COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF BIOCHEMISTRY

Evaluation of anti-hyperglycemic effect of hydroethanolic extract of *Foeniculum Vulgare* (“Ensilal”) seed on alloxan-induced Diabetic male Wistar albino rats.

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LIST OF ABBREVIATIONS

ADA	American diabetic association
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CE	Cholesterol esterase
CHOD	Cholesterol oxidase
DCCT	Diabetes control and complications trial
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
DNSA	Dinitrosalicylic acid
DPPH	2,2- diphenyl-1-picryl hydrazyl
DPX	Dibutyl phthalate in xylene
EDTA	Ethylene dimethyl tetra acetate
FBG	Fasting blood glucose
GDM	Gestational Diabetes Mellitus
GLUT	Glucose transporter
GSH	Glutathione
H_2O_2	Hydrogen peroxide
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HLA	Human Leukocyte Antigen
HEEFVS	Hydro-ethanolic extract of Foeniculum vulgare seed
HSS	Hyperosmolar syndrome
IC 50	Inhibition concentration
IDF	International diabetic federation
LDH	Lactate dehydrogenase
LD 50	Lethal dose

LPL	Lipoprotein lipase
MDH	Malate dehydrogenase
MODY	Maturity-onset diabetes of the young \NAD
	Nicotinamide adenine dinucleotide
NCD	Non - communicable diseases
NAFLD	Non - alcoholic fatty liver disease
NGSP	National Glycohemoglobin Standardization Program
OECD	Organization for Economic Co-operation Devlopment
OGTT	Oral glucose tolerance test
MDA	Malondialdehyde
PCOS	Polycystic ovary syndrome
POD	Peroxidase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SST	Serum separator tube
STZ	Striptozotochin
T1DM	Type one diabetes mellitus
T2DM	Type two diabetes mellitus
TC	Total cholesterol
TG	Triglyceride
TZDs	Thiazolidinediones

ABSTRACT

Background: - Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from inherited and acquired deficiency in production of insulin by the pancreas, and due to resistance to insulin's effects. DM is one of the leading causes of death, disability, illness, and economic loss in the world. Nowadays herbal medicines have become a good alternatives for the treatment of hyperglycemia. Ethiopia has many plants that are useful in the treatment of different diseases from those fennel has a potential in reducing the hyperglycemic state during diabetes.

Objective: - To evaluate the anti-hyperglycemic effect of *Foeniculum vulgare* (fennel) seed hydro-ethanolic extracts on alloxan-induced diabetic male Wistar albino rats.

Method: -Thirty-six male Wistar albino rats with a weight of 180-250 grams were induced with diabetes using 175mg/kg alloxan monohyderate. After grouping, experimental rats were given daily oral administration of hydro - ethanolic extract of *Foeniculum vulgare* seed (100, 200 and 400mg/kg) for 21consecuative days. Fasting blood sugar, total cholestrol, triglyceride, alanine aminotransferase, aspartate aminotransferase and histopatology of liver was assessed. Glibenclamide was used as a standard reference drug. Additionally in vitro study conducted on Free radical scavenging and α amylase inhibitory activity of the *Foeniculum vulgare* seed hydroethanolic extract was also investigated.

Result: - *Foeniculum vulgare* seed hydro-ethanolic extract showed a highly significant blood-glucose-lowering effect. After induction of diabetes, rats treated with 400mg/kg extract respond in a better, gradual, and statistically significant way to reduce mean blood glucose levels from 352 mg/dl on day 0 to 189.1 mg/dl on 21st day. In addition, a significant reduction was observed in total cholesterol, triglyceride, alanine aminotransferase and aspartate aminotransferase analysis also, liver abnormality restoration were seen in histopatological study. Besides, good anti-oxidative and α amylase inhibitory activity was shown from the in vitro lab tests.

Conclusion: - The result of the experiment showed that *Foeniculum vulgare* seeds may have several bioactive secondary metabolites effective in lowering blood glucose levels and increasing body antioxidants.

Keywords: - Diabetes mellitus, Alloxan, *Foeniculum vulgare*, Wistar albino rats

1. INTRODUCTION

1.1Background

Diabetes Mellitus (DM) is a chronic disease caused by inherited and acquired deficiency in production of insulin by the pancreas, and due to resistance to insulin's effects (Evcimen and King, 2007) commonly presenting with episodes of hyperglycemia and glucose intolerance. Untreated chronic hyperglycemia makes DM a heterogeneous disorder due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids, and proteins. (International Diabetes Atlas, 2009, Peiro *et al.*, 2012).

The long-term effects of diabetes mellitus include the progressive development of retinopathy with potential blindness, nephropathy that may lead to renal failure, and neuropathy with risk of foot ulcers, amputation resulting in increasing disability, reduced life expectancy and enormous health costs for virtually every society (International Diabetes Atlas, 2009).

Diabetes is a public health problem and one of the four priority area of non - communicable diseases (NCDs) targeted for action by world leaders (Chan, 2014). According to WHO, the estimated worldwide prevalence of diabetes among adults in 2010 was 285 million and this value is predicted to rise to around 439 million by 2030 (World Health Organization, 2016). But, the predicted number became almost true in 2017. So, diabetes mellitus becomes a highly increasing chronic disease by doubling its prevalence within few years among adults aged above 18. From this point, the International diabetes federation, 2017 estimated this number could potentially rise to 628 million cases by 2045. DM-related complications were a major cause of disability and reduced quality of life and an estimated 5 million people aged 20–79 years worldwide died prematurely from the disease (International Diabetes Federation, 2017).

Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries and this is intimately linked to changes towards a Western lifestyle in developing countries and rise in prevalence of obesity (Chan, 2014). Around 15.9 million adults in Africa affected by DM with the highest rate of undiagnosed cases (International Diabetes

Federation, 2017). When it comes to Ethiopia currently, the country is also facing an increase in DM among its population both in rural and urban areas. 4.8% of the population live with DM and 1 % of country deaths occur because of it. IDF report indicates that because of this number Ethiopia become the number one country from the top five-listed African country's (World Health Organization, 2016, International Diabetes Federation, 2017). A study conducted in Ethiopia reports that DM, especially type 2 DM, is an important cause of admission to Ethiopia's largest referral hospital. Many patients had already developed disease-related complications at admission, and mortality was high. This shows that there is a need to improve awareness about and care for DM in Ethiopia (Gizaw *et al.*, 2015).

1.2 Literature Review

1.2.1 Normal well-fed state metabolism of fuel

Immediately after a calorie-rich meal, glucose, fatty acids, and amino acids enter the liver. Insulin released in response to the high blood glucose concentration stimulates glucose uptake by the tissues. Some glucose is exported to the brain for its energy and some to fat and muscle tissue. In the liver, excess glucose is oxidized to acetyl-CoA, which is used to synthesize fatty acids for export as triacylglycerols in VLDLs to fat and muscle tissue. The NADPH necessary for lipid synthesis is obtained by oxidation of glucose in the pentose phosphate pathway. Excess amino acids are converted to pyruvate and acetyl-CoA, which are also used for lipid synthesis. Dietary fats move via the lymphatic system, as chylomicrons, from the intestine to muscle and fat tissues (Lehninger, 2005).

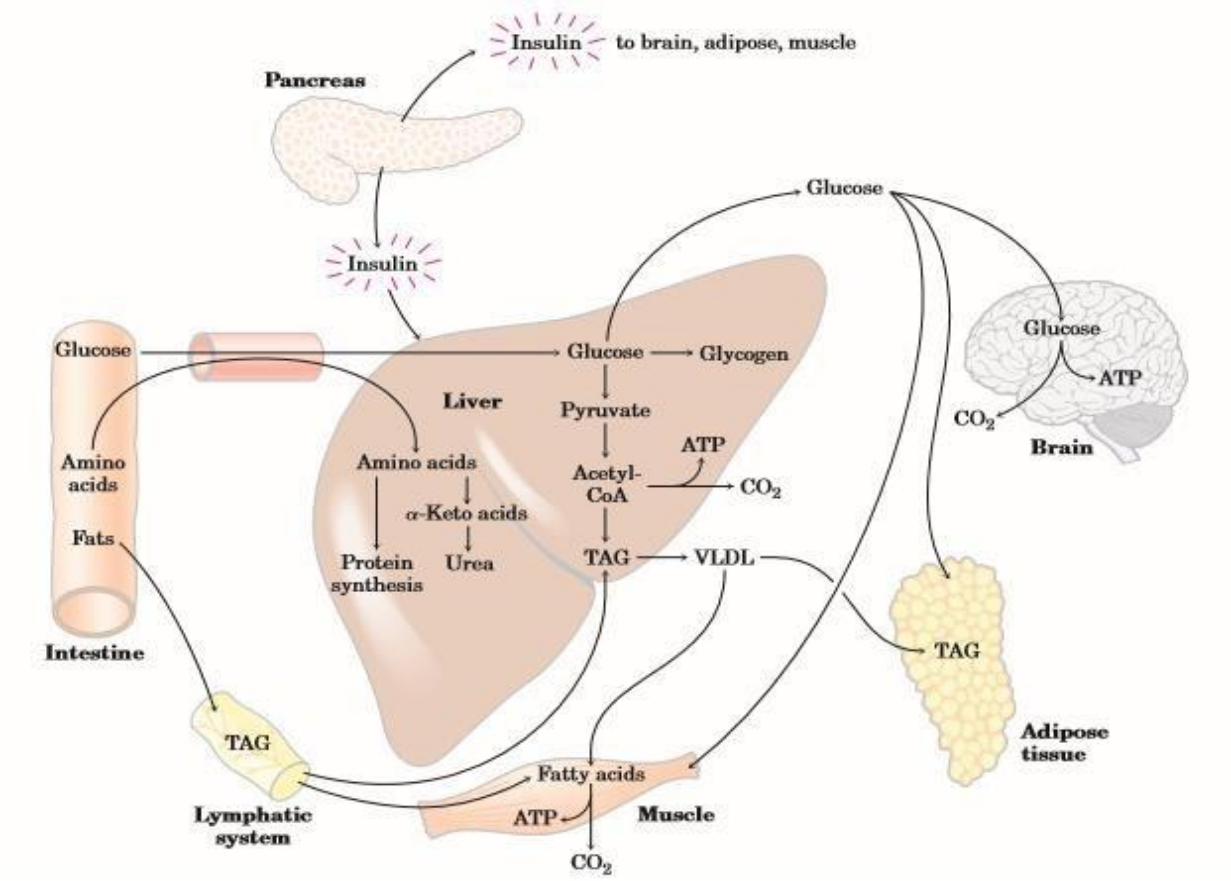


Figure 1 : The fuel metabolism in wel fed state (Adopted from Lehninger biochemistry, 2005)

1.2.2 Classification of Diabetes Mellitus

Classification of diabetes mellitus is based on its etiology, clinical presentation and pathogenic process that leads to hyperglycemia. Diabetes can be classified into the following general categories:

1. **Type 1 Diabetes Mellitus**
2. **Type 2 Diabetes Mellitus**
3. **Gestational Diabetes Mellitus (GDM)**
4. **Specific types of Diabetes Mellitus due to other causes** (American diabetes association 2018).

1.2.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes accounts for 5 - 10% of diagnosed cases. This occurs due to autoimmune destruction of the pancreatic β-cells leading to insulin deficiency hence, the deficiency of insulin cause characteristic symptoms like hyperglycemia, polydipsia, polyuria, and glucosuria. The rate of b-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Individuals with Type 1 must take insulin to live and are at risk for ketoacidosis. The etiology of Type 1 is a combination of genetic and environmental factors. Recently, Type 1 DM was divided into two categories, (a) immune-mediated diabetes (resulting from autoimmune destruction of ß-cells) and (b) idiopathic diabetes (rare forms without a known cause) (American Diabetic Association, 2015).

1.2.2.2 Type 2 Diabetes Mellitus

Type 2 diabetes is the most common form and accounts for 90 – 95% of diagnosed cases. A progressive loss of insulin secretion on the background of insulin resistance. Insulin resistance is defined as improper utilization of insulin by target cells and tissues usually relative (rather than absolute) insulin deficiency (American Diabetic Association, 2015). Most, but not all, patients with type 2 diabetes are obese. Obesity itself causes some degree of insulin resistance. It is often associated with a strong genetic predisposition, more than type 1 diabetes (American Diabetic Association, 2015).

The first phase of insulin resistance stimulates an increase in insulin production by ß-cells. But, in progress ß-cells become unable to take burden this ensues. ß-cell failure due to “exhaustion” but autoimmune ß-cell destruction cannot be the reason for destruction (Thomassian,2017).

1.2.2.3 Gestational Diabetes Mellitus (GDM)

GDM is a temporary condition that occurs in pregnancy and carries long term risk of type 2 diabetes (Chan, 2014) "GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy" This form of diabetes complicates 2-4% of all pregnancies. The pathophysiologic process of GDM is similar to that of Type 2. Weight gain and presence of placental hormones increase insulin resistance. Blood glucose levels should be reassessed following delivery (American Diabetic Association, 2018).

1.2.2.4 Specific types of Diabetes (Monogenic Diabetes)

Types of diabetes mellitus of various known etiologies are grouped together to form the classification called "Other Specific Types" represent a small fraction of patients with diabetes frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years) examples for monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation) (American Diabetic Association,2015).

1.2.3 Pathophysiology of Diabetes Mellitus

Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite its fluctuation. The body has tight regulation behavior and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and insulin secretion (Ozougwu *et al.*, 2013).

1.2.3.1 Pathophysiology of type 1 Diabetes Mellitus

In type 1 diabetes Mellitus the autoimmune destruction of pancreatic β-cells which leads to a deficiency of insulin secretion followed by the abnormal pancreatic α-cells function which responds by excessive secretion of glucagon. This impairs glucose utilization and decreases the expression of a number of genes necessary for target tissues to respond normally to insulin such as glucokinase in the liver and the GLUT 4 class of glucose transporters in adipose tissue. This metabolic derangement promotes glycogenolysis and gluconeogenesis in the liver, uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle. Without insulin, muscle, and adipose cells cannot access glucose to

meet energy requirements. A combination of increased hepatic glucose production, reduced peripheral tissue metabolism, increased availability of free fatty acids and ketone bodies and increase Glucogenic amino acids as a result of proteolysis contributes to the hyperglycemia seen in type 1 Diabetes Mellitus. Mitochondrial oxidation of fatty acids generates acetyl COA that can be further oxidized in the TCA cycle. However, in hepatocytes, the majority of the acetyl COA is not oxidized by the TCA cycle but is metabolized into the ketone bodies (acetoacetate and β - hydroxybutyrate). These ketone bodies are used for energy production by the brain, heart, and skeletal muscle. Production of ketone bodies in excess of the body's ability to utilize them leads to ketoacidosis. A spontaneous breakdown product of acetoacetate is the acetone that is exhaled by the lungs, which gives a distinctive odor to the breath. The kidneys cannot absorb the ever-increasing glucose. So, the excess is excreted in the urine (polyuria). The brain, prompted by this loss of fluid, signals thirst (polydipsia) and hunger (polyphagia). If this process continues, stored fats are metabolized and transformed by the liver into ketoacids, which lower pH levels and cause acidosis. The drop in pH level and loss of ketones in urine signals the onset of ketoacidosis (Ozougwu *et al.,* 2013).

