ASSESSMENT OF ANTIDIABETIC PROPERTIES OF THE PRICKLY PEAR CACTUS IN SWISS WHITE MICE

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A Thesis Submitted to the Graduate School in Partial Fulfilment for the Requirements of the Award of Master of Science Degree in Biochemistry of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

DECLARATION

This is my original work and has not been submitted or presented for examination in any institution either in part or as a whole.

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RECOMMENDATION

This thesis has been submitted with our approval as the supervisors for examination according to Egerton University regulations.

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DEDICATION

I dedicate this thesis to my dear parents, siblings and friends for their love, support and encouragement during my studies. Thank you and God bless you.

ABSTRACT

Diabetes mellitus, a metabolic disorder that affects the body's ability to make or use insulin, has become a major public-health issue globally. This is because of the numerous health problems it causes to humans including: brain damage, heart and renal diseases, circulatory problems, and death. The onset of diabetes mellitus can be due to various causes including genetic that leads mainly to development of Type I diabetes mellitus and the lifestyle of an individual that leads to obesity, a common cause of the Type II diabetes. Various management strategies have been proposed and implemented with the most recent one being the use of extracts of the "prickly pear cactus" *Opuntia* species, a shrub that grows mainly in semi-arid regions of America, Asia and Africa. The current study aimed at assessing the efficacy of prickly pear cactus cladode extracts in managing diabetes mellitus in diabetic mice and its possible cytotoxic effects. Healthy, adult Swiss white albino male mice weighing 20-30 g were induced with diabetes mellitus using Alloxan (150 mg/kg body weight) administered intra-peritoneally. Prickly pear cactus cladode extracts were administered orally at daily dosages of 0.6 ml and 0.8 ml for pre-determined periods. Fasting blood sugar levels, live body weights and packed cell volume values were monitored during and after termination of feeding on cactus cladode extracts. Liver and kidney tissues were obtained at the end of the experiment and processed for histopathological examination. Alloxan administration caused a 3- to 4-fold increase in blood sugar levels. Diabetic animals treated with cactus cladode extracts showed a decline in blood sugar levels, however, the levels varied with the period of treatment. Diabetic animals treated with cactus cladode extracts for 10 days showed a significant decline in blood sugar levels on the 7th ($p=0.012$) and 10th ($p=0.001$) days of feeding on the extracts when compared to the diabetic control animals. Histopathological examination revealed kidneys sections characterised with normal renal architecture. Mild degenerative changes were observed in liver sections of diabetic treated animals. No mortality was reported throughout the experiment. This study has demonstrated that extracts from prickly pear cactus cladode from Kenya have potential in managing blood sugar in alloxan-induced diabetic mice. This study has also demonstrated that cactus cladode extracts minimises the effects of diabetes mellitus on kidneys and liver of diabetic mice.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADA	American Diabetes Association
BMI	Body Mass Index
DM	Diabetes Mellitus
ESRD	End Stage Renal Disease
FBG	Fasting Blood Glucose
FPG	Fasting Plasma Glucose
GFR	Glomerular Filtration Rate
GISD	Global Invasive Species Database
GLP	Glucagon Like Peptides
H & E	Haematoxylin and Eosin
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IPR	Institute of Primate Research
KEFRI	Kenya Forestry Research Institute
LADA	Latent Autoimmune Diabetes of Adulthood
NIDDM	Non-Insulin Dependent Diabetes Mellitus
PCV	Packed Cell Volume
ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
TID	Type I Diabetes
UTI	Urinary Tract Infection

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background of the study

Diabetes mellitus (DM) is a metabolic disorder that affects the body's ability to make or use insulin, a hormone crucial in the uptake of glucose from the bloodstream into the cells (American Diabetes Association, 2007; Samreen, 2009). The words "diabetes" and "mellitus" have different origins. "Diabetes" originated from a Greek term for passing through with reference to the common symptom of the disease, polyuria, while "mellitus" is a Latin word for honeyed with reference to increased glucose observed in the urine of diabetic patients (Samreen, 2009). The disease is characterised by chronic hyperglycaemia with disorders in protein, fat and carbohydrate metabolism resulting from defects in insulin secretion and/or action (World Health Organization, 1999; Rachid *et al.,* 2012). Imbalances in glucose homeostasis as a result of defects in insulin metabolism leads to abnormal levels of glucose in the blood stream, which can cause brain damage, renal, heart and visual impairments, and this may lead to permanent health alterations and even death (Samreen, 2009; Paiz *et al*., 2010).

Two major types of diabetes mellitus have been characterized namely Type I diabetes mellitus and Type II diabetes mellitus. Type I diabetes mellitus is mainly genetic while Type II diabetes mellitus is associated with lifestyle. Type I diabetes occurs when the beta cells responsible for making and secreting insulin die as a result of autoimmune attacks hence leading to absolute lack of insulin in the body. On the other hand, Type II diabetes occurs when insulin producing beta cells are present but they are defective and produce less than the required amounts of insulin in the body. The insulin produced may not also work properly when it gets in the cells, which leads to insulin resistance in the individual (Pereira *et al.*, 2010). Risk factors associated with this type of diabetes include excess body weight, old age, physical inactivity, family history of diabetes, unhealthy diet and impaired glucose tolerance (WHO, 2013). Of the two types, Type II is the most prevalent accounting for 90% of all the diabetic cases across the world while Type I diabetes mellitus accounts for the remaining 10% (Hernandez-Avila and Olaiz-Fernandez, 2002; Rachid *et al.,* 2012).

Studies by the World Health Organization (WHO) and International Diabetes Federation (IDF) indicate that the number of people suffering from diabetes across the world is increasing significantly over recent years.

For instance, in the year 2000 171 million cases of diabetes were recorded worldwide (Wild *et al.*, 2004; Whiting *et al.*, 2011; WHO, 2011). Recent studies show there are 382 million diabetic patients globally with this figure likely to increase to 592 million by the year 2035. This will represent a prevalence of 10.1%, an increase from 8.3% in 2013 (IDF, 2013b) (Figure 1.1).

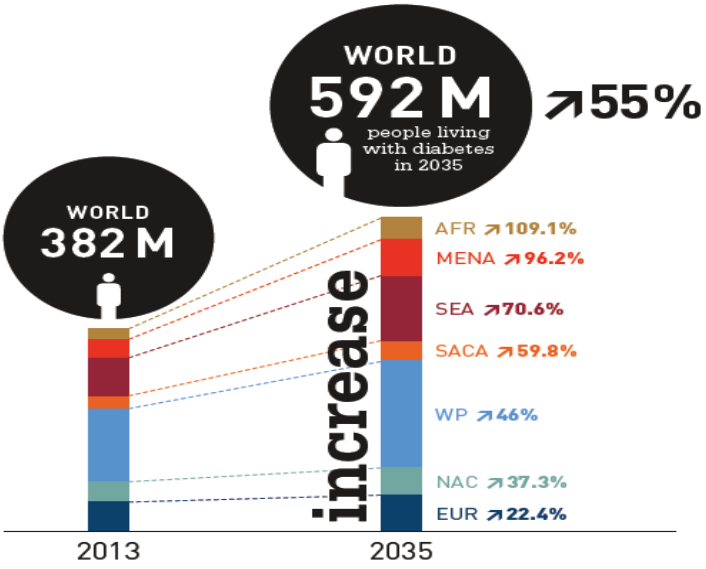

Figure 1.1: People with diabetes in 2013 and 2035 projection (IDF, 2013b)

Management of diabetes mellitus is a lifelong process that requires constant medication and specialized health care (Jones, 2013). Some of the therapeutic strategies used in prevention and management of the disease in the early stages include regular exercise, proper diet as well as administration of glucose-lowering agents. There are two major classes of oral drugs prescribed for diabetic patients because of their affordability and mild side effects: the biguanides (such as metformin and phenformin), and sulphonylureas (such as gilbenclamide, diabenese, orinase, glucotrol, and Amaryl) (David *et al.*, 2014). Other classes of oral pills include: Thiazolidinediones, Alpha-glucosidase inhibitors, Glucagon Like Peptides-1(GLP-1) receptor agonists and Megilitinides. While these approaches are promising in management of diabetes mellitus, they are costly and can pose severe side effects to the patients (Awanish *et al.*, 2011).

For that reason, various formulations of traditional herbal plant extracts including those of the *Cactaceae* family are being used widely in resource-poor developing countries with little access to public health care services (Andrade-Cetto and Heinrich, 2005).
Misuse of these drugs may however lead to adverse health effects (Castillo, 2002). Therefore, there is need to assess the benefits and risks associated with the use of these herbal extracts as anti-diabetic agents.

1.2 Statement of the problem

Diabetes mellitus as one of the most prevalent chronic degenerative diseases causes various health impacts ranging from brain damage, heart and renal diseases, which sometimes lead to permanent alterations in the health of the individual and eventually death. This disease has therefore become a major public health concern globally. Diabetes mellitus can be managed using insulin-based medications in addition to herbal extracts of different plant species including those of *Opuntia*. Previous studies in other parts of the world (Mexico and Egypt) on the use of extracts of this plant species as an anti-diabetic drug have shown that it is promising in management of diabetes mellitus. However, there is lack of information on the possible negative effects associated with its use. This study therefore assessed the role played by prickly pear cactus cladodes in the management of diabetes mellitus and the associated cytotoxic effects.

1.3 Objectives.

1.3.1 General objective

To assess the anti-diabetic properties of the prickly pear cactus in Swiss white mice.

1.3.2 Specific objectives

1. To determine efficacy of the prickly pear cactus extracts in controlling blood sugar in Swiss white mice.
2. To assess cytotoxic effects of the prickly pear cactus extracts on the Swiss white mice.

1.4 Hypotheses

1. Extracts of the prickly pear cactus have no significant effect on blood sugar level reduction in diabetic mice.
2. Prickly pear cactus extract extracts have no cytotoxic effects on the mice.

1.5 Justification

Sedentary lifestyles and improper diets have led to increased incidences of obesity, a leading risk to the onset of Type II diabetes mellitus. Diet and exercise play a crucial role in management of the disease but these approaches become ineffective when the disease has reached a degenerative stage that requires medication. The oral pills and insulin-based drugs used for the management of diabetes mellitus are however very expensive making them unavailable to people of low income countries. This has therefore led to the increased use of herbal therapy of different plants including those of the *Opuntia* species to manage the disease because they are affordable and readily available.

Studies on the hypoglycaemic properties of this plant extracts has been done elsewhere in the world but there is little information on the possible negative health effects that may arise from the continued use of these plant extracts as an anti-diabetic drug. We are also not aware if these properties are confined within one location. This information is important given that plant phytochemicals tend to vary depending on environmental conditions. Therefore, this study aimed to fill this gap by providing information on hypoglycaemic and cytotoxic effects of prickly pear cactus cladodes from Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes mellitus is defined as a metabolic disorder that affects the body's ability to make or use insulin (ADA, 2007; Carlos *et al.,* 2013). It causes glucose intolerance that leads to abnormal levels of glucose in the blood stream observed as chronic hyperglycaemia characterised by disturbances in carbohydrate, protein and fat metabolism (Boon *et al*., 2006; Al-Saadi *et al*., 2011; Rachid *et al*., 2012). Traditionally, diabetes mellitus is described as hyperglycaemia or sugar diabetes due to absolute or relative deficiency of insulin secretion and/or insulin action (Mbaya and Ramiaya, 2006; Ukwueze and Anene, 2010).

Diabetes mellitus is one of the most important chronic degenerative diseases across the world and is ranked 4th among non-communicable diseases (WHO, 2010). It is chronic and can affect any organ of the body (Balisa *et al*., 2012; Faraji *et al*., 2012) causing serious short-term and long-term consequences ranging from brain damage to cardiovascular diseases (Warmeille *et al*., 2004; ADA, 2007; Balisa *et al*., 2012). According to IDF, the disease currently affects more than 382 million people across the world (IDF, 2013). The high morbidity and mortality rates associated with diabetes mellitus have made it to become a major public health concern globally (Carlos *et al*., 2013).

2.2 Types of diabetes

Diabetes can be classified into major and minor types depending on their mortality and morbidity levels across the world (Carlos *et al.,* 2013). The major types include Type I and Type II diabetes mellitus while the minor types include gestational diabetes, Latent Autoimmune Diabetes of Adulthood (LADA), secondary diabetes, double diabetes and the less commonly known type, diabetes insipidus (Samreen, 2009; Al- Saadi *et al*., 2011; IDF, 2013).Type I diabetes (TID) is among the top leading disorders of childhood affecting 0.04% of people below 30 years (Patterson *et al*., 2009). It usually develops rapidly and can affect people of any age but is more prevalent in children and young adults hence sometimes referred to as juvenile diabetes. TID is also called insulin-dependent diabetes mellitus (IDDM).

The term IDDM arises from the fact that people suffering from this type of diabetes produce very little insulin due to deficiency of insulin-producing beta cells in the pancreas hence need daily insulin injections to control their blood glucose levels (Clement *et al*., 2004; Samreen, 2009; Benseffaj *et al*., 2012; IDF, 2013). On the other hand, Type II diabetes is a diverse group of disorders usually characterised by insulin resistance and relative insulin deficiency. This disease is sometimes referred to as adult-onset diabetes because it mostly affects middle age and old people but it also becoming more common in the young people due to increased rates of obesity (Al-Saadi *et al.,* 2011; IDF, 2013). Type II diabetes mellitus is also called non-insulin dependent diabetes mellitus (NIDDM) because most people suffering from it do not necessarily require insulin injections as their beta cells still produce insulin (Samreen, 2009). However, they may require it for control of blood glucose levels if diet or oral hypoglycaemic drugs cannot normalize the blood glucose (Thevenod, 2008; Taylor, 2013). This type of diabetes may take many years to develop and is normally preceded by pre-diabetes whereby levels of blood sugar are above normal but not high enough for diagnosis of diabetes (Samreen, 2009).

There also exist other types of diabetes which are less common due to their low levels of mortality and morbidity. Some of them like Latent Autoimmune Diabetes of Adulthood (LADA) and double diabetes are variations of TID. LADA develops later in life as a result of pancreatic beta cell destruction by autoreactive T cells. Double diabetes on the other hand develops in children and young adults who overeat without exercising. It may also be genetic that is, the person has genes for obesity making them to develop tissue-wide insulin resistance (Parry and Brooks, 2008).

Hormonal changes in pregnant women, overweight and sometimes genetic reasons (the mother has a family history of diabetes) can also lead to a form of diabetes called gestational diabetes, a metabolic disorder that usually occurs in the third trimester. The disease can cause complications both for the mother and the child making them to be more susceptible to developing diabetes later in life. Gestational diabetes is usually temporary and ends when the pregnancy ends (Al-Saadi *et al*., 2011; IDF, 2013).

Other forms of diabetes such as secondary diabetes and diabetes insipidus are neither autoimmune nor metabolic. Secondary diabetes arises from another condition mainly diseases such as pancreatitis, Down's syndrome and cystic fibrosis. Medical treatments with corticosteroids, diuretics and pancreatectomy can also lead to development of this diabetes type while diabetes insipidus is a condition whereby the kidneys release excess water.

It is sometimes referred to as water diabetes and the major symptom observed in patients suffering from this disease is polyuria (Samreen, 2009).

2.3 Causes of diabetes mellitus

The major cause of diabetes in people and animals is deficiency of insulin secretion, and/or action (Rachid *et al*., 2012). Most of the diabetic cases arise from either metabolic or autoimmune disorders (Samreen, 2009).

2.3.1 Autoimmune causes of diabetes mellitus

Autoimmune causes include destruction of the pancreatic beta cells that produce insulin by the person's immune system (Samreen, 2009; Al- Saadi *et al*., 2011). This is a leading cause of the Type I diabetes mellitus. Autoimmune causes of diabetes can also be due to genetic make-up of the individual, family history for example, inheritance of some diabetes causing disorders such as maturity-onset diabetes of the young and Wolfram syndrome, age and viruses or other environmental factors that cause the immune system of a person to mistakenly destroy its pancreatic cells and hence no insulin production. Type I diabetes can also result from other diseases such as pancreatitis that may lead to pancreatectomy (surgical removal of the pancreas). When this is the case, it is called secondary Type I diabetes mellitus (WHO, 1999; Samreen, 2009).

2.3.2 Metabolic causes of diabetes mellitus

Metabolic causes of diabetes mellitus are mainly lifestyle based and they give rise to development of Type II diabetes. Unhealthy lifestyles such as over-eating with physical inactivity (Cefalu *et al*., 2007) leads to obesity that produce undesirable metabolic effects in the body of the individual (Ferreira *et al*., 2010; Pereira *et al.*, 2010). This therefore impairs the ability of the body to use insulin causing hepatic and peripheral tissue resistance and dysfunction of pancreatic beta cells (William and Pickup, 2004; Kasper, 2005) and hence Type II diabetes.

2.4 Signs and symptoms of diabetes mellitus

Diabetes in most cases goes undetected because its symptoms closely resemble many other diseases that can be attributed to different causes. For instance, Type I diabetes mellitus symptoms may be mistaken for the flu or other common diseases as it develops rapidly and often occurs after an illness.

Some people, especially the young with Type I diabetes often go undiagnosed until they get a condition called diabetic ketoacidosis with the following signs; confusion and difficulty in breathing, and sweet fruity-smelling breath. Type II diabetes mellitus, on the other hand, can take several years to develop and often becomes noticeable after the person suffers long-term serious complications such as sexual dysfunction, leg pains that may be due to diabetic neuropathy, insulin shock or diabetic coma (Samreen, 2009; ADA, 2010).

The common clinical signs of untreated diabetes are related to increased blood glucose, loss of glucose in the urine (glucosuria), polydpsia (excessive thirst), polyphagia (excessive appetite) and polyuria (excessive urination) (ADA, 2007; Samreen, 2009; Gupta and De, 2012; Clara, 2013). Summary of metabolic conditions arising from uncontrolled diabetes mellitus is demonstrated in Figure 2.1 below (Ophardit, 2003).

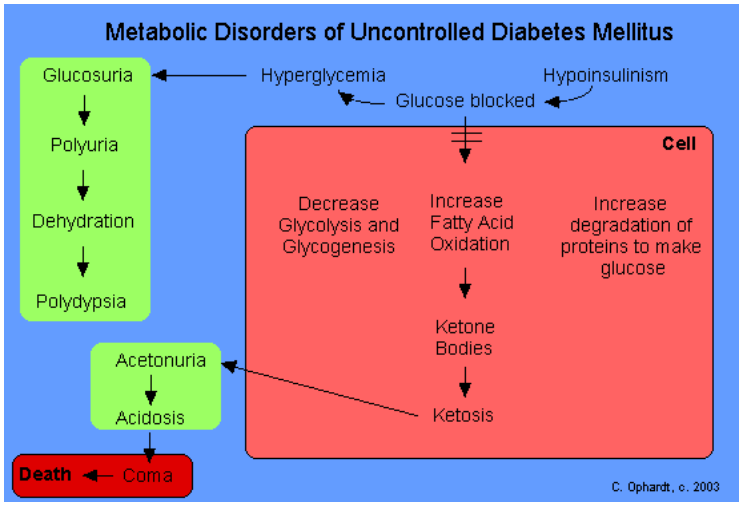

Figure 2.1: Summary of metabolic disorders arising from uncontrolled diabetes mellitus (Source: Ophardit, 2003).

Other signs include: vision problems such as blurred vision and nearsightedness, slow healing of wounds and sores, unexplained weight loss, fatigue, drowsiness, irritability, numbness, pain in the feet, legs or hands, frequent infections, including skin infections, urinary tract infections and yeast infections (Samreen, 2009; WHO, 2013; Health.com, *n.d*).

2.5 Diagnosis of diabetes mellitus

Diagnosis of diabetes mellitus is through performing glucose tests on patients. These blood tests measure the level of blood sugar in a person's blood stream. Screening of a person for diabetes is usually done using a fasting plasma glucose test (FPG). This is usually done in

the morning before one takes breakfast as it allows the patient to fast for the required eight hours.

Blood samples from the patient are drawn and measured for glucose concentrations in milligrams per decilitre (mg/dl) of blood. FPG results below 100 mg/dl are considered normal, between 100 and 125 mg/dl pre-diabetes and above 125 mg/dl indicates diabetes. Confirmation of the diagnosis is done by performing another glucose test on the patient on another day (WHO, 1999; Health.com, *n.d*).

If the glucose test confirms diagnosis of diabetes in the patient, another test usually the C-peptide test is done to establish the type of diabetes in the individual that is, whether the person has Type I or Type II diabetes. A C-peptide test easily distinguishes the diabetes types because people with Type II diabetes have the C-peptide in their blood, a by-product of insulin production, while people with Type I diabetes lack the C-peptide in their blood. Autoantibody testing can also be done to establish the diabetes type as it can easily reveal misguided antibodies present in Type I diabetes but absent in Type II diabetes (Peters *et al*., 1996; Samreen, 2009).

There are other tests that may be done on the patient after establishing the type of diabetes. Thyroid blood tests can be done to find the cause of Type II diabetes while genetic tests can be done to help diagnose conditions such as maturity onset diabetes of the young (ADA, 2007a).

2.6 Global burden of diabetes mellitus

Diabetes is among the common endocrine diseases and it not only affects humans but it can also affect other animals such as dogs, cats (Kim *et al*., 2006; Samreen, 2009), pigs, horse, cattle and sheep (Ukwueze and Anene, 2011). Studies by the International Diabetes Federation show that there are 382million people living with diabetes globally, which represents a prevalence rate of 8.3%. In Africa, it is estimated that 20 million people live with diabetes corresponding to a prevalence of 4.8% with this figure projected to double in the next 20 years (IDF, 2013b).

The epidemiology of diabetes in Kenya has not been studied to a larger scope although evidence from health care services suggests that the incidence of diabetes is on the increase (Christensen *et al*., 2009). IDF studies on diabetes in Kenya show prevalence of diabetes is 3.58% with 20,350 diabetes related deaths of patients between the age of 20-79 in 2013 (IDF, 2013b). 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).

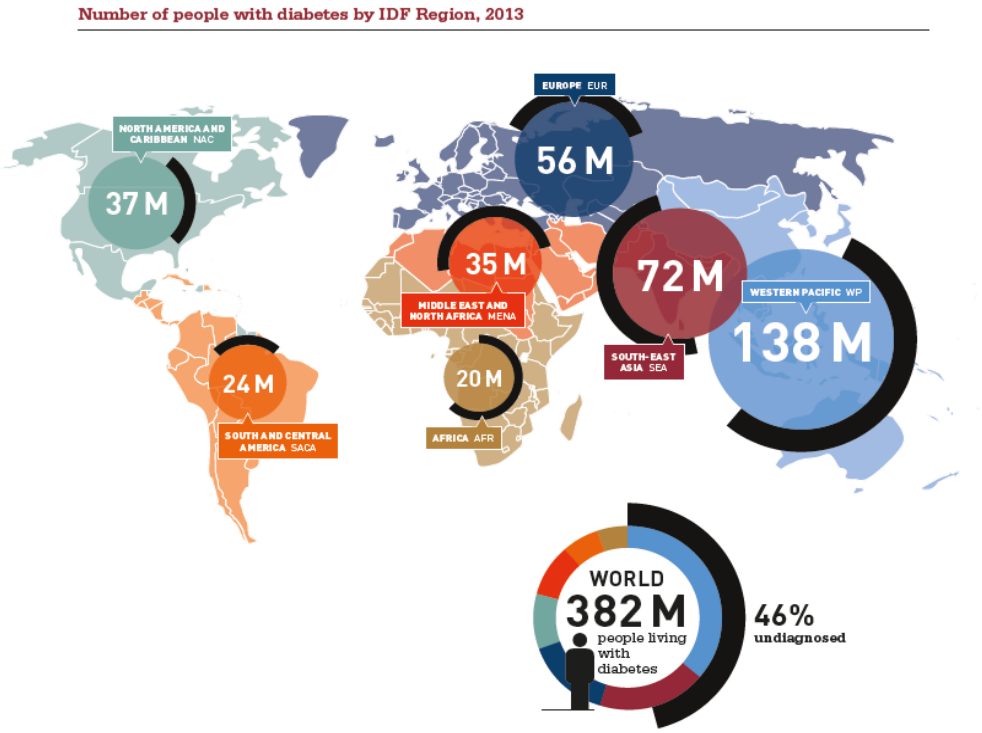

Figure 2.2: Global distribution of diabetes (IDF, 2013b)

2.7 Progression of Type II diabetes mellitus

Progression of Type II diabetes mellitus involves at least two primary pathogenic mechanisms which include progressive decrease in pancreatic islet cell function resulting in reduced insulin secretion and peripheral insulin resistance resulting in a decline in the metabolic responses to insulin (ADA, 2010; Boada and Moreno, 2013). Various factors are believed to increase the progression of diabetes in people across the world. For instance, worldwide spread of Western-style habits such as consumption of food rich in calories with physical inactivity has led to development of Type II diabetes mostly among people in developing countries (Mohan *et al*., 2007).

Age and gender differences are also other contributing factors to development of Type II diabetes mellitus. Studies have shown that aging brings about many health complications among them being diabetes. It is also believed that men are more prone to developing diabetes as they advance in age than their female counterparts (Williams *et al*., 2003; Perreault *et al.*, 2008; Kaur *et al.*, 2010). People with elevated blood pressure are also at a

higher risk of developing Type II diabetes and such individuals have high mortality rates as they become susceptible to cardiovascular diseases (Pancha *et al*., 2012).

According to a report by the WHO, it is believed that diabetes kills more than one million people annually across the globe and this figure is expected to keep growing with majority of the cases being of the metabolic form (Samreen, 2009; WHO, 2011a). More than 80% of such deaths occur in low- and middle-income countries (Mathers and Loncar, 2006). World Health Organisation projects diabetes will be the number 7 leading cause of death in the world by 2030 (WHO, 2011a).

2.8 Health risks associated with diabetes mellitus

People with diabetes mellitus have an increased risk of developing a number of serious health problems ranging from cardiovascular diseases, kidney diseases to even complications in pregnancy (IDF, 2013a). Diabetic complications arising from diabetes mellitus includes the following organs: kidney diseases, cardiovascular diseases, nerve disease, eye diseases, urinary tract infections and other infections, and pregnancy complications

2.8.1 Kidney diseases

Kidney diseases as a result of diabetes are also called diabetic nephropathy. Diabetic nephropathy is a type of progressive kidney disease characterised by persistent albuminuria (increased urinary excretion of albumin) and decline in the glomerular filtration rate (GFR).This disease is caused by increased blood pressure on the small blood vessels in kidneys that damages the vessels leading to the kidneys becoming less efficient in their work or total kidney damage (IDF, 2013a). Development of diabetic nephropathy is usually slow and it affects 20-30% of people with Type I diabetes mellitus. Symptoms of diabetic nephropathy are however not easily noticed until it reaches end-stage renal disease (ESRD), final stage of the disease (ADA, 2010).Diabetic patients with this disease can be treated by performing dialysis or kidney transplant (Engelgau and Gueiss, 2002; Samreen, 2009; Samanta, *et al*.,, 2012).

2.8.2 Cardiovascular diseases

High blood glucose, blood pressure and cholesterol levels in diabetic patients increase their risk of contracting cardiovascular diseases. Most deaths in people with Type II diabetes are caused by these diseases. The common cardiovascular diseases in diabetic patients

include atherosclerosis, diabetic angiopathy, heart conditions and stroke (Yorek, 2003; Samreen, 2009; IDF, 2013a).

2.8.3 Nerve diseases (Diabetic neuropathy)

Diabetic neuropathy is caused when blood glucose and pressure levels are very high in the body leading to nerve damage. It can occur in the posterior parts of the body and this causes pain, tingling and numbness to the feet. Severe peripheral neuropathy may lead to foot infections, which may later lead to amputation of the leg and the foot. Autonomic neuropathy can also occur and it leads to sexual dysfunction and impaired digestion. Diabetic nephropathy can also cause impaired hearing and thinking which leads to memory loss, a common problem observed in most diabetic patients (Samreen, 2009; IDF, 2013a).

