumarin, phenolic compound, isolated from the bark of Marine plant *Rhizophora mucronata* showed anti-diabetic activity both *in vitro* and *in vivo* antidiabetic activity. Thus, the good antidiabetic effect of the extract of *T. schimperi* may be associated with the presence of these different secondary metabolites with possible synergistic effects.

Antidiabetic studies regarding the claimed pharmacological effect have done on thymus plants. It was found that the aqueous extract of *T.serpyllum* in alloxan-induced diabetic rabbits significantly reduced the blood glucose level in diabetic rabbits and the aqueous extract also maintained the body weight of alloxanized rabbits (Alamgeer *et al*., 2012). The author concludes that the possible mechanism for this effect could be due antioxidant activity and α-glucosidase inhibitory effect *in vitro* of the plant. Our study agrees with this study as our study of plant has those effects and the two plants are under the same family and genus. Other mechanisms cannot be ruled out at this stage and more comprehensive studies are, however, required in order to ascertain the actual mechanism of this plant.

In this study, there is the weight reduction in diabetic control mice. Weight loss has been known to be one of the symptoms of DM. Alloxan -induced diabetes was characterized by severe loss in body weight. Alloxan has been reported to cause massive reduction in insulin release by the destruction of the β-cells of islets of Langerhans and inducing hyperglycaemia in animals. This

deficiency of insulin led to decreased amino acids uptake by tissues with a consequent reduction in the level of protein synthesis and also results in lipolysis in adipose tissues and protein breakdown (Mohamed and Nassier, 2013). Similar observations were detected in many experimental studies (Ijaola *et al*., 2014; Akter *et al.,* 2014).

When diabetic mice were treated with *T.schimperi* extract, the weight loss was normalized. The capability of *T.schimperi* to protect the body from weight loss seems to be a result of its ability to reduce hyperglycemia and the bioactive compounds of *T.schimperi* leaves 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 (Dessalegn *et al*, 2015) and ultimately lead to normalize the level of body weight. 0.66mg/kg of glibenclamide treated mice gained weight 9.3% in comparison with the diabetic group after 21 days treatment.

The results of the phytochemical screening carried out on the methanol and aqueous extracts of *T.schimperi* leaves have revealed the presence of flavonoids, saponins, tannins, phenols alkaloids and steroids. Alkaloids and saponins have been detected only in high concentration in the methanol extract. These results were in agreement with that obtained by Haji and co-workers (2016) who reported that saponins, alkaloids, tannins, phenols, and flavonoids were present in the leaf of *T.schimperi.* This finding was against the study which reported that the leaves aqueous extract has not detected of alkaloids and saponins (Haji *et al*., 2016).

Initially acute oral toxicity was evaluated and found to be safe at dose 2000mg/kg per body weight. In the acute toxicity test of the aqueous extract of *T.schimperi*, there was no mortality or any signs of behavioral changes or toxicity observed after oral administration of extract at the dose level of 2000mg/kg body weight in mice. This finding supports the study which presents the strong evidence of the non-toxic effect of the aqueous extract of the plant (Debelo *et al.,* 2016) and also the herbal extracts proved to be safe with the limit dose of 2000 mg/kg per body weight.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In conclusion, this study revealed that the aqueous and methanolic extracts of *T. schimperi* leaves showed significant lowering of blood glucose level on diabetic mice, but lack significant reduction effect on normoglycemic mice and prevented body weight loss of diabetic. Methanolic extract showed pronounced reduction of blood glucose level as compared to respective dose of aqueous extract. The phytochemical screening indicated the presence of alkaloids, phenols, flavonoids, tannins and saponins. It's antihyperglycemic and free radical scavenging property has potential of preventing diabetic-associated complications in mice. The LD50 value of the extract is greater than 2,000 mg/kg which indicates the plant material has a wider safety margin in animal models. Therefore, based on the results obtained from both the hypoglycemic as well as antihyperglycemic studies and acute toxicity study, after further investigation it is possible to say that *T. schimperi* leaves can be exploited as an alternative herbal supplement for the management of diabetes.

