ADDIS ABABA UNIVERSITY
SCHOOL OF GRAGUATE STUDIES
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CENTER OF FOOD SCIENECE AND NUTRITION

A comparison of Hypoglycemic effect in Diabetic Mice and Total Phenolic Content of leaves extracts of *Moringa stenopetala* and *Moringa oleifera*

A Thesis submitted to the school of graduate studies of Addis Ababa University in the partial fulfillment of the requirements for the degree of Master of Science degree in Food Science and Nutrition

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June, 2014

Addis Ababa University

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Acknowledgment

I would like to express my sincere gratitude to my advisors Professor Yalemtsehay Mekonnen and Professor Negussie Retta, without whom this research wouldn't have been accomplished. Their provision of relevant materials, unreserved, constructive and valuable comments from inception of the research till its conclusion has facilitated my work very remarkably.

I also extend my gratitude for their critical comment on the manuscript of my thesis. I am also very much grateful to Ato Yehuna Center of Food Science and Nutrition for sharing his vast and comprehensive knowledge in basic laboratory techniques.

I also wish to thank the Research and Publication Office of Addis Ababa University for the financial assistance and Ethiopian Nutrition and Health Research Institute for sponsoring clinical chemistry test and providing laboratory animals.

Finally and primarily, I would like to express my great appreciation to my family. I am particularly indebted to my parents who inculcated in me the love of education, the respect for study and hard work at a very early age. It would have been impossible for me to accomplish any of my academic pursuits without their love, inspiration, and continued support.

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Lists of Abbreviations

AGE	Advanced Glycation End-product
ANOVA	Analysis of Variance
BGL	Blood Glucose Level
CVD	Cardiovascular Disease
DNA	Di Nucleic Acid
DAL	Diacylglycerol
DPPH	Diphenylpicrylhydrazine
FA	Ferulic Acid
FBG	Fasting Blood Glucose
HPLC	High Performance Liquid Chromatography
HSD	Honestly Significance Difference
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGF	Insulin Growth Factor
IGT	Impaired Glucose in Tolerance
MO	Moringa Oleifera
MO-MW	Moringa Oleifera Methanol-Water Extract
MOEF	Moringa Oleifera Ethyl Acetate Fraction
MS	Moringa Stenopetala
MS-MW	Moringa stenopetala Methanol Water extract
MSEF	Moringa Stenopetala Ethyl Acetate Fraction
mRNA	Messenger Ribonucleic Acid

NIDDM	Non-Insulin Dependent Diabetes Mellitus
NADH	Nicotinamide Adenine Diniuclotide
OGTT	Oral Glucose Tolerance Test
PKC	Protein Kinases C
PLA2	Phospholipase A2
ROS	Reactive Oxygen Species
SPSS	
STZ	Streptozotocin
TPC	Total Phenolic Content
UK	United Kingdome
USA	United State of America
Vit.C	Vitamin C
Vit. E	Vitamin E

Abstract

Diabetes is a defect in the body's ability to convert glucose (sugar) to energy. It develops either when the pancreas fails to produce sufficient quantities of insulin or the insulin produced is defective. This metabolic disorder is characterized by hyperglycemia (fasting blood glucose level greater than 126 mg/dl taken on at least two separate occasions).

Medicinal plants are used in the traditional medical practice to treat diabetes mellitus in different part of the world. Moringa sp. has been documented to have medicinal importance against various illnesses. The present study was conducted to compare the anti-diabetic activity and total phenolic content of two species of Moringa; namely *M. stenopetala* and *M. oleifera* leaves extracted with methanol-water and further with ethyl acetate as fraction. The anti-diabetic activity was studied by repeated oral dose administration of the extract of the leaves and the fraction in Streptozotocin induced mice. The mice were grouped into six groups; *Moringa stenopetala* methanol-water extract treated, *Moringa stenopetala* ethyl acetate fraction treated, *Moringa oleifera* methanol-water extract treated, *Moringa oleifera* ethyl acetate fraction treated, Standard diabetic drug treated and Diabetic control. The diabetic mice received the extract and the fraction of both leave daily for 21 days. The result showed that ethyl acetate fraction of Moringa stenopetala treatment resulted in significant reduction of fasting blood glucose level initially was 190.6±3.4351 mg/dl and it reduced to 129±1.8708mg/dl and 109±4.6368mg/dl respectively after 14 and 21 day administration. Also this fraction has potential antioxidant activity and remarkable amount of phenolic concentration. The result indicated that M. stenopetala leaves are suitable source of green leafy vegetable to reduce the diabetic complications in diabetic patients.

Key words: anti-hyperglycemic, phenolic, diabetes, antioxidant, *Moringa stenopetala, Moringa oleifera*

1. Introduction

1.1. Background

The Family Moringaceae consists of a single genus with about 14 species and it is indigenous to several countries. Among that *Moringa oleifera* is native to northern India, Pakistan and Nepal. It is cultivated and become naturalized well beyond its native range including south Asia, the Arabian Peninsula, tropical Africa, Central America, the Carrabin and South America (A. Roloffr, 2009). This tree also known as horseradish tree, drum stick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nebeday or ben oil tree.

The other species *Moringa stenopetala* is a native tree in south Ethiopia, Northern Kenya and Eastern Somalia. It is often named as African moringa (Beyene, 2005). In Ethiopia it exists in the arid and semi-arid regions in the southern Rift valley. It serves as a major arable tree inter-crop multi-storey system in agriculture practice (Dechasa Jiru, 2006). Also the local farmer widely grown for its edible leaves and used traditionally for treating diabetes mellitus and had already been explored (Makonnen, 1997). It is known by the Gamo name *Haleko* and *Shelaqta* in Konso (Mekonnen & Gessese, 1998).

All parts of the plant are utilized by human. It is reach in nutrients and show pharmacological properties, recognized by popular use and corroborated by scientific community. Aqueous extract of *Moringa oleifera* leaves has significant hypoglycemic and anti-diabetic potential in a rat model (Dolly Jaiswal, 2009).

Dehydrated Drumstick leaf has positive impact on the lipid profile of hyperlipidemics (Vanisha S Nambiar, 2009). It is also reported as anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic, and antihelmintic (Fozia Farooq, 2012).

Different morphology of *Moringa oleifera* extracts such as leaf, pod and fruit has a potential in preventive of Newcastle Disease virus, effective chemotherapy in renal carcinogenesis and strong reducing power and free radical scavenging capacity respectively (Didacus Chukwuemeka Eze, 2012) (Paliwal, 2011) (Suaib Luqman, 2011).

In developing countries such as Senegal, India, Benin and Zimbabwe (Armelle de Saint Sauveur, 2006) Moringa leaves used for preventing malnutrition. *Moringa oleifera* is the significant source of β-carotine, ascorbate (Vit.C), a-tocopherol (Vit. E), iron (Ray-Yu Yang, 2006), potassium, calcium and protein (Suchada Jongrungruangchok, 2010). In *Moringa stenopetala* leaves vitamins are present at nutritionally significant level and minerals exist in significant concentration (Abuye C, 2003). Moringa seed is a good source of essential oil (Tsaknis, 2001) and purpose full in water purification due to its coagulant property (B. García-Fayos, 2010). The seed meal flour can be used as meat extender (A.M. Sharaf, 2009) also it can be functional for biodiesel production (Umer Rashid, 2008)

Increased intake of anti-oxidant may protect against chronic disease, which include cancers, cardiovascular disease and cerebrovascular disease (Prior, 2002). The content of such essential compound is significantly present in moringa, a high content of γ-tocopherol has been found in practically the whole plant, ranging from 5.7µg/g (adult leaves) to 27.8µg/g (6 month-old leaves) of dry mass and xanthins (neoxanthin 219mg/kg, violaxanthin 76.5mg/kg, zeaxanthin 19.4mg/kg) are also found (Paulo Michel Pinheiro FERREIRA, 2008). Major Polyphenols in Moringa Oleifera leaf powder are quercetin glucosides, rutin, kaempferol glycosides and chlorogenic acids (Ndong M, 2007).

Inhibition of α-amylase and α-glucosidases involved in the digestion and absorption of carbohydrates can decrease the postprandial increase of blood glucose level after a mixed carbohydrate diet. Among the tested three flavonols, quercetin had the highest maltase, glucoamylase, and isomaltase inhibitory activities (Jo S-H, 2009-2010). Chlorogenic acid is an important intermediate in lignin biosynthesis. This compound, long known as an antioxidant, also slows the release of glucose into the bloodstream after a meal (Johnston, Clifford, & Morgan, 2003). It has also been demonstrated that flavonoids can act *per se* as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications; besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway (Brahmachari, 2011)

1.2. Statement of the problem

Non communicable disease such as diabetes is considered as life style disease, unfortunately the problem is becoming common in developing countries in a complex and alarming rate. It has been shown that diabetes complication may be caused indirectly by increased oxidative stress and impaired anti-oxidant defense system induced by prolonged hyperglycemia (A. Kutan Fenerciogul, 2010).

Oxygen free radicals and "reactive oxygen species" such as hydroxyl radical, hydrogen peroxide and superoxide anion are constantly produced in human body. When they are in quantity that overwhelm the endogenous oxidant defense system is referred to as oxidative stress (V.Veeranan Arun GIRIDHARI, 2011). Oxidative stress is believed to be the main cause for several chronic diseases including diabetes. Through hyperglycemia, hyperlipidemia, hypertension and possible iron dyshomeostasis, diabetes induces oxidative stress that causes damage to multiple organs, leading to various complications. Therefore, antioxidant therapy may be an interesting approach to prevent diabetes and diabetic complications (Wei W, 2009).

Polyphones are the most abundant antioxidants in the diet and experimental studies on animals or cultured human cell lines support a role of polyphenols in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and osteoporosis. As antioxidants, polyphenols may protect cell

constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated to oxidative stress (Augstin Scalbert, 2005).

Polyphenols may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues. According to (Suaib L. et, al. 2012) the ethanolic and aqueous extract of *Moringa Oleifera* fruit and leaf significantly maintains the basal levels of Glutathione and Malondialdehyde content in a concentration and dose-dependent manner. The ethanolic extract of fruit showed highest phenolic content along with strong reducing power and free radical scavenging capacity.

Hyperglycemia can be handled initially with oral agents and insulin therapy, which is sometimes required to achieve targeted glycemic levels. However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries. Therefore, searching for effective, low cost and less side effected hypoglycemic agents is important. Herbal remedies for diabetes are known since ancient times in different societies. In Ethiopia the Konso people use Moringa leaves for traditionally for traditionally diabetes mellitus (Makonnen, 1997).

This research is intended to investigate the possible effect of *Moringa stenopetala* and *Moringa oleifera* methanol-water extract and ethyl acetate fraction in hyperglycemic mice and compare total phenolic content.

