PHYTOCHEMICAL COMPOSITION, SAFETY AND HYPOGLYCEMIC ACTIVITY OF PURPLE TEA AND GUAVA EXTRACTS IN A MOUSE MODEL OF DIABETES MELLITUS

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DECLARATION

This thesis is my original work and has not been presented to any other university for the award of degree.

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DEDICATION

I dedicate this thesis to my dear mother Mrs. Jane Cherop, Aunties Jelagat Cherop and Nancy Cherop for their love, support and encouragement during my studies. To my uncles Benson Cherop, Albert Cherop, late Samuel Cherop and late grand mum Grace Kong'ato Cherop for their great source of inspiration to me. Thank you and God bless you.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADA	American Diabetes Association
AFR	Africa
ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
BUN	Blood urea nitrogen
CVDs	Cardiovascular diseases
DM	Diabetes mellitus
EUR	Europe
FDA	Food and Drug Administration
GCMS	Gas Chromatography Mass Spectrometry
IDDM	Insulin dependent diabetes mellitus
IDF	International Diabetes Federation
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
KDHS	Kenya Demographic and Health Survey
LCMS	Liquid Chromatography Mass Spectrometry
LD$_{50}$	Lethal dose fifty
MENA	Middle East & North Africa
MDGs	Millennium Development Goals
MTP	Mid-term plans
NAC	North America & Caribbean

NCD	Non communicable diseases
NIIDDM	Non insulin dependent diabetes mellitus
OECD	Organization for Economic Cooperation and Development
PAUISTI	Pan African University Institute for Basic Sciences, Technology and Innovation
RPM	Revolution per minute
SACA	South and Central America
SEA	South East Asia
SPSS	Statistical Package for Social Scientist
TRC	Trypanosomiasis Research Centre
WHO	World Health Organization
WP	Western Pacific

ABSTRACT

Diabetes mellitus is a metabolic disorder presenting with hyperglycemia and affects 382 million people worldwide. It is managed by insulin and oral hypoglycemic drugs, exercise and diet. Oral hypoglycemic drugs and insulin are very costly, unavailable and have severe side effects like hypoglycemia. Medicinal plants have been used for the management of diabetes and many other diseases. This is because of their reported efficacy, affordability and accessibility. However, anti-diabetic activity and safety of medicinal plants extracts are not well documented. Tea (*Camellia sinensis*) and Guava (*Psidium quajava*) are commonly used in traditional medicine in Kenya and elsewhere with increased acclaimed efficacy against diabetes mellitus. Therefore, this study is aimed at determining the safety and anti-diabetic activity of leave extracts of newly bred Kenyan purple tea and guava. Activity guided extraction was done using aqueous, ethanol, acetone, ethyl acetate and chloroform solvents. The dose levels of plant extracts tested was 300mg/kg body weight on alloxan induced diabetic mice treated for a period of 14 days. The standard drug Glibenclamide (Daonil[®]) 5mg/kg was used as positive control. Biochemical indicators including Alanine transaminase (ALT) and Blood Urea Nitrogen (BUN) were evaluated to assay any toxic effect of the plant extracts on the liver and kidneys. Histological examinations of liver, kidney and pancreas were analyzed for *in vivo* toxicity. The results were analyzed using one way ANOVA with Tukey's post hoc analysis and comparison between groups considered significant at $P<0.05$ level. The data was expressed as mean $\pm$ S.E.M (standard Error of mean) using SPSS version 16. Results of phytochemical screening of purple tea and guava leave extracts showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids. The acute oral toxicity test did not reveal any significant toxic effect resulting from purple tea and guava leave extracts. ALT and BUN levels in mice treated with the two plant extracts did not

have any significant changes compared to the mice in the control group. There were no major histopathological changes in the liver, kidneys and the pancreas and most features compared well with the control mice. *Camellia sinensis* (purple tea) and *Psidium guajava* (guava) leaf extracts showed significant ($P<0.05$) blood glucose lowering ability compared to the diabetic control group treated with normal saline. In addition, the assay of purple tea indicated that ethanolic and aqueous leaf extract (300mg/kg) were effective in lowering blood glucose from the 4th hour and 6th hour respectively after administration. Acetone extract of purple tea at a dose of 300mg/kg significantly ($P<0.05$) reduced blood glucose after 8th hour only. Both aqueous and ethanolic extracts of *Psidium guajava* at 300mg/kg were effective from the 6th hour post administration. Acetyl acetate extract of *Psidium guajava* at a dose of 300mg/kg, significantly ($P<0.05$) reduced blood glucose after 4th and 6th hour after administration. Long term reduction of blood glucose up to 14 days was demonstrated by aqueous and ethanolic leave extracts of the two plants. This was achieved by daily oral administration of the leave extracts to the mice and blood glucose assessed on day 7 and day 14 after overnight fasting. The standard drug Glibenclamide significantly ($P<0.05$) reduced blood glucose from the 4th hour upon oral administration to diabetic mice. In conclusion, purple tea and guava leaves extracts are safe and significantly reduced blood glucose in diabetic mice. Isolation of bioactive compounds responsible for the anti-diabetic activity and studying hypoglycemic activity using higher doses of the extracts is recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Diabetes is a metabolic disorder that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar (Speilman, 1993). According to World Health Organization, there are two main types of diabetes mellitus (WHO, 2013), type 1 diabetes mellitus also known as insulin dependent diabetes mellitus (IDDM) and type 2 known as non-insulin dependent diabetes mellitus (NIDDM).

Type 1 diabetes mellitus (Insulin-dependent, juvenile or childhood-onset) is characterized by deficiency in insulin production and requires daily administration of insulin. Risk factors of type 1 diabetes mellitus include genetic susceptibility, chemicals, drugs and autoimmune diseases. Symptoms normally include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, loss of weight, changes in vision and fatigue. These symptoms may occur suddenly or after some time. Type 2 diabetes mellitus (Non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes mellitus affects 90% of people with diabetes around the world (WHO, 1999), and is largely the result of excess body weight, old age, physical inactivity, family history of diabetes, unhealthy diet and impaired glucose tolerance (IGT). Symptoms may be similar to those of Type 1 diabetes mellitus, but are often less marked. As a result, the disease may be diagnosed several years after onset, once complications have already arisen (WHO, 2013).
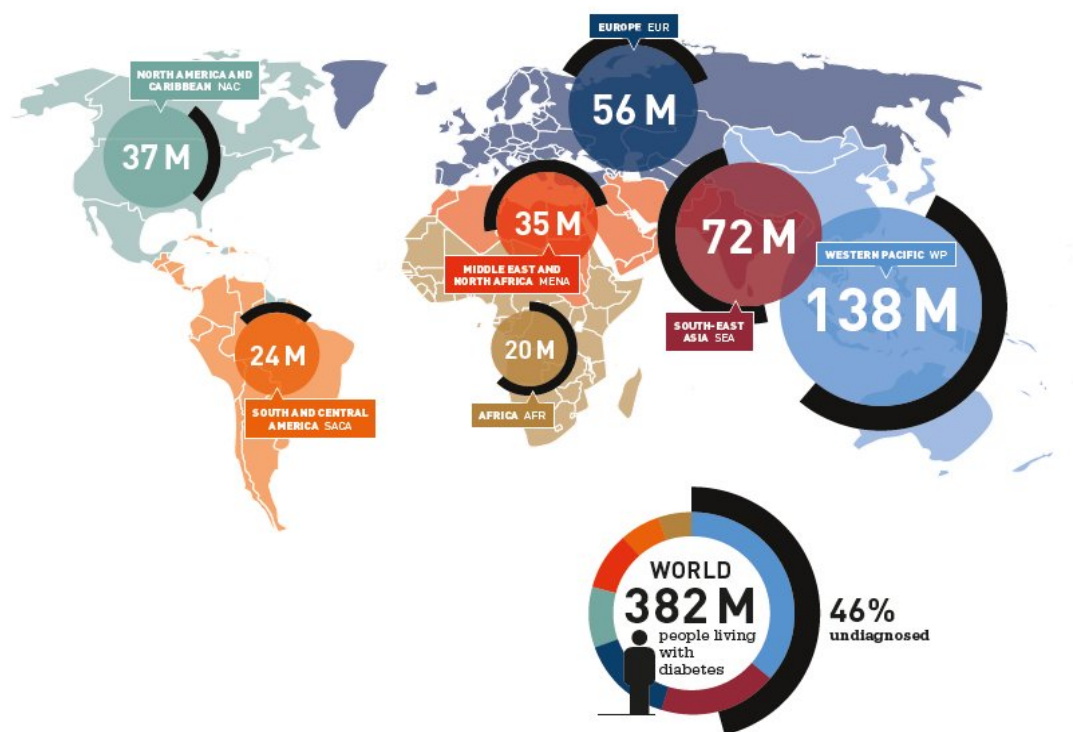
Figure 1.1: Global distribution of diabetes mellitus (IDF, 2013)

It is estimated that 382 million people live with diabetes in the world which represents prevalence rate of 8.3% (International Diabetes Federation, 2013). More than 80% of diabetes associated deaths occur in low- and middle-income countries (Mathers & Loncar, 2006). In Africa there are 20 million people with diabetes corresponding to a prevalence of 4.8% (IDF, 2013). It is estimated that in the next 20 years the number of people with diabetes in Africa will almost double. In Kenya, the prevalence of diabetes is 3.58% with 20,350 diabetes related deaths of patients between the age of 20-79 in 2013 (International Diabetes Federation, 2013). It is projected that diabetes will be the 7th leading cause of death in the world by 2030 (WHO, 2011).

Management of diabetes involves lowering blood glucose and risk factors associated with it. Healthy diet, maintaining healthy body weight, physical activity as well as tobacco use cessation

is important to avoid complications. Feasible interventions include moderate blood glucose control. Those people having type 1 diabetes require insulin. Type 2 diabetic patients can be treated with oral medication, but may also need insulin, blood pressure control and foot care (WHO, 2013). There are two main classes of oral hypoglycemic drugs, the sulphonylureas such as Tolbutamide and Glibenclamide, and the Biguanides such as Phenformin and Metformin. Other classes are Meglitinides, Thiazolidinediones and Alpha-glucosidase inhibitors.

1.2 Statement of the problem

Diabetes increases the risk of heart disease and stroke. About 50% of people with diabetes die of cardiovascular diseases (CVDs) primarily heart disease and stroke (Morrish et al., 2001). Combined with reduced blood flow, neuropathy in the feet increases the chance of foot ulcers, infection and eventual need for limb amputation. Diabetic retinopathy is an important cause of blindness and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. One percent of global blindness can be attributed to diabetes (WHO, 2012), while the disease is among the leading causes of kidney failure (WHO, 2011). The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes (Roglic et al., 2005). In 2013, 5.1 million people in the world died of diabetes accounting to 8.4% of global all-cause mortality and overall expenditure on diabetes was 548 billion US dollars (IDF, 2013).

By 2035, the number of individuals with diabetes worldwide is expected to rise to 592 million from 382 million in 2013, almost 80% of whom will be in low-income and middle-income countries (Mathers & Loncar, 2006; IDF, 2013). In these regions, diabetes drugs are often inaccessible or are too expensive and the problem is further compounded by the necessity to maintain a cold chain for instance refrigeration for purposes of preserving insulin. Use of insulin and oral hypoglycemic agents is bedeviled by the fact that they are costly and have numerous

adverse effects. This include weight gain, drug induced hypoglycemia, gastrointestinal upsets, lactic acidosis, liver toxicity, primary and secondary failure (Beatriz & Mark, 2001). Another limiting factor for insulin is that patients need injections and frequent self-monitoring of blood glucose (Reinhard, 2009). The local health care systems also do not have the personnel and financial capacity to cope with increased cases of diabetes (Lancet, 2011). The economic burden of diabetes and disability from complications can push poor families into destitution and poverty. At a national level the diabetes epidemic threatens to overwhelm health systems, and potentially reverse development gains made in low-income countries (Hu, 2011).Recently, interest has increased in using natural products for pharmacological purposes. For diabetes, few published reports show some plant extracts are effective in reducing hyperglycemia, has few side effects and cheap compared to usual antidiabetic agents (Sohn et al., 2010). In Kenya, there are plants which have shown significant antidiabetic activity which include *Bidens pilosa, Aspilia pluriseta, Erythrina abyssinica, Catha edulis* and *Srychnos henningsii* (Pierro et al., 2011).

1.3 Justification

Plant based medicinal products have been known since ancient times (Subbulakshmi & Naik , 2001). Plants have been the primary source of drugs and many of the currently available drugs have been directly or indirectly derived from plants extracts. For example, the popular hypoglycemic drug Glucophage (Metformin) is derived from *Galega officinalis* (Grover et al., 2002). Plant products and derivatives used by the pharmaceutical industry for treatment of various diseases include paclitaxel, vincristine, vinblastine, artemisinin, camptothecin and podophyllotoxin. Other drugs developed from plants are Morphine from the plant Opium Poppy (*Papaver somniferum*) and Quinine from Cinchona tree (Bhushan et al., 2004). The

recommendation of WHO on Diabetes encouraging research on hypoglycemic agents of plant origin used in traditional medicine has greatly motivated research in this area (WHO, 1980). Medicinal plants have for long been used for treatment of many diseases including management of diabetes mellitus. They have the benefit of affordability and accessibility (Njagi et al., 2012). Without proper management of diabetes mellitus, it will affect achievement of the Vision 2030 where Kenya intends to provide high quality life to its citizens and become middle income country by the year 2030.

The 2 selected plants were chosen because of their wide use in traditional medicine without proper scientific proofs in Kenya and globally. It has recently been reported that the major green tea polyphenolic constituent, epigallocatechin 3-gallate, mimics the cellular effects of insulin (Koyama et al., 2004). Green tea has been shown to have hypoglycemic effects (Tsuneki et al., 2004). Purple tea, a new variety of tea has recently been introduced to farmers by Kenya Tea Research Foundation (Tea Board of Kenya, 2009; Kerio, 2012). Although the anthocyanin rich purple tea is being promoted to have higher medicinal value than green tea, little research has been done to investigate the hypothesis. *Psidium guajava* is also used as a hypoglycemic agent in folk medicine (Conway, 2001). Guava juice has been proved to exhibit hypoglycemic effects in mice (Cheng & Yang, 1983). However, the safety of the extracts has not been documented. Therefore, this scientific study aimed at investigating the safety and hypoglycemic activity of purple tea and guava in Kenya.

1.4 Null Hypothesis

Extracts of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) are unsafe and lack hypoglycemic activity in a mouse model of diabetes mellitus.

1.5 Research questions

i.   What are the major phytochemical constituents of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) leaves?

ii. What is the safety of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) leave extracts in normal Swiss white mice?

iii. What is the hypoglycemic activity of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) in diabetic Swiss white mice?

1.6 Broad objective

To determine the phytochemical composition, safety and hypoglycemic activity of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) leave extracts in a mouse model of diabetes mellitus.

1.7 Specific objectives

i.   To determine the phytochemical constituents of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) leaves

ii. To determine the safety of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) leave extracts in normal Swiss white mice

iii. To determine the hypoglycemic activity of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Guava) leave extracts in diabetic Swiss white mice

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes epidemiology in Kenya and globally

The epidemiology of diabetes in Kenya has not been studied to any great extent. Anecdotal evidence from health care services suggests that the incidence of diabetes is on the increase. The best estimate of diabetes is from an opportunity sample of an urban and rural population that reported a non-age adjusted prevalence of 4.2% (Christensen et al., 2009). But in 2013, IDF posted diabetes prevalence of 3.58% in Kenya and 8.3 % globally representing 382 million people living with diabetes. However, local studies have shown a prevalence of 4.2% in the general population with a prevalence rate of 2.2% in the rural areas and as high as 12.2% in urban areas (Christensen et al., 2009). The prevalence of impaired glucose tolerance in Kenya is equally high at 8.6% in the rural population and 13.2% in the urban population (Christensen et al., 2009). Urbanization in Kenya has led to adoption of western lifestyles like vehicle transport, unhealthy diets rich in carbohydrates, fats, sugars leaving traditional lifestyles like high fiber whole grain foods, vegetables and fruits (IDF, 2009). This western lifestyle has led to rise in obesity and overweight people increasing the risk of diabetes. In 2003, Kenya Demographic and Health Survey showed 20% of women and 7% of men were obese or overweight (KDHS, 2003). Recent studies have shown higher overweight figures of 60.3% and 19.5% for women and men, respectively in urban areas compared to 22.6% and 10% in women and men respectively, in rural areas (Christensen, 2008).

