INVESTIGATION OF THE *IN VITRO* ANTIOXIDANT ACTIVITY, *IN VIVO* ANTIDIABETIC EFFICACY AND SAFETY OF *CAPPARIS TOMENTOSA* ROOTS AQUEOUS EXTRACTS

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Investigation of the *in vitro* antioxidant activity, *in vivo* antidiabetic efficacy and safety of *Capparis tomentosa* roots aqueous extracts

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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ABBREVIATIONS AND ACRONYMS

AAE	Ascorbic – acid equivalent
AGEs	Advanced glycation end products
ALP	Alkaline phosphatase
ALT/GPT	Alanine aminotransferase/ glutamic pyruvic transaminase
ANOVA	Analysis of variance
AST/GOT	Aspartate aminotransferase/glutamic – oxaloacetic transaminase
ATP	Adenosine triphosphate
BG	Biguanides
CD4 count	Cluster differential count
CO_2	Carbon IV oxide
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DPPH	1, 1 –dipheny – 2 – picrylhydrazyl
eNOS	Endothelial nitric oxide synthase
GAPDH	Glycraldehyde – 3 – phosphate dehydrogenase
HbA1c	Glycated haemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte asntigen

IDF	International Diabetes Federation
iNOS	Inducible nitric oxide synthase
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
NAD	β-nicotinamide adenine dinucleotide
NIDDM	Non insulin dependent diabetes mellitus
NO	Nitric oxide
OHD	Oral hypoglycaemic drugs
PKC	Protein kinase C
PVPP	Polyvinyl polypyrrolidine
ROS	Reactive oxygen species
SPSS	Statistical package for social scientist
SU	Sulphonylureas
TAE	Tannic acid equivalent
USD	United states dollar
UV/vis	Ultraviolet/visible
WHO	World Health Organization

ABSTRACT

Capparis tomentosa has been used traditionally to manage several diseases including diabetes, however, its efficacy and safety is not well evaluated. The aim of this study was to determine the *in vitro* antioxidant activity, *in vivo* antidiabetic efficacy and safety of the aqueous root extract of *C. tomentosa*. The *in vitro* antioxidant activity was assessed using 1,1 – dipheny – 2 – picrylhydrazyl method,phosphomolybdate assay and by total reducing power assay. The *in vivo* antidiabetic efficacy was performed in alloxan – induced diabetic male Balb/C mice using oral route of administration of the plant extract and reference drug (glibenclamide). The safety of the extract was studied in mice that were grouped into two; one group orally administered with 1g/kg body weight of plant extract daily while the second group orally administered with 0.1ml physiological saline daily for 28 days and changes in body weight recorded weekly. Comparison in organ weights and biochemical parameters were also studied. Phytochemical screening of the aqueous root extract was also done using standard procedures. *C. tomentosa* aqueous root extracts displayed antioxidant activity. Antioxidant activity by 1,1 – dipheny – 2 – picrylhydrazyl was 35.50 ± 0.02%, phosphomolybdate assay was 41.22± 0.17mg/kg ascorbic acid equivalent and the total reducing power increased with increase in extract concentration up to a maximum of 800µg/ml. The extract showed hypoglycemic activity at dose levels of 50,100 and 200mg/kg body weight. Administration of 1g/kg body weight of the extract decreased body weight gain in Balb/C mice and also altered organ weights of the mice, such as reduction in kidney, liver and increase in size of spleen. *C. tomentosa* at 1g/kg body weight also caused increased levels of Alkaline phosphatase and Aspartate aminotransferase/Glutamic – oxaloacetic transaminase and decreased levels of creatinine and Alanine aminotransferase. The extracts contained alkaloids, tannins, flavonoids, terpenoids and saponins. The observed antioxidant activity, hypoglycemic activity and slight toxicity could be associated with the phytochemicals present in this plant extract.

CHAPTER ONE

INTRODUCTION

1.1 Background

Diabetes is a chronic physiological metabolic disorder that is characterised by elevated blood glucose levels resulting from insulin secretion, action or both (WHO, 1999) Insulin is a hormone produced by the beta cells within the islets. The insulin after production is released into the blood to facilitate glucose absorption by the cells from the blood when blood glucose levels are elevated above normal (Bastaki, 2005). In a case where beta cells do not produce sufficient insulin hormone or the body fails to respond to the insulin produced this would result in the accumulation of glucose in the blood above normal and lack of or insufficient glucose uptake by cells of the body leading to pre – diabetes or diabetes. Pre – diabetes refers to blood glucose levels above normal range but are not high enough for the diagnosis of type 2 diabetes (Smallwood, 2009). The normal fasting blood glucose levels range between 70 mg/dl – 100 mg/dl, thus blood glucose level below 70 mg/dl indicates low blood sugar “hypoglycemia" while blood glucose level above 200 mg/dl indicates "hyperglycemia". Fasting blood glucose higher than 100 mg/dl and less than 126mg/dl (7.0mmol/l) would indicate pre-diabetes or diabetes (Expert Committee on the Diagnosis and Classification of Diabetes, 1997).

Diabetes mellitus posses as a major health problem, affecting about 5% of the total population in the U.S. and 3% of the world population. Epidemiological studies (Liu *et al*., 1993) and clinical trials (Abraira *et al*., 1995), strongly support the notion that hyperglycemia is the principal cause of complications. In Sub – Saharan Africa, type 2 diabetes accounts for over 90% of diabetes while type 1, gestational diabetes and variant forms such as malnutrition – related diabetes constitute the remainder (Levitt, 2008). The prevalence of type 2 diabetes recorded in a survey in Kenya ranged from 2% in rural areas to 12% in urban areas (Christensens *et al*., 2009). Kenya has a population of about 40 million people. Half of the population is comprised of adults aged between 20 and 79 years (Mwenda, 2012). The prevalence rate of diabetes in this age group is 4.66% (720,730 cases). In 2013, 20,350 Kenyans died of diabetes

related causes and 562,570 remained undiagnosed (International Diabetes Federation, 2013).

Medicinal plant products can be used together with prescribed medication in the management of many diseases such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and cancer, among others (Hasan *et al.,* 2009). Some of the documented plants with antidiabetic activity include *Aegle marmelos (L) Correa* which have an alkaloidal-amide that posseses antihyperglycemic activity and *Agrimonia pilosa Ledeb* which has been demonstrated experimentally to effectively lower blood glucose in normal and alloxan-induced diabetic mice (Abu-Zaiton, 2010). Several studies have also been done in Kenya using various plants to determine their hypoglycemic effects. A research done to determine the hypoglycemic activity of Some Kenyan plants used traditionally to manage *Diabetes mellitus* in Eastern Province revealed that these plants namely *Bidens pilosa L., Erythrina abyssinica DC., Catha edulis Forsk., Aspilia pluriseta Schweinf.,* and *Strychnos henningsii Gilg.,* are effective and safe as antidiabetic medicines and further emphasize the large potential of traditional plants in management of diabetes (Ngugi *et al.,* 2011).

In Kitale, Kenya, the roots of *C. tomentosa* are boiled in water and taken as medicinal herb for management of various conditions such as diabetes mellitus, goiter, high blood pressure, boosting of CD4 count for HIV+ patients (Wandeto, 2013). In spite of the medicinal uses of *C. tomentosa* there is little information on its phytochemical profile, antioxidant potential, efficacy and safety when used for medicinal purposes. This study is aimed at evaluating the phytochemical composition, antioxidant activity, antidiabetic activity and safety of *C. tomentosa* roots, to ascertain the claim that it is a potential herb capable of managing diabetes mellitus.

1.2 Problem statement

Kenya has a population of about 40 million people where half of the population is comprised of adults aged between 20 and 79 years (Mwenda, 2012). The prevalence rate of diabetes in this age group which is a productive group is 4.66%. In 2013, 20,350 Kenyans died of diabetes related causes and 562,570 remained undiagnosed (International Diabetes Federation, 2013). The management of diabetes mellitus is therefore a great concern for the development of the nation. The management of this disease using prescription medication is expensive and may lead to increased toxicity and/or long periods of hospitalization all of which are unaffordable to the poor people and as a result some opt for herbal remedies. However, there is no sufficient preclinical data on antioxidant activity, efficacy and safety of *C. tomentosa* roots in the management of diabetes mellitus. Thus this study is expected to provide information on analysis of phytochemicals, *in vitro* antioxidant activity, *in vivo* efficacy and safety of *C. tomentosa* roots as a medicinal herb.

1.3 Justification

Diabetes mellitus is a metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by pancreas, or by the ineffectiveness of the insulin produced. This disease can be considered a major cause of high economic loss which can in turn hinder national development. When left uncontrolled, diabetes leads to many chronic complications such as renal failure, heart failure, blindness and even premature deaths. In an effort to prevent this alarming health problem, the development of research into new hypoglycemic and potentially antidiabetic agents is of great interest. There are several known antidiabetic medicines in pharmaceutical markets such as metformin and sulfonylureas based drugs which play a critical role in management of diabetes mellitus.However these conventional drugs may lead to adverse side effects such as lactic acidosis in the elderly, hypoglycemia, anorexia and gastro intestinal tract side effects such as bloating. Moreover,these drugs are expensive targeting the affluent while the poor in the society are not able to afford and thus opt for cheap herbal remedies. Therefore, screening for new diabetic sources from natural plants is still attractive as natural plants contain substances that

have an alternative and safe effect on diabetes mellitus management. The aqueous roots of *C. tomentosa* are being used by the local community in Kitale county in management of diabetes mellitus (Wandeto, 2013) thus there arises a need to investigate the phytochemical composition of *C. tomentosa* roots. In addition there is also a need to determine the antioxidant activity, the efficacy and safety of the aqueous root extract of this plant which is already being used in management of diabetes mellitus by traditional herbalists.

1.4 Research Questions

The following research questions guided the study:

1. What are the phytochemicals present in *C.tomentosa* roots?
2. What is the *in vitro* antioxidant activity of aqueous root extract of *C. tomentosa?*
3. What is the antidiabetic activity of aqueous root extracts of *C. tomentosa* in Balb/C mice?
4. What is the *in vivo* safety of aqueous root extracts of *C. tomentosa?*

1.5 Hypothesis

H_O *C. tomentosa* root extracts have no antioxidant and antidiabetic activity and may not be safe to humans.

1.6 Objectives

1.6.1 General objective

To investigate the phytochemicals, antioxidant activity and *in vivo* antidiabetic efficacy and safety of *C. tomentosa* roots aqueous extracts.

1.6.2 Specific objectives

1. To determine qualitative phytochemical composition of *C. tomentosa* root extracts.
2. To determine *in vitro* antioxidant activity of aqueous root extract of *C. tomentosa*.
3. To determine antidiabetic activity of aqueous root extracts of *C. tomentosa* in Balb/C mice.
4. To determine *in vivo* safety of aqueous root extracts of *C. tomentosa.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Types of diabetes, aetiology and risk factors

According to WHO(2016), there are two main types of diabetes namely type 1 diabetes and type 2 diabetes. Gestational diabetes is also a type of diabetes but develops only during pregnancy. Type 1 diabetes commonly occurs in children and young adults. It is caused by lack of insulin which can be due to beta cell destruction that often leads to complete insulin deficiency. This can result from a cellular mediated autoimmune destruction of the beta cells of the pancreas (American diabetes association, 2012). Genetic susceptibility can also cause type 1 diabetes as genes are passed down from one generation to the next. Genes often carry instructions required for making proteins needed by cells of the body in order to perform its functions. Some gene variants carry human leukocyte antigen (HLA) that are linked to developing type 1 diabetes. The proteins produced by HLA combinations often determine whether the immune system can recognize body cells as part of itself or as foreign material(American diabetes association, 2012).

Type 2 diabetes typically occurs in adults and its causes range from mainly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance. Environmental factors such as food, virus and toxins may play a role as contributing factors to developing diabetes, though their exact mode is not well established. Other causes include genetic defects in insulin action, endocrinopathies whereby several hormones such as growth hormone, glucagon antagonize insulin action and also drug or chemical induced diabetes (American diabetes association, 2012).

2.2 Diagnosis of Diabetes

According to WHO 2016, Diabetes is diagnosed by measuring glucose in a blood sample taken while the patient is in a fasting state, or 2 hours after a 75 g oral load of glucose has been taken. Diabetes can also be diagnosed by measuring glycated

haemoglobin (HbA1c), even if the patient is not in a fasting state. HbA1c reflects the average blood glucose concentration over the past few weeks, rather than the blood glucose concentration at that moment (reflected fasting and 2-hour blood glucose measurements mentioned above). However, the test is more costly than blood glucose measurement. Blood glucose measurements showing Fasting plasma glucose $\geq$ 7.0mmol/L or 2-hour plasma glucose $\geq$ 11.1mmol/L or HbA1c $\geq$ 6.5% are indicative of presence of diabetes in a patient.

