ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOCHEMISTRY

ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC EFFECT OF *Persea americana mill* FRUIT JUICE IN HIGH FAT DIET AND LOW DOSE STREPTOZOTOCIN (STZ) INDUCED TYPE 2 DIABETIC MALE ALBINO WISTAR RATS

By: Ture Girma

A Thesis Submitted to Addis Ababa University School of Graduate Studies, Department of Biochemistry in Partial Fulfillment of the Requirement for the Degree of Master of Sciences in Medical Biochemistry.

June, 2020

Addis Ababa, Ethiopia

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This is to certify that thesis prepared by Ture Girma Aduna entitled, "**Antihyperglycemic and antihyperlipidemic effect of *Persea americana mill* fruit juice in high fat diet and low dose streptozotocin (STZ) induced type 2 diabetic male albino Wistar rats**" is submitted in partial fulfillment of the Requirement for the Degree of Master of Sciences in Medical Biochemistry and complies with the regulations of the university and meets the accepted standards with respect to the originality and quality.

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ACKNOWLEDGEMENTS

Before all, I would like to thanks my creator almighty God with his mother St' Virgin Marry who strengthen me in times of hardship.

Next, I would like to present my sincere thanks and great appreciation to my supervisors doctor Solomon Genet, Associate professor of Biochemistry, and my co-advisor Mr. Abdisa Tufa, scholar of Biochemistry, for being my scientific mentor over the past one year, for their unreserved, timely follow-ups, critical and constructive comments and also encouragements during their supervisions while this study was in progress. I have great honour for their invaluable contribution from the beginning to end of this thesis.

My warmest gratitude goes to, Dr. Menakath Menon and Dr. Maria Degife, for their material and ideal support and Mr.Teka Obsa for his cooperation during the experiment.

Special thanks go to Addis Ababa University for its financial support and provision of facilities to conduct this research. I am grateful to the Department of Biochemistry and all the staff for providing laboratory instruments and reagents.

I wish also to express my profound thanks and appreciation to, Dilla University for allowing me to enrol in M.Sc. Program by covering my living expense throughout the study period.

I would also like to thank department of Pharmacology for providing laboratory animals and materials, Ato Mohammed Abba fuggi for providing plant material and Ethiopian Public Health Institute for biochemical tests. My thanks extend to my best freinds Mrs.Alemnesh Yirda and Mr.Tolcha Tajeba who beside me during I face problem.

Lastly but not least, my heartfelt thank is going to my families,freinds,classmates and collegues for their support always.

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ADA	American Diabetes Association
ANOVA	Analysis of Varience
ATP	Adenosine Triphosphate
DF	Dietary Fiber
DM	Diabetes Mellitus
EPH	Ethiopian Public Health Institution
FBG	Fasting BloodGlucose
GDM	Gestational Diabetes Mellitus
HbA1c	Glycated hemoglobin
HDL	High Density Lipoprotein
HFD	High Fat Diet
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetic Federation
LDL	Low Density Lipoprotien
MUFA	Monounsaturated Fatty Acid
NIDDM	Noninsulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
STZ	Streptozotocin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TC	Total Cholesterol
TG	Triglyceride
WHO	World Health Organization
GLUT-4	Glucose Transport-4
DNA	Deoxyribonucleic acid
AED	Animal Equivalent Dose
Rpm	revolution per minute
ISR	Insulin Substrate Receptor
GIT	Gastrointestinal Tract
GIP	Glucose dependent Insulinotropic Peptide

ABSTRACT

Introduction:Type II diabetes is an alarming rate problem globally and in Ethiopia due to change in diatery habits and sedentary life style. Serious complications are associated with diabetes, particularly macrovascular and microvascular complications. Early controlling blood glucose significantly reduces the risk of complications of diabetes, but yet no effective cure for it, even though available drugs currently used in managing the disease are associated with several undesirable side effects. Traditional treatment with low cost and minimum side effect is used to treat diabetes across the world due to the different types of ingredients present in medicinal plants that act on a variety of targets by various modes and mechanisms. Ethiopia is rich in traditional medicinal plants and *Persea americana mill* is also part of this which people comsume as food.

Objectives: In this study the effect of *Persea americana mill* fruit juice on FBG, weight, LDL-c, TG, TC, HDL-c and total protein in HFD/ low dose STZ indued type II diabetes mellitus has been evaluated in male albino wistar rats.

Materials and Methods: Thirty six male albino wistar rats of 150-200 g weight were divided in to six different groups. Except six rats, the rest thirty fed on HFD for one month to induce prediabetes and insulin resistance, next followed by 35mg/Kg of STZ injection. Group I served as normal control; Group II served as diabetic control; Group III recieved 7mg/Kg of metformin; Group IV, V and VI were given 856 mg/kg, 1712 mg/kg and 2568 mg/kg of *Persea americana mill* fruit pulp juice for six weeks respectivily. The effect of fruit pulp juice on food intake and weight was measured by triple beam balance. After forty-five days treatment, the rats were fasted overnight (12 to 14 hours), anaesthetized and blood sample was collected by cardiac puncture for biochemical tests. Results were analyzed using one way ANOVA at a 5% level of significance.

Results: In higher dose (2568 mg/Kg) of *Persea americana mill* fruit pulp treated group food consumption,weight, FBG, LDL-c were signifacantly reduced and HDL-c increased ($P<0.005$) compared to diabetic control. Middle dose (1712mg/Kg) treated group showed a decrease in FBG on 6^{th} week and improve HDL-c. Treatement of rats with *Persea americana* fruit juice change TG ,total protien and creatinine but not significant. Oral antidiabetes drug metformin showed significant reduction on pellet consumption, weight, FBG and lipid profile.

Conclusion:These results indicated that *Persea americana mill* fruit juice has antihyperglycemic and antilipidemic effect possibly through reduction of fasting blood glucose, LDL-C and increasing HDL-C, in T2D induced rats. .

Key words: T2DM, *Persea americana mill*, HFD, STZ, hyperglycemea, dyslipidemea

1. INTRODUCTION

1.1. Diabetes Mellitus

Diabetes mellitus (DM) is a metabolic disorder which either results from deficiency in insulin production by the pancreas or inability of the insulin produced to bind effectively to its receptor on the cell surface. Either of these conditions leads to accumulation of glucose in the blood which results in chronic hyperglycaemia and often impacts negatively, damage, dysfuncition and failure on a number of organs in the body, like blood vessels, heart, eyes, kidneys and nerves (Marrero-Faz *et al*., 2014;Ighodaro *et al*.,2012). Untreated chronic hyperglycaemia can lead to long-term complications including micro-vascular and macro-vascular problems that cause disturbances of carbohydrate, fat and protein metabolism, and it covers a wide range of heterogeneous diseases (Ambachew *et al*., 2017;Sherita and Tamar, 2012).

Diabetes is on the rise. WHO estimates that, globally, 425 million adults aged over 18 years were living with diabetes in 2017 and this will be increased to 629 million in 2045 (WHO, 2017).The diabetes prevalence in sub-Saharan Africa, which is 12.1 million, in 2016 is expected to rise to 23.9 million by 2030 (Kamagate *et al*., 2016). Similarly, the prevalence of DM in Ethiopia was 5.2% in the year 2017 (IDF. 2017). From this perspective, diabetes mellitus constitutes a global health concern.

Diabetes mellitus could be categorized into several groups, like type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types, but the vast majority cases of diabetes fall into two broad categories. These are type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (Yigazu and Desse, 2017).Type 1 diabetes arise from absolute deficiency of insulin secretion while the more prevalent category, type 2 diabetes, is due to combination of resistance to insulin action and an inadequate compensatory insulin secretory response (ADA, 2014). According to Edem *et al*. (2009), both T1DM and T2DM lead to hyperglycaemia, excessive urine production, compensatory thirst, increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism.

Type-2 diabetes mellitus is characterized by a significant insulin production ranging from less than normal to above normal but always in quantities insufficient to maintain glucose homeostasis and organ resistance to insulin (Alhassan *et al*., 2012). In T2DM, the pancreas may produce adequate amounts of insulin to metabolize glucose, but the body is unable to utilize it efficiently. Over time, insulin production decreases and blood glucose levels rise. T2DM patients do not require insulin treatment to remain alive. T2DM is the most common type of diabetes and

accounts for 90-95% of all diabetes patients and most common in people older than 45 years who are overweight. However, as a consequence of increased obesity among the young, it is becoming more common in children and young adults (Tama *et al.*, 2013).

The change in dietary habits and a sedentary lifestyle are the two main causes responsible for the development of Type 2 diabetes mellitus, when insulin resistance is established as a previous condition. Insulin resistance is a metabolic condition associated not only with diabetes mellitus but also with multiple disorders such as high blood pressure, dyslipidaemias, and cardiovascular complications (Toro-Equihua *et al*., 2016; Park *et al*., 2013).

There is yet no effective cure for diabetes, the available drugs and insulin currently used in managing the disease are associated with several undesirable side effects. The use of oral anti-diabetic drugs is limited due to their adverse side effects including the haematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and impairment of liver and kidney functions. In addition, they are not suitable for use during pregnancy (Ighodaro *et al*., 2012; Edem *et al*., 2009).

Currently T2DM managed by the combination of oral hypoglycaemic drugs with insulin, diet and exercise. But those conventional drugs are associated with side-effect or diminution in response after prolonged use. Moreover, providing modern medical healthcare across the world is still a far-off goal due to economic constraints (Rao and Haque, 2011).

Apart from currently available therapeutic options for diabetes like oral hypoglycaemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications (Arokiyaraj *et al*., 2011).

Medicinal plants play an important role in the management of diabetes mellitus, especially in developing countries where resources are limited. The treatment of diabetes mellitus relies heavily on dietary measures, which includes the use of traditional plant therapies. Many important drugs used in medicine today are directly or indirectly derived from plants due to its bioactive constituents such as; alkaloids, steroids, tannins and some plant phytochemicals such as cafestol, flavonoids and carotenoids (Dusane and Joshi, 2013).

These traditions are still booming, while approximately 80% of the people in the developing countries rely on these systems of medicine for their primary health care needs (Rashid *et al*., 2014).

Persea americana is the tree originally from Mexico and nowadays cultivated indifferent parts of the world including Ethiopia (Rao and Haque, 2011; Etissa *et al., 2003*). *Persea americana* is one from the most commonly sold fruits in the world and its nutritional composition depends strongly on fruit variety and the season of the year (Monika and Geetha, 2016). Phytochemical constituents of *Persea americana* have been studied and analysis had shown the presence of polyphenols that has one to two times more protein than any other fruit, high in manganese, phosphorous, iron and potassium, but low in sodium, Vitamin E, vitamin C, carotene, thiamin, riboflavin, nicotinic acid and folate. Not only this, it also contains low amount of simple sugar and appreciable amount of dietary fiber (DF) (Naveh *et al*., 2002).

Persea ameriana has been reported to exhibit several pharmacological effects in ethno medicine, ranging from treatment for diarrhoea, intestinal parasites, skin and seed oil for weight loss (Soong and Barlow, 2004; Imafidon and Amaechina, 2010). The edible part or fruit has health promoting fats (Lu *et al*., 2005), anti-carcinogenic effects (Ranade and Thiagarajan, 2015; Butt *et al.,* 2006), suppress liver injury (Kawagishi *et al.,* 2001) and wound healing activity (Nayak *et al*., 2008).