1.3. Significance of the study

To use the natural resource of Moringa plant for diabetics' treatment efficiently. Therefore, the outcome of this research will generate baseline information for the community who use Moringa for food and medicine, (as consumer) manufacturers, researchers and policy maker.

1.4. Hypothesis

Ho: There is no significant difference between *Moringa oleifera* and *Moringa stenopetala* extract and fraction of leaves in lowering the high glucose level in hyperglycemic mice.

H_A: There is significant difference between *Moringa oleifera* and *Moringa stenopetala* extract and fraction of the leaves in lowering the high glucose level in hyperglycemic mice.

1.5. Objective

1.5.1. General Objective

To investigate the ant diabetic and antioxidant activity of the two Moringa spices extract and fraction in a hyperglycemic mice and evaluate the total phenolic content.

1.5.2. Specific Objective

- To evaluate the sugar lowering effect of *Moringa stenopetala* and *Moringa oleifera* methanol-water extract and ethyl acetate fraction in hyperglycemic mice
- To compare total phenolic content in both *Moringa Oleifera* and *Moringa Stenopetala* leaves

2. Literature Review

2.1. Diabetes

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin

action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (WHO, 1999).

Type 1 indicates the processes of beta-cell destruction that may ultimately lead to diabetes mellitus in which "insulin is required for survival" to prevent the development of ke toacidosis, coma and death. An individual with a Type 1 diabetes may be metabolically normal before the disease is clinically manifest, but the process of beta-cell destruction can be detected. Type 1 is usually characterized by the presence of anti-GAD, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction. In some subjects with this clinical form of diabetes, particularly non-Caucasians, no evidence of an autoimmune disorder is demonstrable and these are classified as "Type 1 idiopathic". Aetiological classification may be possible in some circumstances and not in others. Thus, the aetiological Type 1 process can be identified and sub-categorized if appropriate antibody determinations are performed. It is recognized that such measurements may be available only in certain centers at the present time. If these measurements are performed, then the classification of individual patients should reflect this. (WHO, 1999)

Type 2 is the most common form of diabetes and is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Both are usually present at the time that this form of diabetes is clinically

manifest. By definition, the specific reasons for the development of these abnormalities are not yet known. (WHO, 1999)

2.1.1.1. Other specific type

Other specific types are currently less common causes of diabetes mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include, for example, fibrocalculous pancreatopathy, a form of diabetes which was formerly classified as one type of malnutrition-related diabetes mellitus. (WHO, 1999)

2.2. Molecular insights into insulin action and secretion

Insulin affects a wide range of physiological processes, although it is best known for its important regulatory role in glucose homeostasis. In response to elevations in plasma glucose, insulin secretion is increased and it stimulates glucose uptake and glycogen synthesis and inhibits glycogenolysis and gluconeogenesis, thus maintaining normoglycaemia. (M.F.White, 2002)

In addition to its major role in glucose homeostasis, also influences a number of other cellular processes such as glycolysis, glycogenesis, transport of ions and amino acid, lipid metabolism, DNA synthesis, gene transcription, mRNA turnover, protein synthesis and degradation, etc. (Fig. 1). The physiological importance of insulin become prominent in the case of diabetes mellitus, related ketoacidosis and other complication (Gupta, 1997)

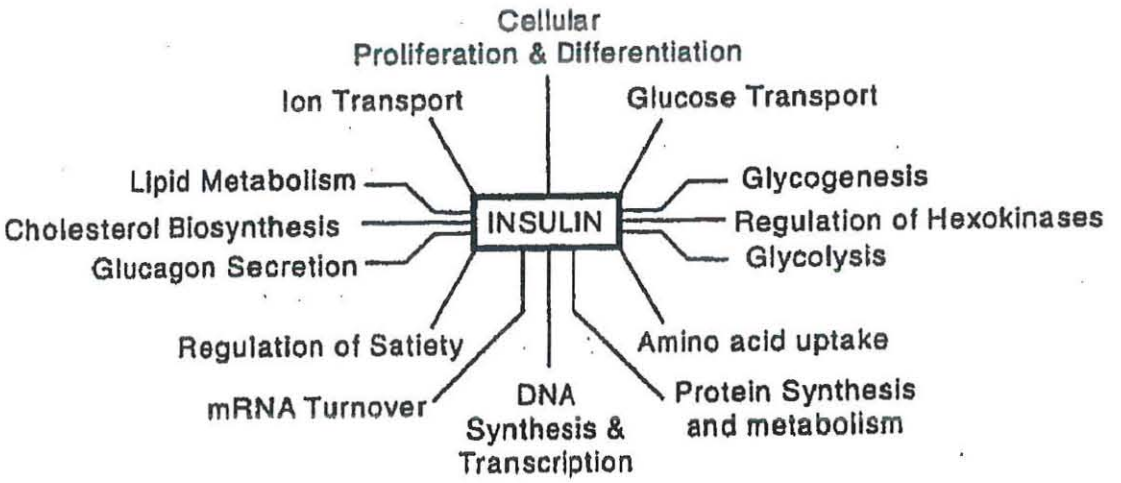


Fig. 1 Insulin is involved in the regulation of the wide range of vital metabolic pathways and cellular functions. Its deficiency leads to diabetes and related disorder (Gupta, 1997)

2.2.1. The molecular mechanism of insulin action

The actions of insulin at the cellular level are initiated by insulin binding to its plasma membrane receptor. This receptor is present on virtually all mammalian tissues, although the concentration varies from as few as 40 receptors on circulating erythrocytes to more than 200,000 receptors on adipocytes and hepatocytes. Like the surface membrane receptors for other hormones and growth factors, the insulin receptor serves at least two functions. The first is to recognize the hormone among all other substances in the blood. This is accomplished by binding the hormone with high affinity and a high degree of specificity. The second function is to produce a trans membrane signal that alters intracellular metabolism and mediates the action of the hormone. Recognition of the insulin molecule by its receptor is a complex molecular event and is closely

linked to signal transmission. The binding domain of the insulin molecule is composed of distant portions of the A and B chains, which come together on one surface as a result of three-dimensional folding to form the receptor binding region (White, 1988). Both genetic and acquired abnormalities in the number of insulin steps in insulin action occur in disease states leading to tissue resistance to insulin action. (Kahn, 1985)

2.2.2. Biosynthesis of Insulin

Insulin is synthesized in the beta cells of pancreas in the form of preproinsulin which is the ultimate precursor and gene for the same is located on chromosome 11 close to that for insulin like growth factor - 2 (IGF-2). Within a minute after synthesis it is discharged into cisternal space of rough endoplasmic reticulum where it is cleaved into proinsulin by proteolytic enzymes. Proinsulin with a C (connecting) chain linking A and B chains is then transported by microvesicles to the Golgi apparatus. Proinsulin is released in vesicles. Conversion of proinsulin to insulin continues in maturing granules through the action prohormone converatse 2 and 3 and carboxy peptidase H. Maturing granules are translocated with the help of microtubules and microfilaments. (Shashank R. Joshi, 2007)

2.2.3. Insulin secretion

Insulin is secreted from the beta cells in response to various stimuli like glucose, Arginine, sulphonylureas though physiologically glucose is the major determinant. Various neural, endocrine and pharmacological agents can also exert stimulator effect. Glucose is taken up by beta cells through GLUT-2 receptors. After entering the beta cell, glucose is oxidized by glucokinase, which acts as a glucose sensor. Glucose concentration below 90 mg/dl does not cause any insulin release. At such sbstimulatory glucose concentrations, K^+ efflux through open K_{ATP} channels keeps the β cell membrane at a negative potential at which voltage-gated Ca^{2+} channels are closed. As there is increase in plasma glucose, glucose uptake and metabolism by the β cell is enhanced. Rise in ATP concentration result in closure of K_{ATP} channels, leading to a membrane depolarization, opening of voltage-gated Ca^{2+} channels, Ca^{2+} influx, a rise in intracellular calcium concentration, and ultimately exocytosis of insulin granules. Structurally, the pancreatic K_{ATP} channel consists of two unrelated subunits: a sulfonylurea receptor (the SUR1 isoform) and a potassium channel subunit (Kir6.2) that forms the central ion-conductin pathway. The mature K_{ATP} channel exists as an octamer of Kir6.2 and SUR1 subunits in a 4:4 stoichiometry. A sub unit specific site specific to pancreatic K_{ATP} channel, confers glimepirirde an advantage over the other sulfonylurea secretagogues. Sulfonylurea and non-sulphonylurea drugs act as inulin secretagogues by closing K_{ATP} Channels by

passing the β cell metabolism. Diazoxide is a K channel opener and ingibits insulin secretion, independent of blood glucose levels. (Shashank R. Joshi, 2007)

2.3. Clinical staging of diabetes mellitus and other glucose tolerance

2.3.1. Diabetes mellitus

Diabetes mellitus, regardless of underlying cause, is sub-divided into: *Insulin requiring for survival* (corresponding to the former clinical class of "Insulin Dependent Diabetes Mellitus - IDDM"), e.g. C-peptide deficient; *Insulin requiring for control*, i.e. metabolic control, rather than for survival, e.g. some endogenous insulin secretion but insufficient to achieve normoglycaemia without added exogenous insulin; and *Not insulin requiring*, i.e. those who may be controlled satisfactorily by non-pharmacological methods or drugs other than insulin. Together, the latter two sub-divisions constitute the former class of NIDDM. (WHO, 1999)

2.3.2. Impaired glucose regulation - Impaired Glucose Tolerance (IGT) and Impaired Fasting Glycaemia (IFG)

Impaired glucose regulation (IGT and IFG) refers to a metabolic state intermediate between normal glucose homeostasis and diabetes. It should be stated unequivocally, however, that IFG and IGT are not interchangeable and represent different abnormalities of glucose regulation, one in the fasting state

and one post-prandial. IGT, rather than being a class as in the previous classification, is categorized as a stage in the natural history of disordered carbohydrate metabolism. A stage of IFG is also recognized because such subjects, like those with IGT, have increased risks of progressing to diabetes and macrovascular disease, although prospective data are sparse and early data suggest a lower risk of progression than IGT, although a similar CVD risk factor profile has been shown in IFG and IGT subjects. IFG refers to fasting glucose concentrations which are lower than those required to diagnose diabetes mellitus but higher than the "normal" reference range.

The values for IFG are a fasting plasma glucose concentration of 6.1 mmol l–1 (110 mg dl^{-1}) or greater (whole blood 5.6 mmol l^{-1}; 100 mg dl^{-1}), but less than 7.0 mmol l^{-1} (126 mg dl^{-1}) (whole blood 6.1 mmol l^{-1}; 110 mg dl^{-1}). If an OGTT is performed, some individuals with IFG will have IGT or diabetes, but this cannot be determined without an OGTT. If resources allow, it is recommended that all those with IFG have an OGTT to exclude the diagnosis of diabetes.