Globally, people with diabetes were 382 million in 2013 and it is projected that in 2035, there will be 55% increase to 592 million diabetic patients which will represent a prevalence of 10.1%,

an increase from 8.3% in 2013. About 80% of diabetic patients in the world live in low and middle-income countries (WHO, 2013). There are more diabetic patients living in the urban (246 million) than in rural (136 million) areas although the number for rural areas are on the increase (IDF, 2013).
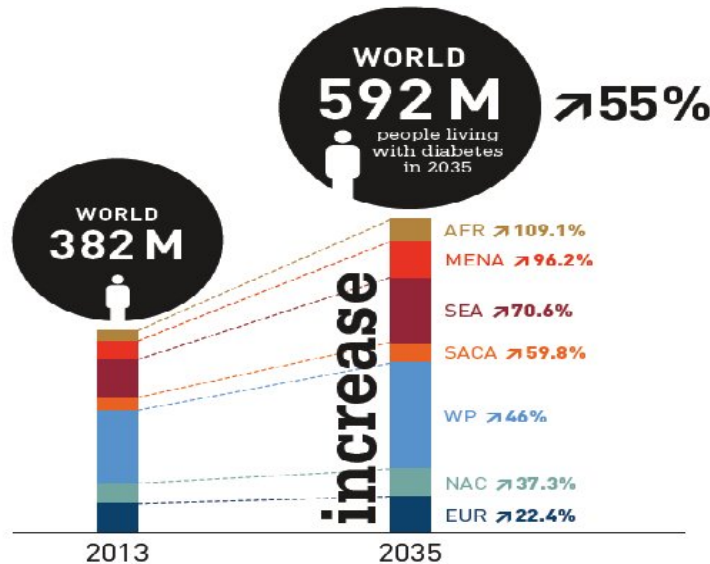
Figure 2.2: People with diabetes in 2013 and 2035 projection (IDF, 2013)

Legend: AFR-Africa, MENA-Middle East & North Africa, SEA-South East Asia, SACA-South and Central America, WP-Western Pacific, NAC-North America & Caribbean, EUR-Europe

2.2 Aetiology of diabetes mellitus

Diabetes mellitus is a chronic disease that occurs when the body cannot produce enough insulin or do not utilize insulin effectively (Harris et al., 1997). Insulin is a hormone produced by the pancreas that allows glucose from the food to enter the body's cells where it is converted to energy needed by muscles and tissues to function. Depending on the aetiology, diabetes mellitus can be divided into four principal forms. These are Type 1 diabetes mellitus, Type 2 diabetes mellitus, Gestational diabetes mellitus and pre-diabetes (IDF, 2013). Type 1 diabetes mellitus

occurs in childhood and is primarily due to autoimmune-mediated destruction of pancreatic beta cells resulting in absolute insulin deficiency. People with type 1 diabetes mellitus must take exogenous insulin for survival (Clement et al., 2004).

Type 2 diabetes mellitus is characterized by insulin resistance and/or abnormal insulin secretion. It is caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, lack of physical activity, stress, as well as aging (Kaku, 2010;Basu et al.,2003). People with type 2 diabetes mellitus are not dependent on exogenous insulin, but may require it for control of blood glucose levels if diet or oral hypoglycemics cannot normalize the blood glucose (Taylor, 2013; Thevenod, 2008). Gestation diabetes manifest in women who develop a resistance to insulin and subsequent high blood glucose during pregnancy. Pre-diabetes occurs when blood glucose levels are higher than normal but not high enough to be diagnosed as diabetes mellitus (IDF, 2013).
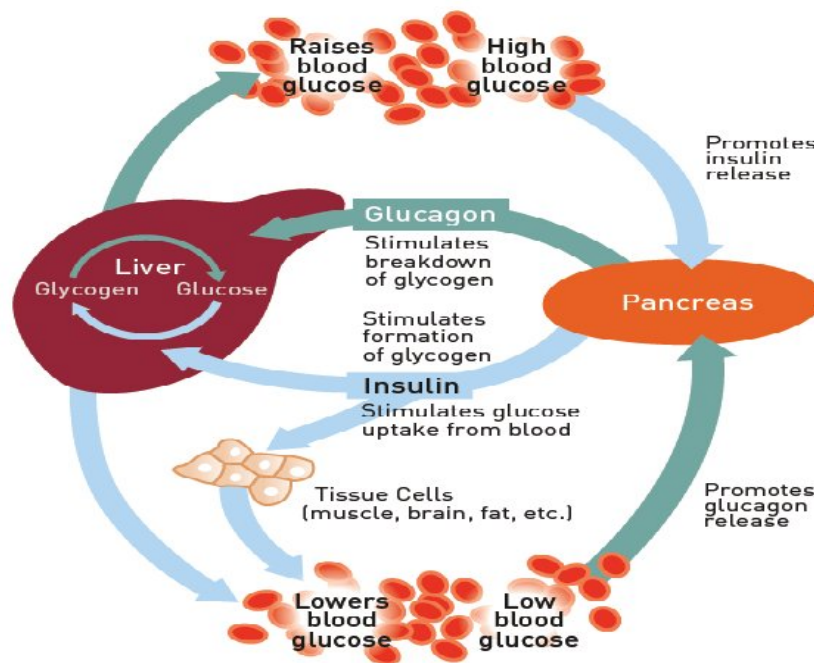
Figure 2.1: Insulin production and action (IDF, 2013)

2.3 Pathogenesis of diabetes mellitus

Type 2 diabetes mellitus involves at least two primary pathogenic mechanisms. These are progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and peripheral insulin resistance resulting in a decrease in the metabolic responses to insulin (Boada & Moreno, 2013; American Diabetes Association, 2010). The transition from normal control of glucose metabolism to type 2 diabetes mellitus occurs through intermediated states of altered metabolism that worsen over time. As the blood glucose levels rises even a small amount above normal, then acquired defects in the glucose homeostasis system occur initially to impair the beta cell's glucose responsiveness to meals by impairing the first phase insulin response and cause increase in blood glucose into the range of impaired glucose tolerance (IGT). This rise in blood glucose and insulin resistance cause deterioration in beta-cell function and the blood glucose levels rise to full-blown diabetes (Thevenod, 2008; Leahy, 2005).

2.4 Clinical signs of diabetes mellitus

The clinical signs of untreated diabetes are related to increased blood glucose and loss of glucose in the urine. A high amount of glucose in the urine causes increased urine output and lead to dehydration which causes increased thirst and water consumption. Other symptoms of untreated diabetes include weight loss, fatigue, nausea, vomiting and patients are prone to infections of the bladder, skin and vaginal areas. Fluctuations in blood glucose levels can also lead to blurred vision and extremely elevated glucose levels can lead to lethargy and coma (WHO, 2013; Gupta & De, 2012; American Diabetes Association, 2010).

2.5 Diabetes management

Blood glucose control is often the theme of diabetes management to help a patient live long and healthier. People with type I diabetes require insulin while with type II diabetes can be treated

with oral medication but may also require insulin. WHO also recommends blood pressure control, foot care, screening and treatment of retinopathy, blood lipid control and screening for early signs of diabetes related kidney disease (WHO, 2013). The two main classes of oral hypoglycemic drugs are Sulphonylureas such as Tolbutamide and Glibenclamide, and Biguanides such as Phenformin and Metformin (Beatriz & Mark, 2001). The major components of diabetes management are diet combined with exercise, oral hypoglycaemic therapy and insulin treatment. Diet treatment aims at ensuring weight control, providing nutritional requirements, allow good glycemic control to normal. Physical activity promotes weight reduction and improves insulin sensitivity thus lowering blood glucose levels. Together with dietary treatment, a programme of regular physical activity and exercise should be considered for patients with diabetes. Oral hypoglycemic drugs are considered only after a regimen of dietary treatment combined with exercise has failed to reduce the blood glucose to targets levels. Sulphonylureas act by stimulating insulin release from beta cells and by promoting insulin action through extrapancreatic mechanisms (Gribble et al., 1998). Biguanides exert their action by decreasing gluconeogenesis and by increasing the peripheral utilization of glucose. Selection of specific sulfonylurea preparation depends on factors such as availability, cost and physician's experience. Glibenclamide can be administered in an initial dose of 1.25-2.5mg which can be increased up to a maximum of 15 mg. For tolbutamide like Metformin, the initial dose is 0.5g which can be increased if necessary to 1.5g in divided doses. Metformin is primarily used in diabetic obese patients not responding to dietary therapy (Defronzo, 1999; WHO, 1994). Insulin is indicated in management of diabetes mellitus when diet and oral hypoglycemic drugs fail control hyperglycemia and achieve therapy targets. Insulin is also indicated in diabetes during pregnancy when diet alone is inadequate and during stressful conditions such as infection or

surgery (Clement et al., 2004). In the current study, Glibenclamide was chosen as the standard oral diabetic drug due to its availability, cost effective, efficacious and recommended by World Health Organization to be used in management of type 2 diabetes mellitus (WHO, 2013).

2.6 Medicinal plants used in management of diabetes mellitus

Since time immemorial many plants have been used for treatment of diabetes because they help lower the blood glucose levels of the diabetic patient.

2.6.1 *Cucurbita pepo* (pumpkin)

Pumpkins (genus; Cucurbita) belong to the family of Cucurbitaceae. They are classified as *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita maxima* and *Cucurbita mixta* according to the texture and shape of plants like stems. Various studies have concluded that the ethanol extracts of Cucurbitaceae family fruits, cucumber, white pumpkin and ridge gourd has significant antihyperglycemic effects in alloxan induced diabetic rats. Cucumber, white pumpkin and ridge gourd 200mg/kg ethanolic fruit extracts reduced blood glucose level by 67, 65 and 51% respectively at 12 hours after single intraperitoneal injection (Sharmin et al., 2013). They also have the capacity to reduce the elevated lipid profiles in alloxan induced diabetic rats. Ridge gourd has also significant effects to restore the depressed hepatic glycogen levels in diabetic rats (Sharmini et al., 2013). Pumpkin fruit powder at a dose of 1g/kg on alloxan induced diabetic rats significantly decreased blood glucose after 4 weeks upon administration to the diabetic rats (Sedighen, 2011).

2.6.2 *Psidium guajava* (guava)

Psidium guajava is a common tree widely distributed as a native plant in Africa, Asia and America from Mexico to Brazil. The bark, leaves, fruit and root have been evaluated

pharmacologically for the treatment of gastrointestinal diseases. Guava possesses antimicrobial, antimutagenic and hypoglycemic properties (Gutierrez et al., 2008)

In traditional folk medicine extracts of roots, bark and leaves of *Psidium guava* (family: Myrtaceae) have been used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat and inflamed gums (Morton, 1987). It is widely cultivated plant in Kenya because of its edible fruit, wood, animal feed and medicinal value. The fruit has several carotenoids such as phytofluene, ß-carotene, ß cryptoxanthin, lycopene, rubixanthin and lutein (Thaipong et al., 2006). The extract of the whole plant of *Psidium guajava* excluding roots was reported to have antibacterial, antifungal, antiviral, hypoglycemic, diuretic and anti-inflammatory activities (Tripathy et al., 1981). One study showed that guava juice exhibited hypoglycemic effects in mice (Cheng & Yang, 1983). However, its effects on other biochemical parameters as well as its safety have not been well assessed. Further, the hypoglycemic properties of other parts of the plants like leaves have not been investigated.

2.6.2.1 Morphology of *Psidium guajava*

Guava is a low evergreen tree or shrub of 6 to 25 feet high, with wide spreading branches and square, downy twigs, is a native of tropical America. It is a common vegetation cover by roads and in waste places in Asia and Africa. Guava is a tropical and semitropical plant and well known in the islands for edible fruits. The plant is common in the backyards with branches which are usually crooked. The flowers are white, incurved petals, 2 or 3 in the leaf axils; they are fragrant, with four to six petals and yellow anthers.

The fruit is small, 3 to 6 cm long, pear shaped and reddish-yellow when ripe (Kavimani & Das, 1997).

Figure 2.3: *Psidium guajava* **leaves and fruit (Dana et al., 2008)**

2.6.2.2 Chemical constituent of *Psidium guajava*

Guava fruit contain vitamin A, vitamin C, iron, calcium and phosphous. Manganese is also present in the plant in combination with phosphoric acid, malic acid and oxalic acid. In addition, the fruit contains saponin combined with oleanolic acid, guaijavarin and quercetin (Joseph, 2011).

The leaves contain essential oil with major chemical components being alpha-pinene, beta-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, caryophyllene oxide, curcumene, crategolic and guayavolic acids (Siddha, 1999). The roots of guava are rich in tannin while the whole plant contains leukocyanidins, sterols and salts. Root stem, bark and leaves contain a large percentage of tannic acid. Guavas also contain high percentage of carbohydrates and salts (Deo & Shasri, 2003).

2.6.3 *Camellia sinensis* (purple tea)

Purple tea (*Camellia sinensis*) belongs to the family Theaceae.

It was developed by Tea Research Foundation (TRF) as a strategic approach to diversify and add value to the tea products for the domestic and international markets. It contains red and purple anthocyanins which are related to similar anthocyanin compounds found in blueberries, raspberries, purple grapes and other common foods that contribute to their characteristic colors and health benefits (Rivero-pevez, 2008). Tea is one of the most ancient and popular therapeutic beverages consumed around the world. It can be prepared as a drink, which can have many systemic health effects or an "extract" can be made from the leaves for use as medicine.

Figure 2.4: Purple tea leaves (Kerio et al., 2012)

The new tea variety has been pre-released in response to the government's vision 2030 and Medium-Term Plan 2008-2012 (MTP), targeting new tea products diversification and value addition in order to enhance productivity of teas as well as to boost economic growth in the agricultural sector (Tea Board of Kenya, 2009).

2.6.3.1 Chemical composition of *Camellia sinensis*

Purple tea contains bioactive compounds of which one third is polyphenols with high antioxidant potency (Kerio, 2013; Tariq, 2010) with anti-proliferation and radiation sensitizing effects

important in cancer management (Linn et al., 2012). In addition, tea has alkaloids (caffeine, theophylline and theobromine), amino acids, carbohydrates, proteins, chlorophyll, fluoride, aluminium, minerals and trace elements (Cabrera, 2003). The polyphenols in purple tea includes catechins thought to be responsible for the health benefits that have traditionally been attributed to tea (Kerio, 2013; Cabrere, 2006).

Long term administration of green tea extract to normal rats have been shown to increase insulin sensitivity (Wu, 2004a).When tea extracts was administered to rats fed on fructose, it was found to prevent development of insulin resistance, hyperglycemia and other metabolic defects in rats (Wu, 2004b). Studies have shown that green tea promote glucose metabolism in healthy human volunteers at 1.5g/body weight in oral glucose tolerance tests (Tsuneki, 2004). Green tea extract for 16 weeks at a daily dose of 856mg of epigallocatechin gallate on obese individuals with type two diabetes significantly reduced glycosylated hemoglobin (HbA1C) and had significant reduction in waist circumference (Hsu et al., 2011).

It is important to note that tea farming is the largest agribusiness in Kenya, a major foreign exchange earner and a source of livelihood for 3 million Kenyans along its value chain. It contributes over 26% of total foreign exchange earnings and over 4% of GDP (Economic survey, 2005). To increase the marketability of the new purple variety, it will be important to determine its medicinal value. The current study aims at establishing the hypoglycemic activity and safety of the newly bred anthocyanin rich purple tea cultivars grown in Kenya.

2.7 Importance of herbal medicines

Herbal medicines are cost effective, more readily available to people but a lot needs to be done to establish their safety, which in common practice is presumed from historical traditional use.

Besides, many plants are being rendered extinct by human activity, which makes it necessary to study them before they become extinct and advocate for their conservation; aside from the disintegration of traditional knowledge on their use with changing culture (Piero et al., 2011)

About 200,000 natural products from plants have been known and many more are being identified from higher plants and microorganisms (Kinghorn et al., 2011). Major current available drugs have been derived from plants. This includes the current oral antidiabetic drug Metformin from the plant *Galega officinalis* (Grover et al., 2002). Arthemeter and Quinine which are important drugs in treatment of malaria are also derived from the plant *Artemisia annua* (Klayman, 1985) and *Cinchona officinalis* respectively (Bhushan et al., 2004).

In recent years, there has been increased interest in development of definitive curative options of diabetes as well as its management (Shafrir, 1997; Bailey, 2001).

