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DEPARTMENT OF ANATOMY

EVALUATION OF ANTIHYPERGLYCEMIC EFFECT OF MORINGA STENOPETALA AQUOEOUS LEAVES EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

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This is to certify that the thesis prepared by Mr Samuel W/kidan entitled: Evaluation of antihyperglycemic and pancreatic damage effect of*Moringa stenopetala* aqueous leaves extract in ratsand submitted in partial fulfilment of the requirements for the degree of Master of Science in Human Anatomy complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

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

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LIST OF ABBREVIATIONS

AAU: ……Addis Ababa University

ADA……..American Diabetes Association

ALT……...Alanine aminotransferase

ALP……...Alkaline phosphatase

ANOVA……Analysisof variance

AST……...Aspartate amino-transferase

DM……Diabetes Mellitus

EPHI: ……Ethiopian Public Health Institute

FBS……Fasted Blood Sugar

GD……Gestational Diabetes

GL……..Glibenclamide

H & E……Haematoxylin and Eosin

HDL……..……High Density Lipoprotein

HCL……...……Hydro Chloric acid

LDL……...……Low Density Lipoprotein

µm……...……...micro meter

MS……*Moringa stenopetala*

NC…… Normal Control

OECD: ……. Organization for Economic Cooperation and Development

ROS……reactive oxygen species

rpm……rotation per minute

SEM: ……Standard Error of Mean

SPSS: ……Statistical package for social sciences

H2SO4……...Sulphuric acid

T1DM……Type 1 Diabetes Mellitus

T2DM……Type 2 Diabetes Mellitus

W/V……...Weight by Volume

WHO: ……...World Health Organization

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ABSTRACT

Background: Diabetes is a serious metabolic disorder with complications that results in significant morbidity and mortality. Current drugs used for diabetes therapy are not free from side effects and do not restore normal glucose homeostasis. Therefore, the purpose of this study is to evaluate antidiabetic, and pancreatic damage effect of *Moringa stenopetala* aqueous extract on alloxan induced diabetic rats.

Methods:-The aqueous extract of *Moringa stenopetala* leaves 250 and 500mg/kg and Glibenclimide 5mg/kg/ body weight were administered to alloxan induced diabetic rats. All were administered orally using intragastric gavage for 28 days. Blood glucose level, Histopathological examination, liver and kidney functions, preliminary phytochemical screening tests were done.

Result:-After IP administration of Alloxan monohydrate, the fasted blood glucose level rose in all experimentally induced diabetic groups. Administration of *M. stenopetala* leaves aqueous extract to diabetic rats showed remarkably reduction in blood glucose concentration, there were significant ($P<0.05$) difference on Day21 and 28 when compared with diabetic group. Moreover, treatment with both doses of *M. Stenopetala* extract to diabetic rats produced significant reduction in the blood glucose levels of rats when compared with diabetic group. Continuous oral administration for 28 days of the plant for diabetic rats led to a significant decrease in serum Urea, Creatinine, ALT, AST and ALP ($P < 0.05$) as compared with diabetic group who showed significant concentrations. This significant reduction was equivalent that occurred on application of glibenclamide. Levels of serum cholesterol remained unaltered in the experimental groups when compared with diabetic control. Histopathology of diabetic untreated rats revealed degeneration of pancreatic islet cells, which was due to alloxan used in this experiment. However, signs of regeneration of β-cells observed following consumption of *Moringa stenopetala* aqueous leaves extract as reported.

Various phytochemical tests performed on the plant revealed the presence of different secondary metabolites. *M. stenopetala* leaves aqueous extract (250 and 500mg/kg) were improved the body weight of rats significantly from Day14-Day28 when compared with the Diabetic group.

Conclusion:-The present study demonstrated that repeated oral administration of *Moringa stenopetala* aqueous leaf extracts (250mg/kg and 500mg/kg) for 28 days has shown beneficial effects on antihyperglycemia, body weight improvement restore biochemical changes of blood, improve alloxan pancreatic damage. *Moringa stenopetala* aqueous leaves extract have active ingredients responsible for antihyperglycemic effect. The phytochemical screening indicated the presence of alkaloids, phenols, flavonoids, tannins, terpenoids, steroids and saponins.

Keywords: *Moringa stenopetala; Diabetes; Alloxan monohydrate*

1. INTRODUCTION

1.1.Background

Majority of population of the world use traditional medicinal plants in primary medical problems (Grover and Yadav, 2004). Hence, medicinal plants become interest of many scientists and researchers to investigate pharmacology and active ingredients of plants (Thatte and Dahanukar, 1986; Cordell and Colvard, 2005).

Lack of equitable distribution and unaffordability of modern drugs to the majority of the population are still serious problems. Therefore, in Ethiopia the use of plant medicine both in rural and urban population is common (Abebe, 1996). These practices could be attributed to cultural acceptability, and economic affordability as compared to modern medicine (Addis et al., 2001).

In view of this, the development and the ultimate integration of traditional medicine with the modern health care system have significant impact on the expansion of the health care coverage (Abebe, 1996).

Therefore, most of the world´s populations are using medicinal plants as an alternative treatment for diabetes. Among these plants *Moringa stenopetala* is the one which showed antihyperglycemic effect as reported by many researchers (Abebe, 1996)

1.2.Diabetes Mellitus

Diabetes mellitus is still one of the most important causes of death and disability in both developed and developing countries. According to the report by World Health Organization (WHO, 2015), 9% of adults in the world suffer from diabetes and this disease will be the 7th leading cause of death in 2030.

DM is a serious micro and macro metabolic disorder described by HBG levels, and also no/ partial response to physiological processes due to problem in insulin secretion and/or insulin action. Majorly known two types of it.Type1 and Type2. Both have genetic origins and also may be environmental and cultural influences such as diet and lifestyle. Type2 develops slowly, typically becoming more serious and difficult to treat over time (Riddle, 1997). This metabolic disorder leads to abnormality of carbohydrates, proteins and lipids metabolism (Andrade et al., 2005; Schoenfelder et al., 2006).

Fasting blood glucose level/ concentration of 126mg/dl and above is diagnosed as DM in human beings. After discovery of insulin by Banting and Best in 1912, makes less easy and transformed the killer disease into a chronic health problem (Hurwitz, 1990). Findings showed

that Type2 diabetes and hyperglycemia are causes for different disorders (Deedwanea, 2000) like kidney, eye, heart, and nerve complications.

With early diagnosis and tight glycemic control, complications may be significantly delayed or even prevented. Initially, lifestyle modifications may be sufficient to achieve glycemic control, but long term adherence to such changes is unusual; even patients, who successfully maintain programs of diet, exercise, and weight control may experience recurrent hyperglycemia because of declining insulin secretion. Most patients require drug therapy with antihyperglycemic agents soon after Type2 diabetes is diagnosed (Riddle, 1997).

1.2.1. Types of Diabetes Mellitus

Diabetes can be classified based on the etiology and clinical symptoms into the following general categories:

Type 1

Type 1 diabetes is a result of cellular mediated autoimmune destruction of the insulin secreting β-cells of the pancreas, which results in an absolute deficiency of insulin for the body. Patients become prone to ketoacidosis. It occurs in children and young, usually before 40 years of age, although can occur at any age. Type I diabetes make the patients insulin dependent medication for survival. It may account for 5 -10 % of all diagnosed cases of diabetes. Autoimmune, genetic and environmental factors are the major risk factors for type I diabetes (NDFS, 2005; Abebe et al., 2003 and Cavallerano).

Type 2

Type 2 diabetes causes is multifactorial. It is primarily due to lifestyle factors, obesity, living a sedentary lifestyle, bad diet and genetics which is characterized by hyperglycemia and lipoprotein abnormalities. It also associated with increased risk for developing premature atherosclerosis due to an increased in triglycerides (TG) and low density lipoproteins (LDL), and decrease in high density lipoproteins (HDL). It accounts about 90% of the chronic diseases.

The rise in prevalence is predicted to be much greater in developing than in developed countries (69 versus 20%, respectively) (Shaw et al., 2010). In developing countries, people aged 40 to 60 years (working age) are vulnerable compared with those older than 60 years in developed countries (Shaw et al., 2010).

It involves at least two primary pathogenic mechanisms: (1) a progressive decline in pancreatic islet cell functions resulting in reduced insulin secretion and inadequate

suppression of glucagon secretion3, 4. (2) peripheral insulin resistance resulting in a decrease in the metabolic responses to insulin1.

It is widely recognized that both insulin secretion and insulin resistance are important elements in the pathogenesis of type 2 diabetes. Subjects with insulin resistance require more insulin to promote glucose uptake by peripheral tissues, and genetically predisposed individuals may lack the necessary β-cell secretory capacity. The resulting insulin deficiency disrupts the regulation of glucose production in the liver and is a clue element in the pathogenesis of glucose intolerance.

Gestational Diabetes (GD) Mellitus

GD refers to the onset or initial recognition of glucose intolerance during pregnancy, usually in the second or third trimester. It occurs in about 4% of all pregnancies.

It is temporary and fully treatable, but if untreated, may cause problems with the pregnancy such as macrosomia (high birth weight), fetal malformation and congenital anomalies such as cardiac, central nervous system and skeletal malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinaemia may result from red blood cell destruction in this type of diabetes. In severe cases prenatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment (Kenneth, 2006).

1.2.2. Pathophysiology of DM

Clinical evidence of Type1 diabetes mostly involves symptoms such as polyuria, polyphagia, and polydipsia are thought to occur after autoimmune destruction of most of the pancreatic cells which results in sever insulin shortage and fasting hyperglycemia (Greenbaum et al.,2001).

Pathology of Type2 diabetes is impaired insulin secretion and reduced ability of insulin to act on the major insulin sensitive tissues (Davis and Granner, 1996). The combination of this defects results in an inability of the body to maintain glucose homeostasis leading to hyperglycemia and other metabolic disturbances. The decreased responsiveness to insulin termed “insulin resistance “is due to a reduced ability of insulin to activate it’s signaling pathways (Goldfine, 1999).

