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Effects of *Acacia nilotica subalata* on plasma glucose, lipids, protein and hemoglobin levels in normal and type 2 diabetic male rats

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Reg. Number: H56/65812/2010

A thesis submitted in partial fulfillment for the Degree of Master of Science in Medical Physiology.

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DECLARATION

This research project is my original work and has not been presented for award of a degree in any other university.

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This research project has been submitted with our approval as university supervisors.

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DEDICATION

This thesis is dedicated to my son NIYODUSENGA M. Bruce, to my wife UMUFASHA Christa and to my parents KARAHANYUZE J.B and ICYITEGETSE M.

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ABBREVIATIONS AND ACRONYMS

AAHA: American Animal Hospital Association

ACE: Angiotensin Converting Enzyme

ADA: American Diabetes Association

A.n: Acacia nilotica

ANOVA: Analysis Of Variance

ATP: Adenosine Triphosphate

BP: Blood Pressure

CO_2: Carbon dioxide gas

CoA: Coenzyme A

Cu^{2+}: Copper ions

CVD: Cardiovascular Disease

DNA: Deoxyribonucleic Acid

EDTA: Ethylene-Diamine-Tetraacetic Acid

Fe^{2+}: Ferrous iron

Fe^{3+}: Ferric iron

GLUT: Glucose Transporter

Hb: Hemoglobin

Hb A: Hemoglobin A

Hb A_{1C}: Glycated Hemoglobin A

HDL: High Density Lipoprotein

H_2O_2 : Hydrogen peroxide

IDF: International Diabetes Federation

IFG: Impaired Fasting Glucose

IGT: Impaired Glucose Tolerance

IUPAC: International Union of Pure and Applied Chemistry

LDL: Low Density Lipoprotein

NAD: Nicotinamide Adenine Dinucleotide

NCEPEP: National Cholesterol Education Program Expert Panel

O_2: Oxygen gas

SPSS: Statistical Package for Social Sciences

T2DM: Type 2 Diabetes Mellitus

USA: United States of America

VLDL: Very Low Density Lipoprotein

WHO: World Health Organization

ABSTRACT

Introduction: Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycemia resulting from defective insulin secretion by the pancreas, insulin action, or sometimes both. Its management is very expensive. Herbal products have gained worldwide popularity due to milder side effects, low cost and easy accessibility. One such product is *Acacia* which has also been shown to have some cholesterol-lowering and antidiabetic effects. However there is insufficient evidence in support of these observations.

Objective: To demonstrate the effects of *Acacia nilotica subalata* on plasma glucose, lipids, protein and hemoglobin levels of normal and type 2 diabetic male rats.

Study design: An experimental study

Setting: Department of Medical Physiology, University of Nairobi

Methodology: Diabetes was induced in 18 out of 30 rats by administering alloxan (150 mg/kg body weight). Induction was confirmed if fasting blood glucose level was > 7 mmol/l after one week. The rats were in five groups: Group A normal control, group B diabetic control, group C diabetic rats reveived *Acacia nilotica subalata* (800mg/kg body weight), group D normal rats received *Acacia nilotica subalata* (800mg/kg body weight) and group E diabetic rats received metformin (100 mg/kg body weight). The rats received treatment for 42 consecutive days. Once a week blood glucose was measured by glucometer. Levels of total protein, lipids and hemoglobin were essayed by colorimetric methods at the end of experiment. Body weight was measured at the beginning, thereafter every two weeks. Data were presented as mean ± standard

error of mean and analysis of variance performed. Results were considered statistically significant if p value $< 5\%$.

Results: *Acacia nilotica subalata* leaf extract or metformin significantly decreased the blood glucose of diabetic groups C and E compared to diabetic control group B (7.08 ± 1.451 and 6.50 ± 1.10 vs 18.10 ± 1.378 mmol/l, $p < 0.05$). Administration of either *A. nilotica subalata* extract or metformin showed no difference in total protein levels between control diabetic group B and diabetic groups treated with those products respectively ($p > 0.05$). *A. n. subalata* extract and metformin decreased total cholesterol in group C and E compared to diabetic control group B (109.05 ± 9.134 and 90.69 ± 6.838 vs 153.89 ± 18.829 mg/dl, $p < 0.05$). A statistically significant elevation of high density lipoproteins (HDL) was shown in group E treated with metformin compared to diabetic control B ($p < 0.05$). *A. n. subalata* extract and metformin in groups C and E respectively, significantly decreased low density lipoproteins (LDL) levels compared to diabetic control group B (59.62 ± 6.532 and 42.32 ± 4.844 vs 105.56 ± 15.14 mg/dl, $p < 0.05$) . The total hemoglobin level reduction was significant in diabetic group C compared to normal control group A (104.40 ± 5.50 vs 135.67 ± 5.76g/l, $p<0.05$). Treatment with *A. n. subalata* decreased weight in group D (normal treated with *Acacia*) compared to normal control A (no treatment) (324.06 ± 9.58 vs 372.50 ± 13.32 g, $p < 0.05$).

Conclusion: *A. n. subalata* leaf extract produced antihyperglycemic effect in diabetic rats. At the dose of 800mg/kg body weight it reduced significantly total cholesterol, LDL cholesterol, body weight and total hemoglobin. But increase of HDL cholesterol was not statistically significant. However, there were no significant changes of triglycerides and total plasma protein. Consequently *A. n. subalata* leaf extract may be considered as beneficial against hyperlipidemia induced by diabetes mellitus.

CHAPTER ONE

1.1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by persistent hyperglycemia resulting from defective insulin secretion by the pancreas, insulin action, or sometimes both. Uncontrolled chronic hyperglycemia results in long-term damage to body organs such as the eyes, heart, nerves, blood vessels and kidneys. According to International Diabetes Federation (IDF), type 2 diabetes mellitus has become a serious public health problem (IDF, 2003). It is the main cause of disability and premature death due to cardiovascular disease and other chronic complications (Roglic *et al*., 2005). Each year more than four million people die of diabetes mellitus and associated complications (IDF, 2009). The World Health Organization (WHO) estimates that in the year 2000 at least 171 million people worldwide had diabetes, which is equivalent to 2.8% of the population (Wild *et al*., 2004). The number of people with diabetes mellitus continues to rise. In 2010 at least 285 million people worldwide had the disease. This number is projected to rise to 439 million by 2030 (Shaw *et al*., 2010).

Diabetes mellitus requires long- term treatment which is very expensive. Worldwide its management annually requires about 480 billion US dollars (IDF, 2005). A large number of synthetic antidiabetic drugs are available which reduce the effects of diabetes mellitus and its related complications, but no cure is available yet. In addition diabetic patients suffer adverse effects associated with various synthetic antidiabetic drugs. For instance sulfonylureas have been associated with weight gain and hypoglycemia. The WHO expert committee on diabetes has recommended investigating traditional herbal medicines. Information from ethnobotanical databases indicates that more than 800 medicinal plant species with antidiabetic effects are in use worldwide. These herbal products are gaining popularity in developing and developed countries due to their fewer side effects and low cost (Modak *et al*., 2007). Diabetes mellitus causes an impairment of control of blood glucose, high level of cholesterol and hypoproteinemia. The present study is designed to determine the effects of *Acacia nilotica subalata* leaf extract on plasma glucose, hemoglobin, protein and lipid profile in normal and alloxan-induced diabetic male rats.

1.2. PROBLEM STATEMENT

The use of medicinal plants to treat diseases can be traced back over five millennia to written documents of the early civilization in China, India and the Near East. The ancient Greeks and Romans used medicinal plants. Greek physicians wrote the first European treatise on the properties and uses of medicinal plants, the Materia Medica (Zohara and Bachrach, 2005). These days people of all continents use indigenous plants for treatment of various diseases. One such disease is diabetes mellitus which leads to abnormalities in carbohydrate, protein and lipid metabolism. It is one of the most common diseases with a worldwide prevalence estimated to be over 6 per cent of the world population. This is a high prevalence rate and diabetes mellitus requires long-term treatment. Synthetic drugs are very expensive for diabetic patients. In the search for means of control, man uses medicinal plants to combat this alarming metabolic disease. Due to various challenges in management of diabetes mellitus using synthetic drugs, the plant products are gaining popularity because they are believed to have fewer side effects, low cost and easier accessibility (Modak *et al.*, 2007).

Several herbal products have been used as potential therapeutic agents in the management of diabetes mellitus and its related complications. One such product is *Acacia.* This plant has been reported to have cholesterol-lowering and antidiabetic effects, although there is insufficient evidence in support of those observations (Batra *et al.*, 2011). Aqueous extract of *Acacia nilotica* (A.n) pods, fruits, bark and seeds have traditionally been used to treat diarrhea, leprosy, asthma, skin diseases, ulcer, cancers of eye and ear, tuberculosis and small pox (Sundaram and Mitra, 2007). Extract of *Acacia nilotica* had shown analgesic and antipyretic activities (Dafallah *et al.*, 1996). Extract of *A nilotica* blocked platelet aggregation (Shah *et al.*, 1997). Aqueous extracts of fruits and stem bark showed molluscicidal activity (Hussein-Ayoub, 1985). *Acacia nilotica nilotica* has been reported to have many biological activities including antihypertensive and antispasmodic effects. *Acacia nilotica indica* leaves are rich in tannins and polyphenols (Carter *et al.,* 1988). Polyphenols decrease blood glucose levels (Sabu *et al.*, 2002). Some phenolic compounds with dihydroxyl groups can form complexes with transition metals, preventing metal- induced free radical formation. This prevents the interaction between hydrogen peroxide (H_2O_2) and redox active metallic ions such as Cu^+ or Fe^{++} (Yoshino *et al.*, 1998). Tannins bind

to proteins in the gastrointestinal pathway and subsequently decrease feed intake. In addition tannins have the ability to bind and inhibit the digestive enzyme activities (Kumar and Singh, 1984).

Diabetes mellitus causes an impairment of control of blood glucose, high level of cholesterol and hypoproteinemia. The best way of research on antidiabetic drugs must be oriented toward the control of those metabolic disorders. In this regard the present study will determine the effects of *Acacia nilotica subalata* leaf extract on plasma glucose, hemoglobin, total protein and lipid profile in normal and alloxan-induced type 2 diabetic male rats.

1.3. JUSTIFICATION OF STUDY

Diabetes mellitus is a chronic severe disease which affects many people. Because of the high cost and adverse effects of synthetic drugs there is a need for man to discover more effective, available and safer antidiabetic agents by using medicinal plants.

Diabetes mellitus causes an impairment of control of blood glucose, high level of cholesterol and hypoproteinemia. The best way of research on antidiabetic drugs must be oriented toward the control of those metabolic disorders. *Acacia* have been reported to have some antidiabetic effects, although there is insufficient evidence in support of these uses. In this regard the present study will determine if *Acacia nilotica subalata* leaf extract has effects on plasma glucose, lipids, total protein and hemoglobin levels in normal and alloxan-induced type 2 diabetic male rats.