2.8 Phytochemical screening of medicinal plants

 Medicinal plants are the richest biological resources of drugs in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for many synthetic drugs (Ncube et al., 2008).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are natural bioactive compounds found in plant food, leaves, roots, flowers or other parts of plants that interplay with nutrients and dietary fiber to protect them. Recent research demonstrates that they can protect humans against diseases as well as, in risk reduction for a variety of chronic or inflammatory conditions. Some of the well known phytochemicals include lycopenes found in tomatoes, isoflavones in soy and flavonoids in fruits and Tea. There are different types of flavonoids and each appears to have protective effects including anti-inflammatory, anti-oxidant, antiviral and even anti-carcinogenic properties. Flavonoids are

generally found in a variety of foods, such as oranges, tangerines, berries, apples and onions (Middleton et al., 2000). The high flavonoids content in tea is thought to induce anti-inflammatory effects in disease models in mice (Karori et al., 2008).

The purpose of standardized extraction procedures of crude drugs from medicinal plant parts is to achieve therapeutically desired portions and eliminate unwanted material by treatment with a selective solvent. The extract obtained after standardization, may be used as medicinal agent in form of tinctures or fluid extracts or can be further processed to be incorporated in any dosage form such as tablets or capsules. These plant products contain complex mixture of many medicinal plant metabolites such as alkaloids, glycosides, terpenoids, saponins, flavonoids and lignans (Handa et al., 2008).

Plants have active compounds which are important components of phytomedicine for a very long time. It is possible to obtain wonderful assortment of industrial chemicals from plants. Plant based natural constituents may be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds since any part of the plant may contain active components. The systematic screening of plants species with the purpose of discovering new bioactive compounds is a routine activity in many research laboratories. Scientific analysis of plant components follows a systematic logical pathway. Plants are normally collected either randomly or by following leads supplied by local healers/herbalist in geographical areas where the plants are found (Parekh et al., 2006).

Most results of phytochemical screening of plants having hypoglycemic effects like *Blighia sapida* root bark have shown the presence of alkaloids, saponins, cardiac glycosides, reducing sugar, carbohydrates, flavonoids, phenol and tannin. These metabolites are known to show biological activity as well as exhibiting physiological activity (Sofowara, 1993). It is reported

that *Blighia sapida* contain two hypoglycins A and B that have strong hypoglycemic action in most animals and man (Bever, 1980). Reduction in blood glucose by these bioactive compounds from plants might act by one of several many mechanisms. Some of them may inhibit endogenous glucose production (Eddouks et al., 2003) or interfere with gastrointestinal glucose absorption (Musabayane et al., 2006). Other bioactive compounds may also have insulin-like activity (Gray & Flat, 1999) or may inhibit insulinase activity. They may also increase secretion of insulin from the ß cells of the pancreas that is pancreatotropic action (Khan et al., 1990; Trevedi et al., 2004; Yadav et al., 2008). Bioactive compounds may increase beta cells in pancreas by activating regeneration of beta cells (Jelodar et al., 2007).

There are various solvent which are normally used in extraction procedures. These include water, acetone, alcohol, ethanol, chloroform and ether. Water is a universal solvent used to extract plant products with medicinal activity. Though traditional healers use primary water, plant extracts from organic solvents have been found to yield more consistent antimicrobial and hypoglycemic activity compared to water extract. Water soluble flavonoids especially anthocyanins and phenols which are antioxidants have been extracted using aqueous solvents (Das et al., 2010).

Acetone dissolves many hydrophilic and lipophilic components from plants extracts. It is miscible with water, volatile and has low toxicity to the bioassay used. It is very useful extractant especially for antimicrobial and hypoglycemic studies where more phenolic compounds are required to be extracted. Various studies have reported that extraction of tannins and other phenolics was better in aqueous acetone than in aqueous methanol (Das et al., 2010, Eloff, 1998). Indeed both acetone and methanol have been found to extract saponins which have antimicrobial activity (Ncube et al., 2008).

The other solvent which is commonly used in extraction of bioactive components from plants is alcohol. The higher activity of the ethanolic extracts as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols compared to aqueous extracts. This means that they are more efficient in cell walls and seeds degradation which have non polar character and cause polyphenols to be released from cells. A more useful explanation for the decrease in activity of aqueous extract can be attributed to the enzyme polyphenol oxidase, which degrade polyphenols in water extracts but the enzyme is inactive in methanol and ethanol. Moreover, water is usually a better medium for the occurrence of the micro-organisms as compared to ethanol (Larpornik et al., 2005). The higher concentrations of more bioactive flavonoid compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding more water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent was increased (Bimakr, 2010).

Phytochemical screening can involve techniques using chloroform extraction. Terpenoid lactones have been obtained by successive extraction of dried barks with hexane, chloroform and methanol with activity concentrating in chloroform fraction (Cowan, 1999). Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999). Ether is used selectively for the extraction of courmarins and fatty acids.

2.9 Safety of medicinal plants

The earliest report of the toxicity of herbs originated from Galen who was a Greek pharmacist and physician and showed that herbs do not contain only medicinally beneficial constituents, but may also be constituted with harmful substances (Cheng et al., 2004).

Drug management of diabetes mellitus without associated side effects has also remained a challenge for medical practice in the world. This has necessitated exploration and screening of medicinal plants with acclaimed therapeutic efficacies in diabetes management as recommended by the WHO Expert Committee on Diabetes Mellitus (WHO, 1980; WHO, 2002).

By 2003 in the United States, over 1500 herbal products sold were nutraceuticals which are exempt from extensive preclinical efficacy and toxicity testing by the U.S. Food and Drug Administration (Bent, 2004). This increased usage, which has been reported in other countries, has led to increased concerns about potential harmful effect of these plant products, which has resulted in efforts to globally harmonize standards of toxicity testing methods. Methods used for herbal medicine toxicological characterization include tests for acute high dose exposure effects, chronic low dose toxicity tests and specific cellular, organ and system based toxicity assays.

Because herbs are plants, they are often perceived as "natural" and therefore very safe (Ernst, 1998). However, different side effects of herbs have been reported and recently reviewed (Achila et al., 2013; De Smet, 2002) including effects from biologically active constituents from herbs, side effects caused by contaminants and herb–drug interactions. A particularly morbid case series describes 105 patients in Belgium who had been taking a Chinese herbal product for weight loss and eventually developed nephropathy caused by the herb *Aristolochia fangchi*. Forty three patients developed end stage renal failure and 39 had prophylactic kidney removal. Eighteen of these patients were found to have urothelial carcinoma which was shown to be related to the formation of many DNA adducts from the aristolochic acid in this herb (Nortier et al., 2000).

Another common toxicity to herbal medicines involves pyrrolizidine alkaloids which are complex molecules found in certain plants that may be used or inadvertently added to herbal medicines. These alkaloids produce hepatotoxicity through a characteristic veno occlusive disease that may be rapidly progressive and fatal (Stickel et al., 2005).

In Kenya, despite the growing market demand for herbal medicines, there are still concerns associated with not only their use, but their safety (Achila et al., 2013). Less than 10% of herbal products in the world market are truly standardized to known active components and strict quality control measures are not always diligently adhered to (Winston, 2007). For majority of these products in use, very little is known about their toxic constituents. In Kenya, herbal medicines are not subjected to the same regulatory standards as conventional drugs in terms of efficacy and safety (Achila et al., 2013). This raises concern on their safety and implications for their use as medicines. Toxicity testing can reveal some of the risks that may be associated with use of herbs, therefore avoiding potential harmful effects when used as medicine.

Another implication in the toxicity of certain herbs is the presence of toxic minerals and heavy metals like mercury, arsenic, lead and cadmium (Achila et al., 2013; Dwivedi, 2002). Lead and mercury can cause serious neurological impairment when herbal medicinal product contaminated with these metals is ingested.

The primary aim of toxicological assessment of any herbal medicine is to identify adverse effects and to determine limits of exposure level at which such effects occur. Two important factors which are taken into consideration in evaluating the safety of any herbal drug are the nature and significance of the adverse effect and in addition, the exposure level where the effect is observed. Toxicity testing can reveal some of the risks that may be associated with use of herbs especially in sensitive populations. An equally important objective of toxicity testing is the detection of

toxic plant extracts or compounds derived thereof in the early (pre-clinical) and late (clinical) stages of drug discovery and development from plant sources. This will facilitate the identification of toxicants which can be discarded or modified during the process and create an opportunity for extensive evaluation of safer, promising alternatives (Gamaniel, 2000).

Brine shrimp (Artemia sp.) is a small invertebrate that dwell in sea water and other saline ecosystems. They are frequently used in laboratory studies to evaluate toxicity values as a measure of median lethal concentration values or LC50. They offer a simple, quick and cost effective way to test plant extracts toxity (McLaughlin, 1991).

Acute toxicity analysis of many medicinal plants extracts is been researched to obtain safer drugs. The animal models used include Wister albino rats, mice, non-human primates (Rees, 2004). Animals of each species are randomly divided into two equal groups, control group and experimental groups. The experimental animals are dosed orally with extracts at various and closely observed for 24hrs for any mortality and next 10 or 14 days for any delayed toxic effects on gross behavioral activities (Mowla et al., 2009).

Changes in plasma or serum enzymes and isoenzymes are useful indicators of tissue damage in many diseases including kidney failure, liver toxicities, hepatitis and cardiovascular diseases. Enzyme increase is usually related to leakage of enzymes from damaged cells. The amount of increase depends on factors such as the concentration of enzyme in the cells, the rate of leakage of enzyme from cells and the rate of clearance of enzyme from plasma (Boyd, 1983).

Alanine aminotransferase (ALT) is an important enzyme in the intermediary metabolism of glucose and protein catalyzing the reversible transamination between alanine and 2-oxoglutarate

to form pyruvate and glutamate. It is used as a marker for liver injury and in preclinical toxicity studies. Serum ALT activity is significantly elevated in a variety of liver conditions, including viral infection, cirrhosis, non-alcoholic steatohepatitis and drug toxicity (Knapen et al., 2000).

The most frequently determined clinical biomarker for estimating renal function is blood urea nitrogen (BUN). Blood urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. In kidneys, urea is filtered out of blood by glomerulli and is partially being reabsorbed with water depending upon concentration in the serum (Mitchel, 2006). The markers of renal function test assess the normal functioning of kidneys. They indicate the glomerular filtration rate, concentrating and diluting capacity of kidneys (tubular function). An increase or decrease in the level of blood urea nitrogen indicates kidney dysfunction associated with kidney disease or failure, blockage of the urinary tract by a kidney stone or drug induced toxicities. (Gowda et al., 2010). If the BUN level is higher than 100 mg/dL, it points to severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma, surgery, opioids, malnutrition and anabolic steroid use (Pagana & Kathleen, 2002).

Because many herbs contain pharmacologically active compounds, some may cause severe side effects through excessive biological activities. Therefore, the need to determine the safety of the plants extracts is important.

2.10 Analysis of herbal products efficacy

State-of-the-art screening for bioactivity in natural products is a 'top down' approach. This normally starts with the larger plant units like whole plant material and working down to the specific fractions or combinations responsible for the bioactivity of interest. Briefly, the source plant materials are collected and preprocessed for ease of obtaining the 'working mixture'. This

mixture refer to the form administered for therapeutic purposes and can be in the form of powders, teas or infusions, decoction, juice, tincture, or poultice (Campel, 2012).

There have been many studies on hypoglycemic plants and a great variety of compounds have been isolated for instance alkaloids, glycosides, terpenes and flavonoids. The main challenge is further development of such 'leads' into clinically useful drugs and especially phytomedicines or adequate nutritional supplements, which would be of direct benefits to patients. Modern drug Metformin (a biguanide) is a derivate of an active natural product is used for diabetes management. Galegine, a guanidine isolated from *Galega officinalis* L. is very important plant extract used in medieval times to relieve the intense urination in diabetic people (Witters, 2001).

There are numerous traditional medicinal plants which have significant hypoglycemic properties such as *Allium sativum* (Garlic), *Azadirachta indica* (Neem), *Vinca rosea* (Nayantara), *Trigonella foenum* (Fenugreek), *Momordica charantia* (Bitter ground), *Ocimum santum* (Tulsi). Many other plant extracts are less effective in lowering glucose levels in severe diabetes (Ahmed et al, 2010) and their use have not been validated in scientific studies.

For instance, it has been found that methanolic whole plant extract of *Vinca rosea* at high dose of 500mg/Kg is more effective than whole plant extract at low dose of 300mg/kg after 14 days of treatment. The methanolic whole plant extract of *Vinca rosea* at high dose (500mg/kg) shows similar curative effect as the standard drug Glibenclamide (5mg/kg) (Mohammed et al., 2010).

Extensive animal and human clinical evaluations of efficacy and toxicity of herbal medicines should be done before diabetic patients can rely solely on the plant-based therapies for controlling type 2 diabetes. Some of these plant-derived medicines offer potential for cost-effective management of diabetes through dietary interventions, nutrient supplementation and

combination therapies with synthetic drugs in the short term and as sole medication from natural sources over the long term period (Srinivasan et al., 2007).

Unfortunately, after the introduction of Sulfonylurea and Metformin about 50 years ago, no major lead has been obtained in this direction with an objective of finding a very safe and efficacious drug for diabetes mellitus. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered to be good candidates for new drugs or a lead to make a new drug. Plant extract or different folk plant preparations are being prescribed by the traditional practitioners and accepted by diabetic patients in many countries especially in third world countries Kenya included. Currently, more than 400 plants are being used in different forms for hypoglycemic effects although they have not been validated scientifically (Nahar, 1993).

2.11 Animal models of diabetes mellitus

Animal models have been used extensively in diabetes research. Early studies used pancreatectomised dogs to confirm the central role of the pancreas in glucose homeostasis, culminating in the discovery and purification of insulin (Rees, 2004). Most studies are carried out on rodents like Swiss white mice and on larger animals like baboons. Several toxins with diabetogenic effects in experimental animals include streptozotocin and alloxan have been used in induction of diabetes in rats and mice (Sengupta et al., 2011; Prasad, 2009). Selective inbreeding has produced many strains of animal models that are considered reasonable models of type 1 and type 2 diabetes mellitus. Apart from their use in studying the pathogenesis of diabetes mellitus and its complications, all new treatments including islet cell transplantation and preventive strategies are initially investigated in animal models. The most common models for type 1 diabetes include non-obese diabetic (NOD) mouse and bio breeding (BB) rat which

spontaneously develop diabetes similar to human type 1 diabtes (Nakhooda et al., 1977). Other type 1 model includes Long Evans Tokushima Lean (LETL) rat, New Zealand white rabbit, keeshond dog, Chinese hamster and Celebes black ape (Rees, 2004). Animal models of type 2 diabetes are as complex and heterogeneous as the human condition. The models for type 2 diabetes include Ob/Ob mouse, db/db mouse, Zucker rat, KK mouse, NSY mouse, alloxan/streptozotocin treated mouse, Diabetic Torri rat and New Zealand Obese mouse (Zhang et al., 1994; Nakamura & Yamada, 1967).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study site

The study was carried out at in the Departments of Biochemistry and Chemistry, in Jomo Kenyatta University of Agriculture and Technology. The site was chosen because of the availability of facilities and equipments of great significance to the study. These include, drying house, laboratory safety hoods important during extraction, Reflotron machine for biochemical analysis, and animal facility for the laboratory animals among others.

3.2 Collection of *Camellia sinensis* and *Psidium guajava*

The leaves of *Camellia sinensis* (Purple tea variety, TRFK 306/1) and *Psidium guajava* (Guava) were collected during the month of January 2014 and authenticated by a Botanist in the Department of Botany of the Jomo Kenyatta University of Agriculture and Technology. The leaves of *Camellia sinensis* were collected from a farm in Brooke village, Kericho County while the leaves of *Psidium guava* were collected from a farm in Mumol village, Baringo central sub-county, Baringo County. Voucher specimens were deposited at the herbarium in Jomo Kenyatta University of Agriculture and Technology.

 Two kilograms of purple tea leaves and a bud were hand-picked and transported to drying house at Botany laboratory in Jomo Kenyatta University of Agriculture and Technology. The leaves were then dried under a shade at room temperatures. Similarly, the leaves of guava were harvested by cutting the huge branches for easy picking of the leaves on the ground. Two kilograms of fresh leaves were then transported to drying house located at Botany department of

the Jomo Kenyatta University of Agriculture and Technology and kept at room temperatures to dry under a shade.