Pancreas is an organ that mainly involved in metabolism of glucose by secreting insulin and glucagon. The endocrine portion of the pancreas secretes the insulin and glucagon directly in

to the blood. Insufficient secretion of insulin, inadequate structure or function of insulin or its receptors results in impaired metabolism of carbohydrates, proteins and fats, described by hyperglycemia (ADA, 1984; Hurwitz, 1990; ADA, 2005).

An existence /absence of insulin or ineffective insulin activity prevent glucose from entering into the cell. As the glucose level close to 180 mg/dl, the ability of the kidney to reabsorb glucose will decrease and glucose is excreted into the urine causing frequent urination in large quantities leading to dehydration, hunger, and fatigue.

Therefore, classic symptoms of DM include polyuria, polydipsia, and polyphagia (ADA, 1984; Harwits, 1990; ADA, 2005). Hence, the body begins to break stored protein, and leading to a negative nitrogen balance due to inability of glucose to generate energy.

In the continued absence or ineffectiveness of insulin, fat are mobilized into free fatty acids. However, excessive amounts of fatty acids cannot enter into the Krebs cycle and instead condensed into ketone bodies called acetoacetic acid and beta hydroxybutyrate. Then these acids are excreted and buffered with body base reservoir like bicarbonate. As these complication continues production of ketone bodies, however, causes a drop in plasma PH and fatal if left untreated. Hyperglycemia is most frequently observed to cause metabolic acidosis (Herwits, 1990).

1.2.3. Epidemiology of DM

Occurrence of DM increases with aging of the population and life style change associated with urbanization and westernization (Sobngwi, et al., 2001).

In Ethiopia no population based prevalence study exist but hospital based studies show that the prevalence of diabetes admission has increased from 1.9% in 1970 to 9.5% in 1999 of all medical admissions. It accounts about 7% of all deaths over the age of 55 years in the medical wards of referral hospitals. According to WHO estimate, the number of diabetic cases in Ethiopia in the year 2000 were 800,000 and is expected to increase to 1.8 million by 2030 (Feleke and Enquselassie, 2005).

1.2.4. Prevalence and incidence of DM

Globally, the prevalence of DM, independent of type, was estimated to be 2.8% in 2000 and expected to raise 4.4% in 2030 (Wild et al., 2004). The reasons of increment in prevalence rate are due to higher life expectancy, urbanization, population growth, physical inactivity, and obesity (Hayes and Unwin, 2001; Sobngwi et al., 2001; Wild et al., 2004).

In Ethiopia, even though the prevalence of diabetes is not well studied, the number of people seeking medical attention due to diabetes has been increasing for the past three decades (Abdulkadir and Reja, 2001). Incidence rates of diabetes from hospital admissions in Ethiopia vary from 0.5% to 8.4%.

The reasons for the increasing number of diabetes in Ethiopia possibly due to the effects of urbanization, westernization and higher life expectancy. And also possibly, diabetes in Ethiopia is considered to be far less important than infectious diseases because of limited public health awareness about the disease prevalence (Habtuet al., 1999).

1.2.5. Complications due to DM

Untreated diabetes can cause many complications in many parts of the body. Serious long-term complications include heart disease, kidney failure and damage to the eyes (Alberti et al., 1998; WHO, 2014). In pregnancy, poorly controlled diabetes increases the risk of fetal death and other complications.

Metabolic syndrome

A majority of patients with T2DM have features of metabolic syndrome, which has also been called “Syndrome X” (Reaven and Banting, 1988). The major components of the metabolic syndrome include abdominal obesity, glucose intolerance/T2DM, dyslipidemia and hypertension (Hauner, 2002).

Cardiovascular disease

Macrovascular complications of DM are due to accelerated atherosclerosis and have an important role in the increased morbidity and mortality suffered by these individuals (Wingard et al., 1995). Cardiovascular disease (CVD) has for a long time been among the worldwide public health problem and remained the leading cause of death in many countries. CVD includes several types of vascular and heart diseases, atherosclerosis is the cause of approximately 75% of all CVD-related deaths (Thom et al., 2006].

The clinical manifestations of CVD include coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease. CVD mechanism is accelerated atherosclerosis. The atherosclerotic process starts from fatty streaks, consisting of intimal deposits of lipids and macrophages with lipid droplets (foam cells), gradually developing into more advanced plaques. The process ends up in complicated atherosclerotic lesions, which through a plaque rupture and thrombosis can cause an acute myocardial infarction (Stary et al, 1995).

The severity of cardiovascular complications in diabetes is demonstrated by the statistic that diabetics are 2 to 4 times more likely to have a stroke or die of heart disease than non-diabetics. Cardiovascular disease accounts for about 70% of all deaths in patients with diabetes (Laakso, 1999).

Hyperglycemia

A strong consistent relationship has been postulated be-tween hyperglycemia and the incidence and progression of micro- and macrovascular complications in people with diabetes (Hanssen, 1997). Epidemiological data have revealed hyperglycemia to be a major player in the development of the macrovascular complications such as CAD and stroke. Prospective clinical studies in T2DM patients have shown an association between level of hyperglycemia and increased risk for mortality due to macrovascular disease (Standl et al, 1996; Lehto et al., 1997).

Diabetic retinopathy

Diabetes results in characteristic lesions in the retinal blood vessels. This can result in formation of microaneurysms (minimal retinopathy), haemorrhages and increased leakage, causing retinal edema and lipid exudates (background retinopathy). When pathological development of new vessels in the retina or abnormal blood vessels and fibrous tissue (neovascularisation) occurs, the retinopathy is classified as proliferative retinopathy (Aiello et al., 1998). The formation of fibrous tissue may eventually cause retinal detachment and severe visual impairment (Forrester et al., 1997).

Diabetic nephropathy

Diabetic nephropathy is estimated to develop in one third of both main types of diabetes (O`BRYAN et al., 1997). Nephropathy is characterized by glomerular basement membrane thickening and arteriosclerosis of small arterioles. The hall-mark of renal damage in diabetes is increased excretion of albumin in the urine. The natural history of diabetic nephropathy has been viewed as a descending path from normoalbuminuria to microalbuminuria, clinically overt diabetic nephropathy; i.emacroalbuminuria, and eventually to end-stage renal disease. The term microalbu-miuria; i.e. incipient diabetic nephropathy, has been de-fined as urine albumin excretion rate 20-200 µg/min in a timed overnight or 30-300mg/24h urine collection (Mogensen et al., 1986) as determined by sensitive laboratory measurements. Urine albumin excretion rate exceeding these values is called macroalbuminuria and considered a sign of manifest diabetic nephropathy. It has been estimated that approximately half the patients with microalbuminuria will progress to overt nephropathy (Krolewski et al., 1996).

Diabetic neuropathy

The term diabetic neuropathy includes either a clinical or subclinical disorder without any additional causes of peripheral neuropathy other than diabetes. In fact, damage to the microvasculature in peripheral nerves is now becoming recognized as a major pathogenic factor in diabetic neuropathy (Vinik et al., 1992).

The incidence of neuropathy increases with duration of diabetes and is accelerated by poor control (Feldman et al., 2002). Additionally, the death rate is as high as 50% at three years after diagnosis of overt autonomic neuropathy (KFPIR, 1996).

1.2.6. Management of DM

Consumption of energy rich, high fat, low fiber diets, obesity physical inactivity, age and genetic predisposition are among the risk factors for DM(Hayes and Unwin, 2001; Sobngwi et al., 2001; Shaw and Chrisholm, 2003; Wild et al., 2004; ADA, 2004).

As stated in one of the above subtitles, the symptoms associated with hyperglycemia include polyuria, polydypsia, polyphagia, weight loss and blurred vision (Committee Report, 2003a) while the long term complications include increased risk of retinopathy and cataract, renal disease, neuropathy, heart disease, ischemic foot disease, cerebrovascular disease and an increase in risk of infection (Ang and Lumsden, 2001; Pari and Saravanan, 2004).

It has no cure and its management includes diet, exercise, and modern drugs such as insulin and oral anti-hyperglycemic (insulin secretagogues, biguanides, thiazolidinediones and alpha-glucosidase inhibitors (Nolte and Karam, 2001) drugs as well as traditionally used medicinal plants (News and Views, 2001).

Insulin secretagogues: sulfonylureas

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

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

Glibenclamide is a potent anti-diabetic and it improves glucose control by acting both on insulin secretion and insulin action. The drug inhibits ATP sensitive K+ channels in pancreatic beta cells. This inhibition causes cell membrane depolarization, opening of voltage-dependent calcium channels, thus triggering an increase in intracellular calcium into the β cell which stimulates insulin release (Girani et al., 2016). Glibenclamide has also role on insulin action at the level of different organ/tissues and it has action at the liver, skeletal muscle, heart muscle and smooth muscle sites. In liver the drug has a positive action on glycogen deposition with direct action on the synthesis of glucose transport 2 proteins (Moore, 2007).

Insulin secretagogues: Meglitinides

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

Biguanides

Metformin is one of the most commonly used of biguanides, which is used in overweight and obese patients. Even though the molecular mechanisms of action have not as yet been clearly established it is thought that it suppresses hepatic glucose production, primarily by decreasing gluconeogenesis, as a lesser effect, it increase glucose uptake by skeletal muscles, increases insulin sensitivity, enhances glucose uptake by phosphorylation GLUT-enhancer factor,

increases fatty acid oxidation, and decreases the absorption of glucose from the gastrointestinal tract (Olokoba et al., 2012)

Thiazolidinediones

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

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

Alpha-glucosidase inhibitors

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

1.2.7. Role of Alloxan in Type 2 DM

It is possible to produce different grades of severity of the disease by varying the dose of alloxan used. Moderate diabetic animals are recommended for use in testing drugs for use in Non-insulin dependent diabetes mellitus (Williamson et al., 1996). For all animals a single dose of alloxan, 140 – 180 mg/kg (usually 150 mg/kg) is administered as a5% w/v in distilled water injected intravenously into the marginal ear vein of rabbit or intraperitoneally in case of mice and rats.