1.2.3.2 Pathophysiology of type 2 Diabetes Mellitus

In type 2 diabetes the two main pathological defects are the impaired insulin action through insulin resistance followed by impaired insulin secretion through a dysfunction of the pancreatic β-cell. Resistance to the action of insulin will result in impaired insulin-mediated glucose uptake in the periphery (by muscle and fat cell), incomplete suppression of hepatic glucose output and impaired triglyceride uptake by adipose tissue(Baynest, 2015). To overcome this condition β -cells increase in capacity of insulin production even if, its unable to make enough insulin to meet the body's perceived need sustainably. As the insulin resistance continues hyperglycemia results in β -cell failure due to "exhaustion" or "glucose toxicity" (American Diabetic Association, 2017). The presence of insulin prevents the lipolysis that would lead to the production of ketones. Hyperglycemic Hyperosmolar Syndrome (HHS) is a life-threatening outcome of neglected hyperglycemia in Type 2 diabetes. Severe hyperglycemia and extreme dehydration characterize this condition (Thomassian, 2017).

1.2.4 Pathogenesis of Diabetes Mellitus

1.2.4.1 Pathogenesis of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is characterized by autoimmune destruction of insulin-producing cells in the pancreas by $CD4^+$ and $CD8^+$ T cells and macrophages infiltrating the islet. Several features characterize type 1 diabetes mellitus as an autoimmune disease such as the presence of immuno-competent and accessory cells in infiltrated pancreatic islets, Presence of islet cell-specific autoantibodies, response to immune therapy and others (Thomassian, 2017).

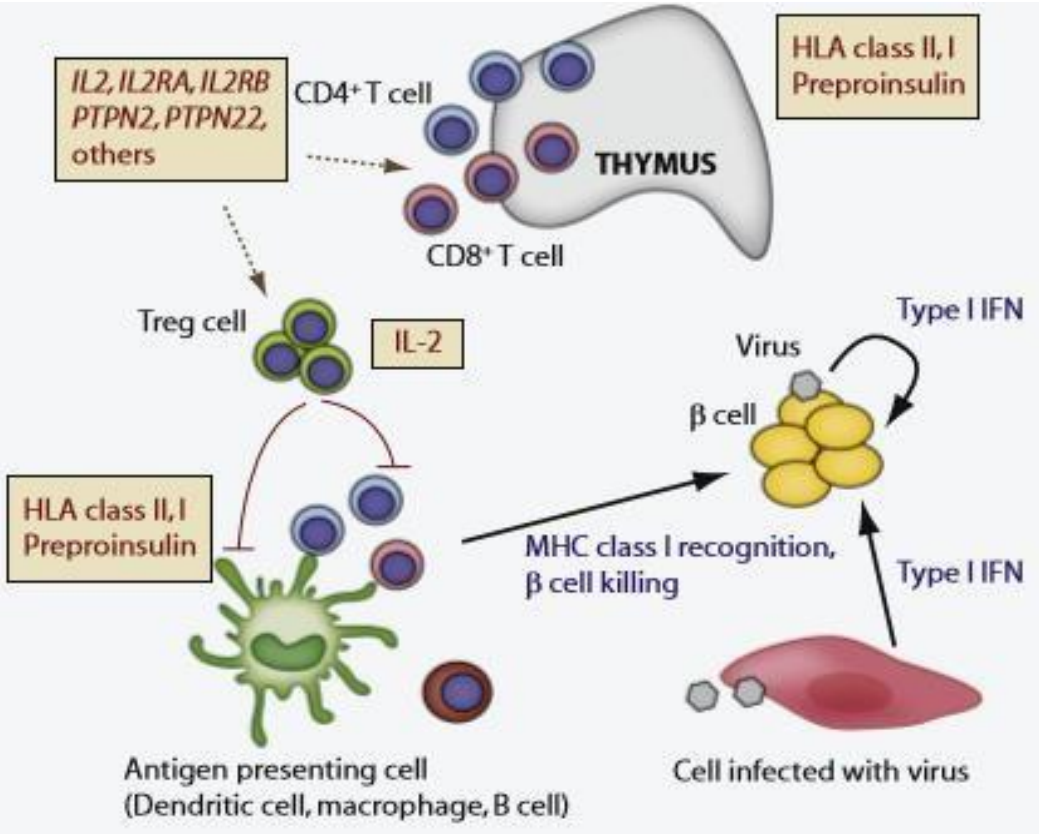


Figure 2: Pathogenesis of type 1 diabetes mellitus

Based on Genetic Etiological Studies in Humans Shown genes (e.g. those that encode MHC HLA class II and class I, antigen-presenting cells, preproinsulin (INS) in the thymus, CTLA-4 in Treg cells, cytokines) believed to be causal in the process of type I diabetes. Also shown are influences of viral infections and type 1 interferon (IFN) production and effects; autoimmune repertoire development in the thymus; and the main immune cell types, CD4+ and CD8+ T cell subsets, T

regulatory (Treg) cells, and B cells and various other antigen-presenting cells, acting together to kill pancreatic b cells. (Adapted from Todd. JA., 2010)

1.2.4.2 Pathogenesis of Type 2 Diabetes Mellitus

Type 2 Diabetes is a defect in insulin-mediated glucose uptake inperipheal cells, a disruption of secretory function of adipocytes, a dysfunction of pancreatic b-cells, impaired sensing and response to hyperglycemia in the CNS, an excessive accumulation of lipids, and impaired fatty acid oxidation due to obesity, physical inactivity, and genetic predisposition. (lin & sun, 2010).

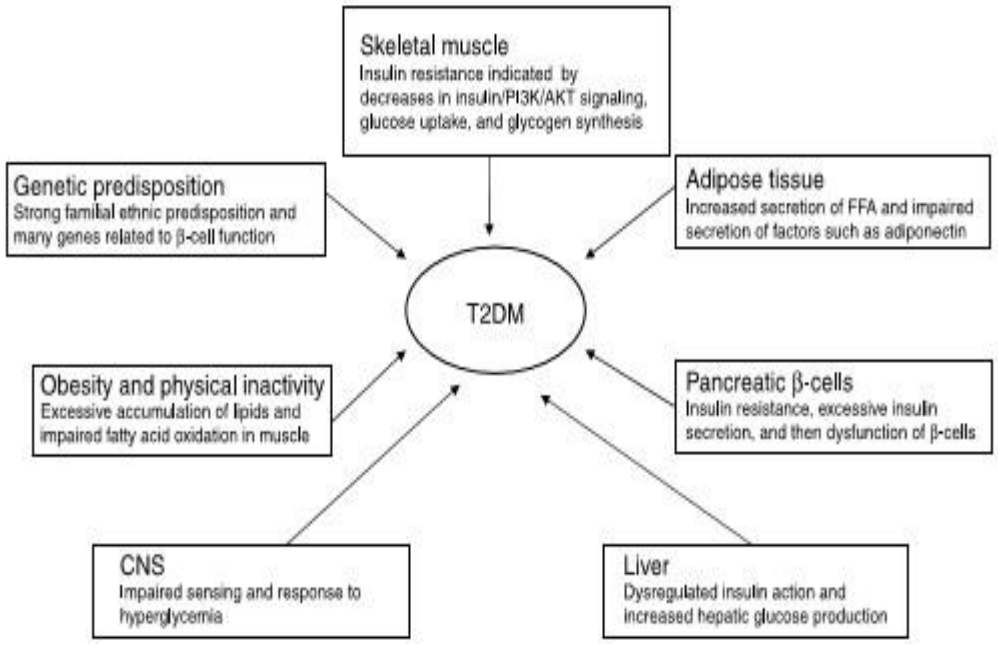


Figure 3: Pathogenesis of type 2 diabetes mellitus (adopted from lin & sun, 2010)

1.2.5 Complication of Diabetes Mellitus

Diabetes of all types can lead to complications in many parts of the body and increase the overall risk of dying prematurely (Chan, 2014) The complications include macrovascular diseases (hypertension, hyperlipidemia, heart attacks, coronary artery disease, strokes, cerebral vascular disease, and peripheral vascular disease), microvascular diseases (retinopathy, nephropathy, and neuropathy) (Volpe *et al*, 2018).

Abnormally high blood glucose can have a life-threatening impact if it triggers conditions such as diabetic ketoacidosis (DKA) in types 1 and 2, and hyperosmolar coma in type 2. Abnormally low blood glucose can occur in all types of diabetes and may result in seizures or loss of consciousness.

It may happen after skipping a meal or exercising more than usual, or if the dosage of anti-diabetic medication is too high (Chan, 2014).

Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids, and proteins emanating from defective insulin secretion, insulin action, or both (Baynest, 2015). Oxidative stress plays a central role in the development of diabetes complications(Brownlee *et al*., 1984). Possible mechanisms for Diabetes complications can be :

1.2.5.1 Excessive Glycosylation

The major consequence of hyperglycemia is excessive nonenzymatic glycosylation of proteins. The stable products thus formed accumulate inside insulin-independent cells, and outside on cell membrane proteins, circulating proteins, and structural proteins. Nonenzymatic glycosylation begins in all cases with glucose attachment to protein amino groups via nucleophilic addition with the formation of unstable Schiff base. Schiff base further chemical rearrangement for days form the second unstable non-enzymatic early glycation products(Amadori), best example is glycosylated hemoglobin. Then Amadori products form very slowly series of further reactions, rearrangements, and dehydrations for months to form a stable advanced glycation end product (AGE). In long term, uncontrolled hyperglycemia (either Schiff base or Amadori product) may be sufficient to significantly impair important functional properties of several critical proteins (Brownlee *et al*.,1984).

1.2.5.2 Sorbitol Accumulation

Aldose reductase normally reduces toxic aldehydes in the cell to inactive alcohols. (Mathebula, 2015). But, in the case of hyperglycemia, 30% of glucose channeled into plyol pathway which cause aldose reductase activity to also goes to reducing glucose to sorbitol by using cofactor NADPH, which is important cofactor to generate GSH(Critical cellular antioxidant)by enzyme glutathione reductase. Oxidation of sorbitol to fructose by sorbitol dehydrogenase causes oxidative stress because its cofactor NAD+ is converted to NADH in the process, and NADH is a substrate for NADH oxidase which generate ROS. Polyol pathway converts glucose to fructose and its metabolites fructose-3- phosphate, and 3-deoxyglucosone. This metabolites are more potent non enzymatic glycation agent than glucose so this all above mentioned contribute to increase in oxidative stress

(Chung, *et al*,2003). sorbitol is a hydrophilic compound that can not diffuse through cell membranes consequently, cause osmotic damage which leads to retinopathy and neuropathy (Mathebula, 2015). Hence, drugs targeting enzyme aldose reductase are important to reduce risk of diabetic complication

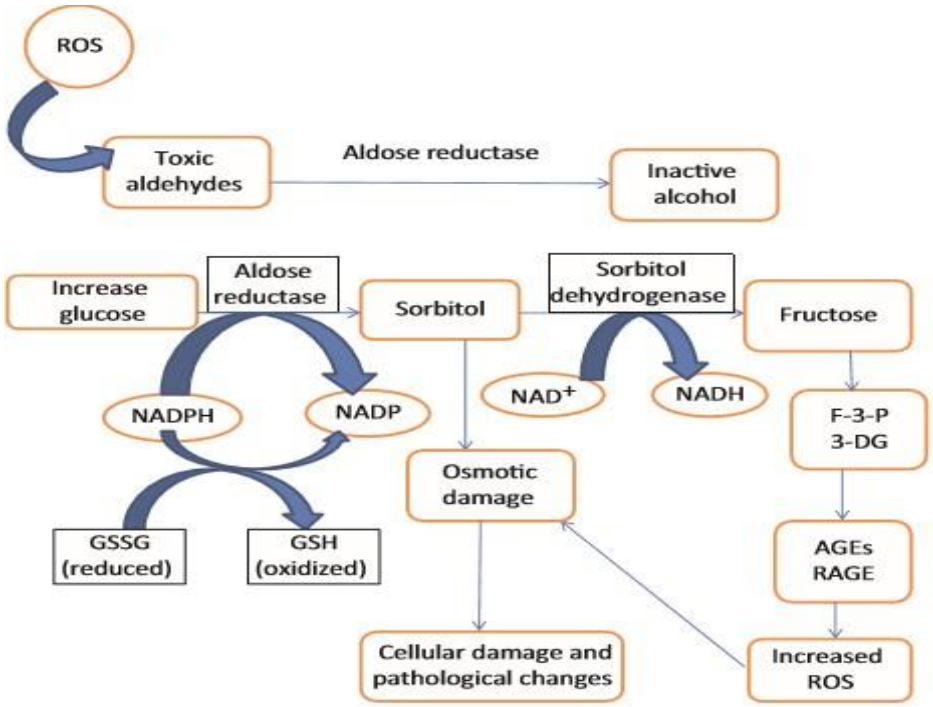


Figure 4: The polyol pathway (adopted from Mathebula, 2015).

1.2.6 Diagnosis of Diabetes Mellitus

Diabetes mellitus is characterized by persistent hyperglycemia, and it can be diagnosed by demonstrating one of the following tests:-

Fasting blood glucose test ≥126 mg/dL (7.0 mmol/L) Fasting is defined as no caloric intake for at least 8 hours. (American diabetes association, 2018).

Two-hour plasma glucose ≥200 mg/dL (11.1mmol/L) during an Oral glucose tolerance test (OGTT), the test should be performed as described by WHO, using glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. (American diabetes association, 2018).

Hemoglobin A1c ≥ 6.5% (48 mmol/L)**.** The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay(American diabetes association, 2018). Glycated hemoglobin level provides a reliable measure of chronic glycemic control without

the need for fasting or timed sample and it correlates well with the risk of long term diabetes complications and mortality. Measures how much glucose is stuck to red blood cells (World Health Organization, 2011).

Random plasma glucose ≥ 200mg/dL (11.1 mmol/L).In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis (American diabetes association, 2018).

1.2.7 Pathophysiology of oxidative stress in Diabetes Mellitus

The pathogenesis of diabetes in both types 1 and 2 is supported by oxidative stress as different researches reports indicate. The main source of oxidative stress in DM is during oxidative metabolism in mitochondria. A component of the utilized oxygen is reduced to water and the remaining oxygen is transformed to oxygen Free radical (O^-) which is an important ROS that is converted to other Reactive species such as ONOO, OH, and H_2O_2. Insulin signaling is modulated by ROS/RNS in two ways. On one side, in response to insulin, the ROS/RNS are produced to exert its full physiological function and on the other side, the ROS and RNS have got negative regulation on insulin signaling, interpreting them to develop insulin resistance which is a risk factor for diabetes type 2 (Asmat *et al.,* 2016).