3.3 Activity guided extraction of the medicinal plants

Activity guided extraction was carried out as described by Mohamed, (2011) and Larpornik, (2005) using chloroform, ethyl acetate, acetone, ethanol and water as solvents. Briefly, the fresh leaves of *Psidium guajava* and *Camellia sinensis* were dried for two weeks under a shade at 25ºC at low humidity. After drying, the dried leaves were milled using an electric mill (GB-36, Henan, China). The milled plant material was weighed and kept at room temperatures away from direct sunlight in closed dry plastic bags.

For successful isolation of the active compounds from the plants leaves, the crude extracts were extracted with various organic solvents differing in their polarity, from highly polar to non-polar, and each obtained extract was subjected to bioassay**.**

Extraction was performed with organic solvents in increasing order of polarity from chloroform (chloroform extract), ethyl acetate(ethyl acetate extract), acetone (acetone extract), ethanol (ethanol extract) and water (aqueous extract) crude leaf extract so that the active components soluble in that particular solvent could be extracted and further assayed for their safety and hypoglycemic activity.

Cold extraction was done in which the coarsely powdered plant material was placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter was dissolved. The powdered leaves extracts of *Psidium guajava* were divided into 5 portions of 150g each. The 5 portions were then each subjected to cold extraction with 900mls chloroform, ethyl acetate, acetone, ethanol and water at a ratio of 1:6 (w/v) respectively.

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The same procedure of cold extraction was repeated for *Camellia sinensis*, the purple tea variety TRFK 306/1. The powdered tea leaves were divided into 5 portions of 90g and each portion was subjected to cold extraction with 540mls of chloroform, ethyl acetate, acetone, ethanol and aqueous at a ratio of 1:6 (w/v) respectively. They were then allowed to stand at room temperature for a period of 3 days with frequent agitation. The difference in weights of purple tea and guava used was based on the final weights obtained from the ground extracts after milling in which guava yielded more than purple tea.

After 3 days of extraction, the solvent with the extract was filtered with Whatman filter paper Grade 1, pore size 11 µm (Sigma-Aldrich®, USA) and the extract concentrated at 40°C using rotavapor (Buchi R-215, Switzerland). The extracts obtained were weighed to obtain the percentage yields and stored at 4°C in the refrigerator for subsequent assays.

3.4 Qualitative phytochemical screening

Preliminary qualitative phytochemical screening of purple tea and guava extracts was carried out using standard methods described below.

3.4.1 Test for alkaloids

This method was carried out as described by Trease and Evans, (1997). Briefly, one ml of crude extract was dissolved in 5ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayers reagent separately. Formation of white or creamy precipitates with the reagents indicated the presence of alkaloids (Harborne, 1998; Trease & Evans, 1997).

3.4.2 Test for flavonoids

This method was carried out as described by Trease and Evans, (1997). Briefly, one ml of each of the plant extract in a test tube was added 1ml of 5% lead acetate and the mixture was allowed

to stand at room temperature (25°C) for two minutes. The formation of white precipitates in any of the samples showed that the extract contained flavonoids (Harborne, 1998; Trease & Evans, 1997).

3.4.3 Test for phenolic compounds and tannins

This method was done as described by Trease and Evans, (1997).Briefly, five mls of the extract was dissolved in distilled water. To this solution, 3ml of 10% lead acetate solution was added. A dark-green colour indicated the presence of phenolic compounds (Harborne, 1998; Trease & Evans, 1997).

3.4.4 Test for saponins (frothing test)

This method was done as described by Trease and Evans, (1997). Briefly, two mls of the extract was added to 6mls of water in a graduated cylinder. The mixture was shaken vigorously and observed for formation of persistent 2cm foam that indicated the presence of saponins (Trease & Evans, 1997).

3.4.5 Test for steroids and terpenoids (Liebermann-Burchard's test)

This method was carried out as described by Sofowara, (1993). Five mls of the extract was treated with 2 drops of chloroform, acetic anhydride and concentrated H_2SO_4. The mixture was observed for the formation of dark pink or red colour which indicated presence of steroids and terpenoids (Sofowara, 1993).

3.4.6 Test for cardiac glycosides (Keller-Killani test)

This method was done as described by Sofowara, (1993). Briefly five mls of each plant extract was treated with 2 mls of glacial acetic acid in a test tube and one drop of ferric chloride solution was added to it. One ml of concentrated H_2SO_4 was carefully added to form separate layer. A

brown ring at the interface due to the presence of deoxy sugar characteristic of cardenolides and a pale green colour in the upper layer indicated the presence of cardiac glycosides (Sofowara, 1993).

3.5 Experimental animals

The experimental animals used in this study were female Swiss white albino mice weighing between 20-30g. The 6 weeks old mice were sourced from Kenya Agricultural Research Institute-Trypanosomiasis Research Centre (KARI-TRC) and transported to the animal house facility at Jomo Kenyatta University of Agriculture and Technology. The animals were housed under standard environmental conditions with the temperature ranging between 20-25ºC, relative humidity was 50% and exposed to natural day and night cycles. They were randomly allocated to different sterilized steel cages of five mice per cage with wood shavings as beddings. The mice were feed on a standard pellet (Mice cubes, Unga Ltd, Kenya) diet and water *ad libitum* and acclimatized to handlers and laboratory environment for 7 days before the start of the experiment.

3.6 Study design

The study involved two key arms which are *in vivo* safety evaluation of purple tea and guava extracts in normal Swiss white mice and hypoglycemic activity test in diabetic Swiss white mice. *In vivo* safety profile of the leaves extracts of the plants were established first prior to the hypoglycemic test.

3.7 *In vivo* safety evaluation

The oral sub-acute toxicity test of the extracts was determined in accordance with OECD (Organization for Economic Co-operation and Development) guidelines 425 and Food and Drug

Administration (FDA) Safety Testing of Drug Metabolites (FDA, 2008). Swiss white mice were used in this study. Baseline body weights were measured using a digital balance EW-10000-14 (Cole-Parmer, USA) before test and weight changes were monitored on day 7 and day 14 after extract administration.

Mice were grouped into fifteen (15) experimental groups of five mice per group and one control group (1) of five mice. They were fasted overnight for 12 hours prior to dosing but had access to water *ad libitum*. The control group was administered orally with normal saline while the experimental groups were administered with purple tea and guava extracts (Table 3.1).

Table 3.1: Experimental groups for *in vivo* safety study of purple tea and guava extracts

Groups	*Camellia sinensis* (purple tea)	*Psidium guajava* (guava)
1	Control group	Control group
2	Aqueous100mg/kg	Aqueous100mg/kg
3	Aqueous300mg/kg	Aqueous300mg/kg
4	Aqueous1000mg/kg	Aqueous1000mg/kg
5	Ethanol100mg/kg	Ethanol100mg/kg
6	Ethanol300mg/kg	Ethanol300mg/kg
7	Ethanol1000mg/kg	Ethanol1000mg/kg
8	Acetone100mg/kg	Acetone100mg/kg
9	Acetone300mg/kg	Acetone300mg/kg
10	Acetone1000mg/kg	Acetone1000mg/kg
11	Ethylacetate100mg/kg	Ethylacetate100mg/kg
12	Ethylacetate300mg/kg	Ethylacetate300mg/kg
13	Ethylacetate1000mg/kg	Ethylacetate1000mg/kg
14	Chloroform 100mg/kg	Chloroform 100mg/kg
15	Chloroform300mg/kg	Chloroform300mg/kg
16	Chloroform1000mg/kg	Chloroform1000mg/kg

Oral administration using gastric gavage of chloroform, ethyl acetate, acetone, ethanol and aqueous extracts reconstituted in normal saline was done at a dose of 100mg/kg, 300mg/kg and 1000mg/kg daily for 14 days (Table 3.1).

The animals were closely observed for clinical signs of toxicity like paw licking, reduced activity, sedation, raised hair coat and convulsions. The clinical observations were done for the first 4 hours and thereafter daily for 14 days.

At 14 days of treatment, mice were euthanized using carbon dioxide to obtain blood and organs for biochemical test and histopathology respectively. One ml of blood was harvested from cardiac puncture and centrifuged at 3,000rpm for 10mins. Biochemical analysis of alanine transaminase (ALT) and blood urea nitrogen (BUN) was done using Reflotron[R] Plus (Roche, Switzerland) with compatible Reflotron test strips. The major organs (liver, kidneys and pancreas) were preserved in 10% (v/v) formalin. The organs were processed for histopathology and slides stained with Hematoxylin and Eosin as described by Cardiff et al., (2014). Microscopy and photomicrography was also done using light microscope (Leica DM1000, Germany).

3.8 Induction of diabetes

After the *in vivo* safety study of the leaves extracts of purple tea and guava, normal healthy Swiss white mice were induced with diabetes for hypoglycemic activity test.

Mice were treated with alloxan monohydrate (Sigma-Aldrich, St. Louise, MO, USA) to induce diabetes mellitus. They were injected intraperitoneously with a single dose of alloxan at 150mg/kg freshly dissolved in normal saline. After injection, the animals were allowed free access to food, water and 5% glucose to drink overnight to counter hypoglycemic shock. The level of blood glucose in tail snip blood was measured daily using a GlucoPlus™ glucometer (GlucoPlus Inc. Canada) with compatible test strips. After 3 days, the fasting blood glucose

levels were determined and mice showing fasting blood glucose more than 200 mg/dL (WHO and IDF, 2005) were considered diabetic and selected for the hypoglycemic activity test.

3.9 Experimental groups and protocol for hypoglycemic activity test

The diabetic Swiss white mice were divided into eight (8) groups of five mice and administered with purple tea and guava extracts (Table 3.2). Diabetic negative control group and positive control group were treated with normal saline and standard diabetic drug respectively. The doses of five extracts of purple tea and guava were orally administered daily for 14 days and baseline blood glucose determined before treatments.

Table 3.2: Experimental protocol for hypoglycemic activity test

Groups	Purple tea treatments	Guava treatments
1	Normal + saline	Normal + saline
2	Diabetic +saline	Diabetic + saline
3	Diabetic + 5mg/kg Glibenclamide	Diabetic + 5mg/kg Glibenclamide
4	Diabetic + 300mg/kg aqueous tea extract	Diabetic + 300mg/kg aqueous guava extract
5	Diabetic + 300mg/kg ethanolic tea extract	Diabetic + 300mg/kg ethanolic guava extract
6	Diabetic + 300mg/kg acetone tea extract	Diabetic + 300mg/kg acetone guava extract
7	Diabetic + 300mg/kg ethylacetate tea extract	Diabetic + 300mg/kg ethylacetate guava extract
8	Diabetic + 300mg/kg chloroform tea extract	Diabetic + 300mg/kg chloroform guava extract

3.10 Collection of blood samples and blood glucose determination

Blood samples were collected from the tails of the Swiss white mice after wiping the tail with surgical spirit. The tail was snipped with a pair of sharp scissors, a drop of blood was then squeezed into a GlucoPlus glucometer. After collection of blood, the tail was rubbed with cotton wool soaked in absolute ethanol to arrest further bleeding and protect the mice from infection.

The fasting blood glucose level (Mg/dl) in the mice was determined by reading using GlucoPlus glucometer and compatible test strips from GlucoPlus Canada. Fasting blood glucose samples were collected from all the groups of mice.

Baseline blood glucose level was first taken for all the animal groups before oral treatment with normal saline, Glibenclamide 5mg/kg and the plant extracts for the respective groups.

After the treatment and the tail blood was collected, fasting blood glucose levels were monitored after 2, 4, 6 and 8 hours upon single dose treatment. Prolonged treatment was also monitored up to 14 days where mice were daily treated orally with the plant extracts and the blood glucose measured at day 7 and day 14 post first day of treatment. During the experiment, the animals were fasted 12 hours before blood collection but were allowed free access to water.

3.11 Ethical care and use of laboratory animals

All animal use protocols were carried out according to the International Council for Laboratory Animal Science (ICLAS) and Kenya Veterinary Association (KVA) guidelines on care and use of laboratory animals.

3.12 Data management and analysis

A database was designed in Microsoft access (Microsoft™, USA) and secured using a password. Descriptive statistics was used to analyze data from experimental groups and also from the control groups. The data output included mean, mode, median, standard deviation,

standard error of the mean and variance using SPPS version 16. Analysis of multiples means from the different experimental groups was analyzed using one way Analysis of Variance (ANOVA) followed by Tukey's post hoc test.

One way ANOVA with Tukey's post hoc analysis helped to determine whether there was any significant differences between the data obtained using the plant extracts, diabetic control and Glibenclamide treated groups. A value of $P<0.05$ was considered statistically significant. Data representation used involved tables, graphs and photomicrographs.

CHAPTER FOUR

4.0 RESULTS

4.1 Solvent extraction of purple tea and guava leaves

The percentage yield of the crude plant extracts obtained showed that different solvents yielded different quantities of the crude extract. The water or aqueous solvent yielded the highest percentage of 22.18% of crude tea extract and 16.35% of guava crude extracts (Table 4.1 and Table 4.2).

The leaves of purple tea and guava were extracted using chloroform, ethyl acetate, acetone ethanol, aqueous and their percentage yield were recorded. Aqueous (water) extracts yielded more crude extract (22.18%) of purple tea extract while ethyl acetate extract yielded the lowest extract of 5.47% (Table 4.1).

Table 4.1: Percentage yield of purple tea leaves extracts

Solvent	Amount of purple tea used (g)	Volume of the solvent used (ml)	Extract yield(g)	Percentage (%) yield
Water	90	540	19.96 ± 2.54	22.18
Ethanol	90	540	6.25 ± 1.13	6.94
Acetone	90	540	6.38 ± 0.60	7.09
Ethyl acetate	90	540	4.92 ± 0.62	5.47
Chloroform	90	540	5.33 ± 0.83	5.92

Mean values of yields are presented as mean ± SEM from two replicates.

The aqueous extract of guava leaves yielded the highest percentage of crude extract (16.35%) while acetone extract yielded the lowest percentage (5.66%) of guava leaves crude extract (Table 4.2).

Table 4.2: Percentage yield of guava leaves extracts

Solvent	Amount of Guava used (g)	Volume of the solvent used (ml)	Extract yield (g)	Percentage (%) yield
Water	150	900	24.52±4.41	16.35
Ethanol	150	900	18.54±1.57	12.36
Acetone	150	900	8.49±2.05	5.66
Ethyl acetate	150	900	15.35±4.59	10.23
Chloroform	150	900	12.46±1.25	8.31

Mean values of yields are presented as mean ± SEM from two replicates.

4.2 Qualitative phytochemical screening

The preliminary phytochemical screening results of *Camellia sinensis* (purple tea) and *Psidium guajava* (Guava) showed the presence of various phytochemical constituents. These wide array of phytochemicals include alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids. Cardiac glycosides were absent in both extracts of purple tea and guava (Table 4.3).

Table 4.3: Phytochemicals constituents of leaves extracts of *Camellia sinensis* (purple tea) and *Psidium guajava* (guava)

	Aqueous extract		Ethanol extract		Acetone extract		Ethyl acetate extract		Chloroform extract	
	Tea	Guava	Tea	Guava	Tea	Guava	Tea	Guava	Tea	Guava
Alkaloids	+	+	+	+	+	+	-	-	-	-
Flavonoids	+	+	+	+	+	+	-	-	-	-
Phenols	+	+	+	+	+	+	-	-	-	-
Tannins	+	+	+	+	+	+	-	-	-	-
Saponins	+	+	-	-	+	-	-	-	-	-
Steroids & terpenoids	+	+	-	-	-	-	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

From the results of phytochemical screening, water or aqueous extract of both purple tea and common guava had the highest presence of phytochemical constituents. This was followed by acetone, ethanol, ethyl acetate and chloroform extracts having few phytochemicals.

4.3 Acute oral toxicity test

A toxicity study was done to evaluate the appropriate safe dose range that could be used for subsequent assays including hypoglycemic activity test. Acute toxicity study conducted revealed that administration of various doses of crude plant extracts extracted from the five solvents did

not show any clinical signs. No death was observed up to the dose of 1000mg/kg during experimental period of 14 days.

4.4 Changes in body weights

Mice in experimental and control groups gained weight over the course of the study. The change in body weights of mice showed a weight increase in control group of mice treated with normal saline and the experimental groups treated with purple tea and guava leaves extracts at a dose of 100mg/kg, 300mg/kg and 1000mg/kg. The mean body weights of mice treated with purple tea extracts increased similar to the control groups administered with normal saline for the 14 days studied. At day 0 (Pretreatment,) the control group of mice treated with purple tea extracts had a mean weight of 24.4±0.5g while the same control group at day 14 had a mean weight of 27.2±0.5g (Table 4.4).