1.4. OBJECTIVES

1. 4. 1. GENERAL OBJECTIVE

To demonstrate the effects of *Acacia nilotica subalata* on plasma glucose, lipids, total protein and hemoglobin in normal and type 2 diabetic male rats.

1. 4. 2. SPECIFIC OBJECTIVES

1. To compare the effects of *Acacia nilotica subalata* leaf extract and metformin (glucophage) on blood glucose levels in diabetic rats.
2. To determine the plasma lipids and total protein levels in rats treated with *Acacia nilotica subalata* leaf extract.
3. To determine the total plasma hemoglobin levels in normal and diabetic rats treated with *Acacia nilotica subalata* leaf extract.
4. To investigate the effect of *Acacia nilotica subalata* leaf extract on rat body weight.

CHAPTER TWO

LITERATURE REVIEW

2. 1. Type 2 Diabetes mellitus

2. 1.1. Definition

Type 2 diabetes mellitus (T2DM) refers to a group of metabolic diseases in which a person has hyperglycemia, either because the pancreas does not produce enough insulin, or because peripheral cells do not respond to the insulin that is produced.

The symptoms of diabetes mellitus include: polyuria, polydipsia and polyphagia. The presence of large amount of glucose in the urine causes osmotic diuresis which leads to dehydration, increased thirst and water consumption. Diabetics experience excessive hunger despite hyperglycemia because in diabetes mellitus there is limited glucose utilization by peripheral body tissues (Guyton and Hall, 2006).

2.1.2. Prevalence

Type 2 diabetes mellitus affects millions of people worldwide. The number of people with T2DM is projected to rise rapidly from 285 million in 2010 to 439 by 2030. This increase of 54% represents the prevalence of 7.7% of the adult people aged 20 to 79 years (Shaw *et al*., 2010). Type 2 diabetes mellitus mainly affects the elderly population and the situation is projected to be more serious in the future due to increasing life expectancy (IDF, 2009). Although T2DM was considered as a metabolic disease of adults, it has become more common in adolescents and occasionally in children (Pinhas- Hamiel *et al.,* 2005). In addition to the rising rate of T2DM in youth, the number of pre-diabetics among adolescents has also increased. For instance, about 16.1% of US adolescents had impaired fasting glucose (IFG) and/ or impaired glucose tolerance (IGT) in 2005-2006. And as in adult people some risk factors such as obesity, hyperinsulinemia or a family history of diabetes mellitus are contributors of pre-diabetes in youth (Li *et al*., 2009).

2. 1.3. Pathophysiology and complications

Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin involves the insulin receptor resistance. Insulin has varied effects in the body; in the liver it stimulates glycolysis and glycogen storage. It also stimulates chylomicrons and very low density lipoproteins (VLDL) uptake. In the skeletal muscle insulin stimulates glucose uptake via glucose transporter (GLUT) - 4 receptors and glucogen synthesis. In addition it stimulates amino-acid uptake and protein synthesis. At the level of adipocytes it facilitates glucose transport via GLUT -4 receptors. Insulin induces activity of the lipoprotein lipase which hydrolyzes circulating triglycerides leading to uptake of free fatty acid and glycerol (Barrett *et al.*, 2010). Resistance to the action of insulin results in impaired insulin mediated glucose uptake in the skeletal muscle and adipocytes, incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. To counter the insulin resistance beta islet cells of pancreas increase the secretion of the insulin. Hyperinsulinemia removes the impedance to the action of insulin. This state of high insulin levels with normal glycemia persists for many years. Hyperglycemia and dyslipidemia occur when there is a mismatch between insulin requirements, as in insulin receptor resistance and insulin supply consequent to beta cell exhaustion (Guyton and Hall, 2006).

Type 2 diabetes mellitus is associated with obesity (Moore *et al.*, 2000). In the majority of cases T2DM widely is associated with components of metabolic syndrome. The metabolic syndrome is characterized by abdominal obesity, dyslipidemia, hypertension, insulin resistance, prothrombotic and proinflammatory features (Tenenbaum *et al.*, 2003). Other risk factors associated with development of diabetes mellitus include: physical inactivity, excess dietary fat intake, smoking, family history of diabetes mellitus and race (Kelestimur *et al.*, 1999). The disease induces hyperosmolar nonketotic coma which is usually an acute complication and is less common but accounts for about 10 percent of all hyperglycemic emergencies in developing countries (Zouvanis *et al.*, 1997). In addition the other severe acute complication is hypoglycemia induced by treatment of diabetes with drugs such as sulfonylureas.

Over time, uncontrolled T2DM can lead to blindness, kidney failure and nerve damage. These types of damage are the result of damage to small vessels and nerves, referred to as

microvascular disease. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries which is the cause of atherosclerosis, leading to strokes, coronary heart disease and other large blood vessel diseases. This is referred to as macrovascular disease. The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia (Fowler, 2008).

Diabetic retinopathy is the most common microvascular complication associated with T2DM. It may begin to develop as early as seven years before the diagnosis of T2DM (Fong *et al*., 2004). The pathological mechanism leading to development of retinopathy seems to be the conversion of glucose into sorbitol. Probably due to aldose reductase, hyperglycemia leads to overproduction of sorbitol and its accumulation in the cells. Accumulation of sorbitol is associated with microneurysms, the thickening of the basement membrane and loss of pericytes at the level of the kidneys (Gabbay, 2004). Hyperglycemia can induce the nonenzymatic formation of glycosylated end products. Large amounts of glycoproteins can also promote injury to the cells. In addition oxidative stress also plays a major role in cellular lesion from hyperglycemia. In fact high blood glucose levels can stimulate free radical production and formation of reactive oxygen species (Baynes *et al*., 1999).

Diabetic nephropathy is the major cause of renal failure. About 7 per cent of patients with T2DM may already have microalbuminuria at the time they are diagnosed with diabetes (Gross *et al*., 2005). The onset of microalbuminuria is due to pathological changes to the kidneys. These are mainly thickening of the glomerular basement, microneurysm formation and mesengial nodule formation. In T2DM peripheral neuropathies appear in several forms; they may be sensory, sensorimotor, which can be focal or multifocal and autonomic neuropathies. The peripheral neuropathies are also frequent. More than 80 per cent of amputations that occur following foot ulceration are attributed to diabetes mellitus (Boulton *et al*., 2005).

The main pathological mechanism in the genesis of macrovascular complications is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Due to chronic inflammation and damage of arteries oxidized LDL cholesterol accumulates in the endothelial

wall of arteries. The inflammatory process stimulates macrophage proliferation and attraction of T lymphocytes at the site of inflammation, the net result being the onset of atherosclerosis.
In addition, in T2DM there is increased platelet adhesion and hypercoagulability, which increase the risk of vascular occlusion and cardiovascular disease. Cardiovascular disease is the primary cause of death in people with T2DM (Laing *et al.*, 2003). Patients of T2DM also have high risk of developing of stroke. They manifest an increased risk of stroke related dementia and recurrence of stroke at the rate of 150-400 per cent compared to non-diabetics (Beckman, 2002).

2.2. Treatment of Type 2 Diabetes Mellitus

Diabetes mellitus is a chronic disease which cannot be cured. Management concentrates on controlling blood sugar levels as close to normal as possible, without causing hypoglycemia. This can usually be accomplished through selective diet, physical exercise and use of appropriate oral medications as well as insulin injections in type I diabetes. Efforts also aim to control other health problems that may exacerbate the complications of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure and lack of regular exercise.

To reduce cardiovascular events that occur in type 2 diabetes patients, blood pressure needs to be monitored routinely. Type 2 diabetic patients with hypertension (BP > 140/90 mmHg), in addition to antidiabetic drug, should be treated with angiotensin converting enzyme (ACE) inhibitor and advised on lifestyle modification. They should be tested annually for lipid profile to maintain low density lipoproteins (LDL) below 100 mg/dl and fasting triglycerides at 150 mg/dl. Combined therapy of statin and other drugs such as fibrate or niacin may be useful in the control of lipid levels. The use of aspirin or other antiplatelet adhesion agents are indicated in secondary prevention of cardiovascular disease (CVD). The American Diabetes Association (ADA) considers the use of aspirin as helpful in diabetics who are over 40 years old or between 30-40 years of age presenting other risk factors (ADA, 2007).

2.2.1. Metformin

The International Union of Pure and Applied Chemistry (IUPAC) name for metformin is N, N dimethyl imido dicarbonimidic diamine. Its chemical formula is $\mathbf{C_4H_{11}N_5}$.

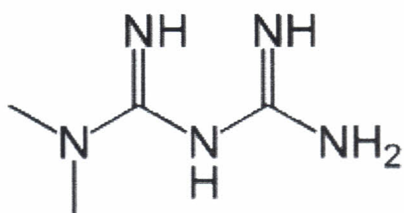


Figure 1: Chemical formula of Metformin

(Source: http://enc.Wikipedia.org/metformin)

Metformin (also known as glucophage) is an oral antidiabetic drug in the biguanide class. It is the first line drug of choice for treatment of type 2 diabetes, particularly in overweight, obese people and those with normal kidney function. Metformin acts by suppressing glucose production by the liver. It activates AMP – activated protein kinase (AMPK), an enzyme which plays a role in insulin signaling, whole body energy and the metabolism of glucose and fats. The activation of AMPK produces inhibitory effect on the production of glucose by liver cells (Zhou *et al.*, 2001). In addition to suppressing gluconeogenesis, metformin increases insulin sensitivity, and enhances peripheral glucose uptake by phosphorylating GLUT- 4. Metformin increases fatty acid oxidation and reduces glucose absorption from gastrointestinal tract (Collier *et al.*, 2006). It is predominantly absorbed in the small intestine. Complete absorption requires 6 hours. Its excretion occurs in the kidney without chemical modification.

Metformin prevents cardiovascular complications of diabetes. It reduces LDL cholesterol and triglyceride levels and is not associated with weight gain. It reduces mortality by about 30 per cent when compared with insulin and sulfonylureas and by about 40 per cent when compared with application of dietary advice as means of control of diabetes (Bolen *et al.*, 2007). Metformin is contraindicated in people with any condition which can increase the risk of lactic acidosis, such as kidney failure, lung and liver diseases (Jones *et al.*, 2003).

The most common adverse effects of metformin is gastrointestinal upset, including diarrhea, cramps, nausea, and flatulence. The most severe side effect of metformin is lactic acidosis but this complication is rare (Khumar *et al.*, 2010). Long-term use of metformin has been associated

with malabsorption of vitamin B_{12} leading to increased incidence of its deficiency (Jager *et al.*, 2010).

2.3. Definition of Acacia

The name *Acacia* derives from the Greek word *akis* for its characteristic thorns. The species name *nilotica* was given by Linnaeus from trees located along the Nile river. *Acacia nilotica* belongs to order of *Fabales*, family of Fabacae, subfamily of Mimosoideae, Tribe of Acacieae, and Genus of Acacia. *Acacia* is a tree that is widely distributed all over the world. It is distributed throughout tropical and warm temperate areas of the world, with many species in Australia, but also with high number of species in America, Africa and Asia (Maslin *et al.*, 2003).