2.3 Classification of Type of Diabetes

Classification of the type of diabetes can then be determined after diagnosis is confirmed. Type 1 diabetes (insulin dependent diabetes mellitus) presents with symptoms that prompt a patient to contact health services. These symptoms include rapid weight loss, copious urination, thirst, constant hunger, vision changes and fatigue. Type 2 diabetes (non – insulin dependent diabetes mellitus) is characterized by presence of obesity, absence of classical symptoms of diabetes with an onset at 30 years and above. Type 2 diabetes develops slowly showing no symptoms over a long period of time thus most patients would go to a health service center due to a complication such as loss of vision, heart attack or limb gangrene.

2.4 Treatment of Diabetes

Effective blood glucose control is the critical intervention measure in management of diabetic complications and improving quality of life in patients with diabetes (DeFronzo, 1999). Thus, sustained reductions in hyperglycemia will lower the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications (Gaster & Hirsch, 1998).

Treatment of diabetes can be grouped into three forms; prescribed diet, oral hypoglycemic therapy and insulin treatment. Patients with Type 1 diabetes require daily administration of insulin to regulate the amount of glucose in their blood, in order to live (WHO, 2016). For Type 2 diabetes, diet can be combined with exercise with an aim of ensuring weight control and providing nutritional requirements to the diabetic. Oral hypoglycemic drugs (OHDs) are given when diet and exercises have

not achieved the target. Two major OHDs are sulphonylureas (SUs) and biguanides (BGs). The SUs work by stimulating insulin release from the beta cells and also by promoting its action through extrapancreatic mechanisms (WHO, 1994).

2.4.1 Mode of Action of Sulfonylureas.

a) Pancreatic Mechanism:

All sulfonylurea hypoglycemics inhibit the efflux of K^+ (K^+channel blockers) from pancreatic ß-cells via a sulfonylurea receptor which is closely linked to an ATP-sensitive K^+channel. The inhibition of efflux of K_+ leads to depolarization of the ß cell membrane and, as a consequence, voltage-dependent Ca^{++}channels on the ß-cell membrane open to permit entry ofCa^{++}. The resultant increased binding of Ca^{++} to calmodulin results in activation of kinases associated with endocrine secretory granules thereby promoting the exocytosis of insulin-containing secretory granules (DeRuiter, 2003).

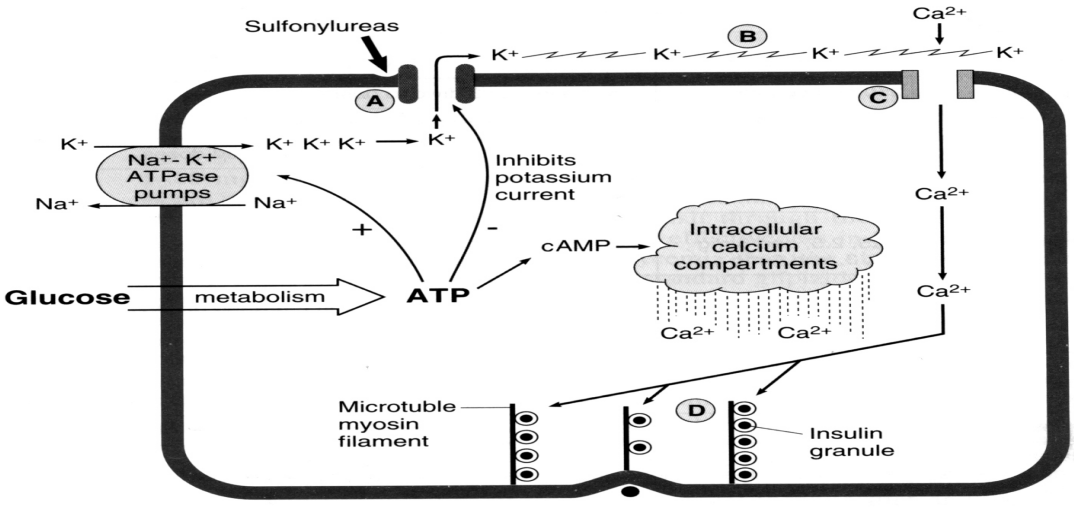

Figure 2.1: Pancreatic mechanism of sulfonylurea

b) Extra-Pancreatic Mechanisms:

Sulfonylureas reduce serum glucagon levels therefore contributing to its hypoglycemic effects. The precise mechanism by which this occurs remains unclear but may result from indirect (secondary) inhibition due to enhanced release of both somatostatin and insulin(DeRuiter, 2003).

Examples of SUs include glibenclamide and tolbutamide. Tolbutamide is a slow acting SU thus can be suitable for patients with renal impairment.

Glibenclamide(Glyburide)

This is a high potency sulfonylurea having high receptor binding capability. It is extensively bound by plasma proteins and is recycled in the hepatic hence its prolonged duration of action. Glibenclamide is metabolized in the liver by oxidation of the cyclohexyl rings with *cis*-3-OH and *trans*-4-OH compounds being the major isomeric metabolites being formed (DeRuiter, 2003). It has a short plasma half-life of 2 – 10 hours but a prolonged biological effect due to the formation of active metabolites. Apart from hypoglycemia, which may be as a result of the drug's prolonged therapeutic action, Glibenclamide does not cause water retention as does chlorpropamide (also a sulfonylurea). Dose reduction, however, is essential in the elderly (2.5mg/day – 1.25mg/day) to avoid hypoglycemia due to the drug (WHO, 1994).

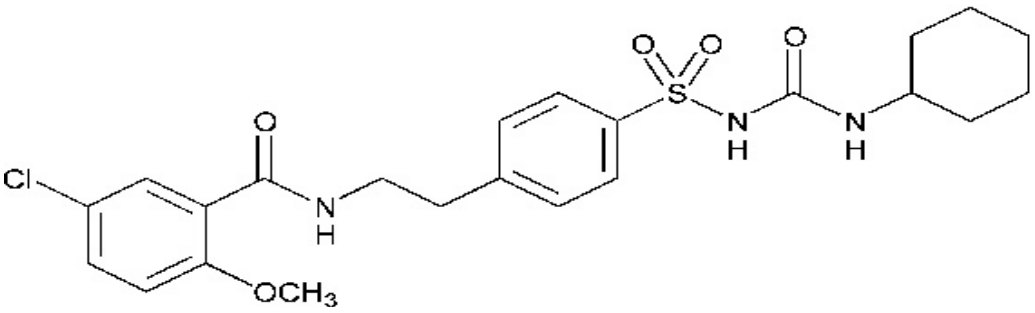

Figure 2.2: Structural formula of glibenclamide. (Molecular mass = 494; Molecular formula: $C_{23}H_{28}C1N_3O_5S$)

2.4.2 Mode of Action of Biguanides

Biguanides work by decreasing gluconeogenesis and by increasing peripheral utilization of glucose. Metformin is a commonly used BG and it is mainly used in the obese who fail to respond to dietary therapy. The initial daily dose is 500 – 850mg with or after food and it can be increased to 500mg tds or 850mg bd (WHO, 1994). However it is contraindicated in the following situations because of the risk of lactic acidosis: elderly people above 70 years, patients of impaired renal function, patients with predisposition to lactic acidosis, patients with heart failure or hepatic impairment. Metformin may also cause adverse reactions such as anorexia, vomiting and gastrointestinal tract side effects (bloating) (DeRuiter, 2003). These effects may be overcome by discontinuing use of drug, lowering dosage or when drug is used in combination with other drugs. Metformin may be used together with a sulfonylurea (glibenclamide) when diet and metformin or a sulphonylurea alone does not result in adequate glycemic control. For instance Glucovance tablets (metformin/glucovance combination) (DeRuiter, 2003).

2.5 Cost of Treatment and Availability of Drugs.

The use of OHDs to manage diabetes depends on availability of the drugs both in the private and public sectors, affordability of OHDs and the physician experience. Both generic and originator forms of these drugs are available in private sector retail pharmacies but are not easily available in the public sector. In addition, they are extremely unaffordable to most poor people and thus limited to the affluent (WHO, 2006). Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes mellitus (Singh, Singh, & Saxena, 2010).

2.6 Herbal alternative

The use of medicinal herbs and herbal medicine is an age – old tradition and the recent progress in modern therapeutics has stimulated the use of natural product worldwide for diverse ailments and diseases. According to WHO, traditional medicine is popular in all regions of the world and its use is rapidly expanding even in developed countries. For instance, in China, traditional herbal preparations account for 30-50% of the total medicinal consumption and the annual market for herbal medicine is over 60 billion USD (Eddouks, Chattopadhyay,Vincenzo & Cho., 2012).

Herbal medications are preferred in management of diabetes since they can target multiple mechanisms including enhancement of insulin sensitivity, stimulation of insulin secretion, reduction of carbohydrate absorption, inhibition of protein glycation and polyol pathway and inhibition of oxidative stress (Karau *et al*., 2013). This contrasts with Western medicine which usually contains a single active ingredient that targets a specific mechanism (Ceylan-Isik *et al.,* 2010)

Several studies on medicinal plants have documented presence of phytochemicals which may contribute to the ability of these plants to possess antioxidant and antidiabetic activity. For instance, the antidiabetic effect of *Moringa oleifera* seed powder on Streptozotocin – induced diabetic rats is said to be due to the antioxidant activity of *Moringa oleifera* seed powder which is due to its content of phenolics and

flavonoids that have scavenging effect on free radicals (Kalyan *et al*., 2015). The ability of *Durio zibethinus* fruit peels ethanolic extracts to reduce blood glucose was presumed to be due to the flavonoids constituents present (Kalyan *et al*., 2015).

2.6.1 Capparis tomentosa

Capparis tomentosa Lam., also known as African Caper, mbada paka(Swahili), woolly caper – bush(English), gombor lik (Somali) "Wonder plant", is a plant belonging to the Capparaceae family. It is a small spiny tree or scrambling shrub found in tropical or other warm regions and sometimes can develop into a tree that can grow as high as 10 meters tall (Windadri, 2001). It is native to Africa where it is found in Zimbabwe, Senegal, South Africa and in Kenya where it is used for medicinal purposes, as food spice, in ritual cleansing and for decorative purposes (Kokwaro, 2009). *C. tomentosa* is documented as a popular medicine for rheumatism, snakebite, chest pain, jaundice, malaria, headache, coughs, pneumonia, constipation, infertility and to prevent abortions. It is also used to treat leprosy, tuberculosis and gonorrhea (Van Wyk & Gericke, 2003). The roots are boiled in water and this infusion is drunk three times a day for coughs and chest pains (Van Wyk *et al*., 2002; Van Wyk & Gericke, 2003). In Kenya it is alleged to heal patients suffering from asthma, infertility/ sterility, high blood pressure, bleeding gums, gout, arthritis, diabetes mellitus, as an immune booster for HIV/AIDS as it boosts CD4 counts within a short period of using it (Wandeto, 2013). The plant is accessible (Mander, 1998) and may contribute to new bioactive compounds that are safe and effective. *C. tomentosa* is used in Kenya by local communities to manage several ailments including diabetes without scientific screening on its efficacy and safety.

Figure 2.3: Photo of *C.tomentosa*: Flowers, leaves and buds

Previous study done to determine the medicinal and food value of Capparis revealed the presence of alkaloids namely L – stachydrin and 3 – hydroxyl – 4 – methoxy – 3 – methyl – oxindole from the roots of *C. tomentosa.* The study demonstrated that L – stachydrin found in *C.tomentosa Lamm* root barks and in the fruits of *C. mooni Wight* possessed anti – tuberculosis property in *in vivo,* and this compound was found to increase blood coagulation thus shortening bleeding time and blood loss (Mishra *et al.,* 2007). A study done to determine toxicity of *C.tomentosa* to sheep and goats being orally administered with a mixture of *C. tomentosa* fruits and leaves dosed at 3g/kg body weight revealed that the animals were anaemic and concluded that the plant was toxic to sheep and goats at high doses by causing structural and functional changes in various organs (Salih *et al*., 1980).

2.7 Phytochemicals

2.7.1 Examples of Phytochemicals possessing antioxidant activity

Flavonoids

Flavonoids belong to a group of polyphenols which are widely distributed among the plant flora. Flavonoids are derived from flavans, which are the parent compounds. Their structure consists of more than one benzene ring in its structure (a range of C15 aromatic compounds). Several reports support the use of flavonoids as

antioxidants or as free radical scavengers (Kar, 2007). Some of the most common flavonoids include Quercetin, quercitrin and kaempferol which are found in nearly 70% of plants. Other group of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones and catechin and leucoanthocyanidins(Doughari,2012).