1.2.8 Treatment of Diabetes Mellitus

1.2.8.1 Insulin

Insulin is a polypeptide hormone synthesized in humans and other mammals within the beta cells of the islets of Langerhans in the pancreas. The islets of Langerhans from the endocrine part of the pancreas, accounting for 2% of the total mass of the pancreas (Peiro *et al*.,2012). Insulin is important in keeping blood glucose levels within acceptable limits by enabling glucose to enter cells and provide energy (International Diabetes Federation, 2015). Insulin is more of an anabolic hormone rather than catabolic. Insulin production is directly proportional to the amount of sugar (carbohydrate) consumed. Higher insulin levels upregulate various anabolic processes including cell growth, cellular protein synthesis, and fat storage. Insufficient amounts of insulin or poor cellular response to insulin as well as defective insulin lead to improper handling of glucose by body cells which leads to metabolic derangement (Peiro *et al*.,2012).

Insulin is injected into the body by people with type 1 diabetes in whom the cells that produce insulin have been destroyed so it is life-saving and life long. Insulin may also be used by people

with type 2 diabetes when the body needs more insulin than it can produce (International Diabetes Federation, 2015).

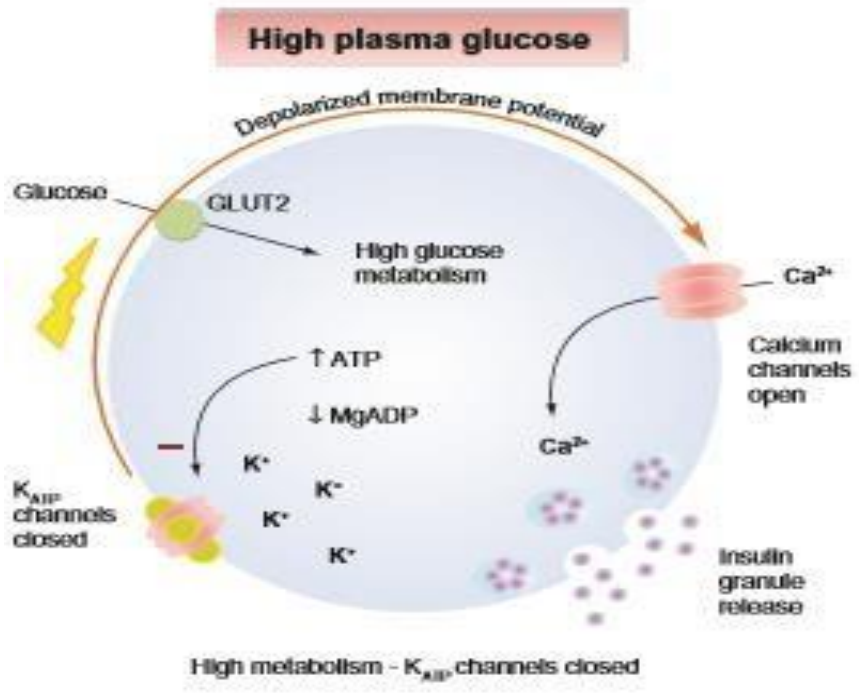


Figure 5: Insulin secretion by pancreatic beta cells to regulate blood glucose

In fed state, β cell glucose metabolism raises intracellular [ATP], which leads to the closing of K^+ channels that depolarizing the membrane potential. And opening of voltage-gated Ca^{2+} channels. Cytosolic increase in Ca^{2+} triggers insulin release by exocytosis (adopted from Lang and Light, 2010).

1.2.8.2 Life style management

It is apparently the cornerstone of the management of diabetes mellitus. It is recognized as being an essential part of diabetes and cardiovascular disease prevention. Meta-analyses demonstrate that lifestyle interventions, including diet and physical activity, led to a 63% reduction in diabetes incidence in those at high risk. Dietary management has a positive effect on long term health and quality of life. Dietary management aims at optimal metabolic control by establishing a balance between food intake, physical activity, and medication to avoid complications. Plant-derived medications have also found immense use in the management of diabetes mellitus.

1.2.8.3 Drugs

Sulfonylureas like glibenclamide establish normoglycemia by upregulating endogenous insulin secretion, thiazolidinediones (TZDs) maintain normoglycemia by enhancing insulin sensitivity in the peripheral tissue, α-glucosidase and α-amylase inhibitory drugs delay intestinal carbohydrate absorption and minimize postprandial hyperglycemia, metformin works by decreasing hepatic gluconeogenesis while at times also increases peripheral glucose mobilization. Despite many effective oral anti- hyperglycemic agents available to manage type 2 diabetes, 5% to 10% of the population with diabetes experience diabetic complication (Piero *et al*., 2012).

1.2.9 Medicinal plant

Plants have played a significant role in maintaining human health and improving the quality of life since ancient times. According to WHO about three-quarters of the world's population rely upon traditional medicine when it comes to their primary healthcare needs, and most of these treatments involve the use of plant extracts or their active components. The medicinal value of these herbs lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Pagan *et al*., 2005).

Plant derivatives with anti- hyperglycemic properties had been used in folk medicine and traditional healing systems around the world from very ancient times. If treatment means to cure the disease, there is no drug that can cure diabetes completely. The primary target for a diabetic patient is to bring blood glucose levels close to the normal value (Mohamed *et al.*, 2013). Some of blood glucose lowering plants used in ethiopia are Allium sativum (Thomson *et al*., 2007) , Curcuma longa (Masry, 2012), Moringa-stenoptela(Nardos*et al*.,2011),Nigella sativa(Salama,2011), Zingiber- officinale(Jafri *et al.*, 2011). Fennel (*Foeniculum vulgare*) is also one of the medicinal plant with blood glucose reducing effect.

1.2.9.1 *Foeniculum vulgare*(Fennel)

1.2.9.1.1 Botanical and common names of *Foeniculum vulgare*

Fennel (*Foeniculum vulgare*) is a biennial or short-lived perennial herb attaining a height of up to 2 meters. Stems are erect, furrowed and branched. Leaves are pinnate, decompound and finely divided. The small yellow flowers appear in terminal compound umbels. The seeds are oval, ribbed, 5 - 10 mm long and with strong and sweet smell and are blue-green at first turning into greenish-brown on ripening (Vinodhini, 2016). In Ethiopia, the plant is known by name "ensilal" in Amharic. Commonly used as additional ingredient for traditional alcohol drinks like"areke",

additional ingredient spice for preparation of perper (mitmita), mouth refershiner, traditional medicine as diuretic agent and as anti-hypertensive agent.

Figure 6: Flower, bulb, seed, and population of *Foeniculum Vulgare*

It is generally considered indigenous to the shores of the Mediterranean Sea but has become widely naturalized in many parts of the world especially on dry soils near the seacoast and on the river banks. Fennel has been used as folk medicine according to available researches that show itspharmacological properties in the treatment of glaucoma, hypertension, respiratory tract disorders, and indigestion. Also, used as a relaxant, diuretic, estrogenic, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-cancer and anti-stress. The medicinal properties of the plant is due to its different bioactive phytochemicals (Elizabeth *et al.*, 2014, Kooti *et al.*, 2015).

1.2.9.1.2 History of *Foeniculum vulgare*

The ancient Chinese, Indian, Egyptian and Greek civilizations knew the herb fennel. Some historical notes say the name *Foeniculum* is derived from the Latin word meaning fragrant hay naming because of its smell. Word fennel was also a place name Marathon (meaning "place of fennel"), naming after Greeks fought in 490 BC in the field of fennel and succeed at the Battle of Marathon. During the 13th century in England, fennel was considered a royal spice due to its property of increasing milk secretion, promoting menstruation, facilitating birth, and increasing the libido then in1930 researchers became interested to develop synthetic estrogens from it (El-Soud *et al.*, 2011).

1.2.9.1.3 Classification and composition of *Foeniculum vulgare*

The genus *Foeniculum* (fennel) belongs to Kingdom: Plantae; Order: Apiales; the family Apiaceae (Umbelliferae); Genus: *Foeniculum* Species:Vulgare; Binomial name: *Foeniculum Vulgare*. There are two sub-species of fennel piperitum, and Vulgare: *Foeniculum piperitum* has

bitter seeds and the later one *Foeniculum Vulgare* has sweet seeds (anise odor is due to this characteristic). Sweet fennel used as flavoring agents in baked goods, meat and fish dishes, ice creams, alcoholic beverages, etc. for centuries (Rather *et al.*,2016). Bitter fennel contains 50 % trans-anethole,10–20 % fenchone (which contributes to the bitter flavor). The sweeter variety has 50–80 % trans-anethole, little (5 %) or no fenchone (Vinodhini, 2016). Different Researchers identify 28 bioactive components that give fennel its medicinal value through different techniques. Trans-anethole and estragole were found in the first and second major components respectively followed by limonene, fenchone and other minor components. Fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid) were also detected (Rather *et al.*, 2016, Osman *et al.*, 2017).

Foeniculum Vulgare has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals,18.5% fibre and 42.3% carbohydrates. The minerals and vitamins present in *Foeniculum Vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C and vitamin E (Rather *et al.*, 2016). Ethiopian fennel fruit is rich in Ca and Mg and other essential elements Fe, Cu, Co and Zn and toxic element Cd is at trace level with undetected Pb (Endalamaw and Chandravanshi, 2015).

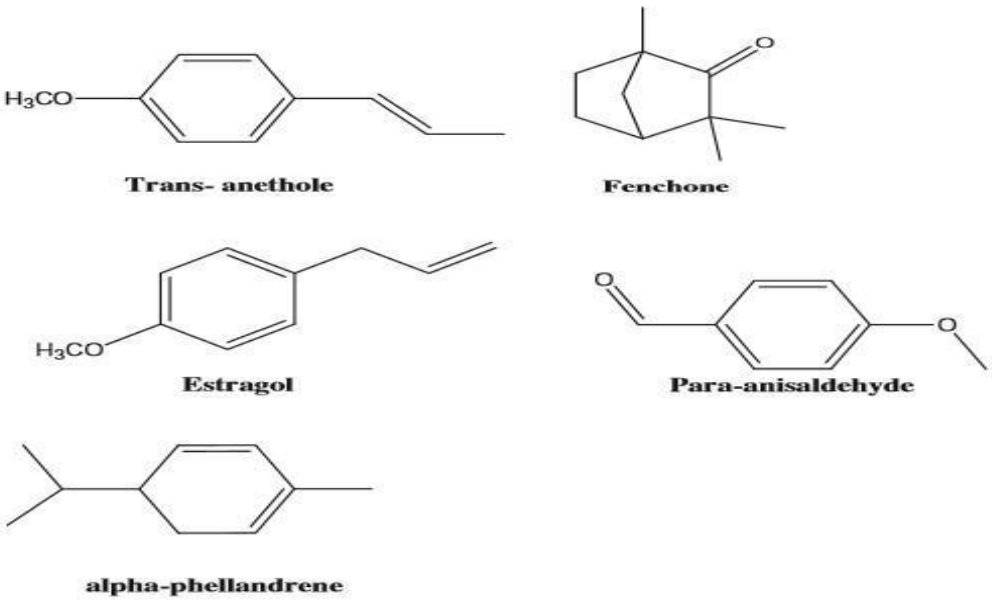


Figure 7: The molocular structure of the major bioactive components of *Foeniculum Vulgare* (adopted from Rather *et al.*, 2016)

1.2.9.1.4 Absorption, metabolism and excretion of *Foeniculum vulgare*

No data were available for fennel's metabolism in human beings or animals. However, the study was done on trans-anethole (the main active compound found in fennel in large amounts) by oral administration of radioactively labeled trans-anethole to five healthy volunteers. It was rapidly absorbed, 54-69% of the dose was eliminated in the urine and 13-17% in exhaled as carbon dioxide; none was detected in the feces. The bulk of elimination occurred within 8 hours (EMA, 2008).

1.2.9.1.5 Mechanism of action of *Foeniculum vulgare*

Studies showed the different mechanism of action for *Foeniculum vulgare* to act as an anti-hyperglycemic agent such as, antidiabetic action of fennel is by its active component trans-anethole which is isolated from fennel with potent aldose reductase inhibition reaction and anti-cataract activity through the increase in soluble lens protein, reduced glutathione, catalase, and SOD activity. Prolonged treatment with fennel shows improvement in blood glucose, lipid profile, glycated hemoglobin (Dongare *et al.,* 2012). Antioxidant property of fennel active components such astriterpens, steroids, saponins and may relieve diabetes and its complications via their ability to stimulate insulin secretion" (El-Soud *et al.,* 2011) and increased levels of liver and muscle glycogen (Kooti *et al.,* 2015) .

1.2.9.1.6 Therapeutic effect of *Foeniculum Vulgare*

Fennel used as an important ingredient in various folk medicines throughout the world. The leaves, stalks, and seeds (fruits) of the plant are edible (Kooti *et al.,* 2015). A series of studies showed that *Foeniculum.vulgare* has high antioxidant potency so this proved that all parts of this plant act as strong phytotherapeutic agent in disease processes like coronary vascular disease, inflammatory disease, carcinogenesis and aging (Rather *et al.,* 2016, Sharopov *et al.,* 2017) also, have hepatoprotective, antiinflammatory, antidepressant, anti-tumor activity, anti-microbial and anti-diabetic. But in Ethiopia, this plant commonly used as a diuretic, mosquito repellant, mouth refreshing and anti-hypertensive (Vinodhini, 2016).

Table 1 Some of the theraputic potential of *Foeniculum Vulgare*

Therapeutic effect	Extraction solvent	Conclusion of the study	Reference
Anti-cancer	Methanol	Exhibited antitumor, antioxidant, cytotoxic effect and have a capacity to serve as a nontoxic radioprotector in Swiss albino mice.	Mohamad *et al*., 2011
Anti-helminthic	Essential oil	Displayed moderate in vitro schistosomicidal activity against adult S. mansoni worms, exerted remarkable inhibitory effects on the egg development and was of low toxicity.	Wakabayashi *et al*., 2015
Anti-inflammatory	Essential oil	Show significant anti-inflammatory effect in Sprague-Dawley rats and swiss albino mice.	Ozbek, 2005
Renoprotective effect	Aqueous	used with phytoestrogens compounds for polycystic ovary syndrome (PCOS) treatment. and show renoprotective effect in experimental PCOS female Wistar rats.	Sadrefozalayi et al., 2014
Anti-bacterial	Aqueous Chloroform Methanol	Exhibit good inhibitory effect on the growth of gram-positive and gram-negative bacteria.	Abebie et al.,2017
Hepatoprotective	Methanol	Fennel has a potent hepatoprotective action against CCl4-induced liver fibrosis in Wister rats.	Özbek et al.,2004

1.2.9.1.7 Fennel (*Foeniculum vulgare*) and Diabetes mellitus

More than 400 plants with glucose-lowering effects are known. Fennel is one of the important spices that cure many diseases. It has been supported by different researches that explored different chemical extrac and essential oil of *Foeniculum vulgare* to correct hyperglycemia reduces chronic complications associated with diabetes, restore dyslipidemia, enhance levels of liver and muscle glycogen and preserving the integrity of beta-cells of the pancreas and oxidative stress (Kamble*et al.*, 2015).