Persia ameriana fruit pulp is consumed, not only for its flavor, but also for its high nutritional value and beneficial health effects (Meyer and Terry, 2010) including, anti-obesity (Monika and Geetha, 2016), hepatoprotective (Mahmoed and Rezq, 2013), antiosteoarthritis (Christiansen *et al*., 2015) and chemo-protective activities (Paul *et al*., 2011).

1.2. Literature Review

Diabetes mellitus is a multi-factorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances. The hyperglycaemia in diabetes mellitus can result from an absolute deficiency in insulin secretion (type 1 DM), insulin action (type 2 DM) or both. A number of pathogenic processes are involved in the development of diabetes. These range from an autoimmune destruction of the β-cells of the pancreas, with consequent insulin deficiency, to abnormalities that result in resistance to insulin action (Satyawali *et al*., 2016; El-Yassin, 2012).

Insulin resistance is a clinical state in which the patients can present with normal or elevated insulin levels in plasma, but their biological response is diminished. There is decreased ability of target tissues, such as liver, adipose tissue and muscle to respond properly to normal circulating concentrations of insulin, accompanied by an increase in insulin synthesis, which in turn, causes the compensatory hyperinsulinemia that tries to maintain glucose levels within the normal range. That means, insulin resistance is characterized by uncontrolled adipose hepatic glucose production and decreased glucose uptake by muscle and tissue (Toro-Equihua *et al*., 2016).

In the later stage of diabetes, lipid metabolism is affected and seen as hyperlipidaemia and hypercholesterolemia which is risk factor in atherosclerosis. In Diabetes mellitus, the insulin defect reflects in elevated gluconeogenic metabolite accumulation, which leads to excess acetyl Co-A storage, and, in turn, the acetyl Co-A acts as a precursor of lipids and lipoprotein synthesis directly or indirectly (Mahadeva *et al*., 2011).

1.2.1. Glucose metabolism and role of insulin

Glucose is the most important carbohydrate fuel in the body. In the fed state, the majority of circulating glucose comes from diet; in the fasting state, gluconeogenesis and glycogenolysis maintain a glucose concentration that is endpoint breakdown product of carbohydrate digestion that is used by all living organisms as an important energy substrate and metabolic intermediate in many pathways. Uptake and metabolism of glucose is crucial for cellular functioning and is tightly regulated by insulin (Mashili, 2013).

First, insulin secretion is exquisitely sensitive to changes in blood glucose. This is achieved by coupling glucose metabolism with insulin secretion via changes in intracellular ATP levels, β-cell electrical activity and insulin vesicle release. When blood glucose rises, most of the glucose

taken up by the β-cell is metabolized via oxidative phosphorylation, thereby elevating intracellular adenosine triphosphate (ATP) which closes K-ATP channels. This closure of K-ATP channels trigger an influx of calcium through voltage-gated calcium channels that in turn, stimulates insulin release. Conversely, when blood glucose levels fall, insulin secretion is rapidly switched off due to a reduction in intracellular ATP in β-cells, leading to opening of K-ATP channels, membrane hyperpolarization, reduced calcium entry and thus inhibit insulin secretion (Cantley and Ashcroft, 2015).

1.2.2. Prevalence of Diabetes mellitus

Diabetes mellitus is now one of the prominent public health problems of twenty first century. The greatest burden of the disease is felt by underdeveloped and developing countries, which account for 80% of all diabetes cases (Satyawali *et al.,* 2016). It is a heterogeneous disorder with varying prevalence among different ethnic groups. It affects large number of people around the world. Data from the IDF indicates that DM affects 366 million people worldwide in 2011 and this is likely to increase to 552 million or even more by the year 2030 (Adejoh *et al*., 2016). This number of patients affected by diabetes is expected to increase to 642 million by the year 2040 (Satyawali *et al*., 2016).

The diabetes prevalence in sub-Saharan Africa, which was 12.1 million, will be rise to 23.9 million by 2030 (Keter and Mutiso, 2012). WHO reported that, by the year 2017, there were about 2.6 million people having diabetes in Ethiopia, will be one from top ten countries at 2045 with 14.1 million people between 20-79 years with impaired glucose tolerance. Worldwide, approximately 4.0 million people aged between 20-79 years are estimated to die from diabetes in 2017, which is equivalent to one death every eight second (IDF, 2017). From this perspective, diabetes mellitus constitutes a global health concern (Kamagate *et al*., 2016).

The prevalence of diabetes is rising all over the world due to aging, urbanisation and an increase of obesity and physical inactivity. Unlike in the West, where older person are most affected, diabetes in Asian countries is disproportionately high in young to middle-aged adults. This could have long-lasting adverse effects on a nation's health and economy, especially for developing countries. Diabetes not only increasing morbidity and mortality, it decreases the quality of life which its complications are causing heavy economic burden on patients suffering from it (Borle *et al.,* 2016).

1.2.3. Classification of Diabetes Mellitus

According to (ADA, 2017) DM is classified into four broad categories: type 1 DM, type 2 DM, gestational DM and other specific types.

1.2.3.1.Type 1 Diabetes Mellitus

Type 1 diabetes mellitus is called insulin dependent diabetes (IDDM) or juvenile-onset diabetes, which accounts for 5-10% of diabetes and is due to cellular-mediated autoimmune destruction of the pancreatic β-cells. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors (ADA, 2016). The disease can affect people of any age, but onset usually occurs in children or young adults. People with this form of diabetes need insulin every day in order to control the levels of glucose in their blood. Patients with untreated T1DM often present with dehydration, which is caused by osmotic diuresis when the rate of glucose filtration at the kidney exceeds the maximum rate of renal glucose reabsorption. A complication of type 1 diabetes mellitus is diabetic ketoacidosis due to ketone formation, which cause metabolic acidosis (IDF, 2015).

1.2.3.2. Type 2 Diabetes Mellitus

Type 2 diabetes mellitus also named as noninsulin-dependent diabetes (NIDDM) or adult-onset diabetes, which accounts for 90-95% of all diabetes. Those patients have relative not absolute insulin deficiency and have peripheral insulin resistance which mean individuals may not need insulin treatment to survive. The risk of developing type 2 diabetes mellitus increases with age, obesity, and lack of physical activity (ADA, 2016).

Type 2 diabetes is the most common type of diabetes, usually occurs in adults, but is increasingly seen in children and adolescents. In type 2 diabetes, the body is able to produce insulin but becomes resistant, so that the insulin is in effective. The two metabolic defects characterizing type 2 diabetes are derangement of insulin secretion, which is delayed or is insufficient relative to glucose load and inability of peripheral tissues respond to insulin called insulin resistance. Over time, insulin levels may subsequently become insufficient. Both the insulin resistance and deficiency lead to high blood glucose levels (IDF, 2015; Bayne, 2015; Atanasovska *et al.*, 2014).

1.2.3.3. Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM), resembles T2DM in several aspects, involve a combination of relatively inadequate insulin secretion and action during pregnancy. It occurs in about 2-5% of all pregnancies and may improve or disappear after delivery (Amreen *et al*., 2012). However, women who have been previously diagnosed are at higher risk of developing gestational diabetes in subsequent pregnancies and type 2 diabetes later in life (IDF, 2015).

1.2.3.4. Other Specific Types of Diabetes Mellitus

Other specific types of diabetes result from specific genetic conditions such as maturity-onset diabetes of youth, surgery, drugs, malnutrition, infections, and other illnesses. Such types of diabetes may account for 1% to 5% of all diagnosed cases of diabetes (ADA, 2016).

1.2.4. Diagnostic Tests for Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is characterized by recurrent or persistent hyperglycaemia, and it can be diagnosed by demonstrating one of the following tests:-

1. Fasting blood glucose test- is most common. Fasting is defined as no caloric intake for at least 8 hours and fasting blood glucose level checked after fasting. FPG≥126 mg/dL (7.0 mmol/L) is taken as diabetic.

2. Random blood glucose test - blood glucose levels are checked at various times during the day. In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L) is diabetic.

3. Oral glucose tolerance test (OGTT) - a high-glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water drink is given. Blood samples are checked at regular intervals for two hours. Two-hour plasma glucose≥200 mg/dL (11.1mmol/L) during an OGTT indicates diabetes.

4. Glycohemoglobin HbA1c - measures how much glucose is stuck to red blood cells. HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. The A1C has several advantages compared with the FPG and OGTT, including greater convenience (fasting not required), greater preanalytical stability, and less day-to-day perturbations during stress and illness. However, these advantages may be offset by the lower sensitivity of A1C at the

designated cut point, greater cost, limited availability of A1C testing in certain regions of the developing world, and the imperfect correlation between A1C and average glucose in certain individuals (ADA, 2017).

Table 1.Diagnostic test to perceive type II diabetes mellitus

Diagnostic test	Normal	Pre-diabetes	Diabetes
Hemoglobin A1C	<5.7%	5.7-6.4%	≥6.5%
Fasting plasma glucose	< 100 mg/dL	100-125 mg/dL	≥ 126 mg/dL
Random plasma glucose	<130 mg/dL	130-199 mg/dL	≥ 200 mg/dL
Oral glucose tolerance test (OGTT)	<140 mg/dL	140-199 mg/dL	≥ 200 mg/dL

Patients with fasting plasma glucose ≥126 mg/dL, oral glucose tolerance test (75 g glucose load) ≥200mg/dL or glycated haemoglobin≥ 6.5% are diagnosed with T2DM (Weschenfelder et al.,2015).

1.2.5. Complication of Type 2 Diabetes Mellitus

The two metabolic defects characterizing type 2 diabetes are derangement of insulin secretion that is delayed or insufficient relative to glucose load and inability of peripheral tissues to respond to insulin called insulin résistance. Either of these conditions leads to accumulation of glucose in the blood or hyperglycaemia often impacts negatively on a number of organs in the body, especially the blood vessels. Microvascular and macro vascular complications are strongly seen in diabetes (Atanasovska *et al*., 2014).

Patients with diabetes are at high risk for microvascular like nephropathy, retinopathy and neuropathy and macrovascular complications like peripheral vascular disease, stroke and cardiovascular disease. In the later stage of diabetes, lipid metabolism is affected and seen as hyperlipidemia and hypercholesterolemia which is risk factor in atherosclerosis. There is also the possibility of liver damage due to increased gluconeogenesis**.**

The complications associated with diabetes are likely connected to oxidative stress induced by hyperglycaemia which overcomes the body natural's antioxidant system. The hyperglycemia-induced oxidative stress in DM is believed to be the major cause of the development and progression of diabetic microvascular complications. The antioxidant defence mechanisms are overwhelmed in diabetic patients (Ighodaro, 2012).

1.2.6. Dyslipidaemia and Type II Diabetes Mellitus

Dyslipidemia is one of the major risk factors for cardiovascular disease in diabetes mellitus. The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, reduced HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles (Dayaa *et al.,* 2017; Mooradian, 2009).