Figure 2: Map of global distribution of *Acacia* tree

(Source: http://enc.Wikipedia.org/acacia nilotica)

Acacia is widely used as a chewing stick. Its antimicrobial properties against *Streptococcus fecalis* may contribute to controlling gum related disease (Almas, 2001). *Acacia* has also shown some cholesterol-lowering and antidiabetic effects, although there is insufficient evidence in

support of these uses. *Acacia* gum is used as a food additive. *Acacia concinna* is often used in cosmetics (Pandey *et al*., 2010).

Figure 3: Picture of leaves and flowers of *Acacia nilotica subalata*

(Source: http:// herbaria.plant.ex.ac.uk/vfh/image/index.item2029)

Acacia nilotica subalata is a tree 2.5 - 5 m high with a dense spherical crown. Its stems and branches are usually dark to black. The tree has thin, straight, light and grey spines in axillary pairs. Spines of young trees are 5 to 7.5 cm long. Mature trees commonly have no thorns. The leaves are bipinnate. Flowers in globulous heads measure 1.2 to 1.5 cm in diameter and have bright golden-yellow color. Pods are strongly constricted, white-grey and thick. This subspecies is tolerant to heat and dryness. It occurs in eastern Africa in Sudan, Ethiopia, Kenya and Tanzania.

2.3.1. Medicinal uses and pharmacological activities of *acacia*

The *Acacia nilotica* has been used in traditional medicine in many situations. The aqueous extract of *Acacia nilotica* (AN) pods, fruits, bark and seeds have been used traditionally for

treatment of diarrhea, leprosy, asthma, skin diseases, ulcer, cancers of eye and ear, tuberculosis and small pox (Sundaram and Mitra, 2007). *Acacia nilotica* is considered a remedy that is helpful for treating premature ejaculation (Pankhurst *et al*., 1990). *Acacia nilotica* has been used to treat gingivitis, stomatitis, pharyngitis and indigestion in children. The young seed pods and young foliage are edible. The raw or dried seeds are eaten when food is scarce. Extract of *Acacia nilotica* had shown Analgesic and antipyretic activities (Dafallah *et al*., 1996). Extract of *Acacia nilotica* blocks platelet aggregation (Shah *et al*., 1997). Aqueous extracts of fruits and stem bark showed molluscicidal activity (Hussein-Ayoub, 1985). Aqueous methanolic extract of *Acacia nilotica nilotica* pods has been reported to have antidiabetic and hypolipidemic effect in diabetes rabbits (Maqsood *et al*., 2008).

2.3.2. Biological activities of key components of *acacia* species

Acacia leaves contain 14-20% protein. The seed pods of *A. nilotica sensu lato* subsp. *nilotica* have a tannin content of about 25-33.8%. Pods without seeds have a tannin content of about 50% (Bargali *et al*., 2009). Tannins which are in hydrolysable polyphenol group form precipitation with proteins. Applications to medicine, food and other fields are associated with low molecular weight polyphenols. Tannin exists in other medicinal plants and plant foods such as legumes, sorghum, beans, fruits and vegetables such as onions, grapes and tea. Tannins bind to proteins, vitamins and minerals to form complexes ((Kumar and Singh, 1984). Some medicinal plants mostly contain tannin, and they are used to treat inflammation, to stop bleeding, protect the gastrointestinal mucosa and in the treatment of cardiovascular disease. Tannin content in the food is mainly in condensed form. Lower molecular weight tannins can be absorbed into the blood capillaries and have antioxidant effects. Polymers of more than three monomers cannot be absorbed (Rice *et al.,* 1995).

Too much tannin in food may produce some adverse effects on the human body by reducing the nutritional value of protein. Long-term consumption of tannin reduces absorption of calcium and iron (Dai and Mumper, 2010). Tannin has antimicrobial and antiviral properties (Mahesh and Satish, 2008). Larger molecular weight tannins have strong antioxidant activity. The excess of free radicals can damage the biological macromolecules, affecting protein conformation and may also induce destruction of tissues and organs. They are believed to promote the aging process and

lead to development of many diseases (Dai and Mumper, 2010). Tannin herbs can significantly reduce high serum cholesterol and tannin can antagonize calcium-induced contraction of smooth and heart muscle, thereby reducing blood pressure (Gilani *et al.,* 1999).

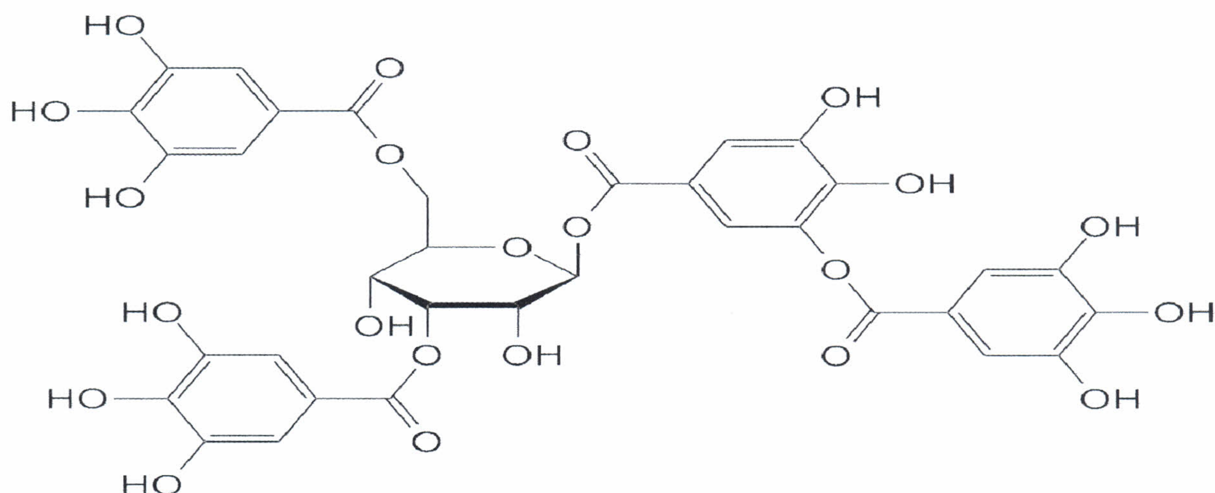


Figure 4: Chemical structure of Tannic acid

(Source: http://upload.wikimedia.org/wikipedia/commons/9/96/Tannic_acid.png)

2.3.3. Polyphenols

Polyphenols are a structural class of natural, synthetic and semisynthetic organic chemicals characterized by the presence of multiple phenol units. The number and characteristics of these phenol substructures underlie the unique physical, chemical and biological properties of particular members of the class (Quidieu *et al*., 2011). Phenol refers to a chemical compound formed by an aromatic phenyl or benzenoid ring having an alcohol-type hydroxyl (-OH) group giving rise to the "-ol" suffix. The polyphenols act as antioxidants (Rice *et al.,* 1995). They protect cells and body chemicals against damage caused by free radicals, reactive atoms that contribute to tissue damage. For example, when low-density lipoprotein (LDL) cholesterol is oxidized, it can damage arteries and cause coronary heart disease. Polyphenols can also block the action of enzymes that promote cancer growth and they can deactivate substances that cause the growth of cancers (Dai and Mumper, 2010). Polyphenols are found in all tea plants. Polyphenols

isolated from tea have been shown to act as scavengers for oxygen and nitrogen-free radicals, protecting the fatty membranes of cells, proteins and DNA (Gupta *et al.*, 2008).

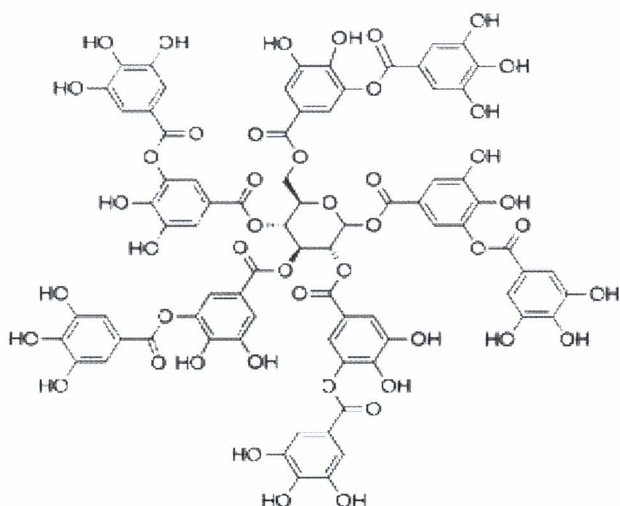


Figure 5: Chemical structure of Polyphenols.

(Source: http://enc.wikipedia.org/wiki/file:tanic_acid.svg)

2.4. Cholesterol

2.4.1. Definition of cholesterol

The name cholesterol originates from the Greek chole- (bile) and *stereos* (solid), and the chemical suffix *–ol* for alcohol. Cholesterol was first discovered in solid form in gallstones by Francois Poulletier de la Salle in 1769 (Olson, 1998). However, it was only in 1815 that chemist Eugene Chevreul named the compound cholesterine (Olson, 1998). Cholesterol is a chemical compound that is naturally produced by the body. It is a building block for cell membranes and steroid hormones namely glucocorticoids, mineralocorticoids, androgens and estrogens. About 80% of the body's cholesterol is produced by the liver, while the rest comes from diet. Following a meal, dietary cholesterol is absorbed in the intestine and stored in the liver. The liver regulates

cholesterol levels in the blood stream and can secrete cholesterol if needed by the body (Guyton and Hall, 2006). Cholesterol is required to establish proper membrane permeability and fluidity. In addition, it is an important precursor of bile acids and vitamin D (Barrett *et al.*, 2010).

2.4.2. Metabolism of cholesterol

Cholesterol is susceptible to oxidation and easily forms oxygenated derivatives known as oxysterols. Three different mechanisms are involved: autoxidation, secondary oxidation to lipid peroxidation and cholesterol metabolizing enzyme oxidation. The oxysterols play a major role in cholesterol regulation in the body because they exert inhibitory actions on cholesterol biosynthesis. Oxysterols in human physiology participate in bile acid biosynthesis, function as transport forms of cholesterol and regulation of gene transcription (Russell, 2000). Cholesterol is oxidized in the liver into a variety of bile acids. In turn bile acids are conjugated with glycine, taurine, glucuronic acid, or sulfate in the liver. Approximately 95% of the bile acids are reabsorbed in the intestines and the remainder lost in feces (Wolkoff and Cohen, 2003). The excretion and reabsorption of bile acids form the basis of the enterohepatic circulation, which is essential for the digestion and absorption of dietary fats. Up to 1g of cholesterol enters the colon daily (Guyton and Hall, 2006). This cholesterol originates from diet, bile and desquamated intestinal cells and can be metabolized by colonic bacteria. Cholesterol is mainly converted into coprostanol, a non absorbable sterol which is excreted in the feces (Lye *et al.*, 2010).