2.8.4 Eye diseases (Diabetic retinopathy)

Eye diseases in diabetic patients are caused by consistently increasing blood sugar, pressure and cholesterol. Common eye diseases in diabetic patients include cataracts and glaucoma that cause impaired vision, which may eventually lead to blindness (Samreen, 2009; IDF, 2013a).

2.8.5 Urinary tract infections and other infections

People with diabetes mellitus are at an increased rate of contracting many infections including UTIs, thrush, bacterial and yeast infections. Diabetic women are at a greater risk of contracting urinary tract infections (UTIs) and the most common UTI in the women is vulvovaginal candidiasis (Nicolle, 2005; Goswami *et al*., 2006). This is caused by excessive growth of *Candida albicans* in the mouth, digestive tract, vagina and other tissues due to poor control of sugar levels in the body (Nowakowska *et al*., 2004).

Bacterial infections in diabetic patients result from immunosuppression caused by increased blood sugar in the blood stream (Samreen, 2009). The common bacterial infections in women include bacteriuria and bacteraemia. Various factors such as sexual intercourse, high body mass index (BMI), age and degree of glycosuria predispose diabetic women to bacteriuria (Lyamuya *et al*., 2011). Bacteraemia, on the other hand, is caused by lack of antimicrobial activity in the body due to inhibition by hyperglycaemia that favours growth of microbes (James, 2000).

2.8.6 Pregnancy complications

Increased blood sugars in pregnant women can lead to pregnancy complications such as preeclampsia and problems during delivery. Diabetic pregnant women are also at a higher risk of having miscarriages, stillbirths and giving birth to babies with defects such as overweight babies (Samreen, 2009; IDF, 2013a).

2.9 Management of diabetes mellitus

Diabetes mellitus is becoming one of the major concerns in the public health sector across the world. Therefore there is need to manage occurrence and progression of the disease. Diabetes management involves lifestyle changes, taking medications and close monitoring of the diabetic patients for any adverse chronic complications such as cardiovascular, renal and eye diseases (Sandra *et al.,* 2009). Early screening and diagnosis are of great importance in the management of all types of diabetes mellitus.

Many types of diabetes including Type II diabetes, gestational diabetes and some metabolic forms of diabetes can be easily managed through lifestyle changes such as physical exercises that enhance glucose consumption by the body thus reducing blood sugar levels, and proper diets (Ferreira *et al*., 2010; Kaur *et al.,* 2010; Paiz *et al*., 2010) with no or reduced alcohol consumption (Samreen, 2009). People with TID and latent autoimmune diabetes of adulthood require regular insulin medications, which are administered in various forms including insulin pumps, syringe injections, jet injections, insulin pens, and inhaled insulin. Some people with Type II diabetes can also be prescribed with insulin therapy at some stages of their disease (Samreen, 2009). Most of the diabetes therapeutic measures taken are aimed at reducing blood glucose levels to attain a normal glycemic level in the body (Agius, 2007).

2.9.1 Drugs used in the management of diabetes mellitus

Treatment of diabetes is a life-long process that requires regular health care to achieve good control of blood sugar levels and to prevent further health complications that may cause death of the patient (ADA, 2007; Sandra *et al.*, 2009). Diabetes medication is aimed at lowering blood glucose levels as it does not cure diabetes and most of the people with diabetes have to take the medication throughout their lives. The type of medication prescribed depends mostly on the health status of the patient and individual needs (Diabetes UK, 2015). Two types of diabetes medications are used; oral pills and injections (insulin therapy) (Talking diabetes, 2012; Cicero *et al.*, 2013).

Several classes of oral drugs are used to manage the disease. They are classified into first-choice and second-choice medications depending on affordability, availability and side effects they cause:

1. First-choice diabetes medications- These drugs include Biguanides and Sulfonylureas. They are affordable and easily available to many people.
2. Second-choice medications-They include Thiazolidinediones, DPP-4 inhibitors, Alpha-glucosidase inhibitors, Glucagon Like Peptides-1(GLP-1) receptor agonists and Megilitinides.

Biguanides mostly metformin is the drug that is usually prescribed to patients after diagnosis with diabetes mellitus. Metformin reduces blood sugar levels in three ways; inhibiting glucose release from the liver, decreasing the rate of glucose absorption from the intestines and increasing sensitivity of body tissues to insulin (Talking diabetes, 2012). The first dose is taken in the evening with a meal followed by another dose after one or two weeks taken in the morning with breakfast to counteract side effects such as nausea and diarrhoea (Talking diabetes, 2012; David *et al.,* 2014). Sulphonylureas are prescribed when metformin fail to adequately lower blood sugar levels on its own. This class of drugs manage sugar levels by stimulating extrapancreatic mechanisms that increase insulin release from beta cells and promote insulin action (Gribble *et al.,* 1998; Talking diabetes, 2012; David *et al.,* 2014).

Sulphonylureas are known to cause hypoglycaemia. They are taken with metformin and other classes of diabetes drugs or insulin. The patient is required to take them just before having a meal and to also have regular meals throughout the day to prevent the hypoglycaemic risk (Talking diabetes, 2012; David *et al.,* 2014). There are a number of sulphonylureas used to lower blood sugar levels. They have the same mode of action and the choice between them depends on availability and affordability (WHO, 1994; David *et al.,* 2014). Examples include; Diabinese, Orinase, Glucotrol, DiaBeta, Micronase, Glynase and Amaryl (David *et al.,* 2014).

The other classes of diabetes oral drugs are usually prescribed to patients who do not respond to metformin and sulphonylureas well (WHO, 1994; Talking diabetes, 2012; Cicero *et al.,* 2013 David *et al.,* 2014). These drugs are taken in combination with either of the two. They are rarely prescribed as first-choice diabetes medicines because they are costly and pose severe side effects to the patient (Talking diabetes, 2012; David *et al.,* 2014).

Insulin therapy is indicated when diet and oral medications become inefficient in controlling blood sugar levels. Insulin is injected into the body as normal digestion interferes

with insulin taken orally (WHO, 1994; Cicero *et al.*, 2013). Three groups of insulin are used in the management of hyperglycaemia; animal insulin, human insulin and analogues of insulin. Human insulin is synthetic insulin made to mimic human insulin whereas insulin analogues are a group of human insulin whose chemical structure has been altered to make it more efficient. An example of human insulin is Incretin mimetics (Talking diabetes, 2012).

2.9.2 Medicinal plants used in management of diabetes mellitus

Diabetes mellitus can also be managed using extracts of various plants. The use of traditional medicinal plants for treatment of diabetes mellitus is becoming common among people of low income groups without access to public health care services (Andrade-Cetto and Heinrich, 2005). Various plant species are being used as anti-diabetic agents in the management of diabetes worldwide among them being species of Arctium such as *Arctium minus* (Hill) and *Arctium lappa* (Ferreira *et al*., 2010), *Aegle marmelos, Eugenia jambolana* (Gohil *et al*., 2010) and *Opuntia* species (Paiz *et al*., 2010). These plant species belong to the *Cactaceae* family and they are believed to have compounds with hypoglycaemic properties that reduce blood glucose levels when taken by diabetic patients (Ferreira *et al*., 2010; Gohil *et al*., 2010; Paiz *et al*., 2010).

Studies on the *Arctium* species, a plant found in Portuguese and some Asian countries show that roots of *A. minus* (Hill) and *A. lappa* have a fructan compound called inulin that is hard to digest in the upper digestive system (Duke *et al*., 2002; Li *et al*., 2008). The compound has also a negative effect on absorption of lipids and cholesterol in the small intestines (Causey *et al*., 2000). This therefore prevents release of glucose into the blood thus maintaining a normal blood sugar level in the body. Extracts of *A. minus* (Hill) have also shown to contain flavonoids, which are anti-oxidants that reduce oxidative stress associated with elevated blood glucose levels (Erdemoglu *et al*., 2009; Ferreira *et al*., 2010).

Extracts from seeds and pulp of *Eugenia jambolana*, a plant belonging to the *Myrtaceae* family, have also been used in Asian countries for management of diabetes. These extracts contain flavonoids that act as anti-oxidants reducing oxidative stress caused by hyperglycaemia (Ravi *et al.,* 2004). Extracts from the fresh seeds of these plants show that it is very effective in treating diabetes as it reduces concentration of glucose in urine of diabetics rapidly (Gohil *et al*., 2010). The other plant species whose extracts are believed to reduce blood glucose levels is the *Aegle marmelos* species, used widely among the Asian communities (Gohil *et al*., 2010). Use of the above species of plants is however limited due

to inadequate information on the mechanism of action and the therapeutic effects of the plants with accredited anti-diabetic action by traditional medicine.

Opuntia species on the other hand have been studied extensively and this has led to its widespread use as a hypoglycaemic agent in several parts of the world. These cacti are however native to the Americas and their occurrence in African and Asian countries is due to introduction of exotic seeds (Plagens, 2011).

In Kenya, there are four major species mainly found in semi-arid regions and they include; *Opuntia ficus-indica (L.), Opuntia valgaris*, *Opuntia monocantha* and *Opuntia occidontalis* (Plagens, 2011).

2.10 Morphology of *Opuntia*

Opuntia ficus-indica is a cactus that belongs to order Caryophyllales and family Cactaceae (Kang'ara and Gitari, no date (n.d)). The shrub, usually succulent, has flattened branches, grey to green-grey in colour. The branches vary in size from 30-60cm long and 6-15cm wide. Leaves, if developed, are tiny and shed during early growth stages of the shrub. *Opuntia* has bright yellow or orange, red coloured flowers making them very conscipicous (Orwa *et al*., 2009; Kang'ara and Gitari, n.d). The plant also develops fruits, about 8 cm long and covered with clusters of tiny spines, green when unripe and red when ripe (Kang'ara and Gitari, n.d; Norris and Media, n.d) .

Figure 2.3: Picture of Prickly pear cactus with leaves (pads), flowers and unripe fruits (Source: PhotoStock-Israel, 2013).

Figure 2.4: Ripe fruits of *Opuntia* (Norris, n.d)

Opuntia is easily propagated from the cladodes (oval stems) when they are detached from the main plant and drop on the ground where they develop roots and more pads (cladodes). These succulent pads rarely fail to grow except when they are disturbed frequently during the rooting (Kang'ara and Gitari, n.d). The shrub length ranges from 1.5metres to 3 metres but it can grow up to 5m tall forming a strong trunk as time goes by (GISD, 2005; Orwa *et al*., 2009; Kang'ara and Gitari, n.d). These plants are also called Prickly pear cactus because of the pricks they bear on their leaves, stems and fruit covering (Plagens, 2011).

2.11 Traditional uses of *Opuntia*

2.11.1 Food

Prickly pear cactus pads and fruits have been used as food in Mexico and other Central American countries, Australia, India, North Africa, the Middle East and parts of Europe for centuries (Orwa *et al.,* 2009). The fruit also called sabra in Arabic and tuna in Spanish ranges in flavour from sour to very sweet, and in colour from yellow to red when ripe (Orwa *et al.,* 2009). The fruit can be eaten raw or cooked to make sweets and jellies after peeling the outer covering (Rave, 2012). In Mexico, it is also used to make alcoholic drinks mostly spirits known as colonche (Plants for a future, 2012). The fruit is also served as a

whole fruit or syrup made and used as a mixer in cocktails at fancy tourist lodges in some parts of Kenya (Factsheet, 2011).

Edible pads consumed as vegetables are usually the tender young pads also known as nopales. They are characterised by fleshy oval leaves with a soft but crispy texture that is a bit sticky when cooked (Hahm *et al.,* 2011). Consumption of these nopales have great health benefits as they are rich in important biochemical compounds such as pectin, fiber, calcium, magnesium and beta carotenes (Rave, 2012). They are good sources of proteins, vitamins A, C, B6 and K (Hahm *et al.,* 2011). Apart from *Opuntia* being used as food for humans, it is also used as feed for dairy animals (DeFelice, 2004; Saleem *et al.,* 2006; Orwa *et al.,* 2009). The tender young pads (nopales) are believed to enhance milk flavour and quality and hence better quality for butter and other dairy products. The cactus is also used as a dietary supplement to stimulate growth in young sheep (Conrad, 2005).

2.11.2 Ornamental

Prickly pear cactus is planted as an ornamental crop to reclaim land, mark boundaries, control soil erosion and fence homesteads (Factsheet, 2011). The shrubs are planted in degraded lands which cannot be reclaimed through the usual agricultural methods to rehabilitate them because of their easy propagation through vegetative means. This has been used to rehabilitate steep, stony and rocky landscapes (Orwa *et al.,* 2009). Planting of cactus along edges also controls soil erosion (Synman, 2006). The cactus hedges act as physical barriers to top soil run-off in arid areas prone to wind erosion thereby improving soil fertility in the areas. These shrubs are also used as live hedges to mark boundaries and protect gardens in North America and some parts of Italy and Spain (Orwa *et al.,* 2009). In Kenya it grows as a wild plant in semi arid areas such as Baringo and Laikipia. It is however used as a live fence for protection of agricultural lands from invasion by grazers. It is also used to mark boundaries and fence homesteads to protect their animals from predators at night (Dena, 2008).

2.11.3 Medicine (Medicinal Uses)

Prickly pear cactus has long been used to treat ailments in North America and some Asian countries. These cacti are rich in antioxidants such as flavonoids, known to prevent and/or treat various diseases (Herbs, 2012; Rave, 2012; Osuna-Martinez *et al.,* 2014). The fruit is used to treat asthma, diarrhoea, indigestion and alcohol hangovers while the fleshy pads are used to treat ailments such as gastric ulcers, edema, high blood pressure and elevated

cholesterol levels (Gurrieri *et al.,* 2000; DeFelice, 2004; Rave, 2012; Osuna-Martinez *et al.,* 2014).

The pads can also be applied topically to prevent wound infection that may result from burns and insect bites (DeFelice, 2004; Rave, 2012). The Fruits and young pads of this plant have been widely used to manage diabetes mellitus in Mexico, South Korea and Sicily (Drugs.com, 2015). Several species are used to manage the disease. For example, in Mexico *Opuntia* species used include *Opuntia joconostle*, *Opuntia streptacantha*, *Opuntia leucotricha, Opuntia matudae* Scheinvar and *Opuntia ficus-indica* (L.) (Paiz *et al.,* 2010).

Different parts of the plant can be chewed or fresh extract prepared from its parts and administered to patients. The extracts can be from a combination of any of the parts or just a single part and can be taken at anytime of the day (Paiz *et al.,* 2010). Although, the WHO has acknowledged the use of these plant extracts as a cheaper and an alternative drug for the management of diabetes mellitus (Paiz *et al.,* 2010), prickly pear cactus is still viewed as a weed in farms by many Kenyans (Dena, 2008; Plagens, 2011). Additionally, there lacks data on its safety for use as an anti-diabetic agent. Therefore, there was need to assess the hypoglycaemic potential of prickly pear cactus from Kenya and to evaluate safety of these plant extracts for use as anti-diabetic agents.

CHAPTER THREE

THE PRICKLY PEAR CACTUS CLADODES (OPUNTIA SPECIES) MODULATE BLOOD SUGAR IN SWISS WHITE ALBINO MICE

3.1 Abstract

Medicinal plants including the prickly pear cactus have been reported to modulate blood sugar levels. Extracts of prickly pear cactus have been used in various parts of the world to manage diabetes mellitus. However the cactus is viewed as a weed in Kenya. The current study therefore aimed at evaluating the efficacy of prickly pear cactus cladode extracts from Kenya in managing diabetes mellitus in induced mice. Healthy, adult Swiss white albino male mice weighing 20-30 g were induced with diabetes mellitus using Alloxan (150 mg/kg body weight) administered intra-peritoneally. Prickly pear cactus cladode extracts were administered orally at daily dosage of 0.6 ml for pre-determined periods. Fasting blood sugar levels were monitored at intervals of 72 hours throughout the experimental period of 30 days. Alloxan administration resulted in about 3- to 4-fold increase in blood sugar levels. Treatment of diabetic mice with cactus cladode extracts led to decline in blood sugar levels of the animals, however, the levels varied with the period of treatment. Diabetic animals treated with cactus cladode extracts for 10 days showed a significant decline in blood sugar levels on the 7th ($p=0.012$) and 10th ($p=0.001$) days of feeding on the extracts when compared to the positive control (diabetic, not treated) animals. This study has demonstrated that extracts of prickly pear cactus cladodes from Kenya have potential in managing blood sugar in diabetic mice.

3.2 Introduction

Diabetes mellitus has been considered as one of the major public health concerns globally (Rohilla and Ali, 2012) and ranks 4th among non-communicable diseases (WHO, 2010) especially because its effects reach multiple organs causing serious heath complications ranging from brain damage, cardiovascular diseases, renal failure and even death (Samreen, 2009). The disease is characterised by hyperglycaemia associated with abnormal lipid, protein and carbohydrate metabolism (WHO, 1999).

Two major types of diabetes mellitus have been characterised by the World Health Organisation based on their morbidity and mortality rates, Type I diabetes mellitus which

accounts for 10% and Type II which accounts for 90% of all the diabetic cases in the world (Hernandez-Avila and Olaiz-Fernandez, 2002; Rachid *et al.*, 2012; WHO, 2013).
Type 1 diabetes is often caused by deficiency in insulin production while Type II occurs as a result of ineffective use of insulin by the body (Pereira *et al.*, 2010).

Management of diabetes mellitus is a life-long process that requires regular medication and specialized health care (Jones, 2013) to achieve good control of blood sugar levels and to prevent further health complications which may cause death of the patient (ADA, 2007). While people with type 1 diabetes mellitus require regular insulin administration, lifestyle changes to include healthy diet and physical exercise is key to managing type 2 diabetes mellitus. Two major classes of oral drugs are prescribed for diabetic patients due to their affordability and mild side effects, the biguanides such as metformin and phenformin; and the sulphonylureas such as gilbenclamide, diabenese, orinase and glucotrol Amaryl (David *et al.*, 2014). Other classes of oral pills include Thiazolidinediones, Alpha-glucosidase inhibitors, Glucagon Like Peptides-1(GLP-1) receptor agonists and Megilitinides. While these approaches are promising in management of diabetes mellitus, they are costly and can pose severe side effects to the patients.

This challenge has therefore encouraged use of herbal therapy including use of prickly pear cactus extracts in managing diabetes mellitus. Prickly pear cactus is a shrub that thrives in arid and semi-arid areas in most parts of the world. Extracts of this plant have been used to treat various ailments among them being diabetes mellitus **(**DeFelice, 2004; Paiz *et al.*, 2010; Rave, 2012**).** In Kenya it is however viewed as a weed (Dena, 2008) that grows in the wild in semi-arid areas where it is used as a live fence for protection of agricultural lands from invasion by grazers and also to mark boundaries and fence homesteads to protect their animals from predators (Dena, 2008).

So far, there has been no assessment of the anti-hyperglycaemic potential of prickly pear cactus in Kenya. The present study assessed the efficacy of prickly pear cactus cladode extracts in controlling blood sugars of alloxan-induced diabetic mice.

3.3 Materials and Methods

3.3.1 Plant collection, preservation and identification

Plant samples which were used in the study were collected from Baringo County of Kenya. The cactus pads (cladodes) were pruned and collected in sterile sampling bags, sealed and stored in cool boxes filled with ice cubes. The plant samples were then transported to

Kenya Forestry Research Institute (KEFRI) Plant Pathology laboratories for preparation and another set of similar sample to the National Museums of Kenya for taxonomic identification.

3.3.2 Plant sample preparation

Freshly collected prickly pear cactus pads were cut into small pieces and blended using an extract blender. The extract was centrifuged at 10,000 rpm for 10 minutes and then filtered in a Whatman #.1 filter paper. The filtrate was concentrated to a third of the total volume in a freeze drier (El-Razek *et al*., 2012). The concentrated extract was stored in -20^{0}C and later used in oral feeding of the mice using gastric gavages.

Figure 3.1: Lyophiliser concentrating cactus cladode extracts.

3.3.3 Ethical consideration

The animal experimental protocol was approved by the Kenya Agricultural Research Institute- Trypanosomiasis Research Centre Animal Care and Ethics Committee.

3.3.4 Experimental animals

The experimental animals used were male adult (6-8 weeks old) Swiss White albino mice weighing between 20-30 g. The mice were sourced from Biotechnology Research Institute's small animal breeding unit. The animals were housed under controlled temperatures of 21-24^{o}C in natural light. They were randomly allocated to different sterilized mice cages with saw dust as beddings.

For ease of identification, the mice were numbered using wet Picric Acid then de-wormed using a single dose of 0.01 ml Ivermectin (Shanghai Gongyi Veterinary Medicine Plant, Huancheng East Road, Fengxian Town, Shanghai, China) administered subcutaneously. The mice were maintained on a standard feed (Mice cubes, Unga Ltd, Kenya) and water *ad libitum,* and acclimatized to laboratory environment for two weeks before the start of the experiments.

3.3.5 Induction of diabetes

Fresh alloxan was prepared by dissolving alloxan monohydrate (Sigma-Aldrich, St. Louise, MO, USA) in distilled water. Diabetes was induced in the mice that had been fasted overnight with free access to water. A single intraperitoneal injection of 150 mg/kg body weight of the alloxan solution was used (Etuk, 2010). After injection, the mice were allowed free access to feed and water. After 3 days, the mice were fasted overnight and blood collected through a tail snip. Fasting blood glucose (FBG) levels were measured using SD CodeFree^{TM} glucometer (SD Biosensor, Inc. Korea) with compatible test strips. Only the mice with FBG levels above 200 mg/dl (WHO, 2005) were considered diabetic and used for subsequent study.

To determine the optimal volume of the cactus cladode extract for hypoglycaemic test, Alloxan induced diabetic mice were fed once with cactus extract at volumes of 0.2 ml (Group1), 0.4 ml (Group 2), 0.6 ml (Group 3) and 0.8 ml (Group 4), and their blood sugar levels monitored for 24 hours.

3.3.6 Hypoglycaemic activity test

Four groups of 6 mice each were used: Group 5 (healthy control on tap water only), Group 6 (diabetic control induced with diabetes, on tap water only), Group 7 (Induced with diabetes, fed with 0.6 ml cactus cladode extract daily for 5 days), and Group 8 (Induced with diabetes, fed with 0.6 ml cactus cladode extract daily for 10 days).

Baseline blood glucose levels of all the animals were taken before oral treatment with cactus cladode extract. Blood samples for glucose test were collected from the tails of all animals. A drop of tail blood was placed into the glucometer and glucose levels read and recorded. Fasting blood glucose levels (mg/dl) in the mice were determined at intervals of 72 hours during administration of cactus cladode extract (ED *et al.,* 2015).

The same procedure was repeated for all groups for a period of 15 days following termination of cactus extract administration. All the animals were fasted overnight prior to blood collection, during which time they had free access to water.

3.3.7 Data Analysis

Mean recordings of changes in blood sugar levels were determined for each group and expressed as the mean ± standard error of the mean (mean±SEM). One way ANOVA followed by Turkey post hoc tests was performed to determine whether the differences of means between the treatment groups and diabetic control group were statistically different. *P* values of <0.05 were considered to be statistically significant. SPSS (IBM SPSS Statistics version 20.0) was used in the analysis of data.

3.4 Results

3.4.1 Determination of optimal volume of cactus extract for hypoglycaemic activity test

A three- to four-fold increase in blood sugar levels was recorded following induction of mice with diabetes. The lowest mean (±SE) level of blood sugar before induction of mice with diabetes was 126.3±8.7 mg/dl and the highest was 140.7±11.25 mg/dl. Following induction, these values were 317.3±20.90 mg/dl and 495.0±20.17 mg/dl, respectively. No decline in blood sugar levels was observed in animals that were treated orally with 0.2 and 0.4 ml cactus extract. However, 66.7% of diabetic animals died within 12 hours following oral administration of 0.8 ml cactus cladode extract. Animals given 0.6 ml extract showed a decline in mean (±SE) blood sugar levels from 477.6±15.13 mg/dl to 420.9±16.70 mg/dl 24 hours after feeding on cactus cladode extract. Based on these findings, 0.6 ml of the cactus cladode extract concentrate was selected as the optimal dose for the hypoglycaemic study.

3.4.2 Hypoglycaemic activity

Following establishment of the optimal volume of cactus extracts, the animals were grouped and the study carried out as described under Section 3.3.6 of Materials and Methods. Group 5 animals (healthy control not induced with diabetes, on tap water only) had a mean (±SE) blood sugar levels fluctuating from 137.2±5.96 mg/dl to 172±9.93 mg/dl. These levels were generally maintained throughout the period of study.

Approximately 53% of all the animals administered with alloxan in Groups 6, 7 and 8, developed diabetes mellitus (blood sugar levels above 200 mg/dl) after 24 hours and they maintained the diabetic state all through for 72 hours up to the start of feeding on cactus cladode extract. Group 6 animals (diabetic control induced with diabetes, not given the cactus cladode extracts) had blood sugar levels of 164.9±4.98 mg/dl before administration of alloxan. These levels were elevated to mean (±SE) blood sugar levels of 413.3±43.64 mg/dl 72 hours after administration with alloxan. The group recorded a gradual increase in mean (±SE) blood sugar levels throughout the study attaining levels of 423.4±41.38 mg/dl at the end of the observation period.

Group 7 animals (induced with diabetes, fed on cactus cladode extracts daily for 5 days) had a mean (±SE) blood sugar levels of 138.0±8.88 mg/dl before administration with alloxan which increased to 451.2±40.07 mg/dl 72 hours after administration of alloxan. A decline in mean (±SE) blood sugar levels was observed in the group throughout the 5 day period of feeding on cactus cladode extract. However, this decline was not significant ($p=0.638$) when compared to the diabetic control group. Group 8 animals (induced with diabetes, given cactus cladode extracts daily for 10 days) had a mean (±SE) blood sugar level of 140.2±10.95 mg/dl before administration with alloxan. An elevation in mean (±SE) blood sugar levels to 484.5±20.61 mg/dl was recorded in this group following injection with alloxan. Significant decline ($p=0.012$ and $p=0.001$) in mean (±SE) blood sugar levels was observed in this group on the 7^{th} and 10^{th} days respectively of treatment with cactus cladode extracts (Table 3.1) (Appendix 1 and 2 respectively).

Table 3.1: Blood sugar levels (mean± S.E.M) of alloxan-induced diabetic mice following administration of cactus cladode extract

	Blood sugar levels (mg/dl)			
Days post cactus extract administration	**Group 5**	**Group 6**	**Group 7**	**Group 8**
[#]-3 Days	153.3±4.87	164.9±4.98	138.0±8.88	140.2±10.95
[##]0 Days	164.0±5.91	413.3±43.64	451.2±40.07	484.5±20.61
Day 3	162.8±6.22	415.6±44.26	387.8±39.85	375.0±9.36
Day 5	156.8±6.23	448.4±55.12	386.7±46.48	377.8±10.88
Day 7	160.8±8.43	426.7±41.05	N.D	267.0±12.77[*](p=0.012)
Day 10	150.0±10.17	457.1±25.14	N.D	337.2±15.76[*](p=0.001)

Values are statistically different compared to diabetic control group at: [*]$p<0.05$. Group 5 (Healthy control mice, not induced with diabetes not fed with cactus extract), Group 6 (Diabetic control mice, induced with diabetes not fed with cactus extract), Group 7 (Diabetic mice fed with 0.6ml cactus extract for 5 days), Group 8 (Diabetic mice fed with cactus extract for 10 days). [#]-3 days denotes blood sugar levels before administration with alloxan. [##]0 days denotes blood sugar levels at the start of treatment with cactus extract. N.D (Not determined).

Fasting blood sugar levels of the alloxan-induced diabetic mice were also monitored at intervals of 3 days for 15 days following termination of cactus extracts administration. Figure 3.1 shows changes in mean (±SE) blood sugar levels of the animals after treatment with cactus extract was terminated. Diabetic animals previously given cactus cladode extract (Group 7 and Group 8) recorded a decline in their blood sugar levels compared to pre-treatment period. Group 7 animals had a mean (±SE) blood sugar levels of 364.8±48.77 mg/dl whereas Group 8 animals had mean (±SE) blood sugar levels of 383.0±15.93 mg/dl at the end of the observation period. The changes in mean (±SE) blood sugar levels observed were however not statistically different when compared to those of the diabetic control group.