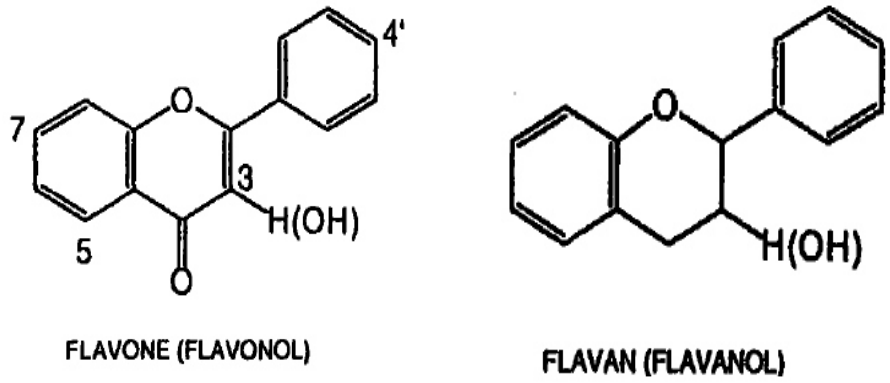

Figure 2.4: Structural formula of some flavonoids

Phenolics (phenols)

Phenols occur ubiquitously as natural colour pigments. They are responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase . The most important role of phenols in plants is defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (Doughari, 2012). Phenols are classified into three: a) phenolic acids b) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and c) non-flavonoid polyphenolies. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007). Phenolics represent a host of natural antioxidants, used as nutraceuticals, and are found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments and are also used as anti-inflammatory agents (Doughari, 2012).

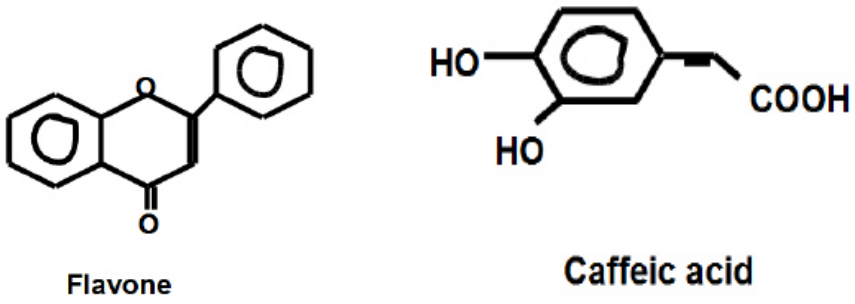

Figure 2.5: Structural formula of some phenols (flavone and caffeic acid)

2.7.2 Phytochemicals mode of action – Antioxidants.

The role of antioxidants is to protect cells against the damaging effects of reactive oxygen species (free radicals) such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage (Mattson & Cheng, 2006). Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Uddin *et al*., 2008; Jayasri *et al*., 2009). Antioxidants exert their activity by scavenging the 'free-oxygen radicals' thereby giving rise to a 'stable radical'. The free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate surroundings. These radicals if not scavenged effectively, they damage essential biomolecules such as lipids, proteins including those present in all membranes, mitochondria and, the DNA resulting in abnormalities leading to disease conditions (Uddin *et al.,* 2008). Thus, free radicals are involved in a number of diseases including: tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's diseases), AIDS and even early senescence (Chen *et al*., 2006; Uddin *et al*., 2008). Free radicals generated in the body can be removed by the body's own natural antioxidant defenses such as glutathione or catalases (Sen, 1995). However, the human body produces insufficient amounts of antioxidants essential for

preventing oxidative stress. Thus this deficiency is compensated by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, beta-carotene and natural products in plants such as phenols, flavonoids, terpenoids which contain free radical scavenging potential hence rich in antioxidant activity (Madsen & Bertelsen, 1995; Rice-Evans *et al*., 1997; Diplock *et al*., 1998; Cai & Sun, 2003). These vitamins are involved in synthesis of enzymes that are essential to metabolic cell activity, synthesis of hormones, repairing genetic materials, and maintaining normal functioning of the nervous system, processes critical in alleviating the effects of diabetes mellitus (Chehade *et al*., 2009). Antioxidant bioactive compounds from plant sources are commercially promoted as nutraceuticals, and have been shown to reduce the incidence of diseases (Hermans *et al.*, 2007). Many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and therefore may contribute significantly to protective effects *in vivo* (Rice-Evans *et al*., 1997; Jayasri *et al.,* 2009). In the food industries, antioxidants are added to foods to prevent the radical chain reactions of oxidation. Here, they act by inhibiting the initiation and propagation step leading to the termination of the reaction and therefore delay the oxidation process.

2.8 Relationship between Antioxidant activity and Diabetes.

Type 2 diabetes is often characterized by development of increased morbidity and mortality for cardiovascular disease (CVD), and also by microangiophatic complications, such as retinopathy, nephropathy, and neuropathy (Chaturvedi, 2007). Previous studies suggests that glucose overload may result in damaging of cells via oxidative stress (Brownlee, 2001).

Four key biochemical changes induced by hyperglycemia have been linked to the overproduction of superoxide radicals resulting in hyperglycemia – induced oxidative tissue damage (Brownlee, 2001). They include:

a) Increased flux through the polyol pathway. Here glucose is reduced to sorbitol, levels of both NADPH and reduced glutathione are reduced.
b) Increased formation of advanced glycation end products (AGEs)

c) Activation of protein kinase C. This may result to effects ranging from vascular occlusion to expression of proinflammatory genes.

d) Increased shunting of excess glucose through the hexosamine pathway. This mediates increased transcription of genes for inflammatory cytokines. Excess plasma glucose drives excess production of electron donors (NADH/H) from the tricarboxylic acid cycle; in turn, this surfeit results in the transfer of single electrons (instead of the usual electron pairs) to oxygen, producing superoxide radicals and other reactive oxygen species (instead of the usual H_2O end product). The superoxide anion itself inhibits the key glycolytic enzyme glyceraldehyde-3- phosphate dehydrogenase (GADPH), and consequently, glucose and glycolytic intermediates spill into the polyol and hexosamine pathways, as well as additional pathways that culminate in protein kinase C activation and intracellular AGE formation(Brownlee, 2001).

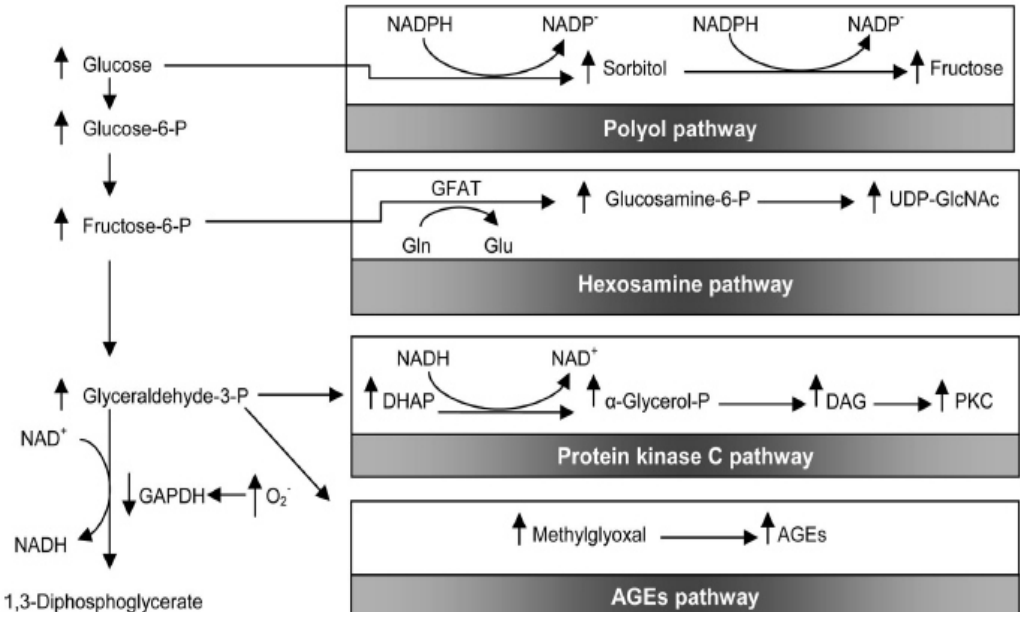

Figure 2.6: Hyperglycemia induced biochemical changes linked to overproduction of superoxide radicals

The overproduction of superoxide is often accompanied by increased nitric oxide generation. This is due to endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) uncoupled state (Ceriello, 2003),which favors formation of the strong oxidant peroxynitrite, which then damages DNA(Ceriello,2003).

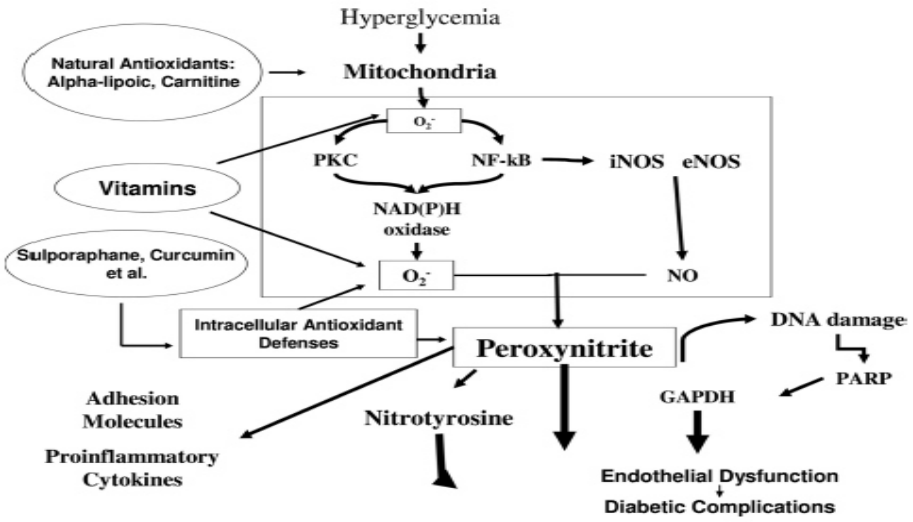

Figure 2.7: Effect of hyperglycemia in the cells at mitochondrial level

Hyperglycemia in the cells induces the overproduction of superoxide at the mitochondrial level. The overproduction of nitric oxide, also at the mitochondrial level, through eNOS and iNOS also takes place. Protein kinase C (PKC) and nuclear factor –kB(NF – kB) are activated and this favors the overexpression of NAD(P)H oxidase enzyme which generates greater amounts of superoxide radicals. This overproduction together with increased nitric oxide (NO) leads to formation of a strong oxidant peroxynitrite which damages DNA. DNA damage stimulates activation of nuclear enzyme poly (ADP – ribose) polymerase (PARP) which reduces activity of glyceraldehyde – 3 –phosphate dehydrogenase (GAPDH) resulting in endothelial dysfunction, which contributes to development of diabetic complications (Ceriello, 2003).

The use of herbal remedies possessing natural antioxidants may be crucial in the management of diabetes mellitus and its complications. These antioxidants may contribute by balancing free radical production at the cell or even mitochondrial level by scavenging free radicals produced in the cells. This therefore makes it critical to determine the antioxidant activity and anti-diabetic efficacy of *C. tomentosa* aqueous roots extracts which are already being used by local communities as herbal remedy for management of diabetes mellitus.

2.9 Mode of Action of Diabetes Inducer Alloxan

The use of alloxan (chemical induction) to induce diabetes appears to be the most popularly used procedure in inducing diabetes mellitus in many experimental animals. Several experimental studies have demonstrated that alloxan causes a sudden rise in insulin secretion in the presence or absence of glucose, which appeared just after alloxan treatment (Szkudelski *et al*., 1998; Lachin & Reza, 2012). Alloxan being hydrophilic and unstable, it has similar shape as that of glucose. This property makes it responsible for its selective uptake into the cytosol by glucose transporter (GLUT2) found in cell membrane of beta cells where it accumulates in the cytosol (Gorus *et al*., 1982). This particular alloxan-induced insulin release occurs for short duration followed by complete suppression of the islet response to glucose even when high concentrations of glucose were used (Kliber *et al*., 1996). In the pancreatic beta cells, the reduction process occurs in the presence of different reducing agents such as reduced glutathione (GSH), cysteine, ascorbate and protein-bound sulfhydryl (-SH) groups (Lenzen *et al*.,1998; Zhang *et al*.,1992). Alloxan reacts with two -SH groups in the sugar binding site of glucokinase resulting in the formation of the disulfide bond and inactivation of the enzyme. As a result of alloxan reduction, dialuric acid is formed which is then re-oxidized back to alloxan establishing a redox cycle for the generation of reactive oxygen species (ROS) and superoxide radicals(Munday,1998;Das *et al*., 2012). The superoxide radicals liberate ferric ions from ferritin and reduce them to ferrous and ferric ions (Sakurai & Ogiso, 1995). In addition, superoxide radicals undergo dismutation to yield hydrogen peroxide (H_2O_2) in the presence of superoxide dismutase. As a result, highly reactive hydroxyl radicals are formed according to the Fenton reaction in the presence of

ferrous and H_2O_2 . Antioxidants like superoxide dismutase, catalase and the non-enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity (Ebelt *et al*., 2000).