2.4.3. Types of cholesterol.

Low density lipoprotein (LDL) cholesterol is referred to as "bad" cholesterol, because its elevation is associated with an increased risk of coronary heart disease. Deposits of LDL on artery walls, cause formation of a hard, thick substance called cholesterol plaque. Over time, cholesterol plaque causes thickening of artery walls and narrowing of their lumen, a process called atherosclerosis. High density lipoprotein (HDL) cholesterol is considered the "good cholesterol" because it prevents atherosclerosis by extracting cholesterol from the plasma and transporting it to the liver. Thus, high levels of LDL and low levels of HDL cholesterol (high LDL/HDL ratios) are risk factors for atherosclerosis, while low levels of LDL and high level of HDL cholesterol (low LDL/HDL ratios) are a good index for preventing atherosclerosis (Guyton and Hall, 2006). Very low density lipoproteins (VLDL) carry cholesterol from the liver to organs

and tissues in the body. They are formed by a combination of cholesterol and triglycerides. VLDLs are heavier than LDL, but are also associated with atherosclerosis and heart disease. Their quantity is derived by dividing triglyceride levels by a factor of 5 (Fridewald *et al.*, 1979). Total cholesterol is the sum of all types of cholesterol in circulation.

2.4.4. Factors that affect plasma cholesterol concentration.

The concentration of cholesterol in plasma is mainly affected by the following factors: increased cholesterol intake slightly increases plasma concentration of cholesterol. Diet rich in saturated fat increases blood cholesterol concentration by about 20%. Consumption of unsaturated fatty acids decreases the blood cholesterol concentration by moderate amounts. Lack of insulin or thyroid hormone increases the plasma cholesterol concentration, whereas excess thyroid hormone has the opposite effect. Being overweight can also increase blood cholesterol. Losing weight can help lower LDL and total cholesterol levels, as well as increase HDL cholesterol. Regular exercise can lower LDL cholesterol and raise HDL cholesterol. As people get older, cholesterol levels rise. Before menopause, women tend to have lower total cholesterol levels than men of the same age. After menopause, however, women's LDL levels tend to rise. Poorly controlled diabetes increases cholesterol levels. Genes partly determine how much cholesterol the body makes. Certain medications and medical conditions can cause high cholesterol (Guyton and Hall, 2006).

2.4.5. Interpretation of levels of cholesterol in the plasma.

According to the 1987 report of National cholesterol education program expert panel (NCEPEP), normal adult total blood cholesterol level should be < 200 mg/dl. A range of 200–239 mg/dl is considered borderline-high and a level > 240 mg/dl considered high (NCEPEP, 1987). The following guidelines have been set forth by the NCEPEP: LDL levels less than 100 mg/dl (2.6 mmol/l) are considered optimal. LDL levels between 100 – 129 mg/dl (2.6–3.34 mmol/l) are near or above optimal. LDL levels between 130 – 159 mg/dl (3.36–4.13 mmol/l) are borderline high. LDL levels between 160 – 189 mg/dl (4.14 - 4.90 mmol/l) are considered high. LDL levels at or above 190 mg/dl (4.91 mmol/l) are very high. Any HDL level above 60 mg/dl (1.56 mmol/l) is considered high. A high HDL level is considered very healthy, since it has a protective role in guarding against heart disease. An acceptable HDL range is between 40- 60

mg/dl (1.04–1.56 mmol/l). Undesirable level of HDL is below 40 mg/dl (1.04 mmol/l). In this case, low HDL levels may contribute to heart disease. Possible complications of high cholesterol include: Atherosclerosis, Coronary artery disease, Stroke and Heart attack or death.

Table 1: Levels of cholesterol and risk for heart disease

Level mg/dl	**Level** mmol/l	**Interpretation**
< 200	< 5.0	Desirable level corresponding to lower risk for heart disease
200–240	5.2 – 6.2	Borderline high risk
> 240	> 6.2	High risk

2.4.6. Lipid disorders in Type 2 Diabetes Mellitus

Type 2 diabetes mellitus affects all types of lipids and lipoproteins. Chylomicron and VLDL remnants accumulate, and triglycerides increase HDL and LDL, leading to high levels of potentially atherogenic particles and low levels of HDL cholesterol. Lipid metabolism in T2DM is modulated by a series of factors, the key ones being the degree of glycemic control and insulin resistance. Insulin resistance is at the basis of the pathophysiologic mechanisms of diabetic dyslipidemia, being the major cause of hypertriglyceridaemia and postprandial lipemia. In normal subjects, insulin inhibits the assembly and secretion of VLDL particles by increasing apolipoprotein B (apoB) degradation and decreasing the expression of the microsomal transfer protein (MTP) in hepatocytes (Chirieac *et al.*, 2000). Failure of insulin to suppress VLDL release in the postprandial phase can saturate the lipolytic pathways and contribute to postprandial lipemia.

2.4.7. Prevention and treatment of excess cholesterol

There are important measures that improve cholesterol levels and thus help prevent heart disease and heart attack. Among them include eating plenty of fiber-rich fruits and vegetables and avoiding animal products and commercially baked products as they contain saturated fats and trans-fatty acids. Regular exercise help raise HDL. Periodic health checkups, cholesterol screenings, Weight reduction if overweight and avoiding smoking help in the control of cholesterol levels (Guyton and Hall, 2006).

There are several types of drugs available to help lower blood cholesterol levels and they work in different ways. Some are better at lowering LDL cholesterol, some are good at lowering triglycerides, while others help raise HDL cholesterol. The most commonly used and most effective drugs for treating high LDL cholesterol are called statins. Examples are: lovastatin (Mevacor), pravastatin (Pravachol), simvastatin (Zocor), fluvastatin (Lescol), torvastatin (Lipitor) and rosuvastatin (Crestor). Other drugs that may be used include bile acid sequestering resins, cholesterol absorption inhibitors, fibrates and nicotinic acid (niacin) (Guyton and Hall, 2006).

2.5. Proteins

2.5.1. Definition of proteins

Proteins are biochemical compounds made of one or more polypeptides. A polypeptide is a linear polymer of amino acids bound together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues (Francis, 2008). Together with other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of organisms and participate in virtually every process within cells. Many proteins are enzymes that catalyze biochemical reactions. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which maintains cell shape. Other proteins are important in cell signaling, immune responses, cell adhesion and the cell cycle. Proteins are also necessary in animal diets, since animals cannot synthesize all the amino acids they need and must obtain essential amino acids from food. Due to the process of digestion and intestinal absorption animals break down ingested protein into free amino acids that are then used in metabolism (Barrett *et al*., 2010).

2.5.2. Protein catabolism

Protein catabolism is the breakdown of proteins into amino acids. This process involves specific enzymes that cleave proteins. The amino acids produced by catabolism may be directly recycled, used to make new amino acids or undergo amino acid catabolism to be converted to other compounds via the Krebs cycle (Guyton and Hall, 2006). Blood proteins are those found in

plasma. The total plasma protein is 7g/dl. They serve many different functions including: circulatory transport of molecules for lipids, hormones, vitamins and minerals. Some are enzymes, complement components and protease inhibitors. Some function as regulators of cellular activity.

Table 2: Levels of blood proteins and their functions

Blood protein	**Normal level**	**%**	**Function**
Albumins	3.5-5.0 g/dl	60%	create oncotic pressure and transports other molecules
Immunoglobulins	1.0-1.5 g/dl	35%	participate in immune system
Fibrinogens	0.2-0.45 g/dl	4%	coagulation
Alpha 1-antitrypsin			neutralize trypsin that has leaked from the digestive system
Regulatory proteins		<1%	Regulation of gene expression

All plasma proteins are synthesized in liver except gamma globulins. Albumin which makes up 60% of the plasma proteins is the major contributor to osmotic pressure. Globulins make up 35% of plasma proteins and are used in the transport of ions, hormones and lipids assisting in immune function. Fibrinogen contributes 4% which is essential in the clotting of blood and can be converted into insoluble fibrin. Regulatory proteins which make up less than 1% of plasma proteins are proteins such as enzymes, proenzymes and hormones (Anderson *et al.*, 1977).

2.5.3. Protein decrease in Type 2 Diabetes Mellitus

Normally insulin stimulates transport of amino acids into the cells. It promotes synthesis and storage of proteins. In the liver, insulin suppresses gluconeogenesis. In uncontrolled diabetes muscle proteins are broken down into amino acids. Part of those amino acids is used directly as source of energy, the other is converted into glucose by the liver. Since the rate of protein breakdown is greater than the rate of protein synthesis, decreased utilization of glucose for energy leads to increased utilization and decreased storage of proteins (Guyton and Hall, 2006).

2.6. Hemoglobin

2.6.1. Definition of hemoglobin

Hemoglobin (Hb) is the iron-containing oxygen-transport metalloprotein inside red blood cells. Hemoglobin in the blood carries oxygen from the lungs to the rest of the body tissues where it releases the gas required for respiration. The resultant carbon dioxide is returned to the lungs for expiration. In mammals, hemoglobin makes up about 97% of the red blood cells' dry content, and around 35% of the total content. Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin (Dominguez de Villota *et al.*, 1981). This increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind four oxygen molecules.

Hemoglobin is involved in the transport of carbon dioxide bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen (Connie, 1988). Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells and mesengial cells in the kidneys. In these tissues, hemoglobin has a non-oxygen-carrying function, acting as antioxidant and regulator of iron metabolism (Biagioli *et al*, 2009). In 1959 Max Perutz determined the molecular structure of hemoglobin (Perutz *et al.*, 1960). The name hemoglobin is derived from the words heme and globin.

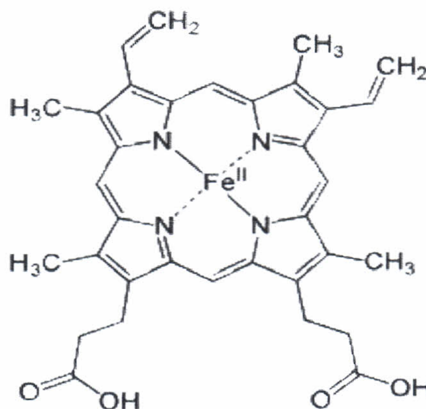


Figure 6: Chemical structure of Heme

(Source: http://en.wikipedia.org/hemoglobin)

2.6.2. Structure of hemoglobin

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape (Linberg, 1998). Hemoglobin quaternary structure comes from its four subunits in roughly tetrahedral arrangement. In man hemoglobin molecule is formed by an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges into a set of alpha-helix structural segments bound to a globin arrangement.