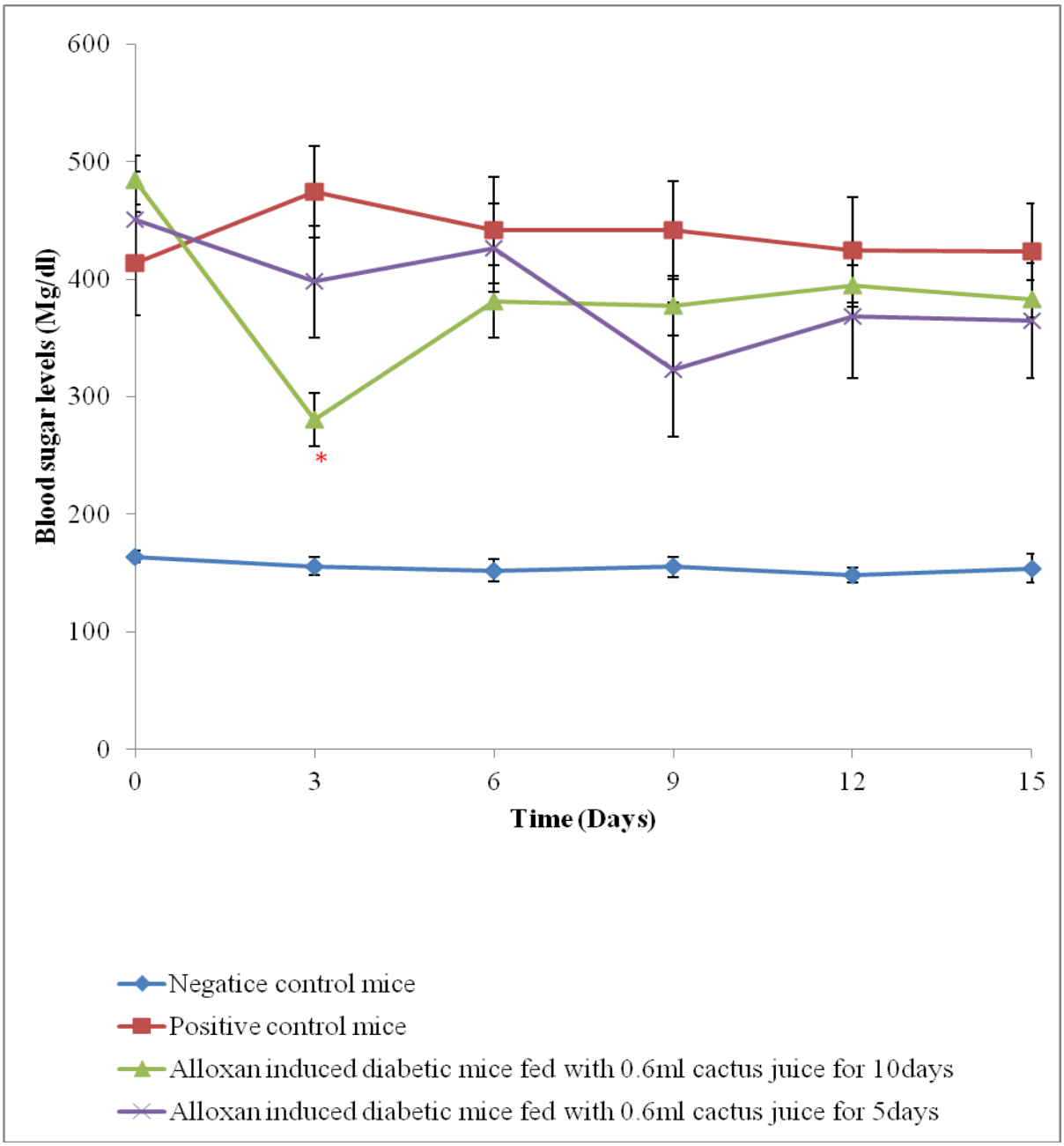

Figure 3.1: Effect of prickly pear cactus cladodes on blood sugar levels of diabetic mice after termination of feeding on cactus cladode extracts. *Indicates significant difference at $p<0.05$.

3.5 Discussion

The findings of this study show that the diabetic status (blood sugar levels above 200 mg/dl) was achieved by administration of alloxan to mice. Alloxan is known to induce experimental diabetes by selective destruction of the insulin-producing pancreatic beta cells of islet of Langerhans (Rohilla and Ali, 2012; Pankaj and Varma, 2013). The basic mechanism of action of alloxan involves the selective cellular uptake of the compound due to its specific chemical properties including structural similarity to glucose in addition to a highly efficient uptake and accumulation of alloxan by the pancreatic beta cells. Alloxan is a urea derivative which causes selective necrosis of the pancreatic beta cells. The cytotoxic action of alloxan on β cells of pancreatic islets involves the following pathyway: oxidation of essential sulphydryl (-SH) groups, selective inhibition of glucose-induced secretion through specific inhibition of glucokinase enzyme, generation of reactive oxygen species (ROS) and disturbances in intracellular calcium haemostasis leading to an immense increase in cytosolic calcium concentration eventually causing rapid destruction of pancreatic beta cells. This results in the reduction of insulin levels and increased blood glucose eventually leading to diabetes mellitus (Lenzen, 2008; Rohilla and Ali, 2012).

Our results also demonstrated that extracts of *Opuntia ficus*-Indica cladodes from Kenya significantly reduced blood sugar levels in experimental diabetic mice when administered orally at a volume of 0.6 ml daily for a period of 10 days compared to 5 days, thereby showing its potential in the management of diabetes. These results corroborate those of Meckes-Lazyoa and Roman-Ramos who reported significant reductions in serum glucose levels of non-insulin dependent patients given *Opuntia streptacantha* cladode extracts for 10 days (Meckes-Lazyoa and Roman-Ramos, 1986). In another study, Frati *et al* gave crude extracts of *Opuntia streptacantha Lemaire* to one group of type 2 diabetes mellitus patients and to the other group of patients he gave boiled stems of *Opuntia streptacantha Lemaire*. He, however, reported an insignificant decrease in blood sugar levels of non-insulin dependent patients that received crude extracts compared to those who received boiled stems of *Opuntia streptacantha Lemaire* (Frati *et al*., 1990). Our findings suggest that *Opuntia fiscus-Indica* cladodes from Kenya can cause hypoglycemic effects when consumed in their crude form. We also reported a decline in blood sugar levels of the diabetic treated animals following termination of feeding on cactus cladode extracts.

However, the decline in blood sugar levels of the animals was not significant when compared to the diabetic control, which also suggests that a daily intake of cactus cladodes extracts is required to maintain the hypoglycemic effect and that the plant could also be consumed as a cooked vegetable.

Prickly pear cactus cladodes are believed to contain pectin, which are soluble fibers that interfere with carbohydrate metabolism (Nelson *et al.*, 1991; El-Razek and Hassan, 2011), stabilize the movement of intestinal food and slow down the absorption of glucose (Wolfram *et al.*, 2002), which in turn reduce blood sugar levels (Sloan, 1994*;* Kaur *et al.*, 2012). The anti-hyperglycemic effect observed from our results may be attributed to pectin and other insulin-like compounds present (Gray and Flatt, 1999) in the cladodes that may have enhanced the sensitivity of pancreatic β-cells stimulating them to produce more insulin (Khan *et al.*, 1990) and enhance insulin activity, which eventually led to improved glucose utilization by the cells (Broadhurst, 1997; Wolfram *et al.*, 2002) and the reduced blood sugar levels. The anti-hyperglycemic effect observed could also be attributed to phenols and flavonoids that have been reported in prickly pear cactus cladodes (El-Mostafa *et al.*, 2014; Osuna-Martinez *et al.*, 2014). Phenols have been reported to improve sensitivity of the tissues to insulin through their ability to scavenge free radicals (Faure *et al.*, 1999; Stull *et al.*, 2010). Flavonoids, on the other hand, are known to promote the uptake of glucose by peripheral tissues (Gupta, 1994) thus reducing the glucose levels in blood.

3.6 References

American Diabetes Association (2007). Standards of medical care in diabetes. *Diabetes Care,* **30,** 4–41.

Broadhurst, C. L. (1997). Nutrition and non-insulin dependent diabetes mellitus from an anthropological perspective. *Alternative Medicine Review*, **2**, 378-399.

David, K.M., David, M.N. and Jean, E.M. (2014). Patient information: Diabetes mellitus type 2: Treatment (Beyond the basics). Accessed 2015 from http://www.uptodate.com/contents/diabetes-mellitus-type-2-treatment-beyond-the basics

DeFelice, M.S. (2004). Prickly pear cactus, *Opuntia spp*. A spine-tingling tale. *Weed Technology*, **18**, 869-877.

Dena, C. (2008). Prickly Pear Cactus in Kenya. Accessed on 15th February, 2015 from http://www.denacrain.com/blog/prickly-pear-cactus-in-kenya/

ED, E., Atsukwei, D., Adams, M.D., Tende, J.A., Malgwi, I.S. and Onuoha, T.N. (2015). Effects of alpha lipoic acid on blood glucose, body weight and haematological profile of streptozotocin-induced hyperglycaemia in wistar rats. *European Journal of Research in Medical Sciences*, **3**, 25-33

El-Mostafa, K., Kharrassi, Y.E., Badreddine, A., Andreoletti, P.,Vamecq, J., Kebbaj,M'H.S.E., Latruffe, N., Lizard, G., Nasser, B. and Cherkaoui-Malki, M. (2014). Nopal cactus (*Opuntia ficus-indica*) as a source of bioactive compounds for nutrition, health and disease. *Molecules*, **19**, 14879-14901.

El-Razek, A.H.F and Hassan, A.A. (2011). Nutritional value and hypoglycemic effect of prickly cactus pear (*Opuntia Ficus-Indica*) fruit extract in alloxan-induced diabetic rats. *Australian Journal of Basic and Applied Sciences*, **5**, 356-377.

El-Razek, A.H.F, El-Metwally, E.M., Shehab, G.M., Hassan, A.A. and Gomaa, A.M. (2012). Effects of cactus pear (*Opuntia ficus indica*) extract on oxidative stress in diabetic cataract mice. *Saudi Journal of Health Science*, **1**, 23-29.

Faure, P., Rossini, E., Wiernsperger, N., Richard, Mi., Favier, A., and Halimi, S. (1999). An insulin sensitizer improves the free radical defense system potential and insulin sensitivity in high fructose-fed rats. *Diabetes*, **48**, 353-357.

Frati, A.C., Jiménez, E. and Ariza, C.R. (1990). Hypoglycemic effect of *Opuntia ficus-indica* in non-insulin dependent diabetes mellitus patients. *Phytotherapy Research*, **4**, 195-197.

Gray, A.M. and Flatt, P.R. (1999). Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant coriander (*Coriandrum sativum*). *Brazilian Journal of Nutrition*, **81**, 203-208.

Gupta, S.S. (1994). Prospects and perspectives of natural plant products in medicine. *Indian Journal of Pharmacology*, **26**, 1-12.

Hernández-Ávila, M. and Olaíz-Fernández, G. (2002). Diabetes and Mexico: a public health challenge. *Science,* **53,** 8-17.

Jones, L.E.T. (2013). Diabetes mellitus: the increasing burden of disease in Kenya.

Kaur,M., Kaur, A. and Sharma, R. (2012). Pharmacological actions of *Opuntia ficus indica*: A review. *Journal of Applied Pharmaceutical Science*, **02**, 15-18

Khan, A., Bryden, N.A., Polansky, M.M and Anderson, R.A. (1990). Insulin potentiating factor and chromium content of selected food and spices. *Biology of Trace Elements Research*, **24**, 183-188.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, **51,** 216. doi:10.1007/s00125-007-0886-7

Meckes-Lozyoa, M., Roman-Ramos, R., (1986). *Opuntia streptacantha*, a coadjutor in the treatment of Diabetes mellitus. *American Journal of Chinese Medicine,* **14**, 116-118

Nelson, R.W., Ihle,S.L., Lewis, L.D., Salisbury, S.K., Miller, T., Bergdall, V. and Bottoms, G.D. (1991). Effects of dietary fiber supplementation on glycemic control in dogs with alloxan-induced diabetes mellitus. *American Journal of Veterinary Research,* **52**, 2060-2066.

Osuna-Martínez, U., Reyes-Esparza. J. and Rodríguez-Fragoso. L. (2014). Cactus (*Opuntia ficus-indica*): A review on its antioxidants properties and potential pharmacological use in chronic diseases. *Natural Products Chemistry and Research*, **2**, 153.doi:10.4172/2329-6836.1000153

Paiz, R.C., Juárez-Flores, B.I., Rivera, J.R.A., Ortega, N.C.C., Agüero, J.A.R., Chávez, E.G. and Fuentes, G.A. (2010). Glucose-lowering effect of xoconostle (*Opuntia joconostle A.* Web., Cactaceae) in diabetic mice. *Journal of Medicinal Plants Research,* **4,** 2326-2333.

Pankaj, P.P, and Varma, M.C. (2013). Potential role of *Spirulina platensis* in maintaining blood parameters in Alloxan induced diabetic mice. *International Journal of Pharmacy and Pharmaceutical Sciences,* **5**, 450-456.

Pereira, C.A., Pereira, L.L.S. and Corrêa, A.D. (2010). *Hoodia gordonii* in the treatment of obesity: A review. *Journal of Medicinal Plants Research*, **4,** 2305-2312.

Rachid, A., Rabah, D., Farid, L., Zohra, S.F., Houcine, B. and Nacéra, B. (2012). Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. *Journal of Medicinal Plants Research,* **6,** 2041-2050.

Rave, U. (2012). Medicinal Uses of Cactus Plants. Accessed on 4th May, 2015 from http://www.buzzle.com/articles/medicinal-uses-of-cactus-plants.html

Rohilla, A. and Ali, S. (2012). Alloxan induced diabetes: *Mechanisms and Effects. International Journal of Research in Pharmaceutical and Biomedical Sciences*, **3**, 819-823.

Samreen, R. (2009). Diabetes mellitus. *Scientific Research and Essay*, **4,** 367-373

Sloan, E. (1994). Top ten trends to watch and work on. *Food Technology*, **7**, 89-100.

Stull, A.J., Cash. K. C., Johnson, W.D., Champagne, C.M. and Cefalu, W.T. (2010). Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *Journal of Nutrition*, **140**, 1764-68.

Wolfram, R.M., Kritz, H., Schmid, P., Efthimiou, Y., Stamatopoulos, Y. and Sinzinger, H. (2002). Effect of prickly pear (*Opuntia robusta*) on glucose- and lipid-metabolism in non-diabetics with hyperlipidemia.

World Health Organization. (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO Consultation, Part 1: *Diagnosis and Classification of Diabetes Mellitus*, 1- 49.

World Health Organization (2005). Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia, Report of a WHO/IDF consulttion.

World Health Organization (2010). Global status report on non-communicable disease. Accessed 2015 from http://www.who.int/nmh/publications/ncd_report_full_en.pdf.

World Health Organization, (2013). Diabetes facts sheet number 312. Accessed 2015 from http://www.who.int/mediacentre/factsheets/fs312/en/

CHAPTER FOUR

SAFETY EVALUATION OF PRICKLY PEAR CACTUS CLADODES IN SWISS WHITE ALBINO MICE

4.1 Abstract

Prickly pear is a cactus belonging to the genus *Opuntia spp.* and family Cactaceae family. These cacti are indigenous to Mexico but they are also widespread in all the semi-arid countries. Pads (cladodes) and fruits of the prickly pear have been used traditionally in folk medicine to manage various diseases including diabetes. Studies on the health benefits of prickly pear cactus have been done but there is no information on the adverse health effects that may arise from use of these cacti for medicinal purposes. In this study we assessed the cytotoxic effects of prickly pear cactus cladode extracts in Swiss white albino mice. Healthy and alloxan-induced (150 mg/kg body weight) diabetic mice were used for our study. Mice were fed on varied volumes of cactus cladode extracts for pre-determined period. Live body weights of all animals were assessed at intervals of 3 days throughout the experiment. Packed cell volumes were assessed at intervals of 5 days during feeding on cactus cladode extracts and once per week after termination of feeding. Kidney and liver tissues of the animals were taken at the end of the experiment and processed for histopathological examination. Cactus cladode extracts administration produced insignificant changes ($p>0.05$) in body weights and packed cell volume values of both the healthy and diabetic induced mice. Histopathological examination of kidney tissues of the animals presented normal renal architecture with minimal pathological changes. Severe degeneration was observed in liver tissues of healthy animals fed on cactus cladode extracts and the diabetic control animals. However, mild degenerative changes were observed in liver tissues of diabetic animals fed on cactus cladode extracts. No cases of mortality were reported throughout the experiment. These findings suggest that the cactus cladode extracts minimises the effects of diabetes mellitus on the kidneys and liver.

4.2 Introduction

In recent years, interest on plant research has increased across the world as many plant extracts including those of prickly pear cactus have shown potent application in

treatment of various ailments. Prickly pear also known as *Opuntia* is a cactus that belongs to the Cactaceae family.

Opuntia is native to Mexico but is also spread widely in all the semi-arid countries (Brahmi *et al.,* 2011). However its occurrence in Africa is due to introduction of exotic seeds (Plagens, 2011).

The prickly pear cactus is easily cultivated from the pads when they are detached from the main plant and drop on the ground where they develop roots and more pads (Kang'ara and Gitari, n.d). The shrub, usually succulent, has flattened branches, formed by groups of cladodes (pads) grey to green-grey in colour. The branches vary in size from 30-60 cm long and 6-15 cm wide and produce large yellow flowers, followed by fruits about 8 cm long covered with clusters of tiny spines which are green when unripe and yellow or reddish-purple when ripe. The shrub length ranges from 1.5 m to 3 m but it can grow up to 5 m tall forming a strong trunk with time (Orwa *et al*., 2009; Boutakiout *et al.,* 2015).

Different parts of the prickly pear cactus have been used in traditional medicine in several countries for numerous purposes (Hunt *et al.,* 2006). Fruits, pads or flower infusions have been traditionally used as traditional medicine to treat a number of illnesses such as ulcers, allergies, diarrhoea, indigestion, fatigue, and edema, and to alleviate alcoholic hangover (Nefzaoui *et al.,* 2007; Madrigal-Santillan *et al.,* 2013). Studies on the health benefits of cactus fruits and cladodes have demonstrated their anti-oxidant, anti-cancer, and hypoglycaemic and anti-diabetic properties (Nefzaoui *et al.,* 2007; Osuna-Martínez *et al.,* 2014). However, information on the possible negative health effects associated with use of these cacti is limited.

Although herbal medicines and formulations are believed to be safe and free from side effects because they are natural, they may contain contaminants such as heavy metals and other undeclared pharmaceuticals illegally added to the herbs to produce a desired effect. Genetic factors and microbial toxins may also affect the contents of bio-active substances in the herbal products causing undesirable side effects (Gagnier *et al*., 2006). Moreover, a large number of medicinal plants have been reported to possess some degree of toxicity (Bnouham *et al.,* 2006). Therefore, in this study we assessed the cytotoxic effects of the prickly pear cactus cladodes extracts in Swiss white albino mice.

4.3 Materials and Methods

4.3.1 Plant sample collection and preparation

Prickly pear cactus cladodes were collected from Baringo County of Kenya and prepared as described earlier in chapter three.

4.3.2 Experimental animals

All the animals were healthy, male adult (6-8 weeks old) Swiss White albino mice weighing between 20-30 g. They were sourced from Biotechnology Research Institute's small animal breeding unit and maintained under standard conditions for 2 weeks to acclimatize to the laboratory environment as described earlier in Section 3. 3.4.

Following two week acclimatization period, the animals were divided into two broad groups. Group I comprised of 72 healthy mice and Group II comprised of 18 diabetic mice (induced with diabetes as described in Section 3.3.5). Thirty mice from Group I were further sub-divided into 5 groups of six mice each: Group 1 (healthy control mice) were fed on water, Group 2 were fed on 0.6 ml cactus cladode extract for 5 days, Group 3 were fed on 0.8 ml cactus cladode extract for 5 days, Group 4 were fed on 0.6 ml cactus cladode extract for 10 days and Group 5 fed on 0.8 ml cactus cladode extract for 10 days.

4.3.3 Determination of maximum tolerated volume of cactus cladode extract

To determine maximum tolerated volume of cactus cladode extract, 42 healthy mice (from Group I above) were fasted overnight and separated into 7 groups of 6 mice each, and orally given varying volumes of cactus cladode extract ranging from 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml and 1.4 ml. The animals were monitored for signs of toxicity such as convulsions, raised fur, paw licking, reduced activity and death within a period of 24 hours giving special attention to the behaviour of the individual animals during the first 4 hours (Chauhan *et al.*, 2014).

4.3.4 Determination of live body weights and packed cell volumes

Baseline data on body weights of the animals were measured for a period of two weeks using a digital mice balance D-72336 (KERN®, Germany) before assigning them to the various experimental groups of 6 animals per group. The body weights of all the animals were taken at intervals of 3 days throughout the experimental period of 30 days.

Packed cell volume (PCV) values of all animals was assessed at intervals of 5 days during cactus cladode extract administration and once weekly after termination of feeding on cactus cladode extract. 75 µl of blood sample was collected from each animal for this experiment. All the animals were fasted overnight with free access to water before blood collection.

Tail blood was collected into heparinised haematocrit capillary tubes and centrifuged at 12,000 rpm for 5 minutes in a micro-haematocrit centrifuge (Haematokrit 210 Hettich Zentrifugen, Germany) following the method described by Bull and Rittenbach (1990). PCVs were read with a haematocrit reader and expressed as a percentage (%) of the total blood volume (Bull and Rittenbach, 1990).

4.3.5 Histopathology Analysis

At the end of the experiment, mice were euthanized using carbon dioxide to obtain liver and kidneys for histopathology. The organs were fixed and preserved in 10% (v/v) formalin. They were then processed by paraffin embedding procedures. Paraffin embedded sections (7 µm thick) were cut, mounted onto slides, and stained with haematoxylin and eosin (H and E) for light microscopy and photomicrography (Leica DM500, Germany) evaluation.

4.3.6 Data Analysis

Data on body weights and PCV values were expressed as (mean±SEM) and analyzed using SPSS (IBM SPSS Statistics version 20.0) software. Significance of difference between means for body weights and packed cell volumes were determined by one way ANOVA followed by Turkey post hoc tests to determine if means between treatment groups and control groups were statistically different. *P* values of <0.05 were considered to be statistically significant.

4.4 Results

4.4.1 Maximum tolerated volume of cactus cladode extract

Oral administration of cactus cladode extract at volumes of 0.2 ml, 0.4 ml, 0.6 ml and 0.8 ml given as a single dose did not show any toxicity signs in the mice. No deaths were reported in these animal groups. However, approximately 70% of mice given 1.0 ml cactus

cladode extract died while 100% mortality rate was observed in mice given 1.2 ml and 1.4 ml cactus cladode extract concentrate 24 hours post-administration. Therefore, 0.8 ml was established as the maximum tolerated volume given as a single oral dose of cactus cladode extract in mice.

4.4.2 Live body weights and packed cell volumes

4.4.2.1 Live body weights of healthy mice fed on 0.6 ml and 0.8 ml cactus cladode extract for 5 days

Group 1 animals (healthy controls) had a mean (±S.E) body weight of 26.8±0.31 g at the start of the experiment. Animals from this group showed an increase in mean (±S.E) body weight throughout the experiment attaining values of 29.8±0.60 g at the end of the 30 day observation period. Healthy mice fed on 0.6 ml (Group 2) cactus cladode extract recorded a consistent drop in mean (±S.E) body weight from 27.3±0.93 g to 23.8±0.64 g during feeding on cactus cladode extract. A significant decline (p=0.036 and p=0.030) in mean (±S.E) live body weights was observed in this group on the 3rd and 5th day respectively of feeding on cactus cladode extract (Appendix 4 and 5 respectively). A gradual decline in mean (±S.E) live body weight was observed in healthy mice on 0.8 ml (Group 3) cactus cladode extract from 25.8±0.91 g to 24.7±1.15 g. The decline in body weight observed was however not significant when compared to that of the healthy control group (Table 4.1).

Table 4.1: Effect of prickly pear cactus cladode extracts on live body weights of healthy mice fed on cactus cladode extracts for 5 days.

Live body weights (g)			
	Group 1	**Group 2**	**Group 3**
-1 Days	26.8±0.31	27.0±1.03	26.1±0.60
#0 Days	26.8±0.31	27.3±0.93	25.8±0.91
Day 3 Treatment	26.8±0.40	23.3±0.73*(p=0.036)	24.7±1.26
Day 5 Treatment	27.1±0.48	23.8±0.64*(p=0.030)	24.7±1.15
Day 3 Post-treatment	28.8±0.17	24.8±0.72*(p=0.008)	26.0±1.13
Day 6 Post-treatment	28.8±0.17	26.5±0.74	27.5±0.99
Day 9 Post-treatment	30.5±0.34	27.8±0.83	28.5±0.85
Day 12 Post-treatment	29.3±0.42	27.8±0.83	28.7±0.92

Day 15 Post-treatment	29.8±0.60	29.3±0.87	29.5±0.99

Values are expressed as Mean ± SEM (n=6). Values are statistically different from control group at, *p<0.05. Group 1(Healthy control mice not fed with cactus extract), Group 2 (Healthy mice fed with 0.6 ml cactus extract for 5 days), Group 3 (Healthy mice fed with 0.8 ml cactus extract for 5 days). #0 Days denote body weights at the start of feeding on cactus cladode extracts.

All the animals recorded a gradual increase in mean (±S.E) live body weight following termination of feeding on cactus cladode extracts. Healthy animals fed on 0.6 ml (Group 2) cactus cladode extract had a mean (±S.E) live body weight of 29.3±0.87 g from 23.8±0.64 g whereas those fed on 0.8 ml (Group 3) cactus cladode extract recorded an increase in mean (±S.E) live body weight from 24.7±1.15 g to 29.5±0.99 g at the end of the observation period (Table 4.1) (Figure 4.1).

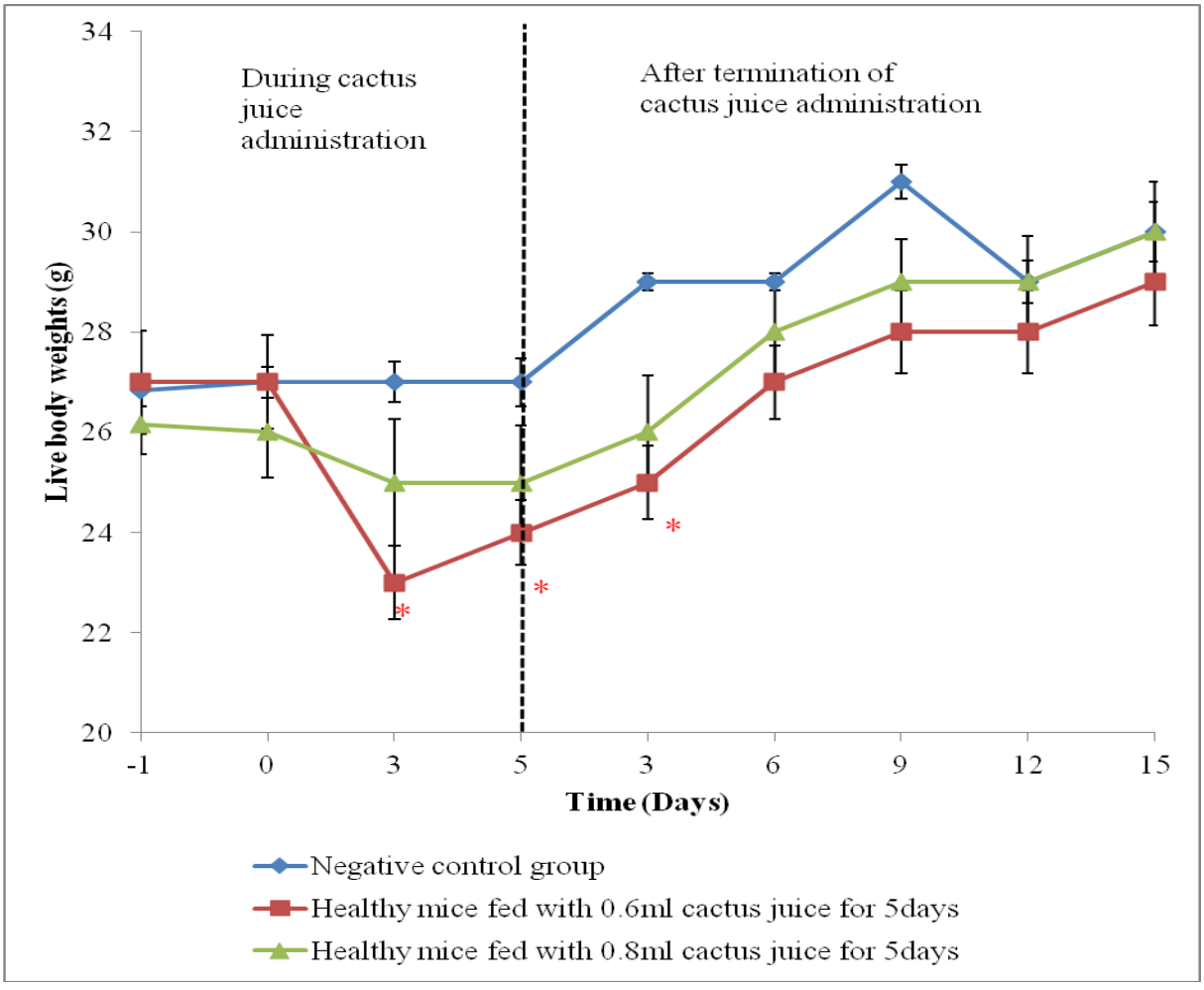

Figure 4.1: Effect of prickly pear cactus cladode extracts on live body weights of healthy mice fed on cactus cladode extracts for 5 days. *Indicates significant difference at $p<0.05$.