5.2. Recommendations

- It is essential that further investigation can be carried out to fractionate and purify using different advanced technologies like HPLC to isolate and identify the active principle(s) present in the leaves of *T. schimperi.*
- More research is needed to elucidation the appropriate mechanism (s) of action of the extract.
- Moreover, more experiments should be done with other animal models of diabetes and researches have to be done on the other parts of the *T. schimperi*
- Further, it is proposed that researches on histopathology of pancreas should be undertaken to make the study more comprehensive.

6. REFERENCES

Abate, G., (1989). *Etse Debdabe*. Biology Department, Science Faculty, Addis Ababa University

Abebe, D., (1996). In Proceedings of the Workshop on Development Utilization of Herbal Remedies in Ethiopia. *AddisAbaba: University Academic Press.*

Abebe, S., Berhane, Y., and Worku, A., (2014). Barriers to diabetes medication adherence in North West Ethiopia. *Springer plus*, 3(1).

Abdulfatai, B., Olusegun, A., and Lateefat, B., (2012). Type 2 Diabetes Mellitus: A Review of Current Trends. *Oman Medical Journal*, 27(4): 269-273.

Akter, R., Islam, F., Khatun, A., *et al*. (2013). Comparative studies on antidiabetic effect with phytochemical screening of Azadirachta indicia and Andrographis paniculata. IOSR Journal of Pharmacy and Biological Sciences, 5(2): 122-128.

Akter, F., Rahman, M., Mostofa, M., and Chowdhury, E., (2014). Anti-diabetic Effect of Neem and Spirulina in Alloxan Induced Diabetic Mice. Int.J.Curr.Res.Aca.Rev, 2(4): 124-134.

Alamgeer, Bashir, S., Muhammad Rashid, M., *et al* (2012). Hypoglycemic and hematological effects of aqueous extract of *Thymus serpyllum* in alloxan-induced diabetic rabbits. Afr. J. Pharm. Pharmacol, 6(40): 2845-2850.

Aloulou, A., Hamden, K., Elloumi, D., (2012). Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. Complementary and Alternative Medicine, 12(63):1-9.

American Diabetes Association (2010). Diagnosis and classification of diabetes mellitus. Diabetes Care, 33(1): S62-S69.

American Diabetes Association (2015). Standards of medical care in diabetes—2015: summary of revisions. Diabetes Care, 38(1): S4-S4.

American diabetes association (2016). Standards of medical care in diabetes. The journal of clinical and applied research and education, 39 (1):1-199.

Aseffa, H., Giday, T/K., and Kidanemariam, A., (2014). Assessment of Risk Factors Associated with Type 2 Diabetes Mellitus in Central Zone of Tigray, North Ethiopia. IJPBSF International Journal of Pharmaceutical and Biological Sciences Fundamentals, 07(01):1-20.

Ashwini, N., (2015). Type 2 Diabetes: A Review Of Current Trends. International Journal of Current Research and Review, **7**(18): 61.

Bastaki, A., (2005). Diabetes mellitus and its treatment. International journal of Diabetes and Metabolism, 13(3): 111.

Bhatia, E., and Aggarwal, A., (2007). Insulin therapy for patients with type 1 diabetes. Journal-Association Of Physicians Of India, 55(L): 29-40

Boada, C., and Martínez-Moreno, J., (2013). "Pathophysiology of diabetes mellitus type 2: beyond the duo insulin resistance-secretion deficit. Nutr hosp, 28(2): 78-87.

Bösenberg, L. H., and G. van Zyl, D., (2008). The mechanism of action of oral antidiabetic drugs: a review of recent literature. Journal of Endocrinology, Metabolism and Diabetes of South Africa, 13(3): 80-88.