A heme group consists of an iron (Fe) ion held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly to the globular protein via the imidazole ring of the histidine residue below the porphyrin ring. The iron ion may be either in the ferrous (Fe^{2+}) or ferric ($Fe3^{+}$) state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily and reversibly oxidizes Fe^{2+} to Fe^{3+} while oxygen temporally turns into superoxide, thus iron must be in the ferrous state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable to bind oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin A contains 4 protein subunits. It consists of two alpha (α) and two beta (β) subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of about 17,000 daltons, for a total molecular weight of the tetramer of about 68,000 daltons (Jensen, 2009). In infants, the hemoglobin molecule is made up of 2 α chains and 2 gamma chains. The gamma chains are gradually replaced by β chains as the infant grows (Perutz, 1960). The four polypeptide chains are bound to

each other by salt bridges, hydrogen bonds, and the hydrophobic effect. There are two kinds of contacts between the α_1 and β chains: $\alpha_1\beta_1$ and $\alpha_1\beta_2$.

2.6.3. Hemoglobin in diabetic patients

Hemoglobin A slowly combines with glucose at the terminal valine of each β chain. The resulting molecule is often referred to as glycosylated hemoglobin (HbA_{1c}). With chronic hyperglycemia found in diabetics, the percentage of Hb A that is converted into Hb A_{1c} increases. The HbA_{1c} percentage increases with the duration of diabetes because of the slow rate of HbA combination with glucose. This form of hemoglobin reduces the lifespan of red blood cells which is normally 120 days. Levels of glycosylated hemoglobin are therefore measured in order to monitor the long-term control of the chronic T2DM. Poor control of T2DM leads to high levels of glycosylated hemoglobin in the red blood cells. The normal reference range is approximately 4–5.9 %. Though difficult to obtain, values less than 7 % are acceptable for people with T2DM. According to Asian-Pacific Type 2 Diabetes Policy Group (APTDPG), levels greater than 7.5 % are associated with poor control of the glucose level, and levels greater than 12 % are associated with very poor control. Diabetics who have glycosylated hemoglobin levels close to 7 % have a much better chance of avoiding the complications of diabetes than those whose levels are higher (APTDPG, 2002).

2.6.4. Clinical uses of hemoglobin

Hemoglobin concentration measurement is among the most commonly performed blood tests, usually as part of a complete blood count. Results are reported in g/dl or mol/l. 1 g/dl equals about 0.6206 mmol/l. Normal levels are for men: 13.8 to 18.0 g/dl (8.56 to 11.3 mmol/l) and 12.1 to 15.1 g/dl (7.51 to 9.37mmol/l) for women (Amber, 2012).

2.7. Induction of type II diabetes mellitus in animals

Causes of type II diabetes mellitus in animals include: spontaneous or genetic, nutritional, chemical, surgical and transgenic. In this study, the chemical method with alloxan was applied because it induces selective loss of β cells of the pancreas leaving α and δ cells of the pancreas unaffected. The occurrence of ketosis as the main cause of mortality is relatively reduced. The animals can survive long time without insulin therapy due to residual insulin secretion. The

method is also cheaper and easier to manipulate during the course of the experiment (Srinivasan *et al.*, 2007).

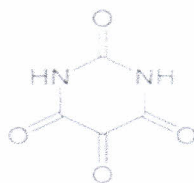


Figure 7: Chemical structure of alloxan

(Source: http://enc.wikipedia.org/wiki/file:alloxan.png)

2.7.1. Alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative. It is present as alloxan hydrate in aqueous solution. It is the chemical agent commonly used to induce type II diabetes mellitus in animals (Viana *et al.*, 2004). Alloxan, urea derivative provokes selective destruction of pancreatic islet B cells. The degree of pancreatic B cell damage depends on the doses of alloxan administered. The response of pancreas to the dose of alloxan is determined by the levels of fasting blood glucose.

In rabbits moderate diabetes has been defined as a fasting blood glucose level of 180-250 mg/dl, and severe diabetes as above 250 mg/dl (Huralikuppi, 1991). The destruction of pancreatic B cells causes hyperglycemia due to decreased production of insulin. Hence drugs such glibenclamide which stimulate insulin release from B cells have no significant effect. A single dose of alloxan, 140-180 mg/kg body weight is administered intravenously or intraperitoneally. Alloxan and its reduction product dialuric acid form superoxide free radicals. These radicals undergo dismutation to hydrogen peroxide. In addition highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen free radicals with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells (Szkudelski, 2001). The DNA of pancreatic islets is the target of the reactive oxygen species. Its fragmentation principally occurs in B cells exposed to alloxan (Takasu *et al.*, 1991). The most frequently used intravenous dose of alloxan in rats is 65 mg/ kg body weight, but when it is administered intraperitoneally or subcutaneously its effective dose must be higher (Frideriuk *et al.*, 2004). In all cases the experimental dose of alloxan must be strictly precised to avoid excessive pancreatic tissue damage leading to type I diabetes mellitus.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Collection of *Acacia nilotica subalata* leaves

Acacia nilotica subalata leaves were collected in February 2012, from Athi river area, near Daystar University, Mombasa road for identification by Botany Department, University of Nairobi.

3.2. Extraction

The *Acacia nilotica subalata* leaves were washed free of debris and dust particles and air dried at room temperature for three days. The leaves were ground using electrical grinder (Wiley Mill, model 2, Arthur H. Thomas company, Philadelphia, USA). The ground product obtained was mixed with 97% ethanol from Department of Chemistry, University of Nairobi, and soaked for two days. Thereafter it was filtered using cotton inserted in the filter funnel. The filtrate was concentrated using rotary evaporator (ROVA-2L, mrc).

3.3. Experimental animals

Thirty healthy male Wistar rats of about 6 to 8 months, weighing 200 - 350g, living in the same conditions were procured from the Department of Zoology, Kenyata University and the Department of Biochemistry, University of Nairobi. All rats with detectable abnormalities were excluded from this study. The rats were housed in the animal house of Department of Medical Physiology, University of Nairobi. Individual animals were marked and each group assigned its own cage. The animals were allowed to acclimatize to the laboratory conditions for one week. Standard laboratory conditions of room temperature 25 ± 2 ° C and a 12 h light- 12h dark cycle were maintained. The rats received standard rat feed (mice pencils supplied by Unga Farm care Ltd) and had unlimited access to water. Each morning the cages were cleaned. Basic data and weight of different groups of rats were taken. The weight was measured with the balance (Triple

beam balance, US. Pat. N° 2.729.439, Ohaus scale corporation, Florham Park, N.J. USA) at the beginning, thereafter every two weeks, until the end of experiment.

3.4. Induction of experimental type II Diabetes Mellitus and experimental design

This is a comparative experimental study. The animals were fasted for 16- 18 hours with free access to water before induction. Diabetes mellitus was induced in 18 out of 30 rats by administering 150 mg/kg body weight alloxan 4% weight/ volume (Yanarday and Colac, 1998) from the Department of Medical Physiology. Induction was confirmed by measuring fasting blood glucose level > 7 mmol/l after one week (WHO, 2006). A repeat dose of alloxan 100 mg/kg was injected to animals that were not diabetic to increase the sample size. The levels of total hemoglobin, cholesterol and protein were determined after 6 weeks of treatment with *Acaci*a. The rats were assigned into five groups as follows:

1. Group A normal control received normal saline
2. Group B diabetic control received normal saline
3. Group C diabetic rats received *Acacia nilotica subalata* extract.
4. Group D normal rats received *Acacia nilotica subalata* extract.
5. Group E diabetic rats received metformin.

Groups B, C, and E received intraperitoneally (150 mg/kg body weight) alloxan 4% w/v prepared with 20 ml of normal saline. The same volume of normal saline was administered to group A and D. Each morning the rats of group C, D received orally (800 mg/kg body weight) *acacia nilotica subalata* leaf extract dissolved in 30 ml of normal saline. Group E received orally (100 mg /kg body weight) metformin (M- FORLIN 500, LINCOLN Pharmaceuticals LTD, Gujarat, India) dissolved in 10ml of normal saline. The course of treatment in each group was 6 weeks.

Blood for glucose measurements was collected from the study rats by tail amputation using a tail snip. Once a week blood glucose was measured by glucometer (On Call Plus, ACON Laboratories Inc. 4108 Sorrento Valley Boulevard, San Diego, CA 92121, USA). This method

involves measuring the electrical current produced by chemical reactions between glucose and glucose dehydrogenase, Nicotinamide Adenine dinucleotide (NAD) and phenanthelin quinine present in compatible glucose strip.

By sacrificing rats at the end of the experiment, the animals were anesthetized with inhalation of diethyl ether 0.706g/l at 25% from Medical Physiology Department, University of Nairobi. Blood samples (2 ml) of each rat in all groups drawn by cardiac puncture and taken in ethylene - diamine-tetraacetic acid (EDTA) tubes were analyzed for lipids, total protein and hemoglobin in hematology and clinical chemistry units, University of Nairobi.

3.5. Total protein assay

Total protein was assayed by colorimetric methods using commercial kits (total protein liquicolor, Human, Max-Plank-Ring 21.65205 Wiesbaden. Germany) supplied by Chem Labs Ltd, using biuret reaction. Proteins form a purple coloured complex with cupric ions in alkaline solution. The intensity of purple color produced was measured at 545 nm with yellow/ green filter and compared with standard plasma of known protein concentration (Reinhold, 1953).

Table 3: Doses of reagents in appropriate tubes

	Blank	S1	S2	S3	Test
Sodium Chloride Diluents (ml)	2.5	2.45	2.4	2.35	2.4
Standard (ml)	-	0.05	0.1	0.15	-
Test Sample/ QC (ml)	-	-	-	-	0.1
Mix well					
Biuret reagent (ml)	3.0	3.0	3.0	3.0	3.0
Mix well					

Reagents involved in Biuret reaction were introduced in appropriately labeled tubes (standard S1, S2, and S3) and sample (test). The mixture was incubated at room temperature 25° C for 15 minutes. Then the spectrophotometer was set to 545 nm and standardized using blank. Total

plasma concentration was calculated automatically by the machine Humalyzer 2000 (Human, SI. 2500 – 3723, Germany) in this study.

The reference range for total plasma protein in rats is 5.6-7.6g/dl or 56 -76g/l (Sarah and Maggie, 1998). The level below the reference range reflects hypoproteinemia. Values higher than the reference range reflect hyperproteinemia.

3.6. Assay for total cholesterol

Total cholesterol was assayed by colorimetric method using commercial kits (Enzymax), made by Vitro Scient, in Egypt and locally supplied by In Vitro Diagnostics East Africa Ltd. This method involves enzymatic reactions: Cholesterol esterase hydrolyzes cholesterol esters to cholesterol and free fatty acids. Cholesterol oxidase oxidizes free cholesterol to cholest-4-en-3-one and H_2O_2, and finally in the presence of peroxidase the oxidative coupling of phenol and 4-aminoantipyrine with hydrogen peroxide (H_2O_2) form a red-colored quinoneimine dye (Alain et al., 1974). The intensity of the color produced is directly proportional to cholesterol concentration. It was determined by measuring the increase in absorbance at 545 nm by Humalyzer 2000 (Human, SI. 2500 -3723, Germany).