4.4.2.2 Live body weights of healthy mice fed on 0.6 ml and 0.8 ml cactus cladode extract for 10 days

Table 4.2 shows changes in mean (±S.E) live body weights of healthy animals fed on 0.6 ml and 0.8 ml cactus extract for 10 days. Healthy control (Group 1) animals recorded a gradual increase in mean (±S.E) live body weight throughout the experiment.

All animals fed on cactus cladode extract recorded a decline in mean (±S.E) live body weight following administration with cactus cladode extract. Healthy animals fed on 0.6 ml (Group 2) cactus cladode extract showed significant decline (p=0.018 and p=0.025) in mean (±S.E) live body weight on the 7th and 10th days respectively of feeding on cactus cladode extract (Appendix 7 and 8 respectively). A significant decline (p=0.042) in mean (±S.E) live body weight was also observed in Group 3 animals (fed on 0.8 ml cactus cladode extract) on the 10th day of administration with cactus cladode extract (Appendix 8) (Table 4.2).

Table 4.2: Effect of prickly pear cactus cladodes on live body weights (g) of healthy mice fed on cactus cladode extracts for 10 days

Live body weights (g)			
Day/Treatment	**Group 1**	**Group 2**	**Group 3**
-1Days	26.8±0.31	27.3±0.49	26.7±0.80
#0 Days	26.8±0.31	27.3±0.33	27.0±0.89
Day 3 Treatment	26.8±0.40	25.5±0.76	24.5±1.26
Day 5 Treatment	27.7±0.33	24.3±0.99	25.2±1.35
Day 7 Treatment	27.7±0.42	23.5±1.28*(p=0.018)	24.5±0.92
Day 10 Treatment	27.7±0.33	24.6±1.03*(p=0.025)	25.0±0.73*(p=0.042)
Day 3 Post-treatment	28.8±0.17	26.6±0.75	27.2±0.75

Day 6 Post-treatment	28.8±0.17	27.0±1.05	27.8±0.87
Day 9 Post-treatment	30.5±0.34	28.8±0.73	29.0±0.97
Day 12 Post-treatment	29.3±0.42	29.4±0.87	29.3±0.95
Day 15 Post-treatment	29.8±0.60	30.2±0.80	30.2±1.08

Values are expressed as Mean ± SEM (n=6). Values are statistically different from control group at, $^{*}p<0.05$. Group 1 (Healthy control mice not fed with cactus extract), Group 2 (Healthy mice fed on 0.6 ml cactus extract for 10 days), Group 3 (Healthy mice fed on 0.8 ml cactus extract for 10 days). $^{\#}$0 Days denote body weights at the start of feeding on cactus cladode extracts.

Following termination of feeding on cactus cladode extracts, a consistent increase in mean (±S.E) live body weight was observed in all animals throughout the observation period of 30 days. Animals on 0.6 ml (Group 2) cactus cladode extract recorded a mean (±S.E) live body weight of 30.2±0.80 g from 24.6±1.03 g whereas animals fed on 0.8 ml (Group 3) cactus cladode extract recorded an in increase in mean (±S.E) live body weight from 25.0±0.73 g to 30.2±1.08 g at the end of the observation period. The increase in mean (±S.E) live body weight observed in both groups was however not significant when compared to that of the negative control group (Figure 4.2).

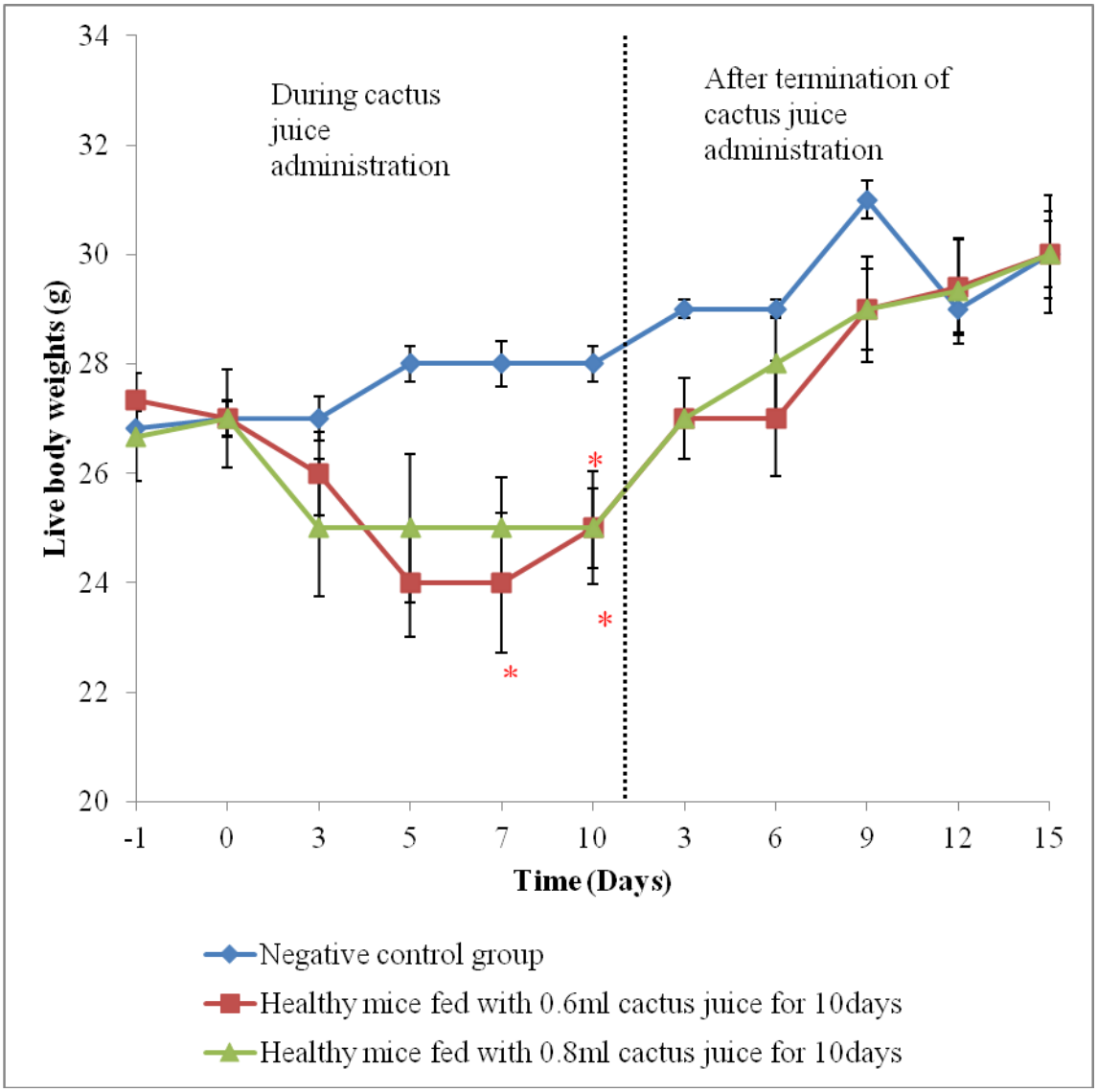

Figure 4.2: Effect of prickly pear cactus cladodes extracts on live body weights (g) of healthy mice fed on cactus extracts for 10 days. *Indicates significant differences at $p<0.05$.

4.4.2.3 Live body weights of alloxan-induced diabetic animals

The animals recorded a decline in mean (±S.E) live body weight following administration with alloxan. Healthy control (Group 1) animals showed a gradual increase in mean (±S.E) live body weight throughout the observation period from 26.8±0.31 g to 29.8±0.60 g (Table 4.3). Prior to treatment with alloxan, Group 2/diabetic control animals (induced with diabetes not given cactus caldode extract) had a mean (±S.E) live body weight of 29.0±0.86 g. A decline in mean (±S.E) live body was observed in this group following injection with alloxan and throughout the experiment reaching 24.4±0.72 g at the end of the experiment (Table 4.3).

Table 4.3: Effect of prickly pear cactus extract on live body weights (g) of alloxan-induced diabetic mice fed on cactus cladode extracts

Live body weights (g)				
Time/Treatment	**Group 1**	**Group 2**	**Group 3**	**Group 4**
#-3 Days	26.8±0.31	29.0±0.86	29.8±1.17	29.7±1.56
##0 Days	26.8±0.31	26.3±0.64	26.5±1.18	27.7±1.02

Day 3 Treatment	26.8±0.40	27.7±0.63	26.7±1.36	26.8±1.54
Day 5 Treatment	26.8±0.40	24.6±0.90	26.0±1.06	24.7±1.36
Day 7 Treatment	27.8±0.40	25.7±0.47	25.8±1.24	N.D
Day 10 Treatment	27.7±0.33	25.3±0.57	25.0±1.05	N.D
Day 3 Post-treatment	28.8±0.17	23.6±1.11	24.8±1.38	27.3±1.26
Day 6 Post-treatment	28.8±0.17	23.1±1.50	25.5±1.32	27.8±1.17
Day 9 Post-treatment	30.5±0.34	24.0±1.21	25.0±1.47	25.7±1.48
Day 12 Post-treatment	29.3±0.42	24.6±0.61	26.5±0.96	25.4±1.12
Day 15 Post-treatment	29.8±0.54	24.4±0.72	26.8±1.11	27.0±1.34

Values are expressed as Mean ± SEM (n=6). Group 1 (Healthy control mice not induced with diabetes not fed with cactus extract), Group 2 (Diabetic control mice induced with diabetes mot fed with cactus extract), Group 3 (Alloxan-induced diabetic mice fed with 0.6 ml cactus extracts for 10 days), Group 4 (Alloxan-induced diabetic mice fed with 0.6 ml cactus extracts for 5 days). [#]-3 days denote body weights at induction of diabetes. [##]0 days denotes body weights at the start of treatment with cactus extract. N.D (Not determined).

Figure 4.3 shows effect of cactus cladode extracts on body weights of diabetic mice fed on 0.6 ml cactus cladode extracts for 10 days (Group 3). The animals had a mean (±S.E) live body weight of 29.8±1.17 g before administration with alloxan, which dropped to 26.5±1.18 g three days after injection with alloxan.

A decline in mean (±S.E) live body weight was observed in this group during feeding on cactus cladode extract reaching 25.0±1.05 g on the last (10^{th}) day of feeding. A gradual increase in mean (±S.E) live body weight was however observed in these animals throughout the observation period following termination of feeding on cactus extract cladode. The changes in body weights observed were however not significant when compared to the diabetic control group.

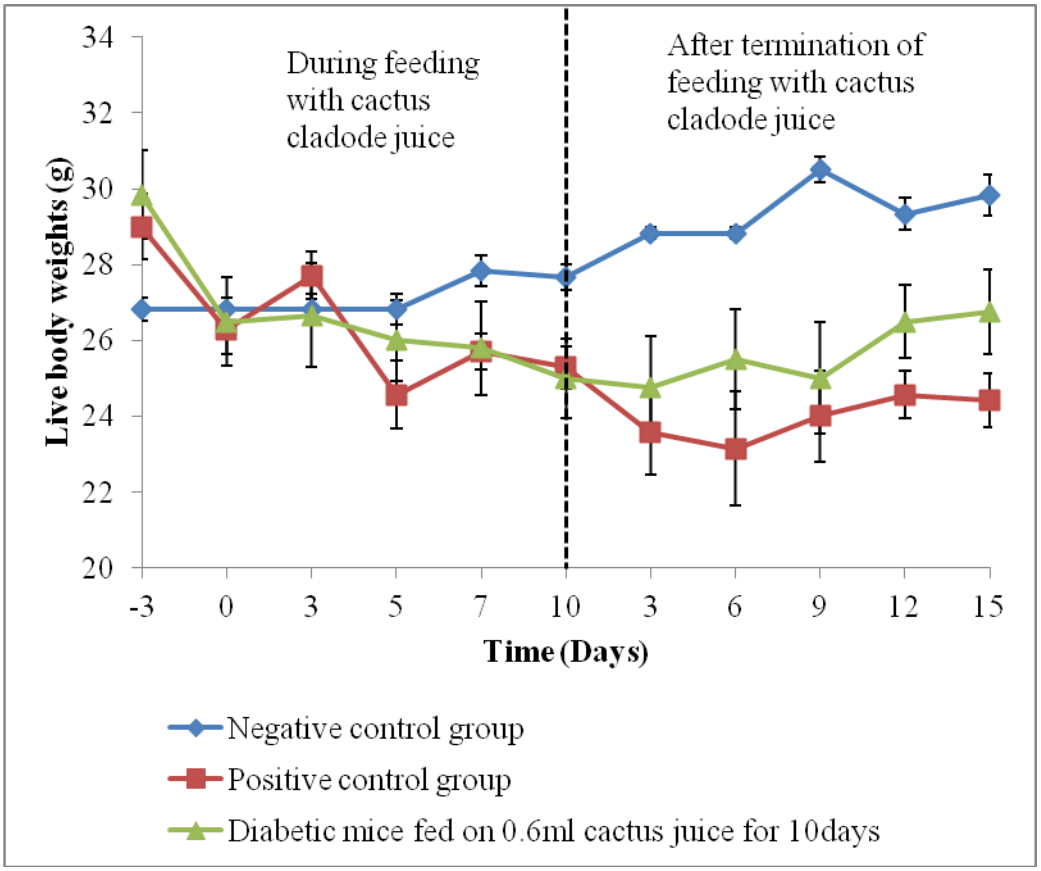

Figure 4.3: Effect of prickly pear cactus cladodes on live body weights of alloxan-induced diabetic mice fed on cactus cladode extracts for 10 days

Group 4 animals (induced with diabetes fed on 0.6 ml cactus cladode extracts) also showed a decline in mean (±S.E) body weight from 29.7±1.56 g to 27.7±1.02 g following administration with alloxan. The animals recorded an insignificant decline in mean (±S.E) body weight during feeding on cactus cladode extract. After termination of feeding on cactus cladode extract, an insignificant increase in mean (±S.E) body weight was observed in the animals throughout the experiment reaching 27.0±1.34 g at the end of the experiment (Figure 4.4).

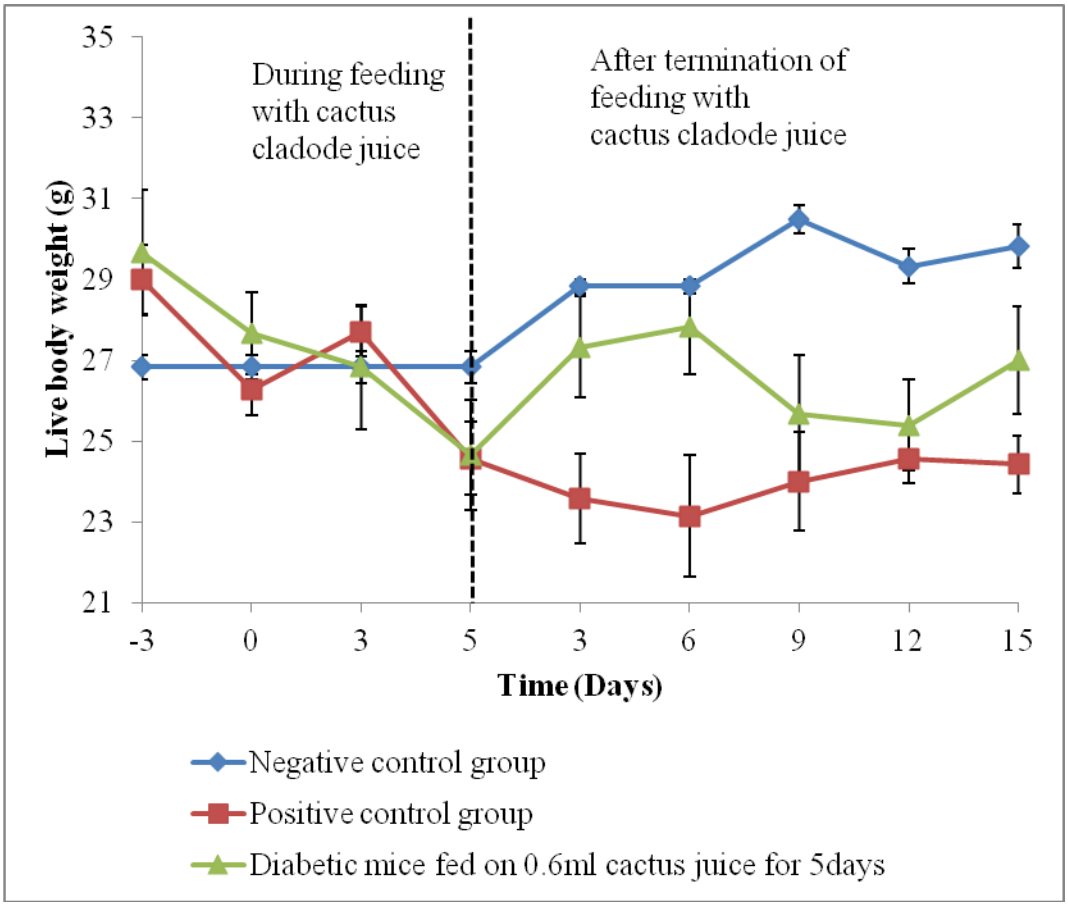

Figure 4.4: Effect of prickly pear cactus on live body weights of alloxan-induced diabetic mice fed on cactus cladode extracts for 5 days

4.4.2.4 Packed cell volumes of healthy animals fed on 0.6 ml and 0.8 ml cactus cladode extract for 5 days

Healthy control (Group 1) animals had mean (±S.E) PCV value of 60.0±1.57% before start of the experiment. This group showed an increase in mean (±S.E) PCV to 61.7±1.11% at the start of the experiment. A decline in mean (±S.E) PCV was observed in this group reaching values of 54.2±0.79% at the end of observation period of 30 days. Healthy animals on 0.6 ml (Group 2) cactus cladode extract recorded an insignificant increase in mean (±S.E) PCV values from 60.0±1.00% to 63.5±1.80% following feeding on cactus cladode extract for 5 days. Group 3 animals (healthy mice fed on 0.8 ml cactus cladode extract) had a mean (±S.E) PCV value of 60.8±1.17% at the start of the experiment. Following feeding on cactus cladode extract for 5 days, a decline in mean (±S.E) PCV to values of 55.8±3.87% was observed in this group. The change in PCV values was however not significant when compared to those of the healthy control group (Table 4.4).

Table 4.4: Effect of prickly pear cactus extract on packed cell volumes of healthy mice fed on cactus cladode extracts for 5 days

Packed cell volumes (%)

Time/Treatment	**Group 1**	**Group 2**	**Group 3**
-3 Days	60.0±1.57	61.0±0.82	60.0±1.29
#0 Days	61.7±1.11	60.0±1.00	60.8±1.17
5 Days Treatment	61.5±2.64	63.5±1.80	55.8±3.87
7 Days Post-treatment	54.5±0.85	59.3±2.33	58.3±0.61
14 Days Post-treatment	54.2±0.79	57.0±1.12	56.5±1.06

Values are expressed as Mean ± SEM (n=6). Group 1 (Healthy control mice not fed on cactus extract), Group 2 (Healthy mice fed on 0.6 ml cactus extract for 5 days), Group 3 (Healthy mice fed on 0.8 ml cactus extract for 5 days). #0 Days denote PCV values at the start of feeding on cactus cladode extracts.

Treatment with cactus cladode extract was terminated after 5 days and changes in mean (±S.E) PCV monitored once weekly for 2 weeks. Effect of cactus cladode extracts on PCV values of the animals are presented in Figure 4.6. Group 2 animals recorded a gradual decline in their mean (±S.E) PCV values throughout the observation period following termination of feeding with cactus cladode extract. An insignificant increase and decline in mean (±S.E) PCV was observed in Group 3 animals on the 7^{th} and 14^{th} day after feeding on cactus cladode extracts was stopped (Figure 4.5).

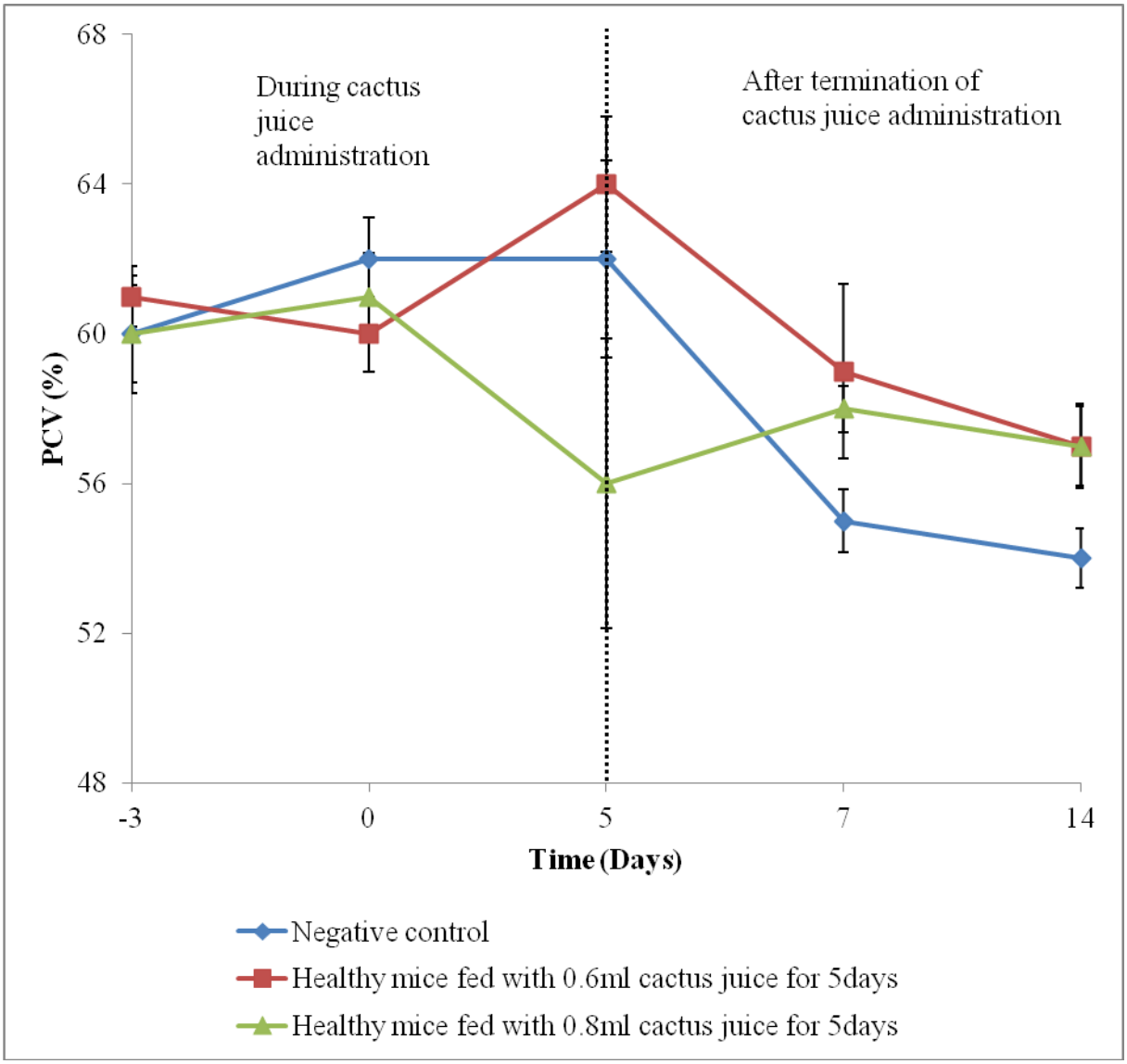

Figure 4.5: Effect of prickly pear cactus extract on packed cell volumes of healthy mice fed on cactus cladode extracts for 5 days

4.4.2.5 Packed cell volumes of healthy mice fed on cactus cladode extracts for 10 days

Group 1(healthy control group) animals had mean (±S.E) PCV value of 61.7±1.11% at the start of the experiment. The animals recorded a gradual decline in mean (±S.E) PCV values throughout the experiment reaching values of 54.2±0.79% at the end of observation period of 30 days. Group 2 animals (healthy mice fed on 0.6 ml cactus cladode extract for 10 days) showed an increase in mean (±S.E) PCV on the 5th day of monitoring after start of feeding on cactus cladode extract. A decline in mean (±S.E) PCV value from 60.2±3.23% to 56.4±1.44% was observed in this group on the last (10th) day of feeding on cactus cladode extract.

Healthy animals fed on 0.8 ml cactus extract for 10 days (Group 3) recorded a drastic drop in mean (±S.E) PCV values (51.7±4.47% from 67.2±1.42%) on the 5th day of feeding on cactus cladode extract. However, the animals later recorded an increase in mean (±S.E) PCV reaching values of 56.0±1.67% at the end of the 10 days treatment period. The changes in PCV values observed were however not significant when compared to the healthy control group (Table 4.5).

Table 4.5: Effect of prickly pear cactus extract on packed cell volumes of healthy mice fed on cactus cladode extracts for 10 days

	Packed cell volumes (%)		
Time/Treatment	**Group 1**	**Group 2**	**Group 3**
-3 Days	60.0±1.57	59.5±0.34	67.0±1.00
#0 Days	61.7±1.11	59.3±0.49	67.2±1.42
5 Days Treatment	61.5±2.64	60.2±3.23	51.7±4.47
10 Days Treatment	56.7±0.61	56.4±1.44	56.0±1.67
7 Days Post-treatment	54.5±0.85	52.2±4.85	56.3±2.21
14 Days Post-treatment	54.2±0.79	53.4±1.25	52.5±2.02

Values are expressed as Mean ± SEM (n=6). Group 1 (Negative control mice not fed on cactus extracts), Group 2 (Healthy mice fed with 0.6 ml cactus extracts for 10 days), Group 3 (Healthy mice fed on 0.8 ml cactus extracts for 10 days). #0 Days denote PCV values at the start of feeding on cactus cladode extracts.

Changes in PCV values were also monitored in the animals after feeding on cactus cladode extract was terminated. An insignificant change in mean (±S.E) PCV was observed in all animals following termination of feeding on cactus cladode extract. Healthy animals on 0.6 ml (Group 2) cactus cladode extract recorded a mean (±S.E) PCV of 53.4±1.25% whereas Group 3 animals (fed on 0.8 ml cactus cladode extracts) had a mean (±S.E) PCV of 52.5±2.02% at the end of the observation period of 30 days (Figure 4.6).