Borgohain, R., Lahon, K., Das, S., and Gohain, K., (2012). Evaluation of mechanism of anti-diabetic activity of *terminalia chebula* on alloxan and adrenaline induced diabetic albino rats. Int J Pharm Bio Sci, 3(3): 256 – 266.

Brahmachari, G., (2011). Flavonoids with promising antidiabetic potentials: A critical survey. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry: 187-212.

Bürger, C., Fischer, D. R., Cordenunzzi, D. A., *et al.*, (2005). Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (Acmela brasilinsis) (Asteraceae) in mice. J Pharm Sci , 8(2): 370-373.

Cheng, A. Y., and Fantus, I.G., (2005). Oral antihyperglycemic therapy for type 2 diabetes mellitus. Canadian Medical Association Journal, 172(2): 213-226.

Colagiuri, R., Colagiuri, S., Yach, D., and Pramming, S., (2006). The answer to diabetes prevention: Science, surgery, service delivery, or social policy? A J of Public Health, 96:1562–1569

Control, C. D., and Prevention (2014). National diabetes statistics report: estimates of diabetes and its burden in the United States. Atlanta, GA: US Department of Health and Human Services.

Deacon, C. F., (2011). Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes, Obesity and Metabolism, 13(1): 7-18.

Debelo, N., Afework, M., Debella, A., Makonnen, E., Ergete, W., *et al.* (2016) Assessment of Hematological, Biochemical and Histopathological Effects of Acute and Sub-chronic Administration of the Aqueous Leaves Extract of *T.schimperi* in Rats. J Clin Toxicol, 6: 286.

DEMSEW, S., (1993). The GenusThymus (Labiatae) in Ethiopia, Opera. Bot.121: 57-60, 121

Deshmukh, C., and Jain, A., (2015). Diabetes Mellitus: A Review. Int. J. Pure App. Biosci, 3(3): 224-230.

Dessalegn, E., Bultosa, G., Desse, G., and Vasantha, H., (2015). Antioxidant and α-amylase inhibition activities *in vitro* of various solvent extracts of *T.schimperi*. Journal of Medicinal Plants Research, 9(15):515-524

El-Desouk, N., Basyony, M., Hegazi, M., and El –Aama, M., (2015). *Moringa oleifera* Leaf Extract Ameliorates Glucose, Insulin and Pancreatic Beta Cells Disorder in Alloxan-Induced Diabetic Rats. RJPBCS,6(3):642-653.

Enyew, A., Asfaw Z., Kelbessa, E., and Nagappan, R., (2014). Ethnobotanical study of traditional medicinal plants in and around Fiche District, central Ethiopia. Current Research Journal of Biological Sciences, 6(4): 154-167.

Erasto, P., Adebola, P., Grierson, D., and Afolayan, A., (2005). An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. African Journal of Biotechnology, 4 (12): 1458-1460.

Ermias, Bisrat, Worku and Hailu (1998). Constituents of the essential of *T.schimperi,* AA, 1-2.

Etuk, E.U., (2010). Animals models for studying diabetes mellitus. Agric. Biol. J. N. Am., 1(2): 130-134.

Flint, A., and Arslanian, S., (2011). Treatment of type 2 diabetes in youth. Diabetes Care, 34(2): S177-S183.

Feleke, Y., and Enquselassie, F., (2005). An assessment of the health care system for diabetes in Addis Ababa, Ethiopia. Ethiop.J.Health Dev, 19(3): 204-210.

Fraser, M. H., Cuerrier, A., Haddad, P. S., *et al* (2007). Medicinal plants of Creecommunities (Quebec, Canada): Antioxidant activity of plants used to treat type 2 diabetes symptoms. *can.J. pharmacol.*, 85**:**1200-1214.

Gaikwad, B., Krishna Mohan, G., and Sandhya Rani, M., (2014). Phytochemicals for Diabetes Management. *Pharmaceutical Crops*, 5(1):11-28.