So anti-oxidative effect of fennel makes researchers suggest fennel as a new alternative for clinical management of diabetic patients, and preventive measure for uncontrolled diabetic complication (Rezq, 2012)

1.2.10 Alloxan

1.2.10.1 Alloxan and its mechanism of action to induce Diabetes mellitus

Alloxan is cytotoxic; diabetogenic chemical used in experimental diabetes research most of the time. Alloxan is selectively toxic to β-cells of the islets of Langerhans by structurally mimicking glucose. Three mechanisms are used by alloxan act on pancreatic beta cells that are inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Rohilla and Ali, 2012). As a result, alloxan causes a massive reduction in insulin release to meet its goal of inducing hyperglycemia (Guria *et al*., 2014, Cheekati *et al.*,2017).

Alloxan, transport through GLUT 2 transport system and enter in beta cells to react with two SH groups in the super binding site of glucokinase and breakdown disulfide bond this inactivate glucokinase and alloxan become reduced to dialuric acid . In cyclic redox reaction autoxidation of dialuric acid generates superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) (Rohilla and Ali, 2012, Guria *et al*., 2014). In addition, Alloxan has the ability to depolarize pancreatic beta cells that further open voltage-dependent calcium channels and enhances calcium entry into pancreatic cells this further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause cellular damage β-cells and final death of beta cells of pancreatic islets (Rohilla and Ali., 2012).

Alloxan taking rats show metabolic derangements like an increase in blood glucose, cholesterol, alkaline phosphate, transaminase, bilirubin and a decrease in the total protein (Cheekati *et al.*,2017). The resultant metabolic derangement affects the liver. Development of atrophy of pancreatic islets, pyknosis of islets cells, central vein congestion of liver with significant dilatation of sinusoidal spaces and pyknosis of nuclei of hepatocytes are observed derangements of pathological observation (Guria *et al*., 2014).

1.2.10.2 Phases of Diabetes induction by alloxan

Alloxan induces a multiphasic blood glucose response when injected into an experimental animal, the first phase that comes into view within 30 minutes after alloxan injection is a transient hypoglycemic phase. Transient hyperinsulinemia may be attributed to a temporary increase in ATP availability due to inhibition of glucose phosphorylation through glucokinase inhibition. The second phase appearing

one hour after administration of alloxan leads to a rise in blood glucose concentration lasts for 2-4 hours. Accompanied by decreased plasma insulin concentration. These changes are a result of inhibition of insulin secretion from the pancreatic beta cells that are attributed to the induction due to their beta-cell toxicity.The third phase is again a hypoglycemic phase that is noted 4-8 hours after the alloxan injection, which lasts for several hours. The flooding of circulation with insulin occurs as a result of the alloxan-induced secretory granule and cell membrane rupture resulting in severe transitional hypoglycemia. In addition, other subcellular organelles loose structural integrity, which include cisternae of rough endoplasmic reticulum, Golgi complex and mitochondria. These changes are irreversible and highly characteristic for necrotic cell death of pancreatic islets. Fourth phase is the final permanent hyperglycemic phase during which complete degranulation and loss of the integrity of the beta cells within 24-48 h after administration of the alloxan takes place (Rohilla and Ali,2012).

1.3 Statement of the problem

Diabetes Mellitus (DM) is now one of the most common non-communicable diseases with an estimated global prevalence of 425 million and it will reach 628 million by 2045. From this type 1 diabetes account 1 million children and adolescents. Diabetes is the fourth leading cause of death in most high-income countries and there is substantial evidence that it is epidemic in many economically developing and newly industrialized nations. Diabetes mellitus is a very expensive disease with a total health expenditure of 727 billion US dollars and has profound implications interms of long-term microvascular and macrovascular complications. These complications reduce both life expectancy and quality of life (International Diabetes Atlas, 2009, World health organization,2016).

In Africa 15.9 million peoples are living with diabetes in 2017 from this number Ethiopia takes 4.8 % and became the first from top 5 listed African countries with a high number of diabetic population and1% of death in the country caused by increasing blood sugar and its complication (International Diabetes federation, 2017).

People with type 1 diabetes, can live healthy and fulfilling lives with the provision of an uninterrupted supply of insulin and blood glucose testing equipment, when combined with a healthy lifestyle but, in Ethiopia the needs of the patients is increasing as the prevalence of the disease is rising even 66,000cases are undiagnosed yet (International Diabetes Atlas, 2009). Inadequate provision of basic social needs like health care service and costs for the treatment plays roll in increasing the prevalence of life-threatening complications as a result of these it is necessary to develop additional efficient, easily available, therapeutic strategies and appropriate prevention measures for the control of diabetes and its complication.

Therefore, this study is aimed to investigate the anti- hyperglycemic effect of hydro-ethanolic extract of Ethiopian Fennel seed (*Foeniculum vulgare*) in a diabetic rat model. The anti-hyperglycemic effect of this plant has not been investigated yet in our country.

1.4 Significance of the study

In Ethiopia, there is a high prevalence of diabetes mellitus and it is increasing from time to time. If left uncontrolled, it can cause damage to various body organs which is leading to the development of disability and life-threatening health complications. On the other hand, if appropriate management of diabetes is achieved, these serious complications can be delayed or prevented (Piero *et al*., 2012). . It is estimated that 70 to 80% of people worldwide rely on traditional herbal medicine to meet primary health care needs. WHO has encouraged and recommended the use of herbs as an alternative therapy for diabetes mellitus (Assefa *et al*., 2017). There are a number of synthetic drugs that are available in the market they are life long, expensive and associated health side effects are a challenge that worsen the health and economic burden of the disease especially in developing nations. So, the search for improved, cheaper, available and safer natural anti-diabetic agent should be consolidate in order to alleviate the above mentioned problems. Fennel is a widely available medicinal plant also used as food in different dishes in the world (EMA, 2008) this research project aims to evaluate, the anti-hyperglycemic effect of fennel seed extract in a diabetic rat model. This might be useful for encouraging fennel as one of the possible traditional medicine, which helps to reduce blood glucose and lower risk of diabetic complications in Ethiopia because fennel is well known by it's effectiveness in delaying or lowering diabetic complications. Many countries use fennel as part of food and they got benefit from it. In Ethiopia, this plant is available but not well known. This research aims to promote use of fennel and make people especially DM patients to get benefit. Therefore, the present study provides information on the anti- hyperglycemic effect of fennel in addition to providing a base line data for future researches.

1.5 Hypothesis

Foeniculum v*ulgare* seed hydro-ethanolic extract has Improving effect on fasting plasma glucose, serum biochemical parameters and histo pathological changes in liver of alloxan-induced diabetic male Wistar albino rats.

2. OBJECTIVES

2.1. General Objective

To evaluate the anti-hyperglycemic effect of the hydro-ethanolic extracts of *Foeniculum vulgare* (Fennel) seed on alloxan-induced diabetic male Wistar albino rats.

2.2. Specific Objectives

- ✓ To assess the in vitro antioxidant effect of *Foeniculum vulgare* hydro-ethanolic seed extract.
- ✓ To assess the in vitro alpha-amylase inhibitory effect of *Foeniculum vulgare* hydro-ethanolic seed extract.
- ✓ To assess the effect of *Foeniculum vulgare* seed hydro-ethanolic extract on the fasting blood glucose levels of alloxan-induced diabetic rat models
- ✓ To evaluate diabetes-induced changes in Aspartate Transaminase and Alanine Transaminase and reversal using *Foeniculum vulgare* hydro-ethanolic extract
- ✓ To evaluate the effect of *Foeniculum vulgare* hydro-ethanolic seed extract on diabetes-induced change in total cholesterol and triglyceride
- ✓ To assess the effect of *Foeniculum vulgare* seed hydro-ethanolic extract on the body weight of alloxan-induced diabetic rat models
- ✓ To examine the effect of hydro-ethanolic extract of *Foeniculum Vulgare* seed treatment on diabetes-induced changes of liver histology.

3. MATERIALS AND METHODS

3.1 Study design

Randomized experimental study.

3.2 Study Area

The study was conducted at the Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University.

3.3 Drugs, Chemicals, and Instruments

Alloxan monohydrate & 2,2-diphenyl-1-picryl-hydroxyl (Sigma-Aldrich, Germany), Ascorbic acid (BDH chemical Ltd, UAE), α-amylase & 3,5-dinitrosalicylic acid (Cisco Ltd company, India), Hydrochloric acid ,Sulfuric acid, Ethanol, Methanol, Iodine, Potassium iodide, Chloroform, Ferric chloride and Formalin from (Research –Lab fine chem industries, India) .

Lyophilizer (OPR-FDU-5012,Korea), Rotary evaporator (Heizbad Hei-VAP, Germany), Light microscope (LEICA DM750), Centrifuge (PLC 012E ,Taiwan), High definition colored camera (PC1732,China), Deep freezer(- 80o C), Shaker (G25,USA) , Spectrophotometer (UV-1600 PC, Germany), SensoCard glucometer and strip (77 Electronic Kft, Hungary) , Auto lab 18 clinical chemistry analyzer (Italy).

3.4 Study animals

The experimental animals used in this study were adult Wistar albino rats (*Rattus norvegicus Domestica*). Thirty six male and ten female Wistar albino rats weighing 180-220 gram (initial) were obtained from the Department of Pharmacology, School of Pharmacy, CHS, AAU. Female rats were used for acute toxicity tests of the extract but excluded from the main study because of their cyclic hormonal variations, which may result in a change in biochemical parameters. The rats were placed in the animal house of the Department of Physiology, SOM, CHS, and AAU and acclimatized with rat pellets and water provided *ad libitum* for two weeks. Rat pellet supplied by Kality Animal Nutrition Production Ltd., Addis Ababa, Ethiopia.

The room was well- ventilated, maintained at room temperature and natural 12/12hr light and dark period. The animals were housed 6 rats per cage (male rats) and 5 rats per cage (females) in polypropylene cages (size 47 cm x 34 cm and 20 cm hight) with a wire mesh top and a

hygienic bed of husk (regularly changed every three days). Cages were labeled and specific code was given (on the tail) for all rats.

3.5 Plant materials and authentication

The seed of *Foeniculum vulgare* specimen was collected from wild area of Gara muleta. Gara muleta is located in the East Harargee (one zone of Oromia regional state). It is located 335 km away from Addis Ababa at Latitude of 9^{o} 4' 60" N and Longitude 41^{o} 45' 0"E and at altitude of 2057 meters above see level. The site receives mean annual rainfall of between 1270 and 1280 mm with average anuall temperature 16^{o}C (http://www.et.gov.view.info/gara-muleta) .The specimen was kept in a plastic bag and transported to Addis Ababa within a day of collection. The Specimen was pressed, identified and authenticated at National Herbarium, College of Natural science, Adiss Ababa university using Ethiopian flora volumes. It was identified as "*Foeniculum vulgare* Miller'' as the family of "Apiaceae'' and numbered as F-001 and deposited at National herbarium AAU for further reference.

3.6 Extraction of plant material

The plant material was brought to the department of biochemistry laboratory where the study was conducted. Then carefully washed with distilled water to remove any dirt materials, dried under shade at room temperature, grounded to a coarse powder using electronic grinder and the hydro-ethanolic extract of the seeds of the plant was prepared as follows:300g of the *Foeniculum vulgare* powdered seed was weighed by electrical balance and macerated in 1000mL of 80% ethanol (v/v) for 24 hours in a shaker (Anwar *et al*., 2009). After a 24 hour maceration supernatant was poured into other flask and further filtered through a Whatman No. 2 filter paper. The filtrate was evaporated to dryness under reduced pressure by Rotary evaporator and further concentrated by water bath at 45°C then freeze-dried at 4°C. On the next day, the frozen extract was allowed to dry in a freeze dryer (lyophilizer) until the freeze-dried product was obtained. Then the extract was dried, collected in airtight plastic containers, weighed, labeled and put in a desiccator until used for subsequent experimental tests (Anitha *et al*.,2014). The percentage yield of extraction was calculated by the formula:

$$\text{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

3.7 Qualitative phytochemical analysis

Phytochemical screening tests were carried out for the 80% ethanol extracts of the *Foeniculum vulgare* seed using standard procedures to identify the presence of secondary metabolites like tannins, saponins, flavonoids, alkaloids, glycosides, phenol and terpenoids. .

Test for tannins :- 0.5 g of samples was boiled in 20 ml of water in a test tube and then filtered. Two drops of 0.1% ferric chloride was added and brownish green or a blueblack coloration indicatie the presence of tannins (Ade-Ajayi *et al*., 2011).

Test for saponins :- 5g of extract was mixed with 20ml of distilled water and then vigorously shaken in a test tube and left to stand. Foaming (frothing) was observed which lasted for 15 minutes. This showed the presence of saponins (Mujeeb et al., 2014).

Test for flavonoids :- 2g of the extract was heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture was filtered and the filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for appearance of light yellow color, light yellow colour indicates the presence of flavonoid and when drops of diluted NaOH and HCl was added the yellow solution turned colorless (Sheel *et al*.,2014).

Test for alkaloids (Wagner's test) :- Extract was dissolved in diluted hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids. Filtrates were treated with Wagner's reagent (potassium iodide solution). The formation of brown or reddish brown precipitate indicated the presence of alkaloids (Anyasor *et al*., 2014).

Test for Phenols (**Ferric chloride test)** : - 10 mg of plant extracts were treated with few drops of ferric chloride solution. The formation of bluish-black color indicated the presence of phenols(Usman *et al*., 2009).

Test for glycosides :- 5 mL of plant extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. Then 1 mL of concentrated sulphuric acids added
. A brown ring at the interface indicated the presence of glycosides (Sheel *et al*., 2014).

Test for terpenoids :- 5 mL of plant extract were mixed with 2 mL of chloroform and 3 mL of concentrated H_2So_4 to form a layer. A reddish-brown precipitate coloration formed at the interface indicated the presence of terpenoids (Ade-Ajayi *et al*., 2011).

3.8 Extract preparation for the experiment

The concentrations of 100 mg/kg, 200 mg/kg and 400 mg/kg were prepared from hydro-ethanolic extract of *Foeniculum vulgare* seed and water in different container for experimental groups. Glibenclamide mixed with water in other container for diabetic control group. All were freshly prepared daily before administration.