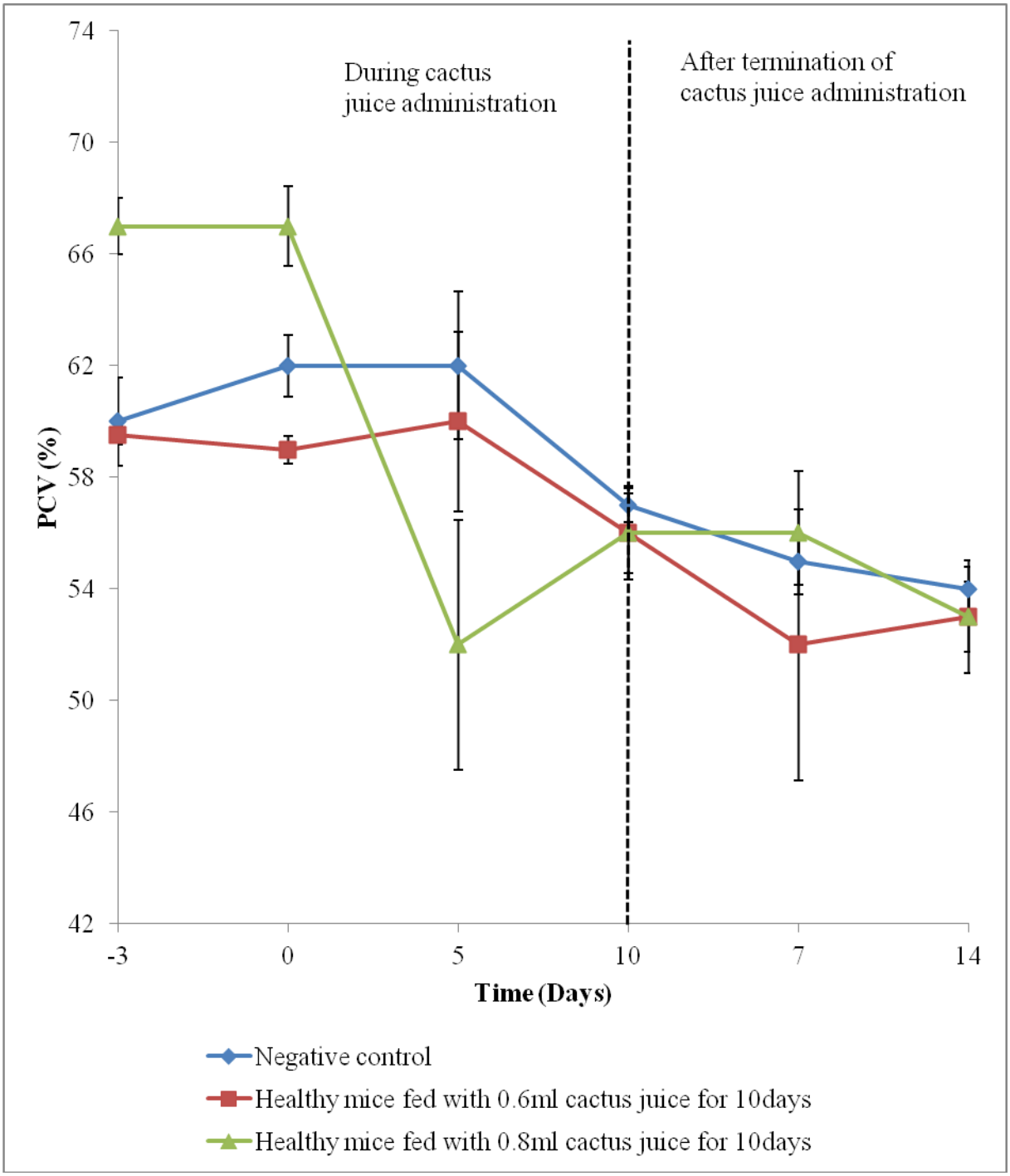

Figure 4.6: Effect of prickly pear cactus extracts on packed cell volumes of healthy mice fed on cactus cladode extract for 10 days

4.4.2.6 Packed cell volumes of alloxan-induced diabetic mice fed on 0.6 ml cactus cladode extracts

Healthy control (Group 2) animals had mean (±S.E) PCV value of 61.7±1.11% at the start of the experiment. The group showed a decline in mean (±S.E) PCV values throughout the experiment reaching values of 54.2±0.79% at the end of observation period. A decline in mean (±S.E) PCV was recorded in all the animals (Group 2, Group 3 and Group 4) following injection with alloxan. Diabetic control animals (Group 2) showed a consistent drop in mean (±S.E) PCV throughout the experiment reaching values of 48.9±0.74% from 58.9±1.37% at the end of the observation period of 30 days (Table 4.6).

Table 4.6: Effect of prickly pear cactus extract on packed cell volumes of alloxan-induced diabetic mice fed on cactus cladode extracts

Packed cell volumes (%)				
Time/Treatment	**Group 1**	**Group 2**	**Group 3**	**Group 4**
#-3 Days	60.0±1.57	59.6±1.57	63.3±3.29	60.3±1.05
##0 Days	61.7±1.12	58.9±1.37	56.7±1.54	57.3±1.12
Day 5 Treatment	61.5±2.64	53.3±0.57	56.8±1.83	54.8±1.54
Day 10 Treatment	56.7±0.61	55.3±2.34	52.8±2.75	N.D
Day 7 Post-treatment	54.5±0.85	50.1±0.59	51.0±2.48	58.0±1.30
Day 14 Post-treatment	54.2±0.79	48.9±0.74	52.8±2.06	53.0±1.87

Values are expressed as Mean ± SEM (n=6). Group 1 (Healthy control mice not induced with diabetes not fed with cactus extract), Group 2: (Diabetic control mice induced with diabetes not fed with cactus extracts), Group 3 (Alloxan-induced diabetic mice fed with 0.6 ml cactus extracts for 10 days), Group 4 (Alloxan-induced diabetic mice fed with 0.6 ml cactus extracts for 5 days). #-3 days denote PCV values at induction diabetes. ##0 days denotes PCV values at the start of treatment with cactus extracts. N.D (Not determined).

Group 3 animals (diabetic fed on 0.6 ml cactus cladode extracts for 10 days) showed a gradual decline in mean (±S.E) PCV values during feeding on cactus cladode extracts reaching values of 52.8±2.75% from 56.7±1.54% at the end of the 10 days feeding period. An isignificant change in mean (±S.E) PCV was observed in this group throughout the observation period following termination of feeding on cactus cladode extracts (Table 4.6)(Figure 4.7).

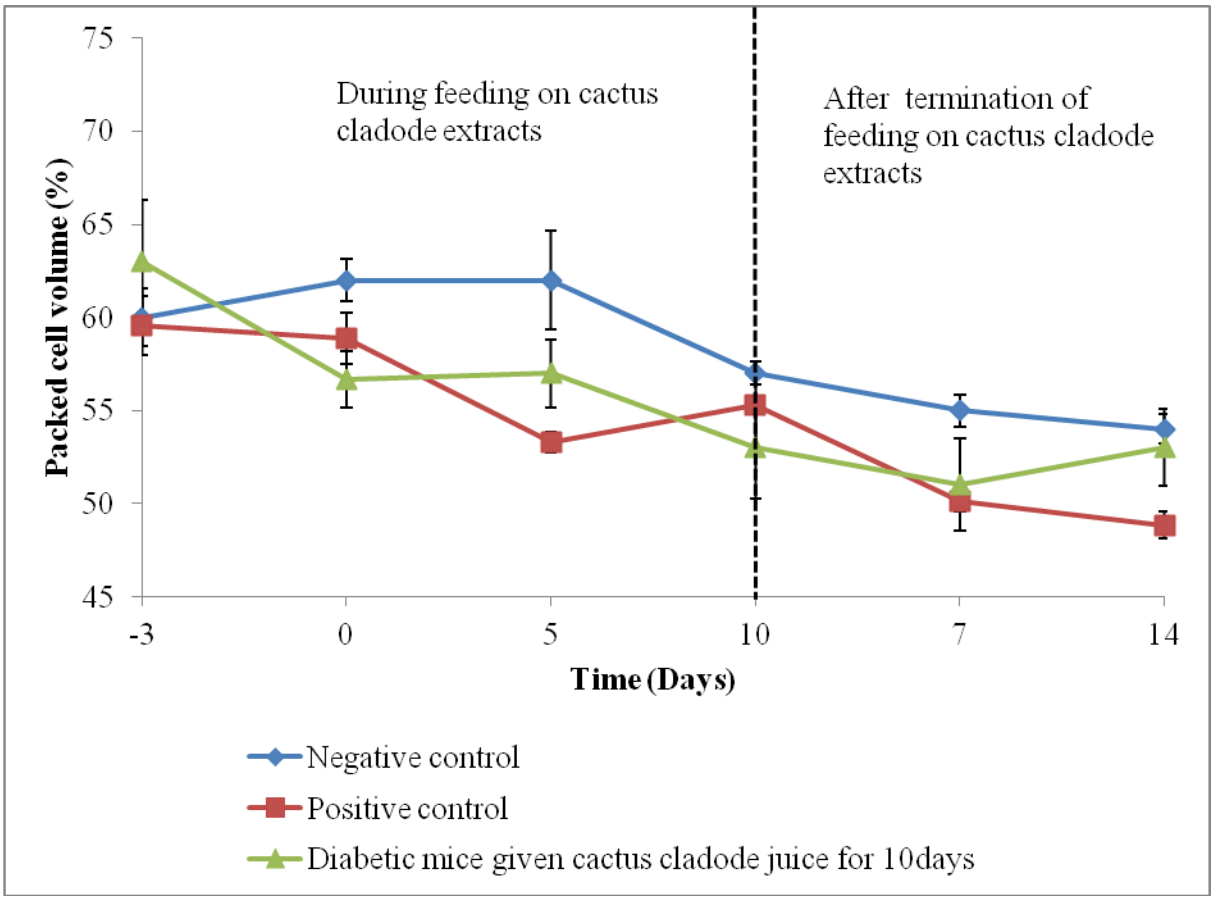

Figure 4.7: Effect of prickly pear cactus extract on packed cell volumes of diabetic mice fed on cactus cladode extracts for 10 days

Figure 4.8 shows effect of cactus cladode extracts on PCV values of diabetic animals fed on 0.6 ml cactus cladodes for 5 days (Group 4). The animals recorded a decline in mean (±S.E) PCV value from 57.3±1.12% to 54.8±1.4% at the end of 5 day feeding period. Following termination of feeding on cactus cladode extract, the animals showed an increase in mean (±S.E) PCV values to 58.0±1.30% in the first week but later declined to 52.8±2.06% at the end of the observation period. The changes observed were however not significant compared to the positive control group (Table 4.6).

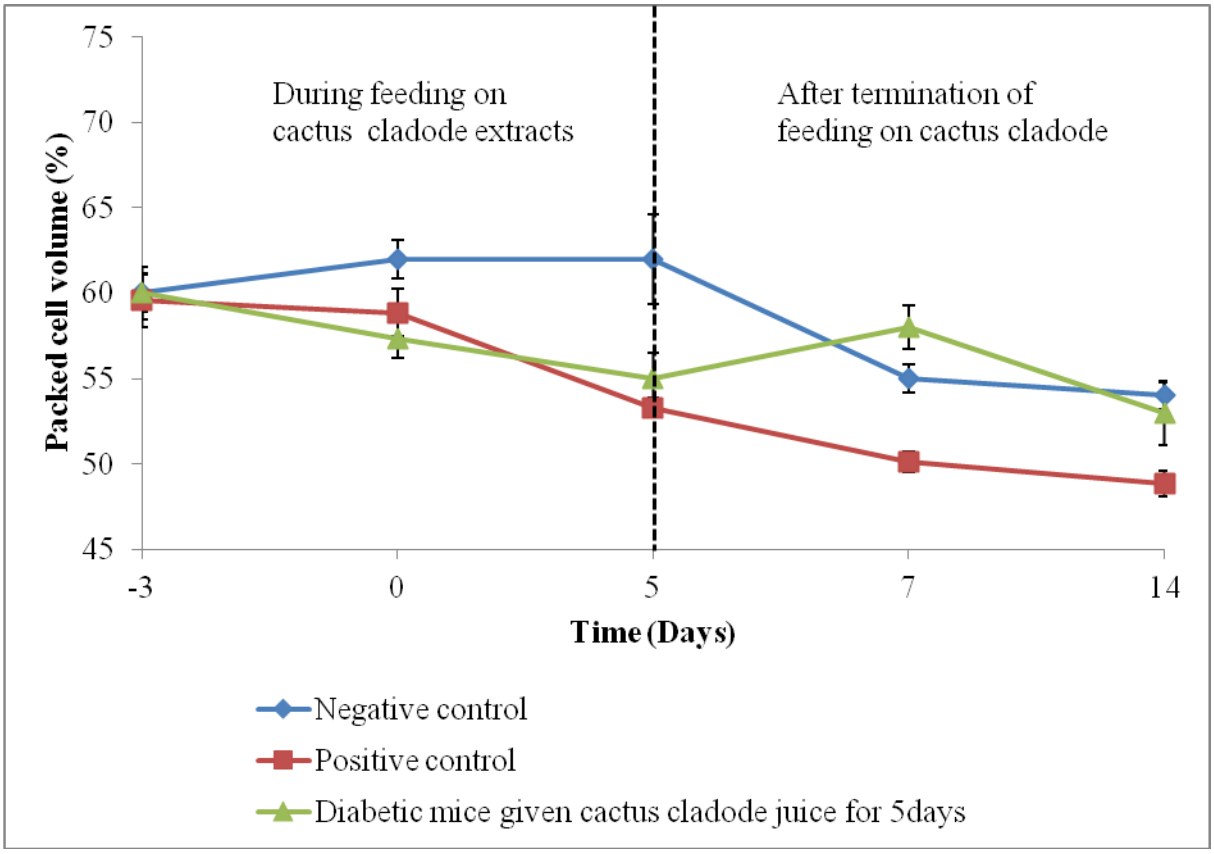

Figure 4.8: Effect of prickly pear cactus extract on packed cell volumes of diabetic mice fed on cactus cladode extracts for 5 days

4.4.3 Histopathology Analysis

4.4.3.1 Liver histopathology

Histopathology results of liver sections from the control groups and experimental groups are presented below. Liver cells of healthy control (not given cactus cladode extracts) showed normal cellular architecture characterised with normal hepatic cells with distinct nuclei and normal eosinophilic cytoplasm with normal sinusoids (Plate 4.1). Severe cellular degenerative changes were observed in liver cells of diabetic control mice (Plate 4.2).

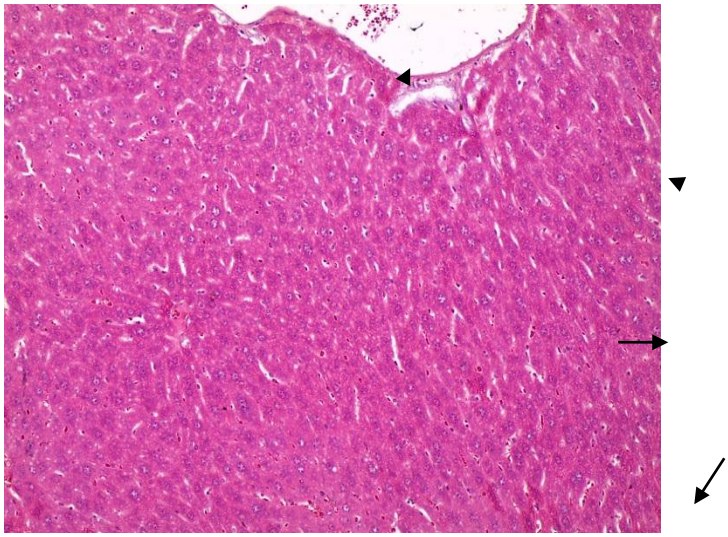

Plate 4.1: Liver section of healthy control mice showing normal hepatocytes (arrow heads) with normal sinusoids (arrows) (H&E, ×40)

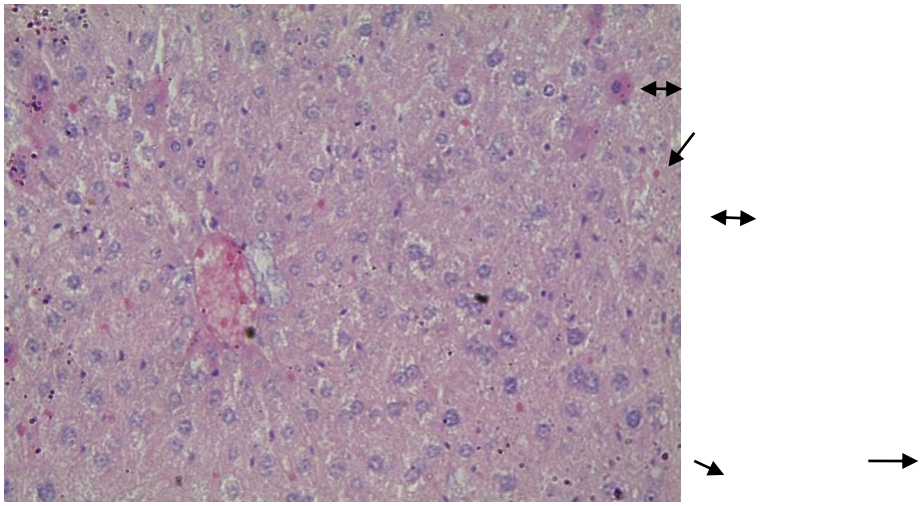

Plate 4.2: Liver section of diabetic control mice showing severe hapatocellular degeneration (double arrows) and fatty liver degeneration (arrows) (H&E, ×40)

Liver sections of healthy mice fed on various volumes of cactus cladode extract for varied days showed severe fatty liver and hepatocellular degeneration (Plate 4.3 to Plate 4. 6). Mild perivascular cuffing was observed in healthy mice fed on 0.6 ml (Plate 4.3) and 0.8 ml (Plate 4.4) cactus cactus cladode extract daily for 5 days. A focus of necrosis was also observed in healthy animals fed on 0.8 ml cactus cladode extract for 5 days (Plate 4.4) and 0.6 ml cactus cladode extract for 10 days (Plate 4.5). However, half of the liver sections of healthy animals fed on 0.8ml cactus cladode extracts for 10 days showed mild degenerative changes (Plate 4.6A).

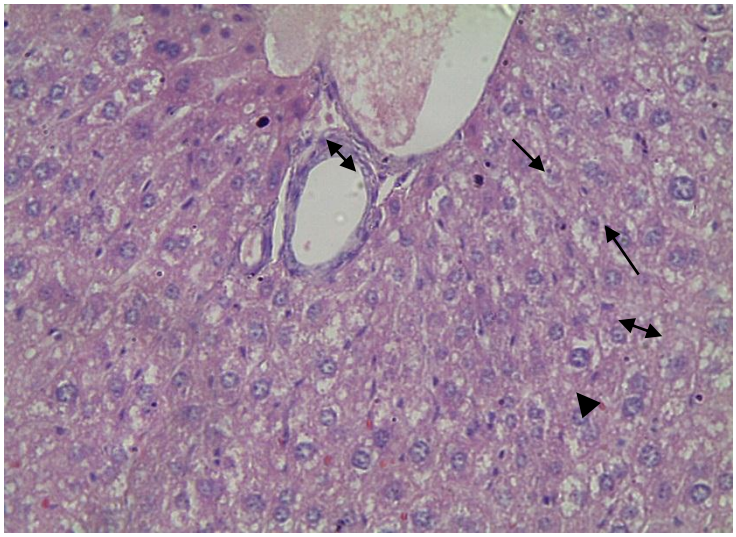

Plate 4.3: Liver section of healthy mice fed on 0.6 ml cactus cladode extract for five days showing perivascular cuffing (arrows), hepatocellular degeneration (double arrow) and fatty liver degeneration (arrow heads) (H&E, ×40)

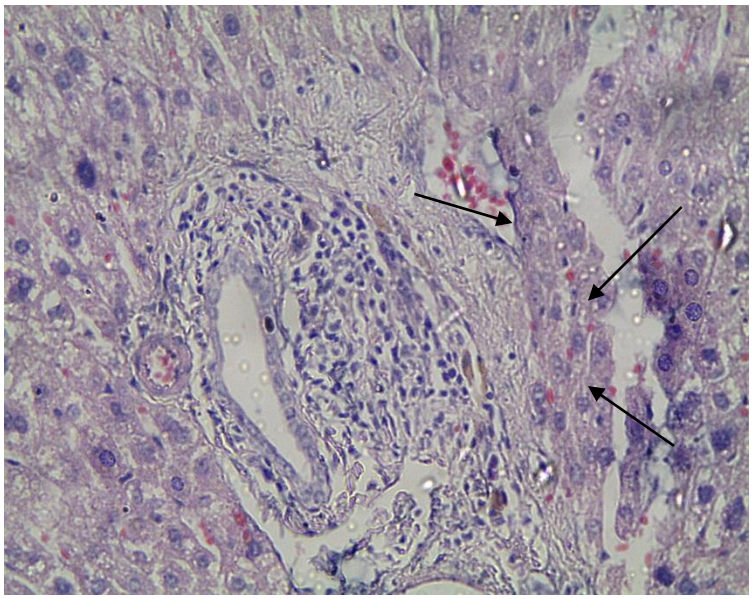

Plate 4.4: Liver section of healthy mice fed on 0.8 ml cactus cladode extract for five days showing focal necrosis (arrows) (H&E, ×40)

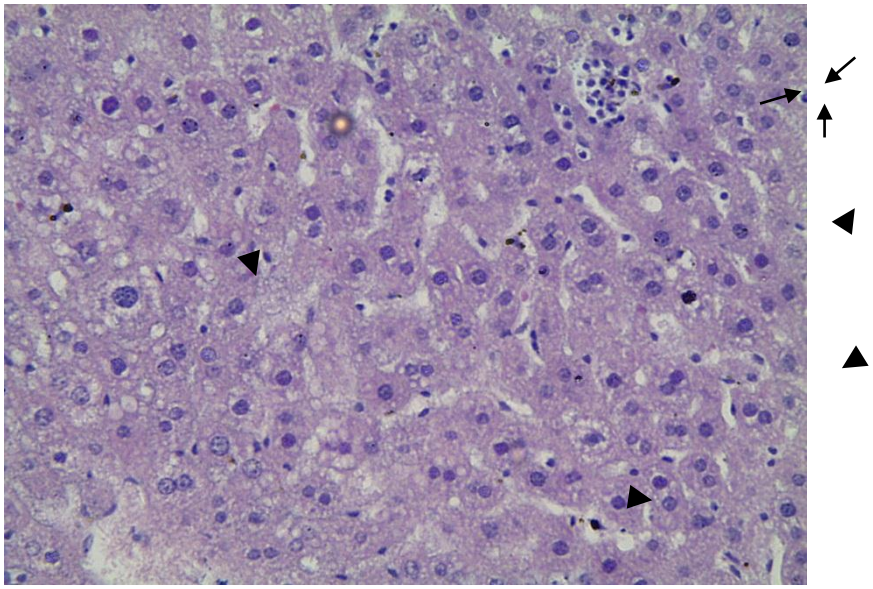

Plate 4.5: Liver section of healthy mice fed on 0.6 ml cactus cladode extract for ten days showing a focus of necrosis (arrows) and fatty liver degenaration (arrow heads) (H&E, ×40)

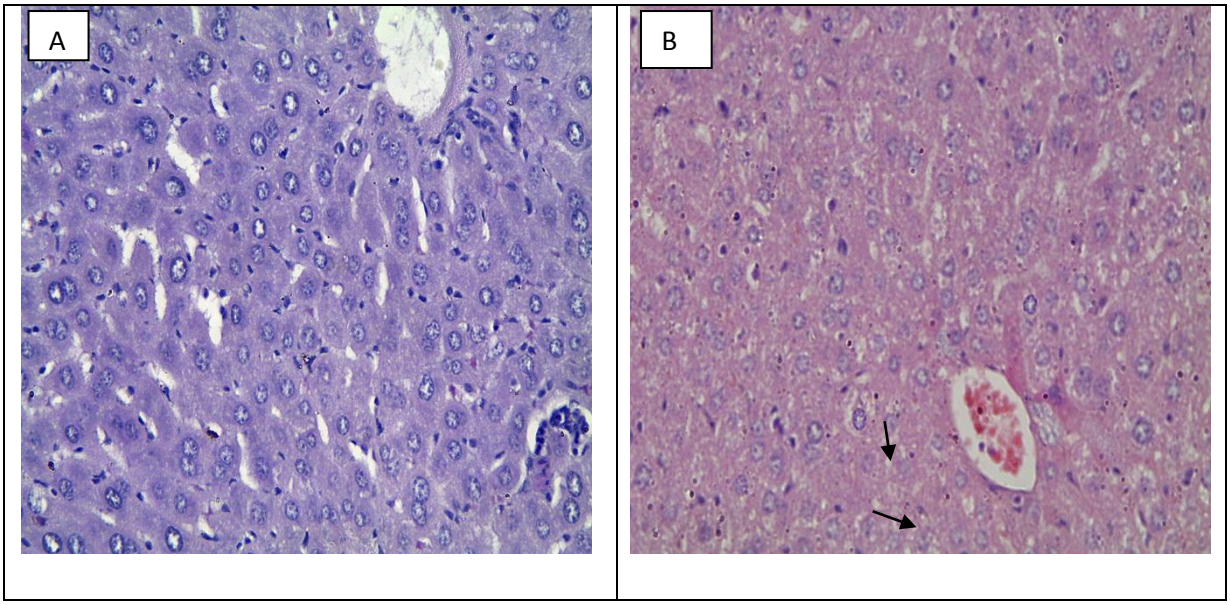

Plate 4.6: Liver sections of healthy mice fed on 0.8 ml cactus cladode extracts for ten days (H&E, ×40).

Plate 4.6A shows liver section characterised with mild degenaration of hepatic cells and mild steatosis. Plate 4.6B is a section of liver from the same animal group showing severe hepatocellular and fatty liver degeneration (arrows).

The liver sections of diabetic animals fed on cactus extracts showed mild cellular changes characterised with mildly dilated sinusoids. Mild hepatocellular degeneration and perivascular cuffing with lymphocytes (shown with arrows) was also observed in liver sections of diabetic mice fed on cactus extract for 5 days (Plate 4.7). Liver sections of diabetic animals fed on cactus extract for 10 days showed mild fatty degeneration and cytoplasmic vacuolation (Plate 4.8B). In contrast, two of the diabetic animals fed on cactus cladode extracts for 10 days showed normal liver histology with no morphological changes (Plate 4.8A).