These changes are caused by increased free fatty acid flux secondary to insulin resistance and aggravated by increased inflammatory adipokines. The availability of several lipid lowering drugs and nutritional supplements offers novel and effective options for achieving target lipid levels in people with diabetes (Chehade *et al*., 2013). In type 2 diabetics insulin resistance causes unrestricted lipolysis leading to increased fatty acid flux in liver and ends in higher hepatic triglyceride synthesis. Also the activity of endothelial insulin dependent lipoprotein lipase is less resulting in decreased triglyceride clearance. Other processes involving apoprotein production and action of cholesteryl ester also get affected (Borle *et al*., 2016).

Lipid abnormalities observed in patients with type 2 diabetes play a central role in the development of atherosclerosis. These lipid abnormalities are not only quantitative, but also qualitative and kinetic in nature. Increased triacylglycerols and reduced HDL cholesterol are the main quantitative lipid abnormalities of diabetic dyslipidaemia. In addition, patients with type 2 diabetes show qualitative and kinetic abnormalities for all lipoproteins. All of these abnormalities are known to be risk factors for the development of atherosclerosis (Verges, 2015).

1.2.7. Treatment of type II Diabetes Mellitus

Diabetes is one of the five leading causes of death in the world, with type 2 diabetes occurring more frequently than other type. Management of diabetes without side effects is still a challenge and therefore new strategies need to be examined (Ghasemi *et al.,* 2014).

Patients with T2DM develop both micro- and macro-vascular complications contribute in the increasing morbidity and mortality. So the primary goal of treatment of diabetes mellitus is to prevent both micro- and macro-vascular complications and permit the patient to live out their natural life span by maintaining near normal glycaemic control (Thenmozhi *et al*., 2012).

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

(Molitch, 2013). Current convectional drugs available for T2DM include sulfonylureas, biguanides, thiazolidenediones and α-glucosidase inhibitors.

Metformin hydrochloride is a biguanide that is an amino group-rich compound like aminoguanidine currently used first-line hypoglycemic agent in treatment of diabetes. It is a rather safe drug and its anti-hyperglycemic property has been generally attributed to combination of a decreased rate of intestinal absorption of carbohydrate, decreased hepatic gluconeogenesis and improvement of peripheral glucose utilization. Besides glucose-lowering action, there is increasingly interest in the potential anti-inflammatory action of this drug (Han *et al*., 2017; Pournaghi *et al*., 2012).

A protein, adenosine 5'-monophosphate protein kinase is target of metformin. The mainstay of action of this class of drug can be attributed to its hepatic effects. Hepatic sensitivity to insulin is increased, thereby reducing gluconeogenesis as well as glycogenolysis, which contribute to the post-prandial plasma glucose lowering effects. Skeletal muscle and adipocytes undergo up-regulation of the insulin-sensitive GLUT-4 and GLUT-1 transporters to the cell membranes, thereby increasing glucose uptake. Glucose metabolism in the splanchnic bed also increases. Further metabolic effects include suppression of fatty acid oxidation as well as triglyceride lowering (Bosenberg *et al*., 2008).

Metformin use is associated with several gastro-intestinal adverse effects such as diarrhea, vomiting, flatulence and abdominal discomfort and other effect like headache (Bouza *et al.*,2012; Cheong, 2013; Refuerzo *et al.*, 2015; Wang *et al.*, 2012,).

Most oral anti-diabetic treatments target insulin resistance or β-cell dysfunction as their primary mechanisms of action. But, they are not affordable by low income earners and all these factors have led to the need for plants with hypoglycaemic properties and their employment in the management of diabetes (Ighodaro *et al*., 2012).

No satisfactory effective therapy has been available till date in modern medicine to cure DM. There are several drawbacks like insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment using insulin therapy for the management of DM. The use of amylin analogues, inhibitors of α- glycosidase, sulphonylureas and biguanides have certain effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhoea. Due to the above side effects of currently-used drugs, there is a need for safe agents with minimal adverse effects, which can be consumed for long duration. The undesirable side effects, high cost and low availability of synthetic drugs have led to a strong preference for

hypoglycemic drugs of plant origin, which are believed to be suitable for chronic treatments (Mhaidat *et al*., 2015; Edem, 2010).

1.3. Medicinal Plants

According to world health organization (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. About 85% of world population uses herbal medicines for prevention and treatment of diseases, and the demand is increasing in developed and developing countries (Abera, 2014).Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource for poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old (Hosseinzadeh *et al*., 2015).

In Africa about 90% and in Ethiopia about 80% of the population depend on traditional medicine to help meet their health care needs. Ethiopia is blessed with biodiversity in which many medicinal plants are found due to its geographical location in tropical and subtropical climate and its known that plants accumulate important secondary metabolites through evolution as natural means of surviving in hostile enviroment (Tadele, 2017). Plants have played a significant role in maintaining human health and improving the quality of life. In particular, herbs have been used for centuries as food and medicine. In herbal medicine, parts of plants used for medicine are bark, roots, leaves, seeds, flowers and fruit (Rao and Adinew, 2011).

There has been increasing demand for the use of natural products with antidiabetic activity. The undesirable side effects of synthetic drugs, easier consumption or availability and the fact that they are not suitable for use during pregnancy, have been some of the factors leading to the strong desire to use hypoglycaemic agents of plant origin (Edem *et al*., 2009).

Many traditional plants are used for the management of diabetes throughout the world. Plant drugs or polyhebral formulations are frequently considered to be less toxic and free from side effects than synthetic one. Based on the WHO recommendations, hypoglycaemic agents of plant origin used in traditional medicine are important. Nowadays global attention has been taken to develop herbal traditional medicine in more effective manner for the treatment of metabolic disorders like diabetes, hypertension, and gout (Bera *et al*., 2015).

1.3.1. *Persea americana*

Persea americana mill is edible fruit of the Lauraceae family commonly known as avocado, butter fruits or alligator pear fruits which are native to Central American (Mexico, guamala, Antalles) and easily adapt in other tropical regions. The *Persea americana* tree is erect, evergreen, perennial and usually tall 9 m to 18 m and 30 to 60 cm in diameter. *Persea americana* has different names in various languages; avocado (Ahmaric), avucado(Afan Oromo),avocado (English),abucato(Sidamuffa).Its' fruits are rich in vitamin A (β-carotene), C, E, thiamine, riboflavin, nicotinic acid, folate and natural antioxidants which protect the cells from the harmful effects of free radicals (Weschenfelder *et al.*, 2015).

Persea americana is a nutrition rich protective fruit that gained subtantial popularity and is often marketed as 'superfood' because of its unique nutritional compositional, antioxidant content and biochemical profile which enxtensively used in food,nutraceutical, pharmaceutical and cosmetic industries (Bhuyan *et al*.,2019). The variety, grade of ripening,climate, the composition of soil and fertilizers are the major factors that largely influence the nutritional profiles of avocado fruit (Duester, 2000).

Persea americana mill extract possess antiprolifrative property when tested in human cancer cell lines. Avocados are rich in phytochemicals that have anti-diabetic, antioxidant, antimicrobials, antivenom and chemopreventive properties. *Persea americana* fruit leaves and seeds are used for different purposes. Fruit pulp contain one or two times more protein than any other fruits and high in manganese, phosphorous, iron and potassium, but low in sodium and are rich source of MUFA (Monika and Geetha, 2016).

The health benefits of *Persea americana* fruit pulp may be due to its contents of over 20 essential nutrients and various disease-curing potential phytochemicals. *Persea americana* fruit and leaves have been used in Latin American folk medicine, including Mexico to treat a variety of diseases. Hot water infusion from its leaves has been used as a diuretic, to induce menstruation and to treat hypertension (Elbadrawy and Shelbaya, 2013). Furthermore, *Persea americana* juice made from ripe fruit was very popular due to its numerous health benefits.

Persea americana mill fruit contains high levels of DF from fruits and this impose positive modifications on viscosity, motility, nutrient absorption, content, transit time, empting, and probiotic properties of the entire digestive track which resolve constipation, reduce fat absorption, lower glycaemic index and plasma insulin levels, alter colon fermentation and microbial proliferation, and reduce plasma cholesterol (Rao and Haque, 2011; Naveh *et al*., 2002).

Phytosterol is another substance found in avocado whose structure is very similar to cholesterol. Its mechanism of action in the body involves the inhibition of intestinal cholesterol absorption and decreased hepatic cholesterol synthesis. The benefit of cholesterol reduction also comes from replacing saturated by unsaturated fats, which promote a decrease in total cholesterol and LDL and an increase in HDL levels. The β-sitosterol in *Persea americana* suppressing carcinogenesis, strengthening the immune system by enhancing lymphocytes proliferation and natural killer cell activity and aid weight loss by reducing compulsive eating binge by increase satiety and fat accumulation in the abdominal region (Duarte *et al*.,2016 ; Weschenfelder *et al*., *2015*).

The phenolic compounds of plant origin act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers, and metal chelators (Carpena *et al*., 2011).

Persea americana has traditionally been used due to its antibacterial, anti-fungal, hypotensive, anti-inflammatory, and immune-enhancing effect (Adeyemi *et al*., 2002 ; Rao and Adinew, 2011). Furthermore, *Persea americana* juice made from ripe fruit pulp was very popular due to its numerous health benefits (Rao and Adinew,2011).In Ethiopia, traditionally the fruit pulp of *Persea americana* is used for antifungal, reduce constipation, gastric and for cosmetical value.

1.3.1.1. Phytochemistry of *Persea americana* and their health benefits

The chemical composition of *Persea americana mill* fruit pulp analysis has shown that it is nutritionally valuable.The most important bioactive phytochemicals of *Persea americana* are categorized into: Carotenoids, fatty acids, minerals, phenolics and polyphenolic compounds, phytosterols and phytostanols, proteins, seven-carbon sugars, and vitamins. (Lu *et al*., 2005; Sudhir, 2005; Tabeshpour *et al*., 2017).

The β-sitosterol in avocados has a special effect on immunity, contributing to the treatment of diseases such as cancer, by suppressing carcinogenesis. It enhances lymphocytes proliferation and natural killer cell activity, and takes action in weight loss by reducing compulsive eating binge and fat accumulation in the abdominal region (Ranade and Thiagarajan, 2015).

Treatment of rats with the extract of *Persea americana* resulted in increase in the phospho-PKB expression in the soleus muscle. The activation of this enzyme leads to the translocation of the GLUT-2 molecule from the cytoplasm to the cell membrane in the uptake of glucose (Ranade and Thiagarajan, 2015).

Phenolic and flavonoids are bioactive compounds that have been related with a decrement of different deteriorative processes in the human body owing to their ability to reduce the formation and to scavenge free radicals. The hepatoprotective capacity of *Persea americana* fruit due its flavonoid and phenolic content has been reported (Vinha, 2013; Mozaffarian and Wu, 2018).

The phenolic compounds of plant origin act as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers, and metal chelators. The principal function of antioxidants is delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and, therefore, reducing oxidative damage. Antioxidants act in various ways, which include complexation of redox-catalytic metal ions, scavenging of free radicals and decomposition of peroxides (Carpena, 2011).

The Polyphenols Vitamin C, carotenoids, vitamin E are compounds with antioxidant effects that help to protect cells from free radical harm. These compounds also have anti-inflammatory effects that may help prevent atherosclerosis or the thickening and hardening of the arteries associated with heart disease. Reducing sodium and maintaining an adequate intake of potassium can help to guard against high blood pressure, heart disease and stroke (Noorul *et al*., 2016).