Table 4.4: Distribution of weights (g) of mice treated with different doses of purple tea extracts

Groups	Day 0	Day 7	Day 14
Control group	24.4±0.5	26.0±0.6	27.2±0.5
Aqueous100mg/kg	26.3±1.2	26.0±1.4	28.6±1.3
Aqueous300mg/kg	28.9±0.5	30.4±0.7	30.8±0.5
Aqueous1000mg/kg	25.2±2.0	26.0±2.3	28.0±2.1
Ethanol100mg/kg	24.3±1.2	25.1±1.3	27.7±1.3
Ethanol300mg/kg	25.2±1.6	26.6±1.6	28.8±3.1
Ethanol1000mg/kg	24.5±1.2	25.3±1.2	27.5±1.0
Acetone100mg/kg	23.5±1.2	24.4±1.4	27.1±1.4
Acetone300mg/kg	25.3±0.8	26.1±0.7	28.3±0.8
Acetone1000mg/kg	21.2±0.6	22.0±1.1	24.4±0.8
Ethylacetate100mg/kg	24.8±1.4	25.9±1.5	28.1±1.4
Ethylacetate300mg/kg	25.0±1.7	26.5±1.4	27.9±1.9
Ethylacetate1000mg/kg	24.0±1.0	26.9±0.7	27.9±1.2
Chloroform 100mg/kg	24.4±1.5	26.0±1.6	27.4±1.7
Chloroform300mg/kg	24.1±2.4	25.1±0.5	27.4±1.1
Chloroform1000mg/kg	25.0±1.2	26.5±1.0	28.1±1.3

Values are expressed as mean ± S.E.M (n=5).

The increase in body weights of mice treated with purple tea extracts were not statistically significant (Day 0, p=0.143, Day 7, p=0.112, Day 14, p=0.593) when the control and experimental groups were compared using One way ANOVA followed by Tukey's post hoc test.

The experimental group treated with 100mg/kg of purple tea aqueous extracts lost weight in day 7 but by day 14 they had gained weight (Figure 4.1).

Figure 4.1: Distribution of weights (g) of mice treated with different doses of purple tea extracts

The changes in body weights of mice treated with guava leaves extracts was also studied for a period of 14 days. There was an increase in weight of mice treated with guava extracts similar to the control group administered with normal saline over the 14 day study period. Before treatments, the control group of mice treated with guava extracts had a mean weight of 21.4 ± 0.5g while the same control group at day 14 had a mean weight of 24.2 ± 0.5g (Table 4.5).

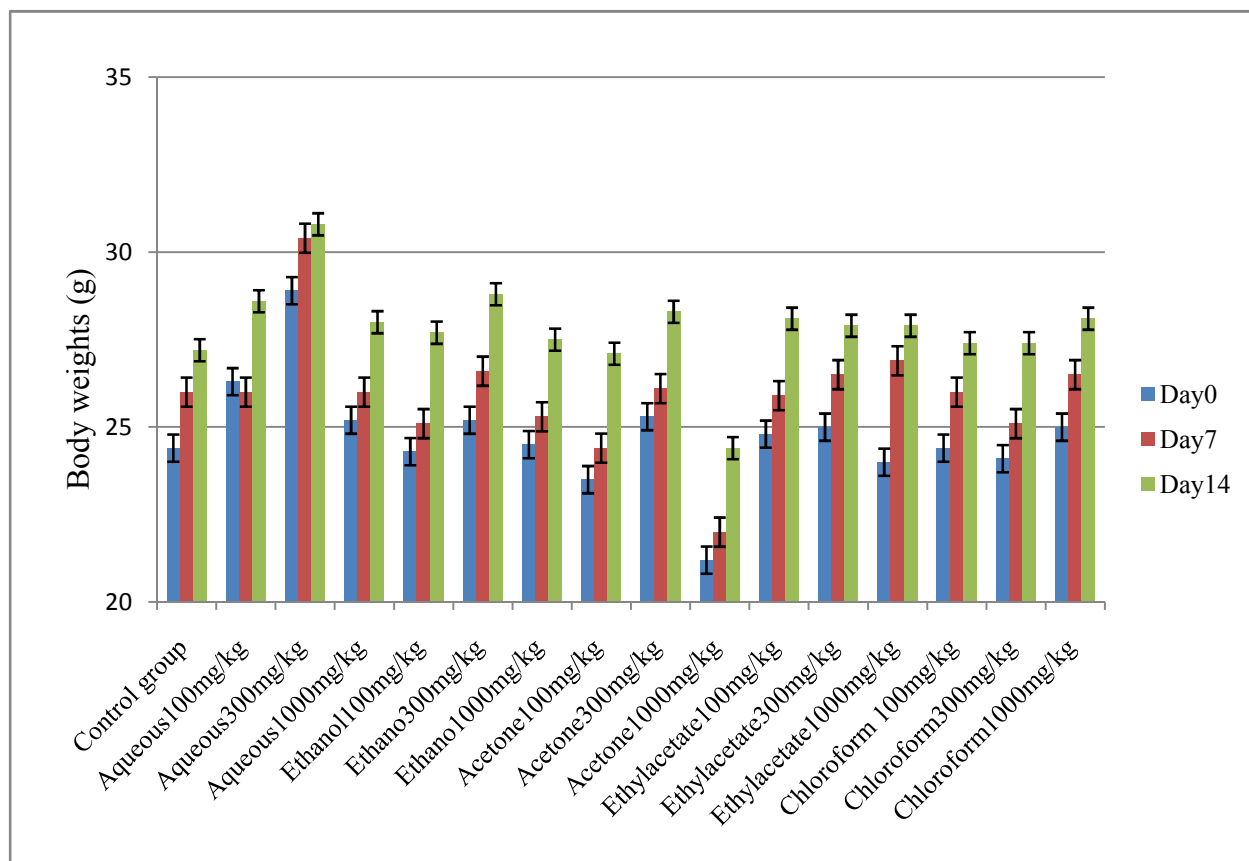**Table 4.5: Distribution of weights (g) of mice treated with different doses of guava extracts**

Groups	Day 0	Day 7	Day 14
Control group	21.4±0.5	22.84±0.3	24.54±0.5
Aqueous100mg/kg	21.4±1.2	22.24±0.6	22.54±0.9
Aqueous300mg/kg	21.04±1.2	21.84±1.4	24.14±1.4
Aqueous1000mg/kg	22.24±0.4	23.54±0.2	24.54±0.6
Ethanol100mg/kg	23.54±2.8	23.14±0.4	26.24±0.8
Ethanol300mg/kg	20.94±0.9	21.84±0.7	24.94±0.7
Ethanol1000mg/kg	22.04±0.4	24.14±0.7	23.44±1.1
Acetone100mg/kg	21.64±0.5	22.14±0.6	24.84±0.9
Acetone300mg/kg	20.24±1.0	21.94±0.4	24.24±1.0
Acetone1000mg/kg	22.84±0.6	23.34±0.9	23.74±0.6
Ethylacetate100mg/kg	19.94±0.4	21.94±0.8	25.84±0.5
Ethylacetate300mg/kg	22.04±1.2	22.84±0.8	24.24±1.3
Ethylacetate1000mg/kg	22.14±0.8	24.04±0.4	24.34±0.9
Chloroform 100mg/kg	21.04±0.4	23.14±0.4	26.34±1.0
Chloroform300mg/kg	22.74±0.9	22.64±0.8	24.14±0.6
Chloroform1000mg/kg	22.04±0.9	22.44±1.2	25.84±0.5

Values are expressed as mean ± S.E.M (n=5)

There was an insignificant (Day 0, p=0.252, Day 7, p=0.505, Day 14, p=0.326) increase in body weights of mice treated with leaves extracts of *Psidium guajava* and mice treated with normal saline in the control group.

Mice treated with 100mg/kg of ethanolic extract and 300mg/kg chloroform extract at day 7 lost weight but eventually gained weight by day 14 (Figure 4.2).
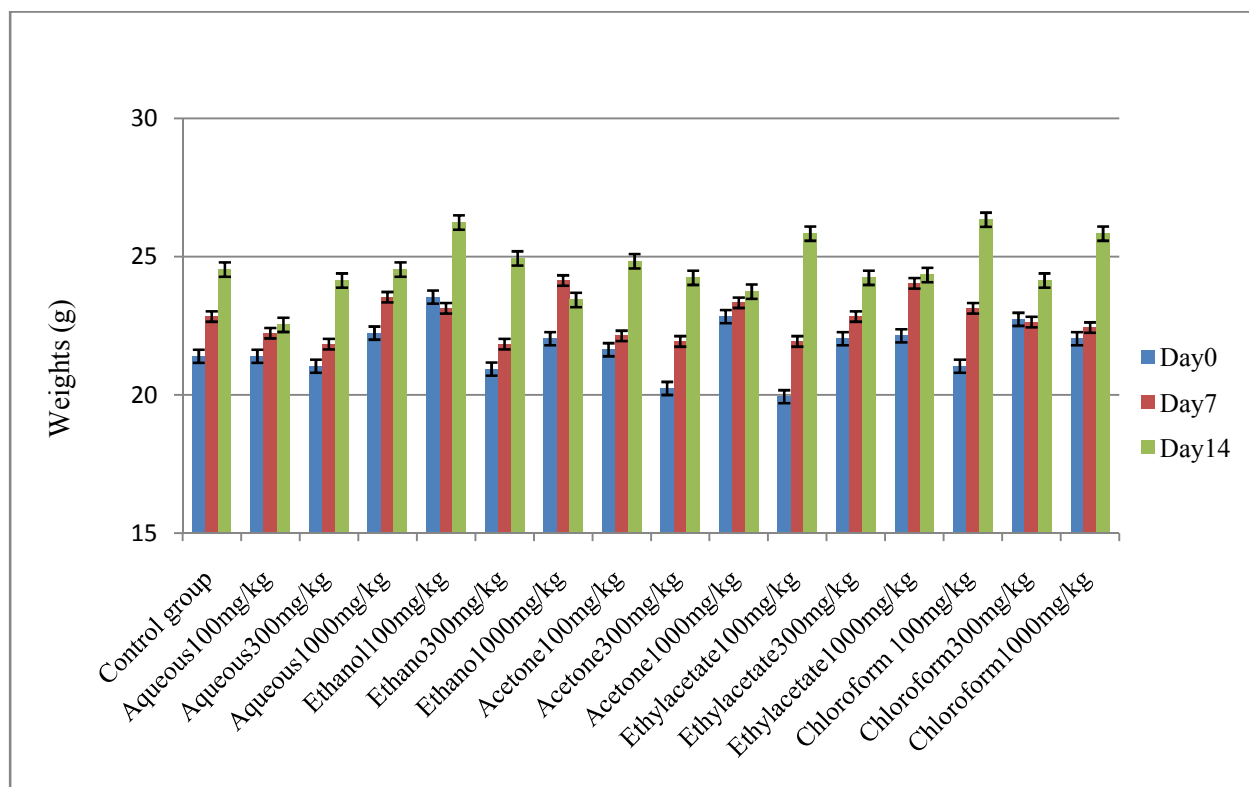
Figure 4.2: Distribution of weights (g) of mice treated with different doses of guava extracts.

Guava extracts did not show any significant (P>0.05) weights changes in mice treated with the three doses of 100mg/kg, 300mg/kg and 1000mg/kg over a period of 14 days. The weights of the mice in the control and experimental groups treated with guava extracts were increased during the study (Figure 4.2).

4.5. Biochemical analysis

After 14 days of treatment with purple tea and guava extracts, alanine transaminase (ALT) and blood urea nitrogen (BUN) were measured. The mean value of ALT for the control group was 49.69±7.0 U/L. The highest ALT mean value of mice treated with purple tea extracts was

64.21±14.56 U/L (Chloroform 1000mg/kg) while the lowest value was 32.11±5.87 U/L (Aqueous 300mg/kg) (Table 4.6). Compared statistically to the control group treated with normal saline, there were no significant (p=0.533) changes in ALT levels in experimental mice treated with the purple tea extracts.

Table 4.6: Alanine transaminase and blood urea nitrogen levels of mice treated with different doses of purple tea extracts.

Groups	Purple tea extracts	ALT (U/L)	BUN (mmol/L)
1	Control-Normal saline	49.69±7.00	6.03±0.31
2	Aqueous100mg/kg	34.86±3.94	5.68±0.53
3	Aqueous300mg/kg	32.11±5.87	6.31±0.32
4	Aqueous1000mg/kg	47.14±8.77	6.38±0.42
5	Ethanol100mg/kg	33.25±6.15	5.64±0.52
6	Ethanol300mg/kg	43.55±3.37	5.84±0.18
7	Ethanol1000mg/kg	48.37±6.55	6.09±0.60
8	Acetone100mg/kg	50.09±3.30	6.12±0.58
9	Acetone300mg/kg	51.15±12.28	6.53±0.53
10	Acetone1000mg/kg	46.15±16.18	6.49±0.37
11	Ethylacetate100mg/kg	45.20±5.30	6.00±0.26
12	Ethylacetate300mg/kg	41.42±5.25	5.70±0.24
13	Ethylacetate1000mg/kg	50.08±8.18	5.75±0.87
14	Chloroform 100mg/kg	44.49±3.66	4.98±0.33
15	Chloroform300mg/kg	39.93±6.36	5.89±0.33
16	Chloroform1000mg/kg	64.21±14.56	5.91±0.38

All values are expressed as mean ± S.E.M (n=5).

Legend: ALT-Alanine transaminase, BUN- Blood Urea Nitrogen

When the experimental animals were treated with purple tea extract and control group treated with normal saline, there were no significant (p=0.776) change in BUN between the experimental groups and the control group. The mean value of BUN of control mice treated with purple tea extracts was 6.03±0.31mmol/L, and the highest mean value was 6.53±0.53mmol/L (Acetone 300mg/kg) while the lowest mean value was 4.98±0.33mmol/l (Chloroform 100mg/kg) (Table 4.6).

Guava extracts administered to mice and compared with control group treated with normal saline did not show any significant (P>0.05) changes in the level of ALT and BUN.

The leaves extracts of *Psidium guava* did not significantly (p=0.958) increase alanine transaminase (ALT) enzyme in experimental groups compared statistically to the normal control group treated with normal saline. The mean value of ALT for the control group was 47.44±4.00 U/L. The highest ALT mean value of mice treated with guava extracts was 54.68±6.17 U/L (Ethyl acetate 1000mg/kg) while the lowest value was 40.56±6.62 U/L (Ethyl acetate 100mg/kg) (Table 4.7).

Table 4.7: Alanine transaminase and blood urea nitrogen levels of mice treated with different doses of guava extracts.

Groups	Guava Extracts	ALT (U/L)	BUN (mmol/L)
1	Control-Normal saline	47.44±4.00	5.69±0.35
2	Aqueous100mg/kg	42.00±3.00	4.97±0.60
3	Aqueous300mg/kg	49.90±6.91	4.94±0.54
4	Aqueous1000mg/kg	51.80±5.83	6.17±0.50
5	Ethanol100mg/kg	42.54±5.90	6.26±0.48
6	Ethanol300mg/kg	47.48±11.45	6.60±1.16
7	Ethanol1000mg/kg	47.44±7.07	5.70±0.96
8	Acetone100mg/kg	47.50±4.40	5.72±0.42
9	Acetone300mg/kg	50.82±2.98	5.57±0.73
10	Acetone1000mg/kg	47.86±2.87	6.03±0.77
11	Ethylacetate100mg/kg	40.56±6.62	5.35±0.57
12	Ethylacetate300mg/kg	48.62±2.88	6.12±1.17
13	Ethylacetate1000mg/kg	54.68±6.17	6.18±0.69
14	Chloroform 100mg/kg	48.88±4.56	5.44±0.18
15	Chloroform300mg/kg	48.92±4.49	5.81±0.49
16	Chloroform1000mg/kg	42.42±6.16	5.57±0.82

All values are expressed as mean ± S.E.M (n=5).

Legend: ALT-Alanine transaminase, BUN- Blood Urea Nitrogen

The mean value of BUN of control mice treated with guava leave extracts was 5.69±0.35mmol/L, and the highest mean value was 6.6±1.16mmol/L (Ethanol 300mg/kg) while the lowest mean value was 4.94±0.54mmol/L (Aqueous 300mg/kg) (Table 4.7).