Galuppo, M., Giacoppo, S., Bramanti, P., and Mazzon, E., (2014).Use of Natural Compounds in the Management of Diabetic Peripheral Neuropathy. *Molecules*, 19, 2877-2895.

García Mesa, M., (2014). Antidiabetic potential of plants used in Cuba. PhOL 1: 52-62.

Gebrehana, A., And Shimelis, A., (2013). Antioxidant Activity and Preservative Effect of Thyme (*Thymus schimperi R.*). *British Journal of Applied Science & Technology*. 3(4): 1311-1326.

Gidado, A., Ameh, D. A., and Atawodi, S. E., (2005). Effect of *Nauclea latifolia* leaves aqueous extracts on blood glucose levels of normal and alloxan-induced diabetic rats. *African Journal of Biotechnology*, 4: 91-93

Girani, S., Patel, D., Kavatekar, M., *et al (2016).* Formulation and evaluation of matrix type transdermal therapeutic system containing glibenclamide. *ejpmr ,3(5), 556-569*

Gupta, A., Sharma, M., and Sharma, J., (2015). "A Role of Insulin in different types of Diabetes." Int. J. Curr. Microbiol. App. Sci, 4(1): 58-77.

Haile, K., (2013). Antimicrobial activity and phytochemical screening of crude extracts of medicinal plants grown in eastern Ethiopia. Int J Pharm Bio Sci, 4(4): (P) 326 – 33

Haji, H., Makonnen, E., Debella, A., and Geleta, B., (2016). Evaluation of Diuretic and Antihypertensive Activity of Leaf Extracts of *T. Schimperi* in Rats. British Journal of Pharmacology and Toxicology, 7(1): 1-8.

Harborne, J.B., (1998) Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. 3rd Edition, Chapman and Hill, London, 285.

Hasani-Ranjbar, SH., Larijani, B., and Abdollahi, M., (2008). A systematic review of Iranian medicinal plants useful in diabetes mellitus. Arch Med Sci; 4(3): 285–292.

Hashemnia, M., Oryan A., Hamidi, A., and Mohammadalipour, A., (2012). Blood glucose levels and pathology of organs in alloxan-induced diabetic rats treated with hydro-ethanol extracts of Allium sativum and Capparis spinosa. African Journal of Pharmacy and Pharmacology, 6(21): 1559-1564.

Hemalatha, S., Patel, D., Kumar, R., and Laloo, D., (2012). Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pac J Trop Biomed; 2(5): 411-420

Hosseini, A., Shafiee-Nick, R., and Ghorbani, A., (2015). Pancreatic beta cell protection/regeneration with phytotherapy. Brazilian Journal of Pharmaceutical Sciences, 51(1): 1-16.

Ibrahim, R., (2010). Diabetes mellitus type II: review of oral treatment options. Int J Pharm Pharmaceut Sci, 2(1): 21-30.

Ijaola, T.O., Osunkiyesi, A., Taiwo, A., *et al* (2014). Antidiabetic Effect of Ipomoea Batatas in Normal and Alloxan-Induced Diabetic Rats. *I* IOSR-JAC, **7**(5): 16-25

Inzucchi, S., Bergenstal, R., Buse, J., *et al* (2012). Management of Hyperglycemia in Type 2 Diabetes: *Diabetes Care*, 35: 1364- 1379.

Jadhav, R., and Puchchakayala, G., (2012). Hypoglycemic and antidiabetic activity of flavonoids: boswellic acid, Ellagic acid, quercetin, rutin on streptozotocin-nicotinamide induced type 2 diabetic rats. *Int J Pharm Pharm Sci, 4(2): 251-256.*

Jarald, E., Balakrishna, S., Joshi, A., and Jain, D. C., (2008). Diabetes and Herbal Medicines. *Iranian Journal of Pharmacology & Therapeutics,* 7: 97-106.