The mechanisms by which Alloxan monohydrate brings about its diabetic state includes selective destruction of pancreatic insulin secreting beta cells, which make cells less active (Junod et al., 1969) and lead to poor glucose utilization by tissues (Marles and Farnsworth, 1995).

Alloxan, in the presence of intracellular thiols, especially glutathione, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product is dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and hydroxyl radical. These free radicals are ultimately responsible for the death of the beta cells.

Alloxan selectively inhibits glucose induced insulin secretion through its ability to specifically inhibit the glucokinase through oxidation of functionally essential thiol groups in this protein, thereby impairing oxidative metabolism and the glucose sensor function of this signaling enzyme of beta cell.

Thus alloxan induced diabetes mellitus served as a pathological bio model for testing a substance with supposed antioxidant activities in vivo (Bartosikova et al., 2003). Its fragmentation takes place in beta cells exposed to alloxan (Takasu, et al., 1991,). The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent of alloxan.

1.3.Role of Medicinal Plant

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

Plants may act on blood glucose through different mechanisms including facilitating insulin's activity, acting as potential insulin-like substances, inhibiting insulinase activity and increasing the quality and/or quantity of the β- cells in the pancreas by enhancing regeneration of these cells. The fiber of plants may also interfere with carbohydrate absorption; thus affecting blood glucose (Shanmugasundaram et al., 1990; Nelson et al., 1991; Jelodar et al., 2005).

1.4.*Moringa stenopetala*

Moringa stenopetala (locally called "halleku") is a green, drought-resistant plant where leaves are commonly used in cooking for human consumption (Berhe et al., 2007). It is a deciduous plant eaten as a vegetable in the daily diet (Mekonnen and Gessesse, 1998). In Ethiopia, Leaves from the *moringa* tree are very important vegetable source in which more than 5 million people depend on, especially during dry seasons (Abuye et al., 2003; Bosch, 2004).

M. stenopetala is endemic to East Africa, where it predominantly occurs in northern Kenya and in Ethiopia. The coverage includes South Ethiopia, North Kenya and East Somalia (Mayer, 1990). *M. stenopetala* is often referred to as the African *Moringa* tree because it is native only to southern Ethiopia and northern Kenya. Though, it grows in many other parts of the tropics, it is not as widely known as its close relative *Moringa oleifera,* but often considered more desirable than *M. oleifera* (Mark, 1998).

According to Sutherland et al (1994), the two most common English vernacular names for the tree are 'drumstick' (describing the shape of its pods) and 'horseradish' (describing the taste of its roots).

M. stenopetala differs from *M. oleifera* in that its leaves are made up of leaflets (3.3-6.5 cm) with a pointed rather than a rounded tip; its pods larger than those of *M. oleifera* are twisted when the fruit is fresh; its seeds are ellipsoidal and not spherical, and cream-colored rather than dark brown (Jiru, 1995).It belongs to the family Moringaceae that is represented by gene *Moringa*. It is a branched tree that grows 6-10m tall, thick white to pale gray or silvery color and smooth soft bark (Abuye et al., 2003). The species is known by name of Shiferaw in Amharic, Halleko in Wollayitegna and Gamogna, and Cabbage tree in English. It grows widely at an altitude range of 1000-1800m.

Different parts of the plant have been studied of nutritional and medical impacts. The leaves are used as vegetable foods (Abuye et al, 2003). The flowers are good nectar sources for honey; the seeds are used in clearing muddy water, the grinded wet or dried root used to treat malaria (Mekonnen and Gessesse, 1998).

There is also report on medicinal value for stomach pain and to expel retained placenta following birth (Mekonnen, 1999), antileshimanial effect (Mekonnen and Gessesse, 1998), antitrypanosomial effect (Mekonnen et al., 1999) and antimicrobial effect (Biffa, 2005). Its crude aqueous extract of leaves showed hypoglycemic effect on rabbit and mice (Mekonnen et al., 1997; Mussa et al., 2008).

1.4.1. Taxonomic position of MS

M. stenopetala belongs to family *Moringaceae* that is represented only by a single genus *Moringa*and 14 species. It has some features similar to those of *Brassicaceae* and *Capparidaceae* but the seed structure is not similar with either of the above families (Edwards et al., 2000). These indicate that the taxonomic position of the family is not yet settled and is open for further studies. But, some literatures suggest and place the species in the following taxonomic distribution (Table 1).

Table 1: Taxonomic distribution of *Moringa stenopetala*

Taxonomic rank	Nomenclature
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Capparales*
Family	*Moringaceae*
Gene	*Moringa*
Species	*Stenopetala*

1.4.2. Nutritional Role of MS

The leaves are one of the best vegetable foods that can be found in the locality. In fact, all parts of the tree except the wood are edible, providing a highly nutritious food for both humans and animals. It was reported that *Moringa* foliage and fruit pods are rich sources of calcium and iron, and good sources of vitamins A, B, and C with good amounts of the sulphur-containing amino acids, methionine and cystine (Rams, 1994).

Both young and older leaves are edible, though older ones are milder and tender, and can be either cooked in soups or boiled, while dried leaves can be stored as future soup or sauce supplements. Often, the green pods and surrounding white material can be removed from larger pods and cooked in various ways. Likewise, immature seeds are often cooked and eaten as a fresh vegetable, while mature seeds can be dried and roasted. Usually, edible oil can be extracted from its seeds. On the other hand, the flowers can be cooked or dried and steeped as tea. The roots are often used as flavoring in poultices. In southern Ethiopia, *M. stenopetala* is very widely grown for its edible leaves, especially in the Konso special district (Rams, 1994).

1.4.3. Therapeutic uses of MS

Currently there are fourteen known species of *Moringa* trees in the family *Moringaceae*, and a study that evaluated the antioxidant effect and nutritional content of four types (*Moringa oleifera, Moringa peregrina, Moringa stenopetala* and *Moringa drouhardii)* showed that all have a high content of antioxidants (Yang et al., 2006).

In many of its ecological areas, several parts of the plant have been used in medicinal preparations. It is reported that the leaves and roots, mixed with the water are used to treat malaria, hypertension, stomach disorders, asthma and the leaves, boiled in water, can cure malaria, hypertension and stomach pain, whereas the roots, chopped and mixed with water, are also used for treating severe malaria (Yang et al., 2006).

In southern Ethiopia, *M. stenopetala* is used as herbal medicine in areas where visceral leishmaniasisorkala-azarprevails (Mekonnen and Dräger, 2003). Furthermore, *M. stenopetala* leaves are used to expel the retained placenta in women who have just given birth (Mekonnen and Gessesse, 1998). In Arba Minch area, the leaves are also used by local people to treat hypertension and diabetes (Padayachee and Baijnath, 2012).

In many areas of Ethiopia, the leaves and roots are also used to treat malaria, hypertension, colds, asthma stomach problems and diabetes (Bosch, 2004). Compared to other fruit and vegetables rich in antioxidants, *Moringa* has a high content of antioxidants and is also rich in protein, calcium and iron (Yang et al., 2006).

A study that fed mice with aqueous leaf extract of *Moringa stenopetala* showed a significant decrease in both blood cholesterol and glucose levels after treatment with *Moringa* leaf extract (Ghebreselassie et al., 2011).

In this regard, ethanol extracts of the leaves and roots of *M. stenopetala* have shown effect against *Trypanosoma brucei* and *Leishmania donovani.* Furthermore, crude seed extracts have shown anti-microbial activity strongly inhibiting the growth of *Staphylococcus aureus, Salmonella typhi, Shigella species* and *Candida albicans* due to the presence of benzyl isothiocyanate which is an active bactericide and fungicide (Bosch, 2004; Padayachee and Baijnath, 2012).

The presence of glucosinolates determined by (Eilert et al. 1981) as 4(á-lrhamnosyloxy) benzyl isothiocyanate in the seeds of *M. stenopetala*found to exert many biological activities, such as anti-cancer activity due to their ability to kill cancer cells by inducing apoptosis, depleting ATP and leading the cells to oxidative stress (Padayachee and Baijnath, 2012).

On the other hand, (Bennett et al. 2003) analyzed the major secondary metabolites in the tissues of *M. stenopetala* and determined the presence of low amounts of 4-monoacetyl-4-(R-Lrhamnopyranosyloxy)-benzylglucosinolate isomers, but significant amounts of 4-(R-L

rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate are found in the stem tissue.

The root tissues found to contained both 4-(R-Lrhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate. The leaves of *M. stenopetala* contained quercetin 3-O-rhamnosylglucoside (rutin) and traces of quercetin 3-O-glucoside. On the other hand, the raw leaves of *M. stenopetala* are also known to contain isothiocyanates (cyanogenicglucosides), which is a known goitrogenic factor that can be detrimental to humans.

Previous studies conducted in Ethiopia have shown a significant correlation between the prevalence of goiter and the frequency of consumption of the leaves. For instance, (Abuye et al. 2003) determined the presence of cyanogenicglucosides in the raw leaves (88.8 mg/100 g) and cooked leaves (79 mg/100 g) of *M. stenopetala* and suggested that significant and frequent consumption of the leaves may exacerbate hypothyroidism since it is widely consumed by populations living in areas of incidence of endemic goiter though these concentrations are less than what is expected to cause goiter.

1.5.Statement of the Problem

Diabetes is a serious metabolic disorder with micro- and macrovascular complications that results in significant morbidity and mortality. Current drugs used for diabetes therapy are not free from side effects and do not restore normal glucose homeostasis (Rang et al. 1991).

Ethno botanical information indicates that more than 800 plants including *Moringa stenopetala* have been used as traditional remedies for the treatment of diabetes (Ajgaonkar 1979, Alarcon-Aguilara et al. 1998).

The antihyperglycemic effect of a large number of these plants has been evaluated and confirmed in different animal models (Karawya et al. 1984, Farjou et al. 1987, Swanston-Flatt et al. 1991a, 1991b, Jouard et al.2000). Therefore, the present research planned to investigate antihyperglycemic, antihyperlipidemic, and regeneration effect of *Moringa stenopetala* on Alloxan induced rat pancreas.