3.9 DPPH Assay for testing the anti-oxidant activity of HEEFVS

3.9.1 Qualitativly assay for anti- oxidant property

Qualitative assay for antioxidant property HEEFV was done on the procedure of (Soler- Rivas et al., 2000) with modification. Hydro-ethanolic extract of *Foeniculum vulgare* (HEEFVS) seed was dissolved in 80% ethanol for the preparation of 250mg/100mL, 500mg /100mL and 750mg/100ml in separate test tubes. 50ml of 1mM DPPH solution was prepared in absolute methanol. Then, this adequate amount of DPPH solution was poured to a wide Petri dish for easy immersion of the TLC plates. After these preparations, 2µL of HEEFVS from the different test tubes were load into 3cm×4cm size of individual TLC plates and waited for 30 minutes until TLC plates completely dried. Then the TLC plates with the dry spot were placed upside down and gently immersed in 0.1mM DPPH solution for 10 seconds. Then the mixture was allowed to stand at room temperature for 30 minutes. As a control, the TLC paper was inserted into the DPPH solution without any extract. After that, the TLC paper was captured by photo depicting the antioxidant activity of HEEFVS at various doses. The wider the zone of inhibition the higher the antioxidant capacity of the plant.

3.9.2 Quantitative assay for anti-oxidant property

Free radical scavenging activity of HEEFVS was examined using the DPPH assay as in (Wickramaratne *et al*., 2016) with slight modifications. The plant extract was dissolved in methanol with different concentrations ranging from 50 to 250 µg/ml in a separate test tube. Ascorbic acid (vitamin C) used as a positive control, prepared with the same concentration as exract. 100 ml of the 0.1mM DPPH in methanol was freshly prepared and kept in dark at 4 °C until use. A volume of 5 ml of methanolic DPPH solution was added into 1 ml of the extract and ascorbic acid solution.the mixture was then incubated at a temperature 37^0c for 30 min in hot oven. Blank solution was prepred by replacing 5ml of DPPH with 1ml of absolute methanol instead of extract or positive standared. Finally , the absorbance of each sample was measured at

517nm using UV-Visible spectrophotometer model 1600 PC. Radical scavenging activity was expressed as the inhibition concentration (IC50). IC50 value of each sample, which is the concentration of sample required to inhibit 50% of DPPH free radical, was obtained from dose vs.inhibition curve using the following formula.

$$\% \text{ Inhibtion} = \frac{Ao - As}{Ao} \times 100$$

Where, Ao is absorbance of the negative control (0.1mM of DPPH solution without test sample) and Abs sample is the absorbance of the solution in the precence of sample extract or ascorbic acid)

3.10 Determination of in vitro α-amylase inhibitory activity of HEEFVS

The α-amylase inhibition assay of HEEFVS was performed using the 3,5-dinitrosalicylic acid (DNSA) method which is described by (Wickramaratne *et al*., 2016) with a slight modification. The hydro-ethanolic extract of *Foeniculum vulgare* seed was first dissolved in buffer((Na_2HPO_4/NaH_2PO_4 (0.02M), NaCl (0.006M) at PH 6)) and concentration range from 62.5 to 1000 μg/ml by serial dilution were prepred. Likewise, a positive standared drug, acarbose solution with similar concentrations was prepared. A volume of 200 μl of the α-amylase solution was mixed with 200 μl of the each concentration of extract and acarbose and incubated for 10 min at 30 °C. Thereafter 200 μl of the starch solution (1% in water (w/v)) was added to each tube. The reaction was terminated 3 mins later by adding 200 μl DNSA reagent (DNSA reagent was made by mixing 12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) test tubes containing all the above added solution were then boiled for 10 min in a water bath at 85 –90 °C. The mixture was cooled to ambient temperature and diluted with 5 ml of distilled water then the absorbance was measured at 540 nm using a UV-Visible spectrophotometer model 1600 PC. Sample blank with 100% enzyme activity was prepared by replacing the plant extract with 200 μl of buffer in a separate test tube. A reagent blank reaction was similarly prepared using the plant extract/acarbose at each concentration in the absence of the enzyme solution was prepared in the same procedure as mentioned above. The α-amylase inhibitory activity is expressed as percent inhibition and was calculated using the equation given below. Finally, the % α-amylase inhibition was plotted

against the extract and acarbose concentration and the IC50 values were obtained from the graph for both extract and standared using the following equation.

$$\% \ \alpha \text{ amylase inhibition} = \frac{(Ac - Acb) - (As - Asb)}{Ac - Acb} \times 100$$

Where Ac refers to the absorbance of control (enzyme and buffer); Acb refers to the absorbance of control blank(buffer without enzyme);As refers to the absorbance of sample(enzyme and inhibitor); and Asb is the absorbance of sample blank(inhibitor without enzyme).

3.11 Acute toxicity test

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance and then animals were individually observed for changes in skin, general behavioral pattern, tremors, convulsions, salivation, postural abnormalities, hair loss, diarrhea, dizziness, impairment in food intake and water consumption, coma and death for a period of 12 hours with special attention and conntinous follow up to 14days. Test doses of hydro-ethanolic extract of *Foeniculum vulgare* seed were calculated in relation to the bodyweight of every fasted female rats and administered via oral gavage at 2000mg/kg body weight (OECD-425, 2008). Then the five rats were observed for any change from the above- mentioned changes if they didn't show any wthin 14 days the test further continued by the dose
3500mg/kg body weight on the other five female rats similarly.

3.12 Induction of Diabetes Mellitus in experimental Rats

In order to induce diabetes, two weeks acclimatized rats were fasted overnight weighed and injected intraperitoneally with freshly prepared alloxan monohydrate 175 mg/ kg of body weight, which was diluted in normal saline in a single dose (Abdel-barry *et al*., 1997). After four-hour rats were allowed to 10% glucose solution for 24 hours to reduce Alloxan induced hypoglycemia (Rohilla and Ali, 2012). At the 48 hours after the induction procedure rats were measured for fasting blood glucose after overnight (12-15) hours fasting. The rats with blood glucose levels above 200 mg/dl were considered diabetic (Carvalho *et al*., 2003). The treatment was started on the 5^{th} day of the final check-up on FBG level and rats above 200 mg/dl were used for further experiment and considered as zero-day of treatment (Jafri *et al.*, 2000).

3.13 Experimental protocol

Treatment with hydro-ethanolic extract of *Foeniculum vulgare* was started after the final diabetes confirmation in the 5^{th} day of induction with diabetes and random grouping of animals. *Foeniculum vulgare* seed extract was given by oral gavage once daily for 21 days. Each rat in the given group was identified by giving a number on its tail by a permanent marker. Prior to the initiation of the experiment, their body weight was measured by an electrical balance and ranged from 180g to 220g.

3.14 Treatment and Animal grouping

These experimental rats were randomly divided into five groups consisting of 6 rats in each group. The experimental animals in different groups were as follows

Group I (Healthy control group)	Rats treated with distilled water only
Group II (Negative control group)	Diabetic rat treated with distilled water
Group III (Treatment group)	Diabetic rats treated with 100mg/kg body weight per day of HEEFVS
Group IV (Treatment group)	Diabetic rats treated with 200mg/kg body weight per day of HEEFVS
Group V (Treatment group)	Diabetic rats treated with 400mg/kg body weight per day of HEEFVS
Group VI (Positive control group)	Diabetic rats treated with 5 mg/kg body weight per day Standard drug (glibenclamide)

Hydro-ethanolic extract of *Foeniculum vulgare* seed (HEEFVS*)* and glibenclamide was administered every morning for 21 days by an oral gavage.

3.15 Sample collection

Fasting blood glucose measurement was done on day 0, 7^{th}, $14^{th,}$ and 21^{st} after the collection of blood samples from the tail vein of the overnight (12-15) fasted rats.

At the end of the experiment, animals were fasted overnight and anesthetized with diethyl ether and 1.5 ml of blood was collected through cardiac puncture using a sterile 3ml syringe. To

prepare serum, the blood sample was transferred into a serum separator tube (SST) and left to clot at room temperature for 30 min immediately following collection. Subsequently, the clotted blood sample was centrifuged at 2000 rpm for 15 min. Finally, the serum was transferred into Nunc tube and stored at -20 ℃ deep freezer until the analyses were performed.

For the histopathological study, rats were killed by cervical dislocation and the extremities of the animals were stretched and fixed on a dissecting board. The abdominal cavity was opened to isolate a sample of the liver then tissue allowed to be fixed in 10% formalin by inserting it in tissue cassette until other procedures were continued in the pathology department.

3.16 Determination of the bodyweight of rats

Bodyweight gain or loss in each experimental rat was measured and recorded before induction of diabetes and on the final experimental day using an electrical balance

3.17 Sample anlysis

3.17.1 Principle of glucometer

Fasting blood glucose was measured with SensoCard glucometer, which applies a glucose oxidase method for the analysis of capillary whole blood glucose. It is specific for β-D-glucose measurement. In the reaction zone, there is enzyme glucose oxidase, which triggers the oxidation of glucose in the blood. Then the intensity of formed electrons measured by the meter correlates well with the concentration of glucose in the blood sample. Result expressed in mg/100 ml of blood.

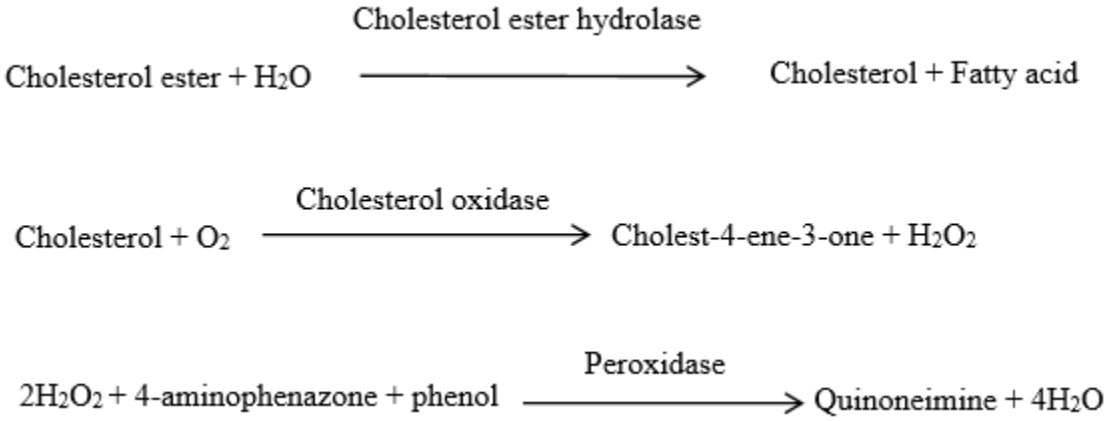
3.17.2 Biochemical analysis

Biochemical parameters measured were total cholesterol (TC), triglyceride(TG), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). All biochemical analyses were carried out by COBAS 6000 (Roche Diagnostics GmbH, Mannheim, Germany)clinical chemistry analyzer in National Reference Laboratory for Clinical Chemistry, at Ethiopian Public health Institute (EPHI).

3.17.2.1 Test principle for Total Cholesterol

Cholesterol esters are cleaved by the action of cholesterol esterase (CE) to yield free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of

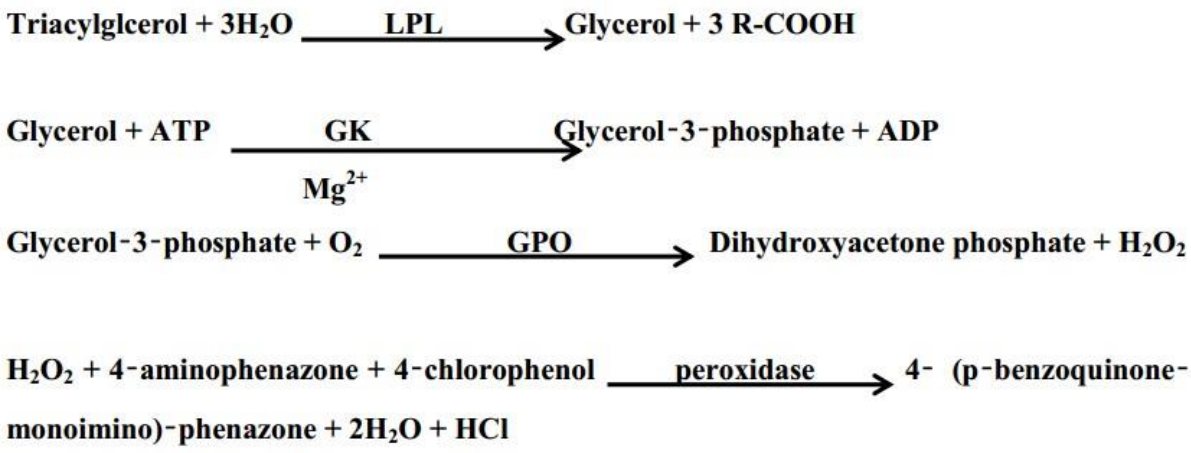
cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine to form a red Quinone- imine dye. The absorbance is measured at 500 nm



3.17.2.2 Test principle for triacylglycerol

Triacylglycerol present in the sample is hydrolyzed by lipoprotein lipase (LPL) to glycerol and free fatty acids. In the presence of ATP, the glycerol is phosphorylated by glycerol kinase to form glycerol-3-phosphate. In the presence of oxygen, glycerol-3-phosphate is oxidized by glycerol phosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red-colored compound. The intensity of the red color is directly proportional to the concentration of triacylglycerol present in the sample. The absorbance is measured at 700/705nm.

Roche/Hitachi Cobas c system automatically calculate the analyte concentration of each sample



3.17.2.3 Test principle for ALT

ALT present in the sample catalyzes the reaction between L- alanine, and 2-oxoglutarate forming L-glutamate and pyruvate. NADH reduces the pyruvate formed in a reaction catalyzed by lactate dehydrogenase (LDH) to form L- Lactate and NAD+. The ALT activity is determined by assaying the rate of NADH oxidation, which is proportional to the reduction in absorbance at 340 nm over time. The rate of the NADH oxidation is directly proportional to the catalytic activity of ALT.

Conversion factor: U/L x 0.0167 = µkat/L

3.17.2.4 Test principle for AST

AST present in the sample catalyzes the transfer of an amino group from L aspartate to 2-oxoglutarate to form oxaloacetate and L- glutamate. In the presence of malate dehydrogenase (MDH), the oxaloacetate is reduced by NADH, to form malate and NAD+. The rate of NADH oxidation is directly proportional to the catalytic activity of AST. It is determined by measuring the decrease in absorbance at 340 nm over time.

Conversion factor : U/L x 0.0167 = µkat / L

Finally the absorbance of the sample (ΔA sample) and the standard (ΔA standard) against the reagent blank were calculated. Concentration of cholestrol, triglyceride , AST and ALT calculated by the formula in mg/dL

$$= \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \quad X \text{ C of standard in mg/dL}$$

3.17.3 Histopathological examination

After blood samples were withdrawn, the rats were sacrificed by cervical dislocation.The liver was taken, rinsed with saline collected under labeled cassettes. Slide preparation and reading were done with a magnification power of 10x and 40x in the Department of Pathology, SOM,

CHS, AAU. Slide picture was taken by a binocular microscope fitted with a camera (Leica ICC50 HD, UK).