Individuals who meet criteria for IGT or IFG may be euglycaemic in their daily lives as shown by normal or near- normal glycated haemoglobin levels. IGT and IFG are not clinical entities in their own right, but rather risk categories for future diabetes and/or cardiovascular disease. IGT is often associated with the Metabolic Syndrome (Insulin Resistance Syndrome). Thus, IGT may not be directly involved in the pathogenesis of cardiovascular disease, but rather may serve as an indicator or marker of enhanced risk by virtue of its correlation with

the other elements of the Metabolic Syndrome that are cardiovascular risk factors. Self-evidently, those individuals with IGT manifest glucose intolerance only when challenged with an oral glucose load. (WHO, 1999)

2.3.3. Normoglyceamia

A fasting venous plasma glucose concentration of less than 6.1 mmol l^{-1} (110 mg dl^{-1}) has been chosen as "normal". Although this choice is arbitrary, such values are observed in people with proven normal glucose tolerance, although some may have IGT if an OGTT is performed. Values above this are associated with a progressively greater risk of developing micro- and macrovascular complications.

The pathological or aetiological processes which often lead to diabetes mellitus begin, and may be recognizable, in some subjects who have normal glucose tolerance. Recognition of the pathological process at an early stage may be useful if progression to more advanced stages can be prevented. Conversely, effective treatments, or occasionally the natural history of some forms of diabetes mellitus, may result in reversion of hyperglycemia to a state of normoglycaemia. The proposed classification includes a stage of normoglycaemia in which persons who have evidence of the pathological processes which may lead to diabetes mellitus, or in whom a reversal of the hyperglycemia has occurred, are classified. (WHO, 1999)

2.4. The Global Burden and Prevalence of Diabetes

The global burden of diabetes has been estimated several times. In 1994, the International Diabetes Federation (IDF) Directory included type 1 and type 2 diabetes estimates supplied by member nations. Using these data, IDF estimated that over 100 million people worldwide had diabetes. Also in 1994, McCarty et al used data from population-based epidemiological studies and estimated that the global burden of diabetes was 110 million in 1994 and that it would likely more than double to 239 million by 2010.

WHO also produced a report using epidemiological information and estimated the global burden at 135 million in 1995, with the number reaching 299 million by the year 2025. In 1997, Amos et al estimated the global burden of diabetes to be 124 million people, and projected that this would increase to 221 million people by the year 2010. In the 2006 3rd edition of the Diabetes Atlas the estimates were of 246 million people worldwide with diabetes for 2007, and an anticipated 380 million for 2025 (Richard Sicree, 2010)

Table 1. The prevalence of diabetes mellitus and IGT

Top 10: Countries/territories of number of people with diabetes (20-79 years), 2011 and 2030

	COUNTRY /TERRITORY	2011 MILLIONS
1	China	90.0
2	India	61.3
3	United States of America	23.7
4	Russian Federation	12.6
5	Brazil	12.4
6	Japan	10.7
7	Mexico	10.3
8	Bangladesh	8.4
9	Egypt	7.3
10	Indonesia	7.3

	COUNTRY /TERRITORY	2030 MILLIONS
1	China	129.7
2	India	101.2
3	United States of America	29.6
4	Brazil	19.6
5	Bangladesh	16.8
6	Mexico	16.4
7	Russian Federation	14.1
8	Egypt	12.4
9	Indonesia	11.8
10	Pakistan	11.4

AT A GLANCE	2011	2030
Total world population (billions)	7.0	8.3
Adult population (20-79 years, billions)	4.4	5.6
DIABETES AND IGT (20-79 YEARS)		
Diabetes		
Global prevalence (%)	8.3	9.9
Comparative prevalence (%)	8.5	8.9
Number of people with diabetes (millions)	366	552
IGT		
Global prevalence (%)	6.4	7.1
Comparative prevalence (%)	6.5	6.7
Number of people with IGT (millions)	280	398

Sources; International Diabetes Federation, IDF Diabetes Atlas, Fiveth edition

http://www.idf.org/diabetesatlas/5e/the-global-burden

The prevalence of diabetes in African communities is increasing with ageing of the population and lifestyle changes associated with rapid urbanization and westernization. Traditional rural communities still have very low prevalence, at most 1-2%, except in some specific high-risk groups, whereas 1-13% or more adults in urban communities have diabetes. Type - 2 diabetes is the predominant form (70-90%), the rest being represented by typical type 1 patients and patients and patients with a typical presentation that require more path physiological insight. Due to the high urban growth rate, dietary changes, reduction in physical activity, and increasing obesity, it is estimated that the prevalence of diabetes id due to triple within the next 25 years. In addition, long-term complications occur early in the course of diabetes and concern a high proportion of patients, probably higher than in other ethnic groups, and that could be partly explained by uncontrolled hypertension, poor metabolic control and possible ethnic predisposition. The combination of the rising prevalence of diabetes and the high rate of long-term complications in Africans will lead to a drastic increase of the burden of diabetes on health systems of countries. (E. Sobngwi, 2001)

2.5. Complication of Diabetes mellitus and Oxidative stress

Diabetes mellitus is accompanied by vascular disorders, which is relatively specific to diabetes, and caused primarily by hyperglycemia. In contrast, atherosclerosis in diabetes advances 10 years earlier in diabetic patients as

compared to patients not suffering from diabetes and is accelerated by hyperglycemia, hypertension, hyperlipidemia, obesity, and smoking, which are commonly observed in diabetic patients. Recent studies have reported that the various mechanisms accompanying hyperglycemia cause more oxidative damage (increased oxidative stress) in the blood and tissue of diabetic patients, as compared with healthy individuals. (KASHIWAGI, 2000)

Oxidative stress may be defined as a measure of the steasy-state level of reactive oxygen or oxygen radicals in a biological system. A hypothetical sequence of events by which oxidative stress may be linked to tissue damage and the development of pathophysiology is outlined in Fig 4. According to this scheme, increased oxidative stress may result from over or decreased efficiency of inhibitory and scavenger systems. The stress then may be amplified and propagated by and autocatalytic cycle of metabolic stress, tissue damage, and cell death, leading to a simultaneous increase in free radical production and compromised inhibitory and scavenger mechanisms, which further exacerbate the oxidative stress. (Baynes, 1991)

It has been reported that oxidative stress is enhanced in response to hyperglycemia in vascular tissues of patients with diabetes mellitus, leading to the peroxidation of cellular membrane lipids as well as the increased oxidative modification of amino acids and DNA. This can be explained by the molecular mechanisms of active oxygen overproduction and impaired antioxidant defense. Overproduction of active oxygen under hyperglycemic conditions is reported to

be associated with the following factors: 1) Auto-oxidation of glucose; 2) effects of protein glycation products; 3) activation of protein kinase C; and 4) active oxygen produced by mitochondria. On the other hand, regarding the antioxidant defense system, decreases in SOD activity in hydrogen peroxide detoxifying activity in the glutathione redox cycle, or in the contents of the ascorbate and glutathione (GSH) lead to impaired antioxidant function. These abnormalities initiate oxidative stress to vascular tissues resulting in the activation of transcriptional factors (NF-B, AP-1) in vascular cells, which subsequently induces the expression of genes of adhesion molecules and growth factors, leading to the progression of atherosclerosis. (KASHIWAGI, 2000)

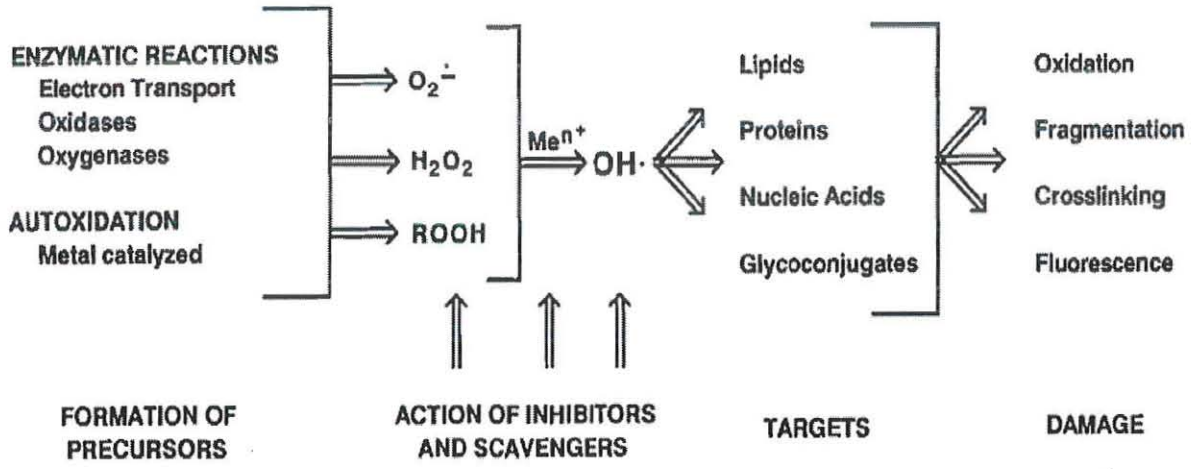


FIG. 3. General pathway by which increased oxidative stress may contribute to development of complications in diabetes. (Baynes, 1991)

2.5.1. Over production of Active oxygen due to Hyperglycemia

2.5.1.1. Glucose autoxidation

The increased metabolism of glucose due to intracellular hyperglycemia leads to the overproduction of nicotinamide adenine dinucleotide (NADH) and flavin adenosine dinucleotide, which are used by the electron transport chain to generate adenosine triphosphate. When NADH is in excess, an increase in the mitochondrial proton gradient is produced and electrons are transferred to oxygen, producing superoxide. Production of superoxide by the electron transport chain occurs at two main sites: the NADH dehydrogenase of complex I and the interface between ubiquinone and complex III. It is thought that mitochondrial-derived superoxide causes increased diacylglycerol (DAG) synthesis and subsequent protein kinase C (PKC) activation. However, some investigators have shown that hyperglycemia induces de novo synthesis of DAG, independent of mitochondrial metabolism. (Desmond Jay, 2006)

2.5.1.2. Advanced glycation end-products

The formation of AGE begins with non-enzymatic covalent bonding of ketone or aldehyde groups of reducing sugars to the free amino groups of proteins and other molecules. A series of rearrangements and reactions occurs to irreversibly produce AGE. AGE has been demonstrated in atherosclerotic lesions from patients with diabetes and their tissue concentration increases with disease severity. They are proposed to contribute to atherosclerosis by modifying the

extracellular matrix and circulating lipoproteins, as well as binding to and activating the receptor for AGE (RAGE), which is present on many vascular cells. It is through their receptormediated effects that AGE have been shown to induce ROS production. Stimulation of the RAGE causes the production of ROS, perhaps via an NAD(P)H oxidase, and subsequent activation of redox-sensitive transcription factors and expression of inflammatory mediators. (Desmond Jay, 2006)