In addition, the disturbance in intracellular calcium homeostasis has also been reported to constitute an important step in the diabetogenic action of alloxan. It has been noted that alloxan elevates cytosolic free Ca^{2+} concentration in the beta cells of pancreatic islets (Park *et al*., 1995). The calcium influx is resulted from the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. The increased concentration of Ca^{2+} ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets.

In conclusion, the alloxan-induce pancreatic beta cell toxicity and resultant diabetogenicity can be attributed to the redox cycling and the toxic ROS generation in combination with the hydrophilicity and the glucose similarity of the molecular shape of alloxan (Rohilla & Ali, 2012).

CHAPTER THREE

MATERIALS AND METHODS

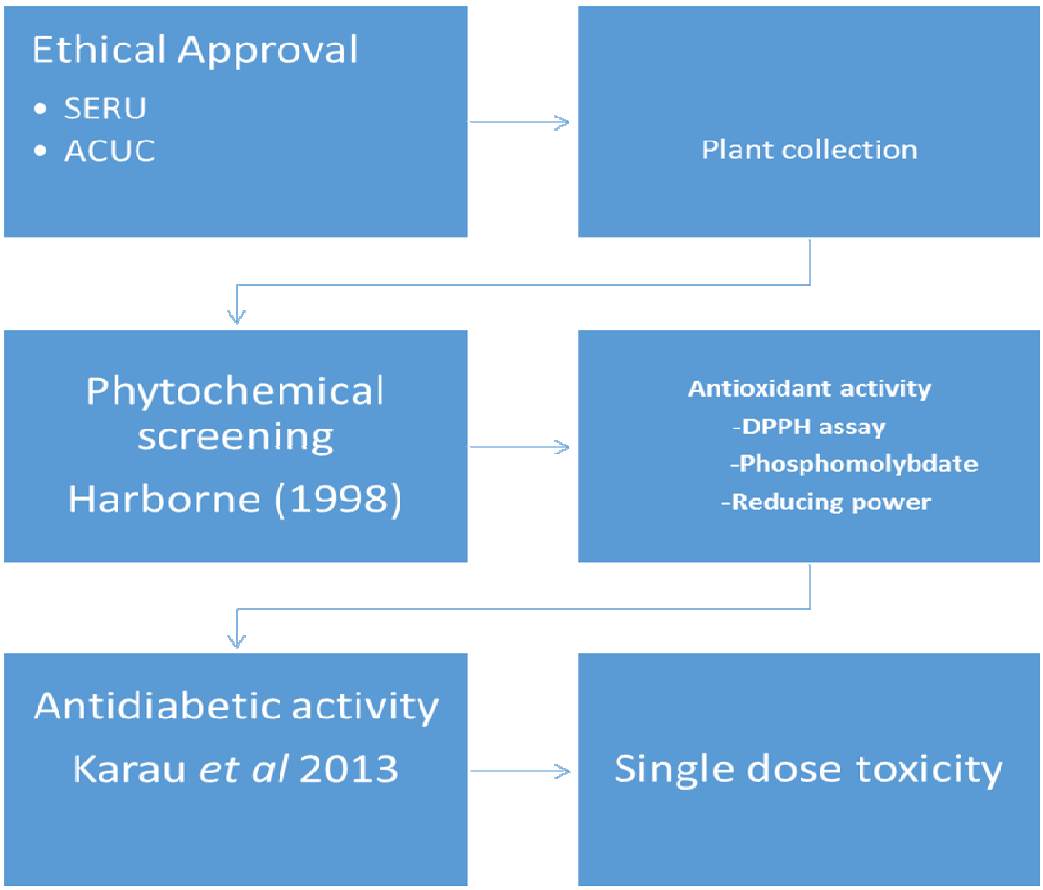

Figure 3.1: Layout of experiment in a flow chart for ease of illustration

3.1 Collection and Preparation of the aqueous roots extracts

Fresh roots of *C. tomentosa* were collected from Kitale Nature conservancy in the month of December, 2013. The fresh roots were washed with clean tap water, cut into small pieces and dried under shade at room temperature for 4 weeks. They were then ground into fine powder using electric mill, and the powder kept at room temperature(23°C± 2°C) away from direct sunlight in dry air-tight plastic bags. 100 g of the roots powder was extracted in 1 liter of distilled water at 60ºC in a metabolic

shaker for 6 hours. The extract was decanted into a clean dry conical flask and then filtered through cotton gauze into another clean dry conical flask. The filtrate was freeze dried in 200 ml portions in a Modulyo Freeze Dryer (Edward, England) for 48 hours. These aqueous extracts were weighed and stored in air-tight, amber containers at 4ºC ready for use.

3.2 Qualitative Phytochemical screening techniques

The methods of Mandal *et al*. (2013), Zohra *et al*. (2012) and Harborne (1998) were used in the qualitative phytochemical screening.

3.2.1 Determination of alkaloids

The methods used to test for alkaloids was as described by Harborne (1998). Briefly, a few drops (3 drops) of Wagner's reagent was added by the sides of the test tube containing 0.5mL of the aqueous root extract. A red-brown precipitate confirmed the presence of alkaloids. For Mayer's test, a drop of Mayer's reagent was added by the side of the test tube containing 0.5ml of the aqueous root extract. A white creamy precipitate indicated presence of alkaloids.

3.2.2 Determination of carbohydrates and reducing sugars

Two methods were used to test for carbohydrates and reducing sugars, according to the method by Harborne (1998). For the Fehlings test, the aqueous extract amounting 1mL was boiled on a water bath with 1mL each of Fehling solution A and B and heated in a water bath for 10 minutes at 50^{o}C. A red precipitate indicated the presence of reducing sugars in the aqueous root extract. For the Benedict's test, 0.5mL of Benedict's reagent was added to 0.5mL of the aqueous root extract and mixed thoroughly then heated on a boiling water bath for two minutes and appearance of a brick red colored precipitate indicated the presence of reducing sugars in the aqueous root extract.

3.2.3 Glycosides (Keller-Killian test)

The extraction of glycosides was carried out using the method of Harborne (1998). To the plant extract of one milliliter, 1mL of 3.5% ferric chloride in glacial acetic acid was added and 1.5mL of concentrated sulphuric acid carefully added by the sides of the test tube to form separate layer at the bottom. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides, and a pale green colour in the upper layer indicated the presence of steroidal nucleus, thus presence of cardiac glycosides.

3.2.4 Determination of phenolic compounds and tannins

Two methods were used to test for phenolic compounds and tannins according to the method of Zohra *et al* (2013). For the ferric chloride test, a few drops of neutral 5% ferric chloride solution (three drops) was added to 1ml of the extract. Observation of a dark-green colour indicated presence of phenolic compounds in the aqueous root extract. For the lead acetate test, three milliliter of 10% lead acetate solution was added to one milliliter of the aqueous root extract. Observation of a bulky white precipitate indicated the presence of phenolic compounds in the aqueous root extract.

3.2.5 Flavonoids

The method of Harborne (1998) was used. Ethyl acetate amounting to five milliliter was added to one milliliter of sample and heated in a water bath for three minutes. Three milliliter of the filtrate was then shaken with one milliliter of dilute ammonia solution. The observation of yellow colour indicated presence of flavonoids in the aqueous root extract. For alkaline reagent test, one milliliter of aqueous root extract was treated with 10% ammonium hydroxide solution. Observation of yellow fluorescence indicated the presence of flavonoids in the aqueous root extract. For the magnesium and hydrochloride reduction test, one milliliter of aqueous root extract was dissolved in 5mL of alcohol then few fragments of magnesium ribbon was added. Concentrated hydrochloric acid was then added drop wise. Development of any pink to crimson colour indicated presence of flavonol glycosides in the aqueous root extract.

3.2.6 Determination of phytosterols (Liebermann-Burchard's test)

The method of Mandal *et al.*(2013) was used. Acetic anhydride of two milliliter was added to one milliliter of sample extract. Concentrated sulphuric acid (2 drops) was then carefully added along the sides of the test tube. A red-brown colour at the interface indicated the presence of phytosterols.

3.2.7 Saponins

The method of Mandal *et al.* (2013) was used whereby the aqueous root extract amounting one milliliter was diluted with distilled water and made up to 20mL. The suspension was shaken in a graduated cylinder for 15 minutes and allowed to stand for 15 minutes. A persistent foam observed for 15 minutes indicated presence of saponins.

3.2.8 Terpenoids

The determination of terpenoids was done as described by Mandal *et al.* (2013) . The extract of 2mL was added to 2mL of chloroform. Two milliliter of concentrated sulphuric acid was carefully added by the sides of the test tube to form a bottom layer for observation of a brown ring at the interface.

3.3 In vitro Antioxidant activity assay

3.3.1 Free radical scavenging activity by DPPH assay

The free radical scavenging activity of the root powder was determined by 1, 1-dipheny-2-picrylhydrazyl (DPPH) method (Brand-Williams *et al*., 1995). In this method, a stock solution was prepared by dissolving 2.4 mg of DPPH free radical in 100 mL of distilled water. The solution was kept at 20ºC until required. The working solution was prepared by diluting DPPH stock solution with distilled water till the absorbance was 0.980 ± 0.02 at 517 nm. Then, 3 ml of the working solution was mixed with 100 µl of aqueous root extract (1 mg/mL). After incubating the mixture in the dark for 30 min, absorbance was read at 517 nm using a UV/vis spectrophotometer. The blank contained all reagents except the roots extract.

Ascorbic acid at a concentration of 1 mg/ml was used as reference. The scavenging activity was calculated by using the formula shown in the equation:

Percent scavenging activity =

$$\frac{(Absorbance\ of\ the\ blank - Absorbance\ of\ the\ sample)}{Absorbance\ of\ the\ blank} \times 100\%$$

3.3.2 Total antioxidant activity by phosphomolybdate assay

This was carried out according to the procedure described by Umamaheswari & Chatterjee (2008). The phosphomolybdate reagent was prepared by mixing equal volumes of 100 ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Test samples were prepared by dissolving 1 mg of aqueous root extract in 1 ml of distilled water. Then, 0.1 ml of the test sample was dissolved in 1 ml of reagent solution in a test tube which was capped with silver foil and incubated in water bath for 90 min at 95ºC. After cooling the sample to room temperature, the absorbance was read at 765 nm against a blank. Ascorbic acid was used as a standard antioxidant with concentrations ranging from 10 to 50 mg/L. The ascorbic acid absorbances were used in the construction of the standard curve. The results were expressed as µg of ascorbic acid equivalent (AAE) per mg of the dried weight of the root extracts of *C. tomentosa*. The AAE was determined according to the expression:

$$\text{Ascorbic acid equivalent} = \frac{Absorbance\ at\ 765nm}{0.0084}\ (\mu g/mg\ \text{of dried matter}).$$

3.3.3 Reducing power assay

The reducing power assay was carried out by the method of (Oyaizu, 1986). The root extract or gallic acid solution ranging from 25 to 800 µg/ml, each 2.5 ml was mixed with 2.5 ml of 0.2 M sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min and 2.5 ml of trichloroacetic acid solution (100 mg/L) was added. The mixture was centrifuged at 650 rpm for 10 min, and 5 ml of the supernatant was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride solution. The absorbance was measured at 700 nm.

3.4 Preparation of dosage for in vivo assay

The extracts for *in vivo* antidiabetic studies were prepared according to the protocol by Karau *et al*. (2013). Briefly, 125 mg to make an oral dose of 50; 250 mg to make 100, and 500 mg to make 200 mg/kg body weight, respectively, were each dissolved in 10 ml of physiological saline. Glibenclamide, a sulfonylurea conventionally used in managing diabetes mellitus was prepared by dissolving 7.5 mg to make a dose of 3 mg/kg body weight was dissolved in 10 ml of physiological saline.

3.5 Experimental animals

The experiment was designed as previously described by Karau *et al.* (2013) to determine hypoglycemic effects of aqueous and ethyl extracts of *Senna spectabilis* in alloxan induced diabetic male mice. This study employed 3-5 weeks old male BALB/c mice of weights 20-30 g bred in the animal house at the department of Biochemistry and Biotechnology of Kenyatta University. This study was conducted according to the "Principles of Laboratory Animal Care" (World Health Organization, 1985), and all the experimental protocols were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenya Medical Research Institute. The mice were housed at a temperature of 25ºC with 12 hours light /12 hours darkness / photoperiod and fed on rodent pellets (Unga Feeds Limited, Kenya) and water *ad libitum*.