Analysis of these results of blood urea from mice treated with *Psidium guajava* at the three dose level of 100mg/kg, 300mg/kg and 1000mg/kg resulted in insignificant (p=0.968) change in BUN of mice compared to the mice in the control group.

4.6 Histopathology results

Histopathology examination of mice liver, kidney and pancreas were observed for pathological features which may manifest when extracts of purple tea and guava are administered to mice.

4.6.1 Effect of purple tea extracts on mice liver

The liver cells from the mice treated with extracts of purple tea were characterized with normal hepatic cells with distinct nuclei, normal eosinophilic cytoplasm with normal sinusoids (Plate 4.1).
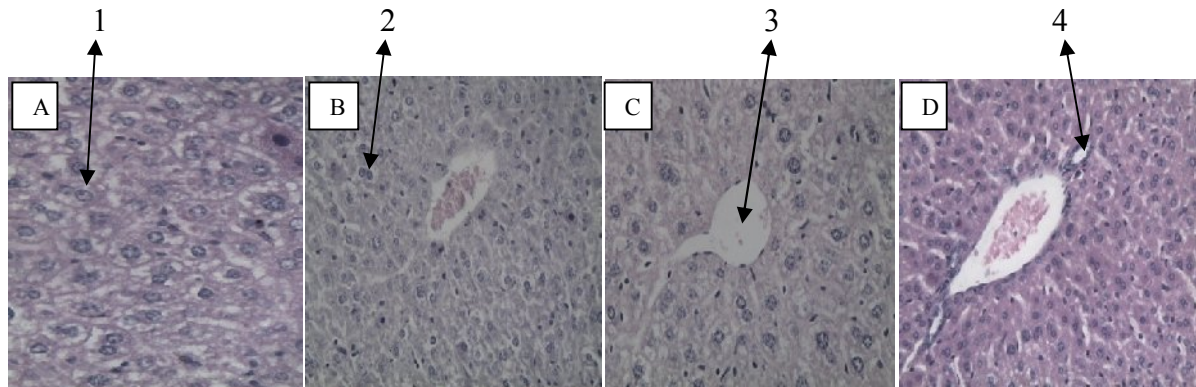
Plate 4.1: Sections of liver: A) Liver of normal control mice. B, C and D are sections of mice liver treated with 100mg/kg, 300mg/kg and 1000mg/kg of purple tea extracts respectively. (H & E, X40).
Legend: 1-Normal hepatic cells, 2-Eosinophilic cytoplasm, 3-Portal vein, 4-Sinusoids

4.6.2 Effect of purple tea extracts on mice kidneys

The kidneys from the control group displayed normal renal architecture with normal glomeruli, proximal tubules and collecting ducts. In addition, it has normal cellularity with capillary loops which are well-defined and thin.

Bowman's space is observed along parietal epithelial cells. Similar features to the control group were observed in the experimental groups (Plate 4.2).

Plate 4.2: Sections of kidney: A) Kidney of normal control mice. B, C and D are sections of mice kidneys treated with 100mg/kg, 300mg/kg and 1000mg/kg of purple tea extracts respectively. (H & E, X40).
Legend: 1-Glomerullus, 2-Bowman's space, 3-Epithelial cells, 4-Proximal tubules

4.6.3 Effect of purple tea extracts on mice pancreas

The Islets of Langerhans of mice treated with extracts of purple tea (Plate 4.3, B, C & D) showed normal cells compactly arranged with no inflammatory cells similar to the control group (Plate 4.3, A).

Plate 4.3: Sections of pancreas: A) Pancreas of normal control mice. B, C and D are sections of mice pancreas treated with 100mg/kg, 300mg/kg and 1000mg/kg of purple tea extracts respectively. (H & E, X40).
Legend: 1-Islets of Langerhann's, 2-Pancreatic lobule

4.6.4 Effect of guava extracts on mice liver

Histopathology results of organs of mice treated with the three dosages of guava leaf extracts are presented below.

The liver cells in the mice treated with extracts of guava were characterized with normal hepatic cells with normal cellular architecture (Plate 4.4).
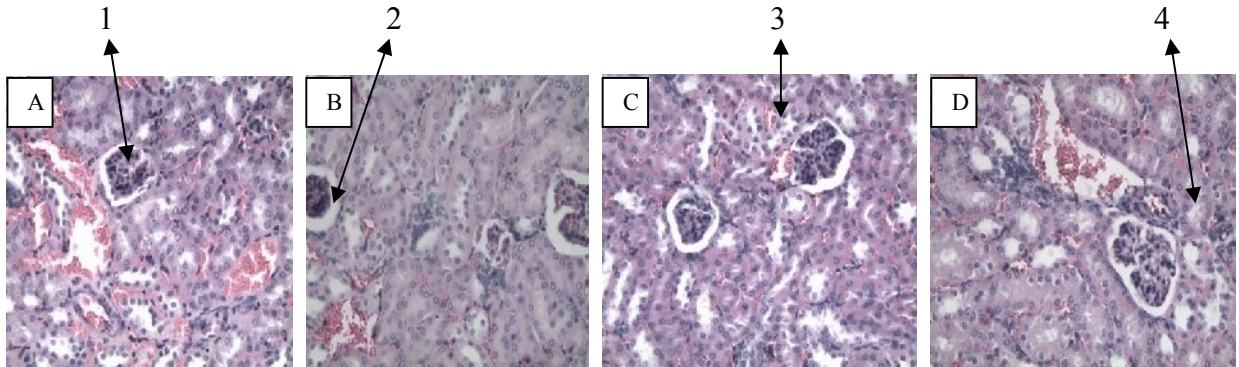

Plate 4.4: Sections of liver: A) Liver of normal control mice. B, C and D are sections of mice liver treated with 100mg/kg, 300mg/kg and 1000mg/kg of guava extracts respectively. (H & E, X40).

Legend: 1-Normal hepatic cells, 2-Portal vein, 3 and 4 - Normal hepatic cells

4.6.5 Effect of guava extracts on mice kidneys

The cortical area of the renal tissue appear as a dense round mass separated from surrounding structures by Bowmans's space (Plate 4.5).

The kidneys of the treated mice with the plant extracts and the control group showed normal appearance with no morphological changes except mice treated with chloroform extract at 1000mg/kg.
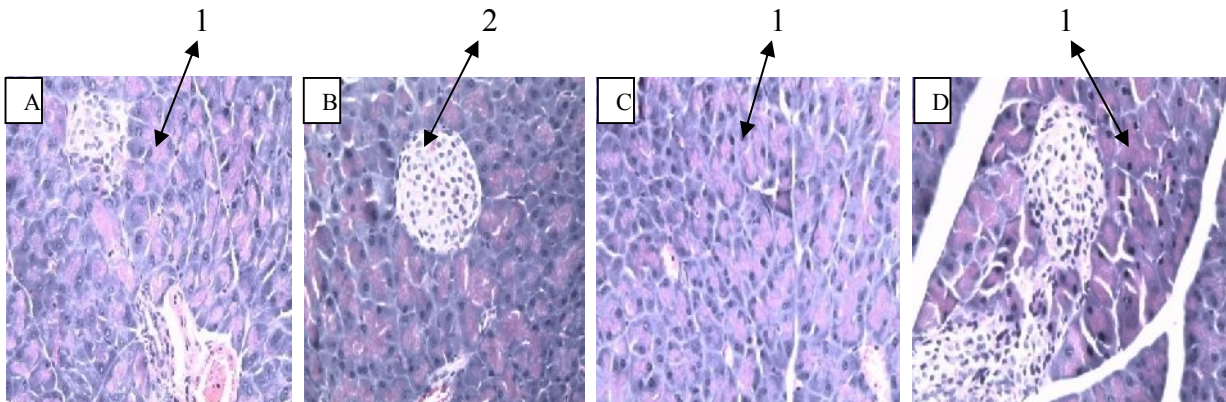
Plate 4.5: Sections of kidney :A) Kidney of normal control mice. B, C and D are sections of mice kidneys treated with 100mg/kg, 300mg/kg and 1000mg/kg of guava extracts respectively. (H & E, X40).
Legend: 1-Glomerullus, 2-Distal tubules, 3-Bowman's space, 4-Mild lympholytic infiltration

Kidneys of mice treated with 1000mg/kg (Plate 4.5, D) of chloroform extract showed mild lymphocytic infiltration and mild focal tubular casts.

4.6.6 Effect of guava extracts on mice pancreas

Histopathology of mice pancreas treated with guava extracts at the three dose level appeared normal. The Islets of Langerhans of normal control compared to the experimental groups showed normal islets with negligible intercellular space with no inflammatory cells (Plate 4.6).
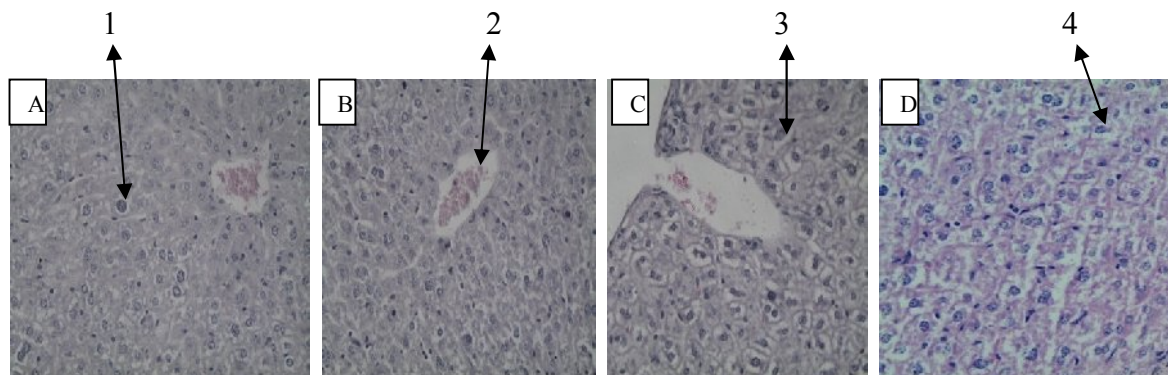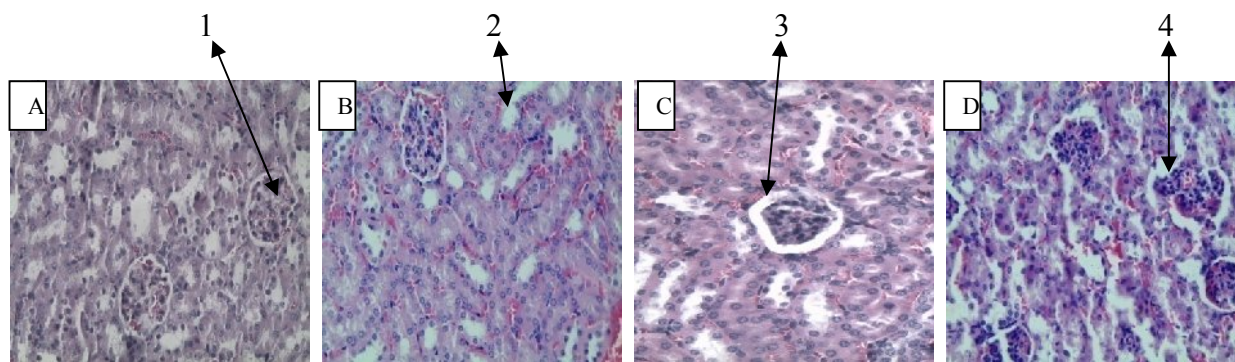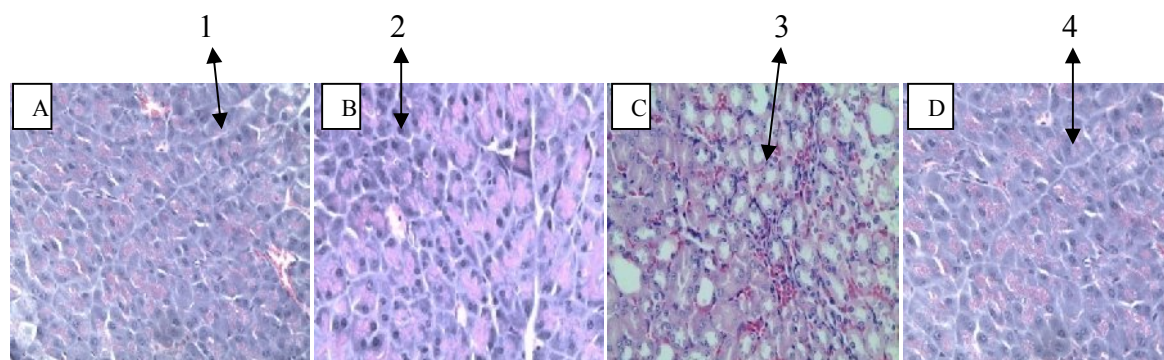

Plate 4.6: Sections of pancreas: A) Pancreas of normal control mice. B, C and D are sections of mice pancreas treated with 100mg/kg, 300mg/kg and 1000mg/kg of guava extracts respectively. (H & E, X40).
Legend: 1-4, Normal Islets of Langerhanns

4.7 *In vivo* hypoglycemic activity of purple tea and guava extracts

Diabetes mellitus type II was induced using alloxan monohydrate and mice having blood glucose level of above 200mg/dl were selected for the study. Seventy mice were induced with diabetes mellitus while ten mice were not induced to serve as the control.

Aqueous, ethanolic and acetone extract of purple tea significantly (P<0.05) lowered the blood glucose in diabetic mice. Ethyl acetate and chloroform extracts of purple tea did not significantly lower the blood glucose in diabetic mice (Table 4.8).

Table 4.8: Blood glucose levels of diabetic mice treated with purple tea extracts.

Treatments	Blood glucose (mg/dl)						
	0 hr	2 hr	4 hr	6 hr	8 hr	Day 7	Day 14
Normal+saline	87±3.8	86±1.9	80±4.3	81±5.3	86±8.6	91±7.0	80±3.3
Diabetic+saline	264±15.7	270±18.1	279±20.3	284±17.6	271±25.9	322±19.4	294±24.2
Diabetic+Glibenclamide	365±67.9	250±72.1	61±8.0[a]	114±24.2[a]	98±8.6[a]	94±17.4[a]	92±6.8[a]
Diabetic+300mg/kg aqueous extract	257±18.8	222±28.8	198±23.2	122±19.8[a]	92±11.4[a]	124±12.6[a]	117±15.9[a]
Diabetic+300mg/kg ethanolic extract	264±16.6	117±15.1	83±13.2[a]	110±5.9[a]	201±27.0	155±26.85[a]	162±24.5[a]
Diabetic+300mg/kg acetone extract	282±33.9	338±59.3	267±39.4	197±39.0	116±24.8[a]	242±32.1	293±27.5
Diabetic+300mg/kg ethylacetate extract	280±38.3	308±65.2	345±70.9	307±50.0	302±45.6	262±63.2	313±29.6
Diabetic+300mg/kg chloroform extract	296±111.6	290±49.2	251±74.4	306±45.8	292±61.6	310±45.2	338±36.5

All values are expressed as mean ± S.E.M (n=5).Data was analyzed using One way ANOVA followed by Tukey multiple comparison test. [a] Indicates a significant difference (p<0.05) between diabetic control and purple tea treated groups.

The standard diabetes drug Glibenclamide 5mg/kg significantly (p=0.013) reduced blood glucose in diabetic mice 4 hours after oral administration (Table 4.8). Aqueous extracts of purple tea at a dose of 300mg/kg significantly (P<0.05) reduced blood glucose after 6th (p=0.11) and 8th hr (p=0.008) upon oral administration to diabetic mice. Ethanolic extract at a dose of 300mg/kg significantly reduced blood glucose levels in diabetic mice after 4 hrs (p=0.034) and 6 hrs (p=0.007) after oral administration of the extract.

However, ethanolic extract of purple tea leaves did not significantly reduced blood glucose in diabetic mice at 8th hr upon administration. Acetone extract of purple tea at a dose of 300mg/kg significantly (p=0.034) reduced blood glucose after 8th hour upon oral administration to the diabetic mice.

The standard diabetic drug Glibenclamide reduced blood glucose in diabetic mice to the lowest value of 61± 8.0 mg/dl after 4th hour. Aqueous, ethanolic and acetone extracts of *Camellia sinensis* (purple tea) significantly reduced blood glucose in diabetic mice. Aqueous extract of purple tea at hour 8 recorded the lowest (92±11.42 mg/dl) reduction of blood glucose while ethanolic extract at 4th hour, had the lowest blood glucose reduction (83±13.2 mg/dl) (Table 4.8). The blood glucose reduction of purple tea can be observed and compared to different treatment groups (Figure 4.3). The standard diabetes drug Glibenclamide 5mg/kg significantly (p=0.013) reduced blood glucose after 4th hour upon administration to the diabetic mice (Table 4.8).