2.5.1.3. PKC activation and active oxygen production

PKC (protein kinase C) is a phospholipid dependent serine/threonine kinase. In diabetes mellitus, diacylglycerol (DAG) is synthesized *de novo* utilizing excess glucose taken up by cells, and activates PKC via the glycolysis system. It has been reported that PKC activation is observed in many vascular tissues such as retina, heart, aorta, and glomeruli which are isolated from diabetic animals. PKC activation is related to vasoconstriction, proliferation and overgrowth of smooth muscle cells as well as accelerated synthesis of extracellular matrix proteins, and thus plays significant roles in the onset and progression of vascular cell dysfunction in diabetes mellitus. Recently, it has been reported that, a PKC_ isoform-specific inhibitor (LY 333531) has been developed and its usefulness in inhibiting the onset and progression of diabetic complications has been demonstrated. It is also indicated that PKC is activated by generated active

oxygen, and that the activated PKC induces the activation of phospholipase A2 (PLA2) resulting in enhanced prostaglandin metabolism, which is associated with increased production of active oxygen. (KASHIWAGI, 2000)

2.5.1.4. Abnormal mitochondria and active oxygen production

In a recent study, it has been reported that the production of active oxygen is increased when the oxidative phosphorylation in mitochondria is enhanced. The mitochondrion has been shown to play an important role in active oxygen production particularly under hyperglycemic conditions. Hyperglycemia-induced activation of PKC, AGE production, sorbitol accumulation and activation of NF-_B (nuclear factor-_B) have been reported to be reversed after inhibiting active oxygen production caused by mitochondria in aortic endothelial cells, suggesting that mitochondria plays an important role in the production of active oxygen under high glucose conditions. (KASHIWAGI, 2000)

2.6. Ant-Diabetic effect of antioxidants

Impairment in glucose metabolism leads to physiological imbalance with the onset of the hyperglycemia and subsequently diabetes mellitus. There are two main categories of diabetes; type-1 and type-2. Studies have shown that several physiological parameters of the body get altered in the diabetic conditions. (Rizvi SI, 2001) Long term effects of diabetes include progressive development of specific complements such as retinopathy, which affects eyes and lead to

blindness; nephropathy in which the renal functions are altered or disturbed and neuropathy which is associated with the risks of amputations, foot ulcers and features of autonomic disturbance including sexual dysfunctions. Numerous studies report the ant diabetic effects of polyphenols. Tea catechins have been investigated for their anti-diabetic potential. (Rizvi SI, 2001) Polyphenols may affect glycemia through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues. The hypoglycemic effects of diacetylated anthocyanins at a 10 mg/kg diet dosage were observed with maltose as a glucose source, but not with sucrose or glucose (Matsui T, 2001). This suggests that these effects are due to an inhibition of α-glucosidase in the gut mucosa. Inhibition of α-amylase and sucrase in rats by catechin at a dose of about 50 mg/kg diet or higher was also observed.

The inhibition of intestinal glycosidases and glucose transporter by polyphenols has been studied.66 Individual polyphenols, such as (+) catechin, (-) epicatechin, (-) epigallocatechin, epicatechin gallate, isoflavones from soyabeans, tannic acid, glycyrrhizin from licorice root, chlorogenic acid and saponins also decrease S-Glut-1 mediated intestinal transport of glucose.

Saponins additionally delay the transfer of glucose from stomach to the small intestine (Dembinska-Kiec A, 2008). Resveratrol has also been reported to act as an anti-diabetic agent. Many mechanisms have been proposed to explain the anti-diabetic action of this stilbene, modulation of SIRT1 is one of them which improves whole-body glucose homeostasis and insulin sensitivity in diabetic

rats. It is reported that in cultured LLC-PK1 cells, high glucose induced cytotoxicity and oxidative stress was inhibited by grape seed polyphenols. Resveratrol inhibits diabetes-induced changes in the kidney (diabetic nephropathy) and significantly ameliorates renal dysfunction and oxidative stress in diabetic rats. Treatment with resveratrol also decreased insulin secretion and delayed the onset of insulin resistance. A possible mechanism was thought to be related to the inhibition of K + ATP and K + V channel in beta cells (Chen WP, 2007).

Onion polyphenols, especially quercetin is known to possess strong anti-diabetic activity. A recent study shows that quercetin has ability to protect the alterations in diabetic patients during oxidative stress. Quercetin significantly protected the lipid peroxidation and inhibition antioxidant system in diabetics (Rizvi SI M. M., 2009). *Hibiscus sabdariffa* extract contains polyphenolic acids, flavonoids, protocatechuic acid and anthocyanins. A study performed by Lee et al. (Lee WC, 2009) showed that polyphenols present in the extracts from *Hibiscus sabdariffa* attenuate diabetic nephropathy including pathology, serum lipid profile and oxidative markers in kidney.

Ferulic acid (FA) is another polyphenol very abundant in vegetables and maize bran. Several lines of evidence have shown that FA acts as a potent anti-diabetic agent by acting at many levels. It was demonstrated that FA lowered blood glucose followed by significantly increased plasma insulin and a negative correlation between blood glucose and plasma insulin (Jung EH, 2007)

3. Materials and Methods

3.1. Materials

3.1.1. Collection of Plant material

Fresh leaves of *Moringa stenopetala* was collected from Melkasa Agricultural Research Center 150km from Addis Ababa and *Moringa oleifera* was collected from Debrezeyet Agricultural Research Center 47km from Addis Ababa. It was identified and authenticated by taxonomist in Ethiopia Agricultural Institutes. It was washed well with water, dried under shade, crushed and powdered for extraction.

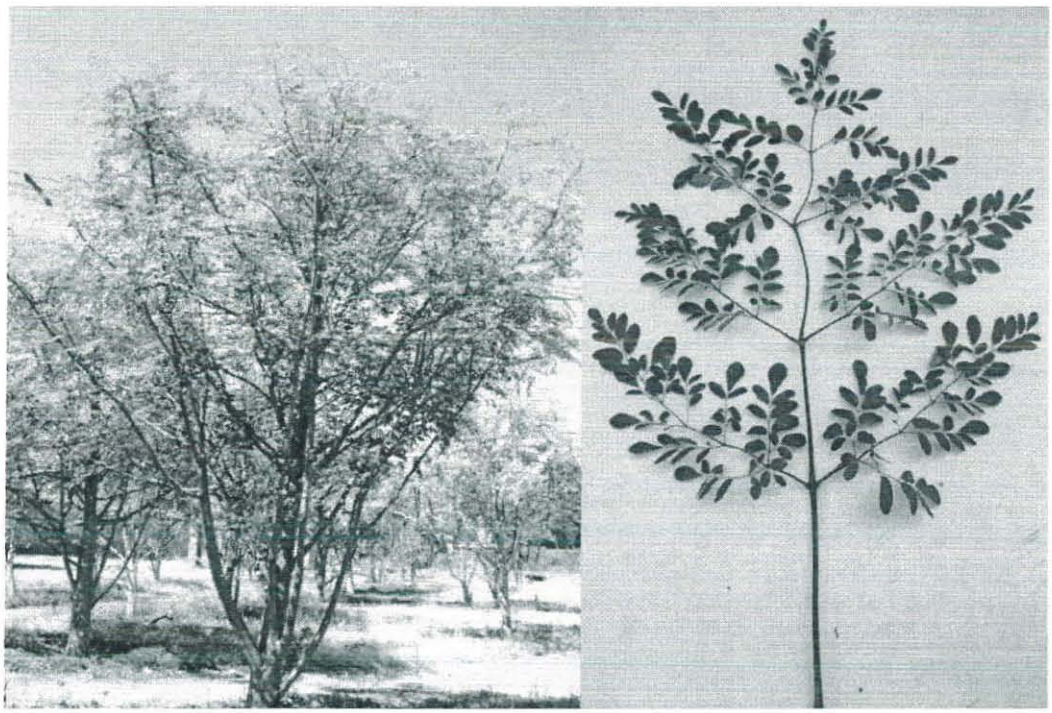


Fig 4. *Moringa oleifera from Ethiopian Agriculture Research Institute, Deberzeyet Research Center, February 16, 2013*

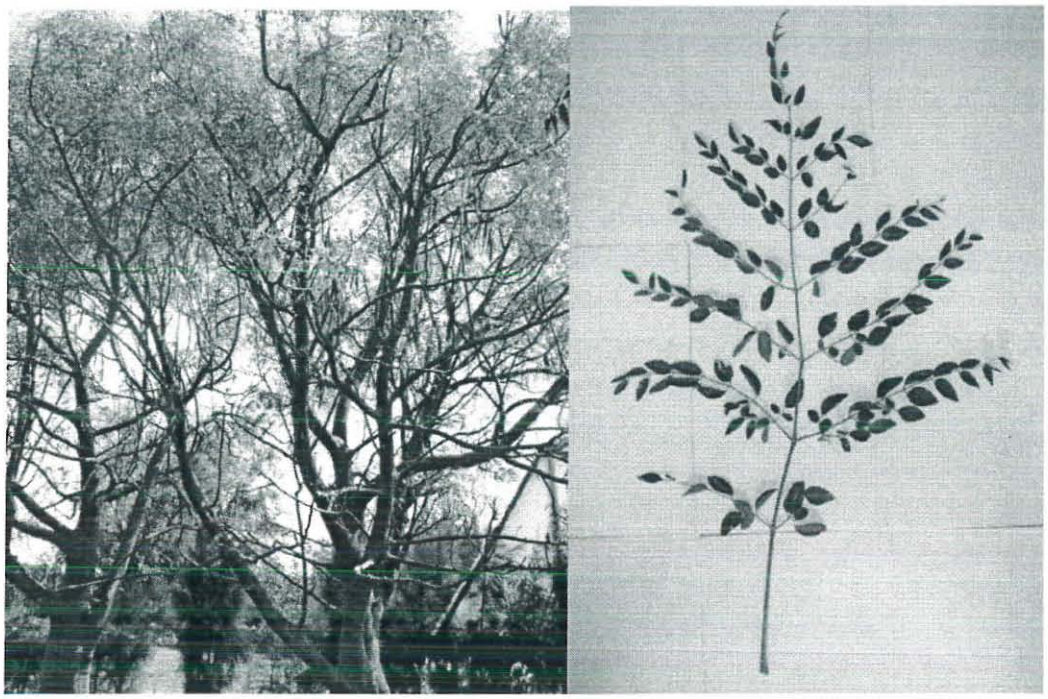


Fig 5. *Moringa stenopetala from Ethiopian Agriculture Research Institute, Melkasa Agricultural Research Center February 20, 2013*

3.1.2. Chemicals and Solvents

The following chemicals and solvents were purchased and used as received. All the chemicals, reagents, and solvents used in the assay protocols are analytical grade. Methanol (Riedel-de Haen, Germany), Petroleum ether (40^0c-60^0c, Fluka, Germany), Ethyl acetate (Park Scientific, Nottingham UK),1, 1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma, USA), sodium hydroxide (Riedel-de Haen, Germany), ascorbic acid (Merck, Germany), Folin Ciocalteu's reagent (Sigma, USA) and sodium bicarbonate (Na_2CO_3) (Park Scientific, Nottingham UK),

3.1.3. Standard Drugs

Streptozotocin (STZ) (Sigma, USA) for induction of hyperglycemia Glibenclamid (Glitisol, Cyprus) as a standard hypoglycemic drug

3.1.4. Instrument

Rota vapor (buchi rota vapor vac R-500, Switzerland), lyophilizer (Labconco, USA) to concentrate the extracts were used. UV/Visible Spectrophotometer (Evolution 220, Thermo Scientific Germany) Senso Card glucometer (77 Elektronic kft, Hungary) and GLAB active glucose test strip(77 Elektronic kft, Hungary) were used in this study.