Joshi, R., Setzer, W., Junior, V., (2015). Aromatic and medicinal plants with anti-diabetic potential from India: A review. American Journal of Essential Oils and Natural Products; 2(4): 22-28.

Kabbaoui, EL., Chda, A., Mejrhit, N., *et al* (2016). Antidiabetic effect of *thymus satureioides* aqueous extract in streptozotocin-induced diabetic rats. *Int J Pharm Pharm Sci, 8(9), 140-145*

Kassaye, KD., Amberbir, A., Getachew, B., Mussema, Y., (2006). A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiop J Health Dev,* 20(2):127-134.

Khanavi, M., Taheri, M., Rajabi, A *et al.,* (2012). Stimulation of hepatic glycogenolysis and inhibiyion of gluconeogenesis are the mechanisms of antidiabetic effect of centaurea bruguierana ssp. belanerana. *Asian Journal of Animal and veternary Advances* 7, 1166-1174.

Kohei, K., (2010). Pathophysiology of type 2 diabetes and its treatment policy. JMAJ, 53(1): 41-46.

Kumar, S., et al. (2014). Review On Some Medicinal Plants With Antidiabetic And Analgesic Activity.

Kumar, R., Prakash, O., Srivaxstava, R., *et al* (2015). Plants Explored with Anti-diabetic Properties: A Review. *American Journal of Pharmacological Sciences*, 3(3): 55-66.

Lavle, N., Shukla, P., and Panchal, A., (2016). Role of Flavonoids and Saponins in the Treatment of Diabetes Mellitus. *J Pharm Sci Bioscientific Res., 6(4):535-541*

Lanjhiyana, S., Garabadu, D., Ahirwar, D., (2011). Hypoglycemic activity studies on root extracts of *Murraya koenigii* root in Alloxan-induced diabetic rats. J. Nat. Prod. Plant Resour, 1 (2): 91-104.

Larbie, C., *et al.* (2014). Anti-diabetic and hypolipidaemic effect of botanicals: a review of medicinal weeds on KNUST campus, Kumasi.

Libman, I. M., and Arslanian, S. A., (2007). Prevention and treatment of Type 2 diabetes in youth. Hormone Research, 67: 22–34.

Madhu, S., and Srivastava, S., (2015). Diabetes Mellitus: Diagnosis and Management Guidelines. Journal International Medical Sciences Academy, 1: 47-50.

Mahomoodally, M., (2013). Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Complementary and Alternative Medicine.

Matheka, DM., Kitua, M., and Alkizim, F., (2012). Peculiar glycemic patterns in alloxan induced diabetes animal model. *Afr. J. Pharmacol. Ther.*, 1(1): 30-34.

Mardanyan, S., *et al.* (2011). Dipeptidyl peptidase IV and adenosine deaminase inhibition by Armenian plants and antidiabetic drugs. Int J Diabetes Metab, 19(2).

Michael, J., And John, A., (2006). Insulin and Oral Drugs for Diabetes Mellitus. In Modern Pharmacology with Clinical Application, Lippincot 5th Edition; 763-777.

Mohammed, S., Yaqub, A., Sanda, K., *et al*. (2013). Review on diabetes, synthetic drugs and glycemic effects of medicinal plants. Journal of Medicinal Plants Research, **7**(36): 2628-2637.

Mohan, Y., Jesuthankaraj, G., and Thangavelu, N., (2013). Antidiabetic and Antioxidant Properties of *Triticum aestivum* in Streptozotocin-Induced Diabetic Rats. Advances in Pharmacological Sciences: 1-9.

Moore, T., (2007) Glyburide for the Treatment of Gestational Diabetes *Diabetes Care*, 30(2): 209-213

Morales (1997). Synopsis of the genus thymus in the mediterranean area. 249-262

Nagappa, A. N., Thakurdesai, P. A., Rao, N. V., and Singh, J., (2003).Antidiabetic activity of *Terminalia catappa Linn* fruits. *Journal of Ethnopharmacology,* 88: 45-50.