Male BALB/c mice were used as mice models because they are dosile, also because male mice are not easily prone to hormonal changes. The BALB/c mice were randomly divided into six experimental groups consisting of five animals each. These groups included: the normal unmanipulated mice (the reference group of the experiment) orally administered with 0.1 ml physiological saline; the alloxan-induced diabetic mice (the negative control roup) orally administered with 0.1 ml physiological saline; alloxan induced diabetic control mice orally administered with 0.06mg of glibenclamide (3 mg/kg body weight, positive control group) in 0.1 ml physiological saline; and alloxan-induced diabetic mice orally administered with 1, 2, and 4 mg of extracts, respectively, in 0.1 ml physiological saline (50, 100, and 200 mg of plant extracts/kg body weight, respectively).

3.5.1 Induction of diabetes

Diabetes was experimentally induced in male BALB/c mice fasted for 8-12 hours, but allowed free access to water by a single intraperitoneal injection of 186.9 mg/kg body weight of a freshly prepared 10% alloxan monohydrate (Sigma Chemicals, St. Louis, OH) in physiological saline. This dose was found to be optimum in inducing stable diabetes in male BALB/c mice (Karau *et al*., 2012). Forty eight hours after injection, blood glucose was determined by use of a glucometer (Contour RTS, Bayer Pty. Ltd; Healthcare Division, Japan), and mice with blood glucose levels above 2000 mg/L (>11.1 mmol/L), were considered diabetic and suitable for use in the study.

3.5.2 Blood glucose determination

Determination of blood glucose was carried out on blood drops obatined by tail bleeding of the mice at predetermined time points. Briefly, the tip of the tail was sterilized with 10% alcohol, and then nipped at the tip. A drop of blood was applied at the glucometer's sample pot. Blood glucose was determined at times 0, 2, 4, 6 and 8 hours after oral administration of aqueous roots extracts of *C. tomentosa*.

3.6 Single dose toxicity study

Ten mice were randomly divided into two groups of five mice each. Group I consisted of untreated control mice orally administered daily for 28 days with 0.1 ml physiological saline. Group II, consisted of normal control mice orally administered with aqueous roots extracts of *C. tomentosa* at 25 mg (1 g/kg body weight) in 0.1 ml physiological saline daily for 28 days. During this period, the mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behaviour and mortality. The body weight of each mouse was assessed after every seven days during the dosing period up to and including the 28^{th} day and the day of sacrifice. On the 28^{th} day, all the study animals were euthanized, organs were removed and the weights determined (Appendix IV). Blood samples were obtained by cardiac puncture and collected in plastic test tubes and allowed to stand for 3 hours to ensure complete clotting. The clotted blood was centrifuged at 3000 rpm for

10 min and clear serum samples aspirated off and stored frozen at -20ºC until required for biochemical analysis.

3.6.1 Determination of Biochemical Parameters for Toxicity

Test for biochemical parameters was done using serum samples previously obtained. An autoanalyser (Olympus 640 chemistry autoanalyser) was used to test for aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), urea and creatinine. The reagents to be used were commercially prepared to fit the concentrations and volumes. For the identification by the machine, the reagent cartridges holding the reagents were bar coded. The machine was then programmed for the selected tests for each sample and the sample sectors then placed into the autoloader assembly. A series of events occurred automatically under the direct control of the autoanalyser's microprocessor. The assays were carried out based on the standard operating procedures of Kenyatta National Hospital, department of laboratory medicine.

3.7 Ethical clearance

Approval by KEMRI Scientific Ethical Review Unit (SERU) and Animal Care and Use Committee (ACUC) was sought before study implementation. Clearance letters are attached (Appendix VII and VIII) respectively.

3.8 Data management and analyses

Phytochemical screening experiments were done in triplicates and the data was entered in a notebook and later transferred into Excel spread sheet. A normality test done on the data showed that the data followed a normal distribution, hence the parametric test that was then employed was analysis of variance. Where applicable the data was subjected to one way Analysis of Variance (ANOVA) and differences between samples determined by Duncan's Multiple Range test using Minitab program (version 12 for windows). Antioxidant assay analyses were done in triplicate and the data statistically evaluated using ANOVA with SPSS15.0. DPPH was calculated in percentage and the significant levels were defined using $p \leq 0.05$.

For phosphomolybdate assay the ascorbic acid absorbances were entered in a notebook and the data used to plot a standard curve and the ascorbic acid equivalence calculated. Blood glucose levels for each group of mice was measured after 0, 2, 4, 6, 8 hours by use of glucometer strips and data entered in a notebook. The means of blood glucose levels were determined and significant levels also determined using $p \leq 0.05$. All the data was recorded as mean ± standard deviation (SD) of the blood glucose levels. One-way ANOVA and post-ANOVA (Bonferroni-Holm) test was used to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug, and diabetic mice treated with plant extracts at doses of 50, 100 and 200mg per kg body weight. Also, the student T test was used to compare mean differences between organ weights, and animal weights in single dose toxicity study. $P \leq 0.05$ was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Qualitative analysis of phytochemicals

The present study carried out on *C. tomentosa* aqueous root extract revealed the presence of phytochemical compounds. The phytochemicals detected in the root powders of *C. tomentosa* were alkaloids, glycosides, phenolic compounds, tannins, phytosterols, flavonoids, saponins and terpenoids. Saponins were detected in trace amounts while carbohydrates and reducing sugars were not detected in the root plant powder (Table 4.1). The phytochemical compounds were qualitatively analyzed and the results presented in Table 4.1.

Table 4.1: Qualitative Phytochemical screening

Class of Phytochemical	**Test**	**Aqueous root extract**
Alkaloids	a) Wagner's	**+**
Carbohydrates & reducing sugars	a) Benedict's	**-**
	b) Fehling's	**-**
Glycosides	a) Keller killian	**+**
Phenolic compounds & tannins	a) Ferric chloride test	**+**
	b) Mg & HCl reduction	**++**
Phytosterols	a) Liebermann - Burchard's	**++**
Flavonoids		**++**
Saponins		**+**
Terpenoids		**++**

Key: + = Present ++ = Present in high concentration - = Absent

4.2 In vitro Antioxidant activity assays

As depicted in figure 4.1, the reducing power of the roots powder of *C. tomentosa* increased with increase in extracts concentrations with 800 µg/mL possessing the highest reducing power. In a similar manner the reducing power of gallic acid used as the standard increased with increase in concentration. At the lower concentrations of the extracts and gallic acid, the reducing powers were low. At 400 µg/mL the gallic acid had completely attained maximum reducing power when compared to the *C. tomentosa* roots powders and even at the maximum concentration tested still the roots powders had not attained the optimum reducing power. This is a significant free radical scavenging activity which accounts for the dose-dependent reducing potential observed in the reducing assay.

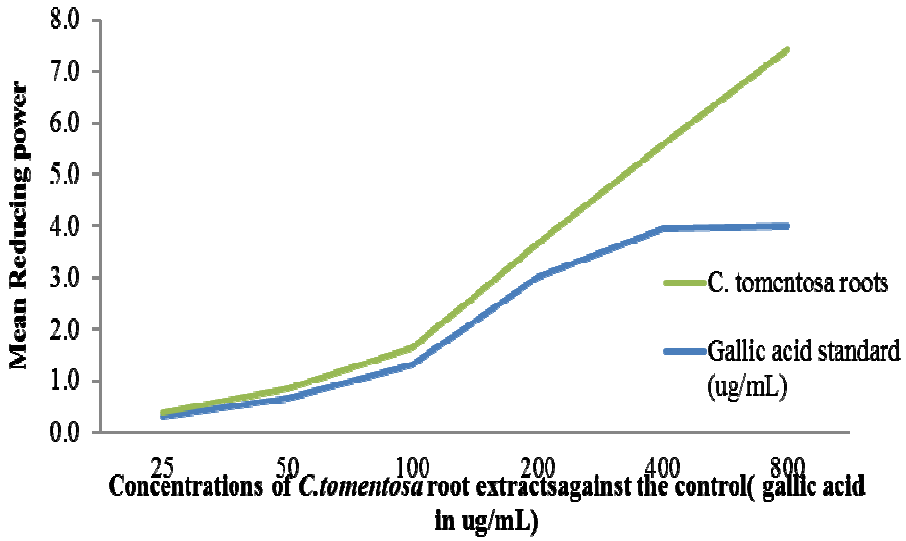

Figure 4.1: The concentration dependent reducing power of C. tomentosa roots compared with gallic acid standard

The radical scavenging activity of the *C. tomentosa* roots according to DPPH method was found to be 35.50 ± 0.02 % compared to the ascorbic acid pure standard which had 96.50 ± 0.02 %. By the phosphomolybdate assay the reducing power was found to be 41. 22 ± 0.17 mg/kg ascorbic acid equivalent. The extract was further found to have 35.50 ± 0.02% free radical scavenging activity by DPPH assay, and this value was significantly different from that observed with ascorbic acid standard at a

concentration of 1 mg/ml. In this case ascorbic acid was used an external standard in a serial dilution ranging from 0.5 to 20 mg/ml.

4.3 *In vivo* Anti-diabetic activity

Prior to the oral administration of the extracts,the mice were all male of same age, their body weights and blood sugars were similar ($p \leq 0.05$). The mice were fed with mice pellets thirty minutes before the experiment. As depicted in figure 4.2, the antidiabetic activity of the aqueous extract is dose-dependent with 200 mg/kg body weight displaying higher activity even after 8 hours. The activities of the three doses was higher than that of the reference drug glibenclamide at 3 mg/kg body weight up to the 6^{th} hour when their activities becomes comparable. At 6 – 8 hours, the activities of the three doses are equal to that of the reference drug glibenclamide at 3mg/kg body weight. As shown in figure 4.2, the blood sugar of the negative group of mice (untreated) significantly increased within 8 hours, while groups treated with conventional drug (glibenclamide) and the extracts at 50, 100 and 200 mg/kg body weight doses, the blood sugar declined significantly ($p \leq 0.05$). At the 2^{nd} hour after treatment except the untreated, those treated with glibenclamide and 200 mg/kg body weight of the extract, the rest had a significant decline in blood sugar which persisted till the 8^{th} hour. The conventional drug, glibenclamide, is most efficacious between 4^{th} and 6^{th} hours where blood glucose levels declined significantly ($p \leq 0.05$) as shown in figure 4.2.

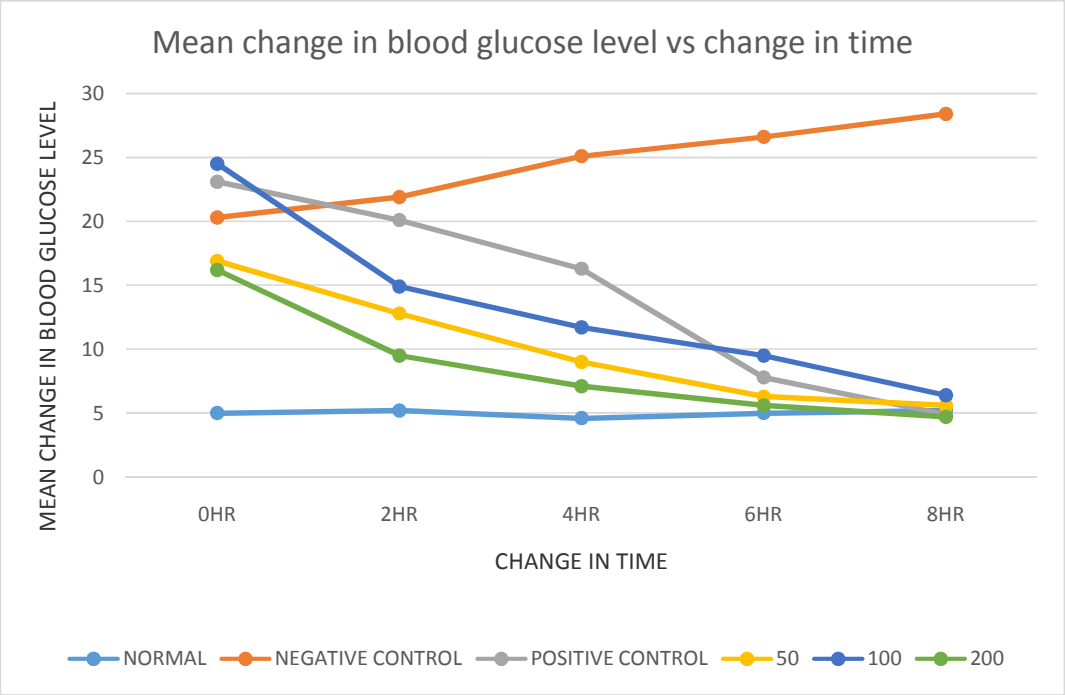

Figure 4.2: Mean change in blood glucose levels after oral administration of aqueous roots extracts of *C. tomentosa* in alloxan-induced diabetic male BALB/c mice. Values are expressed as Means ± SEM for five animals at each time point

4.4 Single dose toxicity study

It was observed that there was significant change in body weights ($p \leq 0.05$) for the animals under treatment compared to the controls over the 28 days administration of the oral extracts at 1000 mg/kg body weight, as shown in figure 4.3,(Appendix IV).The body weight of the experimental mice decreased with oral administration of the aqueuos extract while that of control mice increased. The organ weights were found to be comparable for both experimental and normal control mice ($p \leq 0.05$) as shown in figure 4.4. There was a significant reduction in kidney weight ($p = 0.001$), among the experimental mice compared to the normal control mice. It was also

observed that the lungs and spleen also increased in weight in the mice under treatment compared to the controls.