3.2. Methods

3.2.1. Extraction

Preparation of total ethanol extracts of Moringa stenopetala and Moringa oleifera: Air dried powdered leaves of *Moringa Stenopetala and Moringa oleifera* (400 g) were soaked with (80:20) methanol-water (Arnnok, 2012) for three days successively and shake. This was done three times and filtered with Whatman No.1 filter paper and the filtrates were mixed. The combined filtrates were concentrated using Rota Vapor (Buchi Rota Vapor vac R-500, Switzerland). The aqueous residues were dried in a lyophilizer (Labconco, USA) and kept in desiccators for future use. The methanol-water extract yielded 20.415 % (81.66g)

for *Moringa stenopetala* and 18.753% (75.021g) for *Moringa oleifera* and stored in refrigerator for subsequent experiments.

3.2.2. Fractionation of total Methanol-Water Extract of *Moringa stenopetala* and *Moringa oleifera*

Solvent-solvent partitioning: The procedure for solvent-solvent separation was adopted from Samsam-sharjat (1992). 10 g of methanol-water extract of the plant was dissolved in 100 ml of methanol and distilled and deionized water (80:20). The dissolved extract was separated in a separatory funnel with Ethyl acetate (40-60^{0}c), successively until the extracting solvents became colorless. In all cases of separation, 150 ml of solvents were used. After completing the separation process, the solvents were recovered by Rota Vapor. The separates kept in the refrigerator for the next experiments.

3.3. Pharmacological Evaluation

3.3.1. Laboratory Animals

Laboratory bred Swiss albino mice, 6-8 weeks, weighing 25-30g were obtained from Ethiopian Health and Nutritional Research Institute, Addis Ababa. All animal procedures were in accordance with the standards set forth in guidelines for the care and use of experimental animals by committee for purpose of supervision of experiments on animals. All mice were fasted for 24 hour before diabetes was induced with STZ. The animals were allowed to acclimatize for 2

weeks before the experiment. The animals were housed in polypropylene cages inside a well-ventilated room. Each cage consists of not more than 5 rats. They were maintained under standard laboratory conditions and 12 hour light/dark cycle. They were fed a standard commercial pellet diet and water *ad libitum*. The diet consists of 71% carbohydrate, 18% protein, 7% fat, 4% salt mixture and adequate minerals and vitamins.

3.3.2. Induction of Diabetes Mellitus

Streptozotocin was obtained from Sigma Chemicals Co., St. Louis, MO, USA. STZ was dissolved in cold 0.01 M citrate buffer, pH 4.5 and prepared freshly for immediate use. STZ injections were given intraperitoneally 50mg/kg and the doses were determined according to the body weight of animals. The blood glucose concentration was measured week from the day of STZ injection. The blood samples were collected from the tail vein once a week and the obtained sample was used immediately for the determination of blood glucose by glucose oxidase method.

3.3.3. Assessment for repeated dose anti-Hyperglycemic effect of crude extracts and fraction in Streptozotocin induced mice

Streptozotocin induced diabetic mice fasted for 16 hours were selected and divided into six groups of six mice each. Group 5 and 6 served as positive and negative control and received glibenclamide (0.66 mg/kg) and distilled water (10 ml/kg) daily for 21 days through orally by gavage. Group 1 up to 4 were

administered with 300 mg/kg of 80:20 methanol-water crude extract, and ethyl acetate fraction of *Moringa stenopetala* and *Moringa oleifera* respectively, daily for 21 days through orally by gavage. In all cases of pharmacologic evaluations, the extracts were dissolved in distilled. The blood samples were collected from the tail vein once a week at 0, 7, 14 and 21 day and the obtained sample was used immediately for the determination of blood glucose concentration by glucose oxidase method.

3.4. Phenolic content and DPPH assay

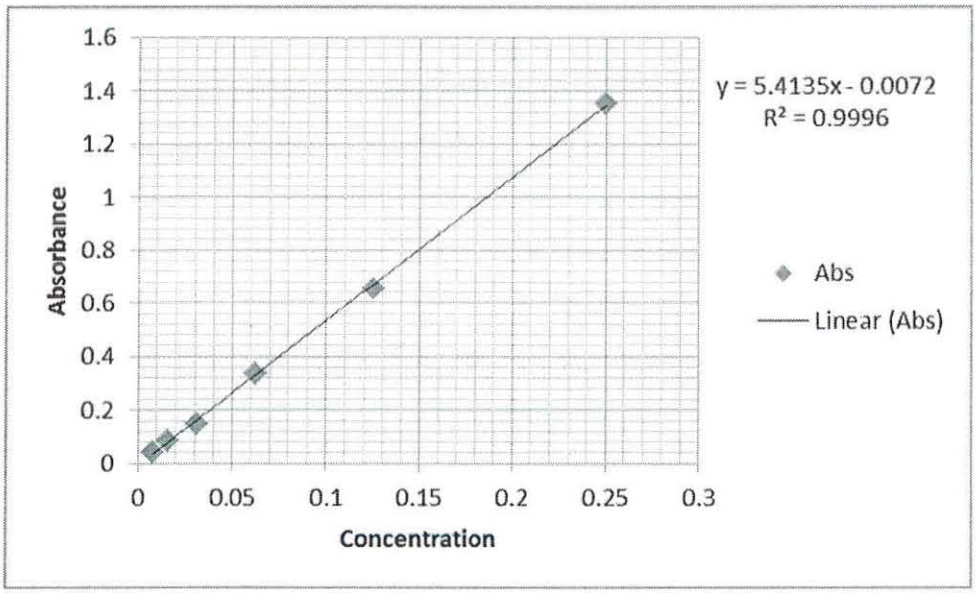
3.4.1. Phenolic content determination

Using the Folin Ciocalteu's Reagent, phenolic content in each Moringa leaves powder sample was measured at an absorbance of 752 nm. The results are expressed as g Gallic Acid Equivalents.

First, 2 mL Folin Ciocalteu's Reagent in 20 mL of water was prepared to form a stock solution of Folin Ciocalteu's reagent solution. Saturated solution of Na2CO3 (15%) was prepared by dissolving 7.5 g of Na2CO3 in 50 mL of water. About 100 mg of the powdered *M. stenopetala* and *M. oleifera* was placed in a 25 mL volumetric flask and 20 mL of 80% methanol in water (v/v) was added and sonicated for 25 minutes. After sonication, the flasks were filled to volume with water and 40 µL of the extract was transferred to a centrifuge tube with 900 µL of Folin Ciocalteu's Reagent solution and set aside for five minutes. 400 µL of 15%

Na2CO3 was added to the mixture, allowed to react for 45 minutes and was measured at 752 nm. To develop the calibration curve, 6.0 mg of gallic acid was added into 25 mL of 60% methanol solution to provide the standard solution. Six dilutions of concentrations ranging from 0.25 mg/mL to 0.0078 mg/mL and a blank were prepared as the plant samples above and gave the equation $y = 5.4135x - 0.007$ ($r^2 = 0.999$). 40 µL of each dilution was also used for the calibration curve.

Fig 6. Calibration curve



3.4.2. DPPH Assay

Free radical scavenging activity of methanol-water extract and the ethyl acetate fractionate of each samples was measured by the modified DPPH method (Manish, 2011). DPPH in methanol is a stable radical, dark violet in color. Its color is bleached by its reaction with a hydrogen donor. DPPH scavenging activity was measured by spectrophotometric method. To 1 ml of various concentrations of extract, 1 ml solution of DPPH (0.1 mM) was added. An equal amount of methanol and DPPH served as control. After 20 min of incubation in dark, absorbance was recorded at 517 nm. The experiment was performed in triplicate and the percentage inhibition calculated by using the formula.

$$\text{Scavenging \%} = \frac{(\text{Abs. of Blank} - \text{Abs. of Sample})}{\text{Abs. of Blank}} \times 100$$

4. Statistical Analysis

The results are expressed as mean±S.D. and all statistical difference between the treatment and the control were tested by one-way analysis of variance (ANOVA) followed by Duncan multiple test comparisons using SPSS version 16. The difference in the mean values showing a *P* level of 0.05 or lower was considered to be statistically significant.

5. Results

5.1. Ant-Hyperglycemic effect of Moringa stenopetala and Moringa oleifera methanol-water crude extract and ethyl acetate fraction on fasting blood glucose level in Streptozotocin induced mice

During the three weeks crude extract and fraction treatment, fasting blood glucose level were measured once weekly. The results are summarized in Table 2. and Fig. 7. Before induction of diabetes, there was no significant difference in fasting blood glucose (FBG) level among the treatment groups. FBG levels in the treatment groups showed no significant differences at 0 day of the administration ($p<0.05$). After repeated oral administration of Moringa stenopetala (MS) ethyl acetate fraction and standard drug showed significant reduction in FBG level at the 7th day ($P<0.05$). MS methanol-water crude extract and Moringa oliefera (MO) ethyl acetate fraction showed similar reduction in FBG level at the 7th day ($P<0.05$). MS ethyl acetate fraction and MS methanol-water crude extract showed significant reduction in FBG level at 14th and 21 th day ($P<0.05$). MO ethyl acetate fraction and MO methanol-water crude extract showed similar reduction in FBG level at 14th day ($P<0.05$).