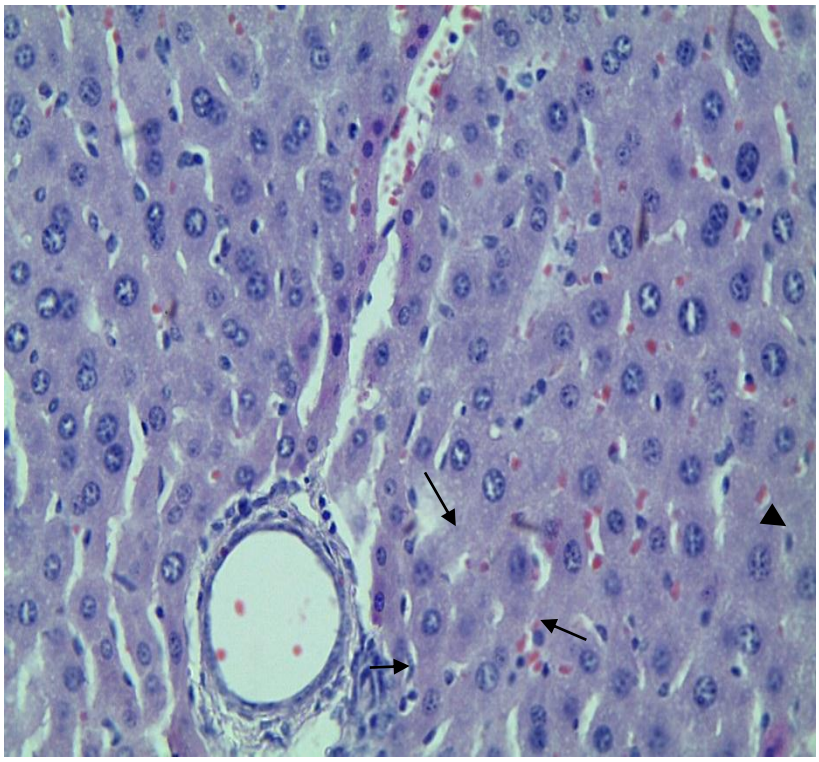

Plate 4.7: Liver section of alloxan-induced diabetic mice fed on 0.6 ml cactus cladode extracts for five days showing perivascular cuffing (arrows) and dilated sinusoids (arrow heads) (H&E, ×40)

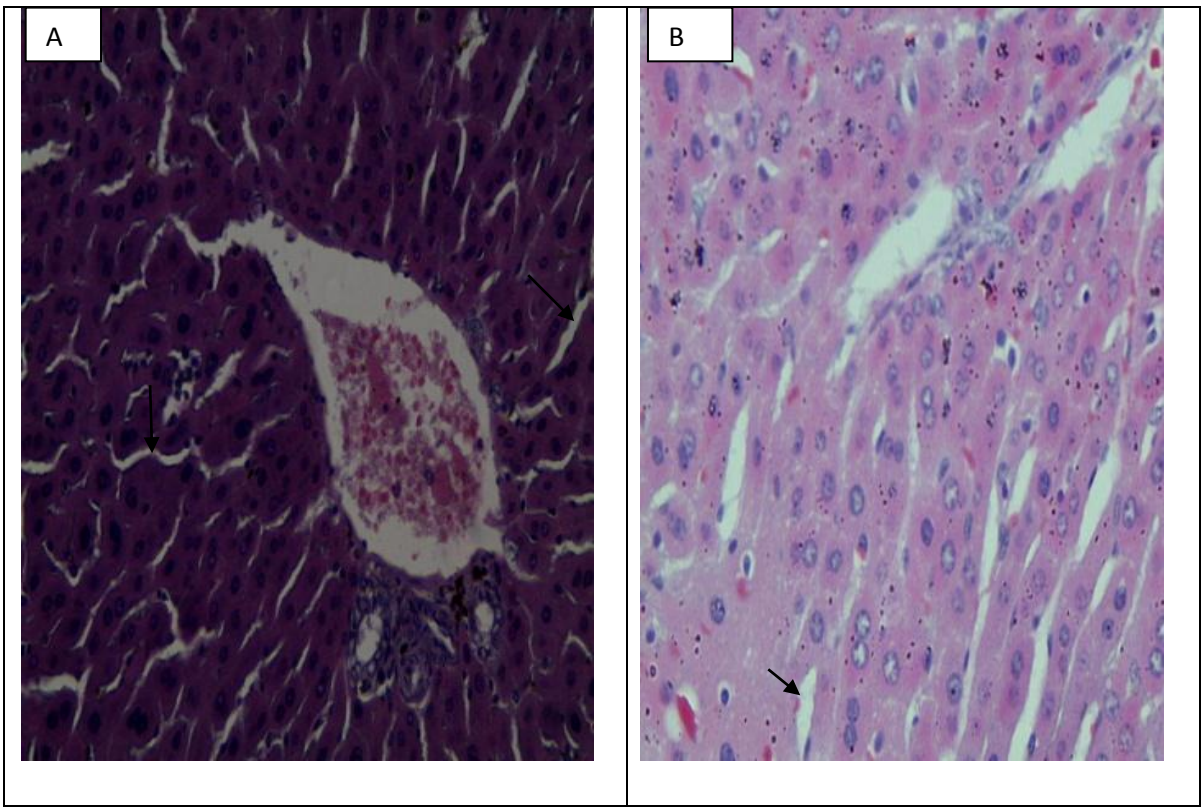

Plate 4,8: Liver section of alloxan-induced diabetic mice fed on 0.6 ml cactus cladode extracts for ten days (H&E, ×40)

Plate 4.8A shows liver section of diabetic mice fed on 10 days showing normal cellular architecture with normal sinusoids (arrows) and Plate 4.8B shows liver from same treatment group showing mildly dilated sinusoids (arrows).

4.4.3.2 Kidney histopathology

The kidney sections from the healthy control group (not given cactus cladode extracts) showed normal renal architecture with distinct glomeruli and tubules (Plate 4.9). The kidneys of diabetic control mice showed severe cellular degeneration characterised with severe degeneration of tubular epithelium (Plate 4.10).

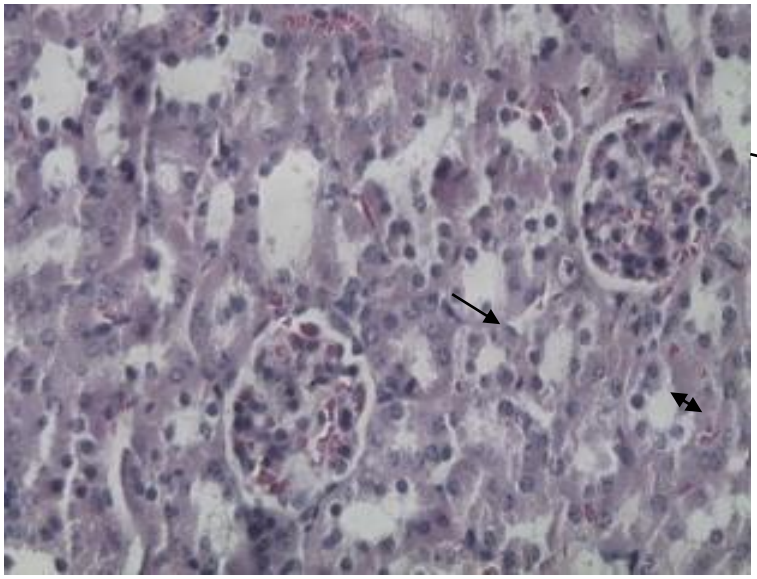

Plate 4.9: Kidney section of healthy control mice showing normal renal architecture with distinct glomeruli (arrows) (H&E, ×40)

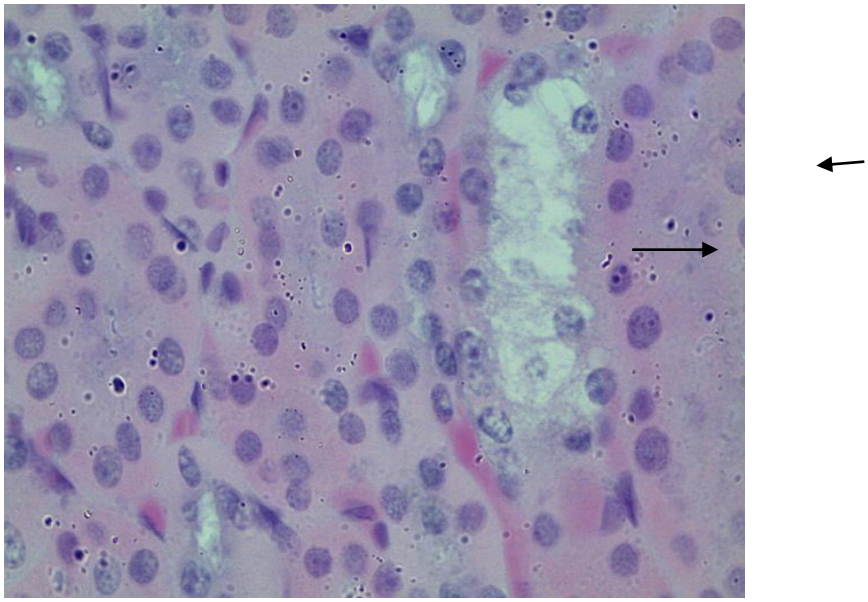

Plate 4.10: Kidney section of diabetic control mice showing severe tubular epithelium degeneration (arrows) (H&E, ×40)

Kidney sections of healthy mice fed on 0.6 ml cactus cladode extract for 5 days showed a focus of necrosis characterised with slight mononuclear infiltration (Plate 4.11). The kidney sections from the other healthy mice groups displayed normal renal architecture with minimal morphological changes (Plate 4.12 to Plate 4.14).

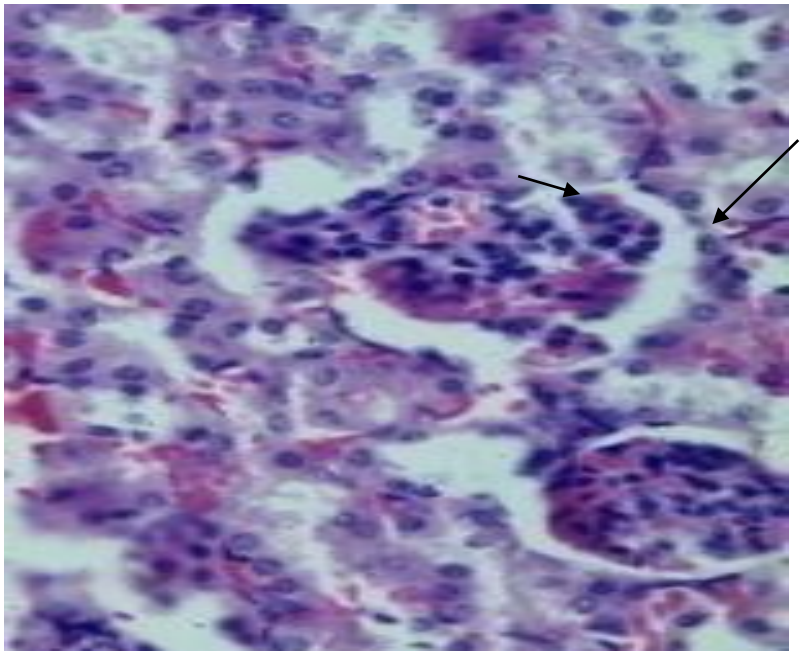

Plate 4.11: Kidney section of healthy mice fed on 0.6 ml cactus cladode extract for five days showing a focus of necrosis (arrows) (H&E, ×40)

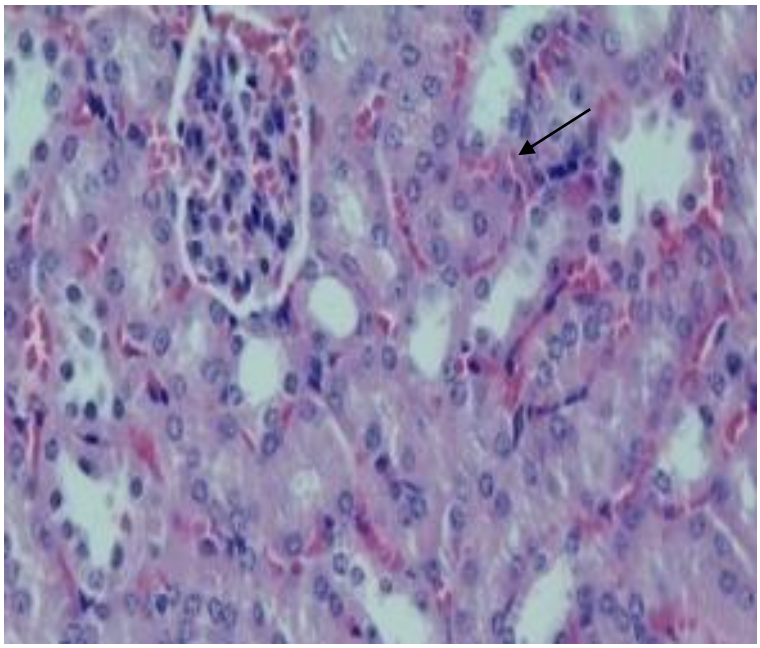

Plate 4.12: Kidney section of healthy mice fed on 0.8 ml cactus extract for five days showing normal renal architecture with distinct glomeruli (arrows) (H&E, ×40)

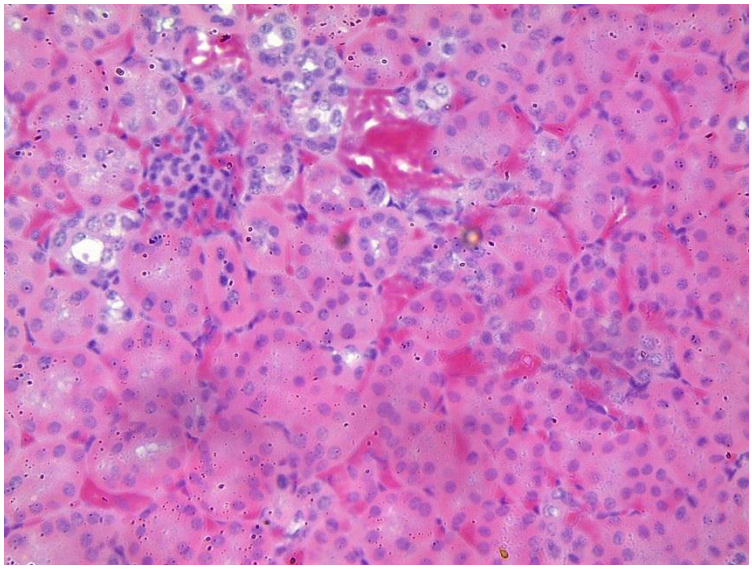

Plate 4.13:Kidney section of healthy mice fed on 0.6 ml cactus cladode extract for ten days (H&E, ×40)

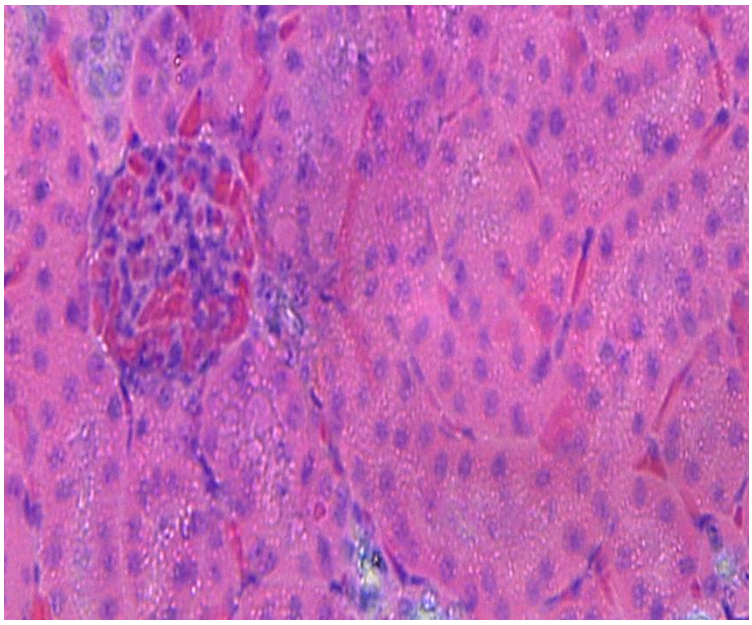

Plate 4.14: Kidney section of healthy mice fed on 0.8 ml cactus cladode extract for ten days (H&E, ×40)

Kidney sections of diabetic mice fed on cactus cladode extract for 5 days displayed normal renal architecture characterised with distinct glomeruli (Plate 4.15). Focal area of necrosis characterised with mild tubular epithelial degeneration and glomerular infiltration was observed in kidney sections from diabetic mice group fed on cactus cladode extract for 10 days (Plate 4.16).

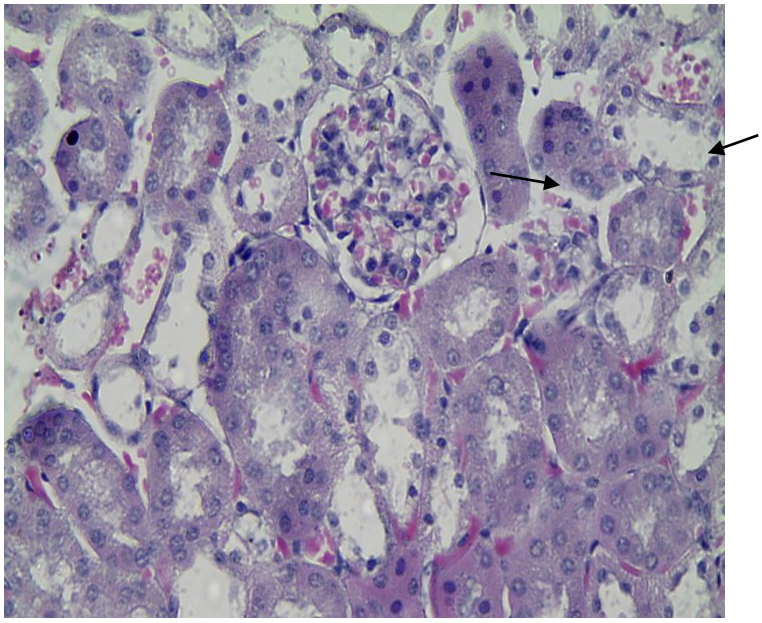

Plate 4.15: Kidney section of alloxan-induced diabetic mice fed on 0.6 ml cactus cladode extracts for five days showing normal renal architectute with distinct glomeruli (arrows) (H&E, ×40)

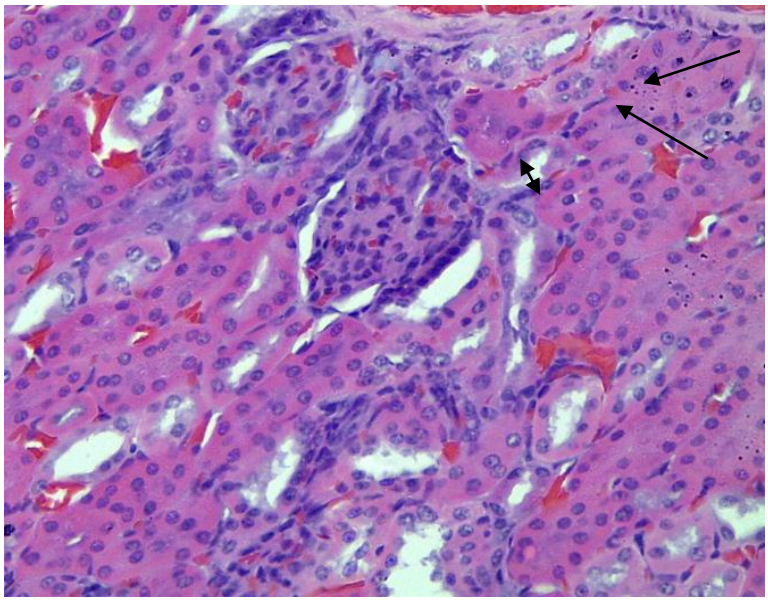

Plate 4.16: Kidney section of alloxan-induced diabetic mice fed on 0.6 ml cactus cladode extract for ten days showing tubular epithelium degenaration (double arrow) and focal necrosis (arrows) (H&E, ×40)

4.5 Discussion

Alloxan-induced diabetic untreated group recorded a consistent drop in their mean body weights throughout the experiment from 26.3±0.64 g to 24.4±0.72 g while the negative control group showed gradual increase in their body weights from 26.8±0.31 g to 29.8±0.54 g. These results are in concurrence with those obtained by El-Razek and Hassan (2011). The loss in body weight observed is likely to have been as a result of muscle wasting due to lack of carbohydrate for utilisation as an energy source that arises from poorly controlled diabetes mellitus (El-safty and Al-Masri, 2009). All the other animal groups fed on cactus extract similarly recorded a decline in their body weights during feeding on the cactus cladode extract but later gained weight when feeding was terminated. The changes in the body weights observed were however not statistically significant when compared to those of their corresponding control (diabetic and healthy) groups.

The weight loss observed in the animals may be attributed to high content dietary fibres that have been reported in prickly pear cactus cladodes (Ayadi *et al.,* 2009). Dietary fibers promote weight loss through reducing energy intake by binding to dietary fats making them unavailable for use in digestion (Uebelhack *et al.,* 2014). It is also believed that the dietary fibres in the cactus cladodes promote a feeling of satiety (Burley *et al.,* 1993; Slavin and Green, 2007) which reduces food intake thus promoting weight loss.

Packed cell volumes is one of the key haematological parameters used to detect defects in metabolic processes of the body arising from stress as a result of injury to the body (Patil and Surana, 2010). Diabetic control animals recorded a gradual decrease in PCV levels throughout the experiment. Animals from these group had a mean (±S.E) PCV of 59.6±1.57% before treatment with alloxan. A drop in mean (±S.E) PCV to 58.9±1.37% was observed in this animal group following alloxan administration which also decreased gradually throughout the experiment to 48.9±0.74%. Diabetes mellitus is usually accompanied by increased production of free radicals which cause damage to membrane lipids and cellular proteins (Pankaj and Varma, 2013). Increased non-enzymatic glycosylation of red cell membrane proteins has been implicated in the occurrence of anaemia associated with diabetes mellitus. Oxidation of these proteins due to hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides resulting in red blood cells haemolysis (Oyedemi *et al.,* 2011; Alamgeer *et al.,* 2012) thus the decreased packed cell volume values.

Diabetic animals given cactus cladode extracts recorded higher PCV values than their positive control group. This can be attributed to lowered blood sugar levels observed in the animals following treatment with cactus cladode extracts. Hyperglycaemia in uncontrolled diabetes mellitus causes generation of free radicals which attacks red blood cells leading to haemolysis. Prickly pear cactus cladodes have been reported to contain anti-oxidants especially flavonoids which scavenge the free radicals (Bensadón *et al.,* 2010) hence protecting the red blood cells by decreasing their susceptibility to haemolysis (Tahmasebpour *et al.,* 2013). Healthy animals fed on cactus extracts showed insignificant changes in PCV values when compared to those of the negative control group. Animals given cactus cladode extracts daily for 5 days had slightly higher PCV values than the negative control animals while those fed for 10 days recorded lower PCV values at the end of the observation period of 30 days. In general, the PCV values remained within the normal range of the animal species throughout the experiment.

Histopathological evaluation of liver tissues of positive control animals revealed severe degenerative changes characterised by severe fatty liver degeneration and hepatocellular degeneration. Uncontrolled diabetes mellitus is accompanied by elevated generation of free radicals that lead to increased mitochondrial oxidative stress. This elevation of mitochondrial oxidative stress leads to liver damage usually characterised by inflammation and cellular necrosis (Garcia-Compean *et al.,* 2009). Administration of cactus cladode extract to the diabetic mice for 10 days led to normalcy of liver structure in 30% of the animals. Mildly dilated sinusoids and mild fatty degeneration were observed in liver tissues of diabetic animals on cactus cladode extract for 5 days and 10 days. A characteristic mild perivascular cuffing with lymphocytes in liver sections of diabetic mice on cactus cladode extract for 5 days was also observed. These results corroborate those of Ncibi *et al* (2008) who reported hapatoprotective effect of *Opuntia ficus indica* cladode in mice against liver damage induced by chlorpyrifos, an organophosphorous insecticide (Ncibi *et al.,* 2008).

Severe hepatocellular and fatty liver degeneration, and focal necrosis with mononuclear cellular infiltration were observed in healthy mice fed on cactus cladode extract. In contrast, liver sections of half of the healthy animals on 0.8 ml cactus cladode extract for 10 days showed mild degenerative changes. Similar to our findings, Almeida da Luz *et al* (2015) reported focal necrosis with lymphocytic infiltration on mice liver treated with diphenyl ditelluride (Almeida da Luz *et al.,* 2015).

However, kidney sections of these animals revealed normal structure with minimal pathological changes. On the other hand, no deaths were observed in these animal groups. It is therefore possible that the liver damages were not fatal for the animals.

Kidney sections of positive control animals showed severe cellular damage. This could be as a result of oxidative stress caused by increased blood sugar levels in diabetes mellitus which facilitate production of free radicals that lead to cellular damage by the reactive oxygen species (El-Razek and Hassan, 2011). Focal necrosis characterised by mild glomerular infiltration and epithelial lining degeneration was observed in kidney sections of diabetic animals on cactus cladode extracts for 10 days. Our findings are similar to those of Oršolić *et al* (2012) who reported tubular dilation and necrosis, and epithelial flattening on diabetic mice following treatment with extracts of propolis (Oršolić *et al.,* 2012). On the other hand, kidney sections of diabetic mice on cactus cladode extract presented normal appearance with minimal pathological changes. Our results show that cactus cladode extract minimises effects of diabetes mellitus on kidneys and liver of diabetic mice. This protective role of cactus cladode extract could be attributed to anti-oxidant activity that has been reported in prickly pear cactus (Osuna-Martínez *et al.,* 2014; Hwang *et al.,* 2015). Opuntia cladodes have been reported to be high in anti-oxidants such as phenols and flavonoids (Jun *et al.,* 2013; Boutakiout *et al.,* 2015; Hwang *et al.,* 2015). These compounds are able to counteract the effects of oxidative free radicals on the cells (Wie, 2000) through their scavenging activity (Brahmi *et al.,* 2009) against hyperglycaemia induced reactive oxygen species production.

4.6 References

Alamgeer, Mushtaq, M.N., Bashir, S., Rashid, M., Malik, M.N.H., Ghumman, S.A., Irfan, H.M., Akram, M., Khan, A.Q. and Rashid, H.U. (2012). Hypoglycemic and hematological effects of aqueous extract of *Thymus serpyllum Linn*. in alloxan induced diabetic rabbits *African Journal of Pharmacy and Pharmacology*, **6**, 2845 2850. DOI: 10.5897/AJPP12.1343

Almeida da Luz, S.C., Daubermaann, M.F., Thomé, G.R., Mülling dos Santos, M., Ramos, A., Salazar, G.T., Teixeira da Rocha, J.B and Barbosa, N.V. (2015). Diphenyl ditelluride intoxication triggers histological changes in liver, kidney, and lung of mice. *Analytical Cellular Pathology*, 784612. Doi: 10.1155/2015/784612

Ayadi, M.A., Abdelmaksoud, W., Ennouri, M. and Attia, H. (2009). Cladodes from *Opuntia ficus indica* as a source of dietary fibre: effect on dough characteristics and cake making. *Industrial Crops and Products*, 40–47.

Bensadón, S, Hervert-Hernández, D, Sáyago-Ayerdi, S.G and Goñi, I. (2010). By-products of *Opuntia ficus-indica* as a source of antioxidant dietary fiber. *Plant Foods for Human Nutrition*, **65**, 210-216.

Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A. and Legssyer, A. (2006). Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). *International Journal of Diabetes and Metabolism,* **14**, 1-25.

Boutakiout, A, Elothmani D.D, Mahrouz,M, Hmid, I. and Hanine, H.(2015). Phytochemical constituents and in vitro radical scavenging activity of different cladodes extract of cactacea cultivars from different areas in Morocco. *International Journal of Technology Enhancements and Emerging Engineering Research,* **3**, 56-66.

Brahmi, D, Bouaziz, C, Ayed, Y, Ben Mansour, H, and Zourgui, L. (2011). Chemopreventive effect of cactus *Opuntia ficus indica* on oxidative stress and genotoxicity of aflatoxin B1.*Nutrition and Metabolism (Lond)*, **8**, 73.

Bull, B.S. and Rittenbach, J.D. (1990). A proposed reference haematocrit derived from multiple MCHC determinations via haemoglobin measurements. *Clinical and Laboratory Haematology*, **12**, 43-53.

Burley, V.J, Paul, A.W. and Blundell, J.E. (1993). Sustained post-ingestive action of dietary fibre: effects of a sugar-beet-fibre-supplemented breakfast on satiety. *Journal of Human Nutrition and Dietetics*, **6**, 253–60.

Chauhan, S.P., Sheth, N.R. and Suhagia, B.N. (2014). Haematinic evaluation of fruits of *Opuntia elatior mill* on mercuric chloride induced anemia in rats. *International Journal of Research in Ayurveda and Pharmacy*, **5**, 115-122.

El-Razek, A.H.F and Hassan, A.A. (2011). Nutritional value and hypoglycemic effect of prickly cactus pear (*Opuntia ficus indica*) fruit extract in alloxan-induced diabetic rats. *Australian Journal of Basic and Applied Sciences*, **5**, 356-377.

El-safty, S.M.S. and Al-Masri, S.A. (2009). Effect of prickly pear consumption on some biological and biochemical changes in hyperglycemic injured rats. *Bulletin of the National Nutrition Institute,* (34), 46-61.