1.6.Significance of the Study

Recently, there has been increasing interest in the use of medicinal plants. The plant kingdom has become a target for multinational drug companies and research institutes for the discovery of new biologically active compounds and potential drugs (Evans, 1996).

Moreover, providing modern medical healthcare across the world, especially in developing countries, is still a far-reaching goal due to economic constraints. Thus, the outcome of this study will serve as premise for further investigation on *Moringa stenopetala* and alternative medicinal plants for diabetes.

2. OBJECTIVES

2.1. General Objectives

- ✓ To evaluate antihyperglycemiceffect of *Moringa stenopetala* aqueous leaves extract on Alloxan induced diabetic rats

2.2. Specific Objectives

- To evaluate anti-hyperglycemic effect of *Moringa stenopetala* aqueous leaves extract on diabetic induced rats
- To evaluate body weight change of experimental rats
- To explore biochemical changes of blood at different doses of extract
- To evaluate the regenerating effect of the extract on pancreas histopathology of rats
- To perform phytochemical screening on *Moringa stenopetala*leaves aqueous extract

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted at AAU, College of Health Science, School of Medicine, Department of Anatomy; Traditional and Modern Medicine Drug Research Directorate, EPHI.

3.2. Study Design

The fresh *M .stenopetala* leaves were collected from Southern Ethiopia around Arbaminch, based on ethno-botanical description. The animals (rats) were divided in to five groups then the aqueous extract of *Moringa stenopetala* leaves 250 and 500mg/kg and Glibenclimide 5mg/kg/ body weight were administered to alloxan induced (180mg/kg) diabetic rats. Blood glucose level and Body weight change measured every week till the end of study (28days). Finally, Histopathological examination, liver and kidney functions, and preliminary phytochemical screening tests were done.

3.3. Study Period

The study was conducted from Jan, 2016- Jan, 2017 (including writ-up of proposal- submission of thesis)

3.4. Collection of Plant Material

The fresh *M .stenopetala* leaves were collected from Southern Ethiopia around Arbaminch, based on ethno-botanical description, about 500km far from Addis Ababa on December 2016.The plant material was authenticated by a taxonomist in the EPHI and a voucher number AL-001was deposited in the herbarium for future reference (Debella, 2002).

3.5. Extraction of Plant Material

Fresh *M. Stenopetala* leaves were cleaned of tiny particles, chopped, dried under shade (at room temperature), grinded to powder using mortar and pestle and stored in cool and dry place. Weighed amounts of 1.208 and2.130Kg powdered leaves were kept in Erlenmeyer flasks and macerated with water (distilled) for 4hrs with intermittent agitation by orbital shaker DS-500. Then, the supernatant part of agitated materials separated from the undissolved portion of plant. The supernatant portion was filtered with 0.1 mm^2 mesh gauze. The filtrate of plant was freeze-dried at lower temperature (-46^oC to -51^oC) and lower pressure ($133x10^{-3}$mbr) to form crude extract. Then kept in a diseccator at room temperature (Debella, 2002)

3.6. Preparation of Experimental Animals

Total of 30 wistar rats (*Rattus norvegicus*) weighing 90 to 150 g were randomly divided into five groups (n = 6 in each). These were labelled as Normal control (NC) and Diabeticcontrol (DC), *M. stenopetala aqueous* leaves extract treated group (MS 250 & 500mg/kg) and diabetic glibinclamide treated (GL 5mg/kg). Each group of rats was kept in a separate cage, in the same room with standard temperature and light period at 21±1°C. They were allowed on standard rat diet and water in the experimental. All the animals used for this study were acclimatized to laboratory conditions for two weeks prior to the experiment to avoid any non-specific stress (OECD, 2008).

3.7. Induction of Experimental Diabetes Mellitus

Diabetes was induced by intra-peritoneal injection of Alloxan monohydrate at a dose of 180mg/kg/ body weight after dissolved in normal saline. Prior diabetes induction, the animals were fasted for 12 hours. Confirmation of diabetes was done seven days after alloxan monohydrate treatment (Fasting Blood Sugar), using One Touch glucometer blood sample for the FBS obtained from tail puncture of the rats, and animals with FBS ≥126mg/dl included in the study as diabetic animals (Aruna*et al.*, 1999).

3.8. Experimental Procedure

The animals (rats) were divided into five groups including NC and DC diabetic *M. stenopetala* treated group (MS 250 & 500mg/kg), diabetic glibinclimide treated group (GL 5mg/kg). The normal control group was kept normal without treatment and the diabetic control group was kept as diabetic without treatment. The rest three groups were treated with 250, 500 mg/kg/day *M. Stenopetala* leaves aqueous extract and 5mg/kg/day glibinclimide, respectively for 28 days orally using oral gavages.

3.9. Blood Glucose Level Determination/ Pharmacological Evaluation

Blood samples were taken from the tail of animals and glucose levels were determined at the beginning of the experiment (0 day) and every seventh day of 12 hours fasted animals using one touch glucometer**.**

3.10. Blood Collection

At the end of treatment period, the animals were sacrificed and blood samples were collected into non anti-coagulated tubes. Blood of approximately 5 ml volume was drawn from the heart with a plastic disposable syringe (3ml) after an overnight fast.

3.11. Blood BiochemicalDeterminations

The blood was then centrifuged at 25000 rpm for 7min and serum was collected. Clinical biochemistry such as aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Urea, Creatinine and Cholesterol level were evaluated to determine the liver and kidney functions of experimental animals (Abbas and Qureshi, 2013)

3.12. Histological Examination

The fixation process started quickly after removal of the sample. On labeling tape, label the plastic sample jar with the names of the experimental group members, date, and type of sample. The vial filled about 2/3 full with the fixative, the experimental rats were sacrificed and pancreas were removed then placed into separate vials. After overnight fixation, pancreases were washed with tap water to remove excess fixatives for several times. The water removed from tissue block by the process of dehydration with increased concentration of ethanol 70% (2hrs), 80% (), 90% (), absolute alcohol (I, II & III for one and half hours for each, and IV overnight). Prior to sectioning, tissue were cleared in two changes of xylene I (one and half hours) and xylene II (for two and half hours). Then tissue were infiltrated with three steps of paraffin wax I, II, & III for one and half hours, two and half hours, and overnight , respectively. After infiltration, the tissue allowed to solidify in a mold, embedded within a small cube of paraffin forming tissue blocks, whereby each tissue block was labelled and stored at room temperature.

The microtome will drive a knife across the surface of the paraffin cube and produce a series of thin sections of very precise thickness. Tissue blocks were sectioned in ribbons at a thickness of 5 µm with Leica rotary microtome (LEICA RM 2125RT, Germany). The ribbons of sections were taken at every 5th sections and put onto the surface of a warm water bath at temperature of 40oC. The floating ribbons over the surface of warm water were mounted onto pre cleaned slides.

The slides containing paraffin wax sections were arranged within the slide holder and placed in an oven with temperature of 40oC for overnight; to fix the tissue to the slides .The next day tissue sections were allowed to cool at room temperature for 30 minutes and stained progressively with routine Harris haematoxylin and eosin staining. For this two series of coupling jars were prepared. One for paraffin removal and hydration, and the other for dehydration and clearing. Sections were placed in xylene- I for 4 minutes and xylene II for 4 minutes to remove the paraffin from tissue and hydrated with decreasing concentrations of absolute alcohol I, II for four minutes each and 95% and , 80% of alcohol for three minutes each. The tissue sections were washed with tap water for five minutes and stained progressively with Harris haematoxylin for 10 minutes, then washed under running tap water

for five minutes. The slides were immersed in acidic alcohol for differentiation and controlling over stained haematoxylin for 1-3 seconds and then put in bluing solution (Sodium bicarbonate) until they became blue. Then, the slides were counter stained with eosin for one minute and then washed in tap water for five minutes. The sections were dehydrated with increasing alcohol concentration of 80%, 95%, absolute II and I for three minutes, each.

The dehydrated sections were cleared with xylene II and I for three minutes each and permanently mounted on microscopic slides using DPX and cover slips and then observed by light microscope for investigations of any histological change, thereby the histology of the treated groups were compared with histology of the control group.(Conn, 1946)

3.13. Light Microscopy and Photomicrography

Finally, Stained tissue sections of pancreas were carefully examined under binocular compound light microscope (LEITZ WETZARE, Germany) and CX41RF, Philippines). Tissue sections from the treated groups were examined for any evidence of histopathological changes with respect to those of the controls. After examination, photomicrograph of selected samples of pancreas sections from both the treated and control rats were taken under a magnification of x40 objective lens by using digital photo camera according to (Conn, 1946) procedure.

3.14. Phytochemical Screening

MS aqueous leaves extract used for the *in vivo* study was subjected to phytochemical screening following methods described by Tiwari et al. (2011). The extracts along with negative controls were tested for the presence of alkaloids, saponins, polyphenols, flavonoids, terpenoids, tannins, phytosterols, and glycosides as follows:

A) Alkaloids: One and half millilitre of 10% HCl was added to 0.5mg of the extracts in a test tube. The mixture was heated for 20min. It was then cooled and filtered. To 1ml of the filtrate five drops Mayers and Draggendorff's reagents each were added. Formation of cream and orange colour precipitates respectively indicates the presence of alkaloids in the extracts.

B) Saponins Froth test: An aqueous solution of 0.5mg of the extract in a test tube was vigorously shaken for 2min. Foam which persisted for 30min and doesn't disappear upon warming was taken as an indication of the presence of saponin in the extract.

C) Polyphenols (Phenolic compounds): Three drops of a mixture of 1ml 1%FeCl3 and 1% K3Fe (CN)6 each were added to 2ml of extracts. Formation of green or blue colour was taken as an indication of the presence of polyphenols.

D) Flavonoids: To 2ml of aqueous solution of the extract four drops of 2% lead acetate solution was added. Development of yellow or orange colour confirms the presence of flavonoids.