Figure 1. Picture of *Persea americana mill* plant taken by (Ture G.,2017)

1.4. Role of high fat diet (HFD) and STZ in development of T2DM

Animal models of type 2 diabetes are currently the first line for investigating disease mechanisms and pharmacological therapies (Mansor *et al*., 2013). Introducing a suitable animal model of T2D for research purposes can be achieved by combining a HFD which produces insulin resistance and a low dose of STZ injection that causes initial β-cell dysfunction. Feeding a HFD leads to development of hyperinsulinemia, obesity and insulin resistance but not frank hyperglycemia or diabetes; therefore, to induce diabetes it would be necessary to administer low dose STZ (Gheibi *et al*., 2017; Zhang *et al*., 2008; Srinivasan *et al*., 2005). The HFD rat model

with low dose of STZ (35 mg kg-1) was therefore considered by the authors to represent the pathophysiological state of type 2 diabetes as it was supplemented by minimal increase in body weight, in contrast to the catabolic loss of body weight, characteristic of diabetic condition produced by high dose of STZ (Eleazu *et al*., 2013).

The nutritional overload, which in the long term leads to obesity, can quickly induce insulin resistance in skeletal muscle as well as in the liver. Insulin resistance in skeletal muscle might reduce the occurrence of lipotoxic effects in muscle by redirecting the excess energy to the adipose tissue stores, and can thus be seen as a normal physiological function in healthy individuals. Severe expansion of the adipose tissue is tightly associated with adipose inflammation and a distorted adipokine profile, marked by high leptin and low adiponectin levels representing dysfunctional adipocytes that lead to ectopic fat accumulation in non-adipose tissue, like muscle, liver and β-cells (fig.2). Insulin-resistant muscles have lower glycogen synthesis and redirect glucose to the liver, where it contributes to hepatic lipid accumulation through de novo lipogenesis. Hepatic fat accumulation can induce hepatic insulin resistance, with decreased glycogen synthesis and increased gluconeogenesis. This impaired insulin-induced suppression of hepatic glucose output may contribute to hyperglycemia (Cantley and Ashcroft, 2015; Skovon, 2014).

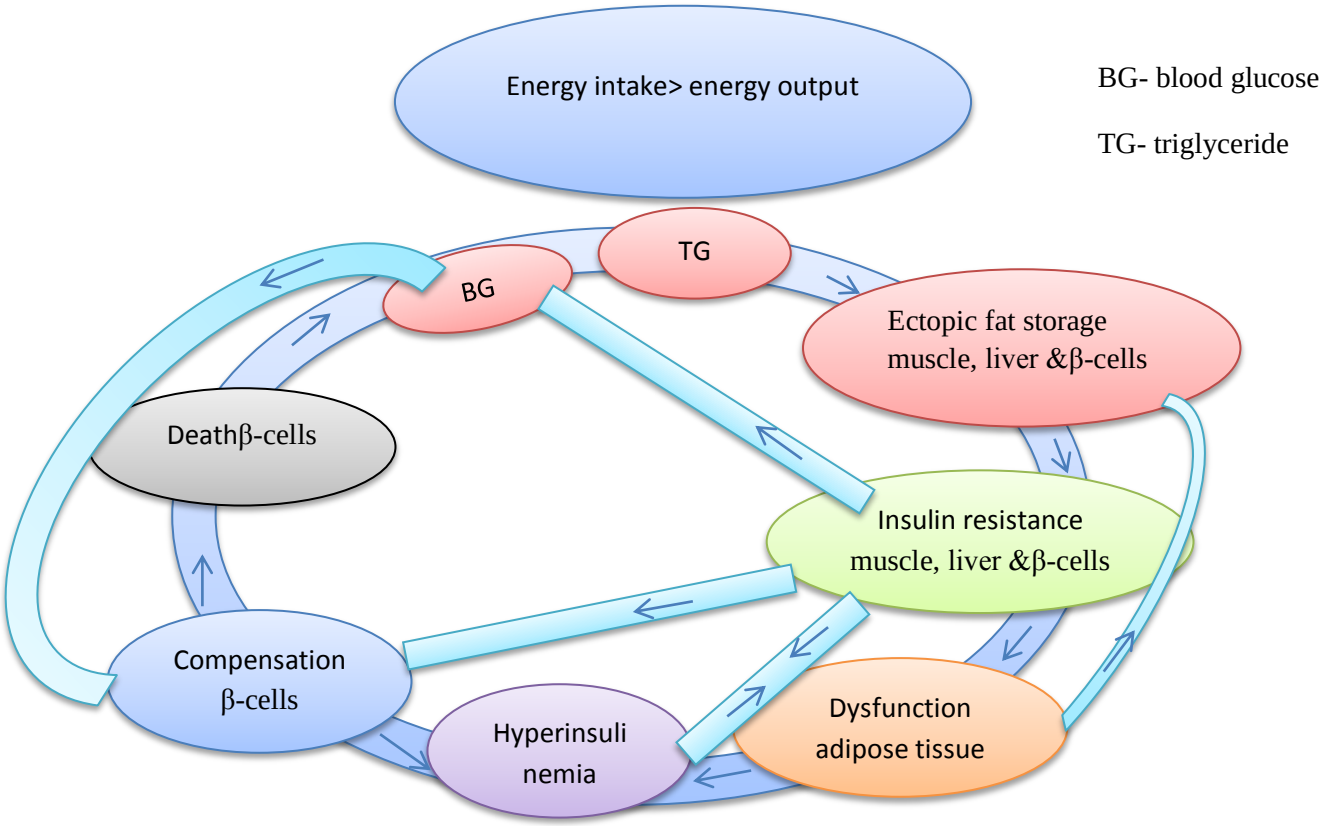

Figure 2. Overview of the interactions between multiple tissues in type 2 diabetes adapted from (Skovon, 2014).

The most common chemicals to induce diabetes in the animal model are alloxan and STZ. Streptozotocin is a permanent diabetes inducing drug which is synthesized by a strain of the soil microbe *Streptomyces achromogenes* (gram^{+}) with broad spectrum of antibacterial properties and essentially a nitrosourea analogue. Its nitrosourea moiety causes β-cell damage, while its deoxyglucose moiety is responsible for transporting the native molecule across the cell membranes. STZ uses GLUT-2 for transportation into the pancreatic β-cells, and accumulates there. It has selective toxic effects on β-cells, because of high affinity for β-cell membrane, low capacity of β-cells to scavenge free radicals and low NAD^{+}/DNA ratio in islets (Goud *et al*.,2015; Etuk, 2010).

Streptozotocin (fig.3) damages cells by alkylation or breakage of DNA strands and a consequent increase in poly-ADP-ribose synthetase activity that leads to depletion of cellular NAD+, which leads to further reduction of the ATP content and subsequent inhibition of insulin synthesis and secretion (Gheibi *et al*., 2017;Islam *et al.*, 2017).

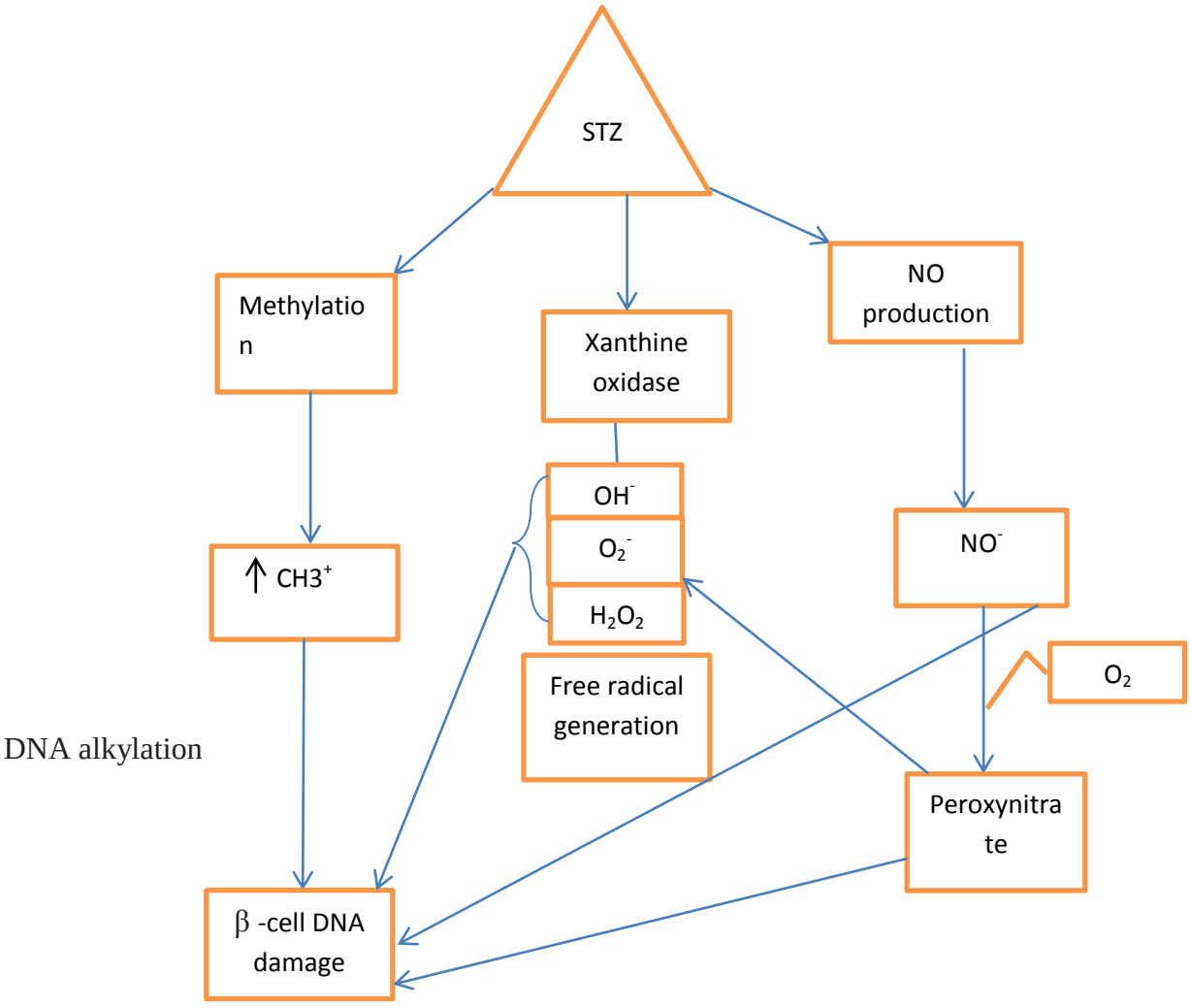

Figure 3.The mechanism of STZ induced toxic events in β- cells of Pancreas: Adopted from (Ghasemi, 2014).

Diabetes can be induced by STZ either by single injection of STZ or by multiple low dose injection of STZ. STZ is the most commonly used drug for induction of diabetes in rats (Singh and Pathak, 2015; Ghasemi *et al*.,2014).

High fat diet-fed, streptozotocin treated rat model combines two different stressors, that is feeding the rats on HFD to induce dyslipidemia, hyperpreinsulinemia, insulin resistance and glucose intolerance followed by administering a β-cell toxin namely, STZ to reduce functional β-cell mass in order to simulate human diabetic condition (Antony *et al*.,2017).