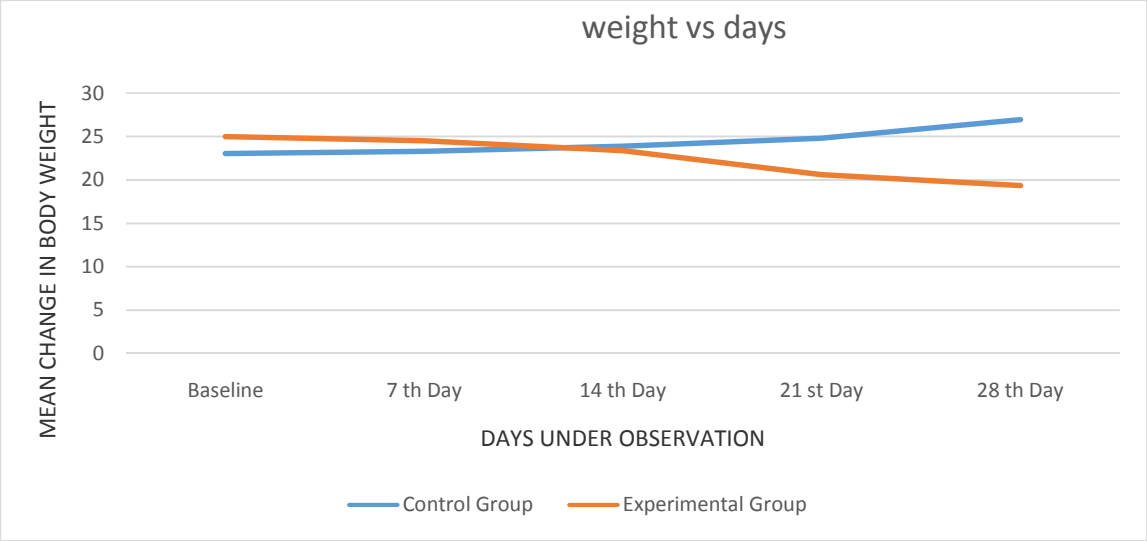

Figure 4.3: A graph on Mean change in body weight of mice orally administered with *C.tomentosa* aqueous roots extracts at 1000mg/kg body weight daily for 28 days. Values are expressed as Mean ± SEM

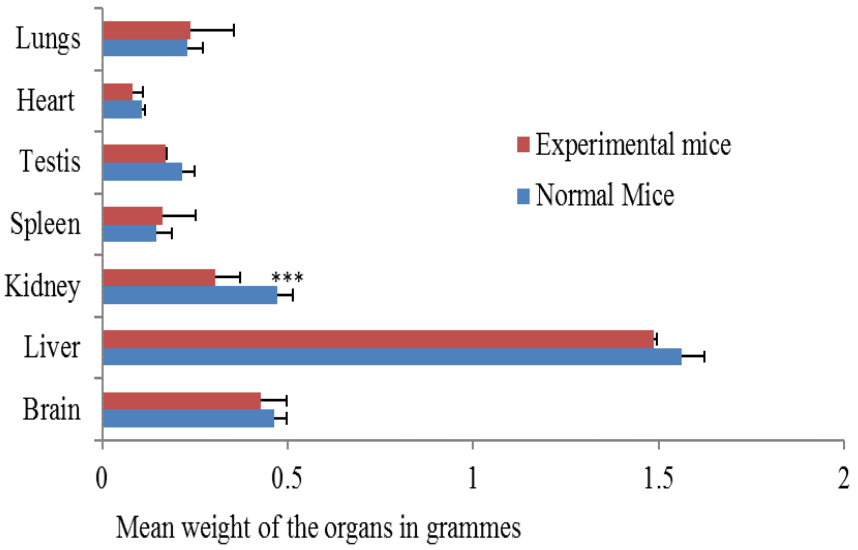

Figure 4.4: The mean weights of various organs in normal control mice and experimental mice in the single dose toxicity assay of *C. tomentosa* at 1000 mg/kg body weight

4.5 Determination of Biochemical parameters.

As shown in Table 4.2, oral administration of aqueous extract of *C.tomentosa* significantly increased the levels of ALP and AST/GOT and significantly decreased the levels of Creatinine and ALT/GPT.The aqueous extract however, had no significant effect on levels of urea compared to the normal control mice (Appendix VI).

Table 4.2: Results on biochemical parameters expressed as Mean ± SEM. *p ≤ 0.05 significantly different from normal control mice by paired mean comparisons by two – way student t – test

Treatment	ALT/GPT	AST/GOT	ALP	UREA	CREATININE
Normal control serum	67.08±19.72	361.46±130.48	711.78±223.42	12.20±3.43	125.66± 22.92
Test serum	61.80±20.71*	378.30±137.05*	795.75±261.68*	11.10±3.27	112.08± 20.84*

CHAPTER FIVE

DISCUSSION

5.1 Qualitative analysis of phytochemicals

Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Alkaloids and tannins have been documented to manage chronic diseases. They are often used as elementary therapeutic agents because of their analgesic, antispasmodic and bactericidal effects (Chukeatirote *et al.,* 2007). Previous study on the antidiabetic and antioxidant properties of alkaloids from *Cantharanthus roseus*(L.) revealed the presence of four indole alkaloids namely: vindoline I, vindolidine II, vindolicine III, and vindolinine IV isolated from the leaves of *Catharanthus roseus* (Tiong *et al.,* 2013).These alkaloids are said to lead to improved glucose uptake in pancreatic (β -TC6) and muscle (C2C12)cells. The alkaloids also inhibited protein tyrosine phosphatase PTP-1B, a down regulator in the insulin signaling pathway (Tiong *et al*., 2013)

Saponins present in the aqueous root extracts may contribute to the hypoglycemic effect of the plant in managing diabetes. In other studies, administration of saponins from the roots of *Garcinia kola* to alloxan – induced diabetic rats is said to cause a significant ($p \leq 0.05$) anti – hyperglycemic effect on the rats (Spasov *et al*., 2008). Phenols are a class of antioxidant agents that act as free radical terminators (Sahidi & Wanasundara, 1992). Flavonoids enhance the effects of vitamin C and both function as antioxidants. The mode of action of flavonoids is often through scavenging or chelating process. They are abundantly found in the root powder of *C.tomentosa* and may be responsible for synergistic antioxidant activity vital in management of diabetes mellitus (Karau *et al.,* 2013). The phytochemicals present may be responsible for the antioxidant activity displayed by the aqueous root extract *of C.tomentosa* in lowering blood glucose levels in the alloxan – induced diabetic Balb/C mice.

5.2 *In vitro* Antioxidant activity

The observation that the reducing power of the aqueous roots extracts of *C. tomentosa* increased with concentration could be explained by the fact that the extracts contained chemical substances capable of reacting with potassium ferricyanide (Fe^{3+}) to form potassium ferricyanide (Fe^{2}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm (Jayanthi & Lalitha, 2011). The observation that the reducing power was linearly proportional with the concentration could be due to the increase in amounts of antioxidants with increase in the amount of crude extracts. Reducing power is associated with antioxidant activity and indicates that primary and secondary antioxidants with ability to donate electrons and reduce oxidized intermediates of lipid peroxidation processes are present in the extracts (Jayanthi & Lalitha, 2011). In the assay, the yellow colour of the test solutions changes to various shades of green and blue and this depend on the reducing power of the extracts concentrations. Similarly, the radical scavenging activity by DPPH and phosphomolybdate assay indicates that the roots extracts contains antioxidants.

In the body, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases, and extensive lyses. Anti-oxidants are important since they are capable of deactivating free radicals, before the radicals are able to attack cells and biological targets. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is uptake of natural antioxidants from food supplements and traditional medicines (Yazdanparast *et al*., 2008). The antioxidant potential of *C.tomentosa* aqueous roots extracts displayed in the present study could be due to the presence of phytochemical constituents in the plant which are capable of donating hydrogen to free radicals generated to scavenge the potential damage.

5.3 *In vivo* anti-diabetic efficacy

This study demonstrates that the aqueous roots extracts of *C. tomentosa* exhibits dose dependent hypoglycemic activity that is significantly higher than that of the reference drug glibenclamide dosed at 3g/kg body weight. These findings are consistent to those of 30% extracts of *C. decidua* fruit powder orally administered to alloxanized rats for 3 weeks and found to significantly reduce the induced lipid peroxidation in erythrocytes, kidneys and heart and also to alter superoxide dismutase and catalases enzymes activities (Modak *et al.,* 2007). The observed hypoglycemic activity of *C. tomentosa* could be due to its strong antioxidant activity and anti-diabetic chemical compounds (Mishra *et al*., 2007). The activities of the three doses (50, 100 and 200mg/kg body weight) was higher than that of glibenclamide (3mg/kg body weight) up to the sixth hour when their activities became equal. This could be indicative of the earlier absorption, onset and peak of the plant extracts in lowering blood glucose levels compared to glibenclamide.

At the second hour after treatment a significant decline in blood sugar which persisted till the 8^{th} hour was observed in groups of mice treated with plant extracts compared to the group treated with glibenclamide. This could also be suggestive that the onset of plant extract is faster compared to that of glibenclamide which begins after $1\frac{1}{2}$ hours (De Reuiter, 2003) and its peak drug levels is at about 4 hours. This

would also give an explanation to the observation between 4^{th} and 6^{th} hours where the levels of blood glucose significantly declined in groups treated with glibenclamide compared to groups treated with plant extracts up to the sixth hour when they were comparable. This could also imply that the efficacy of the conventional drug was achieved at this point. At 6 – 8 hours where the activities of the three doses (50,100 and 200mg/kg body weight) are equal to that of glibenclamide, and also these activities are equal to the activity of physiological saline (0.1ml) administered to normal non – diabetic mice indicated the ability of the plant extracts and the conventional drug to lower blood glucose levels to a normal state.

The observed hypoglycemic activity of *C. tomentosa* extracts may be due to the presence of phytochemicals possessing antioxidant properties which may be crucial in the management of diabetes mellitus and its complications. These antioxidants may contribute by balancing free radical production at the cell or even mitochondrial level by scavenging free radicals produced in the cells (Ceriello, 2003). The hypoglycemic activity could also be due to increased glucose plants transport and uptake, increased glycogen storage and modulation of insulin secretion.

5.4 Single dose toxicity

The single dose toxicity test allowed growth of mice but there was a decrease in body weight of the mice compared to the control group, and this may be due to tissue hypoxia resulting to mass wasting, hence decrease in body weights of experimental mice. The significant reduction in the weight of kidney($p \leq 0.009$) of the experimental mice compared to the normal mice as well as the significant decrease ($p \leq 0.05$) in the levels of creatinine and ALT/GPT in sera of experimental mice compared to the normal control mice sera would indicate a defect in the renal function (renal impairment).The decrease in size of the liver as well as increase in ALP and AST/GOT enzymes in the sera of experimental mice compared to the normal mice indicates there being a defect in the functioning of the liver. Tissue hypoxia often causes most tissues to initially enlarge and as the swollen cells continue rupturing the organ size reduces(Voet & Voet, 2004). This happens when cells relying on glycolysis for the production of ATP rapidly deplete the store of phosphocreatine and glycogen, decreasing the rate of ATP production below the levels required by membrane ion pumps for the maintenance of proper intracellular ionic concentrations. Therefore, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell. These membranes become permeable and leak their enclosed contents. The intracellular pH then becomes acidic allowing release of lysosomal enzymes which then degrade the contents of the cell. Some of the degraded compounds are the initially elevated serum enzymes which are later reduced to values below the control values. The reduced metabolic activity results in irreversible cell damage(Voet & Voet, 2004; Strain & Cashman, 2009; Abdirahman *et al*., 2015).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In this study, the aqueous root extracts of *C. tomentosa* demonstrated antioxidant and anti-diabetic potential. At a dose of 200mg/kg body weight, the aqueous root extract displayed same therapeutic effect as reference drug, glibeclamide in lowering blood glucose levels to normal levels. The aqueous root extracts of *C. tomentosa* at a high dose of 1g/kg body weight which is far from the therapeutic dose (200mg/kg body weight), tends to cause sub – clinical toxicological effects which were well demonstrated by changes in body and organ weights as well as altered levels of biochemical parameters. It can be concluded that both the anti – diabetic and toxic effects of *C. tomentosa* aqueous root extracts may have resulted from its phytochemical constituents.