The procedure of this enzymatic colorimetric method with cholesterol esterase, cholesterol oxidase and 4-aminoantipyrine involved the following steps: One milliliter (1ml) of cholesterol reagent was introduced into each test tube labeled “blank”, “standard” and “specimen”. Then 10µl of cholesterol standard and specimen were drawn into respective tubes. The mixture was incubated for 5 minutes at 37° C. The spectrophotometer was set to 545 nm and standardized to zero with reagent blank. Total cholesterol concentration was calculated automatically by Hmalyzer 2000 (Human, SI. 2500 -3723, Germany).

Conversion factor: mg/dl x0.0259 = mmol/l. Plasma cholesterol level below 200 mg/dl is considered normal, between 200-239 mg/dl borderline high, and >240 mg/dl is considered high cholesterol (NCEPEP, 1987).

3.7. Assay for HDL

The HDL cholesterol was measured by enzymatic colorimetric test using cholesterol liquicolor test kit made by Human campany, in Germany and locally supplied by Chem Labs Ltd. The assay combines two specific steps: in the first step chylomicrons, VLDL and LDL cholesterol are specifically destroyed by cholesterol esterase and cholesterol oxidase in the specific conditions to form cholestenone and H_2O_2. In the presence of catalase H_2O_2 is converted into H_2O and oxygen. In the second step in the presence of specific surfactants, cholesterol esterase and cholesterol oxidase HDL is transformed into cholestenone and H_2O_2. The latter reacts with chromogen in the presence of peroxidase to form quinone pigment. The intensity of red color produced is proportional to HDL concentration. It was determined by measuring the increase of absorbance at wavelength range of 546 nm in our stusy by Humalyzer 2000 (Human, SI. 2500 -3723, Germany).

The procedure of this method involved the following steps: 10 µl of water were introduced into tube labeled reagent blank and 10 µl of calibrator (cholesterol/ sample into tube labeled cal/ sample. The mixture was incubated for 5 minutes at 37° C. Then 250 µl of substrate were added into each tube. The mixture was incubated at 37° C for 5 minutes. The spectrophotometer was set to 546 nm and standardized to zero with reagent blank. The HDL concentrations were calculated automatically by the machine Humalyzer 2000 (Human, SI. 2500 – 3723, Germany).

Conversion factor: concentration (mg/dl) x0.02586 = concentration (mmol/l). HDL levels below 35mg/dl (< 0.9mmol/l) are associated with coronary heart disease. HDL levels above 60mg/dl (> 1.54mmol/l) indicate reduced risk factor for coronary heart disease (NCEPEP, 1987).

3.8. Assay for LDL

The LDL levels were calculated by using the formula: LDL= total cholesterol – HDL– (triglyceride/5) (Fridewald et al., 1979).

In human beings LDL levels less than 100 mg/dl (2.6 mmol) are considered optimal. LDL levels between 130-159 mg/dl (3.36 – 4.13 mmol) are considered borderline high. LDL levels between 160- 189 mg/dl (4.14 – 4.90 mmol) are considered high. LDL levels at or above 190 mg/dl (4.91 mmol) are considered very high (NCEPEP, 1987).

3.9. Assay for triglycerides

The Triglycerides were measured by enzymatic colorimetric test using triglycerides test kit made in Egypt by Vitro Scient and locally supplied by In Vitro Diagnostics East Africa Ltd. The series of reactions involved in measurement of triglycerides is the following: triglycerides are hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol is then phosphorylated to glycerol-3- phosphate by ATP in a reaction catalysed by glycerol kinase. Glycerol phosphate oxidase catalyzes the oxidation of glycerol-3 phosphate to form dihydroacetone phosphate andhydrogen peroxide (H_2O_2). In the presence of peroxidase, the oxidative coupling of 4-chlorophenol and 4-aminoantipyrine with H_2O_2 forms a red-colored quinoneimine dye. The intensity of the color produced is proportional to triglycerides concentration. It was determined by measuring the increase in absorbance at 545 nm by Humalyzer 2000 (Human, SI. 2500 -3723, Germany) in this study.

Procedure of the measurement of triglycerides involved the following steps: One milliliter of triglycerides reagent was drawn into each tube labeled "blank", "standard" and "specimen". Then 10 μl of triglycerides standard and specimen were added into respective tubes. The mixture was incubated for 5 minutes at 37° C. Spectrophotometer was set to 545 nm and standardized to zero with reagent blank. The triglycerides were calculated automatically by measuring the increase in absorbance by Humalyzer 2000 (Human, SI. 2500 -3723).

Conversion factor: mg/dl x 0.0114 = mmol/l. In human beings Plasma triglycerides levels between 40 -160 mg/dl (0.45 – 1.85 mmol) are considered normal. Plasma triglycerides levels above 200 mg/dl (2.28 mmol) are considered elevated (NCEPEP, 1987).

3.10. Assay for hemoglobin

Plasma total hemoglobin was measured using cyanmethemoglobin technique. This method uses separate alkaline ferricyanide and cyanide reagents (Stadie, 1920). In alkaline medium, potassium ferricyanide oxidizes hemoglobin to methemoglobin. The subsequent reaction with potassium cyanide produces the more stable cyanmethemoglobin. The intensity of color produced is proportional to total hemoglobin concentration. It was determined by measuring the increase of absorbance at 540 nm by the machine (the CELL-DYN 1300 Series Hematology systems, Abbott Park, IL 60064. U.S.A).

The results are normal if the total hemoglobin is in the interval of 11g - 18 g/ dl or 110 -180g/l, and they are abnormal if the total hemoglobin is less than 11g or greater than 18 g/ dl (Sarah and Maggie, 1998)

3.11. Ethical consideration

In the present study, we put in consideration care and welfare of laboratory animals as an important factor in influencing the outcome in biomedical research and drug testing. The experiments and procedures presented in this study were performed in accordance with the guidelines for care and use of laboratory animals as prepared by the Federation of the European Laboratory Animal Science Association (FELASA), the European Society of Laboratory animal Veterinarians (ESLAV) and the European College of Laboratory Animal Medicine (ECLAM) (Voipio *et al.*, 2007). In addition, the standard operating procedures of Medical Physiology Department were applied.

3. 12. Data analysis

The control and analysis of data were analyzed on SPSS version 16. The presentation of results of this study was done by tables, and graphs. The data were expressed as mean ± S. E.M (Standard Error of Mean) and statistically analyzed using Analysis of Variance (ANOVA) with multiple comparisons versus control groups by Tukey's method. Results were considered as statistically significant if p value < 0.05.

3.13. Complications

During the course of this study, the overall mortality was evaluated at 13%. Four rats out of 30 died, two rats of them died in three days after induction of diabetes. This may be due to type I diabetes mellitus induced by alloxan in those rats instead of type 2 diabetes mellitus. One rat died due to inhalation syndrome during administration of drug and the last case died due to respiratory infection. All results of died cases were excluded from this study.

CHAPTER FOUR

RESULTS

4.1. Fasting blood glucose profile

The fasting blood glucose levels in diabetic groups B, C and E before treatment (day 0) were significantly high compared to the normal control group A (15.54± 0.580, 16.28 ± 3.321 and 14.93 ± 2.31 vs 4.47 ± 0.114 mmol/l), $p < 0.05$ respectively. Treatment with *A. n. subalata* extract significantly decreased the blood glucose levels in diabetic group C compared to diabetic control group B (7.08 ± 1.451 vs 18.10 ± 1.378 mmol/l, $p < 0.05$). The blood glucose reduction was not statistically different between groups treated with *A. n. subalata* extract (group C) and metformin (group E) (7.08 ± 1.451 vs 6.50 ± 1. 10 mmol/l, $p = 0.992$). When the comparison was done between normal group D treated with *A. n. subalata* extract and normal control group A there was no significant difference in fasting blood glucose levels (4.52 ± 0.188 vs 4.53 ± 0.185 mmol/l, $p = 1$). The blood glucose levels between groups are shown in table 4.

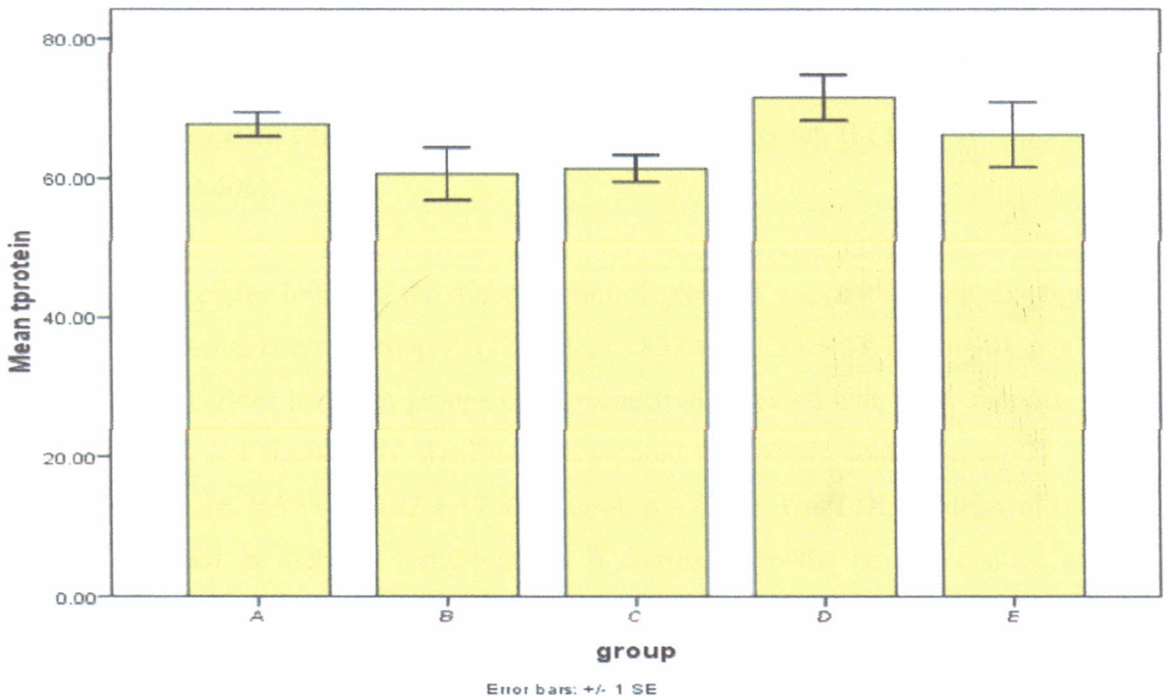
Table 4: Effects of *A. n. subalata* extract on fasting blood glucose (mmol/l) of normal and diabetic rats

Groups	Day0	Day14	Day28	Day35	Day42
	Mean± SEM	Mean ±SEM	Mean ±SEM	Mean± SEM	Mean± SEM
A	4.47 ± 0.11	4.33 ± 0.16	4.53 ± 0.20	4.37 ± 0.14	4.53 ± 0.18
D	4.48 ± 0.24	4.50 ± 0.26	4.48 ± 0.08	4.66 ± 0.43	4.52 ± 0.18
B	15.54 ± 0.58 **ab**	17.27±1.33	18.48 ± 1.69	18.76 ± 1.37	18.10 ± 1.37
C	16.28 ± 3.32 **ac**	9.32 ± 0.27 **bc**	8.22 ± 2.17 **bc**	7.96 ± 1.42 **bc**	7.08 ± 1.45 **bc**
E	14.93 ± 2.31 **ae**	7.68 ± 1.78 **be**	6.38 ± 0.78 **be**	6.32 ± 1.06 **be**	6.50 ± 1.10 **be**

Group A: normal control, Group B: diabetic control, Group C: diabetic treated with plant Extract, Group D: normal group treated with plant Extract, Group E: diabetic rats treated with metformin., **ab**: $p < 0.05$ group B as compared to group A. **ac** : $p < 0.05$ group C as compared to group A. **ae**: $p < 0.05$ group E as compared to group A. **bc**: $p < 0.05$ group C as compared to group B. **be** : $p < 0.05$ group E as compared to group B.