Streptozotocin entering the β-cell via the GLUT-2 transporter and causes DNA damage due to the DNA alkylating activity of its methyl nitrosourea moiety which in turn results in DNA fragmentation. Subsequently, the fragmented DNA activates poly ADP-ribose synthetase to repair DNA. Poly ADP-ribosylation leads to the depletion of cellular NAD+ and ATP. The decreased ATP synthesis is demonstrated by dephosphorylation which provides more substrates for xanthine oxidase, resulting in the formation of hydrogen peroxide and hydroxyl radicals causing oxidative stress. The presence of N-methyl-Nitrosourea side chain has the ability to release nitric oxide that inhibits aconitase activity, resulting in mitochondrial dysfunction. STZ is diabetogenic due to its targeted GLUT 2-dependent action in the pancreatic *β*-cells (Nahdi *et al*., 2017).

The mitochondria appear to be a highly sensitive target for STZ toxicity. The mitochondrial membrane potential and enzyme activities were altered in STZ treated cells resulting in the inhibition of ATP synthesis. Reactive oxygen species sensitive mitochondrial aconitase activity was markedly inhibited suggesting increased oxidative stress in STZ-induced mitochondrial toxicity. These results suggest that STZ-induced cytotoxicity in HepG2cells is mediated, at least in part, by the increase in ROS production, oxidative stress and mitochondrial dysfunction (Raza and John, 2012).

1.5. Statement of the problem

Management of diabetes with less adverse effect is still a challenge. Currently, available therapies for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides. These products are expensive and not easily accessible in developing countries and complementary medicine is important (Upendra *et al.*, 2010).

This leads to an increasing search for improved anti-diabetic drugs. Many efforts have been made to identify new anti-diabetic agents from different sources, especially medicinal plants because of their effectiveness, fewer adverse effects and relatively low cost (Bahman *et al.*, 2009).

In Ethiopia, there is high prevalence of type 2 diabetes mellitus and it increases from time to time. To treat type 2 diabetes mellitus there are many drugs, but those drugs are expensive and not affordable by many. Another drawback is that they have adverse effects on patients and not appropriate to use during pregnancy. To reduce these problems, researchers focus on medicinal plants that are available abundantly, less toxic and cheaper than the manufactured drugs. *Persea americana* is one of the traditional medicine and dietary sources that its fruit extract reduces high fat induced obesity (Monika and Geetha, 2016). Mahadeva *et al*. (2011), reported the efficacy of ethanolic avocado fruit extract on diabetic dyslipidemia. Hence, the present study investigates the anti-hyperglycemic and anti-hyperlipidemic properties of *Persea americana* fruit pulp juice in addition to its food value for community and paves the way for further elaborate studies.

1.6. Significance of the study

According to the World Health Organization, more than 80% of the world's population uses traditional medicine to their primary health needs. A great number of medicinal plants used in the control of diabetes mellitus have been reported. There are numerous medicinal plants in the world, which are the potential sources of the drug.

In Ethiopia, there is high prevalence of type II diabetes mellitus and it increases from time to time. But there is less attention to nearby food which has medical value like *Persea americana* which people believe it increases body weight. This study gives information about the effect of *Persea americana* fruit juice on weight and lipid profile. Besides, it paves the way for further studies and also provides information on the anti-hyperglycemic and anti-hyperlipidemic properties of the fruit juice of *Persea americana* in addition to its routine food value.

1.7. Hypothesis of the study

Fruit juice of *Persea americana* has anti-hyperglycaemic and anti-lipidemic activity on high-fat diet and low dose streptozotocin(STZ) induced type 2 diabetes mellitus male albino wistar rat.

2. OBJECTIVE OF THE STUDY

2.1. General objective

- To investigate the antihyperglycaemic and antilipidemic activity of *Persea americana* fruit juice on high-fat diet and low dose streptozotocin induced type 2 diabetic albino wistar rats.

2.2 specific objectives

- To test the effect of *Persea americana* fruit juice on body weight of HFD & low dose STZ induced diabetes
- To evaluate the effect of *Persea americana* fruit juice on blood glucose of HFD & low dose STZ induced diabetes
- To investigate the effect of *Persea americana* fruit juice on lipid profile of HFD & low dose STZ induced diabetic rats
- To assess the influence of *Persea americana* fruit juice on total protein of HFD & low dose STZ induced diabetic rats
- To estimate the consequence of *Persea americana* fruit juice on serum creatinine of HFD & low dose STZ induced diabetic rats

3. MATERIALS AND METHODS

3.1. Study Design

The study design used was experimental based on the antidiabetic effect of *Persea americana* fruit pulp juice on animal model.

3.2. Study Animals

Male albino Wistar rats of age 10 weeks were used as experimental animals.

3.3. Sample size determination

Apprioprate sample size determination is important since too small size misses the real effect in an experiment while a sample size larger than necessary will lead to wasting resources and ethical issues on sacrified animals (Arifin and Zahiruddin, 2017). Number of animals was calculated according to Federer rule :(k-1) (n-1)≥15 where k, is the number of groups and n, number of subjects per group; and drop-out size(do) was estimated 10%, the minimal size of sample was determined as: ndo= $5/(1\text{-do})^2=5/(1\text{-}0.1)^2=5/0.81=6.2\approx6$ where ndo is minimal sample size. The size of sample in each group was at least 6 rats (Lelyana, 2016).

Allocation of rats was performed in simple random sampling by giving number to the tail of rats. The numbers were taken through lottery. This study was performed on 6 groups (3 treatment groups; single, double, triple dose treated group and 3 control groups; normal conrtol, diabetic control, drug control). Each group consisted of 6 rats, so the number of total sample were 6 groups × 6 rats=36 rats

3.4. Ethical Consideration

The study was conducted after the proposal was evaluated and approved by Department of Biochemistry Research and Ethical Review Committee (DRERC) by protocol no. M.Sc.13/17. All experimental activities conducted in laboratory was in accordance to ethical declaration of national and international standards for experimental animals which protect the right of experimental animal and minimize suffer, hunger, pain, thirst, injury, discomfort and fear to the best minimal level.

3.5. Study variables

Independent variable: administration of *persea Americana* juice

Dependent variables: body weight, food consumption, serum glucose, lipid profiles, createnine and total protein level.

3.6. Materials, reagents, chemicals and drug

Materials- refrigerator, electrical analytical balance, triple beam balance, scissors, mortar and pestle, gavage (oral feeding syringe), syringe(10cc, 5cc, 1cc), spoon, blender, pH metre, one touch glucometer, test strips, spatula, beaker, serum separator tube, nunk tube, ice bag, polypropylene plastic cage, watering bottle, cotton and graduated cylinder (100mL)

Reagents- 5% glucose solution, citric acid, tri-sodium citrate, diethyl ether and deionized water

Drug- Metformin, was purchased from a local drug store (Ghion Pharmaceuticals (Ethiopia) PLC, India)

Chemicals- streptozotocin (STZ) was also purchased from sigma Aldrich Company, India.

3.7. Plant material collection and authentication

Persea americana mill fruit was purchased from Jimma town, 350 Km south west of Addis Ababa, Ethiopia. The plant was identified by taxonomists in national herbarium, Addis Ababa University. The ripe fruit was washed carefully with distilled water to remove external materials. Then the fruit was cut off by spoon, peeled off and the seed removed. After that edible part of fruit was mixed by blender and juice was made.

3.8. Experimental animals

Laboratory male Wistar albino rats (4-5weeks) were obtained from Addis Ababa University Department of Pharmacology. The animals were allowed to grow up till ten weeks (10) and housed in polypropylene plastic cages and maintained under standard laboratory conditions of room/optimum temperature and 12 hour light/dark cycle. The rats were fed a standard commercial pellet diet and water *ad libitum* till this age. After 10weeks age, thirty (30) rats were fed on high fat diet for one month and the rest six were fed on standard rat chow. At the time of the experiment the weight of rats was between 220-280gm.

3.9. Induction of Experimental Type 2 Diabetes Mellitus

There are different methods to induce diabetes mellitus in animal model (Skovso, 2014). In this study high fat diet/streptozotocin treated (HFD/STZ) rat model was used. This model involves a combination of a diet high in fat, to bring about hyperinsulinemia, insulin resistance and glucose intolerance followed by treatment with the β- cell toxin STZ, which results in a severe reduction in functional β- cell mass. Together, these two stressors were designed to mimic the pathology of type 2 diabetes, though on a shorter time scale than found in the human condition.

3.9.1. High Fat Diet (HFD) Preparation

Many experiments have used rats fed with commercial lard (animal fat) as an obesity model (Brainard *et al*., 2013; Louwe *et al*., 2012). Nevertheless, commercial lard is not easily available in Ethiopia. For that reason, a system was developed to prepare lard from bovine fat (choma) from local butchers in Addis Ababa, Ethiopia.

High fat diet preparation, animal fat was melted, liquefied, and then non-fat solid material, including connective tissue and meat were removed and allowed to solidify (lard) to measure weight for mixing with standard pellet. Then the warm (but not hot), liquefied animal fat was mixed with powdered standard pellet and allowed to cool to produce a solid homogeneous mixture of lard/ pellet(40% / 60% w/w) that could be fed to the rats.

Standard rat pellets contain 20% fat, 60 % carbohydrates and 20 % proteins. The powdered pellet was prepared by grinding standard pellet food using pestle and mortar. Therefore, with the addition of 40% lard (which is essentially 100% fat) to the pellets that contains 20 % fat, a food mixture containing 52% fat was produced. Hence, the term, "high fat diet" in this study refers to a diet containing 52% fat by weight (40% from added lard and 12% of the standard pellet), since 20% of the standard pellet is equal to 12% (Srinivasan *et al*., 2005). So, rats were fed on high fat diet (52%) for one month to induce insulin resistance.

Figure 4.The process of high fat diet preparation from choma (lard) and standard pellet.

3.9.2. Stereptozotocin Injection

After 4 weeks of dietary manipulation, rats were fasted overnight and injected intraperitoneally with freshly prepared STZ at a concentration of 35 mg/Kg body weight in 0.1M citrate buffer (pH 4.5)(appendex II). Rats were given 5% glucose solution from 4-8 hours to reduce STZ induced hypoglycaemia (Kripa, 2011) and fasting blood glucose was determined after 72hrs by glucometer (appendex V). The rats with blood glucose level above 200 mg/dL were considered as diabetic and used for further experiment. After 72 hrs rats with normal blood glucose reinjected STZ.