It is envisaged that further studies on the plant may lead to development of new affordable and effective phytomedicines for management of diabetes. This information augments observational studies and forms a basis for validation and development of the extract as an alternative therapy for management of diabetes.

6.2 Recommendations

Thorough toxicity studies involving tissue histology could be done to further demonstrate sub – clinical toxicological effects of the plant extract. Also, further studies could be done on toxicity using the therapeutic dose 200mg/kg body weight but for a prolonged period. Pre – clinical evaluations of the root extract in the human diabetes should be mounted to demonstrate the therapeutic efficacy as seen in the murine model.

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APPENDICES

Appendix I: Composition of reagents used for phytochemical screening

REAGENT	COMPOSITION	RESULT
Mayer's	Potassium mercuric iodide solution	White creamy precipitate
Wagner's	Iodine in potassium iodide	Brown/reddish precipitate
Benedict's	Anhydrous sodium carbonate, sodium citrate and copper(II) sulfate pentahydrate solution	Orange red precipitate
Fehling's solution A	Copper sulphate solution	Red precipitate
Fehling's solution B	Sodium hydroxide and potassium sodium tartarate solution	Red precipitate

Appendix II: Mean change in blood glucose level

		Control					
		Wt	0hr	2hr	4hr	6hr	8 hrs
	1	22	5.1	5.2	4.9	5.2	5.6
	2	23	5.0	4.7	4.3	4.4	4.1
	3	24	5.1	5.3	5.6	5.4	5.5
	4	22	4.8	5.1	4.1	4.9	5.6
	5	24	5.2	5.5	4.3	5	5.3
Mean		**23.0**	**5.0**	**5.2**	**4.6**	**5.0**	**5.2**
SEM		**0.4**	**0.1**	**0.1**	**0.3**	**0.2**	**0.3**

		Negative					
		Wt	0hr	2hr	4hr	6hr	8hrs
	1	23	19.7	21	23.7	25.8	28.6
	2	25	27.4	27.9	28.8	29.9	30.1
	3	24	20.6	22.4	25.6	27.6	28.4
	4	23	16.3	18.6	22.4	23.2	26.3
	5	24	17.5	19.7	21.6	23.4	27.4
Mean		**23.8**	**20.3**	**21.9**	**25.1**	**26.6**	**28.4**
SEM		**0.4**	**1.9**	**1.6**	**1.3**	**1.3**	**0.6**

		Positive					
		Wt	0hr	2hr	4hr	6hr	8hr
	1	21	25.8	22.3	15.6	8.6	5.4
	2	23	27.2	23.1	20	9.3	5.1
	3	22	23.4	21.8	18.2	7.2	4.8
	4	24	18.6	16	13.3	6.4	4.2
	5	25	20.4	17.1	14.6	7.3	4.9
Mean		**23**	**23.1**	**20.1**	**16.3**	**7.8**	**4.9**
SEM		**0.7**	**1.6**	**1.5**	**1.2**	**0.5**	**0.2**

		Wt	0hr	2hr	4hr	6hr	8hr
	1	25	17.4	12.2	8.2	7.2	5.6
	2	24	18.3	14.6	10.1	7.7	6.3
	3	21	16.2	10.2	8.2	5.5	5.4
	4	23	17.5	14.4	9	6.3	5.6
	5	22	15.1	12.8	9.4	4.9	5.2
Mean		**23.0**	**16.9**	**12.8**	**9.0**	**6.3**	**5.6**
SEM		**0.7**	**0.6**	**0.8**	**0.4**	**0.5**	**0.2**

100

		Wt	0hr	2hr	4hr	6hr	8hr
	1	22	21.2	10.9	7.2	5.2	6.3
	2	24	27.9	15.3	11.2	5.2	3.9
	3	23	28.5	16.9	14.3	12.3	6
	4	25	23	12.4	10.1	12.7	7.9
	5	22	22.1	18.8	15.8	11.9	7.7
Mean		**23.2**	**24.5**	**14.9**	**11.7**	**9.5**	**6.4**
SEM		**0.6**	**1.5**	**1.4**	**1.5**	**1.7**	**0.7**

200

		Wt	0hr	2hr	4hr	6hr	8hr
	1	24	19.2	9.5	7.1	6.5	4.1
	2	22	16.4	9.6	7.3	5.6	4.7
	3	23	15.2	9.2	7.6	5.9	5.2
	4	24	14.2	9.3	6.3	3.6	2.5
	5	25	15.9	9.9	7.3	6.2	4.6
Mean		**23.6**	**16.2**	**9.5**	**7.1**	**5.6**	**4.7**
SEM		**0.5**	**0.8**	**0.1**	**0.2**	**0.5**	**0.5**

Appendix III: Hypoglycemic effects of oral administration of aqueous roots extracts of *Capparis tomentosa* in alloxan-induced diabetic BALB/c mice

Mice Group	Treatment	Blood glucose levels (mmol/L)				
		0 hr	2 hr	4 hr	6hr	8 hr
Normal control	Saline	5.0±0.1	5.2±0.1	4.6±0.2	5.0±0.3	5.2±0.3
Diabetic control	Saline	20.3±1.9	21.9±1.6^{A}	25.1±1.3^{A}	26.6±1.3^{A}	28.4±0.6^{Ad}
Diabetic control	Glibenclamide (3 mg/kg body weight)	23.1±1.6	20.1±1.5^{A}	16.3±1.2^{Bb}	7.8±0.5^{Bc}	4.9±0.2^{Bd}
Diabetic treated	50 mg/kg body weight	16.9±0.6	12.8±0.8^{B}	9.0±0.4^{B}	6.3±0.5^{B}	5.6±0.2^{B}
	100 mg/kg body weight	24.5±1.5^{A}	14.9±1.4^{B}	11.7±1.5^{B}	9.5±1.7^{B}	6.4±0.7^{B}
	200 mg/kg body weight	16.2±0.8^{A}	9.5±0.1^{A}	7.1±0.2^{B}	5.6±0.5^{B}	4.7±0.5^{B}

Results were expressed as mean ± standard error of mean (SEM) of five mice per group. Means followed by similar upper case letters in the same column are not significantly different at $P \leq 0.05$ by ANOVA and post ANOVA (Bonferroni-Holm) test. $^{a}p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 2nd hour; $^{b}P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 4th hour; $^{c}P \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at the 6th hour; $^{d}p \leq 0.05$ when blood glucose levels at 0 hour is compared to blood glucose at 8th hour is compared to blood glucose at the 8th hour by ANOVA and post ANOVA (Bonferroni-Holm) test

Appendix IV: Animal weight

Animal weights					
Control group					
	Baseline	7th day	14th	21st	28th
1	23.4	23.8	22.5	24.8	27.1
2	24.5	24.6	24.6	25	28.2
3	24.9	25.2	25.6	25.8	27.2
4	22	22.04	24.5	25.1	28.1
5	20.5	20.9	22.3	23.4	24.2
Mean	**23.06**	**23.31**	**23.90**	**24.82**	**26.96**
Stdev	**1.82**	**1.80**	**1.44**	**0.88**	**1.62**
Experimental group					
	Baseline	7th day	14th	21st	28th
1	27.8	27.1	26.3	d	d
2	24.6	24.3	24	23.6	22.1
3	25.8	25.2	23.1	20.2	19.1
4	24.1	23.8	23.6	22.4	20.3
5	22.6	22	19.7	16.2	15.9
Mean	**24.98**	**24.48**	**23.34**	**20.60**	**19.35**
Stdev	**1.95**	**1.87**	**2.38**	**3.25**	**2.61**

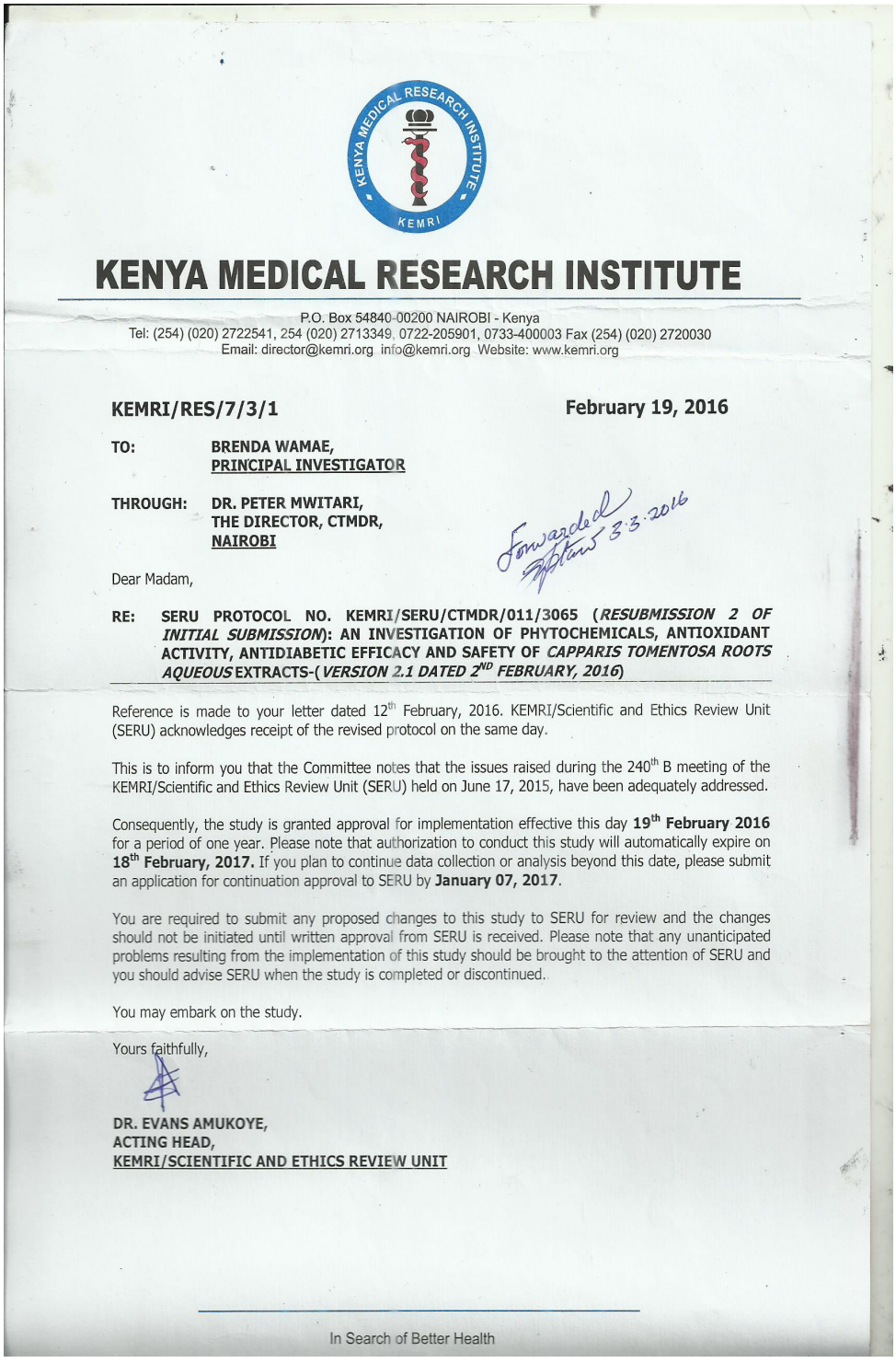
Appendix V: Post mortem organ weights

	Brain	Liver	Kidneys	Spleen	Testis	Heart	Lungs
Control group							
1	0.413	1.55	0.5	0.11	0.264	0.107	0.274
2	0.51	1.645	0.5	0.212	0.191	0.113	0.25
3	0.468	1.55	0.47	0.143	0.187	0.105	0.23
4	0.462	1.499	0.489	0.15	0.253	0.118	0.23
5	0.466	1.274	0.396	0.108	0.232	0.097	0.162
Mean	**0.464**	**1.561**	**0.471**	**0.145**	**0.216**	**0.108**	**0.229**
Stdev	**0.034**	**0.061**	**0.044**	**0.042**	**0.032**	**0.008**	**0.042**
Experimental Group							
1	0.481	1.479	0.358	0.241	0.172	0.112	0.341
2	0.492	1.485	0.367	0.237	0.175	0.1	0.337
3	0.371	1.486	0.26	0.078	0.17	0.061	0.129
4	0.364	1.497	0.233	0.091	0.169	0.058	0.139
Mean	**0.427**	**1.487**	**0.305**	**0.162**	**0.172**	**0.083**	**0.237**
Stdev	**0.069**	**0.008**	**0.068**	**0.089**	**0.003**	**0.027**	**0.118**