Nasir, M., Tafesse, K., and Abate, D., (2015). Antimicrobial potential of the Ethiopian Thymus schimperi essential oil in comparison with others against certain fungal and bacterial species. BMC Complementary and Alternative Medicine 15:260.

NDEP, (2007). The National Diabetes Education Program : Ten Years of Progress 1997 – 2007. *National Diabetes Education Program*.1–38

Obernier, JA., and Baldwin, RL., (2006) Establishing an appropriate period of acclimatization following transportation of laboratory animals. *ILAR Journal*, 47(4):364-370.

O'Connell, PJ., Hawthorne, WJ., Holmes-Walker, DJ., *et al* (2006). Clinical islet transplantation in type 1 diabetes mellitus: results of Australia's first trial. MJA, 184: 221–225.

Oderda, G., *et al*. (2013). Sulfonylurea Agents & Combination Products Drug Class Review.

OECD (2008). Guidelines for Testing of Chemicals, Acute Oral Toxicity, Environmental Health and Safety Monograph. Series on Testing and Adjustment. 425, 1.

Olokoba, A. B., Obateru, O.A., and Olokoba, L. B., (2012). Type 2 diabetes mellitus: a review of current trends. Oman Med J, 27(4): 269-273.

Oliver, B., (1980). Oral hypoglycemic plants in West Africa. J.Ethnopharmacol; 2: 119-127.

Osigwe, C., Akah, P., Nworu, C., *et al*. (2015). Antihyperglycemic Studies on the Leaf Extract and Active Fractions of Newbouldia laevis. Pharmacology & Pharmacy, 6(11): 518.

Patel, D., *et al*. (2012). Natural medicines from plant source used for therapy of diabetes mellitus: An overview of its pharmacological aspects. Asian Pacific Journal of Tropical Disease, 2(3): 239-250.

Patel, DK., Kumar, R., Laloo, D., Hemalatha, S., (2012). Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pac J Trop Biomed; 2(5): 411-420.

Patel, P., Harde, P., Pillai, J., *et al*., (2012). Antidiabetic herbal drugs a review. *Pharmacophore*, 3 (1):18-29.

Piero, M., Joan, N.M., Kibiti, M., Cromwell, K.M., *et al*. (2012). Herbal management of diabetes mellitus: A rapidly expanding research avenue.

Prabhakar, P. K., and Doble, M., (2011). Mechanism of Action of Natural Products Used in the Treatment of Diabetes Mellitus. *Chin J Integr Med*, 17: 563-574.

Qamar, A., and Qureshi, T., (2013). Anti-hyperglycemic and anti-nephropathic effects of Woodfordia fruticosa." Alloxan-induced iabetic ats. ISESCO J. Sci. Technol, 9(15): 33-38.

Rahman, S., Akter, R., Mazumdar, S., *et al* (2015). Antidiabetic and antidiarrhoeal potentials of ethanolic extracts of aerial parts of Cynodon dactylon Pers. Asian Pac J Trop Biomed; 5(8): 658–662.

Ramu, A., and Vijayakumar, V., (2016). Antidiabetic activity of 7-methoxycoumarin from the bark of Marine plant *Rhizophora mucronata. Glo. Adv. Res. J. Med. Plants;* 4(1):001-006.

Rajput, R., (2009). Dipeptidyl Peptidase-IVInhibitors: new drug in the Therapeutic Armamentarium for treatment of T2DM. J Indian Acad Clin Med, 10:128-133.

Rohilla, A., and Ali, S., (2012). Alloxan Induced Diabetes: Mechanisms and Effects. International Journal of Research in Pharmaceutical and Biomedical Sciences; 3(2): 2229-3701

Rupeshkumar, M., Kavitha, K., Haldar, P., (2014). Role of Herbal Plants in Diabetes Mellitus Therapy: An Overview. International Journal of Applied Pharmaceutics; 6(3): 1-3.