3.10.Preparation of *Persea americana* fruit pulp juie and dose calculation

Fruit pulp of *Persea americana mill* was consume when ripe as it is or by juice form. In this expermental study juice of *Persea americana* was used to treat diabetic induced male albino Wistar rats. It is difficult to know individual consumption if the fruit is given to them as it is. As indicated on appendix (V) juice preparation,by taking 80gm fruit pulp which contain it self water and add water up o 200ml. This juice is air dried to know the weight of the fruit powder that is found in juice (44.5gm) and 145.5ml is water. The volume of juice given to rats was also extrapolated from volume of human daily consumption which is (200ml of juice /day in 70Kg).In this study the average body weight (BW) of rats were 250gm. Therefore, the volume of juice given to rats was extrapolated as follows:

70,000gm = 200ml

250gm = **x**

x= (200ml*250gm)/70,000gm

x=0.7ml

The approximate amount of pesea americana fruit pulp powder in 0.7ml of juice was calculated as:

44.5gm (44500mg) = 145.5ml

x = 0.7ml

x= (44500mg*0.7ml)/ 145.5ml

x= 214mg

214mg, 428mg and 642mg /250gm BW/day was considered as a low, middle and higher dose of *Persea americana* fruit pulp juice rescpectivily. Similarly, 0.7, 1.4 and 2.1ml/250gm BW/day

were considered as low,middle and higher volume that is given to rats daily. These doses were converted to standard unit (mg/kg) as 856,1712, and 2568mg/kg /day respectively and used in this thesis document.

3.11. Extrapolation of Metformin Dose

Safe and effective drug dosing is necessary, regardless of its purpose of administration. There are several instances; where in the initial dose of a particular drug is unavailable in a specific species. Therefore, choosing starting dose of such drugs for research, experiments, or clinical trials in animals and humans is a concern. The human dose of metformin drug was extrapolated to animal dose by the formula below (Nair and Jacob, 2016).

$$\text{AED mg / kg} = \text{Human dose in mg / kg} \times \text{Km ratio where Km} = \frac{\text{Human Km}}{\text{Animal Km}}$$

3.12. Design of Experiment

After three days of STZ injection thirty six male albino wistar rats with FBG greater than 200 ml/dL were randomly divided in to six groups comprising of six rats in each group as follows.

- ✓ **Group I** Normal control, were injected citrate buffer and 3.5ml/kg distilled water daily orally
- ✓ **Group II** HFD and STZ induced diabetic rats that served as Diabetic Control and were given distilled water daily
- ✓ **Group III** HFD and STZ induced diabetic rats treated with 7mg/kg of Metformin orally
- ✓ **Group IV** HFD and STZ induced diabetic rats treated with 856mg/kg of *Persea Americana* fruit juice
- ✓ **Group V** HFD and STZ induced diabetic rats treated with 1712mg/kg of *Persea Americana* fruit juice
- ✓ **Group VI** HFD and STZ induced diabetic rats treated with 2568mg/kg-BW/day of *Persea Americana* fruit juice administered for six weeks orally from 9:30-10:30 AM.

3.13. Blood sample collection, serum preparation and storage

At the end of the experimental period, all groups of animals were fasted overnight and euthanized by anesthetizing with diethyl ether and then blood was collected via direct cardiac puncture to serum separator tube. After the blood was coagulated at room temperature for 30 minutes, it was centrifuged for 10 minutes at 3000 rpm. Serum sample was transferred to nunk

tube and stored in deep freezer at -80 °C until further analyses of various biochemical parameters.

3.14. Biochemical Test Assay

Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), total protein (TP) and creatinine were estimated with chemistry analyzer. Low density lipoprotein (LDL) level was calculated using Friedwald equation (Dansethakul *et al.,* 2015).

3.14.1. Fasting Blood Glucose level

Principle;

Fasting blood glucose,after 12 hour fasting was collected from the tail vein of the rats, and estimated with One Touch GlucoSure on 0, 14^{th}, $28^{th}$and 42^{th} days. The method involves two coupled reactions.

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

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

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

3.14.2. Serum Triglyceride Level

Principle;

Triglyceride (TG) level is estimated by the enzymatic colorimetric method. TG is measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol and free fatty acids by the enzyme lipase. The glycerol formed phosphorylated to glycerol-3 phosphate by glycerol kinase. The glycerol-3-phophate is oxidized by glycerol phosphate oxidase producing dihydroxyacetone phosphate and hydrogen peroxide. Then, Peroxidase catalyzes the redox-coupled reactions of H_2O_2 with 4-aminoantipyrine (4-AAP), producing a bright purple colour which is directly proportional to triglyceride in sample. The absorbance is measured at 540 nm.

Reaction;

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol + fatty acid}$$

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

Glycerol-3-phosphate + O2 $\xrightarrow{\text{Glycerol phosphate oxidase}}$ dihydroxyacetone phosphate +H2O2

H2O2 + 4-aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinonimine + $2H_2O$

3.14.3. Total Cholesterol Determination

Principle**;**

Cholesterol esters are hydrolysed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-ene-3-one with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminophenazone and phenol in the presence of peroxidase to yield a chromogen. The intensity of the red color produced is directly proportional to the total cholesterol in the sample when read at 500 nm.

Reaction;

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

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

$2H_2O_2$ +4- aminophenazone+phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + 4 H_2O

3.14.4. High Density Lipoprotein determination

Principle;

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

Reaction;

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

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

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

3.14.5. Determination of low density lipoprotein

Indirect method, TC, TG and HDL are measured and LDL cholesterol is calculated from the primary measurements by use of the empirical Freidewald Formula equation: Concentration of **LDL-c =TC -[TG/5+HDL-c]** in mg/dL.

3.14.6. Total protein determination

Principle;

In alkaline solution, peptide bonds bind with Cu^{2+} ions to form a blue violet coloured complex. This complex is formed between the Cu^{2+} ion, the carbonyl oxygen and amide hydrogen atoms. Each Cu^{2+} ion is combined to six peptide bonds. The intensity of the colour is proportional to the reacting number of peptide bonds, and therefore to the amount of protein present in the medium, at absorbance of 546nm.

Reaction;

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

3.14.7. Serum Creatinine

Principle;

Colorimetric estimation of serum creatinine is done by using the alkaline picrate method. Creatinine in an alkaline medium forms a coloured complex with picric acid. The formation rate of the complex measured colorimetrically through increase of the absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample at 400nm.

Reaction;

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

3.15. Statistical analysis

Data analysis was done using SPSS version 22.The results of various biochemical parameters were expressed as mean ± SEM. Statistical difference between means was done using analysis of variance (ANOVA) followed by Post hoc Tukey's multiple comparisons which is used to determine the particular groups showing significant difference at 5% level of significance ($P<0.05$).

4. RESULTS

To find out the protective effect of *Persea americana* fruit juice extract against HFD and low dose STZ induced T2DM and dyslipidemia in albino wistar rats. The biochemical changes were observed in the experimental groups. The results were given below:

4.1. Effect of *Persea americana* fruit juice on food intake

As indicated on Table (3), Food intake of diabetic control group is significantly increased (p=0.001) when compared to normal control. In higher dose treated group, the mean value of pellet consumption is significantly reduced when compared to diabetic control group (p=0.004). Similarly, the mean food consumption of metformin and low dose treated group were not significantly reduced. In other words, no significant difference was observed between middle and higher dose treated group compared to the normal control group.

Table 2. Effects of *Persea americana mill* fruit juice on food consumption

Variable	Group					ANOVA		
	Nc(n=6)	Dc(n=6)	Mt(n=6)	A(n=6)	B(n=6)	C(n=6)	F	P-Value
FIT(gm/cage/day)	135.88±4.117	149.03±.458[a]	143.45±.228	145.60±.494	142.29±2.458	137.19±1.134[b]	5.868	0.001*

*Values are expressed as mean ±SEM. FIT- food intake; NC(I)- normal control; DC(II)-diabetic control; Mt-(III) metformin control; A(IV)-low dose treated group;B (V)-middle dose treated group;C (VI)-higher dose treated group; *- significant difference at P < 0.05 between all groups as tested by one way ANOVA. The superscript letters "a" and "b" indicate significant difference at P<0.05 compared to normal control and diabetic control groups.*

4.2. Effect of *Persea americana* fruit juice on body weight

Before the induction of diabetes on rats, there was no significant difference between the mean values of body weight in all groups (P>0.05). But at the end of experiment, the mean body weight of diabetic control, low dose and metformin treated group significantly decreased compared to normal control group (p=0.001; 0.007: 0.044) respectivily. Interestingly the mean body weight of higher dose treated group was significantly increased, (p= 0.002) compared to diabetic control group. There was no significant difference between normal control and higher dose treated groups (p>0.05).

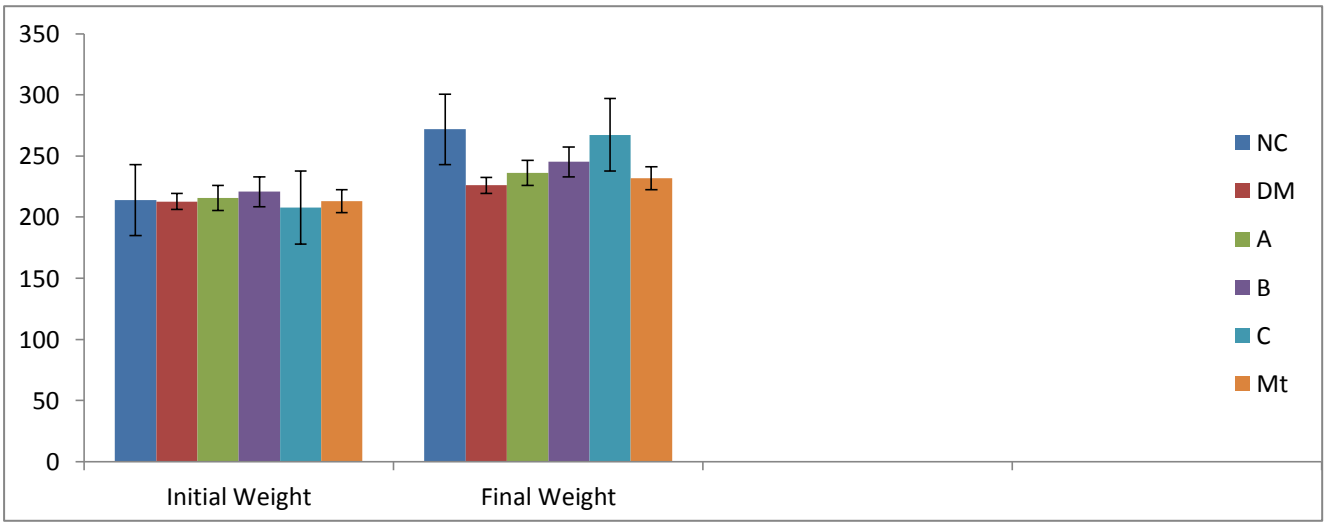

Figure 5.The effects of *Persea americana mill* pulp juice on body weight in STZ and HFD induced diabetic albino wistar rats.

The results are expressed as mean ±SEM (n=6) ; NC- normal control; DC-diabetic control; Mt-metformin control; A-low dose treated group;B -middle dose treated group;C –higher dose treated group.

4.3. Effects of *Persea americana* on fasting serum glucose level

The fasting serum glucose levels were significantly increased compared to normal control initially in all diabetic groups (P< 0.05). However, after treatments of HFD/ low dose STZ diabetic induced wistar rats with *Persea americana* fruit pulp juice, the fasting serum glucose levels were significantly (P<0.05) decreased on 4th and 6th week. Treatment with metformin which is used as standard anti-diabetic drug also led to significant reduction in fasting serum glucose on 2nd, 4th and 6th weeks.