Appendix VI: Toxicity biochemical data of *Capparis tomentosa*

NO	SAMPLE NUMBER	ALT/GPT	AST/GOT	ALP	UREA	CREATININE
1	CONT (3) P	31.4	106	496	3.53	82.6
2	CONT (1) P	60.2	349.3	821	10	138.4
3	CONT (4) P	42.5	76.7	298	6.56	74.6
4	CONT (2) P	33.9	182.4	611	6.4	76.2
5	CONT (5) P	29.4	199.8	641	6.8	84.1
	Mean Control Plasma	**39.48**	**182.84**	**573.40**	**6.66**	**91.18**
	SEM	**5.64**	**47.52**	**86.35**	**1.03**	**11.94**
1	CONT (1) S	35.4	88.6	307	6.9	77
2	CONT (5) S	82.5	516	1020	15.6	136
3	CONT (3)S	68.9	477	905	12.6	129
4	CONT (2) S	17.6	24.6	72.9	3.1	82.3
5	CONT (4) S	131	701.1	1254	22.8	204
	Mean Control Serum	**67.08**	**361.46**	**711.78**	**12.20**	**125.66**
	SEM	**19.72**	**130.48**	**223.42**	**3.43**	**22.92**
1	1000 (5) S	88.7	549	1154	15.98	143
2	1000 (3) S	5	17.2	62	2.4	66
3	1000 (4) S	96.1	628	1189	16.3	151.4
4	1000 (2) S	57.4	319	778	9.7	87.9
	Mean Test serum	**61.80**	**378.30**	**795.75**	**11.10**	**112.08**
	SEM	**20.71**	**137.05**	**261.68**	**3.27**	**20.84**
1	1000 (5) P	5	24.5	71.5	2.9	79.3
2	1000 (4)P	4.6	26.7	84.6	2.1	70.5
3	1000 (2) P	35	92.4	354	6.8	86.6
4	1000 (3) P	41.1	60.3	119	5.6	73.1
	Mean test plasma	**21.43**	**50.98**	**157.28**	**4.35**	**77.38**
	SEM	**9.68**	**16.06**	**66.34**	**1.11**	**3.59**

Appendix VII: Clearance letter, KEMRI Scientific Ethical Review Unit(SERU)



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KEMRI/RES/7/3/1 **February 19, 2016**

TO: **BRENDA WAMAE,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. PETER MWITARI,**
THE DIRECTOR, CTMDR,
NAIROBI

Forwarded 3.3.2016

Dear Madam,

RE: **SERU PROTOCOL NO. KEMRI/SERU/CTMDR/011/3065 (*RESUBMISSION 2 OF INITIAL SUBMISSION*): AN INVESTIGATION OF PHYTOCHEMICALS, ANTIOXIDANT ACTIVITY, ANTIDIABETIC EFFICACY AND SAFETY OF *CAPPARIS TOMENTOSA ROOTS AQUEOUS* EXTRACTS-(*VERSION 2.1 DATED 2ND FEBRUARY, 2016*)**

Reference is made to your letter dated 12th February, 2016. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised protocol on the same day.

This is to inform you that the Committee notes that the issues raised during the 240th B meeting of the KEMRI/Scientific and Ethics Review Unit (SERU) held on June 17, 2015, have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day **19th February 2016** for a period of one year. Please note that authorization to conduct this study will automatically expire on **18th February, 2017.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **January 07, 2017**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

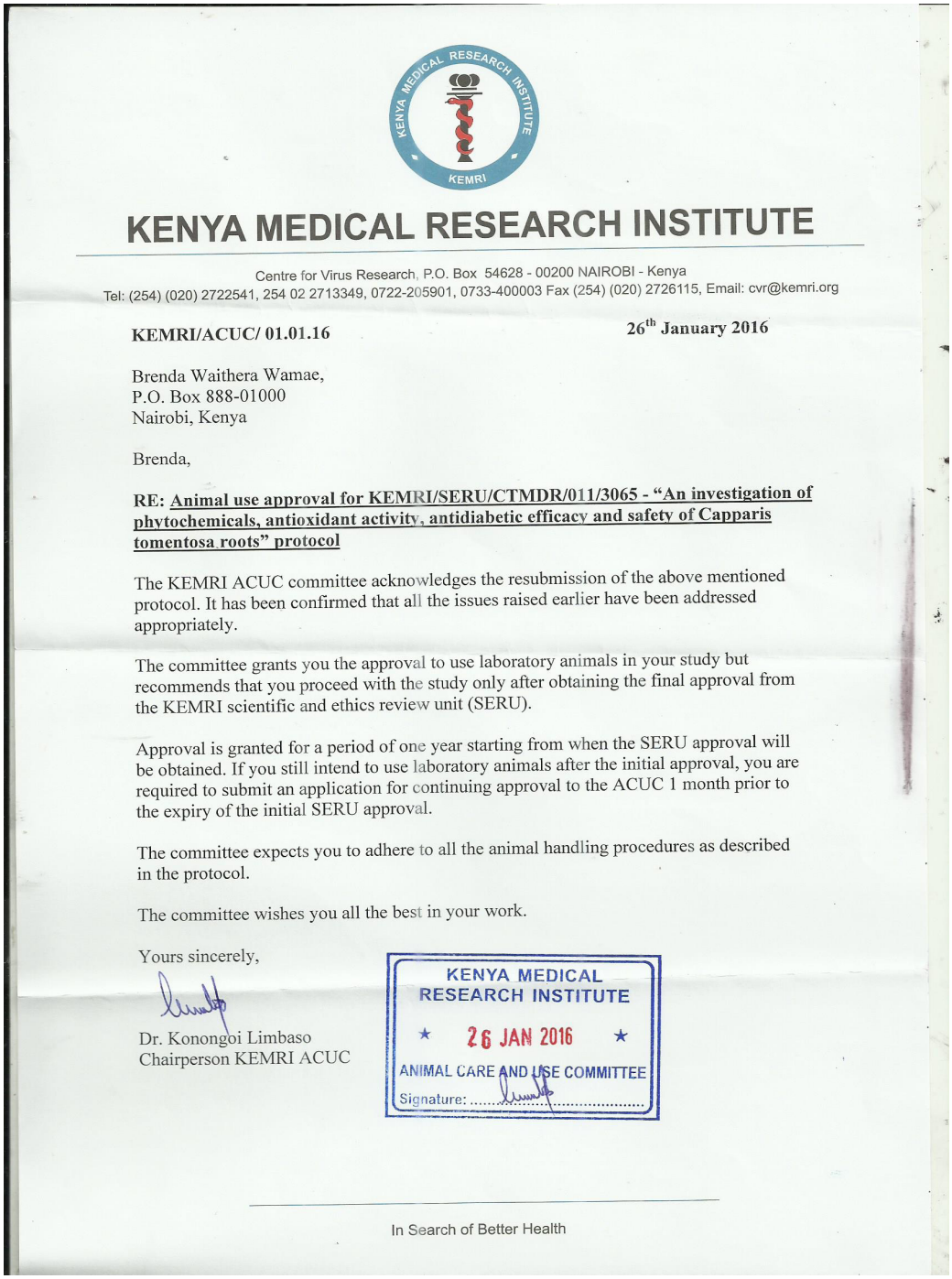
You may embark on the study.

Yours faithfully,

DR. EVANS AMUKOYE,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix VIII: Clearance letter, KEMRI Animal Care and Use Committee(ACUC)



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KEMRI/ACUC/ 01.01.16 **26th January 2016**

Brenda Waithera Wamae,
P.O. Box 888-01000
Nairobi, Kenya

Brenda,

RE: Animal use approval for KEMRI/SERU/CTMDR/011/3065 - "An investigation of phytochemicals, antioxidant activity, antidiabetic efficacy and safety of Capparis tomentosa roots" protocol

The KEMRI ACUC committee acknowledges the resubmission of the above mentioned protocol. It has been confirmed that all the issues raised earlier have been addressed appropriately.

The committee grants you the approval to use laboratory animals in your study but recommends that you proceed with the study only after obtaining the final approval from the KEMRI scientific and ethics review unit (SERU).

Approval is granted for a period of one year starting from when the SERU approval will be obtained. If you still intend to use laboratory animals after the initial approval, you are required to submit an application for continuing approval to the ACUC 1 month prior to the expiry of the initial SERU approval.

The committee expects you to adhere to all the animal handling procedures as described in the protocol.

The committee wishes you all the best in your work.

Yours sincerely,

Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

KENYA MEDICAL RESEARCH INSTITUTE
★ 26 JAN 2016 ★
ANIMAL CARE AND USE COMMITTEE
Signature: .....

In Search of Better Health

Appendix IX: Abstract of the journal on the study on *Capparis tomentosa*

Journal of Medicinal Plants Studies 2015; 3(1): 42-47

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JMPS 2015; 3(1): 42-47

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Investigation of the *in vitro* antioxidant activity, *in vivo* antidiabetic efficacy and safety of *Capparis tomentosa* aqueous roots extracts in male alloxanized mice

Laura Nyawira Wangai, Brenda Wamae Waithera, Muriira Geoffrey Karau, Ndura Boniface Koimburi, Philip Karanja Ndura, Rebecca Karanja, Michael Kimani Gitau, Peter Kirira

Abstract
The current study investigated the *in vitro* antioxidant activity, *in vivo* antidiabetic efficacy and safety of *Capparis tormentosa* aqueous root extracts. Antioxidant activity was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), phosphomolybdate and reducing power assay with ascorbic and gallic acid as references. Six groups of BALB/c mice each comprising of five were used in evaluating the antidiabetic activity. Diabetes mellitus was induced in five groups using 10% alloxan monohydrate at a dose of 186.9 mg/kg body weight. Non-diabetic control mice was orally administered with 0.1 ml physiological saline; diabetic mice with 0.075 mg of reference drug, glibenclamide at 3 mg/kg body weight; 1.25 mg, 2.5 mg, and 5 mg extracts in 0.1 ml physiological saline for 50, 100 and 200 mg/kg body weight, and the other group of diabetic mice was given 0.1 ml physiological saline. The blood glucose level was determined after 0, 2, 4, 6 and 8 hours. Safety was evaluated by daily administration of a single dose of 1000 mg/kg body weight extract to BALB/c male mice of comparable age and weight over a period of one month, while recording body weights every 7 days and organs weights after the 28th day. The antioxidant activity by DPPH was 35.50 ± 0.02%, by phosphomolybdate assay was 41.22 ± 0.17 mg/kg ascorbic acid equivalent, and the reducing power increased with increase in concentration up to a maximum at 800 µg/ml. The antidiabetic activity was dose dependent and significantly higher. There was no significant change in body weights for treated and untreated mice in safety studies ($p = 0.69$), and the weight gain was normal for both experimental and control mice. Except kidneys, which changed significantly ($p = 0.009$), all the other organ weights were not affected. The study supports the claim that *C. tormentosa* is effective and safe in the management of diabetes mellitus.

Keywords: Antidiabetic, BALB/c mice, *Capparis tormentosa*, Safety, Efficacy

1. Introduction
Diabetes mellitus is a chronic metabolic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. This deficiency in insulin production results to increase in the concentration of blood glucose, which in turn causes damage to many of the body's systems particularly the blood vessels and nerves [1]. Diabetes mellitus is a major health problem, affecting about 5% of the total population in the U.S and 3% of the world population. Epidemiological studies [2] and clinical trials [3], strongly support the premise that most of the complications are caused by hyperglycemia. Effective blood glucose control is the critical intervention measure in the management of diabetic complications and improving quality of life [4]. Thus, sustained reduction of hyperglycemia lowers the risk of developing microvascular complications and reduces the risk of macrovascular complications [5].
Capparis tomentosa belongs to the family Capparaceae commonly referred to as Woody Caper Bush in English [6]. It is an indigenous South African plant that grows naturally in the savanna forest of Western, Eastern and Southern Africa [7]. It is a scrambling shrub, sometimes maturing into a tree that can grow as high as 10 meters tall and is covered with scattered spines. It is well branched and the branches are normally covered with thick yellow hairs; even the robust, recurved spines are often hairy. The twigs and leaves are yellow-green in colour and are covered in soft, velvety hairs. The oblong leaves are approximately 50 × 20 mm, with