5.2. Effect of Extract and Fraction of Moringa stenopetala and Moringa oliefera leaves on body weight

Change in body weights in control and experimental groups are shown in Table 3 and Fig. 8. There were no significant differences in the initial body weights among the six groups ($P<0.05$). Both crude extract and fraction improved the weight gain compared with the diabetic control mice

5.3. Total Phenolic Content

The present investigation has been carried out to determine the Total Phenolic Content (TPC) present in Methanol-Water (80:20) extract and Ethyl acetate fraction of *Moringa stenopetala* (MS) and *Moringa oliefera* (MO) leaves. The quantitative analysis of TPC in crude extract and fraction of the leaves of MS and MO revealed that the ethyl acetate fraction of MS leaves containing highest amount of TPC (283.8µg GAE/ml) followed by crude methanol-water extract of MS leaves (211.9 µg GAE/ml) whereas moderate amounts recorded in ethyl acetate fraction of MO leaves (93.56 µg GAE/ml) followed by methanol-water crude extract of MO leaves (58.7 µg GAE/ml). Fig. 9

5.4. DPPH Scavenging activities

The anti-oxidant activity of methanol-water (80:20) crude extract and ethyl acetate fraction of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) leaves were measured by DPPH scavenging activities. The scavenging activity of the DPPH radical was tested by reduction of the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. According to the experiment MS ethyl acetate fraction showed maximum activity 96.22% followed by MS methanol-water crude extract 94.14% whereas MO methanol-water crude extract showed 90.03% scavenging activity followed by MO ethyl acetate fraction 86.09%. Fig. 10

Table 2. Fasting Blood Glucose level Mean±SD (mg/dl)

Group	Treatment	0 day	7 day	14 day	21 day
I	MS M	198.6±6.3087	174.4±3.8471	147±5.1478**	117.2±10.7331***
II	MS F	190.6±3.4351	160.4±12.7397**	129±1.8708***	109±4.6368***
III	MO M	199.8±6.3796	182.6±8.1731	160.6±4.7223	129.8±10.3779***
IV	MO F	189.2±7.4297	173.4±6.2689	156.8±12.9884**	135±4.6368***
V	SD	200.8±6.8337	124.6±4.615***	135.8±8.6718***	115.6±7.4699***
VI	DC	183.8±5.4037	192.2±5.5857	217.2±4.037	253.2±1.304

*=p<0.05, **=p<0.01, ***= p<0.001 as compared to the control

Results are means±SD of *n=5*

Dose of crude extracts and fraction = 300mg/kg

Dose of glibenclamide = 0.66mg/kg

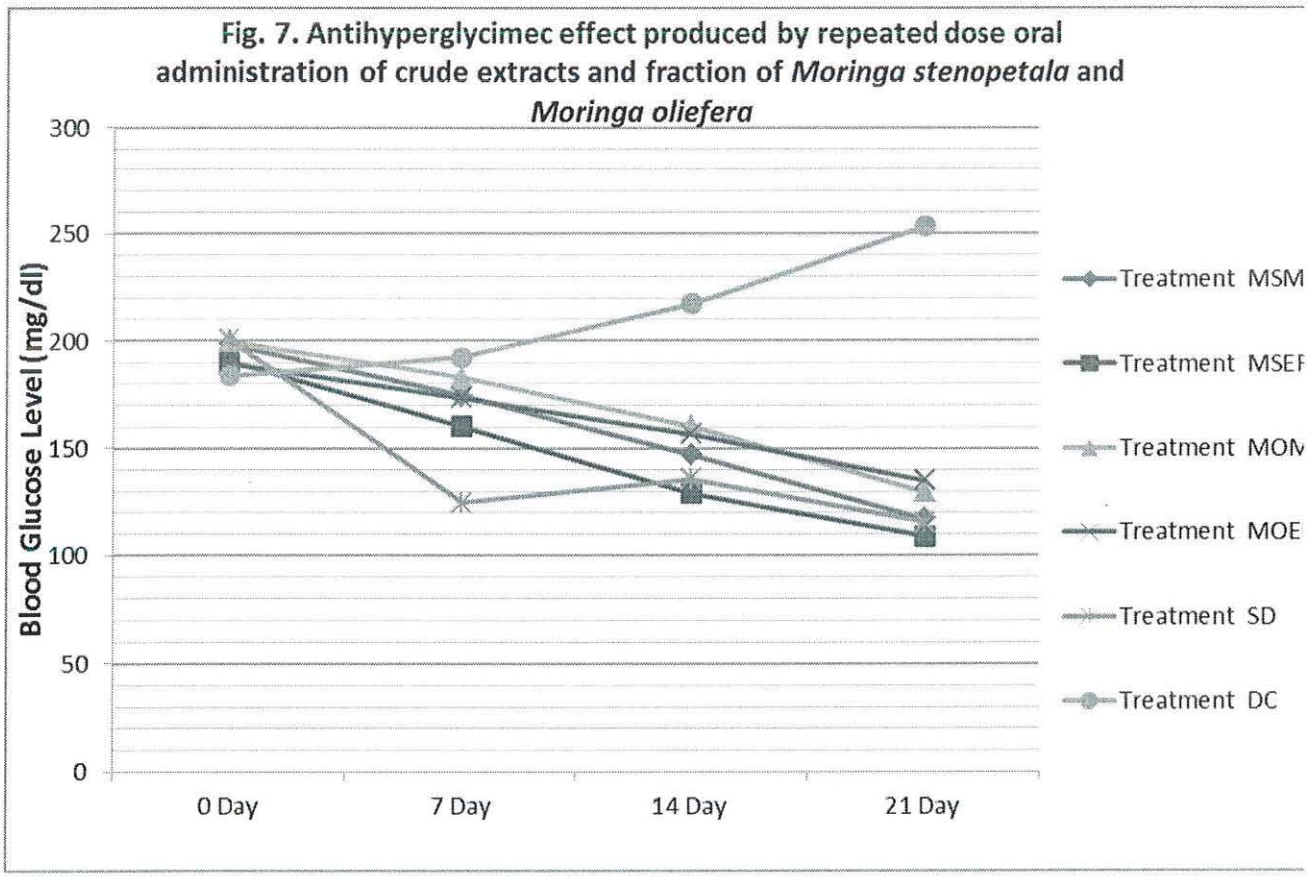
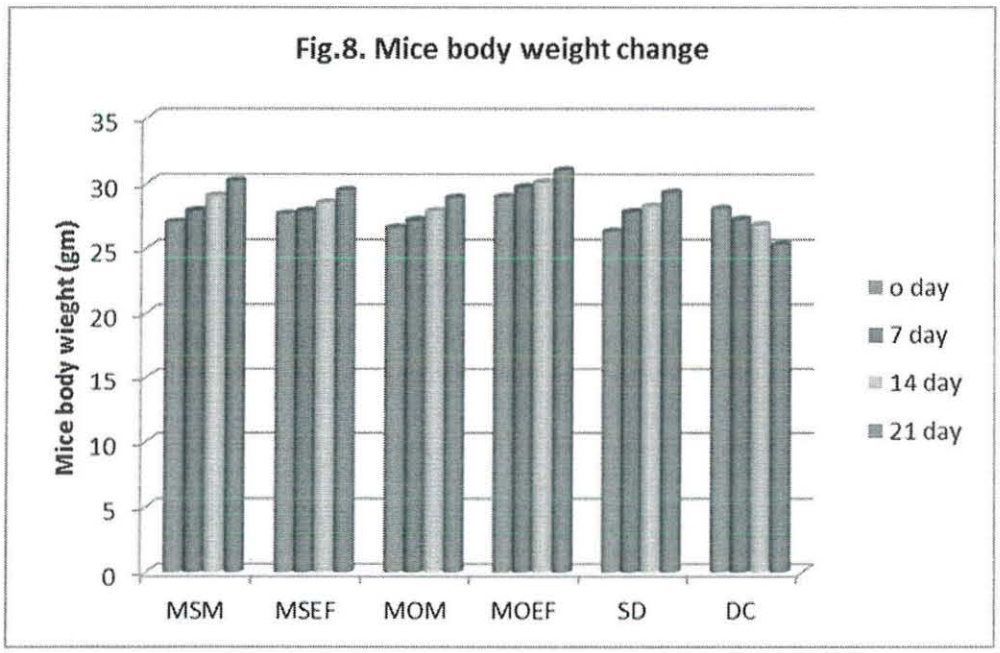
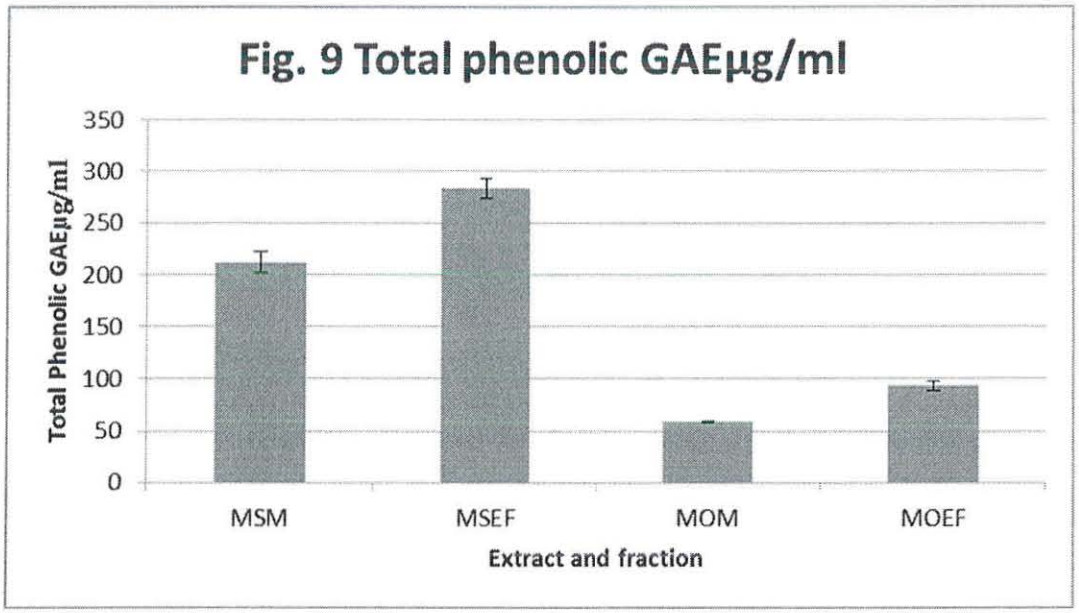
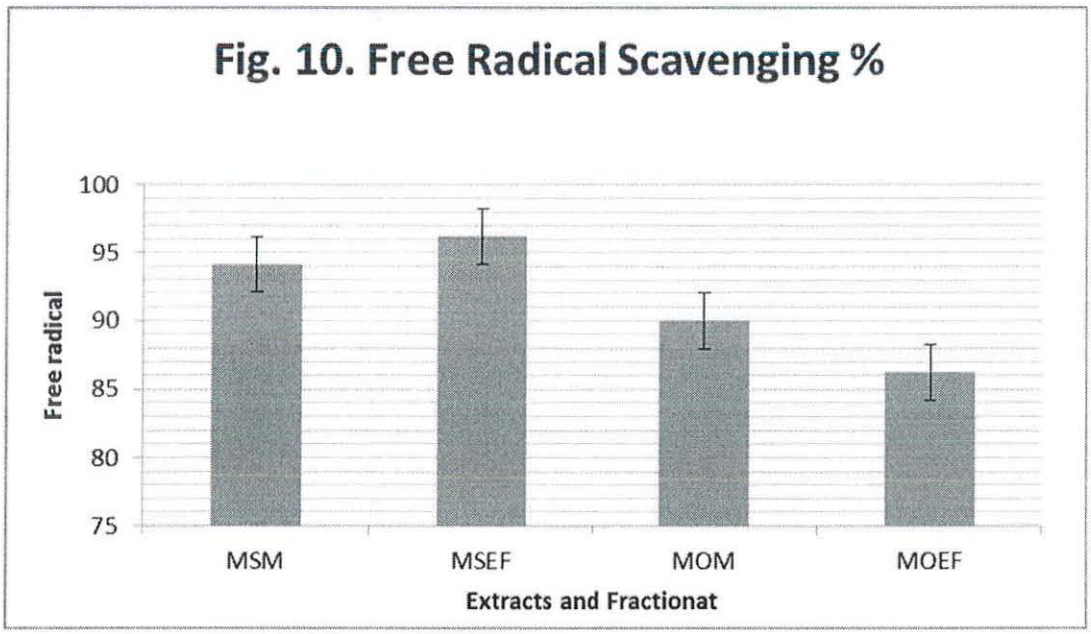