Method of processing

1. **Fixation: -** The tissue was preserved/fixed in 10% formalin solution

2. **Dehydration:** - The preserved tissue was washed in running tap water for 4-6 minutes. Then, they passed through upgraded alcohol as follows:- 70% alcohol – 1hour, 85 % alcohol – 1hour, 96% alcohol – 1hour, Absolute alcohol I -1hour, Absolute alcohol II – 1hour.

3. **Clearing**: - Clearing of tissue was done in xylene,-1hour in xylene -I, then after in xylene II for 1 hour.

4. **Infiltration**: - Tissue was infiltrated with paraffin wax I, for 1and 1/2 hrs, paraffin wax II for 2 and 1/2 hrs and paraffin wax III for overnight.

5. **Embedding**: - The cleared tissue was put in molten wax (melting point 56-58degree Celsius) for 12 hours in the cryostat. The paraffin blocks of tissue were made with the help of embedding cassettes.

6. **Sectioning**: - The serial paraffin sections of 5-micron thickness was cut by rotator microtome and floated in a water bath having temperature 45-50 degree Celsius. The section was made spread on the slide smeared with the adhesive solution (a mixture of equal 55 amount of glycerol and egg albumin). The slide was dried on a hot plate having a temperature 50 degrees Celsius.

7. **Deparaffinisation of sections**: - The slide was put in xylene II, changes made for 5-10 min in order to remove the extracellular and intracellular wax.

8. **Rehydration**: - The slide was put in descending grades of alcohol i.e. absolute, 90 %, 70 % and 50% alcohol for 2 min in each case. The slide was then washed in running tap water for 2 minutes and then taken for routine H & E staining.

Method of staining

The slide was Stained with Hematoxyline for 10 minutes. Then Washed in running tap water until the section becomes blue. Again , Stained in 1% eosin for 7- 10 min and washed in running tap water for 5 minutes. The slide Dehydrated through 70 % and 95% Alcohol for 3 minutes each, then absolute alcohol I and absolute alcohol II for 1 and 1/2 hours each. Finally, Cleaned by - Xylene I and Xylene II for 5 minutes each.

9. **Mounted** – By Dibutyl phthalate in xylene (DPX)

3.18 Ethical statement

The study was performed after the protocol was approved by the department of biochemistry research and ethical review committee, meeting number DRERC: 08/18 and a protocol number of M.SC.05/18. All the animal procedures were performed in accordance with the standared guideline for care and use of laboratory animals.

3.19 Statistical analysis

All statistical analyses were performed using SPSS (version 21 for Windows, SPSS, IBM, Chicago, IL, USA). The results were expressed as mean ± standard deviation (M ± SD) for each group. Statistical differences between groups was analyzed by One-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Statistical significance was accepted when $p < 0.001$, $p < 0.01$ and $p < 0.05$.

4. RESULT

4.1 General Observations

Fourty two animals received Alloxan monohydrate of which 33 had fasting blood suger level ranging to 200mg/dl and 400mg/dl that is 78% of rats . Out of the 33 rats with diabetes mellitus 30 diabetic rats were selected randomly to 5 experimental study groups 6 rats for single group. Out of the 9 rats rejected from the experiment 4 were non diabetic and 5 had the undesired blood glucose level.

4.2 Percentage yield of HEEFV

The amount of crude extracts which was obtained from 300-gram coarse Powder of *Foeniculum Vulgare* seed was 46 grams. Therefore, the percentage yield of these extracts by using Hydro-Ethanol (80/20 v/v) was calculated and given as:

$$\text{Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

$$\frac{46g}{300g} \times 100 = 15\% \text{ (w/w)}$$

Therefore, 15% w/w of crude extract of HEEFVS was obtained from dried powder.

4.3 Acute Toxicity Test of HEEFVS

Rats in both 2000 mg/kg and 3500 mg/kg body weight of hydro- ethanolic extract of *Foeniculum vulgare* seed taking groups were observed for acute toxic effect. As a result the doses are safe or had no toxicity sign in both groups. The median lethal dose (LD_{50}) of *Foeniculum vulgare* seed was greater than 3500mg/kg body weight.

4.4 Qualitative phytochemicals screening test for HEEFVS

The phytochemical screening of HEEFVS shows the extract has positive results on tannins saponins, flavonoids, alkaloids, phenols, glycosides and Terpenoids. The result indicates that phytochemicals are present in the plant as indicated in Table 2

***Table 2 Phytochemical* screening of HEEFVS**

Phytochemical	**HEEFVS**
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Phenols	+
Glycosides	+
Terpenoids	+

+ secondary metabolite present

4.5 DPPH Assay for testing Anti-oxidant Activity of HEEFVS

4.5.1 Qualitative method to assess anti-oxidant activity of HEEFVS by TLC Plate

As shown in Figure 8 qualitative and rapid anti-oxidant activity of *Foeniculum Vulgare* seed hydro-ethanolic extract by the TLC plate was determined. The diameter of the reduced zone (zone of inhibition) increased in direct proportion to the dose of plant extract. Described as the higher the concentration of extract, the larger is the diameter of inhibition. The yellowish pale color observed shows a reduced zone that masks the color of DPPH radical and the unreduced region showing violet color.

Control **250mg/100µl** **500mg/100µl** **750mg/100µl**

Figure 8: Qualitative method to assess antioxident activity of HEEFVSby TLC plate in 0.1mM DPPH

4.5 .2 Quantitative assay for the anti-oxidant activity of HEEFVS by UV- Visible spectrometer

Figure 9 indicates a graph of DPPH radical scavenging activity of 80% ethanolic extract *Foeniculum vulgare* seed and standard antioxidant L- ascorbic acid by using UV- Visible spectrophotometer. The result from the figure indicates that IC50 of HEEFVS is 146.6µg/ml and Ascorbic acid is 97.06µg/ml. As the concentration of the sample increased, the percent inhibition of DPPH radical also increased.

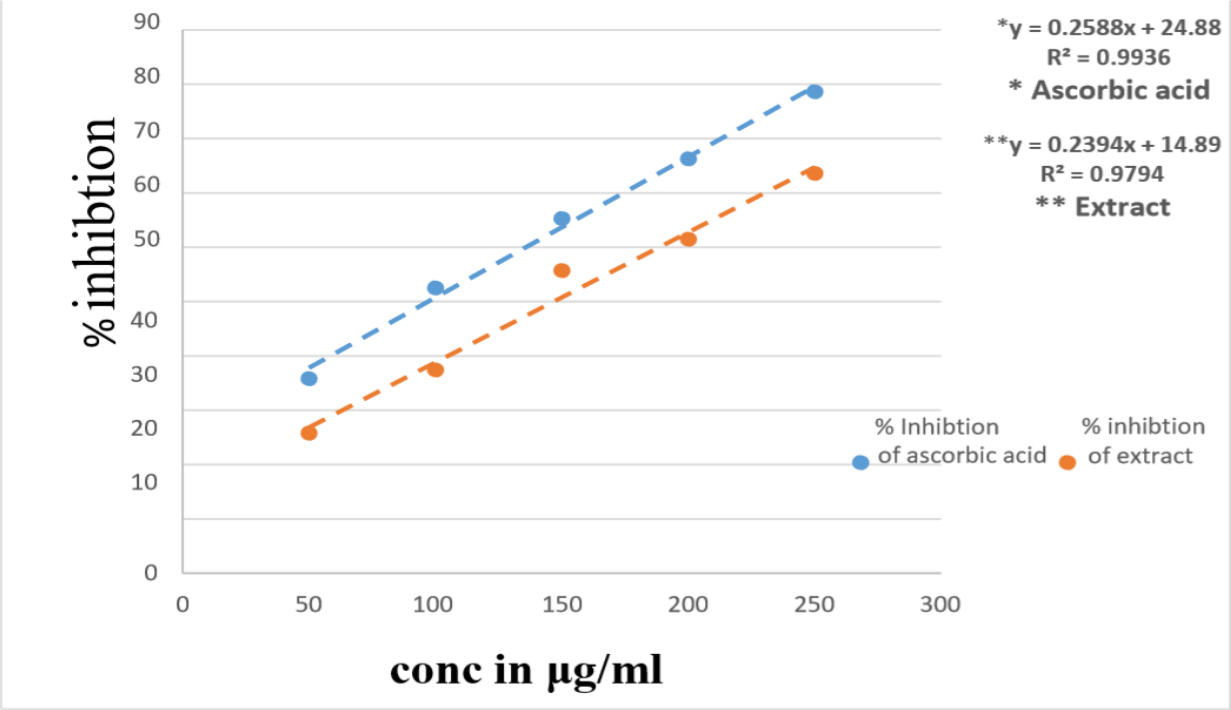


Figure 9*:* Percentage inhibition of DPPH by ascorbic acid and HEEFVS at different concentration

4. 6 In Vitro *α-amylase* inhibitory activity of HEEFVS

α -amylase enzymes inhibition effect of HEEFVS and standard *α- amylase* inhibitory drug acarbose was presented in Figure 10 which, indicates that the IC50 of HEEFVS is 8.47 µg/ml and of the standard acarbose is 1.71µg/ml.

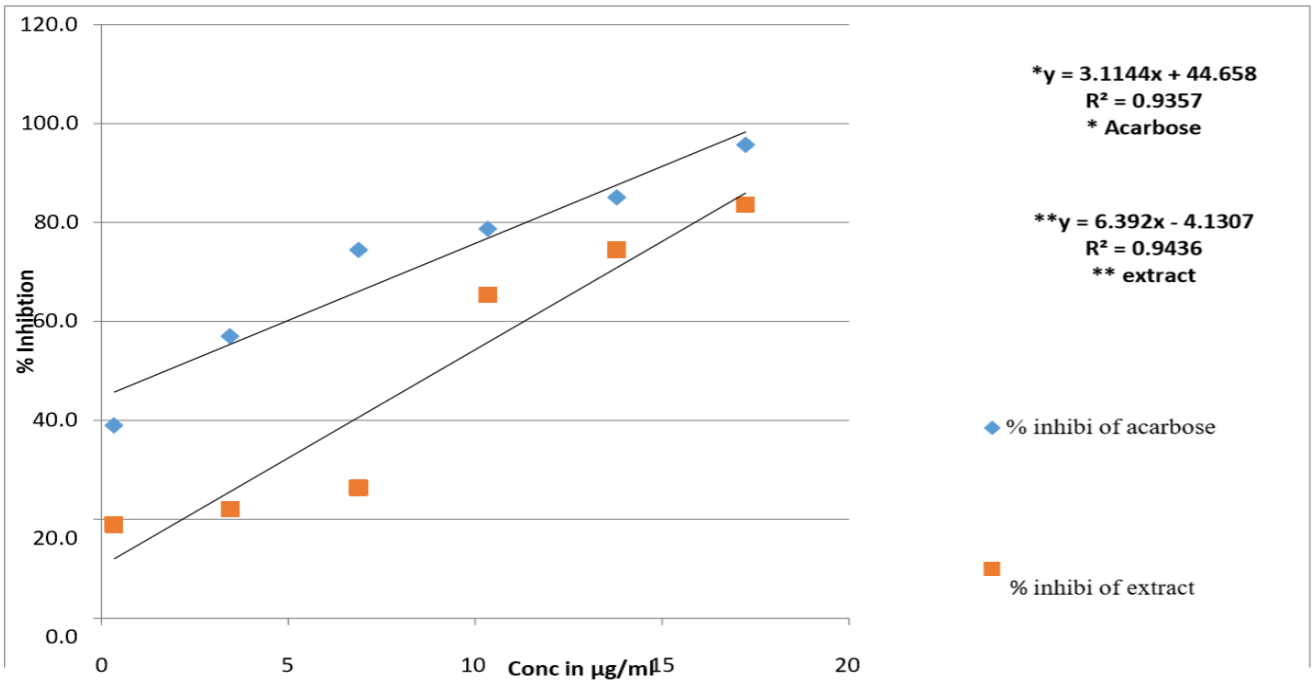


Figure 10*:* percentage inhibition of a α-amylse enzyme by HEEFVS and standared Acarbose at different concentration

4.7 Effects of HEEFVS on the Biochemical parameters

Table 3 shows the effects of hydro-ethanolic extract of *Foeniculum vulgare* seed on the ALT, AST, TC, and TG in diabetic rats compared to healthy and diabetic control rats. The activity of serum ALTand AST were significantly elevated in diabetic control rats compared to healthy controls. Administration of HEEFVS at 200 mg/kg bodyweight and 400mg/kg bodyweight doses for 21 days significantly ($p < 0.001$**)** reduced serum enzyme activity of (ALT and AST) in diabetic rats compared to the diabetic control group. The effect was comparable with 5mg/kg bodyweight glibenclamide dose for the same period of treatment.

Serum total cholesterol and triglyceride increased significantly in diabetic control rats compared to the normal control rats. Serum total cholesterol significantly reduced at ($p < 0.001$**)** with HEEFVS treatment at a dose of 100mg/kg, 200mg/kg and 400mg/kg compared to the diabetic control rats. A similar effect was also observed with 5mg/kg glibenclamide treatment. Serum triacylglycerol only at the dose of 400 mg/kg bodyweight HEEFVS treatment showed a significant reduction ($p < 0.01$**)** compared to the diabetic control rats.

Table 3 Effect of HEEFVS on serum ALT, AST, TC,and TG of alloxan-induced Diabetic rats after 21 days of treatment

Group	ALT(U/L)	AST(U/L)	TC(mg/dl)	TG(mg/dl)
Healthy control	$68.2\pm5.6^{*b}$	$188.5\pm14.6^{*b}$	$49.3\pm3.0^{*b}$	$48.3\pm5.2^{**b}$
Diabetic control	$202.9\pm18.25^{*a}$	$437.8\pm45^{*a}$	$84.0\pm6.6^{*a}$	$66.1\pm8.9^{**a}$
Diabetic +HEEFVS 100 mg/kg	$162.9\pm17.9^{*ab}$	$312.2\pm16.4^{*ab}$	$57.4\pm7.5^{*b}$	59.2 ± 6.8
Diabetic +HEEFVS 200 mg/kg	$84.4\pm11.7^{*b}$	$239.1\pm60.4^{*b}$	$55.5\pm8.01^{*b}$	58.7 ± 8.6
Diabetic +HEEFVS 400 mg/kg	$75.4\pm9.5^{*b}$	$231.01\pm18.1^{*b}$	$49.6\pm6.7^{*b}$	$50.2\pm8.2^{**b}$
Diabetic + glibenclamide 5 mg/kg	$70.31\pm5.62^{*b}$	$192.3\pm16.8^{*b}$	$51.1\pm2.8^{*b}$	54.7 ± 4.8

Values are mean ± SD; n=6 for each group. a = compared with normal control, b= compared with diabetic control, Superscript with asterisk sign indicate significant level * = significant at ($p < 0.001$), ** = significant at ($p < 0.01$) ALT= Alanine aminotransferase, AST= Aspartate amino transferase, TC = Total cholestrol ,TG= Triacylglycerol, HEEFVS= hydro-ethanolic extract of *Foeniculum vulgare* seed

4.8 Effect of HEEFVS on fasting blood glucose (FBS) level of diabetic rats

The dose dependent effect of HEEFVS on the fasting blood glucose level in diabetic rats at 0, 7, 14 and 21 days of the experimental period are given in Table 4. No significant change in fasting blood glucose was observed in healthy control rats treated with distilled water throughout the experiment. The study showed significant hyperglycemia for all rats treated with Alloxan compared to healthy control rats in the whole study period. In diabetic rats, treated with 200 mg/kg and 400 mg/kg HEEFVS and 5mg/kg glibenclamide, fasting blood glucose level was significantly lowered ($p < 0.001$) in the second and third week of treatment compared to initial week fasting blood glucose and diabetic control group fasting blood glucose level. In the diabetic rats treated with 400mg/kg hydro-ethanolic extract of *Foeniculum vulgare* seed there was a better gradual and statistically significant reduction in mean blood glucose levels from 352 mg/dl on day 0 to 189.1 mg/dl similar to rats treated with glibenclamide with a deacrese in FBS from 350.8mg/dl to188.8mg/dl after 21days of

treatment.The decrease in blood glucose with the plant extract treatment was dose dependent

Table 4 Effect of HEEFVS on fasting blood glucseof alloxan induced diabetic rats.