Saganuwan, A., (2009). "Tropical plants with antihypertensive, antiasthmatic, and antidiabetic value." Journal of herbs, spices & medicinal plants *15(*1): 24-44.

Sakthiswary, R., Zakaria, Z., and Srijit, D., *et al.* (2014). Diabetes Mellitus: Treatment Challenges and the Role of Some Herbal Therapies. Middle-East Journal of Scientific Research ; 20(7): 786-798.

Sharma, B., Siddiqui, S., Ram, G., *et al*. (2013). Hypoglycemic and hepatoprotective effects of processed Aloe Vera gel in a mice model of alloxan induced diabetes mellitus. Diabetes Metab, 4(9): 4-9.

Sharma, K. V., Kumar, S., Patel, J.H., and Hugar, S., (2010). Hypoglycemic activity of ficus *glomerata* in alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences Review and Research; 1*(2):18-22.

Shah Ayub, MA., Garg, SK., Garg, KM., (1997). Subacute toxicity studies on Pendimethalin in rats. Indian J Pharmacol; 29: 322-324.

Sheeja, V., Reddy, M., Joseph, J., and Reddy, D., (2010). Insulin therapy in diabetes management. Insulin ; 2(2): 98-105.

Shyam, K., and Kadalmani, B., (2014). Antidiabetic activity of Bruguiera cylindrica (Linn.) leaf in Alloxan induced diabetic rats. Int. J. Curr. Res. Biosci. Plant Biol ; 1: 56-60.

Singab, A. N., et al. (2014). Medicinal plants with potential antidiabetic activity and their assessment. Medicinal & Aromatic Plants.

Singh, A. K., and Singh, J., (2010). Evaluation of anti-diabetic potential of leaves and stem of Flacourtia jangomas in streptozotocin-induced diabetic rats. Indian journal of pharmacology; 42(5): 301.

Sucharitha, E., and Estari, M., (2013). Evaluation of antidiabetic activity of medicinal plant extracts used by tribal communities in rural areas of Warangal district, Andhra Pradesh, India. Biology and Medicine; 5: 20–25

Sunil, C., and Kumar, R., (2014). Diabetes mellitus and medicinal plants-a review. Asian Pac J Trop Dis; 4(5): 337-347.

Teshale, Ch., Hussien, J., and Jemal, A., (2013). Antimicrobial activity of the extracts of selected Ethiopian aromatic medicinal plants. *Spatula DD; 3(4):175-180*

Topi, E., (2014). The Role of Pharmacogenetics in the Treatment of Diabetes Mellitus. Journal of Medical Biochemistry; 33(1): 58-70.

Trease, G.E., and Evans, M.C., (1983). *Textbook of Pharmacognosy*. 12th edition Bailliere, Tindall, London :343-383.

Tsegaye, B., Tesfamaryam, G., Tadesse, T., and Wubete, A., (2015). In vitro Screening of Antibacterial Activities of Selected Ethiopian Medicinal Plants. International Journal of Microbiological Research; 6 (1): 27-33.

Tripathi, V., and Verma, J., (2014). Current updates of indian antidiabetic medicinal plants. International Journal of Research in Pharmacy and Chemistry ;4(1): 114-118

Verspohl, E., (2012). Novel pharmacological approaches to the treatment of type 2 diabetes. Pharmacological reviews 64(2): 188-237.

Vessal, M., Hemmati, M., Vasei, M., (2003). Antidiabetic effects of quercetin in streptozocin induced diabetic rats. Comp. Biochem. Physiol. C.;135: 357-364.

Weiss, J., and Sumpio, B., (2006) Review of prevalence and outcome of vascular disease in patients with diabetes mellitus. *Eur J Vasc Endovasc Surg;* 31 (2): 143–150

Weyer C., et al. (1999). The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. The Journal of clinical investigation ; 104(6): 787-794.