Table 3. Mice Body Weight change Mean±SD (gm) n=5

Group	o day	7 day	14 day	21 day
MSM	27±2.1548	27.88±1.9419	29±1.8339	30.18±1.8523
MSEF	27.62±2.9040	27.86±2.8827	28.48±2.7110	29.44±2.6050
MOM	26.56±1.6823	27.16±1.8293	27.84±2.0382	28.86±2.5687
MOEF	28.92±2.33	29.68±2.1306	30.04±2.0245	30.96±2.0684
SD	26.28±1.6939	27.78±2.0663	28.2±2.0909	29.26±2.0155
DC	28.02±2.4425	27.2±2.7004	26.76±2.1134	25.28±2.337







MSM *Moringa stenopetala* Methanol-Water Extract

MSEF *Moringa stenopetala* Ethyl Acetate Fraction

MOM *Moringa oliefera* Methanol-Water Extract

MOEF *Moriga oliefera* Ethyl Acetate Fraction

SD Standard Drug

DC Diabetic Control

6. Discussion

Diabetes is a defect in the body's ability to convert glucose (sugar) to energy. It develops when the pancreas fails to produce sufficient quantities of insulin - Type 1 diabetes or the insulin produced is defective and cannot move glucose into the cells - Type 2 diabetes. This metabolic disorder is characterized by hyperglycemia (fasting blood glucose level greater than 126 mg/dl taken on at least two separate occasions) and disturbances of carbohydrate, protein and fat metabolisms. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs. (WHO, 1999)

For hundreds of years, traditional healers have prescribed different parts of Moringa for treatment of skin diseases, respiratory illnesses, ear and dental infection, hypertension, diabetes, cancer treatment, and water purification, and they have promoted its use as a nutrient dense food source (Fozia Farooq, 2012) (Makonnen, 1997) (Suchada Jongrungruangchok, 2010). The previous works on hypoglycemic activity evaluation of the crude aqueous extract of *Moringa stenopetala* leaves by Makonnen (1997), hypoglycemic effect evaluation of the cured aqueous extract and n-butanol as well as chloroform fractions of *Moringa stenopetala* leaves by Mussa and coworkers (2008), effect of *Moringa oleifera* leaves aqueous extract therapy on hyperglycemic rats by Dolly and coworkers (2009) and anti-diabetic property of drumstick (*Moringa oleifera*) leaf tablets by Giridhari and coworkers (2011) confirmed this traditional claim. Herein I report a comparative *in vivo* and *in vitro* analysis of anti-hyperglycemic effect and anti-

oxidant activities for methanol-water extract and ethyl acetate fraction in both *Moringa stenopetala* and *Moringa oleifera* leaves.

In this study the anti-hyperglycemic effect was carried out in Streptozotocin induced diabetic mice. Streptozotocin (STZ, *N-nitro* derivative of glucosamine) is a naturally occurring, broad-spectrum antibiotic and cyto-toxic chemical that is particularly toxic to the pancreatic, insulin producing beta cells in mammals. It has been widely used to induce DM in experimental animal models, allowing the investigation of hypoglycemic agents in treatment of diabetes. Streptozotocin injection consistently produces symptoms of DM including hyperglycemia, decreased insulin levels, polyuria, and weight loss. The dose of STZ required for inducing diabetes depends on the animal species, route of administration and nutritional status. In mice, doses vary between 50-60 mg/kg by intraperitoneal route; clinical symptoms of diabetes are clearly seen within 2-4 days. (Abdu, 2009)

In the present study, the diabetic mice were treated for 21 days with methanol-water crude extract and ethyl acetate fraction of *Moringa stenopetala* and *Moringa oleifera* leaves. The result showed that the ethyl acetate fraction of *Moringa stenopetala* administration produced significant hypoglycemia effect starting from the 14th day as compared with the standard drug. Moringa stenopetala methanol-water crude extract and the standard drug caused almost similar significant reduction in blood glucose level at 21th day . *Moringa oleifera* methanol -water

extract and ethyl acetate fraction did not cause any significant change in blood sugar level as compared to the control.

Total phenolic content of the extract and fraction were performed using Folin-Caltue method that indicated Moringa stenopetala ethyl acetate fraction containing the highest amount followed by methanol-water crude extract. However at this stage it is impossible to generalize specific group of compound responsible for ant diabetic activity.

Although at this juncture it is difficult to come up with the exact antidiabetogenic mechanisms of actions of Moringa compared to the action with that of standard ant diabetic drugs like glibenclamide could hint as how the test substance acts. Glibenclamide is a second generation sulfonylurea derivative which acts by stimulating release of insulin from pancreatic-cells and by increasing sensitivity of peripheral tissue to insulin. Glibenclamide is not effective in pancreas ectomized animals or patient with no endogenous insulin (Habibuddin *et al.*, 2008). Plants can have properties similar to known drugs like glibenclamide, which reduce blood sugar level in normoglycemic and hyperglycemic animals. Some other plants do not reduce sugar level in normal states of the animal like metformin (Stumvoll *et al*, 1995). As the extracts of Moringa were observed to reduce sugar level in Streptozotocin induced diabetic mice which glibenclamide dose as well, the extracts perhaps have a mechanism of action similar to insulin secretgouges like glibenclamide.

In in-vitro antioxidant test, methanol-water extract and ethyl acetate fraction showed the presence of antioxidant components as visualized by DPPH reagent on the UV visble spectrophotometer. It is known that the diabetogenic effect of Streptozotocin is due to generation of free radicals that can affect the normal functioning of β-cells of pancreas (Szudelske, 2001). Hence, there might be relationship between the existing antioxidant and antihyperglycemic effect of extracts. As the antioxidants can normalize the β-cells of the pancreas and might renew cells to secrete efficient amount of insulin. However, this must be supported by further study.

7. Conclusion

Based on the observation the ethyl acetate fraction of Moringa stenopetala leaves maintain the fasting blood glucose level in Streptozotocin induced mice.

The result support the potential antioxidant activity of Moringa stenopetala leaves followed by Moringa oleifera which add one more positive attribute to its known pharmacological properties and hence its use in traditional system of medicine.

8. Recommendation

Further studies are required to identify the ant diabetic activity principle present in the Moringa leaves with its molecular mechanism of action.

Since the study showed the blood glucose lowering effects of Moringa leaves on animal model, product development is recommended such as functional foods for people with diabetes mellitus.

Reference

A. Kutan Fenerciogul, T. Saler, E. Genc, H. Sabuncu and Y. Altuntas. "The effect of polyphenol containing antioxidants on oxidative stress and lipid peroxidation in type 2 diabetes mellitus with out complication ." Endocrinol investigation (2010): 118-124.

A. Roloffr, H. Weisgerber, U. Lang and B. Stimm. Moringa Oleifera LAM., 1785. Weinheim: WILEY-VCH Veriag GmbH & Co. KGaA, 2009.

A.M. Sharaf, M.E. Ebrahium, M.S. Ammar and M.E. Abd El-Ghany. "Influence of Using Moringa Meal Flour as Meat Extender on Quality Characteristics of Beef Burger Patties During Frozen Storage." World Journal of Dairy & Food Sciences (2009): 32-40.

Abdu, Mahmoud. "Induction of Diabetes Mellitus in Mice Using Intraperitional Streptozotocin ." Euro Journals (2009): 398-402.

Abuye C, Urga K, Knapp H, Selmar D, Omwega AM, Imungi JK, Winterhalter P. "A compositional study of Moringa Stenopetala leaves." East African Medical Journal (2003): 247-252.

Armelle de Saint Sauveur, Mélanie Broin. "Fighting malnutrition with Moringa oleifera leaves: an untapped resource." Moringa and other highly nutritious plant resources: Strategies, standards and markets for a better impact on nutrition in Africa (2006): 1.

Arnnok, Ruangviriyochai, Mahochi and Chanthas. "Determination of total phenolics and anthocyanin contents in the pericorp of hot chilli pepper (Capsicum annuum L.)." International Food Research Journal (2012): 235-243.

Augstin Scalbert, Claudine Manach, Christin Morand and Christin Remesy. "Dietary Polyphenols and the Prevention of Diseases." Critical Reviews in Food Science and Nutrition (2005): 287–306.

B. García-Fayos, J.M. Arnal, G. Verdú, A. Saur. "Study of moringa oleifera oil extraction and its influence in primary coagulant activity for drinking water." Food Inovva (2010): 1-5.

Barham, D., Trinder, P. "An improved colour reagent for the determination of blood glucose by the oxidase system." Analyst 97 (1972): 142–145.

Baynes, John W. "Role of oxidative strees in develoment of complications in diabetes ." Diabetes (1991): 405-412.

Beyene, Dereje. "Genetic Variation in Moringa Stenopetala Germplasm of Ethiopia By using RAPD as Genetic Marker." (2005).

Brahmachari, Goutam. "Bio-flavonoids with promising antidiabetic potentials: a critical survy ." Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry (2011): 187-212.