	Fasting blood glucose (mg/dl)			
Group	**0 day**	**7th day**	**14th day**	**21st day**
Healthy control	**95.1 ± 3.86*b**	**96.5±3.2*b**	**93.5 ± 5.2*b**	**91.3±5.5*b**
Diabetic control	**354.0±53.3*a**	**361.8±48.8*a**	**372.0± 63.7*a**	**399.3±64.9*a**
Diabetic+HEEFVS 100 mg/kg	**354.5±52.2*a**	**332.3±56.2*a**	**302.7±58.3*a**	**271.6±37.9*ab**
Diabetic+HEEFVS 200 mg/kg	**351.6± 49.5*a**	**334.8±53.9*a**	**252.2±48.2*ab**c**	**228.3±43.5*ab*c**
Diabetic+HEEFVS 400 mg/kg	**352±50.6*a**	**317.8± 60.1*a**	**226.3±19.3*abc**	**189.1±23.1*bc**a**
Diabetic + glybenclamide 5 mg/kg	**350.8± 43.5*a**	**298.6± 40.4*a**	**248.1±28.1*ab**	**188.8±11.5 *b**ac**

Values are mean ± SD; n=6 for each group. a = compared with normal control, b= compared with diabetic control, c = compared with the initial level of fasting blood glucose (0 days) of the rats in the respective group. Superscript with asterisk indicate significant level: * = significant at ($p < 0.001$), ** = significant at ($p < 0.01$), HEEFVS= hydro-ethanolic extract of *Foeniculum vulgare* seed

4.9 Effect of HEEFVS on body weight of diabetic rats

Alloxan treated diabetic group showed a decrease in bodyweight compared to normal control group ($p < 0.05$). The final body weight in both extract taking and standard treatment taking group showed increment in body weight compared to the diabetic control group but the difference was not statistically significant.

T**able 5 Effect of HEEFVS on the bodyweight of alloxan induced diabetic rats**

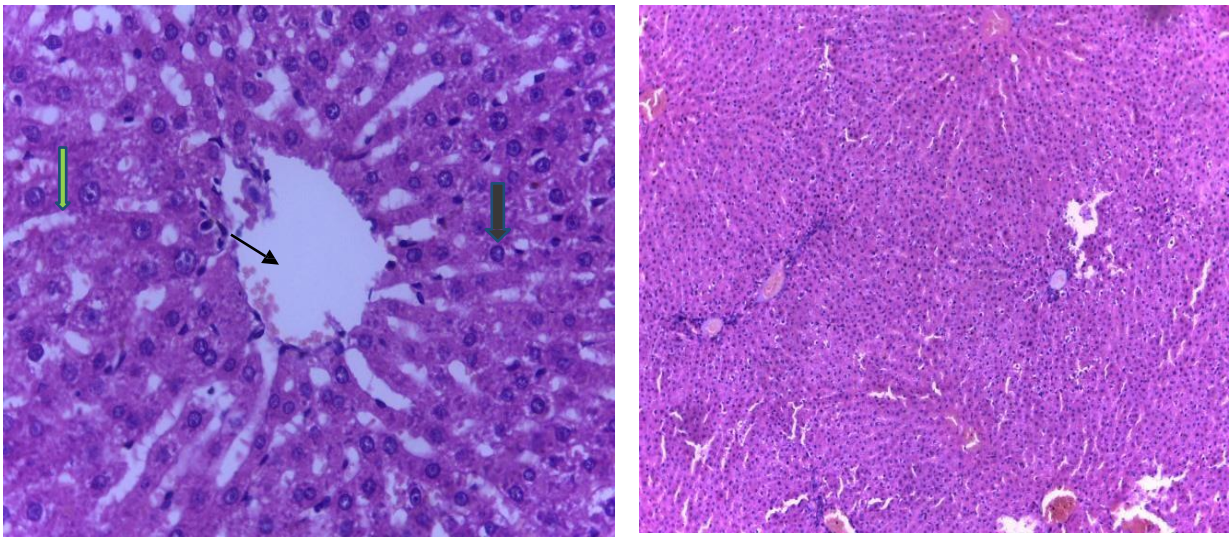
	Bodyweight (g)	
Group	0 day	21 day
Healthy control	**214.9 ±20.1**	**237.6±13.1**
Diabetic control	**215.0 ± 20.8**	**183.9 ± 13.4 [a]**
Diabetic+ HEEFVS 100 mg/kg	**216.8 ±14.4**	**186.5±8.9[a]**
Diabetic+HEEFVS 200 mg/kg	**215.4±22.9**	**186.5±28.8 [a]**
Diabetic+HEEFVS 400 mg/kg	**217.3 ± 24.8**	**190.25 ± 20.3[a]**
Diabetic+ Glybenclamide 5 mg/kg	**212.7 ± 17.0**	**193.5±11.2[a]**

Values are mean ± standard deviation (n=6) [a] $p<0.05$ compared with healthy control values, [b] $p<0.05$

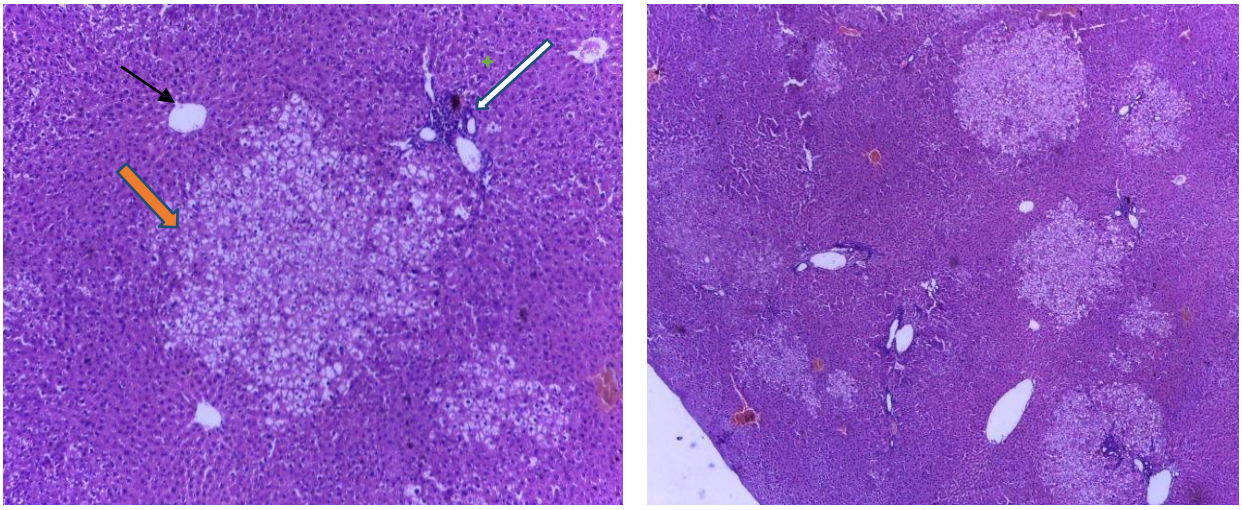
compared with the diabetic control group, HEEFVS= hydro-ethanolic extract of *Foeniculum vulgare* seed

4.10 Histopathological observation of liver in alloxan-induced versus treated with HEEFVS

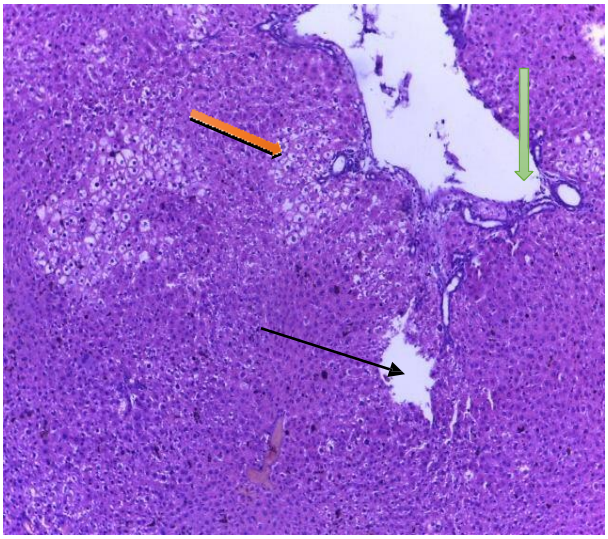
Histopatological result was presented in figure 11 with microscopical observation for hematoxylin-eosin satained liver tissue for normal rats, diabetic rats, and HEEFVS extract treated diabetic rats. Hematoxyline and Eosin stained normal liver result show normal tissue with visible central vein and normal hepatocyte (cell architecture) and 175 mg/kg alloxan trated diabetic rat present with fatty changes, dilated sinosoids, and obvious lymphocytic inflammation in portal areas. But, diabetic rats treated with HEEFVS showed complete regeneration at dose
400 mg/kg with some increasing degree of restortion observed in the 100mg/kg and 200mg/kg taking rats .

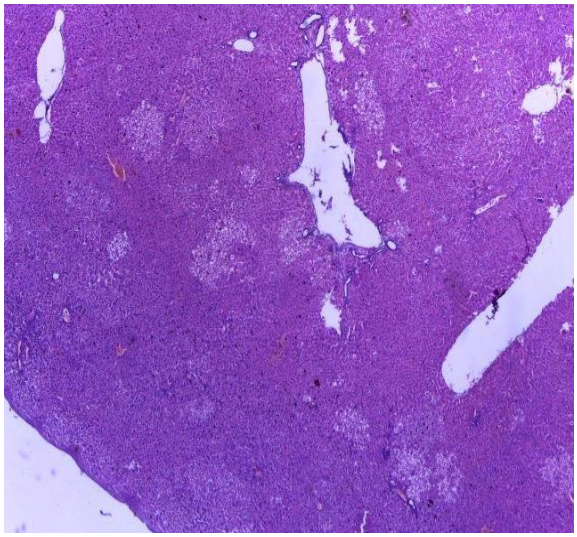


A: Normal control group showing normal liver lobule architecture with → visible central vein ,➡ normal hepatocytes ⇨ sinusoidal space

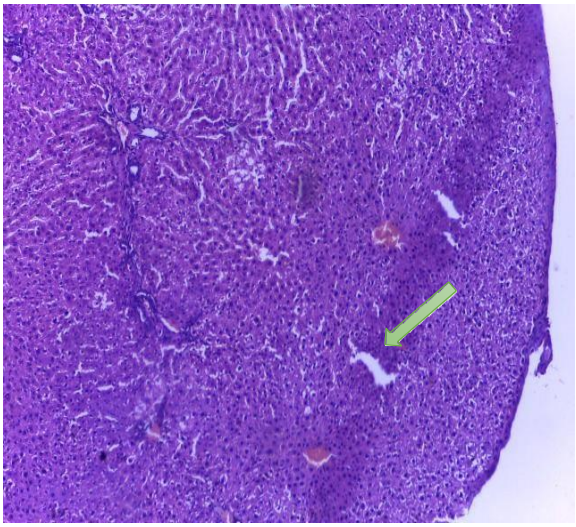


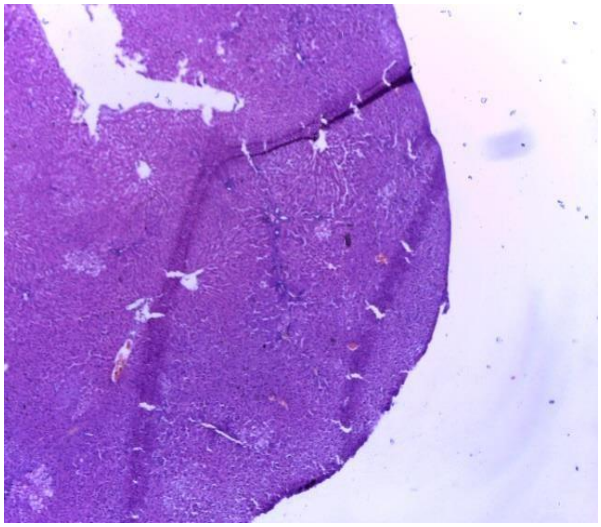
B: Diabetic control group showing ⇨ liver lobule with multiple foci of aggregates of hepatocytes with noticeable multivacuolated cytoplasm (cytoplasmic lipidization), ⇨ Lymphocytic inflamation in portal areas sinusoidal space not observed well



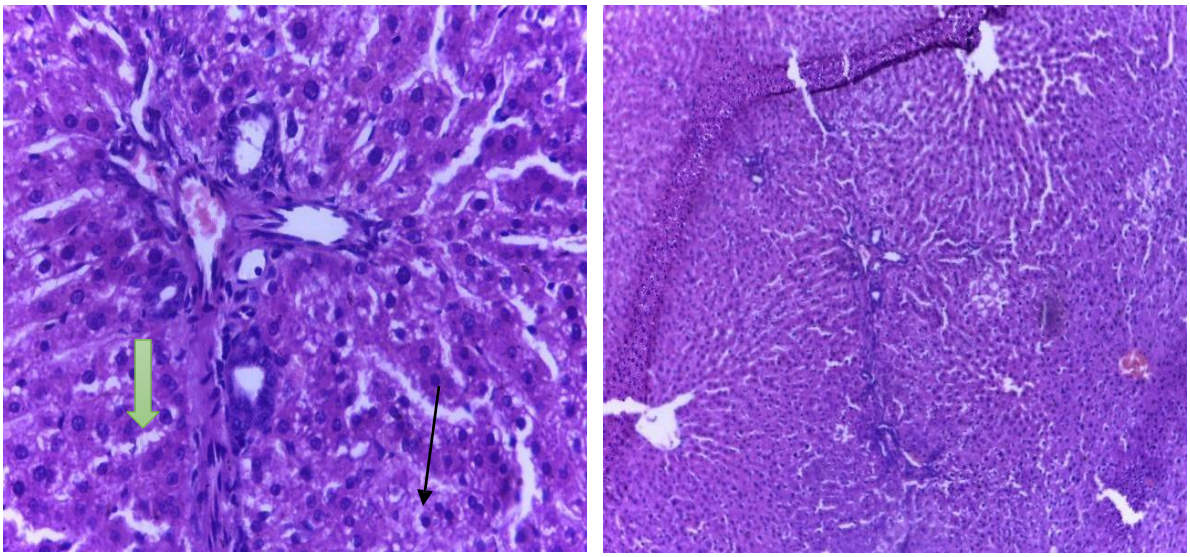


C: 100 mg/kg HEEFVS reciving group showing liver lobule with light and small sized aggergates of fatty change in hepatocytes . no lymphocytic aggregates dilated sinusoidal space





D: 200mg/kg HEEFVS reciving group showing normal liver lobule with dilated sinosoids no fatty change and no lyphocytic inflammation



E. 400mg/kg HEEFVS receiving groups showing⟶ normal liver lobule having normal Hepatocytes (polygonal cells) with abundant granular cytoplasm having centrally located round to oval nuclei with delicate chromatin. ⟹ Prominent sinusoid space.