The mean value of fasting serum glucose was significantly reduced in rats treated with higher dose of *Persea americana* on 4th week (P=0.036) and (P=0.012) on 6th week compared to diabetic control group. Significant change was also observed in double dose *Persea americana* juice treated group after the sixth week of treatment (p =0.02). The mean value of fasting serum glucose was significantly reduced in rats treated with metformin (P=0.044) on 2nd, (P=0.021) on 4th, (P=0.013) on 6th week of treatment compared to diabetic control group. However, there was no significant difference between metformin treated group and normal control on fourth and sixth week. Similarly, no significant difference was observed between higher doses treated and the normal control group on sixth week as mentioned in figure (6).

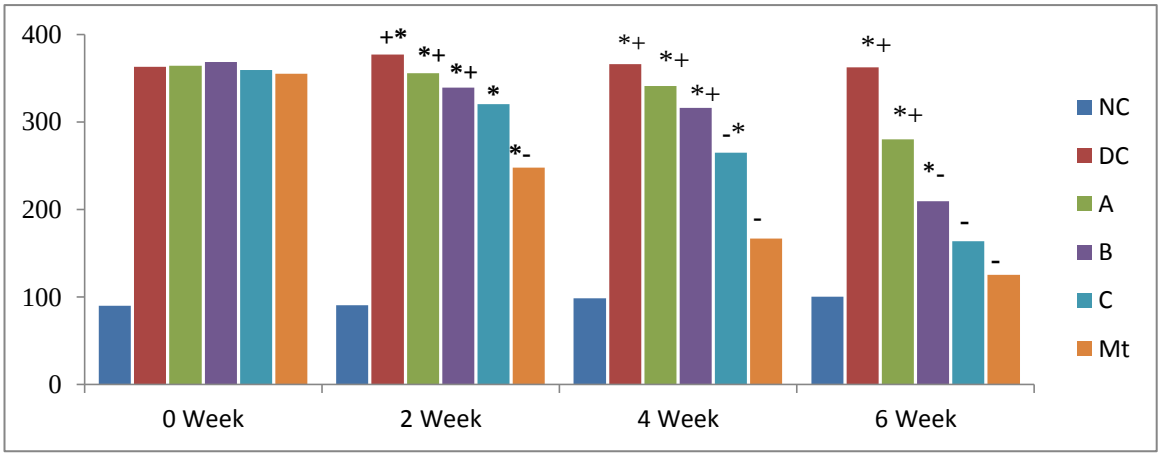

Figure 6. The effects of *Persea americana mill* on fasting serum glucose of HFD &low dose STZ induced diabetic albino wistar rats.

Results indicated mean ±SEM, n=6; NC- normal control; DC-diabetic control; Mt-metformin control; A-low dose treated group;B -middle dose treated group;C -higher dose treated group. "-" Significant when compared with diabetic control, "+" significant when compared with standard drug metformin, "" significant when compared with normal control at P< 0.05.*

4.4. Effects of *Persea americana* on lipid profiles

After the induction of diabetes and subsequent treatment with *Persea americana* juice, the mean value of TC and LDL in higher dose treated diabetic induced rats were significantly reduced when compared with diabetic control group (P=0.006; 0.007). In metformin treated group the mean value of serum TC and LDL were significantly decreased compared to the diabetic control group (P=0.003; 0.006). Interestingly, the mean value of TC and LDL in diabetic control group were significantly increased (p=0.002; 0.004) compared to normal control group. But the mean TC and LDL of double, triple and metformin treated rats were not significantly different when compared with normal control group. Likewise, the mean value of fasting serum HDL was decreased in diabetic control rats, compared to normal control group (P=0.002). in other words, the mean value of HDL treated by middle (P=0.046) and higher (P=0.034) dose was significantly increased compared with diabetic control. Similarly, in metformin treated group the mean value of HDL was significantly increased compared to diabetic control (P=0.014). There were no significant difference of mean HDL level between normal control, metformin and higher dose treated groups. The TG mean value did not show any significant difference among the entire group (P>0.05).

Table 3. Effects of *Persea americana* fruit juice on lipid profile in diabetic rats

Variable	Group						ANOVA	
	NC(n=6)	DC(n=6)	Mt(n=6)	A(n=6)	B(n=6)	C(n=6)	F	**P-value**
TG mg/dL	114.2 ±5.5	185.8 ±49.7	117.8 ±20.9	151±13.6	146.2 ±5.6	139.8 ±10.1	1.247	**.312**
TC mg/dL	109.7 ±4.3	211.7[a] ±13.1	135.2[b] ±11.2	187.8 ±21.4	158.8 ±12.8	141±7.2[b]	4.7	**0.006***
HDL mg/dL	41.2±0.8	26.3±0.6[a]	40.7±0.8[b]	28.7±0.7	34.2±0.6[b]	38.8±0.6[b]	4.3	**0.007***
LDL mg/dL	50.6±4.1	151.4 ±13.1[a]	77.2±10.4[b]	126±23.4	95.4 ±30.5	77.6±9.5[b]	4.7	**0.004***

The results are expressed as Mean ± S.E.M (n=6); NC- normal control; DC-diabetic control; Mt-metformin control; A-low dose treated group;B –middle dose treated group;C -higher dose treated group; TG, Triglyceride; TC, Total Cholesterol; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; [a,b]p< (0.05) compared with normal and diabetic control respectively.

4.5. Effects of *Persea americana* fruit juice on Total protein and Creatinine

As indicated in Table (5), there was no significant difference between each group on serum total protein and creatinine levels.

Table 4.The effects of *Persea americana* fruit juice on total protien and creatinine of diabetic rats

variable	Group						ANOVA	
	NC(n=6)	DC(n=6)	Mt(n=6)	A(n=6)	B(n=6)	C(n=6)	F	P value
TP mg/dL	5.32±.34	3.7±.23	5.23±.65	4.37±.46	4.92±.5	5.05±.54	1.74	.157
Creatinine mg/dL	.53±.06	.90±.13	.54±.09	.61±.07	.60±.08	.59±.09	2.49	.053

The results are expressed as Mean ± S.E.M (n=6); TP, Total Protein; NC- normal control; DC-diabetic control; Mt-metformin control; A-low dose treated group;B -middle dose treated group;C -higher dose treated group.

5. DISCUSSION

Persea americana is a fruit used as human food and treating different diseases. In this study,*Persea americana* is used to treat high fat diet and low dose STZ induced type 2 diabetes mellitus male albino wistar rats for six weeks. At the end of experiment, food consumption,weight, fasting blood glucose, lipid profile and total protien was assessed.

In this expermental study,the food intake of diabetic control rats were higher compared to normal control. Also food consumption of higher dose treated group of rat is lower than diabetic control and metformin treated group. Beside to this, the pellet intake of the middle dose treated group was also reduced when compared to diabetic control.This decreasing appetite of *Persea americana* fruit juice is due to presence of fiber in it, which swells in the stomach and small intestine to achieve early satiety in rats (Naveh *et al.,* 2002).This possibilities also work in human as reported by Wein *et al.,* (2013), among overweight and moderately obese individuals, adding half avocado (70 g) in the lunch increased the satiety in a period of 3 to 5 subsequent hours. Avocados fruit pulp have both a medium energy density of 1.7 kcal/g and viscose water, dietary fibre and fruit oil matrix that appears to enhance satiety (Wien *et al.*, 2011).

Slight body weight Loss observed in low dose HFD/STZ-induced diabetic rats was improved following oral administration of both *Persea americana* fruit juice and metformin. In diabetic rats, decreased body weight may be due to an excessive breakdown of tissue proteins and lipid via unavailability of carbohydrate as an energy source caused by insulin insufficiency (Gouggean *et al*., 2008). The improvement in body weight seen in diabetic rats treated with *Persea americana* extract might be supported by an improved metabolic activity, making the body system more capable of maintaining blood glucose homeostasis (Reo and Adenew 2011). However, 7mg/kg of metfomin treated rats gained weight in comparison with the diabetic group after six weeks of treatment with less effect in normalizing weight than *Persea americana* juice. This may be becuase metformin reduces weight in rats more than *Persea americana* fruit juice but not significantly. This weight loss effect of metformin is due to reducing insulin resistance and hyperinsulinemia (Berstein, 2012; Zhang *et al*., 2014).

In addition, studies done by Duerta *et al.* (2016) showed that the β- sitosterol found in *persea americana* has activity in weight loss by reducing compulsive eating and fat accumulation in the abdominal region.

The bioactive compounds of *Persea americana* fruit juice may help in suppressing free radicals generation due to hyperglycemia and control over muscle wasting that resulted from glycemic control in treated diabetic rats, and ultimately lead to normalize the level of body weight (Auddy *et al.*, 2003).

In the first week of this experiment,fasting blood glucose concentration was elevated in all diabetic induced rats when compared with nomal control.This increase in fasting blood glucose concentration is an important characteristic feature of T2DM. However, for diabetic rats that recieved juice of *Persea americana*, their fasting blood glucose was reduced in second week but not significantly. The fasting blood glucose level was decreased on fourth and sixth week significantly in rats treated with higher dose and middle dose of *Persea americana* pulp juice compared to diabetic control. This study correlated with Rao and Adinew, (2011) who reported that, in albino Wistar rats which received ethanolic extract of *Persea americana* fruit 300 mg/kg/day, orally for 4 weeks, showed a decrease in the fasting blood glucose level, glycosylated hemoglobin, blood urea, and serum creatinine. Similar finding was also reported by Thenmozhi *et al*. (2012) in which treatment of SD rats with n-hexane fraction from hydromethanolic (2:3) extract of *Persea americana* fruit 30mg/Kg orally for 8 weeks showed decrease in fasting blood glucose. Related to this Sabate *et al*. (2015) investigated by randomized clinical trials on 26 healthy overweight individuals revealed that consumption of half avocado significantly reduced the blood insulin and glucagon-like peptide-1 levels. In addition, the results of Wien *et al*. (2013) investigation on healthy overweight adults showed that avocado in lunch meal attenuated the rise in postprandial blood insulin levels 30 min after start of the lunch meal and diminished the desire to eat compared to the avocado-free control group.

The antihyperglycemic effect of avocado fruits may be due to insulin mimetic or stimulatory effect and its ability to stimulate the remaining pancreatic *β*- cells in animal models, making them able to secrete more insulin (Reo and Adinew, 2011). Phytochemicals like flavonoids, saponins, tannins, alkaloids found in avocado act as anti-oxidants and contain insulin stimulating substances such as insulin receptor substrate, glycogen synthase and glucose dependent insulotropic polypeptide. The extract could have acted via increased insulin secretion or increased peripheral glucose utilization in the gut of normal treated rats (Eguaoje *et al*., 2017). D- manno heptulose that is found in *Persea americana* may also be responsible for hypoglycemic effect by decreasing the rate of glycolysis via hexokinase inhibition and weight control via appetite reduction.

In metformin treated group the fasting blood glucose is significantly reduced starting from second week when compared with diabetic control. Metformin reduces hyperglycemia due to reduced gluconeogenesis, increased insulin receptor sensitivity especially in muscle cells, and decreased glucose uptake in the intestine (Derosa and Maffioli, 2011; Harvey, 2012; Quintero and Palacios, 2017). Interestingly the mean valeu of low dose did not cause significant change through six weeks,but decreasing fasting blood glucose to some extent. In other cases, the means of middle,higher dose and metformin treated group did not show significant difference compared to normal control group on the sixth week.