E) Terpenoids (Ketonic): One millilitre of 2, 4-dinitrophenyl hydrazine solutions (0.5g dissolvedin100mlof2MHCl) was added to 2ml aqueous solution of the extract. Formation of yellow-orange coloration indicates the presence of a ketonic terpenoids.

F) Tannins: Three drops of 5% ferric chloride solution was added to 1ml of the extract solution in water. A greenish or blue coloration or precipitation was taken as indication of the presence of tannins.

G) Phytosterols and with anoids: Five drops of 3% vanillinin conc.H2SO4 was added to a concentrated chloroform solution of extracts. Formation of a rose or reddish brown colour indicates the presence of anoids or phytosterols.

3.15. Statistical Analysis

The data was collected and analysed using SPSS version 24. Mean ± S.E.M is given for quantitative variables. One-way analysis of variation (ANOVA) was applied to observe group mean differences. Post Hoc Turkey test was applied to observe which groups mean differs. A *p*-value of < 0.05 was considered as statistically significant.

3.16. Ethical Consideration

The study was conducted in accordance with ethical clearance approved and obtained from Department of Anatomy Graduate Committee; School of Medicine, College of Health Sciences, AAU and Ethiopian Public Health Institute. Animals used in this study were kept from any unnecessary painful and terrifying situations (OECD, 2008). All animals were given appropriate anesthesia to keep from pathogens, pain and suffering minimal during any surgical operation.

4. RESULTS

4.1. Blood Glucose Level Result

After IP administration of alloxan monohydrate, the fasted blood glucose level except the NC group (Normal Control) raised above 126 mg/dl in all experimentally induced diabetic groups. Only those rats with fasted blood glucose level above 126mg/dl were considered diabetic. The level of blood glucose at Day0 significantly increased when compared with NC. Administration of MS leaf aqueous extract to diabetic rats showed remarkably reduction in blood glucose concentration, there were significant ($P<0.05$) difference on Day21 and 28 when compared with DC (diabetic group). Moreover, treatment with both doses of MS extract to diabetic rats produced significant reduction in the blood glucose levels of rats when compared with diabetic control group as shown in table 2.

Table2: Blood glucose level in normal, diabetic and treated rats.

Groups	Initial (mg/dl)	Day7 (mg/dl)	Day14 (mg/dl)	Day21 (mg/dl)	Day28 (mg/dl)
NC	77±1	77±2	78.5±3	76.5±2	79±1
DC	323±12** (0.03)	327±9** (0.009)	318±12** (0.001)	312±16** (0.001)	316±26** (0.001)
GL 5mg/kg	335±91** (0.024)	284±71** (0.02)	199±38^{x},** (0.007,0.015)	156±24^{x} (0.015)	128±17^{x} (0.000)
MS 250mg/kg	340±82** 0.005	312±64** 0.007	233±37** (0.001)	173±46^{x} (0.003)	154±29^{x} (0.003)
MS 500mg/kg	333±19** 0.025	308±18** (0.008)	213±7** (0.006)	154±4^{x} (0.013)	139±3^{x} (0.001)

The mean difference is significant at the $p < 0.05$ level.

^{x} Significant $P<0.05$ when compared with DC.

** Significant $P<0.05$ when compared with NC

Data are expressed as Mean ± Standard Error of Mean (SEM); n=6

4.2. Body Weight Result

Body weight were measured every seventh day of the experiment. The two doses of the extract were improved the body weight of rats significantly from Day14, 21 and Day28 when compared with the DC group as shown in table 3.

Table3: Body weight change in normal, diabetic, and treated rats.

Groups	Initial (gm)	Day7 (gm)	Day14 (gm)	Day21 (gm)	Day28 (gm)
NC	100.5±1.2	115.7±3.7	133.4±5.3	148±6	168±7.9
DC	98.9±1.3	101.6±1	100.8±1.4	113±2.1	110.7±3.9
GL 5mg/kg	99.98±5.9	98.7±6.9	95.7±7.7	100±7.9	105.2±8.7
MS 250mg/kg	101.2±12.6	117.3±16.3	*159.4±18 (0.045)	*177±9 (0.001)	*194±7.5 (0.001)
MS 500mg/kg	100.5±6.8	135.6±13.6	*162±13.6 (0.023)	*174±15 (0.001)	*224±17 (0.001)

The mean difference is significant at the P< 0.05 level.
Data are expressed as Mean ± Standard Error of Mean (SEM); n=6
*significant P<0.05 when compared with DC.

Numbers in bracket show significance level.

4.3. Biochemical Result

Continuous oral administration for 28 days of *M. Stenopetala* leaves aqueous extract for diabetic rats led to a significant decrease in serum Urea, Creatinine, ALT, AST and ALP ($P < 0.05$) as compared with DC showed significantly concentrated. This significant reduction was equivalent that occurred on application of glibenclamide. Levels of serum cholesterol remained unaltered in the extract treated, and glibenclamide treated groups when compared with control rats as shown in table 4.

Table 4: Biochemical results in normal, diabetic and treated rats

Groups	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/L)	ALP (U/L)	AST (U/L)	Cho (mg/dl)
NC	*29.4±2.3 (0.046)	*0.25±0.015 (0.043)	*58.7±5.6 (0.001)	*64±7.3 (0.001)	*88.7±8 (0.001)	106.1±13
DC	90.8±2.2	0.64±0.06	301±1.7	398±2.8	435±25.8	144±10.3
GL 5mg/kg	*26±3.9 (0.032)	*0.14±0.01 (0.046)	*62±12.4 (0.007)	*70.2±10.6 (0.004)	*65±14.4 (0.001)	72.5±7.6
MS 250mg/kg	*31±13.2 (0.034)	*0.29±0.03 (0.04)	*58.7±7.1 (0.021)	*79±6.9 (0.038)	*82.4±20.1 (0.001)	73.4±7.2
MS 500mg/kg	*28.7±12.4 (0.045)	*0.28±0.02 (0.04)	*64.4±7.6 (0.006)	*81.1±9.8 (0.041)	*76.5±19.4 (0.001)	82.6±14.2

*. The mean difference is significant at the 0.05 level.
*significant $P<0.05$ when compared with DC.

Data are expressed as Mean ± Standard Error of Mean (SEM); n=6

Numbers in bracket show significance level

4.4. Effect of *M. stenopetala* aqueous leaves extract of on pancreas histology of rats

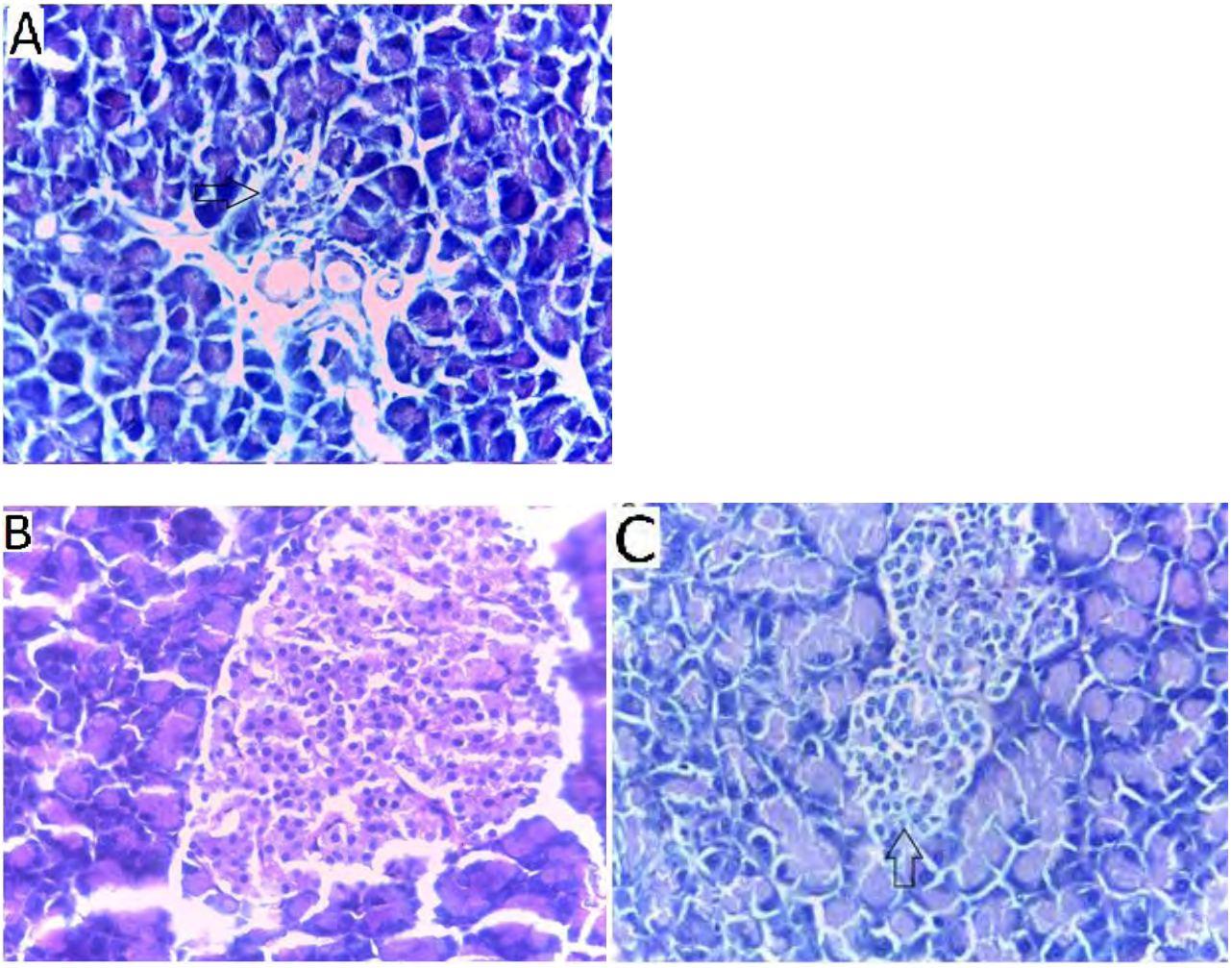

Figure 1: Photomicrograph of Alloxan treated diabetic control (A) compared with Normal control (B), and Glibinclamide treated (C). A: Arrow indicates the alloxan induced damage of islets of Langerhans. B: Arrow indicates healthy islets of Langerhans. C: Arrow indicates Glibinclamide restoration of cellular population size of islets of Langerhans. (Stained with haematoxylin and eosin, Magnification 100x)