Figure 11: The photo micrograph histopatological result for liver sections (H&E) stained presented with magnification power of 40x and 10x respectively

5. DISCUSSION

Alloxan monohydrate has been used to induce diabetes mellitus in experimental rats. Even if, there is a trial to expose animals to uniform conditions to assure consistent alloxan-induced diabetes in this research, drug sensitivity was highly variable in animals. This means a dose that causes severe hyperglycemia in most animals produced mild or no hyperglycemia in other animals in the same strain. So expecting to get a similar result was difficult. Carvalho *et al,* 2003 reported that from the Wister rats intravenusly treated with 40 mg/kg alloxan, 40% developed chronic diabetes mellitus; 20% developed mild or slight degree of diabetes and few died. Similarly, high and low susceptibility to alloxan were observed in this research then finally 78 % of rats receiving alloxan were found to have FBS range between 200–400 mg/dl which was acceptable diabetic range of this research. In addition, other studies reported that 82% of animals developed hyperglycemia with FBS (259 – 292 mg/dl) by using 120 mg/kg of alloxan (Jafri *et al*., 2000). This gaps caused may be because of Animal species, dose, GLUT 2 expression difference, differences in how well the alloxan reached the pancreas after injection, and difference nutritional status of rats (Rohilla and Ali.,
2012).

Antioxidants act as radical scavengers, inhibit lipid peroxidation and other free-radical mediated processes and are able to protect the human body. Currently, the use of plant-based natural antioxidants as preventive and therapeutic medicine is gaining much recognition. (Anwar *et al*., 2009). The parameter IC50 is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that provide a 50% loss of the DPPH activity (Beyazen *et al*., 2017). This research showed that 80% ethanol extract of fennel seed showed IC50 values of 146.6µg/ml. The result is somewhat comparable to the radical scavenging activity of ascorbic acid IC50 value 97 µg/ml. Similarly, a study from Pakistan reported that *Foeniculum vulgare* seed essential oil, 80% ethanol and 80% methanol extract showed radical scavenging activity with IC50 values of 32.32 and 23.61–26.75µg/ml, respectively (Anwar *et al*.,2009). Other study conducted on Ethiopian fennel reported that aqueous extract of the plant show

highest scavenging activity than methanol and chloroform extract (Beyazen *et al*., 2017). The difference in IC50 value from other researches occur may be because of the difference in the laboratory methods used and difference in the concentration of bioactive components like phenol and flavinoids as a result of different geographical location the plant grow. This is well explained by a study on wild and cultivated fennel seed by Conforti *et al*., 2006 which reported that methanol extract of wild fennel showed IC50 value 31 µg /ml and cultivated fennel showed 83 µg /ml. So, as observed from this research it is possible to conclude how the habitat plant grow determine quality of the plant.

Fennel is reported to suppress the nuclear factor-kappa B activation pathway which is linked to several inflammatory diseases such as diabetes by activating pro inflammatory cytokines (Beyazen *et al*., 2017). This plant can inhibit free radicals due to the high content of saponins and phenol compounds.These components especially phenols may alleviate diabetes and its complications via their antioxidantproperties, ability to stimulate insulin secretion and increase liver and muscle glycogen. (Parsaeyan, 2017).

Pancreatic alpha-amylase (PAA) is the principal carbohydrate-hydrolyzing enzyme located in the brush border of the small intestine. It catalyzes the breakdown of the complex dietary carbohydrates (starch) into easily absorbable monosaccharides (glucose) or disaccharides (maltose). Inhibitors of α-amylase activity delay this step to reduce postprandial hyperglycemia. It also plays a role in the management of diabetic complication (Hemlata *et al.*, 2019). In our findings the α-amylase inhibitory activity of hydro-ethanolic extracts of *Foeniculum vulgare* seed had inhibitory potential with the IC50 value (8.47 µg/ml) and standard Acarbose drug with IC50 value of (1.71µg/ml). These result agree with the previous research conducted on methanol extract of phenolic compound from *Foeniculum vulgare* seed and got highest inhibition of α- amylase at 25°C for 8 hrs. 82.43% (Abu-Zaiton *et al*., 2015). But a different result was reported using starch agar plate assay and results were recorded in terms of diameters of starch hydrolysis zones from the six spices namely, cinnamon, cumin, fennel, fenugreek, clove, and nutmeg with a conclusion that fennel is the last in its inhibitory activity than other listed spices in the α-amylase inhibitory activity (Hemlata *et al.,* 2019).

Diabetes mellitus increases oxidative stress in liver which is characterized by increased free redicals and diminished anti-oxidant defence system simultaneously (Maritim *et al*.,2003). Also, hyperglicemia induced by alloxan for experimental study generates reactive oxygen species (ROS) which is capable of initiating tissue damage in the liver along with pancreas. Liver damage by diabetes is known as non alcoholic fatty liver disease if not treated it will aggravate to steatohepatits and cirrhosis step by step(Guria *et al*., 2014).

The liver enzyme analysis and liver histopatological result of this study showed significant increament in the level of AST and ALT enzyme level and lymphocytic inflammation,fatty change and sinusoidal dilation in histological liver morphology when compared to healthy control rats. Similarly, the study on evaluating the long-term effects of alloxan-induced diabetes in rat liver showed the onset of sinusoidal enlargement and small amount of fatty vacuoles presentation in 42mg/kg intravenously alloxan treated rats sacrificed at 6 weeks and for the 14 and 26 weeks sacrificed rats it worsened (Lucchesi *et al*., 2015). Supporting the finding of the present investigation the works of Oyebadejo *et al*., 2014 agree with the histopatological abnormality and liver enzyme increamnet in alloxan induced diabetes rats. Treatment with hydro- ethanolic extract of *Foeniculum vulgare* seed attenuated the significant reduction of AST and ALT level in diabetic rats. This result is in agreement with previous reports (El-Soud *et al*., 2011,Dongare *et al.*, 2012,Parsaeyan 2017,). similarly, 21 days treatment with the HEEFVS showed complete relief of the pathological alteration in 400mg/kg body weight HEEFVS treated groups and decrease in the load of fatty change and relief from inflammation observed in 100mg/kg and 200mg/kg dose taking groups. There is no study to compare the result of diabetic liver trated with *Foeniculum vulgare* hiatopatologicaly. But, study on Streptozotocin-Induced Diabetic Rats reported regeneration effect of *Foeniculum vulgare* seed essential oil in the diabetic rat pancrease and kidney tissue (El-Soud *et al*., 2011). Other researchers reported potent hepatoprotective effect of *Foeniculum vulgare* on carbon tetrachloride-induced liver fibrosis model rats showing the reliefe from the histological alterations and deacrese in AST and ALT enzyme levels in dose dependent manner. They suggested that this could be because of the anti-oxidative effects of phytochemical constituents available in the plant (Özbek *et al*., 2003, El Baz *et al*., 2014) .This study also agreed with the above suggestion and concluded that the hydro-ethanolic extract of *Foeniculum vulgare* seed showed hepatoprotective effect. May be because of bioactive components which acts as

antioxidants to enhance plasma insulin level and reduce oxidative damage caused by hyperglycemia.

One of the major metabolic derangements in uncontrolled diabetes mellitus, is the impairment in lipid metabolism (Ozougwu *et al*.,2013). The mechanism for this is activation of hormone-sensitive lipase during insulin deficiency which causes an increase in free fatty acid mobilization from adipose tissue which leads synthesis of triglyceride in liver. Hence, in this condition a rise in triglyceride will occur. After 21 days of HEEFVS treatment, significant decrease on both serum total cholesterol and triglycerides levels wete seen compared to diabetic control rats. The hypo-lipidemic effect of the plant material showed a dose-dependent trend, indicating that efficacy was proportional to the dose of ethanol extract of *Foeniculum Vulgare* seed. The hypocholesterolemic and hypotriglyceridemic effect of the plant is supported by different researches conducted previously (El-Soud *et al*.,2011, Dongare *et al.*, 2012 , Parsaeyan 2017).Reduction in TC and TG level in hydro-ethanolic extract of *Foeniculum Vulgare* seed treated groups could be due to the inhibition of endogenous triacylglycerol synthesis in liver (Assefa *et al.,* 2017) presence of responsible active compound to suppressed the activity of hormone- sensitive lipase in adipose tissue or increased activity of hepatic lipase or lipoprotein lipase accountable for the hydrolysis of excess lipoprotein bound triacylglycerol into fatty acids (Pritchard *et al*., 1986). Availability of hypocholesterolemic compounds in ethanol extract of *Foeniculum Vulgare* seed that may act as inhibitor for hepatic hydroxyl methyl glutaryl *CoA* (HMG CoA) reductase (Assefa *et al.,20*17) and Improvement in insulin level due to high polyphenols content in fennel scavenge free radicals to reduce lipid peroxidation (Patel *et al*.,2012).This research showed a hydro-ethanolic extract of *Foeniculum Vulgare* seed 400mg/kg bodyweight decreased hyperglycemia from 352 to189 significantly at $p< 0.001$ in comparison to untreated rats. These result coincide with a previous study that reported a significant reduction of blood glucose using *Foeniculum Vulgare* seed essential oil 30mg/kg body weight treatment for a consecutive 21days in STZ induced diabetic rats (El-Soud *et al*., 2011). There are studies conducted on the aqueous extracts of fennel and reported that it's blood glucose reducing effect in diabetic rat model(El-Demerdash *et al*., 2005, Tahraoui *et al*., 2007). The anti-hyperglycemic effect of HEEFVS might be due to the bioactive component. Alkaloids have inhibitiory effect against α- glucosidase and α-amylase enzymes in small intestine which convert disaccharides into monosaccharides for the sake of absorption(Patel *et al*., 2012). The major dominant active component of fennel is trans-anethole which makes fennel an excellent anti-diabetic agent by inhibiting aldose reductase (a key enzyme in the polyol pathway that controls the conversion

of glucose to sorbitol) this goes up to reducing risk of diabetic complications (Dongare *et al*., 2012). Phytochemical investigations on *Foeniculum vulgare* have showed the presence of bioactive compounds such as Saponins and flavonoids that are effective in reducing serum glucose by increasing hepatic glucokinase activity probably by stimulating the release of insulin from pancreatic islets (Patel *et al*., 2012). Higher dose of HEEFVS showed a better result because of having higher concentration of the active components responsible for a better reduction in fasting blood glucose.

Glibenclamide treated diabetic rats also showed reduction in fasting blood glucose after 21 days of the treatment period compared to diabetic control rats. This drug is in a class of sulfonylureas which reduce blood glucose by stimulating beta cells of the pancreas to produce more insulin (Patel *et al*., 2012). The research finding suggested that intraperitoneal administration of alloxan monohydrate at 175 mg/kg might not be sufficient for the complete destruction of β-cells because few cells remained which may have the capability to regenerate and secrete insulin.

Diabetes is associated with weight loss despite polyphagia. This is usually due to muscle wasting resulting from excessive protein catabolism in skeletal muscle because of decreased uptake and utilization of glucose for energy. Significant loss of body weight from the day of intraperitoneal injection of alloxan up to the end experiment was observed compared to control groups that have seen in this research is similar to others (Cheekati *et al*., 2017). However, a hydro-ethanolic extract of *Foeniculum vulgare* seed and glibenclamide treatment improved the body weight in diabetic rats but not stastically significant. This may be because glibenclamide (commonly used oral anti-diabetic drug) may not effectively prevent the excessive weight loss experienced by some diabetics (Obia *et al*., 2016). *Foeniculum vulgare* seed have effect in weight loss in long term application in high fat diet treated obese rats by different mecanisms like trypsin inhibitors in fennel reduce food intake and stimulate cholecystokinin release, by dissolving fat deposits in bloodstream and by allowing them for energy source, and with its natural diuretic effect (Elghazaly *et al*., 2019). So, this might be responsible for this effect of low improvement in weight in this research.

6. CONCLUSION

In this study, it was concluded that hydro-ethanolic extract of *Foeniculum vulgare* seed, especially 400mg/kg was found to significantly reduce FBG, TC, TG, AST, ALT levels and restoration of liver histopathological alteration in a dose-dependent manner in diabetic male Wistar albino rats against alloxan induced abnormality. This indicated that the plant bioactive molecule was involved in the anti-hyperglycaemic, hypo-lipidemic, hepatoprotective activity.

Moreover, the extract has good anti-oxidant and alpha-amylase inhibitory activity that could be due to the presence of phytoconstituents like flavonoids, saponins and phenolics as we have seen from the in vitro study of DPPH free radical scavenging and alpha-amylase inhibitory activity experiments respectively. Generally, the overall findings of the present study suggested that fennel grown in Ethiopia was found to have important secondery metabolites which may be helpful in improving blood glucose levels and daily consumption may help to reduce the risk of diabetic complications by increasing body anti-oxidant level.

7. RECOMMENDATION

The result presented in this study regarding Ethiopia harvested traditional medicinal plant *Foeniculum vulgare* seed should be taken as basis for further investigation. Therefore, we recommend:

- ✓ Additional work needs to isolate and purify the active constituents present in the seed extract of *Foeniculum vulgare*, that are responsible for its anti-diabetic activity.
- ✓ Further investigation should be undertaken in the other parts of the fennel plant, including leaf, stem, and roots.
- ✓ The hypoglycemic effect of *Foeniculum vulgare* seed has to be studied
- ✓ Ethiopian didn't get enough advantage from fennel. It's not well promoted as it has a wide variety of health benefits so, addressing information about fennel and its use is very essential.

8. LIMITATIONS OF THE STUDY

- ✓ Hypoglycemic effect of the hydro-ethanolic extract of *Foeniculum vulgare* seed was not conducted
- ✓ Limited biochemical parameters are conducted
- ✓ Effect of *Foeniculum vulgare* seed in the histopatology of pancrease not studied.

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