4.2. Total plasma protein levels

Figure 10 shows the comparison of total plasma protein levels in the experimental rats. There was no statistically significant decline of plasma protein levels ($p > 0.05$) in diabetic groups (B, C, E) with 60.62g ± 3.774/l, 61.40g ±4.297/l, 66g±10.398/l compared to normal control group A with 67.7g ± 1.718 (mean ± SEM). Administration of *A. n. subalata* extract or metformin showed no difference in plasma total protein levels between control diabetic group B and diabetic groups treated with those products (61.40 ± 1.92 and 66.24 ± 4.65 vs 60.62 ± 1.71 g/l, $p > 0.05$). Treatment with *A. n. subalata* extract slightly increased total plasma protein levels in normal group D compared to normal control group A. Total plasma protein levels of group E treated with metformin were almost comparable to those of normal control group A though they were slightly decreased (66.24 ± 4.65 vs 67.70 ± 1.71g/l). Total plasma protein levels of normal rats treated with extract group D were slightly greater than those of untreated normal control group A (71.52 ± 3.24 vs 67.70 ± 1.71 g/l).



A: Normal control, B: Diabetic control, C: diabetic treated with plant extract, D: Normal group treated with plant extract, E: Diabetic group treated with metformin

Figure 8: Effects of *A. n. subalata* extract on total plasma proteins (g/l) of normal and diabetic rats

4.3. Plasma lipid profile

Table 5 shows the comparison of plasma lipid profile levels in the experimental rats. There was a statistically significant elevation of total plasma cholesterol in diabetic control group B compared to normal control group A (153.89 ± 18.829 vs 98.70 ± 2.643 mg/dl, $p < 0.05$). The administration of *A. n. subalata* extract and metformin statistically decreased total plasma cholesterol as shown in groups C and E compared to diabetic control group B (109.09 ± 9.131 and 90.69 ± 6.838 vs 153.89 ± 18.829 mg/dl, $p < 0.05$) respectively. There was no statistically significant difference between diabetic group C treated with *A. n. subalata* and group E treated with metformin ($p = 0.738$). When the comparison was done between normal group D treated with *A. n. subalata* extract and normal control group A there was no statistical difference in total plasma cholesterol levels (100.05 ± 9.930 vs 98.70 ± 2.643 mg/dl, $p = 1$).

Diabetic control group B had a statistically significant decrease of HDL cholesterol compared to the normal control group A (23.47 ± 2.645 vs 36.08 ± 2.343 mg/dl, $p < 0.05$). A statistically significant elevation of HDL cholesterol was shown in group E treated with metformin compared to diabetic control group B (34.66 ± 4.004 vs 23.47 ± 2.645 mg/dl, $p < 0.05$), but not in group C treated with *A. n. subalata* extract compared to diabetic control group B (30.05 ± 1.976 vs 23.47 ± 2.645 mg/dl, $p = 0.408$).

Elevation of triglycerides levels in the diabetic control group B was not statistically significant compared to the normal control group A (128.02 ±17.837 vs 97.39 ± 17.793 mg/dl, $p = 0.854$). Reduction of triglycerides levels in groups C, E, respectively treated with *A. n. subalata* extract and metformin was not statistically significant compared to diabetic control group B (96.10 ± 35.764 and 74.59 ± 16.765 vs 128.02 ± 17.837 mg/dl, $p > 0.05$). The LDL cholesterol level was statistically increased in diabetic control group B compared to the normal control group A (105.56 ± 15.14 vs 45.15 ± 4.198 mg/dl, $p < 0.05$). Treatment with either *A. n. subalata* extract or metformin in groups C and E, significantly decreased LDL cholesterol levels respectively compared to diabetic control group B (59.62 ± 6.532 and 42.32 ± 4.844 vs 105.56 ± 15.14 mg/dl, $p < 0.05$). There was no statistically significant difference in reduction of LDL cholesterol between diabetic group C treated with *A. n. subalata* extract compared to diabetic group E treated with metformin (59.62 ± 6.532 vs 42.32 ± 4.844 mg/dl, $p = 0.791$). There was no

statistical difference of LDL cholesterol levels between normal group D treated with *A. n. subalata* extract and normal control group A (55.79 ± 17.264 vs 43.15 ± 4.198 mg/dl, p = 0.909).

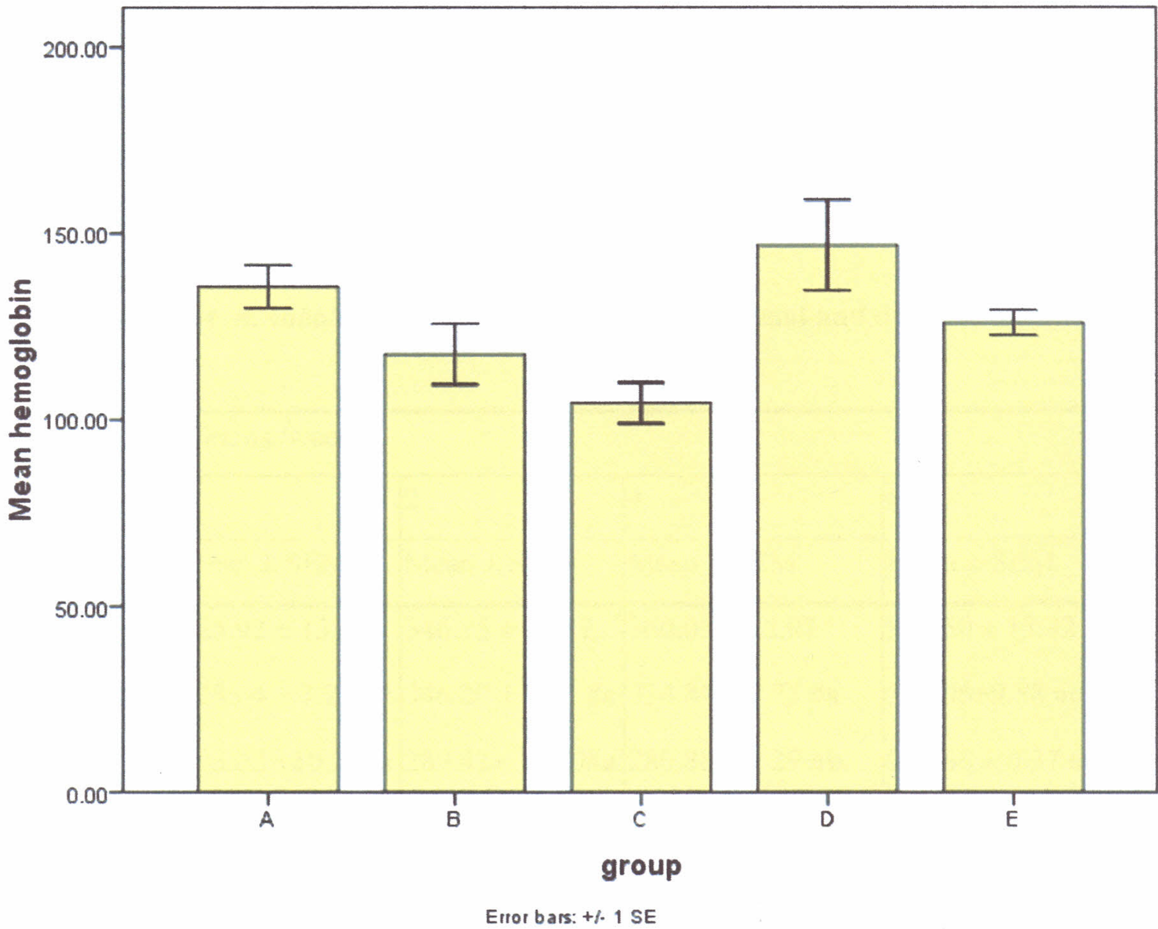
Table 5: Effects of *A. n. subalata* extract on plasma lipid profile (mg/dl) of normal and diabetic rats

	Total cholesterol	HDL	Triglycerides	LDL
	Mean±SEM	Mean± SEM	Mean± SEM	Mean± SEM
group A	98.70 ± 2.64	36.08 ± 2.34	97.39 ± 17.79	43.15 ± 4.19
D	100.05 ± 9.93	38.17 ± 0.69	70.12±18.70	55.79 ± 17.26
B	153.89 ± 18.82 **ab**	23.47 ± 2.64 **ab**	128.02 ± 17.83	105.56± 15.14 **ab**
C	109.09 ± 9.13 **bc**	30.05 ± 1.97	96.10 ± 35.76	59.62 ± 6.53 **bc**
E	90.69 ± 6.83 **be**	34.66 ± 4.00 **be**	74.59±16.76	42.32 ± 4.84 **be**

A: Normal control, B: Diabetic control, C: diabetic treated with plant extract, D: Normal group treated with plant extract, E: Diabetic group treated with metformin. ., **ab**: p < 0.05 group B as compared to group A**.** **bc**: p < 0.05 group C as compared to group B. **be** : p < 0.05 group E as compared to group B.

4.4. Hemoglobin levels

Figure 11 shows the comparison of hemoglobin levels in the experimental rats. From the results, it is evident that there was decline in total hemoglobin across diabetic groups. Total hemoglobin level reduction was significant in group C treated with *A. n. subalata* extract compared to the normal control group A (104.40 ± 5.50 vs 135.67 ± 5.76 g/l, p < 0.05). When the comparison was done between normal group D treated with *A. n. subalata* extract and normal control group A there was no significant difference in total hemoglobin levels (146.80 ± 12.18 vs 135.67 ± 5.76 g/l, p = 0.817). Metformin had a favorable effect on total hemoglobin levels in group E compared to *A. n. subalata* extract in group C (126.60 ± 3.39 vs 104.40 ± 5.50 g/l).



A: Normal control, B: Diabetic control, C: diabetic treated with plant extract, D: Normal group treated with plant extract, E: Diabetic group treated with metformin.