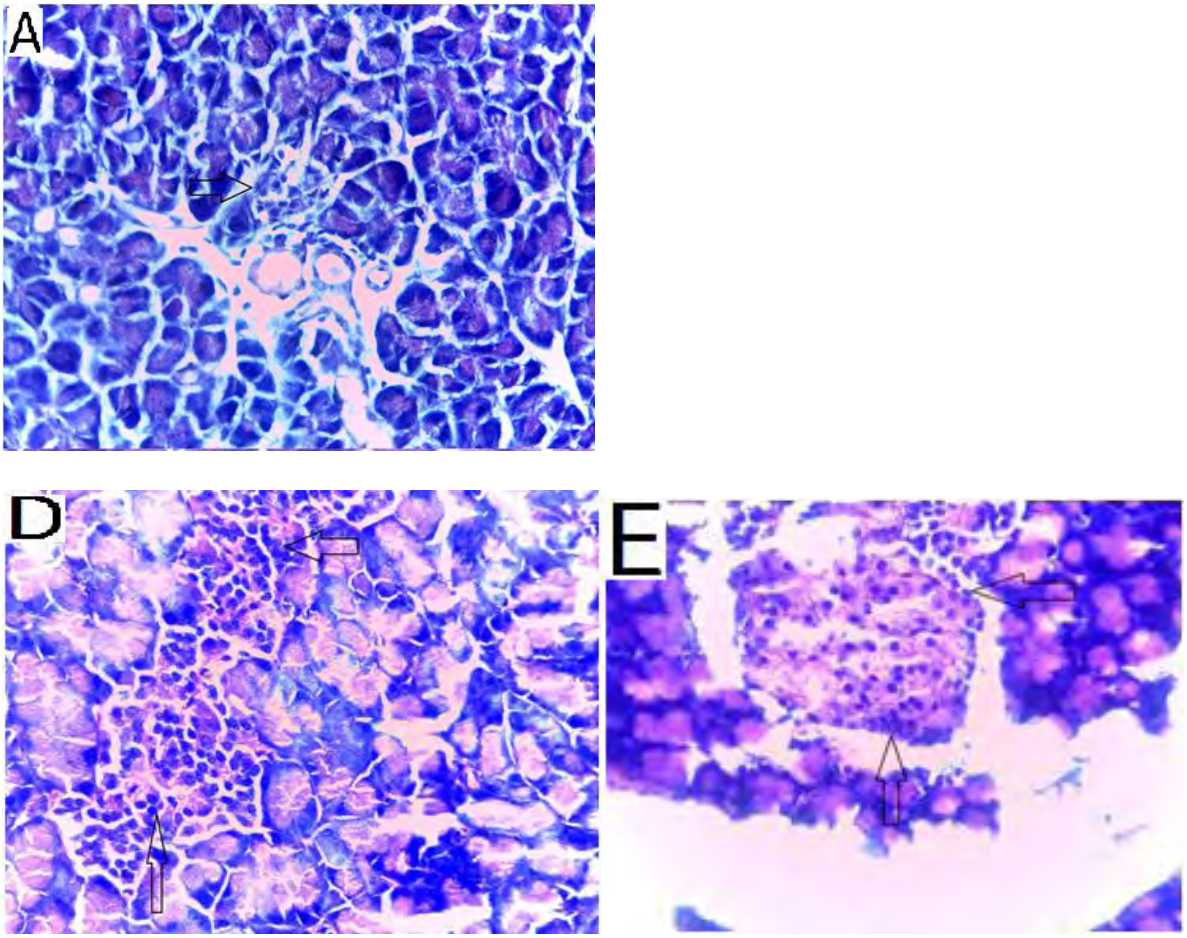

Figure 2: Photomicrograph of Alloxan treated diabetic control (A) compared with *M. stenopetala* 500mg/kg treated (D) and *M. stenopetala* 250mg/kg treated (E). A: Arrow indicates the alloxan induced damage of islets of Langerhans. D and E: Arrow indicates M. stenopetala restoration and sprouting of islets of Langerhans from pre-existing. (Stained with haematoxylin and eosin, Magnification 100x)

4.5. Phytochemical Screening Result

Various phytochemical tests performed on the aqueous extracts of *M. stenopetala* leaves extract revealed the presence of different secondary metabolites as shown in table5

Table 5:phytochemical constituents of *M. stenopetala* leaves aqueous extract

Extract	Alkaloids	Flavonoids	Tannins	Saponins	Phytosteroids	Phenols	Terpenoids
Aq. leaf of MS	+	+	+	+	+	+	+
Negative control	-	-	-	-	-	-	-

Key:"+"indicates presence and" –"Indicates absence

5. DISCUSSIONS

Different mechanisms of action of plant extracts to reduce blood glucose levels are already known. Some plants exhibit properties similar to the well-known sulfonylurea drugs like glibenclamide (Davis and Granner, 1996), while others do not affect blood glucose in normal state and act instead much like biguanides such as metformin which is a known antihyperglycemic compound (De Fronzo and Goodman, 1995; Stumvoll et al., 1995).The use of *Moringa stenopetala* in diabetics has been reported in the literature along with several other traditional claims. Hence, it was thought that investigations of these medicinal properties should be scientifically authenticated to validate the traditional claims. In the present study, in order to establish the scientific basis for the utility of the said plant in the treatment of diabetes, evaluation of antihyperglycemic and pancreatic damage effects of aqueous leaves extracts were performed in alloxan-induced diabetic rats.

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

The increase in fasting blood glucose concentration is an important characteristic feature of diabetes mellitus (DM). In this study, there were elevations in fasting blood glucose (FBG) level in diabetic group. The results of the present study on diabetic rats following daily oral administration for 28days with aqueous leaves extract of *Moringa stenopetala* (dose: 250mg/kg and 500mg/kg) demonstrated significant reduction in fasted blood glucose level after 21 days of treatment. This result is in agreement with (Toma et al, 2015; Sileshi et al, 2014).Therefore, the present study revealed that aqueous leaves extract of *Moringa stenopetala* (dose: 250mg/kg and 500mg/kg) have a significant antihyperglycemic effect on alloxan-induced diabetic rats in dose and time dependent manner.

There was weight reduction in diabetic control rats. Weight loss has been known to be one of the symptoms of DM. Alloxan induced diabetes was characterized by severe loss in body weight. It has been reported to cause massive reduction in insulin release by the destruction of the β-cells of islets of Langerhans and inducing hyperglycaemia in animals. This deficiency of insulin led to decreased amino acids uptake by tissues with a consequent reduction in the level of protein synthesis and also results in lipolysis in adipose tissues and protein

breakdown(Mohan and Nassier, 2013). Similar observations were detected in many experimental studies (Ijaola et al., 2014;Akter et al., 2014). In this study, daily oral treatment with extract showed significant increase ($p<0.05$) in body weight at the end of the experiment when compared with diabetic control. Therefore, these study is in agreement with (Toma et al, 2015; Geleta et al, 2016; Ghebreselassie et al., 2011,; Musa et al, 2015).

Diabetic nephropathy is a microvascular complication of diabetes. A key morphological change associated with sustained hyperglycemia is the accumulation of glycogen granules in distal tubules, which leads to renal hypertrophy (Kang et al., 2005). In this recent study the significant elevations in serum creatinine and urea levels indicate impaired renal function of diabetic animals. Aqueous leaves extract of *Moringa stenopetala* decreased significantly the serum urea and creatinine levels by enhancing the renal function that is generally impaired in diabetic rats and the serum total cholesterol content didn't show significant change. This result is in agreement with (Toma et al, 2014) who reported that *Moringa stenopetala* improved renal functions in diabetic rats by reducing serum urea and creatinine levels

The AST, ALT and ALP are physiologically and clinically important enzymes. Since ALT occurs in much higher concentration in the liver than elsewhere, therefore increased ALT activity specifically reflects hepatic damage, which is a normal occurrence in diabetes may be due to leakage of the enzymes to blood stream. Within limits AST and ALT levels act as indicators of normal liver function (Prince and Menon, 2000). Diabetes and hyperlipidemia also cause cellular damage by altering the cell membrane architecture resulting in enhanced activities of ALP in diabetic rats. Presently in treated diabetic rats, restoration of these enzymes to normal levels indicates restoration of liver normal functioning upon treatment with *M. stenopetala* aqueous leaves extract. The present results appear consistent with a previous report, the restoration of transaminases to their normal levels after treatment indicates revival of insulin secretion and regenerative activities of islets of Langerhans cells of pancreas after administration of the plant material, by (Toma et al, 2015) who studied the effects of *M. stenopetala* extracts in Streptozotocin - induced diabetic rats and (Sileshi et al, 2014) alloxan induced diabetic mice.

It has been suggested that enhanced production of free radicals and oxidative stress are central events to the development of diabetic complications. Use of antioxidants reduces oxidative stress and alleviates diabetic complications (Hasani-Ranjbar et al., 2008). Alloxan produce hyperglycemia by selective cytotoxic effect on pancreatic beta cells, via disruption of the cell membrane integrity. The pancreatic beta - cells are known to be involved in the synthesis, storage, and release of insulin, the peptide hormone regulating carbohydrate, protein, and lipid metabolism. One of the intracellular phenomenons for its cytotoxicity is through generation of

free radicals as reviewed by (Ijaola. 2014). It is equally possible for *M. stenopetala* to have regenerated remnants of the already alloxan- destroyed cells. It probably prevented the destruction of beta cells of islets in the pancreas. There is also interesting finding and suggests are reported of other plant that may have antioxidant or free radical scavenger properties in preventing these changes property of *Thymus schimperi* leaves have been reported earlier by Gebrehana and Shimelis (2013).