The aqueous, ethanolic and acetone extracts of purple tea revealed a sudden reduction in blood glucose in diabetic mice similar to the standard drug Glibenclamide at 8th hour (Figure 4.3).
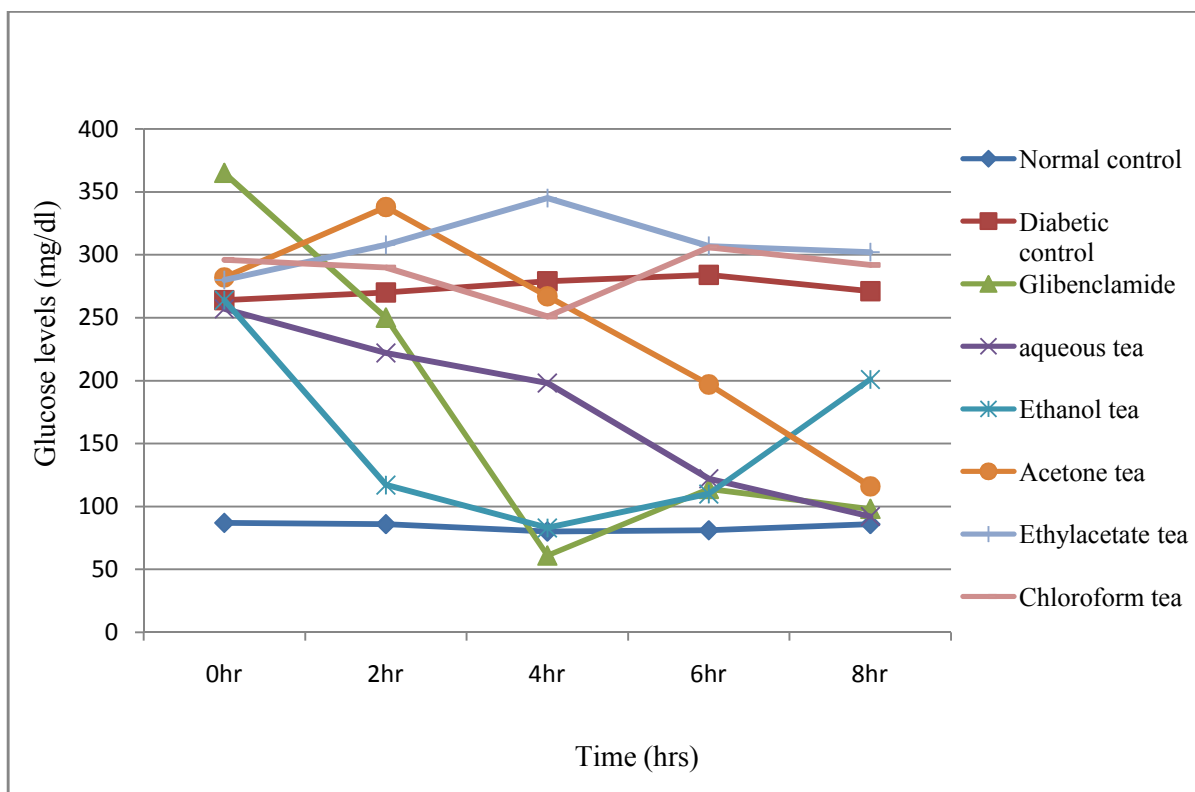
Figure 4.3: Blood glucose levels of diabetic mice treated with purple tea extracts.

Hypoglycemic activity of purple tea was also evaluated after 7 and 14 days. Daily administration of aqueous and ethanolic extracts of purple tea for a period of 14 days resulted in significant reduction of blood glucose in diabetic mice compared statistically to the diabetic control. Both aqueous (Day 7, $p=0.004$, Day 14, $p= 0.001$) and ethanolic extracts (Day 7, $p=0.022$, Day 14, $p= 0.008$) of purple tea at a dose of 300mg/kg significantly reduced blood glucose levels in diabetic mice after 14 days (Figure 4.4).

Figure 4.4: Blood glucose levels of diabetic mice treated with purple tea extracts for a period of 14 days. * Indicates a significant difference (p<0.05) between diabetic control and treated groups.

The hypoglycemic activity of guava extracts showed significant (P<0.05) reduction of blood glucose in diabetic mice (Table 4.9).

Aqueous, ethanolic and ethyl acetate extracts of the leaves of *Psidium guajava* significantly (P<0.05) reduced blood glucose levels. Aqueous extract administered at a dose of 300mg/kg significantly (p=0.011) reduced blood glucose after 6 hour upon oral administration to the diabetic mice. The ethanolic extract of *Psidium guajava* at a dose of 300mg/kg significantly (P<0.05) lowered blood glucose levels after 6 hours (p=0.002) and 8 hours (p=0.003) after oral administration (Table 4.9). The ethyl acetate leaves extract of *Psidium guajava* significantly (P<0.05) reduced blood glucose levels in diabetic mice after 4 (p=0.008) and 6 hours (p=0.005) upon oral administration of the extract.

On the other hand, the standard drug Glibenclamide 5mg/kg significantly (P=0.001) reduced blood glucose in diabetic mice from the 4th hour after administration.

Table 4.9: Blood glucose levels of diabetic mice treated with guava extracts.

Treatments	Blood glucose (mg/dl)						
	0 hr	**2 hr**	**4 hr**	**6 hr**	**8 hr**	**Day 7**	**Day 14**
Normal+ saline	87±3.8	86±1.9	80±4.3	81±5.3	87±8.6	91±7.0	80±3.3
Diabetic+ saline	264±15.7	270±18.1	279±20.3	284±17.6	271±25.9	322±19.4	295±24.2
Diabetic+Glibenclamide	365±67.9	250±72.1	61±8.0[a]	115±24.2[a]	98±8.6[a]	94±17.4[a]	92±6.8[a]
Diabetic+300mg/kg aqueous extract	259±15.9	142±40.4	178±25.0	164±20.4[a]	191±30.2	199±32.3[a]	199±50.8[a]
Diabetic+300mg/kg ethanol extract	259±18.0	215±51.4	232±46.3	142±20.0[a]	105±18.7[a]	104±8.0[a]	108±5.6[a]
Diabetic+300mg/kg acetone extract	234±20.5	165±28.3	190±23.9	235±15.1	255±20.1	259±23.2	296±31.2
Diabetic+300mg/kg ethylacetate extract	255±21.7	216±32.6	134±23.3[a]	153±23.0[a]	195±43.3	268±22.4	257±17.8
Diabetic+300mg/kg chloroform extract	255±25.0	222±26.7	240±29.6	234±38.1	260±39.1	276±23.6	350±23.1

All values are expressed as mean± S.E.M (n=5).Data was analyzed using One way ANOVA followed by Tukey multiple comparison test. [a] Indicates a significant difference (p<0.05) between diabetic control and guava treated groups.

The reduction of blood glucose by aqueous, ethanolic and ethyl acetate extracts of guava revealed a sudden reduction of blood glucose in diabetic mice similar to the standard drug Glibenclamide which significantly reduced the blood glucose to normal level at the 4th Hour (Figure 4.5).

The standard diabetic drug Glibenclamide reduced blood glucose in diabetic mice to the lowest value of 61± 8.0 mg/dl after 4th hour. Aqueous extract of guava leaves at 6th hour recorded the lowest (164±20.4 mg/dl) reduction of blood glucose while ethanolic extract at day 7, had the lowest blood glucose reduction (104±8.0 mg/dl) (Table 4.9).

Glibenclamide significantly (P<0.05) reduced blood glucose in diabetic mice to the normal blood glucose level of the normal non-diabetic mice in the normal control group (Figure 4.5).
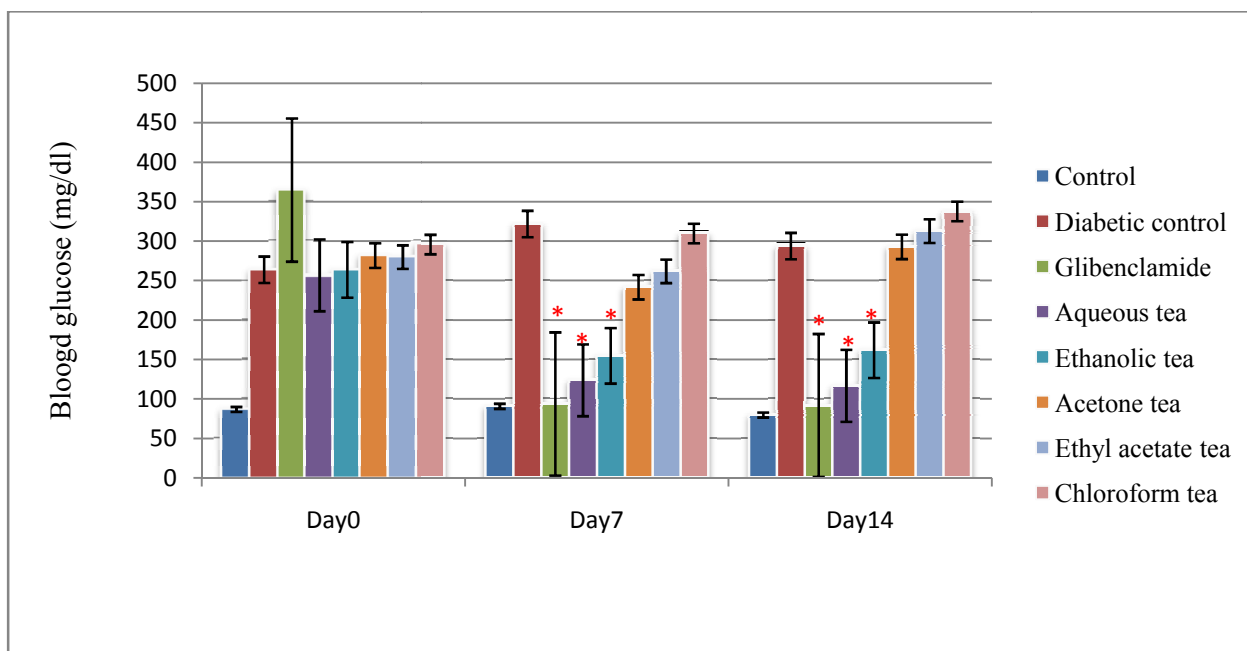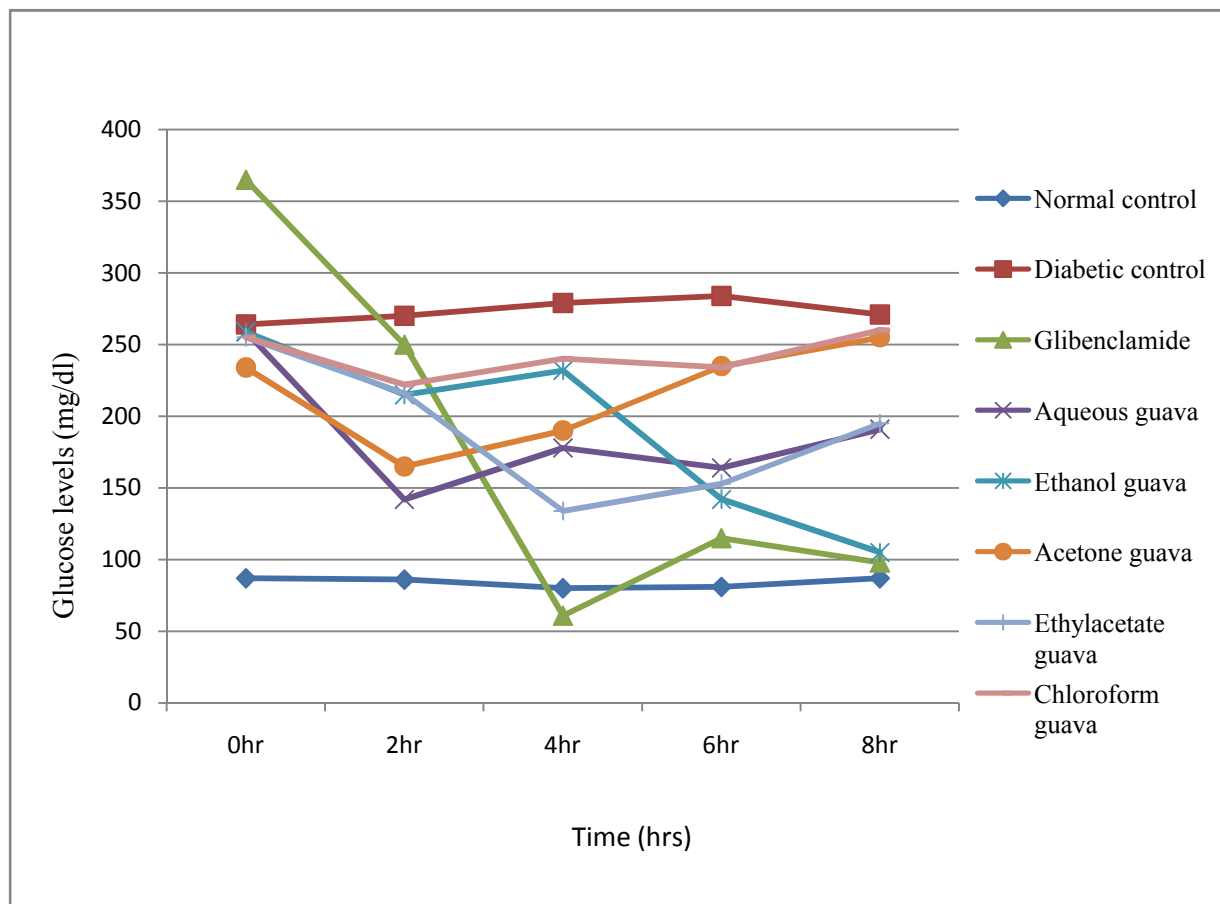

Figure 4.5: Blood glucose levels of diabetic mice treated with guava extracts.

Hypoglycemic activity of *Psidium guajava* was also evaluated for 7 and 14 days. Oral daily administration of the different extracts for a period of 14 days resulted in significant (P<0.05) reduction of blood glucose in diabetic mice compared to the diabetic control.