Figure 9: Effects of *A. n. subalata* extract on total hemoglobin levels (g/l) of normal and diabetic rats

4.5. Changes in the body mass of diabetic and normal rats

The weight changes between control and experimental groups are shown in table 6. At day 0 and 14 in all groups compared to the normal control group A there was no statistically significant difference between body weights (($p > 0.05$). At day 28 and 42 there was statistical difference in reduction of weight between diabetic groups B, C and E compared to normal control group A ($p < 0.05$). There was no statistical difference between C and E compared to diabetic group B (263.76 ± 12.71 and 258.58 ± 6.41 vs 273.48 ± 6.37 g, $p > 0.05$). However, when the comparison was done between normal group D treated with *A. n. subalata* extract and normal control group

A there was significant difference in weight (324.06 ± 9.58 vs 372.50 ± 13.32 g, $p < 0.05$). The control group A increased weight, while the normal group D decreased it. Weight loss in term of percentage: diabetic control group B lost 6.6 % of their initial body mass. Diabetic group C (treated with A. n. subalata extract) and E (treated with metformin) respectively lost 9. 54 % and 11.29%. Normal group D treated with A. n. subalata lost 0.30% of its initial body mass.

Table 6: Effects of *A. n. subalata* extract on body mass of normal and diabetic rats

	Grams /weeks			
	0	2	4	6
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
weight Group A	325.92 ± 13.62	346.25 ± 12.37	360.02 ± 12.97	372.50 ± 13.32
D	328.64 ± 7.22 #**a**	346.20 ± 7.53 #**a**	334.84 ± 9.75 #**a**	324.06±9.58 **ad**
B	293.02 ±10.22#**a**	289.42± 17.70#**a**	280.88 ± 7.29 **ab**	273.48 ± 6.37 **ab**
C	291.60±17.75 #**a**	282.92 ±11.21#**a**	269.70 ± 12.39 **ac**	263.76 ±12.71**ac (#b)**
E	291.50 ± 7.04 #**a**	280.10 ±7.18 #**a**	267.24 ± 6.74 **ae**	258.58 ± 6.41 **ae(#b)**

A: Normal control, B: Diabetic control, C: diabetic treated with plant extract, D: Normal group treated with plant extract, E: Diabetic group treated with metformin, . **#a:** $p > 0.05$ as compared to group A. **#b:** $p > 0.05$ as compared to group B. **ab**: $p < 0.05$ group B as compared to group A. **ac** : $p < 0.05$ group C as compared to group A. **ae**: $p < 0.05$ group E as compared to group A. **ad** $p< 0.05$ group D compared to group A.

CHAPTER FIVE

DISCUSSION

5.1. Introduction

Alloxan monohydrate is commonly used to induce type 2 diabetes mellitus by selective partial destruction of β cells of the pancreas (Cakici *et al.*, 1994). This reduction of β cells decreases insulin levels and results in hyperglycemia leading to type 2 diabetes mellitus.

5.2. Study findings and mechanisms

5.2.1. Fasting blood glucose

In this study *Acacia nilotica subalata* leaf extract had hypoglycemic effect in diabetic rats. Our results are comparable with those of Maqsood *et al.* (2008) who reported *Acacia nilotica nilotica* extract decreased blood glucose levels in diabetic rabbits. The results are also in corroboration with those of Liu et al. (2005) who reported *Acacia nilotica nilotica* to have hypoglycemic effect in diabetic animals. The blood glucose-lowering effect of *Acacia nilotica subalata* extract possibly occurs by stimulating the β cells of the pancreas and/or due to its insulin-like activity. This suggests that *Acacia nilotica subalata* leaf extract at this dose of 800 mg/kg body weight, may induce β cell proliferation to compensate the lost cells. The exact mechanism remains unclear. The hypoglycemic effect of *Acacia nilotica subalata* could reside in the polyphenols found in the plant extract (Martin, 1999). Polyphenols are known to have antioxidant activity (Rice et al., 1995). This could stop the damage of the remaining β cells of the pancreas by removal of circulating reactive oxygen species generated by alloxan. In addition the polyphenols may help regenerate activity of β cells of the pancreas. It has further been reported that polyphenols and tannins have hypoglycemic effect by their inhibitory interactions with α-amylase and α-glucosidase in the gut (Nwosu *et al.*, 2011) hence reducing the amount of absorbable glucose in the small intestine.

However, the euglycemia found in normal group D compared to normal control A may be due to the normal homeostasis of glucose in normal rats which acts through negative feedback systems to maintain blood glucose within the normal range of 70 to 110 mg of glucose per deciliter of

blood. If blood glucose levels fall below normal, insulin secretion is inhibited and the cells of the pancreas respond by secreting glucagon, a hormone that accelerates the breakdown of glycogen to glucose in the liver. It stimulates liver cells to increase glucose synthesis from glycerol and enhances glucose release into the blood. These effects cause an increase in blood glucose levels back to normal levels. In addition, other hormones such epinephrine, cortisol and growth hormones can assist to increase blood glucose levels (Guyton and Hall, 2006). The reduction of blood glucose in group E that received metformin results from the mechanism of action of metformin which acts by suppressing glucose production by the liver (Zhoo *et al*., 2001). In addition to suppressing gluconeogenesis, metformin increases insulin sensitivity, enhances peripheral glucose uptake and reduces glucose absorption from gastrointestinal tract (Collier *et al*., 2006).

5.2.2. Total plasma protein

The present study showed a decline of total plasma protein in diabetic groups B, C, E compared to the normal control group A. This reduction is due to increase in protein breakdown which is greater than the magnitude of the increase of protein synthesis during insulin deprivation found in diabetes mellitus (Charlton *et al*., 1998). The results of our study in diabetic group C differ from the results of Zaki *et al*. in 2000 who reported a significant decrease in the level of total protein. The difference in their results from ours may be due to different subspecies and doses. It seems clear that at the dose of 800mg/kg body weight *Acacia nilotica subalata* extract has no toxic effect on total plasma protein.

5.2.3. Plasma lipid profile

In the present study the levels of LDL, triglycerides and total cholesterol were elevated in diabetic group B whereas plasma HDL level is decreased. These results are similar to the results of Alarcon-Aguilar *et al*. (2002) in diabetic rats and mice. The higher lipid levels found in diabetic rats was due to increased mobilization of free fatty acids from peripheral deposits and also to lipolysis caused by hormones. In addition our study showed a significant reduction of total cholesterol, LDL in diabetic groups C and E, and a significant increase of HDL levels in group E treated with metformin, but the reduction of triglycerides levels is not significant. A number of other plant extracts have been reported to have hypoglycemic and hypolipidemic and insulin stimulatory effects (Fernandes *et al*., 2007). In this regard the *Acacia nilotics subalata*

extract possibly causes regeneration of β cells of the pancreas that yields to increase of insulin secretion from the surviving β cells. The increase in insulin secretion consequently decreases blood glucose level which may lead to inhibition of lipid peroxidation and control of lipolytic hormones. The beneficial effects of *Acacia nilotica subalata* on lipid profile in induced diabetic rats may be secondary to better glycemic control. Metformin causes beneficial effects on lipid profile by correcting abnormal glucose metabolism (Defronzo *et al*., 1995). It also moderately decreases triglycerides levels as a result of decreased hepatic synthesis of very-low-density lipoprotein (Chehade and Mooradian, 2000). Metformin is more beneficial in preventing cardiovascular complications associated with T2DM than *A. n. subalata* extract because it increased HDL, decreased LDL and triglycerides (Bolen *et al*., 2007).

5.2.4. Hemoglobin levels

The present study showed a decrease in total hemoglobin of diabetic rats compared to normal rats. The results of our study are similar to the results of Muhammad *et al*. in 2011. There was a significant decline in total hemoglobin in diabetic group C treated with *Acacia nilotica subalata* extract compared to normal group A. Decline of total hemoglobin in diabetic group C treated with *Acacia nilotica subalata* may be a result of non-enzymatic glycation of hemoglobin commonly found in diabetic rats (Muhammad *et al*., 2011), and the reduction of iron absorption through the gastrointestinal lumen. It has been reported that tannins and polyphenols found in plants inhibit absorption of minerals such as iron, because tannins are metal ion chelators (Bruce *et al*., 1989).

5.2.5. Changes of rat body mass

In our study, alloxan-induced diabetic rats showed a significant loss of weight compared to normal control. This observation is similar to that done by American Animal Hospital Association (AAHA) on diabetic dogs and cats (AAHA, 2010). When the normal rats and diabetic rats were treated with either *Acacia nilotica subalata* extract or metformin, the reduction of weight was noticeable compared to normal and diabetic control rats. The exact mechanism of action of *Acacia nilotica subalata* on weight loss is not known, but the effect of *Acacia nilotica subalata* extract on weight loss seems to be linked to polyphenol fraction found in the plant extract (Martin, 1999). It has been reported that polyphenols reduce glucose absorption through the small intestine by inhibiting the action of α-amylase and α –glucosidase (Nwosu *et al*., 2011).

It has also been reported also that the polyphenols have shown an inhibitory effect on adipose tissue formation in wistar rats (Koichi *et al.*, 2006). In this context the weight loss may be the results of those combined effects. Several mechanisms of metformin for weight loss effects have been evaluated. These include reduction in gastrointestinal absorption of carbohydrates, and insulin resistance, induction of an anorectic and lipolytic effect, and decreased level of leptin (Gluck *et al.*, 2001), a hormone integral to body weight regulation. Both *A. n. subalata* extract and metformin are helpful in weight loss in T2DM.

5.2.6. Study limitations

To induce type 2 diabetes mellitus, alloxan was administered to experimental animals. Alloxan does this by destroying insulin secreting islet cells. The number of cells destroyed depends on the doses of alloxan administered and the sensitivity of the animal to alloxan. In humans Pathophysiology of diabetes is a sequence of events starting with exposure to risk factors which lead to insulin resistance and deficiency. Therefore, use of chemicals to induce T2DM in rats does not act in the same way as in real situation in humans. In the present study the number of rats and duration of follow up are not enough to compare the process of events with that occurred in humans. Thus, use of *Acacia nilotica subalata* leaf extract as hypoglycemic agent on human beings must be taken carefully because treatment takes a long period and side effects are dose and time-dependent. Its use in humans requires other studies done with big samples, in long period and analysis of other parameters to investigate potential side effects.

5.2.7. Conclusion

In conclusion, the present study showed that ethanolic leaf extract of *A. n. subalata* produced antihyperglycemic effect in alloxan-induced diabetic rats. At the dose of 800mg/kg, it reduced significantly total cholesterol, LDL cholesterol, weight and total hemoglobin, but the increase of HDL cholesterol is not statistically significant. However there were no significant changes of triglycerides and total plasma protein. Consequently *Acacia nilotica subalata* leaf extract is a herbal product with good promise and has the potential to lower blood glucose and hyperlipidemia in type 2 diabetes mellitus. This study investigated parameters which are common in diabetes. Since the use of plant extract may change other plasma parameters even damage some internal organs. Further studies are required to investigate the detailed mechanism of action of *Acacia nilotica subalata* leaf extract and its effects on other metabolites.

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