In addition to its anti-hyperglycemic effect, *Persea americana mill* fruit juice was also shown to be able alter the levels of lipid metabolites including TC, LDL, TG, and HDL cholesterol levels in diabetic albino rats. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. Serum FFA concentration are a result of the balance between the release from lipolysis, neosynthesis and disposal and represent the major determinant of insulin effect on FFA oxidation and non-oxidative metabolism (Edem, 2010).

In the current study, there was significant ($P<0.05$) increase in the TC and the LDL levels in diabetic rats. The elevated TG level in diabetic rats might be due to the consequence of increased synthesis of triglyceride rich lipoprotein particles (VLDL) in liver and diminished catabolism. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver (Bays *et al.,* 2013). The increased levels of LDL and VLDL in the HFD/low dose STZ induced diabetic rats might also be due to over production of LDL and VLDL by the liver in turn by the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx (Samatha *et al*., 2012). Increased pool of triacylglycerol-rich lipoproteins mainly VLDL1, observed in type 2 diabetes, promotes CETP-mediated triacylglycerol enrichment of HDL particles and, as a consequence, enhances HDL catabolism, while depleting them from cholestryl esters thus decreasing HDL cholestrol level (Verges , 2015).

The elevated levels of TC and LDL were reduced in *Persea americana* fruit juice treated diabetic rats. Administration of higher dose of *Persea americana* fruit juice decreased the mean of TC and LDL level in HFD and low STZ induced diabetic rats.This study agreed with the report of Pahua-Ramos *et al*., (2014) where administration of reduced-calorie avocado paste 2 g/kg/day for 7 weeks in high cholestrol diet and high fructose diet rats showed a significant reduction in TC, LDL-c and increased insulin sensitivity. Similar study by Elbadrawy and

Shelbaya (2013) using hydro alcoholic extract of avocado130 and 150 mg/kg/day via stomach tube for 8 weeks on HCD rats showed a significant reduction in serum cholesterol, LDL-C, and VLDL-C, while, the serum level of HDL-C was enhanced. In another study by Al-Dosari (2011), administration of *Persea americana* pulp 1 and 2 ml/rat/day, orally for 10 weeks showed a significant decrease in serum cholesterol, LDL-C, VLDL-C and TG. This LDL and TC reducing effect of *Persea americana* fruit pulp juice is due to Phytosterols, phytostanols and dietary fiber which help to reduces cholesterol reabsorption in the intestine and promoting fecal cholestrol excretion which inturn reduces the rate of LDL in plasma, increasing the amount of cholesterol excreted from the body and decrease in hepatic cholesterol synthesis (Ras *et al*., 2014: wang, 2007). In other words soluble diatery fibre found in avocado fruit pulp affects cholesrtol metabolism through binding to bile acid and alter micelle formation which decreases its absorbtion in small intestine and the extract of avocado fruit inhibits the action of acetyl-CoA carboxylase, a key enzyme in the production of fat in the body (Hashimura *et al*.,2001).

Treatment of diabetic albino rats with metformin decreases the serum TC and LDL after six weeks. Metformn increase insulin sensitivity in which decrease rate of lipolysis there by slowing the conversion of FFA to lipoprotein precursor in the liver (Melmed *et al*.,2016). Interestingly the mean value of HDL-C in the HFD and low dose STZ induced diabetic rats was increased in double and triple dose.This study is inline with Ebadrawy and shelbaya (2013), where administration of hydroalcolic extract of avocado pulp 150mg/Kg for 8 weeks increased HDL in HCD rats. The possible mechanism by which avocado inhances HDL serum level may be by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues due to presence of tocopherols ,phytosterols and polyphenols in friuts. Avocado also modifies the structure of the HDL lipoprotein by increasing paraoxonase-1 enzyme activity which is responsible for the hydrolysis of lipid hydroperoxides (Boshtam *et al*., 2013). In the same way, the HDL cholestrol level in diabetic rats treated with metformin drug was significantly increased compared to diabetic control.

Particularly, no dose of avocado showed significant effect on serum TG level in all treatment groups compared to diabetic control group ($p>0.05$). But there is decreasing of mean value of serum TG after treatment. This result is in agreement with Pieterse (2005), In a randomized, controlled parallel study on 61energy-restricted-diet volunteers administration of 200 g/day of avocado for 6 weeks which substituted for 30g of other mixed dietary fats didn't change TG, HDL, LDL and TC-level significantly in the experimental group. But this result contradicts with another study by Shehata and Soltan (2013),where treatment of

hypercholesteromic rat with 30% avocado fruit for 4-weeks reduced TG. This may be due to the difference in extracts.

In type II diabetes mellitus insulin resistance does not alter only glucose and lipid metabolism but also protein metabolism. In this study the total protein level was fall in diabetic rats relative to normal control. This is due to diabetes disturb amino acid metabolism which may increases muscle proteolysis ,reduced protein synthesis and stimulated hepatic gluconeogenesis. Treatment of diabetic rats with avocado fruit juice improve this issues but not significant for all doses. The metformin treated group also did not show significant difference. In diabetic rats breaking down of liver and plasma protein enhance serum creatinine level. Administration of *Persea americana* fruit juice and metformin to diabetic rats decreases total protien to some extent but not significant. This indicates effect of *Persea americana* normalizing metabolic disturbance is comparable to that of metformin.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

The present study has made an attempt to study antihyperglycemic and antilipidemic effects of a locally available plant *Persea americana* fruit juice which is in use for nutritional and therapeutic purpose for different ailments but not scientifically proved against STZ associated T2D and dyslipidemia. Hence, *Persea americana* fruit juice has been selected for the present study. Based up on the present study the following conclusions were arrived: *Persea americana* fruit juice reduces pellet consumption in HFD/low dose STZ induced type II diabetic albino wistar rats. Particularly, higher dose (2568 mg/Kg) of *Persea americana* juice reduces significantly better metformin.This is because it acts by slowing gastric empyting and inreasing satiety in experimental animals.

Similarly, *Persea americana* friut juice has effect in normalizing weight and reduce fasting blood glucose level. Treatment with *Persea americana* juice (1712 mg/kg, 2568mg/kg) showed significant fasting blood glucose reduction on the 4^{th} and 6^{th} weeks.This reducing blood glucose level has inturn great role in normaling other metabolic abnormalities. Not only this, but also *Persea americana* fruit pulp has a potential in reducing effect on LDL,TC and increasing HDL in diabetic induced albino rats. From this *Persea americana* has the ability to solve dyslipidemia and the risk of cardiovascular dieseases.

From all these findings, it can be concluded that *Persea americana* fruit pulp juice has the effect of normalizing metabolic disturbances of carbohydrate and lipid that is caused by diabetes and has antihyperglycemic as well as antilipidemic activities.

6.2. Recommendations

The present study demonstrated that, *Persea americana mill* fruit juice have anti-hperglycemic and anti-dyslipidemic activity in albino male Wistar rats. However,

- More studies are required to investigate the therapeutic effect of *Persea americana* fruit juice, since in Ethiopia this juice has a wide traditional use for different ailments but yet not well known at large

- Investigations are also needed with regard to the isolation, purification, characterization and quantification of active principles components of *Persea americana mill* fruit juice such as alkaloids, tannins, phytates, flavonoids, etc. that are responsible for the antidyslipidemia.

- Further research should be conducted on the effect of *Persea americana* fruit from different part of Ethiopia.

- There is a need for more molecular level studies using another diabetic model like genetic models of diabetic.

7. LIMITATIONS OF THE STUDY

- Because of lack of commercial lard, HFD used in this model was home made and its content was not known.
- *Persea americana* fruit was not fresh because it was collected from jimma and stayed for a week and during storage different biochemical changes may occur.

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Appendix I. HFD preparation

- Animal fat (choma) was bought from local butchers
- 'Choma' was melted and liquidified
- Non-fat material, meat and connective tissue was removed or filtered
- The melted filtrate was solidified to form lard
- The lard was weighted (8300gm)
- The standard pellet was grind to make powder
- The lard was remelt again to mix with standard rat chow
- The melted warm lard was mixed with 12450gm pellet powder and compressed immediately by hand
- The compressed mixture of pellet and lard solidify which was given to experimental animal

Appendix II Citrate buffer preparation

To prepare citrate buffer (0.1M) from citric acid and tri sodium citrate

Given, molar mass of citric acid =210.14

Molar mass of tri-sodium citrate=294.10

Molarity =0.1M

By formula **molarity**=$\frac{number\ of\ mole}{volume\ of\ solution(L)}$

From this No. of mole =$\frac{given\ mass}{molar\ mass}$

Both citric acid and sodium citrate were dissolved in 100mL distilled water.so from above formula by rearranging for No. of mole;

Molarity =$\frac{given\ mass}{molar\ mass}$ x$\frac{1}{V\ of\ solution\ in\ L}$

For sodium citrate

0.1M = $\frac{given\ mass}{294.10x0.1}$ =2.94gm of sodium citrate was dissolved in 100mL distilled water.

For citric acid; molarity 0.1M, molar mass =210.4gm and it was dissolved in 100mL of distilled water.

By the same formula to above 0.1M $=\frac{given\ mass}{210.4gm}$ x $\frac{1}{0.1L}$ =2.104gm of citric acid was dissolved in 100mLof distilled water.

After this pH adjustment of buffer solution was made by slowly adding citric acid solution on 30ml of sodium citrate solution until pH was become 4.5.final volume of buffer was =55ml then stored in cold.

STZ dose calculation

If 35mg/kg is used for low dose induction of diabetes then calculating for individual male rat depends on its weight.so, for example for 255gm of wistar albino rat is;

35mg =1000gm

? = 255gm, =8.93 mg of STZ but in how much volume of buffer it should dissolve?

If 3.5ml/kg, calculating for a single rat which its weight is 255gm,

3.5ml =1000gm

? = 255gm, so 0.89 ml was given to this rat.

Appendix III STZ injection protocol

- After citrate buffer was prepared STZ-citrate buffer was prepared prior to injection to avoid degradation of STZ (15-20min)
- Rats should be fasted over night
- The rats were kept in isoflurane jar(desiccator with cotton)to anesthetize by diethyl ether
- Rats were removed when their breathing is slow
- Appropriate amount of STZ solution was injected (35mg/Kg) into rats regard to their body weight
- Rats were allowed to awaken up and returned to their cage
- 5% glucose solution was given to rats to avoid sudden hypoglycaemia after injection of STZ from 2-6 hours

Appendix IV Fasting Blood Glucose Determination

- First rats should fast over night
- To sterilize tip tail of rats, tail scrubbed with cotton socked into alcohol
- Tail tip cut with sterile scissor
- Blood samples from the tip of the tail were taken to measure circulating glucose levels using glucose-oxidase reagent strips by glucometre
- The displayed result recorded

Appendex V *Persea americana* fruit juice preparation

- After peel and seed removed 80gm of *Persea americana* pulp was measured
- The weighted *Persea americana* was added to graduated cylinder and deionized water was added until the volume become 200ml.
- Mix with blender until homogenous mixture formed
- 856mg/Kg, 1712mg/Kg and 2568mg/Kg was administered as low, middle and higher dose respectivily
- This juice must be fresh and prepared daily