In present study, histopathological study of diabetic untreated rats revealed degeneration of pancreatic islet cells, which was due to alloxan used in this experiment. However, signs of regeneration of β-cells have been reported following consumption of *Moringa stenopetala* leaf extracts as reported by (Toma et al, 2015).

A review on the mode of action of flavonoids(Brahmachari, 2011) discussed about the various effects of the drug candidates in regulating diabetic syndromes. It has been demonstrated that flavonoids act against diabetes mellitus either through their capacity to avoid glucose absorption (inhibition of α-glucosidase activity in the intestine), or to improve glucose tolerance. Moreover, it has also been demonstrated that flavonoids can act as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications, besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. Researchers have found that the antihyperglycemic effect of plant extract may be due to the presence of tannin. Tannins are excellent free radical scavengers, this property arising mainly from the presence of well-known antioxidants (Borgohain et al., 2012).

Reduction of blood glucose level action of saponins is through restoration of insulin response, improvement in insulin signaling, increase plasma insulin levels and induction of insulin release from the pancreas, inhibition of disaccharides activity, activation of glycogen synthesis, inhibition of gluconeogenesis, inhibition of α- glucosidase activity and inhibition of mRNA expression of glycogen phosphorylase and glucose 6 phosphatase (Lavle et al., 2016).

The preliminary phytochemical screening of the extract in this recent study revealed the presence of alkaloids, steroids, terpenoids, tannins, saponins and flavonoids.Hence, the biological effect of aqueous leaf extract of *Moringa stenopetala* are connected with their active principles including flavonoids, tannins and alkaloids which have been reported to have antihyperglycemic properties amongst others, however, required in order to ascertain the actual mechanism of this plant. This finding is in line with those of the previous studies carried out on antidiabetic and antihyperglycemic activity of different solvent extracts of *M. stenopetala* (Bakerf.) Cufod. Leaves using various models: 70% EtOH and its fractions in alloxan induced

diabetic mice (Sileshietal., 2014), n-butanol fraction of 70% EtOH in alloxan induced diabetic mice (Toma et al., 2012) and AQ ,70% EtOH and n-butanol fractions in STZ induced diabetic rats (Toma et al., 2015; Geleta et al, 2016)

6. CONCLUSION

- ✓ Repeated oral administration of *Moringa stenopetala* aqueous leaf extracts (250mg/kg and 500mg/kg) for 28 days has shown beneficial effects on antihyperglycemia, improved body weight restore biochemical changes of blood, improve alloxan pancreatic damage.
- ✓ The phytochemical screening of *Moringa stenopetala* aqueous leaves extract indicated the presence of alkaloids, phenols, flavonoids, tannins, terpenoids, steroids and saponins, which has antihyperglycemic properties.
- ✓ After further investigation it is possible to say that *Moringa stenopetala* leaves can be exploited as an alternative herbal supplement for the management of diabetes.

7. RECOMMENDATIONS

- Further investigation should be carried out using advanced technologies to isolate and identify the active ingredient present in the leaves of *M. stenopetala.*
- Further researches need to elucidate the mechanism action of *M. stenopetala*.
- Experiments should also be conducted with non-rodent species.

8. REFERENCES

Aiello LP, Gardner TW, King GL, et al. Diabetic reti-nopathy. Diabetes Care 1998; 21: 143-56.

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

Alberti, K.G.M.M., Zimmett, P. Definition, diagnosis and classification of diabetes mellitus and its complications part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med, 1998. 15: 539-553.

American Diabetes Association (1984). Pathophysiology of insulin secretion in non-insulin dependent diabetes mellitus. Diabetes Care17: 491-502.

American Diabetes Association (2004). Diagnosis and classification of diabetes mellitus. Diabetes care 27: S5-S10.

American Diabetes Association (2005). Hypoglycemia in Type 2 Diabetes: Pathophysiology, frequency, and effects of different treatment modalities. Diabetes Care 28: 2948-2961.

Andrade, R.R.O., Lopez, V.R., Ramirez, M.L.G., Espana, P.C. and Soto, S.E. (2005).Antidiabetic effect on alloxanized and normoglycemic rats and some pharmacologic evaluation of Tournefortiahartwegiana.Journal of Ethinopharmacology101: 37-42

Aruna R V, Ramesh B, and Kartha V N, Effect of beta carotene on protein glycosylaion in alloxan induced diabetic rats. Ind. J. Exp. Biol, 37: 399-401, 1999.Bartosikova L, Nieces J, Succhy V, Kubinov R, Vesala D, Benes L (2003). Monitoring of antoxidative effect of Agric. Biol. J. N. Am., 2010, 1(2): 130-134 *morine*in alloxan-induced diabetes mellitus in the laboratory rat. Acta Vet. Brno. 72:191-20.

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

Biffa, D. (2005). In Vitro antimicrobial activities of bark and leaf extracts of Moringa.

Szkudelski, T. The mechanism of alloxan and Streptozotocin action in . cells of the rat pancreas. Physiol. Res., 2001, 50, 536-546.

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

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

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

Cavallerano, J. O. D. and Cooppan, R. (2002). *Optometric clinical practice guideline care of the patient with diabetes mellitus*. American Optometric Association 243 N.Lindbergh Blvd., St. Louis, MO 63141-7881, 3rd ed., U.S.A

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

Conn, H. J. (1946). Biological Stains: A handbook on the nature and uses of the dyes employed in the biological laboratory. 5th edition, Geneva, N.Y., Biotech publication, pg 24-29.

Committee Report (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 26: S5-S20.

Dapar, L. P. Maxwell , Aguiyi, C. John , Wannang, N. Noel, Gyang, S. Steven and Tanko, M. Naanlep (2007). The histopathologic effects of Securidacalongepedunculata on heart, liver, kidney and lungs of rats.

Davis S N, and Granner D K, "Insulin, Oral Hypoglycemic Agents, and the Pharmacology of the Endocrine Pancreas," 9th ed., Chap. 60, by J. G., Hardman, L. E., Limbird, P. B. Molinoff, R. W. Ruddon, A. G. Gilman. (eds). McGraw-Hill, New York. 1487, 1996.

De Fronzo R A, and Goodman A M, Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. N. Eng. J. Med., 333: 550-554, 1995.

Debella, A. (2002). Manual for phytochemical screening of medicinal plants. EHNRI, Addis Ababa, Ethiopia, pp: 26-71.

Deedwania PC. (2000). Mechanism of the deadly quartet. Can J Cardiol. 16 (suppl E): 17E-20E.

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

Esmaeili M. A. and Yazdanparast R. (2004). Hypoglycemic effect of Teucrium polium: studies with rat pancreatic islets. Journal of Ethnopharmacology95: 27–30.

Feldman EL, Stevens MJ, Russell JW. Diabetic peripheral and autonomic neuropathy. In: Sperling MA eds. Contemporary endocrinology. Totowa, N.J, Humana Press 2002; pp 437-461.

Feleke, Y. and Enquselassie, F. (2005).An assessment of the health care system for diabetes in Addis Ababa, Ethiopia. *Ethiopian Journal of Health Development,* 19:203-210.

Forrester JV, Knott RM. Pathogenesis of diabetic reti-nopathy and cataract. In: Pickup J, Williams G eds. Textbook of Diabetes. Blackwell Science, Oxford 1997; pp 45.1 -45.19.

Geleta B, Makonnen E, Debella A and Tadele A (2016) In vivo Antihypertensive and Antihyperlipidemic Effects of the Crude Extracts and Fractions of Moringa stenopetala (Baker f.) Cufod. Leaves in Rats. Front. Pharmacol. 7:97. doi: 10.3389/fphar.2016.00097.

Goldfine ID. (1999). Unraveling the riddle of insulin resistance. J Clin Lab Med. 134: 100 – 102.

Greenbaum, C. J., Cuthbertson D., Krischer J.P., and the Diabetes Prevention Trial of Type1 Diabetes Study Group, (2001). Diabetes Vol. 50.

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

Grover J.K. and Yadav S.P. (2004). Pharmacological actions and potential uses of Momordica charantia: a review. Journal of Ethnopharmacology93: 123–132.

Habtu, E., Gill, G. and Tesfay, S. (1999). Characteristics of insulin requiring diabetes in rural northern Ethiopia- a possible link with malnutrition? Ethiopia Medical Journal 37: 263-267.

Hanssen KF. Blood glucose control and microvascular and macrovascular complications in diabetes. Diabetes 1997; 46 (Suppl.2): S101-S103.

Hauner H. Insulin resistance and the metabolic syn-drome-a challenge of the new millenium. Eur J ClinNutr 2002; 56 (Suppl 1): S25-S29.

Hayes, L. and Unwin, #. (2001). Addressing the emerging pandemic of diabetes and cardiovascular disease in Sub-Saharan Africa. Report of a workshop held in Nairobi, Kenya 16-19 April 2001.

Hurtwitz, L.S. (1990). Diabetes mellitus. In: Pathophysiology, Concepts of Altered Health States.Third edition, Carol Mattson Porth J.B. Lippincott Company, East Washington Square,Philadelphia, Pennsylvania 19105.pp:797-812.

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

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

Jelodar GA, Maleki M, Motadayen SS (2005). Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. Indian J. Med. Sci., 52: 54-69.

Junod A, Lambert A, Stauffacher W and Renold A (1969). Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J. Clin. Invest. 48: 2129-39.

Kang J, Dai X S, Yu T B, Wen B, and Yang Z W, Glycogen accumulation in renal tubules, a key morphological change in the diabetic rat kidney. Acta. Diabetol, 42: 110-116, 2005.

Karam, J.H. (1995). Pancreatic hormones and antidiabetic drugs. In: Basic and Clinical Pharmacology, Sixth edition. Paramount publishing Business and professional group. Prentice Hall international Inc.