Chen WP, Chi T C, Chuang LM, Su MJ. "Resveratrol enhances insulin secretion by blocking K(ATP) and K(V) channels of beta cells." Eur Journal of Pharmacol (2007): 404-419.

Dechasa Jiru, Kai Sonder, Lalisa Alemayehu, Yalemtshay Mekonen and Agena Anjulo. "Leaf yield and Nutritive value of Moringa Stenopetala and Moringa Oleifera Accessions: Its potential role in food security in constrained dry farming agroforesty system ." Moringa and other highly nutritious plant resources: Strategies, standards and markets for a better impact on nutrition in Africa (2006): 2.

Dembinska-Kiec A, Mykkänen O, Kiec-Wilk B, Mykkänen H. "Antioxidant phytochemicals against type 2 diabetes." Jouranla of Nutrition (2008): 712-718.

Desmond Jay, Hirofumi Hitomi and Kathy K. Griendling. "Oxidative stress and diabetic cardiovascular complications." Free Radical Biology and Medicine (2006): 183-192.

Didacus Chukwuemeka Eze, Emmanuel Chukwudi Okwor, Okoye John Osita A., Onah Denis Nnabuike and Shoyinka S. Vincent Olu. "Effects of Moringa oleifera methanolic leaf extract on the morbidity and mortality of chickens experimentally infected with Newcastle disease virus (Kudu 113) strain." Journal of Medicinal Plants Research (2012): 4443-4449.

Dolly Jaiswal, Prashant Kumar Rai, Amit Kumar, Shikha Mehta, GeetaWatal. "Effect of Moringa Oleifera Lam. leaves aqueous extract therapy on hyperglycemic rats." Journal of Ethnopharmacology (2009): 392-396.

E. Sobngwi, F. Mauvais-Jarvis, P. Vexiau, J.C. Mbanya and J.F. Gautier. "Diabetes In Africans ." Diabetes Metab (Paris) (2001): 628-634.

E., Demeulenaere. "Moringa Stenopetala, A subsistence resource in the Konso district." Development potential for moringa product (2001): 1.

El-Hilaly, J., Tahraoui, A., Israili, Z.H., Lyoussi, B. "Hypolipidemic effects of acute and sub-chronic administration of an aqueous extract of Ajugaiva L. whole plant in normal and diabetic rats." Journal of Ethnopharmacology (2006): 441-448.

Fozia Farooq, Meenu Rai, Avinash Tiwari, Abdul Arif Khan and Shaila Farooq. "Medicinal properties of Moringa oleifera: An overview of promising healer." Journal of Medicinal Plants Research (2012): 4368-4374.

Gupta, B.B.P. "Mechanism of insulin action." Current Science (1997): 993.

Jo S-H, Ka E-H, Lee H-S, Apostolidis E, Jang H-D and Kwon Y-I. "Comparison of Antioxidant Potential and Rat intestinal α-Glucosidases inhibitory Activities of Quercetin, Rutin, and Isoquercetin." International Journal of Applied Research in Natural Products (2009-2010): 52-60.

Johnston, K. L., M.N. Clifford and L.M. Morgan. "Coffee acutely modifies gastrointestinal hormone secretion and glucose tolerance in humans: glycemic effects of chlorogenic acid and caffeine." American Journal of Clinical Nutrition (2003): 728–733.

Jung EH, Kim SR, Hwang IK, Ha TY. "Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice." Journal of Agricultural Food Chemistry (2007): 9800-9804.

Kahn, C. Ronald. "The molecular mechanism of insulin action ." Annual Review of Med. (1985): 429-451.

KASHIWAGI, Atsunori. "Complications of Diabetes Mellitus and Oxidative Stress." Journal of the Japan Medical Association (2000): 1559–1564.

Lee WC, Wang CJ, Chen YH, Hsu JD, Cheng SY,. "Polyphenol extracts from Hibiscus sabdariffa Linnaeus attenuate nephropathy in experimental sabdariffa Linnaeus attenuate nephropathy in experimental." Journal of Agricultural Food Chemistry (2009): 2206-2210.

M.F.White, C.J.Rhodes and. "Molecular insights into insulin action and secretion." European Journal of Clinical Investigation (2002): 3-13.

Makonnen, E., Hunde, A., Damecha, G.,. "Hypoglycaemic effect of moringa stenopetala aqueous extract in rabbits." phytotherapy research (1997): 147-148.

Manish, Nehetete Jetendra and Bhatia. "Correlation of antioxidant activity with phenolic content and isolation of antioxidants compound from Lygodium Flexuosum(L.)Sw. extract." International Journal of Pharmacy and Pharmceutical Science (2011): 48-52.

Matsui T, Ebuchi S, Kobayashi M, Fukui K, Sugita. "Anti-hyperglycemic effect of diacylated anthocyanin derived from Ipomoea batatas cultivar Ayamurasaki can be achieved through the alpha-glucosidase inhibitory action ." Agric Food Chem (2001): 1952-1958.

Matsui, T., Ebuchi, S., Kobayashi, M., Fukui, K., Sugita, K., Terahara, N., and Matsumoto, K. "Anti-hyperglycemic effect of diacylated anthocyanin derived from Ipomoea batatas cultivar Ayamurasaki can be achieved through the alpha-glucosidase inhibitory action." Journal of Agriculture and Food Chemistry (2002): 7244–7248.

Matsumoto, N., Ishigaki, F., Ishigaki, A., Iwashina, H., and Hara, Y. "Reduction of blood glucose levels by tea catechin." Bioscience, Biotechenology and Biochemistry (1993): 525–527.

Natalia Bellostas, Jens Christian Sørensen, Albert Nikiema, Hilmer Sørensen, Dov Pasternak and Sanjeet Kumar. "Glucosinolates in leaves of Moringa species grown and disseminated in Niger." African Journal of Agricultural Research (2010): 1338.

Ndong M, Uehara M, Katsumata S, Suzuki K. "Effects of Oral Administration of Moringa oleifera Lam on Glucose Tolerance in Goto-Kakizaki and Wistar Rats." Journal of Clin Biochem Nutr. (2007): 229-33.

Paliwal, Veena Sharma and Ritu. "Potential chemoprevention of 7, 12-dimethylbenz[a]anthracene induced renal carcinogenesis by Moringa oleifera pods and its isolated saponin." Asian Pacific Journal of Tropical Biomedicine (2011): 1-6.

Paulo Michel Pinheiro FERREIRA, Davi Felipe FARIAS, José Tadeu de Abreu OLIVEIRA andAna de Fátima Urano CARVALHO. "Moringa oleifera: bioactive compounds and nurtitional potential." Review Nutrition (2008): 433.

Prior, Guohua Cao and Ronald L. Measurement of Total Antioxidant Capacity in Nutritional and Clinical Studies. Boston, Massachusetts : Taylor & Francis Group, LLC, 2002.

Ray-Yu Yang, Lien-Chung Chang, Jenn-Chung Hsu, Brian B. C. Weng, Manuel C. Palada, M. L. Chadha and Virginie Levasseur. "Nutritional and Functional Properties of Moringa Leaves- From Germplasem, to Plant, to Food, to Health." Moringa and other highly nutritious plant resources: Strategies, standards and markets for better impact on nutirition in Africa (2006): 3.

Richard Sicree, Jonathan Shaw, Paul Zimmet. "Diabetes and Impaired Glucose Tolerance." IDF Diabetes Atlas fourth edition. IDF, 2010.

Rizvi SI, Mishra M. "Anti-oxidant effect of quercetin on type 2 diabetic erythrocytes." Journal of Food Biochem (2009): 269-277.

Rizvi SI, Zaid MA. "Interacellular reduced glatathione contnte in normal and type 2 diabetic erythrocytyes: effect of Insulin and (-) epicatechin ." Jouranal of Physiolpharmacol (2001): 483-491.

Rizvi, Kanti Bhooshan Pandey and Syed Ibrahim. "Plant polyphenols as dietary antioxidants in human health and disease." Oxidative Medicine and Cellular Longevity (2009): 270-278.

Rossi, V. L. Singleton and J. A. "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents." American Journal of Enology and Viticulture (1965): 144–158.

Shanmugasundaram, E.R., Gopinath, K.L., Shanmugasundaram, K.R., Rajendaran, V.M. "Possible regeneration of the islets of Langerhans in streptozotocin diabetic rats given Gymnema sylvestre leaf extracts." Journal of Ethnopharmacology (1990): 265–279.

Shashank R. Joshi, Rakesh M. Parikh and A. K. Das. "Insulin - History, Biochemistry, Physiology and Pharmacology ." Journal of Associtión of Physician India (2007): 19-25.

Singh, S.K.,Kesari,A.N.,Gupta, R.K., Jaiswal, D.,Watal, G. "Assessment of antidiabetic potential of Cynodon dactylon extract in streptozotocin diabetic rats." Journal of Ethnopharmacology (2007): 174–179.

Suaib Luqman, Suchita Srivastava, Ritesh Kumar, Anil KumarMaurya, and Debabrata Chanda. "Experimental Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays." Evidence-Based Complementary and Alternative Medicine (2011): 1-12.

Suchada Jongrungruangchok, Supawan Bunrathep and Thanapat Songsak. "Nutrients and Minerals Content of Eleven Different Sample of Moringa Oleifera Caltivated in Thailand." Journal of Health Research (2010): 123.

Szudelske, Tawlberk. "The mechanisim of alloxan and streptozotocin action in cells of the rat pancreas." physiology research (2001): 536-546.

Tsaknis, Stavros Lalas and John. "Characterization of Moringa oleifera Seed Oil Variety." Journal of Food Composition and Analysis (2001): 65–77.

Umer Rashid, Farooq Anwar , Bryan R. Moser and Gerhard Knothe. "Moringa oleifera oil: A possible source of biodiesel." Bioresource Technology (2008): 8175–8179.

V.Veeranan Arun GIRIDHARI, D. MALATHI and K. GEETHA. "Anti diabetic property of drumstick (Moringa oleifera) leaf tablets." International Journal of Health & Nutrition (2011): 1-5.

Vanisha S Nambiar, Parul Guin, Shilpa Parnami, Mammen Daniel. "Impact of Antioxidants from Drumstick leaves on the Lipid profile of Hyperlipidemics." Journal of Herbal Medicine and Toxicology (2009).

Wei W, Liu Q, Tan Y, Liu L, Li X, Cai L. "Oxidative stress, diabetes, and diabetic complications." US National Library of Medicine National Institutes of Health (http://www.ncbi.nlm.nih.gov/pubmed/19821780) (2009): 370-377.

White, C. Ronald Kahn and Morris F. "The Insulin Receptor and the Molecular Mechanism of Insulin Action ." Journal of Clinical Investigation (1988): 1151-1156.

WHO. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Geneva: World Health Organization, 1999.