Widyawati, T., Yusoff, N., Asmawi, M., & Ahmad, M., (2015). Antihyperglycemic Effect of Methanol Extract of Syzygium polyanthum (Wight.) Leaf in Streptozotocin-Induced Diabetic Rats. Nutrients ; 7: 7764-7780.

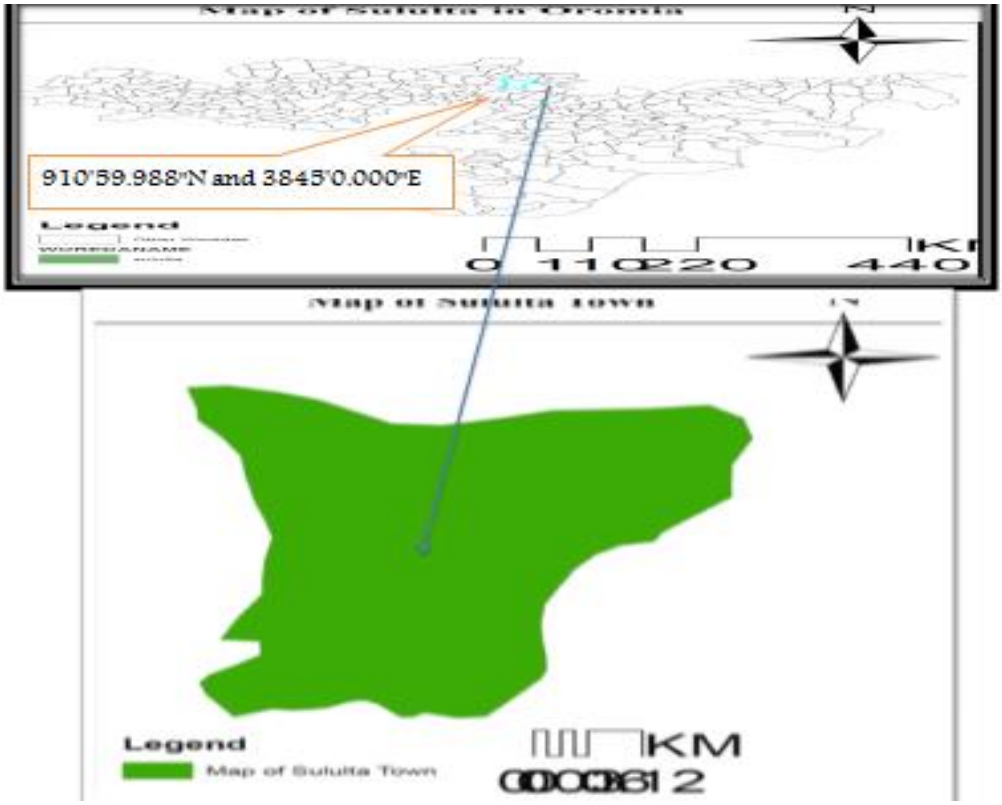
World health organization (2106): global report on diabetes: 1-88.

Yadav, M., Chatterji, S., Kumar, SG., and Watal, G., (2014). Preliminary phytochemical screening of six medicinal plants used intraditional medicine. Int J Pharm Pharm Sci, 6(5), 539-542.

Yadav, R., and Agarwala, M., (2011).Phytochemical analysis of some medicinal plants. *Journal of Phytology*; 3(12): 10-14.

7. APPENDIXES

7.1. **Map showing *T.schimperi* collection site**



7.2. **Photo of some materials used during experiment**

glucometer | Rota vapour | lyophilizer

7.3. **Photos of animal grouping and some groups of the experiment**

Grouping of mice

Extract treated group of mice

Diabetic control group

7.4. **Photo showing extracts adminstration to laboratory mice**

Measuring of required volume

Calibration to required volume

Administration of extracts

7.5. **Picture indicating steps of fasting blood glucose measuring**

Selecting mice for FBL measuring

Blood dropping on glucose strip

Fasting blood glucose reading

Recording the values