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

Kesari, A., Gupta, R.K., Singh, S.K., Diwaker, S., and Watal, G. (2006). Hypoglycemic and antihyperglycemic activity of Aegle Marmelos seed extract in normal and diabetic rats.Journal of Ethinopharmacology107: 374-379.

King's Fund Policy Institute Report, Commissioned by the British Diabetic Association. Counting the cost: the real impact of noninsulin dependent diabetes. London, King's Fund 1996.

Krolewski AS, Warram JH, Freire MB. Epidemiology of late diabetic complications. A basis for the devel-opment and evaluation of preventive programs. Endo-crinolMetabClin North Am 1996; 25: 217-242

Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes 1999; 48: 937-942.

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

Lehto S, Ronnemaa T, Haffner SM, et al. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. Diabetes 1997; 46: 1354-1359.

Lemhadri, A., Hajji, L., Michel, J.B., and Eddouks, M., (2006). Cholesterol and triglycerides lowering activities of caraway fruits in normal and Streptozoticine diabetic rats. Journal of Ethinopharmacology106: 321-326.

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

Makonnene, E., Hunde, A. and Damecha, G. (1997). Hypoglycemic effect of MoringaStenopetalaaqueous extracts in Rabbits. Phytotherapy Research 11: 147-148.

Marles RJ, Farnsworth NR (1995) Antidiabetic plants and their active constituents. Phytomedicine 2: 133-169.

Mekonnen, Y.andGessesse, A., (1998). Documentation on the use of Moringa Stenopetala and its possible antileshimanial and antifertility effects. SINET: Ethiopian Journal of Sciences 21: 287-295.

Mekonnen, Y., (1999). Effects of ethanol extracts of Moringa Stenopetala leave on guinea pig and mouse smooth muscle. Phytotherapy research 13: 442-444.

Mekonnen, Y, Yardely, V., Rock, P. and Croft, S. (1999). In vitro antitrypanosomal activity of Moringa Stenopetala leaves and roots. Phytotherapy research 13: 538-539.

Mekonnen, A. and Gebreyesus, T. (2000). Chemical investigation of the leaves of Moringa Stenopetala. Bulletin of the chemical Society of Ethiopia 14 (1): 51-56.

Mogensen CE, Chachati A, Christensen CK, et al. Mi-croalbuminuria: an early marker of renal involvement in diabetes. Uremia Invest 1986; 9: 85-95.

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

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

Mussa A, Makonnen E, and Urga K. (2008). Effects of the crude aqueous exract and isolated fraction of moringa stenopetala leaves in normal and diabetic mice; pharmacologyonline 3:1049-1055.

National Diabetes Fact Sheet (NDFS), United States, (2005). (http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2005.pdf) (Accessed on 10/11/06)

Nelson RW, Ihle SL, Lewis LD, Salisbury SK, Miller T, Bergdall V, Bottoms GD (1991). Effects of dietary fiber supplementation on glycemic control in dogs with alloxan-induced diabetes mellitus. Am. J. Vet. Res., 52: 2060-2066.

O'Bryan GT, Hostetter TH. The renal hemodynamic basis of diabetic nephropathy. Semin Nephrol 1997; 17: 93-100.

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

OECD Guidelines for the testing of chemicals, (2008). Acute Oral Toxicity – Up-and-Down-POlokoba, A. B., Obateru, O.A., and Olokoba, L. B., (2012). Type 2 diabetes mellitus: a review of current trends. Oman Med J, 27(4): 269-273.rocedure (UDP).

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

Qamar Abbas and Irfan Zia Qureshi / ISESCO Journal of Science and Technology - Volume 9, Number 15 (May 2013)(33-38)

Rahman, A.U.andZaman, K. (1989). Medicinal plants with hypoglycemic activity. Journal of Ethinopharmacology26:1-55.

Reaven GM. Banting lecture 1988. Role of insulin re-sistance in human disease. Diabetes 1988; 37: 1595-1607.

Riddle MC (1997). Tactics for type II diabetes EndocrinolMetabClin ,orth Am. 26:659–677.

Schoenfelder, T., Cirimbelli, T.M., and Zanette, V.C. (2006). Acute effect of Tremami cranthaon serum glucose levels in normal and diabetic rats. Journal of Ethinopharmacology 107:456-459.

Shanmugasundaram ER, Gopith KL, Radha SK et al. Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given Gymnema sylvestre leaf extract. J Ethnopharmacol 30: 265-9, 1990.

Sharma P, Kumari P, Srivastava MM, Srivastava S. 2006. Removal of cadmium from aqueous system by shelled Moringa oleifera Lam. seed powder. BioresourTechnol97: 299–305.

Shaw, JE. And Chrisholm, D.J. (2003). Epidemiology and prevention of type 2 diabetes and the metabolic disorder. Medical journal of Australia 179:379-383.

Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res ClinPract 2010; 87(1):4-14.

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

Sileshi,T., Makonnen,E., Debella,A., and Tesfaye,B. (2014). Antihyperglycemic and subchronic toxicity study of Moringastenopetalaleaves in mice. J.CoastalLifeMed. 2,214–221.doi:10.12980/JCLM.2.2014C300.

Sobngwi, E., Mauvais-Jarvis, F., Vexiau, P., Mbanya, J.C. and Gautier, J.F. (2001). Diabetes in Africans. Diabetes Metabolism 27:628-546.

Standl E, Balletshofer B, Dahl B, et al. Predictors of 10-year macrovascular and overall mortality in patients with NIDDM: the Munich General Practitioner Project. Diabetologia 1996; 39: 1540-1545.

Stary HC, Chandler AB, Dinsmore RE, et al. A defini-tion of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: A report from the Committee on Vascular Lesions of the Coun-cil on Atherosclerosis, American Heart Association. Circulation 1995; 92: 1355-1374.

Stumvoll M, Nurjan W, Periello G, Dailey G, and Gerich J E, Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. N. Engl. J. Med, 333: 541-549, 1995.

Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50: 537-546.

Takasu N, Asawa T, Komiya I, Nagasawa Y, Yamada T. (1991) Alloxan induced DNA strand breaks in pancreatic islets. Journal of Biochemistry 266: 2112- 2114.

Thatte, U.M. and Dahanukar, S.A. (1986). Ayurveda and Contemporary Scientific Thought. Trends in Pharmacological Sciences 7:247-251.

Thom T, Haase N, Rosamond W, et al. Heart Disease and Stroke Statistics--2006 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2006; 113: e85-e151.

Toma,A., Makonnen,E., Mekonnen,Y., Debella,A., and Adisakwattana, S. (2015). Antidiabetic activities of aqueous ethanol and n-butanol fraction of Moringa stenopetala leaves in streptozotocin-induced diabetic rats. BMC Comp. Alt. Med. 15,242–249.doi:10.1186/s12906-015- 0779-0.

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

Vinik AI, Holland MT, Le Beau JM, et al. Diabetic neuropathies. Diabetes Care 1992; 15: 1926 -75.

Wang, H.X., (1999). National products with hypoglycemic, hypocholestromic, antiatherosclerotic and antithrombotic activities. Minireview, Life Science 65:2663-2677.

WHO Expert Committee on Diabetes Mellitus (1980). Technical Report Series 646.World Health Organization, Geneva.PP.61.

Wild, S., Roglic, G., Green, A., Sicree, R.and King, H. (2004). Global prevalence of diabetes. Diabetes Care 27:1047-1053.

Williamson E.M, Okpoko D.T, Evans F.J (1996). Pharmacological methods in phytotherapy research. John Wiley and sons, Inc. Third Avenue, New York, USA. ISBN 0471 94216 2. pp. 155-167.

Wingard DL, Barrett-Connor E. Heart disease and dia-betes. In: Harris M eds. Diabetes in America. 2nd ed. Bethesda, NIH NIDDK 1995; pp 429–456.

9. APPENDICES

Appendix I: Preparation of working solutions

10% Neutral Buffered Formalin

40% Formaldehyde	100 ml
Distilled water	900 ml
Sodium dihydrogen phosphate monohydrate	4 gm
Disodium hydrogen phosphate anhydrous	6.5 gm

Harris's Hematoxylin (H)

Hematoxylin crystals	2.5 gm
Absolute alcohol	25 ml
Potassium alum	50 gm
Distilled water	500 ml
Sodium iodate	0.5 gm
Glacial acetic acid	20 ml

1% Alcoholic Eosin (E)

Eosin Y, water soluble (CI 45380)	1 gm
95% Ethanol	100 ml
Glacial acetic acid 0.5 ml	

1% Acidic alcohol

70% alcohol	500 ml
Hydochloric acid, concentrated	5 ml

Bluing solution

Sodium bicarbonate	2.5 gm
Distilled water	1000 ml

Appendix II: Tissue processing procedures

Fixation

10% Neutral Buffered Formalin	24 hrs

Washing

Tap water	several changes

Dehydration

70% Ethanol	2 hrs
80% Ethanol	2 hrs
90% Ethanol	2 hrs
Absolute alcohol I	11/2 hrs
Absolute alcohol II	11/2 hrs
Absolute alcohol III	11/2 hrs
Absolute alcohol IV	overnight

Clearing

Xylene I	11/2 hrs
Xylene II	21/2 hrs

Infiltration

Paraffin wax I	11/2 hrs
Paraffin wax II	21/2 hrs
Paraffin wax III	overnight

Appendix III: Heamatoxylin and Eosin (H & E) Staining Protocol

Xylene I	5 min
Xylene II	5 min
Rehydration	
Absolute alcohol I	3 min
Absolute alcohol II	3 min
95% Ethanol	3 min
70% Ethanol	3 min
Rinse in distilled water	5 min
Stain in Hematoxylin 15 min	
Rinse in running tap water	5 min
Decolorize in acid alcohol	1-3 sec
Rinse in running tap water	5 min
Immerse in Sodium bicarbonate solution	1 min
Rinse in running tap water	5 min
Counter stain in Eosin	1 min
Dehydration	
70% Ethanol	3 min
95% Ethanol	3 min
Absolute alcohol II	3 min
Absolute alcohol I	3 min
Clearing	
Xylene II	5 min
Xylene I	5 min