Factsheet (2011). *Opuntia ficus indica* (Sweet Prickly Pear). Accessed 2015 from http://keys.lucidcentral.org/keys/v3/eafrinet/weeds/key/weeds/Media/Html/Opuntia_ficus-indica (Sweet_Prickly_Pear).htm

Gagnier, J.J., DeMelo, J., Boon, H., Rochon. P. and Bombardier, C. (2006) Quality of reporting of randomized controlled trials of herbal medicine interventions. *American Journal of Medicine,* **119**, 800.

Garcia-Compean, D., Jaquez-Quintana, J.O., Gonzalez-Gonzalez, J.A. and Maldonado-Garza, H. (2009). Liver cirrhosis and diabetes: Risk factors, pathophysiology, clinical implications and management. *World Journal of Gastroenterology*, **15**, 280-288.doi: 10.3748/wjg.15.280

Hahm, S.W., Park, J. and Son, Y.S. (2011). *Opuntia humifusa* stems lower blood glucose and cholesterol levels in streptozotocin-induced diabetic rats. *Nutrition Research,* **31**, 479 87.

Hunt D, Taylor, N.P. and Charles, G. (2006). The new cactus lexicon. DH Books, Milborne Port, UK.

Hwang, J-H, Yi, M-R, Kim, J-W, Bu, H-J, Kang, C-H. and Lim, S-B. (2015). Quality characteristics and antioxidant activity of prickly pear cactus cladodes. *Journal of the Korean Society of Food Science and Nutrition,* **44**, 356-362. DOI: 10.3746/jkfn.2015.44.3.356

Jun, H, Cha, E-I, Yang, E-I, Choi, D.G. and Kim, Y-S. (2013). Physicochemical properties and antioxidant activity of Korean cactus (*Opuntia humifusa*) cladodes. *Horticulture, Environment and Biotechnology*, **54**, 288-295.

Kang'ara, J.N.N. and Gitari, J.N. (n.d). Exploiting fodder potentials of cactus (*Opuntia* spp) in Kenya for pastoral livestock feeding under a changing climate.

Madrigal-Santillán, E., Garcia-Melo, F., Morales-González, J.A., Vázquez-Alvarado, P., Muñoz-Juárez, S., Zuñiga-Pérez, C., Maria Teresa Sumaya-Martínez, M.T., Madrigal-Bujaidar, E. and Hernández-Ceruelos, A. (2013). Antioxidant and anticlastogenic capacity of prickly pear extract. *Nutrition*, **5**, 4145–4158. Doi: 10.3390/nu5104145

Ncibi, S, Othman, M.B, Akacha, A, Krifi, M.N. and Zorgui, L. (2008). *Opuntia ficus indica* extract protects against chlorpyrifos-induced damage on mice liver. *Food and Chemical Toxicology*, **46**, 797–802.

Nefzaoui, A., Nazareno, M. and El Mourid, M. (2007). Review of medicinal uses of cactus. *Cactusnet*, **11**, 3-17.

Oršolić, N., Sirovina, D., Končić, M.Z., Lacković, G. and Gregorović, G. (2012).Effect of Croatian propolis on diabetic nephropathy and liver toxicity in mice. *BMC Complementary and Alternative Medicine*, **12**, 117.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. (2009). Agroforestry database: a tree reference and selection guide version 4.0. Accessed on 13th may, 2015 from http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp

Osuna-Martínez, U., Reyes-Esparza, J. and Rodríguez-Fragoso, L. (2014). Cactus (*Opuntia ficus indica*): A review on its antioxidants properties and potential pharmacological use in chronic diseases. *Natural Products Chemistry and Research*, **2**, 153.

Oyedemi, S.O., Adewusi, E.A., Aiyegoro, O.A. and Akinpelu, D.A. (2011). Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pacific *Journal of Tropical Biomedicine*, **1**, 353–358. Doi: 10.1016/S2221-1691(11)60079-8

Pankaj, P.P, and Varma, M.C. (2013). Potential role of *Spirulina platensis* in maintaining blood parameters in alloxan induced diabetic mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, **5**, 450-456.

Patil, P.H. and Dr. Surana S.J. (2010).Toxicological e valuations of *Eranthemum roseum R.* Br Linn root extracts. *International Journal of PharmTech Research*, **2**, 753-759.

Plagens, M.J. (2011). Prickly pear cactus in Kenya. Accessed on 21st April, 2015 from http://www.ngkenya.com/flora/Opuntia-01.html

Slavin, J. and Green, H. (2007), Dietary fibre and satiety. *Nutrition Bulletin*, **32**, 32–42. doi: 10.1111/j.1467-3010.2007.00603.x

Tahmasebpour, N., Dehghan, G., Feizi, M.A.H. and Esmaeili, H.A. (2013). Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with *Teucrium orientale*. *Pharmacology OnLine*, **3**, 32 – 36.

Uebelhack, R., Busch, R., Alt, F., Beah, Z-M. and Chong, P-W. (2014). Effects of cactus fiber on the excretion of dietary fat in healthy Subjects: A double blind, randomized, placebo-controlled, crossover clinical investigation. *Current Therapeutic Research*, **76**, 39–44.

Wie, M.B (2000). Protective effects of *Opuntia ficus indica* and *Saururus chinensis* on free radical induced neuronal injury in mouse cortical cell cultures. *Journal of the Pharmaceutical Society of Korea*, **44**, 613-619.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 General discussion

Oxidative stress, through the production of reactive oxygen species (ROS) has been reported as the major cause in the development of insulin resistance which leads to cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus. It has also been suggested to be a common pathway linking diverse mechanisms involved in the progression of long-term complications in diabetes mellitus (Nirmala *et al*., 2009; ED *et al.,* 2015). Over the last few decades, use of traditional plant medicines for treatment of various ailments, including diabetes mellitus, has increased worldwide due to its therapeutic efficacy, minimal side effects and low costs (Alamgeer *et al.,* 2012). Several plant drugs and herbal formulations among them being those of prickly pear cactus (*Opuntia* species) have been used since ancient times to manage diabetes mellitus. Different studies using European and Asian varieties of prickly pear cactus fruits and/or cladodes have shown remarkable antioxidant activities that reduce significantly the oxidative stress in patients and may prevent development of chronic pathologies such as diabetes mellitus (Madrigal-Santillan *et al.*, 2013).

In the present study, oral administration of 0.6 ml cactus cladode extracts daily for 10 days showed significant reductions in blood sugar levels of the alloxan-induced diabetic mice when compared to the diabetic (positive) control group. Our present results corroborate the findings of Meckes-Lazyoa and Roman-Ramos (1986) who reported that intake of cladode extracts for 10 days significantly reduced serum glucose levels in type 2 diabetes mellitus patients. Cactus cladodes have been reported to be rich in anti-oxidants and pectin (Nefzaoui *et al.,* 2007). Anti-oxidants have been shown to reduce blood glucose levels by improving sensitivity of the tissues to insulin and by promoting uptake of glucose by peripheral tissues whereas pectins reduce blood glucose levels by increasing sensitivity of pancreatic ß cells to produce more insulin (Bnouham *et al.,* 2006).

Body weight is usually investigated as a sensitive indicator of chemically induced changes (ED *et al.,* 2015). Injection of alloxan led to a decrease in body weights of the animals. Similar to our findings, Orsolic *et al* (2012) reported reduction in body weights of animals treated with alloxan.

A reduction in body weight was similarly observed in all the animals during feeding on cactus cladode extracts but later gained weight insignificantly when feeding was stopped compared to the negative control animals which gained weight consistently throughout the observation period. The decrease in body weight could have been due to the action of dietary fibers which have been reported in cactus cladodes. Dietary fibers promote weight loss through reducing energy intake by binding to dietary fats making them unavailable for use in digestion and by reducing food intake through promoting a feeling of fullness (Slavin and Green, 2007).

Diabetic untreated animals recorded consistent decline in packed cell volume values throughout the experiment. Increased non-enzymatic glycosylation of red cell membrane proteins has been implicated in the occurrence of anaemia associated with hyperglycaemia during diabetes mellitus. When these proteins undergo oxidation due to hyperglycaemia in diabetes mellitus, an increase in production of lipid peroxidase occurs which result in haemolysis of red blood cells (Oyedemi *et al.,* 2011). Diabetic animals fed on cactus cladode extracts recorded higher packed cell volume values compared to the diabetic untreated animals. This can be attributed to lowered haemolysis of erythrocytes. Cactus cladodes have been reported to contain anti-oxidants particularly flavonoids that scavenge free radicals (Nefzaoui *et al.,* 2007) generated in hyperglycaemia reducing susceptibility of the red blood cells to haemolysis. Healthy animals fed on cactus cladode extracts exhibited an insignificant change in packed cell volume values compared to the negative control animals. This could suggest that the cactus cladode extracts did not alter the packed cell volume values of the animals but rather play a preventive role in the mice.

Histolopathological examination of liver tissues of diabetic untreated group revealed severe degenerative changes characterised by severe fatty liver degeneration and hepatocellular degeneration. This could have been due to increased accumulation of fat within the cells as a result of impaired fatty acids metabolism (Orsolic *et al.,* 2012). It could also have been due to increased mitochondrial oxidative stress caused by reactive oxygen species in diabetes mellitus that lead to liver damage (Garcia-Compean *et al.,* 2009). Mild degenerative changes characterised by mildly dilated sinusoids and fatty liver degeneration was observed in liver tissues of diabetic animals fed on cactus cladode extracts. Moreover, liver sections of 30% of diabetic animals fed on cladode extracts for 10 days revealed normal liver histology. This could suggest the cactus cladodes may have a hepatoprotective effect on the liver tissues as reported earlier by Ncibi *et al* (2008).

Severe degenerative changes characterised by fatty liver degeneration and focular necrosis with lymphocytic infiltration was observed in liver sections of healthy animals fed on various volumes of cactus cladode extracts for varied period. Histopathology examination of kidney tissues of the healthy animals fed on cladode extracts showed minimal pathological changes in the renal architecture. Severe cellular damage was observed in kidney sections of diabetic untreated group which could have been due to oxidative stress by free radicals released during hyperglycaemia (El-Razek and Hassan, 2011). Diabetic animals fed on cladodes extracts for 10 days showed kidney tissues with a focal of necrosis characterised by mild glomerular infiltration and epithelial lining degeneration. No mortality was reported in the animals throughout the observation period of 30 days. This could suggest the damages observed in the hepatic and renal tissues may not have been fatal to the animals.

5.2 Conclusions

In the present study, there were significant reductions in blood sugar levels in alloxan-induced diabetic mice following treatment with cactus cladode extracts for 10 days as compared to the diabetic animals given cactus cladode extracts for 5 days. This would therefore imply that the efficacy of cactus cladode extract in managing diabetes mellitus is dependent on the duration of treatment.

Histopathalogical studies on the liver sections of healthy animals on cactus cladode extract revealed severe degenerative changes. In contrast, there was minimal alterations in the morphology kidney sections of these animals and also in liver and kidney sections of diabetic animals on cactus cladode extract. On the other hand, no mortality cases were reported in the present study. This would suggest that liver damages observed were not lethal to the animals.
The results of this study also showed insignificant changes in live body weights and packed cell volumes of the experimental animals. Consumption of herbal plants and/or drugs has been reported to alter the normal values of blood parameters (Ajagbonna *et al.,* 1999). Changes in blood parameters such as packed cell volumes are therefore used in safety evaluation studies of therapeutic agents including medicinal plants (Pankaj and Varma, 2013) as the haematological system has a higher predictive value for toxicity in humans especially when rodents and non-rodents are used in the study (Patil and Surana, 2010). So, from the data in the study it is concluded that prickly pear cactus cladode extracts from Kenya have potential to manage diabetes mellitus in humans in a dose- and time-dependent manner with minimal toxic effects on liver and kidneys of the animal.

5.3 Recommendations

The present study has demonstrated hypoglycaemic effect of prickly pear cactus cladode extracts on diabetic mice. Significant reductions in blood sugar levels of diabetic animals on cactus cladode extracts for 10 days were also reported. Based on these observations, it is recommended that an identification and analysis of bioactive substances present in prickly pear cactus cladodes responsible for the hypoglycaemic effect to be done. It is also recommended that the mode of action of these cladode extracts to be established.

The results have also shown a possible hepatoprotective potential of prickly pear cactus cladode extracts on liver and kidney tissues of the diabetic animals. The anti-oxidant activity that has been reported in prickly pear cactus might have contributed towards this effect by providing protection against damage caused by free radicals generated during diabetes mellitus. It is therefore suggested that analyses and quantification of the anti-oxidant compounds in the prickly pear cactus cladode should be done. It is also recommended that a biochemical analysis of parameters involved in kidney and liver functions to be done to ascertain our findings on histopathology studies. Lastly, since phytochemicals tend to vary from one region to another it is recommended another study on hypoglycaemic effect and cytotoxic evaluation to be done with prickly pear cactus cladodes from other regions in Kenya to assess whether they have the same effect as the ones from Baringo County.

REFERENCES

Abdulazeez, M.A., Kassim, I., Kenpia, B., Hope B. B. and Abdullahi,Y. (2013). Effect of combined use of *Ocimum gratissimum* and *Vernonia amygdalina* extract on the activity of angiotensin converting enzyme, hypolipidemic and antioxidant parameters in streptozotocin-induced diabetic mice. *African Journal of Biochemistry*, **7,**165 173.

Agius, L. (2007). New hepatic targets for glycaemic control in diabetes. *Clinical Endocrinology Metabolism*, **21**, 587-605.

Ajagbonna, O.P, Onifade, K.I. and Suleiman, U. (1999). Haematological and biochemical changes in rats given extract of *Calotropis procera. Sokoto Journal of Veterinary Science*, **1**, 36-42.

Alamgeer, Mushtaq, M.N., Bashir, S., Rashid, M., Malik, M.N.H., Ghumman, S.A., Irfan, H.M., Akram, M., Khan, A.Q. and Rashid, H.U. (2012). Hypoglycemic and hematological effects of aqueous extract of *Thymus serpyllum Linn*. in alloxan induced diabetic rabbits . *African Journal of Pharmacy and Pharmacology*, **6**, 2845 2850. DOI: 10.5897/AJPP12.1343

Almeida da Luz, S.C., Daubermaann, M.F., Thomé, G.R., Mülling dos Santos, M., Ramos, A., Salazar, G.T., Teixeira da Rocha, J.B and Barbosa, N.V. (2015). Diphenyl ditelluride intoxication triggers histological changes in liver, kidney, and lung of mice. *Analytical cellular pathology*, 784612. Doi: 10.1155/2015/784612

Al-Saadi, M.A.K., Al-Charrakh, A.H. and Al-Greti, S.H.H. (2011). Prevalence of bacteraemia in patients with diabetes mellitus in Karbala, Iraq. *Journal of Bacteriology Research*, **3**, 108-116

American Diabetes Association (2007). Standards of medical care in diabetes. *Diabetes Care,* **30,** 4–41.

American Diabetes Association (2007a). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **30,** S42-47.

American Diabetes Association (2010). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **33**, S62-S69

Andrade-Cetto, A. and Heinrich, M. (2005). Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *Journal of Ethnopharmacolology*, **99,** 325-348.

Awanish, P., Poonam, T., Rishabh, P., Rashmi, S. and Shambaditya, G. (2011). Alternative therapies useful in the management of diabetes: A systematic review. *Journal of Pharmacology and Bioallied Sciences,* **3,** 504–512.

Ayadi, M.A., Abdelmaksoud, W., Ennouri, M. and Attia, H. (2009). Cladodes from *Opuntia ficus indica* as a source of dietary fibre: effect on dough characteristics and cake making. *Industrial Crops and Products,* 40–47.

Balisa-Rocha, B.J., Guimarães, V.G., Mesquita, A.R., Aguiar, P.M., Krass, I. and Divaldo Pereira de Lyra, J.D.P. (2012). Enhancing health care for Type II diabetes in Northern Brazil: A pilot study of pharmaceutical care in community pharmacy. *AfricanJournal of Pharmacy and Pharmacology,* **6,** 2584-2591.

Bensadón, S, Hervert-Hernández, D, Sáyago-Ayerdi, S.G and Goñi, I. (2010). By-products of *Opuntia ficus indica* as a source of antioxidant dietary fiber. *Plant Foods for Human Nutrition,* **65**, 210-216.

Benseffaj, N., Brick, C., Atouf, O., Bourhanbour, D.A., Sanae, O. and Essakalli, M. (2012). Human leukocyte antigen (HLA) polymorphism and Type I diabetes in the Moroccan population. *African Journal of Biotechnology,* **11,** 16126-16131.

Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A. and Legssyer, A. (2006). Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). *International Journal of Diabetes and Metabolism,* **14**, 1-25.

Boada, C.A.C., & Martinez-Moreno, J.M. (2013). Pathophysiology of diabetes mellitus type 2: beyond the duo "insulin resistance-secretion deficit". *Nutricion Hospitalaria,* **28**, 78-87.

Boon, N.A., Colledge, N.R., Walker, B.R. and Hunter, J.A.A. (2006). *Principles and practice Davidson's medicine*. 20th edition, Churchill Livingstone, London.

Boutakiout,A, Elothmani D.D, Mahrouz,M, Hmid, I. and Hanine, H.(2015). Phytochemical constituents and in vitro radical scavenging activity of different cladodes extract of cultivars from different areas in Morocco . *International Journal of Technology Enhancements and Emerging Engineering Research,* **3**, 56-66.

Brahmi, D, Bouaziz, C, Ayed, Y, Ben Mansour, H, and Zourgui, L. (2011). Chemopreventive effect of cactus *Opuntia ficus indica* on oxidative stress and genotoxicity of aflatoxin B1.*Nutrition and Metabolism (Lond),* **8**, 73.

Broadhurst, C. L. (1997). Nutrition and non-insulin dependent diabetes mellitus from an anthropological perspective. *Alternative Medicine Review,* **2**, 378-399.

Bull, B.S. and Rittenbach, J.D. (1990). A proposed reference haematocrit derived from multiple MCHC determinations via haemoglobin measurements. *Clinical and Laboratory Haematology*, **12**, 43-53.

Burley, V.J, Paul, A.W. and Blundell, J.E. (1993). Sustained post-ingestive action of dietary fibre: effects of a sugar-beet-fibre-supplemented breakfast on satiety. *Journal of Human Nutrition and Dietetics*, **6**, 253–60.

Carlos, G.L., Anakaren, G., Nemesio, E., Israel, G.M. and Patricia, G. (2013). Incidence of Candida albicans in diabetic patients with a dental prosthesis in Northeast Mexico. *African Journal of Microbiology Research*, **7,** 4844-4847.

Castillo, G.E. (2002). Natural products in diabetes mellitus treatment. In: Lopez Castellano AC, Silvestre-Castellano MD, Villagrasa-Sebastian V. (Eds.) *Diabetes mellitus, Pathophysiology, therapeutic and social*, 49-61.

Causey, J.L., Feirtag, J.M., Gallaher, D.D., Tungland, B.C. and Slavin, J.L. (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research*, **20,** 191-201**.**

Cefalu,W.T., Waldman, S. and Ryder, S. (2007). Pharmacotherapy for the treatment of patients with type 2 diabetes mellitus: rationale and specific agents. *Clinical Pharmacology and Theraphy***, 81,** 636-649.

Chauhan, S.P., Sheth, N.R. and Suhagia, B.N. (2014). Haematinic evaluation of fruits of *Opuntia elatior mill* on mercuric chloride induced anemia in rats. *International Journal of Research in Ayurveda and Pharmacy*, **5**, 115-122.

Christensen, D.I., Friss, H., Mwaniki, D.L., Kilonzo, B., Tetens, I., Boit, M.K., Omondi, B., Kaduka, L. and Borch-Johnsen, K. (2009). Prevalence of glucose intolerance and associated risk factors in rural and urban populations of different ethnic groups in Kenya. *Diabetes Research and Clinical Practice***, 84**, 303–310.

Cicero, L. T. C., Yenshou, L., Arlene, P. B., Yi-Ching, C., Shao-Chih, C. and Wen-Chin, Y. (2013). Herbal therapies for type 2 diabetes mellitus: Chemistry, biology, and potential application of selected plants and compounds. *Evidence-Based Complementary and Alternative Medicine*, **2013**, 33.

Clara, S. (2013). Diabetes signs and symptoms. Accessed on 28th October, 2013 from http://www.diabetescare.com

Clement, S., Braithwaite, S.S., Magee, M.F., Ahmann, A., Smith, P.E., Rebecca, G.S. and Hirsch I.B. (2004). Management of diabetes and hyperglycemia in hospitals. *Diabetes Care*, **27**, 553-591.

Conrad, R. (2005). Medicinal herbs and their uses: Health benefits of cactus plants. Accesssed on 4th May, 2015 from https://www.richters.com/show.cgi?page=QandA/Medicinal/20050618-1.html

David, K.M., David, M.N. and Jean, E.M. (2014). Patient information: Diabetes mellitus type 2: Treatment (beyond the basics). Accessed on 3rd May, 2015 from http://www.uptodate.com/contents/diabetes-mellitus-type-2-treatment-beyond-the basics

DeFelice, M.S. (2004). Prickly pear cactus, *Opuntia* spp. A spine-tingling tale. *Weed Technology*, **18**, 869-877.

Dena, C. (2008). Prickly pear cactus in Kenya. Accessed on 15th February, 2015 from http://www.denacrain.com/blog/prickly-pear-cactus-in-kenya/

Diabetes UK (2015). Diabetes treatment. Accessed on 3rd May, 2015 from http://www.diabetes.org.uk/Guide-to-diabetes/What-is-diabetes/Diabetes-treatments/

Drugs.com (2015). Prickly pear uses, benefits and dosages. Accessed on 4th April, 2015 from http://www.drugs.com/npp/prickly-pear.html

Duke, J.A., Bogenschutz-Godwin, M.J., duCellier, J. and Duke, P.A.K. (2002). *Handbook of medicinal herbs.* CRC Press, Boca Raton, Florida (U.S.A.)

ED, E., Atsukwei, D., Adams, M.D., Tende, J.A., Malgwi, I.S. and Onuoha, T.N. (2015). Effects of alpha lipoic acid on blood glucose, body weight and haematological profile of streptozotocin-induced hyperglycaemia in wistar rats. *European Journal of Research in Medical Sciences*, **3**, 25-33.

Eduardo, M.S., Eduardo, M.B., Sandra, C.J., María, T. S, Karla, G. P. and José, A.M. (2013). The chemoprevention of chronic degenerative disease through dietary antioxidants: progress, promise and evidences.

El-Mostafa, K., Kharrassi, Y.E., Badreddine, A., Andreoletti, P.,Vamecq, J., Kebbaj,M'H.S.E., Latruffe, N., Lizard, G., Nasser, B. and Cherkaoui-Malki, M. (2014). Nopal cactus (*Opuntia ficus indica*) as a source of bioactive compounds for nutrition, health and disease. *Molecules*, **19**, 14879-14901.

El-Razek, A.H.F and Hassan, A.A. (2011). Nutritional value and hypoglycaemic effect of prickly cactus pear (*Opuntia ficus indica*) fruit extract in alloxan-induced diabetic rats. *Australian Journal of Basic and Applied Sciences*, **5**, 356-377.

El-Razek, A.H.F, El-Metwally, E.M., Shehab, G.M., Hassan, A.A. and Gomaa, A.M. (2012). Effects of cactus pear (*Opuntia ficus indica*) extract on oxidative stress in diabetic cataract mice. *Saudi Journal of Health Science*, **1**, 23-29.

El-safty, S.M.S. and Al-Masri, S.A. (2009). Effect of prickly pear consumption on some biological and biochemical changes in hyperglycemic injured rats. *Bulletin of the National Nutrition Institute,* (34), 46-61.

Engelgau, M.M. and Geiss, L.S. (2000). *The burden of diabetes mellitus. Medical management of diabetes mellitus*. Leahy, J.L., Clark, N.G. and Cefalu, W.T. (Eds.). New York, Mark Dekker, Inc.: 1-17.

Erdemoglu, N., Turan, N.N., Akkol, E.K., Sener, B. and AbacIoglu, N. (2009). Estimation of anti-inflammatory, antinociceptive and antioxidant activities on *Arctium minus (Hill) Bernh. ssp. minus. Journal of Ethnopharmacology,* **121,** 318-323.

Etuk, E.U. (2010). Animals models for studying diabetes mellitus. *Agriculture and Biology Journal of North America*, **1**, 130-134.

Factsheet (2011). *Opuntia ficus-indica* (sweet prickly pear). Accessed 2015 from http://keys.lucidcentral.org/keys/v3/eafrinet/weeds/key/weeds/Media/Html/Opuntia_ficus-indica (Sweet_Prickly_Pear).htm

Faraji, R., Rahimi, M.A., Rezvanmadani, F. and Hashemi, M. (2012). Prevalence of vaginal candidiasis infection in diabetic women. *African Journal of Microbiology Research,* **6,** 2773-2778.

Faure, P., Rossini, E., Wiernsperger, N., Richard, Mi., Favier, A., and Halimi, S. (1999). An insulin sensitizer improves the free radical defense system potential and insulin sensitivity in high fructose-fed rats. *Diabetes*, **48**,353-357.

Ferreira, F.M., Peixoto, F.P., Nunes, E., Sena, C., Seiça, R. and Santos, M.S. (2010). Inhibitory effect of *Arctium minus* on mitochondrial bioenergetics in diabetic Goto Kakizaki mice. *Scientific Research and Essay,* **5,** 2136-2142.

Frati, A.C., Jiménez, E. and Ariza, C.R. (1990). Hypoglycemic effect of *Opuntia ficus-indica* in non-insulin dependent diabetes mellitus patients. *Phytotherapy Research,* **4**,195-197.

Gagnier, J.J., DeMelo, J., Boon, H., Rochon. P. and Bombardier, C. (2006) Quality of reporting of randomized controlled trials of herbal medicine interventions. *American Journal of Medicine,* 119, 800.

Garcia-Compean, D., Jaquez-Quintana, J.O., Gonzalez-Gonzalez, J.A. and Maldonado-Garza, H. (2009). Liver cirrhosis and diabetes: Risk factors, pathophysiology, clinical implications and management. *World Journal of Gastroenterology*, **15**, 280-288.doi: 10.3748/wjg.15.280.

GISD (2005). Global invasive species database online data sheet. *Opuntia ficus-indica* (interim profile). Accessed on July, 2014 from http://www.issg.org/database.

Gohil, T., Pathak, N., Jivani, N., Devmurari, V. and Patel, J. (2010)Treatment with extracts of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts prevents hyperglycemia and hyperlipidemia in alloxan induced diabetic mice. *African Journal of Pharmacy and Pharmacology,* **4**, 270-275.

Goswami, R., Dadhwal, V., Tejaswi, S., Datta, K., Paul, A., Haricharan, R.N., Banerjee, U. and Kochupillai, N. (2000). Species-specific prevalence of vaginal candidiasis among patients with diabetes mellitus and its relation to their glycaemic status. *Infection Journal,* **41,** 6-162.

Gray, A.M. and Flatt, P.R. (1999). Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant coriander (*Coriandrum sativum*). *Brazilian Journal of Nutrition*, **81**,203-208.

Gribble, F.M., Tucker, S.J. and Seino, S. (1998). Tissue specificity of sulfonylureas: studies on cloned cardiac and beta-cell KATP channels. *Diabetes,* **47**, 1412–18.

Gupta, P.D.P. and De, A. (2012). Diabetes mellitus and its herbal treatment. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, **3**, 706-721.

Gurrieri, S., Miceli, L., Lanza, C.M., Tomaselli, F., Bonomo, R.P. and Rizzarelli, E. (2000). Chemical characterization of Sicilian prickly pear (*Opuntia ficus indica*) and perspectives for the storage of its extract. *Journal of Agriculture and Food Chemistry*, **48**, 5424-5431.

Hahm, S.W., Park, J. and Son, Y.S. (2011). *Opuntia humifusa* stems lower blood glucose and cholesterol levels in streptozotocin-induced diabetic rats. *Nutrition Research,* **31**, 479 87.