Both aqueous (Day 7, p=0.005, Day 14, p= 0.004) and ethanolic extract (Day 7, p=0.001, Day 14, p= 0.001) of *Psidium guajava* at a dose of 300mg/kg significantly (P<0.05) reduced blood glucose levels in diabetic mice compared to the control group treated with normal saline (Figure 4.6).
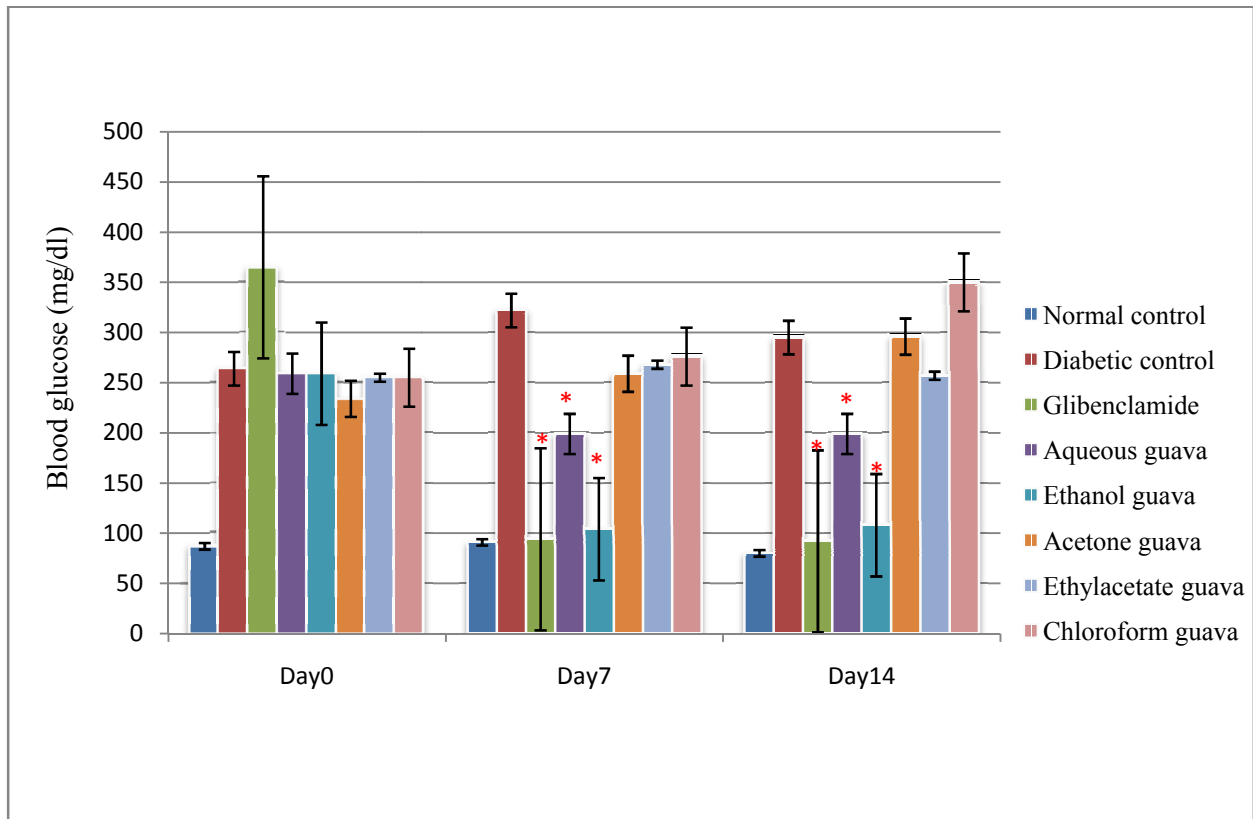
Figure 4.6: Blood glucose levels of diabetic mice treated with guava extracts for a period of 14 days. * Indicates a significant difference (p<0.05) between diabetic control and treated groups.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Yield of the plant extracts

From the above results, it can be noted that the percentage yields of crude extracts of both purple tea and guava leaf extracts was high in polar aqueous and ethanol solvents than the non-polar solvents. This therefore, explains that different solvents with different polarities extract specific phytochemicals in plants (Tiwari et al., 2011). The present study demonstrates that the leaves extracts had high amounts of polar bioactive compounds than non-polar compounds as revealed by the extraction yields from the solvents. Similar results have been obtained where polar solvents like water yielded more crude extracts than non-polar petroleum ether (Jeyaseelan, 2012). This is a good observation since communities using traditional herbal medicines usually use water as a common available solvent in extraction. Water has been shown to extract antioxidants which have health enhancing properties which are known to manage various diseases (Larpornik, 2005). In modern pharmaceutical industry, the various solvents ranging from polar and non-polar are critical in isolating specific bioactive compounds of interest if prior knowledge of their polarities is known.

5.2 Phytochemical screening of purple tea and guava leave extract

Qualitative phytochemical screening of the purple tea showed the presence of various phytochemicals from the five different solvents used in extraction. The aqueous extract of purple tea had alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids. This compares well with a study by Cabrera (2003) who demonstrated that green tea have alkaloids and polyphenols with antioxidant activity (Cabrera, 2003). These results are also in tandem to

another study which showed that flavonoids and phenols are usually extracted by polar solvents like water and ethanol (Das et al., 2010; Chaitanya, 2014). Aqueous extracts have been shown to have anthocyanins, tannins, saponins, terpenoids and steroids (Savithramma et al., 2011). This study also presents similar results that ethanolic extract of tea has tannins and saponins (Katoc et al., 2013). Chloroform extracts showed the presence of steroids and terpenoids since the low polarity of terpenoids made them easily extracted by chloroform. This has also been demonstrated in a different study where chloroform was used to extract fatty acids and terpenoids while ethyl acetate and acetone was used to extract less polar and polar flavonoids, tannins and terpenoids (Cowan, 1999; Eloff 2001).

Qualitative phytochemical screening of *Psidium guajava* revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids. Each solvent did extract different phytochemicals groups based on the polarity of the solvent. Aqueous and ethanolic extracts had the highest presence of secondary metabolites. This mirrors another study which showed that ethanolic extracts of *Psidiam guajava* fruits had terpenoids, flavovoids, saponins and tannins (Ayoola et al., 2008, Cheruiyot et al., 2009; Kateregga et al., 2014). Other studies have indicated that polar solvents had the highest presence of phytochemicals than non-polar solvents for instance chloroform which extract steroids and terpenoids (Chaitanya et al., 2013).

5.3 *In vivo* safety evaluation

The weights of mice in the experimental group treated with extracts of both purple tea and guava did not show any statistically significant changes in body weight over a period of 14 days. Body weight changes are an indicator of adverse side effects of drugs (Feres et al., 2006). Therefore, the absence of any significant differences in the body weights of the experimental animals compared to the normal control group provides support for the safety of *Camellia sinensis* and

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Psidium gujava extracts. In other studies, the decrease in body weights in animals clearly have been attributed to loss or degradation of structural proteins due to toxic compounds or diseases like diabetes (Rajkumar & Govindarajulu, 1991). These results are similar to those of another study where aqueous extracts of *Psidium guajava* was shown to increase body weights of mice (Prasad, 2009, Al-Attar, 2010).

The acute toxicity test of purple tea and guava extracts during the study period of 24 hour at a dose of 100mg/kg, 300kg/kg and 1000mg/kg did not exhibit any clinical signs. The mice survived throughout the 14 days for the sub-acute toxicity and there were no drug related changes noted in the behavior, activity and posture or external appearance in mice that received the plant extracts. This has also been demonstrated in another experiment that aqueous extracts of *Psidium quajava* leaves up to a dose of 2000mg/kg did not result in any mortality in Wistar albino rats (Chachal et al., 2006).

The LD_{50} of purple tea and guava leaf extracts were higher than 1000mg/kg since no animal died during the study period of 14 days. The medium lethal dose (LD_{50}) of the extracts is higher than 1000mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effects. The oral LD_{50} was inderterminable since a higher dose of 1000mg/kg as per OECD guidelines 423 (OECD, 2008) did not result in any mortality and thus the extracts were non toxic and thus safe. This indicates that the medium lethal dose (LD_{50}) could be greater than 1000mg/kg body weight of mice. Similar study conducted by Kateregga et al., (2014) showed that the LD_{50} of ethanolic leaf extracts of guava in mice was 9922mg/kg and LD_{50} of aqueous and chloroform extract of guava leaf extracts was 5,000mg/kg (Jaiarj et al., 1999). The implication of this acute toxicity study is that high doses of up to 1000mg/kg of the purple tea and guava leaves extracts can be safely used for efficacy studies.

5.4 Biochemical parameters of mice treated with purple tea and guava extracts

There were no statistically significant differences between control and treated groups in biochemical parameters alanine transaminase and blood urea nitrogen measured. Few changes in biochemical parameters were observed where the values of either ALT or BUN were higher or lower than those of normal control groups but were not statistically significant. The biochemical evaluation is important since there are several reports of liver and kidney toxicity as a result of using phytotherapeutic products (Rhiouani et al., 2008; Corns, 2003). The results of this study concur with another study which showed that aqueous extracts of *Psidium guajava* leaves at a dose of 500mg/kg in Wistar rats had no significant changes in biochemical parameter ALT, AST and bilirubin (Chanchal, 2005). Similar studies have shown that the fruit peel of *Psidium guajava* had no significant changes in ALT and AST levels (Rai, 2010) in streptozotocin induced diabetic rats. This shows that the leave extracts of *Psidium guajava* (Guava) extracted from the five solvents (water, ethanol, acetone, ethyl acetate and chloroform) does not have any toxic effect on liver and kidneys of mice at a dose of 100mg/kg, 300mg/kg and 1000mg/kg.

The absence of significant change in biochemical parameters tested is an indication that the extracts did not have a toxic effect on the liver and the kidneys. Administration of purple tea extracts to mice did not reveal any significant change in biochemical indices measured. ALT and blood urea levels were not significantly changed by the effect of the extracts. This was similar to another study by Al Attah et al., (2010) who demonstrated that aqueous extracts of green tea did not have any significant changes in blood urea in mice. Furthermore, these results concur with a study on antioxidant properties of aqueous extract of green tea, which when administered at a dose of 400mg/kg showed insignificant change in ALT level in normal mice (Al-Attar, 2013). It

has also been shown that green tea extracts does not significantly affect ALT levels in rats compared with the control groups not administered with the tea extracts (Avwioro et al., 2010). A study by Jabber, (2012) demonstrated that administration of aqueous green tea extracts at a dose of 300mg/kg to normal mice did not significantly affect blood urea levels (Jabber, 2012). This indicates that administration of aqueous tea extracts does not have any toxic effect on the kidneys. These results therefore demonstrate that purple tea extracts can be safely used at a dose of 100mg/kg, 300mg/kg and 1000mg/kg in mice for efficacy studies.

5.5 Histopathology of liver, kidney and pancreas

Histopathology of liver, kidneys and pancreas of experimental mice treated with purple tea extracts had no adverse effects on liver, kidneys and pancreas compared to the control group. Liver histology of mice treated with all the five extracts of purple tea showed similar appearance with no indication of cellular lesion or distortion. The sections of normal hepatic tissue showed the portal tracts at the periphery of indistinct hepatic lobule. In the liver of mice treated with purple tea extracts, there was normal appearance of tissue histology observed. This study shows that aqueous, ethanol, acetone, ethylacetate and chloroform extracts of purple tea do not have any adverse effect on the visceral organs as shown. Similar to these observations, Avwioro (2010) showed that administration of aqueous extracts of green tea did not reveal any toxic effect in Wistar rats.

The lack of significant changes in kidney and pancreas in mice treated with aqueous extracts of guava has been reported in albino rats (Prasad, 2009). A study by Kobayashi et al.,(2005) demonstrated that oral administration of guava extracts at a dose of 200 and 2000mg/kg/day caused no abnormal effects in rats, indicating lack of acute nor chronic toxicity in the leaves of guava plant (Kobayashi et al., 2005). Histopathology of liver from mice treated with butanol

soluble fraction of guava leaves have been shown to have preventive effect in diabetic mice alongside its anti-diabetic potential (Oh et al., 2005). Similar histopathology study of rats treated with 200mg/kg of aqueous leaves extracts of guava did not show any adverse alteration in the morphological architecture of the liver tissues of rats (Uboh et al., 2010). This implies that the leaves extracts of guava can be safely be used in the hypoglycemic efficacy study.

5.6 Hypoglycemic activity

In hypoglycemic activity test, diabetic control mice were compared statistically with the diabetic mice that were treated with the crude leaf extracts of purple tea and guava.

The reduction of blood glucose by purple tea is in agreement with other studies which showed that administration of aqueous green tea extracts at a dose of 300mg/kg significantly reduced blood glucose in diabetic mice (Miura et al., 2005, Tsuneki et al., 2004). The results are in tandem with another study in mice which revealed that aqueous extracts of *Camellia sinensis* significantly reduced blood glucose after 2nd, 4th and 6th hour (Tsuneki et al., 2004). A study on anti-diabetic effects of purple potatoes rich in anthocyanins, also found in purple tea, was shown to lower blood glucose levels in diabetic rats (Mi-Kyeong Choi et al., 2013). Similar findings were found when ethanolic extract of green tea extracts at a dose of 200mg/kg reduced blood glucose level in diabetic models (Haidari, 2013; Nickavar & Yousefia, 2012).

In the current study, the mechanism by which the administration of purple tea leaves extracts could exert hypoglycemic activity on diabetic mice may be by antioxidant effect. The traditional green tea (*Camellia sinensis*) infusion is characterized by a high content of flavonoids that have unique biological properties and may be responsible for many of the health benefits attributed to tea (Rietveld & Wiseman, 2003). Phytochemical results of purple tea leaves extracts from this study revealed the presence of saponins, flavonoids and alkaloids which have been reported to

have hypoglycemic activity (Sabu et al., 2002). It has been found that consumption of green tea significantly decreased blood glucose levels by increasing hepatic glycogen level in alloxan diabetic rats (Moram, 2001). This is possibly through reactivation of the glycogen synthase system due to increased insulin secretion and decreasing liver glucose-6-phosphatase activity, which is mainly responsible for releasing glucose molecules to the blood by converting glucose-6-phosphate to glucose (Shimizu, 2000; Sabu et al., 2002). Other studies have revealed that tea epigallocatechin gallate promotes pancreatic β-cells regeneration with insulin-like insulinotropic activities and inhibits gluconeogenesis through inhibition of liver phosphoenolpyruvate kinase synthesis (Chemler et al., 2007). Another possible mechanism by which crude purple tea leaves extracts mediates hypoglycemic effect may be by reduction of glucose absorption in small intestine and the serum insulin effect by either pancreatic secretion of insulin from β-cells (Al-attar, 2010). It has also been demonstrated that green tea increases insulin sensitivity in rats and that tea polyphenol is one of the active components (Wu, 2004a).

From the current study, aqueous extracts of guava leaves significantly reduced blood glucose in diabetic mice. Similar results have been reported when aqueous leaves of guava were given to volunteer human subjects at a dose of 400mg/kg resulting in significant reduction of fasting blood glucose after 30,90 and 120 minutes (Deguchi, 2010). Another study involving human subjects demonstrated that consecutive ingestion of Guava Leaf Tea (Bansoureicha®) with every meal improved hypoadiponectinemia and hyperglycemia, showing an increase in adiponectin level and a decrease in HbA1c% in blood (Asano et al., 2013). Other investigations of methanolic guava leaves extracts at a dose of 250mg/kg on diabetic Swiss albino mice showed a positive trend in regulating blood glucose after 4th and 6th hour and even after 24hrs (Sengupta et al.,2011). The antidiabetic activity of the guava leave extracts could partly be attributed to the

phytochemicals present in the plant leaf extract. Alkaloids, tannins and flavonoids have been reported to have hypoglycemic activity (Ragavan & Krishnakumari, 2006; Oubre et al., 1997), hypolipidemic, hypotensive and antioxidant properties (Begum et al., 2004). The leaf extracts of guava contain flavonoids, mainly quercetin derivatives which have antioxidants properties that prevent oxidative stress in tissues (Nakamura et al., 2000). Other studies have also shown that phytoconstituents of guava leaves are potent antiglycation agents in a glucose model system and prevent glycation-associated complications in diabetes (Wu et al., 2009). The active components of guava leaf extract inhibit alpha-glucosidase enzymes *in vitro* resulting in reduction of postprandial blood glucose elevation and improvement of hyperglycemia in murine models (Deguchi & Miyazachi, 2010). The fruits of *Psidium guajava* (guava) plant are rich in flavonoids, lectins, carotenoids and saponins proven to have antidiabetic activity (Kamath et al., 2008). Flavonoids and saponins were found to be present in *Psidium guajava* leaves extracts in the current study which are associated with the hypoglycemic activity observed.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

6.1.1 Phytochemical composition of purple tea and guava

This study showed that the leaves of *Camellia sinensis* and *Psidium guajava* had major phytochemical constituents including alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids. These bioactive compounds have been reported to be antidiabetic, anticancer, antibiotic and anti-inflammatory among other diseases. Identification and isolation of these bioactive compounds can lead to a good source of pharmaceutical drugs to manage such diseases.

6.1.2 *In vivo* safety evaluation of purple tea and guava

In vivo sub acute toxicity results from this study showed that the leave extracts of *Camellia sinensis* and *Psidium guajava* are safe in mice at a dose of 100mg/kg, 300mg/kg and 1000mg/kg. Mice administered with the extracts of purple tea and guava did not show any clinical signs associated with toxicity and biochemical markers of liver and kidney toxicity did not reveal any significant change compared to the control group.

6.1.3 Hypoglycemic activity of purple tea and guava

The aqueous, ethanolic and acetone leave extracts of *Camellia sinensis* (purple tea) and the aqueous, ethanolic and ethyl acetate leaves extracts of *Psidium guajava* (guava) significantly ($p < 0.05$) reduced blood glucose in diabetic mice. This therefore, has provided a scientific basis for their local usage as medicinal plants used to manage diabetes mellitus.

6.2 RECOMMENDATIONS

1. Quantification using GCMS or LCMS of the phytochemicals present in purple tea and quava leaves is recommended.

2. Higher doses greater than 1000mg/kg and chronic toxicity evaluation of the purple tea and guava extracts should be done to ascertain its long term toxicity.

3. A higher dose of more than 300mg/kg should be analyzed for hypoglycemic activity of purple tea and guava extracts.

4. There is need to identify and isolate the bioactive compounds responsible for the hypoglycemic activity of the leaves extract of *Camellia sinensis* (purple tea) and *Psidium guajava* (guava).

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