Health.com (n.d). Signs of diabetes. Accessed on 28th October, 2013 from http://www.health.com/health/m/gallery/0,,20442821_8,00.html/

Herbs (2012). Medicinal Value of Cactus. Accessed on 4th May, 2015 from http://herbs.ygoy.com/medicinal-value-of cactus/

Hernández-Ávila, M. and Olaíz-Fernández, G. (2002). Diabetes and Mexico: a public health challenge. *Science,* **53,** 8-17.

Houacine, C., Elkhawad, A.O. and Ayoub, S.M.H. (2012). A comparative study on the anti-diabetic activity of extracts of some Algerian and Sudanese plants. *Journal of Diabetes and Endocrinology*, **3,** 25-28**.**

Hunt D, Taylor, N.P. and Charles, G. (2006). The new cactus lexicon. DH Books, Milborne Port, UK.

Hwang, J-H, Yi, M-R, Kim, J-W, Bu, H-J, Kang, C-H. and Lim, S-B. (2015). Quality characteristics and antioxidant activity of prickly pear cactus cladodes. *Journal of the Korean Society of Food Science and Nutrition,* **44**, 356-362. DOI: 10.3746/jkfn.2015.44.3.356

International Diabetes Federation (IDF) (2013). Types of diabetes. Accessed on 28th October, 2013 from http://www.idf.org/types-diabetes

International Diabetes Federation (IDF) (2013a). Complications of diabetes. Accessed on 28th October, 2013 from http://www.idf.org/complications

International Diabetes Federation (IDF), (2013b). *Diabetes Atlas*, Sixth edition. Accessed on 23rd January, 2015 from http://www.idf.org/.../IDF%20Diabetes%2013

James, S.T. (2000). Infectious complications in patients with diabetes mellitus. *International Diabetes Monitor*, **12,** 33-39.

Jones, L.E.T. (2013). Diabetes mellitus: the increasing burden of disease in Kenya.

Jun, H, Cha, E-I, Yang, E-I, Choi, D.G. and Kim, Y-S. (2013). Physicochemical properties and antioxidant activity of Korean cactus (*Opuntia humifusa*) cladodes. *Horticulture, Environment and Biotechnology*, **54**, 288-295.

Kang'ara, J.N.N. and Gitari, J.N. (n.d). Exploiting fodder potentials of Cactus (*Opuntia* spp) in Kenya for pastoral livestock feeding under a changing climate.

Kasper, D.L., Braunwald, E., Fauci, S.A., Hanser, S.L., Longo, D.L. and Jameson, J.L. (2005). *Harrison's Principle of internal medicine*. 2nd edition, volume 16, 2067, McGraw-Hill Medical Publishing Division, London.

Kaur, T., Bishnoi, D. and Badaruddoza (2010). Effect of sex on prevalence of Type II diabetes mellitus (T2DM) with respect to blood pressure, BMI and WHR among Punjabi population. *International Journal of Medicine and Medical Sciences,* **2,** 263-270.

Kaur,M., Kaur, A. and Sharma, R. (2012). Pharmacological actions of *Opuntia ficus indica*:A review. *Journal of Applied Pharmaceutical Science*, **02**, 15-18.

Khan, A., Bryden, N.A., Polansky, M.M and Anderson, R.A. (1990). Insulin potentiating factor and chromium content of selected food and spices. *Biology of Trace Elements Research*, **24**, 183-188.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, **51,** 216. doi:10.1007/s00125-007-0886-7

Li, D., Kim, J.M., Jin, Z. and Zhou, J. (2008). Prebiotic effectiveness of inulin extracted from edible burdock. *Anaerobe*, **14,** 29-34.

Lyamuya, E.F., Moyo, S.J., Komba, E.V. and Haule, M. (2011). Prevalence, antimicrobial resistance and associated risk factors for bacteriuria in diabetic women in Dar es Salaam, Tanzania. *African Journal of Microbiology Research*, **5,** 683-689.

Madrigal-Santillán, E., Garcia-Melo, F., Morales-González, J.A., Vázquez-Alvarado, P., Muñoz-Juárez, S., Zuñiga-Pérez, C., Maria Teresa Sumaya-Martínez, M.T., Madrigal-Bujaidar, E. and Hernández-Ceruelos, A. (2013)**.** Antioxidant and anticlastogenic capacity of prickly pear extract. *Nutrition*, **5**, 4145–4158. Doi: 10.3390/nu5104145

Mathers, C.D. and Loncar, D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Medicine*, **3**, 442

Mbaya, J. and Ramiaya, K. (2006) *Disease and mortality in sub-Saharan Africa*. The World Bank publication, 19.

Meckes-Lozyoa, M., Roman-Ramos, R., (1986). *Opuntia streptacantha,* a coadjutor in the treatment of diabetes mellitus. *American Journal of Chinese Medicine*, **14**, 116-118.

Ncibi, S, Othman, M.B, Akacha, A, Krifi, M.N. and Zorgui, L. (2008). *Opuntia ficus indica* extract protects against chlorpyrifos-induced damage on mice liver. *Food and Chemical Toxicology*, **46**, 797–802.

Nefzaoui, A., Nazareno, M. and El Mourid, M. (2007). Review of medicinal uses of cactus. *Cactusnet*, **11**, 3-17.

Nelson, R.W., Ihle, S.L.,Lewis, L.D., Salisbury, S.K., Miller, T., Bergdall, V. and Bottoms, G.D. (1991). Effects of dietary fiber supplementation on glycemic control in dogs with alloxan-induced diabetes mellitus. *American Journal of Veterinary Research,* **52**, 2060-2066.

Nicolle, L.E. (2005). Urinary tract infection in diabetes. *Current Opinion on Infectious Diseases*, **18,** 49-53.

Nirmala, A., Saroja, S., Vasanthi, H.R. and Lalitha, G. (2009). Hypoglycemic effect of *Basella rubra* in streptozotocin – induced diabetic albino Mice. *Journal of Pharmacognosy and Phytotherapy,* **1,** 025-030.

Norris, L. and Media, D. (n.d.).Types of Prickly Pear Cactus. Accessed 4th April, 2015 from http://homeguides.sfgate.com/types-prickly-pear-cactus-40430.html

Nowakowska, D., Kurnatowska, A., Stray-Pedersen, B. and Wilczyński, J. (2004). Species distribution and influence of glycemic control on fungal infections in pregnant women with diabetes. *Journal of Infection*, **48,** 339-346.

Ophardt, C.E. (2003). Diabetes - Errors of metabolism.

Oršolić, N., Sirovina, D., Končić, M.Z., Lacković, G. and Gregorović, G. (2012).Effect of *Croatian propolis* on diabetic nephropathy and liver toxicity in mice. *BMC Complementary and Alternative Medicine*, **12**,117.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. (2009). Agroforestry Database: a tree reference and selection guide version 4.0. Accessed on 13th may, 2015 from http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp

Osuna-Martínez, U., Reyes-Esparza. J. and Rodríguez-Fragoso. L. (2014). Cactus (*Opuntia ficus-indica*): A review on its antioxidants properties and potential pharmacological use in chronic diseases. *Natural Products Chemistry and Research,* **2**, 153.doi:10.4172/2329-6836.1000153

Oyedemi, S.O., Adewusi, E.A., Aiyegoro, O.A. and Akinpelu, D.A. (2011). Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pacific *Journal of Tropical Biomedicine*, 1, 353–358. Doi: 10.1016/S2221-1691(11)60079-8

Paiz, R.C., Juárez-Flores, B.I., Rivera, J.R.A., Ortega, N.C.C., Agüero, J.A.R., Chávez, E.G. and Fuentes, G.A. (2010). Glucose-lowering effect of xoconostle (*Opuntia joconostle* A. Web., Cactaceae) in diabetic mice. *Journal of Medicinal Plants Research,* **4,**2326-2333.

Pankaj, P.P, and Varma, M.C. (2013). Potential role of *Spirulina platensis* in maintaining blood parameters in alloxan induced diabetic mice. *International Journal of Pharmacy and Pharmaceutical Sciences,* **5**,450-456.

Patil, P.H. and Dr. Surana S.J. (2010).Toxicological evaluations of *Eranthemum roseum R.* Br Linn root extracts. *International Journal of PharmTech Research*, **2**, 753-759.

Patterson, C.C., Dahlquist, G.G., Gyürüs, E., Green, A. and Soltész, G. (2009). Study Group Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. *Lancet*, **373,** 2027-2033.

Pereira, C.A., Pereira, L.L.S. and Corrêa, A.D. (2010). *Hoodia gordonii* in the treatment of obesity: A review. *Journal of Medicinal Plants Research*, **4,** 2305-2312.

Peters, A.L., Davidson, M.B., Schriger, D.L. and Hasselblad, V. (1996). A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated haemoglobin levels. Meta-analysis research group on the diagnosis of diabetes using glycated hemoglobin levels. *Journal of American Medical Association*, **276,** 1246-1252.

PhotoStock-Israel and World of Stock (2013). Kenya, Lake Naivasha, Kenya, Blooming prickly pear cactus cactaceae *Opuntia* stock photography-world of stock.

Plagens, M.J. (2011). Prickly pear cactus in Kenya. Accessed on 21st April, 2015 from http://www.ngkenya.com/flora/Opuntia-01.html

Plants for a future (2012). *Opuntia ficus-indica* prickly pear, barbary fig PFAF plant database.Accessed2015fromhttp://www.pfaf.org/user/Plant.aspx?LatinName=Opuntia+ficus indica

Rachid, A., Rabah, D., Farid, L., Zohra, S.F., Houcine, B. and Nacéra, B. (2012). Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. *Journal of Medicinal Plants Research,* **6,** 2041-2050.

Rave, U. (2012). Medicinal uses of cactus plants. Accessed on 4th May, 2015 from http://www.buzzle.com/articles/medicinal-uses-of-cactus-plants.html

Ravi, K., Sivagnanam, K. and Subramanian, S. (2004c). Anti-diabetic activity of *Eugenia jambolana* seed kernels on streptozotocin-induced diabetic mice. *Journal of Medicinal Food,* 7**,** 187–191.

Rohilla, A. and Ali, S. (2012). Alloxan induced diabetes: *Mechanisms and Effects. International Journal of Research in Pharmaceutical and Biomedical Sciences*, **3**, 819-823.

Saleem, M., Kim, H.J., Han, C.K., Jin, C. and Lee, Y.S. (2006). Secondary metabolites from *Opuntia ficus-indica* var. saboten . *Phytochemistry*, **67**, 1390-1394.

Samanta, D., Mallika, V. and Yadav, S. (2012). Early microalbuminuria in adolescent Type 1 diabetic patients: Experience from a paediatric endocrine clinic in a developing country Depobam. *Journal of Diabetes and Endocrinology,* **3,** 6-10.

Samreen, R. (2009). Diabetes mellitus. *Scientific Research and Essay,* **4,** 367-373.

Sandra G.S.R, Omar G. and Ruy, L.R. (2009). Diabetes treatment and control: the effect of public health insurance for the poor in Mexico. *Bull World Health Organ,* **87,** 512 519.

Slavin, J. and Green, H. (2007), Dietary fibre and satiety. *Nutrition Bulletin*, **32**, 32–42. doi: 10.1111/j.1467-3010.2007.00603.x

Sloan, E. (1994). Top ten trends to watch and work on. *Food Technology***, 7**, 89-100.

Stull, A.J., Cash. K. C., Johnson, W.D., Champagne, C.M. and Cefalu, W.T. (2010). Bioactives in blueberries improve insulin sensitivity in obese, insulin-resistant men and women. *Journal of Nutrition*, **140**, 1764-68.

Synman, H.A. (2006). A greenhouse study on root dynamics of cactus pears, Opuntia ficus indica and O. robusta. *Journal of Arid Environment*, **65**, 529-542.

Tahmasebpour, N., Dehghan, G., Feizi, M.A.H. and Esmaeili, H.A. (2013). Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with *Teucrium orientale*. *PharmacologyOnLine*, **3**, 32 – 36.

Talking diabetes (2012). Medications for Type 2 diabetes. A diabetes information series from Diabetes State/Territory Organisations. Issue Number 25. Accessed on 22nd April, 2015 from http://www.diabetesvic.org.au

Taylor, R. (2013). Type 2 Diabetes: Etiology and reversibility. *Diabetes care*, **36**, 1047-1055.

Thevenod, F. (2008). Pathophysiology of diabetes mellitus type 2: Roles of obesity, insulin resistance and Beta-cell dysfunction. *Diabetes and Cancer,* **19**, 1-18.

Uebelhack, R., Busch, R., Alt, F., Beah, Z-M. and Chong, P-W. (2014). Effects of Cactus Fiber on the Excretion of Dietary Fat in Healthy Subjects: A double blind, randomized, placebo-controlled, crossover clinical investigation. *Current Therapeutic Research*, **76**, 39–44.

Ukwueze, S.C. and Anene, B.M. (2010). The effects of diabetes mellitus on the pathogenicityof trypanosomosis in mice. *Journal of Veterinary Medicine and Animal Health*, **3,** 40- 44.

Wermeille, J., Bennie, M., Brown, I. and McKnight, J. (2004). Pharmaceutical care model for patients with type 2 diabetes: integration of the community pharmacist into the diabetes team - a pilot study. *Pharmaceutical World Science*, **26,**18-25.

Whiting, D.R., Guariguata, L., Weil, C. and Shaw, J. (2011). IDF Diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetic Researchers and Clinical Practitioners,* **94,** 311-321.

Wie, M.B (2000). Protective effects of *Opuntia ficus-indica* and *Saururus chinensis* on free radical induced neuronal injury in mouse cortical cell cultures. *Journal of the Pharmaceutical Society of Korea,* **44**, 613-619.

Wild, S., Roglic, G.A., Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, **27,** 1047 1053.

William, G. and Pickup, J.C. (2004). *Handbook of Diabetes*: Blackwell Publishing.

Wolfram, R.M., Kritz, H., Schmid, P., Efthimiou, Y., Stamatopoulos, Y. and Sinzinger, H. (2002). Effect of prickly pear (*Opuntia robusta*) on glucose- and lipid-metabolism in non-diabetics with hyperlipidemia.

World Health Organization (1994). Management of Diabetes Mellitus Standards of Care and Clinical Practice Guidelines. WHO-EM/DIN6/E/G. Accessed on 23rd January, 2015 from http://www.emro.who.int/dsaf/dsa509.pdf.

World Health Organization (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Report of a WHO Consultation, Part 1: *Diagnosis and Classification of Diabetes Mellitus*, 1- 49.

World Health Organization (2005). Definition and Diagnosis of Diabetes Mellitus and Intermediate hyperglycemia, Report of a WHO/IDF consultation.

World Health Organization (2010). Global Status Report on non-communicable disease. Accessed on 23rd January, 2015 from http://www.who.int/nmh/publications/ncd_report_full_en.pdf.

World Health Organization (2011). Diabetes. *Fact sheet* Number 312.

World Health Organization, (2011a). Global status report on Non-Communicable Diseases.WHO, Geneva. Accessed on 23rd January, 2015 from http://*www.who.int/nmh/.../ncd_report_full_en.pdf.*

World Health Organization, (2013) Diabetes Facts sheet Number 312. Accessed on 23rd, January, 2015 from http://www.who.int/mediacentre/factsheets/fs312/en/

Yorek, M.A. (2003). The role of oxidative stress in diabetic vascular and neural disease. *Free Radical Research*, **37,**471-480.

APPENDICES

Appendix 1: ANOVA table of blood sugar levels of diabetic animals on the 7th day of feeding on cladode extracts.

ANOVA

Blood sugar

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	63656.333	1	63656.333	9.449	.012
Within Groups	67367.333	10	6736.733		
Total	131023.667	11			

Appendix 2: ANOVA table of blood sugar levels of diabetic animals on the 10th day of feeding on cladode extracts.

ANOVA

Blood sugar

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	64848.109	1	64848.109	23.167	.001
Within Groups	25192.800	9	2799.200		
Total	90040.909	10			

Appendix 3: (a) ANOVA table, (b) Post hoc tests table, of blood sugar levels of diabetic animals on the 3rd day after termination of feeding on cladode extracts.

ANOVA

Blood sugar

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	104517.717	2	52258.859	6.419	.011
Within Groups	113972.048	14	8140.861		
Total	218489.765	16			

Multiple Comparisons

Dependent Variable: Blood sugar

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	2	3	118.095	50.198	.081	-13.29	249.48
		4	193.929*	56.553	.011	45.91	341.94
	3	2	-118.095	50.198	.081	-249.48	13.29
		4	75.833	58.241	.417	-76.60	228.27
	4	2	-193.929*	56.553	.011	-341.94	-45.91
		3	-75.833	58.241	.417	-228.27	76.60

*. The mean difference is significant at the 0.05 level.

Group 2 (Positive/diabetic control animals, induced with diabetes not given cladode extracts), Group 3 (Diabetic animals fed on cladode extracts for 5 days) and Group 4 (Diabetic animals fed on cladode extracts for 10 days).

Appendix 4: (a) ANOVA table and (b) Post hoc tests table of live body weights of healthy mice fed on cactus cladode extracts for 5 days on the 3rd day of feeding.

ANOVA

Body weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	37.444	2	18.722	3.928	.042
Within Groups	71.500	15	4.767		
Total	108.944	17			

Multiple Comparisons

Dependent Variable: Body weight

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	3.500*	1.261	.036	.23	6.77
	3	2.167	1.261	.231	-1.11	5.44
2	1	-3.500*	1.261	.036	-6.77	-.23
	3	-1.333	1.261	.554	-4.61	1.94
3	1	-2.167	1.261	.231	-5.44	1.11
	2	1.333	1.261	.554	-1.94	4.61

*. The mean difference is significant at the 0.05 level.

Group 1 (Negative control animals, not given cladode extracts), Group 2 (Healthy animals fed on 0.6 ml cladode extracts for 5 days) and Group 3 (Healthy animals fed on 0.8 ml cladode extracts for 5 days).

Appendix 5: (a) ANOVA table and (b) Post hoc tests table of live body weights of healthy mice fed on cactus cladode extracts for 5 days on the 5th day of feeding

ANOVA

Body weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	36.111	2	18.056	4.440	.031
Within Groups	61.000	15	4.067		
Total	97.111	17			

Multiple Comparisons

Dependent Variable: Body weight

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	3.333*	1.164	.030	.31	6.36
	3	2.500	1.164	.114	-.52	5.52
2	1	-3.333*	1.164	.030	-6.36	-.31
	3	-.833	1.164	.758	-3.86	2.19
3	1	-2.500	1.164	.114	-5.52	.52
	2	.833	1.164	.758	-2.19	3.86

*. The mean difference is significant at the 0.05 level.

Group 1 (Negative control animals, not given cladode extracts), Group 2 (Healthy animals fed on 0.6 ml cladode extracts for 5 days) and Group 3 (Healthy animals fed on 0.8 ml cladode extracts for 5 days).

Appendix 6: (a) ANOVA table and (b) Post hoc tests table of live body weights of healthy mice fed on cactus cladode extracts for 5 days on the 3rd day after termination of feeding

ANOVA

Body weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.778	2	25.389	6.604	.009
Within Groups	57.667	15	3.844		
Total	108.444	17			

Multiple Comparisons

Dependent Variable: Body weight

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	4.000*	1.132	.008	1.06	6.94
	3	2.833	1.132	.060	-.11	5.77
2	1	-4.000*	1.132	.008	-6.94	-1.06
	3	-1.167	1.132	.570	-4.11	1.77
3	1	-2.833	1.132	.060	-5.77	.11
	2	1.167	1.132	.570	-1.77	4.11

*. The mean difference is significant at the 0.05 level.

Group 1 (Negative control animals, not given cladode extracts), Group 2 (Healthy animals fed on 0.6 ml cladode extracts for 5 days) and Group 3 (Healthy animals fed on 0.8 ml cladode extracts for 5 days).

Appendix 7: (a) ANOVA table and (b) Post hoc tests table of live body weights of healthy mice fed on cactus cladode extracts for 10 days on the 7th day of feeding

ANOVA

Body weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	56.778	2	28.389	5.301	.018
Within Groups	80.333	15	5.356		
Total	137.111	17			

Multiple Comparisons

Dependent Variable: Body weight

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	4.167*	1.336	.018	.70	7.64
	3	3.167	1.336	.076	-.30	6.64
2	1	-4.167*	1.336	.018	-7.64	-.70
	3	-1.000	1.336	.739	-4.47	2.47
3	1	-3.167	1.336	.076	-6.64	.30
	2	1.000	1.336	.739	-2.47	4.47

*. The mean difference is significant at the 0.05 level.

Group 1 (Negative control animals, not given cladode extracts), Group 2 (Healthy animals fed on 0.6 ml cladode extracts for 10 days) and Group 3 (Healthy animals fed on 0.8 ml cladode extracts for 10 days).

Appendix 8: (a) ANOVA table and (b) Post hoc tests table of live body weights of healthy mice fed on cactus cladode extracts for 10 days on the 10th day of feeding

ANOVA

Body weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.937	2	15.969	5.515	.017
Within Groups	40.533	14	2.895		
Total	72.471	16			

Multiple Comparisons

Dependent Variable: Body weight

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	3.067*	1.030	.025	.37	5.76
	3	2.667*	.982	.042	.10	5.24
2	1	-3.067*	1.030	.025	-5.76	-.37
	3	-.400	1.030	.921	-3.10	2.30
3	1	-2.667*	.982	.042	-5.24	-.10
	2	.400	1.030	.921	-2.30	3.10

*. The mean difference is significant at the 0.05 level.

Group 1 (Negative control animals, not given cladode extracts), Group 2 (Healthy animals fed on 0.6 ml cladode extracts for 10 days) and Group 3 (Healthy animals fed on 0.8 ml cladode extracts for 10 days).

Appendix 9: List of publications

1. The Prickly Pear Cactus (*Opuntia Ficus-Indica*) Cladode Extracts Modulate Blood Sugar in Swiss white Albino Mice.

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The Prickly Pear Cactus (Opuntia Fiscus-Indica) Cladode Extracts Modulate Blood Sugar in Swiss White Albino Mice

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Abstract **Background and purpose**: Medicinal plants including the prickly pear cactus have been reported to modulate blood sugar levels. Extracts of prickly pear cactus have been used in various parts of the world to manage diabetes mellitus. However the cactus is viewed as a weed in Kenya. The current study therefore aimed at evaluating the efficacy of prickly pear cactus cladode extracts from Kenya in managing diabetes mellitus in induced mice. **Methods**: Healthy, adult Swiss white albino male mice weighing 20-30g were induced with diabetes mellitus using Alloxan (150 mg/kg body weight) administered intra-peritoneally. Prickly pear cactus cladode extracts were administered at daily dosage of 0.6 ml for pre-determined periods. Fasting blood sugar levels and live body weights were monitored at intervals of 72 hours throughout the experimental period of 30 days. **Results**: Alloxan administration resulted in about 3- to 4-fold increase in blood sugar levels. Treatment of diabetic mice with cactus cladode extracts led to decline in blood sugar levels of the animals, however, the levels varied with the period of treatment. Diabetic animals treated with cactus cladode extracts for 10 days showed a significant decline in blood sugar levels on the 7th ($p=0.012$) and 10th ($p=0.001$) days of feeding on the extracts when compared to the positive control (diabetic, not treated) animals. **Conclusions**: This study has demonstrated that extracts of prickly pear cactus cladodes from Kenya have potential in managing blood sugar in diabetic mice.

Keywords Alloxan, Opuntia, Prickly pear cactus, Cactus cladodes, Diabetes

1. Introduction

Diabetes mellitus is considered one of the major public health concerns globally [1] and ranks 4th among non-communicable diseases [2] because its effects reach multiple organs causing serious heath complications ranging from brain damage, cardiovascular diseases, renal failure and death [3]. The disease is characterized by hyperglycemia associated with abnormal lipid, protein and carbohydrate metabolism [4]. Two major types of diabetes mellitus have been characterized by the World Health Organization based on their morbidity and mortality rates: Type I diabetes mellitus, which accounts for 10% and Type II that accounts for 90% of all the diabetic cases in the world [5] [6] [7]. Type 1 diabetes is often caused by a deficiency in insulin production while Type II occurs as a result of ineffective use of insulin by the body [8]. Studies by the World Health Organization and International Diabetes Federation indicate that the number of people suffering from diabetes across the world has increased significantly over recent years. For instance, in the year 2000,171 million cases of diabetes were recorded worldwide [9]. Recent studies show there are 382 million diabetic patients globally. This figure is likely to increase to 592 million by the year 2035. This will represent a prevalence of 10.1%, an increase from 8.3% in 2013 [10].

The management of diabetes mellitus is a lifelong process that requires constant medication and specialized health care [11]. While people with type 1 diabetes mellitus require regular insulin intake, a lifestyle change to include healthy diet and physical exercise is key to managing type 2 diabetes mellitus. Two major classes of oral drugs are prescribed for diabetic patients because of their affordability and mild side effects: the biguanides such as metformin and phenformin and the sulphonylureas such as gilbenclamide, diabenese, orinase and glucotrol Amaryl [12]. Other classes of oral pills include thiazolidinediones, alpha-glucosidase inhibitors, glucagon like peptides-1(GLP-1) receptor agonists and megilitinides. While these approaches are promising in management of diabetes mellitus, they are costly and can

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3. Safety Evaluation of Prickly Pear Cactus Cladodes in Swiss White Albino Mice (In press).

Appendix 10: Institute of Animal Care and Use Committee approval letter

KENYA AGRICULTURAL RESEARCH INSTITUTE

Trypanosomiasis Research Centre, MUGUGA

P.O. BOX 362, KIKUYU, 00902, TEL: 020-2700604, 2700654, FAX: 066-32397

KIKUYU

Email:Centre@ Kari-trc.org, trccd@kari-trc.org

ef: C/TR/4/325/149 4th March 2014

ARI-TRC Animal Care and Use Committee (IACUC)

M/S Peris Mokua and Dr Grace Murilla (Principal Investigators)

Dear Peris and Grace

RE: IACUC Approval for research on Antidiabetic Properties of Pricky Pear Cactus

The Institutional Animal Care and Use Committee (IACUC) of the Kenya Agricultural Research Institute-Trypanosomiasis Research Centre (KARI-TRC) has reviewed your MSc proposal entitled "**Assessment of anti-diabetic properties of the Pricky Pear Cactus in Swiss White Mice**". The committee notes that this MSc project addresses one component in a broader project "Analysis of anti-diabetic compounds in Opuntia species and camel milk in arid and semi-arid lands in Kenya" that was reviewed and approved for implementation in August 2013 (C/TR/4/325/142). The committee has further found that the methods described for the in vivo component of your study are appropriate and are consistent with its recommendations as well as National (KVA) standards of animal welfare. The committee has therefore approved your study and further wishes to emphasize that it will hold you (the PIs) personally responsible to ensure that high standards of animal welfare are observed at all times of the project. The committee may make impromptu visits to ensure compliance with its regulations.

Yours Sincerely

for

Charles Otieno

Ag IACUC Chairman, KARI-TRC

Appendix 11: Research Permit

Kenya Agricultural & Livestock Research Organization
Biotechnology Research Institute Headquarters, Muguga
P.O. Box 362, Kikuyu – 00902

Our Ref: KARI/1/1 October 30, 2016

TO WHOM IT MAY CONCERN

RE: COLLECTION OF PRICKLY PEAR CACTUS (OPUNTIA) CLADODE SAMPLES FROM BARINGO COUNTY

This is to confirm that the Opuntia samples used by the student (Peris Moraa Mokua-SM14/3374/12) were obtained from Baringo County through Kenya Forestry Research Institute (KEFRI). Mr. Oscar Mayunzu, a research scientist at KEFRI, and co-author on Ms. Mokua's published paper, has an on-going project exploring the nutritional aspects of Opuntia collected from different parts of the country. This research falls within KEFRI's institutional mandate 'to undertake research in forestry and allied natural resources'. Ms. Mokua used some of the samples which were collected by Mr. Mayunzu to enable her explore the medicinal value of Opuntia. The work carried out by the two was complementary. Mr Mayunzu is in the process of preparing a manuscript on Opuntia fruit as a nutritional supplement.

Ms. Mokua, therefore did not require a permit for sample collection. The work was done as collaborative project with KEFRI.

Thank you

Dr. Grace Murilla
Director, Biotechnology Research Institute - KALRO